Antiangiogenic Metabolites from a Marine-Derived Fungus, *Hypocrea vinosa*

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The aims of this study were to investigate the role of tyrosine kinase in intracellular signaling and to search for lead compounds with tyrosine kinase inhibitory activity from metabolites of marine-derived fungi. We initially prepared 400 extracts from 200 species of marine fungi and then subjected them to a tyrosine kinase screening assay using human umbilical vein endothelial cell lysate. Tyrosine kinase inhibitory activity was observed among certain metabolites of *Hypocrea vinosa*. We isolated one known compound, SC2051 (**1**), as well as two new compounds, hypochromins A (**2**) and B (**3**), which have a bis(naphtho-γ-pyrone) skeleton. Compounds **1**−**3** showed tyrosine kinase inhibitory activity, with IC$_{50}$ values of 42.1, 58.7, and 18.0 μM, respectively. Furthermore, compounds **1**−**3** exhibited inhibitory effects on proliferation, migration, and tubule formation.

More than 70% of the earth's surface is covered by oceans, and numerous fungi have been identified in sponges, algae, marine wood, mollusks, tunicates, and oceanic sediments. Marine fungi have yielded a variety of novel chemical compounds, with the highest rate being reported in specimens collected from sponges and algae. We previously identified a new compound, phomactin (phomactin H), with a novel skeleton, isolated from a marine-derived fungus in brown algae.[1] In recent years, natural products isolated from marine-derived fungi have received interest for their potential biological activity as antibiotics and anticancer agents.[2,3] Such investigations of marine organisms may yield novel bioactive compounds for disease therapy in humans.[4]

Angiogenesis plays a critical role in the growth of tumor cells, and tumor angiogenesis inhibitors represent a promising strategy for the treatment of cancer. Epigenetic changes in the tumor environment, such as increased CO_2 and hypoxia, induce angiogenesis by increasing the production and secretion of angiogenic factors such as vascular endothelial growth factor (VEGF). Binding of VEGF to its receptor (a tyrosine kinase receptor), which is present on the surface of endothelial cells, induces dimerization and activation of the receptor's tyrosine kinase domain, resulting in autophosphorylation of cytoplasmic tyrosine residues, which are used as docking sites for signaling proteins that relay signals for cell proliferation, migration, permeability, and tubule formation.[5,6]

Tyrosine phosphorylation sites of the VEGF receptor are important targets for the development of antiangiogenic agents in cancer chemotherapy. Several tyrosine kinase inhibitors have been tested in preclinical studies and clinical trials. ZD6474 (Zactima, Astra Zenaca) is a novel orally available inhibitor of VEGFR-2 (KDR) tyrosine kinase, with additional activity against epidermal growth factor receptor (EGFR) tyrosine kinase. ZD6474 has been shown to inhibit angiogenesis and tumor growth in a range of tumor models and may be promising for the treatment of non-small-cell lung cancer.[7]

In this paper, we report our investigations of natural products produced by marine-derived fungi. We prepared 400 extracts from 200 species of marine fungi obtained from algae, mollusks, sand, and seawater. We then tested these extracts using a tyrosine kinase screening assay with human umbilical vein endothelial cell (HUVEC) lysates. Extracts from one species of fungi, *Hypocrea vinosa*, showed inhibitory activity. A known compound, SC2051 (**1**),[8] and two novel compounds, hypochromins A (**2**) and B (**3**), which have bis(naphtho-γ-pyrone) skeletons, were isolated from EtOAc extracts of *H. vinosa*. Structures for the new metabolites **2** and **3** were confirmed by spectroscopic analysis. Furthermore we examined the inhibition of proliferation, migration, and tube formation by compounds **1**−**3**; the results of these studies are described below.

