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Synthesis and *in vitro* Antitumor Activity of 2-Alkyl, 2-Aryl, and 2-Piperazinyl Benzimidazole-4,7-dione Derivatives

Chan Mug Ahn[1], Jung Ae Tak[1], and Sun Ju Choi[2]

[1]Department of Basic Sciences, [2]Department of Microbiology, and [1,2]Institute of Basic Medical Science, Wonju College of Medicine, Yonsei University, Wonju 220-701, Korea

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A series of 2-alkyl, 2-aryl, and 2-piperazinyl benzimidazole-4,7-dione derivatives (**7a-h**) and **16m-o**) were prepared, and their cytotoxicities were tested against three cancer cell lines (mouse lymphocytic leukemia cell line P388, and human gastric carcinoma cell lines SNU-1 and SNU-16). These compounds showed potent cytotoxicity against all of three cell lines tested, and especially SNU-16 was sensitive to them. 2-Aryl (**7g,h**) and 2-piperazinyl benzimidazole-4,7-dione derivative (**16 m**) were more potent than mitomycin C against P388 and SNU-16. Among benzimidazole-4,7-dione derivatives with alkyl group at position 2, **7a** had the most potent cytotoxicity against all of the cell lines tested.

Key words: Benzimidazole-4,7-dione, Cytotoxicity, Mouse lymphocytic cells, Human gastric carcinoma cells

INTRODUCTION

Pyrrolobenzimidazoles (azamitosenes) are analogues of mitomycin C which acts as reductive cross-linker of DNA. (Skibo and Schulz, 1993; Schulz *et al.*, 1993; Boruah and Skibo, 1995; Skibo *et al.*, 1997; Moore and Czerniak, 1981; Zhou and Skibo, 1996) These agents has been reported to have potent antitumor activities against a variety of cancer cell lines, being especially more active than mitomycin C against ovarian cancer cell lines.

Recently, we reported that these azamitosene derivatives showed strong cytotoxicities against gastric cancer cell line (SNU-16). (Ahn and Kim, 1996; Kim *et al.*, 1997) Aziridinyl pyrrolo[1,2-a]benzimidazole derivatives (AAP) which should be expected to act as a cross-linker were prepared. However, structure-activity relationship studies revealed that they bound but did not cross-link to DNA. In the cytotoxic reaction, aziridinyl group was alkylated and cleaved to bind DNA. The presence of acetoxy group at position 2 of the pyrrolo[1,2-a]benzimidazole did not result in the increase of *in vitro* antitumor activity, suggesting that it was not used as a binding site with DNA.

In the following studies, 2-acetoxymethylbenzimidazole derivatives (AAB) which were devoid of pyrrolo ring from the azamitosene were found to be equal to or slightly more cytotoxic than aziridinyl azamitosene (AAP) against mouse and human cancer cells, showing especially strong activity against human gastric tumor cells. Pyrrole ring in the structure of azamitosenes did not appear to be essential for their cytotoxicity. Also, increase in the lipophilicity of these benzimidazole derivatives at position 1 did not affect cytotoxicities of them against tumor cell lines. (Ahn *et al.*, 1998)

In search for new antitumor agents, we synthesized benzimidazole-4,7-dione derivatives with a variety of alkyl, aryl, and piperazinyl group at position 2, and examined the effect of these substituents on cancer cell lines.

Correspondence to: Chan Mug Ahn, Ph.D., Department of Basic Sciences, Wonju College of Medicine, Yonsei University, Wonju 220-701, Korea
E-mail: ahn0707@wonju.yonsei.ac.kr

MATERIALS AND METHODS

Cancer cell lines

Cancer cell lines tested for cytotoxicity were P388, SNU-1, and SNU-16 (Korean Cell Line Bank, KCLB). Each cell line was maintained in RPMI 1640 medium supplemented with 10% fetal calf serum and incubated in a humidified 5% CO_2 at 37°C.

Determination of cytotoxicity

For determination of cytotoxicity by the synthesized compounds, MTT methods was used (Carmichel *et al.*, 1987). Tumor cells were maintained in plastic plates in RPMI1640 medium supplemented with 10% fetal calf

serum and antibiotics. For the drugs treatment, tumor cells (210 × 3) were incubated at 37°C for 24 h in a 96 well imcroplate containing 200 μl/well of growth medium in a humidified atmosphere of 5% CO_2. Compounds were dissloved in dimethyl sulfoxide (2 mg/ml), diluted with medium (20 μg/ml) and introduced to the cell culture by gradual dilution. The cells were exposed to compounds for 3 days at 37°C in air containing 5% CO_2. MTT was added to each well. The plates were incubates for an additional 4 h, and the medium was removed. The formazan product was dissolved in DMSO and the absorbance was measured at 540 nm using a Microplate RReader. The 50% growth inhibitory concentration (IC_{50}) was calculated by regression utilizing Origin 5.0 (Microsoft).

Synthesis

Melting points were determined on a Electrothermal 9200 and are uncorrected. IR spectra were obtained on a Shimadzu IR-435 spectrometer. ^{1}H NMR spectra were recorded on a Bruker AM-300 (250 MHz) and/ or Varian Gemini 200 (200 MHz) NMR spectrometer. The chemical shifts were reported in parts per million (ppm) downfield from tetramethylsilane (TMS) as a internal standard and *J*-values were in Hz. When necessary, chemicals were purified according to the reported procedure (Perrin *et al.*, 1980).

4-(*N*-Methylacetamido)-3-nitrotoluene (1a)

To a solution of 4-methylamino-3-nitrotoluene (1.00 g, 6.02 mmol) of dry dichloromethane (65 ml) containing pyridine (0.54 ml, 6.62 mmol) was added acetyl chloride (3.13 ml, 7.29 eq). After reaction mixture was refluxed for 8 h, the solvent was evaporated *in vacuo* and the residue was dissolved in 300 ml. The chloroform solution was washed with 1 M HCl, washed with 10% $NaHCO_3$, washed with water, dried over sodium sulfate, and concentrated under reduced pressure to afford the crude product. Chromatography of the crude product with ethyl acetate/ methanol (95:5) on a silica gel column gave **1a** (1.04 g, 83%) as a yellow solid. mp 65°C; TLC (chloroform/methanol =90:10) *Rf*=0.58; IR (KBr pellet); 3037, 2998, 1660, 1529, 1384, 1356, 1310, 1067, 975 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.81 (1H, s, C(2)H), 7.51 and 7.26 (2H, 2d, *J* =7.9Hz, C(5)H and C(6)H), 3.20 (3H, s, NCH_3), 2.50 (3H, s, C(1)-methyl), 1.82 (3H, s, acetamido methyl).

4-(*N*-Methylpropionamido)-3-nitrotoluene (1b)

The same procedure described above was employed for the preparation of **1b** by using 4-methylamino-3-nitrotoluene (2.00 g, 12.04 mmol) and propionyl chloride 7.3 ml as starting material. Chromatography of the crude product on silica gel column with chloroform/methanol (98/2) as a eluent gave **1b** (2.50 g, 94%) as a viscous orange liquid; TLC (chloroform/methanol=98/2) *Rf*=0.37; IR (NaCl) 3042, 2980, 2939, 2880, 1671, 1320, 1284, 1150, 1130, 1074, cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.80 (1H, s, C(2)H), 7.48 and 7.24 (2H, 2d, *J*=8.1Hz, C(5)H and C (6)H), 3.20 (3H, s, NCH_3), 2.49 (3H, s, C(1)-methyl), 1.98 (2H, q, *J*=7.49Hz, -NCO**CH$_2$**CH$_3$), 1.04 (3H, t, *J*=7.29Hz, -NCOCH$_2$**CH$_3$**).

4-(*N*-Methyl-*n*-butyramido)-3-nitrotoluene (1c)

The same procedure described above was employed for the preparation of **1c** by using 4-methylamino-3-nitrotoluene (2.00 g, 12.04 mmol) and butyryl chloride 9.1 ml as a starting material. Chromatography of the crude product on silica gel column with chloroform/methanol (98/2) as a eluent gave **1c** (2.70 g, 95%) as a viscous orange liquid; TLC (chloroform/methanol=98/2) *Rf*=0.36; IR (NaCl) 3040, 2965, 2875, 1668, 1533, 1383, 1348, 1132, 1079 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.81 (1H, s, C(2)H), 7.48 and 7.24 (2H, 2d, *J*=8.10Hz, C(5)H and C(6)H), 3.20 (3H, s, NCH_3), 2.50 (3H, s, C(1)-methyl), 1.94 (2H, t, *J*=7.69Hz, -NCO**CH$_2$**CH$_2$CH$_3$), 1.64 (2H, sextet, *J*=6.99 Hz, -NCOCH$_2$ **CH$_2$**CH$_3$), 0.81 (3H, t, *J*=7.15Hz, -NCOCH$_2$CH$_2$**CH$_3$**).

4-(*N*-Methylisobutyramido)-3-nitrotoluene (1d)

The same procedure described above was employed for the preparation of **1d** by using 4-methylamino-3-nitrotoluene (3.50 g, 21.07 mmol) and isobutyryl chloride 15.45 ml as a starting material. Chromatography of the crude product on silica gel column with chloroform/ methanol (98/2) as a eluent gave **1d** (4.70 g, 94%) as a viscous yellow liquid; TLC (chloroform/methanol=98/2) *Rf*=0.49; IR (NaCl) 3019, 2975, 2935, 1668, 1534, 1503, 1387, 1354, 1270, 1084 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.80 (1H, s, C(2)H), 7.48 and 7.24 (2H, 2d, J_{ortho}=7.86 Hz, C(5)H and C(6)H), 3.18 (3H, s, NCH_3), 2.48 (3H, s, C (1)-methyl), 2.26 (1H, septet, *J*=6.69Hz, -NCO**CH**$(CH_3)_2$), 1.01 (6H, d, *J*=9.89Hz, -NCOCH(**CH$_3$**)$_2$).

