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Articles

Synthesis and Evaluation of the Antitumor Activity of 4,5-Diamino-Substituted 1,2-Benzoquinones

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A series of 4,5-diamino-substituted-1,2-benzoquinones were prepared from catechol and the corresponding secondary amines in high yield in a single step using copper complex formation to stabilize the intermediate. The cytotoxicity of the products under various conditions was evaluated using the EMT-6 mammary carcinoma cell line, and antitumor activity was tested in the L1210 murine leukemia. The 4,5-diaziridinyl-1,2-benzoquinone was a more potent cytotoxic agent than diaziquone (AZQ) and was very effective against the L1210 leukemia. The azetidine, pyrrolidine, and diethylamine derivatives were not effective antitumor agents.

Introduction

The quinone moiety occurs abundantly in nature, playing vital roles in normal biochemical processes and in the action of many cytotoxins.[1-9] More than 1000 naturally occurring quinones have been tested for antitumor activity, and several of these including doxorubicin, daunorubicin, and mitomycin C are in current clinical use.[8-10] Aziridinyl-1,4-benzoquinones were among the earliest rationally designed synthetic anticancer agents.[10,11] Of these, diaziquone [3,6-bis[(ethylcarboxy)amino]-2,5-diaziridinyl-1,4-benzoquinone (AZQ; NSC-182986)] is in clinical use primarily for the treatment of brain tumors.[10,11] Although many aziridinyl-1,4-benzoquinone derivatives have been prepared and have been shown to be active against a variety of animal tumors, there has been very little exploration of 1,2-benzoquinone-containing compounds.[12,13] Like aziridinyl-1,4-benzoquinones, aziridinyl-1,2-benzoquinones have the potential to form highly reactive species with the capability of cross-linking DNA and thus have potential as antitumor agents. It has become clear from the extensive study of aziridinyl-1,4-benzoquinone derivatives that small changes in the structure of these molecules can lead to different chemical reactivity and to different cytotoxic properties.[14-17]

The 1,2-benzoquinone derivatives have been studied to a much lesser extent than have 1,4-benzoquinone derivatives because they have been much more difficult to prepare even in moderate yield.[18,19] We now report a new synthetic strategy involving the oxidation of catechol and amine substitution to reach 4,5-diamino-substituted 1,2-benzoquinone derivatives in a single step. The cytotoxicity under several environmental conditions and antitumor activity of a series of 4,5-diamino-substituted 1,2-benzoquinones are described.

Synthetic Chemistry

Based upon the 1,2-benzoquinone moiety, 4,5-diamino-substituted 1,2-benzoquinone derivatives can be prepared readily in a single reaction, accomplishing both oxidation of catechol and the secondary amine substitution at the 4- and 5-positions of the 1,2-benzoquinone. Many oxidizing agents have been used for the preparation of quinones, including sodium dichromate,[20] chromic acid,[21] iodic acid,[22] silver oxide,[22,23] lead dioxide,[24] and barium ferrate.[25] Most of these reagents are of limited use for the preparation of reactive quinones, such as 1,2-benzoquinones, because of the tendency of the 1,2-benzoquinones toward decomposition and polymerization.[19] Even when the oxidation of catechol was carried out using ceric ammonium nitrate coated on silica the labile 1,2-benzoquinone was reported at high yield in the cold, but at ambient temperature, the product 1,2-benzoquinone easily decomposed to a tarry substance.[19]

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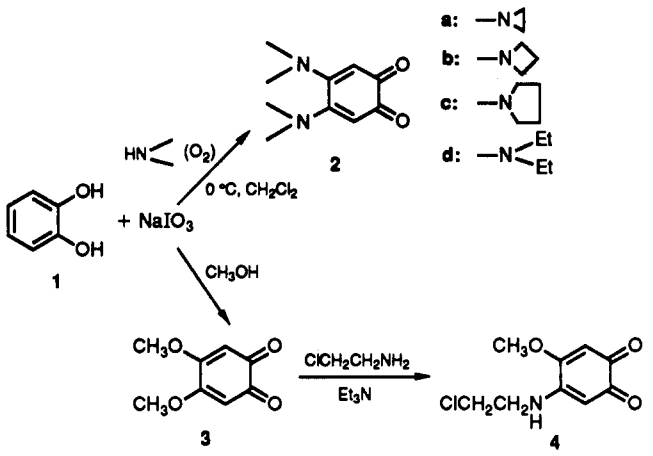
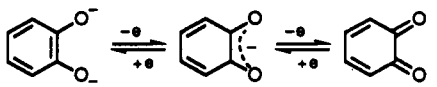
Scheme I



