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Synthesis and Structure–Activity Relationship of Diarylamide Urea Derivatives as Selective Inhibitors of the Proliferation of Human Coronary Artery Smooth Muscle Cells

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Abstract—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 **2o** was superior to the lead compound, Tranilast, in terms of its potency of the inhibitory activity and cell selectivity.

Introduction

Proliferation of SMCs plays a cardinal important part in restenosis and in the progression of atherosclerosis.[1] Many growth factors have been reported to induce the proliferation of SMCs in vitro and in vivo.[2] Among them, platelet-derived growth factor (PDGF) is a momentous regulator of SMCs action.[3] Recently, Tranilast ([*N*-(3,4-dimethoxycinnamoyl]) anthranilic acid) was reported to noticeably inhibit the proliferation of SMCs and ECs in vitro.[4] Besides, a double-blind, large-scale, multicenter trial demonstrated the potent preventive effect of Tranilast on restenosis [restenosis rate 14.7% (Tranilast 600 mg/day for 3 months, $n=68$) versus 46.5% (placebo, $n=71$); $P<0.001$] after percutaneous transluminal coronary angioplasty.[5] However, some of the patients had been liver dysfunction in this trial. We speculated that these frequent and severe side effects may have been caused by the high dose of Tranilast. In addition, the selective inhibitiors 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] Thus, we have tried to develop more potent and SMCs-selective compounds by the modifying Tranilast.

Previously, we reported a series of diarylamide derivatives, **1a** and **1b** (Fig. 1), exhibiting potent and highly selective inhibition of SMCs proliferation.[7] According to the report of Tranilast analogues by the Kissei group[8] and our research,[7] methoxy groups substitution was more potent than the other substituents such as halogen, hydroxy, nitro, alkyl and amino groups. On the other hand, Kirin Brewery research group also reported a series of quinazoline analogues exhibiting potent inhibition of PDGF receptor autophosphorylation.[9,10] According to their reports, over 100-fold elevation of the activity was observed when a ureide group replaced the methoxy groups on the phenoxy moiety.[10] In fact, we actually evaluated the inhibitory activities of Kirin compounds (**3a**–**b**) on PDGF-induced proliferation of SMCs, and the elevation of the activity was also observed (**3a**: $IC_{50}=0.5$ μM, **3b**: 55% inhibition @ 0.016 μM). In view of the similarity of the structure between their compounds and ours, we speculated that the introduction of a ureide group to the B ring of our diarylamide derivatives might make the inhibitory activity more potent (Fig. 1). In this paper, we report the results of our preliminary study on a series of these diarylamide urea derivatives.

Chemistry

The synthesis of urea derivatives **2a**–**2s** are outlined in Scheme 1.

Acid-catalytic ester formation with sulfuric acid or amide formation with thionyl chloride ($SOCl_2$) of 4,5-

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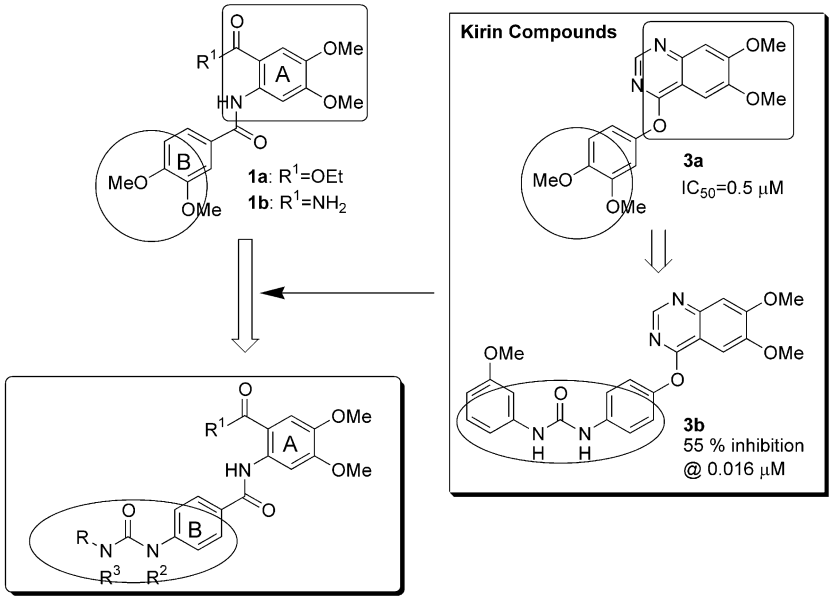


Figure 1.

dimethoxy-2-nitro benzoic acid, followed by catalytic hydrogenation with 5% Pd/C gave the corresponding compounds **4a**–**b** in a good yield (70–75%). Condensation of **4a**–**b** with 4-nitrobenzoyl chloride in the presence of triethylamine, followed by catalytic hydrogenation with 5% Pd/C gave **5a**–**b** in a good yield (70–90%), and **5c** was quantitatively obtained by the condensation of **4b** with 4-methylaminobenzoic acid by use of 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide hydrochloride salt (WSC·Cl) as a condensation reagent. Finally, condensation of **5a**–**c** with the corresponding isocyanate or *N*-phenyl-*N*-methylcarbamoylchloride gave the desired urea derivatives.

