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Published

2011

Journal Title

Journal of Natural Products

Version

Accepted Manuscript (AM)

DOI

10.1021/np200700f

Alkaloids from the Chinese Vine *Gnetum montanum*

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During a high-throughput screening campaign of a prefractionated natural product library, fractions prepared from the Chinese vine *Gnetum montanum* (Gnetaceae) showed *in vitro* activity against *Pseudomonas aeruginosa* wild type strain PAO1. UV-directed isolation of the organic extract from the leaves resulted in the purification of the TFA salts of the new natural products *N*-methyl-laudanosolinium (**1**), 3'-hydroxy-*N*,*N*-dimethyl coclaurinium (**2**), 1,9,10-trihydroxy-2-methoxy-6-methyl aporphinium (**3**) and 6a,7-didehydro-1,9,10-trihydroxy-2-methoxy-6-methyl aporphinium (**4**). Compound **4** is described here for the first time, and this is the first report of compounds **1**-**3** as natural products. We also report the known natural products latifolian A (**5**) and magnocurarine (**6**). When tested for their effect on the viability of this bacterial strain, **5** displayed an IC_{50} value of 9.8 μM, while **3** and **4** displayed minimal activity, and **1**, **2** and **6** were inactive. Based upon a 55% inhibitory activity displayed against *Staphylococcus aureus* culture at 350μM of compound, **5** was estimated to have a moderate selectivity index to *P.aeruginosa* of 36 times.

Species that comprise the group of non-fermenting, Gram-negative bacteria, pose a major risk for healthcare as they possess multidrug resistance.[1] Such species include *P. aeruginosa*, *S. maltophilia*, and *Acinetobacter* spp, which are opportunistic pathogens and prominent in critically ill, hospitalised patients.[2] The multidrug, genetically acquired resistance of these pathogens is primarily due to the active transport of drugs out of the cell by multidrug transporters in efflux pump systems.[3, 4] Intrinsic resistance by these bacteria also decreases those antibiotics that can be used for treatment, including ampicillin, cephalosporins and macrolide antibiotics, mainly due to impermeability.[1, 5] Because of resistance displayed by these and other, particularly Gram-negative bacteria, there has been further reliance upon more toxic drugs than those commonly applied.[6] These facts highlight the urgent need to discover new, selective antibiotics, with low toxicity and new mechanisms of action. Natural products constitute a possible source of new leads for this search as most existing antibiotics are based on natural chemotypes.[7]

We undertook a high throughput screening (HTS) campaign to identify potential leads with an antibiotic effect against the wild type strain of *P. aeruginosa* (PAO1). Initial hits were identified in an *in vitro*, whole cell, optical density based 384 well survival assay. The *P. aeruginosa* efflux pump knockout strain, PAO200 (MexAB-OprM deficient mutant) was employed to screen a pre-fractionated natural product library, this strain was used to potentially increase the initial hits identified by decreasing efflux clearance. HTS hits were re-tested against the wild type strain PAO1 and a Gram-positive bacterium, *Staphylococcus aureus* (strain 01A1095) for estimation of Gram selectivity. Three fractions prepared from the Chinese plant *Gnetum montanum* Markgr. showed in vitro activity against *P. aeruginosa* PAO200. These fractions were early eluting ones and showed characteristic UV profiles. Thus, compounds with a short retention time obtained from a chromatographical separation of the combined CH_2Cl_2/MeOH plant extract were isolated. This afforded the benzylisoquinoline alkaloids *N*-methyl-laudanosolinium (**1**), and 3'-hydroxy-*N*,*N*-dimethyl coclaurinium (**2**), the aporphine alkaloids 1,9,10-trihydroxy-2-methoxy-6-methyl

aporphinium (**3**) and 6a,7-didehydro-1,9,10-trihydroxy-2-methoxy-6-methyl aporphinium (**4**), as well as the known alkaloids latifolian A (**5**) and magnocurarine (**6**). The antibacterial activities for compounds **1**-**6** against *P. aeruginosa* PAO1 and *S.aureus* 01A1095 are also reported.

