Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry 14 (2006) 5795–5801
Bioorganic & Medicinal Chemistry

# Synthesis and biological evaluation of 5-arylamino-1*H*-benzo[*d*]imidazole-4,7-diones as inhibitor of endothelial cell proliferation

Kwang-Hoe Chung,[b] Sung-Yu Hong,[b] Hea-Jung You,[a] Rae-Eun Park[a] and Chung-Kyu Ryu[a,*]

[a]*College of Pharmacy, Ewha Womans University, Seodaemun-ku, Seoul 120-750, Republic of Korea*
[b]*National Research Lab for Cardiovascular Nanotechnology, BioBud Co. Ltd, Seodaemun-ku, Seoul 120-110, Republic of Korea*

Received 11 April 2006; revised 13 May 2006; accepted 16 May 2006
Available online 19 June 2006

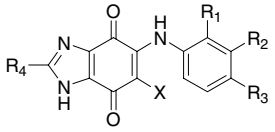
**Abstract**—5-Arylamino-1*H*-benzo[*d*]imidazole-4,7-diones were synthesized and tested for their inhibitory activities on the proliferation of human umbilical vein endothelial cells (HUVECs) and the smooth muscle cells (SMCs). Among them, several 1*H*-benzo[*d*]imidazole-4,7-diones exhibited the selective antiproliferative activity on the HUVECs. Further mechanistic study revealed that the inhibitory effect of one representative 1*H*-benzo[*d*]imidazole-4,7-dione **2b** on HUVEC proliferation was mediated by the activation of p38 signaling pathway in the HUVECs.


## 1. Introduction

The angiogenesis involves the proliferation of endothelial cells (ECs) in response to specific growth stimuli such as vascular endothelial growth factor (VEGF) of basic fibroblast growth factor (bFGF). Each step of the process is controlled by these regulatory growth factors that stimulate or inhibit angiogenesis.[1] However, these control mechanisms are often disordered in several pathologic diseases including cancer. The growth and maintenance of solid tumors are highly dependent on neovascularization and can be regulated by compounds that interfere with either the stimulation or proliferation of ECs.[2] The identification of agents with selective antiproliferative or cytostatic activity against the ECs has a significant value for the treatment of diseases associated with angiogenesis, including solid tumors.[3]

Heterocyclic quinone class represents an important class of biologically active molecules.[4] Inhibitory activity of quinone class on the proliferation of ECs has not been reported to the best of our knowledge. In order to search for angiogenesis inhibitors, we synthesized and tested various promising quinone derivatives to elucidate their contribution to the antiproliferative effects on proliferation of human umbilical vein endothelial cells (HUVECs) in response to the bFGF. Among the quinones tested, 5-arylamino-6-halo-1*H*-benzo[*d*]imidazole-4,7-dione series **1** (Fig. 1) showed the potent antiproliferative effect on the HUVECs.

Herein, we describe a 5,6-disubstituted-1*H*-benzo[*d*]imidazole-4,7-dione scaffold, exemplified by structures **2**–**4** (Scheme 1), and report preliminary in vitro activity data on the HUVECs indicating that this scaffold is a promising lead for the development of inhibitors of the HUVEC proliferation.

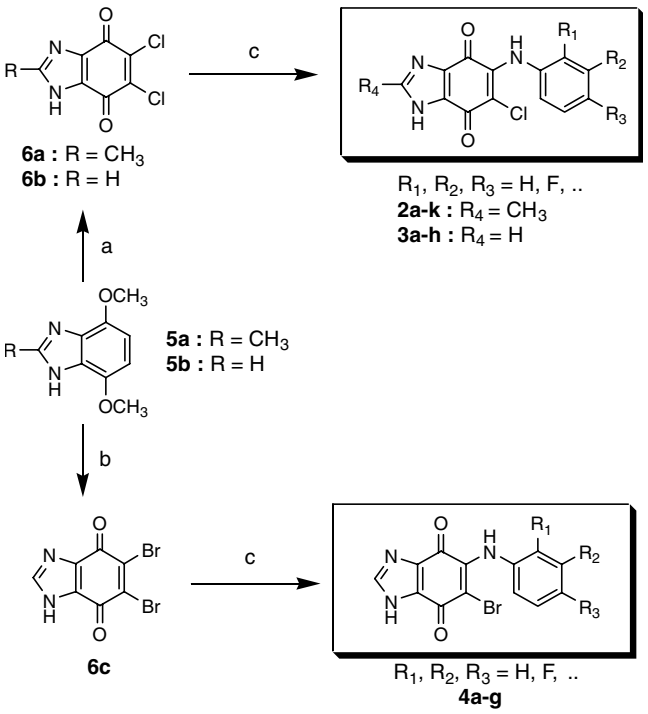



$R_1$, $R_2$, $R_3$ = H, F,..
$R_4$= H, Al or Ar
X= Cl or Br
**1**

**Figure 1.** 1*H*-Benzo[*d*]imidazole-4,7-dione derivatives.

*Keywords*: 1*H*-Benzo[*d*]imidazole-4,7-dione; Endothelial cells; Antiproliferative activity; Substitution effects.
* Corresponding author. Tel.: +82 2 3277 3027; fax: +82 2 3277 3051; e-mail: ckryu@ewha.ac.kr

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.05.059




**Scheme 1.** Synthesis of 1*H*-benzo[*d*]imidazole-4,7-diones **2**–**4**. Reagents and conditions: (a) HCl/$HNO_3$/reflux/0.5 h; (b) HBr/reflux/6 h and then; HBr/$NaBrO_3$/reflux/1 h; (c) arylamine (1 equiv)/EtOH/reflux/5 h.

Additional data for the mechanism of HUVEC antiproliferative activity of one representative 1*H*-benzo[*d*]imidazole-4,7-dione **2b** was also performed. The mitogen-activated protein kinase (MAPK) pathway has a critical role in the proliferation of the HUVECs.[5] A central function of the MAPK pathway is the activation of gene expression, mediated through phosphorylation of transcription factors. The MAPK pathway consists of a kinase cascade that includes a MAPK, a MAPK/ERK kinase (MEK) and a MEK kinase (MEKK). p38 is a 38 kDa stress-activated protein kinase/MAP kinase (SAPK/MAPK) that is fully activated by dual phosphorylation of threonine and tyrosin.[6] The p38 pathways function to modulate cell cycle, apoptotic and transcriptional responses to stress.[6] In order to investigate the effect of compound **2b** on the intracellular signaling of HUVECs, we examined whether the compound **2b** activates the p38 or not. Additional data for antiproliferative potential of benzimidazoledione derivatives on smooth muscle cells (SMCs) are provided.

