Nitrobenzyl Derivatives as Bioreductive Alkylating Agents: Evidence for the Reductive Formation of a Reactive Intermediate

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o- and *p*-nitrobenzyl chlorides and carbamates were chemically and electrochemically reduced in the presence and absence of the nucleophile morpholine; activation of these compounds by reduction was required to produce an intermediate capable of alkylation. The reduction products formed by the catalytic hydrogenation of each compound were examined by gas chromatography–mass spectrometry. In addition, the products generated by controlled-potential electrolysis were examined by ESR and NMR spectrometry. After a one-electron reduction, *o*- and *p*-nitrobenzyl chlorides were activated to the nitrobenzyl radicals, which subsequently dimerized to the dinitrobibenzyl derivatives or reacted with morpholine when present in the reaction medium to form the (nitrobenzyl)morpholine adducts. The nitrobenzyl carbamates were not activated after a one-electron reduction; however, the morpholine and the ether adducts of these agents were observed after catalytic hydrogenation. It was assumed that an intermediate or intermediates formed after the one-electron reduction product, or the full reduction product of the carbamates, were capable of alkylating various nucleophiles. Chemical reduction of the potential bioreductive alkylating agent (*o*-nitrobenzyl)-6-thioguanine produced (*o*-aminobenzyl)-6-thioguanine, indicating a lack of formation of a reactive electrophile by reduction. (*o*-, (*m*-, and (*p*-nitrobenzyl)-6-thioguanine analogues were also examined for cytotoxic activity toward EMT6 tumor cells under aerobic and hypoxic conditions. In agreement with the inability of (*o*-nitrobenzyl)-6-thioguanine to form a reactive species after chemical reduction, no decrease in the survival of neoplastic cells exposed to 10^{-4} M drug occurred under either aerobic or hypoxic conditions.

Hypoxia is known to protect cells from the cytotoxic effects of ionizing radiation; therefore, the hypoxic malignant cells in solid tumors are considered to be capable of limiting the curability of localized cancer by radiotherapy.[1] Nitroaromatic heterocycles have been demonstrated to have the ability to sensitize hypoxic tumor cells to the lethal effects of ionizing radiation.[2] Compounds of this type have also been shown to be preferentially toxic to hypoxic cells.[3-13] This differential toxicity appears to depend upon the more complete reductive activation of the nitro heterocyclic drugs by enzyme systems under conditions of hypoxia.[13,14] In an effort to exploit this phenomenon by the design of more efficacious antineoplastic agents that require reductive activation, this laboratory synthesized a number of nitrobenzyl halides and carbamates that were more cytotoxic to hypoxic tumor cells than to their oxygenated counterparts.[15] These relatively simple molecules were regarded as prototypes of nitroreductase-catalyzed bioreductive alkylating agents. It was hypothesized[15] that the toxicity of *o*-nitrobenzyl halides and carbamates to EMT6 tumor cells was the result of the formation of a reactive methide-containing species following bioreduction by a "nitroreductase" enzyme system. It has been postulated that the nitroreductase activity involved in the reductive activation of the analogous nitroimidazoles may be cytochrome P-450,[16] NADPH-cytochrome P-450 reductase,[17] xanthine oxidase,[18] or DT-diaphorase.[19]

A relatively large number of studies have been conducted to characterize the reductive intermediates of nitro-substituted compounds produced by alkali metals,[20] pulse radiolysis,[21-25] electrochemical,[26-28] and enzymatic systems.[29,30] These investigations have provided evidence that the transient production of the nitro radical anion is important to the mechanism of action of the biologically active nitro sensitizers.

For these reasons, it was of interest to examine the reductive activation of the *o*- and *p*-nitrobenzyl halides and carbamates to determine possible reasons for their different biological efficacies against hypoxic tumor cells. This report describes the chemical and electrochemical reduction of a series of nitrobenzyl compounds and discusses possible reasons for differences in the cytotoxicities of these derivatives. In addition, the cytotoxicity of three (nitrobenzyl)thioguanine derivatives toward oxygenated

