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Synthesis and *in vitro* Antitumor Activity of Isoazamitosene and Isoiminoazamitosene Derivatives

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Seven isoazamitosene derivatives, mitomycin analogues, were synthesized and tested for cytotoxicities against leukemia and gastric cancer cell lines. Preparation of a pyrrolo[1,2-a]benzimidazole (**3**) (azamitosene ring system) was completed by utilizing the Lewis acid-catalized cyclization, with *o*-chloronitrotoluene as the starting material. Nitration of **3** produced a mixture of two isomers (5-nitro isomer (**4**) and 7-nitro isomer (**5**)) in product ratio of 36 : 52. **4** was directly converted into quinone (**7**) by reduction and Fremy oxidaton. Finally, quinone derivatives (**8**, **9**, **10**, and **11**) were synthesized by 1,4-addition of **7** with cyclic secondary amines. From above-mentioned **5**, 8-nitro compound (**15**) was prepared in 4 steps. At pH 3, Fremy oxidation of **15** produced quinone (**16**), whereas iminoquinone derivatives (**17a** and **17b**) at pH 7. Isoazamitosene derivatives (**8**, **9**, **10**, and **11**), containing cyclic amino groups at the 7-position, showed potent cytotoxicity on P388, SNU-1, and KHH tumor cell lines. Among them, **8** had stronger cytotoxicity against SNU-1 cell line than mitomycin and adriamycin. Considering these results, isoazamitosene derivatives may had unique cytotoxicity profiles. However, isoiminoazamitosene derivatives (**17a** and **17b**) revealed very weak cytotoxicity.

Key words : Mitomycin, Isoazamitosene, Cytotoxicity, Reductive alkylating agent

INTRODUCTION

Mitomycins and mitosenes are well-known examples of reductive alkylating quinones (Moore, 1977; Moore and Czerniak, 1981; Andrews *et al.*, 1986; Keyes *et al.*, 1985; Tomasz *et al.*, 1986) (Fig. 1). Alkylating quinone methide species which are formed upon reduction of the quinones and subsequent elimination of leaving groups are highly reactive and produce cytotoxic effects by reacting with DNA (nucleophile) of tumor cells. Because tumor cells possess a low reduction potential environment, reductive alkylating quinones such as mitomycins and mitosenes can be more selectively cytotoxic within rapidly-dividing tumor cells than within normal cells (Kennedy *et al.*, 1980).

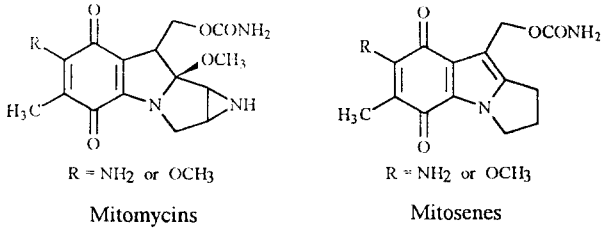

Fig. 1. Structures of mitomycins and mitosenes.

The reductive alkylating quinones can generate toxic oxygen species by cycling between the quinone and hydroquinone forms of the agent (Doroshow, 1983; Begleiter, 1983). The formation of these toxic substances have made a clinical application less effective. To reduce toxicity of quinones and optimize their antitumor activity, many mitomycin and mitosene derivatives have been synthesized (Iyengar *et al.*, 1983; Sami *et al.*, 1984; Vyas *et al.*, 1987; Fishbein, 1987). Recently, pyrrolo[1,2-a]benzimidazole (PBI)-based antitumor agents (azamitosenes) designed

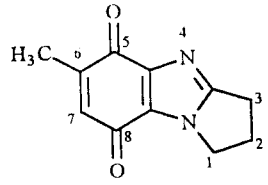

Fig. 2. Skeleton of isoazamitosene.

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as new DNA cross-linkers, mimicking the mitomycin antitumor agents, were reported to show potent cytostatic activity (Islam and Skibo, 1990; Islam and Skibo, 1991). Some of them were much more active than mitomycin C against human tumor cell line. In particular, azamitosenes had a stronger cytotoxicity against solid tumor cell line than against leukemia cell line (Skibo and Schulz, 1993). However, there is few reports on the inhibition of gastric cancer cell growth by azamitosene. The purposes of this study were to synthesize isoazamitosene and isoiminoazamitosene derivatives, and to evaluate their antigastric cancer activity (Fig. 2).

MATERIALS AND METHODS

Cancer cell lines

Tested cancer cell lines for cytotoxicity test were as follows : P388 (lymphocytic leukemia, mouse), SNU-1 (gastric adenocarcinoma, human), KHH (gastric adenocarcinoma, human). 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.

Measurement of cytotoxicity

For measurement of cytotoxicity by isoazamitosene, MTT method (Carmichel *et al.*, 1987) was used. To compare cytotoxicity among compounds, IC_{50} value, 50% inhibition of cell growth, was calculated by regression analysis (plotting the viability versus the concentration of the test compound).

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 (300 MHz) and/ or Varian Gemini 200 (200 MHz) NMR spectrometer. The chemical shifts were recorded as δ values in parts per million relative to tetramethylsilane as an internal standard and *J*-values were in Hz. When necessary, chemicals were purified according to the reported procedures (Perrin *et al.*, 1980).

