Hypoxia-Selective Nitrobenzyloxycarbonyl Derivatives of 1,2-Bis(methylsulfonyl)-1-(2-chloroethyl)hydrazines

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Some 4- and 2-(nitrobenzyloxycarbonyl)-1,2-bis(methylsulfonyl)-1-(2-chloroethyl)hydrazines (**4**, **6**, and **7**) were synthesized and evaluated for their ability to exert preferential toxicity to hypoxic EMT6 mammary carcinoma cells using a colony-forming assay. Of these, the 4,5-dimethoxy-2-nitro analogue **6** (50 μM, 1-h exposure) caused greater than 3 logs of kill of hypoxic cells, with relatively minor toxicity to corresponding aerobic cells. The ability of 4-nitro (**4**) and 4,5-dimethoxy-2-nitro (**6**) analogues to reach and kill hypoxic cells of solid tumors was also demonstrated using intradermally implanted EMT6 solid tumors in mice. In addition, a possible source of toxicity to normal tissue, i.e., the activation of the 4-nitrobenzyl derivative **4** by glutathione *S*-transferase-catalyzed thiolysis, was essentially eliminated by replacing one of the benzylic methylene protons by a methyl group. The 4-nitro (**4**) and 4,5-dimethoxy-2-nitro (**6**) analogues also appear to be reduced more easily under acidic conditions (pH 6.0) than under neutral conditions, as measured by differential pulse polarography. Since the pH in hypoxic regions is often lower than that in adjacent aerobic regions, this property should aid in the cytotoxic action of these agents against hypoxic cells of solid tumors.

Solid tumors contain regions of hypoxia which frequently constitute 5–30% of the total viable tumor cell population. These oxygen-deficient tumor cells limit the curability of many solid tumors by radiation and most chemotherapeutic agents. Thus, hypoxic cancer cells may be capable of proliferating and causing tumor regrowth after treatments that produce tumor regression. However, hypoxia also creates an environment conducive to reductive processes, which results in an important exploitable difference between normal and neoplastic tissues.[1] This observation has led to extensive efforts to design and develop several classes of synthetic nitro-containing hypoxia-selective agents, including analogues of nitroimidazoles (for a review, see ref 2), nitroacridines,[3] benzotriazine *N*-oxides,[4] nitrobenzyl halides and carbamates,[5,6] nitrobenzyl mustard quaternary salts,[7,8] and nitrobenzyl phosphorodiamidates.[9] This report describes our efforts to employ the exceedingly efficacious and broad-spectrum activity of the 1,2-bis(sulfonyl)-1-(2-chloroethyl)hydrazine family of cytotoxic agents[10–14] to develop prodrugs of this class with preferential toxicity to hypoxic cells of solid tumors relative to their aerobic counterparts.

Several classes of prodrugs of 1,2-bis(methylsulfonyl)-1-(2-chloroethyl)hydrazine (**1**) have been synthesized by our laboratory and evaluated for antineoplastic activity against a number of transplanted tumors in mice.[11–14] Of these, compounds **2** and **3** produced 80–100% cures of mice bearing the L1210 leukemia and were active against a spectrum of transplanted murine and human tumors, with activities that compared favorably with those of the clinically useful agents, mitomycin C,

