Structural Modification of an Angiogenesis Inhibitor Discovered from Traditional Chinese Medicine and a Structure–Activity Relationship Study

Sheng-Ping Yang, Yu-Jun Cai, Bang-Le Zhang, Lin-Jiang Tong, Hua Xie, Yan Wu, Li-Ping Lin, Jian Ding,* and Jian-Min Yue*

State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Zhangjiang Hi-Tech Park, Shanghai, 201203, People's Republic of China

Received July 26, 2007

Pseudolaric acid B (PAB), discovered as a promising angiogensis inhibitor, was served as the anticancer drug lead, and a series of its derivatives were synthesized. Among them, some derivatives, such as **13c**−**13k**, exhibited potent inhibition on the HMEC-1 cell proliferation and strong cytotoxic activities against the tested six tumor cell lines. The PAB derivatives **13c**−**13k** also showed significant and specific inhibition on HMEC-1 cell migration in vitro, and only **13d** expressed moderate activity against HMEC-1 cell tube formation. The in vitro anticancer tests of the selected natural PAB analogs and the structurally modified PAB derivatives have led to the establishment of a clear structure–activity relationship.

Introduction

Cancer chemotherapy has now produced cures in at least some categories of human cancers. There are still urgent needs for more potent, specific, and low toxicity anticancer drugs. The growth of solid tumors and the formation of metastases are strictly reliant on the formation of new blood vessels. Without these blood vessels, tumors cannot grow beyond a critical size or metastasize to other organs.[1] The target of antiangiogenesis therapy is normal endothelial cells that form the blood vessel, other than the unstable cancer cells, which can mutate and acquire drug resistance. Endothelial cells, on the other hand, divide normally without mutation and do not develop resistance to drugs. The inhibition of the endothelial cell proliferation, migration, and tube formation could in fact be an effective strategy for blocking tumor progression and metastasis.[2] Therefore, endothelial cells present good targets for new anticancer drug discoveries, and the inhibitors of the endothelium cells abnormal proliferation and migration offer hope for a new alternative to cancer chemotherapy.

Pseudolaric acid B (**1**; PAB[a]), with a unique scaffold, was initially isolated as the major compound (0.5–0.8% in the bark) from the root and trunk bark of *Pseudolarix kaempferi* Gordon (Pinaceae), which is a well-known traditional Chinese medicine. PAB (**1**) exhibited significant cytotoxic activities against numerous tumor cell lines[3] and strong antifungal activity.[4] PAB is also an early pregnancy terminating agent.[5] It has been an attractive structure for synthetic chemists.[6]>

Recently, PAB (**1**) was initially demonstrated by our research group to inhibit angiogenesis via antagonizing the vascular endothelial growth factor-mediated antiapoptotic effect[7] and to have dual functions of direct inhibiting endothelial cells and abrogating paracrine stimulation of VEGF (vascular endothelial growth factor) from tumor cells due to the reduction of HIF-1α protein by promoting its proteasome-mediated degradation in MDA-MB-468 cells, which has potential clinical relevance.[8] Further to this, other research groups reported that PAB induced apoptosis of tumor cells through Bax/Bcl-2 pathways on the basis of the elevated levels of Bax expression and down-regulation of Bcl-2,[9] and PAB-induced tumor cells arrest at the G_2/M phase of the cell cycle, leading to apoptosis.[9a,10]

Due to the unique structural scaffold and the significant antiangiogenesis activity, PAB (**1**) has potential value as an anticancer drug or a drug lead. However, there has been a very limited study on the structural modification and structure–activity relationship (SAR) of PAB analogs related to the anticancer purpose,[3] largely due to the protracted and complex nature of their structures for modification and synthesis. Fortunately, a series of pseudolaric acid analogs with diversified structures has been isolated from *P. kaempferi*[11] or semisynthesized from PAB (**1**) in our laboratory,[4d] providing a chance to initially outline the gross anticancer SAR. Subsequently, a refined structural modification of PAB (**1**) and the SAR study were conducted, and the HMEC-1 (human microvascular endothelial cell) cell line relevant to angiogenesis was selected as the indicator for the antiangiogenesis evaluation. The in vitro anticancer activities of the selected naturally occurring pseudolaric acids and the structurally modified PAB derivatives have led to the establishment of a clear SAR. Some PAB derivatives, such as **13c**−**13k**, exhibited more potent inhibition (e.g., **13c**, $IC_{50} = 0.199$ μM; **13d**, $IC_{50} = 0.238$ μM; **13f**, $IC_{50} = 0.195$ μM) than PAB ($IC_{50} = 0.803$ μM) on the proliferation of HMEC-1 cell and also displayed strong cytotoxicities against six tumor cell lines. The in vitro cell migration assay showed that the six selected PAB derivatives **13c**−**13k** were potent and specific inhibitors of HMEC-1 cell migration, and these derivatives were statistically more active than PAB. The in vitro tuber formation assay revealed that one of the PAB derivatives (**13d**) also showed moderate inhibition on HMEC-1 cell tube formation. We present herein the structural modification of the

* To whom correspondence should be addressed. Phone: +86-21-50806718. Fax: +86-21-50807088. E-mail: jding@mail.shcnc.ac.cn; jmyue@mail.shcnc.ac.cn.

[a]Abbreviations: PAB, pseudolaric acid B; SAR, structure–activity relationship; VEGF, vascular endothelial growth factor; HMEC-1, human microvascular endothelial cell; HL-60, human premyelocytic leukemia; A-549, human lung cancer; MDA-MB-468, human breast cancer; BEL-7402, human liver cancer; Hela, human cervical cancer; HCT116, human colon cancer; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; SRB, sulforhodamine B; EGF, epithelial growth factor.

10.1021/jm070906g CCC: $40.75
Published on Web 12/14/2007

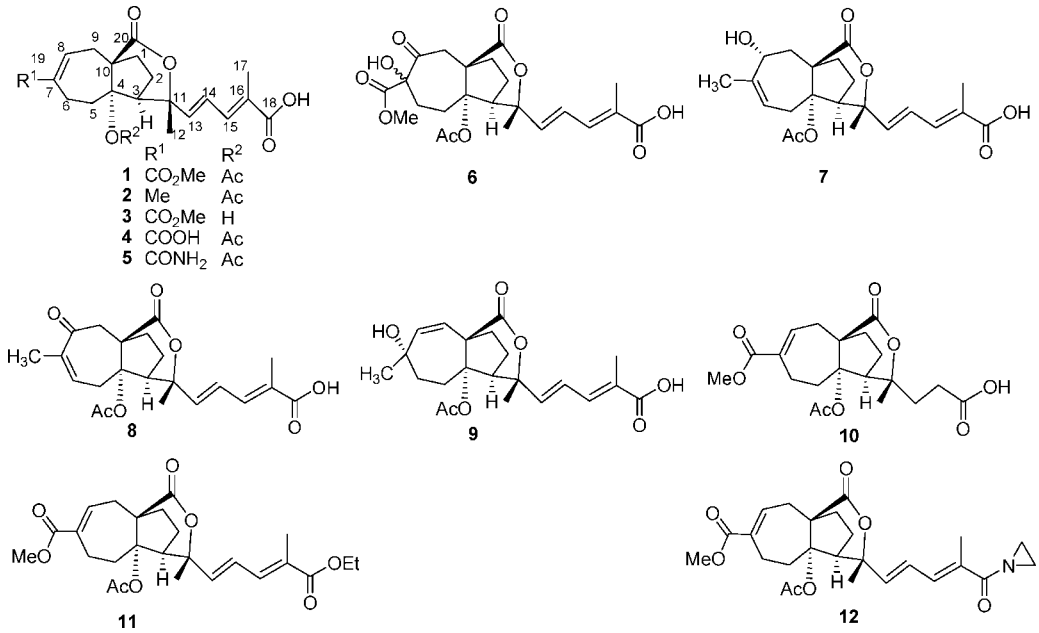
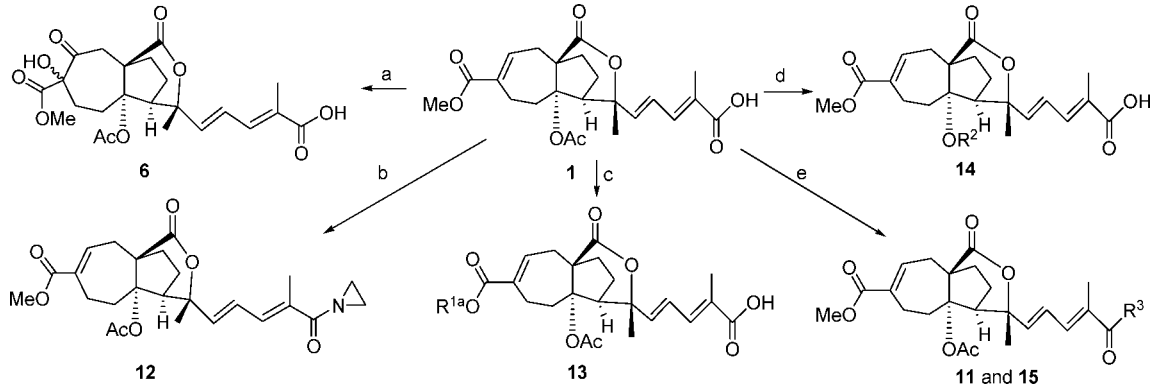


Figure 1. Stuctures selected for the gross SAR of PAB analogs.