In order to overcome this obstacle, we developed a new synthetic method which involves combination of the oxidation of catechol and the secondary amine substitution reaction onto the 1,2-benzoquinone ring in one reaction vessel. To enable both reactions to be carried on at the same time the reaction mixture is maintained at 0 °C without separation of the labile 1,2-benzoquinone. The oxidation of catechol proceeds in two one-electron transfer steps with a semiquinone intermediate.[26] The semiquino-



ne can form a stable copper complex in the presence of copper(II).[27] Therefore, copper(II) acetate or copper(I) chloride is used as a catalyst in the presence of anhydrous magnesium sulfate which absorbs the water formed *in situ*.[19]

Briefly, synthesis of 4,5-diamino-substituted 1,2-benzoquinones from catechol is carried out as follows: Oxidation of catechol is performed at 0 °C using sodium iodate as a mild oxidizing agent[28] in the presence of anhydrous magnesium sulfate as a drying agent, copper(II) acetate or copper(I) chloride, and a secondary amine while introducing dry oxygen gas into the reaction mixture. The reaction leads to formation of a copper semiquinone complex which is hydrolyzed and then purified by column chromatography to give the desired product: 4,5-diaziridinyl-1,2-benzoquinone with a yield of 62% or other 4,5-diamino-substituted 1,2-benzoquinones with yields of about 50% (Scheme I).

In contrast to the reaction described above, the nucleophilic substitution reaction of ethyleneamine on 4,5-dimethoxy-1,2-benzoquinone cannot be carried out to completion to give pure 4,5-diaziridinyl-1,2-benzoquinone. Repeated attempts under a variety of reaction conditions resulted in relatively low yields of a mixture of products which were very difficult to separate by column chromatography.

Biological Evaluation

Cytotoxicity studies with a series of 4,5-diamino-substituted 1,2-benzoquinones were carried out using EMT-6 murine mammary carcinoma cells growing in culture (Table I). The cytotoxicity of these compounds was compared with that of AZQ, an aziridinyl-1,4-benzoquinone. As can be seen from Table I, 4,5-diaziridinyl-1,2-benzoquinone was markedly cytotoxic under both normally oxygenated and hypoxic conditions while the 4,5-diazetidinyl-, 4,5-dipyrrolidinyl-, 4,5-bis(diethylamino)- and 4,5-dimethoxy-1,2-benzoquinone derivatives showed very limited cytotoxicity. The 4-[(chloroethyl)amino]-5-methoxy-1,2-benzoquinone was moderately cytotoxic but only the 4.5-diaziridinyl-1,2-benzoquinone was more cytotoxic than AZQ.

Regions of relatively acidic pH exist in solid tumors; therefore the cytotoxicity of 4,5-diaziridinyl-1,2-benzoquinone was compared with the cytotoxicity of AZQ under normally oxygenated and hypoxic conditions at normal and acidic (pH 6.45) pH through several logs of cell killing (Figure 1). At normal pH 4,5-diaziridinyl-1,2-benzoquinone was selectively cytotoxic toward normally oxygenated cells at levels of cell killing of greater than 2 logs while AZQ was equally cytotoxic toward normally oxygenated and hypoxic cells over the concentration range tested. The 4,5-diaziridinyl-1,2-benzoquinone was markedly more cytotoxic at acidic pH (pH 6.45) than at normal pH under both normally oxygenated and hypoxic conditions. When the extracellular pH was 6.45 the 4,5-diaziridinyl-1,2-benzoquinone was selectively cytotoxic toward hypoxic cells such that 1 μM of the drug killed about 3.5 logs of hypoxic EMT-6 cells. The cytotoxicity of AZQ was not significantly altered when the cells were exposed to the drug under acidic pH conditions; however, there was a trend toward increased cytotoxicity toward hypoxic cells at the lower pH.

