Arch Pharm Res Vol 29, No 4, 276-281, 2006

Archives of Pharmacal Research
http://apr.psk.or.kr

Synthesis and Cytotoxic Activities of 8-Alkyl or 8-Aryl-8,9-dihydro-7*H*-isoindolo[5,6-*g*]quinoxaline-7,9-diones

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(Received January 20, 2006)

A series of 8-alkyl- and 8-aryl-8,9-dihydro-7*H*-isoindolo[5,6- *g*]quinoxaline-7,9-diones were synthesized using sultine chemistry as a key step in good yield. These compounds were evaluated for their *in vitro* cytotoxicity against six human cancer cell lines (HCT15, SK-OV-3, A549, SNB19, MCF7 and MCF7/ADR).

Key words: Cytotoxic activity, Isoindoloquinoxaline

INTRODUCTION

Doxorubicin (**1**) is a well-known anthracycline antibiotic and the most commonly prescribed intercalating agent for the treatment of cancer (Wakelin and Waring, 1990). Doxorubicin (**1**) (Fig. 1) has a broad spectrum of activity, being particularly effective against solid tumors. However its clinical efficacy is limited due to cardiotoxicity, which develops during extended therapy, and the appearance of an acquired resistance (Priebe, 1995). The development of drug resistance is one of the main limitations to its use as chemotherapy for cancer patients.

In an effort to develop novel antitumor agents that can overcome the shortcomings of anthracyclines, the synthesis and biological evaluation of a series of azaanthraquinone derivatives **2** were recently reported (Lee *et al.*, 2003, 2004). Recently, a free radical hypothesis was proposed to explain the anthracycline-induced cardiotoxicity. NADPH-dependent reductases can produce a one-electron reduction of anthracyclines to anthracycline semiquinone free radicals. It was suggested that the cardiotoxicity of doxorubicin (**1**) is related to its ketol side chain or quinone moiety (Olsen *et al.*, 1990; Evert *et al.*, 2001). Therefore, the target compounds (**3**) were designed to avoid these structural features.

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This paper describes the synthesis of isoindoloquinoxalinedione derivatives **3**. The quinone group of the azaanthraquinone derivatives was removed in the target compounds. The aim was to reduce the cardiotoxicity of doxorubicin. The target compounds **3** were designed to incorporate the structural features of *N*-[2-(dimethylamino) ethyl]acridine-4-carboxamide (DACA) (**4**) (Atwell *et al.*, 1987; Baguley *et al.*, 1995; Dittrich *et al.*, 2003) and amonafide (**5**) (Brana *et al.*, 2001). DACA (**4**) has a neutral chromophore and acridine moiety, and poisons mixed topoisomerases I and II with DNA intercalating activity. Topoisomerase I and II inhibitors have been reported to have a synergistic effect against resistant tumor cells (Cortes and Pinero, 1994; Bonner and Kozelsky, 1996). Amonafide (**5**) is an imide derivative of naphthalic acid that intercalates into DNA and inhibits topoisomerase II, resulting in protein-associated strand breaks and impaired DNA and RNA synthesis (Brana and Ramos, 2001). Various alkyl and arylsubstituents were introduced to the imide nitrogen of the target compounds in order to delineate the SAR of isoindoloquinoxalinedione derivatives **3**.

MATERIALS AND METHODS

The melting points were recorded on a Electrothermal IA9100 digital melting point apparatus and were uncorrected. The IR spectra were obtained using a Jasco FT/IR-300E spectrophotometer and absorbance peaks are reported as cm^{-1}. The ^{1}H-NMR spectra were recorded

