# 2,3-Dimethyl-1,4-naphthoquinone Derivatives as Bioreductive Alkylating Agents with Cross-Linking Potential[1]

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Bioreducible 2,3-disubstituted 1,4-naphthoquinones have been synthesized and evaluated for anticancer activity by measuring their capacity to prolong the life span of Sarcoma 180 tumor bearing mice. The leaving group in the 2- and 3-positions of these agents significantly influenced the degree of antineoplastic activity, with the most active agents being the methyl sulfonate (**5**), the methyl carbamate (**9**), and the 2-chloroethyl carbamate (**10**) derivatives; when these quinones were administered daily for 6 consecutive days, they produced maximum T/C × 100 values of 232, 266, and 230, respectively.

The clonogenic hypoxic malignant cells of solid tumors are an obstacle to the potential cure of these malignancies by conventional anticancer agents, primarily because these cells are relatively insensitive to drugs, such as bleomycin, that require oxygen for maximum expression of cytotoxic potential, as well as to those agents that require cellular replication to exert anticancer activity, since hypoxic cells may have prolonged cell-cycle times or may be blocked in their progression through the cycle.[3,4] Oxygen-deficient tumor cells may also exhibit resistance to chemotherapeutic agents due to pharmacodynamic considerations, such as penetration in adequate concentration into hypoxic regions of solid tumors.[5] Teicher et al.[6] have demonstrated that the quinone antibiotic mitomycin C, which may be considered a prototype bioreductive alkylating agent, is preferentially cytotoxic to hypoxic tumor cells in vitro, and Kennedy et al.[7] have shown that this antibiotic is metabolically activated by reduction to a reactive species by tumor cell enzyme systems under anaerobic conditions. Based on these concepts, various quinone derivatives have been synthesized and evaluated as potential bioreductive alkylating agents by this laboratory.[8-15] Since the potential for cross-linking of cellular structures appears to be a requisite for the optimum activity of conventional alkylating agents, we have synthesized a series of bioreducible 2,3-disubstituted 1,4-naphthoquinone derivatives, with the potential to be activated preferentially by hypoxic cells to produce highly reactive "double-armed" quinone methides with the capacity to cross-link cellular components (Scheme I). In addition, although earlier studies[16] suggested that the leaving group had little influence on anticancer activity, these findings seemed to be inconsistent with chemical and pharmacological considerations, and since the studies were conducted on a limited series, we also examined the influence of a variety of leaving groups on the biological activity of the series generated in this report; this was evaluated by measuring the capacities of the synthesized quinones to prolong the survival time of Sarcoma 180 tumor bearing mice.

Scheme I

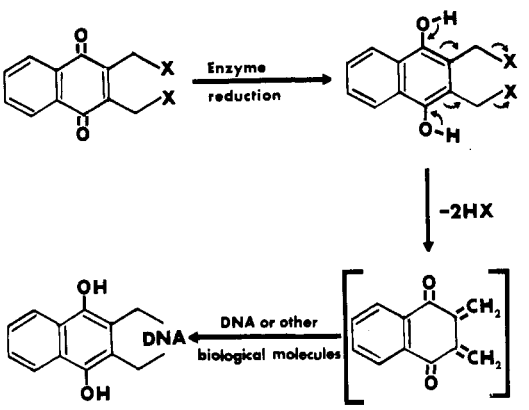
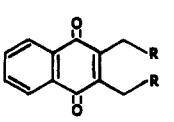



Chart I



| | | | |
|---|---|---|---|
| 1 | R = H | 6 | R = $OSO_2C_6H_4CH_3$-p |
| 2 | R = Cl | 7 | R = $OCOCH_3$ |
| 3 | R = Br | 8 | R = $OCH_3$ |
| 4 | R = OH | 9 | R = $OCONHCH_3$ |
| 5 | R = $OSO_2CH_3$ | 10 | R = $OCONHCH_2CH_2Cl$ |

**Chemistry.** A series of 2,3-disubstituted 1,4-naphthoquinones has been synthesized (Chart I). Treatment of 1,4-naphthoquinone with aqueous formaldehyde in glacial acetic acid saturated with hydrogen chloride gas yielded the 2,3-bis(chloromethyl) derivative **2**.[17] Bromination of 2,3-dimethyl-1,4-naphthoquinone[18] with *N*-bromosuccin-