## 2. Chemistry

The method used to synthesize 5-arylamino-6-chloro-2-methyl-1*H*-benzo[*d*]imidazole-4,7-diones **2** is shown in Scheme 1. 4,7-Dimethoxy-2-methyl-benzimidazole (**5a**) was prepared according to the known method[7] with minor modification. 5,6-Dichloro-2-methyl-1*H*-benzo[*d*]-imidazole-4,7-dione (**6a**) was synthesized by oxidizing compound **5a** with $HNO_3$/HCl combination in 46% yield. 2-Methyl-1*H*-benzo[*d*]imidazole-4,7-dione series **2a**–**k** (Table 1) were prepared by nucleophilic substitution on compound **6a** with appropriate arylamines. Most of these substitutions went as expected and had overall yields of 76–94%.

In a similar manner, 5-arylamino-6-chloro-1*H*-benzo[*d*]imidazole-4,7-diones **3** were prepared from 5,6-dichloro-1*H*-benzo[*d*]imidazole-4,7-dione (**6b**). Oxidation of 4,7-dimethoxybenzimidazole (**5b**)[7] with the $HNO_3$/HCl combination yielded compound **6b**. 1*H*-benzo[*d*]imidazole-4,7-dione series **3a**–**h** (Table 1) were synthesized by the substitution on compound **6b** with arylamines.

Demethylating compound **5b** with HBr gave 4,7-dihydroxybenzimidazole, which was oxidized to 5,6-dibromo-1*H*-benzo[*d*]imidazole-4,7-dione (**6c**) in a HBr/$NaBrO_3$ solution, in 49% yield. 5-Arylamino-6-bromo-1*H*-benzo[*d*]imidazole-4,7-diones **4a**–**g** (Table 1) were synthesized by the substitution on compound **6c** with arylamines.

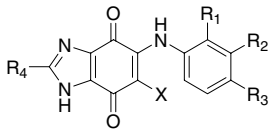
## 3. Biological activities

Synthesized 1*H*-benzo[*d*]imidazole-4,7-diones **2**–**4** were tested in vitro for their antiproliferative activity on the HUVEC proliferation. Inhibition of proliferation of these cells was determined by WST colorimetric assay with a minor modification.[8] The $IC_{50}$ values were determined by comparison to mycophenolic acid (MPA)[9] as a standard agent. As represented in Table 1, the most active potential among the 1*H*-benzo[*d*]imidazole-4,7-dione series **2**–**4** was found for 5-arylamino-6-chloro-2-methyl-1*H*-benzo[*d*]imidazole-4,7-diones **2a**–**k**, which generally showed good activity. In fact, many of 5-arylamino-6-halo-1*H*-benzo[*d*]imidazole-4,7-diones **3a**–**h** and **4a-g** showed actually good antiproliferative activity. 1*H*-Benzo[*d*]imidazole-4,7-diones **2b**, **2d**, and **2g** inhibited the bFGF-stimulated proliferation of the HUVECs tested at the level with the $IC_{50}$ values of 0.4 μM. The activity of these compounds is approximately 3 times more potent than MPA. The structure–activity relationship may not exist between properties of substituents (R: F, Cl, Br, ...) for the 5-arylamino moieties of 1*H*-benzo[*d*]imidazole-4,7-diones **2**–**4**.

In terms of structure–activity relationship, 5-arylamino-6-chloro-2-methyl-1*H*-benzo[*d*]imidazole-4,7-diones **2** showed, in general, more potent antifungal activity than the other 5-arylamino-1*H*-benzo[*d*]imidazole-4,7-diones **3** and **4**. The 2-methyl-1*H*-benzo[*d*]imidazole-4,7-diones **2** exhibited the greatest activity, indicating a correlation that may offer insight into the mode of action of these compounds. The 6-chloro- and 6-bromo-moieties of compounds **3** and **4** appear to contribute partially toward biological potency.

In addition, the quinone moiety in 1*H*-benzo[*d*]imidazole-4,7-diones **2** might be essential for the antiproliferative activity. For example, non-quinonoid compounds **5a** and **5b** lost activity. The results of their QSAR study would imply that alteration of X, $R_1$, $R_2$, and $R_3$ on 1*H*-benzo[*d*]imidazole-4,7-diones **2**–**4** did not greatly influ-

**Table 1.** Structures and $IC_{50}$ values of 1*H*-benzo[*d*]imidazole-4,7-diones for inhibition of HUVEC/SMC proliferation



| Compound | X | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $IC_{50}$[a] (μM) | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | HUVECs[b] | SMCs | [HUVECs]/[SMCs] |
| **2a** | Cl | H | H | F | $CH_3$ | 1.0 | 3.0 | 3.0 |
| **2b** | Cl | H | H | Br | $CH_3$ | 0.4 | 5.5 | 13.8 |
| **2c** | Cl | H | H | Cl | $CH_3$ | 0.8 | 2.8 | 3.5 |
| **2d** | Cl | H | H | $OCF_3$ | $CH_3$ | 0.4 | 3.0 | 7.5 |
| **2e** | Cl | H | H | $CH_2CH_3$ | $CH_3$ | 1.0 | 3.0 | 3.0 |
| **2f** | Cl | H | Br | H | $CH_3$ | 0.8 | 40.0 | 50.0 |
| **2g** | Cl | Cl | Cl | H | $CH_3$ | 0.3 | 0.6 | 2.0 |
| **2h** | Cl | F | F | F | $CH_3$ | 0.5 | 0.8 | 1.6 |
| **2i** | Cl | F | H | F | $CH_3$ | 1.0 | 1.2 | 1.2 |
| **2j** | Cl | H | Cl | H | $CH_3$ | 0.6 | 0.9 | 1.5 |
| **2k** | Cl | H | H | $CF_3$ | $CH_3$ | 0.6 | 0.6 | 1.0 |
| **3a** | Cl | H | H | F | H | 0.8 | 1.2 | 1.5 |
| **3b** | Cl | H | H | Br | H | 1.0 | 2.0 | 2.0 |
| **3c** | Cl | H | H | $OCF_3$ | H | 1.6 | 2.5 | 1.6 |
| **3d** | Cl | H | H | CN | H | 1.4 | 2.7 | 1.9 |
| **3e** | Cl | F | H | F | H | 1.4 | 2.7 | 1.9 |
| **3f** | Cl | H | Cl | H | H | 1.0 | 1.4 | 1.4 |
| **3g** | Cl | H | F | F | H | 0.8 | 1.1 | 1.4 |
| **3h** | Cl | F | H | H | H | 0.8 | 1.7 | 2.1 |
| **4a** | Br | H | H | F | H | 2.0 | 1.0 | 0.5 |
| **4b** | Br | H | H | Cl | H | 2.0 | 4.0 | 2.0 |
| **4c** | Br | H | Br | H | H | 0.8 | 5.3 | 6.6 |
| **4d** | Br | H | Cl | H | H | 0.7 | 3.0 | 4.3 |
| **4e** | Br | H | I | H | H | 1.0 | 2.5 | 2.5 |
| **4f** | Br | H | H | H | H | 1.0 | 1.1 | 1.1 |
| **4g** | Br | H | $CF_3$ | H | H | 1.0 | 3.0 | 3.0 |
| **5a** | | | | | | >100 | >100 | nt[c] |
| **5b** | | | | | | >100 | >100 | nt |
| MPA | | | | | | 1.0 | 1.1 | 1.1 |