Scheme 1. Preparation of Compounds **4**, **11**, **12**, **13a**−**13n**, **14a**−**14d**, and **15a**−**15g**[a]



[a] Reagents and conditions: (a) $KMnO_4$, 20% $NaHCO_3$ in water, rt, 1 h; (b) i. $SOCl_2$, 40–50 °C, 20 min; ii. 2-chloroethanaminium chloride, rt, 1 h; (c) i. $R^{1a}OH$, *t*-BuOK, rt, 24 h; ii. acetyl chloride, rt, 24 h; (d) i. methanol, *t*-BuOK, rt, 24 h; ii. acyl chloride, rt, 24 h; (e) i. $SOCl_2$, 40–50 °C, 20 min; ii. R^3H, rt, 1 h.

angiogenesis inhibitor PAB (**1**), the in vitro anticancer evaluation of PAB derivatives, and their SAR study.

Results and Discussion

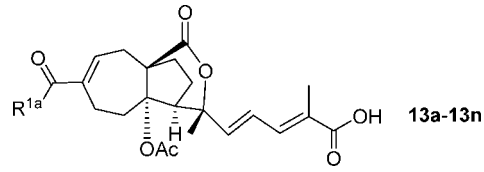
PAB (**1**), possessing an early stage pregnancy terminating activity, is believed to be associated with the inhibition of the new blood vessel formation of embryo,[5] and this has endowed us with an inspiration to evaluate its angiogenesis inhibitory activity. To our delight, PAB (**1**) exhibited significant inhibitory activity against the proliferation of HMEC-1 ($IC_{50} = 0.73\ \mu M$) in our preliminary screening and showed the unique mode-of-action of anticancer in the latter study.[7–10] To comprehend the gross SAR of anticancer PAB analogs, 12 structurally diverse compounds, including eight natural isolates pseudolaric acids B (**1**),[4d] A (**2**),[4d] C (**3**),[4d] C_2 (**4**)[4a], G (**7**),[4d] F (**8**),[4d] A_2 (**9**),[11] and B_3 (**10**)[11] and four semisynthesized derivatives **5**,[4d] **6**, **11**, and **12** (Figure 1, Scheme 1), were initially selected for anticancer evaluation (Table 1). Pseudolaric acids B (**1**) and A (**2**) showed comparable activity against HMEC-1 and two tumor cell lines, while other compounds were almost inactive (IC_{50} > 10.0 μM was defined as inactive). These results primarily indicated that a hydrophobic group (−CO_2Me or −Me) at C-7, a Δ^7 double bond (reduction or migration will render the compounds **6**−**9** inactive), an acyloxy (OAc) at C-4,[3] and the side chain with a conjugated double bond and a hydrophilic terminal group are essential for the anticancer activity. With this gross SAR study in mind, PAB (**1**), the major component of *P. kaempferi* (Tu-Jin-Pi), with a isolated yield of 0.5–0.8%,

Table 1. Inhibition on Tumor and Endothelial Cells of Compounds **1**−**12**[a]

	IC_{50} (μM)		
	HMEC-1	HL-60	A-549
1	0.73	0.50	5.3
2	0.95	0.82	5.7
3	>10.0	>10.0	>10.0
4	>10.0	>10.0	>10.0
5	>10.0	>10.0	>10.0
6	>10.0	>10.0	>10.0
7	>10.0	>10.0	>10.0
8	>10.0	>10.0	>10.0
9	>10.0	>10.0	>10.0
10	>10.0	>10.0	>10.0
11	7.44	>10.0	>10.0
12	>10.0	>10.0	>10.0

[a] IC_{50} > 10.0 μM was defined as inactive; PAB (**1**) also served as control.

Table 2. Inhibition on Tumor and Endothelial Cells of Compounds **13a**−**13n**[a]



	R^{1a}	IC_{50} (μM)						
		HMEC-1	HL-60	A-549	MB-MDA-468	BEL-7402	HCT116	Hela
1	H_3CO—	0.803±0.003	0.235±0.074	1.162±0.368	0.596±0.222	0.704±0.040	0.136±0.037	0.497±0.150
13a	H_3CHN—	>5.00	>5.00	>5.00	-	-	-	-
13b	H_3CO—	1.047±0.012	0.500±0.266	1.992±0.022	0.587±0.111	0.804±0.014	0.670±0.020	0.756±0.112
13c	H_3CO(CH$_3$)—	0.199±0.008	0.242±0.010	0.608±0.013	0.332±0.046	0.612±0.011	0.367±0.048	0.427±0.230
13d	H_3CO—	0.238±0.042	0.190±0.036	0.677±0.113	0.243±0.026	0.464±0.176	0.234±0.069	0.213±0.020
13e	H_3CO(CH$_3$)—	0.278±0.093	0.171±0.004	0.649±0.006	0.239±0.026	0.489±0.138	0.243±0.037	0.262±0.018
13f	H_3CO—	0.195±0.008	0.198±0.052	0.567±0.028	0.245±0.074	0.367±0.042	0.257±0.040	0.163±0.083
13g	H_3CO(CH$_3$)—	0.757±0.03	0.316±0.017	0.726±0.011	0.270±0.098	0.630±0.014	0.394±0.060	0.426±0.250
13h	H_3CO—	0.216±0.011	0.264±0.000	0.676±0.114	0.298±0.086	0.541±0.063	0.304±0.030	0.212±0.052
13i	H_3CO—	0.569±0.143	0.658±0.020	0.756±0.004	0.657±0.083	0.673±0.001	0.641±0.004	0.565±0.157
13j	O—	0.562±0.045	0.459±0.311	0.740±0.030	0.377±0.076	0.588±0.109	0.419±0.096	0.550±0.127
13k	O—	0.335±0.023	0.421±0.245	0.666±0.064	1.973±0.127	2.935±0.996	1.563±0.599	2.291±0.389
13l	HO—	>5.00	>5.00	>5.00	-	-	-	-
13m	H_3CO—	>5.00	>5.00	>5.00	-	-	-	-
13n	H_3CO—	>5.00	>5.00	>5.00	-	-	-	-

[a] PAB (**1**) served as both the lead compound and the positive control.

was thus selected as the lead compound for structural modification. Our strategy thus focused on the modification of the C-7 methylcarboxyl group, C-4 acyloxy group, and the hydrophilic terminal moiety of the side chain. Three categories of PAB derivatives were prepared subsequently (Table 2, Supporting Information S3 and S4).

Modification of the C-7 Methylcarboxyl Group. A methylamino-derivative (**13a**)[4d] was first prepared by the replacement of the hydrophobic ester group with a hydrophilic amide group, and it was inactive in the tested cell lines, further indicating that a hydrophobic group at C-7 is required for the anticancer activity. From this assumption, compounds **13b**−**13k** were prepared from PAB (**1**) via transesterification under mild conditions (Scheme 1, Table 2). Among them, compounds **13b**, **13d**, and **13f** were semisynthesized according to the literature procedures.[4d] The transesterfication of PAB with anhydrous alcohol catalyzed by potassium *tetra*-butoxide gave a mixture of the desired products and the C-4 deacetyl byproducts (monitored by TLC), which then underwent a direct esterification (the mixture was not separated) with acetyl chloride to finally provide the desired products in good yields. Surprisingly, the commonly used catalysts for esterification, such as triethylamine, pyridine, and DMAP, did not accelerate the reactions, but retarded the transesterfication.

Modification of the C-4 Acyl Group. PAB (**1**) was first treated with potassium *tetra*-butoxide in the solvent of anhydrous methanol to give its C-4 deacetyl intermediate, pseudolaric acid C (**4**), which was then converted to the derivatives **14a**−**14d** (Figure 2) with different C-4 acyloxy groups by using an excessive amount of acyl chloride at room temperature. For the

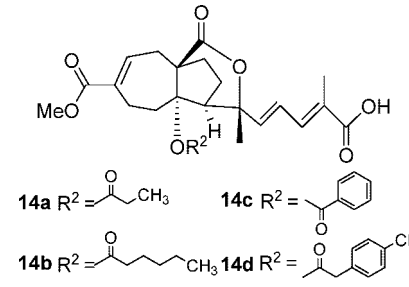


Figure 2. Stuctures of compounds **14a**−**14d**.

preparation of compounds **14c** and **14d**, AgCN powder as catalyst was required in the acylation procedure to complete the reaction.[12]

Modification of the C-18 Carboxylic Acid Group. PAB derivatives **15a**−**15g** (Figure 3) were semisynthesized by the treatment of pseudolaric acid B (**1**) with thionyl chloride, followed by the acylation or amidation with alcohol or amine, respectively (Scheme 1).

Anticancer Activity and SAR Study. The inhibition of PAB (**1**) and its derivatives on human microvascular endothelial cell (HMEC-1) growth were measured by using the SRB assay. The cytotoxic activities of PAB (**1**) and its derivatives against six tumor cell lines, including HL-60 (human premyelocytic leukemia), A-549 (human lung cancer), MDA-MB-468 (human breast cancer), BEL-7402 (human liver cancer), Hela (human cervical cancer), and HCT116 (human colon cancer) were also tested. For a quick and comprehensive understanding of the SAR of PAB derivatives, the inhibitory activities of these compounds

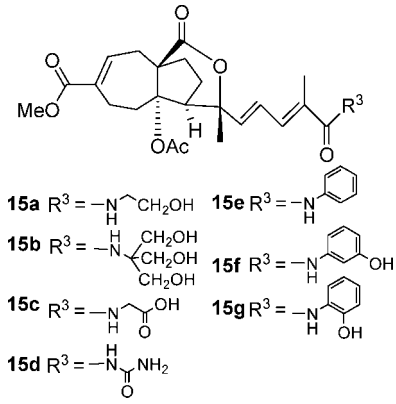
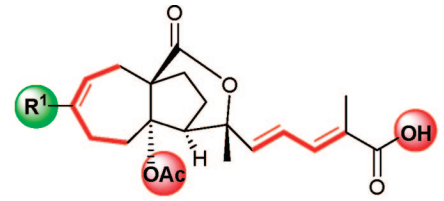


Figure 3. Stuctures of compounds **15a**−**15g**.

on HMEC-1 cell line were selected as the activity index and involved in the SAR discussion, and the cytotoxic activities of PAB derivatives against six tumor cell lines were also kept for reference. The inhibitory activities on the proliferation of the HMEC-1 cell line and the cytotoxic activities against six tumor cell lines of PAB derivatives showed consistent tendencies.