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Table I. Reduction Products of Nitrobenzyl Derivatives

no.	substrate	reactn conditions[a]	products[b] 6		7
1	*o*-$NO_2C_6H_4CH_2Cl$	A	+++	++	NP
		B	++++	N/A	++
2	*p*-$NO_2C_6H_4CH_2Cl$	A	+	++++	NP
		B	++++	N/A	NP
3	*o*-$NO_2C_6H_4CH_2OCONHCH_3$	A	+	++++	+
		B	+++	NA	++
4	*p*-$NO_2C_6H_4CH_2OCONHCH_3$	A	NP	++++	NP
		B	+++	N/A	+++
5	*o*-$NO_2C_6H_4CH_2S$-thioguanine[c]	A	NP	NP	NP
		B	+	N/A	NP

[a] Reaction conditions: A = substrate reduced in the presence of morpholine; B = substrate reduced in the absence of morpholine. Since the catalytic hydrogenation conditions employed also produced hydrogenolysis of the C–Cl and C–O bonds, the (aminobenzyl)toluene was observed as a product in most reactions. [b] The percentage of each product formed is a semiquantitative estimate obtained from the total ion mass spectrum. Percentages are represented by the following symbols: +, <10%; ++, 10–49%; +++, 50–80%; ++++, >80%; N/A, not applicable; NP, the product was not present. [c] (*o*-Aminobenzyl)-6-thioguanine was the major product (80%, under both reaction conditions).

Table II. Cyclic Voltammetric Data for Nitrobenzyl Derivatives in Me_2SO with 0.1 M TEAP

compd	$E_{p,c}$, V	$E_{p,a}$, V	compd	$E_{p,c}$, V	$E_{p,a}$, V
1	−1.21		**2**	−1.24	
	−1.51	−1.43		−1.55	−1.41
	−1.62	−1.54	**3**	−1.56	−1.40
1 + morpholine	−1.21		**4**	−1.50	−1.37
	−1.56	−1.47			

and hypoxic EMT6 tumor cells was measured, in an effort to determine whether these agents could exploit the reductive environment of solid tumors and express preferential toxicity to hypoxic tumor cells.

Chemistry

Ethanolic solutions of *o*- or *p*-nitrobenzyl chloride or *o*- or *p*-nitrobenzyl carbamate (**1–4**), morpholine, and PtO_2 at room temperature were monitored by HPLC for 24 h without hydrogenation; the mixtures of nitro compounds and morpholine did not undergo reaction, and the nitro compounds remained unchanged. In contrast, reduction of *o*-nitrobenzyl chloride with Sn/HCl in the presence of morpholine[31] resulted in readily isolable quantities of the white crystalline product, (*o*-aminobenzyl)morpholine (**6**), which was prepared as a reference compound for subsequent studies. The catalytic hydrogenation of compounds **1–5** was monitored by HPLC; the products formed when these compounds were reduced in the presence or absence of morpholine are presented in Table I. The concomitant hydrogenolyses of the C–Cl and C–O bonds to produce compounds **6** and **7**[37] during the catalytic hydrogenation of the nitro function interfered with the analyses of products and prompted the use of electrochemical methods to selectively reduce the nitro function on these compounds.

The cyclic voltammetric behavior of the *o*- and *p*-nitrobenzyl halides and carbamates in Me_2SO is illustrated in Figures 1 and 2, with the corresponding anodic and cathodic peak potentials listed in Table II. The electrochemical reduction of the *o*- and *p*-nitrobenzyl chlorides has been reported by Lawless et al.[32] to involve a one-electron reduction to the corresponding nitro anion radical. These investigators demonstrated that dimerization fol-

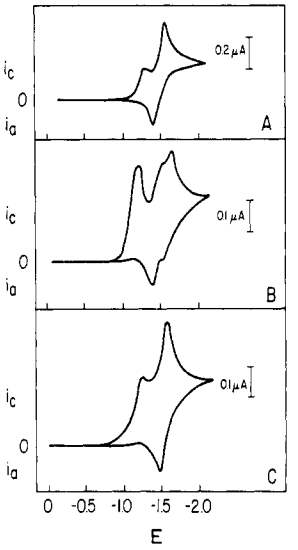

Figure 1. Cyclic voltammetric curves: (A) 2×10^{-2} M *p*-nitrobenzyl chloride; (B) 2×10^{-2} M *o*-nitrobenzyl chloride; (C) 2×10^{-2} M *o*-nitrobenzyl chloride and 2.3 mmol of morpholine in Me_2SO containing 0.1 M TEAP.

