Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 3473–3480

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis and Structure–Activity Relationship of Diarylamide Derivatives as Selective Inhibitors of the Proliferation of Human Endothelial Cells

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Received 15 May 2002; accepted 18 June 2002

Abstract—A series of diarylamide urea derivatives were synthesized and evaluated for their inhibitory activities against human coronary artery endothelial cells (ECs) and human coronary artery smooth muscle cells (SMCs). Compound **5k** was superior to Tranilast, in terms of both cell selectivity and the potency of its inhibitory activity toward the proliferation and angiogenesis of ECs.

Introduction

Tranilast [*N*-(3,4-dimethoxycinnamoyl) anthranilic acid], an anti-allergic drug, has been clinically used for patients with allergic diseases such as bronchial asthma, allergic rhinitis, and atopic dermatitis.[1a–c] On the other hand, it has been reported that Tranilast had inhibitory activity toward angiogenesis of dermal vascular endothelial cells in vitro and in experimental animal models.[2a,b] Angiogenesis is a phenomenon involving degradation of the membrane by proteolytic enzymes, chemotaxis and proliferation of endothelial cells, tube formation by endothelial cell differentiation, and reorganization of blood vessels, and is associated with various diseases such as diabetic retinopathy, rheumatic arthritis, psoriasis, cancer, and so on.[3] So, we have had a great interest in modifying Tranilast in order to develop potent and cell-selective anti-angiogenetic compounds.

Previously, we reported a series of diarylamide urea derivatives (exemplified by **1**) that exhibited a potent and highly selective inhibition for vascular smooth muscle cells (SMCs) proliferation.[4a,b] In the course of a recent study on substitutions at the 2-position of the A ring, we found that ethoxycarbonyl and carbamoyl groups were suitable substituents for this position and considered that the direct substituted carbonyl group might be required to exert a potent activity. Therefore, we introduced an acetyl group (**5a**) to the A ring of **1** instead of the ethoxycarbonyl group (Fig. 1). The inhibitory activity of **5a** toward the proliferation of SMCs was weaker than that of **1**; but unexpectedly and surprisingly, the selectivity for SMCs had disappeared, or rather this compound displayed selectivity for vascular endothelial cells (ECs). That is, the acetyl group substitution increased the potency of inhibition against ECs proliferation.

As selective inhibitors of ECs proliferation can be considered as a good drug candidate for diseases associated with angiogenesis, and in view of our findings on **5a**, we sought to develop more potent and selective inhibitors of ECs proliferation by modifying **5a**. In this paper, we report the results of the structure–activity relationship (SAR) study on a series of these acetyl-type diarylamide derivatives with respect to ECs proliferation.

Chemistry

The synthesis of diarylamide derivatives **5a**–**x** is outlined in Scheme 1. Condensation of 2-amino-4,5-dimethoxyacetophenone (**2**) with corresponding acid halides in the presence of triethylamine, followed by catalytic hydrogenation with 5% Pd/C gave **4a**–**d** in a good yield (70∼90%) and **4e** was obtained by reduction of **3c** with

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0968-0896/02/$ - see front matter
PII: S0968-0896(02)00258-4

Fe/AcOH. Finally, condensation of **4a–e** with the corresponding isocyanates or acid halides gave the desired urea and amide derivatives **5a–x** (except **5h**). Compound **5h** was obtained by the condensation of **4c** with phenol by using 1,1-carbonyldiimidazole (CDI) as a condensation reagent.

Biology

A series of these compounds were evaluated for their inhibitory activities toward platelet derived growth factor (PDGF)-induced proliferation of SMCs and fetal bovine serum (FBS)-induced proliferation of ECs.[4b] The inhibitory activity toward tube formation of human umbilical vein endothelial cells (HUVECs) was determined by a previously reported method.[2b]

Results and Discussion

The inhibitory activities of **5a–x** toward PDGF-induced proliferation of SMCs and FBS-induced proliferation of ECs are shown in Tables 1–3. Table 1 presents the results of the SAR study on the effect of the distance between the A and B rings. In the case of the diarylamide derivatives in our previous work,[4a] the distance did not much influence the inhibitory activities. But in the case of these urea derivatives, the insertion of an alkyl chain increased the activity; and an ethylene group (**5d**) at the X position showed the most potent inhibition of ECs proliferation. But the insertion of a vinyl group (**5c**) instead of an ethylene group (**5d**) significantly decreased the activity although the both had the same number of carbon atoms. This result suggested that the linker between these rings might need to be flexible to some extent. So, further modification of **5d** was conducted.

Table 2 shows the results of the SAR study when the urea moiety was changed to an alternate linkage. As we expected, the ureido group (**5d**) was more suitable for the activity than the others such as carbonylamino (**5f–g**) and alkoxycarbonylamino (**5h**) groups. In our previous work,[4b] methylation at the N atom in ureido