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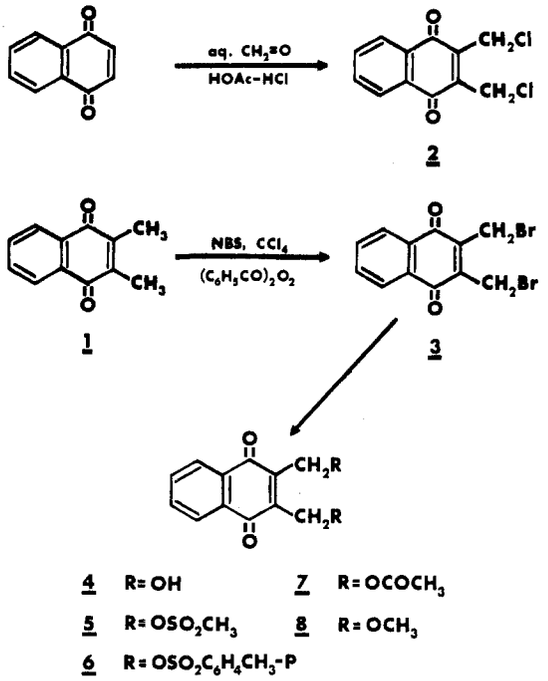
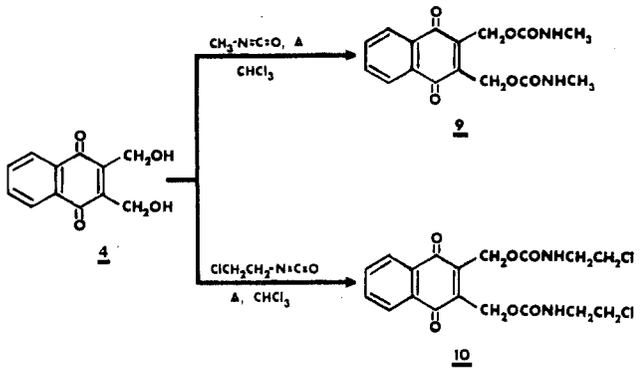
Scheme II



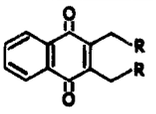

Scheme III




imide and a trace of benzoyl peroxide in dry carbon tetrachloride afforded 2,3-bis(bromomethyl)-1,4-naphthoquinone (**3**).[19] The mesylate **5** and the tosylate **6** were obtained by stirring **3** in acetonitrile with silver mesylate or silver tosylate, respectively,[20] and the acetate **7** was formed by refluxing **3** in a mixture of chloroform and acetic acid containing silver acetate.[20] The ether **8** was synthesized by refluxing **3** in methanol.[9] Refluxing **3** in water–dioxane[14] (3:2, v/v) gave the alcohol **4**. Reaction of **4** with methyl isocyanate and 2-chloroethyl isocyanate[14] yielded the corresponding carbamates **9** and **10** (Schemes II and III).

**Biological Evaluation.** The 2,3-disubstituted 1,4-naphthoquinone derivatives were evaluated for anticancer activity against Sarcoma 180 tumor bearing mice; the results of these tests are summarized in Table I. Several compounds of this series showed significant antineoplastic activity in this system. The leaving group influenced significantly the degree of tumor-inhibitory activity, with the best leaving groups, in general, giving the highest T/C × 100 values. Thus, compounds such as the mesylate **5** and the carbamates **9** and **10** exhibited marked anticancer activity against Sarcoma 180, producing maximum T/C × 100 values of 232, 266, and 230, respectively. The poor antineoplastic activity produced by the tosylate **6** is speculated to be due to the pronounced instability of this compound, which was observed to decompose gradually over a period of a few weeks, even when stored at 0 °C. Compounds **7** and **8** did not show any anticancer activity. These findings are consistent with the mechanism of activation proposed in Scheme I.

