JOURNAL OF NATURAL PRODUCTS

NOTE

pubs.acs.org/jnp

Synthesis and Antifungal Activity of Natural Product-Based 6-Alkyl-2,3,4,5-tetrahydropyridines

Liyan Dai,[†,‡] Melissa R. Jacob,[†] Shabana I. Khan,[†,§] Ikhlas A. Khan,[†,§] Alice M. Clark,[†,§] and Xing-Cong Li*[,†,§]

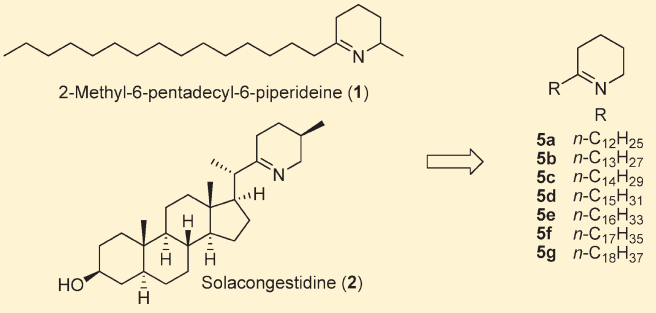
[†]National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, The University of Mississippi, University, Mississippi 38677, United States

[‡]Department of Chemical and Biological Engineering, Zhejiang University, Hangzhou 310027, People's Republic of China

[§]Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, Mississippi 38677, United States

Supporting Information

ABSTRACT: Seven 6-alkyl-2,3,4,5-tetrahydropyridines (**5a**−**5g**) that mimic the natural piperideines that were recently identified in fire ant venom have been synthesized. Compounds **5c**−**5g** with C-6 alkyl chain lengths from C14 to C18 showed varying degrees of antifungal activities, with **5e** (6-hexadecyl-2,3,4,5-tetrahydropyridine) and **5f** (6-heptadecyl-2,3,4,5-tetrahydropyridine) being the most active. Compound **5e** exhibited minimum fungicidal concentrations of 3.8, 15.0, 7.5, and 7.5 μg/mL against *Cryptococcus neoformans*, *Candida albicans*, *Candida glabrata*, and *Candida krusei*, respectively. The antifungal activities of these compounds appear to be associated with the C-6 side chain length. This study represents the first effort to evaluate antifungal activities of synthetic analogues of the newly identified fire ant venom alkaloids.



Recent studies have shown that the venom of the red imported fire ant *Solenopsis invicta* contains a series of 2,6-dialkylpiperideine alkaloids that were identified by GC-MS,[1−4] with 2-methyl-6-pentadecyl-2,3,4,5-tetrahydropyridine (**1**) being a major constituent.[1] Since previous studies on chemical constituents of fire ants (*Solenopsis* spp.)[5] and their biological activities,[5−7] especially antimicrobial properties,[8−11] have focused on 2,6-dialkylpiperidine derivatives (such as solenopsins and dehydrosolenopsins), the identification of these new piperideine alkaloids in fire ants is significant, and these compounds may possess interesting biological activities.

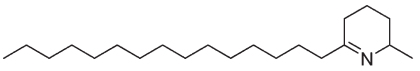
Coincidently, the steroidal alkaloid solacongestidine (**2**) isolated from the higher plant *Solanum congestiflorum* contains a 2,3,4,5-tetrahydropyridine structural moiety and has demonstrated significant antifungal activities against opportunistic fungal pathogens that may cause life-threatening disseminated mycoses.[12] The analogue 25β-hydroxyverazine isolated from *S. surinamense* also showed potent antifungal activities and low cytotoxicities.[13] Assuming the antifungal activities of these steroidal alkaloids are associated with their heterocyclic ring, simple 6-alkyl-2,3,4,5-tetrahydropyridine derivatives with an alkyl length up to nine carbons as well as 6-phenyl-2,3,4,5-tetrahydropyridine and 6-(4-chlorophenyl)-2,3,4,5-tetrahydropyridine were synthesized for structure−activity relationship (SAR) studies. However, these compounds failed to exhibit antifungal activity.

