15 h. The solvent was removed in vacuo, and the residue was treated with 150 mL of H_2O and filtered. The filtrate was acidified to pH 2 with concentrated HCl, and the product was collected, washed with H_2O, and dried.

B. Curtius Rearrangement. A rapidly stirred mixture of the above crude hydrazide hydrochloride (0.0033 mol), 6 mL of 5 N HCl, and 30 mL of Et_2O was cooled to −10 °C and treated over several minutes with a solution of $NaNO_2$ (0.0034 mol) in 2 mL of H_2O. After 10 min, the organic layer was separated and the aqueous portion was washed with another portion of Et_2O. The ethereal extracts were combined and dried ($MgSO_4$) briefly. The solution was treated with 40 mL of absolute EtOH and gently distilled until the temperature of the distillate reached 78 °C, at which point the solution was refluxed for an addition hour and then allowed to stand at ambient temperature for 1 h. The solvent was removed in vacuo, and the residue was crystallized to give **13**.

Saponification of Urethanes. Method K. A mixture of the above urethane (0.016 mol) and 26 mL of 3 N NaOH was refluxed 1.5 h. The solution was cooled, slowly acidified to pH 2 with concentrated HCl, and filtered. The filtrate was adjusted to pH 5.0 with NH_4OH, diluted to 300 mL with H_2O, heated to boiling, treated with Nuchar, and filtered while hot. Upon allowing the filtrate to cool slowly, the product crystallized out and was collected and washed with ice–water to give **14**.

α-Acetyl-α-(5-methoxy-3-indolyl)acetonitrile (16). Method L. A solution of NaOEt was prepared from Na (6 g, 0.26 mol) and 100 mL of absolute EtOH. After removal of the solvent in vacuo, the alkoxide cake was rapidly treated with vigorous stirring with a solution of 5-methoxy-3-indolylacetonitrile (20.6 g, 0.12 mol) in 120 mL of EtOAc. The mixture was refluxed for 6 h and then allowed to stand at ambient temperature for 18 h, diluted with 200 mL of Et_2O, cooled for 1 h, and filtered. The filter cake was washed with Et_2O and dissolved in 300 mL of H_2O, and the solution was cooled and treated slowly with HOAc to pH 4–5. The mixture was extracted into Et_2O, the organic layer was washed three times with $NaHCO_3$ solution and dried ($MgSO_4$), and the solvent was removed in vacuo to give 15.6 g (61%) of **16** as an oil, which was used directly for the subsequent reaction.

3-Amino-2-(5-methoxy-3-indolyl)butyronitrile (17). Method M. A mixture of **16** (12.5 g, 0.063 mol), NH_4OAc (49 g, 0.63 mol), and $NaBH_3CN$ (4 g, 0.063 mol) in 170 mL of MeOH was stirred at ambient temperature for 5 days. The dark mixture was cooled, slowly treated with concentrated HCl to pH 2, and stirred for 15 min at ambient temperature. The mixture was evaporated in vacuo to dryness, and the residue was partitioned between 300 mL of H_2O and 200 mL of EtOAc. The aqueous layer was washed three times with EtOAc and was then treated slowly with 10 N NaOH to pH 10. The turbid mixture was extracted twice with EtOAc, and the organic extracts were combined, dried ($MgSO_4$), treated with Nuchar, and filtered. The solvent was evaporated in vacuo to give 8.5 g of **17** as an oil, which was pure enough for use in the subsequent reaction.

Methyl 3-Amino-2-(5-methoxy-3-indolyl)butyrate (18). Method N. A mixture of 8.5 g of the above crude amine, 40 mL of 95% EtOH, 10 mL of H_2O, and 10 g of KOH was refluxed for 22 h. After cooling, the mixture was diluted to 250 mL with H_2O and extracted three times with Et_2O. The aqueous layer was adjusted to pH 5.2 with dilute HCl, diluted to 300 mL with H_2O, heated to boiling, treated with Nuchar, and filtered while hot. The filtrate was evaporated in vacuo to dryness and the residue desicated for 2 h. The dried residue was slurried in 50 mL of MeOH, and the mixture was cooled to −10 °C and treated dropwise with $SOCl_2$ (2.3 mL, 0.0325 mol). After the addition, the mixture was stirred for 16 h at ambient temperature, and the solvent was removed in vacuo. The residue was partitioned between 50 mL of EtOAc and 20 mL of 5% K_2CO_3, and the organic layer was dried ($MgSO_4$) and treated with a solution of 2.9 g of anhydrous oxalic acid in 4 mL of MeOH. The solid was collected, affording 0.9 g of **18**, mp 145–147 °C dec. Anal. ($C_{16}H_{20}N_2O_7$) H, N; C: calcd, 54.54; found, 55.62.

Pharmacology. Antihypertensive Effects in Rats. Rats were made hypertensive by applying a figure-of-eight ligature to one kidney and removing the other kidney 2 weeks later. At least 4 weeks were allowed to elapse after the second operation before experimental studies were performed. Indirect systolic blood pressure measurements were made with an occluding cuff and pulse sensor system fitted to the rat's tail. Control blood pressure measurements were made before any compounds were administered. Blood pressure measurements were then made 1, 2, 4, 6, and 8 h after the oral administration of the test compounds at the dose level of 10 mg/kg. Statistical significance of the difference between the control and posttreatment value was determined by Wilcoxon's signed rank test.[20]

Hypotensive Effects in Anesthetized Cats. Cats of either sex (2–4 kg) were anesthetized with α-chloralose (80 mg/kg, iv). The trachea, a femoral artery, and a jugular vein were cannulated for adequate ventilation, for drug administration, and to record blood pressure, respectively. Blood pressure was measured with a pressure transducer and was obtained before and 30 min after the administration of an iv dose of 10 mg/kg of compound.

