Alkaloids from *Pachysandra terminalis* Inhibit Breast Cancer Invasion and Have Potential for Development As Antimetastasis Therapeutic Agents

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S *Supporting Information*
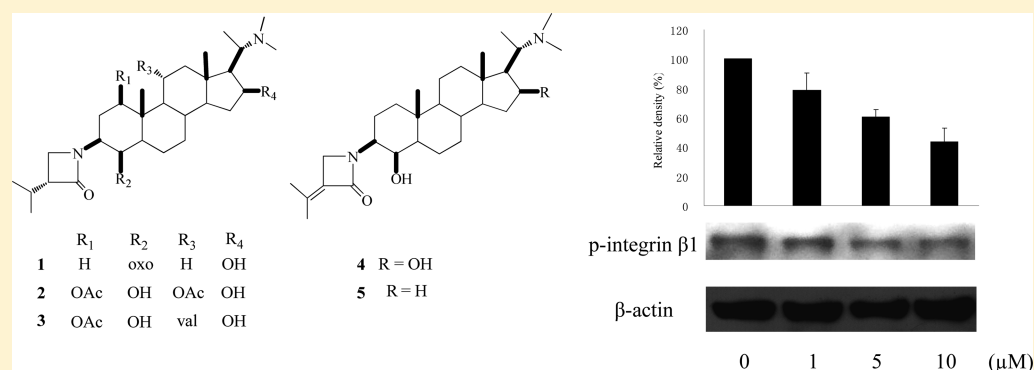
ABSTRACT: The aim of the present study was to identify potentially useful natural compounds for the development of novel therapeutic agents to inhibit metastasis. A phytochemical investigation of *Pachysandra terminalis* resulted in the isolation of seven new pregnane alkaloids, terminamines A–G (**1**–**7**), and seven known alkaloids (**8**–**14**). The structures of **1**–**7** were elucidated by 1D- and 2D-NMR spectroscopic and mass spectrometric methods. Compounds **1**–**5** and **8**–**14** inhibited the migration of MB-MDA-231 breast cancer cells induced by the chemokine epithelial growth factor. In addition, compound **1** inhibited phosphorylation of integrin β_1, which plays an important role in MB-MDA-231 cell adhesion and metastasis.

Pachysandra terminalis Sieb. et Zucc. (Buxaceae) is a small shrub, distributed in southwestern mainland China and Japan, which has been used extensively in Hubei Province, People's Republic of China, as a Tujia traditional medicine against pain and stomach problems.[1] Phytochemical investigations of this species by Kikuchi et al. revealed the presence of several steroidal alkaloids as well as triterpenes and sterols. Members of this class of alkaloids are reported to possess antiulcer[2] and cytotoxic[3] properties. In recent years, a large number of steroidal alkaloids have been isolated from *Pachysandra axillaris*, *Pachysandra procumbens,* and species of the genus *Sarcococca*.[4,5] Research on the bioactivity of these compounds has focused on acetyl- and butylcholinesterase inhibition,[6] anti-estrogen-binding site inhibition,[7] and cytotoxicity.[5] In the present study, preliminary screening revealed that an ethanol extract of *P. terminalis* inhibited the migration of human MDA-MB-231 breast cancer cells induced by the chemokine epidermal growth factor (EGF), with an inhibitory rate of 84.7% at 5 μg/mL. Bioassay-guided phytochemical investigation led to the isolation of seven new (**1**–**7**) and eight known (**8**–**15**) pregnane alkaloids.
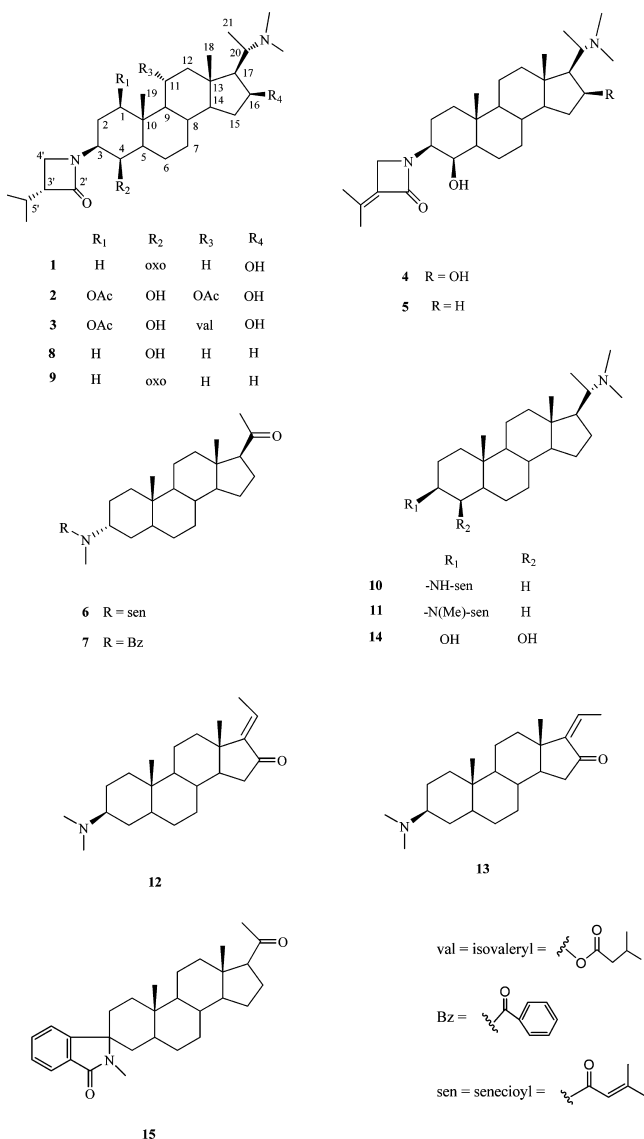
Metastasis is the major cause of mortality in breast cancer. Primary tumor cells detach from the tissue, intravasate into the circulatory system, extravasate into secondary tissues, and begin to proliferate.[8] Increasing evidence suggests that epidermal growth factor receptor (EGFR)-mediated chemotaxis plays a pivotal role in the process of metastasis in breast cancer.[9−11] Hence, a search for novel and specific natural products that inhibit migration and invasion may be beneficial for the prevention and therapy of cancer metastasis.

Integrins are a family of heterodimers comprised of α and β subunits. Integrin $\beta1$ combines with the α subunits to form 12 different integrin receptors, which bind extracellular matrix (ECM) molecules such as collagen and fibronectin. A large number of reports have shown that integrin $\beta1$ is closely involved with cancer cell adhesion and metastasis.[12−15]

This paper describes the isolation, identification, and effects of compounds **1−15** from *P. terminalis* on the invasion of MDA-MB-231 breast cancer cells in vitro. In addition, the effect of **1** on integrin $\beta1$ phosphorylation was investigated in MDA-MB-231 cells.