Our recent studies on antifungal acetylenic acids indicated that the chain lengths of these fatty acids play a pivotal role in antifungal activity. Within the series, only 6-octadecynoic acid and 6-nonadecynoic acid showed potent antifungal activity against *Candida albicans*.[14] Synthetic analogues of the marine-derived phloeoedictine alkaloids that show potent antifungal activity must possess an aliphatic side chain with a chain length of C12 to C16.[15] Thus, we hypothesized that 6-alkyl-2,3,4,5-tetrahydropyridine derivatives with a relatively long C-6 side chain length, greater than C12, would possess antifungal activities. In this study, we have synthesized seven 6-alkyl-2,3,4,5-tetrahydropyridine derivatives with a C-6 side chain length from C12 to C18 and evaluated their antifungal activity against several clinically relevant opportunistic fungal pathogens as well as cytotoxicity against several mammalian cell lines using our published protocols.[14,16]

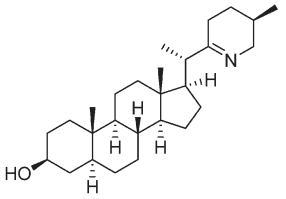
The methodology for the synthesis of our designed compounds followed reported procedures[13,17,18] for preparation of similar compounds and is shown in Scheme 1. Thus, the commercially available *N*-Boc-pyrrolidinone (**3**) reacted with a Grignard reagent (RMgBr or RMgCl) and TMEDA in THF or hexanes to afford *N*-Boc-ω-amininoketones (**4a**−**4g**). Treatment of the resultant aminoketone with TFA and then NaOH in DCM furnished the product 6-alkyl-2,3,4,5-tetrahydropyridines (**5a**−**5g**). The seven synthesized compounds are 6-dodecyl-2,3,4,5-tetrahydropyridine (**5a**), 6-tridecyl-2,3,4,5-tetrahydropyridine (**5b**), 6-tetradecyl-2,3,4,5-tetrahydropyridine (**5c**), 6-pentadecyl-2,3,4,5-tetrahydropyridine (**5d**), 6-hexadecyl-2,3,4,5-tetrahydropyridine (**5e**), 6-heptadecyl-2,3,4,5-tetrahydropyridine (**5f**), and

Received: August 1, 2011
Published: September 09, 2011

6-octadecyl-2,3,4,5-tetrahydropyridine (**5g**), providing a basis for SAR analysis.



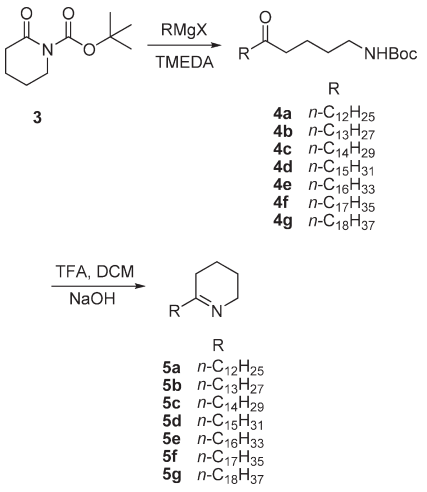
2-Methyl-6-pentadecyl-6-piperideine (**1**)



Solacongestidine (**2**)

