Alkoxybenzylcyanoguanidine Analogs as a Novel Class of Inhibitors for Restenosis

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Received February 25, 2004

A novel class of alkoxybenzylcyanoguanidine analogs as the inhibitors of restenosis was discovered, which showed the inhibitory effects on angiotensin II-induced cell proliferation, determined by [^{3}H]thymidine incorporation method. The compound**,** *N'*-(4-nitrophenyl)guanidine analog **19**, showed 62% inhibition of [^{3}H]thymidine incorporation at 1 μM concentration. In addition, the compound **19** inhibited intimal thickening dose-dependently after balloon injury, which suggests the therapeutic potential for restenosis.

Key Words : Alkoxybenzylcyanoguanidine, PTCA, Restenosis, Cell proliferation, Intimal hyperplasia

Introduction

Coronary artery stenosis can often be successfully treated through percutaneous coronary intervention (PCI),[1] including conventional balloon angioplasty or percutaneous transluminal coronary angioplasty (PTCA).[2] However, restenosis is still the major limitation of the procedure, despite continuing improvement in technical equipment and medical therapy.[3-6] Restenosis appears to be influenced by multiple mechanisms, therefore its prevention and treatment remain as a major challenge in the cardiovascular research.[7-11] Although the complete biology and pathology of restenosis are not fully understood, identification of the factors thought to play a role in the progression of restenosis provides a number of pharmacological targets including platelets, inflammation, smooth muscle cell (SMC) proliferation and migration, and endothelial cellular matrix (ECM) synthesis.[12,13] Some drug classes, including glycoprotein IIb/IIIa antagonists, nitric oxide donors, and antioxidant probucol, have been demonstrated potential benefits in clinical trials by local administration. Agents that alter ECM, such as matrics metalloproteinase inhibitors (MMPIs), integrin antagonists, and hyaluronan mimetics, might have therapeutic efficacy against restenosis. Although the relationship between apoptosis, cell proliferation, and restenosis after balloon angioplasty has not been clearly demonstrated, a cellular mass at the arterial injury site depends on the balance between cell death including apoptosis and cell proliferation.[7]

For several years, we have been working on the identification of ATP sensitive potassium channel (K_{ATP}) openers to protect cardiac cells from ischemic cell death.[14] Simultaneously, we investigated inhibitory effect on angiotensin II-induced smooth muscle cell proliferation of K_{ATP} openers besides their anti-ischemic properties. From these studies, several alkoxybenzylcyanoguanidine analogs, which were modified by the ring opening of benzopyranylcyanoguanidines previously studied as K_{ATP} openers (Figure 1), were identified to inhibit cell proliferation. We also examined *in vivo* effects on intimal hyperplasia by balloon injury in rats to confirm the therapeutic potential of this series of compounds.

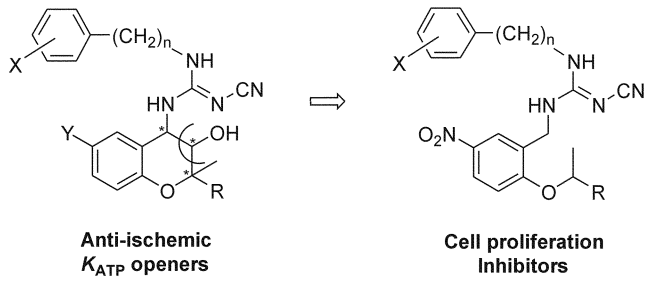

Figure 1

Chemistry

2-Hydoxy-5-nitrobenzyl bromide was reacted with sodium azide to give the azide **1**, and then the phenolic hydroxy was alkylated by the treatment of ethyl 2-bromopropionate to provide the ester **2**. Hydrolysis of **2** and subsequent reduction with borane in THF afforded the alcohol **4**. The alcohol **4** was oxidized to the aldehyde **5** by Swern oxidation,[15] followed by acetalisation to yield the dimethyl acetal **6** (Scheme 1).

Azidomethylbenzene compounds (**2**, **4**, **6**) were reduced to the corresponding benzylamines (**7**, **8**, **9**) with triphenylphosphine. The alcohol **8** was alternatively obtained by the reduction of **2** with lithium aluminum hydride. The alkoxybenzylcyanoguanidine compounds (**10**-**20**) were synthesized by the treatment of benzylamines (**7**-**9**) with *N*-cyanothioureas,[16] using a water soluble coupling reagent (WSC) in DMF (Scheme 2). *N*-Cyanothioureas were prepared independently from the corresponding isothiocyanates and preformed monosodium cyanamide.