For initial *in vivo* testing a series of 4,5-diamino-substituted 1,2-benzoquinones were examined for their ability to increase the life span of animals bearing L1210 leukemia. The azetidinyl, pyrrolidinyl, and diethylamino derivatives of 1,2-benzoquinones were not active against L1210 leukemia over the dosage range from 1 to 10 mg/kg administered ip daily on days 1–5. The 4,5-diaziridinyl-1,2-benzoquinone, however, was a highly effective antitumor agent over the dosage range from 0.5 to 2.0 mg/kg administered ip daily for 5 days (Table II).

Conclusions

The successful synthesis of 4,5-diamino-substituted 1,2-benzoquinones from catechol oxidation and secondary amine substitution is dependent upon using a copper metal catalyst to form a stable semiquinato complex intermediate. Triethylamine and copper(I) chloride or copper(II) acetate used in combination as catalyst in the proceeding reaction makes this synthetic method an improvement over previously published methods[27,29] in which substituted catechol oxidation using specially prepared a $[CuCl\cdot Py]_n$ complex as catalyst is not convenient. The $[CuCl\cdot Py]_n$ complex catalyst was only suitable for preparation of 3,5-di-*tert*-butyl-substituted 1,2-benzoquinone; other substituted-catechol oxidation reactions produced mixtures of coupled products.[29]

Our synthetic method can generally produce 4,5-diamino-substituted 1,2-benzoquinone products in high yield (50–60%). Only in the case of the pyrrolidine derivative when an excess of the amine (8 mol) was used did the reaction (method A) produce a lower yield (10%), but use of a small excess of amine (2.2 mol) in reaction method C resulted in a 46% yield of the desired product.

All of the 1,2-benzoquinones were unstable, undergoing polymerization and decomposition, and therefore were stored dry in the dark under a vacuum or under nitrogen.

Table I. Cytotoxic Concentrations for 4,5-Diamino-Substituted 1,2-Benzoquinones in EMT-6 Tumor Cells in Culture

compound	condition			
	normal oxygenation		hypoxia	
	IC_{50}, μM	IC_{90}, μM	IC_{50}, μM	IC_{90}, μM
4,5-diaziridinyl-1,2-benzoquinone	0.4	1.1	0.2	1.0
4,5-diazetidinyl-1,2-benzoquinone	500	≫500	400	≫500
4,5-dipyrrolindinyl-1,2-benzoquinone	>500	≫500	>500	≫500
4,5-bis(diethylamino)-1,2-benzoquinone	500	≫500	400	≫500
4,5-dimethoxy-1,2-benzoquinone	500	≫500	≫500	≫500
4-[(Chloroethyl)amino]-5-methoxy-1,2-benzoquinone	125	455	110	190
3,6-bis[(ethylcarboxy)amino]-2,5-diaziridinyl-1,4-benzoquinone (AZQ)	5	15.5	7.5	17.5