Result and discussion

The air-dried and ground leaves of *G. montanum* were sequentially extracted with *n*-hexane, CH_2Cl_2, and MeOH. The *n*-hexane extract was discarded, and the remaining organic extracts combined and prepurified on polyamide gel using MeOH as eluent. The resulting crude MeOH wash was subjected to several steps of C_{18} semi-preparative HPLC with different gradients of MeOH/H_2O (0.1% TFA) to yield **1** (6.1 mg, 0.24% dry wt), **2** (7.2 mg, 0.17% dry wt), **3** (6.3 mg, 0.15% dry wt), **4** (1.3 mg, 0.03% dry wt), **5** (3.6 mg, 0.09% dry wt) and **6** (6.4 mg, 0.15% dry wt). All alkaloids were isolated as their TFA salts. Compounds **5** and **6** were assigned to known compounds latifolian A and magnocurarine[8], respectively, after spectroscopic data comparison. Latifolian A was previously isolated from *Gnetum latifolium*[9] and exhibited inhibition in an assay against the JNK3 kinase. Magnocurarine, isolated from a variety of plants, has been shown to display weak antinociceptive activity.[10]

Compound **1** was isolated as an optically active brown gum. Its molecular formula was shown to be $C_{18}H_{22}NO_4^+$ by HRESIMS ($[M]^+$: *m/z* 316.15437). Analysis of the ^1H NMR and gCOSY spectra (Table 1) revealed the presence of two aromatic isolated singlets at δ_H 6.58 and 5.94, one aromatic ABX system (δ_H 6.65, d, J = 8.0 Hz; δ_H 6.53, d, J = 1.9 Hz; and δ_H 6.35, dd, J = 8.0, 1.9 Hz), two N^+Me groups (δ_H 3.23 and 3.03), two contiguous methylene groups (δ_H 3.68/3.54 and 2.97) and finally one methine-methylene system (δ_H 4.55/3.44, 2.68). Furthermore, correlations observed in the gHSQC and gHMBC spectra showed the presence of two different substituted aromatic rings (rings A and B), both of which possessed a catechol function [δ_C 143.6

and 145.5 (first ring), 145.2 and 144.1 (second ring)]. The two isolated aromatic singlets were found to be *para* to each other on the aromatic ring A (δ_C 114.7 and 114.5 respectively), while the aromatic protons in the ABX system could be placed on the aromatic ring B (δ_C 115.2, 116.6 and 119.9). The two last carbons belonging to the aromatic ring A were quaternary (δ_C 118.9 and 121.6). The carbon at δ_C 118.9 showed HMBC correlations with the two contiguous methylenes (with carbons at δ_C 54.3 and 22.4), while the carbon at δ_C 121.6 displayed HMBC correlations with the methine-methylene system (with carbons at δ_C 71.3, 35.6). Moreover the protons in both N^+Me groups showed HMBC correlations with the carbons at δ_C 71.3 and 54.3, thus allowing the ring to be closed, creating an isoquinolinium moiety. Furthermore, correlations between the carbon at δ_C 35.6, present in the methine-methylene system, with protons belonging to the aromatic ring B confirmed that **1** was a benzylisoquinoline derivative. These two systems were connected through a quaternary carbon at δ_C 127.7. Compound **1** is thus *N*-methyl-laudanosolinium trifluoroacetate. The synthetic preparation of the iodide salt has already been reported.[11] We report here the full NMR characterisation of **1**.

In order to determine the absolute stereochemistry of (+)-**1** we compared the optical rotation data for (+)-*N*-methyl-laudanosolinium with literature values of related natural products, such as (+)-roefractine,[12] (+)-laudanosine[13] and (+)-reticuline[14] whose absolute stereochemistry had been unequivocally determined, either by CD measurements, or by asymmetrical synthesis. These three compounds all showed positive optical rotations, and their absolute configuration at C-1 was determined as *S*. The optical rotation of compound **1** was also positive, thus suggesting that it shares the same absolute stereochemistry (*S*) as (+)-roefractine, (+)-laudanosine and (+)-reticuline.