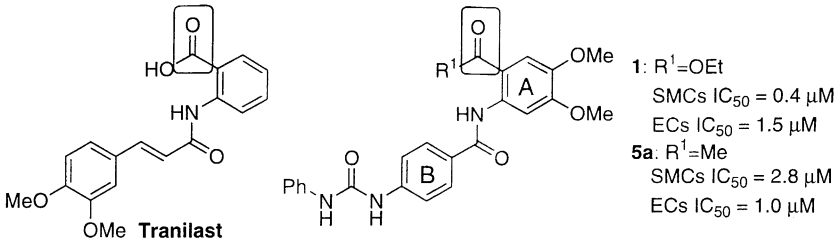
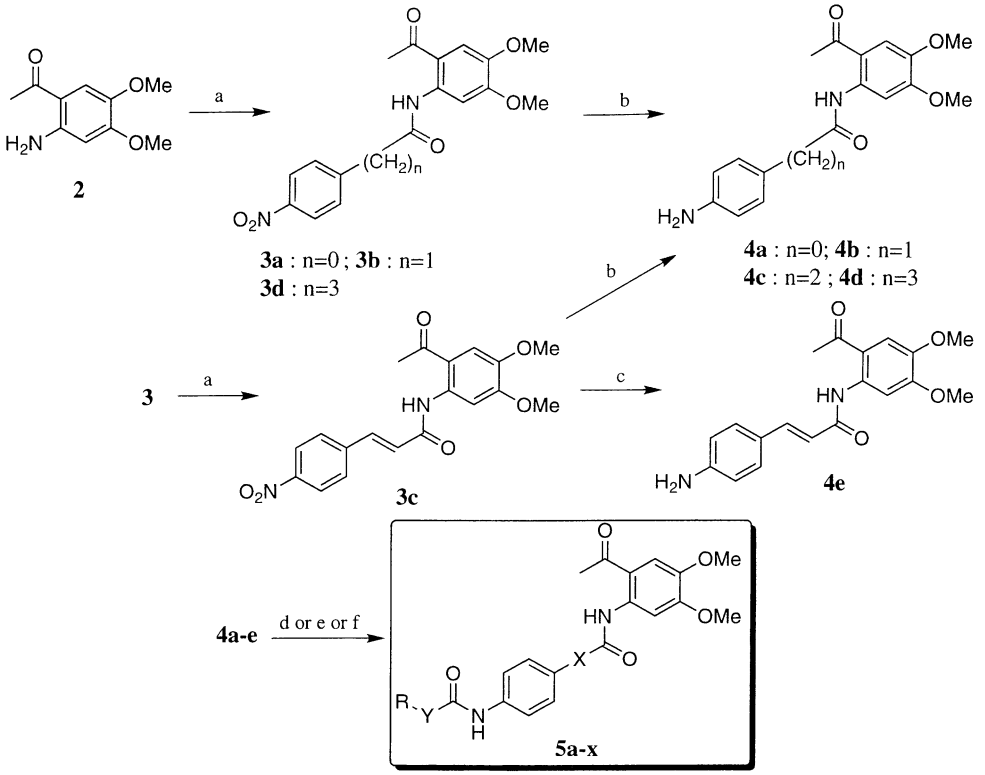


Figure 1. Structure of Tranilast and diarylamide urea derivatives.



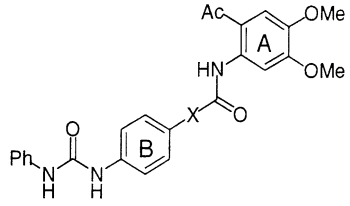
Scheme 1. Synthesis of compounds **5a–x**. Reagents and conditions: (a) (4-NO_2–Ph)-X-COCl, Et_3N/CH_2Cl_2; (b) H_2, 5% Pd/C, EtOH; (c) Fe, AcOH; (d) RNCO, DMAP/THF; (e) Ph-Y-COCl, Et_3N/CH_2Cl_2; (f) PhOH, CDI/THF then DBU.

group resulted in decreased activities. This result suggested that the presence of two NH groups was essential to exhibit the activity.

Table 3 presents the results of the SAR study on substituents of the urea moiety. First, we examined the position of an electron-donating group (amino group) and an electron-withdrawing group (nitro group) on the phenyl ring and found that the *para* position was the most suitable for the activity in both cases (**5i**–**n**). Next, we determined the effect of substituents at the *para* position of the phenyl ring and found the order of potency to be nitro (**5k**) > amino (**5n**), fluorine (**5o**), hydrogen (**5d**) > methyl (**5q**), methoxy (**5r**) > acetyl (**5p**). We previously reported that in the case of the ethoxycarbonyl substitution at the A ring, the 3,4,5-trimethoxyphenyl compound showed the most potent inhibitory activity toward SMCs.[4b] However, in this case, it decreased the activity toward not only ECs but also SMCs (**5s**). Cyclohexyl (**5x**), *n*-butyl (**5w**), and 4-pyridyl (**5t**) groups instead of the phenyl ring gave only weak activities. However, the benzyl group (**5u**) afforded almost the same activity as **5d**. As a result, **5k** was the most potent (IC_{50} = 2.4 nM for ECs), and was about 8000-fold more potent than Tranilast. In terms of cell selectivity, most of the evaluated compounds displayed a low selectivity of with the [SMCs]/[ECs] value ranging from 0.1 to 0.8 times for ECs. However **5k** and **5u** showed good selectivity by one order of magnitude toward ECs. We further evaluated the relation between the inhibition of ECs proliferation and anti-angiogenic activity of **5k**, which showed the most potent inhibitory activity toward ECs proliferation and had much higher selectivity than Tranilast.

To evaluate the activity for the angiogenesis, we used the method of tube formation by HUVECs on Matrigel. Before this analysis, we confirmed the inhibitory activity toward HUVECs in the same manner as used with SMCs and ECs. The IC_{50} of **5k** was 0.7 nM, indicating a little greater potency toward HUVECs than toward ECs. As shown in Figure 2, Tranilast had marginal inhibitory activity against the tube formation even at 100 μM. However, the tube formation was significantly reduced by the treatment of compound **5k** in a dose-dependent manner, and it was completely inhibited at 10 μM of **5k**. Thus, we confirmed the superior inhibitory activity of **5k** against angiogenesis, as we expected, compared with that of Tranilast.

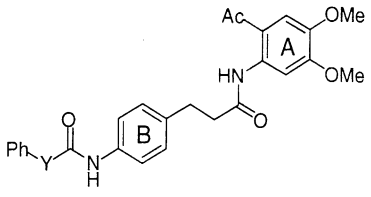
Table 1. IC_{50} values of the compounds **5a**–**e** for inhibition of proliferation of SMCs and Ecs



Compd	X	ECs IC_{50} (μM)[a]	SMCs IC_{50} (μM)[b]	[SMCs]/[ECs]
Tranilast		19	25	1.3
5a	—	1	2.8	2.8
5b	CH_2	0.43	0.26	0.6
5c	(*E*)-CH=CH	1.8	2.7	1.5
5d	$(CH_2)_2$	0.052	0.07	1.3
5e	$(CH_2)_3$	0.13	0.05	0.38

[a]Inhibitory activity against the proliferation of ECs induced by 5% FBS.
[b]Inhibitory activity against the proliferation of SMCs induced by PDGF-BB (20 ng/mL).

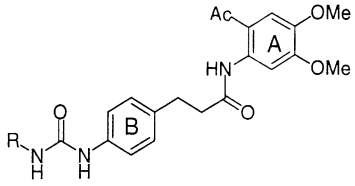
Table 2. IC_{50} values of the compounds **5d**, **f**–**h** for inhibition of proliferation of SMCs and Ecs



Compd	Y	ECs IC_{50} (μM)[a]	SMCs IC_{50} (μM)[b]	[SMCs]/[ECs]
Tranilast		19	25	1.3
5d	NH	0.052	0.07	1.3
5f	—	1.7	0.62	0.37
5g	CH_2	1.6	0.22	0.14
5h	O	1.9	3.2	1.7

[a]Inhibitory activity against the proliferation of ECs induced by 5% FBS.
[b]Inhibitory activity against the proliferation of SMCs induced by PDGF-BB (20 ng/mL).