SC2051 (**1**)

Hypochromin A (**2**)

Hypochromin B (**3**)
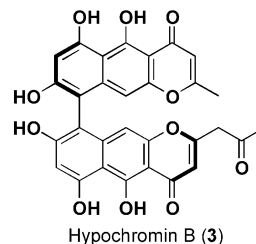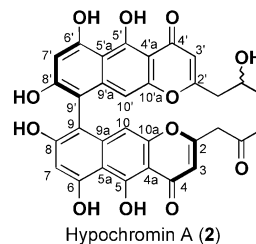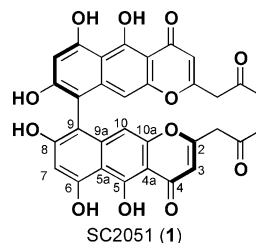
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Table 1. NMR Spectroscopic Data (500 MHz, DMSO-d_6) for Hypochromin A (**2**)

position	δ_C, mult.	δ_H, (J in Hz)	HMBC
2	165.8, C		
2′	170.8, C		
3	108.1, CH	6.18, s	2, 4, 4a
3′	106.6, CH	6.15, s	2′, 4′, 4′a
4, 4′	183.2, C, 183.2, C		
4a, 4′a	101.8, C, 101.8, C		
5, 5′	162.5, C, 162.6, C		
5a, 5′a	106.2, C, 106.3, C		
6, 6′	159.0, C, 159.2, C		
7, 7′	100.8, CH, 100.7, CH	6.58, s, 6.61, s	9, 9′
8, 8′	157.9, C, 157.9, C		
9, 9′	105.8, C, 105.9, C		
9a, 9′a	139.7, C, 139.8, C		
10, 10′	97.9, CH, 98.1, CH	6.15, s, 6.19, s	4a, 9, 10a, 4′a, 9′, 10′a
10a, 10′a	151.8, C, 152.0, C		
6-OH, 6′-OH		9.77, s, 9.81, s	5a, 6, 7, 5′a, 6′, 7′
8-OH, 8′-OH		9.97, s, 10.00, s	7, 8, 9, 7′, 8′, 9′
2-*CH₂*-CO-CH₃	47.9, CH₂	3.83, d (17.1)	2, 3, 2-CH₂CO
		3.88, d (17.1)	2, 3, 2-CH₂CO
2-CH₂-*CO*-CH₃	202.0, C		
2-CH₂−CO-*CH₃*	29.9, CH₃	2.14, s	2-CH₂-CO, 2-CH₂-CO
2′-*CH₂*-CHOH-CH₃	43.6, CH₂	2.51, m	2′, 2′-CH₂CHOH
		2.60, dd (14.2, 4.4)	2′, 2′-CH₂CHOH
2′-CH₂-*CHOH*-CH₃	64.1, CH	3.96, m	
2′-CH₂-CHOH-*CH₃*	23.5, CH₃	1.07, d (6.1)	2′-CH₂, 2′-CH₂-CHOH

Table 2. NMR Spectroscopic Data (500 MHz, DMSO-d_6) for Hypochromin B (**3**)

position	δ_C, mult.	δ_H, (J in Hz)	HMBC
2	165.6, C		
2′	169.5, C		
3	108.0, CH	6.19, s	2, 2-CH₂-CO
3′	105.7, CH	6.16, s	2′, 2′-CH₃
4, 4′	183.0, C, 183.0, C		
4a, 4′a	101.5, C, 101.7, C		
5, 5′	162.3, C, 162.4, C		
5a, 5′a	106.2, C, 106.2, C		
6, 6′	158.9, C, 159.0, C		
7, 7′	100.6, CH, 100.7, CH	6.56, s, 6.59, s	8, 9; 8′, 9′
8, 8′	157.8, C, 157.8, C		
9, 9′	105.7, C, 105.7, C		
9a, 9′a	139.6, C, 139.7, C		
10, 10′	97.9, CH, 98.1, CH	6.15, s, 6.15, s	4a, 9, 10a; 4′a, 9′a, 10′a
10a, 10′a	151.6, C, 151.7, C		
6-OH, 6′-OH		9.76, s, 9.78, s	5a, 6, 7; 5′a, 6′, 7′
8-OH, 8′-OH		9.95, s, 9.95, s	7, 8, 9; 7′, 8′
2-*CH₂*-CO-CH₃	47.9, CH₂	3.83, d (17.2)	2, 3, 2-CH₂CO
		3.88, d (17.2)	2, 3, 2-CH₂CO
2-CH₂-*CO*-CH₃	202.0, C		
2-CH₂-CO-*CH₃*	29.9, CH₃	2.13, s	2-CH₂-CO, 2-CH₂-CO
2′-CH₃	20.1, CH₃	2.22, s	2′, 3′

