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Synthesis of 6-Aziridinylbenzimidazole Derivatives and Their *In Vitro* Antitumor Activities

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In search for new antitumor agents, twelve 6-aziridinylbenzimidazole derivatives were synthesized and their cytotoxicities were tested against three cancer cell lines (mouse lymphocytic leukemia P388 and B16, and human gastric carcinoma SNU-16). From 4-amino-3-nitrotoluene as the starting material, 2-(acetoxymethyl)benzimidazoles (**5a-d**) were obtained by Phillips reaction. These benzimidazoles were then reacted with Fremy's salt to give a mixture of three 2-(acetoxymethyl) (**8a-c**) and four 2-(hydroxymethyl)benzimidazole-4,7-diones (**9a-d**). Addition of these quinones with aziridine afforded 6-aziridinyl-2-(acetoxymethyl) (**10a-c**) and 6-aziridinyl-2-(hydroxymethyl)benzimidazole-4,7-diones (**11a-d**). Utilizing 2-(hydroxymethyl)benzimidazole-4,7-diones (**9b,d**), esters **10d** and **13e-h** were prepared by the sequential reactions of esterification and addition. The synthesized compounds show potent cytotoxicity against all of three cell lines tested. The cytotoxicities of **10a-d** or **11a-d** against SNU-16 were superior to those of **13e-h**, and were equal to or slightly higher than that of mitomycin C. Compounds **11a-d** were slightly more cytotoxic than **10a-d** in all cell lines tested.

Key words : Benzimidazole, Cytotoxicity, Azamitosene

INTRODUCTION

Recently, 2,3-dihydro-1H-pyrrolo[1,2-a]benzimidazole-5,8-diones (azamitosenes), which have a benzimidazole nucleus instead of the indole nucleus in mitosene, were reported as a new class of antitumor agents, mimicking mitomycins and mitosenes (Islam and Skibo, 1990; Islam and Skibo, 1991) (Fig. 1). The azamitosenes were designed as reductive cross-linkers of DNA. Accordingly, the presence of a leaving group at the 3-position of the pyrrolo[1,2-a]benzimidazole-5,8-dione should permit the formation of an alkylating quinone methide species on quinone reduction followed by elimination of the leaving group (Moore, 1977; Moore and Czerniak, 1981; Tomasz *et al.*, 1986). The aziridinyl group at the 6-position of the pyrrolo[1,2-a] benzimidazole-5,8-dione was expected to be used for another alkylating center. However, structure-activity relationship studies on the azamitosene derivatives revealed that they did not cross-link, but alkylated and cleaved DNA in the cytotoxic reaction at the position of aziridinyl group (Skibo and Schulz, 1993). Some of them showed potent cytostatic activity against a variety of cancer cell lines, and were especially active against solid tumor cell lines.

To optimize their antitumor activity, many azamitosene derivatives have been synthesized (Ahn and Baek, 1993; Schulz *et al.*, 1993; Boruah and Skibo, 1995; Zhou and Skibo, 1996; Kim *et al.*, 1997; Skibo

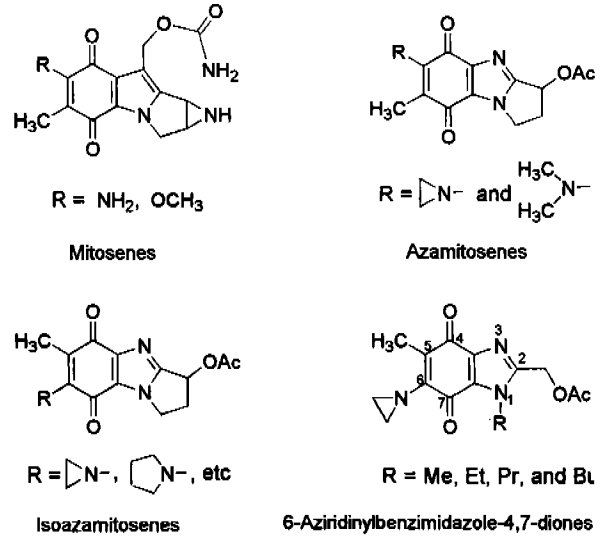

Fig. 1. Structures of mitosenes, azamitosenes, isoazamitosenes, and 6-aziridinylbenzimidazole derivatives.

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et al., 1997). In our previous studies, our isoazamitosene derivatives showed potent anticancer activity against gastric cancer (Ahn and Kim, 1996). Among them, the compound with aziridine ring showed the most potent antitumor activity *in vitro*. This fact suggests that the aziridine ring of isoazamitosene may be mainly responsible for their potent antitumor activity. Thus, the present study was directed to synthesize new 6-aziridinyl benzimidazole derivatives without the pyrrole ring and to evaluate structure-activity relationships.

MATERIALS AND METHODS

Cancer cell lines

Cancer cell lines tested for cytotoxicity were P388, B16 (mouse lymphocytic leukemia) and SNU-16 (human gastric adenocarcinoma). 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 method was used (Carmichel *et al.*, 1987). To compare cytotoxicities among compounds, the IC_{50} value which is the concentration that produces 50% inhibition of cell growth, was determined by regression analysis utilizing GraphPad Prism 2.0 (GraphPad, CA. U.S.A.)

Synthesis

Melting points were determined on a Fisher melting point apparatus and are uncorrected. IR spectra were obtained on a Shimadzu IR-435 spectrometer. ^{1}H NMR spectra were recorded on a Bruker AM-300 (400 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)

3-Nitro-4-(trifluoroacetylamino)toluene (1): A solution of 3-nitro-4-aminotoluene (15.2 g, 0.1 mol) and trifluoroacetic anhydride (16 ml) in trifluoroacetic acid (20 ml) was stirred at room temperature for 2 h. The solution was then poured over cracked ice. Collection of the resulting precipitate by filtration followed by washing with cold water and drying *in vacuo* gave **1** (22.6 g, 91%).

m.p 117°C; TLC (chloroform/n-hexane=80/20) Rf= 0.59; IR (KBr) 3305, 2933, 1735, 1595, 1473, 1356, 1311, 1276, 1168 cm^{-1}; ^{1}H NMR ($CDCl_3$) 11.27 (1H, br s, amide proton), 8.62~7.54 (3H, aromatic protons), 2.45 (3H, s, methyl).

4-(Methylamino)-3-nitrotoluene (2a): A mixture of 1 (3.04 g, 20 mmol), iodomethane (3.74 ml, 60 mmol), KOH (3.37, 60 mmol), and acetone (100 ml) was refluxed for 8 h. Then, the reaction solution was decanted from the solids while still hot, concentrated *in vacuo*, and triturated with 50 ml of benzene. The resulting solid was filtered off. The filtrate was concentrated again to give a red-colored oil, recrystallization of which from n-hexane gave the pure N-methylated compound (**2a**):

m.p 75~78°C; TLC (chloroform/n-hexane=80/20) Rf =0.44; IR (KBr) 3388, 3079, 2919, 1638, 1571, 1526, 1512, 1393, 1352, 1273, 1222, 1177, 1051 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.06 (1H, br s, amine proton), 7.86~ 6.89 (3H, aromatic protons), 3.31 (3H, $NHCH_3$), 2.22 (3H, s, methyl).

4-(Ethylamino)-3-nitrotoluene (2b): The same procedure described above was employed for the preparation of **2b** by using iodoethane (4.80 ml, 60 mmol). Pale pink-colored solid (2.66 g, 74%), m.p 55~59°C; TLC (chloroform/n-hexane=80/20) Rf=0.49; IR (KBr) 3385, 2972, 1632, 1567, 1523, 1403, 1353, 1277, 1229, 1167 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.95 (1H, br s, amine proton), 7.84~6.92 (3H, aromatic protons), 3.33 (3H, q, *J*=6.67 Hz, $NHC\mathbf{H}_2CH_3$), 2.20 (3H, s, methyl), 1.20 (3H, t, *J*=7.19 Hz, $NHC\mathbf{H}_2CH_3$).

3-Nitro-4-(propylamino)toluene (2c): The same procedure described above was employed for the preparation of **2c** by using 1-iodopropane (5.85 ml, 60 mmol). Chromatography of the crude product on silica gel column with chloroform/n-hexane (80:20) gave a viscous liquid, **2c** (2.75 g, 71%).; TLC (chloroform/n-hexane=80/20) Rf=0.51; IR (NaCl) 3382, 2963, 1634, 1568, 1525, 1407, 1350, 1271, 1232, 1161 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.03 (1H, br s, amine proton), 7.82~6.89 (3H, aromatic protons), 3.25 (2H, q, *J*=6.52 Hz, $NHC\mathbf{H}_2CH_2CH_3$), 2.18 (3H, s, methyl), 1.60 (2H, sextet, *J*=7.21 Hz, $NHCH_2C\mathbf{H}_2CH_3$), 0.91 (2H, t, *J*=7.36 Hz, $NHCH_2CH_2C\mathbf{H}_3$.