Pseudolaric acids B (**1**) and A (**2**) showed comparable activity against the proliferation of the HMEC-1 cell line with IC_{50} values at 0.73 and 0.95 μM, respectively, in the preliminary screening (Table 1), while other analogs **3**−**12** were almost inactive (IC_{50} > 10.0 μM was defined as inactive in this study). Compounds **1** and **2** have the same basic scaffold; the only structural difference is the C-7 substituents. The former has a C-7-CO_2Me and the latter bears a C-7-Me. Compound **3** that is the deacetyl derivative of **1** was inactive against the HMEC-1 cell line, indicating that an acyloxy (OAc) at C-4 is necessary for the activity. In comparison with **1** and **2**, compounds **4** and **5** were inactive, suggesting that a hydrophobic group at C-7 is important for the activity. Inspection of the structures of compounds **6**−**9** and their inhibitory activities against the HMEC-1 cell line indicated that the seven-membered ring with a Δ^7 double bond is crucial for the activity; any changes in this domain, for example, substituted with oxygenated functional groups at C-7 and C-8, and the Δ^7double bond migration, will render the analogues inactive. Compound **10**, with a degraded and saturated side chain, was inactive, suggesting that the chain length and the conjugated double bonds are essential for the activity. Esterification or amidation of PAB (**1**) into compounds **11** or **12** is also detrimental to the inhibitory activity against the HMEC-1 cell line.

In addition to these, the structural modification of PAB (**1**) confirmed and refined the aforementioned SAR discussion in detail (Table 2, Supporting Information S3 and S4). As shown in Table 2, compound **13a** with a hydrophilic amide group at C-7 was inactive, while compounds **13b**−**13k** showed potent activities against the HMEC-1 cell line. This observation further demonstrated that a hydrophobic group at C-7 is necessary for the anticancer activity. Compounds **13b**−**13k** with a trend of increasing hydrophobicity of the R^1 group exhibited an enhancement against HMEC-1 cell line, especially compounds **13c** and **13f** were 4-fold more potent than PAB (**1**). Furthermore, when one oxygen atom was inserted into the fatty alcohol chain of R^1, for example, compounds **13m** and **13n**, it will largely attenuate the activity. Compounds **14a**−**14d** (Figure 2, Supporting Information S3) were inactive against the HMEC-1 cell line (IC_{50} > 5.0 μM was defined as inactive), suggesting that the C-4 acetoxyl is the essential group for the activity, and the replacement of the C-4 acetoxyl with bulky acyloxys will



Green: Modification with hydrophobic R^1 is allowed
Red: Modification is forbidden

Figure 4. SAR of pseudolaric acid B analogs.

significantly attenuate the activity. Compounds **15a**−**15g** were almost inactive (IC_{50} > 5.0 μM was defined as inactive) in the tested cell lines, except that **15b** showed moderate activities against HL-60 and A-549, with the IC_{50} at 1.010 and 3.846 μM, respectively (Figure 3, Supporting Information S4), indicating that the free carboxylic acid group in the terminal of the side chain is essential for the anticancer activity, and acylation or amidation of this group with either hydrophilic or hydrophobic group is detrimental to the anticancer activity.

Taken together, the above analysis has outlined a clear SAR for PAB analogs (Figure 4) as follows: (1) All the active compounds **1** and **13b**−**13k** tested in our study are of amphipathic properties and possess a hydrophobic domain of a constrained-rings system and a hydrophilic domain of the side chain possessing a conjugated double bond and a terminal carboxylic acid. (2) A hydrophobic group R^1 at C-7 and a Δ^7 double bond are necessary for the anticancer activity, and the bulk and steric factor of R^1 seem also relevant to the activity. (3) The chain length and the conjugated double bonds in the side chain are essential for the anticancer activity. If the side chain becomes shortened or loses the conjugated double bonds, the compound becomes inactive. (4) Any structural changes in the seven-membered ring, for example, the Δ^7 double bond migration and oxygenation at C-7 or C-8 will render the analogs inactive. (5) The C-4 acetoxyl group is crucial for the activity, and the removal or replacement with bulky acyloxy group will significantly attenuated the activity. (6) The free carboxylic acid group at the terminal of the side chain is necessary for the anticancer activity, and acylation or amidation of this group with either a hydrophilic or a hydrophobic group is detrimental to the activity.

PAB (**1**) is also a strong antifungal agent.[4] The anticancer SAR of PAB analogs aforementioned is very similar to the antifungal SAR reported by our group,[4d] except for the difference occurring in the modification of the R^1 group. Modification on the R^1 group of PAB for the anticancer activity showed a very exciting result, while in the antifungal test, all the structurally modified PAB analogs showed attenuated activities or were totally inactive.[4d] The aforementioned result suggests that PAB as an anticancer drug lead is very promising, but as an antifungal lead, it is limited by its modification forbidden feature and strong cytotoxic side effect.

Effects of PAB and the Derivatives on the HMEC-1 Migration. Cell migration is essential for endothelial cells to generate new blood vessels. Inhibition on this process will prevent the formation of new blood vessels. To characterize the effects of PAB and its derivatives (**13c**,**13d**, **13f**, **13h**, **13j**, and **13k**) on HMEC-1 cell migration, an in vitro migration assay by using a slightly modified Boyden chamber[13] was thus performed. As illustrated in Figure 5, HMEC-1 actively migrated to the serum-containing lower chamber within 6 h under the compound-free condition (blank control). While when PAB or its derivatives **13c**, **13d**, **13f**, **13h**, **13j**, and **13k** were served at

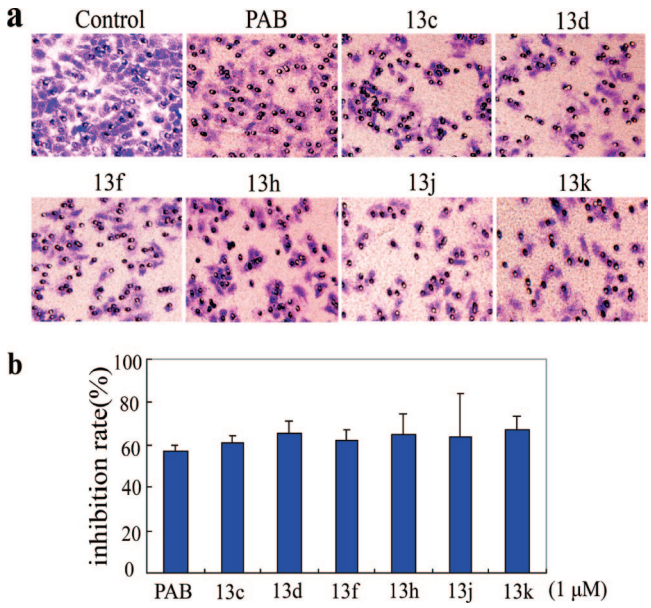


Figure 5. Effects of PAB and its derivatives on the HMEC-1 migration. (a) HMEC-1 seeded in transwell Boyden Chambers were incubated for 6 h with medium alone (control) or contained 1 μM of tested compounds (**13c**−**13k**); the photographs (100×) showed the cells on the lower surface of the filter (migrated) stained with 1% crystal violet. (b) Inhibition rates of the compounds (**13c**−**13k**) on HEMC-1 migration, bars representing the mean ± SD of three independent experiments.

the concentration of 1 μM, the migration of HMEC-1 was inhibited by 57.1, 60.8, 65.3, 62.0, 64.8, 63.8, and 67.5%, respectively. This result indicated that PAB and its derivatives were potent inhibitors against the migration of HMEC-1 cell, and the derivatives statistically showed slightly increased activity than PAB.

Effects of PAB and its Derivatives on HMEC-1 Tube Formation. In the later stages of angiogenesis, endothelial cells will self-assemble into tubes to form new blood vessels. Inhibition on the tube formation will also terminate the construction of new blood vessels. An in vitro HMEC-1 tube formation assay was therefore applied to evaluate the effects of PAB and its derivatives in this process.[14] In the blank control group, HMEC-1 cells displayed high mobility on matrigel and developed into an intact network of tubes in 8 h (Figure 6a). After the treatment with PAB at the concentration of 1 μM, the tube formation was inhibited by 50.0%, and the disrupted tube structure was incomplete and sparse (Figure 6b,d). For the PAB derivatives, only **13d** exerted 31.0% inhibition on the tube formation of HMEC-1 at the concentration of 1 μM (Figure 6c,d), the others, including **13c**, **13f**, **13h**, **13j**, and **13k**, were inactive up to the concentration of 5 μM.