4-(N-Pyrrolidino)-3-nitrotoluene (1)

A solution of 4-chloro-3-nitrotoluene 17.2 g (0.1 mol) and pyrrolidine 25 ml (21.3 g, 0.3 mol) was heated at reflux for 3 hour. The reaction mixture was cooled and poured over 300 g of cracked ice. The resulting mixture was extracted three times with 200 ml of chloroform. The combined extracts was dried over magnesium sulfate and then concentrated under reduced pressure. The residue was purified by chromatography on silica gel (eluent, chloroform) to give **1** (19 g, 91%). mp 62-64°C; TLC (ethyl acetate/ methanol=95/5) *Rf*=0.61; IR(KBr) 3015, 2883, 2693, 1626, 1552, 1471, 1368, 1269, 1183 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 7.53-6.94 (3H, aromatic protons), 3.10 (4H, m, pyrrolidine methylene adjacent to nitrogen), 2.24 (3H, s, methyl), 1.90 (4H, m, other pyrrolidine methylene).

3-Acetoxy-6-methyl-2,3-dihydro-1H-pyrrolo[1,2-a] benzimidazole (3)

A mixture of **1** (18.0 g, 87 mmol), anhydrous $ZnCl_2$ 12.4 g (91 mmol), and acetic anhydride 100 ml was stirred at 90°C for 1 hour. The solvent was evaporated *in vacuo*, and the brown residue was dissolved in 800 ml of chloroform. The chloroform solution was washed with water, dried over sodium sulfate, and concentrated under reduced pressure to afford the crude product. Chromatography of the crude product on silica gel (eluent, ethyl acetate/methanol= 95/5) gave **2**. A solution of **2** in acetic anhydride 20 mL was stirred at room temperature for 2 hour. The excess acetic anhydride was removed *in vacuo*, and the solid residue was recrystallized from n-hexane-chloroform to give **3** (12.6 g, 63%). mp 199-201°C; TLC (chloroform/methanol=95/5) *Rf*=0.59; IR(KBr) 3031, 2901, 1745, 1537, 1496, 1370, 1290, 1232, 1266, 1248, 1082, 1052 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 7.65-7.11 (3H, aromatic protons), 6.13 (1H, dd, *J*=7.7 Hz and *J*=3.3 Hz, C(3)-proton coupled with C(2)-methylene), 4.23 (1H, m, one of C(1)-protons), 4.16 (1H, m, other one of C(1)-protons), 3.13 (1H, m, one of C(2)-protons), 2.58 (1H, m, other one of C(2)-protons), 2.43 (3H, s, C(6)-methyl), 2.09 (3H, s, acetate methyl).

Compound 2 : mp 181-183°C; TLC (chloroform/methanol=8/2) *Rf*=0.35; IR(KBr) 3027, 2957, 1494, 1451, 1363, 1283, 1243, 1208, 1023, 827 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 7.62-7.26 (3H, aromatic protons), 4.29 (2H, t, C(3)-methylene), 3.26 (2H, t, C(1)-methylene), 2.70 (2H, m, C(2)-methylene), 2.41 (C(6)-methyl).

3-Acetoxy-6-methyl-5(and 7)-nitro-2,3-dihydro-1H-pyrrolo[1,2-a]benzimidazole (4 and 5)

A solution of **3** (9.2 g, 40 mmol) in 40 ml of a 9 : 1 mixture of fuming nitric acid and concentrated sulfuric acid was stirred in an ice bath for 10 min. The reaction mixture was poured over cracked ice, and the pH of the resulting solution was adjusted to pH 6. 5 with ammonia water. The reaction mixture was extracted three times with 300 ml of chloroform. The combined extracts was dried over magnesium sulfate, and then concentrated under reduced pressure. The resulting mixture was chromatographed on silica gel

(eluent, ethyl acetate/methanol=95/5) to give **4** (3.96 g, 36%) and **5** (5.72 g, 52%).

Compound 4 : mp 133-135°C; TLC (chloroform/methanol=95/5) *Rf*=0.56; IR (KBr) 3042, 2912, 1743, 1534, 1436, 1367, 1328, 1232, 1083, 1036 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.42 (1H, d, one of aromatic protons), 7.16 (1H, d, other one of aromatic protons), 6.18 (1H, dd, *J*=7.48 Hz and *J*=3.19 Hz, C(3)-proton coupled with C(2)-methylene), 4.31 (1H, m, one of C(1)-protons), 4.17 (1H, m, other one of C(1)-protons), 3.21 (1H, m, one of C(2)-protons), 2.68 (1H, m, other one of C(2)-protons), 2.52 (3H, s, C(6)-methyl), 2.10 (3H, s, acetate methyl).

Compound 5 : mp 182-184°C; TLC (chloroform/methanol=95/5) *Rf*=0.63; IR (KBr) 3056, 2885, 1748, 1523, 1364, 1230, 1067, 821 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.13 and 7.63 (2H, 2s, aromatic protons), 6.14 (1H, dd, *J*=7.69 Hz and *J*=3.60 Hz, C(3)-proton coupled with C(2)-methylene), 4.31 (1H, m, one of C(1)-protons), 4.17 (1H, m, other one of C(1)-protons), 3.21 (1H, m, one of C(2)-protons), 2.68 (1H, m, other one of C(2)-protons), 2.52 (3H, s, C(6)-methyl), 2.10 (3H, s, acetate methyl).

