Nitric Oxide Scavenging Lignans from *Vitex negundo* Seeds

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A new phenyldihydronaphthalene-type lignan, vitexdoin A (**1**), a new phenylnaphthalene-type lignan alkaloid, vitedoamine B (**2**), four new phenylnaphthalene-type lignans, vitexdoins B–E (**3**–**6**), and four known lignan derivatives (**7**–**10**) were isolated from *Vitex negundo* seeds. Their structures were elucidated by detailed analyses of NMR, IR, and MS data. The ability of the isolates to prevent nitric oxide (NO) production by LPS-stimulated RAW 264.7 macrophages in a concentration-dependent manner was also studied. Compounds **5**, **6**, and **9** were among the most potent NO production inhibitors, with IC_{50} values of 0.13, 0.15, and 0.11 μM, respectively. The introduction of free hydroxy groups plays a vital role in the potency of these compounds.

Vitex negundo L. (Verbenaceae) flourishes abundantly in wastelands and is widely distributed in tropical to temperate regions, being a native of South Asia, China, Japan, Indonesia, East Africa, and South America. The seeds have been claimed to possess anti-inflammatory,[1] analgesic,[2] antioxidant,[3] and antiandrogenic activity.[4] The presence of lignans,[3,5] flavonoids,[4,6] and terpenoids[1,3] has been previously reported. In the course of our studies on *V. negundo* seeds,[2] the EtOH extract showed potent anti-inflammatory activity in the xylene-induced ear edema test. Since nitric oxide (NO) plays an important role in the inflammatory process,[7] inhibitors of NO release may be accordingly considered as therapeutic agents in inflammatory diseases. The present study was therefore designed to further extract, purify, and identify the bioactive components in these medicinal seeds and resulted in the isolation of six new compounds (**1**–**6**) and four known lignan derivatives (**7**–**10**). We report herein the isolation, structure elucidation, and NO production inhibitory activity of the isolated compounds.

Results and Discussion

The EtOH extract of *V. negundo* seeds was subjected to a succession of chromatographic procedures to yield six new lignans, named vitexdoins A–E (**1**, **3**–**6**) and vitedoamine B (**2**), along with four known lignan derivatives (**7**–**10**), 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde,[5] vitedomamine A,[3] vitrofolal F,[8] and vitrofolal E,[8] respectively, on the basis of their spectroscopic data.

Compound **1**, trivially named vitexdoin A, was obtained as a yellowish powder. The negative HRESIMS data indicated the molecular formula of **1** to be $C_{19}H_{18}O_6$. Its ^{1}H and ^{13}C NMR spectra were analogous to those of **7**, except for the disappearance of the signal corresponding to an *O*-methyl group (δ_H 3.70 and δ_C 56.6 in **1**; δ_H 3.64, 3.82 and δ_C 55.7, 55.5 in **7**) and with the signals due to the B-ring protons slightly shifted. From this data, **1** was considered to be a 7-*O*-demethyl derivative of **7**. This was confirmed by the HMBC and NOESY spectra. In the NOESY spectra of **1**, key correlations were observed between H-4 and H-6′, H-4 and H-2′, H-4 and H-5, H-3 and H-2′, and H-2′ and OCH$_3$-3′. The relative configuration at C-4 and C-3 could be assigned in analogy with that of **7** and confirmed by the signal of H-4 [δ_H 4.28 (s)], which did not couple with H-3, revealing a dihedral angle of ca. 90° between H-4 and H-3. The structure of vitexdoin A (**1**) was therefore defined as 6,7-dihydroxy-4β-(4-hydroxy-3-methoxyphenyl)-3α-hydroxymethyl-3,4-dihydro-2-naphthaldehyde.

