Synthesis of Potent and Selective Inhibitors against the Proliferation of Human Coronary Artery Smooth Muscle Cells

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A series of diarylamide urea derivatives were synthesized and evaluated for their inhibitory activities against human coronary artery smooth muscle cells (SMCs) and human coronary artery endothelial cells (ECs). Compound 2h was much superior to Tranilast, in terms of both the potency of its inhibitory activity toward the proliferation of SMCs and the cell selectivity.

Key words diarylamide urea; proliferation; coronary artery smooth muscle cell; coronary artery endothelial cell

Proliferation of smooth muscle cells (SMCs) plays an important role in restenosis and in progression of atherosclerosis.[2)] Tranilast has been shown to inhibit platelet-derived growth factor (PDGF)-induced migration and proliferation in both SMCs[3)] and endothelial cells (ECs). And the multicenter, randomized, double-blinded placebo-controlled trials demonstrated the potent preventive effect on restenosis after percutaneous transluminal coronary angioplasty (PTCA) in Japanese patients.[4,5)] [at the phase III trial,[5)] restenosis rate 18.8% (Tranilast 600 mg/d for 3 months, $n=112$) versus 44.1% (placebo, $n=127$); $p=0.00005$] But some patients had suffered liver dysfunction in these trials. We speculated that these frequent and severe side effects in these trials might have been caused by the high dose of Tranilast. In addition, the selective inhibitors of the proliferation of SMCs over that of ECs was more preferable for the treatment of restenosis than the non-selective inhibitors like Tranilast.[6)] So, we had been trying to search more potent and SMCs-selective compounds by modification of Tranilast.

We previously reported a series of diarylamide derivatives, exhibiting potent and highly selective inhibition against SMCs proliferation induced by PDGF-BB.[7)] We conducted that structure–activity relationship (SAR) study of the substituents at the A and B rings, and found potent inhibitor, **1a**, which had about 30-fold stronger activity than Tranilast. In that case, the linker between A and B rings was not so influenced on the inhibitory activities (**1a**—**c**). In order to find more potent inhibitor, we also reported that the introduction of a ureido group to the B ring of our diarylamide derivatives make the inhibitory activity more potent and keep the selectivity for SMCs and **2b** showed the most potent inhibitory activity for SMCs (IC_{50} = 40 nM), which was 20- and 600-fold stronger than **1a** and Tranilast respectively.[8)] On the basis of these results (Fig. 1), we tried to confirm the effect of the linker between these rings against the proliferation of SMCs. In this paper, we report the results of the further modification of these urea derivatives.

Chemistry

The syntheses of desired urea derivatives **2c**—**s** are outlined in Chart 1. The condensation of **3**[8)] with corresponding acid halides was performed in the presence of triethylamine to provide **4a**—**c**. Acid halide **10** was prepared from **9** with thionyl chloride, which was obtained by the condensation of **8** with ethyl bromoacetate followed by basic hydrolysis. Then, catalytic hydrogenation, followed by the condensation of **5a**—**c** with the corresponding isocyanates gave the desired urea derivatives **2c**—**s**. Compound **2e** was obtained by the condensation of **3** with **7** by using 2-chloro-1,3-dimethylimidazolidium hexafluorophosphate (CIP)[9,10)] as a condensation reagent.

Biology

A series of these compounds were evaluated for their inhibitory activities on PDGF-induced proliferation of human coronary artery SMCs and fetal bovine serum (FBS)-induced the proliferation of human coronary artery ECs. Inhibition of the proliferation of these cells was determined by ^{3}H-thymidine incorporation as previously reported method.[8)]

Results and Discussion

The inhibitory activities of **2c**—**s** on PDGF-induced proliferation of SMCs and FBS-induced proliferation of ECs were shown in Tables 1 and 2.

Table 1 presented the results of the SAR study on the linker X between A and B rings. A methylene (**2c**) or vinyl (**2e**) groups at X position showed almost the same activity with **2a**. This tendency was easily expected from the result of