An experiment was finally designed to demonstrate the effects of these compounds on HMEC-1 migration and tube formation, which are specific rather than resulting from their cytotoxic activities. As the exposure time of these compounds was 6 h in the migration assay and 8 h in the tube formation assay, the effect of these compounds on HMEC-1 proliferation was then tested for an 8 h incubation by using the SRB method. The results indicated that PAB and its six derivatives exhibited no obvious effects on HMEC-1 cell growth in an 8 h exposure; even the concentration was up to 5 μM. This result is not contrary to the cytotoxic activities of these compounds on HMEC-1, observed in a 72 h incubation, because in two experiments, the exposure time of the HMEC-1 cells to the

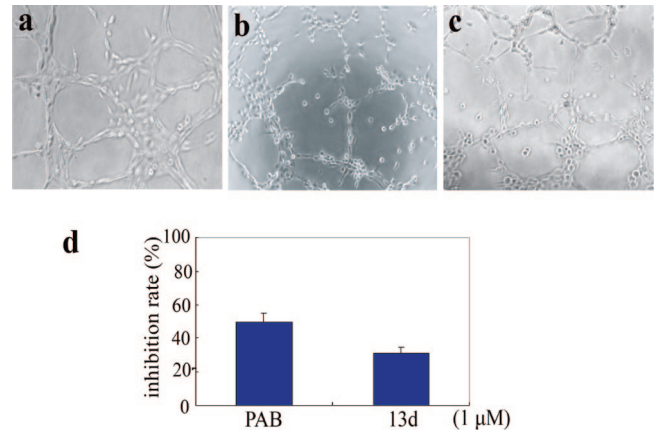


Figure 6. Effects of PAB and **13d** on HMEC-1 tube formation. HMEC-1 cells were seeded in matrigel-coated 96-well plates: (a) HMEC-1 cells formed the network of intact tubes in 8 h on matrigel (control). After treatment with 1 μM of PAB (b) or **13d** (c), the tube structures were severely disrupted (magnification: 100×) in 8 h on matrigel. (d) Inhibition rates of PAB and **13d** on HEMC-1 tube formation. The numbers of intact tubes were counted in five randomly chosen regions and expressed as the percentage of the control, and the results were expressed as mean ± SD.

tested compounds was quite different. Taken together, it suggests that PAB and its six tested derivatives are specific inhibitors of HMEC-1 cell migration, and PAB and its derivative **13d** are also specific inhibitors of HMEC-1 cell tube formation.

Conclusions

Pseudolaric acid B and its derivatives represent a novel structural scaffold and possess a unique mode-of-action of anticancer. Their strong inhibitory activity against HMEC-1 cell proliferation, migration, and tube formation (only **13d**), featuring the antianiogenesis property, and their significant cytotoxic activity against the selected tumor cell lines suggest that these compounds have dual functions, and a further investigation into this structure class is warranted. The in vivo tests of some PAB derivatives are in progress. We view the pseudolaric acid B and its derivatives as a promising new class of anticancer leads deserving of further studies.

Experimental Section

General Methods. IR spectra were recorded on a Perkin-Elmer 577 spectrometer with KBr disk. ^{1}H NMR spectra were measured on a Varian Mercury-400 spectrometer with TMS as internal standard. EIMS (70 eV) was carried out on a Finnigan MAT 95 instrument. ESI-MS was recorded on a Finnigan LCQ^{DECA} or a Bruker Esquire 3000plus Mass spectrometer. All solvents used were of analytical grade (Shanghai Chemical Plant). Silica gel (200–300 mesh) was used for column chromatography, and the precoated silica gel GF_{254} plate (Qingdao Haiyang Chemical Plant, Qingdao, People's Republic of China) was used for TLC. The purity checks for all the compounds **1**−**12**, **13a**−**13n**, **14a**−**14d**, and **15a**−**15g** were carried out on an Agilent 1100 Series HPLC system with two different mobile phases. The HPLC column was a Phenomenex Luna C18 silica gel (5 μm, 150 mm × 4.60 mm). The mobile phases were 70−90% methanol in water and aqueous CH_3CN (70−90%), and the flow rate was 1 mL/min. The column chamber was kept at around 20 °C for the analysis of all the compounds.

Biological Materials. The human microvascular endothelial cell line (HMEC-1), human premyelocytic leukemia cell line

(HL-60), human lung cancer cell line (A-549), human breast cancer cell line (MDA-MB-468), human colon cancer cell line (HCT116), and human cervical cancer cell line (Hela) were obtained from the American type Culture and Collection (Rockville, MD). Human liver cancer cell line BEL-7402 was obtained from the Cell Bank of type Culture Collection of the Chinese Academy of Sciences. HMEC-1 cells were propagated in MCDB131 (Sigma) and supplemented with 20% fetal bovine serum, 30 μg/mL endothelial cell growth supplements (ECGS), 10 ng/mL epithelial growth factor (EGF), and 1 μg/mL hydrocortisone. HL-60, A-549, MDA-MB-468, HCT116, Hela, and BEL-7402 cells were maintained in RPMI-1640 medium (GIBCO, Grand Island, NE, U.S.A.) and supplemented with 10% heat-inactivated fetal bovine serum (GIBCO), L-glutamine (2 mM), penicillin (100 IU/mL), streptomycin (100 μg/mL), and HEPES (10 mM), pH 7.4. All the cells were incubated in a humidified atmosphere of 95% air plus 5% CO_2 at 37 °C. 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and sulforhodamine B (SRB) were obtained from Sigma (St. Louise, MO, U.S.A.).

Inhibitory Activities of PAB Analogs against HMEC-1 Cell Growth. Inhibition on human microvascular endothelial cell growth was measured by using the SRB assay. Briefly, HMEC-1 cells were seeded at the density of 6000 cells/well in 96-well plates (Falcon, CA) and allowed to attach overnight. The cells were treated in triplicate with grade concentrations of compounds at 37 °C for 72 h and were then fixed with 10% trichloroacetic acid and incubated at 4 °C for 1 h. The culture plates were washed and dried, and SRB solution (0.4 wt %/vol in 1% acetic acid) was added and incubated for an additional 15 min. The culture plates were washed and dried again, the bound cell stains were solubilized with Tris buffer, and the optical density of each well was read on a plate reader (model VERSA Max, Molecular Devices) at the wavelength of 515 nm. The growth inhibitory rate of treated cells was calculated by the formula of $[1 - (A_{515\ \text{treated}}/A_{515\ \text{control}})] \times 100\%$. The inhibitory activity was finally expressed in IC_{50} (the compound concentration required for 50% growth inhibition of tumor cells), which was calculated by using Logit method. The mean IC_{50} was determined from the results of three independent tests.

Inhibitory Activities of PAB Analogs against Six Tumor Cell Lines. Six tumor cell lines, including HL-60, A-549, MDA-MB-468, HCT116, Hela, and BEL-7402, were used to evaluate the cytotoxic activities of PAB analogs. The growth inhibitory effect of compounds on the cell lines of HL-60 was measured by MTT assay (microculture tetrazolium 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide). Briefly, cells in 100 μL of culture medium were plated in each well of 96-well plates (Falcon, CA). Cells were treated in triplicate with grade concentrations of compounds at 37 °C for 72 h. A 20 μL aliquot of MTT solution (5 mg/mL) was added directly to all the appropriate wells. The culture was then incubated for 4 h, and then 100 μL of "triplex solution" (10% SDS/5% isobutanol/12 mM HCl) was added. The plates were incubated at 37 °C overnight and then measured by using a plate reader at 570 nm (VERSA Max, Molecular Devices). For A-549, MDA-MB-468, HCT116, Hela, and BEL-7402 human cancer cell lines, the growth inhibition was tested by sulforhodamine B (SRB) assay as described above. The growth inhibitory rate of treated cells was calculated by the formula of $[1 - (A_{515\ \text{treated}}/A_{515\ \text{control}})] \times 100\%$. The results were also expressed in IC_{50}, as calculated by the Logit method. The mean IC_{50} was determined from the results of three independent tests.

Cell Migration Assay. The migration assay was performed by using a 24-well chamber (Costar, Cambridge, MA, U.S.A.) as the outer chamber, and the inner chambers contained 1% gelatin-coated polycarbonate filters (8 μm pores). HMEC-1 cells (1.5×10^5/well) were seeded into the inner chamber in MCDB-131 medium containing tested compounds (each 1 μM) or vehicle (control). The outer chamber contained the same medium with FBS (10%). After incubation for 6 h at 37 °C, the cells on the filter were fixed in 90% ethanol for 10 min. Nonmigrated cells on the upper surface of the filter were removed by gentle scraping with a cotton swab. Migrated cells on the lower surface of the filter were stained with 1% crystal violet and washed with PBS several times. Images of migrated cells were captured by phase contrast microscope (Olympus, IX70, Japan). Stained migrant cells were then extracted with 10% acetic acid, and the absorbance was measured at 600 nm by using a multiwell spectrophotometer (VERSAmax, Molecular Devices, Sunnyvale, CA, U.S.A.). The inhibition of migration was calculated as $[1 - (A_{600\ \text{treated}}/A_{600\ \text{control}})] \times 100\%$.

Matrigel Tube Formation Assay In Vitro. To a 96-well plate, 0.1 mL of growth factor-reduced matrigel (Becon Dickinson, Bedford, MA, U.S.A.) was coated and left to solidify at 37 °C for 1 h. HMEC-1 cells (2.0×10^4/well) in 100 μL of medium containing tested compounds (each 1 μM) or vehicle (control) were then seeded. The plates were incubated at 37 °C for 8 h, and the image was recorded by using a phase contrast microscope (Olympus, IX70, Japan). The network of intact tubes from five randomly chosen areas were counted, and the inhibition rate of the tube formation was calculated as $[1 - (\text{tube number}_{\text{treated}}/\text{tube number}_{\text{control}})] \times 100\%$.

