DOI 10.5012/bkcs.2010.31.11.3353

Anti-Cancer Activity of T-Type Calcium Channel Blocker *In Vivo*

Hang Ah Park, Soo Yeon Jung, So Hyung Lee, Han Byul Kang, Min Sik Min, Jungahn Kim, Dong Joon Choo, Chun Rim Oh,† Young Deuk Kim,† Kyung-Tae Lee,‡ and Jae Yeol Lee*

*Research Institute for Basic Sciences and Department of Chemistry, College of Sciences, Kyung Hee University, Seoul 130-701, Korea. *E-mail: ljy@khu.ac.kr*
†DongWoo Syntech Co., Ltd., Bondae-Ri Geumwang-Eup, Eumsung-Goon, Choongcheungbook-Do 369-901, Korea
‡Kyung Hee East-West Pharmaceutical Research Institute and Department of Pharmaceutical Biochemistry, College of Pharmacy, Kyung Hee University, Seoul 130-701, Korea
Received August 2, 2010, Accepted September 15, 2010

3,4-Dihydroquinazoline **1** as T-type calcium channel blocker was *in vivo* evaluated against A549 xenograft in BALB/c-nu Slc mice, which exhibited 54% tumor growth inhibition through oral administration of 8 mg/kg of body weight and was slightly less active than doxorubicin (68%). In addition, this compound was also profiled for its acute toxicity to ICR mice to afford oral LD_{50} value of 1,038 mg/kg of body weight.

Key Words: 3,4-Dihydroquinazoline, Acute toxicity, A549 Xenograft, Anti-cancer

Introduction

Calcium is an important activator or inhibitor of numerous intracellular enzymes in the cytosol, organelles and nucleus.[1] Proliferation of tumors and non-tumor cells is regulated, in part, by the second messenger calcium.[2-3] In particular, T-type (low voltage activated) calcium channels are well recognized to play a role in regulating important cellular phenotype transitions leading to cell proliferation, differentiation, growth and death.[4-5] Recently, the anti-cancer effects of a T-type Ca^{2+} channel antagonists (or blockers) including mibefradil and pimozide[6] on tumor cells *in vivo* have been reported by many researcher groups.[7] Our group have also reported the identification of 3,4-dihydroquinazoline compound **1**, which exhibits both selective/potent T-type calcium channel blocking effect and *in vitro* strong anti-cancer effect on cancer cell lines comparable to doxorubicin as shown in Table 1 and 2.[8-9]

Based on these interesting biological data, we have further carried out A549 xenograft study for 3,4-dihydroquinazoline compound **1** and its acute toxicity study. In addition, we have re-designed the scale-up synthetic procedure of compound **1** for *in vivo* animal study. All results of these studies are reported herein.

Table 1. *In vitro* calcium channel blocking effects of 3,4-dihydroquinazoline **1**[8]

Compounds	HEK293 cell (T-Type: α_{1G})	HEK293 cell (N-Type: α_{1B})	Selectivity (T/N-Type)[b]
	IC_{50} (μM)[a]	IC_{50} (μM)[a]	
1	0.041 ± 0.001	4.9 ± 0.0	119.5
mibefradil	1.34 ± 0.49	1.34 ± 0.02	1.0

[a]IC_{50} value was determined from the dose-response curve by using patch-clamp method; [b]Selectivity index based on IC_{50} values.

Table 2. *In vitro* anti-cancer effects of 3,4-dihydroquinazoline **1**.[9]

Compound	Growth inhibition of cancer cell (GI_{50}: μM)[a]				
	A549[b]	DU 145[c]	HT-29[d]	SK-MEL-2[e]	SK-OV-3[f]
1	0.17 ± 0.02	0.19 ± 0.02	0.04 ± 0.01	0.48 ± 0.13	0.66 ± 0.13
Doxorubicin	0.16 ± 0.01	0.06 ± 0.01	0.21 ± 0.04	0.11 ± 0.01	0.12± 0.02

[a]value was determined from dose-response curve and obtained from three independent experiments; [b]human lung carcinoma (A549); [c]human prostate cancer (DU 145); [d]human colon cancer (HT-29); [e]human malignant melanoma (SK-MEL-2); [f]human ovarian cancer (SK-OV-3).

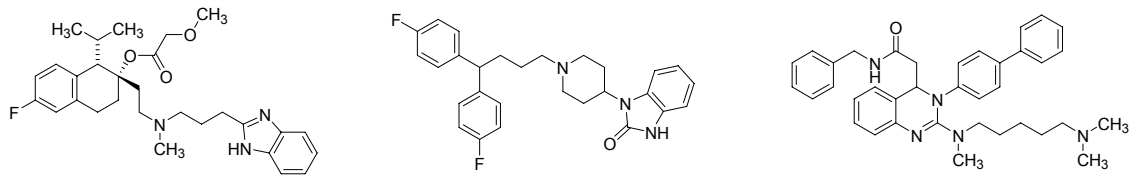


Figure 1. Structures of mibefradil, pimozide, and 3,4-dihydroquinazoline (**1**).

