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Synthesis and biological evaluation of benzimidazole-4,7-diones that inhibit vascular smooth muscle cell proliferation

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Abstract—A series of 6-arylamino-5-chloro-benzimidazole-4,7-diones were synthesized and tested for their inhibitory activity on the rat aortic smooth muscle cell (RAoSMC) proliferation. Among them, 6-arylamino-5-chloro-2-methyl-benzimidazole-4,7-diones exhibited potent antiproliferative activity. Benzimidazole-4,7-dione **2c** activated SAPK/JNK signaling pathway in the RAoSMCs.

1. Introduction

The abnormal proliferation and migration of vascular smooth muscle cells (SMCs) play an important role in the pathology of coronary artery atherosclerosis and restenosis following angioplasty.[1] Arterial injury results in the migration of SMCs into the intimal layer of the arterial wall, where they proliferate and synthesize extracellular matrix components. Several growth factors induce the proliferation and migration of arterial SMCs.[2] Platelet-derived growth factor (PDGF) is one of the most potent promoters of the proliferation and migration of SMCs.[3]

Compounds containing the heterocyclic quinone group represent an important class of biologically active molecules.[4] However, the inhibitory activity of quinone classes on the proliferation of SMC has not been reported to the best of our knowledge. Therefore, we synthesized and tested various quinone derivatives to elucidate their contribution to the antiproliferative effects on PDGF-stimulated SMC proliferation. Among the quinones tested, benzimidazole-4,7-dione derivatives **1** (Fig. 1) showed the potent antiproliferative activity. There have been several reports[5–10] on some benzimidazole-4,7-dione derivatives, which exhibited antiproliferative effect[5] on human lymphoblastic leukemia and non-Hodgkin lymphoma, cytotoxic activity[6,7] against cancer cell lines, inhibitory effect[8] on protozoal purine nucleoside phosphorylase and antifungal activity.[9]

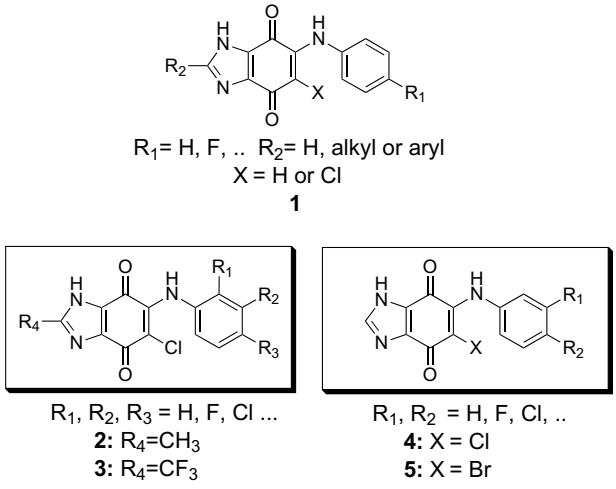

Figure 1. Benzimidazole-4,7-dione derivatives.

We describe herein our preliminary results on the synthesis of 6-arylamino-benzimidazole-4,7-diones **2**–**5** and their antiproliferative activity on the rat aortic SMCs (RAoSMCs).

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Additional data for the mechanism of SMC antiproliferative activity of compound **2c** is provided. Among the signal transduction pathways, mitogen-activated protein kinase (MAPK) pathways have a critical role in the proliferation of the SMCs.[11,12] Activation of MAPK pathways is observed on treating SMCs with growth factors, tumor necrosis factor, interleukin-1, and upon stress exposure. A central function of the MAPK pathways is the activation of gene expression, mediated through phosphorylation of transcription factors. A stress-activated protein kinase/Jun-N-terminal kinase (SAPK/JNK) is a kind of MAPK. The SAPK/JNK is required for cell cycle arrest, apotosis, and growth of the SMCs.[12] In order to investigate the effect of benzimidazole-4,7-dione **2c** on the intracellular signaling of SMCs, we examined whether the compound **2c** activates the SAPK/JNK.

