Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 2948–2951
Bioorganic & Medicinal Chemistry Letters

Design, synthesis and evaluation of 2-phenyl-1*H*-benzo[*d*]-imidazole-4,7-diones as vascular smooth muscle cell proliferation inhibitors

Chung-Kyu Ryu,[a,*] Ra-Young Lee,[a] Seung-Yon Lee,[a] Hwa-Jin Chung,[a] Sang Kook Lee[a] and Kwang-Hoe Chung[b]

[a]*College of Pharmacy, Ewha Womans University, Seodaemun-ku, Seoul 120-750, Republic of Korea*
[b]*Thrombosis & Vascular Biochemistry Laboratory, Department of Biochemistry, College of Medicine, Pochon CHA University, Sungnam 463-836, Republic of Korea*

Received 17 December 2007; revised 5 March 2008; accepted 24 March 2008
Available online 27 March 2008

Abstract—A series of 2-phenyl-1*H*-benzo[*d*]imidazole-4,7-diones were synthesized and tested for their inhibitory activity on the PDGF-stimulated proliferation of rat aortic vascular smooth muscle cells. Among the tested compounds, 6-arylthio-5-chloro-2-phenyl-1*H*-benzo[*d*]imidazole-4,7-diones exhibited an potent antiproliferative activity.

The proliferation and migration of vascular smooth muscle cells (SMCs) play an important role in the progression of atherosclerosis and restenosis.[1] Abnormal arterial injury results in the migration of SMCs into the intimal layer of the arterial wall, where they proliferate and synthesize extracellular matrix components. Many growth factors induced the proliferation and migration of arterial SMCs.[2] Among them, platelet-derived growth factor (PDGF) is one of the most potent promoters of the proliferation and migration of SMCs.[3]

Quinonoid compounds represent an important class of biologically active molecules.[4] Therefore, we designed, synthesized and evaluated the antiproliferative effects of various quinone derivatives on PDGF-stimulated SMC proliferation. Previously, we reported that benzimidazole-4,7-dione derivatives **1** exhibited a potent inhibition for the smooth muscle cells (SMCs) proliferation as preliminary results. The arylamino, arylthio- or phenyl-substituents of quinones have been improved several biological activities.[5] On this line, we further extended to synthesize 1*H*-benzo[*d*]imidazole-4,7-diones **2**–**4**, which would be analogues of quinones **1**, and evaluated their antiproliferative activity on the rat aortic SMCs (Fig. 1). We describe herein our results on the synthesis of 2-phenyl-1*H*-benzo[*d*]imidazole-4,7-dione series **2**–**3** and their antiproliferative activity on the rat aortic SMCs. Additional data for the antiproliferative activity of other 2-pyridyl-1*H*-benzo[*d*]imidazole-4,7-diones **4** are also provided.

Additional data for the mechanism of SMCs antiproliferative activity of one representative 1*H*-benzo[*d*]imidazole-4,7-dione **3a** was also performed. The mitogen-activated protein kinase (MAPK) cascade known as the extracellular signal-regulated kinase (ERK) pathway mediates mitogenic responses induced by a wide variety of growth factor receptors in many cell types,

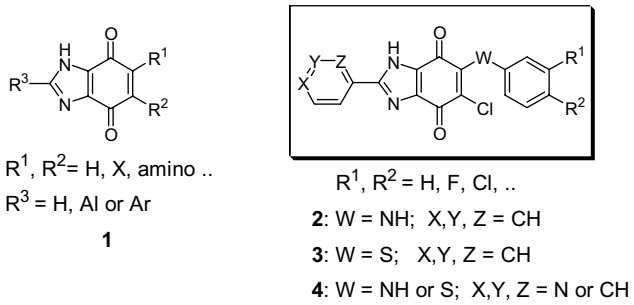
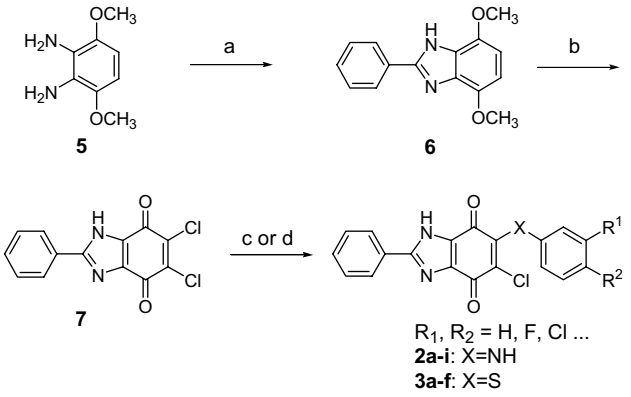