3-Acetoxy-5-amino-6-methyl-2,3-dihydro-1H-pyrrolo [1,2-a]benzimidazole (6)

A solution of **4** (3.30 g, 12 mmol) in 200 ml of methanol containing 0.24 mg of 10% Pd on charcoal was stirred under H_2 for 4 hour. The mixture solution was filtered through Celite, and was washed with methanol. The filtrate was concentrated under reduced pressure. The crude residue was recrystallized from n-hexane-chloroform to give **6** (1.97 g, 67%). mp 197-199°C; TLC (ethyl acetate/methanol=95/5) *Rf*= 0.46; IR (KBr) 3443, 2920, 1737, 1631, 1500, 1300, 1241, 1083, 1032 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.97 (1H, d, one of aromatic protons), 6.68 (1H, d, other one of aromatic protons), 6.11 (1H, dd, *J*=7.35 Hz and *J*= 2.98 Hz, C(3)-proton coupled with C(2)-methylene), 4.28 (2H, amine protons), 4.21 (1H, m, one of C(1)-protons), 4.05 (1H, m, other one of C(1)-protons), 3.17 (1H, m, one of C(2)-protons), 2.62 (1H, m, other one of C(2)-protons), 2.52 (3H, s, C(6)-methyl), 2.11 (3H, s, acetate methyl).

3-Acetoxy-6-methyl-2,3-dihydro-1H-pyrrolo[1,2-a] benzimidazole-5,8-dione (7)

A solution of **6** (1.65 g, 6.7 mmol) in methanol 20 ml containing a few drops of 1N HCl was evaporated to give a salt. To a suspension of the salt in 90 ml of water containing 0.89 g of monobasic potassium phosphate was added dropwisely a solution of 13.2 g of Fremy's salt in 450 ml of water containing 4.47 g of monobasic potassium phosphate. The reaction mixture was stirred at room temperature for 1.5 hour and then extracted three times with 500 ml of chloroform. The combined extracts were dried over magnesium sulfate, and then concentrated under reduced pressure to afford the crude product. Flash chromatography of the crude product on silica gel (eluent, ethyl acetate/methanol=95/5) gave **7** (0.66 g, 38%). The product was recrystallized from n-hexane-chloroform. mp 136-137°C; TLC (ethyl acetate/ methanol= 95/5) *Rf*=0.54; IR (KBr) 2859, 1738, 1675, 1666, 1657, 1516, 1375, 1229, 1137, 1028, 980 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.49 (1H, q, *J*=1.6 Hz, C(7)-proton split by methyl), 6.10 (1H, dd, *J*=7.63 Hz and *J*=3.12 Hz, C(3)-proton coupled with C(2)-methylene), 4.35 (2H, m, C(1)-diastereomeric methylene), 3.17 (1H, m, one of C(2)-protons), 2.65 (1H, m, other one of C(2)-protons), 2.16 (3H, d, *J*=1.6 Hz, C(6)-methyl), 2.11 (3H, s, acetate methyl).

3-Acetoxy-7-N-aziridinyl-6-methyl-2,3-dihydro-1H-pyrrolo[1,2-a]benzimidazole-5,8-dione (8)

To a solution of **7** (0.04 g, 0.15 mmol) in 5 ml of dry methanol was added 0.16 ml of ethylenimine. The reaction mixture was stirred at ice salt bath for 1 hour and then at room temperature for 2 hour. The solvent was then removed *in vacuo*, and the crude residue was flash chromatographed on silica gel (eluent, ethyl actate/methanol=95/5). Recrystallization of the purified product with n-hexane-chloroform gave **8** (8 mg, 18%). mp 75-76°C; TLC (chloroform/ methanol=90/10) *Rf*=0.51; IR (KBr) 2913, 1745, 1669, 1647, 1529, 1378, 1344, 1248, 1144, 1080 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.06 (1H, dd, *J*=7.27 Hz and *J*=3.24 Hz, C(3)-proton coupled with C(2)-methylene), 4.32 (2H, m, C(1)-diastereomeric methylene), 3.16 (1H, m, one of C(2)-protons), 2.63 (1H, m, other one of C(2)-protons), 2.31 (4H, s, aziridinyl protons), 2.12 and 2. 09 (6H, 2s, C(6)-methyl and acetate methyl).

3-Acetoxy-6-methyl-7-N-pyrrolidinyl-2,3-dihydro-1H-pyrrolo[1,2-a]benzimidazole-5,8-dione (9)

The same procedure described above was employed for the preparation of **9** by using pyrrolidine (0.27 ml) as a amine. (12 mg, 24%). mp 99-101°C; TLC (ethyl acetate/ methanol=95/5) *Rf*=0.38; IR (KBr) 2938, 2360, 1744, 1663, 1633, 1626, 1520, 1374, 1241, 1080 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.05 (1H, dd, *J*= 7.43 Hz and *J*=3.36 Hz, C(3)-proton coupled with C (2)-methylene), 4.33 (2H, m, C(1)-diastereomeric methylene), 3.66 (4H, t, pyrrolidinyl methylene adjacent to nitrogen), 3.16 (1H, m, one of C(2)-protons), 2.62 (1H, m, other one of C(2)-protons), 2.12 and 2. 08 (6H, 2s, C(6)-methyl and acetate methyl), 1.92 (4H, t, other pyrrolidinyl methylene).