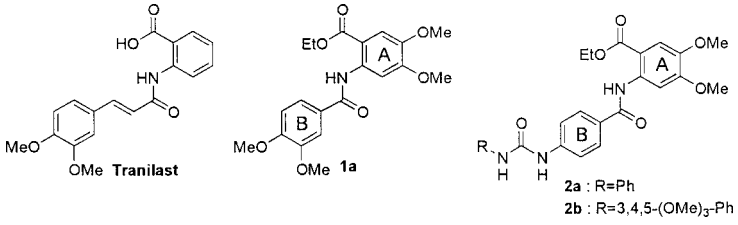

Fig 1. Structure of Tranilast and Diarylamide Derivatives

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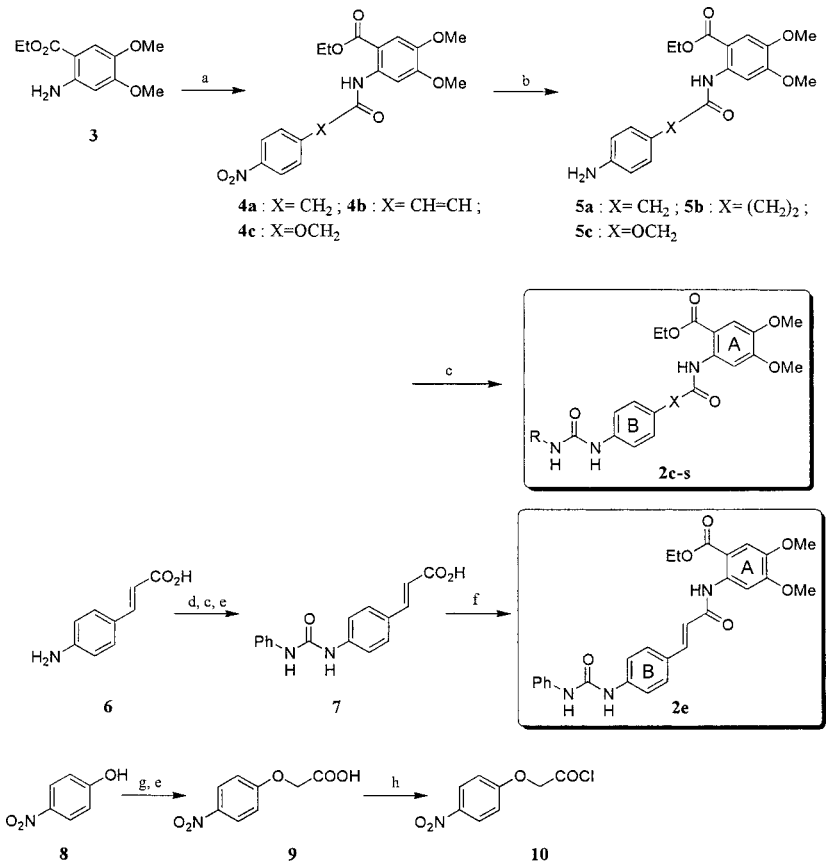
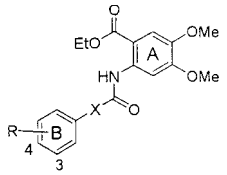


Chart 1. Synthesis of the Compounds **2a**—**s**

Reagents and conditions: a) (4-NO_2–Ph)–X–COCl, Et_3N, CH_2Cl_2; b) H_2, 5% Pd/C, EtOH; c) RNCO, DMAP, THF; d) EtOH, H_2SO_4; e) NaOH, H_2O, MeOH; f) CIP, HOBt, $^i Pr_2NEt$, CH_2Cl_2 then **3**; g) $BrCH_2CO_2Et$, K_2CO_3, DMF; h) $SOCl_2$.

Table 1. IC_{50} Values of the Diarylamide Derivatives for the Inhibition of the Proliferation of SMCs and ECs

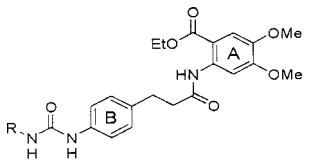


No.	X	R	SMCs IC_{50} (nM)[a]	ECs IC_{50} (nM)[b]	[ECs]/[SMCs]
Tranilast			24500	19100	0.8
1a	—	3,4-$(OMe)_2$	850	6000	7
1b	CH_2	3,4-$(OMe)_2$	800	4700	6
1c	(*E*)-CH=CH	3,4-$(OMe)_2$	1200	4100	4
2a	—	4-NHCONHPh	400	1500	4
2b	—	4-NHCONH(3,4,5-$(OMe)_3$-Ph)	40	54000	140
2c	CH_2	4-NHCONHPh	260	290	1
2d	$(CH_2)_2$	4-NHCONHPh	12	14	1
2e	(*E*)-CH=CH	4-NHCONHPh	410	1200	3
2f	OCH_2	4-NHCONHPh	10000	3800	0.4

