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Indolizidine, Antiinfective and Antiparasitic Compounds from *Prosopis glandulosa* Torr. Var. *glandulosa*

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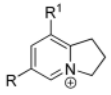
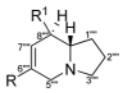
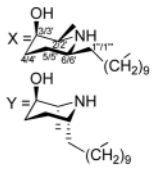
Abstract

A new potent antiinfective and antiparasitic 2,3-dihydro-1*H*-indolizinium chloride, (**1**), was isolated from *Prosopis glandulosa* Torr. *var. glandulosa*. Three additional new (**2–4**) and one known (**5**), indolizidines were also isolated, and the dihydrochloride salts of **1–3** (compounds **6**, **7** and **8**) were prepared. Structures were determined by 1D and 2D NMR and mass spectra. Compound **1** showed potent *in vitro* antifungal activity against *Cryptococcus neoformans* and *Aspergillus fumigatus* (IC_{50} values = 0.4 and 3.0 μg/mL, respectively), and antibacterial activity against methicillin-resistant *Staphylococcus aureus* and *Mycobacterium intracellulare* (IC_{50} values of 0.35 and 0.9 μg/mL, respectively). The remarkable *in vitro* fungicidal activity of **1–4** against *C. neoformans* (MFCs = 0.63→1.25 μg/mL) and **2**, **3**, and **5** against *A. fumigatus* (MFCs = 0.63→2.5 μg/mL) were similar to amphotericin B, but >2–4-fold more potent than **6–8**. Prosopilosidine (**1**) showed potent *in vivo* activity at 0.0625 mg/Kg/day/ip for 5 days in a murine model of cryptococcosis by eliminating ~76% of *C. neoformans* infection from brain tissue compared to ~83% with amphotericin B at 1.5 mg/Kg/day. Compounds **1** and **4** exhibited potent activity and high selectivity index (SI) values against chloroquine sensitive (D6) and chloroquine resistant (W2) strains of *Plasmodium falciparum* with IC_{50} values of 39 and 95 ng/mL, and 42 and 120 ng/mL, respectively; (chloroquine, IC_{50} = 17 and 140 ng/mL). Prosopilosine (**1**) also showed *in vivo* antimalarial activity with an ED_{50} value of ~2 mg/Kg/day/ip against *Plasmodium berghei*-infected mice after 3 days of treatment.







(**1**) R = R[1] = Y
(**4/4a**) R = X/Y; R[1] = Y/X
(**6**) R = R[1] = Y·HCl
(**9**) R = X; R[1] = X

(**2**) R = R[1] = Y
(**3/3a**) R = X/Y; R[1] = Y/X
(**5**) R = X; R[1] = X
(**7**) R = R[1] = Y·HCl
(**8/8a**) R = X·HCl/Y·HCl; R[1] = Y·HCl/X·HCl

Plants of the genus *Prosopis* are trees or shrubs distributed in arid and semiarid tropical and subtropical regions. *P. glandulosa* Torrey var. *glandulosa* (Leguminosae), a medium-sized

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tree, is one of the two varieties of honey mesquite available in North America.[1,2] Generally, mesquite is a popular adjuvant for preparing smoked cuisine in the southern part of the U.S. Many tribes of the southwestern United States and Mexico have long utilized the medicinal values of this plant, including treatment of eye infections, open wounds, dermatological ailments and stomach problems.[3,4] Decoctions of leaves and pods are generally used to make eye washes to treat pink eye.[5] An ethanolic extract of *P. glandulosa* from Pakistan yielded triterpenes, flavonoids, glycosides and the indolizidine alkaloid juliprosopine.[6–10] Among the indolizidines reported from *Prosopis* species to date,[6–11] the stereochemistry of juliprosine and juliprosopine were established by chemical synthesis.[12] The piperidinyl indolizidines, such as juliprosopine, and their analogs exhibited *in vitro* antibmicrobial, antidermatophytic, pesticidal, and amebicidal activities.[11,13–20] In addition, their toxicity,[20–22] DNA binding activity[23], inhibitory effects on β-glucosidase enzymes[23] and plant growth inhibitory activities were also reported.[10,24] This paper describes the isolation of the new potent antifungal and antimalarial dihydroindolizinium chloride prosopilosidine (**1**) from honey mesquite, together with three new analogs prosopilosine (**2**), isoprosopilosine (**3**) and isoprosopilosidine (**4**), and the known juliprosopine (**5**).[25,26] *P. glandulosa* Torrey var. *glandulosa*, has not previously been subjected to chemical or biological investigations. Prosopilosidine (**1**) possesses the pharmacophore 2,3-dihydro-1*H*-indolizinium, a quaternary salt substituted with two units of 10-(5*R*-hydroxy-6*R*-methylpiperidin-2*R*-yl)decyl at C-6‴ and C-8‴ positions. In this paper, we report the isolation, structure elucidation, and *in vitro* and *in vivo* antiinfective, antimalarial and cytoxic evaluation of these compounds.

Results and Discussion

An EtOH extract of *P. glandulosa* var. *glandulosa* leaves showed weak *in vitro* antiinfective and antiparasitic activities, but the presence of alkaloids warranted bioassay-guided fractionation. The bioactivity of the EtOH extract was significantly increased in the alkaloid-enriched CH_2Cl_2 fraction. Column chromatography followed by centrifugal preparative TLC of the CH_2Cl_2 fraction resulted in the isolation of compounds **1–4**, juliprosopine (**5**)[25,26] and tryptamine,[23] in the yields of 0.07%, 0.015%, 0.08%, 0.03%, 0.04% and 0.11%, respectively.

Prosopilosidine (**1**) was analyzed for $C_{40}H_{72}N_3O_2$ by electro-spray isonization high-resolution mass spectroscopy (ESIHRMS). The UV spectrum of **1** demonstrated absorption bands at λ_{max} 198 and 230 nm, typical of indolizinium chromophore.[8] The NMR spectra of **1** revealed a 2,3-dihydro-1*H*-indolizinium nucleus[8,9] consisting of two trisubstituted and a tetrasubstituted olefin [$\delta_{H\text{-}5'}$8.64 and $\delta_{H\text{-}7''''}$ 8.18, each 1H, s; δ_C 137.3, 144.3 (2 × d); δ_C 139.1, 141.3, 155.0 (3 × s)], and three methylenes at $\delta_{C\text{-}1''''}$ 31.6, $\delta_{C\text{-}2''''}$ 20.9 and $\delta_{C\text{-}3''''}$ 59.4. In addition, NMR spectra displayed oxymethine, methine adjacent to nitrogen, methylene, and methyl signals, each accounting for 2 carbons and assigned to two piperidinyl rings, as well as ten methylenes for two decanyl moieties. Comparison of the NMR spectra of **1** and (2*S*,3*R*,6*S*)-juliprosine (**9**)[8,12] suggested close similarities for the dihydroindolizidinium ring and attached decanyl residues, but showed notable differences for the piperidinyl rings (δ_C 51.5, 68.4, 26.7, 28.2, 49.0, 11.9 and 29.3; C-2/2′-C-7/7′, and C-1″/1‴; *vs.* δ_C 57.2, 67.6, 31.4, 25.6, 55.8, 18.1, and 36.0/36.1 for **9**), thereby suggesting that **1** was a diastereoisomer of **9**. Strong shielding of ^{13}C chemical shift values for C-2/2′, C-6/6′, C-7/7′ and C-1″/1‴ of the piperidine ring and attached methylene carbons indicated that the relative configurations of C-2/2′ and C-6/6′ were different (ie. *R* and *R*, respectively, *vide infra*), compared to **9** (ie. 2/2′*S*, 6/6′*S*). The COSY, HMBC and NOESY correlations, together with ^{13}C assignments of C-2/2′-C-7/7′ confirmed the presence of two 2α-methyl-3β-hydroxy-6α-decanyl-piperidinyls with the same configuration for both rings (2/2′*R*, 3/3′*R*, 6/6′*R*). The HMBC spectrum of **1** demonstrated ^3J- correlations between