Table 3. Biological Assay Data of Compounds **1−3**[a]

compound	tyrosine kinase assay	growth inhibition assay	migration assay
1	42.1	17.4	1.09
2	58.7	50.0	0.87
3	18.0	13.1	1.51

[a] Data expressed in IC$_{50}$ values (μM).

Results and Discussion

H. vinosa grown on wheat medium was extracted with CHCl$_3$ followed by EtOAc. The EtOAc extract was subjected to silica gel column chromatography and HPLC, providing compounds **1−3**. The known compound **1** was identified as SC2051 on the basis of its spectroscopic profile (NMR, UV, IR, MS) in comparison with published data.[8] SC2051 (**1**) consists of two naphtho-γ-pyrone skeletons coupled at the 9-position, with hydroxy groups at the 8-position, which create a barrier to rotation, yielding atropisomers. We were unable to find any previously published reports regarding the absolute configuration of SC2051. The absolute configuration of this compound was determined using a combination of circular dichroism (CD) data and the exciton chirality method.[9] When this method is applied, the CD spectrum of **1** exhibits intense bisignate Cotton effects of positive (299 nm) first and negative second signs at 267 nm due to the two long axes of the naphthalene chromophores, indicating a clockwise helicity in SC2051. The CD spectroscopic data for SC2051 were in agreement with the data that were reported for a related bis(naphtho-γ-pyrone) model.[9] Therefore, compound **1** was indicated to have an *S*-configuration.

The molecular formula of hypochromin A (**2**) was determined to be C$_{32}$H$_{24}$O$_{12}$ on the basis of HREIMS, *m/z* 600.1273 [M]$^+$. The IR spectrum exhibited absorptions at 3440 (hydroxy) and 1658, 1738 cm^{-1} (carbonyl). The ^{13}C NMR spectrum exhibited the presence of three carbonyl carbon signals at δ 183.2, 183.2, and 202.0 and two methyl carbon signals at δ 23.5 and 29.9 (Table 1). Furthermore the ^{1}H NMR spectrum showed six singlet methine protons (δ 6.15, 6.15, 6.18, 6.19, 6.58, 6.61) and two methyl protons [δ 1.07 (d), 2.14 (s)]. These spectroscopic data indicated hypochromin A (**2**) also has the bis(naphtho-γ-pyrone) skeleton analogous to **1**. The main difference from **1** was the replacement of the carbonyl group in the C-2′ side chain by a hydroxy group. A hydroxymethine carbon at δ 64.1, a methylene carbon at δ 43.6, and a methyl carbon at δ 23.5 indicated the presence of an isopropyl alcohol moiety from the HMBC correlations. Unambiguous ^{1}H and ^{13}C NMR assignments were supported by HMQC and HMBC observations. The CD spectrum was also recorded, and the data showed positive first (296 nm) and negative second (267 nm) Cotton effects; hence the absolute configuration of the hindered ring system of hypochromin A (**2**) was proposed to be the *S*-configuration. The absolute configuration of the C-2′ side chain hydroxy-bearing center is unknown. Consequently, the overall absolute configuration of hypochromin A (**2**) remains to be determined.