4-(*N*-Methyl-*n*-valeramido)-3-nitrotoluene (1e)

The same procedure described above was employed for the preparation of **1e** by using 4-methylamino-3-nitrotoluene (2.00 g, 12.04 mmol) and valeryl chloride 9.00 ml as a starting material. Chromatography of the crude product on silica gel column with ethyl acetate/n-hexane (90/10) as a eluent gave **1e** (2.80 g, 93%) as a viscous orange liquid; TLC (ethyl acetate/n-hexane=90/10) *Rf*= 0.57; IR (NaCl) 3044, 2959, 2932, 2871, 1709, 1670, 1533, 1352, 1201, 1152, 1081 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.80 (1H, s, C(2)H), 7.49 and 7.23 (2H, 2d, *J*=9.55Hz, C(5)H and C(6)H), 3.19 (3H, s, NCH_3), 2.49 (3H, s, C(1)-methyl), 1.95 (2H, t, *J*=7.13Hz, NHCO**CH$_2$**CH$_2$CH$_2$CH$_3$), 1.53 (2H, qintet, *J*=7.55Hz, NHCOCH$_2$**CH$_2$**CH$_2$CH$_3$), 1.19 (2H, sextet, *J*=7.18Hz, NHCOCH$_2$CH$_2$**CH$_2$**CH$_3$), 0.80 (3H, t, *J*=7.25Hz, NHCOCH$_2$CH$_2$CH$_2$**CH$_3$**).

4-(*N*-Methylcyclohexanecarboxamido)-3-nitrotoluene(1f)

The same procedure described above was employed for the preparation of **1f** by using 4-methylamino-3-nitrotoluene (2.00 g, 12.04 mmol) and cyclohexanecarbonyl chloride 12.08 ml as a starting material. Chromatography of the crude product on silica gel column with ethyl acetate/n-hexane (50/50) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **1f** (2.90 g, 87%) as a yellow solid. mp 76°C; TLC (ethyl acetate/n-hexane=50/50) *Rf*=0.44; IR (KBr pellet) 3037, 2944, 2924, 1661, 1537, 1448, 1427, 1385, 1362, 1260, 1084 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.81 (1H, s, C(2)H), 7.47 and 7.23 (2H, 2d, *J*=9.58Hz, C(5)H and C(6)H), 3.18 (3H, s, NCH_3), 2.50 (3H, s, C(1)-methyl), 1.59, 1.17, and 0.94 (10H, 2m, cyclohexyl).

4-(*N*-Methylbenzamido)-3-nitrotoluene (1g)

The same procedure described above was employed for the preparation of **1 g** by using 4-methylamino-3-nitrotoluene (3.10 g, 18.66 mmol) and benzoyl chloride 15.0 ml as a starting material. Chromatography of the crude product on silica gel column with ethyl acetate/n-hexane (80/20) as a eluent gave **1 g** (4.80 g, 95%) as a viscous brown liquid; TLC (ethyl acetate/n-hexane=80/20) *Rf*=0.6; IR (NaCl) 3049, 2951, 1657, 1532, 1499, 1357, 1311, 1119, 1075 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.77 and 7.53 (5H, 2m, phenyl protons), 7.61 (1H, s, C(2)H), 7.29 and 7.16 (2H, 2d, *J*=7.85Hz, C(5)H and C(6)H), 3.85 (3H, s, NCH_3), 2.51 (3H, s, C(1)-methyl).

3-Amino-4-(methylacetamido)toluene (2a)

A suspension of **1a** (1.00 g, 4.81 mmol) in methanol (200 ml) containing 10% Pd charcoal (50 mg) was shaken under hydrogen atmosphere for 4 h. The catalyst was removed by filtration of the reaction mixture through Celite. The filtrate was evaporated *in vacuo* to give a residue, which was chromatographed from ethyl acetate/methanol (95/5) to afford a white solid, **2a** (0.65 g, 76%). mp 140°C; TLC (chloroform/methanol=90/10) *Rf*=0.49; IR (KBr pellet) 3429, 3348, 3036, 2916, 1629, 1426, 1385, 1299, 1140 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.27 (1H, s, C(2)H), 6.89 and 6.59 (2H, 2d, J_{ortho}=7.74Hz, C(5)H and C(6)H), 3.74 (2H, br s, amine protons), 3.17 (3H, s, NCH_3), 2.29 (3H, s, C(1)-methyl), 1.87 (3H, s, acetamido methyl).

3-Amino-4-(methylpropionamido)toluene (2b)

The same procedure described above was employed for the preparation of **2b** by using **1b** (2.00 g, 9.0 mmol) as a starting material. Chromatography of the crude product on silica gel column with ethyl acetate/n-hexane (80/20) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **2b** (1.23 g, 71%) as a pale yellow solid. mp 115°C; TLC (ethyl acetate/*n*-hexane=80/20) *Rf* =0.44; IR (KBr pellet) 3435, 3349, 3145, 2980, 1634, 1512, 1461, 1385,1296, 1074 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.27 (1H, s, C(2)H), 6.87 and 6.58 (2H, 2d, J_{ortho}=7.75Hz, C(5)H and C(6)H), 3.72 (2H, br s, amine protons), 3.17 (3H, s, NCH_3), 2.28 (3H, s, C(1)-methyl), 2.08 (2H, q, *J*=7.70 Hz, -NCO**CH_2**CH_3), 1.04 (3H, t, *J*=7.28Hz, -NCOCH$_2$**CH_3**).

3-Amino-4-(methylbutyramido)toluene (2c)

The same procedure described above was employed for the preparation of **2c** by using **1c** (1.70 g, 7.20 mmol) as a starting material. Chromatography of the crude product on silica gel column with ethyl acetate/n-hexane (80/20) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **2c** (1.20 g, 81%) as a pale yellow solid. mp 98°C; TLC (ethyl acetate/n-hexane=80/20) *Rf*=0.38; IR (KBr pellet) 3442, 3352, 3023, 2963, 1633, 1512, 1452, 1390, 1264, 1127 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.86 and 6.59 (2H, 2d, *J*=7.83Hz, C(5)H and C(6)H), 6.55 (1H, s, C(2)H), 3.70 (2H, br s, amine protons), 3.16 (3H, s, NCH_3), 2.28 (3H, s, C(1)-methyl), 2.01 (2H, t, *J*=7.43 Hz, -NCO**CH_2**CH_2CH_3), 1.58 (2H, sextet, *J*=7.40Hz, -NCOCH$_2$**CH_2**CH_3), 0.83 (3H, t, *J*=7.35Hz, -NCOCH$_2$CH$_2$**CH_3**).

3-Amino-4-(methylisobutyramido)toluene (2d)

The same procedure described above was employed for the preparation of **2d** by using **1d** (4.00 g, 16.93 mmol) as an starting material. Chromatography of the crude product on silica gel column with ethyl acetate/n-hexane (50/50) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **2d** (3.31 g, 95%) as an orange solid. mp 149°C; TLC (ethyl acetate/*n*-hexane=50/50) *Rf*=0.33; IR (KBr pellet) 3437, 3352, 3060, 2967, 1629, 1511, 1471, 1427, 1391, 1275, 1114 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.88 and 6.57 (2H, 2d, *J*=7.80 Hz, C(5)H and C(6)H), 6.61 (1H, s, C(2)H), 3.69 (2H, br s, amine protons), 3.16 (3H, s, NCH_3), 2.29 (3H, s, C(1)-methyl), 2.50 (1H, septet, *J*= 6.68Hz, -NCO**CH**$(CH_3)_2$), 1.06 (6H, d, *J*=6.73Hz, -NCOCH(**CH_3**)$_2$).

3-Amino-4-(methylvaleramido)toluene (2e)

The same procedure described above was employed for the preparation of **2e** by using **1e** (3.00 g, 12.0 mmol) as a starting material. Chromatography of the crude product on silica gel column with ethyl acetate/n-hexane (90/10) as a eluent gave **2e** (2.43 g, 92%) as a viscous brown liquid; TLC (ethyl acetate/n-hexane=90/10) *Rf*= 0.44; IR (NaCl) 3372, 3027, 2957, 2931, 1511, 1492, 1455, 1271, 1185, 1132, 1086 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.51 (1H, s, C(2)H), 7.17 and 7.07 (2H, 2d, *J*=8.18Hz, C(5)H and C(6)H), 3.91 (2H, br s, amine protons), 3.71 (3H, s, NCH_3), 2.46 (3H, s, C(1)-methyl), 2.32 (2H, t, *J*=9.32 Hz, NHCO**CH_2** $CH_2CH_2CH_3$), 1.83 (2H, qintet, *J*=7.78Hz, NHCOCH$_2$**CH_2** CH_2CH_3), 1.46 (2H, sextet, *J*=7.36Hz, NHCOCH$_2$CH$_2$**CH_2** CH_3), 0.97 (3H, t, *J*=7.33Hz, NHCOCH$_2$CH$_2$CH$_2$**CH_3**).

3-Amino-4-(*N*-methylcyclohexanecarboxamido)toluene (2f)

The same procedure described above was employed

for the preparation of **2f** by using **1f** (2.90 g, 10.5 mmol) as a starting material. Chromatography of the crude product on silica gel column with ethyl acetate/n-hexane (50/50) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **2f** (2.06 g, 80%) as a white solid. mp 130°C; TLC (ethyl acetate/*n*-hexane=50/50) *Rf*=0.43; IR (KBr pellet) 3447, 3350, 3055, 2927, 2854, 1626, 1581, 1511, 1447, 1391, 1113 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.85 and 6.56 (2H, 2d, *J*=9.58Hz, C(5)H and C(6)H), 6.60 (1H, s, C(2)H), 3.67 (2H, br s, amine protons), 3.14 (3H, s, NCH_3), 2.29 (3H, s, C(1)-methyl), 2.18 (1H, quintet, *J*=3.43Hz, methine proton of cyclohexyl), 1.59, 1.52, and 1.07 (10H, 2m, other protons of cyclohexyl).

3-Amino-4-(methylbenzamido)toluene (2g)

The same procedure described above was employed for the preparation of **2g** by using **1g** (4.50 g, 16.7 mmol) as a starting material. Chromatography of the crude product on silica gel column with chloroform/methanol (94/6) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **2g** (3.77 g, 94%) as a white solid. mp 159°C; TLC(chloroform/methanol=90/10) *Rf*=0.54; IR (KBr pellet) 3378, 3332, 3043, 2930, 1614, 1561, 1380, 1289, 1068 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.38 and 7.23 (5H, 2m, 2-phenyl protons), 6.63 and 6.35 (2H, 2d, J_{ortho}=7.92Hz, C(5)H and C(6)H), 6.51 (1H, s, C(2)H), 3.85 (2H, br s, amine protons), 3.32 (3H, s, NCH_3), 2.18 (3H, s, C(1)-methyl).