Preparation of Compound 6. PAB (**1**; 1.00 g) was dissolved in 20 mL of acetone, 20 mL of 20% $NaHCO_3$ was added, and the mixture was stirred at room temperature. Then 0.35 g of $KMnO_4$ powder was added slowly, and the reaction continued for 1 h. The reaction mixture was adjusted to pH $\approx$ 6 with formic acid and was filtrated. After workup, the crude product was purified by column chromatography over silica gel (petroleum/ethyl acetate/formic acid = 3:1:0.1) to obtain compound **6** 0.27 g (25%). ^{1}H NMR δ_H (in $CDCl_3$): 7.27 (H-15, d, 11.4), 6.58 (H-14, dd, 14.9, 11.4), 5.95 (H-13, d, 14.9), 3.84 (H-9a, d, 17.2), 3.80 (OMe, s), 3.35 (H-3, br d, 3.3), 3.09 (H-5a, ddd, 15.4, 5.8, 3.3), 2.91 (H-9b, d, 17.2), 2.56 (H-6a, ddd, 15.4, 12.3, 3.3), 2.41 (H-5b, ddd, 15.0, 6.0, 3.5), 2.06−1.82 (4H, m), 2.04 (OAc, s), 1.95 (H_3-17, d, 1.0), 1.70 (H_3-12, s), 1.52 (H-6b, ddd, 14.9, 12.3, 3.1). ESI-MS *m/z*: 487 $[M + Na]^+$, 951 $[2M + Na]^+$. IR: (cm^{-1}) 3439, 2958, 1743, 1709, 1641, 1441, 1371, 1219, 1169, 1022, 949, 808.

Preparation of Compound 12. PAB (**1**; 0.050 g, 0.10 mmol) and 3 mL of $SOCl_2$ were added to a flask, and the solution was heated at 40–50 °C for 20 min. After removal of the remaining $SOCl_2$ under reduced pressure, the resultant was dissolved in 1 mL of anhydrous ethyl ether to form a solution of the chloride of PAB. To a flask, 0.05 g of 2-chloroethanaminium chloride, 0.05 g of $NaHCO_3$, 1 mL of ethyl ether, and 2 mL of water were added and stirred at −5 °C for 10 min, and then the solution of the chloride of PAB was added dropwise. The reaction was stirred for 1 h at room temperature. After workup, the resulting product was purified by a silica gel column (chloroform/methanol, 10/1) to give 0.050 g of compound **12** (95%). ^{1}H NMR δ_H (in $CDCl_3$): 7.17 (H-8, m), 6.96 (H-15, d, 11.3), 6.49 (H-14, dd, 14.8, 11.3), 5.83 (H-13, d, 14.8), 3.70 (19-OMe, s), 3.66 (18-N(CH_2)$_2$, s), 3.27 (H-3, br d, 4.6), 3.05 (H-5a, dd, 14.0, 6.0), 2.87 (H-6a, dd, 15.3, 6.0), 2.73 (H-9a, dd, 15.0, 8.7), 2.59 (H-9b, br d, 14.8), 2.13 (H-6b, m), 2.11

(OAc, s), 1.98 (H_3-17, s), 1.85−1.69 (5H, m), 1.57 (H_3-12, s). ESI-MS *m/z*: 458 $[M + H]^+$. IR: (cm^{-1}) 3429, 2953, 1740, 1709, 1643, 1444, 1371, 1279, 1232, 1209, 1165, 1072, 1030, 951.

General Procedure for Preparation of Compounds 13c, 13e, 13g−13n. To 5 mL of an alcoholic solution of PAB (**1**; 0.18 mmol), 0.025 g of powder *t*-BuOK was added, and the mixture was stirred for 24 h at room temperature. The reaction mixture was then acidified with 0.010 g of formic acid. After removal of the alcohol under reduced pressure, the residue was dissolved in 1 mL of anhydrous ethyl ether, and then 3 mL of acety chloride was added at room temperature. The reaction mixture was stirred for 24 h at room temperature, and the solvent was evaporated under reduced pressure. The resulting mixture was partioned between water (2 mL) and EtOAc, and the crude product obtained from the organic phases was purified by column chromatography over silica gel eluted with petroleum/ethyl acetate/formic acid (3:1:0.1) to give the desired products (**13c**, **13e**, **13g−13n**), respectively.

Compound 13c. ^{1}H NMR δ_H (in $CDCl_3$): 7.27 (H-15, d, 11.3), 7.17 (H-8, m), 6.56 (H-14, dd, 15.1, 11.3), 5.92 (H-13, d, 15.1), 5.01 (19-OC<u>H</u>Me_2, m), 3.31 (H-3, br d, 3.5), 3.08 (H-5a, dd, 13.9, 6.3), 2.90 (H-6a, dd, 15.3, 6.3), 2.75 (H-9a, dd, 15.0, 8.7), 2.59 (H-9b, br d, 15.0), 2.14 (OAc, s), 2.12 (H-6b, m), 1.96 (H_3-17, s), 1.89−1.71 (5H, m), 1.60 (H_3-12, s), 1.26 (3H, d, 6.1), 1.25 (3H, d, 5.9). ESI-MS *m/z*: 483 $[M + Na]^+$, 943 $[2M + Na]^+$. IR: (cm^{-1}) 3446, 2981, 1741, 1703, 1645, 1447, 1373, 1257, 1244, 1209, 1165, 1109, 1072, 959. HRESIMS *m/z*: 483.2035 $[M + Na]^+$ ($C_{25}H_{32}NaO_8$: calcd, 483.1995).

Compound 13e. ^{1}H NMR δ_H (in $CDCl_3$): 7.27 (H-15, d, 11.5), 7.22 (H-8, m), 6.57 (H-14, dd, 15.0, 11.5), 5.92 (H-13, d, 15.0), 3.90 (2H, m), 3.32 (H-3, br d, 4.4), 3.09 (H-5a, dd, 14.0, 6.2), 2.91 (H-6a, dd, 15.5, 6.2), 2.76 (H-9a, dd, 14.9, 8.7), 2.62 (H-9b, ddd, 15.4, 3.8, 1.8), 2.15 (H-6b, m), 2.14 (OAc, s), 1.97 (H_3-17, s), 2.01−1.72 (6H, m), 1.61 (H_3-12, s), 0.95 (6H, d, 6.6). ESI-MS *m/z*: 497 $[M + Na]^+$, 971 $[2M + Na]^+$. IR: (cm^{-1}) 3421, 2962, 2875, 1743, 1707, 1645, 1446, 1371, 1244, 1207, 1165, 1072, 1030, 957, 752. HRESIMS *m/z*: 497.2154 $[M + Na]^+$ ($C_{26}H_{34}NaO_8$: calcd, 497.2151).

Compound 13g. ^{1}H NMR δ_H (in $CDCl_3$): 7.27 (H-15, d, 11.8), 7.19 (H-8, m), 6.56 (H-14, dd, 15.1, 11.8), 5.92 (H-13, d, 15.1), 4.13 (2H, m), 3.31 (H-3, br d, 4.1), 3.08 (H-5a, dd, 13.7, 6.3), 2.90 (H-6a, dd, 15.5, 6.3), 2.75 (H-9a, dd, 15.0, 8.7), 2.60 (H-9b, ddd, 15.0, 3.4, 1.6), 2.13 (OAc, s), 2.12 (H-6b, m), 1.96 (H_3-17, d, 1.0), 1.87−1.67 (6H, m), 1.59 (H_3-12, s), 1.55 (2H, m), 0.92 (3H, d, 6.6), 0.92 (3H, d, 6.7). ESI-MS *m/z*: 511 $[M + Na]^+$, 999 $[2M + Na]^+$. IR: (cm^{-1}) 3435, 2958, 1743, 1707, 1643, 1446, 1369, 1279, 1244, 1205, 1165, 1072, 984. HRESIMS *m/z*: 511.2319 $[M + Na]^+$ ($C_{27}H_{36}NaO_8$: calcd, 511.2308).

Compound 13h. ^{1}H NMR δ_H (in $CDCl_3$): 7.27 (H-15, d, 11.5), 7.20 (H-8, m), 6.56 (H-14, dd, 15.0, 11.5), 5.92 (H-13, d, 15.0), 4.11 (2H, m), 3.31 (H-3, br d, 3.5), 3.08 (H-5a, dd, 13.8, 6.2), 2.90 (H-6a, dd, 15.4, 6.2), 2.76 (H-9a, dd, 15.0, 8.7), 2.60 (H-9b, m), 2.13 (H-6b, m), 2.13 (OAc, s), 1.97 (H_3-17, d, 1.2), 1.90−1.63 (7H, m), 1.60 (H_3-12, s), 1.38−1.30 (4H, m), 0.91 (3H, m). ESI-MS *m/z*: 511 $[M + Na]^+$, 999 $[2M + Na]^+$, 487 $[M - H]^-$, 997 $[2M + Na - H]^-$. IR: (cm^{-1}) 3458, 2956, 2872, 1740, 1701, 1645, 1610, 1448, 1371, 1242, 1205, 1165, 1072, 980, 752. HRESIMS *m/z*: 511.2327 $[M + Na]^+$ ($C_{27}H_{36}NaO_8$: calcd, 511.2308).