H-5″″ and C-7″″, C-8a″″, C-3″″, and C-10″; H-7″″ and C-8a″″, C-10″, and C-10″″; H-3″″ and C-8a″″ and C-1″″; H-1″″ and C-8″″; H-2″″ and C-8a″″, and ^{2}J-correlations between H-2″″ and C-3″″, and C-1″″, confirming that **1** possessed a 6,8-dialkylated dihydroindolizinium ring. Finally, a 2D NMR NOESY experiment on **1** showed correlation between H-3/3′, H-7/7′and H-1″/1‴, suggesting that these protons were *cis* and on the α-face of the molecule, thereby the C-3/3′ OH group was on the β-face (3*R*) of the molecule. Thus, the structure of **1** was deduced as shown, and it was named prosopilosidine.

Prosopilosine (**2**) was isolated as gum and analyzed for $C_{40}H_{75}N_3O_2$ by ESIHRMS. The ^{1}H and ^{13}C NMR spectra of **2** (Table 1) were similar to those observed for **1**, except for differences associated with the presence of a dehydroindolizidine carbon skeleton,[25,26] consisting of four triplets (δ_C 33.4, 21.8, 54.7, 55.5), three doublets (δC 123.8, 42.8, 65.8) and a singlet (δ_C 136.1), instead of a 2,3-dihydro-1*H*-indolizinium nucleus[8,9] in **1** (Table 1). Comparison of NMR spectra of **2** with spectra of other dehydroindolizidines such as juliprosopine (**5**) and related analogs[6,9] led to the conclusion that **2** contained two identical units of 10-(2-methyl-3-hydroxy-6-alkylpiperidinyl)decane at C-6″″ and C-8″″ positions of the dehydroindolizidine nucleus, suggesting that **2** was a diastereoisomer of **5**. A 2D NMR HMBC experiment of **2** (Figure 1) showed correlations between H-7″″ and C-5″″, C-8″″, C-8a″″ and C-10″; H-10″ and C-5″″, C-6″″, and C-7″″; H_{eq}-3″″ and C-8a″″; H_{ax}-3″″ and C-2″″, and H_{eq}-5″″ and C-10″ and C-8a″″, confirming a 6,8-dialkylated 6,7-dehydroindolizidine nucleus. The configuration of 6,7-dehydroindolizidine nucleus of **2** was assigned as *trans*-(8*R*, 8a*S*) due to its close similarities of ^{1}H NMR data and ^{13}C chemical shift values for C-1″″, C-8″″ and C-8a″″ (δ_C 33.4, 42.8 and 65.8, respectively) with those of **5**.[12] These chemical shift values complies well with the literature values for a *trans* (8*R*, 8a*S*)-configuration, while the *cis*- configuration was reported as $\delta_{C\text{-}1'}$27.9, $\delta_{C\text{-}8'}$36.9 and $\delta_{C\text{-}8a'}$63.6.[12] The COSY and HMBC correlations, together with ^{13}C assignments of C-2/2′ - C-7/7′ confirmed the presence of two 2α-methyl-3β-hydroxy-6α-piperidinyl rings. The shielding of ^{13}C chemical shift values for C-2/2′, C-6/6′, C-7/7′ and C-1″/1‴ (δ_C 50.5, 50.1, 15.3, and 28.3 *vs.* δ_C 57.2, 55.8, 18.4, and 37.0 for **5**) indicated that the relative configuration of C-2/2′ and C-6/6′ was *R* and *R*, respectively, compared to 2/2′*S*, 6/6′*S* of **5**. A NOESY experiment on **2** (Figure 1) further showed correlation between H-3/3′, H-7/7′and H-1″/1‴, like **1**, suggesting these protons were *cis* and on the α-face of the molecule; thus, the C-3/3′ OH group was on the β-face (3*R*) of the molecule.

The structure of isoprosopilosine (**3**), $C_{40}H_{75}N_3O_2$, also showed the presence of two 2-methyl-3-hydroxy-6-piperidinyl rings, and a (8*R*, 8a*S*)-dehydroindolizidine nucleus, as observed for **2** and **5**. Its NMR data (Table 2) were similar to those of **2**, except for the presence of a 2β-methyl-3β-hydroxy-6β-piperidinyl ring (δ_C 56.0, 68.0, 32.5, 26.5, 57.4 and 18.8, C-2′-C-7′, respectively), instead of 2α-methyl-3β-hydroxy-6α-piperidinyl rings for **2**. COSY and HMBC correlations led to the ^{13}C assignments for C-2/2′-C-7/7′ which indicated two different configurations for the alkylpiperidine rings. This was substantiated by comparing the ^{13}C data of the $^{2}\beta$-methyl-3β-hydroxy-6β-piperidine ring of isoprosopilosine and juliprosopine (**5**),[25, 26] suggesting two alternative structures [**3** (R = X, R^1 = Y) or **3a** (R = Y, R^1 = X)] for isoprosopilosine. This compound contained two different decanyl- substituted piperidine rings, where the configurations of the C-2 methyl and C-6 methylene groups were different compared to **2** or **5**. The ^{13}C NMR signals for the piperidine ring carbons C-2/2′ - C-7/7′ of **2** (2*R*, 3*R*, 6*R*) and **5** (2*S*, 3*R*, 6*S*) were assigned to δ 50.5, 68.9, 27.9, 26.9, 50.1 and 15.3, and δ_C 57.2, 67.8, 26.1, 25.8, 55.8 and 18.4, respectively, and were in agreement with **3** or **3a**. Based on above discussion, the configurations of two piperidine rings of isoprosopilosine were determined to be 2*S*, 3*R*, 6*S* and 2*R*, 3*R*, 6*R*.

The NMR spectral data (Table 2) of isoprosopilosidine (**4**), $C_{40}H_{72}N_3O_2$, were similar to those observed for **1**, except for the presence of one 2β-methyl-3β-hydroxy-6β-piperidinyl ring (δ_C 55.0, 66.9, 31.6, 25.2, 56.5 and 18.8; C-2′-C-7′, respectively), instead of one of the 2α-methyl-3β-hydroxy-6α-piperidinyl rings for **1**. This was substantiated by comparing the ^{13}C NMR data for the 2β-methyl-3β-hydroxy-6β-piperidine ring with **5**, suggesting two alternative structures, **4** (R = X, R^1 = Y) or **4a** (R = Y, R^1 = X), due to two different substituted piperidine rings, one with 2*S*, 3*R*, 6*S* and the other with 2*R*, 3*R*, 6*R* configurations. The ^{13}C NMR signals for the piperidine ring carbons (C-2/2′ - C-7/7′) of isoprosopilosidine, with 2*R*, 3*R*, 6*R* and 2*S*, 3*R*, 6*S* configurations, respectively, were assigned to δ 51.6, 69.0, 26.9, 29.1, 48.4 and 16.0, and δ_C 55.0, 66.9, 31.6, 25.2, 56.5 and 18.4. These values were in agreement with those observed for **3** or **3a**, and thus isoprosopilosidine (**4** or **4a**) was established as a diastereoisomer of **1**.