1,2,5-Trimethylbenzimidazole (3a)

To a solution of **2a** (1.50 g, 8.42 mmol) in acetic acid (10 ml) was added 2 drops of concentrated sulfuric acid, and the reaction mixture was stirred at 110°C for 4 h. The reaction mixture was then cooled to room temperature and the pH of the resulting solution was adjusted to 7 with aqueous $NaHCO_3$. The neutralized mixture was extracted three times with chloroform (50 ml). The combined extracts was dried over Na_2SO_4, and then concentrated under reduced pressure to give a residue, which was chromatographed from ethyl acetate/methanol (90/10) to afford a white solid, **3a** (1.30 g, 96%); mp 140°C; TLC (ethyl acetate/methanol=90/10) *Rf*=0.31; IR (KBr pellet) 3039, 2934, 1512, 1455, 1397, 1159, 1037 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.46 (1H, s, C(4)H), 7.16 and 7.06 (2H, 2d, J_{ortho}=8.19Hz, C(6)H and C(7)H), 3.69 (3H, s, NCH_3), 2.58 (3H, s, C(5)-methyl), 2.47 (3H, s, 2-methyl).

2-Ethyl-1,5-dimethylbenzimidazole (3b)

The same procedure described above was employed for the preparation of **3b** by using **2b** (0.92 g, 4.79 mmol) as a starting material. Chromatography of the crude product on silica gel column with ethyl aceate/methanol (95/5) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **3b** (0.63 g, 76%) as a white solid. mp 54°C; TLC (ethyl acetate/methanol=95/5) *Rf*=0.45; IR (KBr pellet) 3033, 2972, 2933, 1632, 1508, 1428, 1241, 1148, 1068 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.50 (1H, s, C(4)H), 7.16 and 7.06 (2H, 2d, J_{ortho}=8.20 Hz, C(6)H and C(7)H), 3.70 (3H, s, NCH_3), 2.89 (2H, q, *J*=7.58Hz, $\mathbf{CH_2}CH_3$ at position 2), 2.46 (3H, s, C(5)-methyl), 1.43 (3H, t, *J*=7.53Hz, $CH_2\mathbf{CH_3}$ at position 2).

2-Propyl-1,5-dimethylbenzimidazole (3c)

The same procedure described above was employed for the preparation of **3c** by using **2c** (0.43 g, 2.08 mmol) as a starting material. Chromatography of the crude product on silica gel column with ethyl aceatate/n-hexane (80/20) as a eluent gave **3c** (0.35 g, 89%) as a pale brown solid. mp 84°C; TLC (ethyl acetate/n-hexane =80/20) *Rf*=0.25; IR (KBr pellet) 3038, 2952, 2868, 1508, 1460, 1321, 1275, 1151 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.50 (1H, s, C(4)H), 7.16 and 7.06 (2H, 2d, J_{ortho}=8.15 Hz, C(6)H and C(7)H), 3.70 (3H, s, NCH_3), 2.84 (2H, t, *J*=7.43Hz, $\mathbf{CH_2}CH_2CH_3$ at position 2), 2.47 (3H, s, C(5)-methyl), 1.88 (2H, sextet, *J*=7.53Hz, $CH_2\mathbf{CH_2}CH_3$ at position 2), 1.05 (3H, t, *J*=7.33Hz, $CH_2CH_2\mathbf{CH_3}$ at position 2).

2-Isopropyl-1,5-dimethylbenzimidazole (3d)

The same procedure described above was employed for the preparation of **3d** by using **2d** (2.83 g, 13.7 mmol) as a starting material. Chromatography of the crude product on silica gel column with ethyl acetate/n-hexane (80/20) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **3d** (2.27 g, 88%) as a white solid. mp 84°C; TLC (ethyl acetate/n-hexane=80/20) *Rf*=0.55; IR (KBr pellet) 3088, 2965, 2930, 1509, 1462, 1403, 1307, 1238, 1135, 1089 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.53 (1H, s, C(4)H), 7.17 and 7.05 (2H, 2d, J_{ortho}=8.18Hz, C(6)H and C(7)H), 3.72 (3H, s, NCH_3), 3.19 (1H, septet, *J*=6.75Hz, $\mathbf{CH}(CH_3)_2$ at position 2), 2.46 (3H, s, C(5)-methyl), 1.43 (6H, d, *J*=6.88Hz, $CH(\mathbf{CH_3})_2$ at position 2).

2-Butyl-1,5-dimethylbenzimidazole (3e)

The same procedure described above was employed for the preparation of **3e** by using **2e** (2.00 g, 9.08 mmol) as a starting material. Chromatography of the crude product on silica gel column with ethyl acetate/methanol (95/5) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **3e** (1.50 g, 82%) as a brown solid. mp 50°C; TLC (ethyl acetate/methanol=95/5) *Rf*=0.4; IR (KBr pellet) 3021, 2953, 2866, 1509, 1443, 1314, 1253, 1094 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.50 (1H, s, C(4)H), 7.16 and 7.06 (2H, 2d, J_{ortho}=8.15Hz, C(6)H and C(6)H), 3.70 (3H, s, NCH_3), 2.87 (2H, t, *J*=7.48Hz, $\mathbf{CH_2}CH_2CH_2CH_3$ at position 2), 2.88 (3H, s, C(5)-methyl), 1.83 (2H, qintet, *J*=7.33Hz, $CH_2\mathbf{CH_2}CH_2CH_3$ at position 2), 1.46 (2H, sextet, *J*=7.38Hz, $CH_2CH_2\mathbf{CH_2}CH_3$ at position 2), 0.97 (3H, t, *J*=6.40Hz, $CH_2CH_2CH_2\mathbf{CH_3}$ at position 2).

2-Cyclohexyl-1,5-dimethylbenzimidazole (3f)

The same procedure described above was employed for the preparation of **3f** by using **2f** (1.80 g, 7.34 mmol) as a starting material. Chromatography of the crude product on silica gel column with ethyl acetate/n-hexane (80/20) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **3f** (1.65 g, 99%) as a white solid. mp 139°C; TLC (ethyl acetate/n-hexane=80/20) *Rf*=0.73; IR (KBr pellet) 3021, 1931, 1499, 1447, 1409, 1326, 1152, 1152 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.52 (1H, s, C(4)H), 7.17 and 7.05 (2H, 2d, J_{ortho}=8.15Hz, C(6)H and C(7)H), 3.71 (3H, s, NCH_3), 2.46 (3H, s, C(5)-methyl), 2.82 (1H, quintet, *J*=3,33Hz, methine proton of cyclohexyl), 1.95, 1.79, and 1.36 (10H, 3m, other protons of cyclohexyl).

2-Phenyl-1,5-dimethylbenzimidazole (3g)

The same procedure described above was employed for the preparation of **3g** by using **2g** (1.90 g, 7.91 mmol) as a starting material. Chromatography of the crude product on silica gel column with chloroform/methanol (90/10) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **3g** (1.55 g, 93%) as a white solid. mp 130°C; TLC (chloroform/methanol=90/10) *Rf*=0.69; IR (KBr pellet) 3042, 2917, 1558, 1490, 1469, 1327, 1282, 1142 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.76 and 7.51 (5H, 2m, 2-phenyl), 7.61 (1H, s, C(4)H), 7.28 and 7.15 (2H, 2d, J_{ortho}=8.01Hz, C(6)H and C(7)H), 3.84 (3H, s, NCH_3), 2.51 (3H, s, C(5)-methyl).

6-Bromo-1,2,5-trimethylbenzimidazole (4a)

To a solution of **3a** (1.50 g, 9.36 mmol) in glacial acetic acid (30 ml), heated at 100°C, was added bromine (0.53 ml, 10.3 mmol) in glacial acetic acid (4 ml). After the addition, the reaction mixture was heated at 110°C for 4 h. The cooled reaction mixture was neutralized to pH 6.5 with aqueous sodium bicarbonate and extracted with chloroform (100 ml × 3). The combined extracts were washed with water, dried (sodium sulfate), and concentrated *in vacuo* to give a residue. The residue was purified by column chromatography on silica gel with chloroform/methanol (90/10). Recrystallization of from n-hexane-chloroform gave **4a** (1.65 g, 74%) as a white solid. mp 137°C; TLC (chloroform/methanol=90/10) *Rf*=0.49; IR (KBr pellet) 3043, 2976, 1518, 1443, 1388, 1306, 1247, 1038 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.53 and 7.48 (2H, 2s, C(4)H and C(7)H), 3.68 (3H, s, NCH_3), 2.58 (3H, s, 2-methyl), 2.49 (3H, s, C(5)-methyl).

6-Bromo-2-ethyl-1,5-dimethylbenzimidazole (4b)

The same procedure described above was employed for the preparation of **4b** by using **3b** (0.60 g, 3.44 mmol) as a starting material. Chromatography of the crude product on silica gel column with ethyl acetate/n-hexane (90/10) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **4b** (0.75 g, 86%) as a white solid. mp 98°C; TLC (ethyl acetate/methanol=95/5) *Rf*=0.62; IR (KBr pellet) 3056, 2975, 1510, 1484, 1443, 1303, 1247, 1064 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.55 and 7.47 (2H, 2s, C(4)H and C(7)H), 3.66 (3H, s, NCH_3), 2.86 (2H, q, *J*=7.53Hz, $\mathbf{CH_2}CH_3$ at position 2), 2.48 (3H, s, C(5)-methyl), 1.43 (3H, t, *J*=7.50Hz, $CH_2\mathbf{CH_3}$ at position 2).

6-Bromo-1,5-dimethyl-2-propylbenzimidazole (4c)

The same procedure described above was employed for the preparation of **4c** by using **3c** (1.05 g, 5.58 mmol) as a starting material. Chromatography of the crude product on silica gel column with ethyl acetate/methanol (95/5) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **4c** (1.00 g, 67%) as a pale yellow solid. mp 82°C; TLC (ethyl acetate/methanol =95/5) *Rf*=0.47; IR (KBr pellet) 3074, 2962, 1508, 1444, 1313, 1281, 1246, 1151 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.60 and 7.51 (2H, 2s, C(4)H) and C(7)H), 3.71 (3H, s, NCH_3), 2.89 (2H, t, *J*=7.49Hz, $\mathbf{CH_2}CH_2CH_3$ at position 2), 2.49 (3H, s, C(5)-methyl), 1.90 (2H, sextet, *J*=7.42Hz, $CH_2\mathbf{CH_2}CH_3$ at position 2), 1.06 (3H, t, *J*=7.35Hz, $CH_2CH_2\mathbf{CH_3}$ at position 2).