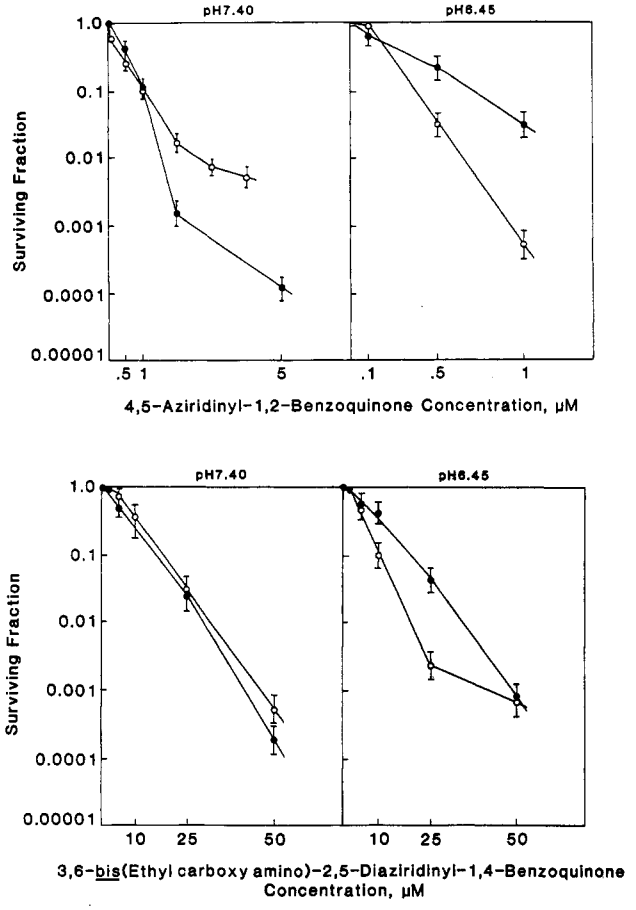


Figure 1. Survival of exponentially growing normally oxygenated (●) and hypoxic (○) EMT-6 cells exposed to various concentrations of 4,5-diaziridinyl-1,2-benzoquinone or AZQ for 1 h at 37 °C and at pH 7.4 or 6.45. *Points*: means of three independent determinations ± SEM (bars).

Table II. Increase in Life Span in Animals Bearing the L1210 Leukemia after Treatment with 4,5-Diaziridinyl-1,2-benzoquinones

treatment	dose, mg/kg[a]	survival, days[b]	% ILS
controls		7.4	
4,5-diaziridinyl-1,2-benzoquinone	0.50	12.6	70
	1.00	13.4	81
	1.25	13.6	84
	1.50	13.8	86
	2.00	11.8	59
	2.50	6.0	toxic

[a] Drug was administered ip daily on days 1–5.

The 4,5-diaziridinyl-1,2-benzoquinone was less stable than the other compounds studied.

Among the six 1,2-benzoquinone derivatives tested for cytotoxicity toward EMT-6 cells under normally oxygenated and hypoxic conditions only the aziridinylbenzoquinones 4,5-diaziridinyl-1,2-benzoquinone and AZQ were markedly cytotoxic (Table I). The two aziridinyl moieties on each of these molecules have nitrogen mustard like character and upon reaction with a biological nucleophile(s) such as DNA can achieve bifunctional alkylation of that molecule. 4,5-Biaziridinyl-1,2-benzoquinone was a more potent cytotoxic agent than AZQ under all of the environmental conditions assayed. Most significantly, 4,5-diaziridinyl-1,2-benzoquinone was markedly cytotoxic toward hypoxic cells at low pH and therefore may be suitable for the treatment of solid tumors. The cytotoxicity of benzoquinones is closely associated with the redox properties of the molecules.[30-33] These results indicate that 4,5-diaziridinyl-1,2-benzoquinones may have greater potential as antitumor agents than the corresponding 1,4-benzoquinones. 1,2-Benzoquinones or the corresponding catechols which lack alkylating capability can also be cytotoxic or cytostatic upon prolonged (continuous) exposure to cells in culture through mechanisms involving cyclic oxidation–reduction reactions with semiquinone as intermediates.[34]

Of the four 1,2-benzoquinone derivatives tested against the L1210 leukemia in mice, only 4,5-diaziridinyl-1,2-benzoquinone demonstrated significant antitumor activity. Treatment of the animals with 1.00–1.50 mg/kg of 4,5-diaziridinyl-1,2-benzoquinone daily for 5 days nearly doubled their life span. AZQ has demonstrated significant activity against intracranial tumors.[35] We are continuing to explore the activity of 4,5-diaziridinyl-1,2-benzoquinone against intracranial tumors and subcutaneously implanted solid tumors.

Experimental Section

Melting points were determined with a Fisher-Johns melting point apparatus. IR spectra were determined on a Perkin-Elmer 781 infrared spectrophotometer as potassium bromide pellets; UV spectra were determined on a Beckman DU-70 spectrophotometer. ^1H NMR spectra were obtained in $CDCl_3$ with tetramethylsilane as an internal reference using a EM360L NMR spectrometer (Varian). Analytical thin-layer chromatography (TLC) was carried out on TLC aluminum sheets of silica gel 60F254 precoated (EM Science). Column chromatography was performed by using silica gel [60–200 mesh (Baker analyzed)] as the chromatographic adsorbent. Chemicals and solvents were purchased from Aldrich Chemical Co. (St. Louis, MO). Dichloromethane was dried over anhydrous calcium chloride, redistilled, and stored over molecular sieves (4 Å), triethylamine was dried over sodium hydroxide and redistilled before using. Anhydrous magnesium sulfate was dried in an oven at 110 °C for 10 h prior to use. Ethyleneamine was synthesized according to the method of Reeves *et al.*[36] Elemental analyses were performed by Quantitative Technologies, Inc., Bound Brook, NJ 08805.