4-(Butylamino)-3-nitrotoluene (2d): The same procedure described above was employed for the preparation of **2d** by using 1-iodobutane (6.83 ml). Chromatography of the crude product on silica gel column with ethyl acetate/n-hexane (80:20) gave viscous liquid, **2d** (2.91 g, 70%); TLC (chloroform/n-hexane= 80/20) Rf=0.55; IR (NaCl) 3382, 2929, 1634, 1568, 1525, 1407, 1350, 1275, 1232, 1159 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.00 (1H, br s, amine proton), 7.85~6.95 (3H, aromatic protons), 3.37 (2H, q, *J*=7.02 Hz, $NHC\mathbf{H}_2$-$CH_2CH_2CH_3$), 2.21 (3H, s, methyl), 1.59 (2H, q, *J*= 7.30 Hz, $NHCH_2C\mathbf{H}_2CH_2CH_3$), 1.37 (2H, sextet, *J*= 7.40 Hz, $NHCH_2CH_2C\mathbf{H}_2CH_3$), 0.91 (2H, t, *J*=7.26 Hz, $NHCH_2CH_2CH_2C\mathbf{H}_3$)

3-Amino-(methylamino)toluene dihydrochloride (3a): A solution of **2a** (1.66 g, 10 mmol) in methanol (150 ml) was shaken in the presence of 0.20 g of 10% Pd

on carbon under hydrogen (1atm) for 2 h. The catalyst was removed by filtration of the reaction mixture through Celite, and then, a few drops of c-HCl were added to the filtrate. The filtrate was evaporated *in vacuo* to give a residue, which was isolated from methanol/ethyl acetate to afford viscous liquid, **3a** (1.48 g, 71%) as the dihydrochloride salt; TLC (ethyl acetate/n-hexane=80/20) Rf=0.56; IR (NaCl) 3345 (br), 3229, 2916, 1615, 1514, 1463, 1304, 1127 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.04~6.70 (3H, aromatic protons), 5.74 (1H, br s, amine proton), 2.74 (3H, s, NCH_3), 2.18 (C(5)-methyl)

3-Amino-(ethylamino)toluene dihydrochloride (3b): The same procedure described above was employed for the preparation of **3b**. Red-white crystal (1.2 g, 68%). m.p 152°C; TLC (ethyl acetate/n-hexane=80/20) Rf=0.62; IR (KBr) 3398, 3373, 3323, 2911, 1638, 1612, 1513, 1449, 1298, 1208, 1015 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.13~6.65 (3H, aromatic protons), 6.40 (1H, br s, amine proton), 3.18 (2H, s, $NC\mathbf{H}_2CH_3$), 2.18 (3H, s, C(5)-methyl), 1.23 (3H, t, $NCH_2C\mathbf{H}_3$).

3-Amino-4-(propylamino)toluene (3c): The same procedure described above was employed for the preparation of **3c**. Viscous liquid (1.1 mg, 64%); TLC (ethyl acetate/n-hexane=80/20) Rf=0.69; IR (NaCl) 3405 (br), 3236, 1615, 1568, 1515, 1456, 1383, 1302, 1215, 1122 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.49 (1H, br s, amine proton), 7.21~6.81 (3H, aromatic protons), 3.11 (2H, s, $NC\mathbf{H}_2CH_2CH_3$), 2.19 (3H, s, C(5)-methyl), 1.69 (2H, sextet, J=7.49 Hz, $NCH_2C\mathbf{H}_2CH_3$), 0.89 (3H, t, J= 7.47 Hz, $NCH_2CH_2C\mathbf{H}_3$).

3-Amino-4-(butylamino)toluene (3d): The same procedure described above was employed for the preparation of **3d**. Viscous liquid (12 g, 63%).; TLC (ethyl acetate/n-hexane=80/20) Rf=0.75; IR (NaCl) 3349 (br), 3227, 1615, 1567, 1517, 1466, 1383, 1302, 1210 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.96~6.67 (3H, aromatic protons), 3.59 (br s, amine protons), 3.37 (2H, q, J=7.04 Hz, $NHC\mathbf{H}_2CH_2CH_2CH_3$), 2.21 (3H, s, methyl), 1.59 (2H, q, J=7.30 Hz, $NHCH_2C\mathbf{H}_2CH_2CH_3$), 1.35 (2H, sextet, J =7.28 Hz, $NHCH_2CH_2C\mathbf{H}_2CH_3$), 0.87 (2H, t, J=7.26 Hz, $NHCH_2CH_2CH_2C\mathbf{H}_3$).

2-(Hydroxymethyl)-1,5-dimethylbenzimidazole (4a): A mixture of **3a** (1.48 g, 7 mmol), 85% glycolic acid (2.51 g, 28 mol), and 10 ml of 4 N HCl was refluxed for 4 h. The reaction mixture was then cooled to room temperature and the pH adjusted to 6.5 with sodium bicarbonate, resulting in crystallization of the crude product. Recrystallization of the crude product from chlorofrom-n-hexane gave a pale pink needles (1.16 g, 94%): m.p 142°C; TLC (chloroform/methanol= 90/10) Rf=0.43; IR (KBr) 3155, 2948, 1497, 1453, 1335, 1218, 1040 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.41~7.03 (3H, aromatic protons), 5.53 (1H, t, J=5.70, hydroxyl), 4.68 (2H, d, J=5.02 Hz, hydroxymethyl methylene), 3.78 (3H, s, NCH_3), 2.39 (3H, s, C(5)-methyl).

1-Ethyl-2-(hydroxymethyl)-5-methylbenzimidazole (4b): The same procedure described above was employed for the preparation of **4b**. A colorless needles (1.12 g, 84%). m.p 194°C; TLC (chloroform/methanol= 90/10) Rf=0.44; IR (KBr) 3186, 2938, 1511, 1495, 1453, 1330, 1168, 1029 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.43~7.02 (3H, aromatic protons), 5.54 (1H, br s, hydroxyl), 4.68 (2H, s, hydroxymethyl methylene), 4.28 (2H, q, J=7.13 Hz, $NC\mathbf{H}_2CH_3$), 2.39 (3H, s, C(5)-methyl), 1.32 (3H, t, J=7.13 Hz, $NCH_2C\mathbf{H}_3$).

2-(Hydroxymethyl)-5-methyl-1-propylbenzimidazole (4c): The same procedure described above was employed for the preparation of **4c**. Chromatography of the crude product with chloroform/methanol (90:10) on a silica gel column gave **12** (1.04 g, 73%) as a colorless needles. m.p 117°C; TLC (chloroform/ methanol=90/10) Rf=0.47; IR (KBr) 3190, 2956, 1459, 1453, 1329, 1198, 1026 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.43~ 7.02 (3H, aromatic protons), 5.54 (1H, br s, hydroxyl), 4.68 (2H, s, hydroxymethyl methylene), 4.28 (2H, q, J=7.13 Hz, $NC\mathbf{H}_2CH_2CH_3$), 2.39 (3H, s, C(5)-methyl), 1.32 (3H, t, J=7.13 Hz, $NCH_2C\mathbf{H}_2CH_3$), 1.32 (3H, t, J= 7.13 Hz, $NCH_2CH_2C\mathbf{H}_3$).

1-Butyl-2-(hydroxymethyl)-5-methylbenzimidazole (4d): The same procedure described above was employed for the preparation of **4d**. Recrystallization of the crude product from chloroform-n-hexane gave (1.08 g, 71%) as a colorless needles. m.p 118°C; TLC (chloroform/methanol=90/10) Rf=0.58; IR (KBr) 3155, 2954, 1459, 1436, 1329, 1203, 1039 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.46~7.04 (3H, aromatic protons), 4.84 (2H, s, hydroxymethyl methylene), 4.28 (2H, q, J=7.46 Hz, $NC\mathbf{H}_2CH_2CH_2CH_3$), 2.44 (3H, s, C(5)-methyl), 1.81 (2H, quintet, J=7.55 Hz, $NCH_2C\mathbf{H}_2CH_2CH_3$), 1.38 (2H, sextet, J=7.44 Hz, $NCH_2CH_2C\mathbf{H}_2CH_3$), 0.94 (3H, t, J= 7.27 Hz, $NCH_2CH_2CH_2C\mathbf{H}_3$).