6-Bromo-2-isopropyl-1,5-dimethylbenzimidazole (4d)

The same procedure described above was employed for the preparation of **4d** by using **3d** (1.92 g, 10.2 mmol) as a starting material. Chromatography of the crude product on silica gel column with ethyl acetate/*n*-hexane (50/50) as a eluent gave a solid, recrystallization of which from *n*-hexane-chloroform gave **4d** (1.80 g, 66%) as a white solid. mp 115°C; TLC (ethyl acetate/*n*-hexane=50/50) *Rf*=0.42; IR (KBr pellet) 3021, 2964, 1512, 1479, 1410, 1294, 1243, 1088 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.58 and 7.48 (2H, 2s, C(4)H and C(7)H), 3.61 (3H, s, NCH_3), 3.19 (1H, septet, *J*=6.85Hz, $\mathbf{CH}(CH_3)_2$ at position 2), 2.48 (3H, s, C(5)-methyl), 1.43 (6H, d, *J*=6.85Hz, $CH(\mathbf{CH_3})_2$ at position 2).

6-Bromo-2-butyl-1,5-dimethylbenzimidazole (4e)

The same procedure described above was employed for the preparation of **4e** by using **3e** (1.00 g, 4.94 mmol) as a starting material. Chromatography of the crude product on silica gel column with ethyl acetate/n-hexane (80/20) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **4e** (1.00 g, 72%) as a pale yellow solid. mp 79°C; TLC (ethyl acetate/n-hexane =80/20) *Rf*=0.45; IR (KBr pellet) 3022, 2959, 2929, 1508, 1461, 1300, 1234, 1150 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.93 and 7.73 (2H, 2s, C(4)H and C(7)H), 3.90 (3H, s, NCH_3), 3.28 (2H, t, *J*=7.67Hz, $\mathbf{CH_2}CH_2CH_2CH_3$ at position 2), 2.53 (3H, s, C(5)-methyl), 1.96 (2H, qintet, *J*=8.11Hz, $CH_2\mathbf{CH_2}CH_2CH_3$ at position 2), 1.51 (2H, sextet, *J*=7.43 Hz, $CH_2CH_2\mathbf{CH_2}CH_3$ at position 2), 1.00 (3H, t, *J*=7.32

Hz, $CH_2CH_2CH_2\mathbf{CH_3}$ at position 2).

6-Bromo-2-cyclohexyl-1,5-dimethylbenzimidazole (4f)
The same procedure described above was employed for the preparation of **4f** by using **3f** (1.56 g, 6.86 mmol) as a starting material. Chromatography of the crude product on silica gel column with ethyl acetate/n-hexane (50/50) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **4f** (1.10 g, 52%) as a pale yellow solid. mp 136°C; TLC (ethyl acetate/n-hexane =50/50) *Rf*=0.62; IR (KBr pellet) 3053, 2926, 2849, 1502, 1478, 1451, 1405, 1288, 1224, 1013 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.58 and 7.48 (2H, 2s, C(4)H and C(7)H), 3.69 (3H, s, NCH_3), 2.80 (1H, quintet *J*=3.43, methine proton of cyclohexyl), 2.48 (3H, s, C(5)-methyl), 1.95, 1.79, and 1.44 (10H, 3m, other protons of cyclohexyl).

6-Bromo-1,5-dimethyl-2-phenylbenzimidazole (4g)
The same procedure described above was employed for the preparation of **4g** by using **3g** (1.44 g, 6.48 mmol) as a starting material. Chromatography of the crude product on silica gel column with methylene chloride/methanol (99/1) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **4g** (1.2 g, 61 %) as a white solid. 161°C; TLC (ethyl acetate/n-hexane =50/50) *Rf*=0.53; IR (KBr pellet) 3061, 2980, 1473, 1374, 1306, 1074 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.74 and 7.50 (5H, 2m, 2-phenyl protons), 7.66 and 7.60 (2H, 2s, C(4)H) and C(7)H), 3.83 (3H, s, NCH_3), 2.53 (3H, s, C(5)-methyl).

6-Bromo-1,2,5-trimethyl-7-nitrobenzimidazole (5a)
To a mixture (20 ml) of fuming nitric acid and sulfuric acid (9:1), cooled in a ice-salt bath, was added **4a** (1.50 g, 6.28 mmol) portionwise. After 12 min, the reaction mixture was poured over cracked ice (300 g), and the pH of the resulting solution was adjusted to 6.5 with saturated aqueous sodium bicarbonate. The reaction mixture was extracted with chloroform (200 ml × 3). The combined extracts were washed with water (100 ml), dried (sodium sulfate), and concentrated *in vacuo* to give a solid, recrystallization of which from chloroform-n-hexane gave a nitro compound, **5a** (0.87 g, 49%), as a pale yellow solid; mp 181°C; TLC (ethyl acetate/methanol=98/2) *Rf*=0.33; IR (KBr pellet) 3042, 2963, 1656, 1526, 1477, 1298, 1064 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.65 (1H, s, C(4)H), 3.60 (3H, s, NCH_3), 2.61 (3H, s, 2-methyl), 2.55 (3H, s, C(5)-methyl).

6-Bromo-1,2,5-trimethyl-4-nitrobenzimidazole mp 184 °C; TLC (ethyl acetate/methanol=98/2) Rf=0.17; IR (KBr pellet) 3042, 2963, 1656, 1526, 1477, 1298, 1064 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.66 (1H, s, C(7)H), 3.73 (3H, s, NCH_3), 2.63 (3H, s, 2-methyl), 2.51 (3H, s, C(5)-methyl).

6-Bromo-2-ethyl-1,5-dimethyl-7-nitrobenzimidazole (5b)
The same procedure described above was employed for the preparation of **5b** by using **4b** (0.60 g, 2.37 mmol) as a starting material. Chromatography of the crude product on silica gel column with ethyl acetate/n-hexane (80/20) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **5b** (0.26 g, 37%) as a yellow solid. mp 164°C ; TLC (ethyl acetate/n-hexane=80/20) *Rf*=0.65; IR (KBr pellet) 3053, 2989, 1527, 1477, 1370, 1281, 1115 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.67 (1H, s, C(4)H), 3.74 (3H, s, NCH_3), 2.94 (2H, q, *J* =7.53Hz, $\mathbf{CH_2}CH_3$ at position 2), 2.51 (3H, s, C(5)-methyl), 1.41 (3H, t, *J*=7.43Hz, $CH_2\mathbf{CH_3}$ at position 2).

6-Bromo-1,5-dimethyl-7-nitro-2-propylbenzimidazole (5c)
The same procedure described above was employed for the preparation of **5c** by using **4c** (0.65 g, 2.43 mmol) as a starting material. Chromatography of the crude product on silica gel column with ethyl acetate/n-hexane (50/50) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **5c** (0.29 g, 38%) as a yellow solid. mp 157°C; TLC (ethyl acetate/n-hexane=80/20) *Rf* =0.62; IR (KBr pellet) 3053, 2966, 1521, 1471, 1368, 1317, 1246, 1050 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.67 (H, s, C(4)H), 3.74 (3H, s, NCH_3), 2.87 (2H, t, *J*=6.57Hz, $\mathbf{CH_2}CH_2CH_3$ at position 2), 2.51 (3H, s, C(5)-methyl), 1.88 (2H, sextet, *J*=7.57Hz, $CH_2\mathbf{CH_2}CH_3$ at position 2), 1.04 (3H, t, *J*=7.37Hz, $CH_2CH_2\mathbf{CH_3}$ at position 2).

6-Bromo-2-isopropyl-1,5-dimethyl-7-nitrobenzimidazole (5d)
The same procedure described above was employed for the preparation of **5d** by using **4d** (1.10 g, 4.12 mmol) as a starting material. Chromatography of the crude product on silica gel column with ethyl acetate/n-hexane (50/50) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **5d** (0.71 g, 55%) as a yellow solid. mp 133°C; TLC (ethyl acetate/n-hexane=50/50) *Rf*=0.45; IR (KBr pellet) 3074, 2973, 2933, 1528, 1476, 1371, 1294, 1254, 1092 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.66 (1H, s, C(4)H), 3.62 (3H, s, NCH_3), 3.19 (1H, septet, *J*=6.58Hz, $\mathbf{CH}(CH_3)_2$ at position 2), 2.50 (3H, s, C(5)-methyl), 1.43 (6H, d, *J*=6.88Hz, $CH(\mathbf{CH_3})_2$ at position 2).

6-Bromo-2-butyl-1,5-dimethyl-7-nitrobenzimidazole (5e)
The same procedure described above was employed for the preparation of **5e** by using **4e** (0.86 g, 3.06 mmol) as a starting material. Chromatography of the crude product on silica gel column with ethyl acetate/n-hexane (40/60) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **5e** (0.45 g, 45%) as a pale brown solid. mp 114°C; TLC (ethyl acetate/n-hexane= 50/50) *Rf*=0.47; IR (KBr pellet) 3029, 2962, 2940, 1525, 1471, 1369, 1310, 1253 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.66 (1H, s, C(4)H), 3.73 (3H, s, NCH_3), 2.89 (2H, t, *J*=7.75Hz,

$\mathbf{CH_2}CH_2CH_2CH_3$ at position 2), 2.51 (3H, s, C(5) -methyl), 1.79 (2H, qintet, J=8.13Hz, $CH_2\mathbf{CH_2}CH_2CH_3$ at position 2), 1.45 (2H, sextet, J=7.60Hz, $CH_2CH_2\mathbf{CH_2}CH_3$ at position 2), 0.96 (3H, t, J=7.35Hz, $CH_2CH_2CH_2\mathbf{CH_3}$ at position 2).

6-Bromo-2-cyclorohexyl-1,5-dimethyl-7-nitrobenzimidazole (5f)

The same procedure described above was employed for the preparation of **5f** by using **4f** (0.80 g, 2.61 mmol) as a starting material. Chromatography of the crude product on silica gel column with ethyl acetate/n-hexane (30/70) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **5f** (0.35 g, 38%) as a white solid. mp 154°C; TLC (ethyl acetate/n-hexane=30/70) *Rf*= 0.44; IR (KBr pellet) 3019, 1527, 1475, 1448, 1370, 1290, 1256, 984 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.65 (1H, s, C(4)H), 3.61 (3H, s, NCH_3), 2.80 (1H, quintet J=3.69, methine proton of cyclohexyl), 2.49 (3H, s, C(5)-methyl), 1.92, 1.77, and 1.39 (10H, 3m, other protons of cyclohexyl).