cyclophosphamide, and the nitrosoureas, evaluated concomitantly.[12,13] More recently, we have reported the synthesis of (alkoxycarbonyl)- and (aryloxycarbonyl)-1,2-bis(methylsulfonyl)-1-(2-chloroethyl)hydrazines, which are preferentially activated by glutathione (GSH) and glutathione *S*-transferase (GST), making these agents potentially useful in the treatment of multidrug-resistant tumors with increased intracellular levels of GSH and/or GST.[14] We have also measured the rates of activation of the synthesized agents (0.2 mM drug concentration) in phosphate buffer (pH 7.4, 37 °C) alone, in 1 mM phosphate buffer containing 5 mM GSH, and in 1 mM phosphate buffer containing 5 mM GSH and 17.5 U/mL GST.[14] The most interesting compound to emerge from this study was the nitrobenzyl analogue **4**, which was found to undergo little or no hydrolysis in phosphate buffer and an extremely slow rate of thiolysis by GSH, but a relatively high rate of activation when thiolysis by GSH was catalyzed by GST. Although this preferential activation occurs to some degree with almost all of the alkoxycarbonyl and aryloxycarbonyl derivatives examined, compound **4** was quite unique in the magnitude of this activation. For example, the GST-catalyzed thiolysis rate for compound **4** was found to be approximately 4 times faster than the second most labile prodrug under these conditions.
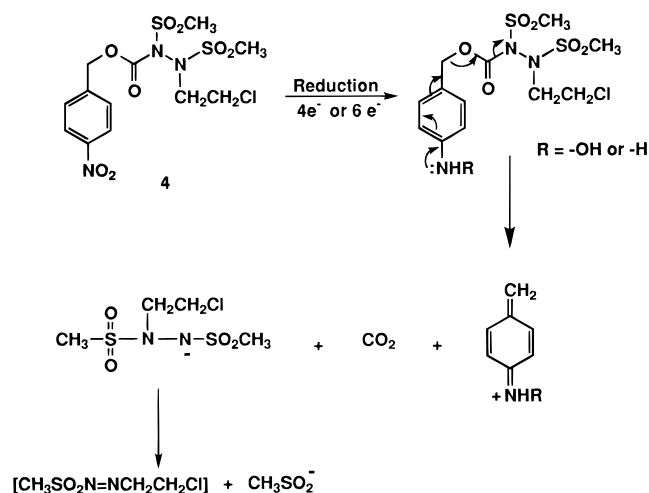
$$CH_3SO_2N(CH_2CH_2Cl)N(R)SO_2CH_3$$

1. R = -H

2. R = -COCH$_3$

3. R = -CONHCH$_2$CH$_2$Cl

4. R = -C(O)OCH$_2$C$_6$H$_4$-4-NO$_2$

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Scheme 1

We reasoned that compound **4** would also have the potential to be activated under hypoxic conditions by a reductive mechanism similar to the one described for nitrobenzyl phosphorodiamidates,[9] whereby lability of the carbamate moiety could be induced by the enzymatic reduction of the electron-withdrawing nitro group to an electron-releasing hydroxylamino or amino group (Scheme 1). Lone pair-assisted expulsion of carbon dioxide and 1,2-bis(methylsulfonyl)-1-(2-chloroethyl)-hydrazine (**1**) as the anion would then be expected to occur, followed by the fragmentation of the latter to generate the putative alkylating species $CH_3SO_2N=N-CH_2CH_2Cl$.

This paper reports the synthesis and biological evaluation of some structural analogues of compound **4** (**5**–**7**). It also reports the successful resolution of the two activation mechanisms to which compound **4** is prone, i.e., thiol-catalyzed activation and bioreductive activation. As observed earlier, activation studies with compound **4** demonstrated that this agent is relatively refractory not only to hydrolysis in phosphate buffer but also to thiolysis by GSH.[14] In contrast, a relatively high rate of activation of compound **4** occurs when thiolysis is catalyzed by GST. Activation by thiols could occur by two possible mechanisms: (a) nucleophilic attack at the carbonyl carbon and/or (b) nucleophilic attack at the benzylic methylene. An S_N1 mechanism involving a carbocation intermediate can be ruled out because of the instability of the 4-nitrobenzyl carbocation. In an earlier study we found that with 2-(alkoxycarbonyl)- and 2-(aryloxycarbonyl)-1,2-bis(methylsulfonyl)-1-(2-chloroethyl)hydrazines lacking the benzylic methylene group, the rate of GST-catalyzed activation ranged from 0 to only 6 times the rate of activation produced by GSH alone.[14] In contrast, for compound **4**, the rate of GST-catalyzed activation is about 340 times greater than that of uncatalyzed thiolysis. This enormous difference in relative rates of activation between compounds lacking the benzylic methylene and compound **4** implicates the benzylic methylene moiety in compound **4** as the predominant site at which the nucleophilic attack takes place. Since activation by an S_N1 mechanism is unlikely, this reaction is probably an S_N2 process in which nucleophilic attack at the benzylic methylene portion of the molecule results in activation (Scheme 2). Compound **4** could be particularly useful in the treatment

of tumors which have become resistant to reactive compounds through elevation of GST. However, GST-catalyzed activation may also result in toxicity to normal tissues because GSTs are not confined to tumor cells.