Biology

A series of these compounds were evaluated for their inhibitory activities on PDGF-induced proliferation of SMCs and FBS-induced the proliferation of ECs. Inhibition of proliferation of these cells was determined by ^{3}H-thymidine incorporation as previously reported with a minor modification.[11]

Results and Discussion

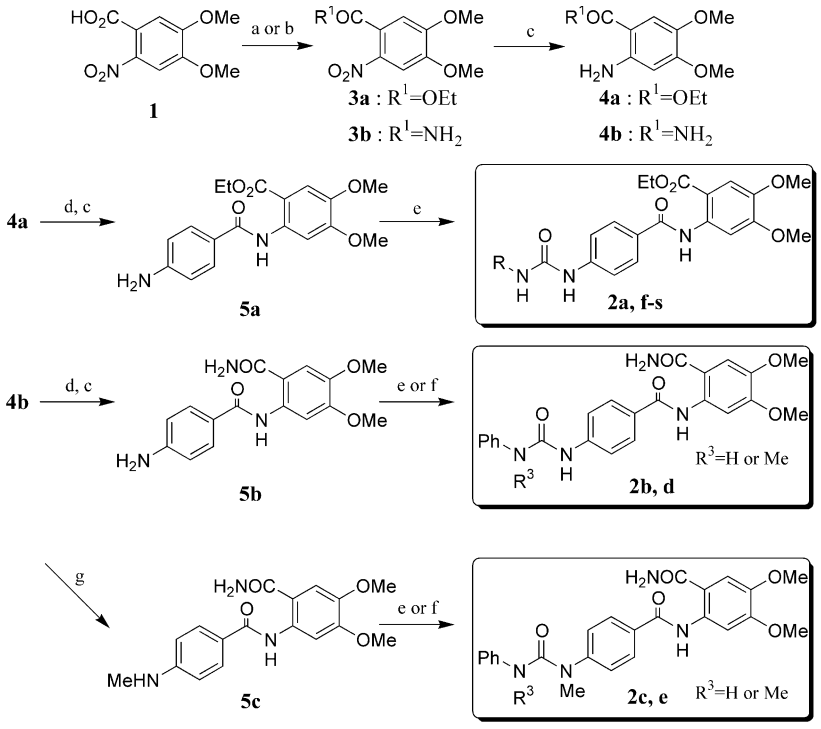
The inhibitory activities of **2a**–**s** on PDGF-induced proliferation of SMCs are shown in Tables 1 and 2.

Table 1 presents the result of the structure–activity relationship (SAR) study on phenyl urea derivatives. As we expected, the introduction of the ureide group (**2a**, **2b**) to the B ring made the inhibitory activity more potent than that of **1a** and **1b**. Similar to the case of diarylamide derivatives **1a** and **1b**, the presence of the ethoxy carbonyl group (**2a**) or the carbamoyl group (**2b**) in the A ring results in almost the same activity. Methyl group substitution at the N atom in the ureide group (**2c**, **2d**, **2e**) resulted in less inhibition. The solubility of **2b** in various organic solvents as well as in water was very poor. Therefore, in view of this property, we concluded that **2b** was not preferable as a drug candidate. On the other hand, the solubility of **2a** was good and the stability of **2a** in the assay medium was confirmed by HPLC analysis although the ester bond was afraid to be cleaved. Thus, the further examination of the substituents of the urea moiety was performed by using **2a** for modification.

Table 2 presents the results of the SAR study on substituents of the ureide moiety. At first, we examined the effect of substituents at the *para* position of the phenyl ring. Acetyl (**2k**) and amino (**2h**) groups there resulted in increased activity. Earlier, Kubo et al. reported that 3-methoxy group substitution on the phenyl ring resulted in the most potent inhibition,[10] and also we found that methoxy group substitution on the B ring increased the activity.[7] Therefore, we examined the effect of the number and position of methoxy group substituents. As a result, 3,4,5-trisubstituted compound (**2o**) was the most potent ($IC_{50} = 0.04$ μM), and was about 600-fold more potent than Tranilast. We also evaluated the effect of most of the compounds against the proliferation of ECs. Most of the evaluated compounds displayed selectivity about 4∼140 times greater for SMCs. However, benzyl (**2p**), *n*-butyl (**2q**), cyclohexyl (**2r**) and 3-pyridyl (**2s**) groups instead of the phenyl ring in the ureide moiety showed low selectivity for SMCs in spite of almost the same inhibitory activity for SMCs. In particular, the IC_{50} values of **2o** for SMCs and for ECs were 0.04 and 5.4 μM, respectively, displaying selectivity about 135 times greater for SMCs. In contrast, the IC_{50} values of Tranilast toward SMCs and ECs were 25 and 19 μM, respectively, indicating a selectivity of about 0.76 times for SMCs. Thus, compound **2o** was superior to Tranilast, as were

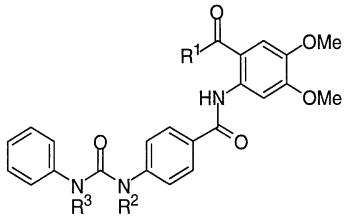
our lead compounds **1a** and **1b**, in the strength of the activity and cell selectivity.