4,5-Diaziridinyl-1,2-benzoquinone (2a). Method A. A solution of catechol (110 mg, 1 mmol) in dried dichloromethane (25 mL) was cooled at 0 °C and well stirred in an ice bath in a 3-necked round-bottomed flask equipped with a condenser, a cylindrical funnel protected by drying tube (calcium chloride),

and a glass tube which was connected to an oxygen cylinder through a drying tube (silica gel).

To this solution the reagents anhydrous magnesium sulfate (3 g), copper(II) acetate (181.69 mg, 1 mmol), and sodium iodate (792 mg, 4 mmol) were added. After the mixture was stirred for 10 min a solution of ethyleneamine (0.41 mL, 8 mmol) and triethylamine (0.56 mL, 4 mmol) in dichloromethane (15 mL) were added dropwise from the cylindrical funnel into the reaction mixture, and at the same time as the addition of the amine, oxygen was introduced through the glass tube. The reaction mixture was then maintained with bubbling oxygen and stirring at 0 °C for 18–20 h until the reaction was complete as determined by disappearance of the catechol on TLC. Over the reaction time course the reaction solution changed color from light blue to deep-green and at last to dark red. The reaction mixture was filtered and washed with dichloromethane. Concentration of the solvent under vacuum with the temperature maintained below 25 °C gave a black red solid which was extracted with chloroform, washed with ice water (40 mL), 10% acetic acid (2-3 mL), and distilled water saturated with sodium chloride (20–30 mL), and then dried over magnesium sulfate. Evaporation of the extracting solvent gave the crude product. Column chromatography with chloroform elution gave 4,5-diaziridinyl-1,2-benzoquinone (119.6 mg, 62.88% yield) as deep red needles from CH_2Cl_2/acetone (1:1): mp 144–45 °C dec (lit.[43] mp 149 °C); IR ν_{max} 3080, 3000, 2920, 1640, 1550, 1370, 870 cm^{-1}; UV λ_{max} (95% EtOH) 220 nm (log ϵ 4.21), 264 (3.68), 344 (4.11); ^1H NMR ($CDCl_3$) δ 2.3 (s, 8H, 4- and 5-aziridinyl), 5.8 (s, 2H, 3,6-H). Anal. $C_{10}H_{10}N_2O_2$: C, H, N.

Method B. Methoxy-1,2-benzoquinone (55 mg, 0.5 mmol) was synthesized according to the literature.[28] The synthetic method was similar to method A, but without use of oxygen gas or the oxidizing agent sodium iodate. The nucleophilic substitution reaction of ethyleneamine was carried out for 19 h at 0 °C. The product mixture consisted of 4,5-diaziridinyl-1,2-benzoquinone (16% yield) and 4-aziridinyl-5-methoxy-1,2-benzoquinone (24% yield) calculated by ^1H NMR. These two products could not be separated by column chromatography.

Method C. The reaction was carried out as described in method A using copper(I) chloride in place of copper(II) acetate. The reaction was allowed to proceed at 0 °C for 5.5 h. The proportion of the reagents was as follows: catechol (1 mmol), sodium iodate (4 mmol), copper(I) chloride (1 mmol), triethylamine (4 mmol), ethyleneamine (4 mmol). After column chromatography this reaction method produced 4,5-diaziridinyl-1,2-benzoquinone (40 mg, 21% yield).