$$CH_3SO_2N(CH_2CH_2Cl)N(R)SO_2CH_3$$

5. R = $-C(O)OCH_2C_6H_5$

6. R = $-C(O)OCH_2C_6H_2\text{-}2\text{-}NO_2\text{-}4,5\text{-}(OCH_3)_2$

7. R = $-C(O)OCH(CH_3)C_6H_4\text{-}4\text{-}NO_2$

Two approaches may be used to minimize normal tissue toxicity created by GST activation. One involves the introduction of different functional groups on the aromatic ring (e.g., compound **6**). A second approach toward minimizing the potential toxicity created by GST activation is to eliminate the structural feature in compound **4** which makes it a good S_N2 substrate. The reactivity of substrates in S_N2 reactions follows the rank order CH_3W (where W is a leaving group) > primary (e.g., CH_3CH_2W) > secondary [e.g., $(CH_3)_2CHW$] > tertiary [e.g., $(CH_3)_3CW$].[15] Since steric factors play an important role in S_N2 reactions, branching at the α-carbon to give compound **7**, which would transform compound **4** into a secondary substrate, should decrease the rate of GST-catalyzed activation appreciably. However, one would still expect compound **7** to be activated in hypoxic cells by bioreduction in a manner analogous to that of compound **4** (Scheme 3). Compound **7** was synthesized and its capacity to undergo GSH/GST-catalyzed activation was evaluated. In addition, the ability of compound **7** to cause preferential cytotoxicity to EMT6 cells was also determined.
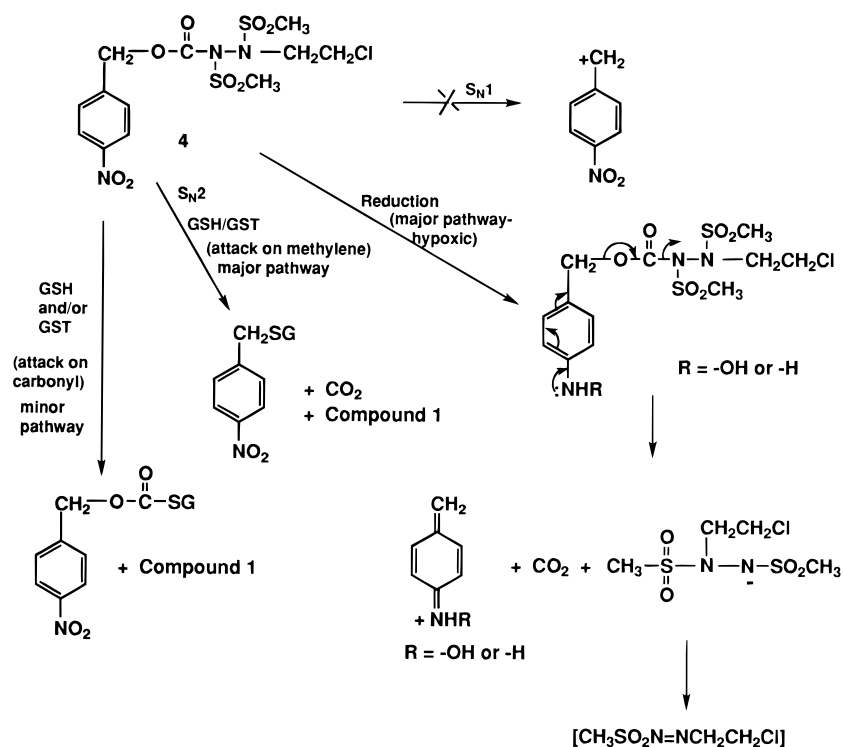
Chemistry

All of the new compounds reported in this paper (**5**–**7**) were prepared by reacting 1,2-bis(methylsulfonyl)-1-(2-chloroethyl)hydrazine (**1**) with the appropriate benzyl chloroformate (Scheme 4) using a procedure similar to the one we have described for the preparation of compound **4**. Where necessary, the chloroformate was synthesized by reacting the appropriate alcohol with a solution of phosgene in toluene.[16] Compound **7** was prepared using 1-(4-nitrobenzyl)ethanol as the starting material. The latter was synthesized by adapting a literature procedure.[17]
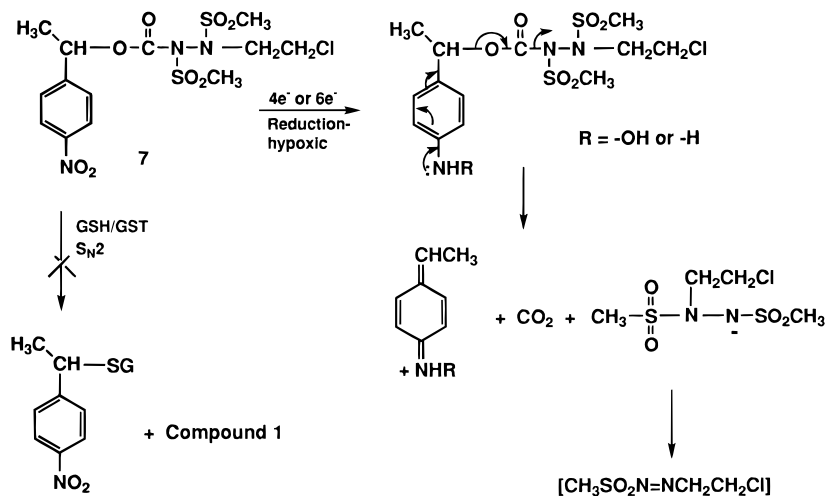
Results and Discussion