Compounds **5a**−**5g** were evaluated for in vitro antifungal activities against *Cryptococcus neoformans*, *C. albicans*, *Candida glabrata*, *Candida krusei*, and *Aspergillus fumigatus*. At the highest test concentration (20 μg/mL) compounds **5a** and **5b**, with C-6 alkyl chain lengths of C12 and C13, respectively, did not show antifungal activities against any fungal species. It was also noted that all compounds were inactive against the filamentous fungus *A. fumigatus*. Compounds **5c**−**5g**, with C-6 alkyl chain lengths from C14 to C18, showed varying degrees of antifungal activities, with compounds **5e** and **5f** being the most active (Table 1). For example, compound **5e**, with a C16 alkyl chain, exhibited minimum fungicidal concentrations (MFCs) of 3.8, 15.0, 7.5, and 7.5 μg/mL against *C. neoformans*, *C. albicans*, *C. glabrata*, and *C. krusei*, respectively, compared to the positive control amphotericin B, with MFCs of 0.47, 0.63, 0.94, and 1.25 μg/mL, respectively. Compound **5e** was the only compound within the series active against *C. albicans*. An interesting trend was that the antifungal activity of these compounds is enhanced with increases in chain length up to C17. Activity was diminished, however, with a chain length of C18, as noted in compound **5g** (Table 1). The results confirmed our hypothesis that a relatively long chain length (C14 to C18) is required for the antifungal activity of the 6-alkyl-2,3,4,5-tetrahydropyridine alkaloids. The aliphatic chain lengths of these antifungal compounds appear to be closely associated with the specificity of their cellular target(s), necessitating optimal spatial interactions to reach maximal binding affinity. Interestingly, the chain lengths of our synthetic antifungal compounds match those of naturally occurring piperideines in fire ants with an alkyl/alkenyl chain length of C13 to C17.[1]

Scheme 1. Synthesis of 6-Alkyl- 2,3,4,5-tetrahydropyridines



Cytotoxicity evaluation of compounds **5c**−**5g** against four human cancerous cell lines (SK-MEL, KB, BT-549, and SK-OV-3) and two noncancerous cell lines (Vero and LLC-PK$_{11}$) showed that they were not toxic to any of the cell lines at the highest tested concentration (10 μg/mL). Therefore the 6-alkyl-2,3,4,5-tetrahydropyridines have a favorable selectivity profile, and of these compound **5e** warrants further preclinical studies as a potential antifungal lead.

It should be pointed out that fire ant venom alkaloids occur in minute quantities, and purified compounds are generally not available for biological studies. Extensive studies[5,19,20] have been conducted to prepare piperidine alkaloids previously identified from fire ant venoms to investigate their biological activities.[6,7,9,11] Natural or synthetic long-chain substituted alkylpyridines,[21] alkylpyridium alkaloids,[22,23] and bis(alkylpyridium) alkanes[24,25] have also been demonstrated to possess antifungal activities. However, the current study represents the first effort to prepare synthetic homologues of the newly discovered piperideine alkaloids in fire ants and to evaluate their antifungal activities. In view of possible physiological and behavioral functions of these small-molecule piperideine alkaloids in fire ants,[1,2,26] further studies on the synthesis, biological activities, and mechanisms of action of this chemotype of compounds should prove to be interesting.

EXPERIMENTAL SECTION

General Experimental Procedures. NMR spectra were recorded on Bruker DRX-400 instruments. Chemical shifts are expressed

Table 1. In Vitro Antifungal Activity of Compounds 5c−5g[a]

	MIC[b] (MFC[c]), μg/mL			
compound	*C. neoformans* ATCC 90113	*C. albicans* ATCC 90028	*C. glabrata* ATCC 90030	*C. krusei* ATCC 6258
5c	10.0 (10.0)	na[d]	na[d]	na[d]
5d	11.2 (12.5)	na[d]	10.0 (15.0)	10.0 (15.0)
5e	3.8 (3.8)	15.0 (15.0)	7.5 (7.5)	7.5 (7.5)
5f	2.5 (3.8)	na[d]	5.0 (7.5)	15.0 (15.0)
5g	10.0 (15.0)	na[d]	10.0 (15.0)	na[d]
amphotericin B	0.47 (0.47)	0.47 (0.63)	0.63 (0.94)	1.25 (1.25)

[a] Data are mean values from two independent experiments, each with two replicates. [b] Minimum inhibitory concentration (the lowest concentration that allows no detectable growth). [c] Minimum fungicidal concentration (the lowest concentration that kills the fungus). [d] Not active at the highest test concentration, 20 μg/mL.