Experimental

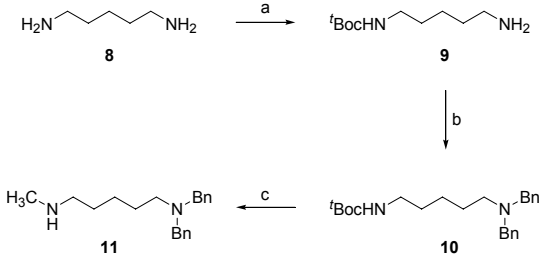
Materials and general methods. Melting points were determined with a MEL-TEMP® capillary melting point apparatus and have not been corrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 (400 MHz) spectrometer, using TMS as the internal standard; the chemical shifts (δ) are reported in parts per million and coupling constant (*J*) values are given in hertz (Hz). Signal multiplicities are represented by: s (singlet), d (doublet), t (triplet), q (quartet), br s (broad singlet), m (multiplet), and dd (double doublet). Low resolution and high-resolution mass spectra (FABMS, positive ion mode) were obtained using a JEOL 700 mass spectrometer. The progress of all reactions was monitored using TLC on precoated silica gel plates (Merck Silica Gel 60 F_{254}). The chromatograms were viewed under UV light at 254 and 365 nm. For column chromatography, Merck Silica Gel (230 - 400 mesh) was used. The chemicals were purchased from Sigma-Aldrich and Tokyo Kasei Chemicals, and the solvents were of analytical grade and used after standard purification.

Synthesis.

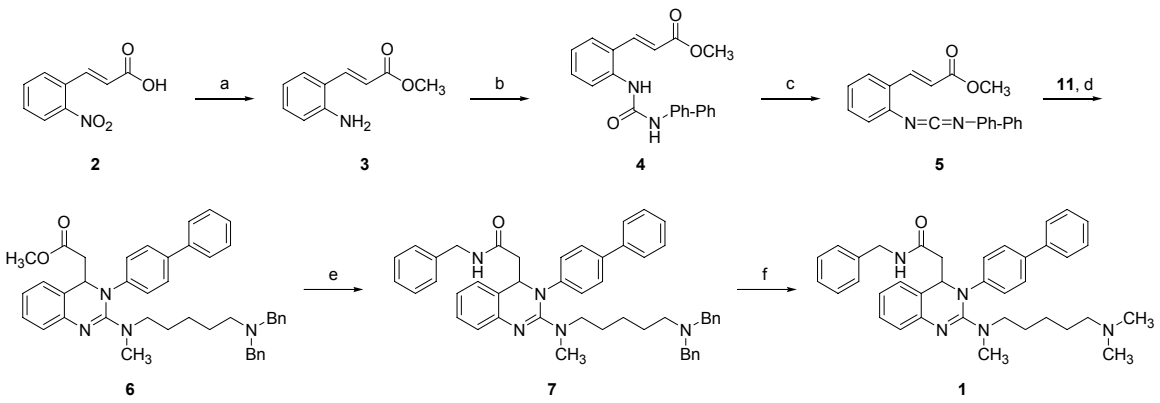
Methyl 2-aminocinnamate (3): To a solution of 2-nitrocinnamic acid (**2**, 3.00 g, 15.53 mmol) in CH_3OH (100 mL) was dropped 95% H_2SO_4 (0.25 mL, 4.66 mmol) at room temperature, and the reaction mixture was stirred at reflux overnight and then allowed to cool to room temperature. A saturated solution of $NaHCO_3$ was added and the mixture was extracted with dichloromethane, dried ($MgSO_4$), and filtered. The organic solvent was concentrated in vacuo to give 3.21 g (99%) of methyl 2-nitrocinnamate as a yellow solid. To a solution of the obtained methyl 2-nitrocinnamate (3.21 g, 15.53 mmol) in EtOAc (100 mL) was added $SnCl_2{\cdot}2H_2O$ (17.52 g, 77.65 mmol) at room temperature, and the reaction mixture was stirred at reflux for 1 h. After cooling to room temperature, the resulting mixture was washed with *aq*. $NaHCO_3$ solution, dried ($MgSO_4$), evaporated in vacuo to give 2.70 g (98%) of compound **3** as a yellow solid: mp 67 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, *J* = 15.9 Hz, 1H, -C-C*H*=CH-), 7.44-6.76 (m, 4H, aromatic), 6.39 (d, *J* = 15.8 Hz, 1H, -C-CH=C*H*-), 3.83 (s, 3H, -OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.7, 145.6, 140.3, 131.3, 128.1, 119.8, 118.9, 117.7, 116.8, 51.7.