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

compounds **1a**—**c**. But an ethylene group (**2d**) showed much more potent inhibition than **2a**. Replacement of the ethylene group by methylenoxy group (**2f**) make it reduced the activity. This result suggested that not only the distance but also the orientation between these rings might be important factors. Since we could not define the most stable conformation from our MOPAC study (data not shown), the linker might need to be flexible to some extent. And we also evaluated the effects of these compounds against the proliferation of ECs but introducing the substituents such as the ethylene group at X position makes the selectivity for SMCs lower. But in the case of **2a** and **2b**, no substitution at X position, 3,4,5-trimethoxyphenyl substitution at the urea moiety enhanced the inhibitory activity and the selectivity as reported previ-

Table 2. IC_{50} Values of the Compounds **2d**, **2g**—**s** for the Inhibition of the Proliferation of SMCs and ECs



No.	R	SMCs IC_{50} (nM)[a]	ECs IC_{50} (nM)[b]	[ECs]/[SMCs]
Tranilast		25000	19000	0.8
2d	Ph	12	14	1
2g	3,4,5-$(OMe)_3$-Ph	580	1100	2
2h	4-NO_2-Ph	0.1	1.7	17
2i	3-NO_2-Ph	15	23	2
2j	2-NO_2-Ph	30	53	2
2k	4-OMe-Ph	40	200	5
2l	3-OMe-Ph	54	45	0.8
2m	2-OMe-Ph	120	200	2
2n	4-NH_2-Ph	57	76	1
2o	4-Me-Ph	28	53	2
2p	4-F-Ph	7	15	2
2q	4-Ac-Ph	67	200	3
2r	Bn	64	300	5
2s	*c*-Hex	11	30	3

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

ously.[8] So, the further modification was conducted based on **2d**.

Table 2 presents the result of the SAR study on the substituents at the urea moiety with the ethylene substitution at X position. Considering the result of **2a** and **2b**,[8] we evaluated **2g** that had the 3,4,5-trimethoxyphenyl group at the urea moiety like **2b**. But in this case, contrary to our expectation, the trimethoxyphenyl substitution resulted in decreased the activity. Therefore, we examined the effect of the substituents at the urea moiety again.

At first, we examined the position of the substituent on the B ring by introducing electron-donating group (methoxy group) and electron-withdrawing group (nitro group), and found that the *para* position was superior to the other positions in both cases (**2h**—**j**, **2k**—**m**). Next, we tried to study the effect of the substituents at the *para* position of the phenyl ring and the order of potency was nitro (**2h**) > fluorine (**2p**), hydrogen (**2d**) > methyl (**2o**), methoxy (**2k**) > acetyl (**2q**), amino groups (**2n**). It seemed that the compact electron-withdrawing group would be preferable to show the potent activity. Benzyl group (**2r**) instead of the phenyl ring gave weak activity. But, cyclohexyl group (**2s**) was almost the same activity with **2d**. These results suggested that the size of the substituents might influence the activity as well as the electronic effects. As a result, **2h** showed the most potent activity (IC_{50}=0.1 nM) with about 250000-fold more potent than Tranilast and about 400-fold much more potent than even **2b** (40 nM), a second lead compound. We also evaluated the effect of these compounds against the proliferation of ECs and most of the evaluated compounds displayed a low selectivity ranging from 2 to 5 times for SMCs. This result showed that the ethylene group substitution at X position had the detrimental effect for the selectivity. But only **2h** displayed the selectivity by one order of magnitude, That is to say, the IC_{50} values of **2h** for SMCs and for ECs were 0.1 nM and 1.7 nM, respectively, displaying selectivity about 17-fold greater for SMCs. In contrast, the IC_{50} values of Tranilast toward SMCs and ECs were 25 μM and 19 μM, respectively, indicating a selectivity of about 0.8-fold for SMCs. So, compound **2h** was much superior to Tranilast in the strength of the activity and cell selectivity.

The mechanism of the cell selectivity between SMCs and ECs and target molecules of our derivatives have been unknown yet. In examining the mechanism of action of Tranilast, several researchers showed that Tranilast increased the levels of p21 and p53 protein and arrested SMCs at the G0/G1 phase.[11—14] On the basis of the structural resemblance between our compounds and Tranilast, the elucidation of target molecules of our derivatives is still underway.

In conclusion, in order to optimize the structure of our diarylamide derivatives, we conducted the SAR study mainly focused on the linker X between the A and B rings, and the substituents at the urea moiety. We found that **2h** had the strongest inhibitory activity against the proliferation of SMCs, which was 250000-fold more potent than Tranilast.

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 Yanako micro 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. TOFMS (Time-of-flight mass spectrometry) were recorded on a Kompact MALDI III spectrometer. Elemental analyses were performed by the Toray Research Center.