Hypochromin B (**3**) was shown to have the molecular formula C$_{30}$H$_{20}$O$_{11}$ on the basis of HREIMS, *m/z* 556.1011 [M]$^+$. The IR spectrum showed absorptions at 3400 (hydroxy) and 1650, 1730 cm^{-1} (carbonyl). The ^{13}C NMR spectrum exhibited three carbonyl carbon signals at δ 183.0, 183.0, and 202.0 and two methyl carbon signals at δ 20.1 and 29.9 (Table 2). Furthermore, the ^{1}H NMR spectrum showed six singlet methine protons (δ 6.15, 6.15, 6.16, 6.19, 6.56, 6.59) and two methyl protons [δ 2.13 (s), 2.22 (s)]. These spectroscopic data indicated hypochromin B (**3**) also has the bis(naphtho-γ-pyrone) skeleton. Unambiguous ^{1}H and ^{13}C NMR assignments were supported by HMQC and HMBC observations. The CD spectrum was also recorded, and the data showed positive first (294 nm) and negative second (266 nm) Cotton effects; hence the absolute configuration of hypochromin B (**3**) was proposed as the *S*-configuration.

Compounds **1−3** were tested using the tyrosine kinase inhibitory assay with HUVEC lysate. Derivatives **1−3** all showed inhibitory activity toward tyrosine kinase. The most active compound was hypochromin B (**3**), which had an IC$_{50}$ value of 18.0 μM. The IC$_{50}$ values for SC2051 (**1**) and hypochromin A (**2**) were 42.1 and 58.7 μM, respectively (Table 3). This result suggested that the bis(naphtho-γ-pyrone) unit was responsible for the inhibitory activity.

Next, we studied the effects of compounds **1−3** on proliferation, migration, and tubule formation in endothelial cells, which are steps in the angiogenic process. First, we tested the cytostatic growth inhibitory activity against HUVECs in the MTT assay. Compounds **1−3** showed cytostatic antiproliferative activity with IC$_{50}$ values of 17.4, 50.0, and 13.1 μM, respectively (Table 3). These data suggested that the activity was not influenced by the side chain at the C-2 position. The bis(naphtho-γ-pyrone) moiety, however, is essential for the antiproliferative activity, just as it was for tyrosine kinase inhibitory activity. The cytotoxicity of SC2051 (**1**) was investigated in HeLa cells and NHDF (normal human dermal fibroblasts). As compared with HUVEC, the selectivity index (SI) was 12.0 for HeLa cells, and no cytotoxicity was observed for NHDF.

The effect of **1−3** on VEGF-induced migration of HUVEC was also examined. HUVECs were cultured on cell culture inserts with
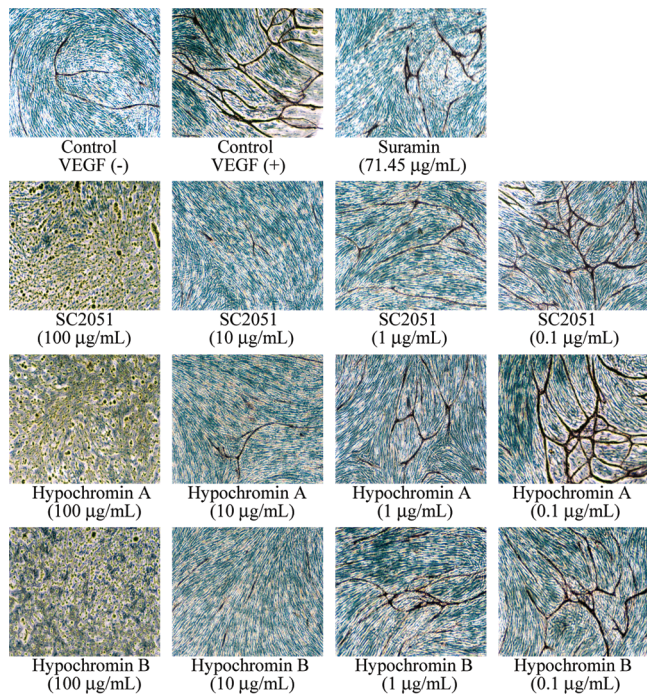
Figure 1. Inhibitory effect of compounds on tubule formation of HUVECs. HUVECs and NHDF cells were admixed onto plates for 3 h. The samples were then added with VEGF (10 ng/mL), and the plates were incubated for 11 days at 37 °C under 5% CO_2. Tubule network patterns were examined microscopically and photographed.

