Antifibrotic Phenanthrenes of *Dendrobium nobile* Stems

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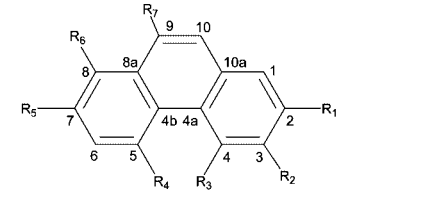
Two new phenanthrenes (**1** and **6**) and four new dihydrophenanthrenes (**2**–**5**) were isolated from a methanolic extract of *Dendrobium nobile* stems, along with 13 known phenanthrenes and dihydrophenanthrenes (**7**–**19**). By spectroscopic analysis, the structures of compounds **1**–**6** were determined as 2,8-dihydroxy-3,4,7-trimethoxyphenanthrene (**1**), 3-hydroxy-2,4,7-trimethoxy-9,10-dihydrophenanthrene (**2**), 2,8-dihydroxy-3,4,7-trimethoxy-9,10-dihydrophenanthrene (**3**), 2-hydroxy-4,7-dimethoxy-9,10-dihydrophenanthrene (**4**), 2,2′-dihydroxy-3,3′,4,4′,7,7′-hexamethoxy-9,9′,10,10′-tetrahydro-1,1′-biphenanthrene (**5**), and 2,3,5-trihydroxy-4,9-dimethoxyphenanthrene (**6**), respectively. Antifibrotic activity of compounds **1**–**19** was evaluated employing HSC-T6, an immortalized rat hepatic stellate cell line, as an in vitro assay system by assessing cell proliferation.

In the course of searching for antifibrotic natural products using HSC-T6 cells, an immortalized rat hepatic stellate cell line as an in vitro assay system,[1] it was found that a methanolic extract of the stems of *Dendrobium nobile* Lindl. (Orchidaceae) inhibited cell proliferation. The stems of *D. nobile* ("Seokgok" in Korean; "Shi Hu" in mainland China) have been used as an analgesic, an antipyretic, and a tonic to nourish the stomach in the Korean and Chinese systems of traditional medicine.[2,3] Alkaloids,[4,5] bibenzyls,[6] fluorenones,[6] phenanthrenes,[7,8] and sesquiterpenoids[9,10] were reported as the constituents of this plant. Some of these compounds have been reported as having cytotoxic,[8] immunoregulatory,[9,10] antimutagenic,[11] and anti-inflammatory[12] activities. To date, however, there has been no report related to the antifibrotic activity of this plant. Thus, we have isolated the antifibrotic constituents from the stems of *D. nobile* through bioactivity-guided fractionation. Two new phenanthrenes (**1** and **6**) and four new dihydrophenanthrenes (**2**–**5**), along with 13 known phenanthrenes and dihydrophenanthrenes (**7**–**19**), were obtained. Herein, we report the isolation and structural elucidation of six new compounds (**1**–**6**) and the antifibrotic activity of the isolated compounds (**1**–**19**).

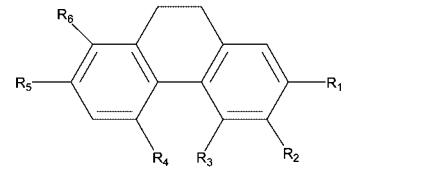
Results and Discussion

The methanolic extract of *D. nobile* stems was successively fractionated into *n*-hexane, $CHCl_3$, EtOAc, *n*-BuOH, and H_2O fractions. The $CHCl_3$ fraction, which showed significant antiproliferative activity in HSC-T6 cells, was further subjected to repeated column chromatography to yield the new phenanthrenes (**1** and **6**) and dihydrophenanthrenes (**2**–**5**) with the known compounds (**7**–**19**).