2-(Acetoxymethyl)-1,5-dimethylbenzimidazole (5a): To a suspension of **4a** (1.06 g, 6 mmol) in dry methylene chloride (60 ml) was added acetic anhydride (0.68 ml, 7.2 mmol) and pyridine (0.58 ml, 7.2 mmol). The resulting mixture was refluxed for 9 h. The solvent were then evaporated *in vacuo*, and the crude product was recrystallized from chloroform-n-hexane to gave 5a (1.19 g, 91%) as a pale pink crystal. m.p 113~114°C; TLC (ethylacetate/methanol= 95/5) Rf=0.54; IR (KBr) 3044, 2919, 1748, 1493, 1478, 1376, 1248, 1031 cm^{-1}; ^1H NMR (DMSO-d_6) 7.46~ 7.08 (3H, aromatic protons), 5.30 (2H, s, acetoxymethyl methylene), 3.77 (3H, s, NCH_3), 2.40 and 2.08 (6H, 2s, C(5)-methyl and acetate methyl).

2-(Acetoxymethyl)-ethyl-5-methylbenzimidazole (5b): This compound was prepared as described above, but using **4b** (0.95 g, 5 mmol), to give a pale pink residue. Chromatography of the residue on a silica gel column with ethyl acetate/methanol (95:5) gave a solid, recrystallization of which from chloroform-n-

hexane gave **5b** (1.02 g, 88%) as a white crystal. m.p 72°C; TLC (ethyl acetate/methanol=95/5) Rf=0.59; IR (KBr) 3044, 2986, 1747, 1438, 1373, 1269, 1237, 1218, 1028 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.49~7.08 (3H, aromatic protons), 5.30 (2H, s, acetoxymethyl methylene), 4.25 (2H, q, J=7.17 Hz, NCH_2CH_3), 2.40 and 2.09 (6H, 2s, C(5)-methyl and acetate methyl), 1.31 (3H, t, J=7.14 Hz, NCH_2CH_3).

2-(Acetoxymethyl)-5-methyl-1-propylbenzimidazole (5c): This compound was prepared as described above, but using **4c** (1.02 g, 5 mmol), to give a brown residue. Chromatography of the residue on a silica gel column with ethyl acetate/methanol (95:5) gave **5c** (1.01 g, 82%) as a viscous liquid; TLC (ethyl acetate/methanol =95/5) Rf=0.61; IR (NaCl) 3035, 2966, 1744, 1458, 1441, 1371, 1229, 1031 cm^{-1}; ^1H NMR ($CDCl_3$) 7.56~7.11 (3H, aromatic protons), 5.36 (2H, s, acetoxymethyl methylene), 4.13 (2H, t, J=7.39 Hz, $NCH_2CH_2CH_3$), 2.47 and 2.13 (6H, 2s, C(5)-methyl and acetate methyl), 1.89 (2H, sextet, J=7.36 Hz, $NCH_2CH_2CH_3$), 0.97 (3H, t, J=7.42 Hz, $NCH_2CH_2CH_3$).

2-(Acetoxymethyl)-1-butyl-5-methylbenzimidazole (5d): This compound was prepared as described above, but using **4d** (0.98 g, 4.5 mmol), to give a brown residue. Chromatography of the residue on a silica gel column with chloroform/methanol (90:10) gave **5d** (0.95 g, 81%) as a viscous liquid; TLC (ethyl acetate/methanol=95/5) Rf=0.64; IR (NaCl) 3031, 2959, 1746, 1455, 1439, 1372, 1229, 1030 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.89~7.12 (3H, aromatic protons), 5.36 (2H, s, acetoxymethyl methylene), 4.17 (2H, t, J=7.46 Hz, $NCH_2CH_2CH_2CH_3$), 2.49 and 2.14 (6H, 2s, C(5)-methyl and acetate methyl), 1.82 (2H, quintet, J=8.50 Hz, $NCH_2CH_2CH_2CH_3$), 1.40 (2H, sextet, J=7.46 Hz, $NCH_2CH_2CH_2CH_3$), 0.97 (3H, t, J=7.31 Hz, $NCH_2CH_2CH_2CH_3$).

2-(Acetoxymethyl)-6-bromo-1,5-dimethyltoluene (6a): To a solution of **5a** (0.98 g, 4.5 mmol) in glacial acetic acid (50 ml), heated at 100°C, was added bromine (0.255 ml, 4.95 mmol) in glacial acetic acid (2 ml). After the addition, the reaction mixture was heated at 100~110°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 solid, recrystallization of which from chloroform-n-hexane gave 6a (1.06 g, 79%) as a yellow solid. m.p 114°C; TLC (chloroform/methanol= 98/2) Rf=0.19; IR (KBr) 3026, 2960, 1740, 1482, 1438, 1375, 1223, 1037, 1003 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.90 and 7.61 (2H, 2s, aromatic protons), 5.30 (2H, s, acetoxymethyl methylene), 3.77 (3H, s, NCH_3), 2.42 and 2.09 (6H, 2s, C(5)-methyl and acetate methyl).

2-(Acetoxymethyl)-6-bromo-1-ethyl-5-methyltoluene (6b): This compound was prepared as described above, but using **5b** (0.93 g, 4 mmol), to give a residue. Chromatography of the residue on a silica gel column with ethylacetate/methanol (95:5) as a eluent gave a solid, recrystallization of which from chloroform-n-hexane gave **6b** (0.97 g, 78%) as a white needles. m. p 104°C; TLC (chloroform/methanol=98/2) Rf=0.20; IR (KBr) 2988, 2921, 1741, 1481, 1375, 1244, 1034 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.93 and 7.62 (2H, 2s, aromatic protons), 5.30 (2H, s, acetoxymethyl methylene), 4.27 (2H, s, NCH_2CH_3), 2.42 and 2.09 (6H, 2s, C(5)-methyl and acetate methyl), 1.30 (3H, t, J=7.40 Hz, NCH_2CH_3).

2-(Acetoxymethyl)-6-bromo-5-methyl-1-propyltoluene (6c): This compound was prepared as described above, but using **5c** (0.98 g, 4 mmol), to give a residue. 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 chloroform-n-hexane gave 6c (1.00 g, 77%) as a white needles. m.p 82°C; TLC (chloroform/methanol=98/2) Rf=0.21; IR (KBr) 2964, 1735, 1476, 1266, 1225, 1038 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.65 and 7.58 (3H, 2s, aromatic protons), 5.35 (2H, s, acetoxymethyl methylene), 4.11 (2H, t, J=7.43 Hz, $NCH_2CH_2CH_3$), 2.51 and 2.15 (6H, 2s, C(5)-methyl and acetate methyl), 1.86 (2H, sextet, J=7.42 Hz, $NCH_2CH_2CH_3$), 0.98 (3H, t, J=7.40 Hz, $NCH_2CH_2CH_3$).

2-(Acetoxymethyl)-6-bromo-1-butyl-5-methyltoluene (6d): This compound was prepared as described above, but using **5d** (0.94 g, 3.6 mmol), to give a residue. Chromatography of the residue on a silica gel column with benzene/ethyl acetate (70:30) as a eluent gave a solid, recrystallization of which from chloroform-n-hexane gave **6d** (0.92 g, 75%) as a white needles; m. p 76~77°C; TLC (chloroform/methanol=98/2) Rf=0.25; IR (KBr) 2968, 1477, 1374, 1266, 1223, 1038 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.62 and 7.56 (2H, 2s, aromatic protons), 5.32 (2H, s, acetoxymethyl methylene), 4.12 (2H, t, J=6.02 Hz, $NCH_2CH_2CH_2CH_3$), 2.50 and 2.14 (6H, 2s, C(5)-methyl and acetate methyl), 1.79 (2H, quintet, J=6.09 Hz, $NCH_2CH_2CH_2CH_3$), 1.39 (3H, sextet, J=6.05 Hz, $NCH_2CH_2CH_2CH_3$), 0.96 (3H, t, J=5.85 Hz, $NCH_2CH_2CH_2CH_3$).

2-(Acetoxymethyl)-6-bromo-1,5-dimethyl-7-nitrotoluene (7a): To a mixture of 20 ml of (9:1) fuming nitric acid and sulfuric acid, cooled in a ice-salt bath, was added **6a** (0.98 g, 3.3 mmol) portionwise. After 10 min, 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 (sodium sulfate), and concentrated *in vacuo* to give a solid, recrystallization of which from chloroform-n-hexane gave a nitro compound 7a (0.86 g, 76%) as a white needles;

m.p 177~178°C; TLC (benzene/ethyl acetate=70/30) Rf =0.33; IR (KBr) 3080, 2996, 2966, 1749, 1530, 1480, 1439, 1366, 1257, 1221, 1037 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.77 (1H, s, C(4)-proton), 5.35 (2H, s, acetoxymethyl methylene), 3.84 (3H, s, NCH_3), 2.52 and 2.13 (6H, 2s, C(6)-methyl and acetate methyl).