In examining the mechanism of action of Tranilast,[12] several researchers showed that Tranilast increased the levels of p21 and p53 protein and arrested SMCs at the G0/G1 phase. However, our compounds had no influence on the regulation of p21 and p53 protein. On the other hand, in the case of quinazoline compounds, the Kirin Brewery group had reported a potent inhibition of PDGF receptor autophosphorylation.[10] Therefore, we also examined their effect on PDGF receptor autophosphorylation but unexpectedly obtained a negative result. Thus, the main mechanism of the action of these urea derivatives is different from that of Tranilast and quinazoline derivatives. Thus, the search for the main



Scheme 1. Synthesis of compounds **2a**–**2s**. Reagents and conditions: (a) EtOH, cH_2SO_4; (b) $SOCl_2$ then NH_3aq/CH_2Cl_2; (c) H_2, 5% Pd/C, EtOH; (d) 4-NO_2–PhCOCl, Et_3N/CH_2Cl_2; (e) RNCO, DMAP/THF; (f) PhNMeCOCl, $^iPr_2NEt$/THF; (g) 4-NHMe–$PhCO_2H$, HOBt, Et_3N, DMF, WSC·HCl.

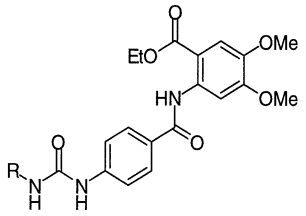
Table 1. IC_{50} values of the compounds **2a**–**e** for inhibition of proliferation of SMCs and ECs



No.	R^1	R^2	R^3	SMCs IC_{50} (μM)[a]	ECs IC_{50} (μM)[b]	[ECs]/[SMCs]
Tranilast				25	19	0.76
3a				0.5	n.t.	
3b				<0.016	n.t.	
1a				0.85	6.0	7.1
1b				0.72	>10	>14
2a	OEt	H	H	0.40	1.5	3.7
2b	NH_2	H	H	0.40	4.4	11
2c	NH_2	Me	H	>2.0	n.t.	
2d	NH_2	H	Me	>2.0	n.t.	
2e	NH_2	Me	Me	>2.0	n.t.	

n.t., not tested.
[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.

Table 2. IC_{50} values of the compounds **2f–s** for inhibition of proliferation of SMCs and ECs



No.	R	SMCs IC_{50} (μM)[a]	ECs IC_{50} (μM)[b]	[ECs]/[SMCs]
Tranilast		25	19	0.76
3a		0.5	n.t.	
3b		<0.016	n.t.	
1a		0.85	6.0	7.1
1b		0.72	>10	>14
2a	Ph	0.40	1.5	3.8
2f	4-OMe–Ph	1.7	n.t.	
2g	4-NO_2-Ph	0.50	n.t.	
2h	4-NH_2–Ph	0.10	1	10
2i	4-Me–Ph	0.33	n.t.	
2j	4-F–Ph	0.40	>2.0	>5.0
2k	4-Ac–Ph	0.090	6.1	6.7
2l	4-CO_2Et–Ph	0.23	3.2	14
2m	2-OMe–Ph	0.28	1.51	5.4
2n	3-OMe–Ph	0.094	1.6	17
2o	3,4,5-$(OMe)_3$–Ph	0.040	5.4	135
2p	Bn	0.10	1.2	12
2q	*n*-Bu	0.23	1.1	4.8
2r	c-Hex	0.34	1.4	4.1
2s	3-Py	0.19	0.030	0.16

n.t., not tested.
[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.

target molecules of our compounds and the further structural optimization of our derivatives are underway.

Experimental

Chemistry

In general, reagents and solvents were used as purchased without further purification. Column chromatography was performed on Walkogel C-200 (WaKo, 70–150 μm) or Walkogel C-300 (WaKo, 45–75 μm). 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. TOFMS were recorded on a Kompact MALDI III spectrometer. Elemental analyses were performed by the Toray Research Center and were within 0.4% of the calculated values unless otherwise noted.

Ethyl 4,5-dimethoxy-2-((4-((*N*-phenylcarbamoyl)amino)-phenyl)carbonylamino)benzoate (2a). To a solution of 4,5-dimethoxy-2-nitro benzoic acid (0.75 g, 3.30 mmol) in ethanol (100 mL) was added H_2SO_4 (3 mL), and the mixture was refluxed for 18 h. To the reaction mixture on ice was added 5% NaOH aq, and adjustment of the pH 8 to save a solid. The obtained solid was washed with H_2O to give 0.53 g (2.08 mmol) of **3a** as a white solid with a yield of 63%.