(20) F. Wilcoxon and R. A. Wilcox, "Some Rapid Approximate Statistical Procedures", Lederle Laboratories, Pearl River, NY, 1964.

2- and 6-Methyl-1,4-naphthoquinone Derivatives as Potential Bioreductive Alkylating Agents

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A number of antineoplastic agents possess both the quinone nucleus and an appropriate substituent that permits them to function as bioreductive alkylating agents. To develop new compounds of this type with unique properties, we have synthesized a series of 2- and 6-methyl-1,4-naphthoquinone derivatives and have evaluated them for antineoplastic activity against Sarcoma 180 ascites cells. Several of these quinones showed antitumor activity, causing significant prolongation of the survival time of tumor-bearing mice. Among the most active agents were the mesylates, tosylates, and *N*-(chloroethyl)carbamates of 2- and 6-methyl-1,4-naphthoquinone. That bioreductive activation to a quinone methide might be involved in the mechanism of action of these agents was shown by the finding that compounds with the best leaving groups were the most efficacious as antineoplastic agents.

Solid tumors contain areas of vascular insufficiency that result in the generation of hypoxic tumor cells. These oxygen-deficient cells are an obstacle to the attainment of curative therapy, since hypoxic stem cells are relatively resistant to X-irradiation and are presumed to be relatively resistant to chemotherapy.[1,2] This laboratory has dem-

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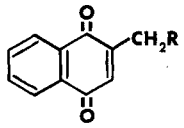
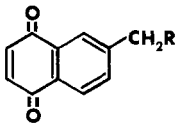
Chart I



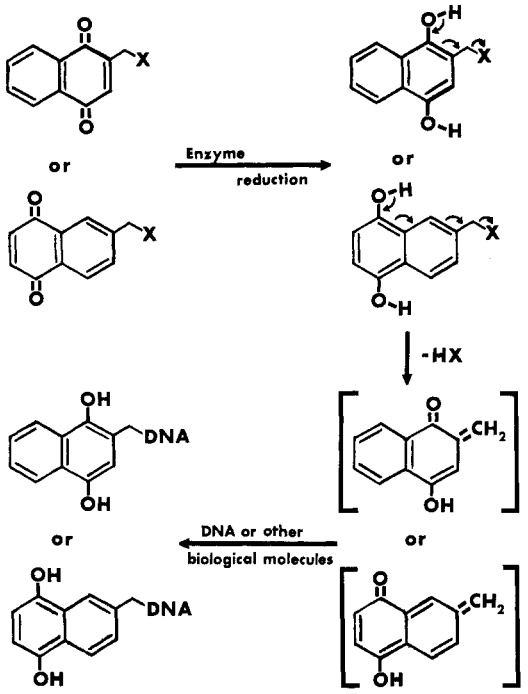
a



b

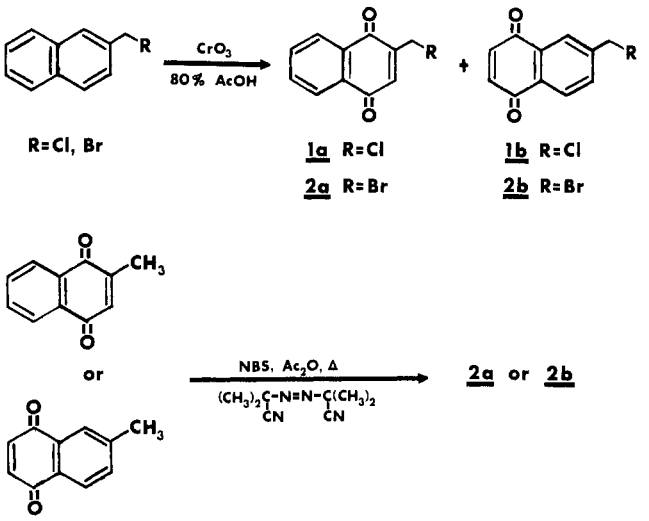
1	R = Cl	6	R = $OSO_2C_6H_4CH_3$-P
2	R = Br	7a	R = OCH_3
3	R = OAc	7b	R = OCH_2CH_3
4	R = OH	8	R = $OCONHCH_3$
5	R = OSO_2CH_3	9	R = $OCONHCH_2CH_2Cl$