	R₁	R₂	R₃	R₄
1	H	oxo	H	OH
2	OAc	OH	OAc	OH
3	OAc	OH	val	OH
8	H	OH	H	H
9	H	oxo	H	H

	R
4	R = OH
5	R = H

	R₁	R₂
10	-NH-sen	H
11	-N(Me)-sen	H
14	OH	OH

6 R = sen
7 R = Bz

12

13

val = isovaleryl =

Bz =

sen = senecioyl =

15

■ RESULTS AND DISCUSSION

Seven new pregnane alkaloids, terminamines A−G (**1−7**), as well as eight known alkaloids (**8−15**) were isolated from the EtOAc-soluble extract of *P. terminalis*. The structures of the isolated compounds were determined by 1D- and 2D-NMR and MS data analysis and comparison with literature values.

Terminamine A (**1**) was obtained as colorless needle crystals and assigned the molecular formula $C_{29}H_{48}N_2O_3$ from its pseudomolecular ion peak at *m/z* 473.3738 [M + H]⁺ in the HRESIMS. The IR spectrum displayed absorption bands at 3418 (OH), 1752 (unconjugated ketone), and 1650 (amide carbonyl) cm⁻¹. The ¹H NMR spectrum of **1** revealed two proton signals [δ_H 4.33 (2H, m)] connected with an electron-withdrawing group, four methine signals [δ_H 3.56 (1H, t, *J* = 5.2 Hz), 3.09 (1H, brt, *J* = 5.3 Hz), 3.02 (1H, brd, *J* = 2.7 Hz), 2.95 (1H, m)], two tertiary methyls [δ_H 0.88 and 0.77 (each 3H, s)], three secondary methyls [δ_H 1.06, 0.96 (each 3H, d, *J* = 6.7 Hz); 0.95 (3H, d, *J* = 5.0 Hz)], and six N(CH₃)₂ protons [δ_H 2.25 (s)].

The ¹³C NMR and DEPT spectra of **1** indicated the presence of seven CH₃, eight CH₂, and 10 CH (including one oxygenated at δ_C 72.4) groups and four quaternary carbons (including two carbonyl carbons at δ_C 170.7 and 207.3). Moreover, a 3-isopropyl β-lactam group was identified from the COSY and HMBC spectra.[7] Considering the previous reports of pregnane alkaloids from species in the genus *Pachysandra*, compound **1** was proposed to have a basic skeleton of 20-(dimethylamino)pregnane, similar to pachystermine A (**9**).[16] The location of the OH proton at C-16 of **1** was confirmed from the HMBC spectrum, in which correlations were observed for the resonance at δ_H 1.24 (H-17) with the signals at δ_C 72.4 (C-16), 41.7 (C-13), and 56.8 (C-20), while the signal at δ_H 0.88 (H-18) correlated with the signals at δ_C 41.7 (C-13) and 59.0 (C-17) (Figure 1). On the other hand, the proton signals at δ_H 2.13 (H-5) and 4.33 (H-3) correlated with the ketone carbonyl resonance at δ_C 207.3 (C-4), and the signal at δ_H 4.33 (H-3) correlated with signals at δ_C 170.7 and 41.7 (3-isopropyl lactam group). Thus, the β-lactam and ketone groups were assigned to C-3 and C-4, respectively.

In the ROESY spectrum, the proton signal of H-16 correlated with H-17 and H-14, and the H-3 signal correlated with H-5 (Figure 1). On comparison of the conformation with that of known steroidal alkaloids, the 3-isopropyl lactam and 16-hydroxy groups were each assigned with a β-orientation. Furthermore, **1** was confirmed structurally by single-crystal X-ray crystallography (Figure 2). Therefore, the structure of **1** was designated as 20α-dimethylamino-16β-hydroxy-3β-(3'α-isopropyl)lactam-5-pregn-4-one.
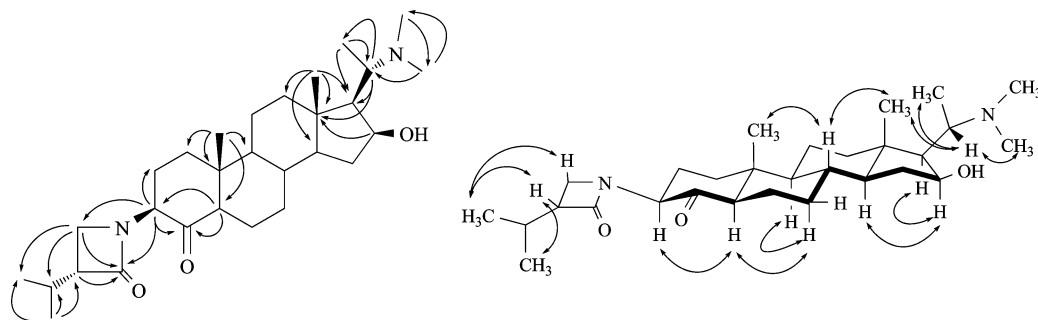

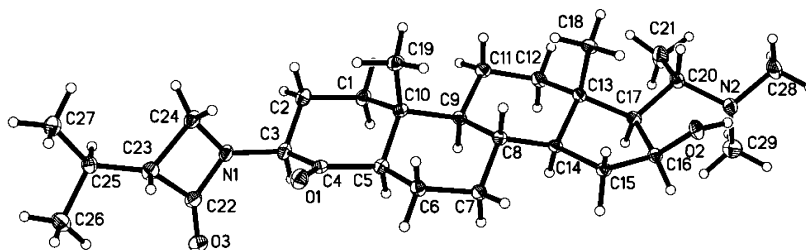**Figure 1.** Key HMBC (→) and ROESY (↔) correlations of **1**.

Figure 2. ORTEP diagram of compound **1**.