Table 3. IC_{50} values of the compounds **5d**, **i**–**x** for inhibition of proliferation of SMCs and Ecs



Compd	R	ECs IC_{50} (μM)[a]	SMCs IC_{50} (μM)[b]	[SMCs]/[ECs]
Tranilast		19	25	1.3
5d	Ph	0.052	0.07	1.3
5i	2-NO_2–Ph	0.017	0.045	2.6
5j	3-NO_2–Ph	0.05	0.039	0.78
5k	4-NO_2–Ph	0.0024	0.026	11
5l	2-NH_2–Ph	0.21	0.061	0.29
5m	3-NH_2–Ph	0.22	0.061	0.28
5n	4-NH_2–Ph	0.074	0.02	0.27
5o	4-F–Ph	0.073	0.031	0.42
5p	4-Ac–Ph	1.1	0.35	0.32
5q	4-Me–Ph	0.2	0.17	0.85
5r	4-OMe–Ph	0.37	0.12	0.31
5s	3,4,5-$(OMe)_3$–Ph	1.4	0.31	0.22
5t	4-Py	1.2	0.16	0.13
5u	Bn	0.02	0.29	15
5w	*n*-Bu	1.2	0.43	0.35
5x	c-Hex	0.2	0.077	0.39

[a]Inhibitory activity against the proliferation of ECs induced by 5% FBS.
[b]Inhibitory activity against the proliferation of SMCs induced by PDGF-BB (20 ng/mL).

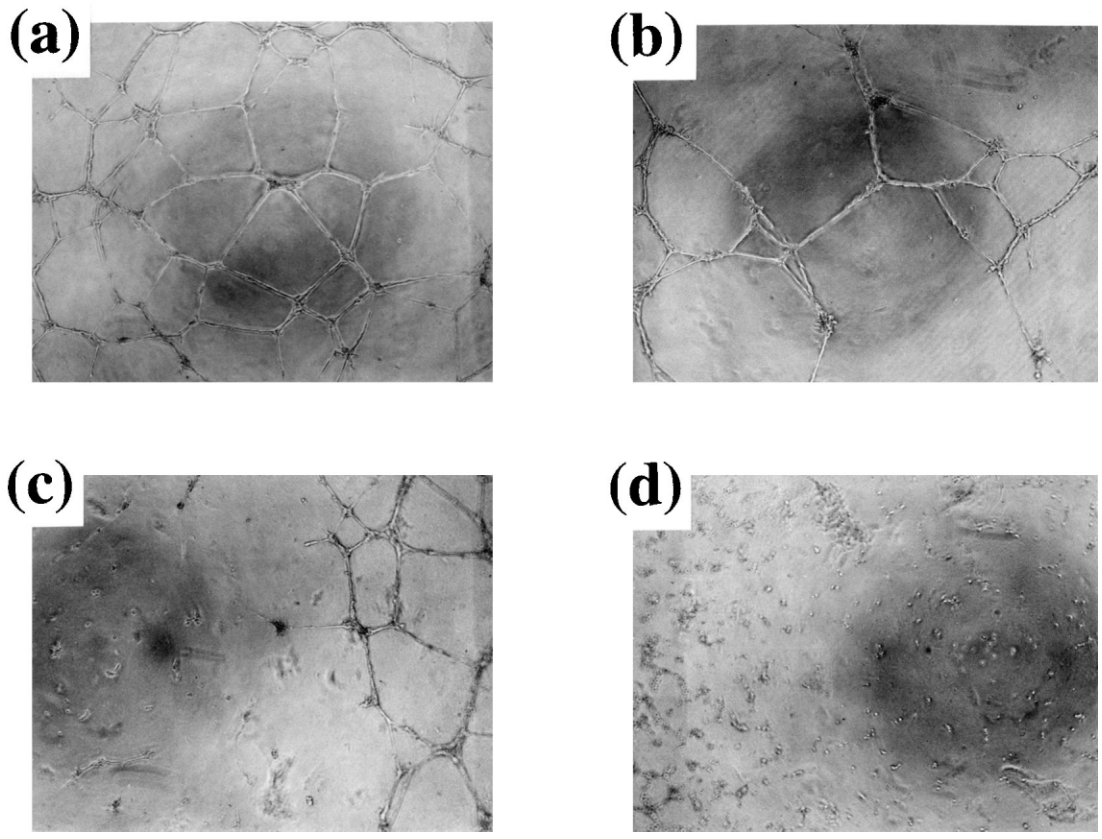


Figure 2. Phase-contrast micrographs of tube formation by HUVECs on Matrigel. Morphological features of HUVECs treated with medium alone (a), 100 μM Tranilast (b), 1 μM **5k** (c), or 10 μM **5k** (d) were photographed at ×40 magnification with a phase-contrast microscope.

By the way, many factors, such as fibroblast growth factor (FGF),[5a] transforming growth factor-β (TGF-β)[5b] and hepatocyte growth factor (HGF),[5c] have been reported to regulate angiogenesis. Among them, vascular endothelial growth factor (VEGF) was known to be a potent and specific angiogenic factor.[6] In fact, the prototype compounds of the Kirin Brewery group,[7a,b] which we also used for modification in our previous study,[4b] are a potent inhibitor of VEGF receptor tyrosine kinase.[8a,b] On the other hand, according to the several studies on the mechanism of the Tranilast, this drug inhibited protein kinase C-dependent signaling pathway linked to angiogenetic activities and did not affect on VEGF binding and tyrosine phosphorylation of the receptor.[9] On the basis of the structural resemblance between our compounds and these two series of compounds, elucidation of the target molecules of our derivatives and the mechanism of the cell selectivity between SMCs and ECs are still underway.

In conclusion, we established the lead compound **5a**, a selective inhibitor for ECs, by introducing acetyl group to the A ring of **1** instead of the ethoxycarbonyl group. From the SAR study of **5a**, we found compound **5k** to be superior to Tranilast in terms of the strength of the activity and cell selectivity. Compound **5k** may thus be a useful compound for the treatment of diabetic retinopathy, rheumatic arthritis, psoriasis and tumors, all of which rely on angiogenesis.