Compound **4** was evaluated against EMT6 mouse carcinoma cells in vitro under aerobic and hypoxic conditions using a colony-forming assay described by our laboratory.[18,19] At a concentration of 40 μM, a 1-h exposure to compound **4** caused 2 logs of kill of hypoxic EMT6 cells, with relatively minor toxicity to corresponding aerobic cells (Figure 1).

To ensure that the nitro group in compound **4** is important to its preferential cytotoxicity to hypoxic cells, the unsubstituted benzyl derivative **5** was synthesized and evaluated against EMT6 cells. As shown in Figure 1, compound **5** not only was considerably less potent than compound **4** but also was essentially equitoxic to EMT6 cells under both conditions of oxygenation. This finding confirmed the importance of the nitro group for the preferential toxicity of compound **4** to hypoxic cells.
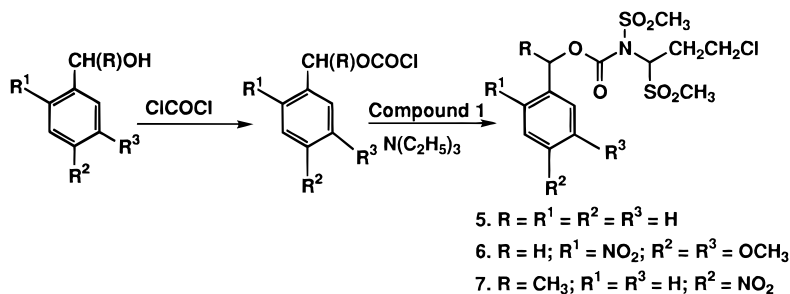
Scheme 2

$CH_2-O-C(=O)-N(SO_2CH_3)-N(SO_2CH_3)-CH_2CH_2Cl$ (4)

S_N1 (crossed out) → $^+CH_2$ (4-nitrobenzyl cation)

S_N2 / GSH/GST (attack on methylene) major pathway → CH_2SG + CO_2 + Compound 1

GSH and/or GST (attack on carbonyl) minor pathway → $CH_2-O-C(=O)-SG$ + Compound 1

Reduction (major pathway-hypoxic) → R = -OH or -H

CH_2 ... $:NHR$ → CH_2 (+ NHR) + CO_2 + $CH_3-S(=O)-N(CH_2CH_2Cl)-N-SO_2CH_3$

R = -OH or -H

$[CH_3SO_2N=NCH_2CH_2Cl]$

Scheme 3

$H_3C-CH-O-C(=O)-N(SO_2CH_3)-N(SO_2CH_3)-CH_2CH_2Cl$ (7)

$4e^-$ or $6e^-$ Reduction-hypoxic → R = -OH or -H

GSH/GST S_N2 (crossed out) → $H_3C-CH-SG$ + Compound 1

$CHCH_3$ (+ NHR) + CO_2 + $CH_3-S(=O)-N(CH_2CH_2Cl)-N-SO_2CH_3$

$[CH_3SO_2N=NCH_2CH_2Cl]$

Scheme 4

$CH(R)OH$ (with R^1, R^2, R^3 substituents) $\xrightarrow{ClCOCl}$ $CH(R)OCOCl$ $\xrightarrow[N(C_2H_5)_3]{Compound\ 1}$ product