Compound **2**, trivially named vitedoamine B, was obtained as a white powder. The molecular formula of **2** was determined as $C_{20}H_{17}NO_5$ by positive HRESIMS. The ^{1}H NMR spectrum of **2** was similar to that of **8**, apart from the splitting patterns and the chemical shifts of two methylene protons [δ_H 4.38 (2H, s) in **2**; δ_H 4.25 (1H, d, J = 16.2 Hz), 4.15 (1H, d, J = 16.2 Hz) in **8**] and the signal due to one aromatic proton, H-1 [δ_H 7.79 (s) in **2**; δ_H 8.06 (s) in **8**]. In comparing the chemical shifts of the ^{13}C NMR signals of **2** and **8**, the signals due to C-2 and C-3 in **2** were shifted by +8.6 and −10.9 ppm, respectively. This allowed us to locate the methylene group at C-1 and the carbonyl group at C-3. Therefore, **2** was considered to be a regioisomer of **8** with an exchange of the positions of the methylene group and the carbonyl group in the γ-lactam ring. This was confirmed by the HMBC and NOESY spectra. In the NOESY spectra of **2**, key correlations were observed between H-1 and H-2α; H-8 and OCH$_3$-7; and H-2′ and OCH$_3$-3′. Thus, vitedoamine B (**2**) was concluded to be 7-hydroxy-9-(4-hydroxy-3-methoxyphenyl)-6-methoxy-2,3-dihydro-1*H*-benzo[*f*]isoindol-1-one.

Compound **3**, trivially named vitexdoin B, was obtained as a yellowish powder and analyzed for the molecular formula $C_{19}H_{16}O_5$ by negative HRESIMS. The ^{1}H NMR spectrum was analogous to that of **10**, except for the splitting patterns and chemical shifts of two aromatic protons [δ_H 7.82 (d, J = 8.4 Hz), 7.38 (d, J = 8.4 Hz) in **3**; δ_H 7.74 (d, J = 1.8 Hz), 7.42 (s) in **10**]. In addition, a higher field signal due to the *O*-methyl protons was observed in **3** (δ_H 3.12) compared to **10** (δ_H 4.06). The ^{13}C NMR spectrum was also similar to that of **10**, apart from the significant low-field shift of an *O*-methyl group (δ_C 61.6 in **3**; δ_C 56.1 in **10**) on the B-ring. Therefore, **3** was a regioisomer of **10** with an *O*-methyl group at C-5 instead of C-7, resulting in considerable steric hindrance that accounts for the notable low-field shift of the 5-OMe carbon signal. This was confirmed by the HMBC and NOESY spectra. In the NOESY spectra of **3**, key correlations were observed between H-3 and H-6′, H-6′ and OCH$_3$-5, H-2′ and OCH$_3$-5, and H-2′ and OCH$_3$-3′. The structure of vitexdoin B (**3**) was therefore defined as 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-5-methoxy-2-naphthaldehyde.

Compound **4**, trivially named vitexdoin C, was obtained as a yellowish powder and gave the molecular formula $C_{19}H_{16}O_5$, as validated by negative HRESIMS. According to the molecular formula and NMR data, **4** was determined to possess the same framework as **3** and **10**, except for two sets of proton doublets of H-2 and H-1 of the A-ring at δ_H 7.89 and 7.74 (J = 8.4 Hz). An

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Table 1. ^{1}H NMR Data of Compounds **1**, **2**, **7**, and **8** (*J* in Hz)

H	1	7	2	8
1	7.38 s	7.51 s	7.79 s	8.06 s
3	3.14 m	3.02 m		
4	4.28 s	4.25 s		
5	6.66 s	6.68 s	7.01 s	7.08 s
8	6.91 s	7.11 s	7.36 s	7.52 s
2′	6.58 d (1.8)	6.62 d (1.8)	6.98 d (1.8)	6.95 d (1.8)
5′	6.57 d (8.2)	6.55 d (8.2)	6.85 d (8.0)	6.93 d (7.8)
6′	6.31 dd (8.2, 1.8)	6.19 dd (8.2, 1.8)	6.64 dd (8.0, 1.8)	6.79 dd (7.8, 1.8)
2α	9.41 s	9.47 s	4.38 s (2H)	
3α	3.47 m, 3.12 m	3.31 m, 2.90 m		4.25 d (1H, 16.2), 4.15 d (1H, 16.2)
7-OCH_3		3.64 s	3.91 s	3.77 s
3′-OCH_3	3.70 s	3.82 s	3.73 s	3.90 s
NH			8.23 s	8.42 s

Table 2. ^{1}H NMR Data of Compounds **3**−**6**, **9**, and **10** (*J* in Hz)