Results and Discussion

The inhibitory effect of alkoxybenzylcyanoguanidine

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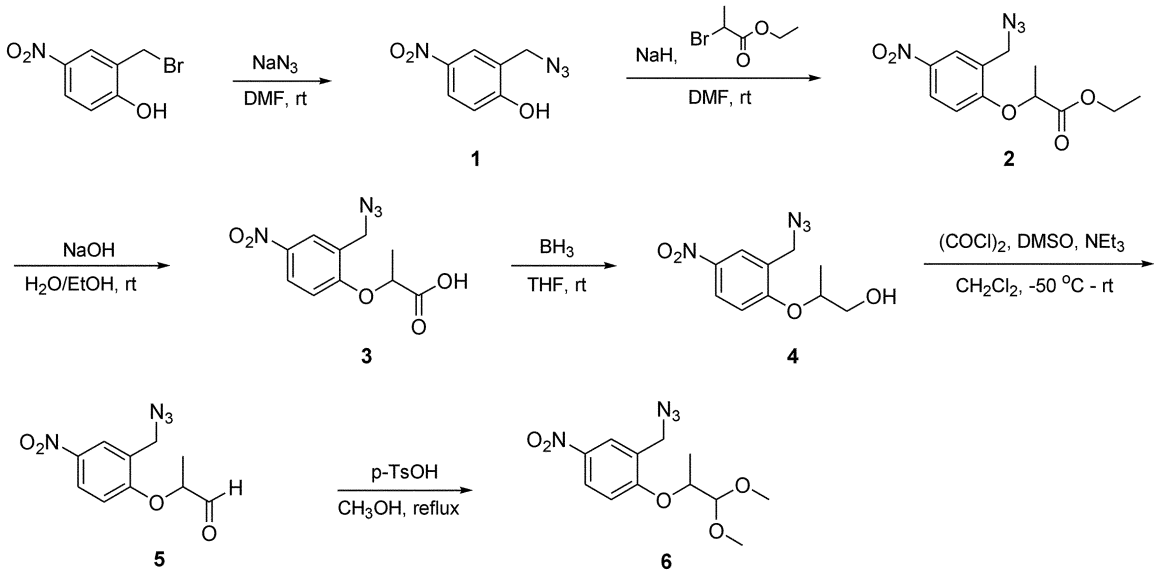
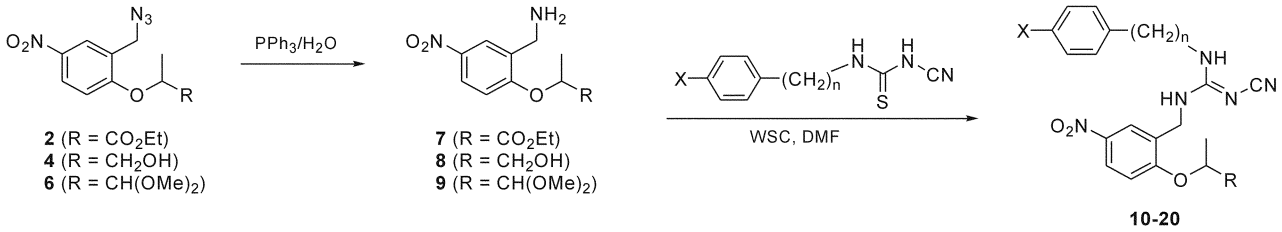


Scheme 1



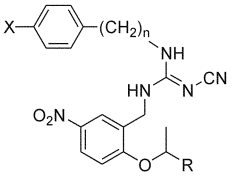
Scheme 2

derivatives on cell proliferation, was determined by [^{3}H]-thymidine incorporation method. [17] As shown in Table 1, some of the compounds strongly inhibited the angiotensin II-induced DNA synthesis at 1 μM.

Primarily, we investigated the effect of sunstituents (R) including ethyl ester, alcohol, or dimethyl acetal. None of the alcohol compounds (**15**-**17**) showed the significant inhibitory effect on angiotensin II-induced increase of DNA synthesis. On the other hand, ethyl ester compounds with *N*-*p*-nitrophenyl **12** and *p*-methylphenyl **13** group inhibited the DNA synthesis greater than 50% at 1 μM, but very weak inhibition (13.6%) was shown by the compound with *p*-methoxyphenyl group **11**. Both acetal compounds with *p*-chlorophenyl **18** and *p*-nitrophenyl **19** substitutents significantly inhibited the DNA synthesis, especially *p*-nitrolphenyl compound **19** showed 62% inhibition of [^{3}H]thymidine incorporation at 1 μM.