1–**3**. The membranes containing migrated cells were stained and cut off from the inserts after incubation for 22 h. Inhibition of migration was determined by counting the number of migrated cells under a microscope. Compounds **1**–**3** were found to have inhibitory effects on HUVEC migration (IC$_{50}$ = 1.09, 0.87, 1.51 μM, respectively) (Table 3).

Inhibition of tubule formation was examined using an angiogenesis kit. In this analysis, HUVECs and NHDF cells were admixed onto plates, and the assay was carried out according to the manufacturer's instructions. Compounds **1**–**3** were added, and the tubule network patterns were photographed using a microscope. Although **1**–**3** were cytotoxic at a concentration of 100 μg/mL, these compounds inhibited tubule formation at 10 μg/mL, which reflected higher activity than suramin, which was used as a positive control (Figure 1).

In conclusion, SC2051 (**1**) (a known phosphodiesterase inhibitor) and two novel compounds, hypochromins A (**2**) and B (**3**), showed inhibitory activity in the tyrosine kinase assay and also inhibited HUVEC proliferation, migration, and tubule formation. These results suggest that **1**–**3** blocked the phosphorylation sites of KDR tyrosine kinase, which when active, induces mitogenesis and differentiation of tumor vessels.[6] It may be crucial to block KDR tyrosine kinase in order to limit the growth and spread of human tumors. In addition, this is the first report of bis(naphtho-γ-pyrone) structures among metabolites of the fungus *H. vinosa*, although bis(naphtho-γ-pyrone)s are known from other fungi. Chaetochromins B–D and ustilaginoidin G, which have naphthodihydropyrone rings, showed marginal antitumor effects on P388 leukemia *in vivo*, but the administration of higher doses reduced the body weights of the mice and the compounds were evaluated as toxic. Chaetochromin A showed cytotoxicity at a dose of 20 mg/kg. However, ustilaginoidin A, which is a bis(naphtho-γ-pyrone) derivative, was not determined to be toxic at a dose of 60 mg/kg to P388 leukemia *in vivo*.[10] Data support the idea that the (naphtho-γ-pyrone) ring regulates the effect of the cytotoxicity. As a result,

bis(naphtho-γ-pyrone) derivatives such as **1**–**3** have the potential to be new antiangiogenic and antitumor molecules.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanaco MP. Optical rotation was measured with a Horiba SEPA-300 polarimeter. UV spectra were recorded with a Shimadzu UV-240 spectrophotometer. Circular dichroism (CD) measurements were carried out under N_2 gas on a Jasco 820 spectropolarimeter. IR spectra were recorded with a Jasco IR Report-100 spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured with JEOL JNM-LA500 MHz spectrometers in DMSO-d_6. Chemical shift values were reported in δ values (ppm) relative to an internal reference of ^{1}H [δ 2.50] and ^{13}C [δ 39.5], respectively. Low- and high-resolution EIMS spectra were measured with a JEOL JMS-700 spectrometer. Column chromatography was performed using silica gel 60N (63-210 μm) from Kanto Chemical. HPLC was performed using a Jasco PU-2080 Plus pump and measured with a Jasco UV-2075 Plus detector.

Fungal Material. The fungus was isolated from beach sand collected at the coast of Okinawa Prefecture, Japan, in March 2002. The isolate was identified by rDNA sequence analysis. The internal transcribed spacer regions 1 and 2 and 5.8S rDNA in the rRNA gene of the isolate were identical to those of the epitype strain of *Hypocrea vinosa* (accession number: AY380904). The DNA sequence data have been deposited at the DDBJ as AB537986.

Fermentation. *H. vinosa* was spread in 500 mL Roux flasks (60 flasks) containing wheat (150 g per flask) and artificial seawater (50 mL per flask). The flasks were incubated at 26 °C in the dark for 14 days.