2-(Acetoxymethyl)-6-bromo-1-ethyl-5-methyl-7-nitrotoluene (7b): This compound was prepared as described above, but using **6b** (0.96 g, 3.1 mmol), to give a residue. Chromatography of the residue on a silica gel column with benzene/ethyl acetate (70:30) as a eluent gave a solid, recrystallization of which from chloroform-n-hexane gave **7b** (0.81 g, 73%) as a white needles; m.p 119~121°C; TLC (benzene/ethyl acetate= 70/30) Rf=0.55; IR (KBr) 2987, 2937, 1741, 1529, 1491, 1458, 1378, 1363, 1260, 1141, 1038 cm^{-1}; ^1H NMR ($CDCl_3$) 7.78 (1H, s, C(4)-proton), 5.36 (2H, s, acetoxymethyl methylene), 4.25 (2H, q, J=6.95 Hz, $NC\mathbf{H}_2CH_3$), 2.53 and 2.14 (6H, 2s, C(6)-methyl and acetate methyl), 1.47 (3H, t, J=7.28 Hz, $NCH_2C\mathbf{H}_3$).

2-(Acetoxymethyl)-6-bromo-5-methyl-7-nitro-1-propyltoluene (7c): This compound was prepared as described above, but using **6c** (0.97 g, 3 mmol), to give a residue. Chromatography of the residue on a silica gel column with benzene/ethyl acetate (70:30) as a eluent gave a solid, recrystallization of which from chloroform-n-hexane gave **7c** (0.80 g, 72%) as a white needles; m.p 119~120°C; TLC (benzene/ethyl acetate= 70/30) Rf=0.61; IR (KBr) 3079, 2995, 2960, 2922, 2851, 1749, 1530, 1479, 1439, 1365, 1259, 1221, 1095, 1037, 1012 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.77 (1H, s, C(4)-proton), 5.35 (2H, s, acetoxymethyl methylene), 4.15 (2H, t, J= 7.49 Hz, $NC\mathbf{H}_2CH_2CH_3$), 2.53 and 2.14 (6H, 2s, C(5)-methyl and acetate methyl), 1.87 (2H, sextet, J=7.42 Hz, $NCH_2C\mathbf{H}_2CH_3$), 1.00 (3H, t, J=7.43 Hz, $NCH_2CH_2C\mathbf{H}_3$).

2-(Acetoxymethyl)-6-bromo-1-butyl-5-methyl-7-nitrotoluene (7d): This compound was prepared as described above, but using **6d** (0.95 g, 2.8 mmol), to give a residue. Chromatography of the residue on a silica gel column with benzene/ethyl acetate (70:30) as a eluent gave a solid, recrystallization of which from chloroform-n-hexane gave **7d** (0.82 g, 76%) as a white needles; (12 mg, 24%). m.p 86°C; TLC (benzene/ethyl acetate=70/30) Rf=0.64; IR (KBr) 2962, 2932, 2877, 1743, 1534, 1459, 1374, 1236, 1033 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.76 (H, s, C(4)-proton), 5.36 (2H, s, acetoxymethyl methylene), 4.18 (2H, t, J=7.39 Hz, $NC\mathbf{H}_2CH_2CH_2CH_3$), 2.53 and 2.14 (6H, 2s, C(5)-methyl and acetate methyl), 1.80 (2H, quintet, J=7.57 Hz, $NCH_2C\mathbf{H}_2CH_2CH_3$)), 1.41 (2H, sextet, J=7.52 Hz, $NCH_2CH_2C\mathbf{H}_2CH_3$), 0.99 (3H, t, J=5.85 Hz, $NCH_2CH_2CH_2C\mathbf{H}_3$).

2-(Acetoxymethyl)-1,5-dimethylbenzimidazole-4,7(1*H*)-dione (8a): A suspension of **7a** (0.85 g, 2.5 mmol) in methanol (100 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 1 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, 0.47 g (61%).

To a suspension of the amine dihydrochloride salt (0.47 g, 1.5 mmol) obtained above in 5.7 ml of water containing 23 mg of monobasic potassium phosphate was added a solution of 0.57 g of Fremy's salt (Zimmer *et al.*, 1971) in 45 ml of water containing 0.23 g of monobasic potassium phosphate. The reaction mixture was stirred at room temperature for 4 h, extracted with chloroform (30 ml×3), dried (sodium sulfate), and concentrated to give a residue. Flash chromatography of the residue on a silica gel column with ethyl acetate/methanol (95:5), recrystallization of which from chloroform-n-hexane gave 22 (57 mg, 47%) as a red solid: m.p 114~115°C; TLC (ethyl acetate/methanol=95/5) Rf=0.56; IR (KBr) 2962, 1741, 1671, 1668, 1486, 1377, 1243, 1037 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.48 (1H, s, C(6)-proton), 5.25 (2H, s, acetoxymethyl methylene), 4.01 (3H, s, NCH_3), 2.17 and 2.14 (6H, 2s, C(5)-methyl and acetate methyl)

2-(Hydroxymethyl)-1,5-dimethylbenzimidazole-4,7(1*H*)-dione (9a): white needle; m.p 179~181°C; TLC (ethyl acetate/methanol=95/5) Rf=0.40; IR (KBr) 3200, 2923, 1663, 1521, 1480, 1377, 1276, 1120, 1041 cm^{-1}; ^1H NMR ($CDCl_3$) 6.46 (1H, d, J=1.38, C(6)-proton), 4.84 (2H, s, hydroxymethyl methylene), 4.01 (3H, s, NCH_3), 2.13 (3H, s, C(5)-methyl).

2-(Acetoxymethyl)-1-ethyl-5-methylbenzimidazole-4,7(1*H*)-dione (8b): Reduction of **7b** (0.56 g, 1.6 mmol) as described above gave the dihydrochloride salt of 7-amino-2-(acetoxymethyl)-1-ethyl-5-methylbenzimidazole (0.28 g, 55%). This salt (0.25 g, 0.8 mmol) was oxidized by using Fremy's salt to give a residue. Chromatography of the residue on a silica gel column with ether/methanol (98:2) as a eluent gave a solid, recrystallization of which from chloroform-n-hexane gave 8b (86 mg, 42%) as white needles; m.p 102~ 104°C; TLC (ethyl acetate/methanol=95/5) Rf=0.61; IR (KBr) 2986, 1743, 1680, 1663, 1512, 1381, 1240, 1039 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.48 (1H, q, J=1.51 Hz, C (6)-proton), 5.26 (2H, s, acetoxymethyl methylene), 4.38 (2H, q, J=5.72 Hz, N(1)-$C\mathbf{H}_2CH_3$), 2.14 and 2.12 (6H, 2s, C(5)-methyl and acetate methyl), 1.43 (3H, t, J=5.73 Hz, N(1)-$CH_2C\mathbf{H}_3$).

2-(Hydroxymethyl)-1-ethyl-5-methylbenzimidazole-4,7(1*H*)-dione (9b): white needles; m.p 159~161°C; TLC (ethyl acetate/methanol=95/5) Rf=0.47; IR (KBr) 3248, 2924, 1659, 1560, 1509, 1297, 1163, 1041 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.45 (1H, d, J=1.57 Hz, C(6)-proton), 4.84 (2H, s, hydroxymethyl methylyene), 4.42 (2H, q, J=5.84 Hz, N(1)-$C\mathbf{H}_2CH_3$), 2.12 (3H, s, C(5)-methyl), 1.44 (3H, t, J=5.73 Hz, N(1)-$CH_2C\mathbf{H}_3$).

2-(Acetoxymethyl)-5-methyl-1-propylbenzimidazole-4,7(1*H*)-dione (8c): Reduction of **7c** (0.62 g, 1.7 mmol)

as described above gave the dihydrochloride salt of 7-amino-2-(acetoxymethyl)-1-propyl-5-methylbenzimidazole (0.32 g, 57%). This salt (0.27 g, 0.81 mmol) was oxidized by using Fremy's salt to give a residue. Chromatography of the residue on a silica gel column with ether/methanol (98:2) as a eluent gave 8c (92 mg, 41%) as a brown viscous liquid; TLC (ethyl acetate/methanol=95/5) Rf=0.63; IR (KBr) 2968, 2938, 2878, 1750, 1661, 1611, 1535, 1475, 1375, 1280, 1231, 1125, 1034 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.48 (1H, q, *J*= 1.43 Hz, C(6)-proton), 5.26 (2H, s, acetoxymethyl methylene), 4.30 (2H, t, *J*=7.56 Hz, N(1)-C**H**$_2$CH$_2$CH$_3$), 2.15 and 2.14 (6H, 2s, C(5)-methyl and acetate methyl), 1.82 (2H, sextet, *J*=7.52 Hz, N(1)-CH$_2$C**H**$_2$CH$_3$), 0.99 (3H, t, *J*=7.35 Hz, N(1)-CH$_2$CH$_2$C**H**$_3$).