H	3	10	4	5	6	9
1	8.25 d (1.2)	8.17 s	7.74 d (8.4)	7.08 s		8.21 s
2			7.89 d (8.4)			
3	7.81 s	7.74 d (1.8)			7.28 s	
5		7.42 s	7.15 s	6.80 s	7.29 s	7.39 s
7	7.38 d (8.4)					
8	7.82 d (8.4)	7.32 s	7.19 s	7.07 s	7.78 s	6.79 s
2′	6.98 d (1.8)	6.95 d (1.8)	6.85 d (1.8)	6.93 d (1.8)	6.92 d (1.8)	6.81 d (1.8)
5′	6.97 d (8.4)	7.03 d (7.8)	7.05 d (7.8)	6.96 d (7.8)	7.02 d (7.8)	6.91 d (7.8)
6′	7.02 dd (8.4, 1.8)	6.97 dd (7.8, 1.8)	6.88 dd (7.8, 1.8)	6.78 dd (7.8, 1.8)	6.93 dd (7.8, 1.8)	6.68 dd (7.8, 1.8)
2α	10.11 s	10.11 s			9.91 s	10.08 s
3α			9.88 s	9.71 s		
5-OCH_3	3.12 s					
7-OCH_3		4.08 s	4.06 s	3.97 s	4.09 s	3.87 s
3′-OCH_3	3.89 s	3.92 s	3.87 s	3.84 s	3.92 s	3.74 s

aldehyde group at C-3 was further established by the HMBC correlation from an aldehyde proton (δ_H 9.88, s) to C-2 (δ_C 120.5). In the NOESY spectra of **4**, key correlations were observed between H-3α and H-2′, H-8 and OCH_3-7, and H-2′ and OCH_3-3′. Thus, vitexdoin C (**4**) was concluded to be 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-7-methoxy-3-naphthaldehyde.

Compound **5**, trivially named vitexdoin D, was obtained as a yellowish powder and analyzed for the molecular formula $C_{19}H_{16}O_6$ by negative HRESIMS. It was recognized as a regioisomer of **9**, with a hydroxy group at C-2 and an aldehyde group at C-3 on the A-ring, as evidenced by the MS data and NMR measurements. A hydroxy group at C-2 may be responsible for the upfield shift of C-1 in **5** (δ_C 110.8) compared to that of **9** (δ_C 134.5), whereas an aldehyde group at C-3 may account for the low-field signal of C-4 in **5** (δ_C 148.0) compared to that of **9** (δ_C 122.4). These explanations were further supported by analyses of HMBC and NOESY spectra. The NOSEY correlations between H-8 and OCH_3-7, H-2′ and OCH_3-3′, and H-5 and H-2′ were also detected. The structure of vitexdoin D (**5**) was therefore deduced as 2,6-dihydroxy-4-(4-hydroxy-3-methoxyphenyl)-7-methoxy-3-naphthaldehyde.

Compound **6**, trivially named vitexdoin E, was obtained as a yellowish powder. The negative HRESIMS data indicated the molecular formula of **6** to be $C_{19}H_{16}O_6$. The MS and NMR data revealed that **6** possessed the same framework as **5** and **9**, differing only in the positions of the hydroxy and the aldehyde groups on the A-ring. The long-range $^{1}H-^{13}C$ correlations observed from H-8 (δ_H 7.78, s) and H-3 (δ_H 7.28, s) to C-1 (δ_C 159.9) indicated the position of the hydroxy group at C-1. In addition, the HMBC correlation from an aldehyde proton (δ_H 9.91, s) to C-1 led to the assignment of the aldehyde group at C-2. In the NOESY spectra of **6**, key correlations were observed between H-3 and H-2α, H-3 and H-2′, H-5 and H-6′, H-8 and OCH_3-7, and H-2′ and OCH_3-3′. Thus, vitexdoin E (**6**) was concluded to be 1,6-dihydroxy-4-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2-naphthaldehyde.

The isolated compounds were evaluated for their ability to inhibit nitric oxide (NO) production by LPS-induced RAW 264.7 cells. The inorganic free radical NO, synthesized by a family of enzymes termed NO-synthase (NOS), acts as a host defense mechanism by damaging pathogenic DNA and also acts as a regulatory molecule with homeostatic activities.[9] However, excess production of NO, due to the reaction with superoxide in biological systems, gives rise to various diseases such as inflammation, carcinogenesis, and atherosclerosis.[10] Therefore, down-regulation of NO production may be of therapeutic benefit in various diseases induced by pathological levels of NO.