6-Bromo-1,5-dimethyl-7-nitro-2-(m-nitrophenyl)benzimidazole (5g)

The same procedure described above was employed for the preparation of **5g** by using **4g** (0.90 g, 2.99 mmol) as a starting material. Chromatography of the crude product on silica gel column with ethyl acetate/n-hexane (30/70) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **5g** (0.38 g, 33%) as a yellow solid. mp 198°C; TLC (ethyl acetate/n-hexane= 30/70) *Rf*=0.27 ; IR (KBr pellet) cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.50-7.94 (4H, 2-phenyl protons), 8.08 (1H, s, C(4)H), 3.70 (3H, s, NCH_3), 2.50 (3H, s, C(5)-methyl).

1,2,5-Trimethylbenzimidazole-4,7(1*H*)-dione (6a)

A suspension of **5a** (0.75 g, 2.64 mmol) in methanol (300 ml) containing 0.12 g of 10% Pd on charcoal was shaken under H_2 for 12 h. The reaction mixture was then filtered through Celite into a flask containing 3 ml of 1 N HCl, concentrated *in vacuo*, and recrystallized from ethyl acetate-methanol to give the dihydrochloride salt of 7-amino-2-(acetoxymethyl)-1,5-dimethylbenzimidazole, 0.60 g (92%). To a suspension of the amine dihydrochloride salt (0.55 g, 2.23 mmol) obtained above in water (25 ml) containing 0.26 mg of monobasic potassium phosphate was added a solution of 2.59 g of Fremy's salt in 125 ml of water containing 0.52 g of monobasic potassium phosphate. The reaction mixture was stirred at room temperature for 4 h, extracted with chloroform (100 ml × 3), dried (sodium sulfate), and concentrated to give a residue. Flash chromatography of the residue on a silica gel column with chloroform/methanol (90/10), recrystallization of which from chloroform-n-hexane gave **6a** (0.28 mg, 56%) as a orange solid: mp 157°C; TLC (chloroform/methanol=90/10) *Rf*=0.51; IR (KBr pellet) 3062, 2930, 1657, 1609, 1528, 1478, 1274, 1196, 1115, 944 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.41 (1H, q, J=1.50Hz, C(6)-proton coupled to C(5)-methyl), 3.89 (3H, s, NCH_3), 2.50 (3H, s, 2-methyl), 2.12 (3H, s, C(5)-methyl).

2-Ethyl-1,5-dimethylbenzimidazole-4,7(1*H*)-dione (6b)

The same procedure described above was employed for the preparation of **6b** by using **5b** (0.80 g, 2.68 mmol) as a strating material. Chromatography of the crude quinone product on silica gel column with ethyl acetate/n-hexane (90/10) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **6b** (0.28 g, 51%) as a orange solid. mp 154°C; TLC (ethyl acetate/n-hexane=90/10) *Rf*=0.35; IR (KBr pellet) 3180, 2985, 2943, 1672, 1656, 1610, 1537, 1478, 1376, 1276, 1194, 1115, 1062 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.40 (1H, q, J=1.48Hz, C(6)-proton coupled to C(5)-methyl), 3.89 (3H, s, NCH_3), 2.79 (2H, q, J=7.50Hz, $\mathbf{CH_2}CH_3$ at position 2), 2.11 (3H, s, C(5)-methyl), 1.39 (3H, t, J=7.50Hz, $CH_2\mathbf{CH_3}$ at position 2).

1,5-Dimethyl-2-propylbenzimidazole-4,7(1*H*)-dione (6c)

The same procedure described above was employed for the preparation of **6c** by using **5c** (0.45 g, 1.44 mmol) as a strating material. Chromatography of the crude quinone product on silica gel column with ethyl acetate/n-hexane (80/20) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **6c** (0.21 g, 67%) as a orange solid. mp 110°C; TLC (ethyl acetate/n-hexane=90/10) *Rf*=0.56; IR (KBr pellet) 3056, 2965, 1660, 1614, 1534, 1468, 1265, 1195, 1117, 955 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.37 (1H, q, J=1.45Hz, C(6)-proton coupled to C(5)-methyl), 3.90 (3H, s, NCH_3), 2.75 (2H, t, J=7.44Hz, $\mathbf{CH_2}CH_2CH_3$ at position 2), 2.12 (3H, s, C(5)-methyl), 1.84 (2H, sextet, J=7.50Hz, $CH_2\mathbf{CH_2}CH_3$ at position 2), 1.02 (3H, t, J=7.34Hz, $CH_2CH_2\mathbf{CH_3}$ at position 2).

1,5-Dimethyl-2-isopropylbenzimidazole-4,7(1*H*)-dione (6d)

The same procedure described above was employed for the preparation of **6d** by using **5d** (1.09 g, 3.79 mmol) as a strating material. Chromatography of the crude quinone product on silica gel column with methylene chloride/methanol (99/1) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **6d** (0.45 g, 59%) as a yellow solid. mp 156°C; TLC (methylene chloride/methanol=94/6) *Rf*=0.71; IR (KBr pellet) 3054, 2970, 1672, 1651, 1607, 1512, 1482, 1312, 1189, 1109 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.40 (1H, q, J=1.32Hz, C(6)-proton coupled to C(5)-methyl), 3.92 (3H, s, NCH_3), 3.08 (1H, septet, J=6.82Hz, $\mathbf{CH}(CH_3)_2$ at position 2), 2.11 (3H, s, 5-methyl), 1.39 (6H, d, J=6.84Hz, $CH(\mathbf{CH_3})_2$ at position 2).

2-Butyl-1,5-dimethylbenzimidazole-4,7(1*H*)-dione (6e)

The same procedure described above was employed for the preparation of an **6e** by using **5e** (0.73 g, 2.24 mmol) as a strating material. Chromatography of the crude quinone product on silica gel column with ethyl acetate/n-hexane (80/20) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **6e** (0.35 g, 67%) as a orange solid. mp 111°C; TLC (ethyl acetate/n-hexane=90/10) *Rf*=0.65; IR (KBr pellet) 3082, 2963, 2942, 1659, 1612, 1472, 1432, 1271, 1116, 957 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.40 (1H, q, *J*=1.53Hz, C(6)-proton coupled to C(5)-methyl), 3.90 (3H, s, NCH_3), 2.76 (2H, t, *J* =7.48Hz, $\mathbf{CH_2}CH_2CH_2CH_3$ at position 2), 2.12 (3H, s, C(5)-methyl), 1.78 (2H, qintet, *J*=7.63Hz, $CH_2\mathbf{CH_2}CH_2CH_3$ at position 2), 1.46 (2H, sextet, *J*=7.53Hz, $CH_2CH_2\mathbf{CH_2}CH_3$ at position 2), 0.95 (3H, t, *J*=7.35Hz, $CH_2CH_2CH_2\mathbf{CH_3}$ at position 2).

2-Cyclohexyl-1,5-dimethylbenzimidazole-4,7(1*H*)-dione (6f)

The same procedure described above was employed for the preparation of **6f** by using **5f** (0.52 g, 1.48 mmol) as a strating material. Chromatography of the crude quinone product on silica gel column with methylene chloride/methanol (99/1) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **6f** (0.20 g, 52%) as a yellow solid. mp 200°C (dec.); TLC (methylene chloride/methanol=94/6) *Rf*=0.82; IR (KBr pellet) 3061, 2929, 2853, 1691, 1675, 1650, 1472, 1366, 1190, 1126 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.40 (1H, q, *J*=1.37Hz, C(6)-proton coupled to C(5)-methyl), 3.91 (3H, s, NCH_3), 2.71 (1H, quintet *J*=3.74, methine proton of cyclohexyl), 2.11 (3H, s, C(5)-methyl), 1.89, 1.77, and 1.35 (10H, 3m, other protons of cyclohexyl).

1,5-Dimethyl-2-(*m*-aminophenyl)benzimidazole-4,7(1*H*) -dione (6g)

The same procedure described above was employed for the preparation of **6g** by using **5g** (0.60 g, 1.53 mmol) as a strating material. Chromatography of the crude quinone product on silica gel column with chloroform/methanol (90/10) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **6g** (0.21 g, 51 %) as a deep brown solid. mp 120°C (dec.); TLC (chloroform/methanol=90/10) *Rf*=0.58; IR (KBr pellet) 3023, 2924, 1656, 1630, 1609, 1492, 1309, 1192, 1115 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.52-6.80 (4H, 2-phenyl protons), 6.48 (1H, q, *J*=1.18Hz, C(6)-proton coupled to C(5)-methyl), 4.06 (3H, s, NCH_3), 2.16 (3H, s, C(5)-methyl)

6-Aziridinyl-1,2,5-trimethylbenzimidazole-4,7(1*H*)-dione (7a)

To a solution of **6a** (0.10 g, 0.53 mmol) in dry methanol (2 ml), cooled at 0°C, was added ethylenimine 0.4 ml. After being stirred at 0°C for 20 min, the reaction mixture was stirred at room temperature for 3 h. The solvent was then removed *in vacuo* to give red residue, and chromatography of the residue on a silica gel column with ethyl acetate/methanol (95/5) as a eluent gave **7a** (25.5 mg, 21 %) as a pale brown solid. mp 192 (dec.); TLC (ethyl acteate/methanol=95/5) *Rf*=0.18; IR (KBr pellet) 3063, 2990, 1661, 1592, 1541, 1332, 1249, 972 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.88 (3H, s, NCH_3), 2.48 (3H, s, 2-methyl), 2.31 (4H, s, aziridine protons), 2.11 (3H, s, C(5)-methyl).

6-Aziridinyl-2-ethy1-1,5-dimethylbenzimidazole-4,7 (1*H*)-dione (7b)

The same procedure as described above, using **6b** (0.10 g, 0.49 mmol), gave a residue after 8 h. Chromatography of the residue on a silica gel column with ethyl acetate/methanol (95:5) as a eluent gave a solid, recrystallization of which from chloroform-n-hexane gave **7b** (24 mg, 20 %) as a yellow-red solid. mp 182°C; TLC (ethyl acetate/methanol=95/5) *Rf*=0.33; IR (KBr pellet) 3062, 2986, 1657, 1538, 1337, 1248, 966 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.89 (3H, s, NCH_3), 2.77 (2H, q, *J*=7.58Hz, $\mathbf{CH_2}CH_3$ at position 2), 2.31 (4H, s, aziridine protons), 2.11 (3H, s, C(5)-methyl), 1.37 (3H, t, *J*=7.53Hz, $CH_2\mathbf{CH_3}$ at position 2).