To a solution of **3a** (0.30 g, 1.18 mmol) in ethanol (20 mL) was added 5% Pd/C (0.06 g), and the mixture was stirred at room temperature under H_2 atmosphere for 14 h. The reaction mixture was then filtered and concentrated to give 0.26 g (1.16 mmol) of **4a** as a white solid with a yield of 98%. To a solution of **4a** (0.26 g, 1.16 mmol) in CH_2Cl_2 (20 mL) 4-nitrobenzoyl chloride (0.27 g, 1.47 mmol) and triethylamine (0.25 mL, 1.79 mmol) were added, 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 redissolved in methanol (100 mL), 5% Pd/C (0.05 g) was added to this solution and the mixture was stirred under H_2 atmosphere for 32 h. The reaction mixture was filtered, and concentrated to give 0.28 g (0.81 mmol) of **5a** as a yellow solid with a yield of 70%, by two steps.

To a solution of **5a** (0.09 g, 0.26 mmol) and dimethylaminopyridine (0.05 g, 0.41 mmol) in THF (20 mL) was added phenylisocyanate (0.05 g, 0.42 mmol), and the mixture was refluxed for 18 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 0.08 g (0.17 mmol) of **2a** as a white solid with a yield of 65%. Mp: 232–236 °C; ^{1}H NMR (DMSO-d_6) δ

ppm: 1.35 (t, $J=7.2$ Hz, 3H), 3.80 (s, 3H), 3.88 (s, 3H), 4.37 (q, $J=7.2$ Hz, 3H), 6.99 (t, $J=7.3$ Hz, 1H), 7.30 (t, $J=8.1$ Hz, 2H), 7.48 (d, $J=7.5$ Hz, 2H), 7.48 (s, 1H), 7.67 (d, $J=7.3$ Hz, 2H), 7.90 (d, $J=8.9$ Hz, 2H), 8.45 (s, 1H), 9.05 (s, 1H), 9.31 (s, 1H), 11.75 (s, 1H); MS (TOF) $m/z=464$ ($M^+ + H$). Anal. calcd for $C_{25}H_{25}N_3O_6$: C 64.8, H 5.5, N 9.1. Found: C 64.8, H 5.4, N 9.4.

4,5-Dimethoxy-2-((4-((*N*-phenylcarbamoyl)amino)phenyl)-carbonylamino)benzamide (2b). To a solution of 4,5-dimethoxy-2-nitro benzoic acid (0.66 g, 2.90 mmol) in $CHCl_3$ (40 mL) was added $SOCl_2$ (5 mL), and the mixture was then refluxed for 6 h. The reaction mixture was concentrated and redissolved in CH_2Cl_2 (20 mL). To this solution was added aqueous NH_3 (20 mL) in an ice bath, and the mixture was vigorously stirred for 10 min at room temperature. After the organic layer had been concentrated and the residue redissolved in methanol (50 mL), 5% Pd/C (0.05 g) was added to this solution, which was then stirred under H_2 atmosphere for 19 h. The reaction mixture was filtered and concentrated to give 0.55 g (0.28 mmol) of **4b** as a white solid with a yield of 96%, by two steps.

Compound **2b** was prepared from **4b** in a manner similar to that described for compound **2a** with a yield of 24%, by three steps. Mp: 232–236 °C; ^{1}H NMR (DMSO-d_6) δ ppm. 81 (s, 3H), 3.84 (s, 3H), 7.00 (t, $J=8.1$ Hz, 1H), 7.30 (t, $J=8.4$ Hz, 2H), 7.44 (s, 1H), 7.47 (d, $J=7.9$ Hz, 2H), 7.64 (m, 3H), 7.87 (d, $J=8.6$ Hz, 2H), 8.31 (s, 1H), 8.53 (s, 1H), 8.87 (s, 1H), 9.13 (s, 1H), 13.21 (s, 1H); MS (TOF) $m/z=435$ ($M^+ + H$). Anal. calcd for $C_{23}H_{22}N_4O_5 \cdot 0.6H_2O$: C 62.5, H 5.2, N 12.7. Found: C 62.7, H 5.1, N 12.9.

4,5-Dimethoxy-2-((4-(methyl(*N*-phenylcarbamoyl)amino)-phenyl)carbonylamino)benzamide (2c). To a solution of **4b** (0.04 g, 0.20 mmol), 1-hydroxybenztriazole (0.03 g, 0.20 mmol), 4-methylaminobenzoic acid (0.03 g, 0.20 mmol), and triethylamine (0.02 g, 0.20 mmol) in DMF (3 mL) was added 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide hydrochloride (0.04 g, 0.20 mmol) in an ice-bath, and the mixture was stirred at room temperature for 50 h. After the reaction mixture had been concentrated, the residue was poured into water, and extracted with CH_2Cl_2, and then 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 = 100:1 ~ 20:1) to give 0.07 g (0.18 mmol) of **5c** as a white solid with a yield of 91%.