In our assay, L-nitroarginine (L-NA), a nitric oxide synthase (NOS) inhibitor, was used as a positive control, and all of the isolates exhibited significant activity in a concentration-dependent manner. As shown in Table 4, compounds **5**, **6**, and **9** were among the most potent inhibitors of NO production, with IC_{50} values of 0.13, 0.15, and 0.11 μM, respectively, much less than that of the positive control L-NA (IC_{50} 43.60 μM), thus suggesting that a hydroxy group on the A-ring may significantly enhance the NO production inhibitory activity of these lignans. Furthermore, compound **1** (IC_{50} 0.38 μM) exhibited higher activity than compound **7** (IC_{50} 3.54 μM), revealing that the introduction of a second hydroxy group to the B-ring may also increase the activity. In addition, compound **3** (IC_{50} 0.20 μM) showed stronger activity than compound **4** (IC_{50} 0.57 μM) and compound **10** (IC_{50} 0.50 μM), indicating that the presence of an *O*-methyl group at C-5 increases the activity. In contrast, the substitution of an aldehyde group at C-2 or C-3 does not have much impact on the inhibition of NO production. Our results support the idea that a high inhibitory activity can be attributed to the free hydroxy groups of these phenylnaphthalene-type lignans. Cell viability was also determined by application of the MTT method in order to evaluate whether inhibition of NO production was due to the cytotoxicity of these tested compounds. It was found that none of the concentrations used in the experiment were cytotoxic (data not shown). Thus, the inhibitory activity of these lignans is not due to their cytotoxic

Table 3. ^{13}C NMR Data of Compounds **1−10**

C	**1**	**7**	**2**	**8**	**3**	**10**	**4**	**5**	**6**	**9**
1	149.7	146.9	119.6	120.4	134.3	131.6	126.6	110.8	159.9	134.5
2	136.2	134.4	137.1	128.5	131.5	132.1	120.5	156.4	113.1	119.8
3	44.2	41.8	124.7	135.6	127.2	122.8	130.4	119.3	125.8	151.1
4	44.5	42.0	135.8	132.1	137.4	139.9	145.1	148.0	130.9	122.4
5	118.7	113.1	108.8	107.6	140.9	108.7	109.7	110.5	108.9	107.0
6	150.7	149.7	147.0	149.0	150.1	148.4	146.2	146.7	149.7	151.5
7	146.1	146.6	150.1	148.6	118.2	147.8	149.4	154.1	147.0	147.8
8	117.6	117.3	106.5	108.1	128.3	107.5	106.1	105.9	102.8	108.5
9	125.3	122.6	128.3	127.8	129.9	128.8	132.3	136.8	119.3	121.4
10	133.5	132.8	130.5	130.1	128.8	132.1	128.8	124.6	133.7	134.5
1′	138.0	135.9	127.2	127.7	133.9	132.1	127.2	128.1	131.9	125.8
2′	112.6	111.5	114.7	113.5	113.2	112.4	113.4	115.6	112.5	114.8
3′	148.9	147.2	146.7	147.7	145.3	146.4	146.3	148.9	146.4	147.5
4′	146.0	144.8	145.7	146.1	145.0	145.3	145.7	148.2	145.1	145.8
5′	116.2	115.1	114.7	115.7	113.0	114.4	114.3	116.1	114.4	115.5
6′	121.2	119.2	122.7	122.0	122.6	122.9	124.1	125.1	123.0	123.2
2α	194.8	192.3	43.2	170.3	191.8	192.2			196.0	196.8
3α	62.9	60.6	169.7	44.3			193.1	199.0		
5-OCH_3					61.6					
7-OCH_3		55.5	55.5	55.5		56.1	56.1	55.4	56.2	55.6
3′-OCH_3	56.6	55.7	55.6	55.7	56.2	56.1	56.0	55.6	56.1	55.6