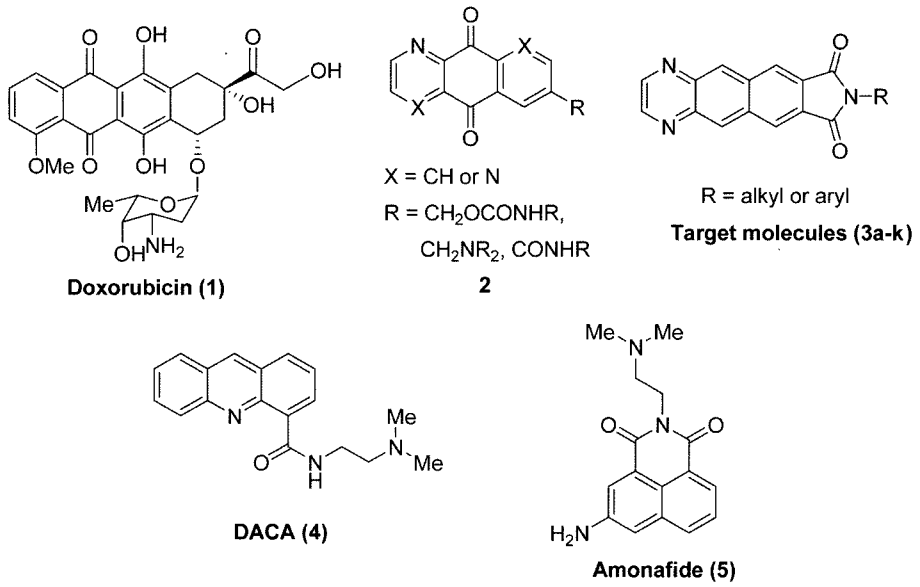


Fig. 1. Structures of compound **1-5**

on a Bruker DPS300 NMR spectrometer using TMS as the internal standard and the chemical shifts are reported as ppm (δ). Unless otherwise stated, the commercially available reagents and solvents were used as received without further purification. The RPMI1640 medium was obtained from Gibco BRL. Dimethyl sulfoxide(DMSO) and the other chemicals were purchased from Sigma.

General procedure for 8-alkyl- and 8-aryl-6a,7,8,9,9a,10-hexahydro-6*H*-isoindolo[5,6-*g*]quinoxaline-7,9-diones (11a-k)

A solution of quinoxalinosultine (**9**) (100 mg, 0.45 mmol) and *N*-alkyl and *N*-arylmaleimides (**10a-k**) (3 equiv) in toluene (4 mL) was sealed in a 40 mL Pyrex tube and heated at 220°C for 12 h. The solution was then cooled to room temperature. The solvent was evaporated under vacuum, and the residue was subjected to flash silica gel column chromatography using dichloromethane/ethyl acetate (from 9:1 to 1:4) as the eluent.

8-Ethyl-6a,7,8,9,9a,10-hexahydro-6*H*-isoindolo[5,6-*g*] quinoxaline-7,9-dione (11a)

The product was obtained as a white solid in 59% yield; mp 189-190°C; IR (KBr) 1699, 1446, 1350 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.80 (s, 2H, hetAr-H), 7.91 (s, 2H, Ar-H), 3.45 - 3.38 (m, 4H, CH_2, CH), 3.30 (q, *J* = 7.2 Hz, 2H, CH_2), 3.15-3.19 (m, 2H, CH_2), 0.71 (t, *J* = 7.2, 3H, CH_3).

8-Propyl-6a,7,8,9,9a,10-hexahydro-6*H*-isoindolo[5,6-*g*] quinoxaline-7,9-dione (11b)

The product was obtained as a white solid in 69% yield; mp 135-136°C; IR (KBr) 1704, 1480, 1399 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.81 (s, 2H, hetAr-H), 7.91 (s, 2H, Ar-H), 3.40 - 3.47 (m, 4H, CH_2, CH), 3.23 (t, *J* = 7.2 Hz, 2H, CH_2), 3.13 - 3.18 (m, 2H, CH_2), 1.16 (m, 2H, CH_2), 0.31 (t, *J* = 7.2 Hz, 3H, CH_3).

8-Butyl-6a,7,8,9,9a,10-hexahydro-6*H*-isoindolo[5,6-*g*] quinoxaline-7,9-dione (11c)

The product was obtained as a white solid in 63% yield; mp 121 - 122°C; IR (KBr) 1693, 1403, 1362 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.80 (s, 2H, hetAr-H), 7.91 (s, 2H, Ar-H), 3.40-3.49 (m, 4H, CH_2, CH), 3.26 (t, *J* = 6.8 Hz, 2H, CH_2), 3.11 - 3.17 (m, 2H, CH_2), 1.04 (m, 2H, CH_2), 0.45 (m, 2H, CH_2), 0.28 (t, *J* = 7.2 Hz, 3H, CH_3).

