(AcOEt); yield 81%. Anal. ($C_{26}H_{30}N_2O_4$) C, H, N. When the solution of **23** in $CHCl_3$ was saturated with HCl and left at room temperature for 5 h, it was converted into compound **24** (TLC, ir, and melting point).

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Potential Bioreductive Alkylating Agents. 7. Antitumor Effects of Phenyl-Substituted 2-Chloromethyl-3-phenyl-1,4-naphthoquinones[1]

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Functional groups such as nitro, chloro, bromo, and methoxy were introduced in the meta and para positions of the phenyl ring of the antineoplastic agent 2-chloromethyl-3-phenyl-1,4-naphthoquinone. Tests for tumor-inhibitory potency of these derivatives against Sarcoma 180 ascites cells in mice indicated that the para-substituted methoxyphenyl, chlorophenyl, and bromophenyl derivatives possessed antitumor activity comparable to that of the parent compound 2-chloromethyl-3-phenyl-1,4-naphthoquinone, whereas meta-substituted nitro and bromo derivatives were either inactive or only weakly active anticancer agents in this system.

This laboratory has synthesized a variety of quinone derivatives with the potential to alkylate biological molecules following reductive activation and has demonstrated their antineoplastic activity against transplantable rodent tumors.[2-6] Studies on the biochemical mechanism of action of a representative member of this series, 2,3-bis(chloromethyl)-1,4-naphthoquinone, have indicated that this agent produces a variety of metabolic lesions including (a) inhibition of the biosynthesis of DNA, with lesser effects on the formation of RNA and protein; (b) inhibition of the coenzyme Q mediated enzyme systems, NADH oxidase and succinoxidase; (c) extensive and prolonged interaction with DNA, RNA, and protein; and (d) fragmentation of DNA.[7] Sodium borohydride reduction of 2,3-dimethyl-5,6-bis(acetoxymethyl)-1,4-benzoquinone in vitro provided chemical evidence for the generation of the proposed reactive *o*-quinone methide intermediate.[8] This finding coupled with evidence that the magnitude of the oxidation–reduction potential of these materials is important for antitumor activity[9] provides evidence in support of the concept of bioreductive alkylation by agents of this class.

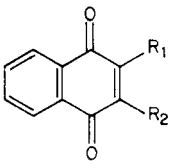
2-Chloromethyl-3-phenyl-1,4-naphthoquinone appeared to be one of the best compounds of this series as an anticancer agent that we have synthesized to date;[6] thus, it appeared important to substitute the phenyl ring system in an effort to determine whether the therapeutic potential of this agent could be enhanced. To accomplish this we have introduced functional groups such as nitro, chloro, bromo, and methoxy onto the phenyl ring of 2-chloromethyl-3-phenyl-1,4-naphthoquinone.

Chemistry. The method of Kvalnes[10] for the synthesis of arylquinones was adapted to the preparation of arylnaphthoquinones **3a–d** (Scheme I). This was accomplished by coupling 1,4-naphthoquinone (**1a**) or 2-methyl-1,4-naphthoquinone (**1b**) to various diazotized anilines (**2a–d**). Attempts to couple diazotized 2-, 3-, and 4-aminopyridines to 1,4-naphthoquinone using this procedure were unsuccessful. Bromination of **3a** with NBS gave the desired bromomethyl derivative **4** in good yield. Chloromethylation of **3b,c,d,** using formaldehyde and hydrogen chloride, gave the appropriate final products **5a–c**, respectively, in moderate yields. Various demethylating reagents, such as BBr_3 and HBr, did not prove to be useful in attempts to demethylate the methoxy group of compound **4**.

Direct coupling of diazotized *m*-nitroaniline (**7**) to 1,4-naphthoquinone gave 2-(*m*-nitrophenyl)-1,4-naphthoquinone (**10**) in poor yield. Thus, an alternate route (Scheme II) was employed[11] for the preparation of **10** which involved the coupling of diazotized *m*-nitroaniline (**7**) to 1,4-benzoquinone (**6**) to give 2-(*m*-nitrophenyl)-1,4-benzoquinone (**8**). Treatment of **8** with butadiene gave the Diels–Alder adduct **9** which was then oxidized with chromic trioxide to give 2-(*m*-nitrophenyl)-1,4-naphthoquinone (**10**) in 60% yield. Chloromethylation of **10** produced the desired product 2-chloromethyl-3-(*m*-nitrophenyl)-1,4-naphthoquinone (**11**).