Table 4. Effects of Isolated Lignans (**1−10**) from *Vitex negundo* Seeds on Nitric Oxide (NO) Production in LPS-Stimulated RAW 264.7 Cells (n = 4)[a]

compound	NO inhibition (%) at dose of 1.0 μM; mean ± SD	IC$_{50}$ (μM)
1	67.00 ± 2.64	0.38
2	56.18 ± 3.58	0.87
3	80.85 ± 3.10	0.20
4	61.71 ± 2.12	0.57
5	91.13 ± 2.29	0.13
6	84.19 ± 2.44	0.15
7	34.44 ± 3.01	3.54
8	43.31 ± 4.28	1.53
9	95.78 ± 1.33	0.11
10	63.23 ± 2.97	0.50
L-NA	2.53 ± 1.84	43.60

[a] LPS: negative control; L-NA: L-nitroarginine, positive control.

properties but to their ability of NO suppression, which merits further studies regarding the precise site and the mechanism of action.

Experimental Section

General Experimental Procedures. Optical rotations were acquired with a Perkin-Elmer 341 polarimeter. UV spectra were run on a Varian Cary Eclipse 300 spectrophotometer, while the CD spectra were recorded on a JASCO J-810 spectropolarimeter. IR spectra were recorded on a Bruker Vector 22 spectrometer with KBr pellets. NMR spectra were recorded on a Bruker Avance 600 or Avance 400 NMR spectrometer with TMS as an internal standard. ESIMS were measured on an Agilent LC/MSD Trap XCT mass spectrometer, whereas HRESIMS were measured using a Q-TOF micro mass spectrometer (Waters, USA). Materials for CC were silica gel (100−200 mesh; Huiyou Silical Gel Development Co. Ltd., Yantai, China), silica gel H (10−40 μm; Yantai), Sephadex LH-20 (40−70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden), and YMC-GEL ODS-A (50 μm; YMC, Milford, MA). Preparative TLC (0.4−0.5 mm) was conducted on glass plates precoated silica gel GF$_{254}$ (Yantai).

Plant Material. The seeds of *V. negundo* (Chinese name "Huang-Jing-Zi") were obtained from Wanglang National Nature Reserve, Sichuan Province, in Oct 2006, and were identified by Prof. Han-Chen Zheng, Second Military Medical University. A voucher specimen (#2006-168) has been deposited in the herbarium of the Department of Pharmacognosy, School of Pharmacy, Second Military Medical University.

Extraction and Isolation. The air-dried and powdered seeds of *V. negundo* (25 kg) were extracted with 80% EtOH (×3), each extraction period lasting 2 h. The solvent was removed under reduced pressure, and the residue was suspended in H_2O and partitioned sequentially with petroleum ether, CH_2Cl_2, EtOAc, and *n*-butanol, respectively. In our previous study,[2] both the CH_2Cl_2 and EtOAc extracted fractions exhibited notable anti-inflammatory activity in vivo, and hence these fractions were selected for investigation in the present study.

The CH_2Cl_2-soluble part (260 g) was subjected to CC on silica gel (200−300 mesh, 1000 g) and eluted successively with gradient petroleum ether−EtOAc mixtures (50:1, 20:1, 10:1, 5:1, 3:1, 1:1, 0:1 v/v) to afford fractions A−G. Fraction C (6.5 g) was further fractionated by ODS column chromatography employing a MeOH−H_2O mixture (80%) as eluent to provide five fractions (C.1−C.5). Fraction C.2 (615 mg) was rechromatographed on Sephadex LH-20 with MeOH−H_2O (80%) followed by preparative TLC to give compounds **3** (5 mg), **4** (16 mg), **5** (11 mg), **6** (5 mg), **9** (35 mg), and **10** (30 mg). Fraction G (8.5 g) was subjected to ODS column chromatography using the gradient MeOH−H_2O from 40% to 80% as eluent to give compounds **2** (18 mg) and **8** (800 mg). The EtOAc-soluble part (70 g) was subjected to silica gel column chromatography using a $CHCl_3$−MeOH mixture (30:1 → 3:1 v/v) as eluent to afford six fractions (H−M). After concentration, crystals of compound **7** (1000 mg) were obtained in fraction I, and these were recrystallized from $CHCl_3$−MeOH (1:1 v/v). Fraction K (2 g) was also subjected to ODS column chromatography and was eluted with 60% MeOH to afford an impure fragment, which was further purified on Sephadex LH-20 using 80% MeOH as eluent to yield compound **1** (32 mg) (Figure 1).