**Table I.** Effects of 2,3-Dimethyl-1,4-naphthoquinone Derivatives on the Survival Time of Mice Bearing Sarcoma 180 Ascites Cells



| no. | R | daily dosage, mg/kg | av. Δ wt., % | T/C × 100[a] |
|---|---|---|---|---|
| 1 | H | 3.25 | +1.4 | 104 |
| | | 7.5 | +1.3 | 78 |
| | | 15.0 | +0.5 | 103 |
| 2 | Cl | 3.25 | −15.1 | 100 |
| | | 7.5 | −10.3 | 158 |
| | | 15.0 | −15.6 | 122 |
| 3 | Br | 3.25 | −8.8 | 165 |
| | | 7.5 | −14.3 | 130 |
| | | 15.0 | −14.1 | 140 |
| 4 | OH | 3.25 | +10.8 | 55 |
| | | 7.5 | −14.3 | 51 |
| | | 15.0 | −9.9 | 37 |
| 5 | $OSO_2CH_3$ | 2.5 | +18.4 | 114 |
| | | 5.0 | +5.9 | 204 |
| | | 10.0 | −2.0 | 232 |
| 6 | $OSO_2C_6H_4CH_3$-*p* | 2.5 | +22.3 | 130 |
| | | 5.0 | +21.0 | 138 |
| | | 10.0 | +13.8 | 126 |
| 9 | $OCONHCH_3$ | 2.5 | +3.8 | 266 |
| | | 5.0 | −7.6 | 177 |
| | | 10.0 | −10.0 | 200 |
| 10 | $OCONHCH_2CH_2Cl$ | 2.5 | 0.0 | 230 |
| | | 5.0 | −4.3 | 209 |
| | | 10.0 | −7.8 | 189 |

[a] T/C × 100 represents the ratio of the survival time of treated to control animals × 100. The average survival time of the untreated tumor-bearing control animals was 12.3 ± 2.6 days. Each value represents the results from 5 to 15 mice.

## Experimental Section

Melting points were determined with a Thomas-Hoover Unimelt apparatus and are uncorrected. $^1$H NMR spectra were recorded at 60 MHz on a Varian T-60 spectrometer or at 270 MHz on a Brucker 270 HX spectrometer, with $Me_4Si$ as the internal reference. TLC was performed on EM precoated silica gel sheets containing a fluorescent indicator. Elemental analyses were carried out by the Baron Consulting Co., Orange, CT. Where analyses are indicated only by symbols of the elements, the analytical results obtained for those elements were within ±0.4% of the theoretical values.

**2,3-Dimethyl-1,4-naphthoquinone (1).** Compound **1** was prepared by the method of Kruber[18] and purified on a silica gel column ($CHCl_3$): yield 50%; mp 124–125 °C (lit.[17] 127 °C); NMR ($CDCl_3$) δ 2.18 (s, 6 H, 2-$CH_3$ and 3-$CH_3$), 7.40–8.10 (m, 4 H, H-5, H-6, H-7, and H-8, aromatic).

**2,3-Bis(chloromethyl)-1,4-naphthoquinone (2).** Compound **2** was synthesized according to the procedure of Thomson.[17] The product was purified by silica gel column chromatography (benzene): 68% yield; mp 136–138 °C (lit.[17] 142–143 °C); NMR ($CDCl_3$) δ 4.65 (s, 4 H, 2- and 3-$CH_2Cl$), 7.60–8.10 (m, 4 H, H-5, H-6, H-7 and H-8, aromatic).

**2,3-Bis(bromomethyl)-1,4-naphthoquinone (3).** The method of Sorrie and Thomson[19] was adopted for the synthesis of **3**. Compound **3** was purified by silica gel column chromatography

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($CHCl_3$–hexane, 4:1, v/v): yield 84%; mp 153–154 °C (lit.[19] 154 °C); NMR ($CDCl_3$) δ 4.51 (s, 4 H, 2- and 3-$CH_2Br$), 7.50–8.20 (m, 4 H, H-5, H-6, H-7, and H-8, aromatic).

**2,3-Bis(hydroxymethyl)-1,4-naphthoquinone (4). Method A.** The bromide **3** (5 g, 15 mmol) in 100 mL of $H_2O$–dioxane (3:2, v/v) was stirred and refluxed for 5 h. The solvents were removed in vacuo. The residue was then dissolved in $CHCl_3$ and chromatographed on a silica gel column (EtOAc–$C_6H_6$, 1:1 v/v) to afford 1.4 g (44%): mp 127–128 °C; TLC $R_f$ 0.36 (EtOAc–$C_6H_6$, 1:1, v/v); NMR ($Me_2SO$-$d_6$) δ 4.52 (s, 4 H, 2- and 3-$CH_2$), 4.85 (m, very broad, 2 H, 2- and 3-OH, $D_2O$ exchangeable), 7.63–8.05 (m, 4 H, H-5, H-6, H-7, and H-8, aromatic). Anal. ($C_{12}H_{10}O_4$) C, H.