8-Isopropyl-6a,7,8,9,9a,10-hexahydro-6*H*-isoindolo[5,6-*g*]quinoxaline-7,9-dione (11d)

The product was obtained as a white solid in 69% yield; mp 193-194°C; IR (KBr) 1691, 1371 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.81 (s, 2H, hetAr-H), 7.91 (s, 2H, Ar-H), 4.08 (m, 1H, CH), 3.33 - 3.45 (m, 4H, CH_2, CH), 3.12 - 3.17 (m, 2H, CH_2), 0.97 (d, *J* = 6.9 Hz, 6H, CH_3).

8-Phenyl-6a,7,8,9,9a,10-hexahydro-6*H*-isoindolo[5,6-*g*] quinoxaline-7,9-dione (11e)

The product was obtained as a white solid in 93% yield; mp 250 - 251°C; IR (KBr) 1706, 1502, 1376 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.82 (s, 2H, hetAr-H), 7.96 (s, 2H, Ar-H), 6.82 - 7.06 (m, 5H, Ar-H), 3.51 - 3.58 (m, 4H, CH_2, CH), 3.25 - 3.28 (m, 2H, CH_2).

8-(3-Chlorophenyl)-6a,7,8,9,9a,10-hexahydro-6*H*-isoindolo [5,6-*g*]quinoxaline-7,9-dione (11f)

The product was obtained as a white solid in 90% yield; m.p. 267 - 268°C; IR (KBr) 1707, 1375 cm^{-1}; ^{1}H-NMR ($CDCl_3$,

300 MHz) δ 8.83 (s, 2H, hetAr-H), 7.97 (s, 2H, Ar-H), 7.20 - 7.25 (m, 2H, Ar-H), 6.94 (s, 1H, Ar-H), 6.77 -6.81 (m, 1H, Ar-H), 3.51 - 3.60 (m, 4H, CH_2, CH), 3.25 - 3.30 (m, 2H, CH_2).

8-(3-Methoxyphenyl)-6a,7,8,9,9a,10-hexahydro-6*H*-isoindolo[5,6-*g*]quinoxaline-7,9-dione (11g)

The product was obtained as a white solid in 91% yield; mp 240-241°C; IR (KBr) 1704, 1491, 1376 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 8.82 (s, 2H, hetAr-H), 7.96 (s, 2H, Ar-H), 7.19 (t, *J* = 8.2 Hz, 1H, Ar-H), 6.80 - 6.82 (m, 1H, Ar-H), 6.42 - 6.44 (m, 1H, Ar-H), 6.34 (t, *J* = 2.2 Hz, 1H, Ar-H), 3.62 (s, 3H, CH_3), 3.51 - 3.58 (m, 4H, CH_2, CH), 3.25 - 3.28 (m, 2H, CH_2).

8-(3-Nitrophenyl)-6a,7,8,9,9a,10-hexahydro-6*H*-isoindolo [5,6-*g*]quinoxaline-7,9-dione (11h)

The product was obtained as a white solid in 85% yield; mp 290-291°C; IR (KBr) 1706, 1531, 1375 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.84 (s, 2H, hetAr-H), 8.14 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.98 (s, 2H, Ar-H), 7.49 (t, *J* = 8.17 Hz, 1H, Ar-H), 7.30 (s, 1H, Ar-H), 3.53 - 3.66 (m, 4H, CH_2, CH), 3.26 - 3.31 (m, 2H, CH_2).

8-(4-Chlorophenyl)-6a,7,8,9,9a,10-hexahydro-6*H*-isoindolo [5,6-*g*]quinoxaline-7,9-dione (11i)

The product was obtained as a white solid in 85% yield; mp 280-281°C; IR (KBr) 1705, 1520, 1395 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.83 (s, 2H, hetAr-H), 7.96 (s, 2H, Ar-H), 7.27 (dd, *J* = 6.7 Hz, *J* = 2.0 Hz, 2H, Ar-H), 6.84 (dd, *J* = 6.7 Hz, *J* = 2.0 Hz, 2H, Ar-H), 3.50 - 3.60 (m, 4 H, CH_2, CH), 3.23 - 3.29 (m, 2 H, CH_2).