4,5-Diazetidinyl-1,2-benzoquinone (2b). Method C. A method similar to that described for preparation of the 4,5-diaziridinyl derivative (**2a**) was employed. The reaction mixture consisted of the following reagents and proportions: catechol (1 mmol), azetidine (2.2 mmol), triethylamine (4 mmol), copper(I) chloride (1 mmol), and sodium iodate (4 mmol). The reaction carried out at 0 °C for 1 h. The product was purified as described above to give 4,5-diazetidinyl-1,2-benzoquinone (98.5 mg, 45% yield) as deep red needles from CH_2Cl_2/acetone (1:1): mp 138–140 °C dec; IR ν_{max} 2960, 2890, 1640, 1570, 1540, 1430, 1340, 1300, 1220, 1140, 1070, 920, 910, 810, 770 cm^{-1}; UV λ_{max} (95% EtOH) 236 nm (log ϵ 4.24), 369 (4.23), 520 (2.99); ^1H NMR ($CDCl_3$) δ 2–2.6 (m, 4H, 2 CH_2), 3.8–4.2 (t, 8H, 2-N(CH_2)$_2$), 5.3 (s, 2H, 3,6-H). Anal. $C_{12}H_{14}N_2O_2$: C, H, N.

4,5-Dipyrrolidinyl-1,2-benzoquinone (2c). Method A. The reaction mixture consisted of the following reagents and proportions: catechol (1 mmol), pyrrolidine (2.2 mmol), triethylamine (2.2 mmol), sodium iodate (4 mmol), and copper(II) acetate (1 mmol). The reaction was allowed to proceed at 0 °C for 20 h. After purification the product 4,5-dipyrrolidinyl-1,2-benzoquinone (26.7 mg, 10.83% yield) was obtained as black red needles from CH_2Cl_2/acetone (1:1): mp 170–172 °C dec; IR ν_{max} 3060, 2960, 2840, 1610, 1560, 1510, 1470, 1440, 1290 cm^{-1}; UV λ_{max} (95% EtOH) 250 nm (log ϵ 4.23), 371 (4.27); ^1H NMR ($CDCl_3$) δ 2.1 (m, 8H, 2-CH_2CH_2-), 3.5 (m, 8H, 2-N(CH_2)$_2$), 5.6 (s, 2H, 3,6-H). Anal. $C_{14}H_{18}N_2O_2$: C, H, N.

Method C. The reaction mixture consisted of the following reagents and proportions: catechol (1 mmol), pyrrolidine (2.2 mmol), triethylamine (4 mmol), sodium iodate (4 mmol), and copper(I) chloride (1 mmol). The reaction was allowed to proceed at 0 °C for 2 h and after purification gave the product 4,5-dipyrrolidinyl-1,2-benzoquinone (114.4 mg, 46.4% yield).

4,5-Bis(diethylamino)-1,2-benzoquinone (2d). Method A was employed. The reaction resulted in a crude product as black oil. Column chromatography with chloroform/ethyl acetate (4:1) elution gave 4,5-bis(diethylamino)-1,2-benzoquinone (131 mg, 52.3% yield) which was recrystallized from CH_2Cl_2/acetone (1:1): mp 100–103 °C; IR ν_{max} 2980, 2940, 1630, 1530, 1360, 820 cm^{-1}; UV λ_{max} (95% EtOH) 252 nm (log ϵ 4.19), 374 (4.23); ^1H NMR ($CDCl_3$) δ 1.1 (t, 12H, 4-CH_3), 3.4 (q, 8H, 4-CH_2), 5.7 (s, 2H, 3,6-H). Anal. $C_{14}H_{22}N_2O_2$: C, H, N.

Method C. A similar reaction was carried out as above, using copper(I) chloride in place of copper(II) acetate at 0 °C for 6.5 h. The reaction product 4,5-bis(diethylamino)-1,2-benzoquinone was obtained in a yield of 40%.