6-Aziridinyl-1,5-dimethyl-3-propylbenzimidazole-4,7 (1*H*)-dione (7c)

The same procedure as described above, using **6c** (0.10 g, 0.46 mmol), gave a residue after 8 h. Chromatography of the residue on a silica gel column with ethyl acetate/methanol (95:5) as a eluent gave a solid, recrystallization of which from chloroform-n-hexane gave **7c** (21.4 mg, 18%) as a yellow-red solid. mp 158°C; TLC (ethyl acetate) *Rf*=0.27; IR (KBr pellet) 3059, 2994, 2962, 1650, 1596, 1537, 1338, 1246, 977 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.89 (3H, s, NCH_3), 2.72 (2H, t, *J*=7.50Hz, $\mathbf{CH_2}CH_2CH_3$ at position 2), 2.30 (4H, s, aziridine protons), 2.03 (3H, s, C(5)-methyl), 1.82 (2H, sextet, *J*=7.57Hz, $CH_2\mathbf{CH_2}CH_3$ at position 2), 1.01 (3H, t, *J*=7.34Hz, $CH_2CH_2\mathbf{CH_3}$ at position 2).

6-Aziridinyl-2-isopropyl-1,5-dimethylbenzimidazole-4,7(1*H*)-dione (7d)

The same procedure as described above, using **6d** (0.10 g, 0.46 mmol), gave a residue after 8 h. Chromatography of the residue on a silica gel column with ethyl acetate/methanol (95:5) as a eluent gave a solid, recrystallization of which from chloroform-n-hexane gave **7d** (25 mg, 21%) as a yellow-red solid. mp 197°C; TLC (ethyl acetate) *Rf*= 0.36; IR (KBr pellet) 3001, 2976, 2927, 1667, 1646, 1538, 1345, 1246, 1143, 1084 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.91 (3H, s, NCH_3), 3.05 (1H, septet, *J*=6.90Hz, $\mathbf{CH}(CH_3)_2$ at position 2), 2.30 (4H, s, aziridine protons), 2.10 (3H, s, C(5)-methyl), 1.38 (6H, d, *J*=6.85Hz, $CH(\mathbf{CH_3})_2$ at position 2).

6-Aziridinyl-2-butyl-1,5-dimethylbenzimidazole-4,7(1*H*)-dione (7e)

The same procedure as described above, using **6e** (0.1 g, 0.43 mmol), gave a residue after 8 h. Chromatography of the residue on a silica gel column with ethyl acetate/methanol (95:5) as a eluent gave a solid, recrystallization of which from chloroform-n-hexane gave **7e** (20 mg, 17 %) as a orange solid. mp 145°C; TLC (ethyl acetate) *Rf*= 0.35; IR (KBr pellet) 3036, 2962, 2945, 1657, 1598, 1540, 1336, 977 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.88 (3H, s, NCH_3), 2.74 (2H, t, *J*=7.57Hz, $\mathbf{CH_2}CH_2CH_2CH_3$ at position 2), 2.30 (4H, s, aziridine protons), 2.10 (3H, s, C(5)-methyl), 1.76 (2H, qintet, *J*=7.34Hz, $CH_2\mathbf{CH_2}CH_2CH_3$ at position 2), 1.41 (2H, sextet, *J*=7.64Hz, $CH_2CH_2\mathbf{CH_2}CH_3$ at position 2), 0.94 (3H, t, *J*=7.25Hz, $CH_2CH_2CH_2\mathbf{CH_3}$ at position 2).

6-Aziridinyl-2-cyclohexyl-1,5-dimethylbenzimidazole-4,7(1*H*)-dione (7f)

The same procedure as described above, using **6f** (0.10 g, 0.39 mmol), gave a residue after 8 h. Chromatography of the residue on a silica gel column with ethyl acetate/methanol (95:5) as a eluent gave a solid, recrystallization of which from chloroform-n-hexane gave **7f** (26 mg, 22%) as a red solid. mp 189°C; TLC (ethyl acetate) *Rf*= 0.51; IR (KBr pellet) 3062, 2921, 2853, 1647, 1588, 1536, 1335, 1139, 968 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.90 (3H, s, NCH_3), 2.68 (1H, quintet *J*=5.75, methine proton of cyclohexyl), 2.29 (4H, s, aziridine protons), 2.10 (3H, s, C(5)-methyl), 1.85, 1.62, and 1.33 (10H, 3m, other protons of cyclohexyl).

2-(m-Amino)phenyl-6-aziridinyl-1,5-dimethylbenzimidazole-4,7(1*H*)-dione (7g)

The same procedure as described above, using **6g** (0.10 g, 0.37 mmol), gave a residue after 8 h. Chromatography of the residue on a silica gel column with ethyl acetate/methanol (95:5) as a eluent gave a solid, recrystallization of which from chloroform-n-hexane gave **7g** (18.5 mg, 16%) as a deep red solid. mp 197°C (dec.); TLC (ethyl acetate) *Rf*=0.22; IR (KBr pellet) 3346, 3357, 3002, 2924, 1670, 1642, 1623, 1578, 1335, 1244 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.16 (2H, s, acetoxymethyl methylene), 4.24 (2H, t, *J*=7.71 Hz, N(1)-$\mathbf{CH_2}CH_2CH_2CH_3$), 2.26 (4H, s, aziridine protons), 2.05 and 2.04 (6H, 2s, C(5)-methyl and acetate methyl), 1.68 (2H, quintet, *J*=7.99 Hz, N(1)-$CH_2\mathbf{CH_2}CH_2CH_3$), 1.34 (3H, sextet, *J*=7.52 Hz, N(1)-$CH_2CH_2\mathbf{CH_2}CH_3$), 0.91 (3H, t, *J*=7.41, N(1)$CH_2CH_2CH_2\mathbf{CH_3}$).

2-(Acetamidophenyl)-6-aziridinyl-1,5-dimethylbenzimidazole-4,7(1*H*)-dione (7h)

A suspension of **6g** (0.27 g, 1 mmol) with in 3ml of acetic anhydride was stirred at room temperature for 2 h. The solvent was removed *in vacuo*, and the crude residue was recrystallized with n-hexane-chloroform to give the acetylated compound (0.23 g, 72%).

The same procedure as described above, using the acetylated compound (0.10 g, 0.32 mmol), gave a residue after 8 h. Chromatography of the residue on a silica gel column with ethyl acetate/methanol (95:5) as a eluent gave a solid, recrystallization of which from chloroform-n-hexane gave **7h** (17.0 mg, 15%) as a red-green solid. mp 130°C (dec.); TLC (ethyl acetate/methanol=95/5) *Rf*=0.25; IR (KBr pellet) 3470, 3002, 2922, 1692, 1671, 1642, 1512, 1379, 1246, 961 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.88 (1H, s, acetamide proton), 7.64-7.41 (4H, phenyl protons), 4.03 (3H, s, NCH_3), 2.35 (4H, s, aziridine protons), 2.19 (3H, s, acetamide methyl), 2.14 (3H, s, C(5)-methyl).

2-Amino-1-(methylamino)toluene (8)

A solution of 1-(methylamino)-2-nitrotoluene (1.50 g, 9.03 mmol) in 200 ml of methanol containing 0.24 mg of 10% Pd on charcoal was stirred under H_2 for 4 h. The mixture solution was filtered through Celite, and was washed with methanol. The filtrate was concentrated under reduced pressure. Chromatography of the residue on a silica gel column with chloroform/methanol (94/6) as a eluent gave a solid, recrystallization of which from chloroform-n-hexane gave **8** (1.16 g, 94%) as a blue-purple solid. mp 42°C; TLC (chloroform/methanol=94/6) *Rf*=0.53; IR (KBr pellet) 3362, 3227, 3022, 2944, 1520, 1450, 1283, 1237 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.56 (1H, s, C(4)H), 6.66 and 6.58 (2H, 2d, *J*=7.69Hz, C(6)H and C((7)H), 3.14 (2H, br s, amine protons), 2.85 (3H, s, NCH_3), 2.33 (3H, s, C(5)-methyl).

1,5-Dimethyl-2-benzimidazolone (9)

A solution of **8** (0.76 g, 5.58 mmol) and urea (0.67 g, 11.16 mmol) was stirred at 150°C for 5 h. The reaction mixture was poured into cold water (300 ml) and extracted with ethyl acetate (150 ml × 3). The extract was washed with 2 N HCl and water, dried ($NaSO_4$), and evaporated *in vacuo*. Chromatography of the residue on a silica gel column with ethyl acetate/methanol (95:5) as a eluent gave a solid, recrystallization of which from chloroform-n-hexane gave **9** (0.60 g, 66%) as a pale yellow solid. mp 196°C; TLC (ethyl acetate/methanol=95/5) *Rf*=0.49; IR (KBr pellet) 3125, 3026, 2949, 1703, 1507, 1294, 1087 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.11 (1H, br s, NH), 6.91-6.83 (3H, aromatic protons), 3.40 (3H, s, NCH_3), 2.38 (3H, s, C(5)-methyl).

2-Chloro-1,5-dimethylbenzimidazole (10)

A mixture of **9** (2 g, 12.33 mmol) and phosphorus oxychloride (3.8 ml, 4.07 mmol) was stirred at 110°C for 30 min and then allowed to cool. The reaction mixture was poured into ice-cold water (50 ml). To this mixture was added 4 N NaOH (65 ml) and the mixture was extracted with ethyl acetate. The extracts were washed with water, dried over Na_2SO_4, and evaporated *in vacuo*. Chromatography of the residue on a silica gel column with ethyl acetate/methanol (95:5) as a eluent gave a solid, recrystallization

of which from chloroform-n-hexane gave **10** (2.07 g, 93 %) as a pale yellow solid. mp 102°C; TLC (ethyl acetate/methanol=95/5) *Rf*=0.65; IR (KBr pellet) 3078, 3051, 2915, 1696, 1472, 1429, 1324, 1120 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.46-6.85 (3H, aromatic protons), 3.75 (3H, s, NCH_3), 2.46 (3H, s, C(5)-methyl).

1,5-Dimethyl-2-(4-methyl-1-piperidinyl)benzimidazole (11)

A solution of 10 (2.70 g, 15.0 mmol) and *N*-methylpiperazine (4.49 g, 44.8 mmol) was stirred at 125°C for 5 h and then allowed to cool. To the reaction mixture was added 2.5 N NaOH (25 ml), and the mixture was extracted with chloroform (50 ml × 3). The extracts were washed with water, dried over Na_2SO_4, and evaporated *in vacuo*. Chromatography of the residue on a silica gel column with chloroform/methanol (85:15) as a eluent gave a solid, recrystallization of which from chloroform-n-hexane gave **11** (2.80 g, 77%) as a white solid. mp 132°C; TLC (chloroform/methanol=85/15) *Rf*=0.51; IR (KBr pellet) 3029, 2931, 1529, 1458, 1397, 1312, 1145, 1009 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.39 (1H, s, C(4)H), 7.05 and 6.97 (2H, 2d, J_{ortho}=8.08Hz, C(6)H and C(7)H), 3.57 (3H, s, NCH_3), 3.34 and 2.60 (8H, 2t, methylene protons of piperazinyl), 2.43 (3H, s, methyl of piperazinyl), 2.36 (3H, s, C(5)-methyl).