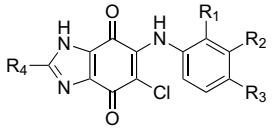
2. Chemistry

The method used to synthesize the 6-arylamino-5-chloro-2-methyl-benzimidazole-4,7-diones **2** is shown in Scheme 1. 4,7-Dimethoxy-2-methyl-benzimidazole (**6a**) was prepared according to the known method.[10] Cyclization of 2,3-diamino-1,4-dimethoxybenzene with CH_3COOH gave the compound **6a**. 5,6-Dichloro-2-methyl-benzimidazole-4,7-dione (**7a)** was synthesized by oxidizing the compound **6a** with HNO_3/HCl variation resulting in 43% yields. The benzimidazole-4,7-diones **2a**–**l** (Table 1) were prepared by nucleophilic substitution on the compound **7a** with appropriate arylamines. Most of these substitutions went as expected and had overall high yields of 76–94%.

In a similar manner, 6-arylamino-5-chloro-2-trifluoromethyl-benzimidazole-4,7-diones **3** were prepared from 4,7-dimethoxy-2-trifluoromethyl-benzimidazole (**6b**),[13] which was oxidized to compound **7b**. The benzimidazole-4,7-diones **3a**–**f** (Table 1) were synthesized by the substitution on the compound **7b** with the arylamines in good yields.

6-Arylamino-5-chloro-benzimidazole-4,7-diones **4** were prepared from the 5,6-dichloro-benzimidazole-4,7-dione (**9**) (Scheme 2). Compound **9** was prepared by oxidizing 4,7-dimethoxybenzimidazole (**8**)[10] with the HNO_3/HCl variation. The benzimidazole-4,7-diones **4a**–**i** (Table 2) were synthesized by the substitution on the compound **9** with the arylamines in good yields.

Table 1. Structures and IC_{50} values of the benzimidazole-4,7-diones for inhibition of SMC proliferation



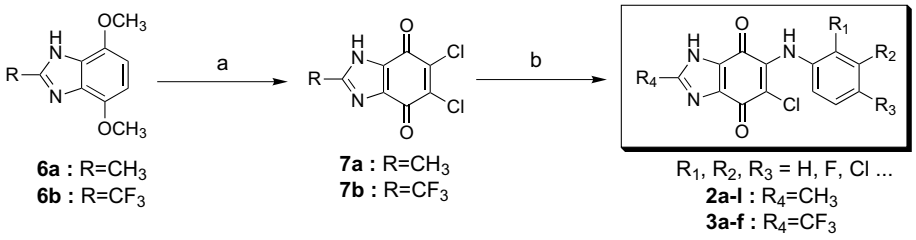
Compds	R_1	R_2	R_3	R_4	SMC[a] IC_{50}[b] (μM)
2a	H	Br	H	CH_3	1.0
2b	H	H	CH_3	CH_3	0.8
2c	H	H	H	CH_3	0.8
2d	H	H	Cl	CH_3	2.8
2e	H	H	F	CH_3	3.0
2f	Cl	Cl	H	CH_3	0.6
2g	Cl	H	Cl	CH_3	0.8
2h	F	H	F	CH_3	1.2
2i	H	Cl	H	CH_3	0.9
2j	F	F	F	CH_3	1.1
2k	H	H	CF_3	CH_3	0.6
2l	H	H	I	CH_3	1.0
3a	H	H	CH_3	CF_3	40.0
3b	H	H	CF_3	CF_3	20.0
3c	H	H	H	CF_3	50.0
3d	H	H	Cl	CF_3	15.0
3e	H	H	I	CF_3	25.0
3f	H	H	F	CF_3	12.0
6a					>100
MPA					1.0