dx.doi.org/10.1021/np200644s |J. Nat. Prod. 2011, 74, 2023–2026

in ppm relative to the solvent residue signals. High-resolution TOFMS were measured on an Agilent series 1100 SL spectrometer equipped with an ESI source. Flash column chromatography was done on silica gel (40 μm, J. T. Baker). TLC was performed on silica gel sheets (silica gel 60 F254, Merck, Germany). *N*-Boc-pyrrolidinone (**3**) was purchased from Santa Cruz Biotech (Santa Cruz, CA). The Grignard reagents *n*-$C_{13}H_{27}MgBr$, *n*-$C_{15}H_{31}MgBr$, *n*-$C_{16}H_{33}MgBr$, and *n*-$C_{17}H_{35}MgBr$ were prepared according to a reported procedure.[27] All other reagents were obtained from commercial vendors in appropriate grades and were used without further purification unless otherwise indicated.

General Procedure for Preparation of *N*-Boc-aminoketones (4a−4g). The Grignard reagent RMgBr or RMgCl (1 mmol) in heptanes or THF (1 mL) and TMEDA (0.15 mL) was added dropwise to a magnetically stirred solution of *N*-Boc-pyrrolidinone (**3**, 0.75 mmol) in THF (3 mL) at −78 °C for **4a** and **4b**, or at −25 °C for **4c**−**4g**, under an argon atmosphere. After stirring at that temperature for 10 h, 2 M HCl was added to quench the reaction. The resultant precipitate was filtered, washed with saturated $NaHCO_3$, brine, and H_2O (20 mL × 2), and dried to give the corresponding *N*-Boc-aminoketone as a white or light yellow solid. The compound was directly used for the next step reaction without purification.

*tert-Butyl 5-oxoheptadecylcarbamate (**4a**):* yield, 82%, white solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.83 (3H, t, J = 6.0 Hz, CH_3), 1.21 (20H, m, CH_2), 1.39 (9H, s, CH_3), 1.42−1.55 (4H, m, CH_2), 2.32−2.40 (4H, m, CH_2), 3.07 (2H, m, CH_2), 4.66 (1H, br s, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.0 (CH_3), 20.2 (CH_2), 22.6 (CH_2), 23.8 (CH_2), 28.4 (CH_3 × 3), 29.6 (CH_2), 31.8 (CH_2), 40.1 (CH_2), 42.0 (CH_2), 42.8 (CH_2), 78.9 (C), 156.0 (CO), 211.0 (NHCO).

*tert-Butyl 5-oxooctadecylcarbamate (**4b**):* yield, 85%, light yellow solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88 (3H, t, J = 6.0 Hz, CH_3), 1.25 (22H, m, CH_2), 1.44 (9H, s, CH_3), 1.45−1.61 (4H, m, CH_2), 2.36−2.44 (4H, m, CH_2), 3.11 (2H, m, CH_2), 4.59 (1H, br s, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1 (CH_3), 20.2 (CH_2), 22.7 (CH_2), 23.9 (CH_2), 28.4 (CH_3 × 3), 29.6 (CH_2), 32.1 (CH_2), 40.1 (CH_2), 42.1 (CH_2), 42.9 (CH_2), 79.1 (C), 156.0 (CO), 211.2 (NHCO).

*tert-Butyl 5-oxononadecylcarbamate (**4c**):* yield, 91%, white solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (3H, t, J = 6.0 Hz, CH_3), 1.24 (24H, m, CH_2), 1.42 (9H, s, CH_3), 1.43−1.60 (4H, m, CH_2), 2.35−2.43 (4H, m, CH_2), 3.10 (2H, m, CH_2), 4.61 (1H, br s, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1 (CH_3), 20.2 (CH_2), 22.6 (CH_2), 23.9 (CH_2), 28.4 (CH_3 × 3), 29.3 (CH_2), 29.58 (CH_2), 29.62 (CH_2), 29.65 (CH_2), 32.1 (CH_2), 40.1 (CH_2), 42.0 (CH_2), 42.9 (CH_2), 79.0 (C), 156.0 (CO), 211.1 (NHCO).