Compound 13i. ^{1}H NMR δ_H (in $CDCl_3$): 7.26 (H-15, d, 11.4), 7.19 (H-8, m), 6.56 (H-14, dd, 15.0, 11.4), 5.92 (H-13, d, 15.0), 4.10 (2H, m), 3.31 (H-3, br d, 3.8), 3.08 (H-5a, dd, 14.1, 6.4), 2.90 (H-6a, dd, 15.5, 6.4), 2.75 (H-9a, dd, 15.3, 8.8), 2.60 (H-9b, dd, 15.3, 2.4), 2.13 (H-6b, m), 2.13 (OAc, s), 1.96 (H_3-17, s), 1.89−1.62 (7H, m), 1.60 (H_3-12, s), 1.39−1.26 (6H, m), 0.89 (3H, t, 6.9). ESI-MS *m/z*: 1027 $[2M + Na]^+$, 501 $[M - H]^-$, 1026 $[2M + Na - H]^-$. IR: (cm^{-1}) 3412, 2956, 2872, 1743, 1705, 1645, 1610, 1446, 1371, 1244, 1205, 1165, 1072, 957, 752, 571. HRESIMS *m/z*: 525.2447 $[M + Na]^+$ ($C_{28}H_{38}NaO_8$: calcd, 525.2464).

Compound 13j. ^{1}H NMR δ_H (in $CDCl_3$): 7.42 (1H, br.s), 7.26 (H-15, d, 11.4), 7.23 (H-8, m), 6.55 (H-14, dd, 15.0, 11.4), 6.41 (1H, d, 3.0), 6.36 (1H, dd, 3.0, 1.8), 5.91 (H-13, d, 15.0), 5.17 (1H, d, 13.2), 5.04 (1H, d, 13.2), 3.30 (H-3, d, 3.6), 3.07 (H-5a, dd, 14.0, 5.9), 2.90 (H-6a, dd, 15.4, 5.9), 2.74 (H-9a, dd, 15.4, 8.9), 2.58 (H-9b, br d, 15.4), 2.14 (H-6b, m), 2.11 (OAc, s), 1.96 (H_3-17, s), 1.88−1.72 (5H, m), 1.59 (H_3-12, s). ESI-MS *m/z*: 521 $[M + Na]^+$, 1019 $[2M + Na]^+$. IR: (cm^{-1}) 3435, 2953, 1740, 1709, 1643, 1446, 1371, 1230, 1203, 1165, 1072, 1018. HRESIMS *m/z*: 521.1811 $[M + Na]^+$ ($C_{27}H_{30}NaO_9$: calcd, 521.1788).

Compound 13k. ^{1}H NMR δ_H (in $CDCl_3$): 7.27 (H-15, d, 11.0), 7.20 (H-8, m), 6.56 (H-14, dd, 15.1, 11.0), 5.92 (H-13, d, 15.1), 3.91 (2H, m), 3.31 (H-3, br d, 3.6), 3.08 (H-5a, dd, 14.3, 6.0), 2.90 (H-6a, dd, 15.4, 6.0), 2.76 (H-9a, dd, 15.1, 8.8), 2.60 (H-9b, dd, 15.1, 2.4), 2.14 (H-6b, m), 2.13 (OAc, s), 1.96 (H_3-17, s), 1.88−1.66 (10H, m), 1.58 (H_3-12, s), 1.30−1.14 (4H, m), 1.01−0.96 (2H, m). ESI-MS (negative) *m/z*: 513 $[M - H]^-$. IR: (cm^{-1}) 2928, 2854, 1743, 1705, 1448, 1371, 1277, 1244, 1205, 1165, 1072, 982, 957, 752. HRESIMS *m/z*: 537.2470 $[M + Na]^+$ ($C_{29}H_{38}NaO_8$: calcd, 537.2464).

Compound 13l. ^{1}H NMR δ_H (in $CDCl_3$): 7.26 (H-15, d, 11.0), 7.26 (H-8, m), 6.55 (H-14, dd, 15.0, 11.0), 5.92 (H-13, d, 15.0), 4.27 (2H, m), 3.86 (2H, m), 3.31 (H-3, br d, 3.4), 3.09 (H-5a, dd, 13.9, 6.2), 2.90 (H-6a, dd, 15.4, 6.2), 2.76 (H-9a, dd, 14.9, 8.8), 2.62 (H-9b, br d, 14.9), 2.16 (H-6b, br.t, 13.9), 2.13 (OAc, s), 1.96 (H_3-17, s), 1.90−1.72 (5H, m), 1.60 (H_3-12, s). ESI-MS *m/z*: 485 $[M + Na]^+$, 947 $[2M + Na]^+$. IR: (cm^{-1}) 3446, 2956, 1741, 1709, 1645, 1446, 1371, 1257, 1207, 1165, 1072, 956, 752.

Compound 13m. ^{1}H NMR δ_H (in $CDCl_3$): 7.36−7.29 (2H, m, H-15, H-8), 6.63 (H-14, dd, 15.1, 11.2), 6.00 (H-13, d, 15.1), 4.38 (1H, m), 4.31 (1H, m), 3.72 (2H, m), 3.48 (3H, s), 3.39 (H-3, br d, 5.2), 3.16 (H-5a, dd, 13.7, 6.1), 2.98 (H-6a, dd, 15.6, 6.1), 2.84 (H-9a, dd, 15.4, 8.9), 2.68 (H-9b, br d, 15.7), 2.23 (H-6b, m), 2.21 (OAc, s), 2.04 (H_3-17, s), 1.96−1.80 (5H, m), 1.68 (H_3-12, s). ESI-MS *m/z*: 499 $[M + Na]^+$, 975 $[2M + Na]^+$. IR: (cm^{-1}) 3419, 2962, 1741, 1707, 1645, 1446, 1371, 1205, 1074, 1034, 956, 802, 752, 571.

Compound 13n. ^{1}H NMR δ_H (in $CDCl_3$): 7.24 (H-15, m), 7.18 (H-8, m), 6.54 (H-14, dd, 15.0, 11.3), 5.90 (H-13, d, 15.0), 3.90 (2H, m), 3.29 (H-3, br d, 4.0), 3.06 (H-5a, dd, 14.3, 6.0), 2.88 (H-6a, dd, 15.4, 6.0), 2.74 (H-9a, dd, 15.3, 9.0), 2.58 (H-9b, br d, 13.2), 2.11 (OAc, s), 2.09 (H-6b, m), 1.94 (H_3-17, s), 1.86−1.64 (10H, m), 1.58 (H_3-12, s), 1.23−1.09 (4H, m), 1.00−0.91 (2H, m). ESI-MS (negative) *m/z*: 513 $[M - H]^-$. IR: (cm^{-1}) 3444, 2928, 1743, 1705, 1448, 1371, 1277, 1244, 1205, 1165, 1072, 982, 957.

General Procedure for the Preparation of Compounds 14a−14d. To 5 mL of a methanolic solution of PAB (**1**; 0.10 mmol), about 0.12 mmol of *t*-BuOK was added (until pH ≈ 10), and the reaction mixture was stirred for 24 h at room temperature. The reaction mixture was then acidified with a certain amount of formic acid. After removal of solvent, 10 equiv of different acyl chloride in 2 mL of anhydrous ethyl ether were added at room temperature. For compounds **14c** and

14d, 1 equiv of AgCN powder (0.10 mmol) was needed. The reaction mixture was stirred for 24 h at room temperature. After workup, the resultant was partioned between water (2 mL) and EtOAc. The crude product obtained from the organic phase was purified by column chromatography over silica gel eluted with petroleum/ethyl acetate/formic acid (3:1:0.1) to give **14a**−**14d**, respectively.

Compound 14a. ^{1}H NMR δ_H (in $CDCl_3$): 7.26 (H-15, d, 11.5), 7.21 (H-8, m), 6.56 (H-14, dd, 15.0, 11.5), 5.92 (H-13, d, 15.0), 3.72 (19-OMe, s), 3.32 (H-3, br d, 5.5), 3.09 (H-5a, dd, 14.3, 6.5), 2.89 (H-6a, dd, 15.8, 6.5), 2.76 (H-9a, dd, 15.0, 8.8), 2.62 (H-9b, br d, 14.3), 2.41 (2H, q, 7.3), 2.13 (H-6b, br.t, 14.9), 1.96 (H_3-17, s), 1.88−1.72 (5H, m), 1.60 (H_3-12, s), 1.17 (3H, t, 7.7). ESI-MS *m/z*: 469 $[M + Na]^+$, 915 $[2M + Na]^+$. IR: (cm^{-1}) 3547, 3442, 2951, 1740, 1716, 1645, 1443, 1279, 1257, 1209, 1167, 1072, 993, 770.

Compound 14b. ^{1}H NMR δ_H (in $CDCl_3$): 7.29 (H-15, d, 10.8), 7.21 (H-8, m), 6.55 (H-14, dd, 15.2, 10.8), 5.92 (H-13, d, 15.2), 3.72 (19-OMe, s), 3.32 (H-3, br d, 5.0), 3.09 (H-5a, dd, 13.4, 6.6), 2.88 (H-6a, dd, 16.4, 6.6), 2.76 (H-9a, dd, 15.0, 8.8), 2.61 (H-9b, br d, 14.3), 2.37 (2H, t, 7.6), 2.13 (H-6b, br.t, 14.9), 1.96 (H_3-17, s), 1.86−1.28 (11H, m), 1.61 (H_3-12, s), 0.89 (3H, t, 7.1). ESI-MS *m/z*: 511 $[M + Na]^+$, 999 $[2M + Na]^+$. IR: (cm^{-1}) 3460, 2955, 1736, 1710, 1686, 1641, 1439, 1275, 1232, 1159, 1076, 978, 750.