Antineoplastic Effects. The anticancer activities of phenyl-substituted 2-halomethyl-3-phenyl-1,4-naphthoquinones were assessed using mice bearing Sarcoma 180 ascites cells; the results obtained are shown in Table I. The para-substituted methoxyphenyl (**4**), chlorophenyl (**5a**), and bromophenyl (**5b**) derivatives possessed maximum antitumor activity comparable to that of the parent compound, 2-chloromethyl-3-phenyl-1,4-naphthoquinone, and the related agent, 2,3-bis(chloromethyl)-1,4-naphthoquinone, which was employed as a positive control,

Table I. Effect of Phenyl-Substituted 2-Halomethyl-1,4-naphthoquinones on the Survival Time of Mice Bearing Sarcoma 180 Ascites Cells



Compd	R_1	R_2	Daily dosage, mg/kg Days 1-6[a]	Days 1,3,5[b]	Av Δ wt, %[c]	Av survival, days ± SE[d]	T/C[e]
	Control				+16.4	13.6 ± 0.3	
	CH_2Cl	CH_2Cl	2.5		+2.0	15.2 ± 0.9	1.12
			5		−1.7	19.2 ± 1.5	1.41
			10		−3.4	22.2 ± 1.2	1.63
			15		−4.9	20.5 ± 1.8	1.50
			20		−6.5	20.2 ± 1.2	1.49
				5	−2.9	21.2 ± 2.8	1.56
				10	−2.7	26.5 ± 2.0	1.94
				20	−1.6	23.7 ± 1.9	1.74
				30	−3.2	25.4 ± 1.7	1.87
				40	−5.8	29.1 ± 1.7	2.14
	CH_2Cl	C_6H_5	5		+6.9	18.1 ± 1.6	1.33
			10		−2.5	23.0 ± 1.7	1.69
			15		−3.4	25.3 ± 2.1	1.86
			20		−7.6	20.0 ± 1.4	1.47
			25		−9.0	20.0 ± 2.4	1.47
				10	0.0	24.3 ± 2.0	1.79
				20	−4.4	28.2 ± 2.5	2.07
				30	−3.2	25.1 ± 2.1	1.84
				40	−7.2	21.9 ± 1.6	1.61
4	CH_2Br	C_6H_4-*p*-OCH_3	10		−3.5	24.2 ± 3.6	1.77
			20		−9.1	21.2 ± 3.7	1.53
			40		−16.1	27.3 ± 3.2	2.00
5a	CH_2Cl	C_6H_4-*p*-Cl	10		−3.8	25.4 ± 6.2	1.86
			20		−9.7	26.4 ± 3.9	1.94
			40		−15.0	28.0 ± 5.9	2.06
5b	CH_2Cl	C_6H_4-*p*-Br	10		+24.4	12.6 ± 1.0	0.92
			20		−7.3	28.2 ± 5.0	2.07
			40		−15.3	22.8 ± 2.1	1.67
5c	CH_2Cl	C_6H_4-*m*-Br	10		+22.7	13.0 ± 2.1	0.95
			20		+31.9	10.0 ± 1.3	0.73
			40		−3.7	18.8 ± 3.1	1.38
11	CH_2Cl	C_6H_4-*m*-NO_2	5		+18.2	11.6 ± 0.5	0.85
			10		+10.5	11.0 ± 0.7	0.80
			20		+8.1	9.6 ± 0.4	0.70

[a] Administered once daily for six consecutive days beginning 24 h after tumor implantation. [b] Administered once daily on days 1, 3, and 5 beginning 24 h after tumor implantation. [c] Average weight change from onset to termination of drug treatment. [d] Each value represents results from 5 to 40 mice. [e] T/C represents the ratio of the survival time of treated to control animals.

prolonging the life span of tumor-bearing mice from 13.6 days for untreated control animals to 25–28 days for animals receiving various drug treatments. In contrast to these findings, the introduction of a nitro or bromo group into the meta position of the phenyl ring of 2-chloromethyl-3-phenyl-1,4-naphthoquinone resulted in a decrease or loss of antitumor activity when these agents were employed at comparable dosage levels.