**Method B.** The chloride **2** (0.2 g, 0.8 mmol) was stirred and refluxed in 15 mL of $H_2O$–dioxane (2:3, v/v) for 24 h. The solvents were evaporated to dryness under diminished pressure. The residue was dissolved in $CHCl_3$ and chromatographed on a silica gel column ($C_6H_6$–EtOAc, 7:3, v/v). The first collected fraction yielded 35 mg (19%) of 2-(hydroxymethyl)-3-(chloromethyl)-1,4-naphthoquinone: mp 113–115 °C; TLC $R_f$ 0.47 ($C_6H_6$–EtOAc, 4:1, v/v); NMR ($CDCl_3$) δ 2.62 (br s, 1 H, OH, $D_2O$ exchangeable), 4.65 (s, 2 H, 2-$CH_2$), 4.75 (s, 2 H, 3-$CH_2Cl$), 7.58–8.08 (m, 4 H, H-5, H-6, H-7, and H-8, aromatic). Anal. ($C_{12}H_9ClO_3$) C, H.

The second fraction collected afforded 70 mg (40%) of **4**.

**2,3-Bis[[(methylsulfonyl)oxy]methyl]-1,4-naphthoquinone (5).** Silver methanesulfonate (1.4 g, 7 mmol) and **3** (0.35 g, 1 mmol) in 20 mL of $CH_3CN$ were stirred overnight at room temperature. The solvent was removed in vacuo, and the solid residue was extracted several times with $CHCl_3$. The combined $CHCl_3$ solutions were concentrated and chromatographed on a silica gel column ($C_6H_6$–EtOAc, 4:1, v/v) to give 0.27 g (71%): mp 134–135 °C; TLC $R_f$ 0.23 ($C_6H_6$–EtOAc, 4:1, v/v); NMR ($CDCl_3$) δ 3.22 (s, 6 H, $OSO_2CH_3$), 5.38 (s, 4 H, 2- and 3-$CH_2$), 7.62–8.07 (m, 4 H, H-5, H-6, H-7, and H-8, aromatic). Anal. ($C_{14}H_{14}O_8S_2$) C, H, S.

**2,3-Bis[[(*p*-tolylsulfonyl)oxy]methyl]-1,4-naphthoquinone (6).** A mixture of **3** (0.7 g, 2 mmol) and silver *p*-toluenesulfonate (2.3 g, 8 mmol) in 25 mL of $CH_3CN$ was stirred for 48 h. The solvent was evaporated under reduced pressure, and the solid residue was extracted several times with $CHCl_3$. The combined $CHCl_3$ solutions were concentrated and chromatographed on a silica gel column ($C_6H_6$–EtOAc, 9:1, v/v) to afford 0.83 g (77%) of **6**: mp 107–108 °C; TLC $R_f$ 0.37 ($C_6H_6$–EtOAc, 9:1, v/v); NMR ($CDCl_3$) δ 2.40 (s, 6 H, $OSO_2C_6H_4CH_3$-*p*), 5.18 (s, 4 H, 2- and 3-$CH_2$), 7.02–8.02 (m, 12 H, H-5, H-6, H-7, and H-8, aromatic, $H_a$ and $H_b$ tolyl ring). Anal. ($C_{26}H_{22}O_8S_2$) C, H, S.

**2,3-Bis(acetoxymethyl)-1,4-naphthoquinone (7).** This compound was synthesized by a modification of the method of Paull et al.[20] The bromide **3** (1.5 g, 4.4 mmol) and silver acetate (2.9 g, 17.4 mmol) in 60 mL of AcOH–$CHCl_3$ (2:1, v/v) were refluxed with stirring overnight. After cooling to room temperature, the mixture was filtered. The filtrate was evaporated to dryness in vacuo. The residue was dissolved in a minimum amount of $CHCl_3$ and chromatographed on a silica gel column ($CHCl_3$) to give 0.77 g (58%), mp 131–132 °C (lit.[9] 129–131 °C).