Table 1. ^{13}C NMR Spectroscopic Data (100 MHz, CDCl$_3$) of Compounds 1−5

position	1	2	3	4	5
1	36.4, CH$_2$	74.7, CH	74.6, CH	36.7, CH$_2$	36.8, CH$_2$
2	27.2, CH$_2$	28.3, CH$_2$	28.2, CH$_2$	24.9, CH$_2$	24.9, CH$_2$
3	58.0, CH	58.0, CH	58.1, CH	57.5, CH	57.6, CH
4	207.3, C	69.6, CH	69.5, CH	78.2, CH	78.3, CH
5	58.1, CH	45.8, CH	45.9, CH	47.2, CH	47.2, CH
6	20.2, CH$_2$	22.7, CH$_2$	22.8, CH$_2$	25.8, CH$_2$	25.8, CH$_2$
7	30.3, CH$_2$	32.2, CH$_2$	32.1, CH$_2$	32.3, CH$_2$	32.4, CH$_2$
8	34.3, CH	34.2, CH	34.2, CH	34.8, CH	35.4, CH
9	54.3, CH	56.0, CH	56.0, CH	55.2, CH	55.4, CH
10	42.5, C	42.2, C	42.1, C	35.7, C	35.8, C
11	21.4, CH$_2$	72.1, CH	71.5, CH	20.4, CH$_2$	20.6, CH$_2$
12	40.1, CH$_2$	44.9, CH$_2$	44.8, CH$_2$	40.2, CH$_2$	39.6, CH$_2$
13	41.7, C	41.2, C	41.4, C	41.9, C	41.7, C
14	53.1, CH	51.5, CH	51.9, CH	53.6, CH	54.8, CH
15	34.7, CH$_2$	35.1, CH$_2$	35.7, CH$_2$	35.1, CH$_2$	24.0, CH$_2$
16	72.4, CH	72.5, CH	71.2, CH	71.6, CH	27.6, CH$_2$
17	59.0, CH	58.5, CH	58.0, CH	58.5, CH	56.6, CH
18	14.2, CH$_3$	14.4, CH$_3$	14.1, CH$_3$	14.1, CH$_3$	12.3, CH$_3$
19	13.8, CH$_3$	10.5, CH$_3$	10.6, CH$_3$	14.2, CH$_3$	14.3, CH$_3$
20	56.8, CH	56.0, CH	57.5, CH	57.6, CH	57.5, CH
21	9.9, CH$_3$	9.82, CH$_3$	10.2, CH$_3$	10.2, CH$_3$	9.88, CH$_3$
N(Me)$_2$	39.8, CH$_3$	39.8, CH$_3$	39.8, CH$_3$	40.1, CH$_3$	39.8, CH$_3$
(Me)$_2$-5′	19.9, CH$_3$	19.9, CH$_3$	19.8, CH$_3$	20.1, CH$_3$	20.0, CH$_3$
				22.6, CH$_3$	22.6, CH$_3$
2′	170.7, C	171.1, C	171.1, C	171.6, C	171.9, C
3′	56.2, CH	56.2, CH	52.6, CH	130.5, C	130.5, C
4′	41.7, CH$_2$	43.0, CH$_2$	43.0, CH$_2$	45.0, CH$_2$	45.0, CH$_2$
5′	28.0, CH	28.2, CH	28.2, CH	137.6, C	137.6, C

Terminamine B (**2**) was isolated as a colorless oil. The HRESIMS showed a molecular ion peak at m/z 591.4004 [M + H]$^+$ (C$_{33}$H$_{53}$N$_2$O$_7$). The ^{1}H NMR spectrum of **2** featured two tertiary methyl signals at δ_H 0.85 (3H, s) and 1.10 (3H, s), three secondary methyls at δ_H 0.92 (3H, d, J = 6.3 Hz), 0.98, and 1.08 (each 3H, J = 6.6 Hz), two acetyl groups at δ_H 1.90 (3H, s) and 1.95 (3H, s), and N(CH$_3$)$_2$ protons at δ_H 2.23 (6H, s). The ^{13}C NMR spectroscopic data for **2** were similar to those of **1**, except for the signals of C-1, C-4, C-5, and C-11 and resonances for an additional two acetyl groups (Table 1). In the HMBC spectrum, the proton signal at δ_H 4.68 (H-1) correlated with the carbon signals at δ_C 42.2 (C-10), 56.0 (C-9), 171.1 (acetyl group), 10.5 (C-19), and 28.3 (C-2), while the signal at δ_H 4.97 (H-11) correlated with those at δ_C 42.2 (C-10), 56.0 (C-9), 41.2 (C-13), and 171.1 (acetyl group). In addition, the signal at δ_H 3.68 (H-3) correlated with the carbon signals at δ_C 69.6 (C-4), 171.1 (β-lactam moiety), and 74.7 (C-1). Furthermore, two separated spin−spin coupling systems (H-3/H-4/H-5 and H-15/H-16/H-17) were observed in the ^{1}H−^{1}H COSY spectrum. Thus, two acetyl and two hydroxy

groups could be located at C-1, C-11, C-4, and C-16, respectively. The β-orientation of the hydroxy groups at C-4 and C-16 and the acetyl group at C-1, the α-oriented acetyl group at C-11, and the β-oriented lactam group at C-3 were determined from the ROESY correlations of H-9α/H-1α/H-3α/H-4α/H-5α, H-16α/H-14α, and H-11β/H-18β and H-19β. Thus, compound **2** was elucidated as 20α-dimethylamino-3β-(3′α-isopropyl)lactam-5α-pregn-4β,16β-diol-1β,11α-diyl acetate.

Terminamine C (**3**) gave a molecular formula of C$_{36}$H$_{60}$N$_2$O$_7$ on the basis of its HRESIMS. There were no obvious differences in the NMR spectra of **3** and **2**, except for the substituent group at C-11. From the ^{13}C NMR, HMBC, and HSQC spectra, an isovaleryl group [δ_C 176.4 (s), 26.6 (t), 40.1 (d), 10.7 (q), and 14.1 (q)] was determined. The HMBC correlations observed from H-11 (δ_H 5.01) to the carbonyl carbon (δ_C 176.4, isovaleryl group) and a signal at δ_C 42.1 (C-10) suggested that the isovaleryl group is located at C-11. Using the same techniques as described for **2**, β-orientations for H-1, H-3, H-4, and H-16 and an α-orientation for H-11 were

elucidated from the ROESY spectrum. Thus, the structure of **3** was designated as 20α-dimethylamino-3β-(3′α-isopropyl)-lactam-1β-acetoxy-11α-isovaleryl-5α-pregn-4β,16β-diol.

Terminamine D (**4**) showed a molecular formula of $C_{29}H_{47}N_2O_3$ from the HRESIMS. Analysis of the ^{1}H and ^{13}C NMR data suggested that compound **4** is a steroidal alkaloid closely related in structure to pachystermine B (**8**), except for the presence of an OH proton at C-16 and the type of substituent group at C-3. The latter functionality was deduced to be a 3′-isopropylidene lactam by comparison with the 3-isopropyl lactum group in compounds **1**−**3**. In addition, two methyls [$δ_H$ 1.70 and 1.85 (each 3H, s)] showed a correlation with double-bond carbons [$δ_C$ 130.5 (s) and 137.6 (s)] in the HMBC spectrum. In turn, the signal at $δ_H$ 4.38 (H-16) showed a HMBC correlation with the carbon signal at $δ_C$ 41.9 (C-13) and [1]H−^{1}H COSY correlations with H-15 ($δ_H$ 2.19) and H-17 ($δ_H$ 1.27). Thus, a 3′-isopropylidene lactam group was determined and located at C-3, and the hydroxy group was assigned to C-16. The ROESY NMR data revealed α-orientations for H-3, H-4, and H-16, consistent with compounds **2** and **3**. Therefore, the structure of **4** was determined as 20α-dimethylamino-3α-(3′-isopropylidene)-lactam-5α-pregn-4β,16β-diol. A molecular formula of $C_{29}H_{49}N_2O_2$ was determined from the HRESIMS data (m/z 457.3749) for terminamine E (**5**). By comparison with the ^{1}H and ^{13}C NMR spectroscopic data of **4** and **5**, compound **5** was determined to be the 16-dehydroxy derivative of **4**. Further analysis of the HSQC, HMBC, and ROESY NMR data for **5** was used to establish the structure of this compound as 20α-dimethylamino-3α-(3′-isopropylidene)lactam-5α-pregn-4β-ol.