The major metabolite, compound **2**, was isolated as an optically active gum. Comparison of the NMR and UV data of **2** with **1** clearly identified that **2** also belonged to the benzylisoquinoline structure class. The major difference in the NMR data of **1** with **2** was that one

of the phenolic moiety present had been replaced by a methoxy group in **2** (δ_H 3.75 / δ_C 55.1). This was confirmed by the HRESIMS ($[M]^+$: *m/z* 330.16914) which indicated a molecular formula of $C_{19}H_{24}NO_4^+$, consistent with **2** being a methyl ether derivative of **1**. The methoxy position was determined following gHMBC analysis. A strong correlation could be observed between the methoxy protons and the carbon C-6 (δ_C 147.7). Moreover the ROESY spectrum showed strong correlation between the methoxy protons and the aromatic proton H-5 (δ_H 6.79). The structure of compound **2** was therefore assigned as the trifluoroacetate salt of 3'-hydroxy-*N*,*N*-dimethyl-coclaurinium. This is the first report of **2** as natural product. As for compound **1**, the synthesis of **2** has been previously reported.[15] Our data is in accordance with the UV spectrum of the literature. Moreover we report here the full NMR data of **2**.

The absolute configuration of C-1 of **2** could be determined using the same arguments as for that of **1**. Since the optical rotation of **2** was also positive, the configuration of C-1 was assigned as *S*.

Compound **3** was isolated as an optically active gum. Its UV spectrum was markedly different compared to **1** and **2**. Moreover, the molecular formula obtained from the HRESIMS ($[M]^+$: *m/z* 328.15320), $C_{19}H_{22}NO_4^+$, indicated that **3** is a reduced form of **2**, the plausible structure being an aporphine derivative. Analysis of the ^{1}H NMR spectrum (Table 2) showed three aromatic singlets at δ_H 7.85, 6.78 and 6.71, but signals were still present for both the two contiguous methylenes (δ_H 3.74/3.65 and 3.18/2.93), the methine-methylene system (δ_H 4.56/3.16, 2.73), the two N^+Me groups (δ_H 3.34 and 2.93) and the methoxy group (δ_H 3.85). These signals are typical for aporphine derivatives[16] and aporphinium salts.[17] The correlations observed in the gHSQC and gHMBC spectra confirmed the structure. The isolated singlet at δ_H 6.78 (δ_C 109.0) was attached to the aromatic ring A. HMBC correlations were observed from this signal to two phenolic carbons (δ_C 148.1 and 142.1) and a quaternary aromatic carbon at δ_C 119.5. The carbon at δ_C 119.5 showed HMBC correlations to both contiguous methylene protons (with carbons at δ_C 23.1 and 60.4) as

well as the methine-methylene system with carbons at δ_C 68.3 and 28.1. The carbon at δ_C 28.1 also displayed correlations with the two other aromatic proton singlets belonging to the aromatic ring B. With the help of the gHMBC spectrum, the four other remaining carbons could be determined to be two phenolic carbons in *ortho* position to each other (δ_C 144.6 and 143.7) and two quaternary carbons (δ_C 123.4 and 122.5). Finally the aromatic proton at δ_H 7.85 displayed a correlation with a quaternary carbon at δ_C 120.4, which belongs to the aromatic ring A, validating the aporphine skeleton. The methoxy group of **3** was positioned at C-2 following 2D NMR data analysis. The methoxy protons (δ_H 3.85) showed a strong HMBC correlation with the C-2 phenolic carbon (δ_C 148.1) and showed a ROESY correlation to the aromatic proton H-3 (δ_H 6.78). Compound **3** is thus the trifluoroacetate salt of 1,9,10-trihydroxy-2-methoxy-6-methyl aporphinium, This is the first report of **3** as a natural product. Its synthesis has been reported by the same group as that of of **2**[15], this latter structure being the starting point to get **3** by oxidative coupling. Our data is in accordance with the ^{1}H NMR and UV spectra from the literature. Moreover we report here the full ^{13}C NMR data of **2**.