5. R = R^1 = R^2 = R^3 = H
6. R = H; R^1 = NO_2; R^2 = R^3 = OCH_3
7. R = CH_3; R^1 = R^3 = H; R^2 = NO_2

As described earlier, the cytotoxicity of compound **4** is postulated to result from the release of a chloroethylating species following preferential 4- or 6-electron reduction of the nitro group to a hydroxylamino or amino function, respectively, under hypoxic conditions. A similar reductive activation mechanism may also be invoked for the 4,5-dimethoxy-2-nitro analogue, compound **6**, a representative of the 2-nitro class. Thus, reduction of the 2-nitro group in compound **6** to the corresponding amino or hydroxylamino substituent would also be expected to result in the generation of a chloroethylating species. In addition, the close proximity
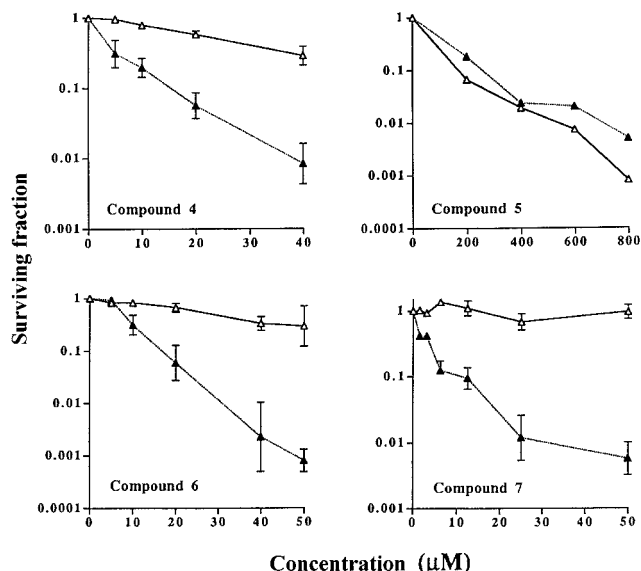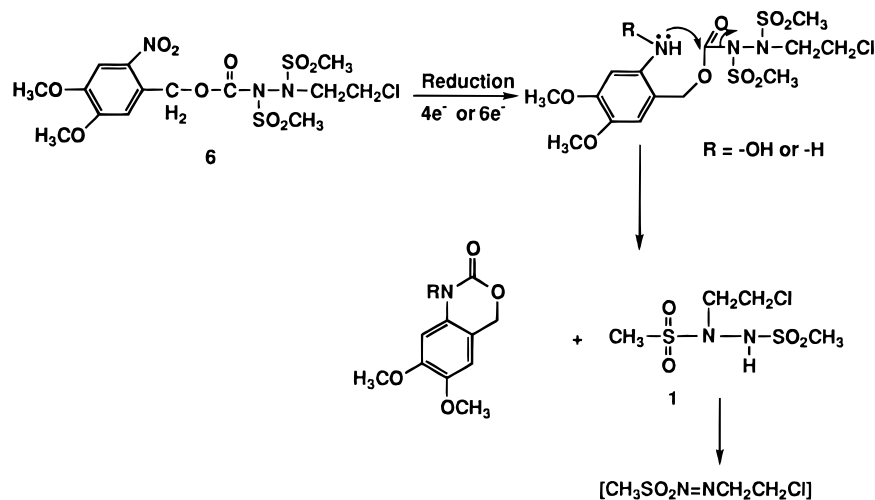
Figure 1. Survival of EMT6 cells exposed to various concentrations of compound **4**, **5**, **6**, or **7** for 1 h under hypoxic or aerobic conditions in vitro. Points are geometric means of two or more independent determinations of surviving fractions. The SEMs are shown where $n = 3$ and where the error bars are larger than the point sizes. Note the differences in scales. Key: aerobic ($\triangle$), hypoxic ($\blacktriangle$).

of the nitro group makes possible other activation mechanisms. Thus, once the nitro group is converted to the nucleophilic amino or hydroxylamino function, activation can be envisioned to occur via a six-membered cyclic transition state (Scheme 5). Activation via a five-membered cyclic transition state may also be envisioned to occur for the hydroxylamino metabolite, whereby nucleophilic attack of the benzylic methylene carbon by the hydroxylamino oxygen results in the generation of compound **1** (not shown). The ability of compound **6** to exert preferential toxicity to hypoxic EMT6 cells was evaluated. As shown in Figure 1, at a concentration of 50 μM, a 1-h exposure to compound **6** caused greater than 3 logs of kill of hypoxic EMT6 cells, with relatively minor toxicity to corresponding aerobic cells.