Vitexdoin A (1): yellowish, amorphous powder; $[\alpha]^{15}_{D}$ −156.0 (c 0.3, MeOH); UV (MeOH) λ_{max} 203, 258, 369 nm; CD (MeOH) λ_{max} ($\Delta\epsilon$) 215 (38.22), 245 (−14.15), 264 (20.85), 291 (0.11), 318 (22.21), 350 (−18.27); IR ν_{max} 3438, 2920, 1650, 1600, 1492, 1451 cm^{-1}; ^{1}H (methanol-d_4, 400 MHz) and ^{13}C NMR (methanol-d_4, 100 MHz) data (see Table 1 and 3); (−)-HRESIMS m/z [M − H]$^-$ 341.0941 (calcd for $C_{19}H_{17}O_6$, 341.1025).

Vitedoamine B (2): white, amorphous powder; UV (MeOH) λ_{max} 201, 256 nm; IR ν_{max} 3340, 2919, 1679, 1515, 1237, 1031 cm^{-1}; ^{1}H (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz) data (see Tables 1 and 3); (+)-HRAPCIMS m/z [M + H]$^+$ 352.1187 (calcd for $C_{20}H_{18}NO_5$, 352.1185).

Vitexdoin B (3): yellowish, amorphous powder; UV (MeOH) λ_{max} 203, 268, 330 nm; IR ν_{max} 3399, 2932, 2837, 1683, 1606, 1513, 1466, 1238, 1155 cm^{-1}; ^{1}H ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) data (see Tables 2 and 3); (−)-HRESIMS m/z [M − H]$^-$ 323.0920 (calcd for $C_{19}H_{15}O_5$, 323.0919).

Vitexdoin C (4): yellowish, amorphous powder; UV (MeOH) λ_{max} 206, 266, 316 nm; IR ν_{max} 3381, 2936, 2840, 1671, 1511, 1480, 1435, 1267, 1021 cm^{-1}; ^{1}H ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) data (see Tables 2 and 3); (−)-HRESIMS m/z [M − H]$^-$ 323.0921 (calcd for $C_{19}H_{15}O_5$, 323.0919).

Vitexdoin D (5): yellowish, amorphous powder; UV (MeOH) λ_{max} 203, 265, 333 nm; IR ν_{max} 3331, 1635, 1516, 1493, 1278, 1031 cm^{-1}; ^{1}H (methanol-d_4, 600 MHz) and ^{13}C NMR (methanol-d_4, 150 MHz)

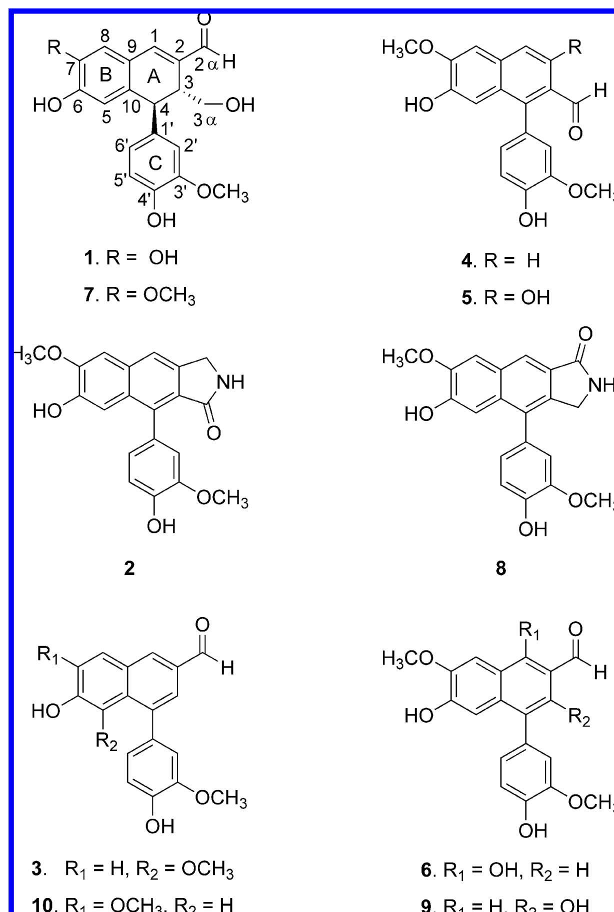

Figure 1. Structures of isolated lignans (**1**−**10**) from *Vitex negundo* seeds.

data (see Tables 2 and 3); (−)-HRESIMS *m/z* [M − H]$^-$ 339.0870 (calcd for $C_{19}H_{15}O_6$, 339.0869).