To determine the absolute stereochemistry of (+)-**3** we compared the optical rotation data with literature values of different aporphines, which have been summarised by Ringdahl *et al.*[18]. They reported values from a set of these alkaloids whose absolute stereochemistry had been unequivocally determined by CD measurements. Furthermore the compounds which showed positive optical rotations were determined to have an absolute configuration of *S* at C-6a. Since the optical rotation of compound **3** was positive, we assigned the C-6a absolute stereochemistry as *S*.

The minor plant metabolite, **4**, was isolated as an optically inactive gum. Its molecular formula of **4** ($C_{19}H_{20}NO_4^+$), given by HRESIMS ($[M]^+$: *m/z* 326.13819), is only different from that of **3** by the loss of two hydrogens, which indicated that **4** was a reduced form of **3**. On analysing the ^{1}H NMR spectrum of **4** (Table 2), the disappearance of the signals belonging to the methine-

methylene system became evident. The aromatic part of the spectrum also showed major modifications. Four aromatic singlets could be detected (δ_H 9.22, 8.10, 7.40 and 7.30), with downfield chemical shifts in comparison with the aromatic proton signals of **3**. These signals suggested a 6a-7-dehydro-aporphine structure, which displayed a three aromatic ring conjugation system. The other proton signals of **4** icluded a N^+Me singlet (δ_H 3.64 which integrated for 6 protons), an OMe singlet (δ_H 3.99) and a methylene-methylene system (δ_H 4.02 and 3.47). The correlations observed in the gHSQC and gHMBC spectra were in accordance with this kind of structure.[19] The OMe group was positioned at C-2 (δ_c 147.6) on the basis of HMBC and ROESY correlations. The structure of **4** was therefore the trifluoroacetate salt of the 6a,7-didehydro-1,9,10-trihydroxy-2-methoxy-6-methyl aporphinium.

Gnetum is currently the only genus belonging to the family Gnetaceae. These gymnosperms have vessel elements in their xylem, which is a feature more commonly found in the angiosperms than in gymnosperms. Plants of this genus include trees, shrubs and lianas and are found in Africa, Asia as well as in Central and South America. The genus is a well known source of polyphenols, mainly stilbenoid derivatives.[20] There are a small number of papers reporting the presence of alkaloids from this genus. To date, only ten alkaloids have been reported.[21] *Gnetum montanum* is a vine which can reach more than 10 m in length. It can be found in the forests of the southern part of China, as well as in other South-Asian countries (Bhutan, India, Laos, Myanmar, Thailand and Vietnam). Fibres from the stem bark have been used for making bags, fishing nets, and ropes. The seeds produce an edible oil, and can also been eaten fried.[22] This plant has been used as a traditional medicine in China to treat arthritis and bronchitis. The plant is a source of stilbenes, dimeric stilbenes, flavonoids and triterpenes.[23]

Table 3 shows the antibacterial activities against *P. aeruginosa* PAO1 and *S. aureus* 01A1095 for compounds **1**-**6**. The activity of the reference antibiotic, ciprofloxacin is also reported. Compounds **3** and **4** displayed weak activities, while **1**, **2**, and **6** were not active. It is