Terminamine F (**6**) was isolated as a white powder, with a molecular formula of $C_{27}H_{43}NO_2$ by HRESIMS. The ^{1}H NMR spectrum showed six tertiary methyl signals at $δ_H$ 0.61, 0.80, 1.83, 1.86, 2.12, and 3.07 (each 3H, s) and an olefinic proton at $δ_H$ 5.78 (1H, s). Comparison of their ^{13}C NMR data (Table 1) showed that compound **6** has the same pregnane skeleton as compound **5**, with different substituent groups at positions C-3 and C-17. In the HMBC spectrum, the methyl protons at $δ_H$ 2.12 correlated with the carbon signals at $δ_C$ 209.7 (C-20) and 63.8 (C-17), the methyl signals at $δ_H$ 1.83 and 1.86 (senecioyl group) correlated with signals at $δ_C$ 144.2 and 119.5, and the signal at $δ_H$ 3.07 (NCH$_3$) correlated with a ketone at $δ_C$ 169.8. Thus, an N-methylsenecioyl moiety was elucidated, with the acetyl found to be connected to C-17 by a C−C bond. However, the C-3 and H-3 signals were not observed in the NMR spectrum, and the absence of an H-3 resonance in several related alkaloids with an 3-amide substituent group has been reported.[5,17] A poikilothermic experiment for the NMR spectrum of **6** was undertaken. In the ^{1}H, ^{13}C, and HSQC NMR spectra (C$_5$D$_5$N, 353 K), a H-3 signal appeared at $δ_H$ 4.59, and C-3 was assigned to $δ_C$ 49.9 from the HSQC spectrum (353 K). Furthermore, the HMBC correlation of NCH$_3$ ($δ_H$ 3.14) with C-3 ($δ_C$ 49.9) clearly demonstrated the amide group to be located at C-3. However, the NOE effect of H-3 ($δ_H$ 4.59)/H-5 ($δ_H$ 1.47) was not observed in the 1D NOE spectrum, indicating that the C-3 configuration is α-oriented. From these observations, compound **6** was determined as 3α-(methylsenecioylamino)pregnan-20-one.

A molecular formula of $C_{29}H_{43}NO_2$ was determined from the HRESIMS data (m/z 436.3216) for terminamine G (**7**). From the comparison of the ^{1}H and ^{13}C NMR data of **6** and **7** (Table 2), compound **7** was assigned as the 3-desenecioyl-3-benzoyl of **6**. After further analysis of its HSQC, HMBC, and ROESY

Table 2. ^{13}C NMR Spectroscopic Data of Compounds 6 and 7

position	6		7	
	CDCl$_3$	pyridine-d_5	CDCl$_3$	pyridine-d_5
1	35.6, CH$_2$	37.4, CH$_2$	35.7, CH$_2$	37.4, CH$_2$
2	24.8, CH$_2$	26.5, CH$_2$	24.4, CH$_2$	26.5, CH$_2$
3	39.0, CH	49.9, CH	41.6, CH	50.8, CH
4	32.1, CH$_2$	34.4, CH$_2$	32.6, CH$_2$	34.0, CH$_2$
5	41.6, CH	43.1, CH	41.8, CH	43.2, CH
6	28.7, CH$_2$	30.4, CH$_2$	28.7, CH$_2$	30.3, CH$_2$
7	31.8, CH$_2$	33.5, CH$_2$	31.8, CH$_2$	33.3, CH$_2$
8	35.5, CH	37.1, CH	35.7, CH	37.2, CH
9	55.0, CH	56.6, CH	55.0, CH	56.6, CH
10	35.2, C	36.6, C	35.1, C	36.5, C
11	20.8, CH$_2$	22.5, CH$_2$	20.9, CH$_2$	22.5, CH$_2$
12	39.1, CH$_2$	40.7, CH$_2$	39.0, CH$_2$	40.6, CH$_2$
13	44.2, C	45.5, C	44.2, C	45.5, C
14	56.7, CH	58.1, CH	56.7, CH	58.1, CH
15	24.8, CH$_2$	25.8, CH$_2$	24.3, CH$_2$	25.8, CH$_2$
16	22.8, CH$_2$	24.7, CH$_2$	22.9, CH$_2$	24.7, CH$_2$
17	63.8, CH	65.0, CH	63.8, CH	65.2, CH
18	13.5, CH$_3$	14.7, CH$_3$	13.4, CH$_3$	14.7, CH$_3$
19	12.6, CH$_3$	14.2, CH$_3$	12.7, CH$_3$	14.3, CH$_3$
20	209.7, C	209.2, C	209.6, C	209.7, C
21	31.5, CH$_3$	32.3, CH$_3$	31.5, CH$_3$	32.3, CH$_3$
N-CH$_3$	29.7, CH$_3$	32.7, CH$_3$	32.6, CH$_3$	31.1, CH$_3$

NMR data (298 and 353 K), the structure of compound **7** was determined as 3α-(methylbenzoylamino)pregnan-20-one.

By comparison of their spectroscopic data with values available in the literature, the known compounds were identified as pachystermine B (**8**),[16] pachystermine A (**9**),[16] epipachysamine E (**10**),[18] epipachysamine B (**11**),[19] E-salignone (**12**),[20] Z-salignone (**13**),[20] terminaline (**14**),[6] and spiropachysine-20-one (**15**).[21]

In a screening experiment, compounds **1**−**5** and **8**−**14** were found to inhibit the EGF-induced invasion of MB-MDA-231 cells (Table 3). Since the dimethylamino and pregnane groups are common moieties among the active compounds, they are assumed to be required for the activity of these compounds in this assay.

Western blotting analysis was used to evaluate the potential mechanism of action of compound **1** on cell invasion. MDA-MB-231 cells were treated with increasing doses of **1** for 24 h. Compound **1** markedly inhibited the phosphorylation of integrin β1 in a dose-dependent fashion (Figure 3). These results suggest that integrin β1 may be a key molecule in the signal transduction pathway targeted by compound **1**.