Methyl 3-[2-(3-biphenylureido)phenyl]acrylate (4): To a solution of biphenyl-4-carboxylic acid (5.00 g, 23.96 mmol) in anhydrous toluene (100 mL) was added Et_3N (6.66 mL, 47.93 mmol) and diphenylphosphoryl azide (DPPA, 10.36 mL, 47.93 mmol) under argon atmosphere. The mixture was stirred at room temperature for 3 h and further stirred at 100 °C for 3 h. After cooling to room temperature, the above solution was treated with compound **3** (4.25 g, 23.96 mmol) and the reaction mixture was stirred to room temperature for 12 h. The reaction mixture was concentrated to afford a solid, which was washed with CH_3OH to give 7.14 g (80%) of compound **4** as a white solid: mp 189 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ 8.54 (s, 1H, -N*H*-CO-), 8.08 (s, 1H, -N*H*-CO-), 7.95-7.17 (m, 13H, aromatic), 7.96 (d, 1H, *J* = 16 Hz, -C-C*H*=CH-), 6.52 (d, 1H, *J* = 16 Hz, -CH=C*H*-CO-), 3.77 (s, 3H, -OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.9, 152.1, 140.4, 139.5, 137.9, 137.0, 128.9, 128.5, 127.8, 127.4, 127.0, 126.6, 125.6, 121.4, 120.9, 113.8, 59.0, 51.4.

Methyl 3-[2-(biphenyliminomethyleneamino)phenyl]acrylate (5): To a solution of compound **4** (5.00 g, 13.43 mmol) and Et_3N (5.62 mL, 40.28 mmol) in CH_2Cl_2 (100 mL) was added dibromotriphenylphosphorane (8.50 g, 20.14 mmol) at 0 °C and the reaction mixture was stirred at the same temperature for 12 h. After treating with water (50 mL), the mixture was extracted with CH_2Cl_2, dried ($MgSO_4$), filtered, and the solvent was evaporated in vacuo to give a solid, which was washed with CH_3OH to give 3.65 g (77%) of compound **5**: mp 105 - 106 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (d, 1H, *J* = 16 Hz, -C-C*H*=



Scheme 2. Reagents and conditions: (a) (^{t}Boc)$_2$O, MeOH, conc-HCl rt, 1 h 53%; (b) 2 equiv of benzyl bromide, Na_2CO_3, CH_2Cl_2, reflux, 12 h, 56%; (c) $LiAlH_4$, THF, reflux, 12 h, 77%.



Scheme 1. Reagents and conditions: (a) i. H_2SO_4, MeOH, reflux, 12 h, 99%; ii. $SnCl_2{\cdot}2H_2O$, EtOAc, reflux, 1 h, 98%; (b) biphenyl-4-carboxylic acid, DPPA, Et_3N, toluene, rt to 100 °C, 6 h, 80%; (c) $PPh_3{\cdot}Br_2$, Et_3N, CH_2Cl_2, 0 °C, 12 h, 77%; (d) toluene, rt, 1 h, 99%; (e) $PhCH_2NH_2$, TBD, 40 °C, 12 h, 89%; (f) HCHO, H_2, 10% Pd/C, CH_3OH, rt, 4 days, 70%.

CH-), 7.61-7.17 (m, 13H, aromatic), 6.53 (d, 1H, J = 16 Hz, -CH=C*H*-CO-), 3.82 (s, 3H, -OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.4, 140.3, 140.2, 138.8, 138.1, 136.9, 131.1, 128.9, 128.7, 128.2, 127.8, 127.5, 127.4, 126.9, 12.8, 125.8, 124.7, 119.3, 51.8.

3-Biphenyl-4-yl-2-[(5-*N*,*N*-dibenzylaminopentyl)-*N*'-methylamino]-4-methoxycarbonyl-methyl-3,4-dihydroquinazoline (6): To a solution of compound **5** (0.53 g, 1.48 mmol) in toluene (20 mL) was added *N*,*N*-dibenzyl-*N*'-methylpentane-1,5-diamine **11** (0.88 g, 2.97 mmol) at room temperature. The reaction solution was stirred at room temperature for 1 h, basified with 1 N NaOH, and extracted with CH_2Cl_2. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. Purification by flash column chromatography (EtOAc/*n*-hexane/CH_2Cl_2 = 1:2:1) gave 0.97 g (99%) of compound **6**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49-6.85 (m, 23H, aromatic), 5.10 (dd, J = 4.4 and 10.4 Hz, 1H, -CH_2-C*H*-N-), 3.68 (s, 3H, -OCH_3), 3.50 (s, 4H, 2 X -NCH_2Ph), 3.05-2.46 (m, 7H, -CO-CH_2-, -NCH_3, -CH_2-NBn$_2$), 2.35 (m, 2H, -CH_2-CH_2-$(CH_2)_3$-NBn$_2$), 1.46-1.18 (m, 6H, -CH_2-CH_2-CH_2-CH_2-NBn$_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0, 153.5, 145.6, 144.4, 140.5, 140.1, 136.9, 128.9, 128.9, 128.7, 128.3, 128.0, 127.3, 127.0, 126.9, 125.6, 124.9, 123.1, 122.8, 122.3, 61.4, 60.5, 58.5, 53.4, 51.9, 50.1, 39.7, 35.6, 27.4, 27.0, 24.8, 21.2, 14.4.