[a] Antiproliferative activity evaluation: WST colorimetric assay. The inhibitory activities against the bFGF-induced proliferation of the HUVECs and the PDGF-induced proliferation of the SMCs.

[b] HUVECs were isolated from newborns. SMCs were isolated from rat thoracic arota.

[c] nt, not tested.

ence the inhibitory activities (Table 1). This suggests that 1*H*-benzo[*d*]imidazole-4,7-dione structure is mainly responsible for the activities.

The 1*H*-benzo[*d*]imidazole-4,7-diones **2**–**4** were further tested in vitro for their antiproliferative activity on the rat aortic SMC proliferation according to the method previously reported.[10] We evaluated the 1*H*-benzo[*d*]imidazole-4,7-diones in order to search selective antiproliferative compounds on the HUVECs. Many compounds **2**–**4** exhibited good activities for both SMCs and HUVECs. In terms of cell selectivity, the compounds **2**–**4** displayed a selectivity with the [SMCs]/[HUVECs] value ranging from 0.5 to 50 times for HUVECs. Among them, compounds **2b**, **2d**, and **2f** displayed good selectivity for HUVECs. $IC_{50}$ values of **2b** for HUVECs and SMCs were 0.4 and 5.5 μM, respectively, displaying selectivity about 13.8 times for HUVECs. $IC_{50}$ values of **2d** for HUVECs and SMCs were 0.4 and 3.0 μM, respectively, displaying selectivity about 7.5 times for HUVECs. Also, $IC_{50}$ values of **2f** for HUVECs and SMCs were 0.8 and 40.0 μM, respectively, displaying selectivity about 50 times for HUVECs. In contrast, $IC_{50}$ values of MPA were 1.0 and 1.1 μM, respectively, displaying selectivity about 1.1 times for HUVECs.

## 4. Effect of 1*H*-benzo[*d*]imidazole-4,7-dione *2b* on SAPK/JNK

Further mechanistic study on the antiproliferative activity was performed using one of potent compounds **2** in the cultured HUVECs. In order to investigate the effect of the compound **2b** on the intracellular signaling of the HUVECs, we examined whether the compound activates the p38 protein in the HUVECs by immunoblot assay or not. As shown in Figure 2, the compound **2b** dramatically increased the phosphorylation of the protein (phospho-p38), while the protein level was slightly increased. It means that the compound **2b** activated

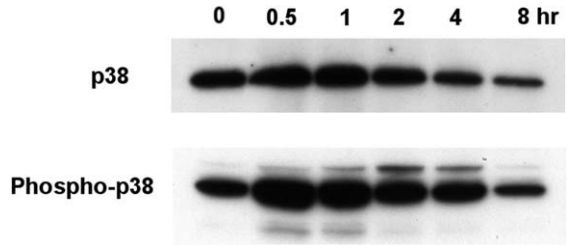



**Figure 2.** Compound **2b** activated p38 protein in the HUVECs. The HUVECs treated with the compound **2b** for 0.5, 1, 2, 4, and 8 h were immunoblotted with the anti-p38 polyclonal antibody and the phospho-p38 polyclonal antibody, respectively.

p38 pathway.The activation of p38 by the compound **2b** started in 30 min and maintained until 2 h after addition of the compound into the cells. The p38, one of various MAPKs, is potentially activated by a variety of environmental stress, including UV and gamma radiation, ceramide, inflammatory cytokines and, some instance, by growth factors.The results suggest that the compound **2b** could induce cell cycle arrest or apoptosis by activating SAPK/MAPK pathway, which resulted in the inhibition of the HUVEC proliferation. Quinone compounds are generally known to have active oxygen species (ROS)-generating ability, since quinones are readily reduced by bio-reductants and the formed semiquinone radicals reduce dioxygen to afford superoxide anion radical and/or hydrogen peroxide. The ROS activates p38 MAPK.[11] Therefore, it can be also supposed that the toxicity of 1*H*-benzo[*d*]imidazole-4,7-diones **2**–**4** would merely suppress the proliferation of the HUVECs.

On the base of the result, we propose that the antiproliferative 1*H*-benzo[*d*]imidazole-4,7-dione series could activate p38 pathway in the HUVECs. Further pharmacological investigations of these compounds and the structural optimization are in progress.

## 5. Conclusion

A series of 5-arylamino-1*H*-benzo[*d*]imidazole-4,7-diones were synthesized and tested for their inhibitory activities on the proliferation of the HUVECs and SMCs. 5-Arylamino-6-halo-2-methyl-1*H*-benzo[*d*]imidazole-4,7-diones **2**–**4** were synthesized by nucleophilic substitution of 1*H*-benzo[*d*]imidazole-4,7-diones **6a**, **6b**, and **6c** with appropriate arylamines. Among them, several 1*H*-benzo[*d*]imidazole-4,7-diones exhibited the selective antiproliferative activity on the HUVECs. The result indicates that compounds **2b**, **2d**, and **2f** are a selective inhibitor for the HUVECs. From the SAR study of 1*H*-benzo[*d*]imidazole-4,7-diones **2**–**4**, we found that most of 1*H*-benzo[*d*]imidazole-4,7-diones **2** were superior to MPA in terms of the strength of the activity and cell selectivity. These compounds may thus be a promising lead for the development of inhibitors of the HUVEC proliferation, all of which rely on angiogenesis.