Prosopilosidine (**1**), its analogs **2–5** and salts **6–8** were tested for *in vitro* antibacterial, antifungal, antimalarial, antileishmanial and cytotoxic activities. Compound **1** showed potent *in vitro* antifungal activity against *Cryptococcus neoformans*, and antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Mycobacterium intracellulare* with IC_{50}/MIC values of 0.4/0.63, 0.35/1.25 and 0.9/2.5 μg/mL, and **2** against *C. neoformans* and *Aspergillus fumigatus* (IC_{50}/MIC = 0.8/1.25 and 0.45/0.63 μg/mL, respectively) (Table 3). The minimum fungicidal concentration (MFC) of **1** against *C. neoformans* was equipotent to amphotericin B, but the selectivity index (SI, ratio of IC_{50} vs. VERO to IC_{50} *C. neoformans*) value was >3-fold more than amphotericin B. Compound **2** was also fungicidal to *C. neoformans* and *A. fumigatus* with MFCs of 1.25 and 0.63 μg/mL, respectively, more potent than amphotericin B. On the other hand, juliprosopine (**5**) exhibited strong activity against *A. fumigatus* (IC_{50}/MIC/MFC = 0.9/1.25/1.25 μg/mL), while the dihydrochloride salts **6–8** showed moderate activity against *C. neoformans*, MRSA, and *M. intracellulare*. Finally, the *in vitro* antifungal and antibacterial activity was not decreased in the presence of 5% human serum (data not shown), indicating that these compounds could be active *in vivo* as well.

In a separate *in vivo* study, experimentally infected mice were treated with compound **1**. At 0.0625 mg/Kg/day, administered intraperitonially for 5 days, it eliminated ~76% of *C. neoformans* infection from brain tissue compared to ~83% with amphotericin B at 1.5 mg/Kg/day. These results showed that **1** is effective against *C. neoformans* infection *in vivo*, and also indicated that the compound passes through the blood brain barrier in sufficient amount to kill the organisms in the brain tissue. The potent *in vivo* anticryptococcal activity of **1** (0.0625 mg/Kg/day/ip) in a mouse model experiment showed the antifungal dose-response effect where **1** is active at doses much lower than its maximum tolerated dose (2.5 mg/Kg/day/ip). This makes it a strong lead candidate for development of a drug for cryptococcosis, cryptococcal meningitis and cryptococcus mediated opportunistic infections in AIDS patients. A full report of the *in vivo* study of **1** together with the toxicological evaluations will be published elsewhere.

Among the compounds (**1–8**) tested for *in vitro* antimalarial activity (Table 4), the dihydroindolizinium salts (**1** and **4**) exhibited the most potent activity and high SI against *P. falciparum*. They showed IC_{50} values of 39 and 95 ng/mL, and 42 and 120 ng/mL, respectively, against chloroquine sensitive (D6) and chloroquine resistant (W2) strains, which were similar to the standard antimalarial drug chloroquine (IC_{50} = 17 and 140 ng/mL). However, the dehydroindolizidine bases prosopilosine (**2**) and isoprosopilosine (**3**) were less potent than **1** and **4** (IC_{50} = 120 and 230 ng/mL against D6 strain, and 83 and 150 ng/mL against W2 strain), but more toxic against mammalian kidney fibroblast (VERO) cells (IC_{50} = 5600 and 1800 ng/mL for **2** and **3** *vs.* >23800 ng/mL for **1** and **4**, respectively). Therefore, **1** and **4** exhibited higher SI values against *P. falciparum* D6 and W2 strains (SI

>610, 567 and SI >250 198, respectively) than the corresponding analogs **2**, **3** and **5** (SI 47, 24, 23 and SI 22, 12, 13 respectively), suggesting that dihydroindolizinium quaternary alkaloids **1** and **4** are better candidates than the tertiary bases, **2**, **3** and **5** for further *in vivo* antimalarial studies. The dihydrochloride salts **6**, **7** and **8**, prepared from compounds **1**, **2** and **3**, respectively, retained weak activities compared to their corresponding bases. Based on investigations of the *in vitro* antimalarial activity profile, cytotoxicity (*vide infra*) and mammalian toxicity of **1–5** (Table 6), compound **1** was selected for preliminary *in vivo* antimalarial screening at two doses, 1 and 2 mg/Kg, in a rodent model for malaria. Compound **1** showed an ED_{50} value of ~2 mg/Kg against *P. berghei* infected mice, thereby exhibiting ~48% suppression of parasitemia after 3 days of treatment (Table 5). Compound **1** also caused 40.5% suppression in parasitemia at 1 mg/Kg/day dose for 3 days, and no significant toxicity was observed due to treatment with **1** at these doses. Higher doses were not tested since the estimated maximum tolerated dose of compound **1** was 2.5 mg/Kg.

Compounds **1–5** also demonstrated potent *in vitro* activity against *Leishmania donavani* promastogotes (IC_{50} 0.26–0.80 μg/mL) and were as potent as the standard control pentamidine (Table 4). Finally, all of the indolizidine compounds were tested for cytotoxic activity against selected human cancer cell lines, namely SK-MEL, KB, BT-549 and SK-OV-3 (Table 6). Both **1** and **4** were weakly active towards all of these cancer cell lines, and were inactive against mammalian VERO (monkey kidney fibroblast and LLC-PK1 (pig kidney epithelial) cells (IC_{50} 21.3–25 μg/mL). However, compounds **2**, **3** and **5** were toxic to VERO (IC_{50} 5.7, 1.8, 5.0 μg/mL) and LLC-PK1 (1.9 and 1.8 μg/mL) cells.

This appears to be the first report of compounds **1–4** from a natural source. In contrast, the only other diastereoisomer of prosopilosidine (**1**), juliprosine (**9**), was reported without biological activity, while several dehydroindolizidine tertiary bases, natural and / or synthetic, showed potential therapeutic value. Compound **1** has favorable drug-like properties since its calculated log $P_{o/w}$ value is 4.4, which is an indication of a lipophilic property, lies within the value set forth by "Lipinski's rule of 5".[27] Additionally, compound **1** was >4-fold less toxic than amphotericin B in regard to hemolytic activity [IC_{50} (RBC lysis) of **1** was 16 μg/mL, *vs*. 3.9 μg/mL for amphotericin B]. The *in vivo* antimalarial assay of prosopilosidine (**1**) in a mouse model showed an ED_{50} value of ~2 mg/Kg/day against *P. berghei*. Indolizidines have not been previously reported as antimalarial agents, against either *P. falciparum* strain *in vitro* or *P. berghei* infected mice *in vivo*, or any other species, suggesting indolizidine to be a new antimalarial pharmacophore. Potent *in vitro* antimalarial activity of **1** against both chloroquine susceptible and resistant strains of *P. falciparum*, low toxicity against mammalian cells and significant activity *in vivo* in the mouse malaria model at low doses indicate **1** to be a promising new antimalarial lead for further optimization.