2-(Hydroxymethyl)-5-methyl-1-propylbenzimidazole-4,7(1*H*)-dione (9c): a brown viscous liquid; TLC (ethyl acetate/methanol=95/5) Rf=0.50; IR (KBr) 2967, 2936, 2877, 1750, 1657, 1543, 1460, 1375, 1307, 1229, 1125, 1040 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.44 (1H, d, *J*=1.34 Hz, C(6)-proton), 4.85 (2H, s, hydroxymethyl methylene), 4.34 (2H, t, *J*=7.53, N(1)-C**H**$_2$CH$_2$CH$_3$), 2.12 (3H, s, C (5)-methyl), 1.84 (2H, sextet, *J*=5.63 Hz, N(1)-CH$_2$C**H**$_2$-CH$_3$), 0.98 (3H, t, *J*=7.38 Hz, N(1)-CH$_2$CH$_2$C**H**$_3$).

1-Butyl-2-(hydroxymethyl)-5-methylbenzimidazole-4,7(1*H*)-dione (9d): Reduction of **7d** (0.55 g, 1.4 mmol) as described above gave the dihydrochloride salt of 7-amino-2-(acetoxymethyl)-1-butyl-5-methylbenzimidazole (0.31 g, 64%). This salt (0.26 g, 0.75 mmol) was oxidized by using Fremy's salt to give a residue. Chromatography of the residue on a silica gel column with diethyl ether/methanol (98:2) as a eluent gave a solid, recrystallization of which from chloroform-n-hexane gave 9d (80 mg, 43%) as white needles; m.p 107°C; TLC (ethyl acetate/methanol=95/5) Rf=0.55; IR (KBr) 3347, 2960, 1662, 1544, 1475, 1376, 1309, 1129, 1041 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.44 (1H, d, *J*=1.49, C(6)-proton), 4.84 (2H, s, hydroxymethyl methylene), 4.35 (2H, t, *J*=7.60 Hz, N(1)-C**H**$_2$CH$_2$CH$_2$CH$_3$), 2.11 (3H, s, C(5)-methyl), 1.76 (2H, quintet, *J*=4.67 Hz, N(1)-CH$_2$C**H**$_2$CH$_2$CH$_3$), 1.40 (2H, sextet, *J*=7.54 Hz, N (1)-CH$_2$CH$_2$C**H**$_2$CH$_3$), 0.97 (3H, t, *J*=7.26, N(1)-CH$_2$CH$_2$-CH$_2$C**H**$_3$).

2-(Acetoxymethyl)-6-(*N*-aziridinyl)-1,5-dimethylbenzimidazole-4,7(1*H*)-dione (10a): To a solution of 2-acetoxymethyl quinone **8a** (25 mg, 0.10 mmol) in dry methanol (2 ml), chilled at 0°C, was added 0.1 ml of ethylenimine. After being stirred at 0°C for 15 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 ethyl acetate/methanol (95:5) as a eluent gave a solid, recrystallization of which from chloroform-n-hexane gave **10a** (6 mg, 21%) as a red solid; m.p 176°C; TLC (ethyl acetate/methanol=95/5) Rf=0.46; IR (KBr) 2924, 1740, 1678, 1661, 1588, 1535, 1378, 1251, 1164, 1041 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.22 (2H, s, acetoxymethyl methylyene), 3.99 (3H, s, N(1)-methyl), 2.31 (4H, s, aziridine protons), 2.11 and 2.10 (6H, 2s, C(5)-methyl and acetate methyl).

2-(Acetoxymethyl)-6-(N-aziridinyl)-1-ethyl-5-methylbenzimidazole-4,7(1*H*)-dione (10b): The same procedure as described above, but using 2-acetoxymethyl quinone **8b** (26 mg, 0.1 mmol) at room temperature gave a residue after 8 h. Chromatography of the residue on a silica gel column with ethyl acetate as a eluent gave a solid, recrystallization of which from chloroform-n-hexane gave **10b** (6.1 mg, 20%) as a red solid: m.p 100~102°C; TLC (ethyl acetate/methanol =95/5) Rf=0.48; IR (KBr) 2921, 1747, 1673, 1653, 1542, 1374, 1340, 1241, 1159, 1033 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.24 (2H, s, acetoxymethyl methylene), 3.38 (2H, q, N(1)-C**H**$_2$CH$_3$), 2.34 (4H, s, aziridine protons), 2.13 and 2.12 (6H, 2s, C(5)-methyl and acetate methyl), 1.44 (3H, t, N(1)-CH$_2$C**H**$_3$).

2-(Acetoxymethyl)-6-(*N*-aziridinyl)-5-methyl-1-propylbenzimidazole-4,7(1*H*)-dione (10c): The same procedure as described above, but using 2-acetoxymethyl quinone **8c** (28 mg, 0.1 mmol) at room temperature 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 **10c** (7.0 mg, 22%) as a red solid: m.p 99~101°C; TLC (ethyl acetate/methanol=95/5) Rf=0.50; IR (KBr) 2998, 2968, 2934, 2878, 1751, 1669, 1647, 1580, 1534, 1475, 1375, 1342, 1253, 1229, 1209, 1141, 1035 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.23 (2H, s, acetoxymethyl methylene), 4.28 (2H, t, *J*=7.58 Hz, N(1)-C**H**$_2$CH$_2$CH$_3$), 2.33 (4H, s, aziridine protons), 2.14 and 2.12 (6H, 2s, C(5)-methyl and acetate methyl), 1.82 (2H, sextet, *J*=7.54 Hz, N(1)-CH$_2$C**H**$_2$CH$_3$), 0.99 (3H, t, *J*=7.44 Hz, N(1)-CH$_2$CH$_2$C**H**$_3$).

6-(N-Aziridinyl)-2-(hydroxymethyl)-1,5-dimethylbenzimidazole-4,7(1*H*)-dione (11a): The same procedure as described above, but using 2-hydroxymethyl quinone **9a** (21 mg, 0.1 mmol) at room temperature 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 **11a** (5.9 mg, 24%) as a red solid: m.p 181~182°C; TLC (ethyl acetate/methanol=95/5) Rf=0.26; IR (KBr) 3202, 2924, 1660, 1591, 1541, 1390, 1335, 1251, 1036 cm^{-1}; ^1H NMR ($CDCl_3$) 4.80 (2H, s, hydroxymethyl methylene), 3.97 (3H, s, N(1)-methyl), 2.30 (4H, s, aziridine protons), 2.06 (3H, s, C(5)-methyl).

6-(N-Aziridinyl)-1-ethyl-2-(hydroxymethyl)-5-methylbenzimidazole-4,7(1*H*)-dione (11b): The same procedure as described above, but using hydroxymethyl quinone **9b** (22 mg, 0.1 mmol) at room temperature 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 **11b** (5.7 mg, 22%) as a red solid: m.p 168°C; TLC (ethyl acetate/methanol=95/5) Rf=0.32; IR (KBr) 3235, 2937, 1658, 1594, 1544, 1336, 1271, 1160, 1037 cm^{-1}; ^1H NMR ($CDCl_3$) 4.82 (2H, s, hydroxymethyl methylyene), 3.38 (2H, q, J=7.12 Hz, N(1)-C**H**$_2$CH$_3$), δ 2.32 (4H, s, aziridine protons), 2.07 (3H, s, C(5)-methyl), 1.44 (3H, t, J=7.15 Hz, N(1)-CH$_2$C**H**$_3$).