## 6. Experimental

All melting points were measured in open capillary tubes with Büchi melting point B-545 and are uncorrected. The TLC was performed on precoated silica gel (60G 254, Merck) using $CHCl_3$ as a solvent. The compounds were detected under UV light (254 nm) or by heating to 110 °C after spraying with a 30% $H_2SO_4$–vanillin solution. Column chromatography was performed on silica gel G60 (70–230 mesh, ASTM, Merck). The IR spectra were taken with a Perkin-Elmer 1420r IR spectrometer with KBr pellets. $^1$H NMR spectra were recorded on Varian Unity INOVA 400 MHz FT-NMR spectrometer using DMSO-$d_6$ as a solvent, and chemical shifts were given in parts per million with TMS as a standard. High-resolution mass spectra (HRMS EI) were taken with a Jeol JMS AX505 WA. Elemental analyses were performed by CE instruments EA1110 with sulfanilamide as a standard material, and analytical results for C, H, and N were within ±0.4% of theoretical values. Arylamines, DMSO-$d_6$, and other reagents were obtained from Aldrich Chemical Co. and Sigma Co.

### 6.1. Synthesis of 5,6-dichloro-1*H*-benzo[*d*]imidazole-4,7-diones 6a and 6b

Briefly, 4,7-dimethoxybenzimidazoles **5a** and **5b** were prepared according to known procedure.[7] Five milliliters of cond. $HNO_3$ was added over a period of 1 h to a stirred suspension of compound **5a** or **5b** (10 mmol) in 15 mL of concd HCl at 80–90 °C. The mixture was stirred at rt for 2 h and was extracted twice with 30 mL of ether. The extract was evaporated and crystallized from EtOH to afford compound **6a** or **6b**, respectively.

**6.1.1. 5,6-Dichloro-2-methyl-1*H*-benzo[*d*]imidazole-4,7-dione (6a).** Yellow powder (yield, 46%); mp 294–295 °C (dec); IR (KBr) 3529 (s, NH), 1703 (s, C=O), 1544–1688, 1100 (s) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.5 (s, 3H, H10); HRMS calcd for $C_8H_4Cl_2N_2O_2$: 229.9650, found: 229.9650.

**6.1.2. 5,6-Dichloro-1*H*-benzo[*d*]imidazole-4,7-dione (6b).** Yellow powder (yield, 56%); mp 270–271 °C (dec); IR (KBr) 3394 (m, NH), 3129 (s), 2352 (m), 1680 (m, C=O), 1422–1552 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 14.2 (s, 1H, NH), 8.3 (s, 1H, H2); HRMS calcd for $C_7H_2Cl_2N_2O_2$: 215.9494, found: 215.9495.

### 6.2. Synthesis of 5,6-dibromo-1*H*-benzo[*d*]imidazole-4,7-dione (6c)

4,7-Dimethoxybenzimidazole (**5b**) (3.4 g, 19 mmol) in 35 mL of 48% HBr was refluxed for 1 h. The mixture was stirred at rt for 1 h and the precipitate was collected by filtration. The product, 4,7-dihydroxybenzimidazole hydrobromide, was purified by recrystallization from EtOH–ether: mp 285 °C (dec), (lit.[7] mp 285–325 °C, dec). The solution of $NaBrO_3$ 3 g in 20 mL of water was added dropwise over a period of 1 h to a stirred suspension of the compound (2 g, 6.45 mmol) in 20 mL of concd HBr at 50–60 °C and

cooled to room temperature. The yellow precipitates were filtered off. The yield was 49%, mp 250 °C (dec); $^1$H NMR (DMSO-$d_6$) $\delta$ 13.9 (s, 1H, NH), 8.2 (s, 1H, H2); HRMS calcd for $C_7H_2Br_2N_2O_2$: 305.8463, found: 305.8462.

### 6.3. General procedure for synthesis of 5-arylamino-6-halo-1*H*-benzo[*d*]imidazole-4,7-diones (2, 3, and 4)

A solution of compounds **6a**, **6b** or **6c** (0.34 mmol) in 15 mL of 95% EtOH was added to the solution of the arylamine (0.35 mmol) in 5 mL of 95% EtOH and stirred at rt for 2 h and then refluxed for 5 h. After the mixture was kept overnight in a refrigerator or poured into 20 mL of ice water, the precipitate was collected by filtration and crystallization from aq EtOH afforded the compounds **2**, **3**, and **4**, respectively.

**6.3.1. 6-Chloro-5-(4-fluorophenyl)amino-2-methyl-1*H*-benzo[*d*]imidazole-4,7-dione (2a).** Dark purple powder (45 mg, 34%); mp 278–279 °C; IR (KBr) 3301 (s, NH), 3061, 1690 (s, C=O), 1518–1632, 1125 (m) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 13.5 (s, 1H, NH), 9.1 (s, 1H, NH), 7.1 (m, 4H, Ph-H), 2.4 (s, 3H, $CH_3$); HRMS calcd for $C_{14}H_9ClFN_3O_2$: 305.0367, found: 305.0368; Anal. Calcd for $C_{14}H_9ClFN_3O_2$ (305.69): C, 55.01; H, 2.97; N, 13.75. Found: C, 55.0; H, 2.99; N, 13.73.

**6.3.2. 5-(4-Bromophenyl)amino-6-chloro-2-methyl-1*H*-benzo[*d*]imidazole-4,7-dione (2b).** Violet powder (72 mg, 45%); mp 311–313 °C; IR (KBr) 3350 (w, NH), 1687 (s, C=O), 1510–1656, 1187 (m), 899 (m) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 13.6 (s, 1H, NH), 9.2 (s, 1H, NH), 7.0–7.5 (m, 4H, Ph-H), 2.4 (s, 3H, $CH_3$); HRMS calcd for $C_{14}H_9BrClN_3O_2$: 364.9567, found: 364.9566; Anal. Calcd for $C_{14}H_9BrClN_3O_2$ (366.6): C, 45.87; H, 2.47; N, 11.46. Found: C, 45.85; H, 2.49; N, 11.45.