8-(4-Methoxyphenyl)-6a,7,8,9,9a,10-hexahydro-6*H*-isoindolo[5,6-*g*]quinoxaline-7,9-dione (11j)

The product was obtained as a white solid in 91% yield; mp 268-269°C; IR (KBr) 1703, 1448, 1386 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.82 (s, 2H, hetAr-H), 7.86 (s, 2H, Ar-H), 6.74 - 6.82 (m, 4H, Ar-H), 3.73 (s, 3H, CH_3), 3.46-3.59 (m, 4H, CH_2, CH), 3.23-3.29 (m, 2H, CH_2).

8-(4-Nitrophenyl)-6a,7,8,9,9a,10-hexahydro-6*H*-isoindolo [5,6-*g*]quinoxaline-7,9-dione (11k)

The product was obtained as a white solid in 73% yield; mp 298-299°C; IR (KBr) 1713, 1520, 1348 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.84 (s, 2H, hetAr-H), 8.17 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.97 (s, 2H, Ar-H), 7.21 (d, *J* = 8.8 Hz, 2H, Ar-H), 3.54 - 3.66 (m, 4H, CH_2, CH), 3.26 - 3.30 (m, 2H, CH_2).

General procedure for 8-alkyl- and 8-aryl-8,9-dihydro-7*H*-isoindolo[5,6-*g*]quinoxaline-7,9-diones (3a-k)

8-Alkyl- and 8-aryl-6a,7,8,9,9a,10-hexahydro-6*H*-isoindolo [5,6-*g*]-quinoxaline-7,9-diones (**11a-k**) (0.275 mmol), *N*-bromosuccinimide (489 mg, 2.75 mmol), and benzoylperoxide (6 mg, 1%) were placed in a 25-mL round bottomed flask containing CCl_4 (25 mL). The stirred reaction mixture was purged with nitrogen and heated under reflux in the dark for 12 h. The reaction mixture turned a yellow color during the course of the reaction. The reaction mixture was cooled to room temperature, triethylamine (1 mL) was added, and the stirring was continued for a further 4 h. The resulting yellow precipitate was collected by filtration and washed with water followed by hot ethanol. The precipitate was subjected to flash silica gel column chromatography using dichloromethane/ethyl acetate (from 1:1 to 1:4) as the eluent.

8-Ethyl-8,9-dihydro-7*H*-isoindolo[5,6-*g*]quinoxaline-7,9-dione (3a)

The product was obtained as a yellow solid in 81% yield; mp 317 - 318°C (dec); IR (KBr) 1704, 1443, 1380 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 9.01 (s, 2H, hetAr-H), 8.94 (s, 2H, Ar-H), 8.64 (s, 2H, Ar-H), 3.87 (q, *J* = 7.2 Hz, 2H, CH_2), 1.36 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 167.2, 147.2, 141.0, 134.6, 131.6, 128.4, 125.6, 33.5, 13.8; MS *m/z* EI 277 (M^+).

8-Propyl-8,9-dihydro-7*H*-isoindolo[5,6-*g*]quinoxaline-7,9-dione (3b)

The product was obtained as a yellow solid in 41% yield; mp 260-261°C (dec); IR (KBr) 1705, 1440, 1388 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 9.01 (s, 2H, hetAr-H), 8.93 (s, 2H, Ar-H), 8.64 (s, 2H, Ar-H), 3.78 (t, *J* = 7.2 Hz, 2H, CH_2), 1.77 - 1.81 (m, 2H, CH_2), 1.01 (t, *J* = 7.4 Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 167.4, 147.2, 141.0, 134.6, 131.6, 128.4, 125.6, 40.2, 21.8, 11.4; MS *m/z* FAB 292 (M^++1).

8-Butyl-8,9-dihydro-7*H*-isoindolo[5,6-*g*]quinoxaline-7,9-dione (3c)

The product was obtained as a yellow solid in 51% yield; mp 189 - 190°C; IR (KBr) 1708, 1442, 1388 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 9.01 (s, 2H, hetAr-H), 8.93 (s, 2H, Ar-H), 8.63 (s, 2H, Ar-H), 3.81 (t, *J* = 7.2 Hz, 2H, CH_2), 1.69 - 1.79 (m, 2H, CH_2), 1.32 - 1.47 (m, 2H, CH_2), 0.98 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 167.4, 147.2, 141.0, 134.6, 131.6, 128.4, 125.6, 38.4, 30.4, 20.2, 13.7; MS *m/z* FAB 306 (M^++1).