4-[(Chloroethyl)amino]-5-methoxy-1,2-benzoquinone (4). 4,5-Dimethoxy-1,2-benzoquinone was prepared as described previously.[28] 4,5-Dimethoxy-1,2-benzoquinone (**3**, 672.4 mg, 4 mmol) was dissolved in dichloromethane (40 mL), and a solution of (chloroethyl)amine hydrochloride (1.2 g, 4.4 mmol) and triethylamine (2.4 mL, 16.8 mmol) in dichloromethane (50 mL) was added dropwise into the solution over the course of 1 h. The reaction mixture was maintained with stirring at room temperature for 21 h. After filtration, the remaining solution was washed with 10% hydrochloric acid, distilled water, and distilled water saturated with sodium chloride and then dried over magnesium sulfate. Evaporation of the solvent gave the black red crude product. Column chromatography with chloroform/methanol (4:0.5, (v/v)) elution gave 4-[(chloroethyl)amino]-5-methoxy-1,2-benzoquinone (**4**) (274.3 mg, 31.8% yield) which was recrystallized from ethanol as red needles: mp 150–52 °C; IR ν_{max} 3400, 2940, 1660, 1640, 1620, 1580, 1510, 1250, 1180, 810 cm^{-1}; UV λ_{max} (95% EtOH) 210 nm (log ϵ 4.35), 304 (4.23), 464 (3.51); ^1H NMR ($CDCl_3$) δ 3.6 (m, 4H, NCH_2CH_2), 3.9 (s, 3H, OCH_3), 5.4 (s, 1H, 6-H), 5.7 (s, 1H, 3-H), 6.2 (bs, 1H, NH). Anal. $C_9H_{10}ClNO_3$: C, H, N, Cl.

Cell Culture Cell Line. EMT-6 mouse mammary tumor cells have been widely used for the study of hypoxia.[37-39] EMT-6 cells in culture were maintained in exponential growth in Waymouth's medium (ISI Corp., Chicago, IL), supplemented with 15% newborn calf serum, penicillin (100 units/mL), and streptomycin (100 μg/mL) (Grand Island Biological Co., Grand Island, NY). The doubling time of these cultures, growing at 37 °C in a 5% CO_2/95% air atmosphere, was 16–19 h. *In vitro* plating efficiencies of control cultures were 65–80%.

Production of Hypoxia. To product hypoxia, the plastic flasks, containing exponentially growing monolayers in complete medium plus serum, were fitted with sterile rubber septums and exposed to a continuously flowing 95% N_2/5% CO_2 humidified atmosphere for 4 h at 37 °C as previously reported.[40,41] Parallel flasks were maintained in 95% air/5% CO_2. At the end of 4 h, the drug or vehicle was added to the flasks by injection through the rubber septum without disturbing the hypoxia.

pH Alterations. The pH of the medium was adjusted using a sodium bicarbonate ($NaHCO_3$)/5% CO_2 buffer system.[42] For altered pH experiments, the flasks were purged with either 95% air/5% CO_2 for 30 min before heating for normally oxygenated conditions or gassed with 95% N_2/5% CO_2 for 4 h at 37 °C for hypoxic experiments as stated above. After completion of the drug treatment, the monolayers were washed with 0.9% phosphate-buffered saline, suspended by trypsinization, and plated in normal pH complete media for colony formation.

Drug Treatments. Exponentially growing cells were exposed to varying concentrations of the drugs for 1 h at 37 °C. Non-drug-treated controls were handled identically. Drugs were prepared in sterile phosphate-buffered saline immediately before use and added to the cells in a small volume (50–100 μL). Addition of the drug solution did not significantly alter the pH of the culture. After treatment, the medium was removed, and the cultures were washed twice with phosphate-buffered saline and suspended by trypsinization.

Cell Viability Measurements. Cell viability was measured by the ability of single cells to form colonies *in vitro*, as described previously.[40,41] Following treatment, suspensions of known cell numbers were plated in plastic Petri dishes and allowed to grow in a 37 °C incubator under standard culture conditions for 8–10 days. After this time interval, macroscopic colonies were stained

with crystal violet in methanol containing 3.7% formaldehyde and were counted manually. Each experiment was repeated three times, and each data point per experiment represents the results of three different dilutions of cells plated in triplicate.

Antitumor Activity. L1210 leukemia (10^6) cells were implanted intraperitoneally in DBA mice (Taconic Farms, Germantown, NY) on day 0. Treatment with each drug was carried out daily for 5 days. The drugs were administered intraperitoneally at the doses shown on Table II. Each treatment group had six animals, and the experiment was carried out twice. The percent increase in lifespan is for the treated animals compared with the untreated controls.

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