The tumor-inhibitory activities of 2,3-bis(chloromethyl)-1,4-naphthoquinone and 2-chloromethyl-3-phenyl-1,4-naphthoquinone were observed to be schedule dependent (Table I). Thus, treatment of tumor-bearing animals with these agents on days 1, 3, and 5 after tumor implantation, at all but the highest doses of 2-chloromethyl-3-phenylnaphthoquinone, resulted in greater prolongations of survival time than occurred in mice treated with comparable total quantities of drug for six consecutive days.

Experimental Section

Assessment of Antineoplastic Activity. Compounds were tested for tumor-inhibitory potency in CD-1 mice bearing Sarcoma 180 ascites cells. Complete details of the biological methods have been described earlier.[12]

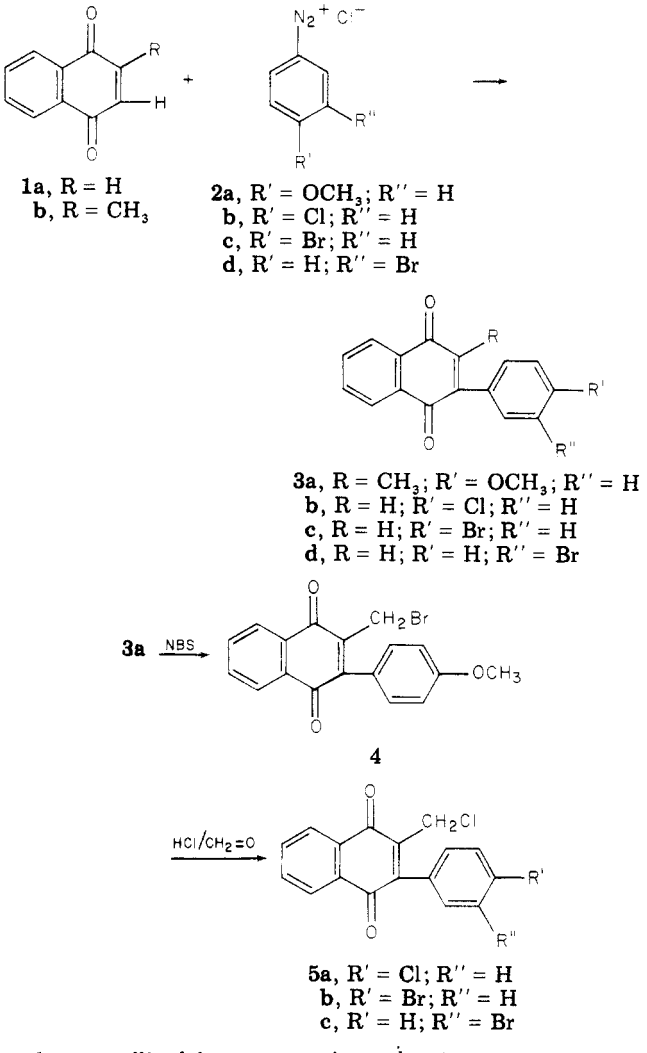
Chemical Methods. All melting points were measured on a calibrated Thomas-Hoover capillary melting point apparatus. Analyses were performed by the Baron Consulting Co., Orange, Conn. Spectral data were obtained using a Perkin-Elmer 257 grating ir spectrophotometer and a Varian T-60A spectrometer. The latter instrument used Me_4Si as an internal standard. Where analyses are indicated only by symbols of elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. The physical properties of newly synthesized compounds are shown in Table II.

(A) General Procedure for the Preparation of Aryl-1,-4-naphthoquinones. Meta- or para-substituted aniline (0.05 mol) was suspended in 24 ml of 6 N HCl. $NaNO_2$ (3.5 g, 0.05 mol) in 10 ml of H_2O was added to the suspension dropwise with cooling. The mixture was stirred at ice-cold temperature for 1 additional hour and then added dropwise to either 1,4-naphthoquinone (7.9 g, 0.05 mol) or 2-methyl-1,4-naphthoquinone (8.6 g, 0.05 mol) in 600 ml of EtOH which contained NaOAc (6.8 g, 0.05 mol) in a small amount of H_2O. The mixture was cooled in an ice bath during the addition and was stirred at room temperature overnight and filtered. The solvent was evaporated to a relatively small volume (100 ml) and the precipitates which formed were collected