Experimental

Chemistry

In general, reagents and solvents were used as purchased without further purification. Column chromatography was performed on FL60D (Fuji Silysia). Melting points were measured with a Buchi 535 melting point apparatus and left uncorrected. Proton NMR spectra were recorded on a Joel GSX270 FT NMR spectrometer. Chemical shifts were expressed in δ (ppm) from internal standard tetramethylsilane. Time-of-flight mass spectrometry (TOFMS) were recorded on a Kompact Maldi III spectrometer. Elemental analyses were performed by the Toray Research Center.

***N*-(2-Acetyl-4,5-dimethoxyphenyl)(4-((phenylamino)carbonylamino)phenyl)formamide (5a).** To a solution of **2** (0.10 g, 0.67 mmol) in CH_2Cl_2 (10 mL) were added 4-nitrobenzoyl chloride (0.13 g, 0.70 mmol) and triethylamine (0.13 g, 0.70 mmol), and the mixture was stirred for 1 h at room temperature. The reaction mixture was poured into saturated $NaHCO_3$ aq and extracted with CH_2Cl_2, after which the organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated to give 0.08 g (0.23 mmol) of **3a** as a yellow solid with a yield of 34%.

To a solution of **3a** (0.08 g, 0.23 mmol) in methanol (10 mL) and tetrahydrofuran (10 mL) was added 5% Pd/C (0.03 g), and the mixture was stirred under a H_2

atmosphere for 18 h. The reaction mixture was filtered, concentrated and washed with methanol to give 0.05 g (0.16 mmol) of **4a** as a yellow solid with a yield of 69%.

To a solution of **4a** (0.05 g, 0.16 mmol) and 4-dimethylaminopyridine (0.02 g, 0.18 mmol) in tetrahydrofuran (5 mL) was added phenylisocyanate (0.05 g, 0.42 mmol), and the mixture was refluxed for 3 h. The reaction mixture was poured into water and extracted with CH_2Cl_2, and the organic layer was then washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2/methanol = 100:1 ~ 20:1) and washed with methanol to give 0.04 g (0.10 mmol) of **5a** as a white solid with a yield of 65%. Mp: 234–236 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.70 (s, 3H), 3.86 (s, 3H), 3.89 (s, 3H), 7.00 (t, J = 7.3 Hz, 1H), 7.30 (t, J = 7.6 Hz, 2H), 7.48 (d, J = 7.8 Hz, 2H), 7.50 (s, 1H), 7.67 (d, J = 8.9 Hz, 2H), 7.92 (d, J = 8.9 Hz, 2H), 8.56 (s, 1H), 8.92 (s, 1H), 9.20 (s, 1H), 12.76 (s, 1H); MS (TOF) m/z = 434 (M^+ + H). Anal. calcd for $C_{24}H_{23}N_3O_5 \cdot 0.8H_2O$: C, 64.36; H, 5.54; N, 9.38. Found: C, 64.06; H, 5.44; N, 9.34.

***N*-(2-Acetyl-4,5-dimethoxyphenyl)-2-(4-((phenylamino)-carbonylamino)phenyl)ethanamide (5b).** Compound **5b** was prepared from **2** in a manner similar to that described for compound **5a** with a yield of 8% by three steps. Mp: 208–210 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.58 (s, 3H), 3.66 (s, 2H), 3.81 (s, 6H), 6.96 (t, J = 7.3 Hz, 1H), 7.27 (m, 4H), 7.43 (m, 5H), 8.28 (s, 1H), 8.66 (s, 1H), 8.68 (s, 1H), 11.72 (s, 1H); MS (TOF) m/z = 448 (M^+ + H). Anal. calcd for $C_{25}H_{25}N_3O_5 \cdot 0.2H_2O$: C, 66.56; H, 5.68; N, 9.31. Found: C, 66.43; H, 5.71; N, 9.26.

***N*-(2-Acetyl-4,5-dimethoxyphenyl)-3-(4-((phenylamino)-carbonylamino)phenyl)prop-2-enamide (5c).** Compound **3c** was prepared from **2** in a manner similar to that described for compound **3a** with a yield of 57%.

To a solution of **3c** (0.13 g, 0.35 mmol) in AcOH (10 mL) was added Fe (0.23 g), and the mixture was heated for 7 h at 70 °C. The reaction mixture was concentrated and the residue was purified by silica gel column chromatography (CH_2Cl_2/methanol = 300:1 ~ 50:1) and washed with methanol to give 0.02 g (0.06 mmol) of **4e** as a yellow solid with a yield of 17%.

Compound **5c** was prepared from **4e** in a manner similar to that described for compound **5a** with a yield of 75%. Mp: 291–292 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.66 (s, 3H), 3.85 (s, 3H), 3.86 (s, 3H), 6.72 (d, J = 15.4 Hz, 1H), 6.98 (t, J = 7.3 Hz, 1H), 7.32 (t, J = 7.6 Hz, 2H), 7.53 (m, 6H), 7.67 (d, J = 8.6 Hz, 2H), 8.43 (s, 1H), 8.87 (s, 1H), 9.06 (s, 1H), 11.89 (s, 1H); MS (TOF) m/z = 459 (M^+ + H). Anal. calcd for $C_{26}H_{25}N_3O_5 \cdot 0.01H_2O$: C, 65.39; H, 5.70; N, 8.80. Found: C, 65.10; H, 5.46; N, 8.68.

***N*-(2-Acetyl-4,5-dimethoxyphenyl)-3-(4-((phenylamino)-carbonylamino)phenyl)propanamide (5d).** Compound **5d** was prepared from **2** in a manner similar to that described for compound **5a** with a yield of 57% by three steps. Mp: 218–219 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.60 (s, 3H), 2.67 (t, J = 7.6 Hz, 2H), 2.89 (t, J = 7.6 Hz, 2H), 3.82 (s, 6H), 6.95 (t, J = 7.3 Hz, 1H), 7.16 (d, J = 8.4 Hz, 2H), 7.27 (t, J = 8.1 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H), 7.43 (s, 1H), 7.44 (d, J = 8.4 Hz, 2H), 8.24 (s, 1H), 8.59 (s, 1H), 8.64 (s, 1H), 11.67 (s, 1H); MS (TOF) m/z = 462 (M^+ + H). Anal. calcd for $C_{24}H_{24}N_4O_5$: C, 67.66; H, 5.90; N, 9.10. Found: C, 67.40; H, 6.07; N, 9.01.

