Antinociceptive Profile of 2,3,6-Trisubstituted Piperidine Alkaloids: 3-*O*-Acetyl-spectaline and Semi-synthetic Derivatives of (−)-Spectaline

Cláudio Viegas, Jr.,[a] Magna Suzana Alexandre-Moreira,[b] Carlos Alberto Manssour Fraga,[c] Eliezer Jesus Barreiro,[c] Vanderlan da Silva Bolzani,[d] and Ana Luísa Palhares de Miranda*,[c]

[a] *Laboratório de Fitoquímica e Química Medicinal, Departamento de Ciências Exatas, Universidade Federal de Alfenas; 37130–000, Alfenas, MG, Brazil:* [b] *Laboratório de Farmacologia e Imunidade, Departamento de Fisiologia, Centro de Ciências Biológicas, Universidade Federal de Alagoas; Maceió, Al, Brazil:* [c] *Laboratório de Avaliação e Síntese de Substâncias Bioativas, Departamento de Fármacos, Faculdade de Farmácia, Universidade Federal do Rio de Janeiro; 21941–902, Rio de Janeiro, RJ, Brazil: and* [d] *Núcleo de Bioensaios, Biossíntese e Ecofisiologia de Produtos Naturais, Instituto de Química, Universidade Estadual Paulista; 14800–900, Araraquara, SP, Brazil.*

Received July 2, 2007; accepted January 24, 2008; published online January 28, 2008

In early studies, we have reported the antinociceptive profile of (−)-spectaline, a piperidine alkaloid from *Cassia spectabilis*. The present study describes the synthesis, the antinociceptive and anti-inflammatory activities of a series of 2,3,6-trialkyl-piperidine alkaloids: the natural (−)-3-*O*-acetyl-spectaline (LASSBio-755) and ten semi-synthetic spectaline derivatives. Structure–activity relationship (SARs) studies were performed. The structures of all synthesized derivatives were confirmed by means of nuclear magnetic resonance. Compounds were evaluated for their analgesic (acetic acid-induced mouse abdominal constrictions, hot-plate test, formalin-induced pain test) and some of them for the anti-inflammatory activities (carrageenan-induced rat paw edema test). The pharmacological results showed that several of the new compounds given orally at a dose of 100 μmol/kg significantly inhibited the acetic acid-induced abdominal constrictions, but they were less active than (−)-spectaline. LASSBio-755 and LASSBio-776 were the most actives with 37% and 31.7% of inhibition. In the formalin-induced pain only LASSBio-776 was able to inhibit by 34.4% the paw licking response of the inflammatory phase, (−)-spectaline and LASSBio-755 did show any activity. In the carrageenan-induced rat paw edema, only (−)-spectaline exhibited an anti-inflammatory profile, showing an ED_{50} value of 56.6 μmol/kg. Our results suggest different mechanisms of action for the analgesic activity observed for LASSBio-776 (3-*O*-Boc-spectaline), LASSBio-755 (3-*O*-acetyl-spectaline) and (−)-spectaline (LASSBio-754). The antinociceptive profile of some of the semi-synthetic spectaline derivatives extends our research concerning the chemical and pharmacological optimization of isolated natural products in the search of new drug candidates from brazilian biodiversity.

Key words *Cassia spectabilis*; piperidine alkaloid; (−)-3-*O*-acetyl-spectaline; antinociceptive; anti-inflammatory

Several species of *Cassia* are widely used in traditional medicine for their antimicrobial, laxative, antiulcerogenic, analgesic and anti-inflammatory properties, which could be correlated with the accumulation of phenolic compounds in their bioactive extracts.[1—5] In previous investigations, we reported the isolation, the structural elucidation and the bioactivity profile of (−)-spectaline and other related piperidine alkaloids from *Cassia spectabilis* (sin. *Senna spectabilis*).[6,7] More recently we have identified (−)-spectaline (**LASSBio-754**, Chart 1) as a potent antinociceptive agent in the

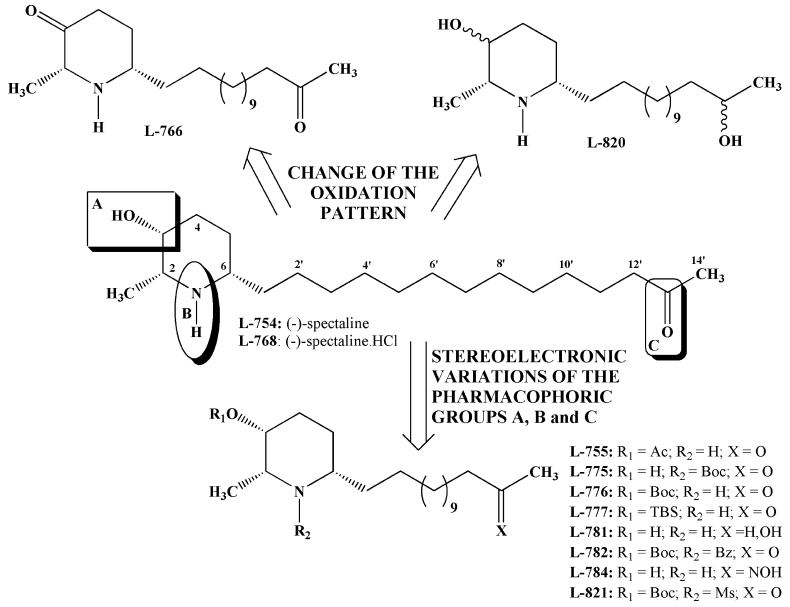

Chart 1. Design Concept of the New 2,3,6-Trisubstituted Piperidine Derivatives

* To whom correspondence should be addressed. e-mail: analu@pharma.ufrj.br

writhing test (ED_{50}=48.5 μmol/kg) and capsaicin induced pain (ED_{50}=20.8 μmol/kg).[8)]

Extending our research program concerning the bioprospection of natural products and its derivatives, we have selected, to conclude this investigation, another abundant piperidine alkaloid, (−)-3-*O*-acetyl-spectaline (**LASSBio-755**, Chart 1) and synthesized other 10 semi-synthetic derivatives prepared from natural (−)-spectaline, *i.e.* a series of 2,3,6-trisubstituted piperidine alkaloids. The present study was focused on the synthesis, evaluation of antinociceptive and anti-inflammatory properties and structure–activity relationship (SAR) analysis of these new derivatives in order to verify the stereoelectronic contributions and requirements of the pharmacophoric groups A, B and C for the antinociceptive and anti-inflammatory activities (Chart 1).