Scheme I

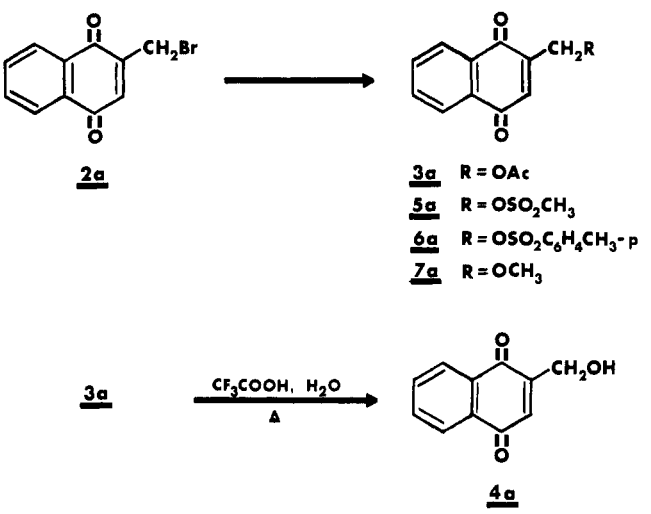


onstrated that the quinone antibiotic mitomycin C is preferentially cytotoxic to hypoxic tumor cells in vitro[3] and is metabolically activated by reduction to an alkylating species by tumor cell enzyme systems under anaerobic conditions.[4] For this reason, a number of quinone derivatives have been evaluated as potential bioreductive alkylating agents.[5-11] The quinones that have been developed were hypothesized to be activated preferentially by hypoxic cells to produce highly reactive quinone methides with the capacity to alkylate cellular components. Early studies suggested that the presence of two side chains capable of generating alkylating moieties was not essential for the expression of antineoplastic activity and that the leaving group could be varied without markedly affecting cancer chemotherapeutic action, although only a limited number of substitutions were evaluated.[12]

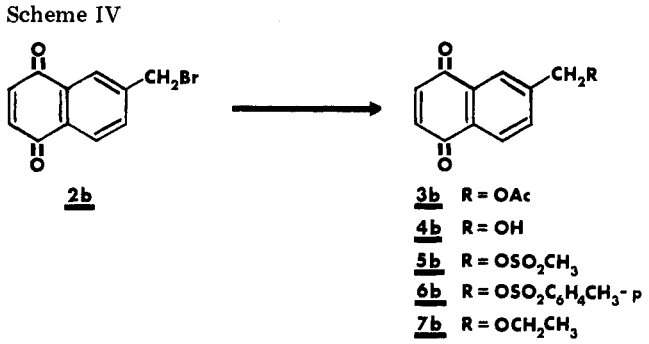
Scheme II



Scheme III



Scheme IV



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(4) K. A. Kennedy, S. Rockwell, and A. C. Sartorelli, *Cancer Res.*, **40**, 2356 (1980).
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(6) A. J. Lin, R. S. Pardini, L. A. Cosby, B. J. Lillis, C. W. Shansky, and A. C. Sartorelli, *J. Med. Chem.*, **16**, 1268 (1973).
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(8) A. J. Lin, R. S. Pardini, B. J. Lillis, and A. C. Sartorelli, *J. Med. Chem.*, **17**, 668 (1974).
(9) A. J. Lin, B. J. Lillis, and A. C. Sartorelli, *J. Med. Chem.*, **18**, 917 (1975).
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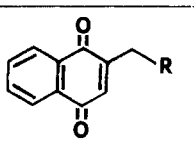
(12) A. J. Lin, L. A. Cosby, and A. C. Sartorelli, in "Cancer Chemotherapy", A. C. Sartorelli, Ed., American Chemical Society, Washington, DC, 1976, p 71.

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



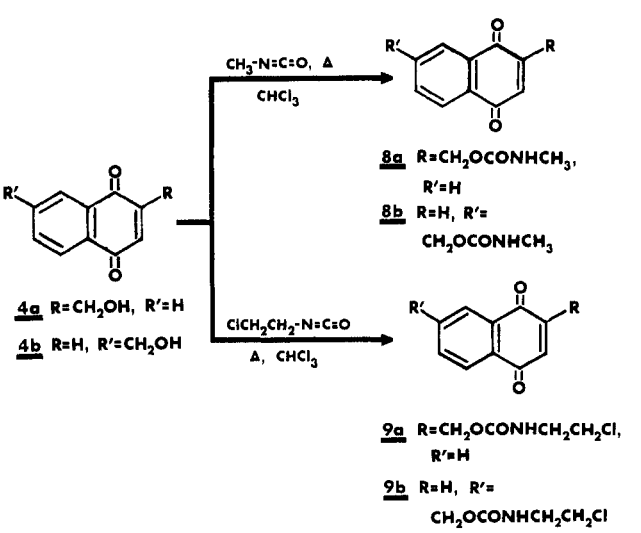
compd	R	daily dosage, mg/kg	av Δ wt,[a] %	T/C[b]
2-methyl-1,4-naphthoquinone	H	5	+18.7	0.85
		10	+17.3	1.03
		20	−2.0	1.34
1a	Cl	5	−2.0	1.40
		10	−9.8	1.47
		20	−16.4	1.30
2a	Br	2.5	+1.7	1.61
		5	−5.4	1.73
		10	−7.7	1.49
		20	−9.1	1.69
3a	OAc	5	−9.3	1.66
		10	−14.4	1.62
		20	−10.2	0.70
4a	OH	5	−6.4	1.16
		10	−15.2	0.45
		20		0.22
5a	OSO_2CH_3	5	−6.3	1.92
		10	−8.3	1.57
		20	−8.8	1.41
6a	$OSO_2C_6H_4CH_3$-*p*	5	+2.8	1.77
		10	−4.4	2.16
		20	−7.4	1.62
7a	OCH_3	5	+17.0	0.98
		10	+1.6	1.38
		20	−19.0	1.07
8a	$OCONHCH_3$	5	−12.6	1.39
		10	−10.5	1.51
		20	−11.4	0.51
9a	$OCONHCH_2CH_2Cl$	5	−4.6	2.08
		10	−11.4	1.71
		20	−13.8	1.96

[a] Average weight change from onset to termination of drug treatments (+16.9 for untreated control tumor-bearing animals). [b] T/C represents the ratio of the survival time of treated to control animals. The average survival time of the untreated tumor-bearing control animals was 11.4 ± 1.4 days. Each value represents results from 5 to 15 mice.