Vitexdoin E (6): yellowish, amorphous powder; UV (MeOH) λ_{max} 201, 233, 278, 333 nm; IR ν_{max} 3419, 2923, 1622, 1506, 1272, 1202 cm^{-1}; ^{1}H (CDCl$_3$, 600 MHz) and ^{13}C NMR (CDCl$_3$, 150 MHz) data (see Tables 2 and 3); (−)-HRESIMS *m/z* [M − H]$^-$ 339.0868 (calcd for $C_{19}H_{15}O_6$, 339.0869).

6-Hydroxy-4-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde (7): yellowish crystals; mp 126−127 °C; $[\alpha]^{26}_{D}$ −176.0 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 255, 359 nm; IR ν_{max} (KBr) 3390, 2840, 1650, 1620, 1565, 1515 cm^{-1}; ^{1}H (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz) data (see Tables 1 and 3); HREIMS *m/z* [M]$^+$ 356.1270 (calcd for $C_{20}H_{20}O_6$, 356.1259).

Vitedomamine A (8): white, amorphous powder; UV (MeOH) λ_{max} 202, 256, 314 nm; IR ν_{max} (KBr) 3340, 1669, 1524, 1455, 1236 cm^{-1}; ^{1}H (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) data (see Tables 1 and 3); HREIMS *m/z* [M]$^+$ 351.1070 (calcd for $C_{20}H_{17}NO_5$, 351.1106).

Vitrofolal F (9): yellowish, amorphous powder; UV (1,4-dioxane) λ_{max} 333, 269, 239 nm; IR ν_{max} (KBr) 3600−2800, 1652, 1509, 1287, 1211 cm^{-1}; ^{1}H (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) data (see Tables 2 and 3); HREIMS *m/z* [M]$^+$ 340.0939 (calcd for $C_{19}H_{16}O_6$, 340.0947).

Vitrofolal E (10): yellowish, amorphous powder; UV (1,4-dioxane) λ_{max} 320, 267 nm; IR ν_{max} (KBr) 3600−3000, 1684, 1508, 1270, 1211 cm^{-1}; ^{1}H (CDCl$_3$, 600 MHz) and ^{13}C NMR (CDCl$_3$, 150 MHz) data (see Tables 2 and 3); HREIMS *m/z* [M]$^+$ 324.0981 (calcd for $C_{19}H_{16}O_5$, 324.0998).

Inhibition Ability against LPS-Induced NO Production. RAW 264.7 macrophages were seeded in 96-well plates (10^5 cells/well). The cells were co-incubated with the isolated compounds and LPS (1 μg/mL) for 24 h. The amount of NO was assessed by determining the nitrite concentration in the cultured RAW 264.7 macrophage supernatants with Griess reagent. Aliquots of supernatants (100 μL) were incubated, in sequence, with 50 μL of 1% sulfanilamide and 50 μL of 0.1% naphthylethylenediamine in 2.5% phosphoric acid solution. The absorbance at 548 nm was read using a microplate reader (POLARstar).

Cell Viability. Cell viability was determined using the mitochondrial respiration-dependent MTT reduction method. After transferring the required supernatant to another plate for the Griess assay, the remaining supernatant was aspirated from the 96-well plates, and 100 μL of fresh medium containing 2 mg/mL of MTT was added to each well. The cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. After incubating for 3 h, the medium was removed and the violet crystals of formazan in viable cells were dissolved in DMSO. Absorbance at 570 nm was measured using a microplate reader.

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Supporting Information Available: Supplementary data associated with this article is available free of charge via the Internet at http://pubs.acs.org.

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