Results and Discussion

The antinociceptive profile of all new 2,3,6-trisubstituted piperidine derivatives developed herein was initially evaluated using classical acetic acid-induced mouse abdominal constriction test,[9)] with indomethacin as standard. The results are disclosed in Fig. 1. Some of the new compounds given orally at a dose of 100 μmol/kg significantly inhibited the acetic acid-induced abdominal constrictions, but they were less active than (−)-spectaline and indomethacin. Among them, the natural acetate derivative (**LASSBio-755**) and its *O*-Boc analogue (**LASSBio-776**) were the most actives with 37% and 31.7% of inhibition. Based on these results some preliminary structure–activity relationships were analysed. Comparing the inhibitory profile of compounds **LASSBio-782**, **LASSBio-821** and **LASSBio-775** with **LASSBio-776** and **LASSBio-755** indicates that the presence of groups that blocked the hydrogen bond donnor character of the piperidine nitrogen seems to be deletery for the analgesic activity. On the other hand, the antinociceptive activity showed to be inversely dependent of the volume of the groups at C-3 (OR_1 group) once that the inhibition of the constrictions follows the order R_1=H>Ac>Boc. The introduction of more polar and hydrogen bond-donating hydroxy groups at C-13′ in the alcohol or oxime derivatives, *i.e.* **LASSBio-781**, **LASSBio-820** and **LASSBio-784**, did not result in a increase of the antinociceptive activity but indicated through the direct comparison of the bioprofile of compounds **LASSBio-781** and **LASSBio-820** that the stereochemistry at C-3 seems to be a pharmacophoric requirement for an adequate fit in the target receptor. Additionally, the introduction of an acetyl group at C-3 of **LASSBio-755** modify drastically the toxicological aspect of these alkaloids, once that it did not show any sign of toxicity when administered at a single dose of 500 μmol/kg (data not shown) in contrast with (−)-spectaline (**LASSBio-754**).[8)]

Considering the significant antinociceptive activity observed for **LASSBio-755** and its analogue **LASSBio-776** we decided to investigate its effects on other models of pain and compare the results with that showed by (−)-spectaline (**LASSBio-754**).[8)] In the formalin-induced pain[10)] only **LASSBio-776** was able to inhibit by 34.4% the paw licking response of the inflammatory phase, (−)-spectaline and **LASSBio-755** did show any activity (Fig. 2). Indomethacin and other nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit the later phase without interfering on the earlier one in the formalin-induced pain test.[11)] These results on the formalin test led us to investigate the anti-inflammatory profile of these same compounds on the carrageenan-induced rat paw edema.[9)] **LASSBio-755** and, unexpectedly, **LASSBio-776** did not show an anti-inflammatory activity at the dose used. Only (−)-spectaline was able to inhibit the edema formation, showing an ED_{50} value of 56.6 μmol/kg (Fig. 3). The same order of potency was observed for both antinociceptive (ED_{50}=48.5 μmol/kg)[8)] and anti-inflammatory (ED_{50}=56.6 μmol/kg) (Fig. 3) activities of (−)-spectaline (**LASSBio-754**), indicating a common pathway of action.

Despite the absence of effect on the neurogenic phase of the formalin test at the dose used, **LASSBio-755** and **LASSBio-776** showed a significant increase in the latency time in the hot-plate test,[12)] similar to morphine, while (−)-spectaline (**LASSBio-754**), even at a dose of 300 μmol/kg, was inactive (Fig. 4).

The hot-plate and formalin test differs in some aspects. Hot-plate test is based on the use of a short-duration nociceptive thermal stimulus (phasic pain) while formalin use a long-duration chemical stimulus (tonic pain). Tissue injury leads to a persistent afferent input in the latter. The nociceptive response in the first phase of formalin and hot-plate models seems to result from direct activation of nociceptors and is inhibited by drugs which act mainly at central sites.[13,14)] Centrally acting analgesics are also effective in the second phase.[10)] This phase is attributed mainly to the development of an inflammatory reaction at the site of injection and increased synaptic transmission in the spinal cord.[13)]

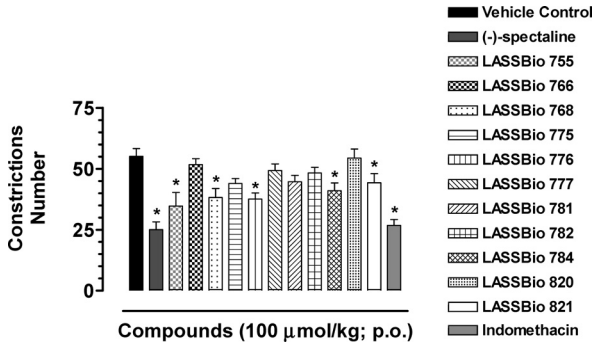

Fig. 1. Effect of the 2,3,6-Trisubstituted Piperidine Alkaloids and Indomethacin in Mice Abdominal Constrictions Induced by Acetic Acid (0.6%, i.p.)

Data are expressed as mean±S.E.M. (n=9—11 animals per group). $*p<0.05$ (Student's t test) as compared to the vehicle control group.