Ethyl 4,5-Dimethoxy-2-(3-(4-((*N*-phenylcarbamoyl)amino)phenyl)-propanoylamino)benzoate (2d) To a solution of **3** (0.10 g, 0.44 mmol) in CH_2Cl_2 (10 ml), 4-nitrocinnamoyl chloride (0.11 g, 0.52 mmol) and triethylamine (0.07 g, 0.70 mmol) were added, and the mixture was stirred for 4 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, concentrated and washed with methanol to give 0.17 g (0.42 mmol) of **4b** as a yellow solid with a yield of 96%. To a solution of **4b** (0.17 g, 0.42 mmol) in tetrahydrofuran (THF) (20 ml) was added 5% Pd/C (0.05 g), and the mixture was vigorously stirred under a H_2 atmosphere for 18 h. The reaction mixture was filtered and concentrated to give 0.15 g (0.40 mmol) of **5b** as a pale yellow solid with a yield of 95%. To a solution of **5b** (0.05 g, 0.13 mmol) and 4-dimethylaminopyridine (DMAP) (0.02 g, 0.16 mmol) in THF (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 (0.08 mmol) of **2d** as a white solid with a yield of 63%. mp 210—212 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.32 (3H, t, J=7.0 Hz), 2.68 (2H, t, J=7.3 Hz), 2.88 (2H, t, J=7.3 Hz), 3.76 (3H, s), 3.81 (3H, s), 4.31 (2H, q, J=7.0 Hz), 6.95 (1H, t, J=7.3 Hz), 7.16 (2H, d, J=8.6 Hz), 7.27 (2H, t, J=8.1 Hz), 7.40 (5H, m), 8.15 (1H, s), 8.65 (1H, s), 8.69 (1H, s), 10.74 (1H, s). *Anal.* Calcd for $C_{27}H_{29}N_3O_6$: C, 65.97; H, 5.95; N, 8.55. Found: C, 65.69; H, 5.99; N, 8.40.

Ethyl 4,5-Dimethoxy-2-(3-(4-((*N*-phenylcarbamoyl)amino)phenyl)prop-2-enoylamino)benzoate (2e) To a solution of **6** (1.12 g, 6.87 mmol) in ethanol (100 ml) was added H_2SO_4 (1 ml), and the mixture was refluxed for 8 h. To the reaction mixture on ice added saturated $NaHCO_3$ aq., adjust to the pH 8 and extracted with CH_2Cl_2, after which the organic layer was dried over $MgSO_4$, filtered and concentrated. To the solution of the resulting residue and DMAP (1.18 g, 9.66 mmol) in THF (30 ml) was added phenylisocyanate (1.15 g, 9.65 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 con-

centrated. The residue was washed with methanol to give 1.39 g (4.48 mmol) of a white solid. To the solution of the resulting solid (0.61 g, 1.97 mmol) in methanol (50 ml) was added 5% NaOH aq. (50 ml) and the mixture was stirred for 18 h. The reaction mixture was extracted with CH_2Cl_2, and the aqueous layer was acidified with 1 N HCl aq. to save a solid. The resulting solid was washed with water to give 0.50 g (1.77 mmol) of **7** as a white solid with a yield of 59% by 3 steps. To the suspension of **7** (0.10 g, 0.35 mmol) and *N*-hydroxybenzotriazole (HOBt) (6 mg, 0.04 mmol) in CH_2Cl_2 (30 ml) was added diisopropylethylamine (0.36 g, 2.79 mmol) on ice. Then the addition of 2-chloro-1,3-dimethylimidazolidium hexafluorophosphate (CIP) (0.20 g, 0.72 mmol) to the mixture was followed by that of **3** (0.13 g, 0.58 mmol). After stirring for 18 h at room temperature, the reaction mixture was poured into the saturated $NaHCO_3$ aq. and extracted with CH_2Cl_2. The organic layer was then washed with 1 N HCl aq. and brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2/methanol=100 : 1) and washed with methanol to give 0.06 g (0.12 mmol) of **2e** as a pale yellow solid with a yield of 21% from **3**. mp 294—298 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.35 (3H, t, J=7.0 Hz), 3.79 (3H, s), 3.85 (3H, s), 4.35 (2H, q, J=7.0 Hz), 6.76 (1H, d, J=15.7 Hz), 6.98 (1H, t, J=7.3 Hz), 7.29 (2H, t, J=8.4 Hz), 7.53 (6H, m), 7.67 (2H, d, J=8.6 Hz), 8.33 (1H, s), 8.91 (1H, s), 9.10 (1H, s), 10.97 (1H, s). *Anal.* Calcd for $C_{27}H_{27}N_3O_6$: C, 66.25; H, 5.58; N, 8.58. Found: C, 65.88; H, 5.64; N, 8.51.