To extend the available information on the relationship between structure and activity in this class of compounds, we have synthesized a variety of 2- and 6-methyl-1,4-naphthoquinone derivatives (Chart I). It was envisioned that both series of agents might be activated in neoplastic cells by the mechanism shown in Scheme I.

Chemistry. A series of 2- and 6-substituted 1,4-naphthoquinones has been synthesized. The oxidation of 2-(chloromethyl)naphthalene with CrO_3 afforded the two products 2- and 6-(chloromethyl)-1,4-naphthoquinone, **1a** and **1b**, respectively[6] (Scheme II). In a similar manner, the oxidation of 2-(bromomethyl)naphthalene yielded the bromo derivatives **2a** and **2b**, which were also synthesized by bromination of 2- or 6-methylnaphthoquinone[13] with *N*-bromosuccinimide[14] (Scheme II). From the key derivative **2a**, the acetate **3a** was obtained by refluxing **2a** in a mixture of chloroform and acetic acid containing silver acetate.[15] The mesylate **5a** and the tosylate **6a** were formed by stirring **2a** in acetonitrile with silver mesylate or silver tosylate, respectively,[15] and the ether **7a** was synthesized by refluxing **2a** in methanol.[6] Hydrolysis of **3a** in trifluoroacetic acid–water (3:1, v/v) under refluxing

Scheme V



conditions[15] yielded the alcohol **4a** (Scheme III).

In an analogous manner, from compound **2b**, the acetate **3b**,[15] the sulfonates **5b** and **6b**,[15] and the ether **7b**[11] were synthesized. The alcohol **4b** was obtained from **2b** by refluxing in water–dioxane[11] (3:2, v/v) (Scheme IV). Reaction of the alcohols **4a** and **4b** with methyl isocyanate and chloroethyl isocyanate gave the corresponding car-

(13) D. B. Bruce and R. H. Thomson, *J. Chem. Soc.*, 2759 (1952).
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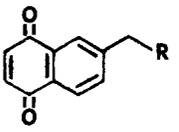
Table II. Effects of 6-Methyl-1,4-naphthoquinone Derivatives on the Survival Time of Mice Bearing Sarcoma 180 Ascites Cells



compd	R	daily dosage, mg/kg	av Δ wt,[a] %	T/C[b]
6-methyl-1,4-naphthoquinone	H	5	−11.5	1.09
		10	−11.1	0.38
		20		0.26
1b	Cl	5	−10.4	1.38
		10	−13.5	1.05
		20		0.22
2b	Br	2.5	−4.2	2.07
		5	−7.8	1.46
		10	−11.3	1.42
		20	−12.5	0.57
3b	OAc	2.5	−2.9	1.35
		5		0.93
		10	−7.4	0.48
4b	OH	2.5	−9.1	1.16
		10		0.17
5b	OSO_2CH_3	5	−6.3	1.71
		10	−11.8	1.43
		20	−13.3	1.44
6b	$OSO_2C_6H_4CH_3$-*p*	5	−3.8	1.44
		10	−11.6	1.82
		20	−15.2	1.64
7b	OCH_2CH_3	2.5	−6.4	1.32
		5	−10.3	0.97
		10		0.42
8b	$OCONHCH_3$	2.5	−7.6	1.71
		5	−7.8	1.61
		10	−10.8	0.81
9b	$OCONHCH_2CH_2Cl$	5	−9.0	1.47
		10	−11.7	1.63
		20	−17.3	0.50

[a,b] See corresponding footnotes in Table I.

bamates **8a**, **9a**, **8b**, and **9b**,[11] respectively (Scheme V).

Biological Evaluation. The results are summarized in Tables I and II. Several derivatives of both series showed significant activity. The leaving group influenced the degree of tumor-inhibitory activity in this system, with the best leaving groups in general giving the highest values of T/C. Thus, compounds such as the mesylates (**5a,b**), the tosylates (**6a,b**), and the *N*-(chloroethyl)carbamates (**9a,b**) were reasonably active agents against Sarcoma 180 ascites cells. These findings appear to be consistent with the mechanism of activation proposed in Scheme I.

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 or at 270 MHz on a Brucker 270 HX spectrometer using 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 value.