worth noting that both aporphine derivatives were slightly active, while the three benzylisoquinolines were inactive. Compound **5** showed promising inhibitory activity against *P. aeruginosa* PAO1, with minimal activity against *S. aureus* 01A1095, displaying a moderate selectivity for *Pseudomonas*, approximated at 36 times. Screening of **5** against other Gram-negative and positive species would be warranted to further determine Gram- selectivity.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco P-1020 polarimeter (Jasco, Tokyo, Japan). UV spectra were recorded on a Jasco V650 UV/vis spectrophotometer. NMR spectra were recorded at 30 °C on either a Varian 500 or 600 MHz Unity INOVA spectrometer (Varian, Walnut Creek, CA, USA). The latter spectrometer was equipped with a triple resonance cold probe. The ^{1}H and ^{13}C NMR chemical shifts were referenced to the solvent peaks for DMSO-d_6 at δ_H 2.49 and δ_C 39.5. LRESIMS were recorded on a Mariner Time-of-Flight spectrometer (Applied BioSystems, Foster City, CA, USA) equipped with a Gilson 215 eight probe injector (Waters, Milford, MA, USA). HRESIMS were recorded on a Bruker Daltronics Apex III 4.7e Fourier-transform mass spectrometer (Bruker, Karlsruhe, Germany). A BIOLINE orbital shaker (Edwards Instrument Company, Narellan, NSW, Australia) was used for the large-scale extraction of plant material. Machery Nagel Polyamide CC6 (0.05-0.016 mm) was used for tannin/polyphenolic removal (Machery Nagel, Düren, Germany). Alltech Davisil 40-60 μm 60 Å C_{18} bonded silica was used for preadsorption work (Alltech, Deerfield, IL, USA). A Waters 600 pump equipped with a Waters 996 PDA detector and a Waters 717 autosampler were used for HPLC. A Thermo-Electron C_{18} Betasil 143 Å column (5 μm, 21.2 × 150 mm) (Thermo Scientific, Los Angeles, CA, USA) or a Phenomenex Luna C_{18} (5 μm, 21.2 × 250 mm) (Phenomenex, Torrance, CA, USA) were used for semipreparative HPLC separations. All solvents

used for chromatography, UV, and MS were Lab-Scan HPLC grade (RCI Lab-Scan, Bangkok, Thailand), and the H_2O was Millipore Milli-Q PF filtered (Millipore, Billerica, MA, USA).

Plant Material. The leaves of *Gnetum montanum* Markgr. (Gnetaceae) were collected by the Zi Yuan Company in the Guangxi province, China, in January 2000 and a voucher sample (17-JAN-2000:12:00_03101699C) is lodged with the Zi Yuan Medicine Company, China.

Extraction and Isolation. The dried and ground leaves of *G. montanum* (10 g) were transferred to a conical flask (1 L), *n*-hexane (250 mL) was added, and the flask was shaken at 200 rpm for 2 h. The *n*-hexane extract was filtered under gravity, and then discarded. CH_2Cl_2 (250 mL) was added to the defatted plant material in the conical flask and shaken at 200 rpm for 2 h. The resulting extract was filtered under gravity and set aside. MeOH (250 mL) was added, and the MeOH/plant mixture was shaken for a further 2 h at 200 rpm. Following gravity filtration the plant material was extracted again with MeOH (250 mL) while being shaken at 200 rpm for 16 h. All CH_2Cl_2/MeOH extracts were combined and dried under reduced pressure to yield a dark green-brown solid (2.88 g). This material was resuspended in MeOH (150 mL), loaded onto MeOH-conditioned polyamide gel (30 g) in a sintered glass column, and washed with MeOH (300 mL) to yield 2.45 g of tannin-free extract. A portion of the this crude (1 g) was preadsorbed to C_{18}-bonded silica, and then packed into a stainless steel cartridge (10 × 30 mm) that was subsequently attached to a C_{18} Betasil HPLC column. Isocratic HPLC conditions of 90% H_2O (0.1% TFA)/10% MeOH (0.1% TFA) were initially employed for 10 min, then a linear gradient to 100% MeOH (0.1% TFA) was run over 40 min, followed by isocratic conditions of 100% MeOH (0.1% TFA) for a further 10 min, all at a flow rate of 9 mL/min. Sixty fractions (60 × 1 min) were collected from time = 0 min, then analysed by (+)-LRESIMS. Fraction 18 afforded 9.8 mg of **1** (0.24% dry wt). Fraction 20 was purified by HPLC using the same Betasil column as before, and a gradient of H_2O (0.1% TFA) / MeOH (0.1% TFA) (80:20 to 50:50 in 60 minutes) to yield 7.2 mg of **2** (0.17% dry wt). Compound **5** (3.6 mg, 0.09% dry wt) was obtained by separating fraction 21 by HPLC with a