4,6-Dibromo-1,5-dimethyl-2-(4-methyl-1-piperazinyl) benzimidazole (12)

To a solution of **11** (1.80 g, 7.37 mmol) in glacial acetic acid (30 ml), heated at 100°C, was added bromine (0.80 ml, 15.5 mmol) in glacial acetic acid (4 ml). After the addition, the reaction mixture was heated at 110°C for 4 h. The cooled reaction mixture was neutralized to pH 6.5 with aqueous sodium bicarbonate and extracted with chloroform (100 ml × 3). The combined extracts were washed with water, dried over sodium sulfate, and evaporated *in vacuo* to give a solid. Chromatography of the residue on a silica gel column with methylene chloride/methanol (90:10) as a eluent gave a solid, recrystallization of which from chloroform-n-hexane gave recrystallization of which from diethyl ether-chloroform gave **12** (1.30 g, 44%) as a white solid. mp 183°C; TLC (methylene chloride/methanol=90/10) *Rf*=0.53; IR (KBr pellet) 3070, 2937, 1525, 1463, 1282, 1145, 1008 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.33 (1H, s, C(7)H), 3.54 (3H, s, NCH_3), 3.41 and 2.62 (8H, 2t, methylene protons of piperazinyl), 2.64 (3H, s, methyl of piperazinyl), 2.36 (3H, s, C(5)-methyl).

4,6-Dibromo-1,5-dimethyl-7-nitro-2-(4-methyl-1-piperazinyl)benzimidazole (13)

To a mixture of 6ml of nitric acid and sulfuric acid (1:1), cooled in a ice-salt bath, was added **12** (0.73 g, 1.81 mmol) portionwise. After 12min, the reaction mixture was poured over cracked ice (100 g), and the pH of the resulting solution was adjusted to 6.5 with saturated aqueous sodium bicarbonate. The reaction mixture was extracted with chloroform (100 ml × 3) The combined extracts were washed with water (100 ml), dried over sodium sulfate, and evaproated *in vacuo* to give a solid. Chromatography of the residue on a silica gel column with chloroform/methanol (90:10) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **13** (0.5 g, 62%) as a yellow-red solid; mp 146°C; TLC (chloroform/methanol=90/10) *Rf*=0.51; IR (KBr pellet) 2938, 1565, 1531, 1452, 1283, 1145, 1006 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.45 and 2.61 (8H, 2t, methylene protons of piperazinyl), 3.43 (3H, s, NCH_3), 2.72 (3H, s, methyl of piperazinyl), 2.37 (3H, s, C(5)-methyl).

7-Amino-1,5-dimethyl-2-(4-methyl-1-piperazinyl)benzimidazoledihydrochloride salt (14)

A solution of **13** (1.00 g, 2.24 mmol) in 300 ml of methanol containing 0.052 g of 10% Pd on charcoal was stirred under H_2 for 8 h. The mixture solution was filtered through Celite, and was washed with methanol. The filtrate was concentrated under reducted pressure. The crude residue was recrystallized from n-hexane-chloroform to give **14** (0.70 g, 94%) as a deep red liquid; TLC (chloroform/methanol=90/10) *Rf*=0.31; IR (NaCl) 3403, 3351, 2927, 1627, 1524, 1461, 1280, 997 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.77 and 6.65 (2H, 2s, C(4)H and C(6)H), 4.24 (br s, amine protons), 3.87 (3H, s, NCH_3), 3.70 and 3.34 (8H, 2t, methylene protons of piperazinyl), 2.49 (3H, s, methyl of piperazinyl), 2.30 (3H, s, C(5)-methyl).

1,5-Dimethyl-2-(4-methyl-1-piperazinyl)benzimidazole-4,7(1*H*)-dione (15)

To a suspension of the amine dihydrochloride salt (**14**, 0.41 g, 1.23 mmol) obtained above in 14 ml of water containing 0.14 g of monobasic potassium phosphate was added a solution of 1.40 g of Fremy's salt in 70 ml of water containing 0.28 g of monobasic potassium phosphate. The reaction mixture was stirred at room temperature for 3.5 h, extracted with chloroform (100 ml × 3), dried over sodium sulfate, and concentrated to give a residue. Flash chromatography of the residue on a silica gel column with diethyl ether/methanol (30:70), recrystallization of which from chloroform-n-hexane gave **15** (0.30 g, 40%) as a deep red liquid; TLC (chloroform/methanol=90/10) *Rf*= 0.56; IR (NaCl) 3065, 2936, 1664, 1652, 1531, 1486, 1281, 1110, 1005 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.35 (1H, q, *J*=1,7Hz, C(6)H), 3.74 (3H, s, NCH_3), 3.30 and 2.57 (8H, 2t, methylene protons of piperazinyl), 2.35 (3H, s, methyl of piperazinyl), 2.09 (3H, s, C(5)-methyl).

6-Aziridinyl-1,5-dimethyl-2-(4-methyl-1-piperazinyl) benzimidazole-4,7(1*H*)-dione (16m)

To a solution of a quinone (**15**, 40 mg, 0.15 mmol) in dry methanol (5 ml), chilled at 0°C was added ethylenimine

(excess). After being stirred at 0°C for 20 min, the reaction mixture was stirred at room temperature for 3 h. The solvent was then removed *in vacuo* to give a red residue, and chromatography of the residue on a silica gel column with methylene chloride/methanol (94/6) as a eluent gave a solid, recrystallization of which from ethyl acetate-n-hexane afforded **16m** (20 mg, 44%) as a red-blue solid. mp 170°C (dec.); TLC (methylene chloride/methanol=94/6) *Rf*=0.36; IR (KBr pellet) 2947, 1650, 1597, 1544, 1478, 1337, 1274, 1150 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.72 (3H, s, NCH_3), 3.30 and 2.58 (8H, 2t, methylene protons of piperazinyl), 2.29 (3H, s, methyl of piperazinyl), 2.07 (3H, s, C(5)-methyl).

1,5-Dimethyl-2-(4-methyl-1-piperazinyl)-6-pyrrolidinyl-benzimidazole-4,7(1*H*)-dione (16n)

The same procedure described above was employed for the preparation of **16n** by using **15** (30 mg, 0.11 mmol) and pyrrolidine 0.21 ml as a strating material. Chromatography of the crude product on silica gel column with chloroform/methanol (90/10) as a eluent gave a solid, recrystallization of which from n-hexane-ethyl acetate gave **16n** (11 mg, 29%) as a deep blue solid. mp 138°C; TLC (chloroform/methanol=90/10) *Rf*=0.33; IR (KBr pellet) 2930, 1655, 1627, 1522, 1375, 1275, 1086 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.69 (3H, s, NCH_3), 3.64 (4H, t, pyrrolidinyl methylene adjacent to nitrogen), 3.31 and 2.57 (8H, 2t, methylene protons of piperazinyl), 2.34 (3H, s, methyl of piperazinyl), 2.00 (3H, s, C(5)-methyl), 1.88 (4H, t, other pyrrolidinyl methylene).

1,5-Dimethyl-2-(4-methyl-1-piperazinyl)-6-morphorinyl-benzimidazole-4,7(1*H*)-dione (16o)

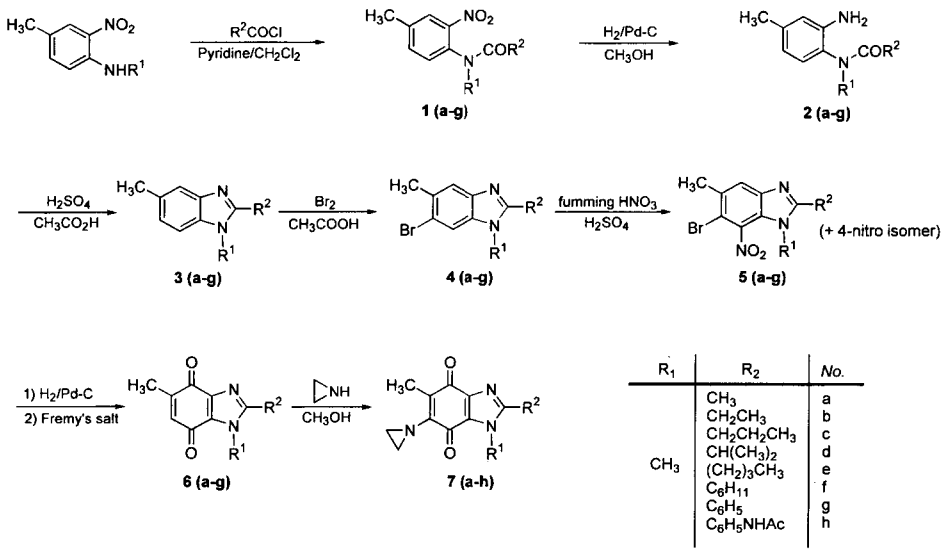
The same procedure described above was employed for the preparation of **16o** by using **15** (30 mg, 0.11 mmol) and morphorine 0.20 ml as a strating material. Chromatography of the crude product on silica gel column with methylene chloride/methanol (94/6) as a eluent gave a solid, recrystallization of which from n-hexane-chloroform gave **16o** (10 mg, 25%) as a red-blue viscous liquid; TLC (methylene chloride/methanol=94/6) *Rf*=0.22; IR (KBr pellet) 2922, 1640, 1541, 1457, 1398, 1295, 1092 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.79 (4H, t, morpholinyl methylene adjacent to nitrogen), 3.71 (3H, s, NCH_3), 3.32 (4H, t, pyrrolidinyl methylene adjacent to oxygen), 3.30 and 2.68 (8H, 2t, methylene protons of piperazinyl), 2.39 (3H, s, methyl of piperazinyl), 2.02 (3H, s, C(5)-methyl).

RESULTS AND DISCUSSION

Chemistry

The preparations of 2-alkyl and 2-aryl benzimidazole-4,7-dione derivatives were carried out in seven steps as shown in scheme 1. Acylation of the 4-methyl amino-3-nitrotoluene with acyl (acetyl, propionyl, butyryl, *i*-butyryl, valeryl, cyclohexanecarbonyl, or benzoyl) chloride gave amido compounds (**1a-g**), which were reduced with hydrogen over 10% palladium on charcoal to afford the amino compounds (**2a-g**) in 71-95% yield. Benzimidazole derivatives (**3a-g**) were obtained by the acid-catalyzed ringformation of these amino compounds in high yield (76 to 99%).