*tert-Butyl 5-oxoicosylcarbamate (**4d**):* yield, 90%, light yellow solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88 (3H, t, J = 8.0 Hz, CH_3), 1.26 (26H, m, CH_2), 1.44 (9H, s, CH_3), 1.45−1.57 (4H, m, CH_2), 2.36−2.44 (4H, m, CH_2), 3.11 (2H, m, CH_2), 4.58 (1H, br s, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1 (CH_3), 20.2 (CH_2), 22.7 (CH_2), 23.9 (CH_2), 28.4 (CH_3 × 3), 29.3 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 31.9 (CH_2), 40.1 (CH_2), 42.1 (CH_2), 42. Nine (CH_2), 79.0 (C), 156.0 (CO), 211.1 (NHCO).

*tert-Butyl 5-oxohenicosylcarbamate (**4e**):* yield, 95%, light yellow solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (3H, t, J = 8.0 Hz, CH_3), 1.25 (28H, m, CH_2), 1.43 (9H, s, CH_3) 1.45−1.56 (4H, m, CH_2), 2.35−2.43 (4H, m, CH_2), 3.10 (2H, m, CH_2), 4.61 (1H, br s, NH).

*tert-Butyl 5-oxodocosylcarbamate (**4f**):* yield, 85%, light yellow solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (3H, t, J = 8.0 Hz, CH_3), 1.23 (30H, m, CH_2), 1.41 (9H, s, CH_3), 1.44−1.57 (4H, m, CH_2), 2.33−2.39 (4H, m, CH_2), 3.08 (2H, m, CH_2), 4.67 (1H, br s, NH).

*tert-Butyl 5-oxotricosylcarbamate (**4g**).* yield, 80%, light yellow solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88 (3H, t, J = 8.0 Hz, CH_3), 1.26 (32H, m, CH_2), 1.44 (9H, s, CH_3), 1.45−1.59 (4H, m, CH_2), 2.36−2.44 (4H, m, CH_2), 3.11 (2H, m, CH_2), 4.68 (1H, br s, NH).

General Procedure for Preparation of 6-Alkyl-2,3,4,5-tetrahydropyridines (5a−5g). To a solution of aminoketone (**4a**−**4g**) (100 mg) in DCM (2 mL) at −10 °C was added TFA (0.45 mL) dropwise in 5 min. The solution was slowly brought to room temperature and stirred for 24 h. The reaction mixture was basified with 2 M NaOH (pH >12), diluted with H_2O, and then extracted with DCM. The organic phase was washed with saturated $NaHCO_3$, brine, and H_2O, dried over $MgSO_4$, and evaporated to dryness to give **5a**−**5g**. Purification by column chromatography using DCM−MeOH (9.5:0.5 to 9.0:1) as eluent yielded the final products.

*6-Dodecyl-2,3,4,5-tetrahydropyridine (**5a**):* 59 mg, yield, 87% from **4a**, light yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.84 (3H, t, J = 7.0 Hz, CH_3), 1.22 (18H, m, CH_2), 1.50−1.62 (6H, m, CH_2), 2.08 (4H, m, CH_2), 3.51 (2H, m, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.0 (CH_3), 19.5 (CH_2), 21.9 (CH_2), 22.4 (CH_2), 26.5 (CH_2), 29.5 (CH_2), 29.59 (CH_2 × 2), 29.62 (CH_2 × 6), 41.0 (CH_2), 49.0 (CH_2), 171.5 (C, imine carbon); HRESIMS m/z 252.3055 (calcd for $[C_{17}H_{33}N + H]^+$, 252.2686).