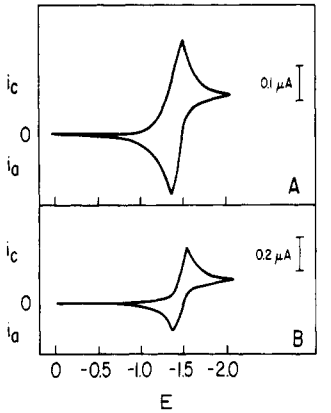

Figure 2. Cyclic voltammetric curves: (A) 2×10^{-2} M *p*-nitrobenzyl *N*-methylcarbamate; (B) 2×10^{-2} M *o*-nitrobenzyl *N*-methylcarbamate in Me_2SO containing 0.1 M TEAP.

lowed the loss of the halide ion from the anion radical to give the dinitrobibenzyl species **8**. The rapid loss of the chloride ion upon one-electron reduction of both the ortho and para isomers is evident in Figure 1 by the absence of an anodic wave, corresponding to the reoxidation of the anion radicals at potentials more positive than −1.21 and

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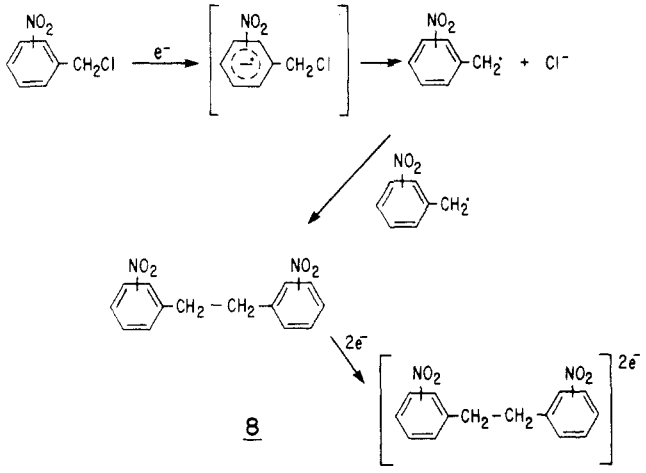
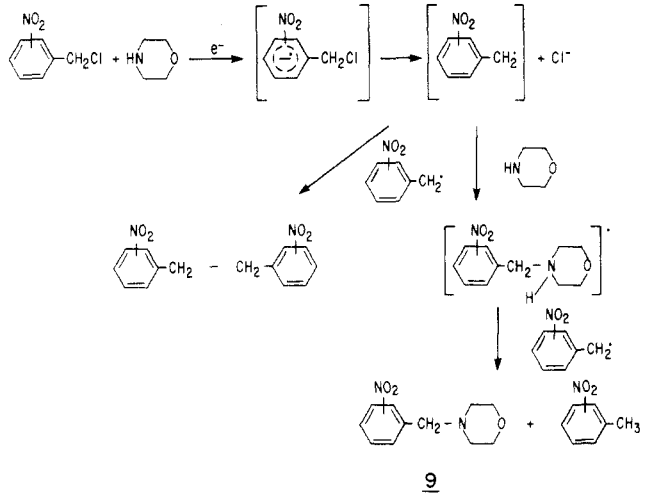
Scheme I



Scheme II



-1.24 V, respectively. As the potential was reduced, the dimerized product was then reduced to the dianion to give an overall two-electron reduction (Scheme I).[32]

Morpholine, which is not reduced in this potential range, was added to the electrolytic solution to investigate the electrophilic reactivity of the intermediate produced by reduction. The voltammogram of *p*-nitrobenzyl chloride was not appreciably altered; however, that of the ortho derivative was noticeably changed. It was presumed that following a one-electron reduction the resulting nitrobenzyl radical was capable of either dimerization or reaction with morpholine to produce **9**. Nitrotoluene could also be produced as a minor product by the abstraction of a hydrogen atom from the morpholine adduct (Scheme II).