The balloon injury model using rats[18] has been the most frequently applied *in vivo* model to study restenosis. Because of their reproducibility of intimal hyperplasia within two weeks after balloon injury, rats are commonly used.[19] Since the compound **19** effectively inhibited angiotensin II-induced cell proliferation, the *in vivo* experiment was then carried out to examine the therapeutic potential of this series of compounds as restenosis inhibitors. Intimal area (0.171 mm^2) in balloon injured rats was approximately 55 times larger than that in sham operated rats (0.0031 mm^2) as shown in Table 2 and Figure 2. The compound **19** significantly blocked the intimal thickening in the rat model in a dose-dependent manner, showing an *in vivo* potential to

Table 1. Inhibitory effects on angiotensin II-induced cell proliferation (1 μM)



Compound	n	X	R	[^{3}H]thymidine incorporation (%)
Basal				0
Control (Ang II)				100
11	0	OCH_3	COOEt	86.4
12	0	NO_2	COOEt	49.6
13	0	CH_3	COOEt	49.6
15	0	Cl	CH_2OH	79.6
16	0	NO_2	CH_2OH	80.9
17	1	H	CH_2OH	82.6
18	0	Cl	$CH(OCH_3)_2$	67.7
19	0	NO_2	$CH(OCH_3)_2$	38.0

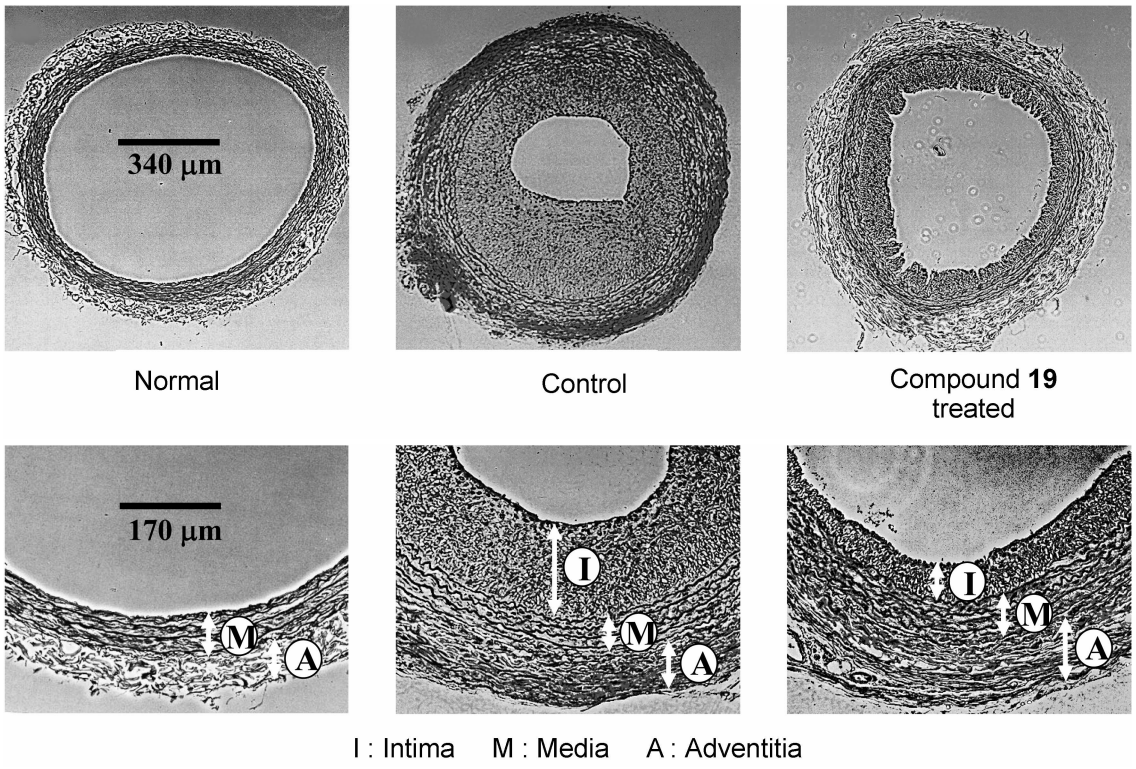


Figure 2. Effects of the compound **19** on restenosis after balloon angioplasty (rat).

Table 2. Effects of the compound **19** on intimal thickening by balloon injury in rats

Compounds (b.i.d.; i. p.)		n	Intimal area (mm^2)	Inhibition (%)
Normal (sham operated)		8	0.0031 ± 0.0004	
Control (balloon injured)		19	0.171 ± 0.024	
Compound **19** Treated	1 mg/Kg/day	10	0.167 ± 0.025	2.4
	3 mg/Kg/day	6	0.125 ± 0.011	27.4
	10 mg/Kg/day	14	0.107 ± 0.019	38.1

treat restenosis. We are going to prepare more analogs of the compound **19** and investigate their antiproliferative properties to study the structure-activity relationships of this novel class of compounds, and to find more potent compounds. In addition, we will continuously study the inhibitory effect of the compound **19** on restenosis in various animal models, as well as its mechanism on antiprolifeative activity, pharmacokinetic profiles, and toxicity.