**6.3.3. 6-Chloro-5-(4-chlorophenyl)amino-2-methyl-1*H*-benzo[*d*]imidazole-4,7-dione (2c).** Dark purple powder (27 mg, 20%); mp 340–342 °C; IR (KBr) 3200 (m, NH), 1687 (s, C=O), 1519–1655, 1125 (m), 903 (m), 557 (m) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 13.6 (s, 1H, NH), 9.0 (s, 1H, NH), 7.0–7.3 (dd, $J$ = 2.4, 8.4 Hz, 4H, Ph-H), 2.2 (s, 3H, $CH_3$); HRMS calcd for $C_{14}H_9Cl_2N_3O_2$: 321.0072, found: 321.0073; Anal. Calcd for $C_{14}H_9Cl_2N_3O_2$ (322.15): C, 52.20; H, 2.82; N, 13.04. Found: C, 52.21; H, 2.82; N, 13.02.

**6.3.4. 6-Chloro-5-(4-trifluoromethoxyphenyl)amino-2-methyl-1*H*-benzo[*d*]imidazole-4,7-dione (2d).** Dark purple powder (95 mg, 59%); mp 280–281 °C; IR (KBr) 3550 (w, NH), 1688 (s, C=O), 1518–1655, 1294 (w), 900 (s), 650 (m) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 13.6 (s, 1H, NH), 9.2 (s, 1H, NH), 7.1–7.3 (m, 4H, Ph-H), 2.4 (s, 3H, $CH_3$); HRMS calcd for $C_{15}H_9ClF_3N_3O_3$: for 371.0284, found: 371.0285; Anal. Calcd for $C_{15}H_9ClF_3N_3O_3$ (371.03): C, 48.47; H, 2.44; N, 11.30. Found: C, 48.42; H, 2.49; N, 11.06.

**6.3.5. 6-Chloro-5-(4-ethylphenyl)amino-2-methyl-1*H*-benzo[*d*]imidazole-4,7-dione (2e).** Dark purple powder (34 mg, 25%); mp 270–271 °C; IR (KBr) 3320 (w, NH), 1687 (s, C=O), 1561–1656, 1125 (m), 836 (m) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 13.6 (s, 1H, NH), 9.0 (s, 1H, NH), 6.9–7.1 (m, 4H, Ph-H), 2.5–2.6 (q, $J$ = 7.6 Hz, 2H, –$CH_2$), 2.4 (s, 3H, $CH_3$), 1.1 (t, $J$ = 7.6 Hz, 3H, $CH_3$); HRMS calcd for $C_{16}H_{14}ClN_3O_2$: 315.0775, found: 315.0775; Anal. Calcd for $C_{16}H_{14}ClN_3O_2$ (315.75): C, 60.86; H, 4.47; N, 13.31. Found: C, 60.82; H, 4.49; N, 13.29.

**6.3.6. 5-(3-Bromophenyl)amino-6-chloro-2-methyl-1*H*-benzo[*d*]imidazole-4,7-dione (2f).** Violet powder (73 mg, 46%); mp 282–293 °C; IR (KBr) 3278 (s, NH), 3008, 1692 (s, C=O), 1432–1556, 781 (m) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 13.6 (s, 1H, NH), 9.1 (s, 1H, NH), 7.0–7.2 (m, 4H, Ph-H), 2.4 (s, 3H, $CH_3$); HRMS calcd for $C_{14}H_9BrClN_3O_2$: 364.9567, found: 364.9567; Anal. Calcd for $C_{14}H_9BrClN_3O_2$ (366.6): C, 45.87; H, 2.47; N, 11.46. Found: C, 45.85; H, 2.49; N, 11.45.

**6.3.7. 6-Chloro-5-(2,3-dichlorophenyl)amino-2-methyl-1*H*-benzo[*d*]imidazole-4,7-dione (2g).** Dark violet powder (45 mg, 30%); mp 282–285 °C; IR (KBr) 3320 (m, NH), 1686 (s, C=O), 1505–1657, 1128 (m), 918 (m) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 13.6 (s, 1H, NH), 9.2 (s, 1H, NH), 7.5 (d, $J$ = 8.4 Hz, 1H, Ph-H), 7.3 (d, $J$ = 8.8 Hz, 1H, Ph-H), 7.0 (d, 1H,$J$ = 8.8 Hz, 1H, Ph-H), 2.3 (s, 3H, $CH_3$); HRMS calcd for $C_{14}H_8Cl_3N_3O_2$: 354.9682, found: 354.9683; Anal. Calcd for $C_{14}H_8Cl_3N_3O_2$ (356.59): C, 47.15; H, 2.26; N, 11.78. Found: C, 47.10; H, 2.29; N, 11.73.

**6.3.8. 6-Chloro-2-methyl-5-[(2,3,4-trifluorophenyl)amino]-1*H*-benzo[*d*]imidazole-4,7-dione (2h).** Dark violet powder (58 mg, 39%); mp 238–240 °C; IR (KBr) 3306 (s, NH), 3108, 1692 (s, C=O), 1464–1636, 1248 (m) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 13.6 (s, 1H, NH), 9.0 (s, 1H, NH), 7.3 (q, 1H, Ph-H), 7.1 (d, 1H, Ph-H), 2.4 (s, 3H, $CH_3$); HRMS calcd for $C_{14}H_7ClF_3N_3O_2$: 341.01788, found: 341.01787; Anal. Calcd for $C_{14}H_7ClF_3N_3O_2$ (341.67): C, 49.21; H, 2.07; N, 12.30; Found: C, 49.19; H, 2.05; N, 12.32.

**6.3.9. 6-Chloro-5-(2,4-difluorophenyl)amino-2-methyl-1*H*-benzo[*d*]imidazole-4,7-dione (2i).** Dark violet needle (56 mg, 40%); mp 261–263 °C p; IR (KBr) 3200 (m, NH), 1690 (s, C=O), 1515–1620, 1098 (m), 960 (s) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 13.6 (s, 1H, NH), 9.0 (s, 1H, NH), 7.0–7.3 (m, 4H, Ph-H), 2.4 (s, 3H, $CH_3$); HRMS calcd for $C_{14}H_8ClF_2N_3O_2$: 323.0273, found: 323.0272; Anal. Calcd for $C_{14}H_8ClF_2N_3O_2$ (323.68): C, 51.95; H, 2.49; N, 12.98. Found: C, 51.93 H, 2.52, N, 12.97.