To date, many natural antimetastasis products have been reported,[22,23] such as the saponin monomer (DT-13) that reduces the adhesion and migration of human breast cancer cells,[24] baicalein, which inhibits the migration and invasiveness of A431 cells,[25] and glabridin, which can inhibit migration, invasion, and angiogenesis in human non-small-cell lung cancer A549 cells.[26] In addition, other alkaloids[27,28] such as nitidine chloride can inhibit the migration and invasion of breast cancer cells,[29] berberine can inhibit the migration of melanoma cells and human SCC-4 tongue squamous cancer cells,[30,31] and sanguinarine can reduce the invasive ability of MDA-MB-231 cells.[32]

Hence, the isolation is reported of pregnane alkaloids (**1**−**15**) that may significantly inhibit the invasion of breast cancer

Table 3. Inhibitory Effects of Compounds 1−15 on the Invasion of MDA-MB-231 Cells

compound	IC_{50} (μM)
1	0.18
2	0.20
3	0.08
4	0.20
5	0.07
6	NA[a]
7	NA[a]
8	0.19
9	0.32
10	0.41
11	0.35
12	0.87
13	1.74
14	0.36
15	NA[a]
LY294002[b]	0.38

[a]NA = not active at a concentration of 10 μM. [b]Positive control.
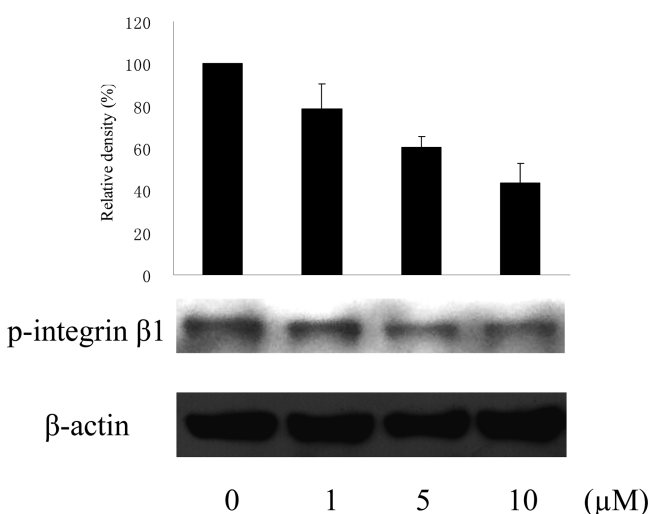

Figure 3. Western blot demonstrating that compound **1** inhibits the phosphorylation of integrin β_1 in MB-MDA-231 cells.

cells in response to EGF. Only four natural products have been reported to suppress integrin β1.[33−36] It was found in the present study that the pregnane alkaloid **1** dose-dependently inhibited the phosphorylation of integrin β1 at lower doses (1−10 μM) than these other compounds.

■ EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on a XT-4 micromelting point apparatus and are uncorrected. Optical rotations were measured with a MC 241 digital polarimeter (Perkin-Elmer, Waltham, MA, USA). UV spectra were recorded in methanol on a Hitachi U-3310 UV−vis spectrophotometer. IR spectra were acquired using a Nicolet 380 FT-IR spectrophotometer (Thermo Electron Corporation, Sugar Land, TX, USA). The NMR spectra were obtained using a Bruker AVANCE III 400 instrument (^{1}H NMR, 400 MHz; ^{13}C NMR, 100 MHz) using TMS as an internal standard. ^{1}H−^{1}H COSY, HSQC, HMBC, and ROESY NMR experiments were also performed on the Bruker AVANCE III 400 instrument, using standard pulse sequences. The high-resolution mass spectra were recorded on a Varian 7.0 T ESI mass spectrometer. HPLC was performed using a JASCO Gulliver Series with a PU-2089 pump and RI-2031 and UV-2075 detectors.

Preparative HPLC column chromatography was carried out on YMC-Pack ODS-A SH-343-5 (ODS), YMC-Pack Polymer-C$_{18}$ (6 μm, 10 × 200 mm; 10 μm, 20 × 300 mm), and Shodex Asahipak GS-310 (GPC). Open column chromatography was performed using silica gel (Qingdao Haiyang Chemical Co., Ltd.) and Toyopearl HW-40 (Tosoh). The chemotaxis chambers and membranes were purchased from Neuroprobe (Gaithersburg, MD, USA), and human EGF was obtained from Peprotech (Rocky Hill, NJ, USA). The rabbit polyclonal antibodies specific for phospho-integrin beta 1 were purchased from Abcam, Inc. (Cambridge, MA, USA).

Plant Material. Entire *Pachysandra terminalis* plants were collected from HeFeng, Hubei Province, People's Republic of China, in April 2005. The plants were identified by Professor D. R. Wan (School of Life Sciences, South Central University for Nationalities, Hubei Province, People's Republic of China), and a voucher specimen (D20051003) was deposited at the School of Pharmacy, Tianjin Medical University, Tianjin, People's Republic of China.

Extraction and Isolation. The dried plant material (8.0 kg) was extracted with 95% ethanol (15 L × 3, 6 h each time) under reflux. The resultant extract was concentrated in vacuo to a gummy residue (800 g) suspended in water, which was partitioned with petroleum ether to defat. The suspension solution was adjusted to pH 2 with 2% HCl. The acid-soluble fraction was alkalinized to pH 10 with NH$_4$OH followed by exhaustive extraction with EtOAc. The invasion assays were performed using petroleum ether and EtOAc-soluble extracts, with IC_{50} values for the inhibition of chemotaxis being >10 and 4.3 μg/mL, respectively. Therefore, the EtOAc extract (29 g) was chromatographed on a silica gel column (300−400 mesh, 10 × 100 cm, 700 g) and eluted using a solvent gradient system (CH$_2$Cl$_2$−MeOH−NH$_4$OH, 98:1:1, 95:4:1, 93:6:1, 90:9:1, 85:15:1, 8:2:1, 7:3:1) to produce 16 fractions (F1−16). Fraction F8 (0.66 g) was separated by semipreparative HPLC (ODS, MeOH−H$_2$O, 98:2) to produce 12 fractions (F8.1−F8.12), and fraction F8.12 was determined to be pure **15** (18.5 mg). Fraction F8.11 provided **6** (12.7 mg) and **7** (3.8 mg) after purification over HPLC (GPC, MeOH). Fraction F10 (0.98 g) was chromatographed on semipreparative HPLC (ODS, MeOH−H$_2$O, 95:5) to produce 13 fractions (F10.1−10.13). Fraction F10.3 was subjected to semipreparative HPLC (Polymer C$_{18}$, 6 μm) and eluted with MeOH−H$_2$O−NH$_4$OH (85:15:0.03, pH 10) to obtain **2** (13.5 mg) and **3** (8.3 mg). Compounds **1** (17.1 mg), **4** (4.4 mg), and **5** (4.9 mg) were obtained from fraction F10.6 by semipreparative HPLC with MeOH−H$_2$O−NH$_4$OH (9:10:0.03, pH 10, Polymer C$_{18}$, 6 μm). Fraction F10.9 and F10.11 were purified by recrystallization (CHCl$_3$−MeOH−H$_2$O, 9:1:0.03) to yield **8** (56.4 mg) and **9** (9.3 mg). Fraction F12 (1.17 g) was chromatographed by semipreparative HPLC (ODS, MeOH−H$_2$O, 9:1) to obtain 16 fractions (F12.1−F12.16). Further purification of fraction F12.11 by recrystallization resulted in **14** (10.8 mg). Fraction F12.16 (50.1 mg) was purified by preparative TLC using petroleum ether−EtOAc−NH$_4$OH (2:1:0.1) to obtain **12** (12.9 mg, R_f 0.3) and **13** (9.4 mg, R_f 0.5). Fraction F14 (1.2 g) was separated by semipreparative HPLC (Polymer C$_{18}$, 10 μm, MeOH−H$_2$O−NH$_4$OH, 9:1:0.03, pH = 10) to obtain 13 fractions (F14.1−F14.13). Of these, fraction F14.6 (176 mg) was purified by semipreparative HPLC (Polymer C$_{18}$, 6 μm, MeOH−H$_2$O−NH$_4$OH, 8:2:0.03, pH 10) to yield compounds **10** (13.7 mg) and **11** (5.8 mg).