8-Isopropyl-8,9-dihydro-7*H*-isoindolo[5,6-*g*]quinoxaline-7,9-dione (3d)

The product was obtained as a yellow solid in 54% yield; mp 235-236°C (dec); IR (KBr) 1704, 1357 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 9.01 (s, 2H, hetAr-H), 8.93 (s, 2H, Ar-H), 8.60 (s, 2H, Ar-H), 4.63 - 4.72 (m, 1H, CH), 1.57 (d, *J* = 7.0 Hz, 6H, CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 167.3, 147.2, 141.0, 134.6, 131.6, 128.4, 125.4, 43.6, 20.0; MS *m/z* FAB 292 (M^++1).

8-Phenyl-8,9-dihydro-7*H*-isoindolo[5,6-*g*]quinoxaline-7,9-dione (3e)

The product was obtained as a yellow solid in 83% yield; mp 298 - 299°C; IR (KBr) 1710, 1369 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 9.03 (s, 2H, hetAr-H), 8.99 (s, 2H, Ar-H), 8.78 (s, 2H, Ar-H), 7.46 - 7.60 (m, 5H, Ar-H); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 165.9, 147.4, 141.2, 134.8, 134.6, 131.9, 130.1, 129.3, 128.5, 126.1, 125.7, 124.2; MS *m/z* EI 325 (M^+).

8-(3-Chlorophenyl)-8,9-dihydro-7*H*-isoindolo[5,6-*g*] quinoxaline-7,9-dione (3f)

The product was obtained as a yellow solid in 89% yield; mp 317°C (dec); IR (KBr) 1716, 1363 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 9.04 (s, 2H, hetAr-H), 9.00 (s, 2H, Ar-H), 8.79 (s, 2H, Ar-H), 7.45 - 7.59 (m, 4H, Ar-H); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 165.9, 147.4, 141.2, 134.8, 134.6, 131.9, 130.1, 128.7, 127.5, 126.8, 126.7, 124.7; MS *m/z* EI 359 (M^+).

8-(3-Methoxyphenyl)-8,9-dihydro-7*H*-isoindolo[5,6-*g*] quinoxaline-7,9-dione (3g)

The product was obtained as a yellow solid in 91% yield; mp 239°c (dec); IR (KBr) 1715, 1491, 1367 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 9.03 (s, 2H, hetAr-H), 8.99 (s, 2H, Ar-H), 8.99 (s, 2H, Ar-H), 8.78 (s, 2H, Ar-H), 7.00 - 7.12 (m, 4H, Ar-H), 3.87 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 166.3, 160.1, 147.4, 143.1, 141.2, 134.7, 132.7, 131.8, 129.9, 127.9, 126.5, 118.9, 114.7, 112.4, 55.5; MS *m/z* EI 355 (M^+).

8-(3-Nitrophenyl)-8,9-dihydro-7*H*-isoindolo[5,6-*g*] quinoxaline-7,9-dione (3h)

The product was obtained as a yellow solid in 75% yield; mp 292-293°C (dec); IR (KBr) 1720, 1532, 1351 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 9.19 (s, 2H, hetAr-H), 9.01 (s, 2H, Ar-H), 9.00 (s, 2H, Ar-H), 7.82 - 8.47 (m, 4H, Ar-H); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 166.9, 147.7, 145.2, 141.6, 139.3, 133.2, 131.5, 130.5, 128.7, 126.9, 123.2, 121.5; MS *m/z* EI 370 (M^+).

8-(4-Chlorophenyl)-8,9-dihydro-7*H*-isoindolo[5,6-*g*] quinoxaline-7,9-dione (3i)

The product was obtained as a yellow solid in 77% yield; mp 314-316°C (dec); IR (KBr) 1717, 1520, 1367 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 9.03 (s, 2H, hetAr-H), 8.99 (s, 2H, Ar-H), 8.78 (s, 2H, Ar-H), 7.52 - 7.60 (m, 4H, Ar-H); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 166.5, 147.5, 144.2, 135.8, 134.6, 132.9, 131.1, 129.7, 128.5, 127.8, 125.7, 124.9; MS *m/z* EI 359 (M^+).