2-(Chloromethyl)- (1a) and 6-(Chloromethyl)-1,4-naphthoquinone (1b). 2-(Chloromethyl)naphthalene (11 g, 62 mmol) in 90 mL of AcOH was added dropwise over a 2-h period to a well-stirred solution of 40 g of CrO_3 in 100 mL of 80% AcOH. The solution was cooled in an ice bath during the addition to keep the temperature from rising above 20 °C. The solution was stirred at room temperature for 24 h and then poured onto 1 L of ice–water to give a yellow solid, which was dissolved in a small volume of chloroform and chromatographed on a silica gel column (benzene–chloroform, 1:1, v/v). The first fraction collected was **1a**: yield 2.5 g (19%); mp 92 °C (lit.[6] mp 86–88 °C). The second fraction collected was **1b**: yield 1.5 g (11.7%); mp 107–108 °C; NMR ($CDCl_3$) δ 4.70 (s, 2 H, 6-CH_2), 6.98 (s, 2 H, H-2 and H-3, aromatic), 7.65–8.10 (m, 3 H, H-5, H-7, and H-8, aromatic). Anal. ($C_{11}H_7ClO_2$) C, H, Cl.

2-(Bromomethyl)- (2a) and 6-(Bromomethyl)-1,4-naphthoquinone (2b). Method A. 2-(Bromomethyl)-naphthalene (22.1 g, 100 mmol) in 200 mL of AcOH was added dropwise over 1 h to a well-stirred solution of CrO_3 (70 g, 700 mmol) in 200 mL of 80% AcOH. The solution was cooled in an ice bath during the addition to keep the temperature from rising above 20 °C and then stirred at room temperature overnight. The reaction mixture was poured onto ice–water (2 L), and the yellow precipitate was collected by filtration, washed with water, and dissolved in $CHCl_3$. The resulting solution was dried (Na_2SO_4), filtered, concentrated, and chromatographed on a silica gel column (benzene) to give **2a** and **2b**. **2a**: yield 4.2 g (17%); mp 94–96 °C; NMR ($CDCl_3$) δ 4.42 (s, 2 H, 2-CH_2), 7.10 (s, 1 H, 3-H, aromatic), 7.60–8.20 (m, 4 H, H-5, H-6, H-7, and H-8 aromatic). **2b**: yield 2.5 g (10%); mp 123–124 °C; NMR ($CDCl_3$) δ 4.52 (s, 2 H, 6-CH_2), 6.93 (s, 2 H, H-2 and H-3, aromatic), 7.60–8.20 (m, 3 H, H-5, H-7, and H-8 aromatic). Anal. ($C_{11}H_7BrO_2$) C, H, Br.

Method B. 2-Methyl-1,4-naphthoquinone (10 g, 59 mmol), *N*-bromosuccinimide (31.5 g, 177 mmol), and azobis(2-methylpropionitrile) (0.5 g, 3 mmol) in 200 mL of Ac_2O were stirred at 120–130 °C for about 0.5 h. When the color lightened, the solution was poured onto ice. The yellow solid was collected and dissolved in $CHCl_3$. The $CHCl_3$ solution was dried (Na_2SO_4), concentrated to a small volume, and chromatographed on a silica gel column (benzene) to yield 7.2 g (49%) of **2a**: mp 94–96 °C (lit.[14] mp 96 °C). 2-(Bromomethyl)-3-bromo-1,4-naphthoquinone was also isolated as a byproduct: yield 5.0 g (26%); mp 119–120 °C (recrystallized from MeOH); NMR ($CDCl_3$) δ 4.62 (s, 2 H, 2-CH_2), 7.70–8.38 (m, 4 H, H-5, H-6, H-7, and H-8, aromatic). Compound **2b** was also prepared in 35% yield from 6-methyl-1,4-naphtho-

quinone[13] using method B as described above.

2-(Acetoxymethyl)-1,4-naphthoquinone (3a). The bromide **2a** (1.0 g, 4.0 mmol) and silver acetate (2.8 g, 17 mmol) in 45 mL of $CHCl_3$–AcOH (1:2, v/v) were stirred at 95–100 °C overnight. The solvent was evaporated in vacuo, and the residue was poured onto ice–water saturated with NaCl. The crude product and AgCl were collected by filtration and washed with water. The solid was then added to $CHCl_3$, and the insoluble AgCl was removed by filtration. The AgCl was washed with $CHCl_3$ until the washings were colorless. The combined $CHCl_3$ solutions were dried (Na_2SO_4), concentrated, and chromatographed on a silica gel column (C_6H_6–$CHCl_3$, 1:1, v/v) to afford 0.55 g (50%) of **3a**: NMR ($CDCl_3$) δ 2.18 (s, 3 H, $COCH_3$), 5.12 (d, 2 H, 2-CH_2), 6.82 (t, 1 H, H-3, aromatic), 7.60–8.10 (m, 4 H, H-5, H-6, H-7, and H-8, aromatic).

6-(Acetoxymethyl)-1,4-naphthoquinone (3b). A mixture of 6-(bromomethyl)-1,4-naphthoquinone (**2b**; 1.5 g, 6 mmol) and silver acetate (2.2 g, 13.2 mmol) in 20 mL of $CHCl_3$ and 40 mL of AcOH was heated at 95–100 °C with vigorous stirring overnight. The product was isolated and purified using the procedure employed in the preparation of **3a** to give 0.6 g (37%): mp 87–88 °C; NMR ($CDCl_3$) δ 2.16 (s, 3 H, $COCH_3$), 5.21 (s, 2 H, 6-CH_2), 6.93 (s, H-2 and H-3, aromatic), 7.58–8.20 (m, 3 H, H-5, H-6, and H-7, aromatic). Anal. ($C_{13}H_{10}O_4$) C, H.