***N*-Benzyl-3-(biphenyl-4-yl)-2-[(5-*N*',*N*'-dibenzylaminopentyl)-*N*''-methylamino]-3,4-dihydroquinazoline 4-ylacetamide (7):** A mixture of compound **6** (0.5 g, 0.77 mmol), benzylamine (0.2 mL, 1.84 mmol), and 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD, 0.03 g, 0.23 mmol) was stirred at 40 °C for 12 h and concentrated under reduced pressure. Flash-column chromatography of residue (CH_2Cl_2/CH_3OH/NH_4OH = 100:9:1) afforded 0.31 g (70%) of compound **7**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63-7.05 (m, 28H, aromatic), 5.40 (dd, J = 5.6 and 9.0 Hz, 1H, -CH_2-C*H*-N-), 4.68-4.64 (m, 2H, PhCH_2-NH-), 3.62 (s, 4H, 2 X -NCH_2Ph), 2.95 (br s, 1H, -CH_2CHN-), 2.74-2.69 (m, 4H, -HCNCH_2- and -CH_2NBn$_2$), 2.46-2.41 (m, 3H, -NCH_3), 1.57-1.19 (m, 7H, -C*H*CH_2N- and -CH_2(CH_2)$_3$$CH_2$-); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 153.6, 145.3, 143.9, 140.4, 139.9, 137.8, 136.9, 128.8, 128.7, 128.6, 128.2, 128.1, 127.9, 127.8, 127.6, 127.1, 126.8, 126.7, 125.9, 125.0, 122.9, 122.6, 122.2, 61.3, 58.3, 53.2, 43.9, 42.0, 35.5, 28.4, 27.1, 26.8, 24.6.

4-(*N*-Benzylacetamino)-3-(biphenyl-4-yl)-2-*N*'-(5-*N*'',*N*''-dimethylaminopentyl)-*N*'-methylamino-3,4-dihydroquinazoline (1): To a solution of compound **7** (3.19 g, 4.40 mmol) in CH_3OH (50 mL) was added 10% Pd/C (0.70 g, 6.60 mmol) and formaldehyde (0.60 mL 21.97 mmol). The reaction mixture was stirred at room temperature for 4 days under H_2 atmosphere. The resulting mixture was filtered with Celite 545 and the solvent was evaporated off in vacuo. Flash column chromatography (CH_2Cl_2/CH_3OH/NH_4OH = 100:14:1) of the residue gave 1.75 g (70%) compound **1**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72-6.98 (18H, m, Ph), 5.32 (1H, dd, J = 9.1 and 4.6 Hz, $COCH_2$C*H*), 4.50 (2H, d, J = 5.8 Hz, PhCH_2-), 3.50-3.20 (2H, m, CH_3N-CH_2), 2.71-2.33 (4H, CH_3-N and COC*H*), 2.44 (1H, dd, J = 14.5 and 5.2 Hz, COC*H*), 2.30-2.29 (2H, m, -NCH_2), 2.21 (6H, s, 2 X NCH_3), 1.65-1.35 (4H, m, 2 xCH_2), 1.25-1.10 (2H, m, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 153.9, 145.3, 143.8, 140.4, 138.4, 136.7, 128.8, 128.6, 128.1, 128.0, 127.8, 127.4, 127.1, 127.0, 126.2, 125.1, 122.8, 122.4, 122.2, 61.2, 59.7, 49.5, 45.2, 43.8, 41.8, 35.3, 27.1, 27.0, 24.7; MS (FAB+), *m*/*z* (relative intensity, %) 596.7 ([M+Na]$^+$, 100), 574.7 ([M+H]$^+$, 30); MS (FAB-), *m*/*z* (relative intensity, %) 572.7 ([M-H]$^+$, 100); HRMS (FAB+) calcd for $C_{37}H_{44}N_5O$: [M+H]$^+$ = 574.3546, found = 574.3516.