Compound 14c. ^{1}H NMR δ_H (in $CDCl_3$): 8.09 (2H, d, 7.6), 7.64 (1H, t, 8.0), 7.50 (2H, t, 8.0), 7.32 (H-15, d, 11.2), 7.24 (H-8, m), 6.61 (H-14, dd, 15.0, 11.2), 6.00 (H-13, d, 15.0), 3.72 (19-OMe, s), 2.88 (H-6a, dd, 14.8, 5.6), 2.67 (H-9a, dd, 14.4, 8.8), 2.58−2.50 (2H, m), 2.07 (H_3-17, s), 2.21−1.69 (7H, m), 1.56 (H_3-12, s). ESI-MS *m/z*: 517 $[M + Na]^+$, 1011 $[2M + Na]^+$. IR: (cm^{-1}) 3458, 2952, 1776, 1712, 1639, 1452, 1273, 1244, 1205, 1175, 1157, 1072, 1036, 1016, 999.

Compound 14d. ^{1}H NMR δ_H (in $CDCl_3$): 7.36−7.19 (6H, m), 6.52 (H-14, dd, 15.2, 11.2), 5.87 (H-13, d, 15.2), 3.74 (19-OMe, s), 3.64 (2H, br.s), 3.22 (br d, 3.2), 3.04 (H-5a, dd, 14.4, 6.2), 2.84 (H-6a, dd, 15.6, 6.2), 2.69 (H-9a, dd, 14.8, 8.8), 2.41 (H-9b, br d, 15.2), 2.04 (H-6b, m), 2.00 (H_3-17, s), 1.87−1.64 (5H, m), 1.56 (H_3-12, s). ESI-MS *m/z*: 565 $[M + Na]^+$, 1107 $[2M + Na]^+$. IR: (cm^{-1}) 3435, 2951, 1738, 1708, 1643, 1493, 1439, 1279, 1257, 1209, 1165, 1092, 1016, 810.

General Procedure for Preparation of Compounds 11, 15a−15g. PAB (**1**; 0.10 mmol) was treated with 3 mL of $SOCl_2$ at 40–50 °C for 20 min. After removal of the remaining $SOCl_2$ under reduced pressure, the residue was dissolved into 1 mL of anhydrous ethyl ether. The above solution of PAB chloride was added to an anhydrous ethyl ether solution containing 5 equiv of alcohol or amine, and the reaction was stirred at room temperature for 1 h. After workup, the crude solid was purified by silica gel column chromatography eluted with petroleum/ethyl acetate/formic acid (3:1:0.1) or chloroform/methanol (10:1) to give desired products **11** or **15a**−**15g**, respectively.

Compound 11. ^{1}H NMR δ_H (in $CDCl_3$): 7.18 (H-8, m), 7.13 (H-15, d, 11.3), 6.51 (H-14, dd, 15.0, 11.3), 5.85 (H-13, d, 15.0), 4.17 (18-OC$\underline{H}_2$CH$_3$, q, 7.0), 3.70 (19-OMe, s), 3.28 (H-3, br d, 5.4), 3.06 (H-5a, dd, 14.1, 6.2), 2.87 (H-6a, dd, 15.2, 6.2), 2.73 (H-9a, dd, 15.0, 8.8), 2.59 (H-9b, br d, 14.3), 2.13 (H-6b, m), 2.11 (OAc, s), 1.94 (H_3-17, s), 1.85−1.69 (5H, m), 1.57 (H_3-12, s), 1.28 (18-OCH$_2$C$\underline{H}_3$, t, 7.3). ESI-MS *m/z*: 483 $[M + Na]^+$, 921 $[2M + H]^+$, 943 $[2M + Na]^+$. IR: (cm^{-1}) 3454, 2953, 1741, 1708, 1643, 1444, 1369, 1277, 1232, 1207, 1165, 1099, 1072, 951.

Compound 15a. ^{1}H NMR δ_H (in $CDCl_3$): 7.18 (H-8, m), 7.05 (H-15, d, 10.4), 6.49 (H-14, dd, 16.2, 10.4), 5.87 (H-13, d, 16.2), 3.79 (18-NHC$\underline{H}_2$CH$_2$OH, s), 3.70 (19-OMe, s), 3.55 (18-NHCH$_2$C$\underline{H}_2$OH, s), 3.28 (H-3, br d, 3.9), 3.06 (H-5a, dd, 14.1, 6.1), 2.87 (H-6a, dd, 15.4, 6.1), 2.72 (H-9a, dd, 14.7, 8.5), 2.59 (H-9b, br d, 13.5), 2.13 (H-6b, m), 2.11 (OAc, s), 1.99 (H_3-17, s), 1.85−1.68 (5H, m), 1.56 (H_3-12, s). ESI-MS *m/z*: 476 $[M + H]^+$, 951 $[2M + H]^+$, 973 $[2M + Na]^+$. IR: (cm^{-1}) 3407, 2951, 1740, 1711, 1649, 1535, 1444, 1279, 1244, 1207, 1167, 1072, 980.

Compound 15b. ^{1}H NMR δ_H (in $CDCl_3$): 7.21 (H-8, m), 6.98 (H-15, d, 11.5), 6.92 (NH, s), 6.51 (H-14, dd, 15.1, 11.5), 5.86 (H-13, d, 15.1), 4.11 (HO, 3H, br s), m), 3.72 (19-OMe, s), 3.67 (18-NHC(C$\underline{H}_2$OH)$_3$), 3.30 (H-3, br d, 3.6), 3.07 (H-5a, dd, 14.6, 5.9), 2.89 (H-6a, dd, 15.3, 5.9), 2.74 (H-9a, dd, 15.3, 8.7), 2.61 (H-9b, m), 2.14 (H-6b, m), 2.13 (OAc, s), 1.99 (H_3-17, s), 1.86−1.71 (5H, m), 1.58 (H_3-12, s). ESI-MS *m/z*: 536 $[M + H]^+$, 1071 $[2M + H]^+$, 1093 $[2M + Na]^+$. IR: (cm^{-1}) 3400, 2926, 1740, 1713, 1630, 1518, 1444, 1371, 1279, 1244, 1209, 1167, 1072, 1051. HRESIMS *m/z*: 558.2300 $[M + Na]^+$ ($C_{27}H_{37}NaO_{10}$: calcd, 558.2315).

Compound 15c. ^{1}H NMR δ_H (in $CDCl_3$): 7.17 (H-8, m), 6.98 (H-15, d, 11.2), 6.48 (H-14, dd, 15.0, 11.2), 5.83 (H-13, d, 15.0), 4.15 (18-NHC$\underline{H}_2$CO$_2$H, m), 3.70 (19-OMe, s), 3.28 (H-3, br.s), 3.05 (H-5a, dd, 13.1, 5.9), 2.86 (H-6a, m), 2.71 (H-9a, dd, 14.2, 8.6), 2.59 (H-9b, br d, 12.7), 2.11 (H-6b, m), 2.11 (OAc, s), 1.99−1.66 (5H, m), 1.56 (H_3-17, s), 1.23 (H_3-12, s). ESI-MS *m/z*: 490 $[M + H]^+$, 512 $[M + Na]^+$, 979 $[2M + H]^+$, 1001 $[2M + Na]^+$. IR: (cm^{-1}) 3415, 2951, 1740, 1709, 1659, 1525, 1443, 1371, 1279, 1244, 1207, 1167, 1072, 1018.

Compound 15d. ^{1}H NMR δ_H (in $CDCl_3$): 7.16 (H-8, m), 7.01 (H-15, d, 11.5), 6.48 (H-14, dd, 14.1, 11.5), 5.88 (H-13, d, 14.1), 3.70 (19-OMe, s), 3.28 (H-3, br.s), 3.05 (H-5a, dd, 13.7, 5.9), 2.87 (H-6a, dd, 15.0, 5.8), 2.72 (H-9a, dd, 15.0, 9.0), 2.59 (H-9b, br d, 14.1), 2.13 (H-6b, m), 2.11 (OAc, s), 1.98 (H_3-17, s), 1.89−1.68 (5H, m), 1.56 (H_3-12, s). ESI-MS *m/z*: 489 $[M + H]^+$, 977 $[2M + H]^+$. IR: (cm^{-1}) 3400, 2953, 1740, 1649, 1537, 1443, 1385, 1279, 1259, 1209, 1182, 1072, 1022, 953.

Compound 15e. ^{1}H NMR δ_H (in $CDCl_3$): 7.54 (2H, d, 8.0), 7.32 (2H, t, 7.8), 7.18 (H-8, m), 7.11 (1H, t, 7.1), 7.02 (H-15, d, 11.1), 6.53 (H-14, dd, 15.0, 11.1), 5.85 (H-13, d, 15.0), 3.70 (19-OMe, s), 3.29 (H-3, br d, 5.2), 3.06 (H-5a, dd, 14.0, 6.1), 2.88 (H-6a, dd, 15.5, 6.1), 2.74 (H-9a, dd, 15.1, 10.8), 2.60 (H-9b, br d, 14.8), 2.14 (H-6b, m), 2.07 (OAc, s), 1.98 (H_3-17, s), 1.85−1.70 (5H, m), 1.58 (H_3-12, s). ESI-MS *m/z*: 508 $[M + H]^+$, 530 $[M + Na]^+$, 1037 $[2M + Na]^+$. IR: (cm^{-1}) 3390, 2980, 1740, 1666, 1599, 1529, 1441, 1317, 1279, 1244, 1207, 1165, 1072, 1030.