2-(Hydroxymethyl)-1,4-naphthoquinone (4a). The acetate **3a** (0.25 g, 1.1 mmol) in 8 mL of CF_3COOH–H_2O (3:1, v/v) was heated under reflux conditions with stirring for 90 min. Upon cooling, the reaction mixture was poured onto ice and chilled for 30 min. The crude product was collected by filtration, washed with H_2O, and dissolved in $CHCl_3$. The $CHCl_3$ solution was dried (Na_2SO_4), concentrated to a small volume, and chromatographed on a silica gel column to yield 40 mg (20%) of **4a**: mp 111–112 °C; NMR ($CDCl_3$) δ 2.60 (t, 1 H, OH, D_2O exchangeable), 4.66 (m, 2 H, 2-CH_2), 7.01 (t, 1 H, H-3, aromatic), 7.50–8.20 (m, 4 H, H-5, H-6, H-7, and H-8, aromatic). Anal. ($C_{11}H_8O_3$) C, H.

6-(Hydroxymethyl)-1,4-naphthoquinone (4b). 6-(Bromomethyl)-1,4-naphthoquinone (**2b**; 1.5 g, 6 mmol) in 50 mL of water–dioxane (3:2, v/v) was refluxed with stirring for 1 h. The solution was then diluted with water (300 mL) and extracted with $CHCl_3$ (3 × 300 mL). The $CHCl_3$ solution was dried (Na_2SO_4), concentrated, and chromatographed on a silica gel column ($CHCl_3$–EtOAc, 7:3, v/v) to yield 0.8 g (71%) of **4b**: mp 81–82 °C; NMR ($CDCl_3$) δ 2.72 (s, 1 H, OH, D_2O exchangeable), 4.82 (s, 2 H, 6-CH_2), 6.92 (s, 2 H, H-2 and H-3, aromatic), 7.58–8.10 (m, 3 H, H-5, H-7, and H-8, aromatic). Anal. ($C_{11}H_8O_3$) C, H.

2-[[(Methanesulfonyl)oxy]methyl]-1,4-naphthoquinone (5a). A mixture of the bromide **2a** (1.0 g, 4.0 mmol) and silver methanesulfonate (3.2 g, 16 mmol) in CH_3CN (20 mL) was stirred at room temperature for 24 h. The solvent was removed in vacuo at 40 °C, and the residue was extracted with $CHCl_3$ (5 × 50 mL). The combined $CHCl_3$ extracts were concentrated and chromatographed on a silica gel column (benzene–EtOAc, 9:1, v/v) to give 0.4 g (38%) of **5a**: mp 121–122 °C; NMR ($CDCl_3$) δ 3.18 (s, 3 H, CH_3), 5.12 (d, 2 H, 2-CH_2), 7.04 (t, 1 H, H-3, aromatic), 7.62–8.20 (m, 4 H, H-5, H-6, H-7, and H-8, aromatic). Anal. ($C_{12}H_{10}O_5S$) C, H, S.

6-[[(Methanesulfonyl)oxy]methyl]-1,4-naphthoquinone (5b). Compound **2b** (1.5 g, 6 mmol) and silver methanesulfonate (4.8 g, 24 mmol) in 30 mL of CH_3CN were stirred at room temperature for 6 h. Compound **5b** was isolated in a manner similar to that described for **5a**: yield 0.41 g (70%); mp 94–95 °C dec; NMR ($CDCl_3$) δ 3.02 (s, 3 H, CH_3), 5.30 (s, 2 H, 6-CH_2), 6.92 (s, 2 H, H-2, and H-3, aromatic), 7.60–8.18 (m, 3 H, H-5, H-7, and H-8, aromatic). Anal. ($C_{12}H_{10}O_5S$) C, H, S.

2-[[(*p*-Toluenesulfonyl)oxy]methyl]-1,4-naphthoquinone (6a). A mixture of 2-(bromomethyl)-1,4-naphthoquinone (1.0 g, 4.0 mmol) and silver *p*-toluenesulfonate (4.5 g, 16 mmol) in 25 mL of CH_3CN was stirred at room temperature for 24 h. The solvent was evaporated to dryness under reduced pressure at 40 °C. The residue was then extracted with $CHCl_3$ (5 × 50 mL), and the combined $CHCl_3$ solution was concentrated to a small volume and chromatographed on a silica gel column ($CHCl_3$) to afford 0.75 g (55%) of **6a**: mp 110–111 °C; NMR ($CDCl_3$) δ 2.42 (s, 3 H, CH_3), 5.15 (d, 2 H, 2-CH_2), 6.92 (t, 1 H, H-3, aromatic), 7.30 (d, 2 H, H_A tolyl ring), 7.59–8.18 (m, 6 H, H-5, H-6, H-7, and H-8, aromatic, H_B tolyl ring). Anal. ($C_{18}H_{14}O_5S$) C, H, S.

6-[[(*p*-Toluenesulfonyl)oxy]methyl]-1,4-naphthoquinone (6b). The bromide **2b** (1.0 g, 4.0 mmol) and silver *p*-toluenesulfonate (4.5 g, 16 mmol) in 25 mL of CH_3CN were stirred at room temperature overnight. The product was isolated as described for the preparation of **6a** to yield 1.27 g (93%) of **6b**: mp 101–102 °C dec; NMR ($CDCl_3$) δ 2.40 (s, 3 H, CH_3), 5.15 (s, 2 H, 6-CH_2), 6.90 (s, 2 H, H-2 and H-3, aromatic), 7.18–8.10 (m, 7 H, H-5, H-7, and H-8, aromatic, H_A and H_B tolyl ring). Anal. ($C_{12}H_{10}O_5S$) C, H, S.