Figure 1. 1*H*-Benzo[*d*]imidazole-4,7-dione derivatives.

Keywords: 2-Phenyl-1*H*-benzo[*d*]imidazole-4,7-dione; Smooth muscle cell; Antiproliferative activity; Substitution effects.
* Corresponding author. E-mail: ckryu@mm.ewha.ac.kr

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.03.066



Scheme 1. Synthesis of 2-phenyl-1*H*-benzo[*d*]imidazole-4,7-diones. Reagents and conditions: (a) benzaldehyde (1 equiv)/toluene/reflux/4 h/72%; (b) concd HCl/concd HNO_3/reflux/0.5 h/46%; (c) arylamine (1 equiv)/EtOH/reflux/5 h/75–92%/; (d) arylthiol (1 equiv)/EtOH/reflux/24 h/65–90%.

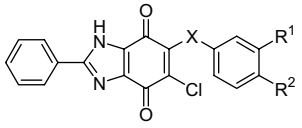
including SMCs.[6] MAPKs play an important role in regulating cell growth and survival, and are also involved in both mitogenic and stress responses of cells.[7] MAPK is activated through a specific phosphorylation cascade. In general, the ERK pathway plays a major role in regulating cell growth and differentiation, being highly induced in response to growth factors and cytokines.[8] The ERK pathway is required for cell cycle arrest, apoptosis and growth of the SMCs.[9] In order to investigate the effect of the compound **3a** on the proliferation of SMCs and its mechanism of action, we examined the effect of the compound on ERK activation and cell cycle regulation.

The method used to synthesize 6-arylamino-5-chloro-2-phenyl-1*H*-benzo[*d*]imidazole-4,7-diones **2** is shown in Scheme 1. 2,3-Diamino-1,4-dimethoxybenzene (**5**) was prepared according to the known method.[10] Cyclizations of compound **5** with benzaldehyde gave 4,7-dimethoxy-2-phenyl-benzimidazole (**6**) resulting in 72% yields. 5,6-Dichloro-2-phenyl-1*H*-benzo[*d*]imidazole-4,7-dione (**7**) was synthesized by oxidizing compound **6** with HNO_3/HCl variation resulting in 46% yields. 2-Phenyl-1*H*-benzo[*d*]imidazole-4,7-diones **2a**–**i** (Table 1) were prepared by nucleophilic substitution on compound **7** with appropriate arylamines. Most of these substitutions went as expected and had overall high yields of 75–92%.

In a similar manner, 6-arylthio-5-chloro-2-phenyl-1*H*-benzo[*d*]imidazole-4,7-diones **3a**–**f** (Table 1) were synthesized by nucleophilic substitution on the compound **7** with appropriate arylthiols in good yields. 2-Pyridyl-1*H*-benzo[*d*]imidazole-4,7-diones **4a**–**t** (Table 2) were prepared according to a method previously reported.[5]

The 1*H*-benzo[*d*]imidazole-4,7-diones **2**–**4** were tested in vitro for their antiproliferative activity on the rat aortic SMC proliferation. Inhibition of PDGF-stimulated proliferation was determined by colorimetric assay.[11] The IC_{50} values were determined by comparison to mycophenolic acid (MPA)[12] as a standard agent. As indicated in Tables 1 and 2, 6-arylthio-2-phenyl-1*H*-

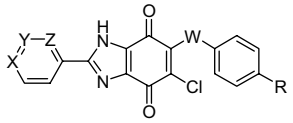
Table 1. Structures and IC_{50} values of 2-phenyl-1*H*-benzo[*d*]imidazole-4,7-diones for inhibition of SMC proliferation