6-(*N*-Aziridinyl)-2-(hydroxymethyl)-5-methyl-1-propylbenzimidazole-4,7(1*H*)-dione (11c): The same procedure as described above, but using **9c** (23 mg, 0.1 mmol) at room temperature 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 **11c** (5.0 mg, 18%) as a red solid: m.p 151~153°C; TLC (ethyl acetate/methanol=95/5) Rf=0.39; IR (KBr) 3237, 2969, 1669, 1667, 1544, 1343, 1260, 1134, 1058 cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.80 (2H, s, hydroxymethyl methylene), 4.31 (2H, t, J=7.53 Hz, N(1)-C**H**$_2$CH$_2$CH$_3$), 2.32 (4H, s, aziridine protons), 2.07 (3H, s, C(5)-methyl), 1.82 (2H, sextet, J=7.50 Hz, N(1)-CH$_2$C**H**$_2$CH$_3$), 0.98 (3H, t, J=7.40 Hz, N(1)-CH$_2$-CH$_2$C**H**$_3$).

6-(N-Aziridinyl)-1-butyl-2-(hydroxymethyl)-5-methylbenzimidazole-4,7(1*H*)-dione (11d): The same procedure as described above, but using **9d** (25 mg, 0.1 mmol) at room temperature 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 **11d** (5.8 mg, 20%) as a red solid: m.p 148°C; TLC (ethyl acetate/methanol=95/5) Rf=0.44; IR (KBr) 3256, 2961, 1655, 1543, 1339, 1250, 1160, 1044 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.80 (2H, d, J=6.24 Hz, hydroxymethyl methylyene), 4.33 (2H, t, J=7.57 Hz, N(1)-C**H**$_2$CH$_2$CH$_2$CH$_3$), 2.33 (4H, s, aziridine protons), 2.08 (3H, s, C(5)-methyl), 1.76 (2H, quintet, J=6.96 Hz, N(1)-CH$_2$C**H**$_2$CH$_2$CH$_3$), 1.40 (2H, sextet, J=7.50 Hz, N(1)-CH$_2$CH$_2$C**H**$_2$CH$_3$), 0.97 (3H, t, J=7.24, N(1)-CH$_2$CH$_2$CH$_2$C**H**$_3$).

2-(Acetoxymethyl)-1-butyl-5-methylbenzimidazole-4,7(1*H*)-dione (8d): A solution of **9d** (10 mg, 0.04 mmol) in excess acetic anhydride (1.5 ml) were stirred at room temperature for 48 h. The reaction mixture was poured into cold water, neutralized by aqueous sodium bicarbonate, and then extracted with chloroform (3×10 ml). The extracts were dried (sodium sulfate), filtered, and concentrated to give a residue. Chromatography of the residue on a silica gel column with ethyl acetate/methanol (95:5) as a eluent gave **8d** (8.7 mg, 75%) as a viscous liquid; TLC (ethyl acetate) Rf=0.64; IR (KBr) 2961, 2931, 2875, 1751, 1681, 1662, 1516, 1475, 1466, 1375, 1227, 1033 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.48 (1H, s, C(6)-proton), 5.26 (2H, s, acetoxymethyl methylene), 4.33 (2H, t, J=7.52 Hz, N(1)-C**H**$_2$CH$_2$CH$_2$CH$_3$), 2.15~2.12 (6H, 2s, C(5)-methyl and acetate methyl), 1.76 (2H, quintet, J=7.79 Hz, N(1)-CH$_2$C**H**$_2$CH$_2$CH$_3$), 1.40 (2H, sextet, J=7.50 Hz, N(1)-CH$_2$CH$_2$C**H**$_2$CH$_3$), 0.97 (3H, t, J=7.33, N(1)-CH$_2$CH$_2$-CH$_2$C**H**$_3$).

2-(Propionyloxymethyl)-1-ethyl-5-methylbenzimidazole-4,7(1*H*)-dione (12e): A solution of **9b** (10 mg, 0.045 mmol) and propanoyl chloride (0.0079 ml, 0.090 mmol) in dry methylene chloride (2 ml) was refluxed for 48 h. The reaction mixture was concentrated *in vacuo,* and chromatography of the residue on a silica gel column with ethyl acetate as a eluent gave **12e** (8.1 mg, 65%) as a red viscous liquid; TLC (ethyl acetate) Rf=0.62; IR (KBr) 2985, 2923, 2852, 1744, 1656, 1532, 1459, 1378, 1342, 1173, 1123, 1083 cm^{-1}; ^1H NMR ($CDCl_3$) 6.48 (H, s, C(6)-proton), 5.27 (2H, s, ethoxymethyl methylene), 4.39 (2H, q, J=7.18 Hz, N(1)-C**H**$_2$CH$_3$), 2.40 (2H, q, J=7.50, COC**H**$_2$CH$_3$), 2.15 (3H, s, C(5)-methyl), 1.43 (3H, t, J=7.20 Hz, N(1)-CH$_2$C**H**$_3$), 1.17 (3H, t, J=7.58 Hz, COCH$_2$C**H**$_3$).

2-(Valeryloxymethyl)-1-ethyl-5-methylbenzimidazole-4,7(1*H*)-dione (12f): A solution of **9b** (10 mg, 0.045 mmol) and valeryl chloride (0.011 ml, 0.090 mmol) in dry methylene chloride (2 ml) was refluxed for 30 h. The reaction mixture was concentrated *in vacuo,* and chromatography of the residue on a silica gel column with ethyl acetate as a eluent gave 12f (9.2 mg, 67%) as a viscous liquid; TLC (ethyl acetate) Rf=0.70; IR (KBr) 2960, 2934, 2870, 1743, 1663, 1524, 1565, 1482, 1459, 1381, 1300, 1269, 1168, 1108 cm^{-1}; ^1H NMR ($CDCl_3$) 6.47 (1H, d, J=1.9, C(6)-proton), 5.26 (2H, s, butoxymethyl methylyene), 4.38 (2H, q, J=7.14 Hz, N(1)-C**H**$_2$CH$_3$), 2.38 (2H, t, J=7.37, COC**H**$_2$CH$_2$-CH$_2$CH$_3$), 2.15 (3H, s, C(5)-methyl), 1.76 (2H, quintet, J=7.41 Hz, COCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.43 (3H, t, J=7.22 Hz, N(1)-CH$_2$C**H**$_3$), 1.31 (2H, sextet, J=7.40, COCH$_2$-CH$_2$C**H**$_2$CH$_3$), 0.91 (3H, t, J=6.96 Hz, COCH$_2$CH$_2$CH$_2$-C**H**$_3$).

2-(Propionyloxymethyl)-1-butyl-5-methylbenzimidazole-4,7(1*H*)-dione (12g): The same procedure as described above, using **9d** (5 mg, 0.02 mmol), gave a residue after 24 h. Chromatography of the residue on a silica gel column with ethyl acetate/methanol (95:5) as a eluent gave **12g** (3.5 mg, 57%) as a red viscous liquid; TLC (ethyl acetate) Rf=0.68; IR (KBr) 2961, 2930, 2874, 1744, 1662, 1509, 1460, 1377, 1274, 1169, 1128, 1081, 1022 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.47 (1H, d, J=1.9 Hz, C(6)-proton), 5.26 (2H, s, ethoxymethyl methylyene), 4.32 (2H, t, J=7.69 Hz, N(1)-C**H**$_2$-CH$_2$CH$_2$CH$_3$), 2.40 (2H, q, J=7.47 Hz, COC**H**$_2$CH$_3$), 2.14 (3H, d, J=1.66 Hz), C(5)-methyl), 1.75 (2H, quintet, J=7.40 Hz, N(1)-CH$_2$C**H**$_2$CH$_2$CH$_3$), 1.40 (2H, sextet, J=7.50 Hz, N(1)-CH$_2$CH$_2$C**H**$_2$CH$_3$), 1.12 (3H, t, J=7.68, N(1)-CH$_2$CH$_2$CH$_2$C**H**$_3$), 0.97 (3H, t, J=7.20 Hz, COCH$_2$-C**H**$_3$).

2-(Valeryloxymethyl)-1-butyl-5-methylbenzimidazole-4,7(1*H*)-dione (12h): The same procedure as described above, using **9d** (10 mg, 0.04 mmol) and valeryl chloride (0.011 ml, 0.090 mmol), gave a residue after 48 h. Chromatography of the residue on a silica gel column with ethyl acetate as a eluent gave **12h** (8.2 mg, 62%) as a viscous liquid; TLC (ethyl acetate) Rf= 0.72; IR (KBr) 2960, 2923, 2853, 1756, 1739, 1655, 1570, 1509, 1459, 1383, 1262, 1165, 1108, 1027 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.47 (1H, d, J=1.97 Hz, C(6)-proton), 5.25 (2H, s, butoxymethyl methylyene), 4.32 (2H, t, J= 7.78 Hz, N(1)-C**H**$_2$CH$_2$CH$_2$CH$_3$), 2.37 (2H, t, J=7.34, COC**H**$_2$CH$_2$CH$_2$CH$_3$), 2.14 (3H, s, C(5)-methyl), 1.73 (2H, quintet, J=7.92 Hz, N(1)-CH$_2$C**H**$_2$CH$_2$CH$_3$), 1.64 (2H, quintet, J=7.46 Hz, COCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.41 (2H, sextet, J=7.70 Hz, N(1)-CH$_2$CH$_2$C**H**$_2$CH$_3$), 1.34 (2H, sextet, J=7.03, COCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.97 (3H, t, J= 7.26, N(1)-CH$_2$CH$_2$CH$_2$C**H**$_3$), 0.91 (3H, t, J=6.91 Hz, COCH$_2$CH$_2$CH$_2$C**H**$_3$).