Terminamine A (1): colorless needles (CHCl$_3$); mp 214−215 °C; $[\alpha]_D^{20}$+56.2 (*c* 0.29, CHCl$_3$); UV (MeOH) λ_{max} (log ε) 245 (2.42) nm; IR (KBr) ν_{max} 3418, 2932, 2875, 1752, 1650, 1449, 1365, 1140 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 0.95 (3H, d, *J* = 5.0 Hz, H$_3$-21), 2.95 (1H, m, H-20), 0.77 (3H, s, H$_3$-19), 0.88 (3H, s, H$_3$-18), 1.24 (1H, m, H-17), 4.33 (1H, m, H-16), 2.17, 1.22 (each 1H, m, H-15), 0.91 (1H, m, H-14), 1.82 and 1.11 (each 1H, m, H-12), 1.53 and 1.27 (each 1H, m, H-11), 0.95 (1H, m, H-9), 1.42 (1H, m, H-8), 1.79 and 0.82 (each 1H, m, H-7), 1.61 and 1.44 (each 1H, m, H-6), 2.13 (1H, m, H-5), 4.33 (1H, m, H-3), 2.12 and 1.82 (each 1H, m, H-2), 1.94 and 1.48 (each 1H, m, H-1), 2.25 [6H, s, N(CH$_3$)$_2$], 3.09 (1H, brt, *J* = 5.3 Hz, H-3′), 3.56 (1H, t, *J* = 5.2 Hz, H-4′ a), 3.02 (1H, brd, *J* = 2.7 Hz, H-4′ b), 1.97 (1H, m, H-5′), 0.96 and 1.06 [each 3H, d, *J* = 6.7 Hz, (Me)$_2$-5′]; ^{13}C NMR (CDCl$_3$, 100 MHz), see Table 1; HRESIMS *m/z* 473.3738 [M + H]$^+$ (calcd for C$_{29}$H$_{48}$N$_2$O$_3$, 473.3743).

Terminamine B (2): colorless oil; $[\alpha]_D^{20}$ +62.1 (*c* 0.37, CHCl$_3$); UV (MeOH) λ_{max} (log ε) 275 (3.58), 321 (2.54) nm; IR (KBr) ν_{max} 3475, 2978, 2850, 1720, 1450, 1374, 1185, 1152 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 0.92 (3H, d, *J* = 6.3 Hz, H$_3$-21), 2.89 (1H, m, H-20), 1.10 (3H, s, H$_3$-19), 0.85 (3H, s, H$_3$-18), 1.23 (1H, m, H-17), 4.34 (1H, m, H-16), 2.23 and 1.19 (each 1H, m, H-15), 0.94 (1H, m, H-14), 2.64 and 0.82 (each 1H, m, H-12), 4.97 (1H, m, H-11), 1.37 (1H, s, H-9), 1.65 (1H, m, H-8), 1.89 and 0.99 (each 1H, m, H-7), 2.20 and 1.46 (1H, m, H-6), 1.63 (1H, m, H-5), 3.39 (1H, br s, H-4), 3.68 (1H, m, H-3), 2.32 and 1.60 (each 1H, m, H-2), 4.68 (1H, m, H-1), 2.23 [6H, s, N(CH$_3$)$_2$], 2.98 (1H, m, H-3′), 3.68 and 2.99 (each 1H, m, H-4′), 1.95 (1H, m, H-5′), 0.98 and 1.08 [each 3H, d, *J* = 6.6 Hz, (Me)$_2$-5′], 1.90 (3H, s, OAc-1), 1.95 (3H, s, OAc-11); ^{13}C NMR (CDCl$_3$, 100 MHz), see Table 1, and δ_C 171.4 (C=O, OAc-1), 22.0 (q, OAc-1), 171.6 (C=O, OAc-11), 22.3 (q, OAc-11); HRESIMS *m/z* 591.4004 [M + H]$^+$ (calcd for C$_{33}$H$_{54}$N$_2$O$_7$, 591.4013).

Terminamine C (3): colorless oil; $[\alpha]_D^{20}$ +46.6 (*c* 0.21, CHCl$_3$); UV (MeOH) λ_{max} (log ε) 276 (3.80) nm; IR (KBr) ν_{max} 3475, 2978, 2850, 1726, 1645, 1450, 1371, 1185, 1152 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 0.98 (3H, d, *J* = 6.4 Hz, H$_3$-21), 3.10 (1H, m, H-20), 1.11 (3H, s, H$_3$-19), 0.86 (3H, s, H$_3$-18), 1.23 (1H, m, H-17), 4.39 (1H, m, H-16), 2.20 and 1.23 (each 1H, m, H-15), 0.96 (1H, m, H-14), 2.57 and 0.76 (each 1H, m, H-12), 5.01 (1H, m, H-11), 1.38 (1H, m, H-9), 1.67 (1H, m, H-8), 1.89 and 1.00 (each 1H, m, H-7), 2.20 and 1.50 (each 1H, m, H-6), 1.63 (1H, m, H-5), 3.38 (1H, m, H-4), 3.68 (1H, m, H-3), 2.30 and 1.60 (each 1H, m, H-2), 4.68 (1H, m, H-1), 2.38 [6H, s, N(CH$_3$)$_2$], 2.95 (1H, m, H-3′), 3.60 and 2.90 (each 1H, m, H-4′), 1.90 (1H, m, H-5′), 1.08 and 0.98 [each 3H, d, *J* = 6.4 Hz, (Me)$_2$-5′], 1.93 (3H, s, OAc-1); 1.60 and 1.40 (each 1H, m, CH$_2$), 2.20 (1H, m, CH), 0.89 and 1.04 (each 3H, d, *J* = 6.8 Hz, Me × 2), isovaleryl-11; ^{13}C NMR (CDCl$_3$, 100 MHz), see Table 1, and δ_C 171.4 (C=O, OAc-1), 21.9 (q, OAc-1), 176.4 (C=O, isovaleryl), 26.6 (t, isovaleryl), 40.1 (d, isovaleryl), 14.1 (q, isovaleryl), 10.7 (q, isovaleryl); HRESIMS *m/z* 633.4476 [M + H]$^+$ (calcd for C$_{36}$H$_{61}$N$_2$O$_7$, 633.4473).