3-Acetoxy-6-methyl-7-N-pyrrolinyl-2,3-dihydro-1H-pyrrolo[1,2-a]benzimidazole-5,8-dione (10)

The same procedure described above was employed for the preparation of **10** by using pyrroline (0.26 ml) as a amine. (9 mg, 18%). mp 65-67°C; TLC (ethyl acetate/ methanol=95/5) *Rf*=0.36; IR (KBr) 2925, 2361, 1667, 1642, 1618, 1519, 1985, 1292, 1081 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.69-6.36 (4H, pyrrolinyl diastereomeric methylenes), 6.09 (1H, dd, *J*=7.35 Hz and *J*=3.3 Hz, C(3)-proton coupled with C(2)-methylene), 5.33 (2H, pyrrolinyl protons), 4.34 (2H, m, C(1)-diastereomeric methylene), 3.16 (1H, m, one of C(2)-protons), 2.65 (1H, m, other one of C(2)-protons), 2.12 and 2.09 (6H, 2s, C(6)-methyl and acetate methyl).

3-Acetoxy-6-methyl-7-N-morpholinyl-2,3-dihydro-1H-pyrrolo[1,2-a]benzimidazole-5,8-dione (11)

The same procedure described above was employed for the preparation of **11** by using morpholine (0.32 ml) as a amine. (7 mg, 13%). mp 97-99°C; TLC (ethyl acetate/ methanol=95/5) *Rf*=0.43; IR (KBr) 2937, 1744, 1653, 1645, 1529, 1367, 1235, 1112, 1048 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.08 (1H, dd, *J*=7.45 Hz and *J*=3.29 Hz, C(3)-proton coupled with C(2)-methylene), 4.26 (2H, m, C(1)-diastereomeric methylene), 3.79 (4H, t, morpholinyl methylene adjacent to nitrogen) and 3.33 (4H, t, morpholinyl methylene adjacent to oxygen), 3.17 (1H, m, one of C(2)-protons), 2.64 (1H, m, other one of C(2)-protons), 2.12 and 2.09 (6H, 2s, C(6)-methyl and acetate methyl).

3-Acetoxy-7-amino-6-methyl-2,3-dihydro-1H-pyrrolo[1,2-a]benzimidazole (12)

A solution of **5** (5.5 g, 20 mmol) in 300 ml of methanol containing 0.5 g of 10% Pd on charcoal was stirred under H_2 for 4 hour. The mixture solution was filtered through Celite, washed with methanol, and then the filtrate was evaporated *in vacuo*. Recrystallization of the crude product with n-hexane-chloroform gave **12** (3.16 g, 65%). mp 193-195°C; TLC (chloroform/ methanol=90/10) *Rf*=0.51; IR (KBr) 2929, 1737, 1637, 1573, 1530, 1462, 1371, 1239, 1021 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 7.19 and 6.63 (2H, 2s, aromatic protons), 5.99 (1H, dd, *J*=7.38 Hz and *J*=2.77 Hz, C(3)-proton coupled with C(2)-methylene), 4.78 (2H, s, amine protons) 4.03 (2H, m, C(1)-diastereomeric methylene), 3.04 (1H, m, one of C(2)-protons), 2.49 (1H, m, other one of C(2)-protons), 2.14 (3H, s, C(6)-methyl), 2.04 (3H, s, acetate methyl)

7-Acetamido-3-acetoxy-6-methyl-2,3-dihydro-1H-pyrrolo[1,2-a]benzimidazole (13)

A suspension of **12** (3.0 g, 12 mmol) in 5 ml of acetic anhydride was stirred at room temperature for 1 hour. The solvent was removed *in vacuo*, and the crude residue was recrystallized with n-hexane-chloroform to give **13** (3.10 g, 90%). mp 250-252°C; TLC(chloroform/methanol=90/10) *Rf*=0.46; IR (KBr) 2906, 1736, 1651, 1526, 1451, 1369, 1248, 1099, 1036 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.09 and 7.58 (2H, 2s, aromatic protons), 7.11 (1H, s, amide proton), 6.17 (1H, dd, *J*=7.40 Hz and *J*=3.16 Hz, C(3)-proton coupled with C(2)-methylene), 4.27 (1H, m, one of C(1)-protons), 4.13 (1H, m, other one of C(1)-protons), 3.18 (1H, m, one of C(2)-protons), 2.91 (1H, m, other one of C(2)-protons), 2.39, 2.26, and 2.13 (9H, 3s, C(6)-methyl, acetate, and acetamido methyls).

7-Acetamido-3-acetoxy-6-methyl-8-nitro-2,3-dihydro-1H-pyrrolo[1,2-a]benzimidazole (14)

A solution of **13** (3.0 g, 10 mmol) in 60 ml of a 9 : 1 mixture of fuming nitric acid and concentrated sulfuric acid was stirred in an ice bath for 10 min. The completed reaction was poured over cracked ice, and the pH of the resulting solution was adjusted to pH 6.5 with ammonia water. This solution was extracted three times with 200 mL of chloroform. The combined extracts was dried over magnesium sulfate, and then concentrated under reduced pressure. Recrystallization of the crude product with *n*-hexane-chloroform gave **14** (2.8 g, 84%). mp 254-255°C; TLC (ethyl acetate/methanol=95/5) *Rf*=0.36; IR (KBr) 3424, 3117, 2936, 1746, 1660, 1528, 1371, 1231, 1099, 1031 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 9.84 (1H, s, amide proton), 7.87 (1H, s, aromatic proton), 6.13 (1H, dd, *J*=7.69 Hz and *J*=3.54 Hz, C(3)-proton coupled with C(2)-methylene), 4.25 (1H, m, one of C(1)-protons), 4.17 (1H, m, other one of C(1)-protons), 3.05 (1H, m, one of C(2)-protons), 2.52 (1H, m, other one of C(2)-protons), 2.33 (3H, s, C(6)-methyl), 2.07 and 2.03 (6H, 2s, acetate and acetamido methyls).