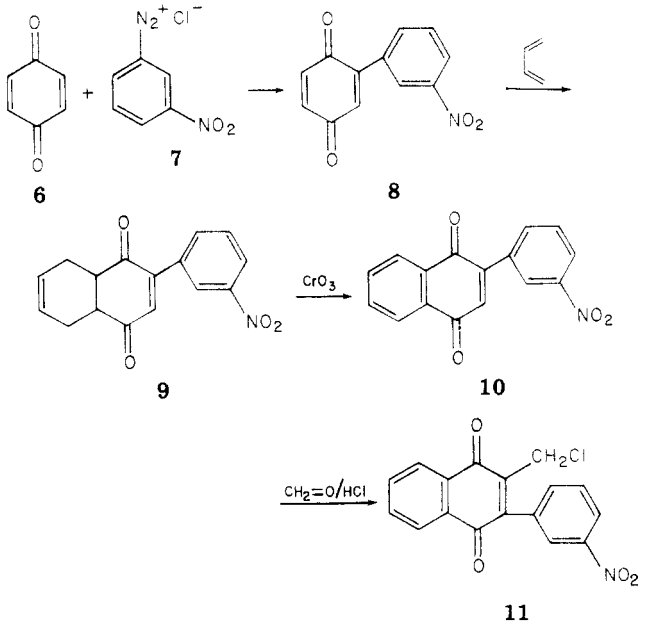
Table II. Physical Properties of Substituted 2-Phenyl-1,4-naphthoquinones

Compd	Method	Recrystn solvent	Yield, %	Mp, °C	Analyses
3a	A	EtOAc	10	173-175	C, H
3b	A	EtOH	22	171-173	C, H, Cl
3c	A	EtOAc	34	164-166	C, H, Br
3d	A	EtOH	11	145-147	C, H, Br
5a	B	EtOAc + ligroine	43	120-122	C, H, Cl
5b	B	EtOAc	22	139-142	C, H, Br, Cl
5c	B	EtOAc + petr ether	20	134-138	C, H, Br, Cl
11	B	AcOH	30	187-190	C, H, N, Cl

Scheme I



Scheme II



and recrystallized from appropriate solvents.

(B) Chloromethylation of Aryl-1,4-naphthoquinones. A suspension of aryl-1,4-naphthoquinone (3 mmol) in 20 ml of glacial AcOH containing aqueous formaldehyde (4 ml, 36%) was cooled in an ice bath. Dry HCl gas was added to the solution for 30 min and the mixture was allowed to stand at room temperature overnight. The reaction mixture was filtered, the solvent evaporated to dryness, and the residue was recrystallized from an appropriate solvent.

2-Bromomethyl-3-(*p*-methoxyphenyl)-1,4-naphthoquinone (4). 2-Methyl-3-(*p*-methoxyphenyl)-1,4-naphthoquinone (0.4 g, 1.4 mmol), NBS (0.25 g, 1.4 mmol), and a catalytic amount of perbenzoate were refluxed in 20 ml of CCl_4 for 2 h. The reaction mixture was filtered and the CCl_4 was removed by distillation under reduced pressure. The residual oil was recrystallized from EtOAc and ligroine to give 0.35 g (70%) of yellow crystals, mp 135–136°. Anal. ($C_{18}H_{13}BrO_3$) C, H, Br.

2-(*m*-Nitrophenyl)-1,4-naphthoquinone. *m*-Nitrophenyl-1,4-benzoquinone (4.7 g, 0.02 mol) was suspended in 100 ml of AcOH and butadiene (2 g) in 10 ml of AcOH was added. The mixture was stirred at room temperature for 2 days in a sealed flask, refluxed for 2 h, and then diluted with AcOH (100 ml). Chromium trioxide (6 g, 0.06 mol) in H_2O was added to the reaction mixture over a 1-h period with the temperature being maintained below 75° during the addition. The solution was then diluted with H_2O to give yellow crystals which were recrystallized from EtOH to give 3.5 g (60%) of yellow needles: mp 208–210°. Anal. ($C_{16}H_9NO_4$) C, H, N.

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