**6.3.10. 6-Chloro-5-(3-chlorophenyl)amino-2-methyl-1*H*-benzo[*d*]imidazole-4,7-dione (2j).** Violet powder (56 mg, 40%); mp 286–289 °C; IR (KBr) $\nu$ 3291 (m, NH), 2991, 1692 (s, C=O), 1432–1660, 1124 (m) cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 14.6 (s, 1H, NH), 9.1 (s, 1H, NH), 7.3 (t, $J$ = 8 Hz, 1H, Ph-H), 7.0 (dd, $J$ = 2, 8.4 Hz, 3H, Ph-H), 2.4 (s, 3H, $CH_3$); HRMS calcd for $C_{14}H_9Cl_2N_3O_2$: 321.0072, found: 321.0072. Anal. Calcd for $C_{14}H_9Cl_2N_3O_2$ (322.15): C, 52.20; H, 2.82; N, 13.04. Found: C, 52.21; H, 2.82; N, 13.02.

**6.3.11. 6-Chloro-2-methyl-5-(4-trifluoromethylphenyl)-amino-1*H*-benzo[*d*]imidazole-4,7-dione (2k).** Violet powder (21 mg, 13%); mp 245–248 °C; IR (KBr) 3271 (m, NH), 3008, 1693 (s, C=O), 1411–1577, 1123 (m) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 13.6 (s, 1H, NH), 9.0 (s, 1H, NH), 7.1–7.6 (m, 4H, Ph-H), 2.4 (s, 3H, $CH_3$); HRMS calcd for $C_{15}H_9ClF_3N_3O_2$: 355.0335, found: 355.0336; Anal. Calcd for $C_{15}H_9ClF_3N_3O_2$ (355.7): C, 50.65; H, 2.55; N, 11.81. Found: C, 50.69; H, 2.58; N, 11.79.

**6.3.12. 6-Chloro-5-(4-fluorophenyl)amino-1*H*-benzo[*d*]imidazole-4,7-dione (3a).** Violet powder (75 mg, 60%); mp 330–331 °C; IR (KBr) 3277 (s, NH), 3126, 1687 (s, C=O), 1464–1594, 1401 (m) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 14 (s, 1H, NH), 9.2 (s, 1H, NH), 8.2 (s, 1H, H2), 7.1 (d, 4H, Ph-H); HRMS calcd for $C_{13}H_7ClFN_3O_2$: 291.0211, found: 291.0210; Anal. Calcd for $C_{13}H_7ClFN_3O_2$ (291.66): C, 53.53; H, 2.42; N, 14.41. Found: C, 53.49; H, 2.44; N, 14.39.

**6.3.13. 6-Chloro-5-(4-bromophenyl)amino-1*H*-benzo[*d*]-imidazole-4,7-dione (3b).** Dark violet powder (62 mg, 40%); mp 263–264 °C; IR (KBr) 3315 (m, NH), 3126, 1669 (s, C=O), 1510–1549 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 14 (s, 1H, NH), 9.2 (s, 1H, NH), 8.3 (s, 1H, H2), 7.5 (d, 2H, Ph-H), 7.0 (d, 2H, Ph-H); HRMS calcd for $C_{13}H_7BrClN_3O_2$: 350.9410, found: 350.9411; Anal. Calcd for $C_{13}H_7BrClN_3O_2$ (352.57): C, 44.29; H, 2.00; N, 11.92. Found: C, 44.20; H, 2.02; N, 11.87; Anal. Calcd for $C_{14}H_9BrClN_3O_2$ (366.6): C, 45.87; H, 2.47;N, 11.46. Found: C, 45.83; H, 2.48; N, 11.46.

**6.3.14. 6-Chloro-5-[(4-trifluoromethoxyphenyl)amino]-1*H*-benzo[*d*]imidazole-4,7-dione (3c).** Dark violet powder (68 mg, 40%); mp 232–233 °C; IR (KBr) 3317 (s, NH), 3126, 1673 (s, C=O), 1475–1545, 1396 (m) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 14 (s, 1H, NH), 9.2 (s, 1H, NH), 8.3 (s, 1H, H2), 7.3 (d, 2H, Ph-H), 7.2 (d, 2H, Ph-H); HRMS calcd for $C_{14}H_7ClF_3N_3O_3$: 357.0128, found: 357.0129; Anal. Calcd for $C_{14}H_7ClF_3N_3O_3$ (357.67): C, 47.01; H, 1.97; N, 11.75. Found: C, 46.98; H, 1.99; N, 11.72.

**6.3.15. 6-Chloro-5-(4-cyanophenyl)amino-1*H*-benzo[*d*]imidazole-4,7-dione (3d).** Dark red powder (46 mg, 30%); mp 199–200 °C; IR (KBr) 3357 (m, NH), 3126, 1674 (s, C=O), 1464–1540, 1395 (s) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 14.2 (s, 1H, NH), 8.3 (s, 1H, H2), 7.7 (d, $J$ = 7.4 Hz, 2H, Ph-H), 7.2 (d, $J$ = 7.4 Hz, 2H, Ph-H); HRMS calcd for $C_{14}H_7ClN_4O_2$: 298.0257, found: 298.0258: Anal. Calcd for $C_{14}H_7ClN_4O_2$ (298.68): C, 56.30; H, 2.36; N, 18.76. Found: C, 56.28; H, 2.36; N, 18.73.

**6.3.16. 6-Chloro-5-(2,4-difluorophenyl)amino-1*H*-benzo[*d*]imidazole-4,7-dione (3e).** Light violet powder (32 mg, 20%); mp 292–293 °C; IR (KBr) 3286 (s, NH), 3126, 1691 (m, C=O), 1432–1594, 1274 (s) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 14 (s, 1H, NH), 9.0 (s, 1H, NH), 8.1 (s, 1H, H2), 7.3 (m, 1H, Ph-H), 7.0 (m, 2H, Ph-H); HRMS calcd for $C_{13}H_6ClF_2N_3O_2$: 309.0117, found: 309.0116; Anal. Calcd for $C_{13}H_6ClF_2N_3O_2$ (309.66): C, 50.42; H, 1.95; N, 13.57. Found: C, 50.40; H, 1.97; N, 13.57.

**6.3.17. 6-Chloro-5-(3-chlorophenyl)amino-1*H*-benzo[*d*]-imidazole-4,7-dione (3f).** Purple powder (20 mg, 15%); mp 324–325 °C; IR (KBr) 3289 (s, NH), 3126, 1687 (s, C=O), 1475–1551 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 14 (s, 1H, NH), 9.2 (s, 1H, NH), 8.2 (s, 1H, H2), 7.3 (t, $J$ = 7.4 Hz, 1H, Ph-H), 7.1 (t, $J$ = 7.4 Hz, 1H, Ph-H), 7.0 (d, $J$ = 7.4 Hz, 1H, Ph-H), 6.5 (s, 1H, Ph-H); HRMS calcd for $C_{13}H_7Cl_2N_3O_2$: 306.9915, found: 306.9916; Anal. Calcd for $C_{13}H_7Cl_2N_3O_2$ (308.12): C, 50.67; H, 2.29; N, 13.64. Found: C, 50.54; H, 2.29; N, 13.57.