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

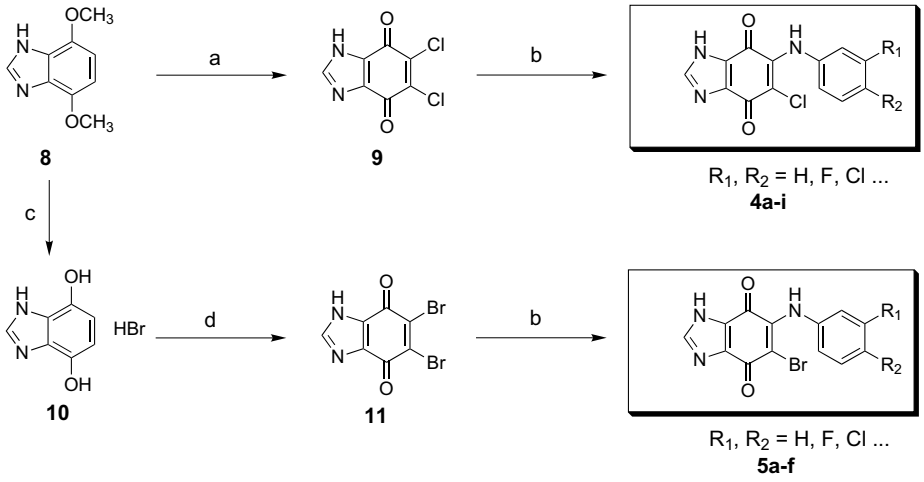
Demethylating the compound **8** with HBr gave 4,7-dihydroxybenzimidazole hydrobromide (**10**), which was oxidized to the 5,6-dibromo-benzimidazole-4,7-dione (**11**)[14] in a HBr/$NaBrO_3$ solution resulting in a 48% yield. The 6-arylamino-5-bromo-benzimidazole-4,7-diones **5a**–**f** (Table 2) were synthesized by nucleophilic substitution on the compound **11** with arylamines in good yields.

3. Antiproliferative activity

The synthesized benzimidazole-4,7-diones **2**–**5** were tested in vitro for their antiproliferative activity on the RAoSMCs. Inhibition of proliferation of these cells was determined by WST colorimetric assay.[15,16] The IC_{50} values were determined by comparison to mycophenolic acid (MPA)[17] as a standard agent. As indicated in the

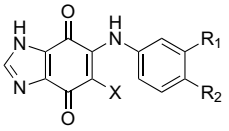


Scheme 1. Synthesis of 2-alkyl-6-arylamino-benzimidazole-4,7-diones. Reagents and conditions: (a) c-HCl/c-HNO_3/reflux/0.5 h/ **7a** 43%; **7b** 56%; (b) arylamine (1 equiv)/EtOH/reflux/5 h/76–94%.



Scheme 2. Synthesis of 6-arylamino-benzimidazole-4,7-diones. Reagents and conditions: (a) c-HCl/c-HNO_3/reflux/0.5 h/52%; (b) arylamine (1 equiv)/EtOH/reflux/5 h/70–91%; (c) c-HBr/reflux/6h/39%; (d) HBr/$NaBrO_3$/reflux/1 h/48%.

Table 2. Structures and IC_{50} values of the benzimidazole-4,7-diones for inhibition of SMC proliferation



Compds	X	R_1	R_2	SMC IC_{50} (μM)
4a	Cl	H	F	1.2
4b	Cl	H	I	1.3
4c	Cl	H	CH_3	1.4
4d	Cl	H	CF_3	2.5
4e	Cl	I	H	1.3
4f	Cl	Cl	H	1.4
4g	Cl	Br	H	1.6
4h	Cl	H	H	5.5
4i	Cl	F	F	1.1
5a	Br	H	F	1.0
5b	Br	H	CH_3	1.0
5c	Br	H	CF_3	3.1
5d	Br	H	Cl	4.0
5e	Br	I	H	2.5
5f	Br	H	H	1.1
8				>100

Tables 1 and 2, the most active potential among the benzimidazole-4,7-dione series **2**–**5** was found for 6-arylamino-5-chloro-2-methyl-benzimidazole-4,7-diones **2a**–**l**, which showed generally good activity. Actually, many compounds of benzimidazole-4,7-diones **4a**–**i** and **5a**–**f** exhibited good activity. The activity of these compounds is superior or comparable to that of MPA. In contrast, compounds **3a**–**f** did not show significant activity.