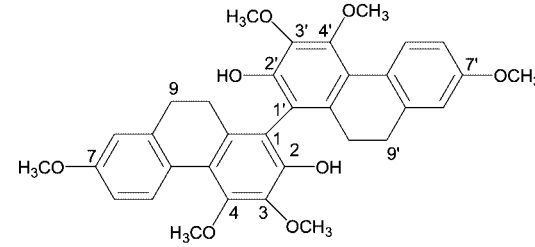
Compound **1** was isolated as a brownish, amorphous powder. The molecular formula of **1** was determined as $C_{17}H_{16}O_5$ from the HREIMS at *m*/*z* 300.0992 $[M]^+$ (calcd *m*/*z* 300.0998) and ^{13}C NMR spectrum. In the ^1H NMR spectrum, two pairs of signals for an *ortho*-coupled AB system [δ_H 8.99 and 7.24 (1H, d, $J = 9.3$ Hz, H-5 and H-6, respectively); δ_H 8.03 and 7.50 (1H, d, $J = 9.1$ Hz, H-9 and H-10, respectively)] and an isolated aromatic proton [δ_H 7.16 (1H, s, H-1)] were observed, which were similar to those of other 2,3,4,7,8-pentasubstituted phenanthrenes.[13] In addition, the signals for two hydroxy groups [δ_H 6.02 (1H, s, OH-8) and 5.98 (1H, s, OH-2), all exchangeable with D_2O] and three aromatic methoxy groups, two of which were *ortho*-disubstituted [δ_H 4.09 (3H, s, OCH_3-3), 4.01 (3H, s, OCH_3-7) and 3.95 (3H, s, OCH_3-4); δ_C 61.3 (OCH_3-3), 56.6 (OCH_3-7), and 59.7 (OCH_3-4)] were observed in the ^1H and ^{13}C NMR spectra of **1**. Locations of the methoxy and hydroxy groups were determined by analysis of the HMBC and NOESY spectra (see Table S1, Supporting Information). The HMBC spectrum exhibited correlations from H-1 to C-2, C-3, C-10, and C-4a, from the methoxy protons at δ 4.09 to C-3, and from the hydroxy proton at δ 5.98 to C-1, C-2, and C-3. In addition, the hydroxy proton at δ 5.98 correlated with H-1 and the methoxy group at δ 4.09 in the NOESY spectrum. These results confirmed that C-2 and C-3 are substituted by a hydroxy group and a methoxy group, respectively. Furthermore, the NOESY correlation between the methoxy group at δ 3.95 and H-5 but not with H-3 suggested that the methoxy group at δ 3.95 should be placed at C-4. In turn, the HMBC correlations from H-5 to C-4a, C-7, and C-8a, from



	R_1	R_2	R_3	R_4	R_5	R_6	R_7
1	OH	OCH_3	OCH_3	H	OCH_3	OH	H
6	OH	OH	OCH_3	OH	H	H	OCH_3



	R_1	R_2	R_3	R_4	R_5	R_6
2	OCH_3	OH	OCH_3	H	OCH_3	H
3	OH	OCH_3	OCH_3	H	OCH_3	OH
4	OH	H	OCH_3	H	OCH_3	H



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H-9 to C-4b, C-8, and C-10a, from the methoxy protons at δ 4.01 to C-7, and from the hydroxy proton at δ 6.02 to C-7, C-8, and C-8a suggested the additional placement of the methoxy and hydroxy groups at C-7 and C-8, respectively. The correlations between the methoxy protons at δ 4.01 and H-6 and between the hydroxy proton at δ 6.02 and H-9 in the NOESY spectrum supported the locations proposed of these functional groups. On the basis of the data obtained, the structure of **1** was determined as 2,8-dihydroxy-3,4,7-trimethoxyphenanthrene.

Compound **2** was isolated as a pale yellowish, amorphous powder. The molecular formula of **2** was determined as $C_{17}H_{18}O_4$ from the HREIMS at *m/z* 286.1211 $[M]^+$ (calcd *m/z* 286.1205) and ^{13}C NMR spectrum. In the ^1H NMR spectrum, the signals for an ABX system [δ_H 8.19 (1H, d, J = 8.7 Hz, H-5), 6.81 (1H, dd, J = 8.7 and 2.7 Hz, H-6), and 6.76 (1H, d, J = 2.7 Hz, H-8)], an isolated aromatic proton at δ_H 6.56 (s, H-1), and two methylene protons [δ_H 2.75 (2H, m, H-9) and 2.69 (2H, m, H-10)] were observed, suggesting that **2** is a 2,3,4,7-tetrasubstituted 9,10-dihydrophenanthrene derivative.[14] In addition, resonances for a hydroxy group [δ_H 5.58 (1H, s, OH-3), exchangeable with D_2O] and three aromatic methoxy groups, one of which was *ortho*-disubstituted [δ_H 3.89 (3H, s, OCH_3-2), 3.82 (3H, s, OCH_3-7) and 3.68 (3H, s, OCH_3-4); δ_C 56.2 (OCH_3-2), 60.0 (OCH_3-4) and 55.2 (OCH_3-7)], were observed in the ^1H and ^{13}C NMR spectra of **2**. Compound **2** gave similar NMR data to those of the co-occurring 3-hydroxy-2,4,7-trimethoxyphenanthrene (**8**),[15] except that two methylene signals in **2** [δ_H 2.75 (2H, m, H-9) and 2.69 (2H, m, H-10); δ_C 29.9 (C-9) and 30.3 (C-10)] were observed. In addition, the chemical shift values for the aromatic protons in **2** were shifted upfield in comparison to those in **8**. From all the data taken, the structure of **2** was elucidated as 3-hydroxy-2,4,7-trimethoxy-9,10-dihydrophenanthrene. The positions of the substituents were confirmed by analysis of the HMBC and NOESY spectra (see Table S2, Supporting Information).