Experimental Section

General Experimental Procedures

Optical rotations were measured using an AUTOPOL® IV instrument at ambient temperature; UV spectra were obtained in MeOH using a Hewlett Packard 8453 UV/VIS Spectrometer; IR spectra were obtained using a Bruker Tensor 27 instrument; The NMR spectra were acquired on a Bruker Avance DRX-500 instrument at 500 MHz (^{1}H), 125 (^{13}C) in $CDCl_3$ or CD_3OD using the residual solvent as internal standard. Multiplicity determinations (DEPT) and 2D NMR spectra (COSY, HMQC, HMBC) were obtained using standard Bruker pulse programs. HRMS were obtained by direct injection using a Bruker Bioapex-FTMS with Electro-Spray Ionization (ESI). TLC was carried out on aluminum oxide IB-F plates (Baker-flex®) using CH_2Cl_2–MeOH-NH_3·H_2O (8:2:0.1), as solvent. For flash column chromatography, basic alumina (Brockman activity I, 60–325 mesh, Fisher Scientific) was used with CH_2Cl_2–MeOH-NH_3·H_2O (9:1:0.1 → 8:2:0.1) mixtures as

solvents. Centrifugal preparative TLC (CPTLC, using a Chromatotron, Harrison Research Inc., model 8924, tagged with a fraction collector) was carried out on 1 or 2 mm alumina (coated with F_{254} indicator) rotors, using CH_2Cl_2-MeOH-NH_3·H_2O (95:5:0.1) as eluant. Samples were dried using a Savant Speed Vac Plus SC210A concentrator. The compounds were visualized by observing under UV light at 254 or 365 nm, followed by spraying, separately, with Dragendorff's and 1% vanillin-H_2SO_4 spray reagents.

Plant Material

Leaves, flowers, stems and aerial parts of *P. glandulosa* were collected from Nevada (voucher # PRGAGL 2884) in June 1998 and May 2004 (and 2006) by Mr. Elray Nixon, Las Vegas, Nevada. Voucher specimens are deposited at the Herbarium of NCNPR, University of Mississippi.

Extraction and Isolation

The powdered air-dried leaves (485 g) were extracted by percolation with 95% EtOH (2 L × 3) for 48 h and the combined extract was evaporated to dryness (70 g). The dried EtOH extract (50 g) was dissolved in aqueous 0.1 N HCl (500 mL, pH 1), and defatted by partitioning successively with *n*-hexane, followed by CH_2Cl_2 (each 100 mL × 3). The aqueous acidic layer was then basified with 0.1 N NH_4OH to pH 11 (based on pKa 10.2 of tryptamine), followed by partitioning successively with CH_2Cl_2 (each 100 mL × 3), and the combined CH_2Cl_2 fractions afforded tryptamine.[23] The aqueous basic layer was then made transparent by adding MeOH (final volume 500 mL) and the pH was adjusted to 12 (based on pKa values 11 of indolizidines **1–5**) by adding conc. NH_4OH, followed by successively with CH_2Cl_2 and EtOAc (each 100 mL × 3). The CH_2Cl_2 and EtOAc fractions were filtered separately over anhydrous Na_2SO_4, and then evaporated under vacuum to dryness (yields 2.35 g and 1.49 g of mixtures **1–5**, respectively). This alkaloid enriched CH_2Cl_2 fraction demonstrated strong *in vitro* activities against *P. falciparum* D6 and W2 strains (Table 1). The CH_2Cl_2 fraction (2.17 g) was subjected to flash chromatography over alumina (Al_2O_3, 80 g), eluted with CH_2Cl_2 and then with increasing concentrations of CH_3OH (1% → 10%) in CH_2Cl_2-NH_3·H_2O mixtures, and 20 mL fractions were collected. Fractions were pooled by TLC, combined, and then evaporated under reduced pressure (total 1.64 g from six combined fractions, ie. A-F). Further purification of fractions A (43 mg), B (62 mg), C (146 mg), D (111 mg), E (43 mg) and F (185 mg) was achieved by repeated CPTLC, using 1 or 2 mm alumina rotors with CH_2Cl_2-MeOH-NH_3·H_2O (99:1:0.1 → 95:5:0.1) as solvent system, which afforded **1** (28 mg), **2** (28 mg), **3** (35 mg), **4** (26 mg) and **5** (13 mg), and tryptamine[23] (39 mg). Using this procedure, bulk quantities of **1–5** (ie. 80–100 mg) were isolated from leaves collected in 2005 and 2006. Compound **5** {gum; $[\alpha]_D^{28}$ +1° (*c* 0.4, MeOH); HRESIMS *m/z* 630.6061 $[M+H]^+$ (calcd. for $C_{40}H_{76}N_3O_2$, 630.5937)} was identified as juliprosopine by comparison of its physical and NMR data with those reported previously.[25,26]

Prosopilosidine (1), (6,8-*bis*-[(2*R*,5*R*,6*R*)-5-hydroxy-6-methylpiperidin-2-yl]decyl-2,3-dihydro-1*H*-indolizinium chloride): colorless gum; $[\alpha]_D^{28}$ +6.0 (c 0.3, MeOH); UV (MeOH) λ_{max}, nm: 204, 220, 276; IR (film) ν_{max}, cm-1: 3309 (OH, NH), 2924, 2853, 1635, 1076; 1H and 13C NMR data, see Table 1; HRESIMS *m/z* 626.5635 $[M]^+$ (calcd. for $C_{40}H_{72}N_3O_2$, 626.5624).

Prosopilosine (2), (6,8-*bis*-[(2*R*,5*R*,6*R*)-5-hydroxy-6-methylpiperidin-2-yl]decyl-[8*R*, 8a*S*]-6,7-dehydroindolizidine): colorless gum; $[\alpha]_D^{28}$ +9.4 (*c* 0.32, MeOH); UV (MeOH) λ_{max}, nm: 204, 224; IR (film) ν_{max}, cm^{-1}: 3284 (OH, NH), 2925, 2853, 1660, 1072; ^1H and ^{13}C NMR data, Table 1; HRESIMS *m/z* 630.5966 $[M+H]^+$ (calcd. for $C_{40}H_{76}N_3O_2$, 630.5937).

Isoprosopilosine (3): colorless gum; $[\alpha]_D^{28}$ +5.3 (*c* 0.45, MeOH); UV (MeOH) λ_{max}, nm: 199, 226; IR (film) ν_{max}, cm^{-1}: 3336 (OH, NH), 2924, 2852, 1661, 1088; ^1H and ^{13}C NMR spectral data see Table 2; HRESIMS *m*/*z* 630.5968 $[M+H]^+$ (calcd. for $C_{40}H_{76}N_3O_2$, 630.5937).

Isoprosopilosidine (4): colorless gum; $[\alpha]_D^{28}$ +5.6 (*c* 0.25, MeOH,); UV (MeOH) λ_{max}, nm: 204, 220, 276; IR (film) ν_{max}, cm^{-1}: 3285 (OH, NH), 2924, 2853, 1624, 1083; ^1H and ^{13}C NMR spectral data see Table 2; HRESIMS *m*/*z* 626.5636 $[M]^+$ (calcd. for $C_{40}H_{72}N_3O_2$, 626.5624).