Ethyl 4,5-Dimethoxy-2-(2-(4-((*N*-phenylcarbamoyl)amino)phenoxy)-acetylamino)benzoate (2f) To a solution of **8** (0.30 g, 2.16 mmol) and K_2CO_3 (0.18 g, 1.30 mmol) in *N*,*N*-dimethylformamide (DMF) (10 ml), ethyl bromoacetate (0.44 g, 2.63 mmol) was added, and the mixture was stirred for 18 h at room temperature. The reaction mixture was evaporated and poured into water to save a solid. The resulting solid was washed with water to give 0.46 g (2.04 mmol) of a white solid. To the solution of the resulting solid (0.20 g, 0.89 mmol) in methanol (10 ml) was added 5% NaOH aq. (10 ml) and the mixture was stirred for 18 h. The reaction mixture was extracted with CH_2Cl_2, and the aqueous layer was acidified to pH 4 with 1 N HCl aq. and extracted with CH_2Cl_2. The organic layer was concentrated to give 0.17 g (0.86 mmol) of **9** as a white solid with a yield of 92% by 2 steps. **9** (0.14 g, 0.71 mmol) was added to $SOCl_2$ (15 ml) and refluxed for 4 h. The reaction mixture was concentrated to give **10** as brown oil. To a solution of **3** (0.11 g, 0.49 mmol) in CH_2Cl_2 (10 ml), this residue **10** and triethylamine (0.08 g, 0.79 mmol) were added, 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, concentrated and washed with methanol to give 0.19 g (0.47 mmol) of **4c** as a yellow solid with a yield of 96%.

Compound **2f** was prepared from **4c** in a manner similar to that described for compound **2d** with a yield of 83% by 2 steps. mp 286—289 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.34 (3H, t, J=7.0 Hz), 3.79 (3H, s), 3.85 (3H, s), 4.35 (2H, q, J=7.0 Hz), 4.68 (2H, s), 6.94 (1H, t, J=7.3 Hz), 7.04 (2H, d, J=8.9 Hz), 7.26 (2H, t, J=7.8 Hz), 7.43 (5H, m), 8.42 (1H, s), 8.74 (1H, s), 8.80 (1H, s), 11.83 (1H, s). *Anal.* Calcd for $C_{26}H_{27}N_3O_7 \cdot 0.3H_2O$: C, 62.59; H, 5.58; N, 8.42. Found: C, 62.84; H, 5.55; N, 8.09.

Ethyl 4,5-Dimethoxy-2-(2-(4-((*N*-phenylamino)carbonylamino)phenyl)-acetylamino)benzoate (2c) Compound **2c** was prepared from **3** in a manner similar to that described for compound **2d** with a yield of 18% by 3 steps. mp 188—190 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.31 (3H, t, J=7.0 Hz), 3.67 (2H, s), 3.76 (3H, s), 3.80 (3H, s), 4.28 (2H, q, J=7.0 Hz), 6.96 (1H, t, J=7.3 Hz), 7.27 (4H, m), 7.43 (5H, m), 8.18 (1H, s), 8.74 (1H, s), 8.75 (1H, s), 10.80 (1H. s). *Anal.* Calcd for $C_{26}H_{27}N_3O_6 \cdot 0.4H_2O$: C, 64.42; H, 5.78; N, 8.67. Found: C, 64.60; H, 5.73; N, 8.60.

Ethyl 4,5-Dimethoxy-2-(3-(4-((*N*-(3,4,5-trimethoxyphenyl)carbamoyl)-amino)phenyl)propanoylamino)benzoate (2g) Compound **2g** was prepared from **5b** in a manner similar to that described for compound **2d** with a yield of 79%. mp 198—202 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.32 (3H, t, J=7.0 Hz), 2.68 (2H, t, J=7.3 Hz), 2.88 (2H, t, J=7.3 Hz), 3.60 (3H, s), 3.70 (6H, s), 3.76 (3H, s), 3.81 (3H, s), 4.30 (2H, q, J=7.0 Hz), 6.79 (2H, s), 7.15 (2H, d, J=8.9 Hz), 7.35 (2H, d, J=8.9 Hz), 7.39 (1H, s), 8.14 (1H, s), 8.58 (1H, s), 8.63 (1H, s), 10.74 (1H, s). MS (TOF) m/z: 582 (M^++H). *Anal.* Calcd for $C_{30}H_{35}N_3O_9$: C, 61.95; H, 6.07; N, 7.22. Found: C, 61.78; H, 6.07; N, 7.06.