Compound **2c** was prepared from **5c** in a manner similar to that described for compound **2a** with a yield of 47%. Mp: 214–218 °C; ^{1}H NMR (DMSO-d_6) δ ppm 3.35 (s, 3H), 3.81 (s, 3H), 3.85 (s, 3H), 6.97 (t, $J=8.5$ Hz, 1H), 7.25 (t, $J=8.4$ Hz, 2H), 7.45 (m, 5H), 7.68 (s,1H), 7.94 (d, $J=8.1$ Hz, 2H), 8.33 (s, 1H), 8.53 (s, 1H), 8.59 (s, 1H), 13.32 (s, 1H); MS (TOF) $m/z=449$ ($M^+ + H$). Anal. calcd for $C_{24}H_{24}N_4O_5 \cdot 2.6H_2O$: C 58.2, H 5.9, N 11.3. Found: C 58.0, H 5.7, N 11.5.

4,5-Dimethoxy-2-((4-((*N*-methyl-*N*-phenylcarbamoyl)-amino)phenyl)carbonylamino)benzamide (2d). To a solution of **5b** (0.11 g, 0.36 mmol) and *N*,*N*-diisopropylethylamine (0.46 g, 3.6 mmol) in THF (10 mL) was added *N*-phenyl-*N*-methylcarbamoyl chloride (0.16 g, 3.6 mmol), and the mixture was refluxed for 16 h. The reaction mixture was poured into water 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 = 100:1 ~ 20:1) and washed with methanol to give 0.05 g (0.15 mmol) of **2d** as a white solid with a yield of 42%. Mp: 196–198 °C; ^{1}H NMR (DMSO-d_6) δ ppm 3.29 (s, 3H), 3.81 (s, 3H), 3.83 (s, 3H), 7.27 (t, $J=6.8$ Hz, 1H), 7.44 (m, 5H), 7.63 (m,3H), 7.80 (d, $J=8.9$ Hz, 2H), 8.30 (s, 1H), 8.52 (s, 1H), 8.53 (s, 1H), 13.18 (s, 1H); MS (TOF) $m/z=449$ ($M^+ + H$). Anal. calcd for $C_{24}H_{24}N_4O_5 \cdot 0.1H_2O$: C 64.0, H 5.4, N 12.5. Found: C 63.7, H 5.5, N 12.5.

4,5-Dimethoxy-2-((4-(methyl(*N*-methyl-*N*-phenylcarbamoyl)amino)phenyl)carbonylamino)benzamide (2e). Compound **2e** was prepared from **5c** in a manner similar to that described for compound **2d** with a yield of 36%. Mp: 176–178 °C; ^{1}H NMR(DMSO-d_6) δ ppm: 3.12 (s, 3H), 3.18 (s, 3H), 3.81 (s, 3H), 3.83 (s, 3H), 7.00 (m, 3H), 7.12 (m, 4H), 7.44 (s,1H), 7.68 (m, 3H), 8.32 (s, 1H), 8.49 (s, 1H), 13.18 (s, 1H); MS (TOF) $m/z=463$ ($M^+ + H$). Anal. calcd for $C_{25}H_{26}N_4O_5 \cdot 1.1H_2O$: C 62.4, H 5.7, N, 11.6. Found: C 62.2, H 5.7, N 11.5.

Ethyl 4,5-dimethoxy-2-((4-((*N*-(4-methoxyphenyl)carbamoyl)amino)phenyl)carbonylamino)benzoate (2f). Compound **2f** was prepared from **5a** in a manner similar to that described for compound **2a** with a yield of 83%. Mp: 244–247 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 1.35 (t, $J=7.2$ Hz, 3H), 3.73 (s, 3H), 3.80 (s, 3H), 3.88 (s, 3H), 4.37 (q, $J=7.2$ Hz, 3H), 6.89 (d, $J=9.2$ Hz, 2H), 7.38 (d, $J=8.6$ Hz, 2H), 7.48 (s, 1H), 7.65 (d, $J=8.9$ Hz, 2H), 7.89 (d, $J=8.9$ Hz, 2H), 8.45 (s, 1H), 8.73 (s, 1H), 9.11 (s, 1H), 11.75 (s, 1H); MS (TOF) $m/z=494$ ($M^+ + H$). Anal. calcd for $C_{26}H_{27}N_3O_7$: C 63.2, H 5.5, N 8.5. Found: C 63.2, H 5.5, N 8.9.