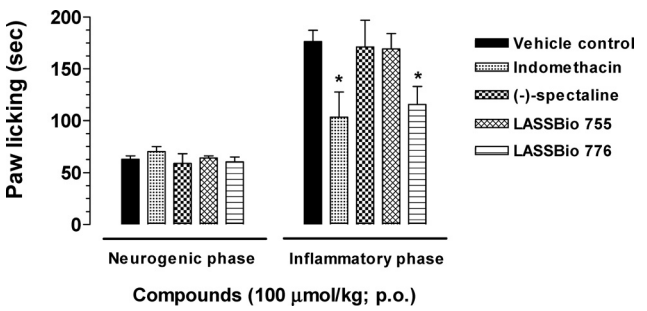

Fig. 2. Effect of (−)-Spectaline (**LASSBio-754**), **LASSBio-755**, **LASSBio-776** and Indomethacin in Mice Formalin-Induced Pain Test

Each column represents the mean±S.E.M. (n=10 animals per group). $*p<0.05$ (Student's t test) as compared to the vehicle control group.

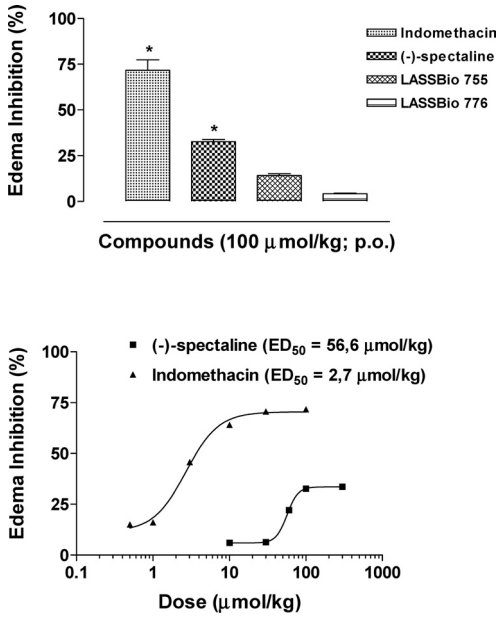

Fig. 3. Effect of (−)-Spectaline (**LASSBio-754**), **LASSBio-755**, **LASSBio-776** and Indomethacin in Carrageenan-Induced Rat Paw Edema

Data are expressed as mean±S.E.M. (n=9—11 animals per group). ED_{50} is the median dose for the anti-inflammatory effect. $*p<0.05$ (Student's t test) as compared to the vehicle control group.

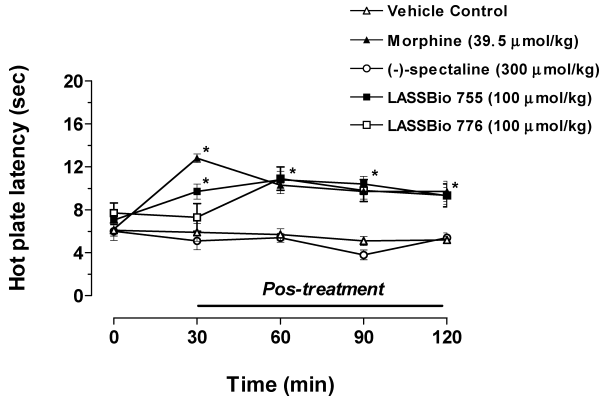

Fig. 4. Time Course Effect of (−)-Spectaline (**LASSBio-754**), **LASSBio-755**, **LASSBio-776** and Morphine in the Hot-Plate Test

Data are expressed as mean±S.E.M. (n=10 animals per group). $*p<0.05$ (Student's t test) as compared to the vehicle control group.

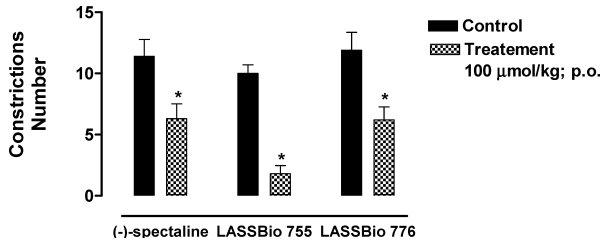

Fig. 5. Effect of (−)-Spectaline (**LASSBio-754**), **LASSBio-755**, **LASSBio-776** in Mice Acetilcholine-Induced Abdominal Constrictions

Each column represents the mean±S.E.M. (n=10 animals per group). $*p<0.05$ (Student's t test) as compared to the vehicle control group.

Considering the central activity observed for **LASSBio-755** and **LASSBio-776** in the hot-plate test, it was expected to observe some activity in the earlier phase of the formalin that could be dependent of the magnitude of the dose. The first phase of formalin is brief and occurs in the first 5 min. The first measure in the hot-plate test was at 30 min and only **LASSBio-755** showed a significant activity. **LASSBio-776** showed a significant activity from 60 min. Since the compounds were administered orally and the dose used was the same in both tests, the lack of activity on the neurogenic phase could be related with the pharmacokinetics profile of the compounds and somewhat with the mechanisms of the pain processing in these different models.

Recently, searching for new candidates compounds useful for treating Alzheimer's disease, the chloride analogues of some of these semi-synthetic piperidine alkaloid derivatives were described as acetylcholinesterase inhibitors.[15] The cholinergic system was involved in the transmission of nociceptive information and cholinergic drugs, such as muscarinic and nicotinic agonists, and also acetylcholinesterase inhibitors were described to induce antinociception.[16—18] The acetylcholine-induced constriction test has been used for screening analgesic compounds.[19] In this model (−)-spectaline and **LASSBio-776** similarly inhibited the constrictions by 44.7% and 47.9%, respectively, while **LASSBio-755** showed a more important antinociceptive effect, inhibiting the constrictions by 82% (Fig. 5). These results in addition with the hot-plate test and the acetylcholinesterase inhibition[15] suggest the involvement of the cholinergic system in the antinociceptive response elicited by **LASSBio-776** and **LASSBio-755**.