Compound **3** was isolated as a pale yellowish, amorphous powder. The molecular formula of **3** was determined as $C_{17}H_{18}O_5$ from the HREIMS at *m/z* 302.1164 $[M]^+$ (calcd *m/z* 302.1154), differing from **1** by two mass units. The ^1H and ^{13}C NMR spectra of **3** were similar to those of **1**, except for the presence of two methylene signals in **3** [δ_H 2.76 (2H, m, H-9) and 2.65 (2H, m, H-10); δ_C 21.3 (C-9) and 29.6 (C-10)], instead of aromatic signals [δ_H 8.03 and 7.50 (1H, d, J = 9.1 Hz, H-9 and H-10, respectively); δ_C 119.9 (C-9) and 126.1 (C-10)] in **1**. Thus, **3** could be considered a 9,10-dihydrophenanthrene derivative of **1**. As in the case of **1**, the positions of the substituents in **3** were confirmed by analysis of the HMBC and NOESY spectra (see Table S3, Supporting Information). From the spectroscopic data obtained, the structure of **3** was established as 2,8-dihydroxy-3,4,7-trimethoxy-9,10-dihydrophenanthrene.

Compound **4** was isolated as a pale brownish gum. The molecular formula of **4** was determined as $C_{16}H_{16}O_3$ from the HREIMS at *m/z* 256.1106 $[M]^+$ (calcd *m/z* 256.1099) and ^{13}C NMR spectrum. In the ^1H NMR spectrum, the signals for an ABX system [δ_H 8.14 (1H, d, J = 8.7 Hz, H-5), 6.78 (1H, dd, J = 8.7 and 2.7 Hz, H-6), and 6.75 (1H, d, J = 2.7 Hz, H-8)], a pair of *meta*-coupled protons [δ_H 6.32 (1H, d, J = 2.0 Hz, H-1) and 6.39 (1H, d, J = 2.0 Hz, H-3)], and four methylene protons [δ_H 2.71 (4H, m, H-9 and H-10)] were observed, suggesting that **4** is a 2,4,7-trisubstitueted 9,10-dihydrophenanthrene derivative.[13] In addition, resonances for two aromatic methoxy groups, both of which were *ortho*-disubstituted [δ_H 3.84 (3H, s, OCH_3-4) and 3.81 (3H, s, OCH_3-7); δ_C 55.5 (OCH_3-4) and 55.2 (OCH_3-7)] were observed in the ^1H and ^{13}C NMR spectra of **4**. By analysis of the HMBC and NOESY spectra, the methoxy groups at δ 3.84 and 3.81 could be suggested to be at C-4 and C-7 (see Table S4, Supporting Information). Since the remaining substituent was not seen in the ^1H NMR spectrum, it was deduced as a hydroxy group from the IR spectrum (3395 cm^{-1}). Furthermore, since H-3 showed a correlation to an oxygenated carbon that did not correlate with any of the methoxy protons, C-2 must be hydroxylated. Thus, the structure of **4** was determined as 2-hydroxy-4,7-dimethoxy-9,10-dihydrophenanthrene.