Phenomenex Luna C_{18} column using a gradient of H_2O (0.1% TFA) / MeOH (0.1% TFA) (80:20 to 50:50 in 30 minutes). Fraction 26 was purified by HPLC using the same Phenomenex Luna C_{18} column as before, and a gradient of H_2O (0.1% TFA) / MeOH (0.1% TFA) (70:30 to 50:50 in 30 minutes) to yield 1.3 mg of **4** (0.03% dry wt). Finally, fraction 22 was separated with the Phenomenex Luna C_{18} column using a gradient of H_2O (0.1% TFA) / MeOH (0.1% TFA) (70:30 to 50:50 in 30 minutes). Thirty fractions (30 × 1 min) were collected from time = 0 min, then analysed by (+)–LRESIMS. Fraction 21 afforded 6.3 mg of **3** (0.15% dry wt), while fraction 23 yielded 6.4 mg of **6** (0.15% dry wt).

***P. aeruginosa* optical density (OD_{620}) viability assay.** *P.aeruginosa* PAO1 or PAO200 strain (supplied by Pfizer Global Research and Development) cultures were prepared at 3.5×10^4 CFU/mL in cation-adjusted Mueller Hinton (caMH) broth (Difco, Detroit, MI, USA), from concentrated frozen stocks of culture. The final bacterial concentration in the assay was 1500 CFU/well. 45 µL of diluted bacteria was added to a 384 well lidded, sterile clear plate (Becton Dickinson, Franklin Lakes, NJ, USA) containing controls/fractions by a Multidrop™ liquid handler (Thermo Scientific, Barrington, USA). Plates were incubated at 37°C in a humidified incubator for 18 hours or until the wells reached an optical density (OD_{620}) of between 0.7-0.8, then allowed to cool for 30 minutes. Clear plate seals (Perkin Elmer, Meriden CT, USA) were placed over the plate surface before reading on a Multiskan Ascent reader (Thermo Scientific) at 620 nm.

Prior to addition of bacteria a 5 µL addition of either test fractions, compounds or control samples were added to the assay plate. Samples were prepared by dilution of stock fractions/compounds/controls in 100% DMSO in to the assay plate with addition of 0.875 µL of stock and 4.125 µL of autoclaved Milli-Q filtered water with a Minitrak™ (Perkin Elmer, Meriden CT, USA) liquid handler . The final concentration of DMSO in the assay was 1.75%. Each assay plate contained both positive and negative controls in columns 23 and 24 respectively. The positive

control, for uninhibited growth, consisted of 5 µL of DMSO/ milli-Q water to a final concentration of 1.75% and the negative control, or 100% cell death, was comprised of 5 µL of the broad spectrum antibiotic ciprofloxacin (Sigma-Aldrich, St Louis, MO, USA) at a final concentration of 5 µg/mL. Whole control plates were included for each assay run, which consisted of duplicate dose response curves of ciprofloxacin, each in triplicate.

***S.aureus* optical density viability assay.** The *S.aureus* assay was carried out as per *P.aeruginosa* assay, with the following modifications: the final bacterial concentration used was 1980 CFU/well and ciprofloxacin for the internal assay control wells was at 500 µg/mL. Incubation was for 19 hours, or until the OD_{620} reached 0.45

***N*-methyl-laudanosolinium trifluoroacetate (1)**. Brown gum; $[\alpha]_D^{24}$ 10.3 (MeOH, *c* 0.1); UV (MeOH) λ_{max} (log ε) 233 sh (3.19), 287 (2.99); ^{1}H and ^{13}C see Table 1; (+)-HRESIMS: *m/z* 316.15437 ($C_{18}H_{22}NO_4$: $[M]^+$, requires 316.15434)