One of the approaches that may be used to minimize normal tissue toxicity created by GST activation is to introduce different functional groups on the aromatic ring. Studies comparing the 4,5-dimethoxy-2-nitro ana-

logue **6** with compound **4** have shown that the functional groups present on the aromatic ring can markedly affect the rate of GST activation. Thus, the rate of activation of compound **6** by GSH/GST was 8.7 compared to 20.5 nmol/mL/min for compound **4**. Both steric and electronic factors may be responsible for the observed differences in the rate of activation.

As anticipated, the rate of activation of compound **7**, which differs from compound **4** in possessing a methyl group in place of one of the benzylic methylene hydrogens, in the presence of 5 mM GSH and 17.5 U/mL GST was considerably slower than that of compound **4**. Thus, the rate of activation of compound **7** (0.1 mM) under these conditions was at least 150 times slower than that of compound **4** (0.1 mM). In addition, compound **7** appeared to be less toxic to aerobic EMT6 cells than compound **4**, while its hypoxic cell toxicity was essentially the same as that of compound **4** (compare graphs for compounds **4** and **7** in Figure 1). In addition, we have found that it is possible to eliminate hypoxia-selective cytotoxicity while retaining the approximate magnitude of the GSH/GST-catalyzed rate of activation of compound **4** by merely removing the nitro group from **4** (data not shown).

The capacity of agents to reach and kill hypoxic cells of solid tumors was tested using the in vivo–in vitro test system we described previously.[18,20,21] The radiation dose used in these studies (15 Gy) was large enough that the tumor cell population surviving after irradiation is composed almost entirely of radioresistant, hypoxic cells. The fact that concomitant treatment of X irradiation with compound **4** or **6** killed cells which survived the radiation treatment showed that these compounds were capable of reaching hypoxic areas of the solid tumor and killing oxygen-deficient cells in these regions (Table 1).

Hypoxic regions have a greater reliance on anaerobic metabolism; as a consequence, the pH in these regions is lower than that generally found in adjacent well-oxygenated tissues.[22] The H$^+$ concentration greatly influences the chemistry in these regions.[23–25] Because of the low oxygen tension, the rate of oxidation of NADH is decreased, resulting in an increase in the ratio of NADH/NAD$^+$, as well as an increase in the redox potential of this couple.[26] However, this effect is par-

Scheme 5

Table 1. Effects of Compounds **4** and **6** on the Survival of Cells from EMT6 Solid Tumors[a]

|	surviving fraction	
treatment	compd **4**	compd **6**
none	1.00	1.00
drug alone	0.841	0.856
X-ray alone	0.043	0.043
drug + X-ray	0.013	0.024

[a] BALB/c mice bearing well-established (ca. 100 mm³) intradermal EMT6 solid tumors were treated with a single 60 mg/kg intraperitoneal injection of compound **4** or **6**, followed 100 min later by 15 Gy of total body radiation to the tumor-bearing animals. Mice were then killed, solid tumors excised, single cell suspensions prepared, and their cloning efficiencies ascertained. Results shown are averages of two experiments.

Table 2. Half-Wave Potentials of Nitrobenzyloxycarbonyl Derivatives of 1,2-Bis(methylsulfonyl)-1-(2-chloroethyl)-hydrazines[a]

	$E_{1/2}$ (mV)		
compd	pH 6.0	pH 7.0	ΔV (mV)
4	−360	−473	−113
6	−467	−545	−78

[a] Half-wave reduction potentials ($E_{1/2}$) were calculated from values of the potentials at the differential pulse polarographic current peak in 100 mM potassium chloride, 50 mM potassium phosphate buffer/electrolyte at pH values of 6.0 and 7.0 as described in the Experimental Section.

tially countered by the increase in H⁺ concentration due to the impact of the H⁺ ion concentration term in the equation below, which describes the redox potential of the NADH/NAD⁺ couple:

$$NAD^+ + H^+ + 2e^- = NADH$$
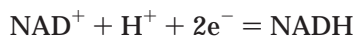
$$E = E° - (RT/2F) \ln [NADH]/[NAD^+][H^+]$$

