Supporting Information

for

A Novel Bis-aziridinylNaphthoquinone with Anti-Solid Tumor Activity in which Caused Apoptosis Is Associated with Altered Expression of Bcl-2 Protein

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Proton and carbon NMR were obtained on a Bruker AMX-500 spectrometer. Chemical shifts were reported in ppm relative to tetramethylsilane (δ units). Elemental analyses were recorded on a Micromass ZAB spectrometer and Perkin-Elmer 2400 elemental analyzer repetitively at the Analytical Facility of The National Taiwan University. IR spectra were obtained on Perkin Elmer Spectrum RXI FT-IR system. All of chemicals were purchased from Across, Aldrich or TCI and used without future purification. Bis-aziridinylnaphthoquinone 1a (CAS #103051-43-0) is prepared according to procedure published by Bayer [19].

General procedure for preparing bis-naphthoquinone thiol ethers 4b-e. A mixture of β,β'-dimercaptodiethyl ether (1.1 mmol) and Ce₂CO₃ (1.2 mmol) in DMF(15 ml) was cool to 5-10 °C under nitrogen, and 1,4-naphthoquinone (2.0 mmol) in DMF (15 ml) was added to mixture drop-wised. The mixture was allowed to warm to room temperature. After 24 hours, the mixture was poured into cold water (100 ml), and acidified with 5 % HCl (30mL). The aqueous solution was extracted with CH₂Cl₂ (50 ml X 4). The combined extracts were dried with MgSO₄ and concentrated under reduced pressure to provide a crude product. The crude product was purified by silica gel chromatography.

1, 2-bis-(3-chloro-1, 4-dioxo-1, 4-dihydro-[2] naphthylmercapto)-butane (4b). As yellow solid (68% yield) M.p. 167-168 IR (KBr) ν max: 1660, 1651 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 1.84(4H, m), 3.41 (4H, t, J = 6 Hz), 7.71 (4H, m), 8.03 (2H, m), 8.11(2H, m); ¹³C NMR
(125MHz, CDCl₃): δ 29.27, 33.52, 127.26, 127.34, 131.20, 132.54, 133.86, 134.19, 140.27, 148.54, 174.96, 179.87; Anal. Calcd for C₂₄H₁₆Cl₂O₄S₂: C, 57.26; H, 3.20. Found: C, 57.37; H, 3.25.

1, 2-bis-(3-chloro-1, 4-dioxo-1, 4-dihydro-[2] naphtylmercaptato)-pentane (4c). As yellow solid (55% yield) M.p. 157-159 IR (KBr) ν max: 1663, 1655 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 1.62 (4H, m), 1.71(4H, q, J = 7.5Hz), 3.39 (4H, t, J = 7.5 Hz), 7.69 (4H, m), 8.03(2H, m), 8.10(2H, m); ¹³C NMR (125MHz, CDCl₃): δ 27.54, 29.80, 34.64, 127.30, 127.49, 130.98, 132.58, 133.70, 134.07, 134.65, 152.62, 174.94, 179.20; Anal. Calcd for C₂₅H₁₈Cl₂O₄S₂: C, 58.03; H, 3.51. Found: C, 58.17; H, 3.59.

1, 2-bis-(3-chloro-1, 4-dioxo-1, 4-dihydro-[2] naphtylmercaptato)-hexane (4d). As yellow solid (62% yield) M.p. 153-154 IR (KBr) ν max: 1663, 1657 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 1.49 (4H, m), 1.68 (4H, t, J = 7.5 Hz), 3.91 (4H, t, J = 7.5 Hz), 7.72 (4H, m), 8.06(2H, m), 8.12(2H, m); ¹³C NMR (125MHz, CDCl₃): δ 28.00, 30.18, 34.07, 127.21, 127.29, 131.23, 132.57, 133.80, 134.12, 139.84, 149.11, 175.00, 178.87; Anal. Calcd for C₂₆H₂₀Cl₂O₄S₂: C, 58.76; H, 3.79. Found: C, 58.88; H, 3.68.

1, 2-bis-(3-chloro-1, 4-dioxo-1, 4-dihydro-[2] naphtylmercaptato)-octane (4e). As yellow solid (70% yield) M.p. 147-149 IR (KBr) ν max: 1663, 1651 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 1.32 (4H, m), 1.44 (4H, m), 1.65 (4H, q, J = 7.5 Hz), 3.38 (4H, t, J = 7.5 Hz), 7.73 (4H, m), 8.02 (2H, m), 8.13 (2H, m); ¹³C NMR (125MHz, CDCl₃): δ 28.46, 28.81, 30.34, 34.24, 127.20, 127.28, 131.25, 132.59, 133.78, 134.10, 139.75, 149.31, 175.03, 179.88; Anal. Calcd for C₂₈H₂₄Cl₂O₄S₂: C, 60.10; H, 4.32. Found: C, 60.11; H, 4.30.