2-(Acetoxymethyl)-6-(*N*-aziridinyl)-1-butyl-5-methylbenzimidazole-4,7(1*H*)-dione (10d): The same procedure as described above, but using 2-acetoxymethyl quinone **8d** (7 mg, 0.024 mmol) at room temperature 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 **10d** (1.7 mg, 21%) as a red viscous liquid; TLC (ethyl acetate/methanol=95/5) Rf=0.61; IR (KBr) 2960, 2928, 2872, 2854, 1752, 1656, 1586, 1535, 1378, 1344, 1227, 1034 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.16 (2H, s, acetoxymethyl methylene), 4.24 (2H, t, J=7.71 Hz, N(1)-C**H**$_2$CH$_2$CH$_2$-CH$_3$), 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$_2$C**H**$_2$CH$_2$CH$_3$), 1.34 (3H, sextet, J=7.52 Hz, N(1)-CH$_2$CH$_2$C**H**$_2$CH$_3$), 0.91 (3H, t, J=7.41, N(1)-CH$_2$CH$_2$CH$_2$C**H**$_3$).

6-(N-Aziridinyl)-2-(propionyloxymethyl)-1-ethyl-5-methylbenzimidazole-4,7(1*H*)-dione (13e): To a solution of 2-ethoxymethyl quinone **12e** (5 mg, 0.018 mmol) in dry methanol (1.5 ml), chilled at 0°C, was added ethylenimine (0.047 ml, 0.9 mmol). 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 ethyl acetate/methanol (95:5) as a eluent gave **13e** (1.4 mg, 24%) as a red viscous liquid; TLC (ethyl acetate/methanol=95/5) Rf=0.57; IR (KBr) 2987, 2924, 2853, 1743, 1655, 1586, 1643, 1378, 1342, 1272, 1163, 1082, 1031 cm^{-1}; ^1H NMR ($CDCl_3$) 5.24 (2H, s, ethoxymethyl methylyene), 4.37 (2H, t, J=7.15 Hz, N(1)-C**H**$_2$CH$_3$), 2.37 (2H, t, J=7.49, COC**H**$_2$CH$_2$CH$_2$CH$_3$), 2.33 (4H, s, aziridine protons), 2.13 (3H, s, C(5)-methyl), 1.66 (2H, quintet, J=6.91 Hz, COCH$_2$CH$_2$C**H**$_2$CH$_3$), 1.43 (3H, t, J=7.13 Hz, N(1)-CH$_2$C**H**$_3$), 1.34 (2H, sextet, J= 7.20, COCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.91 (3H, t, J=7.22 Hz, COCH$_2$CH$_2$CH$_2$C**H**$_3$).

6-Aziridinyl-2-(valeryloxymethyl)-1-ethyl-5-methylbenzimidazole-4,7(1*H*)-dione (13f): To a solution of 2-butoxymethyl quinone **12f** (5 mg, 0.016 mmol) in dry methanol (1.5 ml), chilled at 0°C, was added ethylenimine (0.043 ml, 0.8 mmol). 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 ethyl acetate as a eluent gave **13f** (1.2 mg, 22 %) as a red viscous liquid; TLC (ethyl acetate/methanol=95/5) Rf=0.65; IR (KBr) 2960, 2929, 2873, 1743, 1655, 1540, 1459, 1378, 1342, 1262, 1161 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.29 (2H, s, butoxymethyl methylyene), 4.37 (2H, q, J=7.15 Hz, N(1)-C**H**$_2$CH$_3$), 2.37 (2H, t, J=7.49 Hz, COC**H**$_2$CH$_2$CH$_2$CH$_3$), 2.33 (4H, aziridine protons), 2.13 (3H, s, C(5)-methyl), 1.62 (2H, quintet, J=7.77 Hz, COCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.43 (3H, t, J=7.13, N(1)-CH$_2$C**H**$_3$), 1.34 (2H, sextet, J=6.73 Hz, COCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.91 (3H, t, J=7.22 Hz, COCH$_2$CH$_2$CH$_2$C**H**$_3$).

6-(*N*-Aziridinyl)-1-butyl-2-(propionyloxymethyl)-5-methylbenzimidazole-4,7(1*H*)-dione (13g): The same procedure as described above, using **12g** (5 mg, 0.016 mmol), gave a residue after 4 h. Chromatography of the residue on a silica gel column with ethyl acetate/methanol (95:5) as a eluent gave **13g** (1.2 mg, 21%) as a red viscous liquid; TLC (ethyl acetate/methanol= 95/5) Rf=0.66; IR (KBr) 2961, 2925, 2852, 1736, 1655, 1542, 1459, 1377, 1342, 1250, 1165, 1071, 1030 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.24 (2H, s, ethoxymethyl methylene), 4.31 (2H, t, J=7.71 Hz, N(1)-C**H**$_2$CH$_2$CH$_2$CH$_3$), 2.39 (2H, q, J=7.72, COC**H**$_2$CH$_3$), 2.33 (4H, s, aziridine protons), 2.13 (3H, s, C(5)-methyl), 1.75 (2H, quintet, J=7.60 Hz, N(1)-CH$_2$C**H**$_2$CH$_2$CH$_3$), 1.41 (3H, sextet, J=7.47 Hz, N(1)-CH$_2$CH$_2$C**H**$_2$CH$_3$), 1.16 (3H, t, J=7.41, N(1)-CH$_2$CH$_2$CH$_2$C**H**$_3$), 0.98 (3H, t, J=6.99 Hz, COCH$_2$C**H**$_3$).

6-Aziridinyl-2-(valeryloxymethyl)-1-butyl-5-methylbenzimidazole-4,7(1*H*)-dione (13h): The same procedure as described above, using **12h** (5 mg, 0.015 mmol), gave a residue after 3 h. Chromatography of the residue on a silica gel column with ethyl acetate as a eluent gave **13h** (1.3 mg, 24%) as a red viscous liquid; TLC (ethyl acetate/methanol=95/5) Rf=0.71; IR (KBr) 2958, 2926, 2867, 1743, 1586, 1543, 1459, 1378, 1343, 1249, 1161 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.23 (2H, s, butoxymethyl methylyene), 4.30 (2H, t, J=7.73 Hz, N(1)-C**H**$_2$CH$_2$CH$_2$CH$_3$), 2.36 (2H, t, J=7.10, COC**H**$_2$-CH$_2$CH$_2$CH$_3$), 2.33 (4H, s, aziridine protons), 2.13 (3H, s, C(5)-methyl), 1.75 (2H, quintet, J=6.85 Hz, N(1)-CH$_2$-C**H**$_2$CH$_2$CH$_3$), 1.59 (2H, quintet, J=7.03 Hz, COCH$_2$C**H**$_2$-CH$_2$CH$_3$), 1.41 (2H, sextet, J=7.70 Hz, N(1)-CH$_2$CH$_2$C**H**$_2$-CH$_3$), 1.34 (2H, sextet, J=7.03, COCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.97 (3H, t, J=7.06, N(1)-CH$_2$CH$_2$CH$_2$C**H**$_3$), 0.91 (3H, t, J=7.49 Hz, COCH$_2$CH$_2$CH$_2$C**H**$_3$).

RESULTS AND DISCUSSION

Chemistry

Preparation of the benzimidazole ring was carried out as shown in Fig. 2. Acylation of 3-amino-4-nitrotoluene, the starting material, with trifluoroacetic anhydride gave trifluoroacetylated compound (**1**) in high yield (91%), which was then reacted with alkyl (Me, Et, Pr, and Bu) iodide in the presence of potassium hydroxide to afford **2a-d**. This two-step procedure resulted in monoalkylation on amino group of 3-amino-4-nitrotoluene. Formation of benzimidazole ring was completed by utilizing Phillips reaction (Phillips, 1928). The dihydrochloride salts (**3a-d**) of the amino compounds, which were obtained by catalytic hydrogenation of **2a-d** with hydrogen over 10% palladium on carbon, were reacted with 85% glycolic acid in the presence of acid catalyst to give 2-(hydroxymethyl) benzimidazoles (**4a-d**). Reactions of **4a-d** with acetic anhydride gave 2-(acetoxymethyl)benzimidazoles (**5a-d**) in 81~91% yield.