The redox potential of the NADH/NAD⁺ couple is 30 mV greater (more positive) at pH 6.0 than at pH 7.0. Therefore, a drop in pH of 1 unit would require a 10-fold increase in the NADH/NAD⁺ ratio to maintain the same redox potential. Despite these effects, the redox potential of the NADH/NAD⁺ couple probably still increases significantly under hypoxic conditions, as indicated by a shift in the pyruvate/lactate equilibrium.[27] Many oxidative and reductive processes (or subsequent steps) involve [H⁺]; therefore, how readily these steps occur will be influenced by pH. Ideally, a hypoxia-selective agent should be reduced more readily under more acidic conditions to exploit this difference. We have measured the $E_{1/2}$ of compounds **4** and **6** at both pH 6.0 and pH 7.0 (Table 2). Redox potential shifts of 113 and 78 mV were measured for compounds **4** and **6**, respectively. The shifts in redox potential probably occur as a consequence of the interaction of H⁺ with the initial 1-electron reduction products (and the involvement of H⁺ in subsequent steps), resulting in inhibition of the reverse reaction.[28] Attainment of a maximum differential in the reduction rate between two pH values requires the synthesis of compounds with large shifts in redox potential in the pH range of interest. Studies correlating the rate of reduction of various nitroimidazoles by xanthine oxidase with the redox potential have demonstrated a 10-fold change in the reduction rate for only a 70−80 mV change in redox potential.[29] We believe that the large redox shifts observed with compounds **4**

and **6** (Table 2) will increase the action of these agents in acidic regions of solid tumors and thereby complement the hypoxic/oxic differential activity of these compounds.

Conclusion

The nitrobenzyloxycarbonyl derivatives of 1,2-bis-(methylsulfonyl)-1-(2-chloroethyl)hydrazines described in this paper exhibit exceptional preferential toxicity to hypoxic EMT6 cells relative to their aerobic counterparts. It also appears that their preferential toxicity to hypoxic cells can be improved further by replacing one of the benzylic methylene protons with a methyl group. Two representative agents of this class possess significantly higher (more positive) redox potentials at pH 6.0 than at pH 7.0, a property that should enhance the preferential toxicity of these agents to hypoxic tumor cells, which tend to be in more acidic environments than their well-oxygenated counterparts. Furthermore, the observation that compounds **4** and **6** are capable of reaching and killing hypoxic cells in solid tumors should make these lead agents, and possibly other members of this class, promising new candidates for potential clinical use as adjuvants to radiation therapy.

Experimental Section

Synthesis. Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Proton magnetic resonance spectra were recorded on a Varian EM-390 spectrometer with tetramethylsilane as an internal standard. Elemental analyses were performed by the Baron Consulting Co., Orange, CT, and were within ±0.4% of the calculated values for all compounds reported.

Compounds **5** and **6** were synthesized using procedures analogous to the one reported for compound **4**.[14]

2-(Benzyloxycarbonyl)-1,2-bis(methylsulfonyl)-1-(2-chloroethyl)hydrazine (5). This compound was prepared by reacting compound **1** (1.0 g, 4.0 mmol) with benzyl chloroformate (1.8 g, 10.5 mmol). The title compound (0.64 g, 41.3%) was isolated as an oil. ¹H NMR (acetone-d_6): δ 7.2−7.6 (5H, m, aromatic H), 5.4 (2H, s, ArCH₂), 3.5−4.0 (4H, m, CH₂CH₂-Cl), 3.4 and 3.1 (6H, 2s, 2 CH₃SO₂). Anal. (C₁₂H₁₇ClN₂O₆S₂) C, H, N.

1,2-Bis(methylsulfonyl)-1-(2-chloroethyl)-2-[(4,5-dimethoxy-2-nitrobenzyloxy)carbonyl]hydrazine (6). Compound **1** (1.8 g, 7.2 mmol) was reacted with 4,5-dimethoxy-2-nitrobenzyl chloroformate (2.0 g, 7.3 mmol) to give 0.556 g of the title compound. This compound was recrystallized from ethanol. Mp: 155−155.5 °C. Yield: 15.8%. ¹H NMR (acetone-d_6): δ 7.7 and 7.4 (2H, 2s, aromatic H), 5.8 (2H, d, ArCH₂), 3.7−4.1 (4H, m, CH₂CH₂Cl), 3.9−4.0 (6H, 2s, 2 OCH₃), 3.5 and 3.2 (6H, 2s, 2 CH₃SO₂). Anal. (C₁₄H₂₀ClN₃O₁₀S₂) C, H, N.