Extraction and Isolation. The fermented wheat substrate was extracted with CHCl$_3$ followed by EtOAc. Next the concentrated EtOAc extract (71 g) was redissolved in CHCl$_3$, and 3 g out of 71 g did not dissolve. The bulk (68 g) was soluble in CHCl$_3$, and the material was subjected to step-gradient silica gel column chromatography with a solvent system consisting of 0–100% CHCl$_3$–MeOH (silica gel was treated with 3% oxalic acid in MeOH) to yield six fractions. Fraction 5 was subjected to step-gradient silica gel column chromatography using the aforementioned solvent system to yield five fractions. Fraction 4 was subjected to HPLC (silica gel treated with 3% oxalic acid in MeOH) with CHCl$_3$–MeOH (25:1) to yield SC2051 (**1**) (346.2 mg) and hypochromin A (**2**) (93.3 mg). The insoluble fraction of the EtOAc extract (3 g) was subjected to step-gradient silica gel column chromatography, using the solvent system described above, to yield seven fractions, and SC2051 (**1**) (967.1 mg) was obtained from fraction 5. Fraction 3 was precipitated with CHCl$_3$, and the soluble fraction was subjected to HPLC (using silica gel treated with 3% oxalic acid in MeOH) with CHCl$_3$–MeOH (50:1) to yield hypochromin B (**3**) (24.2 mg).

SC2051 (1): CD (c 7.2 × 10^{-6} M, EtOH) λ_{max} ($\Delta\varepsilon$) 234 (−31.2), 246 (−17.4), 267 (−28.7), 299 (+46.1), 344 (+3.9), 355 (−1.9), 391 (+3.3) nm.

Hypochromin A (2): red, amorphous solid; mp 259−261 °C (dec); [α]$^{25}_D$ +410 (c 0.009, EtOH); UV (EtOH) λ_{max} (log ε) 229 (4.6), 288 (4.7), 335 (3.9), 351 (3.9), 392 (3.9), 435 (4.0) nm; CD (c 2.9 × 10^{-5} M, EtOH) λ_{max} ($\Delta\varepsilon$) 234 (−34.8), 244 (−21.7), 267 (−41.8), 296 (+68.5), 344 (+2.2), 353 (−3.9), 386 (+3.3) nm; IR (KBr) ν_{max} 3440, 2950, 2890, 1738, 1658, 1636, 1600, 1380, 1290, 1165, 1100, 975, 850 cm^{-1}; ^{1}H NMR (DMSO-d_6) see Table 2; ^{13}C NMR (DMSO-d_6) see Table 2; EIMS m/z 600 [M]$^+$ (21), 583 (35), 582 (100), 556 (40); HREIMS m/z 600.1273 [M]$^+$ (calcd for C$_{32}$H$_{24}$O$_{12}$, 600.1268).

Hypochromin B (3): red, amorphous solid; mp 238−240 °C (dec); [α]$^{25}_D$ +340 (c 0.005, EtOH); UV (EtOH) λ_{max} (log ε) 229 (4.6), 288 (4.7), 335 (3.9), 351 (3.9), 392 (3.9), 435 (4.0) nm; CD (c 2.3 × 10^{-5} M, EtOH) λ_{max} ($\Delta\varepsilon$) 234 (−40.9), 244 (−27.8), 266 (−47.0), 294 (+67.9), 341 (+5.2), 352 (−4.3), 387 (+6.1) nm; IR (KBr) ν_{max} 3400, 2950, 2350, 1730, 1650, 1618, 1588, 1360, 1262, 1153, 1082, 959, 840 cm^{-1}; ^{1}H NMR (DMSO-d_6) see Table 3; ^{13}C NMR (DMSO-d_6) see Table 3; EIMS m/z 556 [M]$^+$ (100); HREIMS m/z 556.1011 [M]$^+$ (calcd for C$_{30}$H$_{20}$O$_{11}$, 556.1006).