*6-Tridecyl-2,3,4,5-tetrahydropyridine (**5b**):* 62 mg, yield, 90% from **4b**, light yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (3H, t, J = 7.0 Hz, CH_3), 1.24 (20H, m, CH_2), 1.56−1.67 (6H, m, CH_2), 2.16 (4H, m, CH_2), 3.55 (2H, m, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1 (CH_3), 19.2 (CH_2), 21.5 (CH_2), 22.7 (CH_2), 26.5 (CH_2), 29.62 (CH_2 × 3), 29.65 (CH_2 × 7), 40.7 (CH_2), 48.6 (CH_2), 171.7 (C, imine carbon); HRESIMS m/z 266.2874 (calcd for $[C_{18}H_{35}N + H]^+$, 266.2842).

*6-Tetradecyl-2,3,4,5-tetrahydropyridine (**5c**):* 60 mg, yield, 86% from **4c**, light yellow amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.83 (3H, t, J = 7.0 Hz, CH_3), 1.21 (22H, m, CH_2), 1.47−1.60 (6H, m, CH_2), 2.11 (4H, m, CH_2), 3.50 (2H, m, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.0 (CH_3), 19.3 (CH_2), 21.8 (CH_2), 22.6 (CH_2), 26.5 (CH_2), 29.60 (CH_2), 29.61 (CH_2 × 2), 29.63 (CH_2 × 8), 40.9 (CH_2), 48.8 (CH_2), 172.1 (C, imine carbon); HRESIMS m/z 280.3053 (calcd for $[C_{19}H_{37}N + H]^+$, 280.2999).

*6-Pentadecyl-2,3,4,5-tetrahydropyridine (**5d**):* 66 mg, yield, 92% from **4d**, light yellow amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.89 (3H, t, J = 6.0 Hz, CH_3), 1.26 (24H, m, CH_2), 1.47−1.72 (6H, m, CH_2), 2.20−2.40 (4H, m, CH_2), 3.62 (2H, m, CH_2); ^{13}C NMR ($CDCl_3$) δ 14.1 (CH_3), 18.9 (CH_2), 20.9 (CH_2), 22.7 (CH_2), 26.6 (CH_2), 29.2 (CH_2), 29.3 (CH_2 × 2), 29.6 (CH_2 × 9), 40.0 (CH_2), 47.5 (CH_2), 171.8 (C, imine carbon); HRESIMS m/z 294.3194 (calcd for $[C_{20}H_{39}N + H]^+$, 294.3155).

*6-Hexadecyl-2,3,4,5-tetrahydropyridine (**5e**):* 65 mg, yield, 90% from **4e**, light yellow amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88 (3H, t, J = 7.0 Hz, CH_3), 1.26−1.29 (26H, m, CH_2), 1.55−1.72 (6H, m, CH_2), 2.23−2.31 (4H, m, CH_2), 3.60 (2H, m, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1 (CH_3), 18.9 (CH_2), 21.3 (CH_2), 22.7 (CH_2), 26.8 (CH_2), 29.3 (CH_2), 29.64 (CH_2 × 2), 29.67 (CH_2 × 10), 31.8 (CH_2), 40.3 (CH_2), 48.0 (CH_2), 171.8 (C, imine carbon); HRESIMS m/z 308.3343 (calcd for $[C_{21}H_{41}N + H]^+$, 308.3312).

*6-Heptadecyl-2,3,4,5-tetrahydropyridine (**5f**):* 67 mg, yield, 95% from **4f**, light yellow amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88 (3H, t, J = 7.0 Hz, CH_3), 1.25 (28H, m, CH_2), 1.55−1.72 (6H, m, CH_2), 2.26−2.33 (4H, m, CH_2), 3.60 (2H, m, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1 (CH_3), 18.9 (CH_2), 21.3 (CH_2), 22.7 (CH_2), 26.5 (CH_2), 29.40 (CH_2), 29.63 (CH_2 × 2), 29.67 (CH_2 × 11), 40.2 (CH_2), 47.8 (CH_2), 171.2 (C, imine carbon); HRESIMS m/z 322.3509 (calcd for $[C_{22}H_{43}N + H]^+$, 322.3468).