7-Acetamido-3-acetoxy-8-amino-6-methyl-2,3-dihydro-1H-pyrrolo[1,2-a]benzimidazole (15)

A solution of **14** (2.7 g, 8 mmol) in 500 ml of methanol containing 0.2 g of 10% Pd on charcoal was stirred under H_2 for 4 hour. The mixture solution was filtered through Celite, and washed with methanol. The solvent was concentrated under reduced pressure. Recrystallization of the crude product with *n*-hexane-chloroform gave **15** (1.47 g, 61%). mp 257-258°C; TLC (chloroform/methanol=90/10) *Rf*=0.20; IR (KBr) 3393, 3229, 3001, 2919, 1740, 1659, 1625, 1530, 1439, 1371, 1234, 1080, 1037 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 8.90 (1H, s, amide proton), 6.75 (1H, s, aromatic proton), 6.02 (1H, dd, *J*=7.41 Hz and *J*=2.95 Hz, C(3)-proton coupled with C(2)-methylene), 4.71 (2H, s, amine protons), 4.47 (1H, m, one of C(1)-protons), 4.44 (1H, m, other one of C(1)-

protons), 3.03 (1H, m, one of C(2)-protons), 2.53 (1H, m, other one of C(2)-protons), 2.12-2.05 (9H, 3s, C(6)-methyl, acetate, and acetamido methyls).

7-Acetamido-3-acetoxy-6-methyl-2,3-dihydro-1H-pyrrolo[1,2-a]benzimidazole-5,8-dione (16)

To a suspension of **15** (0.40 g, 1.32 mmol) in 40 ml of water containing 0.26 g of monobasic potassium phosphate was added a solution of 1.98 g of Fremy's salt in 200 ml of water containing 0.66 g of monobasic potassium phosphate. The reaction mixture was stirred at room temperature for 1.5 hour and then extracted three times with 300 ml of chloroform. The extracts were dried over magnesium sulfate, and concentrated under reduced pressure. Flash chromatography of the crude residue on silica gel (eluent, ethyl acetate/methanol=94/6) gave **16** (0.17 g, 41%). The product was recrystallized from *n*-hexane-chloroform. mp 163-165°C; TLC (chloroform/methanol=90/10) *Rf*=0.50; IR (KBr) 3251, 2882, 1741, 1690, 1663, 1622, 1515, 1409, 1370, 1112, 1021 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.39 (1H, s, amide proton), 6.09 (1H, dd, *J*= 7.65 Hz and *J*=3.10 Hz, C(3)-proton coupled with C (2)-methylene), 4.39 (1H, m, one of C(1)-protons), 4. 28 (1H, m, other one of C(1)-protons), 3.21 (1H, m, one of C(2)-protons), 2.66 (1H, m, other one of C(2)-protons), 2.25, 2.11, and 2.03 (9H, 3s, C(6)-methyl, acetate, and acetamido methyls).

syn/anti-7-Acetamido-3-acetoxy-8-imino-6-methyl-2,3-dihydro-1H-pyrrolo[1,2-a]benzimidazole-5-one (17a and 17b)

To a suspension of **15** (0.8 g, 2.52 mmol) in 65 ml of 0.2 M pH 7.0 phosphate buffer (μ=1.0, KCl) was added a solution of 3.31 g of Fremy's salt in 130 ml of water containing 3.31 g of monobasic potassium phosphate. To assist in dissolution of the Fremy's salt, 100 ml of water was then added to the above mixture. While the mixture was stirred at room temperture for 2 hour, red crystal was formed from solution. The crystal was filtered, washed with a small volume of water and recrystallized with *n*-hexane-chloroform to afford **17a** (*syn*) (0.26 g, 32%). The filtrate was extracted three times with 20 ml of chloroform. Drying the combined chloroform extracts (magnesium sulfate), evaporation *in vacuo*, and finally flash chromatography on silica gel (eluent, chloroform/methanol=90/10) afforded **17b** (*anti*) (0.14 g, 17%).

Compound 17a (*syn*): red; mp 142°C; TLC (chloroform /methanol=95/5) *Rf*=0.31; IR (KBr) 3233, 2880, 1721, 1650, 1633, 1597, 1395, 1228, 1202, 1114 cm^{-1}; ^1H NMR ($CDCl_3$) δ 10.61 and 8.17 (2H, 2s, imine protons), 6.06 (1H, dd, C(3)-proton coupled with C(2)-methylene), 4.31 (2H, m, C(1)-diastereomeric methylene), 3.16 (1H, m, one of C(2)-protons), 2.68 (1H, m, other one of C(2)-protons), 2.28 (3H, s, C(6)-methyl), 2.09 and 1.97 (6H, 2s, acetate and acetamido methyls).