The most active potential among the series was found for the compounds **2b**, **2c**, **2f**, **2g**, and **2k**, which showed half maximal inhibition of the PDGF-stimulated proliferation of the RAoSMCs tested at the level of 0.8 μM. The results indicate that the 6-arylamino-5-chloro-2-methyl-benzimidazole-4,7-diones **2** are a promising lead for the development as inhibitors of the SMC proliferations.

In terms of structure–activity relationship, the 6-arylamino-2-methyl-benzimidazole-4,7-diones **2** showed, in general, more potent antiproliferative activity than benzimidazole-4,7-dione series **3**–**5**. The 2-methyl substituted compounds **2** exhibited the greatest activity, indicating a correlation that may offer insight into the mode of action of these compounds. The 2-trifluoromethyl moiety of compounds **3** did not appeared to contribute partially toward biological potency. The structure–activity relationship may not exist between properties of substituents (R: F, Cl, Br,...) for 6-arylamino moieties of benzimidazole-4,7-diones **2**–**5**.

In addition, nonquinonoid compounds **6a** and **8** exhibited no antiproliferative activity. Thus, quinone moiety of benzimidazole-4,7-diones may be essential for the antiproliferative activity.

4. Effect of compound 2c on SAPK/JNK

In order to investigate the effect of the compound **2c** on the intracellular signaling of the SMCs, we examined whether the compound **2c** activates the SAPK/JNK protein in the RAoSMCs by immunoblot assay.[18] As shown in Figure 2, the compound **2c** had little effect on the expression of the SAPK/JNK protein but dramatically increased the phosphorylation of the protein

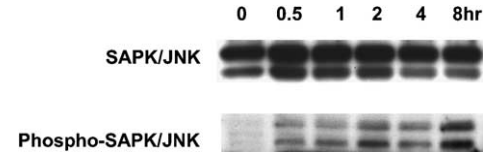


Figure 2. The compound **2c** activated SAPK/JNK protein in rat aortic SMC. The SMCs treated with the compound **2c** for 0.5, 1, 2, 4, and 8 h were immunoblotted with anti-SAPK/JNK polyclonal antibody and phospho-SAPK/JNK 6 monoclonal antibody, respectively.

(phospho-SAPK/JNK), which means that the compound **2c** activated SAPK/JNK pathway. SAPK/JNK, one of various MAPKs is potentially activated by a variety of environmental stress, including UV and gamma radiation, ceramide, inflammatory cytokine and, some instance, by growth factors. The result suggests that the compound **2c** could induce cell cycle arrest or apoptosis by activating SAPK/JNK pathway, which resulted in the inhibition of the SMC proliferation. On the base of the result, we propose that the antiproliferative benzimidazole-4,7-dione series, as derivatives of the compound **2c**, could activate SAPK/JNK pathway in the SMCs. Further pharmacological investigations of these compounds and the structural optimization are in progress.

Acknowledgements

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15. SMC proliferation assay: The RAoSMCs were isolated from rat thoracic aorta by method described previously.[16] The cells were maintained in Dulbeccos's modified eagle medium (DMEM, Gibco BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum, 100 units/mL, and 100 μg/mL streptomycin. The SMCs were seeded in triplicate at a concentration of 1×10^3 cells/well in 200 μL of DMEM containing 10% (v/v) fetal bovine serum in 96-well flat-bottom plates (Costar, Corning, NY, USA). After 24 h incubation, the complete medium was replaced with DMEM containing 0.2% FBS, and incubated for an additional 72 h. And then the cells were treated with test compounds in 100 μL of DMEM containing PDGF (5 ng/mL) and 5% (v/v) fetal bovine serum for 48 h. Proliferation of the cells 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 of tetrazolium salt WST-1, which results in formation of a dark red formazan product, by various mitochondrial dehydrogenase of viable cells.
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18. Immunoblot assay of SMC lysate: SAPK/JNK, phospho-SAPK/JNK antibodies were purchased from Cell Signaling Technology Inc. (Beverly, MA, USA). The RAoSMCs were cultured for 72 h in DMEM containing 0.2% FBS and treated with the compound **2c** (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.