Taken together these results suggest differents mechanisms of action for the analgesic activity observed for **LASSBio-776** (3-*O*-Boc-spectaline) and **LASSBio-755** (3-*O*-acetyl-spectaline), acting mainly by modulation of receptors located at central nervous system, and for (−)-spectaline (**LASSBio-754**) that showed to be a peripheral analgesic and anti-inflammatory agent.

In conclusion, this study revealed new analgesic compounds derived from (−)-spectaline without apparent toxicity and gave support to the strategy of modifications of isolated natural products from brazilian biodiversity seeking the optimization of the biological activities in the search of new drug candidates.

Experimental

Chemistry The natural alkaloids (−)-spectaline (**LASSBio-754**) and (−)-3-*O*-acetyl-spectaline (**LASSBio-755**) were obtained from the ethanolic extract of flowers of *Cassia spectabilis* (DC.) Irwin *et* Barn (Leguminosae), as previously described. [6,7]

For the preparation of the target derivatives from (−)-spectaline (**LASSBio-754**) the reaction sequences outlined in Chart 2 were followed. Reaction of **LASSBio-754** with HCl under anhydrous conditions in CH_2Cl_2 yielded the hidrochloride **LASSBio-768**. Carbamate **LASSBio-775** and carbonate **LASSBio-776** were prepared, as a 1 : 2 mixture, by reaction of **LASSBio-754** with $(BOC)_2O/Et_3N$ in CH_2Cl_2.[20—22] Silyl ether **LASSBio-777** was obtained by reaction of **LASSBio-754** with *tert*-butyl dimethyl silyl triflate (TBSOTf)/2,6-lutidine in CH_2Cl_2.[23,24] Treatment of carbonate **LASSBio-776** with BzCl/4-DMAP/Et_3N[25,26] and MsCl/Et_3N in CH_2Cl_2 afforded *N*-benzoyl carbonate **LASSBio-782** and *N*-methanesulfonyl carbonate **LASSBio-821**, respectively. Oxidation of **LASSBio-754** under Jones conditions yielded the diketone **LASSBio-766**, that was subsequently reduced with $LiAlH_4$ in THF to afford **LASSBio-820**. Reduction of **LASSBio-754** with $NaBH_4$ in MeOH furnished **LASSBio-781**, that differs from **LASSBio-820** at the absolute configuration on C-3. Finally, **LASSBio-754** was converted into the oxime **LASSBio-784** by reaction with $NH_2OH \cdot HCl$ in pyridine/EtOH.[27,28]

Melting points were determined with a Microquimica MQAPF-301 melting point apparatus and are uncorrected. ^1H- and ^{13}C-NMR spectra were

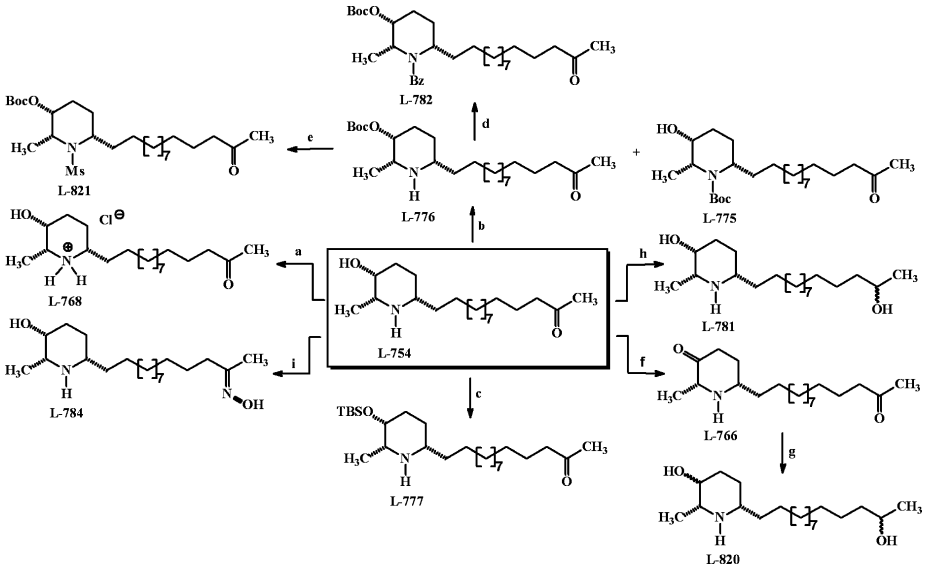

Chart 2. Preparation of 2,3,6-Trisubstituted Piperidine Derivatives

Reagents: (a) anhydrous HCl, CH_2Cl_2, 98%; (b) $(BOC)_2O$, Et_3N, 4-DMAP, CH_2Cl_2 (**LASSBio-775**, 20% and **LASSBIO-776**, 40%); (c) TBSOTf, 2,6-lutidine, CH_2Cl_2, 28%; (d) BzCl, 4-DMAP, Et_3N, CH_2Cl_2, 79%; (e) MsCl, Et_3N, CH_2Cl_2, 79%; (f) Jones conditions, acetone, 68%; (g) $LiAlH_4$, THF, 0ºC, 90%; (h) $NaBH_4$, MeOH, 0 °C, 93%; (i) $NH_2OH \cdot HCl$, pyridine, EtOH, reflux, 94%.

recorded on a Varian Unit 500 spectrometer at 500 and 125.67 MHz, respectively, using DMSO-d_6, $CDCl_3$ and CD_3OD as solvents and TMS as internal standard; gCOSY, gHMQC, gHMBC, and DEPT NMR experiments were performed in the same spectrometer, using standard Varian pulse sequences. All chemical shifts were reported as δ (ppm) values. The chemical reagents used in synthesis were purchased from E. Merck (Darmstadt, FRG) and Aldrich (Milwaukee, U.S.A.). Extracts were dried over $MgSO_4$ and solvents were removed under reduced pressure. Column chromatography was accomplished on Al_2O_3 grade I, type WN-3. TLC visualization was made by spraying with iodochloroplatinate (Merck) and Dragendorff reagents.