General procedure for preparing bis-aziridinylnaphthoquinone 1b-e. To a solution of naphthoquinone 4b-e (1.0 mmol) in benzene (8 ml) was added a solution of triethylamine (4.2 mmol) under stirring at 0 °C. The aziridine (10.0 mmol) was added to mixture dropwise over a period of 5 min. The mixture was stir for 6 hours at room temperature. Ethyl acetate and 1N HCl were added to the reaction mixture, and organic layer was separated. The aqueous layer was further extracted with ethyl acetate, and combined organic layers were dried with MgSO₄ and concentrated. The crude product was purified by silica gel chromatography.

1, 2-bis-(3-aziridin-1, 4-dioxo-1, 4-dihydro-[2] naphtylmercaptato)-butane (1b). As red solid (32% yield) M.p. 112-114 IR (KBr) ν max: 1641, 1667 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 1.71(4H, m), 2.48 (8H, s), 3.03 (4H, t, J = 6.5 Hz), 7.64 (4H, m), 8.02 (4H, m); ¹³C NMR (125MHz, CDCl₃): δ 29.20, 31.10, 32.50, 126.39, 126.63, 129.02, 132.87, 133.00, 133.77, 134.60, 156.10, 179.32, 181.32; Anal. Calcd for C₂₈H₂₄N₂O₄S₂: C, 65.09; H, 4.68; N, 5.42. Found: C, 65.11; H, 4.63; N, 5.40.
1, 2-bis-(3-aziridin-1, 4-dioxo-1, 4-dihydro-[2] naphthylmercapto)-pentane (1c). As red solid (63% yield) M.p. 139-141 IR (KBr) $\nu_{\text{max}}$: 1668, 1651 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 1.49 (2H, m), 1.58 (4H, m), 2.48(8H, s), 2.99 (4H, t, $J = 7.5$Hz), 7.62 (4H, m), 8.01(4H, m); $^{13}$C NMR (125MHz, CDCl$_3$): $\delta$ 27.81, 29.68, 31.14, 32.84, 126.38, 126.64, 129.39, 131, 38, 132.89, 132.99, 133.76, 156.05, 179.36, 181.38; Anal. Calcd for C$_{29}$H$_{26}$N$_2$O$_4$S$_2$: C, 65.64; H, 4.94; N, 5.28. Found: C, 65.58; H, 4.97; N, 5.33.

1, 2-bis-(3-aziridin-1, 4-dioxo-1, 4-dihydro-[2] naphthylmercapto)-hexane (1d). As red solid (65% yield) M.p. 116-117 IR (KBr) $\nu_{\text{max}}$: 1667, 1644 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 1.30-1.43 (8H, m), 2.51 (8H, s), 3.01 (4H, t, $J = 7.5$ Hz), 7.67 (4H, m), 8.04(4H, m); $^{13}$C NMR (125MHz, CDCl$_3$): $\delta$ 28.24, 29.76, 31.14, 32.96, 126.38, 126.64, 128.78, 131.40, 132.90, 133.00, 133.77, 155.99, 179.37, 181.42; Anal. Calcd for C$_{30}$H$_{28}$N$_2$O$_4$S$_2$: C, 66.15; H, 5.18; N, 5.14. Found: C, 66.17; H, 5.19; N, 5.16.

1, 2-bis-(3-aziridin-1, 4-dioxo-1, 4-dihydro-[2] naphthylmercapto)-octane (1e). As red solid (32% yield) Mp99-101 IR (KBr) $\nu_{\text{max}}$: 1667, 1651 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 0.89(4H, m), 1.31 (4H, m), 1.56 (4H, m), 2.49(8H, s), 2.99 (4H, t, $J = 7.5$ Hz), 7.64 (4H, m), 8.01 (4H, m); $^{13}$C NMR (125MHz, CDCl$_3$): $\delta$ 28.54, 28.84, 30.07, 31.08, 32.98, 126.29, 126.53, 128.71, 131.32, 132.92, 133.32, 133.68, 155.88, 179.28, 181.33; Anal. Calcd for C$_{32}$H$_{32}$N$_2$O$_4$S$_2$: C, 67.11; H, 5.63; N, 4.89. Found: C, 67.12; H, 5.66; N, 4.83.