Conclusion

We found a novel class of alkoxybenzylcyanoguanidine analogs, which showed inhibitory effect on cell proliferation by the inhibition of DNA synthesis. Especially, the compound **19** blocked intimal hyperplasia in a dose dependent manner after balloon injury in rats as well as the strong inhibition on cell proliferation, indicating therapeutic potential for treating restenosis after arterial injury.

Experimental Section

Chemistry. Anhydrous solvents were dried by conventional methods. Reagents of commercial quality were used from freshly opened containers unless otherwise stated. ^{1}H NMR spectra were recorded on a Varian Gemini 200.

2-Azidomethyl-4-nitrophenol (1). To a solution of 2-hydroxy-5-nitrobenzyl bromide (10 g, 43 mmol) in DMF (120 mL) was added sodium azide (5.6 g, 86 mmol). The mixture was stirred for 2 h at room temperature. The reaction mixture was poured into water (200 mL), and then extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane : ethyl acetate = 1 : 1) to yield the compound **1** (8.0 g, 96%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.53 (s, 2H), 6.88 (brs, 1H), 6.98 (d, 1H, J = 8.0 Hz), 8.13-8.18 (m, 2H).

2-(2-Azidomethyl-4-nitrophenoxy)propionic acid ethyl ester (2). To a solution of the compound **1** (7.1 g, 36.7 mmol) in DMF (100 mL) were added sodium hydride portionwise (1.76 g, 44.0 mmol, 60% in oil) and ethyl 2-bromopropionate (5.71 mL, 44.0 mmol) at 0 °C. The reaction mixture was stirred for 3 h at room temperature, and neutralized with 1 N HCl, then extracted with ethyl acetate (150 mL × 2). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane : ethyl acetate = 2 : 1) to yield the compound **2** (9.1 g, 90%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.28 (t, 3H,

J = 7.2 Hz), 1.75 (d, 3H, *J* = 6.7 Hz), 4.25 (q, 2H, *J* = 7.2 Hz), 4.48 (d, 1H, *J* = 14.4 Hz), 4.61 (d, 1H, *J* = 14.4 Hz), 4.95 (q, 1H, *J* = 6.7 Hz), 6.83 (d, 1H, *J* = 8.8 Hz), 8.18–8.26 (m, 2H).

2-(2-Azidomethyl-4-nitrophenoxy)propionic acid (3). To a solution of the compound **2** (9.0 g, 31.0 mmol) in ethanol (200 mL) was added 2 N NaOH (100 mL). The reaction mixture was heated at reflux for 2 h, and all volatiles were removed under reduced pressure. The residue was neutralized with 1 N HCl, and extracted with ethyl acetate (200 mL × 2). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (10% methanol in dichloromethane) to yield the compound **3** (7.1 g, 78%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.77 (d, 3H, *J* = 6.7 Hz), 4.48 (d, 1H, *J* = 14.4 Hz), 4.61 (d, 1H, *J* = 14.4 Hz), 4.98 (q, 1H, *J* = 6.7 Hz), 6.83 (d, 1H, *J* = 8.8 Hz), 8.18 –8.26 (m, 2H).

2-(2-Azidomethyl-4-nitrophenoxy)propan-1-ol (4). To a solution of the compound **3** (1.0 g, 3.8 mmol) in THF (3 mL) was added an 1 M solution of BH_3 in THF (11.3 mL, 11.3 mmol) dropwise, and the reaction mixture was stirred for 4 h at room temperature. To the reaction mixture was added a saturated solution of $NaHCO_3$, which was extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane : ethyl acetate = 1 : 1) to yield the compound **4** (593 mg, 63%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.38 (d, 3H, *J* = 6.4 Hz), 2.36 (brs, 1H), 3.82 (d, 2H, *J* = 6.2 Hz), 4.38 (d, 1H, *J* = 13.7 Hz), 4.49 (d, 1H, *J* = 13.7 Hz), 4.72 (m, 1H), 7.05 (d, 1H, *J* = 9.0 Hz), 8.17-8.26 (m, 2H).

2-(2-Azidomethyl-4-nitrophenoxy)propionaldehyde (5). A solution of the compound **4** (1.36 g, 5.4 mmol) in dichloromethane (20 mL) was cooled to -50 °C. To the solution was added oxalyl chloride (0.7 mL, 8.1 mmol), and the reaction mixture was stirred for 10 min, followed by the addition of DMSO (0.91 mL, 12.9 mmol). The reaction mixture was stirred for 2 h at -50 °C, to which was added triethylamine (3.75 mL, 26.9 mmol), then it was continuously stirred at room temperature for 2 h. Water was added to the reaction mixture, which was extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane : ethyl acetate = 1 : 1) to yield the compound **5** (966 mg, 72%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.63(d, 1H, *J* = 6.8 Hz), 4.52 (s, 2H), 4.89 (d, 1H, *J* = 6.8 Hz), 6.83 (d, 1H, *J* = 8.6 Hz), 8.18-8.26 (m, 2H), 9.72 (s, 1H).