2-(*R*)-Methyl-6-(*S*)-(tetradecyl-13′-one)-piperidin-3-(*R*)-ol Hydrochloride (LASSBio-768) To 102.4 mg (0.31 mmol) of 2-(*R*)-methyl-6-(*S*)-(tetradecyl-13′-one)-piperidin-3-(*R*)-ol (**LASSBio-754**) dissolved in dried CH_2Cl_2 (3 ml), was added anhydrous HCl. The acid was added for 30 min at room temperature. The hydrochloride **LASSBio-768** (112 mg) was obtained in 98% yield (mp 151 °C). ^{1}H-NMR (DMSO-d_6) δ: 1.04 (d, 3H, J=6.5 Hz, H-7); 1.19 (m, 18H, H-2′ to H-10′); 1.28 (m, 2H, H-1′); 1.41 (m, 2H, H-4_{ax}, H-5_{ax}); 1.50 (m, 2H, H-11′); 1.81 (m, 1H, H-5_{eq}); 1.84 (m, 1H, H-4_{eq}); 2.06 (s, 3H, CO–CH_3); 2.34 (t, 3H, J=7.5 Hz, H-12′); 2.92 (m, 1H, H-6); 3.12 (dq, 1H, J=6.5, 1.0 Hz, H-2); 3.69 (br s, 1H, H-3).

***N*-*tert*-Butoxycarbonyl-2-(*R*)-methyl-6-(*S*)-(tetradecyl-13′-one)-piperidin-3-(*R*)-ol (LASSBio-775) and 2-(*R*)-Methyl-6-(*S*)-(tetradecyl-13′-one)-3-(*R*)-*O*-*tert*-butoxycarbonyl-piperidine (LASSBio-776)** 1.54 mmol (500.5 mg) of **LASSBio-754** was dissolved in 15 ml of dried CH_2Cl_2, under stirring in N_2 atmosphere. Then, 2.156 mmol of Et_3N and catalytic amount of 4-DMAP were added. After 5 min, a solution of 1.694 mmol of $(Boc)_2O$ in CH_2Cl_2 (15 ml) was added and the mixture was stirred for 72 h period at room temperature, until TLC analysis indicated the total conversion. Next, 10 ml of H_2O was added and the reaction mixture was extracted with $CHCl_3$. The organic extracts were washed with 2 N HCl, brine, dried over $MgSO_4$ and evaporated. The residue was purified by neutral alumina column chromatography using chloroform (6) : hexanes (3) : methanol (1) as eluent. **LASSBio-775** (mp 53 °C) and **LASSBio-776** (mp 59 °C) were obtained in 1 : 2 relative proportion (^{13}C-NMR), yield 60%. ^{1}H-NMR ($CDCl_3$), **LASSBio-775**: δ 1.03 (d, 3H, J=6.0 Hz, H-7); 1.19 (m, 20H, H-1′ to H-10′); 1.38 (s, 9H, $(CH_3)_3$C–OCO); 1.49 (m, 2H, H-11′); 1.78 (m, 2H, H-4); 1.87 (m, 2H, H-5); 2.06 (s, 3H, H-14′); 2.35 (t, 2H, J=6.0 Hz, H-12′); 2.40 (m, 2H, H-6); 2.69 (dq, 1H, J=2.0, 6.0 Hz, H-2); 3.47 (br s, 1H, H-3). **LASSBIO-776**: δ 1.07 (d, 3H, J=8.0 Hz, H-7); 1.30 (m, 20H, H-1′ to H-10′); 1.46 (s, 9H, $(CH_3)_3$C-OCO); 1.56 (m, 6H, H-4, H-5, H-11′); 2.01 (m, 2H, H-12′); 2.12 (s, 3H, H-14′); 2.50 (m, 1H, H-6); 2.84 (dq, 1H, J=2.0, 8.0 Hz, H-2); 4.59 (br s, 1H, H-3).

2-(*R*)-Methyl-6-(*S*)-(tetradecyl-13′-one)-3(*R*)-*O*-*tert*-butyldimethylsilyl-piperidine (LASSBio-777) 0.61 mmol (200 mg) of **LASSBio-754** was dissolved in dried CH_2Cl_2 (0.6 ml), under N_2 atmosphere. The reaction mixture was cooled to −5 °C and 2,6-lutidine (0.15 ml) was added. After stirring for 10 min, 0.4 ml of TBSOTf was added and reaction was carried out for 7.5 h. Reaction was finished by addition of 1 ml saturated NH_4Cl, extracted with CH_2Cl_2 and dried over $MgSO_4$. The residue was purified by alumina column chromatography using EtOAc (9) : hexane (1) as eluent, resulting in 75 mg of an incolor oil. Yield 28%. ^{1}H-NMR ($CDCl_3$) δ: 0.01 (s, 3H, CH_3, TBS group); 0.02 (s, 3H, CH_3, TBS group); 0.88 (s, 9H, *terc*-butyl, TBS group); 1.01 (d, 3H, J=2.5 Hz, H-7); 1.25 (m, 20H, H-1′ to H-10′); 1.40 (m, 1H, H-4_{eq}); 1.52 (m, 4H, H-5, H-11′); 1.79 (m, 1H, H-4_{ax}); 2.08 (s, 3H, H-14′); 2.36 (t, 2H, J=7.5 Hz, H-12′); 2.50 (m, 1H, H-6); 2.69 (dq, 1H, J=2.5, 7.5 Hz, H-2); 3.54 (br s, 1H, H-3).