**2,3-Bis(methoxymethyl)-1,4-naphthoquinone (8).** The bromide **3** (1.32 g, 4 mmol) in 25 mL of $CH_3OH$ was refluxed with stirring for 3 days. The solvent was removed in vacuo. The residue was dissolved in $CHCl_3$ and chromatographed on a silica gel column ($C_6H_6$–EtOAc, 9:1, v/v) to afford 0.9 g of **8** as a syrup, which solidified upon cooling: mp 31–32 °C; TLC $R_f$ 0.28 ($C_6H_6$–EtOAc, 9:1 v/v); NMR ($CDCl_3$) δ 3.40 (s, 6 H, $OCH_3$), 4.51 (s, 4 H, 2- and 3-$CH_2$), 7.40–8.07 (m, 4 H, H-5, H-6, H-7, and H-8, aromatic). Anal. ($C_{14}H_{14}O_4$) C, H.

**2,3-Bis(hydroxymethyl)-1,4-naphthoquinone Bis(*N*-methylcarbamate) (9).** The alcohol **4** (0.35 g, 1.6 mmol) in 20 mL of $CHCl_3$–$CH_3NCO$ (1:2, v/v) was refluxed with stirring for 24 h. The solvents were removed *in vacuo* and the residue was triturated with $C_6H_6$. The product was collected by filtration, washed thoroughly with $C_6H_6$, and dried, to afford 0.35 g (66%); mp 108-110°C (dec); TLC, $R_f$ 0.13 ($C_6H_6$–EtOAc, 3:2, v/v); NMR ($CDCl_3$–$Me_2SO$-$d_6$, 1:1 v/v) δ 2.62 (d, 6 H, NH$CH_3$), 5.08 (s, 4 H, 2- and 3-$CH_2$), 6.30 (m, 2 H, NH, $D_2O$ exchangeable), 7.55–8.05 (m, 4 H, H-5, H-6, H-7 and H-8, aromatic). Anal. ($C_{16}H_{16}N_2O_6$) C, H, N.

**2,3-Bis(hydroxymethyl)-1,4-naphthoquinone Bis[*N*-(2-chloroethyl)carbamate] (10).** Compound **4** (1.2 g, 5.4 mmol) in 60 mL of $CHCl_3$–$ClCH_2CH_2NCO$ (1:2, v/v) was refluxed for 3 days. The mixture was evaporated to dyness in vacuo. The solid residue was washed thoroughly with $C_6H_6$, filtered, and dried to give 1.37 g: mp 122–123 °C; TLC $R_f$ 0.62 ($CHCl_3$–EtOAc, 1:1, v/v); NMR ($Me_2SO$-$d_6$) δ 3.03–3.70 (m, 8 H, NH$CH_2CH_2Cl$), 5.12 (s, 4 H, 2- and 3-$CH_2$), 7.32 (m, 2 H, NH, $D_2O$ exchangeable), 7.60–8.05 (m, 4 H, H-5, H-6, H-7, and H-8, aromatic). Anal. ($C_{18}H_{18}Cl_2N_2O_6$) C, H, Cl, N.

**Biological Test Procedures.** Transplantation of Sarcoma 180 ascites cells was carried out by withdrawing peritoneal fluid from donor $CDF_1$ mice bearing 7-day growths. The suspension was centrifuged for 2 min (1600*g*), the supernatant peritoneal fluid was decanted, and a 10-fold dilution with isotonic saline was made. The cell number was determined with a Coulter particle counter, and the cell population was adjusted to $10^7$ cells/mL. The resulting cell suspension, 0.1 mL (containing approximately $10^6$ cells), was injected intraperitoneally into each animal. Drugs were administered by intraperitoneal injection, beginning 24 h after tumor implantation, once daily for 6 consecutive days. The test compounds were injected as fine suspensions following homogenization in 2 to 3 drops of 20% aqueous Tween 80 and then made up to volume with isotonic saline. All drugs were administered intraperitoneally in a volume of 0.5 mL. For any one experiment, animals were distributed into groups of five mice of comparable weight and maintained throughout the course of the experiment on Purina Laboratory Chow pellets and water "ad libitum". Control tumor-bearing animals given injections of comparable volumes of vehicle were included in each experiment. Mice were weighed during the course of the experiments, and the percent change in body weight from onset to termination of therapy was used as an indication of drug toxicity. Determination of the sensitivity of ascitic neoplasms to these agents was based on the prolongation of survival time afforded by the drug treatments. Each experiment was repeated at least one time.

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