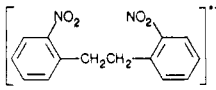
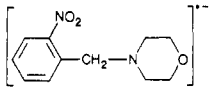
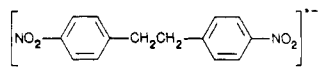
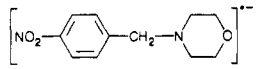
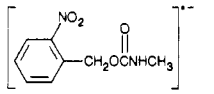
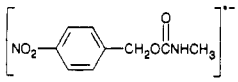
Controlled-potential electrolysis was performed on the *o*- and *p*-nitrobenzyl chlorides at a potential of -1.6 V. Severe electrode fouling prevented an exact determination of the coulometry involved, although it was possible to distinguish when 1 equiv had passed. It was not until this point in the electrolysis of the halides that the production of a colored species was observed. This same delay was also noted from the start of the electrolysis to the detection of an ESR signal when the halides were electrolyzed in situ. Both the delay in color production and the ESR signal detection upon electrolysis support the proposal that the nitrobenzyl radical is extremely reactive and immediately interacts with itself or a nucleophile present in the solution before the initial radical that is produced can be detected. Since the halide is more easily reduced than the dimerized product 8 or the morpholine adduct **9**, reduction of the halide will occur before nitro radical anions of compounds 8 and **9** are produced and detected visually as a colored species or by ESR. The ESR signal produced as a consequence of the reduction of the nitrobenzyl halides is that of the nitro radical anion of dinitrobibenzyl.[32]

The ESR signal observed when morpholine was present during the reduction of the halides appeared to be that of the corresponding morpholine adducts. The similarity in spectra produced by compounds 8 and **9** made it difficult to confirm the absolute structure by ESR. Therefore, after exhaustive electrolyses of the *o*- and *p*-nitrobenzyl halides in the presence of morpholine, the product mixtures were extracted and analyzed by NMR. The morpholine adducts **9** were the only products isolated after exhaustive electrolyses. The distinctive peak of the bibenzyl derivate, which should have appeared at 2.9–3.1 ppm, was absent from the spectra. The methyl absorbance of the possible nitrotoluene products also was not observed, although it is possible that this peak may have been superimposed on the multiplet at 2.45 ppm. The integration in this region, however, was not affected, and it was presumed that the toluene product was not produced in a detectable amount. Thus, the major products produced by the one-electron reduction of the 2,2′ and 4,4′-dinitrobibenzyl chlorides in the presence of morpholine were the corresponding derivatives of compound **9**, and not the dimers *o*- and *p*-nitrobenzyl, which Lawless et al.[32] had identified as the products produced by the reduction of these halides in the absence of a nucleophile.

The voltammograms of the carbamate derivatives (Figure 2) demonstrated only one cathodic and corresponding anodic peak potential (Table II). At these potentials, the *o*- and *p*-nitrobenzyl carbamate compounds underwent a quasi-reversible one-electron reduction and subsequent reoxidation. After exhaustive electrolysis at -1.6 V, the parent carbamate was extracted unchanged from the electrolysis solution and was identified by NMR and melting point (data not shown). The ESR spectra produced while these compounds were electrolyzed in situ correspond to the nitro radical anion of the respective parent compounds (Table III). Morpholine present in the solution during the electrochemical reduction of the *o*- and *p*-nitrobenzyl carbamates did not affect either the cyclic voltammograms or the ESR spectra, and the unchanged parent carbamate could be extracted from the solution after exhaustive electrolysis. Upon reduction of these derivatives, the ESR signal was observed almost immediately, as was the production of a colored species during the controlled-potential electrolysis. Therefore, unlike the halides, the carbamate derivatives underwent a one-electron reduction to nitro radical anions without the subsequent loss of the carbamate portion of the molecule. It is presumed that a reactive intermediate was not produced by the addition of one electron to this system. However, since the morpholine adduct and the ether derivative were observed (Table I) after catalytic hydrogenation, it was assumed that an intermediate or intermediates formed between the one-electron reduction product and the full reduction product of the carbamates were capable of alkylating various nucleophiles. It is also possible that the original hypothesis of full reduction to the amine prior to activation may be operational with the nitro carbamate derivatives. However, the proposed elimination–addition mechanism of activation is questionable under the conditions employed, since the pK_a of the amino derivative relative to water is approximately 25;[33,34] therefore, ab-

(33) Cram, D. J. *Chem. Eng. News* **1963**, *41*, 94.