Compound **5** was isolated as a pale yellowish, amorphous powder. The mass spectrum of **5** exhibited a molecular ion peak $[M]^+$ at *m/z* 570 ($C_{34}H_{34}O_8$) and a significant peak at *m/z* 285 corresponding to the fragment $[M/2]^+$. In addition, the ^{13}C NMR spectrum showed only 17 signals, suggesting **5** to be a symmetrical dimer. In the ^1H NMR spectrum, signals were observed for two ABX systems [δ_H 8.22 (2H, d, J = 8.8 Hz, H-5 and H-5′), 6.81 (2H, dd, J = 8.8 and 2.7 Hz, H-6 and H-6′), and 6.72 (2H, d, J = 2.7 Hz, H-8 and H-8′)], two pairs of multiplets at δ_H 2.60 (4H, H-9 and H-9′) and δ_H 2.42−2.31 (4H, H-10 and H-10′), two hydroxy protons [δ_H 5.60 (2H, s, OH-2 and OH-2′), exchangeable with D_2O], and six aromatic methoxy groups [δ_H 4.01 (6H, s, OCH_3-3 and OCH_3-3′), 3.90 (6H, s, OCH_3-4 and OCH_3-4′), and 3.82 (6H, s, OCH_3-7 and OCH_3-7′)]. By analysis of the HMBC and NOESY spectra, the methoxy groups at δ 4.01, 3.90, and 3.82 and a hydroxy group at δ 5.60 could be placed at C-3, C-4, C-7, and C-2, respectively (see Table S5, Supporting Information). The remaining aromatic ring must bear another identical monomer unit, one hydroxy, and three methoxy groups. The ^1H and ^{13}C NMR spectra of **5** were similar to those of the co-occurring 2-hydroxy-3,4,7-trimethoxy-9,10-dihydrophenanthrene (**7**) in *D. nobile*,[16] except that the signal corresponding to H-1 (δ_H 6.61) of **7** was absent for **5**. In addition, the signal corresponding to H-10 of **7** (δ_H 2.67; δ_C 30.0) was shifted upfield in the spectrum of **5** (δ_H 2.42−2.36; δ_C 26.5), indicating that C-1 (C-1′) of **5** is substituted.[17] In the HMBC spectrum of **5**, H-10 (H-10′) and a hydroxy group at C-2 (C-2′) correlated to a substituted aromatic carbon (δ_C 116.4), which was assigned to C-1 (C-1′). Furthermore, C-1 (C-1′) (δ_C 116.4) of **5** was not oxygenated, but shifted downfield by ca. 6.4 ppm compared with **7** (δ_C 110.0). These observations suggested that two monomeric units in **5** are connected through C-1 and C-1′.[17,18] Taken together, the structure of **5** was determined as 2,2′-dihydroxy-3,3′,4,4′,7,7′-hexamethoxy-9,9′,10,10′-tetrahydro-1,1′-biphenanthrene.

Compound **6** was isolated as a pale brownish, amorphous powder. The molecular formula of **6** was determined as $C_{16}H_{14}O_5$ from the HREIMS at *m/z* 286.0840 $[M]^+$ (calcd *m/z* 286.0841) and ^{13}C NMR spectrum. In the ^1H NMR spectrum, the signals for a 1,2,3-trisubstituted benzene ring [δ_H 7.28 (1H, d, J = 7.7 Hz, H-6), 7.51 (1H, t, J = 7.7, H-7), and 7.96 (1H, d, J = 7.7 Hz, H-8)] and two isolated aromatic protons [δ_H 7.12 (1H, s, H-1) and 6.73 (1H, s, H-10)] were observed. In addition, two aromatic methoxy groups, one of which was *ortho*-disubstituted [δ_H 3.73 (3H, s, OCH_3-4) and 4.00 (3H, s, OCH_3-9); δ_C 62.5 (OCH_3-4) and 55.5 (OCH_3-9)], and a hydroxy group [δ_H 10.32 (1H, s, OH-5), exchangeable with D_2O] were observed in the ^1H and ^{13}C NMR spectra of **6**. However, no signals were apparent for the *ortho*-coupled 9- and 10-protons of a phenanthrene derivative, suggesting that **6** has at least one substituent at the C-9 or C-10 position.[19] The HMBC spectrum exhibited the correlations from methoxy protons at δ 3.73 to C-9 (δ_C 152.6) and from H-10 to C-1, C-4a, C-8a, and C-9. In addition, H-10 correlated with H-1 as well as with the methoxy protons at δ 3.73 in the NOESY spectrum. These results confirmed that C-9 is substituted with a methoxy group. From the HMBC and NOESY spectra, a methoxy group at δ 4.00 and a hydroxy group at δ 10.32 could be suggested to be at C-4 and C-5, respectively (see Table S6, Supporting Information). Although the remaining substituents were not seen in ^1H NMR spectrum, they were deduced from the HMBC spectrum, in which H-1 showed correlations to two oxygenated carbons that did not correlate with any of the methoxy protons, so C-2 and C-3 must be hydroxylated. From these data, the structure of **6** was established as 2,3,5-trihydroxy-4,9-dimethoxyphenanthrene.

Table 1. NMR Spectroscopic Data for Compounds **1**–**4** and **6** ($CDCl_3$)[a]