Compound 17b (*anti*): yellow; mp 214-215°C; TLC (chloroform/methanol=90/10) *Rf*=0.58; IR (KBr) 3224, 3202, 2906, 1743, 1685, 1651, 1622, 1509, 1482, 1354, 1221, 1211, 1044 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.87 and 8.03 (2H, 2s, amide and imine protons), 6.05 (1H, dd, C(3)-proton coupled with C(2)-methylene), 4.72 (2H, m, C(1)-diastereomeric methylene), 3.13 (1H, m, one of C(2)-protons), 2.62 (1H, m, other one of C(2)-protons), 2.49 and 2.34 (6H, 2s, C(6)-methyl and acetamido methyl), 2.13 (3H, s, acetate methyl)

RESULTS AND DISCUSSION

Preparation of the azamitosene ring system was carried out by the Lewis acid-catalyzed cyclization of an *o*-nitropyrrolidinobenzene derivative. (Meth-Cohn and Suschitzky, 1972) (Fig. 3). *o*-Chloronitrotoluene was reacted with pyrrolidine to give *o*-nitropyrrolidino toluene (**1**) in high yield (91%). When **1** was reacted with Ac_2O for 5 hour in the presence of $ZnCl_2$, **3** containing acetoxy group at the 3-position was obtained in 51% yield. This reaction was always accompanied by formation of black-tar, which made it difficult to separate the desired product. However, after **1** was reacted with Ac_2O for 1 hour under the same conditons, an intermediate (*Rf*=0.29) was found on TLC (ethyl acetate/methanol=95/5). The intermediate was separated by chromatography on silica gel (eluent, ethyl acetate/methanol=95/5) and identified as 2,3-dihydro-1H-pyrrolo[1,2-a]benzimidazole (**2**) by ^1H NMR spectroscopy. Acetylation of **2** with Ac_2O for 2 hour at room temperature gave **3**. The latter procedure (two steps) gave **3** in higher yield (63%) than the fomer, and it did not produce black-tar. Nitration of **3** with HNO_3/H_2SO_4 gave a mixture of 5-nitro isomer (**4**) and 7-nitro isomer (**5**). When it was separated on chromatography (eluent, ethyl acetate/methanol=95/5), the product ratio was 36 : 52.

Since the conversion of a pyrrolobenzimidazole to a quinone needs the presence of amino group at 5- or 8-position of pyrrolobenzimidazole, it was expected that **4** could be directly converted to the quinone (Fig. 4). Catalytic reduction of 4 followed by Fremy oxidation afford a quinone (**7**). On the ^1H NMR spectrum, signals of aromatic proton disappeared, and a signal of a quinone proton appeared at δ=6.49. Finally, 1,4-additions of a quinone (**7**) were carried out with various nucleophiles including aliphatic amines, cyclic secondary amines, and thiols, among which cyclic secondary amines were reacted to give isoazamitosene derivatives (**8**, **9**, **10**, and **11**) in low yield (13-24%). These reactions were completed

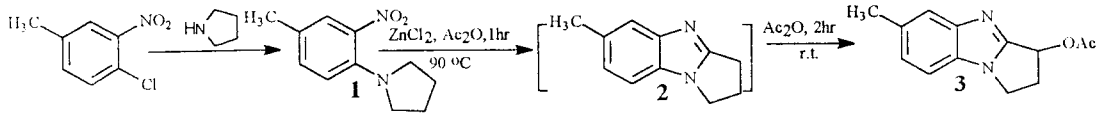

Fig. 3. Preparation of 3-acetoxy-6-methyl-2,3-dihydro-1H-pyrrolo[1,2-a]benzimidazole (**3**) by using the Lewis acid-catalyzed cyclization, with *o*-chloronitrotoluene as the starting material

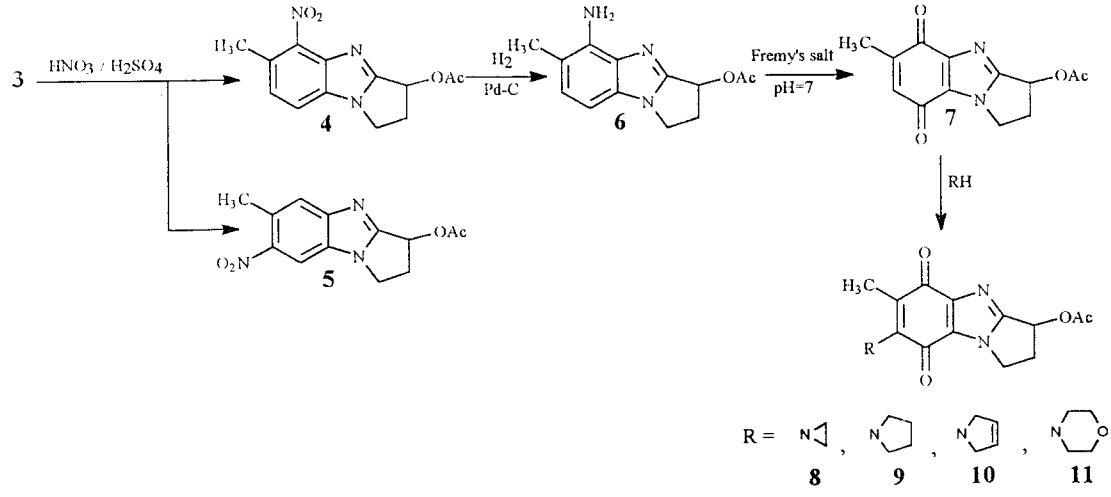

Fig. 4. Preparation of isoazamitosene derivatives (**8**, **9**, **10**, and **11**) from 3-acetoxy-6-methyl-2,3-dihydro-1H-pyrrolo[1,2-a] benzimidazole (**3**)