1,2-Bis(methylsulfonyl)-1-(2-chloroethyl)-2-[[1-(4-nitrophenyl)ethoxy]carbonyl]hydrazine (7). 1-(4-Nitrophenyl)ethanol (2.4 g, 14 mmol) in tetrahydrofuran (5 mL) was added dropwise to a stirred, ice-cold solution of phosgene in toluene (20% w/v, 30 mL). The flask was then wrapped in aluminum foil and allowed to equilibrate to room temperature, and the reaction mixture was stirred for an additional 24 h. A dark oil was obtained upon evaporation of the reaction mixture in vacuo at a temperature not exceeding 30 °C. To this oil were added anhydrous acetonitrile (20 mL) and compound **1** (1.5 g, 6 mmol). After this mixture had cooled in an ice bath, triethylamine (1.7 mL, 12 mmol) was added dropwise and the reaction mixture stirred for 17 h at 4 °C. The reaction mixture was evaporated to dryness on a rotary evaporator at a temperature not exceeding 30 °C. The residue was taken up in ethyl acetate (150 mL) and washed with 2 × 50 and 1 × 100 mL of hydrochloric acid (5%, w/v). The combined aqueous layers were extracted with ethyl acetate (50 mL), and the

extract was combined with the organic (ethyl acetate) layer from the previous step. The combined ethyl acetate extracts were washed with brine (100 mL), dried over anhydrous magnesium sulfate, and filtered. The filtrate was evaporated to dryness to give a viscous oil. The desired product was obtained by column chromatography on silica gel (70−270 mesh, 60 A, chloroform-methylene chloride), followed by recrystallization from ethanol. Yield: 35%. Mp: 94−95 °C. ^{1}H NMR (acetone-d_6): δ 8.3 and 7.8 (4H, 2d, aromatic H), 6.2 (1H, m, ArCH), 3.6−4.1 (4H, m, CH$_2$CH$_2$Cl), 3.5 and 3.2 (6H, 2s, 2 CH$_3$SO$_2$), 1.7 (3H, dd, CH$_3$). Anal. (C$_{13}$H$_{18}$ClN$_3$O$_8$S$_2$) C, H, N.

Cytotoxicity Studies. The ability of compounds **4**−**7** to exert preferential toxicity to hypoxic cells was evaluated using EMT6 mouse mammary carcinoma cells, by methodology described previously.[18,19] Briefly, exponentially growing monolayers of EMT6 cells were exposed to a continuously flowing 95% N$_2$/5% CO$_2$ humidified atmosphere for 2 h to produce radiobiologic hypoxia. Parallel flasks were maintained similarly in humidified 95% air/5% CO$_2$. Without breaking the hypoxia, cells were exposed to various concentrations of the test agent for 1 h. Cell survival was then measured by colony formation.

The capacity of the synthesized agents to reach and kill hypoxic cells of solid tumors was evaluated using the in vivo−in vitro test system described previously.[20,21] BALB/c mice bearing well-established (ca. 100 mm³) intradermal EMT6 solid tumors were treated with a single 60 mg/kg intraperitoneal injection of compound **4** or **6**, followed 100 min later by 15 Gy of total body X-irradiation to the tumor-bearing animals; this irradiation eliminated >99% of the oxygenated tumor cells. Mice were then killed, the solid tumors excised, single-cell suspensions prepared, and cloning efficiencies ascertained.

Decomposition Studies. The rates of decomposition of compounds **4**, **6**, and **7** in the presence and absence of GSH and/or GST were studied by following the acidification of weakly buffered (1 mM potassium phosphate, pH 7.4, at 37 °C) solutions of phenol red (20 mg/L) spectrophotometrically at 560 nm, as descibed previously.[14]

Determination of Half-Wave Potentials. The polarographic half-wave potentials ($E_{1/2}$) for compounds **4** and **6** were calculated from potentials at half-peak current (E_p) measured by differential pulse polarography, using the equation $E_{1/2} = E_p - P_a/2$, where P_a is the pulse amplitude. Polarograms were obtained using a PAR model 174A polarographic analyzer with a PAR model 9346 dropping mercury electrode. A silver/silver chloride half-cell was used as the reference electrode. Solutions of compounds **4** and **6** (2.5 mM) in dimethyl sulfoxide were added to the buffer/electrolyte (100 mM KCl, 50 mM KH$_2$PO$_4$/K$_2$HPO$_4$) at various pH values to give a final concentration of 25 μM. The $E_{1/2}$ values were recorded at room temperature and represent the mean values of 3−5 determinations.

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