Ethyl 4,5-dimethoxy-2-((4-((*N*-(4-nitrophenyl)carbamoyl)-amino)phenyl)carbonylamino)benzoate (2g). Compound **2g** was prepared from **5a** in a manner similar to that described for compound **2a** with a yield of 63%. Mp: 235–236 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 1.35 (t, $J=7.2$ Hz, 3H), 3.80 (s, 3H), 3.88 (s, 3H), 4.37 (q, $J=7.2$ Hz, 3H), 7.48 (s, 1H), 7.71 (m, 4H), 7.92 (d, $J=8.9$ Hz, 2H), 8.22 (d, $J=9.2$ Hz, 2H), 8.43 (s, 1H), 9.40 (s, 1H), 9.65 (s, 1H), 11.76 (s, 1H). Anal. calcd for $C_{25}H_{24}N_4O_8 \cdot 1.6H_2O$: C 55.9, H 5.1, N 10.4. Found: C 56.1, H 5.0, N 10.7.

Ethyl 2-((4-((*N*-(4-aminophenyl)carbamoyl)amino)phenyl)-carbonyl amino)-4,5-dimethoxybenzoate (2h). To a solution of **2g** (0.09 g, 0.18 mmol) in ethanol (10 mL) was added 5% Pd/C (0.02 g), which was then stirred at room temperature under a H_2 atmosphere for 14 h. After the reaction mixture had been 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 0.03 g (0.06 mmol) of **2 h** as a light pink solid with a yield of 33%. Mp: 202–206;

^{1}H NMR (DMSO-d_6) δ ppm: 1.35 (t, J = 7.2 Hz, 3H), 3.80 (s, 3H), 3.87 (s, 3H), 4.37 (q, J = 7.2 Hz, 3H), 4.80 (s, 2H), 6.52 (d, J = 8.1 Hz, 2H), 7.10 (d, J = 8.9 Hz, 2H), 7.48 (s, 1H), 7.63 (d, J = 8.9 Hz, 2H), 7.87 (d, J = 8.9 Hz, 2H), 8.22 (d, J = 9.2 Hz, 2H), 8.42 (s, 1H), 8.45 (s, 1H), 9.03 (s, 1H), 11.74 (s, 1H); MS (TOF) m/z = 479 (M^+ + H). Anal. calcd for $C_{25}H_{26}N_4O_6 \cdot 1.2H_2O$: C 60.0, H 5.7, N 11.2. Found: C 60.0, H 5.5, N 11.1.

Ethyl 4,5-dimethoxy-2-((4-((*N*-(4-methylphenyl)carbamoyl)-amino)phenyl)carbonylamino)benzoate (2i). Compound **2i** was prepared from **5a** in a manner similar to that described for compound **2a** with a yield of 98%. Mp: 236–239 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 1.35 (t, J = 7.0 Hz, 3H), 2.25 (s, 3H), 3.80 (s, 3H), 3.88 (s, 3H), 4.37 (q, J = 7.0 Hz, 3H), 7.10 (d, J = 8.4 Hz, 2H), 7.38 (d, J = 8.4 Hz, 2H), 7.48 (s, 1H), 7.67 (d, J = 8.9 Hz, 2H), 7.89 (d, J = 8.9 Hz, 2H), 8.45 (s, 1H), 9.09 (s, 1H), 9.43 (s, 1H), 11.75 (s, 1H); MS (TOF) m/z = 478 (M^+ + H). Anal. calcd for $C_{26}H_{27}N_3O_6 \cdot 0.2H_2O$: C 64.9, H 5.7, N 8.7. Found: C 64.7, H 5.6, N 8.7.

Ethyl 2-((4-((*N*-(4-fluorophenyl)carbamoyl)amino)phenyl)-carbonylamino)-4,5-dimethoxybenzoate (2j). Compound **2j** was prepared from **5a** in a manner similar to that described for compound **2a** with a yield of 73%. Mp: 243–244 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 1.35 (t, J = 7.2 Hz, 3H), 3.80 (s, 3H), 3.88 (s, 3H), 4.37 (q, J = 7.2 Hz, 3H), 7.14 (t, J = 6.2 Hz, 2H), 7.48 (s, 1H), 7.49 (dd, J = 3.8, 8.6 Hz, 2H), 7.67 (d, J = 8.6 Hz, 2H), 7.89 (d, J = 8.9 Hz, 2H), 8.44 (s, 1H), 9.12 (s, 1H), 9.34 (s, 1H), 11.75 (s, 1H). Anal. calcd for $C_{25}H_{24}FN_3O_6$: C 62.4, H 5.0, N 8.7. Found: C 62.1, H 5.1, N 8.7.

Ethyl 2-((4-((*N*-(4-acetylphenyl)carbamoyl)amino)phenyl)-carbonylamino)-4,5-dimethoxybenzoate (2k). Compound **2k** was prepared from **5a** in a manner similar to that described for compound **2a** with a yield of 47%. Mp: 240–241 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 1.35 (t, J = 7.2 Hz, 3H), 3.80 (s, 3H), 3.88 (s, 3H), 4.37 (q, J = 7.2 Hz, 3H), 7.48 (s, 1H), 7.62 (d, J = 8.9 Hz, 2H), 7.68 (d, J = 8.9 Hz, 2H), 7.93 (m, 4H), 8.44 (s, 1H), 9.34 (s, 1H), 9.38 (s, 1H), 11.76 (s, 1H); MS (TOF) m/z = 506 (M^+ + H). Anal. calcd for $C_{27}H_{27}N_3O_7 \cdot 1.0H_2O$: C 61.9, H 5.6, N 8.0. Found: C 62.0, H 5.5, N 8.0.