***N*-(2-Acetyl-4,5-dimethoxyphenyl)-4-(4-((phenylamino)-carbonylamino)phenyl)butanamide (5e).** Compound **5e** was prepared from **2** in a manner similar to that described for compound **5a** with a yield of 60% by three steps. Mp: 183–185 °C;^{1}H NMR (DMSO-d_6) δ ppm: 1.90 (5, J = 7.3 Hz, 2H), 2.38 (t, J = 7.3 Hz, 2H), 2.51 (t, J = 7.3 Hz, 2H), 2.62 (s, 3H), 3.82 (s, 6H), 6.95 (t, J = 7.3 Hz, 3H), 7.12 (d, J = 8.9 Hz, 2H), 7.26 (t, J = 7.8 Hz, 2H), 7.37 (d, J = 8.4 Hz, 2H), 7.44 (s, 1H), 7.45 (d, J = 8.4 Hz, 2H), 8.27 (s, 1H), 8.80 (s, 1H), 8.85 (s, 1H), 11.68 (s, 1H); MS (TOF) m/z = 476 (M^+ + H). Anal. calcd for $C_{27}H_{29}N_3O_5 \cdot 0.6H_2O$: C, 66.67; H, 6.26; N, 8.64. Found: C, 66.51; H, 6.15; N, 8.62 .

***N*-(2-Acetyl-4,5-dimethoxyphenyl)-3-(4-(phenylcarbonyl-amino)phenyl)propanamide (5f).** To a solution of **4c** (0.05 g, 0.15 mmol) in CH_2Cl_2 (20 mL) were added benzoyl chloride (0.03 g, 0.21 mmol) and triethylamine (0.03 g, 0.30 mmol), and the mixture was stirred for 30 min at room temperature. The reaction mixture was poured into saturated $NaHCO_3$ aq and extracted with CH_2Cl_2, after which the organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2/methanol = 500:1 ~ 200:1) and washed with methanol to give 0.03 g (0.07 mmol) of **5f** as a white solid with a yield of 45%. Mp: 211–213 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.60 (s, 3H), 2.70 (t, J = 7.6 Hz, 2H), 2.95 (t, J = 7.6 Hz, 2H), 3.82 (s, 6H), 7.24 (d, J = 8.6 Hz, 2H), 7.43 (s, 1H), 7.56 (m, 3H), 7.68 (dd, J_1 = 8.6 Hz, J_2 = 1.6 Hz, 2H), 7.93 (dd, J_1 = 8.6 Hz, J_2 = 1.6 Hz, 2H), 8.24 (s, 1H), 10.19 (s, 1H), 11.68 (s, 1H); MS (TOF) m/z = 447 (M^+ + H). Anal. calcd for $C_{26}H_{26}N_2O_5 \cdot 0.2H_2O$: C, 69.38; H, 5.91; N, 6.23. Found: C, 69.11; H, 5.87; N, 6.09.

***N*-(2-Acetyl-4,5-dimethoxyphenyl)-3-(4-(2-phenylacetyl-amino)phenyl)propanamide (5g).** Compound **5g** was prepared from **4c** in a manner similar to that described for compound **5f** with a yield of40%. Mp: 162–165 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.58 (s, 3H), 2.66 (t, J = 7.6 Hz, 2H), 2.88 (t, J = 7.6 Hz, 2H), 3.61 (s, 2H), 3.82 (s, 6H), 7.18 (d, J = 8.4 Hz, 2H), 7.31 (m, 5H), 7.41 (s, 1H), 7.49 (d, J = 8.4 Hz, 2H), 8.21 (s, 1H), 10.10 (s, 1H), 11.65 (s, 1H); MS (TOF) m/z = 461 (M^+ + H). Anal. calcd for $C_{27}H_{28}N_2O_5$: C, 70.42; H, 6.13; N, 6.08. Found: C, 70.40; H, 6.13; N, 6.08.

***N*-(2-Acetyl-4,5-dimethoxyphenyl)-3-(4-(phenoxycarbo-nylamino)phenyl)propanamide (5 h).** To a suspension of 1,1-carbonyldiimidazole (0.34 g, 2.10 mmol) in tetrahydrofuran (5 mL) was added phenol (0.18 g, 0.18 mmol) at 4 °C and the mixture stirred for 3 h at room

temprature. The resulting solution was added to a suspension of **4c** (0.13 g, 0.38 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.06 g, 0.39 mmol) in tetrahydrofuran (5 mL). After heating for 3 h at 50 °C, the mixture was concentrated and the residue was poured into water and extracted with CH_2Cl_2, and the organic layer was then washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2/methanol = 300:1 ~ 100:1) and washed with methanol to give 0.03 g (0.06 mmol) of **5h** as a white solid with a yield of 11%. Mp: 180–182 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.58 (t, J = 7.3 Hz, 2H), 2.59 (s, 3H), 2.76 (t, J = 7.3 Hz, 2H), 3.82 (s, 6H), 6.46 (d, J = 8.1 Hz, 2H), 6.76 (m, 3H), 6.88 (d, J = 8.1 Hz, 2H), 7.15 (t, J = 8.1 Hz, 2H), 7.43 (s, 1H), 8.24 (s, 1H), 9.25 (s, 1H), 11.66 (s, 1H); MS (TOF) m/z = 463 (M^+ + H). Anal. calcd for $C_{26}H_{26}N_2O_6$: C, 67.52; H, 5.67; N, 6.06. Found: C, 67.46; H, 5.71; N, 6.09.