Ethyl 4,5-Dimethoxy-2-(3-(4-((*N*-(4-nitrophenyl)carbamoyl)amino)-phenyl)propanoylamino)benzoate (2h) Compound **2h** was prepared from **5b** in a manner similar to that described for compound **2d** with a yield of 68%. mp 209—211 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.32 (3H, t, J=7.0 Hz), 2.69 (2H, t, J=7.3 Hz), 2.90 (2H, t, J=7.3 Hz), 3.77 (3H, s), 3.82 (3H, s), 4.31 (2H, q, J=7.0 Hz), 7.19 (2H, d, J=8.4 Hz), 7.39 (1H, s), 7.40 (2H, d, J=8.9 Hz), 7.69 (2H, d, J=9.2 Hz), 8.14 (1H, s), 8.18 (2H, d, J=9.2 Hz), 9.12 (1H, s), 9.70 (1H, s), 10.74 (1H, s). *Anal.* Calcd for $C_{27}H_{28}N_4O_8$: C, 60.44; H, 5.28; N, 10.44. Found: C, 60.22; H, 5.37; N, 10.17.

Ethyl 4,5-Dimethoxy-2-(3-(4-((*N*-(3-nitrophenyl)carbamoyl)amino)-phenyl)propanoylamino)benzoate (2i) Compound **2i** was prepared from **5b** in a manner similar to that described for compound **2d** with a yield of 45%. mp 219—220 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.32 (3H, t, J=7.0 Hz), 2.69 (2H, t, J=7.4 Hz), 2.89 (2H, t, J=7.3 Hz), 3.77 (3H, s), 3.82 (3H, s), 4.31 (2H, q, J=7.0 Hz), 7.18 (2H, d, J=8.4 Hz), 7.39 (1H, s), 7.40 (2H, d, J=5.9 Hz), 7.55 (1H, t, J=8.4 Hz), 7.72 (1H, d, J=9.2 Hz), 7.79 (1H, d, J=9.2 Hz), 8.14 (1H, s), 8.57 (1H, s), 9.04 (1H, s), 9.48 (1H, s), 10.74 (1H, s). *Anal.* Calcd for $C_{27}H_{28}N_4O_8$: C, 60.44; H, 5.28; N, 10.44. Found: C, 60.21; H, 5.35; N, 10.21.

Ethyl 4,5-Dimethoxy-2-(3-(4-((*N*-(2-nitrophenyl)carbamoyl)amino)-phenyl)propanoylamino)benzoate (2j) Compound **2j** was prepared from **5b** in a manner similar to that described for compound **2d** with a yield of 69%. mp 109—110 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.32 (3H, t, J=7.0 Hz), 2.69 (2H, t, J=7.3 Hz), 2.89 (2H, t, J=7.3 Hz), 3.77 (3H, s), 3.82 (3H, s), 4.34 (2H, q, J=7.0 Hz), 7.21 (3H, m), 7.39 (3H, m), 7.69 (1H, t, J=7.8 Hz), 8.08 (1H, d, J=8.4 Hz), 8.14 (1H, s), 8.28 (1H, d, J=8.4 Hz), 9.63 (1H, s), 9.82 (1H, s), 10.74 (1H, s). *Anal.* Calcd for $C_{27}H_{28}N_4O_8 \cdot 1.0H_2O$: C, 58.47; H, 5.45; N, 10.11. Found: C, 58.44; H, 5.47; N, 9.81.

Ethyl 4,5-Dimethoxy-2-(3-(4-((*N*-(4-methoxyphenyl)carbamoyl)amino)-phenyl)propanoylamino)benzoate (2k) Compound **2k** was prepared from **5b** in a manner similar to that described for compound **2d** with a yield of 29%. mp 217—220 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.32 (3H, t, J=7.0 Hz), 2.67 (2H, t, J=7.3 Hz), 2.88 (2H, t, J=7.3 Hz), 3.71 (3H, s), 3.77 (3H, s), 3.81 (3H, s), 4.30 (2H, q, J=7.0 Hz), 6.85 (2H, d, J=8.9 Hz), 7.15 (2H, d, J=8.6 Hz), 7.35 (5H, m), 8.14 (1H, s), 8.47 (1H, s), 8.54 (1H, s), 10.74 (1H, s); MS (TOF) m/z: 522 (M^++H). *Anal.* Calcd for $C_{28}H_{31}N_3O_7 \cdot 0.5H_2O$: C, 63.38; H, 6.08; N, 7.92. Found: C, 63.08; H, 6.04; N, 7.64.