Compound	X	R^1	R^2	SMC[a] IC_{50}[b] (μM)
2a	NH	H	OCH_3	2.0
2b	NH	H	CH_3	3.0
2c	NH	H	H	4.0
2d	NH	H	OCF_3	1.5
2e	NH	CH_3	CH_3	3.0
2f	NH	H	CF_3	9.4
2g	NH	H	OCH_2CH_3	50.0
2h	NH	H	Cl	5.3
2i	NH	H	CH_2CH_3	10.7
3a	S	H	OCH_3	1.0
3b	S	H	CH_3	1.0
3c	S	H	H	1.5
3d	S	H	Br	1.5
3e	S	H	OH	2.0
3f	S	CH_3	H	2.0
MPA				1.0

[a] SMCs were isolated from rat thoracic aorta.
[b] The inhibitory activity against the PDGF-induced proliferation of SMCs.

Table 2. Structures and IC_{50} values of 2-pyridyl-1*H*-benzo[*d*]imidazole-4,7-diones for inhibition of SMC proliferation



Compound	X	Y	Z	W	R	SMC[a] IC_{50}[b] (μM)
4a	CH	CH	N	NH	F	9.4
4b	CH	CH	N	NH	Cl	12.1
4c	CH	CH	N	NH	Br	6.5
4d	CH	CH	N	NH	OCH_3	20.0
4e	CH	CH	N	NH	CH_3	50.0
4f	CH	CH	N	NH	CF_3	1.0
4g	CH	CH	N	NH	OCF_3	20.0
4h	CH	N	CH	NH	F	21.0
4i	CH	N	CH	NH	Cl	20.0
4j	CH	N	CH	NH	Br	50.0
4k	CH	N	CH	NH	OCH_3	42.0
4l	N	CH	CH	NH	F	50.0
4m	N	CH	CH	NH	Cl	25.0
4n	N	CH	CH	NH	Br	100.0
4o	N	CH	CH	NH	OCH_3	50.0
4p	CH	CH	N	S	Cl	4.0
4q	CH	CH	N	S	Br	12.0
4r	CH	CH	N	S	OCH_3	4.2
4s	CH	CH	N	S	CH_3	2.5
4t	CH	CH	N	S	CH_2CH_3	7.0
MPA						1.0

[a] SMCs were isolated from rat thoracic aorta.
[b] The inhibitory activity against the PDGF-induced proliferation of SMCs.

benzo[*d*]imidazole-4,7-diones **3a**–**f** showed generally good activity. Actually, many compounds of 6-arylamino-2-phenyl-1*H*-benzo[*d*]imidazole-4,7-diones **2a**–**i** exhibited the potent activity. In contrast, 2-pyridyl-1*H*-benzo[*d*]imidazole-4,7-diones **4a**–**t** did not show significant their antiproliferative activity, although some compounds of them exhibited good antiproliferative activity. 1*H*-Benzo[*d*]imidazole-4,7-diones **3a**, **3b** and **4f** inhibited the PDGF-stimulated proliferation of the SMC tested at the IC_{50} of 1.0 μM. The activity of these compounds is comparable to that of MPA.

In terms of structure–activity relationship, 2-phenyl-1*H*-benzo[*d*]imidazole-4,7-diones **2** and **3** showed, in general, more potent activity than 2-pyridyl-1*H*-benzimidazole-4,7-dione series **4**. The 2-phenyl-substituted compounds **2** and **3** exhibited the greatest activity, indicating a correlation that may offer insight into the mode of action of these compounds. The 2-pyridyl-moiety of compounds **4** did not appeared to contribute partially toward biological potency.

In addition, the quinone moiety in 1*H*-benzo[*d*]imidazole-4,7-diones **2**–**4** might be essential for the antiproliferative activity. For example, non-quinonoid compounds **6** lost the antiproliferative activity. The results of their QSAR study would imply that alteration of R, R_1, and R_2 on 1*H*-benzo[*d*]imidazole-4,7-diones **2**–**4** did not greatly influence the inhibitory activity. This suggests that 1*H*-benzo[*d*]imidazole-4,7-dione structure is mainly responsible for the activities.