Preparation of Dihydrochloride Salts of Indolizidines (6–8)—Excess HCl in Et_2O (2 M, 0.5 mL) was added to compounds 1, 2 and 3 (each 5–6 mg), and dissolved in 2 mL of CH_2Cl_2. The reaction mixtures were kept overnight under nitrogen, and the solutions were dried to give dihydrochloride salts 6, 7 and 8, as yellow gums in yields of 8 mg, 5.5 mg and 6 mg, respectively. The R_f values for 6–8 were found to be 0.16, 0.36, 0.62, respectively (TLC; Alumina, solvent: CH_2Cl_2: MeOH: $NH_3.H_2O$; 8.5:1.5:0.1), *vesus* R_f values of 0.08, 0.43 and 0.49 for 1–3 (TLC; Alumina, solvent: CH_2Cl_2: MeOH: $NH_3.H_2O$; 9:1:0.1).

Prosopilosidine dihydrochloride (6)—$[\alpha]_D^{28}$ −0.7 (c 0.8, MeOH); IR (film) ν_{max}, cm^{-1}: 3474 (OH, NH), 3431 (OH, NH), 3146, 3071, 2928, 2855, 1710, 1292, 1076.

Prosopilosine dihydrochloride (7)—$[\alpha]_D^{28}$ −0.9° (*c* 0.6, MeOH); IR (film) ν_{max}, cm^{-1}: 3531 (OH, NH), 3487 (OH, NH), 3159, 3088, 2927, 2855, 1708, 1295, 1076; ^1H NMR (CD_3OD) δ_H 5.64 (1H, s, H-7″″), 3.87 (2H, m, H-3, 3′), 3.49 (2H, q, *J*= 7.5 Hz, H-2, 2′), 3.37 (2H, m, H-6, 6′), 3.15 (3H, m, H-3″″, 5″″), 2.49 (1H, m, H-3″″), 2.14 (5H, m, H-8″″, 2″″, 10″), 1.88 (2H, m, H-2″″, 8a″″), 1.78 (2H, m, H-4, 4′), 1.68, 1.58, 1.53–1.26 (m), 1.32 (6H, d, *J* = 6 Hz, H-7, 7′).

Isoprosopilosine dihydrochloride (8)—$[\alpha]_D^{28}$ −1.7 (*c* 1.0, MeOH); IR (film) ν_{max}, cm^{-1}: 3473 (OH, NH), 3425 (OH, NH), 3149, 3073, 2928, 2855, 1708, 1295, 1076. However, ^1H NMR signals of 6 and 8 were broad compared to those of 7 in CD_3OD.

Antimicrobial Assay

All organisms were obtained from the American Type Culture Collection (Manassas, VA) and include the fungi *Candida albicans* ATCC 90028, *Cryptococcus neoformans* ATCC 90113, and *Aspergillus fumigatus* ATCC 90906 and the bacteria methicillin-resistant *Staphylococcus aureus* ATCC 43300 (MRS), *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, and *Mycobacterium intracellulare* ATCC 23068. For all organisms excluding *M. intracellulare* and *A. fumigatus*, susceptibility testing was performed using a modified version of the CLSI (formerly NCCLS) methods[28,30], and optical density was used to monitor growth. Media supplemented with 5% Alamar Blue™ (BioSource International, Camarillo, CA) was utilized for growth detection of *M. intracellulare*[31–32] and *A. fumigatus*[29]. Samples were serially-diluted in 20% DMSO/saline and transferred in duplicate to 96 well flat bottom microplates. Microbial inocula were prepared by correcting the OD_{630} of cell/spore suspensions in incubation broth (RPMI @ pH 4.5 for *C. albicans*, Sabouraud Dextrose for *C. neoformans*, cation-adjusted Mueller-Hinton @ pH 7.3 for MRS, and 5% Alamar Blue™ (BioSource International, Camarillo, CA) in Middlebrook 7H9 broth with OADC enrichment, pH = 7.3 for *M. intracellulare* and 5% Alamar Blue in RPMI @ pH 7.3 for *A. fumigatus* to afford final target inocula ($1{\cdot}10^4$, $1{\cdot}10^5$, $5{\cdot}10^5$, $2{\cdot}10^6$, and $3{\cdot}10^4$ CFU/mL, respectively). Drug controls [Ciprofloxacin (ICN Biomedicals, Ohio) for bacteria and Amphotericin B (ICN Biomedicals, Ohio) for fungi] were included in each assay. All organisms were read at either 630 nm using the Biotek

Powerwave XS plate reader (Bio-Tek Instruments, Vermont) or 544ex/590em, (*M. intracellulare, A. fumigatus*) using the Polarstar Galaxy Plate Reader (BMG LabTechnologies, Germany) prior to and after incubation. Minimum fungicidal or bactericidal concentrations were determined by removing 5 μ-L from each clear well, transferring to agar and incubating until growth was seen. The MFC/MBC is defined as the lowest test concentration that kills the organism (allows no growth on agar).

Antimalarial/Parasite LDH Assay

The *in vitro* antimalarial activity was measured by a colorimetric assay that determines the parasitic lactate dehydrogenase (pLDH) activity. [33,34,45] The assay was performed in 96-well microplate and included two *P. falciparum* clones [Sierra Leone D6 (chloroquine-sensitive) and Indochina W2 (chloroquine-resistant)]. For the assay, a suspension of red blood cells infected with *P. falciparum* (D6 or W2) strains (200 μL, with 2% parasitemia and 2% hematocrit in RPMI - 1640 medium supplemented with 10% human serum and 60 μg/mL amikacin) was added to the wells of a 96- well plate containing 10 μL of test samples at various concentrations. The plate was flushed with a gas mixture of 90% N_2, 5% O_2, and 5% CO_2, in a modular incubation chamber (Billups-Rothenberg, 4464 M] and incubated at 37 °C, for 72 h. Plasmodial LDH activity was determined by using Malstat™ reagent (Flow Inc., Portland, OR) as described earlier.[34,35] The IC_{50} values were computed from the dose response curves generated by plotting percent growth against test concentrations. DMSO (0.25%), artemisinin and chloroquine were included in each assay as vehicle and drug controls, respectively. The selectivity indices (SI) were determined by measuring the cytotoxicity of samples towards mammalian cells (VERO; monkey kidney fibroblast).

In vivo Antimalarial Assay[36]

The *in vivo* antimalarial activity of the compounds was determined in mice infected with *P. berghei* (NK-65 strain) according to the Peter's 4-day suppressive test. Male mice (Swiss Webster strain) weighing 18–20 g were intraperitoneally inoculated with $2{\cdot}10^7$ parasitized red blood cells obtained from a highly infected donor mouse. Mice were divided into different groups with 5 mice in each group. Test compounds were prepared in DMSO:PEG:Water (1:3:6) and administered intraperitoneally to the mice about 2 h after the infection (Day 0). The test compounds were administered to the mice once a day for 3 consecutive days (Days 0–2). A control group was treated with an equal volume of vehicle while another control group was treated with the standard antimalarial compound, β-arteether. The mice were closely observed after every dose for any apparent signs of toxicity. Blood smears were prepared on different days (until day 28 post infection) by clipping the tail end, stained with Giemsa and observed under a microscope for determination of the parasitemia. Mice without parasitemia until day 28 post infection were considered as cured.