Ethyl 4,5-Dimethoxy-2-(3-(4-((*N*-(3-methoxyphenyl)carbamoyl)amino)-phenyl)propanoylamino)benzoate (2l) Compound **2l** was prepared from **5b** in a manner similar to that described for compound **2d** with a yield of 45%. mp 185—188 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.32 (3H, t, J=7.0 Hz), 2.68 (2H, t, J=7.3 Hz), 2.88 (2H, t, J=7.3 Hz), 3.72 (3H, s), 3.77 (3H, s), 3.81 (3H, s), 4.30 (2H, q, J=7.0 Hz), 6.53 (1H, dd, J_1=2.4 Hz, J_2=8.1 Hz), 6.92 (1H, d, J=8.4 Hz), 7.16 (4H, m), 7.37 (3H, m), 8.14 (1H, s), 8.67 (1H, s), 8.73 (1H, s), 10.74 (1H, s). MS (TOF) m/z: 522 (M^++H). *Anal.* Calcd for $C_{28}H_{31}N_3O_7$: C, 64.48; H, 5.99; N, 8.06. Found: C, 64.36; H, 6.05; N, 7.94.

Ethyl 4,5-Dimethoxy-2-(3-(4-((*N*-(2-methoxyphenyl)carbamoyl)amino)-phenyl)propanoylamino)benzoate (2m) Compound **2m** was prepared from **5b** in a manner similar to that described for compound **2d** with a yield of 74%. mp 207—208 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.32 (3H, t, J=7.0 Hz), 2.68 (2H, t, J=7.3 Hz), 2.88 (2H, t, J=7.3 Hz), 3.76 (3H, s), 3.81 (3H, s), 3.87 (3H, s), 4.30 (2H, q, J=7.0 Hz), 6.93 (3H, m), 7.15 (2H, d, J=8.4 Hz), 7.35 (3H, m), 8.13 (3H, m), 9.24 (1H, s), 10.74 (1H, s). MS (TOF) m/z: 522 (M^++H). *Anal.* Calcd for $C_{28}H_{31}N_3O_7 \cdot 0.1H_2O$: C, 64.25; H, 6.01; N, 8.03. Found: C, 64.05; H, 5.99; N, 7.80.

Ethyl 2-(3-(4-((*N*-(4-Aminophenyl)carbamoyl)amino)phenyl)propano-ylamino)-4,5-dimethoxybenzoate (2n) To a solution of **2h** (0.17 g, 0.32 mmol) in THF (20 ml) was added 5% Pd/C (0.05 g), which was then stirred at room temperature under a H_2 atmosphere for 18 h. After the reaction mixture had been filtered, concentrated and washed with methanol to give 0.14 g (0.28 mmol) of **2n** as a pale yellow solid with a yield of 86%. mp 294—300 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.32 (3H, t, J=7.0 Hz), 2.67 (2H, t, J=7.3 Hz), 2.87 (2H, t, J=7.3 Hz), 3.77 (3H, s), 3.81 (3H, s), 4.30 (2H, q, J=7.0 Hz), 4.75 (2H, s), 6.49 (2H, d, J=8.9 Hz), 7.05 (2H, d, J=8.6 Hz), 7.13 (2H, d, J=8.1 Hz), 7.32 (2H, d, J=8.1 Hz), 7.39 (1H, s), 8.12 (1H, s), 8.14 (1H, s), 8.43 (1H, s), 10.74 (1H, s). *Anal.* Calcd for $C_{27}H_{30}N_4O_6 \cdot 0.4H_2O$: C, 63.12; H, 6.04; N, 10.91. Found: C, 63.47; H, 6.13; N, 10.54.

Ethyl 4,5-Dimethoxy-2-(3-(4-((*N*-(4-methylphenyl)carbamoyl)amino)-phenyl)propanoylamino)benzoate (2o) Compound **2o** was prepared from **5b** in a manner similar to that described for compound **2d** with a yield of 46%. mp 223—226 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.32 (3H, t, J=7.0 Hz), 2.23 (3H, s), 2.67 (2H, t, J=7.3 Hz), 2.87 (2H, t, J=7.3 Hz), 3.76 (3H, s), 3.81 (3H, s), 4.30 (2H, q, J=7.0 Hz), 7.07 (2H, d, J=8.4 Hz), 7.15 (2H, d, J=8.4 Hz), 7.35 (5H, m), 8.14 (1H, s), 8.57 (1H, s), 8.59 (1H, s), 10.74 (1H, s). *Anal.* Calcd for $C_{28}H_{31}N_3O_6$: C, 66.52; H, 6.18; N, 8.31. Found: C, 66.26; H, 6.19; N, 8.20.