	1		**2**		**3**		**4**		**6**	
position	δ_H (J in Hz)[b]	δ_C[c]	δ_H (J in Hz)[d]	δ_C[e]	δ_H (J in Hz)[d]	δ_C[e]	δ_H (J in Hz)[b]	δ_C[c]	δ_H (J in Hz)[d]	δ_C[e]
1	7.16, s	108.2	6.56, s	107.0	6.62, s	110.0	6.32, d (2.0)	107.2	7.12, s	109.1
2		147.7		145.7		147.6		154.5		145.0
3		140.7		137.6		139.0	6.39, d (2.0)	98.1		135.3
4		151.0		144.8		150.8		157.9		141.2
4a		118.5		120.2		120.4		116.6		111.6
4b		124.9		125.3		126.3		125.5		118.6
5	8.99, d (9.3)	118.2	8.19, d (8.7)	128.1	7.79, d (8.7)	118.8	8.14, d (8.7)	128.9		153.0
6	7.24, d (9.3)	111.4	6.81, dd (8.7, 2.7)	111.8	6.76, d (8.7)	107.9	6.78, dd (8.7, 2.7)	111.1	7.28, d (7.7)	116.3
7		142.1		158.1		144.9		157.6	7.51, t (7.7)	127.1
8		140.1	6.76, d (2.7)	113.3		141.6	6.75, d (2.7)	113.1	7.96, d (7.7)	114.4
8a		121.4		139.4		124.0		139.4		128.7
9	8.03, d (9.1)	119.9	2.75, m	29.9	2.76, m	21.3	2.71, m	30.1		152.6
10	7.50, d (9.1)	126.1	2.69, m	30.3	2.65, m	29.6	2.71, m	30.5	6.73, s	102.7
10a		129.8		129.7		135.1		141.1		128.5
OCH_3-2			3.89, s	56.2						
OCH_3-3	4.09, s	61.3			3.95, s	61.1				
OCH_3-4	3.95, s	59.7	3.68, s	60.0	3.72, s	59.9	3.84, s	55.5	3.73, s	62.5
OCH_3-7	4.01, s	56.6	3.82, s	55.2	3.91, s	55.9	3.81, s	55.2		
OCH_3-9									4.00, s	55.5
OH-2	5.98, s				5.70, s					
OH-3			5.58, s							
OH-5									10.32, s	
OH-8	6.02, s				5.68, s					

[a] Full assignments are based on $^1H-^1H$ COSY, HMQC, and HMBC experiments. Proton coupling constants (J) in Hz are given in parentheses. [b–e] NMR data were measured at 500, 125, 400, and 100 MHz, respectively.

Thirteen other compounds isolated from the methanolic extract of *D. nobile* stems were identified as known compounds, 2-hydroxy-3,4,7-trimethoxy-9,10-dihydrophenanthrene (**7**),[16] 3-hydroxy-2,4,7-trimethoxyphenanthrene (**8**),[15] 2,5-dihydroxy-3,4-trimethoxyphenanthrene (**9**),[20] bulbophyllanthrin (**10**),[21] denbinobin (**11**),[12] erianthridin (**12**),[14,22] nudol (**13**),[23,24] ephemeranthol A (**14**),[22] 4,5-dihydroxy-2-methoxy-9,10-dihydrophenanthrene (**15**),[25] coelonin (**16**),[14,26] lusianthridin (**17**),[27,28] fimbriol B (**18**),[16] and plicatol A (**19**),[29] by comparing their spectroscopic data with previously reported information.

Hepatic stellate cells (HSCs) are considered to play a key role in the pathogenesis of liver fibrosis. During liver fibrogenesis, HSCs are activated and acquire a myofibroblast-like phenotype that is accompanied by increased proliferation and extracellular matrix synthesis.[30] Therefore, suppression of HSC activation has been proposed as a therapeutic target against hepatic fibrosis.[31] In the present study, all isolated compounds were measured for their antifibrotic activities by assessing the effect on the proliferation of HSC-T6 cells using the 3-(4,5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT) assay (Table 3). (−)-Epigallocatechin-3-gallate (EGCG)[32] was used as a positive control in this study (IC_{50} value of 9.9 μM). Compound **6** was the most potent, showing an IC_{50} value of 9.0 μM, similar to the value of the positive control. Compounds **11**, **16**, and **18** showed potent inhibitory activities (IC_{50} values of 15.2, 13.4, and 11.0 μM, respectively). In contrast, compounds **2**, **7**, **8**, **9**, **13**, **15**, and **19** were inactive (IC_{50} > 100 μM). All of the compounds tested in the study were found not to be cytotoxic to primary cultured rat hepatocytes at concentrations ranging from 10 to 100 μM (data not shown). The positions of the hydroxy and methoxy groups on the carbon skeleton seem to be crucial for the inhibition of HSC-T6 cell proliferation (e.g., **10** ≫ **9**, **13**; **16** ≫ **15**, **17**).