The conversion of benzimidazoles into quinones requires the presence of free amino group at 4- or 7-position of benzimidazole. However, the direct nitration of **5a-d** gave predominantly 6-nitro isomers rather than 4-nitro isomers due to steric effect. Therefore, after 6-position of benzimidazoles was protected, nitration of them was carried out. Bromination of these benzimidazoles, as a protecting reaction, gave selectively 6-bromo benzimidazoles (**6a-d**) in 75~79% yield. Nitration of these brominated 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 (**7a-d**), in which more 7-nitro isomers were obtained than 4-nitro isomers. The product ratios were 88:12, 81:19, 82:18 and 85:15. These mixtures 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 chromatograpy 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 was then carried out.

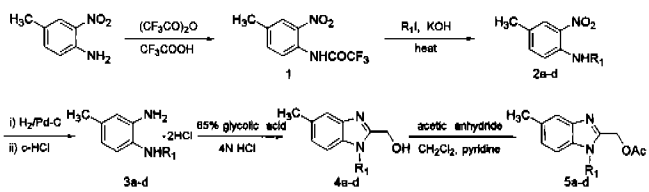

Fig. 2. Synthesis of 2-(acetoxymethyl)benzimidazoles (**5a-d**).

Although we expected only acetylated quinones under these conditions, a mixture of acetylateds and deacetylated quinones started to form before the starting materials were completely consumed. As soon as the spot of starting materials disappeared on TLC (eluent, ethyl acetate), these reactions were quenched. These mixtures were separated by chromatography on silica gel (eluent, ethyl acetate/methanol=95:5) to give acetylateds (**8a-c**) and deacetylated quinones (**9a-d**). The deacetylated quinones were identified as the signal of acetyl group did not appear on the ^{1}H NMR spectrum when compared with those acetylated. However, catalytic hydrogenation of **7d** following Fremy oxidation gave only deacetylated quinone (**9d**) under the same conditions. 1,4-Addition of **8a-c** with aziridine (March and Joullie, 1970) gave aziridinyl quinones (**10a-c**) in low yield (20~22%). These reactions were completed within 3-4 hours. Reaction for more than 4 hour resulted in the formation of deacetylated quinones. **9a-d** were reacted with aziridine to give corresponding aziridinyl quinones (**11a-d**). These unexpected deacetylated aziridinyl quinones, **11a-d**, were utilized to examine whether acetyl group of acetylated aziridinyl quinone would contribute to their biological activity or not (Fig. 3).

To investigate the effect of the 2-substituents on the cytotoxicity of the 6-aziridinylbenzimidazole-4,7-diones, derivatives with increased lipophilicity were synthesized. First, acetylation of **9d** with acetic anhydride afforded the acetylated quinone (**8d**) in 75% yield. Using the procedure as described above, reaction of **8d** with aziridine gave **10d** in 21% yield and then accompanied by deacetylated quinones. On the ^{1}H NMR spectrum, the signal of C(6)-proton disappeared, as the signal of aziridinyl group appeared at 2.33 ppm. Next, esterification of **9b,d** with propanoyl

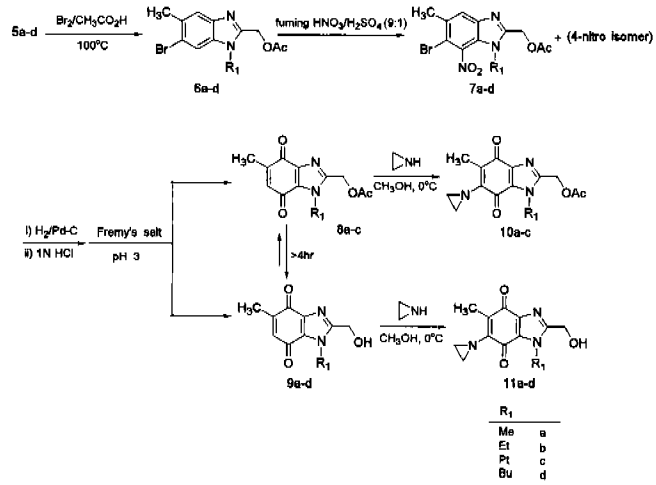

Fig. 3. Synthesis of 6-aziridinyl-2-(acetoxymethyl)benzimidazole-4,7-diones (**10a-c**) and 6-aziridinyl-2-(hydroxymethyl)benzimidazole-4,7-diones (**11a-d**).

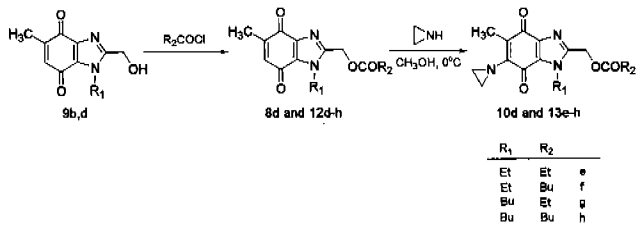

Fig. 4. Synthesis of esters (**10d** and **13e-h**) of 6-aziridinyl-2-(hydroxymethyl)benzimidazole-4,7-diones.

chloride gave **12e,g**, which were followed by reaction with aziridine to give **13e,g** in 24 and 21% yield, respectively. Also, from reactions of **9b,d** with valeryl chloride, aziridinyl quinones (**13f,h**) were obtained by the same two step procedure and identified by the peaks (2.33 ppm) of the aziridine ring, shown on the ^{1}H NMR spectrum (Fig. 4).

Biological activities

Using the MTT method (Carmichel *et al.*, 1986), the cytotoxic activities of the synthesized 6-aziridinyl-benzimidazole-4,7-diones (**10a-d**, **11a-d**, and **13e-h**) were evaluated against P388, B16, and SNU-16. Of these three cell lines, SNU-16 was most sensitive to all synthesized compounds. By contrast, SNU-16 cancer cell line was resistant to mitomycin C. These results suggest that 6-aziridinylbenzimidazoles is more cytotoxic to human cancer cells than to mouse cell lines. Also, this fact is well compatible with the former results that isoazamitosene having common structure with these benzimidazole-4,7-diones were more cytotoxic to KHH (human gastric cancer cell) than to mouse cancer cell lines such as P388 and B16. In general, the cytotoxicities of 6-aziridinyl-2-(acetoxymethyl) (**10a-d**) and 6-aziridinyl-2-(hydroxymethyl)benzimidazole-4,7-dione derivatives (**11a-d**) were higher than those of esters (**13e-h**) of 2-(hydroxymethyl)derivatives. Interestingly, these compounds (**10,11**) have the same or higher cytotoxicities as compared to mitomycin C against SNU-16 cell line. As given in Table I, 6-aziridinyl-2-(hydroxymethyl)benzimidazole-4,7-dione derivatives (**11a-d**) were slightly more cytotoxic than the corresponding 2-(acetoxymethyl) derivatives (**10a-d**). Substitution at 2-position of these benzimidazole-4,7-diones, which was designed to increase lipophilicity, had little effect on cytotoxicity improvement. Despite preferential activity of these benzimidazole-4,7-diones to human gastric cancer cell line, how they could inhibit the cancer cellular growth *in vitro* is yet to be settled. However, the above results indicate that 6-aziridinylbenzimidazole-4,7-dione derivatives shows potent cytotoxicity against mouse and human cancer cells, especially strong activity against human gastric tumor cells. It appears that pyrrole ring in the structure of isoazamitosenes is not essential for their cytotoxicity, since the cytotoxic potencies of benzimidazole derivatives are similar to those of isoazamitosenes which were determined in the previous studies (Ahn and Kim, 1996).

Table I. IC_{50} values of 6-aziridinylbenzimidazole derivatives on various cancer cell lines as determined by MTT assay

Compound	IC_{50} (μg/ml) of tumor cell lines		
	P388	B16	SNU-16
10a	0.304	0.317	0.025
10b	1.136	1.255	0.072
10c	0.908	0.394	0.246
10d	1.196	1.665	0.353
11a	0.334	0.317	0.021
11b	0.320	0.241	0.063
11c	0.825	0.629	0.115
11d	1.368	1.027	0.012
13e	2.149	1.223	0.380
13f	2.204	10.29	0.873
13g	1.948	9.175	0.399
13h	0.635	1.821	0.273
MMC[a]	0.046	0.075	0.369

[a]MMC stands for mitomycin C

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