Cell Culture. HUVECs and NHDF cells were purchased from Lonza Walkersville Inc. and Kurabo Industries Ltd., respectively. HUVECs were cultured using EGM-2 Bulletkit (Lonza Walkersville, Inc.) at 37 °C in 5% CO_2. NHDF cells were grown in α-MEM (Invitrogen Corporation) supplemented with 10% FBS (Invitrogen Corp.) at 37 °C

in 5% CO_2. HeLa cells were grown in MEM (Sigma Aldrich Co.) supplemented with 10% FBS at 37 °C in 5% CO_2.

Tyrosine Kinase Assay. Tyrosine kinase assays were performed on 96-well plates with HUVEC lysates using a Universal Tyrosine Phosphatase Assay Kit (Takara Bio, Inc.). The assay was carried out according to the supplied manual with modifications. Extraction buffer was added to HUVEC, and the lysates were collected. To ensure complete reduction, 2-mercaptoethanol was added. Samples were dissolved in DMSO diluted with kinase reacting solution. Mixtures containing 30 μL of phosphatase reacting solution, 5 μL of HUVEC lysate, 5 μL of test compound, and 10 μL of ATP-Na_2 (40 mM) were incubated for 2 h at 37 °C. Sample solution was removed, and the wells were washed four times with Tween-PBS. Blocking solution (100 μL) was added to each well, and the plates were incubated for 30 min at 37 °C. The blocking solution was then discarded, and 50 μL of antiphosphotyrosine (PY20)-HRP solution was added to each well, followed by a 30 min incubation at 37 °C. The antibody solution was then removed, and the wells were washed four times with Tween-PBS. Washing buffer was completely removed, 100 μL of HRP substrate solution (TMBZ) was added to each well, and the plates were incubated for 30 min at room temperature. Subsequently, 100 μL of stop solution (1 N H_2SO_4) was added to each well, and the absorbance at 450 nm was measured with a plate reader.

Growth Inhibition Assay. HUVECs (3×10^3 cells/well) were seeded in 96-well plates with an EGM-2 Bulletkit for 3 h at 37 °C in 5% CO_2. The medium was removed and replaced with 1% FBS-EBM-2 and incubated for 18 h at 37 °C in 5% CO_2. VEGF (1 nM) and compounds were added to each well and incubated for 72 h at 37 °C in 5% CO_2. Cell proliferation was detected using WST-8 reagent, and the inhibition of proliferation was measured at an absorbance wavelength of 450 nm using a plate reader. The selectivity of the antiproliferative activity (selectivity index, SI) was evaluated from the differences in IC_{50} values as compared with other cell lines.

Migration Assay. Inhibition of cell migration was determined using a PET membrane filter from the inner chamber of an 8 μm Falcon cell culture insert (Becton-Dickson). HUVECs (5×10^4 cells/well) suspended in EBM-2 medium containing 0.2% FBS with various concentrations of compounds dissolved in DMSO were seeded in the inner chamber. Next, the inner chamber was placed into the outer chamber (24-well plate), which was filled with the same medium containing VEGF (20 ng/mL). After a 22 h incubation at 37 °C in 5% CO_2, the nonmigrated cells on the upper surface of the filter were removed by wiping with cotton swabs. The cells were then fixed with MeOH and stained using the Diff-Quik system (Sysmex). The cells, which migrated through the filter to the reverse side, were counted manually at six different microscopic fields (200×).

Tubule Formation Assay. The tubule formation assay was performed using an Angiogenesis Kit (Kurabo, Japan). HUVECs and NHDF cells were cocultured in 24-well plates and incubated with Optimized Medium. After 3 h incubation at 37 °C in 5% CO_2, the medium was replaced with fresh medium containing VEGF (10 ng/mL) and different concentrations of compounds, and the plates were incubated for 11 days at 37 °C in 5% CO_2 (medium was replaced on days 4, 7, 9). After 11 days, formed tubes were fixed with 70% MeOH and stained with mouse anti-human CD31, goat anti-mouse IgG alkaline phosphatase conjugate, and BCIP/NBT. Tubule network patterns were identified under a microscope (200×) and photographed.

Supporting Information Available: ^{1}H/^{13}C NMR and 2D-NMR spectra of compounds and biological assay data are available free of charge via the Internet at http://pubs.acs.org.

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