Experimental Section

General Experimental Procedures. Melting points were measured using a Fisher-Johns 12-144 melting point apparatus with a 12-142T thermometer. The optical rotation was measured with a JASCO DIP-1000 digital polarimeter. UV spectra were obtained on a Shimadzu UV-201 spectrophotometer and IR spectra on a Perkin-Elmer 1710 spectrophotometer. NMR spectra were obtained on a JEOL JNM GSX-400 NMR spectrometer (400 and 100 MHz for ^1H and ^{13}C, respectively) or a Bruker AMX 500 NMR spectrometer (500 and 125 MHz for ^1H and ^{13}C, respectively) in $CDCl_3$ with solvent signals as internal standards. EIMS were obtained on a JEOL JMS 700 spectrometer with a 70 eV ionizing potential. Silica gel 60 (230–400 mesh, Merck), ODS RP (Lichoprep ODS RP-18, 40–63 μm, EM Science), and Sephadex LH-20 (18–110 μm, Pharmacia) were used for column chromatography. TLC was carried out on precoated Kieselgel 60 F254 (0.25 mm, Merck) and RP-18 (0.25 mm, Merck) plates, and spots were visualized by heating after spraying with anisaldehyde-H_2SO_4. HPLC was performed with an L-7100 pump (Hitachi), an L-7400 UV detector (Hitachi), and a Symmetry C_{18} column (Waters, Milford, MA).

Plant Material. The stems of *D. nobile* were purchased from Kyoungdong Oriental Herbal Market, Seoul, Korea, in April 2006 and identified by Prof. Jong Hee Park, College of Pharmacy, Pusan National University. A voucher specimen (SNU-0209) has been deposited in the Herbarium of the Medicinal Plant Garden, College of Pharmacy, Seoul National University.

Extraction and Isolation. The air-dried plant material (25 kg) was extracted three times with 80% MeOH in an ultrasonic apparatus. Removal of the solvent in vacuo yielded a methanolic extract (1.8 kg). This methanolic extract was then suspended in distilled water and partitioned successively with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH. The $CHCl_3$ fraction (299 g), which showed the greatest inhibitory activity on HSC-T6 cell proliferation (8.6 ± 0.89% of control at 100 μM), was subjected to column chromatography on a silica gel column using mixtures of *n*-hexane−$CHCl_3$−MeOH of increasing polarity as eluents to give 12 subfractions (fr. 1–12).

From these 12 fractions, fr. 4 (28.3 g) was chromatographed on a silica gel column eluting with a gradient of *n*-hexane−EtOAc−MeOH to yield 11 fractions (fr. 4-1–4-11). Compound **1** (11.4 mg) was obtained from fr. 4-7 by passage over a Sephadex LH-20 column (*n*-hexane−CH_2Cl_2−MeOH, 5:5:1) and over a ODS column (MeOH−H_2O, 4:6 → 7:3). Fr. 4-4 was further subjected to column chromatography on Sephadex LH-20 (*n*-hexane−CH_2Cl_2−MeOH, 10:10:1) to give seven fractions (fr. 4-4-1−4-4-7), and compound **7** (336.3 mg) was obtained from fr. 4-4-4. Compound **5** (15.8 mg) was obtained from fr. 4-4-1 by column chromatography on silica gel by gradient elution with *n*-hexane−EtOAc (10:1 → 0:100). Fr. 4-5 was chromatographed on a Sephadex LH-20 column (*n*-hexane−CH_2Cl_2−MeOH, 10:10:1) to furnish seven fractions (fr. 4-5-1–4-5-7). Fr. 4-5-4 was applied to an ODS column (MeOH−H_2O, 4:6 → 7:3) and then to HPLC to yield compounds **2** (24 mg, AcCN−H_2O 42:58, 0.9 mL/min, 280 nm, t_R 30.19 min) and **8** (16 mg, AcCN−H_2O 42:58, 0.9 mL/min, 280 nm, t_R 31.36 min). Fr. 4-5-5 was chromatographed on an ODS column (MeOH−H_2O, 4:6 → 7:3) to yield compounds **3** (194 mg) and **9** (70

mg). Fr. 4-5-6 was further purified using on an ODS column (MeOH−H_2O, 4:6 → 7:3) to yield compound **4** (72 mg). Fr. 4-9 was chromatographed over both silica gel ($CHCl_3$−MeOH, 100:0 → 0:100) and Sephadex LH-20 (MeOH) to yield compound **10** (28.8 mg). Compound **11** (1185 mg) was obtained by recrystallization from MeOH of fr. 4-10.

Fr. 5 (3.3 g) was chromatographed on an ODS column (MeOH−H_2O, 4:6 → 7:3) to yield 11 fractions (fr. 5-1–5-11) and afforded compounds **12** (213.5 mg) and **13** (91.2 mg) from fr. 5-5 and fr. 5-6, respectively. Compounds **14** (193.6 mg) and **15** (76.1 mg) were obtained from fr. 5-7 and fr. 5-8, respectively, by column chromatography on Sephadex LH-20 (MeOH).