***tert*-Butyl-5-aminopentylcarbamate (9):** To a solution of 150 mL of MeOH with cooling at 0 °C, 35% HCl solution (6.65 mL, 85.14 mmol) was added with stirring for 15 min. The mixture was stirred for 15 min at room temperature and was carefully added to 1,5-diaminopentane **8** (10 mL, 85.14 mmol) at 0 °C. The mixture was stirred for 15 min at room temperature before adding 50 mL of H_2O and stirring for another 30 min. To the solution Di-*tert*-butyl dicarbonate (18.58 g, 85.14 mmol) in 100 mL of MeOH was added at room temperature for 10 min and the resultant solution was stirred for 1 h. The reaction mixture was concentrated in vacuo and the remaining diamine was removed by diethyl ether. The residue was adjusted to pH 9 - 11 with 2 N NaOH solution and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried ($MgSO_4$) and concentrated in vacuo to provide 9.11 g (53%) of compound **9**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.57 (br, 1H, -NH-), 3.10 (d, J = 6.2 Hz, 2H, -NH-CH_2-), 2.69 (t, J = 6.9 Hz, 2H, -CH_2-NBn$_2$), 1.78 (br, 2H, -NH_2), 1.51-1.44 (m, 4H, -CH_2-CH_2-$(CH_2)_3$-NBn$_2$, and -$(CH_2)_3$-CH_2-CH_2-NBn$_2$), 1.42 (s, 9H, -O(CH_3)$_3$), 1.34 (m, 2H, -CH_2-CH_2-CH_2-NBn$_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.0, 79.1, 42.1, 40.5, 33.4, 30.0, 28.4, 24.1.

***tert*-Butyl-5-(*N*,*N*-dibenzylaminopentyl)carbamate (10):** To a solution of compound **9** (4.22 g, 20.82 mmol) in CH_2Cl_2 was added Na_2CO_3 (11.91 g, 112.43 mmol) and benzyl bromide (5.0 mL, 41.64 mmol). The reaction mixture was stirred at reflux overnight. The solution was extracted with CH_2Cl_2, dried ($MgSO_4$), filtered and purified by flash column chromatography (EtOAc/*n*-hexane/CH_2Cl_2 = 1:5:1) gave 4.50 g (56%) of compound **10** as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.23 (m, 10H, aromatic), 3.57 (s, 4H, 2 X -NCH_2Ph), 3.08 (m, 2H, HN-CH_2-), 2.44 (t, J = 7.1 Hz, 2H, -CH_2-N-), 1.55 (m, 2H, HN-CH_2-CH_2-), 1.48 (s, 9H, -O(CH_3)$_3$), 1.41-1.27 (m, 4H, HN-CH_2-CH_2-CH_2-CH_2-CH_2-); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.0, 140.0, 128.8, 128.2, 126.8, 79.0, 58.4, 53.1, 40.6, 30.0, 28.5, 26.7, 24.4.

***N*,*N*-Dibenzyl-*N*'-methylpentane-1,5-diamine (11):** To a solution of compound **10** (4.50 g, 11.76 mmol) in distilled THF was added $LiAlH_4$ (2.23 g, 58.80 mmol) and stirred at reflux overnight. To a cooled (0 °C) solution was added sodium potassium tartrate (16.59 g, 58.80 mmol) and the mixture was stirred overnight, filtered, and concentrated in vacuo to give 2.68 g (77%) of compound **11** as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42-7.24 (m, 10H, aromatic), 3.59 (s, 4H, -2 X -NCH_2 Ph), 2.56 (t, 2H, -CH_2-NBn$_2$), 2.48-2.44 (m, 5H, CH_3-NH-CH_2-), 1.57 (m, 2H, J = 7.2 Hz, -$(CH_2)_3$-CH_2-CH_2-NBn$_2$), 1.45 (m, 2H, -CH_2-CH_2-$(CH_2)_3$-NBn$_2$), 1.35 (m, 2H, -CH_2-CH_2-CH_2-NBn$_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.1, 128.9, 128.2, 126.8, 58.5, 53.4, 52.2, 36.5, 29.7, 27.1, 25.0.

Animal experiments.