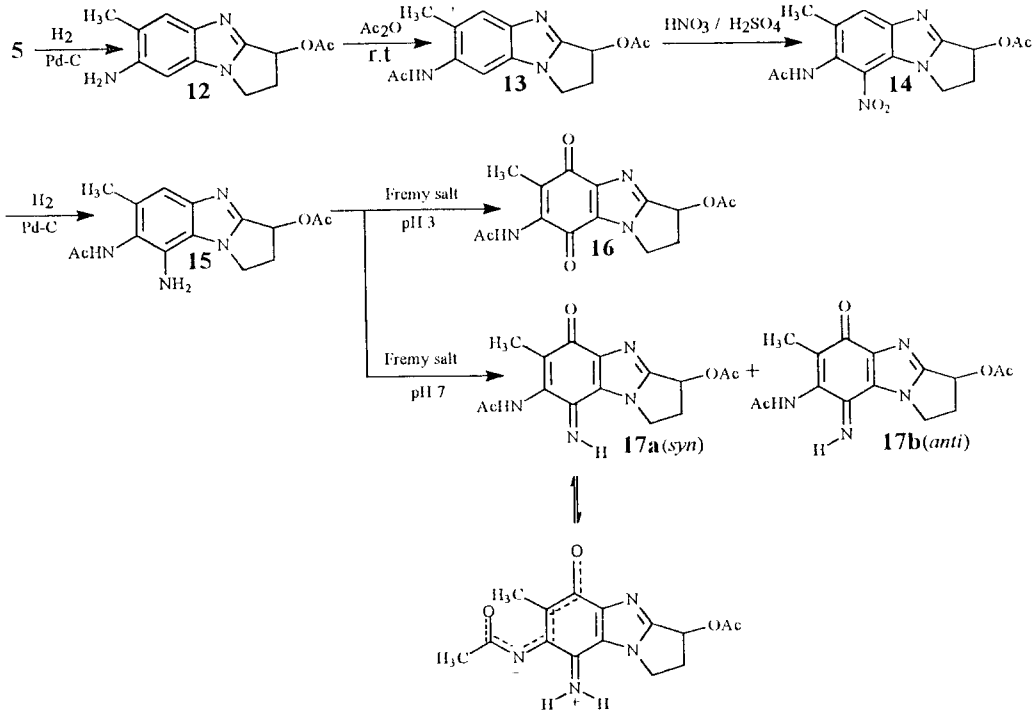

Fig. 5. Preparation of a isoazamitosene derivative (**16**) and isoiminoazamitosene derivatives (**17a** and **17b**) from 3-acetoxy-6-methyl-7-nitro-2,3-dihydro-1H-pyrrolo[1,2-a]benzimidazole (**5**)

within 2 hour, but reaction over 4 hour resulted in the formation of purple-colored unidentified products. Unfortunately, reactions of **7** with other nucleophiles didn't give the desired products.

A synthesis of isoiminoazamitosene derivatives was carried out by using 7-nitro isomer (**5**) (Fig. 5). After **5** was reduced under H_2 atmosphere and acetylated with Ac_2O, **13** was obtained. As done in the Fig. 5,

Table I. *In Vitro* Cytotoxicity of Isoazamitosene Derivatives on Leukemia and Gastric Cancer Cell Lines

compound	IC_{50} (μg/ml) of Tumor Cell Lines		
	P388	SNU-1	KHH
8	0.31	0.026	0.062
9	0.67	4.31	5.1
10	0.72	8.1	18.2
11	0.62	4.02	1.25
16	2.2	85	23.3
17a (*syn*)	46.2	40.4	>100
17b (*anti*)	2.4	20.2	23.1
MMC[a]	0.06	2.32	0.068
ADM[b]	0.094	2.15	0.016

[a]MMC: mitomycin C
[b]ADM: adriamycin

13 was nitrated and reduced to give **15**. Fremy oxidation of a aromatic amine (**15**) in pH 3.0 and in pH 7.0 aqueous buffers gave a quinone (**16**) in 41% yield and iminoquinones (**17a** and **17b**) in 32% and 17% yield, respectively. Structural assignments of the iminoquinones was done by comparing ^{1}H NMR data of Skibo *et al* (Islam and Skibo, 1990). The ^{1}H NMR chemical shifts of the acetoamido methyl and 6-methyl groups of syn isomer (**17a**) (δ=2.28 and 1.97) were shifted upfield relative to those of anti isomer (**17b**) (δ=2.49 and 2.13), because of the formation of a delocalized negative charge at the centers bearing the methyl groups in the syn isomer upon intramolecular proton transfer. On the IR spectrum, the quinone carbonyl stretching frequency of **17b** (1743 cm^{-1}) was greater than that of **17a** (1721 cm^{-1}), due to the decrease in carbonyl bond order in the zwitterion.