Fr. 6 (34.5 g) was chromatographed on an ODS column (MeOH−H_2O, 0:100 →100:0) to give eight fractions (fr. 6-1–fr. 6-8). Fr. 6-3 was chromatographed over a silica gel column ($CHCl_3$−MeOH, 20:1) and a Sephadex LH-20 column ($CHCl_3$−MeOH, 1:1) to yield compound **16** (123.7 mg). Fr. 6-5 was chromatographed on a silica gel column ($CHCl_3$−MeOH, 20:1) to yield compound **17** (188.0 mg). Fr. 6-6 was chromatographed on a silica gel column ($CHCl_3$−MeOH, 30:1) to give seven fractions (fr. 6-6-1–fr. 6-6-7). Compounds **6** (82.0 mg) and **18** (530.1 mg) were obtained from fr. 6-6-4 by passage over a column containing Sephadex LH-20 (MeOH). Finally, compound **19** (167.9 mg) was obtained from fr. 6-8 by separation over Sephadex LH-20 (MeOH).

2,8-Dihydroxy-3,4,7-trimethoxyphenanthrene (1): brownish, amorphous powder; mp 92.6–93.2 °C; UV (MeOH) λ_{max} (log ε) 242 (3.83), 254 (3.80), 265 (3.79), 267 (3.79), 278 (3.79), 287 (3.74), 290 (3.77) nm; IR (KBr) ν_{max} 3499, 2937, 1622, 1572, 1476, 1362, 1281, 1076, 1010, 950, 899, 860, 767 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; EIMS *m/z* 300 $[M]^+$(100), 285 (88), 253 (88), 242 (87), 227 (89), 199 (85), 197 (51), 170 (70), 150 (78), 121 (64), 115 (62), 75 (23), 63 (37), 51 (10); HREIMS *m/z* 300.0992 (calcd for $C_{17}H_{16}O_5$, 300.0998).

3-Hydroxy-2,4,7-trimethoxy-9,10-dihydrophenanthrene (2): pale yellowish, amorphous powder; mp 99.6–99.9 °C; UV (MeOH) λ_{max} (log ε) 240 (3.77), 263 (3.72), 278 (3.78), 279 (3.79), 282 (3,78), 290 (3.72), 297 (3.74), 298 (3.74) nm; IR (KBr) ν_{max} 3435, 2937, 2836, 1613, 1496, 1461, 1244, 1198, 1150, 1117, 1080, 912, 828 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; EIMS *m/z* 286 $[M]^+$ (100), 271 (100), 239 (24), 228 (49), 211 (45), 183 (18), 171 (19), 139 (22), 128 (29), 115 (13), 102 (4), 77 (3), 63 (3), 51 (2); HREIMS *m/z* 286.1211 (calcd for $C_{17}H_{18}O_4$, 286.1205).

2,8-Dihydroxy-3,4,7-trimethoxy-9,10-dihydrophenanthrene (3): pale yellowish, amorphous powder; mp 151.9–153.4 °C; UV (MeOH) λ_{max} (log ε) 222 (3.64), 225 (3.63), 232 (3.72), 277 (3.73), 290 (3.71) nm; IR (KBr) ν_{max} 3433, 2939, 2841, 1590, 1475, 1359, 1277, 1229, 1077, 972, 796, 757 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; EIMS *m/z* 302 $[M]^+$(100), 287 (100), 255 (52), 227 (25), 199 (9), 184 (11), 155 (14), 151 (23), 115 (20), 77 (6), 55 (10); HREIMS *m/z* 302.1164 (calcd for $C_{17}H_{18}O_5$, 302.1154).

2-Hydroxy-4,7-dimethoxy-9,10-dihydrophenanthrene (4): pale brownish gum; UV (MeOH) λ_{max} (log ε) 239 (3.81), 241 (3.83), 256 (3.83), 258 (3.83), 260 (3.82), 262 (3.81), 291 (3.82), 294 (3.81), 307 (3.81) nm; IR (KBr) ν_{max} 3395, 2939, 2836, 1611, 1465, 1262, 1227, 1158, 1084, 1043, 971, 824, 756 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; EIMS *m/z* 256 $[M]^+$ (100), 241 (40), 213 (38), 198 (36), 181 (37), 152 (37), 128 (35), 115 (35), 77 (11), 76 (16), 63 (15); HREIMS *m/z* 256.1106 (calcd for $C_{16}H_{16}O_3$, 256.1099).