Ethyl 2-((4-((*N*-(4-ethoxycarbonylphenyl)carbamoyl)amino)-phenyl)carbonylamino)-4,5-dimethoxybenzoate (2l). Compound **2l** was prepared from **5a** in a manner similar to that described for compound **2a** with a yield of 88%. Mp: 204–205 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 1.32 (m, 6H), 3.80 (s, 3H), 3.88 (s, 3H), 4.33 (m, 4H), 7.48 (s, 1H), 7.62 (d, J = 8.4 Hz, 2H), 7.68 (d, J = 8.6 Hz, 2H), 7.91 (m, 4H), 8.44 (s, 1H), 9.29 (s, 1H), 9.34 (s, 1H), 11.76 (s, 1H). Anal. calcd for $C_{28}H_{29}N_3O_8 \cdot 1.0H_2O$: C 60.8, H 5.6, N 7.6. Found: C 60.5, H 5.6, N 7.6.

Ethyl 4,5-dimethoxy-2-((4-((*N*-(2-methoxyphenyl)carbamoyl)amino)phenyl)carbonylamino)benzoate (2m). Compound **2m** was prepared from **5a** in a manner similar to that described for compound **2a** with a yield of 48%. Mp: 132–134 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 1.35 (t, J = 7.2 Hz, 3H), 3.80 (s, 3H), 3.88 (s, 3H), 3.89 (s, 3H), 4.37 (q, J = 7.2 Hz, 3H), 7.00 (m, 3H), 7.48 (s, 1H), 7.66 (d, J = 8.4 Hz, 2H), 7.90 (d, J = 8.9 Hz, 2H), 8.13 (dd, J = 1.6, 7.3 Hz, 1H), 8.41 (s, 1H), 8.45 (s, 1H), 9.75 (s, 1H), 11.76 (s, 1H); MS (TOF) m/z = 493 (M^+ + H). Anal. calcd for $C_{26}H_{27}N_3O_7 \cdot 1.3H_2O$: C 60.4, H 5.7, N 8.1. Found: C 60.2, H 5.6, N 8.1.

Ethyl 4,5-dimethoxy-2-((4-((*N*-(3-methoxyphenyl)carbamoyl)amino)phenyl)carbonylamino)benzoate (2n). Compound **2n** was prepared from **5a** in a manner similar to that described for compound **2a** with a yield of 82%. Mp: 200–201 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 1.35 (t, J = 7.2 Hz, 3H), 3.74 (s, 3H), 3.80 (s, 3H), 3.88 (s, 3H), 4.37 (q, J = 7.2 Hz, 3H), 6.58 (dd, J = 2.4, 8.1 Hz, 1H), 6.96 (d, J = 9.5 Hz, 1H), 7.20 (m, 2H), 7.48 (s, 1H), 7.66 (d, J = 8.6 Hz, 2H), 7.90 (d, J = 8.9 Hz, 2H), 8.44 (s, 1H), 8.97 (s, 1H), 9.21 (s, 1H), 11.75 (s, 1H); MS (TOF) m/z = 493 (M^+ + H). Anal. calcd for $C_{26}H_{27}N_3O_7$: C 63.2, H 5.5, N 8.5. Found: C 63.1, H 5.5, N 8.5.

Ethyl 4,5-dimethoxy-2-((4-((*N*-(3,4,5-trimethoxyphenyl)-carbamoyl)amino)phenyl)carbonylamino) benzoate (2o). Compound **2o** was prepared from **5a** in a manner similar to that described for compound **2a** with a yield of 45%. Mp: 225–227 °C; ^{1}H NMR(DMSO–d_6) δ ppm: 1.35 (t, J = 7.2 Hz, 3H), 3.61 (s, 3H), 3.76 (s, 6H), 3.80 (s, 3H), 3.88 (s, 3H), 4.37 (q, J = 7.2 Hz, 3H), 6.83 (s, 2H), 7.48 (s, 1H), 7.67 (d, J = 8.4 Hz, 2H), 7.90 (d, J = 8.9 Hz, 2H), 8.44 (s, 1H), 8.93 (s, 1H), 9.19 (s, 1H), 11.74 (s, 1H); MS (TOF) m/z = 554 (M^+ + H). Anal. calcd for $C_{28}H_{31}N_3O_9 \cdot 0.1H_2O$: C 60.6, H 5.7, N 7.6. Found: C 60.3, H 5.6, N 7.3.