Antileishmanial Assay

Antileishmanial activity of the compounds was tested *in vitro* against a culture of *L. donovani* promastigotes, grown in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS, Gibco Chem. Co.) at 26 °C. A 3 day-old culture was diluted to $5{\cdot}10^5$ promastigotes/mL. Drug dilutions (50 – 3.1 μg/mL) were prepared directly in cell suspension in 96-well plates. Plates were incubated at 26 °C for 48 hours, and growth of leishmania promastigotes was determined by Alamar Blue assay.[37] Standard fluorescence was measured on a Fluostar Galaxy plate reader (BMG LabTechnologies) at excitation wavelength of 544 nm and emission wavelength of 590 nm. Pentamidine and amphotericin B were used as the standard antileishmanial agents. Percent growth was calculated and plotted versus test concentration for computing the IC_{50} and IC_{90} values.

Cytotoxicity Assay

The *in vitro* cytotoxic activity was determined against four human cancer cell lines, SK-MEL, KB, BT-549 and SK-OV-3, as well as noncancerous cell lines, VERO and LLC-PK1 (Table 6), obtained from the American Type Culture Collection (ATCC, Rockville, MD). The assay was performed in 96-well tissue culture-treated microplates. Cells (25,000 cells/well) were seeded to the wells of the plate and incubated for 24 hours. Samples were added and plates were again incubated for 48 hours. The number of viable cells was determined using Neutral Red according to a modification of the procedure of Borenfreund *et al.*[38] IC_{50} values were determined from logarithmic graphs of growth inhibition *vs.* concentration. Doxorubicin was used as a positive control, while DMSO was used as the negative (vehicle) control.

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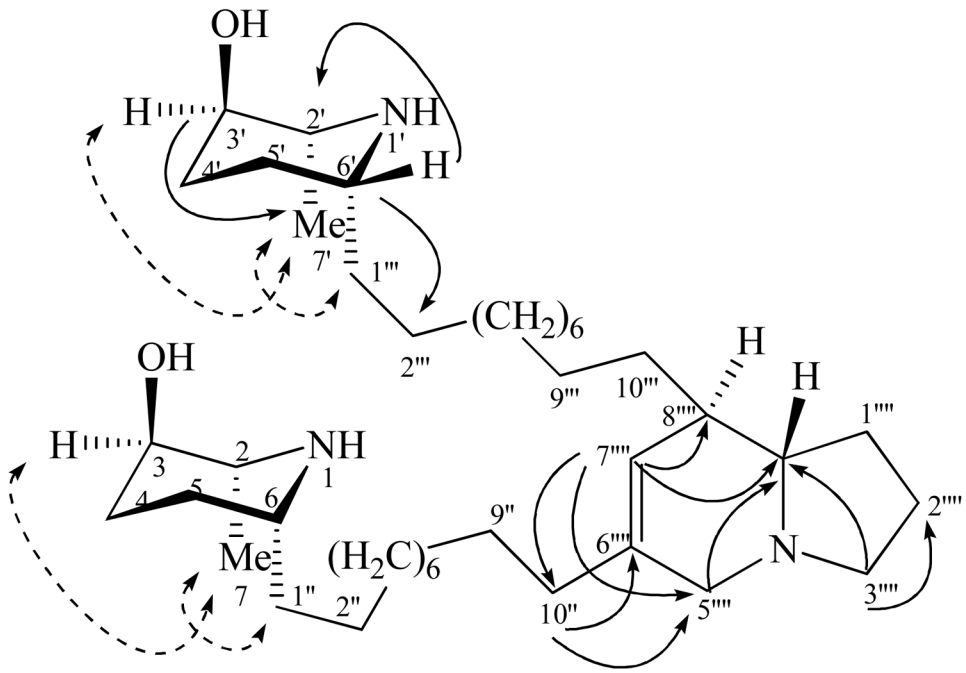
References and Notes

1. Burkhart A. A Monograph of the Genus Prosopis. J Arnold Arboretum. 1976; 57:450–525.
2. Hilu YW, Boyd S, Felker P. Morphological diversity and taxonomy of California mesquites (Prosopis, Lepminosae). Madrono. 1982; 29:237–254.
3. Davidow, J. Infusions of Healing: A Treasury of Mexican-American Herbal Remedies. Simon and Schuster Inc; 1999. p. 1-149.
4. Kay, MA. Healing with Plants in the American and Mexican West. The University of Arizona Press; Tuscon: 1996. p. 221-224.
5. Ali AAN, Al-Rahwi K, Lindequist U. Afr J Trad, Complementary & Alt Med. 2004; 1:72–76.
6. Ahmad VU, Qazi S. J Chem Soc Pak. 1985; 7:347–350.
7. Ahmad VU, Basha A, Haque W. Zeitschrift fuer Naturforschung, Teil B: Anorganische Chemie, Organische Chemie. 1978; 33B:347–348.
8. Daetwyler P, Ott-Longoni R, Schoepp E, Hesse M. Helv Chim Acta. 1981; 64:1959–1963.
9. Ahmad VU, Sultana A, Qazi S. Alkaloids from the leaves of *Prosopis juliflora*. J Nat Prod. 1989; 52:497–501.
10. Nakano H, Nakajima E, Hiradate S, Fujii Y, Yamada K, Shigemori H, Hasegawa K. Phytochem. 2004; 65:587–591.
11. Ahmad, VU.; Ali, MS.; Bano, N.; Bano, S.; Fatima, A.; Fatima, I.; Fizza, K.; Farooqui, TA.; Khan, MA. Nat Prod Chem. Vol. 3. Springer-Verlag; Berlin: 1988. p. 355-358.
12. Snider BB, Neubert BJ. Org Lett. 2005; 7:2715–2718. [PubMed: 15957929]
13. Aqeel A, Khursheed AK, Viqaruddin A, Sabiha Q. Arzneimittel-Forschung. 1989; 39:652–655. [PubMed: 2775330]
14. Ahmad A, Ahmad V, Khalid SM, Ansari FA, Khan KA. Philippine J Sci. 1997; 126:175–182.
15. Ahmad A, Khan KA, Ahmad V. Pak J of Zoology. 1996; 28:365–367.
16. Ahmad A, Khan KA, Ahmad VU, Qazi S. Planta Med. 1986; 4:285–288. [PubMed: 3763718]
17. Ahmad A, Kazmi SU, Khan KA, Ahmad VU, Qazi S. JPMA J Pak Med Asson. 1988; 38:20–24.
18. Khursheed AK, Arshad HF, Viqaruddin A, Sabiha Q, Sheikh AR, Tahir SH. Arzneimittel-Forschung. 1986; 36:17–19. [PubMed: 3513775]
19. Ahmad A, Khursheed AK, Sabiha Q, Viqaruddin A. Fitoterapia. 1989; 60:86–89.