***N*-(2-Acetyl-4,5-dimethoxyphenyl)-3-(4-(((2-nitrophenyl)-amino)carbonylamino)phenyl)propanamide (5i).** Compound **5d** was prepared from **4c** in a manner similar to that described for compound **5a** with a yield of 52%. Mp: 221–222 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.60 (s, 3H), 2.68 (t, J = 7.3 Hz, 2H), 2.90 (t, J = 7.3 Hz, 2H), 3.82 (s, 6H), 7.16 (m, 3H), 7.40 (m, 3H), 7.69 (dt, J_d = 1.6 Hz, J_t = 8.4 Hz, 1H), 8.09 (dd, J_1 = 1.4 Hz, J_2 = 8.4 Hz, 1H), 8.25 (s, 1H), 8.31 (d, J = 8.4 Hz, 1H), 9.58 (s, 1H), 9.79 (s, 1H), 11.69 (s, 1H). Anal. calcd for $C_{26}H_{26}N_4O_7 \cdot 0.3H_2O$: C, 61.00; H, 5.24; N, 10.92. Found: C, 61.05; H, 5.19; N, 10.88.

***N*-(2-Acetyl-4,5-dimethoxyphenyl)-3-(4-(((3-nitrophenyl)-amino)carbonylamino)phenyl)propanamide (5j).** Compound **5j** was prepared from **4c** in a manner similar to that described for compound **5a** with a yield of 80%. Mp: 188–190 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.60 (s, 3H), 2.69 (t, J = 7.3 Hz, 2H), 2.90 (t, J = 7.3 Hz, 2H), 3.82 (s, 6H), 7.19 (d, J = 8.9 Hz, 2H), 7.40 (m, 3H), 7.56 (t, J = 8.1 Hz, 1H), 7.69 (d, J = 8.4 Hz, 1H), 7.80 (dd, J_1 = 1.9 Hz, J_2 = 8.4 Hz, 1H), 8.24 (s, 1H), 8.56 (m, 1H), 8.78 (s, 1H), 9.20 (s, 1H), 11.68 (s, 1H). Anal. calcd for $C_{26}H_{26}N_4O_7$: C, 61.65; H, 5.17; N, 11.06. Found: C, 61.56; H, 5.11; N, 11.05.

***N*-(2-Acetyl-4,5-dimethoxyphenyl)-3-(4-(((4-nitrophenyl)-amino)carbonylamino)phenyl)propanamide (5k).** Compound **5k** was prepared from **4c** in a manner similar to that described for compound **5a** with a yield of 92%. Mp: 195–196 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.51 (s, 3H), 2.68 (t, J = 7.6 Hz, 2H), 2.90 (t, J = 7.3 Hz, 2H), 3.85 (s, 6H), 7.19 (d, J = 7.3 Hz, 2H), 7.40 (m, 3H), 7.68 (d, J = 9.5 Hz, 2H), 8.20 (m, 3H), 8.86 (s, 1H), 9.42 (s, 1H), 11.68 (s, 1H). Anal. calcd for $C_{26}H_{26}N_4O_7 \cdot 0.1H_2O$: C, 61.43; H, 5.20; N, 11.02. Found: C, 61.23; H, 5.17; N, 11.11.

***N*-(2-Acetyl-4,5-dimethoxyphenyl)-3-(4-(((2-aminophenyl)-amino)carbonylamino)phenyl)propanamide (5l).** To a solution of **5i** (0.11 g , 0.22 mmol) in tetrahydrofuran (20 mL) was added 5% Pd/C (0.02 g), which was then stirred at room temperature under a H_2 atmosphere for 14 h. The reaction mixture was filtered, concentrated and washed with methanol to give 0.07 g (0.06 mmol) of **5l** as a white solid with a yield of 66%. Mp: 184–186 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.60 (s, 3H), 2.67 (t, J = 7.3 Hz, 2H), 2.88 (t, J = 7.3 Hz, 2H), 3.82 (s, 6H), 4.76 (s, 2H), 6.56 (dt, J_d = 1.4 Hz, J_t = 7.3 Hz, 1H), 6.72 (dd, J_1 = 1.4 Hz, J_2 = 7.8 Hz, 1H), 6.83 (dt, J_d = 1.4 Hz, J_t = 7.8 Hz, 1H), 7.15 (d, J = 8.4 Hz, 2H), 7.36 (m, 3H), 7.43 (s, 1H), 7.71 (s, 1H), 8.24 (s, 1H), 8.69 (s, 1H), 11.66 (s, 1H); MS (TOF) m/z = 476 (M^+ + H). Anal. calcd for $C_{26}H_{28}N_4O_5$: C, 65.53; H, 5.92; N, 11.76. Found: C, 65.30; H, 5.97; N, 11.83.

***N*-(2-Acetyl-4,5-dimethoxyphenyl)-3-(4-(((3-aminophenyl)-amino)carbonylamino)phenyl)propanamide (5m).** Compound **5m** was prepared from **5j** in a manner similar to that described for compound **5a** with a yield of 73%. Mp: 208–209 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.60 (s, 3H), 2.67 (t, J = 7.3 Hz, 2H), 2.88 (t, J = 7.3 Hz, 2H), 3.82 (s, 6H), 5.01 (s, 2H), 6.17 (d, J = 9.5 Hz, 1H), 6.54 (d, J = 8.6 Hz, 1H), 6.76 (s, 1H), 6.87 (t, J = 7.8 Hz, 1H), 7.15 (d, J = 8.1 Hz, 2H), 7.34 (d, J = 8.1 Hz, 2H), 7.39 (s, 1H), 8.14 (s, 1H), 8.40 (s, 1H), 8.55 (s, 1H), 11.67 (s, 1H); MS (TOF) m/z = 476 (M^+ + H). Anal. calcd for $C_{26}H_{28}N_4O_5 \cdot 0.4H_2O$: C, 64.58; H, 5.96; N, 11.59. Found: C, 64.70; H, 5.71; N, 11.54.

***N*-(2-Acetyl-4,5-dimethoxyphenyl)-3-(4-(((4-aminophenyl)-amino)carbonylamino)phenyl)propanamide (5n).** Compound **5n** was prepared from **5k** in a manner similar to that described for compound **5a** with a yield of 26%. Mp: > 300 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.60 (s, 3H), 2.66 (t, J = 7.6 Hz, 2H), 2.87 (t, J = 7.3 Hz, 2H), 3.82 (s, 6H), 4.57 (s, 2H), 6.49 (d, J = 8.4 Hz, 2H), 7.05 (d, J = 8.1 Hz, 2H), 7.13 (d, J = 8.6 Hz, 2H), 7.32 (d, J = 8.6 Hz, 2H), 7.43 (s, 1H), 8.10 (s, 1H), 8.24 (s, 1H), 9.40 (s, 1H), 11.67 (s, 1H); MS (TOF) m/z = 476 (M^+ + H). Anal. calcd for $C_{26}H_{28}N_4O_5$: C, 63.60; H, 6.08; N, 11.41. Found: C, 63.62; H, 5.92; N, 11.31.