2-(Methoxymethyl)-1,4-naphthoquinone (7a). The bromide **2a** (1.0 g, 4.0 mmol) in 25 mL of absolute methanol was refluxed with stirring until no starting material was detected by TLC (benzene). The solution was then poured onto ice. The crude product was collected by filtration, dissolved in $CHCl_3$, concentrated, and chromatographed on a silica gel column (benzene–$CHCl_3$, 1:1, v/v) to yield 0.4 g (50%) of **7a**: mp 104–105 °C; NMR ($CDCl_3$) δ 3.50 (s, 3 H, OCH_3), 4.42 (d, 2 H, 2-CH_2), 6.97 (t, 1 H, H-3, aromatic), 7.55–8.19 (m, 4 H, H-5, H-6, H-7, and H-8, aromatic). Anal. ($C_{12}H_{10}O_3$) C, H.

6-(Ethoxymethyl)-1,4-naphthoquinone (7b). Compound **2b** (0.5 g, 2.0 mmol) in a mixture of EtOH and H_2O (4:1, v/v) was refluxed for 2 h. The solution was poured onto ice, and the yellow precipitate that formed was collected by filtration. The crude product was dissolved in $CHCl_3$, dried (Na_2SO_4), concentrated to a small volume, and chromatographed on a silica gel column (benzene–EtOAc, 4:1, v/v). The pure product was isolated in 65% yield (0.28 g): mp 73–74 °C; NMR ($CDCl_3$) δ 1.28 (t, 3 H, CH_3), 3.60 (q, 2 H, OCH_2), 4.60 (s, 2 H, 6-CH_2), 6.90 (s, 2 H, H-2 and H-3, aromatic), 7.58–8.11 (m, 3 H, H-5, H-7, and H-8, aromatic). Anal. ($C_{13}H_{12}O_3$) C, H.

2-(Hydroxymethyl)-1,4-naphthoquinone *N*-Methylcarbamate (8a). Methyl isocyanate (23 mL) was added to a solution of **4a** (0.49 g, 2.6 mmol) in 13 mL of CH_2Cl_2. The reaction mixture was refluxed with stirring for 24 h, concentrated to a small volume, and chromatographed on a silica gel column (benzene–EtOAc, 4:1, v/v) to afford 0.23 g (36%) of **8a**: mp 104–105 °C dec; NMR ($CDCl_3$) δ 2.85 (d, 3 H, CH_3), 4.90 (br s, 1 H, D_2O exchangeable), 5.15 (d, 2 H, 2-CH_2), 6.87 (t, 1 H, H-3, aromatic), 7.60–8.20 (m, 4 H, H-5, H-6, H-7, and H-8, aromatic). Anal. ($C_{13}H_{11}NO_4$) C, H, N.

6-(Hydroxymethyl)-1,4-naphthoquinone *N*-Methylcarbamate (8b). Methyl isocyanate (35 mL) was added to a solution of **4b** (0.9 g, 4.8 mmol) in 25 mL of $CHCl_3$. The solution was refluxed with stirring for 30 h and then evaporated to dryness under reduced pressure. The residue was crystallized from $CHCl_3$–EtOAc to give 0.65 g of **8b**. The mother liquor was concentrated and chromatographed on a silica gel column ($CHCl_3$–EtOAc, 4:1, v/v) to afford an additional 0.4 g. The total yield was 1.05 g (90%): mp 142–143 °C; NMR ($CDCl_3$) δ 2.82 (d, 3 H, $NHCH_3$), 4.80 (br s, 1 H, $NHCH_3$, D_2O exchangeable), 5.20 (s, 2 H, 6-CH_2), 6.90 (s, H-2 and H-3, aromatic), 7.51–8.10 (m, 3 H, H-5, H-7, and H-8, aromatic). Anal. ($C_{13}H_{11}NO_4$) C, H, N.

2-(Hydroxymethyl)-1,4-naphthoquinone *N*-(Chloroethyl)carbamate (9a). Chloroethyl isocyanate (20 mL) was added to a solution of **4a** (0.45 g, 2.4 mmol) in 10 mL of $CHCl_3$. The solution was refluxed with stirring for 18 h, and the solvent was then removed in vacuo. The residue was dissolved in a minimum amount of $CHCl_3$ and chromatographed on a silica gel column ($CHCl_3$) to yield 0.6 g (85%) of **9a**: mp 95–96 °C dec; NMR ($CDCl_3$) δ 3.62 (m, 4 H, CH_2CH_2Cl), 5.15 (d, 2 H, 2-CH_2), 5.40 (br s, 1 H, NH, D_2O exchangeable), 6.89 (t, 1 H, H-3, aromatic), 7.60–8.20 (m, 4 H, H-5, H-6, H-7, and H-8, aromatic). Anal. ($C_{14}H_{12}ClNO_4$) C, H, Cl, N.