***N*-Benzoyl-2-(*R*)-methyl-6-(*S*)-(tetradecyl-13′-one)-3-(*R*)-*O*-*tert*-butoxycarbonyl-piperidine (LASSBio-782)** 2-(*R*)-Methyl-6-(*S*)-(tetradecyl-13′-one)-3-(*R*)-*O*-*tert*-butoxycarbonyl-piperidine (0.12 mmol, 52 mg) was dissolved in 3 ml of dried CH_2Cl_2. Then, catalytic amount of 4-DMAP, 0.1 ml Et_3N and 0.36 mmol BzCl were added in sequence. The reaction mixture was stirred at room temperature and N_2 atmosphere for 3.5 h period. After total conversion of starting material, 10 ml CH_2Cl_2 of added and the solution washed with aqueous HCl 10% and saturated $NaHCO_3$. The organic extract was dried over $MgSO_4$, evaporated and purified by preparative TLC using EtOAc (4) : hexane (6) as eluent, furnishing 51.1 mg of a pale yellow oil. Yield 79%. ^{1}H-NMR ($CDCl_3$) δ: 1.16 (m, 3H, H-7); 1.24 (m, 20H, H-1′ to H-10′); 1.47 (s, 9H, $(CH_3)_3$C–O–CO); 1.53 (m, 4H, H-5, H-11′); 1.82 (m, 2H, H-4); 2.11 (s, 3H, H-14′); 2.39 (t, 2H, J=8.0 Hz, H-12′); 3.71 (m, 1H, H-2); 4.15 (m, 1H, H-6); 4.71 (m, 1H, H-3); 7.36 (m, 5H, Ar-H).

***N*-Methanesulfonyl-2-(*R*)-methyl-6-(*S*)-(tetradecyl-13′-one)-3-(*R*)-*O*-*tert*-butoxycarbonyl-piperidine (LASSBio-821)** 0.108 mmol (35 mg) of 2-(*R*)-methyl-6-(*S*)-(tetradecyl-13′-one)-3-(*R*)-*O*-*tert*-butoxycarbonyl-piperidine was dissolved in dried CH_2Cl_2 (1 ml) under N_2 atmosphere. After cooling to −5 °C, 0.640 mmol of Et_3N and 0.640 mmol of MsCl were added. The reaction was carried out with stirring at room temperature for 48 h period. 1 ml aqueous HCl 5% was added and the reaction was extracted with CH_2Cl_2, dried over $MgSO_4$ and evaporated, affording 43.5 mg of the mesylate **LASSBio-821**. Oil, yield 79%. ^{1}H-NMR ($CDCl_3$) δ: 1.29 (m, 20H, H-2′ to H-10′); 1.40 (m, 1H, H-5_{ax}); 1.43 (m, 2H, H-1″); 1.45 (d, 3H, J=8.0 Hz, H-7); 1.48 (s, 9H, $(CH_3)_3$C–O–CO); 1.69 (m, 1H, H-5_{eq}); 1.82 (m, 2H, H-4); 2.13 (s, 3H, H-14″); 2.40 (t, 2H, J=7.5 Hz, H-12′); 2.93 (s, 3H, CH_3–SO_2N); 3.72 (dq, 1H, J=8.0 Hz, J=2.0 Hz, H-2); 3.92 (m, 1H, H-6); 4.78 (m, 1H, H-3).

2-(*R*)-Methyl-6-(*S*)-(tetradecyl-13′-one)-piperidin-3-one (LASSBio-766) 0.32 mmol (104.5 mg) of 2-(*R*)-methyl-6-(*S*)-(tetradecyl-13′-one)-piperidin-3-(*R*)-ol was dissolved in 1 ml of acetone and a yellow $H_2Cr_2O_4$ solution was added until the change of its color was observed to become permanently green. The reaction mixture was stirred for 1 h period, and the precipitated formed was removed by filtration; 5 ml of saturated $NaHCO_3$ was

added to resultant solution, that was kept stirring for additional 10 min up to pH 10. This solution was extracted with EtOAc, dried over $MgSO_4$, evaporated and purified by Al_2O_3 column chromatography using EtOAc (3) : hexane (7) as eluent, furnishing 70.9 mg of a brown oil. Yield 68%. ^{1}H-NMR ($CDCl_3$) δ: 1.07 (d, 3H, J=6.7 Hz, H-7); 1.27 (m, 22H, H-2′ to H-11′); 1.32 (m, 1H, H-1′); 1.42 (m, 1H, H-1′); 1.54 (m, 1H, H-5_{ax}); 1.81 (m, 1H, H-5_{eq}); 2.13 (s, 3H, H-14′); 2.20 (m, 1H, H-4_{ax}); 2.40 (t, 2H, J=8.0 Hz, H-12′); 2.42 (m, 1H, H-4_{eq}); 2.92 (m, 1H, H-6); 3.34 (q, 1H, J=6.7 Hz, H-2).

2-(*R*)-Methyl-6-(*S*)-(tetradecyl-13′-hydroxy)-piperidin-3-ol (LASSBio-820) 1.49 mmol (43.2 mg) of $LiAlH_4$ was suspended in 0.5 ml anhydrous THF, stirred and cooled to 0 °C, under N_2 atmosphere. Then, 0.31 mmol (100 mg) of 2-(*R*)-methyl-6-(*S*)-(tetradecyl-13′-one)-piperidin-3-one, dissolved in 0.5 ml THF, was added, drop to drop, and reaction carried out for 2 h period. Reaction was quenched by the addition of 0.43 ml of H_2O, 0.43 ml of aqueous NaOH 15% and more 1.3 ml of H_2O, subsequently. The mixture was stirred overnight, filtrated, extracted with CH_2Cl_2, dried over $MgSO_4$ and concentrated, resulting in 90 mg of de diol as oil. Yield 90%. ^{1}H-NMR ($CDCl_3$) δ: 1.10 (d, 3H, J=7.3 Hz, H-7); 1.27 (m, 21H, H-1′ to H-11′, 1H-12′); 1.37 (m, 4H, H-12′, H-14″); 1.51 (m, 1H, H-5_{ax}); 1.58 (m, 1H, H-5_{eq}); 2.03 (m, 2H, H-4); 2.70 (m, 1, H-6); 2.89 (m, 1H, H-3); 3.93 (m, 1H, H-13′).