***N*-(2-Acetyl-4,5-dimethoxyphenyl)-3-(4-(((4-fluorophenyl)-amino)carbonylamino)phenyl)propanamide (5o).** Compound **5o** was prepared from **4c** in a manner similar to that described for compound **5a** with a yield of 43%. Mp: 210–211 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.60 (s, 3H), 2.68 (t, J = 7.6 Hz, 2H), 2.89 (t, J = 7.6 Hz, 2H), 3.82 (s, 3H), 3.84 (s, 3H), 7.09 (d, J = 7.3 Hz, 2H), 7.15 (d, J = 8.6 Hz, 2H), 7.35 (d, J = 8.6 Hz, 2H), 7.43 (m, 3H), 8.24 (s, 1H), 8.61 (s, 1H), 8.70 (s, 1H), 11.67 (s, 1H); MS (TOF) m/z = 480 (M^+ + H). Anal. calcd for $C_{26}H_{26}FN_3O_5$: C, 65.13; H, 5.47; N, 8.76. Found: C, 65.12; H, 5.49; N, 8.72.

***N*-(2-Acetyl-4,5-dimethoxyphenyl)-3-(4-(((4-acetylphenyl)-amino)carbonylamino)phenyl)propanamide (5p).** Compound **5p** was prepared from **4c** in a manner similar to that described for compound **5a** with a yield of 98%. Mp: 210–212 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.50 (s, 6H), 2.68 (t, J = 7.6 Hz, 2H), 2.89 (t, J = 7.6 Hz, 2H), 3.82 (s, 6H), 6.18 (d, J = 8.6 Hz, 2H), 7.37 (d, J = 8.6 Hz, 2H), 7.43 (s, 1H), 7.57 (d, J = 8.6 Hz, 2H), 7.90 (d, J = 8.6 Hz, 2H), 8.24 (s, 1H), 8.78 (s, 1H), 9.13 (s, 1H), 11.67 (s, 1H); MS (TOF) m/z = 504 (M^+ + H). Anal.

calcd for $C_{28}H_{29}N_3O_6 \cdot 0.1H_2O$: C, 66.54; H, 5.82; N, 8.32. Found: C, 66.36; H, 5.85; N, 8.24.

***N*-(2-Acetyl-4,5-dimethoxyphenyl)-3-(4-(((4-methylphenyl)-amino)carbonylamino)phenyl)propanamide (5q).** Compound **5q** was prepared from **4c** in a manner similar to that described for compound **5a** with a yield of 74%. Mp: 185–190 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.23 (s, 3H), 2.60 (s, 3H), 2.66 (t, J = 7.6 Hz, 2H), 2.88 (t, J = 7.6 Hz, 2H), 3.84 (s, 6H), 7.07 (d, J = 8.6 Hz, 2H), 7.15 (d, J = 8.6 Hz, 2H), 7.31 (d, J = 6.5 Hz, 2H), 7.34 (d, J = 6.5 Hz, 2H), 7.43 (s, 1H), 8.24 (s, 1H), 8.55 (s, 1H), 8.57 (s, 1H), 11.67 (s, 1H); MS (TOF) m/z = 476 (M^+ + H). Anal. calcd for $C_{27}H_{29}N_3O_5 \cdot 0.6H_2O$: C, 66.68; H, 6.26; N, 8.64. Found: C, 66.83; H, 6.14; N, 8.48.

***N*-(2-Acetyl-4,5-dimethoxyphenyl)-3-(4-(((4-methoxyphenyl)amino)carbonylamino)phenyl)propanamide (5r).** Compound **5r** was prepared from **4c** in a manner similar to that described for compound **5a** with a yield of 56%. Mp: 209–212 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.60 (s, 3H), 2.70 (t, J = 7.6 Hz, 2H), 2.88 (t, J = 7.6 Hz, 2H), 3.71 (s, 3H), 3.84 (s, 6H), 6.85 (d, J = 9.2 Hz, 2H), 7.15 (d, J = 8.6 Hz, 2H), 7.34 (d, J = 8.9 Hz, 4H), 7.43 (s, 1H), 8.24 (s, 1H), 8.50 (s, 1H), 8.56 (s, 1H), 11.67 (s, 1H); MS (TOF) m/z = 492 (M^+ + H). Anal. calcd for $C_{27}H_{29}N_3O_6$: C, 65.97; H, 5.95; N, 8.55. Found: C, 65.95; H, 5.98; N, 8.59.

***N*-(2-Acetyl-4,5-dimethoxyphenyl)-3-(4-(((3,4,5-trimethoxyphenyl)amino)carbonylamino)phenyl)propanamide (5s).** Compound **5s** was prepared from **4c** in a manner similar to that described for compound **5a** with a yield of 23%. Mp: 201–203 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.51 (s, 3H), 2.67 (t, J = 7.6 Hz, 2H), 2.89 (t, J = 7.6 Hz, 2H), 3.60 (s, 3H), 3.74 (s, 6H), 3.82 (s, 6H), 6.78 (s, 2H), 7.15 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H), 7.43 (s, 1H), 8.24 (s, 1H), 8.54 (s, 1H), 8.60 (s, 1H), 11.68 (s, 1H); MS (TOF) m/z = 552 (M^+ + H). Anal. calcd for $C_{29}H_{33}N_3O_8$: C, 63.15; H, 6.03; N, 7.62. Found: C, 62.91; H, 6.09; N, 7.54.

***N*-(2-Acetyl-4,5-dimethoxyphenyl)-3-(4-(((4-pyridyl)amino)carbonylamino)phenyl)propanamide (5t).** Compound **5t** was prepared from **4c** in a manner similar to that described for compound **5a** with a yield of 74%. Mp: 201–202 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.60 (s, 3H), 2.68 (t, J = 7.3 Hz, 2H), 2.90 (t, J = 7.3 Hz, 2H), 3.82 (s, 6H), 7.19 (d, J = 8.4 Hz, 2H), 7.40 (m, 5H), 8.24 (s, 1H), 8.34 (d, J = 6.5 Hz, 2H), 8.83 (s, 1H), 9.11 (s, 1H), 11.66 (s, 1H); MS (TOF) m/z = 463 (M^+ + H). Anal. calcd for $C_{25}H_{26}N_4O_5 \cdot 0.1H_2O$: C, 64.67; H, 5.69; N, 12.07. Found: C, 64.51; H, 5.69; N, 11.98.