Antitumor activity using A549 xenograft nude mice:[10-12] BALB/c-nu Slc mice (5 - 6 weeks old) were purchased from Central Lab. Animal Inc. (Seoul, Korea) and were acclimatized

for 1 or 2 weeks, before the experiment began. During this period and throughout the experiment the mice were housed in polysulfonate cages, and were given access *ad libitum* to water and pellet chow (TEKLAD CERTIFIED GLOBAL 18% PROTEIN RODENT DIET 2918C, Harlan TEKLAD, U.S.A.). The mice were kept under controlled conditions of temperature (20.7 - 22.0 °C) and relative humidity (26.7 - 49.9%), with a 12-hr light/dark cycle. All animal experiments were conducted according to the Guidelines of the Committee on the Care and the Use of Laboratory Animals of Biotoxtech Inc. (Cheongwon, Korea). The A549 cells, which had been grown in Ham's F-12 Medium plus 10% FBS, were collected and reconstituted in Dulbecco's Phosphate Buffered Saline (D-PBS, pH 7.4). Approximately, 5.4×10^7 cells/mL were injected subcutaneously into dorsum region of the BALB/c-nu Slc mice and the tumors were allowed to grow. After 2 weeks, Tumors resulting after 2 weeks in donor animals were aseptically dissected and mechanically minced. Pieces of tumor tissue (3 mm^3 in size) were transplanted by a trocar needle into nude mice. When tumors reached about 95 - 150 mm^3 in size, the mice were randomized into four groups (n = 3 per group). Then the treatments were started, Compound **1** with two doses (2 and 8 mg/kg) and control (methylcellulose from Sigma-Aldrich) with 10 mg/kg dose were administered once daily through oral administration route for consecutive 28 days and doxorubicin (Sigma-Aldrich) with 2 mg/kg was administered once weekly through intravenous administration route for consecutive 4 weeks, respectively. At the end of experiment, all mice were weighed and sacrificed and their tumors were excised. Tumors were weighed and the mean tumor weight was calculated. The tumor growth inhibition rates (TGIR) were calculated as follows:

Tumor growth inhibition rates, TGIR (%) = 100 × (C-T)/C, where T is the average tumor weight of the treated and C the average tumor weight of the control.

Acute toxicity study.[13-15] CD-1 (ICR) male mice (7 weeks old, 30.6 - 32.4 g) were purchased from KOATECH (Pyeongtaek, Korea) and were acclimatized for 1 week before the experiment began (8 weeks old, 32.0 - 35.8 g). During this period and throughout the experiment the mice were housed in stainless cages, and were given access *ad lib.* to water and pellet chow (TEKLAD CERTIFIED GLOBAL 18% PROTEIN RODENT DIET 2918C, Harlan TEKLAD, U.S.A.). The mice were kept under controlled conditions of temperature (23.1 ± 0.17 °C) and relative humidity (50.3 ± 1.14%), with a 12-hr light/dark cycle. All animal experiments were conducted according to the Guidelines of the Committee on the Care and the Use of Laboratory Animals of Chemon Inc. (Suwon, Korea). Compound **1** was suspended in 0.5% CMC-Na (Sodium carboxymethyl cellulose, Daejung Chemicals & Metals Co. Ltd., Korea) and was given by gavages to four groups of five mice at doses of 0 (control: 0.5% CMC-Na), 250, 500 or 1,000 mg/kg body weight once daily. The animals were observed during 14 days. After this period of time, mice were weighed, killed and dissected to detect any macroscopic injuries of organs.

Results and Discussion

Synthesis. We have modified the scale-up synthetic procedure for compound **1** compared with the previously reported procedure[8] as shown in Scheme 1. 2-Nitrocinnamic acid **2** was esterificated under H_2SO_4/MeOH condition followed by a reduction with $SnCl_2 \cdot 2H_2O$/EtOAc condition to provide methyl 2-aminocinnamate **3** in 97% overall yield. In the previous procedure, azaphosphorane (R-N=PPh_3) was used for the preparation of key intermediate, carbodiimide **5**.[8] However, azaphosphorane is too unstable to be completely isolated and stored for long time. Furthermore, 4-biphenylyl isocyanate as a reagent for the reaction with azaphosphorane is expensive reagent and also the purification of their reaction mixture *via* column chromatography has to be avoided for scale-up preparation. As a solution for these problems, we have developed an efficient two-step process for carbodiimide **5**: Curtius rearrangement using cheap reagent biphenyl-4-carboxylic acid/diphenyl phosphorazidate (DPPA)/Et_3N provided the corresponding urea **4** in 80% yield[16] and subsequent dehydration of urea (**4**) using $PPh_3 \cdot Br_2$ and Et_3N afforded the carbodiimide compound **5** in 77% yield as shown in Scheme 1. As an amine nucleophile, *N*,*N*-dibenzyl-*N*′-methylpentane-1,5-diamine **11** was prepared starting from 1,5-diaminopentane **8** as shown in Scheme 2. Monoprotection of diamine **1** with di-*tert*-butyl dicarbonate using known procedure[17] afforded compound **9** in 53% yield and the other amine group was treated with 2 eq. of benzyl bromide and K_2CO_3 to provide dibenzyl compound **10** in 56% yield. The reduction of Boc group with lithium aluminum hydride gave a target amine **11** in 77% yield.[18] The carbodiimide compound **5** was treated with amine **11** to provide 3,4-dihydroquinazoline **6** in 99% yield *via* tandem nucleophilic addition and intramolecular conjugate addition. In the previous procedure,[8] the amide compound **7** was prepared using the traditional two-step procedure: the hydrolysis of ester compound **6** and the subsequent coupling reaction of the corresponding acid with amine. As a modified procedure, however, the reaction of ester compound **6** with benzylamine and 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) as a catalyst under solvent-free condition afforded directly amide **7** in 89% yield.[19] Finally, the hydrogenation of amide compound **7** with 10% Pd/C in the presence of formaldehyde afforded the target compound **1** in 70% yield**.** Therefore, this modified procedure seems to be more efficient and economical than the previously reported method because of higher overall yield (26%) and fewer stages (7 steps). Thus, we have prepared the large quantities of compound **1** using this modified procedure for its following *in vivo* screening.