2-(*R*)-Methyl-6-(*S*)-(tetradecyl-13′-hydroxy)-piperidin-3-(*R*)-ol (LASSBio-781) 0.345 mmol of **LASSBio-754** was dissolved in 1 ml of MeOH, stirred and cooled to 0 °C. After 15 min, 0.245 mmol of $NaBH_4$ in 0.5 ml of MeOH was added, drop to drop. Reaction was carried out for 3 h period and was finished by the addition of aqueous HCl 10% to pH 7. The reaction mixture was extracted with $CHCl_3$, dried over $MgSO_4$ and concentrated yielding 105.8 mg of pure diol (93%) (mp 67 °C). ^{1}H-NMR ($CDCl_3$) δ: 1.12 (d, 3H, J=6.0 Hz, H-7); 1.21 (m, 21H, H-1′ to H-10′, H-11′); 1.32 (m, 3H, H-4_{ax}, H-11′, H-5_{ax}); 1.48 (m, 3H, H-14′); 1.67 (m,2H, H-4_{eq}, H-5_{eq}); 1.91 (m, 2H, H-12′); 2.64 (m, 1H, H-6); 2.86 (m, 1H, H-2); 3.61 (m, 1H, H-13′); 3.73 (m, 1H, H-3′).

2-(*R*)-methyl-6-(*S*)-(tetradecyl-13′-hydroxime)-piperidin-3-(*R*)-ol (LASSBio-784) 0.332 mmol of **LASSBio-754** was dissolved in a mixture of 2 ml of absolute ethanol and 2 ml of anhydrous pyridine, under N_2 atmosphere. 0.76 mmol (53 mg) of $NH_2OH \cdot HCl$ was added and the reaction was refluxed for 2 h. The reaction mixture was diluted with H_2O, alkalinized with NH_4OH (pH 11), extracted with $CHCl_3$, dried over $MgSO_4$ and concentrated to furnish 107.3 mg of the oxime. (mp 82 °C), yield 94%. ^{1}H-NMR ($CDCl_3$) δ: 1.18 (m, 21H, H-2″ to H-10′, H-7); 1.41 (m, 3H, H-5_{ax}, H-11′); 1.48 (m, 4H, H-4_{ax}, H-5_{eq}, H-1′); 1.78 (s, 3H, H-14′); 1.90 (m, 1H, H-4_{eq}); 2.09 (t, 2H, J=8.0 Hz, H-12′); 2.60 (m, 1H, H-6); 2.87 (m, 1H, H-2); 3.60 (br s, 1H, H-3).

Pharmacology Experiments were carried out in albino swiss mice (20—25 g) and rats (150—200 g) of both sexes from LASSBio (Faculty of Pharmacy, UFRJ, Brazil) breeding unit. All animals were kept in standardized conditions, maintained only with water *ad libitum* for 12 h before the experiment. Animal experiments were performed according to the "Principles of Laboratory Animal Care and Use in Research" (Colégio Brasileiro de Experimentação Animal-COBEA/Instituto Brasileiro Carlos Chagas Filho-IBCCF^O, Brazil), based on international guidelines for the care and use of laboratory animals and ethical guidelines for investigation of experimental pain in conscious animals.[29]

All compounds were administered orally (0.1 ml/20 g) as a suspension in arabic gum (0.5% in saline) (vehicle) 1 h before the noxius stimulus. Indomethacin was used as standard. Results are expressed as mean±S.E.M. of "n" animals per group and the activities expressed as percentage of inhibition when compared with the vehicle control group. The data were statistically analyzed by the Student's t test for a significance level of $*p<0.05$. When appropriated, the ED_{50} values (*i.e.* the dose able to elicit 50% of the maximum effect observed) were determined by non-linear regression using GraphPad Prism software.

Acetic Acid-Induced Abdominal Constriction in Mice The antinociceptive activity was determined *in vivo* using the mouse abdominal constriction test induced by acetic acid 0.6% (0.1 ml/10 g; i.p.).[9] Compounds and indomethacin were administered at a dose of 100 μmol/kg. Ten minutes after i.p. injection of the acetic acid the number of constrictions per animal was recorded for 20 min. Control animals received an equal volume of vehicle.

Acetylcholine-Induced Abdominal Constriction in Mice Constrictions were induced by acetylcholine (4.0 mg/kg; i.p.) and the number of constrictions per animal was recorded for 10 min.[30] (−)-Spectaline, **LASSBio-755** and **LASSBio-776** were administered at a dose of 100 μmol/kg. Control animals received an equal volume of vehicle.

Formalin-Induced Pain in Mice The formalin-induced pain test was carried out as described by Hunskaar and Hole.[10] Animals were injected subplantarly with 20 μl of 2.5% formalin in hind paw. (−)-Spectaline, **LASSBio-755**, **LASSBio-776**, indomethacin were administered at a dose of 100 μmol/kg. The time that mice spent licking or biting the injected paw or leg was recorded. Two distinct periods of intensive licking activity were identified and scored separately unless otherwise stated. The first period (earlier or neurogenic phase) was recorded 0—5 min after formalin injection and the second period (later or inflammatory phase) was recorded 15—30 min after injection.

Anti-inflammatory Activity The anti-inflammatory activity was determined *in vivo* using the carrageenan-induced rat paw edema test as previously described.[9] (−)-Spectaline and indomethacin were administered at a range dose of 10—300 μmol/kg and 0.3—100 μmol/kg respectively, **LASSBio-755** and **LASSBio-776** at 100 μmol/kg. Animals were then injected subplantarly with either 0.1 ml of 1% carrageenan solution in saline (0.1 mg/paw) or sterile saline (NaCl 0.9%) into one of the hind paw, respectively. The paw volumes were measured using a glass plethysmometer coupled to a peristaltic pump, 3 h after the subplantar injection. The edema was calculated as the volume difference between the carrageenan and saline-treated paw.