Terminamine D (4): white powder; $[\alpha]_D^{20}$ +56.2 (*c* 0.22, CHCl$_3$); UV (MeOH) λ_{max} (log ε) 228 (4.30) nm; IR (KBr) ν_{max} 3334, 2933, 2850, 1713, 1448, 1197 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.02 (3H, d, *J* = 6.5 Hz, H$_3$-21), 3.1 (1H, m, H-20), 1.05 (3H, s, H$_3$-19), 0.89 (3H, s, H$_3$-18), 1.27 (1H, m, H-17), 4.38 (1H, m, H-16), 2.19 and 1.27 (each 1H, m, H-15), 0.90 (1H, m, H-14), 1.84 and 1.07 (each 1H, m, H-12), 1.46 and 1.33 (each 1H, m, H-11), 0.64 (1H, m, H-9), 1.56 (1H, m, H-8), 1.83 and 0.91 (each 1H, m, H-7), 1.84 and 1.29 (each 1H, m, H-6), 1.23 (1H, m, H-5), 4.32 (1H, m, H-4), 2.75 (1H, m, H-3), 1.60 (2H, m, H-2), 1.76 and 1.01 (each 1H, m, H-1), 3.65 and 3.43 (each 1H, d, *J* = 16.7 Hz, H-4′), 2.35 [6H, s, N(CH$_3$)$_2$], 1.70 and 1.85 [each 3H, s, (Me)$_2$-5′]; ^{13}C NMR (CDCl$_3$, 100 MHz), see Table 1; HRESIMS *m/z* 473.3728 [M + H]$^+$ (calcd for C$_{29}$H$_{49}$N$_2$O$_3$, 473.3743).

Terminamine E (5): white powder; $[\alpha]_D^{20}$ +31.9 (*c* 0.27, CHCl$_3$); UV (MeOH) λ_{max} (log ε) 228 (4.76) nm; IR (KBr) ν_{max} 3400, 3334, 2933, 1713, 1448, 1200 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 0.89 (3H, d, *J* = 6.0 Hz, H$_3$-21), 2.47 (1H, brs, H-20), 1.05 (3H, s, H$_3$-19), 0.68 (3H, s, H$_3$-18), 1.31 (1H, m, H-17), 1.39 and 1.81 (2H, m, H-16), 1.35 and 1.00 (each 1H, m, H-15), 0.90 (1H, m, H-14), 1.84 and 1.07 (each 1H, m, H-12), 1.46 and 1.33 (each 1H, m, H-11), 0.64 (1H, m, H-9), 1.56 (1H, m, H-8), 1.83 and 0.91 (each 1H, m, H-7), 1.84 and 1.29 (each 1H, m, H-6), 1.23 (1H, m, H-5), 4.32 (1H, t, *J* = 3.2 Hz, H-4), 2.75 (1H, m, H-3), 1.60 (2H, m, H-2), 1.76 and 1.01 (each 1H, m, H-1), 3.60 and 2.90 (each 1H, d, *J* = 16.4 Hz, H-4′), 2.21 [6H, s, N(CH$_3$)$_2$], 1.70 and 1.85 [3H, s, (Me)$_2$-5′]; ^{13}C NMR (CDCl$_3$, 100 MHz), see Table 1; HRESIMS *m/z* 457.3749 [M + H]$^+$ (calcd for C$_{29}$H$_{49}$N$_2$O$_2$, 473.3751).

Terminamine F (6): white powder; $[\alpha]_D^{20}$ +43.7 (*c* 0.34, CHCl$_3$); UV (MeOH) λ_{max} (log ε) 210 (4.04) nm; IR (KBr) ν_{max} 2930, 1703, 1621, 1451, 1213 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 2.12 (3H, s, H$_3$-21), 0.80 (3H, s, H$_3$-19), 0.61 (3H, s, H$_3$-18), 2.54 (1H, t, *J* = 8.9 Hz, H-17), 2.15 and 1.63 (each 1H, m, H-16), 1.67 and 1.17 (each 1H, m, H-15), 1.15 (1H, m, H-14), 2.07 and 1.39 (each 1H, m, H-12), 1.61 and 1.35 (each 1H, m, H-11), 0.74 (1H, m, H-9), 1.35 (1H, m, H-8),

1.68 and 0.91 (each 1H, m, H-7), 1.30 and 1.13 (each 1H, m, H-6), 1.38 (1H, m, H-5), 1.61 and 1.45 (each 1H, m, H-4), 2.01 (1H, m, H-3), 1.67 and 1.17 (each 1H, m, H-2), 1.63 (2H, m, H-1), 3.07 (3H, s, NCH$_3$), 5.78 (1H, s, H-3′), 1.83 [3H, s, (Me)$_2$-4′], 1.86 [3H, s, (Me)$_2$-4′], senecioyl-3; ^{1}H NMR (pyridine-*d$_5$* 353 K, 400 MHz) δ 2.12 (3H, s, H$_3$-21), 0.83 (3H, s, H$_3$-19), 0.71 (3H, s, H$_3$-18), 2.54 (1H, t, *J* = 8.8 Hz, H-17), 2.31 and 1.67 (each 1H, m, H-16), 1.67 and 1.19 (each 1H, m, H-15), 1.12 (1H, m, H-14), 2.07 and 1.39 (each 1H, m, H-12), 1.61 and 1.35 (each 1H, m, H-11), 0.75 (1H, m, H-9), 1.35 (1H, m, H-8), 1.64 and 0.95 (each 1H, m, H-7), 1.33 and 1.15 (each 1H, m, H-6), 1.47 (1H, m, H-5), 1.61 and 1.45 (each 1H, m, H-4), 4.59 (1H, br s, H-3), 1.79 (2H, m, H-2), 1.56 and 1.35 (each 1H, m, H-1), 3.14 (3H, s, NCH$_3$); 6.00 (1H, s, CH), 1.82 and 2.05 (each 3H, s, Me × 2)(senecioyl); ^{13}C NMR (CDCl$_3$, 100 MHz), see Table 2, and δ_C 169.8 (NC=O), 119.5 (d), 144.2 (s), 20.1 (q), 26.0 (q)(senecioyl); ^{13}C NMR (pyridine-*d$_5$*, 353 K, 100 MHz), see Table 2, and δ_C 169.5 (NC=O), 121.7 (d), 145.1 (s), 21.2 (q), 27.0 (q)(senecioyl); HRESIMS *m/z* 414.3367 [M + H]$^+$ (calcd for C$_{27}$H$_{43}$NO$_2$, 414.3372).