Table III. Hyperfine Splitting Constants

compd	radical	splitting const,[a] G
1		$a_{NO_2}^N = 10.93$[b] $[a_2^H] = 3.30$ $[a_{3,5}^H] = 1.12$ $[a_4^H] = 4.29$ $[a_{CH_2}^H] = 3.33$
1 + morpholine		$a_{NO_2}^N = 10.43$ $[a_2^H] = 2.67$ $[a_{3,5}^H] = 1.03$ $[a_4^H] = 3.21$ $[a_{CH_2}^H] = 3.13$
2		$a_{NO_2}^N = 10.34$ $[a_{2,6}^H] = 3.55$ $[a_{3,5}^H] = 1.13$ $[a_{CH_2}^H] = 2.87$
2 + morpholine		$a_{NO_2}^N = 10.07$ $[a_{2,6}^H] = 3.65$ $[a_{3,5}^H] = 1.21$ $[a_{CH_2}^H] = 2.34$
3		$a_{NO_2}^H = 9.98$ $[a_2^H] = 3.48$ $[a_{3,5}^H] = 1.05$ $[a_4^H] = 4.45$ $[a_{CH_2}^H] = 3.48$
4		$a_{NO_2}^N = 10.13$ $[a_{2,6}^H] = 3.38$ $[a_{3,5}^H] = 1.13$ $[a_{CH_2}^H] = 2.26$

[a] Assignment of splitting constant to positions not uniquely determined by experimental data are placed in brackets. [b] Values ± 0.05 G.

straction of a proton is unlikely.

Originally, it was postulated that reduction of the nitro function of the *o*-nitrobenzyl halides and carbamates, **1–4**, was a requirement for the conversion of these compounds to a reactive species and that such activation, which was responsible for the cytotoxicity of these agents to oxygen-deficient cells, occurred preferentially in hypoxic tumor cells.[15] The findings in the present report support the concept that reduction of these nitrobenzyl derivatives results in the generation of reactive species.

After a one-electron reduction, both the *o*- and *p*-nitrobenzyl chlorides generate reactive nitrobenzyl radicals. The fact that the rate of intramolecular electron transfer of the *o*-nitrobenzyl halides was 2–2.4 times greater than that of the para isomers[22] may contribute to the differences in biological activities of these compounds. It is possible that enzymatic preferences may also contribute to these differences.

The slightly lower cytotoxicity of the *o*- and *p*-nitrobenzyl carbamates compared to that of the halides[15] may be due to the lack of nitrobenzyl radical formation after a one-electron reduction of the carbamates. Since the para isomers react chemically in a manner analogous to that of the ortho derivatives, other factors must be responsible for the differences in biological activity that these compounds possess.[15] Examination of the products formed after catalytic hydrogenation demonstrates that intermediates, other than the one-electron reduction product or the fully reduced amino derivatives, have sufficient reactivity to alkylate various nucleophiles.

The stability of the thioether linkage of (*o*-nitrobenzyl)-6-thioguanine is illustrated by the isolation of (*o*-aminobenzyl)-6-thioguanine after reduction (Table I), indicating that this agent cannot function as a bioreductive alkylating agent.

(34) Cram, D. J. *Fundamentals of Carbanion Chemistry*; Academic: New York, 1965; p 19.

Table IV. Survival of EMT6 Tumor Cells Exposed to 6-Thioguanine and Nitrobenzyl Derivatives[a]

compd	concn, M	surviving fractn, % aerobic	surviving fractn, % hypoxic
(*S*)-(*o*-nitrobenzyl)-6-thioguanine	10^{-4}	89	85
	10^{-6}	95	82
	10^{-8}	91	94
(*S*)-(*m*-nitrobenzyl)-6-thioguanine	10^{-4}	91	88
	10^{-5}	103	103
	10^{-6}	95	103
(*S*)-(*p*-nitrobenzyl)-6-thioguanine	10^{-4}	78	156
	10^{-6}	106	137
	10^{-8}	79	102
6-thioguanine	10^{-4}	67	106
	10^{-5}	78	107
	10^{-6}	70	90
	10^{-7}	95	89

[a] Survival of aerobic and hypoxic EMT6 tumor cells treated for 2 h with various concentrations of (*o*-, (*m*-, and (*p*-nitrobenzyl)-6-thioguanine in Me_2SO/ethanol (1:1.5, v/v) and 6-thioguanine in 20% poly(ethylene glycol) 400 in ethanol.