**6.3.18. 6-Chloro-5-(3,4-difluorophenyl)amino-1*H*-benzo[*d*]imidazole-4,7-dione (3g).** Light violet powder (95 mg, 67%); mp 261–263 °C; IR (KBr) 3288 (s, NH), 3132, 1690 (s, C=O), 1403–1554, 1210 (s), 1150 (s) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 14 (s, 1H, NH), 9.1 (s, 1H, NH), 8.1 (s, 1H, H2), 7.4 (q, 1H, Ph-H), 7.2 (m, 1H, Ph-H), 7.0 (m, 1H, Ph-H); HRMS calcd for $C_{13}H_6ClF_2N_3O_2$: 309.0117, found: 309.0116; Anal. Calcd for $C_{13}H_6ClF_2N_3O_2$ (309.66): C, 50.42; H, 1.95; N, 13.57. Found: C, 50.39; H, 1.92; N, 13.46.

**6.3.19. 6-Chloro-5-(2-fluorophenyl)amino-1*H*-benzo[*d*]imidazole-4,7-dione (3h).** Dark violet powder (26 mg, 19%); mp 261–264 °C; IR (KBr) 3280 (s, NH), 3130, 1690 (s, C=O), 1402–1556, 1402 (m), 1233 (m) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 14 (s, 1H, NH), 9.1 (s, 1H, NH), 8.1 (s, 1H, H2), 7.1–7.3 (m, 4H, Ph-H); HRMS calcd for $C_{13}H_7ClFN_3O_2$: 291.0211, found: 291.0212; Anal. Calcd for $C_{13}H_7ClFN_3O_2$ (291.66): C, 53.53; H, 2.42; N, 14.41. Found: C, 53.49; H, 2.43; N, 14.37.

**6.3.20. 6-Bromo-5-(4-fluorophenyl)amino-1*H*-benzo[*d*]imidazole-4,7-dione (4a).** Violet powder (89 mg, 80%); mp 293–294 °C; IR (KBr) 3274 (s, NH), 3126 (s), 1687 (s, C=O), 1438–1598, 1400 (s), 1206 (s, F), 892 (m) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 14 (s, 1H, NH), 9.2 (s, 1H, NH), 8.1 (s, 1H, H2), 7.1 (s, 2H, Ph-H), 6.5 (s, 2H, Ph-H); HRMS calcd for $C_{13}H_7BrFN_3O_2$: 334.97057, found: 334.97057; Anal. Calcd for $C_{13}H_7BrFN_3O_2$ (336.12): C, 46.45; H, 2.10; N, 12.50; Found: C, 46.35; H, 2.14; N, 12.43.

**6.3.21. 6-Bromo-5-(4-chlorophenyl)amino-1*H*-benzo[*d*]-imidazole-4,7-dione (4b).** Dark blue powder (90 mg, 80%); mp 82–83 °C; IR (KBr) 3342 (s, NH), 2809, 1669 (s, C=O), 1475–1545, 1398 (s), 830 (m) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 9.2 (s, 1H, NH), 8.2 (s, 1H, H2), 6.7–7.4 (m, 4H, Ph-H); HRMS calcd for $C_{13}H_7BrClN_3O_2$: 350.9410, found: 350.9411; Anal. Calcd for $C_{13}H_7BrClN_3O_2$ (352.57): C, 44.29; H, 2.00; N, 11.92. Found: C, 44.25; H, 2.03; N, 11.95.

**6.3.22. 6-Bromo-5-(3-bromophenyl)amino-1*H*-benzo[*d*]-imidazole-4,7-dione (4c).** Purple powder (80 mg, 60%); mp 285–286 °C; IR (KBr) 3286 (s, NH), 3126, 2368 (m), 1687 (m, C=O), 1443–1545, 1400 (s) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 14 (s, 1H, NH), 9.2 (s, 1H, NH), 8.2 (s, 1H, H2), 7.3 (d, 1H, Ph-H), 7.2 (d, 1H, Ph-H), 7.1 (m, 2H, Ph-H); HRMS calcd for $C_{13}H_7Br_2N_3O_2$:

394.890499, found: 394.890499; Anal. Calcd for $C_{13}H_7Br_2N_3O_2$ (397.02): C, 39.33; H, 1.78; N, 10.58. Found: C, 39.31; H, 1.79; N, 10.56.

**6.3.23. 6-Bromo-5-(3-chlorophenyl)amino-1*H*-benzo[*d*]-imidazole-4,7-dione (4d).** Purple powder (52 mg, 50%); mp 273–274 °C; IR (KBr) 3285 (s, NH), 3126, 1687 (s, C═O), 1470–1545, 1401 (m), 1148 (m) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 14 (s, 1H, NH), 9.2 (s, 1H, NH), 8.2 (s, 1H, H2), 7.0–7.3 (m, 4H, Ph-H); HRMS calcd for $C_{13}H_7BrClN_3O_2$: 350.9410, found: 350.9410; Anal. Calcd for $C_{13}H_7BrClN_3O_2$ (352.57): C, 44.29; H, 2.00; N, 11.92. Found: C, 44.26; H, 2.02; N, 11.94.

**6.3.24. 6-Bromo-5-(3-iodophenyl)amino-1*H*-benzo[*d*]imidazole-4,7-dione (4e).** Purple powder (65 mg, 45%); mp 279–280 °C; IR (KBr) 3289 (m, NH), 3126, 1687 (s, C═O), 1464–1551 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 14 (s, 1H, NH) 9.2 (s, 1H, NH), 8.2 (s, 1H, H2), 6.9–7.5 (m, 4H, Ph-H); HRMS calcd for $C_{13}H_7BrIN_3O_2$: 442.876649, found: 442.876649; Anal. Calcd for $C_{13}H_7BrIN_3O_2$ (444.02): C, 35.16; H, 1.59; N, 9.46. Found: C, 35.27; H, 1.61; N, 9.39.