8-(4-Methoxyphenyl)-8,9-dihydro-7*H*-isoindolo[5,6-*g*] quinoxaline-7,9-dione (3j)

The product was obtained as a yellow solid in 71% yield; mp 242-243°C (dec); IR (KBr) 1721, 1346 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 9.03 (s, 2H, hetAr-H), 8.98 (s, 2H, Ar-H), 8.76 (s, 2H, Ar-H), 7.44 (d, J = 8.9 Hz, 2H, Ar-H), 7.08 (d, J = 8.9 Hz, 2H, Ar-H), 3.88 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 166.6, 159.5, 147.3, 141.1, 134.7, 131.8, 130.5, 129.6, 128.0, 126.4, 114.6, 55.6; MS *m/z* EI 355 (M^+).

8-(4-Nitrophenyl)-8,9-dihydro-7*H*-isoindolo[5,6-*g*]quinoxaline-7,9-dione (3k)

The product was obtained as a yellow solid in 69% yield; mp 279-280°C (dec); IR (KBr) 1721, 1522, 1346 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 9.02 (s, 2H, hetAr-H), 8.99 (s, 2H, Ar-H), 8.83 (s, 2H, Ar-H), 8.43 (d, J = 8.7 Hz, 2H, Ar-H), 7.86 (d, J = 8.7 Hz, 2H, Ar-H); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 166.5, 147.4, 145.6, 142.6, 138.3, 134.2, 131.7, 130.8, 128.5, 126.7, 121.7; MS *m/z* EI 370 (M^+).

Cells

Six human cancer cell lines, HCT15, SK-OV-3, A549, SNB19, MCF7 and MCF7/ADR were used in this study. All the cells were obtained from the national cancer institute, U.S.A. These cells were maintained in RPMI1640 media supplemented with 10% fetal calf serum at 37°C under a humidified atmosphere of 5% CO_2.

In vitro cytotoxicity assay

The number of cells was measured indirectly using the sulforhodamine B (SRB) method according to the NCI (U.S.A.) protocol (Skehan *et al.*, 1990). Briefly, the cells were plated into a 96 well plate at a density of 2×10^3 cells per well. On the next day (day 0), the compounds of interest dissolved in DMSO/media were added in quadruplicate. The final concentration of each compound ranged from 1 nM - 10 μM and the final concentration of DMSO was <0.1%. Seventy-two hours later, the cells were fixed with 10% trichloroacetic acid (TCA) overnight at 4°C. The TCA-treated cells were washed extensively with distilled water and dried in air. A SRB solution (0.4% in 1% acetic acid) was then added to the well at room temperature for one hour. The bound dye was dissolved in 10 mM Tris after washing the wells with 1% acetic acid. The absorbances were measured at 690 nm using a microplate reader. The absorbance of the day 0 sample was subtracted from the absorbance of the day 3 sample.

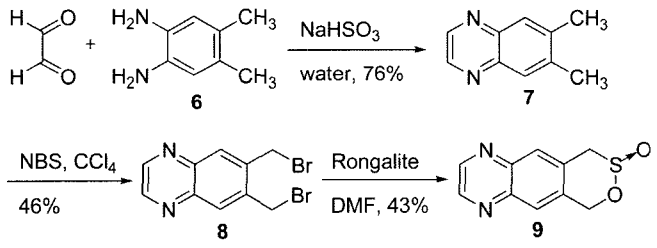
RESULTS AND DISCUSSION

Since it was suggested that the cardiotoxicity of doxorubicin **1** was related to its ketol side chain or quinone moiety (Olsen *et al.*, 1990; Evert *et al.*, 2001), the target compounds **3** were synthesized without these structural features. The removal of the quinone group was intended to reduce the cardiotoxicity of doxorubicin. The

imide ring system of the target compounds **3** was incorporated based on the structure of amonafide (**5**). Various alkyl and arylsubstituents were introduced to the imide nitrogen of the target compounds **3**.

The quinoxalinosultine intermediate **9** was prepared using the reported procedure (Liu *et al.*, 2000), which is outlined in Scheme 1. 4,5-Dimethyl-1,2-phenylenediamine (**6**) was condensed with glyoxal to give 6,7-dimethylquinoxaline (**7**) in aqueous sodium hydrogen sulfite at 80°C. The bromination of compound **7** with NBS afforded 6,7-dibromomethylquinoxaline (**8**) in a 46% yield. The treatment of the compound **8** with Rongalite (sodium formaldehyde sulfoxylate) provided the quinoxalinosultine intermediate **9** in a 43% yield. The dienophiles **10a-k** (*N*-alkyl or *N*-arylmaleimides) were prepared according to the procedure reported elsewhere (Sivaprakasan *et al.*, 2000). The reaction between maleic anhydride with the corresponding alkyl or aryl amines in anhydrous diethyl ether at room temperature furnished crude maleanilic acids. The subsequent cyclization upon treatment with the acetic anhydride-sodium acetate gave the maleimides **10a-k** in a 20-70% yield.