Hot-Plate Test Central analgesic activity was investigated using the hot plate test as described earlier.[12] In these experiments, the hot plate apparatus (Ugo Basile, Model-DS 37) was maintained at 56±1 °C. Mice were placed on the heated surface and the time between placement and the first sign of paw licking or jumping was recorded as latency. Latency was recorded at 0, 30, 60, 90 and 120 min after oral administration of vehicle, morphine (39.5 μmol/kg, used as positive control) or compounds (100 μmol/kg). The basal latencies were found to be 6—10 s. A cut-off time of 30 s was followed to prevent any injury to the paws.

Acknowledgments The authors would like to thank to FAPESP (BR), FAPERJ (BR), FUJB (BR) and CNPq (BR) for financial support and fellowships.

References and Notes

1) Agarkar S. V., Jadge D. R., *Asian J. Chem.*, **11**, 295—299 (1999).
2) Jafri M. A., Subhani M. J., Javed K., Singh S., *J. Ethnopharmacol.*, **66**, 355—361 (1999).
3) Bhakta T., Mukherjee P. K., Mukherjee K., Banerjee S., Mandal S. C., Maity T. K., Pal M., Saha B. P., *J. Ethnopharmacol.*, **66**, 277—282 (1999).
4) Tona L., Ngimbi N. P., Tsakata M., Mesia K., Cimanga K., Apers S., De Bruyne T., Pieters L., Totté J., Vlietinck A. J., *J. Ethnopharmacol.*, **68**, 193—203 (1999).
5) Ibrahim D., Osman H., *J. Ethnopharmacol.*, **45**, 151—156 (1995).
6) Bolzani V. S., Gunatilaka A. A. L., Kingston D. G. I., *Tetrahedron*, **51**, 5929—5934 (1995).
7) Viegas Junior C., Bolzani V. S., Barreiro E. J., Young M. C. M., Furlan M., Tomazela D., Eberlin M. N., *J. Nat. Prod.*, **67**, 908—910 (2004).
8) Alexandre-Moreira M. S., Viegas Junior C., Miranda A. L. P., Bolzani V. S., Barreiro E. J., *Planta Med.*, **69**, 795—799 (2003).
9) Ribeiro I. G., Silva K. C. M., Parrini S. C., Miranda A. L. P., Fraga C. A. M., Barreiro E. J., *Eur. J. Med. Chem.*, **33**, 225—235 (1998).
10) Hunskaar S., Hole K., *Pain*, **30**, 103—114 (1987).
11) Malmberg B., Yaksh T. L., *J. Pharmacol. Exp. Ther.*, **263**, 136—146 (1992).
12) Eddy N. B., Leimback D., *J. Pharmacol. Exp. Ther.*, **107**, 385—393 (1953).
13) Yaksh T. L., *TIPS*, **20**, 329—342 (1999).
14) Le Bars D., Gozariu M., Cadden S. W., *Pharmacol. Rev.*, **53**, 597—652 (2001).
15) Viegas Junior C., Bolzani V. S., Pimentel L. S. B., Castro N. G., Cabral R. F., Costa R. S., Floyd C., Rocha M. S., Young M. C. M., Barreiro E. J., Fraga C. A. M., *Bioorg. Med. Chem.*, **13**, 4184—4190 (2005).
16) Hartvig P., Gillberg P. G., Gordh T., Jr., Post C., *Trends Pharmacol. Sci.*, **10** (Suppl.), 75—79 (1989).
17) Shannon H. E., Sheardown M. J., Bymasters F. P., Calligaro D. O., Dellap N. W., Gidda J., Mitch C. H., Sawyer B. D., Stengel P. W., Ward J. S., Wong D. T., Olesen P. H., Suzdak P. D., Sauerberg P., Swedberg M. D. B., *J. Pharmacol. Exp. Ther.*, **281**, 884—894 (1997).
18) Abelson S. P., Kommalage M., Höglund U., *Neurosci. Lett.*, **368**, 116—120 (2004).
19) Collier H. O. J., Dinneen L. C., Johnson C. A., Schneider C., *Br. J.*

Pharmacol. Chemother., **32**, 295—310 (1968).
20) Greene T. W., Wuts P. G. M., "Protective Groups in Organic Synthesis," 3rd ed., John Wiley & Sons Inc., New York, 1999.
21) Furstner A., Thiel O. R., *J. Org. Chem.*, **65**, 1738—1742 (2000).
22) Ishizuka T., Kunieda T., *Tetrahedron Lett.*, **28**, 4185—4188 (1987).
23) Corey E. J., Venkateswarlu A., *J. Am. Chem. Soc.*, **94**, 6190—6191 (1972).
24) Emde H., Domsch D., Feger H., Frick U., Götz A., Hergott H. H., Hofmann K., Kober W., Krägeloh K., Oesterle T., Steppan W., Westa W., Simchen G., *Synthesis*, **1**, 1—26 (1982).
25) Schlessinger R. H., Lopes A., *J. Org. Chem.*, **46**, 5252—5253 (1981).
26) Corey E. J., Noe M. C., Guzman-Perez A., *J. Am. Chem. Soc.*, **117**, 10817—10824 (1995).
27) Shirner R. L., Hermann C. K. F., Morrill T. C., Curtin D. Y., Fuson R. C., "The Systematic Identification of Organic Compounds," 7th ed., John Wiley & Sons, New York, 1997.
28) DePuy C. H., Ponder B. W., *J. Am. Chem. Soc.*, **81**, 4629—4631 (1959).
29) Zimmerman M., *Pain*, **16**, 109—110 (1983).
30) Matheus M. E., Oliveira L. F., Freitas A. C., Carvalho A. M., Barreiro E. J., *Braz. J. Med. Biol. Res.*, **24**, 1219—1222 (1991).