The cytotoxic activities of synthesized compounds against leukemia and gastric cancer cell lines were evaluated by MTT method (Carmichel *et al*, 1986). As shown in Table 1, isoazamitosene derivatives (**8**, **9**, **10**, and **11**) containing cyclic amino group at the 7-position showed potent cytotoxicity against tumor cell lines tested. Among these derivatives, aziridine substituted-isoazamitosene derivative (**8**) revealed the highest cytotoxicity on tumor cells tested and showed higher cytotoxicity against SNU-1 than that of mitomycin and adrimycin. Isoiminoazamitosene derivatives (**17a** and **17b**), which we expected to have a lower oxygen toxicity, exhibited low cytotoxicity *in vitro*. From our chemosensitivity test, we could infer the possibility of isoazamitosene structure to bind with particular molecule on gastric cancer cell. Differences in the cytotoxicity panels by azamitosene derivatives, adriamycin and mitomycin support the hypothesis that azamitosene derivatives may own cytotoxicity mechanism different from mitomycin C and adriamycin. Recently, we found that cell cycle change induced by isoazamitosene derivatives using flowcytometer was totally different from that by mitomycin C and adriamycin (Unpublished data). From these results, we suggest that isoazamitosene derivatives may be further developed to improve their unique cytotoxicity.

REFERENCES CITED

Andrews, P.A., Pan, S. and Bachur, N.R., Electrochemical reductive activation of mitomycin C. *J. Am. Chem. Soc.*, 108, 4158-4166 (1986).

Begleiter, J.H., Cytocidal action of the quinone group and its relationship to antitumor activity. *Cancer Res.*, 43(2), 481-484 (1983).

Carmichel, J., DeGraff, W.G. and Gazdar, A.F., Evaluation of a tetrazolium-based semiautomated colorimetric assay : Assesment of chemosensitivity testing. *Cancer Res.*, 47, 939-944 (1986).

Doroshow, J.H., Effect of anthracycline antibiotics on oxygen radical formation in rat heart. *Cancer Res.*, 43(2), 460-472 (1983).

Fishbein, P.L., Synthesis and antineoplastic activity of 1a-formyl and 1a-thioformyl derivatives of mitomycin C and 2-methylaziridine. *J. Med. Chem.*, 30, 1767-1773 (1987).

Islam I. and Skibo E.B., Synthesis and physical studies of azamitosene and iminoazamitosene reductive alkylating agents. Iminoquinone hydrolytic stability, syn/anti isomerization, and electrochemistry. *J. Org. Chem.*, 55, 3195-3205 (1990).

Islam I. and Skibo E.B., Structure-activity studies of antitumor agents based on pyrrolo[1,2-a] benzimidazoles : new reductive alkylating DNA cleaving agents. *J. Med. Chem.*, 34, 2954-2961 (1991).

Iyengar, B.S., Sami, S.M., Remers, W.A., Bradner, W. T. and Schurig, J.E., Mitomycin C and porfiromycin analogues with substituted ethylamines at position 7. *J. Med. Chem.*, 26, 16-20 (1983).

Kennedy, K.A., Teicher, B.A., Rockwell, S. and Sartorelli, A.C., The hypoxic tumor cell: a target for selective cancer chemotherapy. *Biochem. Pharm.*, 29, 1-8 (1980).

Keyes, S.R., Heimbrook, D.C., Fracasso, P.M., Rockwell, S., Sligar, S.G. and Sartorelli, A.C., Chemotherapeutic attack of hypoxic tumor cells by the bioreductive alkylating agent mitomycin C. *Adv. Enz. Reg.*, 23, 291-307 (1985).

Math-Cohn, O. and Suschitzky, H., Heterocycles by ring closure of ortho-substituted t-anilines (the t-amino effect), *Adv. Heterocycl. Chem.*, 14, 178-211 (1972).

Moore, H.W., Bioactivation as a model for drug design bioreductive alkylation. *Science*, 197, 527-532 (1977).

Moore, H.W. and Czerniak, P., Naturally occurring quinones as potential bioreductive alkylating agents. *Med. Res. Rev.*, 1, 249-280 (1981).

Perrin, D.D., Armarego, L.F. and Perrin, O.R., *Purification of Laboratory Chemicals. 2nd ed.* Pergamon Press, New York, 1980.

Sami, S.M., Iyengar, B.S., Tarnow, S.E., Remer, W.A., Bradner, W.T. and Schurig, J.E., Mitomycin C analogues with aryl substitutions on the 7-amino group. *J. Med. Chem.*, 27, 701-708 (1984).

Skibo E.B and Schulz W.G., Pyrrolo[1,2-a]benzimidazole-based aziridinyl quinones. a new class of DNA cleaving agent exhibiting G and A Base specificity. *J. Med. Chem.*, 36, 3050-3055 (1993).

Tomasz, M., Lipman, R., Verdine, G.L. and Nakanishi, K., Reassignment of the guanine-binding mode of reduced mitomycin C. *Biochemistry*, 25, 4337-4344 (1986).

Vyas, D.M., Chiang, Y., Benigni, D. and Doyle, T.W., Novel mitomycin C amidines: Synthesis and their reactions with amines. *J. Org. Chem.*, 52, 5601-5605 (1987).