Biology

The survival of hypoxic EMT6 cells (i.e., cells maintained under 95% N_2/5% CO_2 for 2 h prior to the addition of drug) exposed to (*o*-, (*m*-, or (*p*-nitrobenzyl)-6-thioguanine for 2 h was not different from that of normally aerated cells treated with these agents under similar conditions (Table IV). The cytotoxicity of these compounds at the concentrations tested was less than that of 6-thioguanine, which at a level of 10^{-4} M decreased the surviving fraction by 33%. Thus, the lack of reactivity of the three (nitrobenzyl)-6-thioguanine compounds after reduction in vitro was reflected in their lack of preferential cytotoxicity under hypoxic conditions.

Experimental Section

o-Nitrobenzyl chloride and *p*-nitrobenzyl chloride were obtained from commerical sources and recrystallized from ethanol before use. The *o*- and *p*-nitrobenzyl carbamates were synthesized as described previously.[15] (*o*-, (*m*-, and (*p*-nitrobenzyl)-6-thioguanine were obtained from Dr. V. L. Narayanan of the Division of Cancer Treatment of the National Cancer Institute. Dimethyl sulfoxide (Me_2SO) was dried over calcium hydride and distilled under vacuum prior to use. Tetraethylammonium perchlorate (TEAP; Aldrich) was dried under vacuum. Nitrogen gas was passed over hot copper wire in a drying column before use with deaerated sample solutions for the cyclic voltammetry and electrolysis. Melting points, which are uncorrected, were determined on a Thomas-Hoover capillary melting point apparatus. Chemical analyses were performed by the Baron Consulting Co., Orange, CT, and gas chromatography–mass spectrometry (GC-MS) was performed by the Chemical Instrumentation Laboratory, Yale University. NMR spectra were generated on a 300-MHz Bruker spectrometer at the University of Saskatchewan, Saskatoon, Saskatchewan.

Sn/HCl Reduction of *o*-Nitrobenzyl Chloride in the Presence of Morpholine. In a round-bottom flask fitted with a condenser was placed 0.5 g (2.9 mmol) of *o*-nitrobenzyl chloride, 0.45 g (5.2 mmol) of morpholine, and 1 g of tin. While the flask, was shaken over ice, 5 mL of concentrated HCl was added, the mixture was heated for 15–20 min and cooled, and 40% NaOH was added until the formed precipitate dissolved. The solution was filtered, the filtrate extracted with ether and dried over $MgSO_4$, and the solvent evaporated. The resulting yellow oil was triturated with ethanol to yield 270 mg (48%) of a white powder, 4-(*o*-aminobenzyl)morpholine, which was recrystallized from ethanol: mp 56–58 °C (lit.[35] 58–59 °C); δ 6.76 (4 H, m, aromatic

H), 4.57 (2 H, s, NH_2), 3.50 (4 H, m, morpholine H), 3.41 (2 H, s, benzyl H), 2.31 (4 H, m, morpholine H). Anal. ($C_{11}H_{16}N_2O$) C, H, N, O.

Catalytic Reduction of Nitrobenzyl Compounds. General Procedure. The *o*- and *p*-nitrobenzyl chlorides and carbamates (100 mg) were each dissolved in 500 mL of absolute ethanol with or without 0.20 g of morpholine (2.3 mmole); 10 mg of PtO_2 was added, and catalytic reduction was conducted on a Parr pressure reaction apparatus at 30 psi. The reduction was monitored for 24 h by HPLC using a DOS 10/25 PXS column (data not shown). Following completion of the reaction, the ethanolic solutions were filtered, the solvent was evaporated, and the resulting oils were analyzed by GC–MS.

(*o*-Nitrobenzyl)-6-thioguanine was reduced in the presence and absence of morpholine as described above.

Electrochemical Reduction of Nitrobenzyl Compounds. Cyclic voltammetry was carried out on a Metrohm E626 Polarecord with a Metrohm E612 voltage regulator (Herisau, Switzerland), using a platinum working electrode and a saturated calomel electrode as a reference. The nitrobenzyl derivatives were dissolved in concentrations of $(1–2) \times 10^{-2}$ M in Me_2SO containing 0.1 M TEAP, with or without 2.3 mmol of morpholine. These solutions were deaerated with nitrogen gas prior to use.