Ethyl 2-(3-(4-((*N*-(4-Fluorophenyl)carbamoyl)amino)phenyl)propano-ylamino)-4,5-dimethoxybenzoate (2p) Compound **2p** was prepared from **5b** in a manner similar to that described for compound **2d** with a yield of 75%. mp 218—221 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.32 (3H, t, J=7.0 Hz), 2.68 (2H, t, J=7.3 Hz), 2.88 (2H, t, J=7.3 Hz), 3.77 (3H, s), 3.81 (3H, s),

4.30 (2H, q, J=7.0 Hz), 7.12 (4H, m), 7.43 (5H, m), 8.14 (1H, s), 8.71 (1H, s), 8.79 (1H, s), 10.74 (1H, s). *Anal.* Calcd for $C_{27}H_{28}FN_3O_6$: C, 63.65; H, 5.54; N, 8.25. Found: C, 63.42; H, 5.35; N, 8.22.

Ethyl 2-(3-(4-((*N*-(4-Acetylphenyl)carbamoyl)amino)phenyl)propanoylamino)-4,5-dimethoxybenzoate (2q) Compound **2q** was prepared from **5b** in a manner similar to that described for compound **2d** with a yield of 58%. mp 202—204 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.32 (3H, t, J=7.0 Hz), 2.69 (2H, t, J=7.3 Hz), 2.90 (2H, t, J=7.3 Hz), 3.77 (3H, s), 3.82 (3H, s), 4.30 (2H, q, J=7.0 Hz), 7.18 (2H, d, J=8.4 Hz), 7.38 (2H, d, J=8.4 Hz), 7.39 (1H, s), 7.58 (2H, d, J=8.6 Hz), 7.89 (2H, d, J=8.6 Hz), 8.14 (1H, s), 8.82 (1H, s), 9.17 (1H, s), 10.74 (1H, s). *Anal.* Calcd for $C_{29}H_{31}N_3O_7$: C, 65.28; H, 5.86; N, 7.88. Found: C, 65.11; H, 5.74; N, 7.87.

Ethyl 4,5-Dimethoxy-2-(3-(4-((*N*-benzylcarbamoyl)amino)phenyl)-propanoylamino)benzoate (2r) Compound **2r** was prepared from **5b** in a manner similar to that described for compound **2d** with a yield of 46%. mp 200—203 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.32 (3H, t, J=7.0 Hz), 2.66 (2H, t, J=7.3 Hz), 2.85 (2H, t, J=7.3 Hz), 3.76 (3H, s), 3.81 (3H, s), 4.31 (4H, m), 6.60 (1H, t, J=7.3 Hz), 7.09 (2H, d, J=8.1 Hz), 7.30 (7H, m), 7.39 (1H, s), 8.13 (1H, s), 8.50 (1H, s), 10.73 (1H, s). MS (TOF) m/z: 506 (M^++H). *Anal.* Calcd for $C_{28}H_{31}N_3O_6$: C, 66.52; H, 6.18; N, 8.31. Found: C, 66.27; H, 6.22; N, 8.08.

Ethyl 2-(3-(4-((*N*-Cyclohexylcarbamoyl)amino)phenyl)propanoylamino)-4,5-dimethoxybenzoate (2s) Compound **2s** was prepared from **5b** in a manner similar to that described for compound **2d** with a yield of 46%. mp 209—213 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.19 (5H, m), 1.32 (3H, t, J=7.0 Hz), 1.76 (5H, m), 2.68 (2H, t, J=7.3 Hz), 2.88 (2H, t, J=7.3 Hz), 3.33 (1H, m), 3.76 (3H, s), 3.81 (3H, s), 4.30 (2H, q, J=7.0 Hz), 6.05 (1H, d, J=8.4 Hz), 7.09 (2H, d, J=8.4 Hz), 7.26 (2H, d, J=8.4 Hz), 7.39 (1H, s), 8.13 (1H, s), 8.24 (1H, s), 10.73 (1H, s); MS (TOF) m/z: 498 (M^++H). *Anal.* Calcd for $C_{27}H_{35}N_3O_6$: C, 65.17; H, 7.09; N, 8.44. Found: C, 64.79; H, 7.08; N, 8.19.

Primary Culture of Smooth Muscle Cells and Endothelial Cells SMCs, ECs and their culture kits were obtained from Clonetics Corp. (San Diego, CA, U.S.A.). SMCs were cultured in basal medium (SmBM) containing 5% FBS, 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% 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 to 5 d 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, U.S.A.) 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 cultured 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 d, DNA synthesis was determined during the last 4 h of the 3 d culture.

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