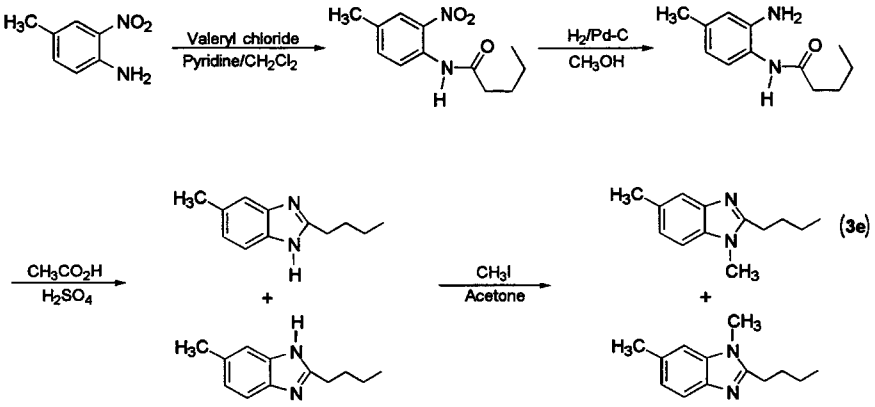
When we utilized the procedure as shown in scheme 2 for synthesis of the benzimidazole, mixtures of isomers were dotained. For example, reaction of 4-amino-3-nitrotoulene with valeryl chloride gave 4-acetamido-3-nitrotoluene, which was reduced with hydrogen over 10% palladium on charchol to afford 3-amino-4-valer-



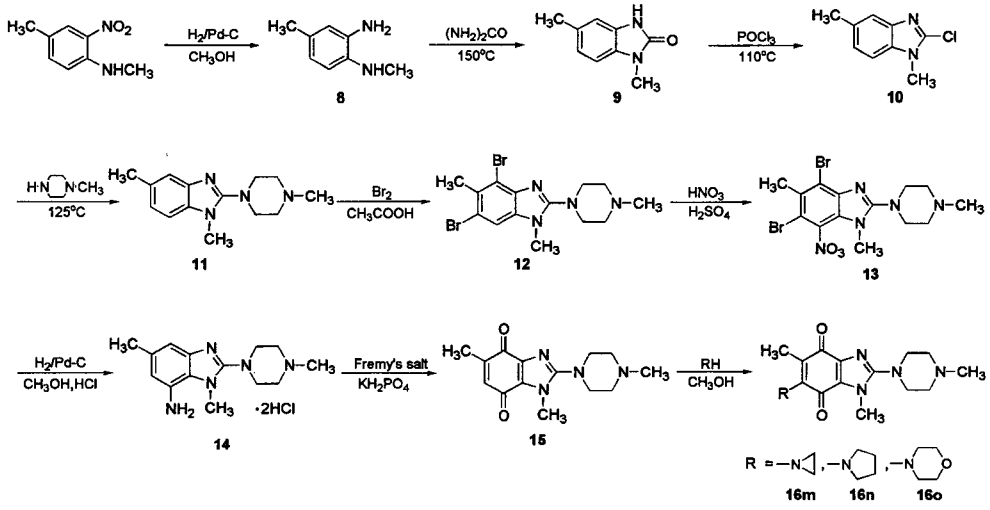
Scheme 1. Synthesis of 2-alkyl, 2-aryl bensimidazole-4,7-diones

amidotoluene. The acid-catalyzed ring fomation of the amino compound resulted in a mixture of 2-butyl-5-methylbenzimidazole and 2-butyl-6-methylbenzimidazole, which was then reacted with methyl iodide in the presence of basic catalyst to give a mixture of 2-butyl-1,5-dimethylbenzimidazole and 2-butyl-1,6-dimethylbenzimidazole with the product ratio of 49:51. On TLC (eluent:methylene chloride/methanol=94/6), two spots (*Rf*=0.58 and 0.47) were obtained. Compound with *Rf*=0.58 was identified as 2-butyl-1,5-dimethylbenzi-midazole (**3e**) by comparing with above results.

The conversion of these benzimidazoles derivatives (**3a-g**) into quinones requires the presence of amino group at 4- or 7-position. To avoid the formation of 6-aminobenzimidazoles, 6-position of the benzimidazole should be protected. Bromination of benzimidazoles were carried out to give 6-bromo benzimidazole derivatives (**4a-g**), which were identified by two singlet peak of aromatic protons on the ^{1}H NMR spectrum. Nitration of 6-bromo benzimidazoles with a mixture of fuming HNO_3 and H_2SO_4 (9:1) in ice salt bath gave mixtures of 4- and 7-nitro benzimidazoles, in which more 7-nitro isomers (**5a-g**) were obtained than 4-nitro isomers. On the ^{1}H NMR spectra, aromatic proton of 7-nitro isomer was deshielded slightly more than 4-nitro isomer. These mixtures of nitro compounds were not separated and utilized in next step. These mixtures of 4- and 7-nitro compounds were reduced with hydrogen over 10% palladium on carbon to give mixtures of 4- and 7-amino compounds. These mixtures were separated by chromatography on a silica gel column with benzene/ethyl acetate (70:30) as a eluent, and, on the ^{1}H NMR spectrum, signals of aromatic protons in major products appeared between 6.5 and 7.0 ppm as two singlets, which established that amino groups were at 7-position. Fremy oxidation of these hydrochloride salt afforded quinones (**6a-g**) in 51-67% yield. 1,4-Addition of **6** with aziridine gave aziridinyl quinones (**7a-h**) in low yield (15 to 23%). These



Scheme 2. Formation of isomers



Scheme 3. Synthesis of 2-piperazinylbenzimidazole-4,7-diones

NMR spectrum, the signal of aziridine protons appeared at 2.26-2.35 as singlet.

Derivatives with piperazinyl group at position 2 were prepared as shown in scheme 3 Reduction of 4-methyl-amino-3-nitrotoluene with hydrogen over 10% palladium on carbon afforded an amino compound (**8**), which was reacted with urea to give a benzimidazolone in 66% yield (**9**). On the ^{1}H NMR spectrum, signal of an amide proton appeared at 9.11 ppm as singlet and carbonyl peak appeared at 1703 cm^{-1} on IR spectrum. This benzimidazolone was reacted with phosphorus oxychloride to 2-chlorobenzimidazole (**10**) in 93% yield. Reaction of **10** with 1-methylpiperazine afforded 2-piperazinyl benzimidazole (**11**) in 77% yield.

By utilizing the same procedure, the benzimidazole was converted into a quinone. In order to protect position 6 of benzimidazole, bromination of **9** in glacial acetic acid at 100°C was carried out. However, 4,6-dibromo compound (**12**) was obtained. On the ^{1}H NMR spectrum, signal of aromatic proton appeared at 7.33 ppm as singlet, which established that dibromination of **10** occurred at position 4 and 6 due to the orientation of 5-methyl as an activating group. Nitration of **12** with a mixture of fuming nitric acid and sulfuric acid gave the nitro compounds (**13**). On the ^{1}H NMR spectrum, signal of aromatic proton disappeared. The 7-nitro compound (**13**) were reduced with hydrogen over 10% palladium on carbon to give 7-amino compound. Fremy oxidation of hydrochloride salt (**14**) of this amino compound gave **15** in 40% yield. 1,4-Addition of **15** with amines (aziridine, pyrrolidine, and morphorine) gave 6-amino quinones (**16m-o**) in low yield (25 to 44%).

Table I. IC_{50} values of benzimidazole-4,7-dione derivatives on various cancer cell lines as determined by MTT assay

Compounds	IC_{50} (μg/ml)[a]		
	P388	SNU-1	SNU-16
7a	0.36	0.34	0.18
7b	1.20	1.00	0.44
7c	1.36	1.05	0.37
7d	1.27	3.34	0.30
7e	2.05	0.95	0.34
7f	1.46	1.43	0.46
7g	0.21	0.32	0.40
7h	0.38	0.26	0.30
16m	0.21	0.30	0.09
16n	> 20	*	> 50
16o	> 20	*	> 50
AAP	0.27	0.43	0.08
AAB	0.27	0.45	0.09
MMC[b]	0.43	0.40	0.86

[a]IC_{50} value which is defined as the concentration that caused 50% inhibition of cell growth
[b]MMC stands for mitomycin C
*Not tested

Biological activities

The cytotoxicity of the synthesized 2-alkyl (**7a-f**), 2-aryl (**7g,h**), and 2-piperazinylbenzimidazole-4,7-diones (**16m-o**) against mouse lymphocytic leukemia (P388) and human gastric carcinoma (SNU-1 and SNU-16) was evaluated by MTT assay (Carmichel *et al.*, 1987). The synthesized compounds showed potent cytotoxicities against all of three cell lines tested. Especially, compounds with aziridinyl group showed potent cytotoxicity, suggesting that aziridinyl group is essential for the antitumor activity.

Among the synthesized compounds, 2-aryl (**7a-h**) and 2-piperidinylbenzimidazole-4,7-dione derivatives (**16m**) showed lower IC_{50} values than mitomycin C against P388 and SNU-16. 2-Acetamidophenyl benzimidazole (**7h**) showed the highest cytotoxicity against SNU-1, and 2-piper-azinylbenzimidazole (**16m**), against SNU-16. Also, the cytotoxicity of these compounds were similar to or slightly higher than those of 2-acetoxymethylbenzimidazoles (AAB) or isoimino-pyrrolo[1,2-a]benzimidazole (AAP) which showed the highest cytotoxicity in our previous studies. These finding suggested us that acetoxy group involved at position of the benzimidazole (A) does not form the quinone methide which was expected to react with DNA. It appears that the formation of quinone methide by electron transfer was not easy after acetoxy group as a leaving group was removed. slight increase of anti-tumor activity is considered due to the increase of polarity.

Also, Table I show that SNU-16 is most sensitive to the synthesized compounds. Considering that in the previous studies AAB and AAP showed the highest cytotoxicity against KHH (human gastric cancer cells) and SNU-16 (human gastric adenocarcinoma) respectively, it is concluded that the benzimidazole-based quinones were more cytotoxic to human cancer cell lines than to mouse cancer cell lines. Among benzimidazole derivatives with alkyl group at position 2, **7a** showed the highest cytotoxicities against all of the cell lines tested.

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