Table 3. Antitumor efficacy of compound **1** against A549 xenograft in nude mice[a]

Compound	Dose (mg/kg)	AR[b]	No. of animal	Sex	Tumor weight (g)	Tumor growth inhibition rate (%)[c]
Control[d]	-	po	3	male	2.10	-
1	2	po	3	male	1.01	52
1	8	po	3	male	0.96	54
Doxorubicin[e]	2	iv	3	male	0.67	68

[a]During 29 days post xenograft; [b]Administration route; [c]TGIR (%) = 100 × (C-T)/C; [d]Vehicle for compound **1**, 10 mL/kg of 5% methylcellulose in CH_2Cl_2; [e]10 mL/kg of doxorubicin hydrochloride in saline solution.

Table 4. Acute toxicity of compound **1** on ICR mice[a]

Compound	Dose (mg/kg)	AR[b]	No. of animal[c]	Sex	Clinical signs	Mortality	LD_{50}
Control[d]	-	po	5	male	normal	0	-
	250	po	5	male	normal	0	
1	500	po	5	male	normal	0	1,038 mg/kg
	1,000	po	5	male	piloerection, soiled perineal region	2	

[a]14 days post single oral administration; [b]Administration route; [c]Number of mice tested in the group; [d]20 mL/kg of 0.5% CMC-Na (sodium carboxymethyl cellulose) for compound **1**.

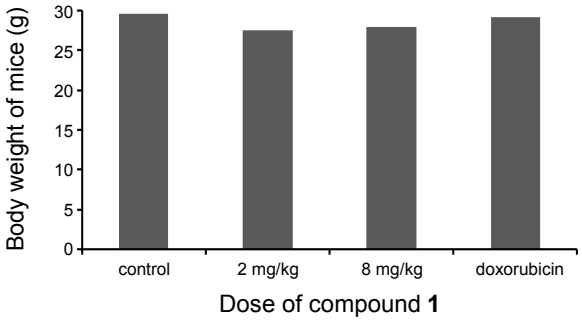


Figure 2. Body weights of mice taken 28 days after daily oral administration of compound **1** and control, and once weekly iv administration of doxorubicin. There were no significant changes in the body weights after administration of compound **1** compared to those of control and doxorubicin. The data was not statistically analyzed because of small animal number (n = 3) in each group.

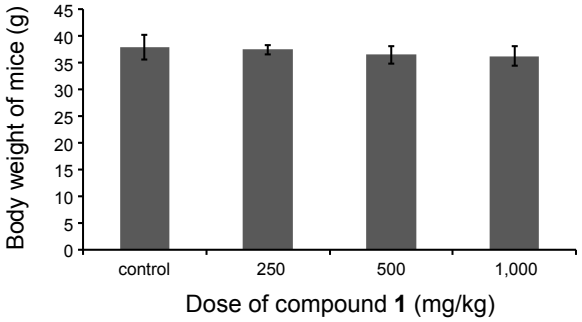


Figure 3. Body weights of mice taken 14 days after daily oral administration of compound **1** and control. There were no significant changes in the body weights after administration of compound **1** compared to that of control. Differences between body weights of each group were tested by one-way ANOVA. Data were analyzed using a commercial statistical program SPSS 10.1 K.

Antitumor activity assay. We have evaluated the *in vivo* antitumor activity of compound **1** (free base) against A549 xenograft in BALB/c nude mice model in comparison with doxorubicin as positive control and 5% methylcellulose in CH_2Cl_2 as a vehicle for compound **1** was used as blank control. The results presented in Table 3 showed that compound **1** through oral administration of 2 and 8 mg/kg of body weight exhibited good experimental therapeutic efficacy *in vivo* against A549 xenograft in mice by 52 and 54%, respectively, compared with control alone. This result was slightly lower than that of doxorubicin (68%). In the previous report, the *in vitro* evaluation against A549 cancer cell revealed that the activity of compound **1** ($GI_{50} = 0.17 \pm 0.02$ μM) was as high as that of doxorubicin ($GI_{50} = 0.16 \pm 0.01$ μM).[9] However, this *in vivo* efficacy comparison was considered to be only a qualitative and preliminary result because of the different administration routes (iv *vs*. po), the difference of total injected amounts, and the shortage of tested animal number per tested group (n = 3). During the experimental period, meanwhile, significant body weight loss was not observed (Figure 2).