2-Azidomethyl-1-[(2,2-dimethoxy-1-methyl)ethoxy]-4-nitrobenzene (6). To a solution of the compound **5** (966 mg, 3.9 mmol) in methanol (16 mL) was added catalytic amounts of *p*-toluenesulfonic acid. The reaction was heated at reflux for 17 h with stirring, and concentrated under reduced pressure after cooling. To the residue a saturated solution of $NaHCO_3$ was added, which was extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane : ethyl acetate = 1 : 1) to yield the compound **6** (1.12 g, 97%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.40 (d, 1H, *J* = 6.0 Hz), 3.42 (s, 3H), 3.49 (s, 3H), 4.35-4.44 (m, 3H), 4.57 (m, 1H), 7.04 (d, 1H, *J* = 8.0 Hz), 8.10-8.24 (m, 2H).

2-(2-Aminomethyl-4-nitrophenoxy)propionic acid ethyl ester (7). To a solution of the compound **2** (500 mg, 1.7 mmol) in THF (8 mL) were added triphenylphosphine (535 mg, 2.0 mmol) and water (36 μL, 2.0 mmol). The reaction mixture was stirred at room temperature for 20 h, then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (20% methanol in dichloromethane) to yield the compound **7** (291 mg, 64%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.26 (t, 3H, *J* = 7.2 Hz), 1.70 (d, 3H, *J* = 6.8 Hz), 3.87 (d, 1H, *J* = 15.0 Hz), 4.02 (d, 1H, *J* = 15.0 Hz), 4.22 (q, 2H, *J* = 7.2 Hz), 4.93 (q, 1H, *J* = 6.8 Hz), 6.76 (d, 1H, *J* = 8.9 Hz), 8.11 (dd, 1H, *J* = 2.8, 8.9 Hz), 8.22 (d, 1H, *J* = 2.8 Hz).

2-(2-Aminomethyl-4-nitrophenoxy)propan-1-ol (8). To a solution of the compound **2** (310 mg, 1.0 mmol) in THF (8 mL) was added lithium aluminum hydride (77 mg, 2.0 mmol) slowly at 0 °C, and the mixture was stirred for 20 min at 0 °C. To the reaction was added 1 N HCl to pH 3-4. An acidic solution was washed with dichloromethane (10 mL), and basified with 2 N NaOH to pH 8-9, then it was extracted with ethyl acetate (15 mL × 2). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (10% methanol in dichloromethane) to give the compound **8** (80 mg, 35%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.41 (d, 3H, *J* = 6.6 Hz), 3.68 (m, 2H), 3.75 (1H, *J* = 12.4 Hz), 4.10 (d, 1H, *J* = 12.4 Hz), 4.55 (m, 1H), 6.99 (d, 1H, *J* = 8.9 Hz), 8.07-8.16 (m, 2H).

2-[(2,2-Dimethoxy-1-methyl)ethoxy]-5-nitrobenzylamine (9). Using the compound **6** (8 g, 27 mmol) as a starting material, the same reaction as the preparation of the compound **7** was proceeded to yield the compound **9** (6.2 g, 85%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.41 (d, 3H, *J* = 6.3 Hz), 3.43 (s, 3H), 3.51 (s, 3H), 3.81-3.98 (m, 2H), 4.44 (d, 1H, *J* = 5.5 Hz), 4.55 (m, 1H), 6.96 (d, 1H, *J* = 9.0 Hz), 8.14 (dd, 1H), 8.22 (d, 1H).

***N′′*-Cyano-*N*-[5-nitro-2-(1-ethoxycarbonyl)ethoxy]benzyl]-*N′*-(4-chlorophenyl)guanidine (10).** The compound **7** (67 mg, 0.25 mmol) was dissolved in DMF (1 mL). To the solution were added *N*-cyano-*N′*-4-chlorophenylthiourea sodium salt (76 mg, 0.32 mmol) and 1-[3-(dimethylamino)-propyl]-2-ethylcarbodiimide hydrochloride (62 mg, 0.32 mmol). The reaction mixture was stirred at room temperature for 3 h, and extracted with ethyl acetate (20 mL × 2). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane : ethyl acetate = 1 : 1) to yield the compound **10** (25 mg, 23%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.23 (t, 3H, *J* = 5.7 Hz), 1.55 (d, 3H, *J* = 6.8 Hz), 4.11 (q, 2H, *J* = 5.7 Hz), 4.53 (dd, 1H, *J* = 5.3, 11.4 Hz), 4.59 (dd, 1H, *J* = 6.7, 11.4 Hz),

4.87 (q, 1H, *J* = 6.8 Hz), 6.02 (brs, 1H), 6.77 (d, 1H, *J* = 8.9 Hz), 7.22-7.48 (m, 4H), 7.83 (brs, 1H), 8.13 (dd, 1H, *J* = 2.8, 7.0 Hz), 8.22 (d, 1H, *J* = 2.8 Hz).