*6-Octadecyl-2,3,4,5-tetrahydropyridine (**5g**):* 73 mg, yield, 99% from **4g**, light yellow amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.89 (3H, t, J = 7.0, CH_3), 1.26−1.29 (30H, m, CH_2), 1.55−1.73 (6H, m, CH_2), 2.22−2.27 (4H, m, CH_2), 3.59 (2H, m, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1 (CH_3), 18.9 (CH_2), 21.5 (CH_2), 22.7 (CH_2), 27.0 (CH_2), 29.42 (CH_2), 29.64 (CH_2 × 2), 29.67 (CH_2 × 12), 40.5 (CH_2), 48.2 (CH_2), 171.8 (C, imine carbon); HRESIMS m/z 336.3641 (calcd for $[C_{23}H_{45}N + H]^+$, 336.3625).

In Vitro Antifungal Assay. A modified version of the CLSI (formerly NCCLS) method was used for susceptibility testing. Organisms

(*C. neoformans* ATCC 90113, *C. albicans* ATCC 90028, *C. glabrata* ATCC 90030, *C. krusei* ATCC 6258, and *A. fumigatus* ATCC 204305) were obtained from the American Type Culture Collection (ATCC) (Manassas, VA). The detailed procedure has been described previously.[14,16]

In Vitro Cytotoxicity Assay. The mammalian cell lines used in this study were obtained from ATCC. They included four human cancerous cell lines [SK-MEL (melanoma), KB (epidermal carcinoma, oral), BT-549 (ductal carcinoma, breast), and SKOV-3 (ovary carcinoma)] and two noncancerous cell lines [Vero (African green monkey kidney fibroblast) and LLC-PK_{11} (pig kidney epithelial cells)]. The assay was an adaptation of the Neutral Red method,[28] and the detailed procedure was described in a previous paper.[16]

■ ASSOCIATED CONTENT

Supporting Information. NMR and MS spectra of compounds **4a** and **5a**−**5g**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: 662-915-6742. Fax: 662-915-7989. E-mail: xcli7@olemiss.edu.

■ ACKNOWLEDGMENT

The authors wish to thank Ms. Marsha Wright and Mr. John Trott for biological testing, and Dr. Bharthi Avula for recording high-resolution ESIMS spectra. This work was supported by the NIH, NIAID, Division of AIDS, Grant No. AI 27094, the USDA Agricultural Research Service Specific Cooperative Agreement No. 58-6408-2-0009, and Zhejiang University, China.