20. Tabassum R, Naqvi SNH, Ahmad VU, Rani S, Jahan M, Azmi MA. Proceedings of Pakistan Congress of Zoology. 1994; 14:326–333.
21. Naqvi SNH, Yasmin N, Mahmood RA, Nizam S, Ahmad VU, Qazi S. Zeitschriftfuer Angewandte Zoologie. 1994; 80:155–164.
22. Aqeel A, Khursheed AK, Viqaruddin A. Toxicological studies of the antimicrobial alkaloid juliflorine. Arzneimittel-Forschung. 1991; 41:151–154. [PubMed: 2043177]
23. Tapia A, Egly Feresin G, Bustos D, Astudillo L, Theoduloz C, Schmeda-Hirschmann G. J of Ethnopharmacol. 2000; 71:241–246. [PubMed: 10904169]
24. Nakano H, Nakajima E, Fujii Y, Shigemori H, Hasegawa K. Plant Growth Regulation. 2004; 44:207–210.
25. Usmanghani K, Saqib QN, Ahmad VU. J Chem Soc Pak. 1982; 4:285–287.
26. Ott-Longoni R, Viswanathan N, Hesse M. Helv Chim Acta. 1980; 6:2119–2129.
27. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Adv Drug Delivery Rev. 1997; 23:3–25.
28. NCCLS. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard. National Committee for Clinical Laboratory Standards (2). 2002; 22:1–51.
29. NCCLS. Reference method for broth dilution antifungal susceptibility testing of conidium forming filamentous fungi. Proposed standard M38-P. National Committee for Clinical Laboratory Standards. 1998; 18:1–39.
30. NCCLS. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. NCCLS Document M7-A5. National Committee for Clinical Laboratory Standards. 2000; 20:1–58.
31. NCCLS. Susceptibility testing of mycobacteria, nocardia, and other aerobic actinomycetes. Tentative standard M24-T2. National Committee for Clinical Laboratory Standards (2). 2000; 20:1–81.
32. Franzblau SG, Witzig RS, McLaughlin JC, Torres P, Madico G, Hernandez A, Degnan MT, Cook MB, Quenzer VK, Ferguson RM, Gilman RH. J Clinical Microbiol. 1998:362–366. [PubMed: 9466742]
33. Dou J, McChesney JD, Sindelar RD, Goins DK, Walker LA. J Nat Prod. 1996; 59:73–76. [PubMed: 8984156]
34. Makler MT, Hinrichs DJ. Am J Trop Med & Hygiene. 1993; 48:205–210.
35. Makler MT, Ries JM, Williams JA, Bancroft JE, Piper RC, Gibbins BL, Hinrichs DJ. Am J Trop Med & Hygiene. 1993; 48:739–741.
36. Peters W, Portus JH, Robinson BL. Annals of Trop Med Parasitol. 1975; 69:155–171.
37. Mikus J, Steverding D. Parasitol Int. 2000; 48:265–269. [PubMed: 11227767]
38. Borenfreund E, Babich H, Martin-Alguacil N. In Vitro Cellular & Developmental Biology: J Tissue Culture Asson. 1990; 26:1030–1034.



2

Figure 1.
2D NMR HMBC (solid lines) and NOESY (dashed lines) correlations of **2**

Table 1

^{1}H and ^{13}C NMR Data† (J values in Hz, in parenthesis) for **1**, **2** and **5**

	Prosopilosidine (1)		**Prosopilosine (2)**		**Juliprosopine (5)**$^{+}$	
H/C	**δ_H**	**δ_C**	**δ_H**	**δ_C**	**δ_H**	**δ_C**
2, 2′	3.19 dq (6)	51.5 d	3.13 q (7.8)	50.5 d	2.75 q (6.5)	57.2 d
3, 3′	3.75 ddd (4.8)	68.4 d	3.66 br s	68.9 d	3.48 br.s	67.8 d
4, 4′	1.70 m 1.59 m	26.7 t	1.72 m 1.62 m	27.9 t	1.84 m 1.46 m	26.1 t
5, 5′	1.82 m 1.21 m	28.2 t	1.49 m 1.22 m	26.9 t	1.40 m 1.20 m*	25.8 t
6, 6′	2.80 m*	49.0 d	2.85 m	50.1 d	2.56 q (8)	55.8 d
7, 7′	1.14 d (7)	11.9 q	1.12 d (6.5)	15.3 q	1.07 d (6.4)	18.4 q
1″, 1‴	1.37 m	29.3 t	1.40 m	28.3 t	1.40–1.20 m*	37.0 t
2″, 2‴		30.7, 29.8,		27.0 t		32.2 t
3″-8″	1.35–1.27 m*	29.6, 29.5,	1.39–1.27 m*	30.5, 30.1,	1.30–1.20 m*	29.3, 29.5,
3‴-8‴		29.4, 29.2,		30.0, 29.8,		29.6, 29.8,
9″, 9‴		29.1, 29.0,		29.8, 29.7*		30.1 (5 × t)
10″	2.80 m*	26.3 (9 × t)	1.43 m	(6 × t)	2.00 t (7.4)	26.6 t
10‴	2.80 m*	32.1 t	2.00 t (7.5)	35.4 t	1.40–1.20 m*	35.1 t
1‴″	2.80 m*	31.7 t	1.39–1.27 m*	29.8*	1.40–1.20 m*	28.0 t
2‴″	2.50 m	31.6 t	1.52 m	33.4 t	1.40–1.20 m*	33.1 t
3‴″	4.88 m*	20.9 t	2.05 m 1.80 m	21.8 t	2.04 m 1.76 m	21.5 t
5‴″	8.64 s	59.4 t	3.13 dd (18, 7.8) 2.10 dd (18, 9)	54.7 t	3.11 ddd (17.6, 7.8) 2.08 dd (17.6, 9)	54.5 t
6‴″	-	137.3 d	3.29 d (15) 2.64 d (15)	55.5 t	3.26 d (15.2) 2.58 d (15.2)	55.3 t
7‴″	8.18 s	139.1 s	-	136.1 s	-	136.3 s
		144.3 d	5.37 s	123.8 d	5.33 s	123.9 d

	Prosopilosidine (1)		Prosopilosine (2)		Juliprosopine (5)[+]	
H/C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
8″″	-	141.3 s	2.00 m	42.8 d	2.00 m	42.6 d
8a″″	-	155.0 s	1.82 m	65.8 d	1.74 m	65.6 d

[†] All spectra recorded in CD_3OD at 500 MHz (^1H) and 125 MHz (^{13}C).

[*] Overlapped signals.

[+] Data recorded during this investigation and the literature data[5,6] was in agreement with recorded values.

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Table 2

^{1}H and ^{13}C NMR Data[†] (*J* values in Hz, in parenthesis) for (**3**/**3a**) and (**4**/**4a**)