3'-hydroxy-*N*,*N*-dimethyl coclaurinium trifluoroacetate (2). Brown gum; $[\alpha]_D^{25}$ 14.5 (MeOH, *c* 0.1); UV (MeOH) λ_{max} (log ε) 231 sh (3.52), 286 (3.29); ^{1}H and ^{13}C see Table 1; (+)-HRESIMS: *m/z* 330.16914 ($C_{19}H_{24}NO_4$: $[M]^+$, requires 330.16999)

1,9,10-trihydroxy-2-methoxy-6-methyl aporphinium trifluoroacetate (3). Brown gum; $[\alpha]_D^{24}$ 8.3 (MeOH, *c* 0.1); UV (MeOH) λ_{max} (log ε) 229 (3.80), 270 sh (3.28), 281 (3.33), 308 (3.42), 317 sh (3.39); ^{1}H and ^{13}C see Table 2; (+)-HRESIMS: *m/z* 328.15320 ($C_{19}H_{22}NO_4$: $[M]^+$, requires 328.15434)

6a,7-didehydro-1,9,10-trihydroxy-2-methoxy-6-methyl aporphinium trifluoroacetate (4). Brown gum; UV (MeOH) λ_{max} (log ε) 222 sh (3.74), 270 (3.88), 288 sh (3.67), 335 (3.35), 353 (3.33), 371 (3.32); ^{1}H and ^{13}C see Table 1; (+)-HRESIMS: *m/z* 326.13819 ($C_{19}H_{20}NO_4$: $[M]^+$, requires 326.13869)

Acknowledgements

The authors would like to acknowledge Pfizer Global Research and Development for financial support. We thank Alita Miller, Doug Fisher and Justin Montgomery at Pfizer Global Research and Development for advice during the screening and isolation campaign. The authors thank C. Lewis and K. Watts from the Molecular Libraries group (Eskitis Institute), for their assistance in the preparation of the screening library. We also thank H. Vu from Griffith University for acquiring the HRESIMS measurements.

Supporting Information Available: ^{1}H and ^{13}C NMR spectrum of compounds **1-3**, ^{1}H and 2D NMR spectrum of compound **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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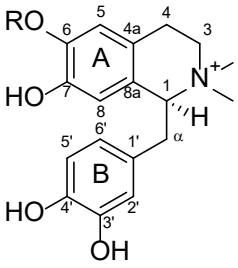
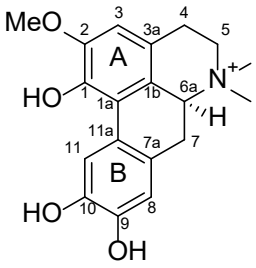
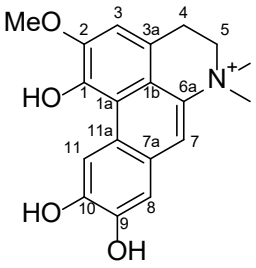


1: R=H

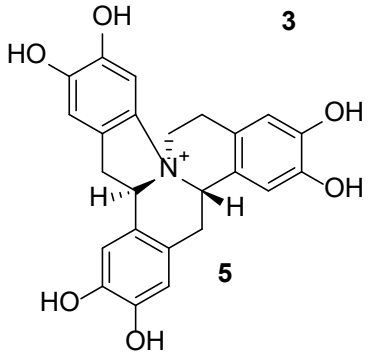
2: R=Me



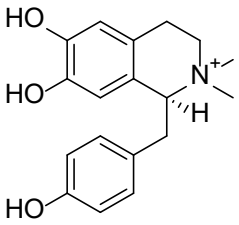
3



4



5



6

Table 1

NMR data for compounds **1** and **2**[*]