2,2′-Dihydroxy-3,3′,4,4′,7,7′-hexamethoxy-9,9′,10,10′-tetrahydro-1,1′-biphenanthrene (5): pale yellowish, amorphous powder; mp 195.2–197.7 °C; $[\alpha]^{25}_D$ −61.6 (*c* 0.1, CH_3OH); UV (MeOH) λ_{max} (log ε) 220 (3.66), 283 (3.59) nm; IR (KBr) ν_{max} 3479, 2935, 1609, 1502, 1459, 1347, 1207, 1072, 756 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; EIMS *m/z* 570 $[M]^+$ (100), 568 (6), 463 (2), 445 (3), 286 (5), 255 (8), 240 (11), 226 (7), 171 (7), 149 (33), 104 (37), 76 (14), 57 (8); HREIMS *m/z* 570.2258 (calcd for $C_{34}H_{34}O_8$, 570.2254).

2,3,5-Trihydroxy-4,9-dimethoxyphenanthrene (6): pale brownish, amorphous powder; mp 121.3–122.0 °C; UV (MeOH) λ_{max} (log ε) 245 (3.61), 250 (3.37), 255 (3.60), 261 (3.59), 270 (3.57), 274 (3.56), 306 (3.37), 317 (3.38), 351 (3.21), 368 (3.17) nm; IR (KBr) ν_{max} 3392, 1628, 1505, 1463, 1318, 1245, 1072, 1032, 874, 757 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; EIMS *m/z* 286 $[M]^+$ (100), 271 (28), 243 (37), 228 (25), 226(18), 200 (13), 199 (8), 143 (10), 126 (16), 115 (8), 100 (3), 63 (4), 51(2); HREIMS *m/z* 286.0840 (calcd for $C_{16}H_{14}O_5$, 286.0841).

Table 2. NMR Spectroscopic Data for Compound **5** ($CDCl_3$)[a]

position	δ_H (*J* in Hz)[b]	δ_C[c]
1 (1′)		116.4
2 (2′)		145.7
3 (3′)		138.9
4 (4′)		150.2
4a (4a′)		120.6
4b (4b′)		125.6
5 (5′)	8.22, d (8.8)	128.7
6 (6′)	6.81, dd (8.8, 2.7)	111.6
7 (7′)		157.9
8 (8′)	6.72, d (2.7)	112.8
8a (8a′)		139.7
9 (9′)	2.60, m	30.0
10 (10′)	2.42−2.31, m	26.5
10a (10a′)		134.3
OCH_3-3 (OCH_3-3′)	4.01, s	61.2
OCH_3-4 (OCH_3-4′)	3.90, s	60.2
OCH_3-7 (OCH_3-7′)	3.82, s	55.2
OH-2 (OH-2′)	5.60, s	

[a] Full assignments are based on ^1H−^1H COSY, HMQC, and HMBC experiments. Proton coupling constants (*J*) in Hz are given in parentheses. [b,c] NMR data were measured at 500 and 125 MHz, respectively.

Table 3. Inhibitory Activities[a] of Compounds **1–19** from *D. nobile* on HSC-T6 Cell Proliferation

compound	IC_{50} (μM)[b]	compound	IC_{50} (μM)[b]
1	54.1	**11**	15.2
2	>100	**12**	73.2
3	35.7	**13**	>100
4	69.1	**14**	79.2
5	88.2	**15**	>100
6	9.0	**16**	13.4
7	>100	**17**	61.2
8	>100	**18**	11.0
9	>100	**19**	>100
10	50.9	EGCG[c]	9.9

[a] Data are expressed as the mean of three independent experiments. [b] Concentration of compound required for 50% inhibition of cell proliferation. [c] EGCG was used as a positive control.

HSC-T6 Cell Culture. An immortalized rat hepatic stellate cell line, HSC-T6, was kindly provided by Prof. S. L. Friedman (Columbia University, New York, NY). HSC-T6 cells were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 100 IU/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere of 95% air−5% CO_2.

Measurement of Inhibitory Activity on Cell Proliferation. Compounds to be tested were dissolved in DMSO (final culture concentration, 0.1%). A preliminary study showed that DMSO at a final concentration of 0.1% in media did not affect the cell viability. For the assay, the cells were seeded in 48-well plates at a density of 5 × 10^4 cells/mL and incubated for 24 h. HSC-T6 cells were treated with vehicle or compounds to be tested for 48 h. Inhibitory activity of compounds on cell proliferation was assessed by the MTT assay.[33] HSC-T6 cells were incubated with 0.5 mg/mL of MTT in the last 2 h of the culture period tested. Reduction of MTT to formazan was assessed in an ELISA plate reader at 540 nm. Inhibitory activity of compounds **1–19** on cell proliferation (% of control) was calculated as 100 × (absorbance of treated compound/absorbance of control). Data were expressed as the mean of three independent experiments. EGCG was used as a positive control.

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Supporting Information Available: Structures of compounds **7**–**19** and 2D NMR data (HMBC and NOESY) of compounds **1**–**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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