■ REFERENCES

(1) Chen, J.; Cantrell, C. L.; Shang, H. W.; Rojas, M. G. *J. Agric. Food. Chem.* **2009**, *57*, 3128–3133.
(2) Chen, J.; Shang, H.; Jin, X. *Toxicon* **2010**, *55*, 1181–1187.
(3) Chen, L.; Fadamiro, H. Y. *Toxicon* **2009**, *53*, 469–478.
(4) Chen, L.; Hu, Q.-B.; Fadmiro, H. Y. *J. Agric. Food. Chem.* **2010**, *58*, 11534–11542.
(5) Jones, T. H.; Blum, M. S.; Fales, H. M. *Tetrahedron* **1982**, *38*, 1949–1958.
(6) Howell, G.; Butler, J.; Deshazo, R. D.; Farley, J. M.; Liu, H. L.; Nanayakkara, N. P.; Yates, A.; Yi, G. B.; Rockhold, R. W. *Ann. Allergy Asthma Immunol.* **2005**, *94*, 380–386.
(7) Yi, G. B.; McClendon, D.; Desaiah, D.; Goddard, J.; Lister, A.; Moffitt, J.; Vander Meer, R. K.; deShazo, R.; Lee, K. S.; Rockhold, R. W. *Int. J. Toxicol.* **2003**, *22*, 81–86.
(8) Blum, M. S.; Walker, J. R.; Callahan, P. S.; Novak, A. F. *Science* **1958**, *128*, 306–307.
(9) Jouvenaz, D. P.; Blum, M. S.; MacConnell, J. G. *Antimicrob. Agents Chemother.* **1972**, *2*, 291–293.
(10) Storey, G. K.; Vander Meer, R. K.; Boucias, D. G.; McCoy, C. W. *J. Invertebr. Pathol.* **1991**, *58*, 88–95.
(11) Sullivan, D. C.; Flowers, H.; Rockhold, R.; Herath, H. M.; Nanayakkara, N. P. *Am. J. Med. Sci.* **2009**, *338*, 287–291.
(12) Kusano, G.; Takahashi, A.; Sugiyama, K.; Nozoe, S. *Chem. Pharm. Bull.* **1987**, *35*, 4862–4867.
(13) Abdel-Kader, M. S.; Bahler, B. D.; Malone, S.; Werkhoven, M. C. M.; vanTroon, F.; David; Wisse, J. H.; Bursuker, I.; Neddermann, K. M.; Mamber; Kingston, D. G. I. *J. Nat. Prod.* **1998**, *61*, 1202–1208.
(14) Li, X.-C.; Jacob, M. R.; Khan, S. I.; Ashfaq, M. K.; Babu, K. S.; Agarwal, A. K.; Elsohly, H. N.; Manly, S. P.; Clark, A. M. *Antimicrob. Agents Chemother.* **2008**, *52*, 2442–2448.
(15) Li, X. C.; Babu, K. S.; Jacob, M. R.; Khan, S. I.; Agarwal, A. K.; Clark, A. M. *ACS Med. Chem. Lett.* **2011**, *2*, 391–395.
(16) Yang, C.-R.; Zhang, Y.; Jacob, M. R.; Khan, S. I.; Zhang, Y.-J.; Li, X.-C. *Antimicrob. Agents Chemother.* **2006**, *50*, 1710–1714.
(17) Giovannini, A.; Savoia, D.; Umani-Ronchi, A. *J. Org. Chem.* **1989**, *54*, 228–234.
(18) Fuganti, C; Gatti, F. G.; Serra, S. *Tetrahedron* **2007**, *63*, 4762–4767.
(19) Leclercq, S.; Daloze, D.; Braekman, J.-C. *Org. Prep. Proced. Int.* **1996**, *28*, 501–543.
(20) Herath, B. H. M. T.; Nanayakkara, N. P. *J. Heterocycl. Chem.* **2008**, *45*, 129–136.
(21) Tsuji, K. *Yakugaku Zasshi* **1976**, *96*, 479–83.
(22) Elersek, T.; Kosi, G.; Turk, T.; Pohleven, F.; Sepcic, K. *Biofouling* **2008**, *24*, 137–143.
(23) Puritch, G. S.; Kondo, E. S. U.S. Patent 4,576,950, 1986.
(24) Obando, D.; Pantarat, N.; Handke, R.; Koda, Y.; Widmer, F.; Djordjevic, J. T.; Ellis, D. H.; Sorrell, T. C.; Jolliffe, K. A. *Bioorg. Med. Chem.* **2009**, *17*, 6329–6339.
(25) Chen, S. C.-A.; Biswas, C.; Bartley, R.; Widmer, F.; Pantarat, N.; Obando, D.; Djordjevic, J. T.; Ellis, D. H.; Jolliffe, K. A.; Sorrell, T. C. *Antimicrob. Agents Chem.* **2010**, *54*, 3233–3240.
(26) Braekman, J. C.; Daloze, D. *J. Brazil. Chem. Soc.* **1996**, *7*, 251–256.
(27) Lai, Y.-H. *Synthesis* **1981**, 585–604.
(28) Borenfreund, E.; Puerner, J. *Toxicol. Lett.* **1985**, *24*, 119–124.