***N''*-Cyano-*N*-[5-nitro-2-(1-ethoxycarbonyl)ethoxy]benzyl]-*N'*-(4-methoxyphenyl)guanidine (11).** Using the compound **7** (67 mg, 0.25 mmol), *N*-cyano-*N'*-4-methoxyphenylthiourea sodium salt (75 mg, 0.33 mmol), and 1-[3-(dimethyl amino)propyl]-2-ethylcarbodiimide hydrochloride (63 mg, 0.33 mmol), the same reaction as the preparation of the compound **10** was proceeded to give the compound **11** (30 mg, 27%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.24 (t, 3H, *J* = 7.1 Hz), 1.54 (d, 3H, *J* = 6.9 Hz), 3.81 (s, 3H), 4.14 (q, 2H, *J* = 7.1 Hz), 4.43-4.68 (m, 2H), 4.85 (q, 1H, *J* = 6.9 Hz), 5.50 (brs, 1H), 6.74 (d, 1H, *J* = 8.8 Hz), 6.93 (d, 2H, *J* = 9.0 Hz), 7.10 (s, 1H), 7.19 (d, 2H, *J* = 9.0 Hz), 8.11-8.17 (m, 2H).

***N''*-Cyano-*N*-[5-nitro-2-(1-ethoxycarbonyl)ethoxy]benzyl]-*N'*-(4-nitrophenyl)guanidine (12).** Using the compound **7** (80 mg, 0.3 mmol), *N*-cyano-*N'*-4-nitrophenylthiourea sodium salt (83 mg, 0.39 mmol), and 1-[3-(dimethyl-amino)propyl]-2-ethylcarbodiimide hydrochloride (74 mg, 0.39 mmol), the same reaction as the preparation of the compound **10** was proceeded to give the compound **12** (41 mg, 32%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.23 (t, 3H, *J* = 6.2 Hz), 1.52 (d, 3H, *J* = 6.6 Hz), 4.14 (q, 2H, *J* = 6.2 Hz), 4.53–4.79 (m, 2H), 4.84 (q, 1H, *J* = 6.6 Hz), 6.74 (d, 1H, *J* = 8.8 Hz), 7.13 (d, 2H, *J* = 7.6 Hz), 7.20 (d, 2H, *J* = 7.6 Hz), 7.47 (s, 1H), 8.09-8.15 (m, 2H).

***N''*-Cyano-*N*-[5-nitro-2-(1-ethoxycarbonyl)ethoxy]benzyl]-*N'*-(4-methylphenyl)guanidine (13).** Using the compound **7** (80 mg, 0.3 mmol), *N*-cyano-*N'*-4-methylphenylthiourea sodium salt (83 mg, 0.39 mmol), and 1-[3-(dimethyl amino)propyl]-2-ethylcarbodiimide hydrochloride (74 mg, 0.39 mmol), the same reaction as the preparation of the compound **10** was proceeded to give the compound **13** (41 mg, 32%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.23 (t, 3H, *J* = 6.2 Hz), 1.52 (d, 3H, *J* = 6.6 Hz), 2.33 (s, 3H), 4.14 (q, 2H, *J* = 6.2 Hz), 4.42-4.68 (m, 2H), 4.84 (q, 1H, *J* = 6.6 Hz), 5.67 (dd, 1H, *J* = 5.8, 5.9 Hz), 6.74 (d, 1H, *J* = 8.8 Hz), 7.13 (d, 2H, *J* = 7.6 Hz), 7.20 (d, 2H, *J* = 7.6 Hz), 7.47 (s, 1H), 8.09-8.15 (m, 2H).

***N''*-Cyano-*N*-[5-nitro-2-(1-ethoxycarbonyl)ethoxy]benzyl]-*N'*-benzylguanidine (14).** Using the compound **7** (87 mg, 0.33 mmol), *N*-cyano-*N'*-benzylthiourea sodium salt (90 mg, 0.42 mmol), and 1-[3-(dimethylamino)propyl]-2-ethyl carbodiimide hydrochloride (81 mg, 0.42 mmol), the same reaction as the preparation of the compound **10** was proceeded to give the compound **14** (32 mg, 23%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.26 (t, 3H, *J* = 7.0 Hz), 1.59 (d, 3H, *J* = 6.5 Hz, *J* = 7.0 Hz), 4.25 (q, 2H), 4.40-4.98 (m, 4H), 5.18 (q, 1H, *J* = 6.5 Hz), 5.40 (brs, 1H), 5.81 (brs, 1H), 6.81 (d, 1H, *J* = 8.7 Hz), 7.04 (d, 1H, *J* = 9.0 Hz), 7.13-7.30 (m, 3H), 8.00-8.17 (m, 3H).