The synthesis of quinoxalino sultine **9** and its application in a Diels-Alder reaction with electron-poor olefins was reported recently (Liu *et al.*, 2000). This methodology was extended to prepare the target compounds. When heated



Scheme 1. Synthesis of quinoxalinosultine (**9**)

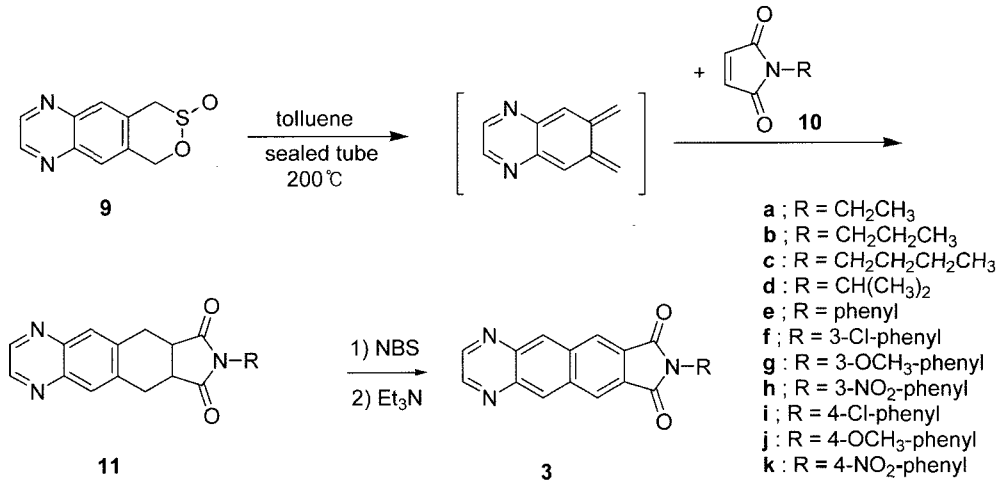
in the presence of 3 equiv of *N*-alkyl or arylmaleimides (**10a-k**) in toluene in a sealed tube for 24 h, the sultine **9** underwent the extrusion of SO_2, and the resulting quinoxalino-6,7-quinodimethanes were intercepted as the 1:1 adducts (**11a-k**). The yields of the Diels-Alder reaction with the *N*-aryl maleimides (**10e-f**) (70-90%) were slightly higher than the yields with *N*-alkylmaleimides (**10a-d**) (60-80%). The cycloaddition adducts (**11a-k**) were then aromatized using NBS, followed by triethylamine to give the target compounds (**3a-k**) (40-90%). The structure of the target compounds was determined from the spectroscopic data.

The biological activity of the compounds was examined *in vitro* according to the protocols developed by the National Cancer Institute (Skehan *et al.*, 1990). The target compounds were evaluated for their *in vitro* cytotoxicity against six human cancer cell lines originating from colon (HCT-15), ovarian (SK-OV-3), lung (A549), CNS (SNB19), and breast (MCF7 and MCF7/ADR) cancer. They all appeared to be inactive (>30 μM). The poor *in vitro* cytotoxic activity might be due to the poor solubility of the target compounds.

The target compounds were designed to have an additional pyrrole ring system to the previous azaanthraquinone analogs **2**, in which the quinone functionality had been removed. Various substituents were introduced on imide nitrogen to delineate the structure activity relationship. This suggests that the quinone functionality may be essential for good activity. Further study aimed at designing, synthesizing, and evaluating additional compounds in this and related systems is currently underway.

ACKNOWLEDGEMENTS

This work was supported by the Korea Research Foundation Grant (KRF-2003-015-E00231).



Scheme 2. Synthesis of the 8-alkyl and 8-aryl-8,9-dihydro-7*H*-isoindolo[5,6-*g*]quinoxaline-7,9-diones (**3a-k**)

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