***N*-(2-Acetyl-4,5-dimethoxyphenyl)-3-(4-((benzylamino)-carbonylamino)phenyl)propanamide (5u).** Compound **5u** was prepared from **4c** in a manner similar to that described for compound **5a** with a yield of 93%. Mp: 189–190 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.59 (s, 3H), 2.65 (t, J = 7.3 Hz, 2H), 2.86 (t, J = 7.3 Hz, 2H), 3.81 (s, 6H), 4.27 (m, 2H), 6.60 (t, J = 7.3 Hz, 1H), 7.10 (d, J = 8.4 Hz, 2H), 7.30 (m, 9H), 7.42 (s, 1H), 8.23 (s, 1H), 8.50 (s, 1H), 11.66 (s, 1H); MS (TOF) m/z = 476 (M^+ + H). Anal. calcd for $C_{27}H_{29}N_3O_5$: C, 68.19; H, 6.15; N, 8.84. Found: C, 67.98; H, 6.21; N, 8.82.

***N*-(2-Acetyl-4,5-dimethoxyphenyl)-3-(4-((butyl amino)carbonylamino)phenyl)propanamide (5w).** Compound **5w** was prepared from **4c** in a manner similar to that described for compound **5a** with a yield of 47%. Mp: 182–184 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.88 (t, J = 7.0 Hz, 3H), 1.32 (m, 4H), 2.60 (s, 3H), 2.70 (t, J = 7.3 Hz, 2H), 2.85 (t, J = 7.3 Hz, 2H), 3.07 (q, J = 6.5 Hz, 2H), 3.82 (s, 6H), 6.08 (t, J = 5.6 Hz, 1H), 7.08 (d, J = 8.6 Hz, 2H), 7.27 (d, J = 8.6 Hz, 2H), 7.42 (s, 1H), 8.23 (s, 1H), 8.31 (s, 1H), 11.66 (s, 1H); MS (TOF) m/z = 442 (M^+ + H). Anal. calcd for $C_{24}H_{31}N_3O_5 \cdot 0.2H_2O$: C, 64.76; H, 7.11; N, 9.44. Found: C, 64.76; H, 7.18; N, 9.30.

***N*-(2-Acetyl-4,5-dimethoxyphenyl)-3-(4-((cyclohexylamino)carbonylamino)phenyl)propanamide (5x).** Compound **5x** was prepared from **4c** in a manner similar to that described for compound **5a** with a yield of 59%. Mp: 204–206 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 1.32 (m, 10H), 2.59 (s, 3H), 2.64 (t, J = 7.3 Hz, 2H), 2.85 (t, J = 7.3 Hz, 2H), 3.43 (m, 1H), 3.82 (s, 6H), 6.03 (d, J = 7.8 Hz, 1H), 7.08 (d, J = 8.4 Hz, 2H), 7.26 (d, J = 8.4 Hz, 2H), 7.42 (s, 1H), 8.21 (s, 1H), 8.23 (s, 1H), 11.65 (s, 1H); MS (TOF) m/z = 468 (M^+ + H). Anal. calcd for $C_{26}H_{33}N_3O_5 \cdot 0.3H_2O$: C, 66.02; H, 7.16; N, 8.89. Found: C, 65.96; H, 7.16; N, 8.83.

Primary culture of smooth muscle cells and endothelial cells

Human coronary artery smooth muscle cells (SMCs), human coronary artery endothelial cells (ECs), and their culture kits were obtained from Clonetics Corp. (San Diego, CA, USA). SMCs were cultured in basal medium (SmBM) containing 5% fetal bovine serum, human epidermal growth factor (0.5 ng/mL), insulin (5 μg/mL), human fibroblast growth factor (2 ng/mL), gentamicin (50 μg/mL), and amphotericin-B (50 pg/mL). ECs were cultured in basal medium (EBM) containing 5% fetal bovine serum (FBS), human epidermal growth factor (10 ng/mL), hydrocortisone (1 μg/mL), bovine brain extract (12 μg/mL), gentamicin (50 μg/mL), and amphotericin-B (50 pg/mL). After 3–5 days in culture at 37 °C in 5% CO_2–95% air, both cell types were subcultured by trypsinization and propagated in each complete medium described above.

Determination of DNA synthesis in smooth muscle cells

SMCs from passage 1-3 were seeded into 96-well plates (3×10^4 cells/well) in the complete medium described above, and cultured for 16–18 h at 37 °C in 5% CO_2–95% air. Then the complete medium was replaced with basal medium (SmBM) containing 20 ng/mL human PDGF-BB (Carbiochem Corp; San Diego, CA, USA) and various concentrations of the test compounds. After 24 h, 1 μCi/mL ^{3}H-thymidine was added to the medium, and the cells were incubated for 4 h at 37 °C in

5% CO_2–95% air. Then the cells were harvested by trypsinization, and the amount of radioactive thymidine incorporated into the DNA was determined by scintillation counting.

Determination of DNA synthesis in endothelial cells

ECs from the second passage were seeded into 96-well plates (3×10^3 cells/well) in the above complete medium, and allowed to attach to the plates for 4 h at 37 °C in 5% CO_2–95% air. Then various concentrations of test compounds were added to the complete medium, and the cells were cultured at 37 °C in 5% CO_2–95% air. After 3 days, DNA synthesis was determined during the last 4 h of the 3-day culture.

HUVECs microcapillary assay

The formation of capillary-like tubular structures was assessed in Matrigel-coated multiwell plates. Aliquots of Matrigel (Becton Dickinson Labware; 100 μL) were distributed as a thin layer in 48-well cell culture plates (Becton Dickinson Labware) and left to polymerize at 37 °C for 30 min. Human umbilical vein endothelial cells (HUVECs, Clonetics; 4×10^4/500 μL) were seeded on the Matrigel and cultured in EGM-2 medium (Clonetics) containing various concentrations of test compounds at 37 °C for 24 h in a humidified atmosphere containing 5% CO_2. After incubation, the tubular formation was observed with a phase-contrast microscope and microphotographed at ×40 magnification.

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