	1		**2**	
position	δ_C, mult	δ_H, mult (*J* in Hz)	δ_C	δ_H, mult (*J* in Hz)
1	71.3, CH	4.55 dd (9.1, 2.9)	71.2	4.59 dd (9.1, 2.6)
3	54.3, CH_2	a 3.68 m b 3.54 m	54.3	a 3.71 m b 3.58 m
4	22.4, CH_2	2.97 m	23.8	3.05 m
4a	118.9, C		119.2	
5	114.7, CH	6.58 s	113.3	6.79
6	143.6, C		147.7	
6-OH		8.84[a]		
7	145.5, C		145.5	
7-OH		9.11[a]		8.89
8	114.5, CH	5.94 s	114.5	5.95
8a	121.6, C		123.3	
α	35.6, CH_2	a 3.44 dd (13.9, 3.1) b 2.68 dd (13.9, 9.4)	36.4	a 3.46 dd (13.9, 2.8) b 2.68 dd (13.9, 9.3)
1′	127.7, C		127.0	
2′	116.6, CH	6.53 d (1.9)	116.5	6.52 d (1.5)
3′	145.2, C		145.7	
3′-OH		8.77[b]		8.75[c]
4′	144.1, C		143.9	
4′-OH		8.92[b]		8.91[c]
5′	115.2, CH	6.65 d (8.0)	115.2	6.65 d (8.0)
6′	119.9, CH	6.35 dd (8, 1.9)	119.9	6.35 dd (8.0, 1.5)
$-N^+Me_2$	50.2, CH_3	3.23 s	50.3	3.25 s
	50.0, CH_3	3.03 s	50.0	3.04 s
-OMe			55.1	3.75 s

[*] Spectra were recorded in DMSO-d_6 at 30 ºC

[a,b,c] Interchangeable signals

Table 2

NMR data for compounds **3** and **4**[*]

	3		**4**	
position	δ_C, mult	δ_H, mult (*J* in Hz)	δ_C, mult	δ_H, mult (*J* in Hz)
1	142.1, C		142.9, C	
1-OH		[a]		9.74
1a	120.4, C		124.3, C	
1b	119.5, C		115.3, C	
2	148.1, C		147.6, C	
3	109.0, CH	6.78 s	111.3, CH	7.40 s
3a	119.1, C		118.8, C	
4	23.1, CH_2	a 3.18 m b 2.93 m	23.7, CH_2	3.47 t (5.9)
5	60.4, CH_2	a 3.74 m b 3.65 td (12.6, 4.3)	60.9, CH_2	4.02, t (5.9)
6a	68.3, CH	4.56 dd (13.7, 2.3)	135.7, C	
7	28.1, CH_2	a 3.16 m b 2.73 t (13.7)	116.3, CH	8.10, s
7a	123.4, C		119.3, C	
8	114.9, CH	6.71 s	112.7, CH	7.30, s
9	144.6, C		143.8, C	
9-OH		[a]		9.73[b]
10	143.7, C		146.5, C	
10-OH		[a]		9.58[b]
11	116.3, CH	7.85 s	113.6, CH	9.22 s
11a	122.5, C		124.1, C	
$-N^+Me_2$	52.9, CH_3 42.8, CH_3	3.34 s 2.93 s	54.5, 2xCH_3	3.64 s
-OMe	55.9, CH_3	3.85 s	56.4, CH_3	3.99 s

[*] Spectra were recorded in DMSO-d_6 at 30 ºC

[a] Signals not observed [b] Interchangeable signals

Table 3
Antibacterial Activities for Compounds **1-6**

	IC_{50} (μM)	
compound	*P. aeruginosa* PAO1	*S. aureus* 01A1095
1	NA	NA
2	NA	NA
3	59%%±5.5 @ 175 μM	NA
4	59%±0.6 @ 87.5 μM	NA
5	9.8±0.78 μM	55%±2 @ 350 μM
6	NA	NA
Ciprofloxacin	0.038±0.01 μM	124.65±19.78 μM

NA not active at a screening dose of 350μM

TOC

Alkaloids from the Chinese Vine *Gnetum montanum*