6-(Hydroxymethyl)-1,4-naphthoquinone *N*-(Chloroethyl)carbamate (9b). Chloroethyl isocyanate (35 mL) was added to **4b** (0.8 g, 4.3 mmol) in $CHCl_3$ (18 mL). The reaction mixture was refluxed with stirring for 6 h and concentrated in vacuo to dryness at 50 °C. The residue was then dissolved in a small amount of $CHCl_3$ and chromatographed on a silica gel column ($CHCl_3$) to afford 1.2 g (96%) of the product: mp 103–104 °C; NMR ($CDCl_3$) δ 3.60 (m, 4 H, CH_2CH_2Cl), 5.20 (s, 2 H, 6-CH_2), 5.45 (br s, 1 H, NH, D_2O exchangeable), 6.92 (s, 2 H, H-2 and H-3, aromatic), 7.55–8.10 (m, 3 H, H-5, H-7, and H-8, aromatic). Anal. ($C_{14}H_{12}ClNO_4$) 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 (1600g), 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. One-tenth milliliter of the resulting cell suspension (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 2–3 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". Controls given injections of a comparable volume of vehicle were included in each experiment. Mice were weighed during the course of the experiments, and the percentage 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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2-(Aminomethyl)phenols, a New Class of Saluretic Agents. 4. Effects of Oxygen and/or Nitrogen Substitution[1]

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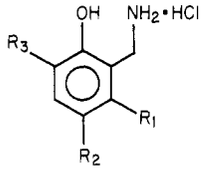
A series of oxygen and/or nitrogen substituted 2-(aminomethyl)phenols was synthesized and tested orally in rats for saluretic and diuretic effects. Intravenous dog data are included as supplementary material to demonstrate diuretic responses, or lack thereof, in a second species. In general, substitution on nitrogen with groups other than lower alkyl or substitution on nitrogen and/or oxygen with groups resistant to hydrolysis substantially diminished or ablated saluretic effects.

We reported previously on a series of 2-(aminomethyl)phenols[3] which were shown to possess a high order of diuretic activity in rats and dogs and discussed the effects of functional-group reorientation and modification.[4] The compounds reported herein were prepared in order to assess the biological consequences of oxygen and/or nitrogen substitution. From those 2-(aminomethyl)phenols described earlier,[3] compounds **1–3**, which exhibit good (1) to excellent (2 and 3) saluretic effects in rats and dogs, were chosen for demonstrating the effects of these substitutions.

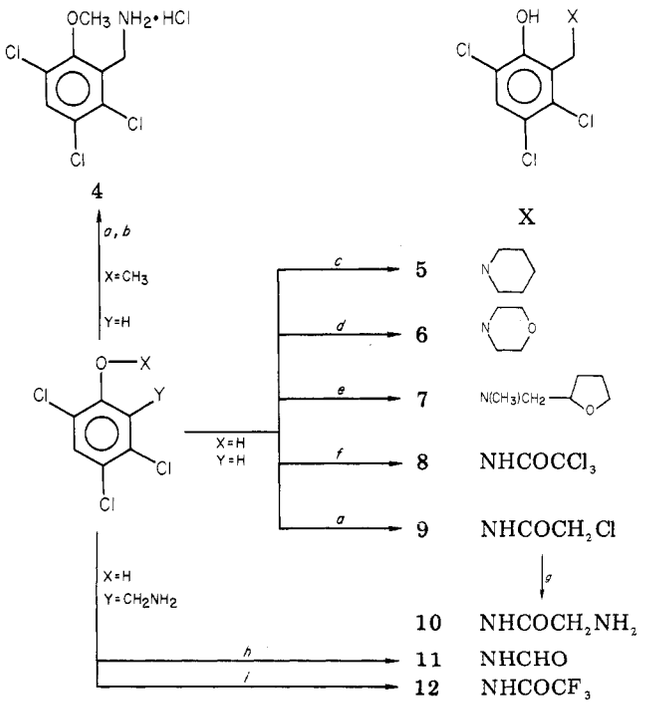


1, $R_1 = R_2 = R_3 = Cl$
2, $R_1 = H$; $R_2 = t\text{-}C_4H_9$; $R_3 = Cl$
3, $R_1 = H$; $R_2 = t\text{-}C_4H_9$; $R_3 = I$

Chemistry. The compounds prepared for this study are listed in Tables I–III, and their syntheses are outlined in Schemes I–IV. The compounds depicted in Schemes I–IV were prepared from the corresponding phenols, with the exception of **4** (methyl ether). Compounds **4**, **8**, and **9** were prepared by amidoalkylation (method A_2; without subsequent hydrolysis in the instances of **8** and **9**) as described in Part 1,[3] while **10** was obtained via subsequent amination of **9**.

Tertiary amines **5–7**, **13**, and **47** and secondary amine **44** (followed by iodination) were prepared under standard Mannich reaction conditions.[5] Diamine **45** was elaborated from dehalo **3a** via transamination with ethylenediamine

Scheme I



[a] $ClCH_2CONHCH_2OH$, H_2SO_4. [b] EtOH, HCl, Δ. [c] CH_2O, c-$NH(CH_2)_5$, Δ. [d] CH_2O, c-$NH(CH_2CH_2)_2O$, Δ. [e] CH_2O, 2-$NH(CH_3)CH_2$-c-C_4H_8O, Δ. [f] $Cl_3CCONHCH_2OH$, H_2SO_4. [g] NaI, NH_4OH. [h] $HCONH_2$, Δ. [i] $(F_3CCO)_2O$.

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