***N''*-Cyano-*N*-[5-nitro-2-(2-hydroxy-1-methyl)ethoxy]benzyl]-*N'*-(4-chlorophenyl)guanidine (15).** Using the compound **8** (77 mg, 0.34 mmol), *N*-cyano-*N'*-(4-chlorophenyl)thiourea sodium salt (103 mg, 0.44 mmol), and 1-[3-(dimethyl amino)propyl]-2-ethylcarbodiimide hydrochloride (85 mg, 0.44 mmol), the same reaction as the preparation of the compound **10** was proceeded to give the compound **15** (87 mg, 64%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.24 (d, 3H, *J* = 6.9 Hz), 3.64 (m, 2H), 4.47-4.57 (m, 3H), 6.17 (dd, 1H, *J* = 5.4, 5.4 Hz), 6.91 (d, 1H, *J* = 8.8 Hz), 7.15 (d, 2H, *J* = 8.9 Hz), 7.28 (d, 2H, *J* = 8.9 Hz), 8.04–8.12 (m, 2H).

***N''*-Cyano-*N*-[5-nitro-2-(2-hydroxy-1-methyl)ethoxy]benzyl]-*N'*-4-nitrophenyl)guanidine (16).** Using the compound **8** (118 mg, 0.52 mmol), *N*-cyano-*N'*-(4-nitrophenyl)thiourea sodium salt (166 mg, 0.68 mmol), and 1-[3-(dimethyl-amino)propyl]-2-ethylcarbodiimide hydrochloride (130 mg, 0.68 mmol), the same reaction as the preparation of the compound **10** was proceeded to give the compound **16** (96 mg, 45%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.36 (d, 3H, *J* = 6.6 Hz), 3.74 (d, 2H, *J* = 4.7 Hz), 4.62 (s, 2H), 4.75 (m, 1H), 7.25 (d, 1H, *J* = 9.0 Hz), 7.46 (d, 2H, *J* = 9.1 Hz), 8.19-8.25 (m, 4H).

***N''*-Cyano-*N*-[5-nitro-2-[(2-hydroxy-1-methyl)ethoxy]-benzyl]-*N'*-benzylguanidine (17).** Using the compound **8** (240 mg, 1.1 mmol), *N*-cyano-*N'*-benzylthiourea sodium salt (294 mg, 1.4 mmol), and 1-[3-(dimethylamino)propyl]-2-ethylcarbodiimide hydrochloride (265 mg, 1.4 mmol), the same reaction as the preparation of the compound **10** was proceeded to give the compound **17** (125 mg, 30%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.29 (d, 3H, *J* = 6.0 Hz), 3.40 (brs, 1H), 3.71 (m, 2H), 4.26–4.44 (m, 4H), 4.59 (m, 1H), 6.06 (dd, 1H, *J* = 5.3, 5.3 Hz), 6.33 (brs, 1H), 6.92 (d, 1H, *J* = 9.6 Hz), 7.18-7.32 (m, 5H), 8.19-8.25 (m, 2H).

***N''*-Cyano-*N*-[5-nitro-2-(2-dimethoxy-1-methyl)ethoxy]-benzyl]-*N'*-(4-chlorophenyl)guanidine (18).** Using the compound **9** (107 mg, 0.39 mmol), *N*-cyano-*N'*-(4-chlroro-phenyl)thiourea sodium salt (120 mg, 0.51 mmol), and 1-[3-(dimethylamino)propyl]-2-ethylcarbodiimide hydrochloride (98 mg, 0.51 mmol), the same reaction as the preparation of the compound **10** was proceeded to give the compound **18** (77 mg, 44%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.28 (d, 3H, *J* = 6.2 Hz), 3.19 (s, 3H), 3.35 (s, 3H), 4.20 (d, 1H, *J* = 5.2 Hz), 4.48 (m, 3H), 5.69 (brs, 1H), 6.95 (d, 1H, *J* = 9.6 Hz), 7.19 (d, 2H, *J* = 8.7 Hz), 7.38 (d, 2H, *J* = 8.7 Hz), 7.92 (brs, 1H), 8.15-8.20 (m, 2H).