Further mechanistic study on the antiproliferative activity was performed using one representative compound **3a** in cultured SMCs. As illustrated in Figure 2, when SMCs were exposed to 1 μg/mL of the compound **3a** for 48 h morphological changes also revealed that the cell density was decreased by observation under the phase-contrast microscope.

To explore whether the antiproliferative effects on the compound **3a** were mediated by the modulation of the cell cycle in SMCs, DNA contents were analyzed by flow cytometry. As shown in Figure 3, when SMCs were treated with the compound **3a** for 48 h, the DNA contents were accumulated in the S phase of the cell cycle compared to vehicle-treated control group.

To investigate whether cell cycle arrest mediated by test compounds was related to the expression of regulatory proteins, Western blot analysis was performed. As shown in Figure 4, when SMCs were treated with

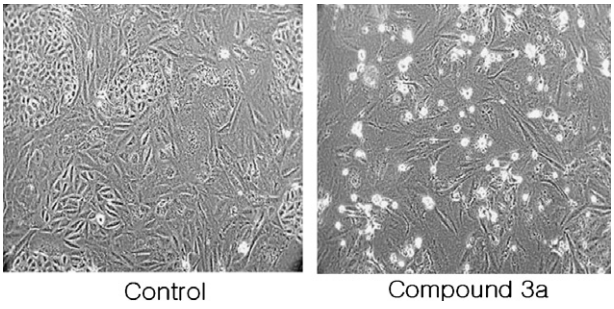


Figure 2. Morphological change in cultured SMCs treated with compound **3a**. SMCs treated with DMSO alone (control) or the compound **3a** (1 μg/mL) for 48 h were observed under the phase-contrast microscope and photographed.

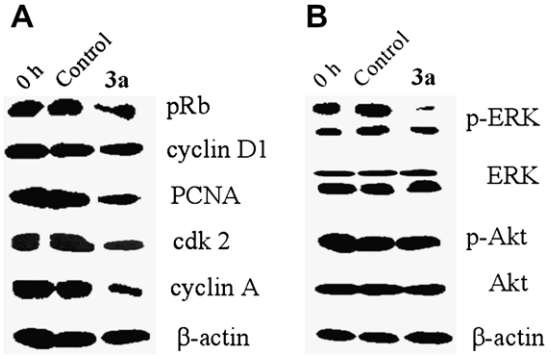


Figure 4. Effect of compound **3a** on protein expression in cultured SMCs. (A) Total cell lysates from SMCs treated with the compound **3a** (1 μg/mL) for 48 h were analyzed for pRb, cyclin D1, PCNA, cdk 2, and cyclin A. (B) The compound **3a** was exposed to SMCs for 1 h, and the expression of phosphorylation of ERK and phosphorylation of Akt was examined.

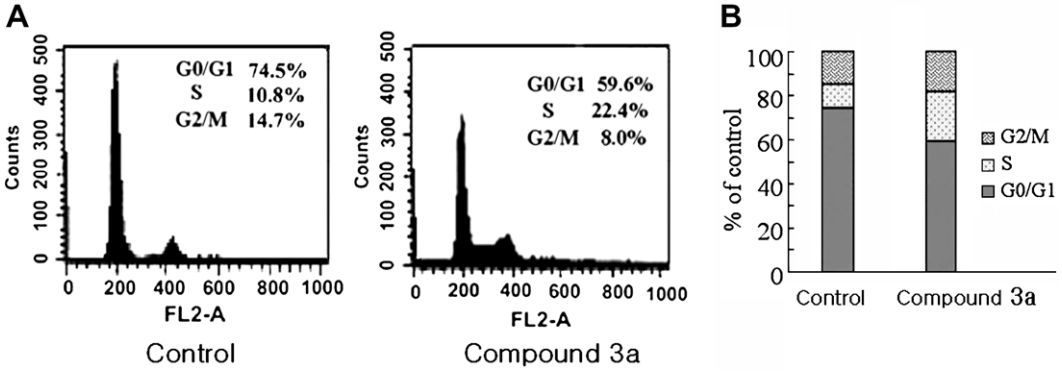


Figure 3. Effect of compound **3a** on cell cycle progression in cultured SMCs. Cells were treated with the compound **3a** (1 μg/mL) for 48 h and then the cell cycle was analyzed by flow cytometry analysis.