	Isoprosopilosine (3/3a)		Isoprosopilosidine (4/4a)	
H/C	**δ_H**	**δ_C**	**δ_H**	**δ_C**
2, 2′	3.12 dq	49.9 d	3.18 dq (6)	51.6 d
	2.77 dq (6)	56.0 d	2.78 dq (6)	55.0 d
3, 3′	3.62 m	69.1 d	3.78 ddd (5, 5, 5)	69.0 d
	3.50 br. s	68.0 d	3.61 br.s	66.9 d
4, 4′	1.63 m	27.0 t	1.72 m, 1.62 m	26.9 t
	1.51 m, 1.86 m	32.5 t	1.93 m, 1.65 m	31.6 t
5, 5′	1.43 m, 1.63 m	28.1 t	1.69 m	29.1 t
	1.23 m	26.5 t	1.53 m	25.2 t
6, 6′	2.84 m	50.4 d	2.84 m	48.4 d
	2.58 m	57.4 d	2.58 m	56.5 d
7, 7′	1.08 d (6)	16.0 q	1.14 d (7)	16.0 q
	1.09 d (6)	18.8 q	1.14 d (7)	18.8 q
1″, 1‴	1.72 m	37.3 t	1.72 m	37.3 t
	1.32 m	28.4 t	1.32 m	28.4 t
2″, 2‴		30.4, 30.2,		30.7, 29.9,
3″-8″		30.0, 29.9,		29.6, 29.4,
3‴-8‴	1.41–1.26 m*	29.9, 29.8,	1.48–1.25 m*	29.2, 26.4,
9″, 9‴		29.6, 26.2 (8 × t)		25.9 (7 × t)
10″	2.00 t (7.5)	35.4 t	2.83 m*	32.1 t
10‴	1.41–1.26 m*	29.8*	2.83 m*	31.7 t
1‴′	1.45 m	33.4 t	2.83 m*	30.7 t
2‴′	1.81 m	21.8 t	2.54 m	21.1 t
3‴′	3.11 dd (18, 7.8) 2.09 dd (18, 9.5)	54.7 t	4.87 m	59.4 t
5‴′	3.29 d (15.5) 2.61 d (15.5)	55.5 t	8.64 s	137.3 d
6‴′	-	136.2 s	-	141.3 s
7‴′	5.36 s	123.8 d	8.17 s	144.3 d
8‴′	2.00 m	42.9 d	-	139.2 s
8a‴′	1.72–1.81 m	65.7 d	-	155.0 s

[†]All spectra recorded in CD_3OD at 500 MHz (^{1}H) and 125 MHz (^{13}C).

*Overlapped signals.

Table 3

Antimicrobial Activity of *P. glandulosa* Extracts and Compounds **1–8**

Extract/Compound	IC_{50}/MIC/MFC or MBC (μg/mL)				
	C. albicans	***C. neoformans***	**MRSA**	***M. intracellulare A. fumigatus***	
P. glandulosa (EtOH extract)	- / - / -	20 / - / -	200 / - / -	- / - / -	- / - / -
P. glandulosa (Alkaloid Fr.)	80 / - / -	<8 / - / -	<8 / - / -	<8 / - / -	<8 / - / -
1	15 / - / -	0.4 / 0.63 / 1.25	0.35/1.25/5.0	0.9 / 2.5 / 20.0	3.0 / 5.0 / -
2	15 / - / -	0.8 / 1.25 / 1.25	1.5 / 2.5 / 10	2.0 / 5.0 / 20.0	0.45 / 0.63 / 0.63
3	- / - / -	0. 4/ 0.63 / 0.63	1.0 / 2.5 / -	1.5 / 5.0 / -	0.1 / 0.16 / 2.5
4	- / - / -	0.55 / 1.25 / 1.25	0.6 / 1.25 / 20.0	1.5 / 2.5 / -	6.5 / 10.0 / 10.0
5	- / - / -	1.5 / 5.0 / 5.0	3.5 / 5.0 / -	6.0 / 20.0 / 20.0	0.9 / 1.25 / 1.25
6	15.0 / 20.0 / -	1.5 / 2.5 / 2.5	1.5 / 2.5 / 5.0	3.5 / 10.0 / -	- / - / -
7	- / - / -	2.0 / 5.0 / 5.0	3.0 / 5.0 / 5.0	9.5 / 20.0 / -	- / - / -
8	7.5 / 10.0 / 10.0	0.8 5/ 1.25 / 2.5	3.0 / 5.0 / 5.0	10.0 / - / -	6.5 / 10.0 / -
Amphotericin B	0.35 / 0.63 / 1.25	0.45 / 1.25 / 2.5	NT	NT	0.40 / 0.63 / 2.5
Ciprofloxacin	NT	NT	0.1 / 0.3 / -	0.25 / 0.63 / -	NT

IC_{50} is the concentration that affords 50% inhibition of growth; MIC (Minimum Inhibitory Concentration) is the lowest test concentration that allows no detectable growth; MFC/MBC (Minimum Fungicidal/Bactericidal Concentration) is the lowest test concentration that kills the organism.

- = Not active at the highest test concentration; NT = Not tested.

Table 4

Antiparasitic Activities of *P. glandulosa* Extracts and Compound **1–8**

Extract/compound	*P. falciparum* (ng/mL)				VERO *L. donovani*		
	D6[a]		W2[b]		(ng/mL)	(μg/mL)	
	IC_{50}	SI[c]	IC_{50}	SI[c]	IC_{50}	IC_{50}	IC_{90}
P. glandulosa (EtOH extr.)	13000	>3.7	10000	>4.8	NC	44	90
P. glandulosa (Alkaloid Fr.)	420	64	630	43	27000	18	35
1	39	>610	95	>250	>23800	0.7	1.9
2	120	47	230	24	5600	0.65	2.0
3	83	22	150	12	1800	0.26	1.2
4	42	>567	120	>198	>23800	0.8	2.5
5	220	23	380	13	5000	0.75	2.4
6	430	>11.1	270	>17.6	NCd	2.8	8.0
7	600	>7.9	560	>8.5	NCd	3.1	7.0
8	380	>12.5	290	>16.4	NCd	0.7	1.2
Chloroquine	17		140		NC	NT	NT
Artemisinin	16		17		NC	NT	NT
Pentamidine	NT		NT		NT	0.5	2.0
Amphotericin B	NT		NT		NT	0.1	0.4

[a] Chloroquine-sensitive clone.

[b] Chloroquine-resistant clone.

[c] Selectivity index = IC_{50} VERO cells/IC_{50} *P. falciparum*.

[d] NC = Not cytotoxic (up to the maximum dose tested; 4760 ng/mL for pure compounds and 47600 ng/mL for crude extracts). NT= Not tested.

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Table 5

In vivo Antimalarial Activity (Suppressive/Curative; *P. berghei* Mouse model) of Compound **1**

Compound/Drug	Animals per group	Dose, mg/kg x days	Suppression in parasitemia (% activity)[a]		Cure[b]
			Day 10	Day 17	
1	5	1 × 3	40.5	nil	0/5
	5	2 × 3	48.4	nil	0/5
β-Arteether	5	10 × 3	100	100	5/5

[a] % suppression in parasitemia = 100 – [Parasitemia in treated group/parasitemia in untreated group × 100].

[b] Number of mice without parasitemia till day 28/Total number of mice in group.

Table 6

Cytotoxic Activities of Compounds **1–5**

Compound	IC_{50} (μg/mL)					
	SK-MEL[a]	KB[b]	BT-549[c]	SK-OV-3[d]	VERO[e]	LLC-PK1[f]
1	4.5	7.5	8.75	7.5	NA	NA
2	<1.1	6.0	6.0	2.0	5.7	1.9
3	<1.1	2.2	2.5	<1.1	1.8	1.8
4	3.3	7.0	6.75	4.5	NA	21.3
5	2.4	6.2	6.0	4.7	5.0	1.8
Artemisinin	NT	NT	NT	NT	>4.76	NT
Amphotericin B	5.0	>25	5.5	>25	7.5	0.6
Doxorubicin	<0.55	<0.55	<0.55	<0.55	3.2	<0.55

[a]SK-MEL: Human malignant melanoma.

[b]KB: Human epidermoid carcinoma.

[c]BT-549: Human ductal carcinoma.

[d]SK-OV-3: Human ovary carcinoma.

[e]VERO: Monkey kidney fibroblast.

[f]LLC-PK1: Pig kidney epithelial.