***N''*-Cyano-*N*-[5-nitro-2-(2-dimethoxy-1-methyl)ethoxy]-benzyl]-*N'*-(4-nitrophenyl)guanidine (19).** Using the compound **9** (107 mg, 0.4 mmol), *N*-cyano-*N'*-(4-nitro-phenyl)thiourea sodium salt (126 mg, 0.52 mmol), and 1-[3-(dimethylamino)propyl]-2-ethylcarbodiimide hydrochloride (99 mg, 0.52 mmol), the same reaction as the preparation of the compound **10** was proceeded to give the compound **19** (73 mg, 42%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.28 (d, 3H, *J* = 6.2 Hz), 3.13 (s, 3H), 3.27 (s, 3H), 4.25 (d, 1H, *J* = 5.2 Hz), 4.45-4.60 (m, 3H), 6.67 (brs, 1H), 6.95 (d, 1H, *J* = 9.2 Hz), 7.43 (d, 2H, *J* = 9.2 Hz), 8.13-8.25 (m, 4H), 8.95 (brs, 1H).

***N''*-Cyano-*N*-[5-nitro-2-(2-dimethoxy-1-methyl)ethoxy]-benzyl]-*N'*-benzylguanidine (20).** Using the compound **9** (126 mg, 0.47 mmol), *N*-cyano-*N'*-benzylthiourea sodium salt (129 mg, 0.6 mmol), and 1-[3-(dimethylamino)propyl]-2-ethylcarbodiimide hydrochloride (116 mg, 0.6 mmol), the

same reaction as the preparation of the compound **10** was proceeded to give the compound **20** (58 mg, 29%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.32 (d, 3H, J = 6.3 Hz), 3.33 (s, 3H), 3.41 (s, 3H), 4.32-4.52 (m, 6H), 6.27 (brs, 1H), 6.94 (d, 1H, J = 9.7 Hz), 7.20-7.61 (m, 5H), 8.09-8.12 (m, 2H).

Biology. *In vitro* inhibitory effects on cell proliferation. Inhibitory effects on cell proliferation of the compounds were evaluated by measurement of incorporation of [^{3}H]-thymidine into DNA. Rat aortic smooth muscle cells were grown in 24 well plate for 3 days to near confluence in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), then DMEM containing FBS was washed out. Cells were cultured again in DMEM without serum for 48 h to be quiescent. Test compounds were added 15 min before addition of angiotensin II (10^{-7} M), which stimulate cell proliferation, then cells were incubated for 72 h. During the last 4 h of incubation, [^{3}H]thymidine (1 μCi/mL) was added. Radioactive medium was removed and cells were washed 3 times with DMEM (3×1 mL) to remove non-incorporated isotopes, and treated with an aqueous solution of 15% TCA (trichloroacetic acid) for at least 2 h, followed by the addition of an aqueous solution of 0.2 N NaOH (0.25 mL). The samples were filtered through glass microfiber filter (GF/B. Whatmann) under vacuum. After washing the filters 3 times with 2 mL of an aqueous solution of 5% TCA, the radioactivity incorporated into DNA was counted by Liquid Scintillation Counter (Packard, TRI-CARB, 2100TR), then calculated incorporation % of [^{3}H]thymidine.

Inhibitory effects on intimal thickening after balloon injury in rats. Male Sprague-Dawley rats (weighing 400-450 g, Bio Genomics, Seoul, Korea) were anesthetized by intraperitoneal injection of pentobarbital sodium (35 mg/Kg), and left common carotid artery angioplasty was performed under aseptic conditions as described previously.[20] Briefly, after a midline incision, the left external carotid artery was identified and cleared of adherent tissue. A Fr Fogarty embolectomy balloon catheter (Baxter Healthcare, Santa Ana, CA, USA) was inserted into the left common carotid artery through an incision made in the wall of the external carotid artery. The balloon catheter was inflated with a fixed volume of fluid to distend the common carotid artery and then pulled back to the site of insertion, deflated completely. This procedure was repeated three times, then the catheter was removed, and the incision on the wall of vessel was sutured. After recovery from the surgery, the animals were housed with controlled 12 h light: 12 h dark cycles and allowed access to food and water ad libitum until the day of experiment. The rats in normal group were sham-operated for the reference of balloon injury. Rats were injected intraperitoneally with vehicle or test compounds (1, 4 and 10 mg/Kg/day, b.i.d.) beginning 6 days before the balloon injury to 14 days after the balloon injury for 20 days. At 14 days after the injury, heparin (100 IU/rat) was injected to rats, and the rats were sacrificed by exsanguination under pentobarbital anesthesia. The left carotid arteries were removed, fixed in 10% neutral solution of formaldehyde until embedding in paraffin, and cross-sectioned into 3 segments (each 500 μm apart) for histological analysis. All samples were stained with hematoxylin-eosin. The cross-sectional intimal, medial, and luminal areas were measured using a computerized image analyzer (Image Pro Plus®, Media Cybernetics, Silver Spring, MD, USA). Inhibition (%) was calculated by following mathematical formula.

$$\text{Inhibition (\%)} = \{(\text{area of positive control} - \text{area of normal}) - (\text{area of treated} - \text{area of normal})\}/(\text{area of positive control} - \text{area of normal}) \times 100$$

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