1 μg/mL of the compound **3a** for 48 h, the level of cdk 2 was markedly reduced. In addition, the down-regulation of cyclin A, which binds to cdk 2 and promotes progression through the S phase of cell cycle, was observed in compound **3a**-treated cells. However, the expression levels of pRb and cyclin D1, which promotes progression through the G1 into S phase of cell cycle, was not observed in compound **3a**-treated cells. The cell-proliferation biomarker PCNA was down-regulated which is well correlated with the antiproliferative effect of the compound **3a**. The expression of protein was not affected by the compound **3a**. These results indicate that the compound **3a** might affect the exit of S phase cell cycle and thus accumulate the DNA contents of S phase in the cells.

To better understand the molecular mechanisms involved in the compound **3a** on the proliferation of SMCs, we investigated the possible involvement of ERK and Akt cell signaling pathways. As shown in Figure 4, a remarkable decrease of ERK phosphorylation, but not Akt, was detected with the treatment of the compound **3a** (1 μg/mL) for 1 h (Fig. 4B), indicating that the ERK signaling pathway might be involved in the inhibition of SMC proliferation.

The ERK and Akt are major signal transduction molecules regulating cell proliferation, differentiation, and apoptosis. In particular, ERK pathway has been known to play pivotal roles in controlling SMC proliferation. Several studies have suggested that the inhibition of SMC proliferation is ERK-dependent. In the present study, the regulation of ERK by test compound **3a** was manifested, but not much related to the regulation of Akt.

In conclusion, the antiproliferative effect of the compound **3a** in SMCs is associated with its blockade of cell cycle progression which appears to be attributable in part to suppression of ERK signaling activation. Further pharmacological investigations of these compounds and the structural optimization are in progress.

Acknowledgments

This study was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MOST: R01-2006-000-10020-0).

References and notes

1. Ross, R. *Nature* **1993**, *362*, 801.
2. Indolfi, C.; Mongiardo, A.; Curcio, A.; Torella, D. *Trends Cardiovas. Med.* **2003**, *13*, 142.
3. Newby, A. C.; George, S. J. *Cardiovasc. Res.* **1993**, *27*, 1173.
4. Middleton, R. W.; Parrick, J.. In *The Chemistry of the Quinonoid Compounds*; Patai, S., Rappoport, Z., Eds.; John Wiley & Sons: London, 1988; Part 2, pp 1019–1066.
5. Ryu, C.-K.; Song, E. H.; Shim, J.-Y.; You, H.-J.; Choi, K. U.; Choi, I. H.; Lee, E. Y.; Chae, M. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 17.
6. Bornfeldt, K. E.; Campbell, J. S.; Koyama, H.; Argast, G. M.; Leslie, C. C.; Raines, E. W.; Krebs, E. G.; Ross, R. *J. Clin. Invest.* **1997**, *100*, 875.
7. Petrache, I.; Choi, M. E.; Otterbein, L. E.; Chin, B. Y.; Mantell, L. L.; Horowitz, S.; Choi, A. M. *Am. J. Physiol.* **1999**, *277*, 589.
8. Roulston, A.; Reinhard, C.; Amiri, P.; Williams, L. T. *J. Biol. Chem.* **1998**, *273*, 10232.
9. Mayr, M.; Xu, Q. *Exp. Gerontol.* **2001**, *36*, 969.
10. Weinberger, L.; Day, A. R. *J. Org. Chem.* **1959**, *24*, 1451.
11. Sohn, Y. D.; Lim, H. J.; Hwang, K. C.; Kwon, J. H.; Park, H. Y.; Chung, K.-H.; Cho, S. Y.; Jang, Y. *Biochem. Biophys. Res. Commun.* **2001**, *284*, 931.
12. Mohacsi, P. J.; Tüller, D.; Hulliger, B.; Wijngaard, P. L. *J. Heart Lung Transplant.* **1997**, *16*, 484.