Compound 15f. ^{1}H NMR δ_H (in acetone-d_6): 7.12−7.06 (3H, m), 6.99 (1H, d, 11.0), 6.58−6.52 (2H, m), 6.12 (H-15, d, 15.1), 3.69 (19-OMe, s), 3.42 (1H, br.s), 3.32 (1H, br.s), 3.09 (H-5a, dd, 14.2, 6.1), 2.82 (H-6a, dd, 15.5, 6.1), 2.70 (2H, d, 6.2), 2.31 (H-6b, m), 2.17 (OAc, s), 2.03 (H_3-17, s), 1.96−1.67 (5H, m), 1.61 (H_3-12, s). ESI-MS *m/z*: 524 $[M + H]^+$, 546 $[M + Na]^+$, 1069 $[2M + Na]^+$. IR: (cm^{-1}) 3390, 2953, 1740, 1716, 1647, 1603, 1539, 1508, 1444, 1317, 1279, 1232, 1209, 1165, 1165, 1072, 1030, 978.

Compound 15g. ^{1}H NMR δ_H (in $CDCl_3$): 7.26−6.87 (6H, m), 6.58 (H-14, dd, 15.5, 11.1), 6.20 (H-13, d, 15.5), 3.69 (OCH$_3$-19, s), 3.51 (H-3, br d, 5.1), 2.78−2.70 (4H, m), 2.13 (H-6b, m), 2.13 (OAc, s), 2.12 (H_3-17, s), 1.45 (H_3-12, s). ESI-MS *m/z*: 523 $[M + H]^+$. IR: (cm^{-1}) 3425, 2951, 1740, 1713, 1647, 1524, 1497, 1454, 1317, 1242, 1196, 1082, 1032, 752.

Acknowledgment. Financial support of the National Natural Science Foundation (Grant No. 30630072; 30572201; 30730103)

and the support of Shanghai Municipal Natural Science Foundation (04ZR14157) are gratefully acknowledged.

Supporting Information Available: ^{1}H NMR, ESIMS, and IR spectra of the active compounds **13c**, **13e**, **13g**−**13k**, and **15b** and the HPLC reports for the purity check of the active compounds **13b**−**13k** and **15b**, as measured in two different mobile phases. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Carmeliet, P.; Jain, R. K. Angiogenesis in cancer and other diseases. *Nature* **2000**, *407*, 249–257. (b) St Croix, B.; Rago, C.; Velculescu, V.; Traverso, G.; Romans, K. E.; Montgomery, E.; Lal, A.; Riggins, G. J.; Lengauer, C.; Vogelstein, B.; Kinzler, K. W. Genes expressed in human tumor endothelium. *Science* **2000**, *289*, 1197–1202.

(2) (a) Unger, C. New therapeutic approaches in cancer treatment. *Drugs Future* **1997**, *22* (12), 1337–1345. (b) Halim, N. S. Small molecules in large proteins. *The Scientist* **2000**, *14*, 20–21.

(3) Pan, D. J.; Li, Z. L.; Hu, C. Q.; Chen, K.; Chang, J. J.; Lee, K. H. The cytotoxic principles of *Pseudolarix kaempferi*: Pseudolaric acid-A and -B and related derivatives. *Planta Med.* **1990**, *56*, 383–385.

(4) (a) Li, Z. L.; Pan, D. J.; Hu, C. Q.; Wu, Q. L.; Yang, S. S.; Xue, G. Y. Studies on the novel diterpenic constituents of Tu-Jin-Pi. *Acta Chim. Sin.* **1982**, *40*, 447–457. (b) Zhou, B. N.; Ying, B. P.; Song, G. Q.; Chen, Z. X.; Han, J.; Yan, Y. F. Pseudolaric acids from *Pseudolarix kaempferi*. *Planta Med.* **1983**, *47*, 35–38. (c) Li, E.; Clark, A. M.; Hufford, C. D. Antifungal evaluation of pseudolaric acid B, a major constituent of. *Pseudolarix kaempferi. J. Nat. Prod.* **1995**, *58*, 57–67. (d) Yang, S. P.; Dong, L.; Wang, Y.; Wu, Y.; Yue, J. M. Antifungal diterpenoids of *Pseudolarix kaempferi* and their structure−activity relationship study. *Bioorg. Med. Chem.* **2003**, *11*, 4577–4584.

(5) (a) Wang, W. C.; Lu, R. F.; Zhao, S. X.; Zhu, Y. Z. Antifertility effect of pseudolaric acid B. *Yaoxue Xuebao* **1982**, *3*, 188–192. (b) Zhang, Y. L.; Lu, R. Z.; Yan, A. L. Inhibition of ova fertilizability by pseudolaric acid B in hamster. *Yaoxue Xuebao* **1990**, *11*, 60–62. (c) Wang, W. C.; Lu, R. F.; Zhao, S. X.; Gu, Z. P. Comparison of early pregnancy-terminating effect and toxicity between pseudolaric acids A and B. *Yaoxue Xuebao* **1988**, *9*, 445–448.

(6) (a) Chen, B.; Ko, R. Y. Y.; Yuen, M. S. M.; Cheng, K. F.; Chiu, P. A tandem metal carbene cyclization-cycloaddition approach to the pseudolaric acids. *J. Org. Chem.* **2003**, *68*, 4195–4205. (b) Chiu, P.; Chen, B.; Cheng, K. F. A rhodium carbene cyclization-cycloaddition cascade strategy toward the pseudolaric acids. *Org. Lett.* **2001**, *3*, 1721–1724. (c) Wu, B.; Karle, J. M.; Watkins, E. B.; Avery, M. A. Toward the total synthesis of pseudolaric acid B. Preparation of a key intermediate by degradation and its use in the reassembly of the natural product. *Tetrahedron Lett.* **2002**, *43*, 4095–4098. (d) Chiu, P.; Chen, B.; Cheng, K. F. A conjugated reduction-intramolecular aldol strategy toward the synthesis of pseudolaric acid A. *Tetrahedron Lett.* **1998**, *39*, 9229–9232. (e) Pan, B. C.; Chang, H. Y.; Cai, G. L.; Guo, Y. S. Sythetic studies on pseudolaric acid A. *Pure Appl. Chem.* **1989**, *61*, 389–392.

(7) (a) Tan, W. F.; Zhang, X. W.; Li, M. H.; Yue, J. M.; Chen, Y.; Lin, L. P.; Ding, J. Pseudolarix acid B inhibits angiogenesis by antagonizing the vascular endothelial growth factor-mediated anti-apoptotic effect. *Eur. J. Pharmacol.* **2004**, *499*, 219–228. (b) Yue, J. M.; Yang, S. P.; Ding, J.; Xiao, D.; Yuan, S. T.; Wu, Y.; Tong, Y. G.; Dong, L. Pseudolaric acid-B derivatives, their preparation and pharmaceutical compositions. U.S. Patent 6,887,895 B2, 2005.

(8) Li, M. H.; Miao, Z. H.; Tan, W. F.; Yue, J. M.; Zhang, C.; Lin, L. P.; Zhang, X. W.; Ding, J. Pseudolaric acid B inhibits angiogenesis and reduces hypoxia-inducible factor 1α by promoting proteasome-mediated degradation. *Clin. Cancer Res.* **2004**, *10*, 8266–8274.

(9) (a) Gong, X. F.; Wang, M. W.; Tashiro, S. I.; Onodera, S.; Ikejima, T. Pseudolaric acid B induces apoptosis through P53 and Bax/Bcl-2 pathways in human melanoma A375-S2 cells. *Arch. Pharm. Res.* **2005**, *28*, 68–72. (b) Gong, X. F.; Wang, M. W.; Wu, Z.; Tashiro, S. I.; Onodera, S.; Ikejima, T. Pseudolaric acid B induces apoptosis *via* activation of c-Jun N-terminal kinase and caspase-3 in Hela cells. *Exp. Mol. Med.* **2004**, *36*, 551–556.

(10) Wong, V. K. W.; Chiu, P.; Chung, S. S. M.; Chow, L. M. C.; Zhao, Y. Z.; Yang, B. B.; Ko, B. C. B. Pseudolaric acid B, a novel microtubule-destabilizing agent that circumvents multidrug resistance phenotype and exhibits antitumor activity in vivo. *Clin. Cancer Res.* **2005**, *11*, 6002–6011.

(11) Yang, S. P.; Wu, Y.; Yue, J. M. Five new diterpenoids from *Pseudolarix kaempferi*. *J. Nat. Prod.* **2002**, *65*, 1041–1044.

(12) (a) Spivey, A. C.; McKendrick, J.; Srikaran, R.; Helm, B. A. Solid-phase synthesis of an A−B loop mimetic of the CE3 domain of human IgE: Macrocyclization by Sonogashira coupling. *J. Org. Chem.* **2003**, *68*, 1843–1851. (b) Takimoto, S.; Inanga, J.; Katsuki, T.; Yamaguchi, M. Preparation of hindered esters from acid chlorides and alcohols in the presence of silver cyanide. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 2335–2336.

(13) Zhang, C.; Yang, F.; Zhang, X. W.; Wang, S. C.; Li, M. H.; Lin, L. P.; Ding, J. *Grateloupia longifolia* polysaccharide inhibits angiogenesis by downregulating tissue factor expression in HMEC-1 endothelial cells. *Br. J. Pharmacol.* **2006**, *148*, 741–751.

(14) Tong, Y. G.; Zhang, X. W.; Tian, F.; Yi, Y. H.; Xu, Q. Z.; Li, L.; Tong, L. J.; Lin, L. P.; Ding, J. Philinopside A, a novel marine-derived compound possessing dual anti-angiogenic and anti-tumor effects. *Int. J. Cancer* **2005**, *114*, 843–853.

JM070906G