Controlled-potential electrolysis was performed with a PAR Model 173 potentiostat/galvanostat and a PAR Model 176 current follower using a platinum electrode and a saturated calomel electrode as a reference, at room temperature. After exhaustive controlled-potential electrolysis (after 1 equiv had passed), the solutions were removed, diluted with water, and extracted with diethyl ether or ligroin. The resulting solids from the carbamate reductions were examined by NMR, and melting points were taken. Oils were recovered from the reduction of the halides and analyzed by NMR: [(*o*-nitrobenzyl)morpholine][35] δ 7.51 (4 H, m, aromatic H), 3.79 (2 H, s, benzyl CH_2), 3.66 (4 H, m, morpholine H), 2.45 (4 H, m, morpholine H); [(*p*-nitrobenzyl)morpholine] δ 8.19 (2 H, m, aromatic H), 7.49 (2 H, m, aromatic H), 3.73 (4 H, m, morpholine H), 3.59 (2 H, s, benzyl CH_2), 2.47 (4 H, m, morpholine H). TLC of the oils on silica (benzene/acetone, 4:1) revealed one spot for both the ortho and para derivatives (R_f 0.64 and 0.51, respectively).

Electron Spin Resonance. The ESR studies were conducted with a Bruker B-R70 spectrometer. The electrolysis was performed on a mercury pool in situ using a Varian V-4556 electrolytic cell. Solutions of the nitro compounds in Me_2SO containing 0.1 M TEAP were deaerated by freeze-thawing under vacuum and were transfered into the electrolytic cell under nitrogen. Each electrolysis was performed at −1.6 V, a potential slightly below those measured from the cyclic voltammetric curves. The signal produced upon reduction of the nitrobenzyl halide compounds in Me_2SO was found to be very unstable; therefore, dimethylformamide was used as a solvent, and the ESR spectra were run at 10 °C for these derivatives.

Biological Methods. The techniques used for culturing EMT6 tumor cells and for measuring the survival of treated cells under hypoxic and oxygenated conditions in culture have been described previously.[15,36] Cells were grown as monolayers in glass bottles in Waymouth's medium supplemented with 15% fetal calf serum. To produce hypoxia, bottles were fitted with sterile rubber sleeve serum stoppers and exposed to a 95% N_2/5% CO_2 atmosphere for 2 h at 37 °C before drug treatment. Parallel bottles were maintained in 95% air/5% CO_2. Drugs were added to each bottle in 25 μL of vehicle by injection through the rubber stopper without breaking the hypoxia. The cells were exposed to each agent for 2 h under hypoxia or normal aeration, and then survival was measured by colony formation.[15] 6-Thioguanine was dissolved in a 20% solution of poly(ethylene glycol) 400 in ethanol. The (*S*)-(*o*-, (*S*)-(*m*-, and (*S*)-(*p*-nitrobenzyl)-6-thioguanines were dissolved in Me_2SO/ethanol (1:1.5, v/v). Each drug was tested a minimum of three times at concentrations that ranged from 10^{-8} to 10^{-4} M.

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Registry No. 1 radical anion, 64178-13-8; 1 + morpholine radical anion, 103240-09-1; 2 radical anion, 64178-14-9; 2 + morpholine radical anion, 103240-10-4; 3 radical anion, 103240-11-5; 4 radical anion, 103240-12-6; morpholine, 110-91-8; 4-(*o*-aminobenzyl)morpholine, 95539-61-0; [(*o*-nitrobenzyl)morpholine], 67589-21-3; [(*p*-nitrobenzyl)morpholine], 6425-46-3; (*S*)-(*m*-nitrobenzyl)-6-thioguanine, 103240-14-8; (*S*)-(*p*-nitrobenzyl)-6-thioguanine, 5069-64-7; 6-thioguanine, 154-42-7; *o*-$NO_2C_6H_4CH_2Cl$, 612-23-9; *p*-$NO_2C_6H_4CH_2Cl$, 100-141; *o*-$NO_2C_6H_4CH_2OCONHCH_3$, 74109-34-5; *p*-$NO_2C_6H_4CH_2OCONHCH_3$, 4287-90-5; *o*-$NO_2C_6H_4CH_2S$-thioguanine, 103240-13-7.

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