Acute toxicity assay. Compound **1** was also profiled for its acute toxicity to ICR mice as shown in Figure 3 and Table 4. First of all, No significant differences either in control or treated groups were observed in body weights as shown in Figure 3. It should be noted that all the mice given a single oral injection of **1** at the dose of 250 and 500 mg/kg of body weight showed no clinical signs and the mortality rate was zero. At 1,000 mg/kg dose, however, symptoms such as piloerection and soiled perineal region were observed and the mortality rate was 2/5 (40%). In addition, a little of body weight loss was also observed but recovered after 3 days on alive 3 mice (Data not shown). As a result of dissection of all mice, finally, there were no macroscopic injuries of organs. Based on these data, LD_{50} value for compound **1** was decided to be 1,038 mg/kg, which means that compound **1** is relatively less toxic compared to another cancer chemotherapeutic agents.

Conclusion

Taking all into account, we suggest that compound **1** would be a promising anti-cancer agent with the lower acute toxicity and the growth inhibition effects on A549 tumors although slightly less active than doxorubicin. For the exact scientific meaning, antitumor activity assay using a number of xenograft nude mice is in progress now.

Acknowledgments. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0028197).

References

1. Mariot, P.; Vanoverberghe, K.; Lalevee, N.; Rossier, M. F.; Prevarskaya, N. *J. Biol. Chem*. **2002**, *277*, 10824.
2. Scheiber, R. *J. Membr. Biol.* **2005**, *205*, 129.
3. Kahl, C. R.; Means, A. R. *Endocr. Rev.* **2003**, *24*, 719.
4. Lory, P.; Bidaud, I.; Chemin, J. *Cell Calcium* **2006**, *40*, 135.

5. Lang, F.; Foller, M.; Lang, K. S.; Lang, P. A.; Ritter, M.; Gulbins, E.; Vereninov, A.; Huber, S. M. *J. Membr. Biol.* **2005**, *205*, 147.
6. Bertolesi, G. E.; Shi, C.; Elbaum, L.; Jollimore, C.; Rozenberg, G.; Barnes, S.; Kelly, M. E. *Mol. Pharmacol.* **2002**, *62*, 210.
7. Lory, P.; Chemin, J. *Expert Opin. Ther. Targets* **2007**, *11*, 717 and references herein.
8. Seo, H. N.; Choi, J. Y.; Choe, Y. J.; Kim, Y.; Rhim, H.; Lee, S. H.; Kim, J.; Joo, D. J.; Lee, J. Y. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5740.
9. Heo, J. H.; Choe, Y. J.; Kim, S.; Oh, C. R.; Kim, Y. D.; Rhim, H.; Choo, D. J.; Kim, J.; Lee, J. Y. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3899.
10. Zhong, G.; Zhang. S.; Li, Y.; Liu, X.; Gao, R.; Miao, Q.; Zhen, Y. *Cancer Lett*. **2010**, *295*, 124.
11. Lee, H. S.; Ryu, J. S.; Jeon, Y. J.; Hong, Y. S.; Lee, J. J.; Hong, S. K.; Kim, T. Y. *Cancer Chemother. Pharmacol.* **2007**, *60*, 197.
12. Labonté, P.; Kadhim, S.; Bowlin, T.; Mounir, S. *Hepatol. Res*. **2000**, *18*, 72.
13. Korea Food and Drug Administration: Good Laboratory Practice, Notification No. 2005-79 (Dec. 21, 2005).
14. Korea Food and Drug Administration: The Standards of Toxicity Study for Medicinal Products, Notification No. 2005-60 (Oct. 21. 2005).
15. Tada, Y.; Fujitani, T.; Yoneyama, M. *Food Chem. Toxicol.* **1992**, *30*, 1021.
16. Sunami, S.; Ohkubo, M. *Tetrahedron* **2009**, *65*, 638.
17. Lee, D. W.; Ha, H.-J.; Lee, W. K. *Synth. Commun*. **2007**, *37*, 737.
18. Phuan, P.-W.; Kozlowski, M. C. *J. Org. Chem*. **2002**, *67*, 6339.
19. Sabot, C.; Kumar, K. A.; Meunier, S.; Mioskowski, C. *Tetrahedron Lett.* **2007**, *48*, 3863.