Ethyl 4,5-dimethoxy-2-((4-((*N*-benzylcarbamoyl)amino)-phenyl)carbonylamino)benzoate (2p). Compound **2p** was prepared from **5a** in a manner similar to that described for compound **2a** with a yield of 86%. Mp: 231–233 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 1.34 (t, J = 7.2 Hz, 3H), 3.80 (s, 3H), 3.87 (s, 3H), 4.37 (m, 4H), 6.99 (t, J = 6.5 Hz, 1H), 7.28 (m, 5H), 7.47 (s, 1H), 7.61 (d, J = 8.6 Hz, 2H), 7.84 (d, J = 8.9 Hz, 1H), 8.44 (s, 1H), 9.18 (s, 1H), 11.72 (s, 1H). Anal. calcd for $C_{26}H_{27}N_3O_6 \cdot 0.2H_2O$: C 65.4, H 5.7, N 8.8. Found: C 65.2, H 5.7, N 8.6.

Ethyl 2-((4-((*N*-butylcarbamoyl)amino)phenyl)carbonylamino)-4,5-dimethoxybenzoate (2q). Compound **2q** was prepared from **5a** in a manner similar to that described for compound **2a** with a yield of 27%. Mp: 218–219 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 0.90 (t, J = 6.7 Hz, 3H), 1.27 (m,4H), 1.34 (t, J = 7.2 Hz, 3H), 3.10 (q, J = 5.7 Hz, 2H), 3.80 (s, 3H), 3.87 (s, 3H), 4.37 (m, 4H), 6.45 (t, J = 5.4 Hz, 1H), 7.47 (s, 1H), 7.59 (d, J = 8.9 Hz, 2H), 7.83 (d, J = 8.6 Hz, 1H), 8.45 (s, 1H), 8.98 (s, 1H), 11.72 (s, 1H); MS (TOF) m/z = 444 (M^+ + H). Anal. calcd for $C_{23}H_{29}N_3O_6 \cdot 0.3H_2O$: C 61.5, H 6.7, N 9.4. Found: C 61.7, H 6.6, N 9.3.

Ethyl 2-((4-((*N*-cyclohexylcarbamoyl)amino)phenyl)carbonylamino)-4,5-dimethoxybenzoate (2r). Compound **2r** was prepared from **5a** in a manner similar to that described for compound **2a** with a yield of 68%. Mp: 231–234 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 1.20 (m, 6H),

1.34 (t, $J=7.2$ Hz, 3H), 1.65 (m, 4H), 3.48 (m, 1H), 3.79 (s, 3H), 3.87 (s, 3H), 4.37 (m, 4H), 6.42 (d, $J=7.8$ Hz, 1H), 7.47 (s, 1H), 7.57 (d, $J=8.9$ Hz, 2H), 7.83 (d, $J=8.9$ Hz, 1H), 8.45 (s, 1H), 8.88 (s, 1H), 11.72 (s, 1H); MS (TOF) $m/z=470$ ($M^+ + H$). Anal. calcd for $C_{25}H_{31}N_3O_6$: C 64.0, H 6.7, N 9.0. Found: C 63.9, H 6.7, N 9.1.

Ethyl 2-((4-((*N*-(3-pyridyl)carbamoyl)amino)phenyl)carbonylamino)-4,5-dimethoxybenzoate (2s). Compound **2s** was prepared from **5a** in a manner similar to that described for compound **2a** with a yield of 88%. Mp: 224–226 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 1.35 (t, $J=7.2$ Hz, 3H), 3.80 (s, 3H), 3.88 (s, 3H), 4.37 (q, $J=7.2$ Hz, 2H), 7.34 (m, 1H), 7.48 (s, 1H), 7.69 (d, $J=8.6$ Hz, 2H), 7.90 (d, $J=8.9$ Hz, 2H), 7.97 (d, $J=8.9$ Hz, 1H), 8.20 (d, $J=4.3$ Hz, 1H), 8.44 (s, 1H), 8.66 (s, 1H), 9.50 (s, 1H), 9.70 (s, 1H), 11.75 (s, 1H); MS (TOF) $m/z=465$ ($M^+ + H$). Anal. calcd for $C_{24}H_{24}N_4O_6 \cdot 0.2H_2O$: C 61.6, H 5.3, N 12.0. Found: C 61.5, H 5.3, N 12.1.

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). And ECs were cultured in basal medium (EBM) containing 5% fetal bovine serum, 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 days in culture at 37 in 5% CO_2–95% air, both cells were subcultured by trypsinization and propagated in each complete medium described above.

Determination of DNA synthesis in smooth muscle cells

SMCs from passages 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 in 5% CO_2–95% air. Then the complete medium was replaced with the basal medium (SmBM) containing 20 ng/mL human PDGF-BB (Carbiochem Corp; San Diego, CA, USA) and various concentration of test compounds. After 24 h, 1 μCi/mL ^{3}H-thymidine was added into the medium, and the cells ware incubated for 4 h at 37 in 5% CO_2–95% air. Then the cells were harvested by trypsinization, and radioactive thymidine incorporation into 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 plates for 4 h at 37 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 in 5% CO_2–95% air. After 3 days, DNA synthesis was determined during the last 4 h of the 3-day culture.

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