Terminamine G (7): white powder; $[\alpha]_D^{20}$ +60.3 (*c* 0.17, CHCl$_3$); UV (MeOH) λ_{max} (log ε) 242 (3.51) nm; IR (KBr) ν_{max} 2929, 1623, 1457, 1380, 1100 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 2.12 (3H, s, H$_3$-21), 0.77 (3H, s, H$_3$-19), 0.63 (3H, s, H$_3$-18), 2.53 (1H, m, H-17), 2.16 and 1.63 (each 1H, m, H-16), 1.66 and 1.19 (each 1H, m, H-15), 1.16 (1H, m, H-14), 2.01 and 1.37 (each 1H, m, H-12), 1.59 and 1.31 (each 1H, m, H-11), 0.75 (1H, m, H-9), 1.35 (1H, m, H-8), 1.68 and 0.93 (each 1H, m, H-7), 1.34 and 1.13 (each 1H, m, H-6), 1.46 (1H, m, H-5), 1.58 (2H, m, H-4), 1.46 (1H, m, H-3), 1.66 and 1.18 (each 1H, m, H-2), 1.63 (2H, m, H-1), 3.07 (3H, s, NCH$_3$), 7.39 (5H, m, benzoyl-3); ^{1}H NMR (pyridine-*d$_5$* 353 K, 400 MHz) δ 2.11 (3H, s, H$_3$-21), 0.75 (3H, s, H$_3$-19), 0.71 (3H, s, H$_3$-18), 2.54 (1H, m, H-17), 2.30 and 1.68 (each 1H, m, H-16), 1.67 and 1.19 (each 1H, m, H-15), 1.15 (1H, m, H-14), 2.07 and 1.36 (each 1H, m, H-12), 1.59 and 1.31 (each 1H, m, H-11), 0.80 (1H, m, H-9), 1.35 (1H, m, H-8), 1.69 and 0.98 (each 1H, m, H-7), 1.37 and 1.14 (each 1H, m, H-6), 1.50 (1H, m, H-5), 1.70 (2H, m, H-4), 4.56 (1H, s, H-3), 1.90 and 1.80 (each 1H, m, H-2), 1.54 and 1.38 (each 1H, m, H-1), 3.14 (3H, s, NCH$_3$), 7.43 (5H, m, benzoyl-3); ^{13}C NMR (CDCl$_3$, 100 MHz), see Table 2, and δ_C 170.3 (C=O), 137.6 (s), 128.4 (d), 128.4 (d), 126.6 (d), 126.6 (d), 129.0 (d)(benzoyl); ^{13}C NMR (pyridine-*d$_5$*, 353 K, 100 MHz), see Table 2, and δ_C 180.2 (C=O), 137.6 (s), 128.4 (d), 128.4 (d), 129.9 (d), 129.9 (d), 130.4 (d)(benzoyl); HRESIMS *m/z* 436.3216 [M + H]$^+$ (calcd for C$_{29}$H$_{43}$NO$_2$, 436.3215).

X-ray Crystallographic Analysis Data of Compound 1. A monoclinic crystal was obtained from a CHCl$_3$−MeOH solvent system (Figure 2). Crystal data: C$_{29}$H$_{48}$N$_2$O$_3$, *M$_r$* = 472.69, monoclinic. Crystal size = 0.20 × 0.18 × 0.10 mm^3. Cell parameters: *a* = 12.3568(12) Å, *b* = 9.7426(9) Å, *c* = 12.6551(13) Å, *V* = 1332.2(2) Å^3, space group *P*2$_1$, β = 119.022(6)°, Z = 2, *D$_x$* = 1.178 Mg m^{-3}, θ_{max} = 27.93, μ = 0.075 mm^{-1}. The Flack parameter is −0.5(8); the wavelength is 0.71073 Å. Data collection was performed on a SMART system (Bruker, 1997), the structure was resolved by direct methods (SHELXS-97), and the final *R* and *R$_w$* values over 3366 observed reflections were 0.0361 and 0.0705, respectively. Crystallographic data (excluding structure factors) have been deposited at the Cambridge Crystallographic Data Center. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 IEZ, UK. Fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk. The CCDC deposition number of **1** is 859138.

Inhibition of Cell Viability Assay for Compounds 1−15. See Supporting Information.

Invasion Assay. The chemotaxis invasion assay was performed as described by Zhang et al.[11] using nontoxic concentrations of each compound. MDA-MB-231 cells were pretreated with the compounds at the indicated concentrations for 24 h at 37 °C in six-well cell culture plates. A chemoattractant (EGF; 1 ng/mL, 30 μL/well) was loaded into the lower chemotaxis chamber. Control (cells only) and pretreated cells were resuspended in binding medium (RPMI 1640 containing 0.1% BSA and 25 mM HEPES) at a density of 0.5 × 10^6 cells/mL and placed into the upper chamber (50 μL/well). The 8 μm filter membranes (Neuroprobe), which had previously been pretreated

with 0.001% fibronectin in serum-free medium at 4 °C overnight and air-dried, were inserted between the upper and lower chambers. The cells were incubated at 37 °C in 5% CO_2 for 3.5 h; then the filter membrane was rinsed, fixed, and stained. The number of migrating cells in three separate fields was counted using light microscopy at 400×. The inhibitory ratio (IR) was calculated as follows: IR% = (1 − number of migrated cells in sample/number of migrated cells in control) × 100%. The potencies of the products were expressed as the median inhibitory concentration (IC_{50}) values. LY294002 (Camarillo, CA, USA) was used as a positive control substance for this assay.[37,38]

Western Blotting. MDA-MB-231 cells were cultured in 12-well plates and lysed on ice in 200 μL of RIPA buffer [100 mM NaCl, 0.25% w/v sodium deoxycholate, 1.0% w/v NP40, 0.1% w/v sodium dodecyl sulfate (SDS), 2 mM ethylenediaminetetraacetic acid, 50 mM NaF, 10 nM okadaic acid, 1 mM sodium orthovanadate, protease inhibitor cocktail, and 50 mM Tris-HCl, pH 7.2]. The samples were electrophoresed on 7.5% SDS−polyacrylamide gels, transferred to polyvinylidene fluoride membranes, blocked for 1 h in 5% (w/v) bovine serum albumin, and then incubated with primary antibodies overnight at 4 °C, followed by incubation with appropriate secondary antibodies for 1 h at room temperature. The bands were detected using chemiluminescent reagent and autoradiographic film.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information
^{1}H NMR, ^{13}C NMR, 2D-NMR, and MS spectra for compounds **1−7** and data on the effects of compounds **1−15** on MDA-MB-231 cell viability. These materials are available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

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