**6.3.25. 6-Bromo-5-phenylamino-1*H*-benzo[*d*]imidazole-4,7-dione (4f).** Black powder (54 mg, 55%); mp 200–201 °C; IR (KBr) 3289 (s, NH), 3127, 1686 (s, C═O), 1448–1587, 1399 (m) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 14 (s, 1H, NH) 9.2 (s, 1H, NH), 8.2 (s, 1H, H2), 7.3 (t, $J$ = 7.9 Hz, 2H, Ph-H), 7.1 (t, $J$ = 7.9 Hz, 3H, Ph-H); MS (*m*/*z*) 317 ($M^+$); HRMS calcd for $C_{13}H_8BrN_3O_2$: 316.9800, found: 316.9801; Anal. Calcd for $C_{13}H_8BrN_3O_2$ (318.13): C, 49.08; H, 2.53; N, 13.21. Found: C, 49.44; H, 2.57; N, 13.24.

**6.3.26. 6-Bromo-5-[(3-trifluoromethylphenyl)amino]-1*H*-benzo[*d*]imidazole-4,7-dione (4g).** Purple powder (87 mg, 65%); mp 265–266 °C; IR (KBr) 3297 (s, NH), 3126, 1687 (s, C═O), 1443–1588, 1400 (s) $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 14 (s, 1H, NH), 9.3 (s, 1H, NH), 8.2 (s, 1H, H2), 7.3-7.5 (m, 4H, Ph-H); HRMS calcd for $C_{14}H_7BrF_3N_3O_2$: 384.9674, found: 384.9673; Anal. Calcd for $C_{14}H_7BrF_3N_3O_2$ (386.12): C, 43.55; H, 1.83; N, 10.88; Found: C, 43.52; H, 1.85; N, 10.92.

### 6.4. HUVEC proliferation assay

HUVECs obtained from umbilical cord veins were prepared by a method described previously[10] and cultured in Medium 199 (Gibco BRL, Grand Island, NY, USA) supplemented with 20% fetal bovine serum, 25 mM HEPES, 10 U/mL heparin, 100 U/mL penicillin, 100 μg/mL streptomycin, and 20 ng/mL bFGF. HUVECs were plated at a density of $5 \times 10^3$ cells/well in 100 μL M199 containing 20% (v/v) fetal bovine serum in gelatin-coated 96-well plates (Costar, Corning, NY, USA). After 24 h incubation, the cells were treated with test compounds in 100 μL M199 containing 5% FBS and 10 ng/mL bFGF for 48 h. HUVEC proliferation was determined using a colorimetric assay kit based on the uptake of WST by viable cells (Premix WST-1 cell proliferation assay system, Takara Bio Inc, Otsu, Japan). The assay kit is dependent on the reduction or tetrazolium salt WST-1, which results in formation of a dark red formazan product, by various mitochondrial dehydrogenase of viable cells.

### 6.5. Immunoblot assay of SMC lysate

The p38 and phospho-p38 antibodies were purchased from BioSource International Inc. (Camarillo, CA, USA). The HUVECs were cultured for 72 h in DMEM containing 0.2% FBS and 5 ng/mL bFGF, and treated with the compound **2b** (1 μM). The cells were pooled and homogenized in RIPA buffer (150 mM NaCl, 50 mM Tris (pH 7.6), 1% Triton X-100, 0.1% SDS, 0.5% sodium deoxycholate, 1 mM PMSF, 1 μg/mL aprotinin, 1 μg/mL leupeptin, and 1 μg/mL pepstatin) at 4 °C. After incubating for 30 min on ice, insoluble materials were removed by centrifugation at 14,000 rpm for 15 min and the protein lysate concentrations were measured by Bradford assay. The same amounts and proportions of proteins from whole cell lysates or precipitated immune complexes were resolved on SDS–PAGE and blotted onto nitrocellulose membranes. The membrane was incubated with primary antibodies overnight at 4 °C, followed by HRP-conjugated secondary antibodies for 50 min at room temperature, and detected by enhance chemiluminescence (ECL) reagent.

## Acknowledgments

This study was supported by a Grant of the Korea Science and Engineering Foundation (KOSEF R01-2006-000-10020-0) and a Grant of National Research Lab from the Ministry of the Science and Technology.

## References and notes

1. Hanahan, D.; Folkman, J. *Cell* **1996**, *86*, 353.
2. Fantl, W. J.; Rosenberg, S.. In *Annual Reports in Medicinal Chemistry*; Doherty, A. M., Ed.; Academic Press: San Diego, CA, 2000; Vol. 35, pp 123–133.
3. Matter, A. *Drug Discovery Today* **2001**, *6*, 1005.
4. Middleton, R. W.; Parrick, J. In *The Chemistry of The Quinonoid Compounds*; Patai, S., Rappoport, Z., Eds.; John Wiley and Sons: London, 1988; Vol. 2, pp 1019–1066.
5. Yoshizumi, M.; Abe, J.; Tsuchiya, K.; Berk, B. C.; Tamaki, T. *J. Pharmacol. Sci.* **2003**, *91*, 172.
6. Zhang, H.; Shi, X.; Hampong, M.; Blanis, L.; Pelech, S. *J. Biol. Chem.* **2001**, *276*, 6905.
7. Weinberger, L.; Day, A. R. *J. Org. Chem.* **1959**, *24*, 1451.
8. Jaffe, E. A.; Nachman, R. L.; Becker, C. G.; Minick, C. R. *J. Clin. Invest.* **1973**, *52*, 2745.
9. Mohacsi, P. J.; Tüller, D.; Hulliger, B.; Wijngaard, P. L. *J. Heart Lung Transplant.* **1997**, *16*, 484.
10. (a) Lee, K.-H.; Lim, S. Y.; Kang, S.-M.; Kim, D. H.; Cho, H. K.; Chung, J. H.; Kwon, H. M.; Chung, K.-H.; Lee, H.; Jang, Y.; Hwang, K.-C. *Eur. J. Pharmacol.* **2004**, *484*, 119; (b) Hong, S.-Y.; Chung, K.-H.; You, H.-J.; Choi, I. H.; Chae, M. J.; Han, J.-Y.; Jung, O.-J.; Kang, S.-J.; Ryu, C.-K. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3563.
11. Ito, K.; Hirao, A.; Arai, F.; Takubo, K.; Matsuoka, S.; Miyamoto, K.; Ohmura, M.; Naka, K.; Hosokawa, K.; Ikeda, Y.; Toshio Suda, T. *Nat. Med.* **2006**, *12*, 446.