A New Class of Analogues of the Bifunctional Radiosensitizer α-(1-Aziridinylmethyl)-2-nitro-1*H*-imidazole-1-ethanol (RSU 1069): The Cycloalkylaziridines[1]

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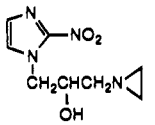
A series of compounds related to α-(1-aziridinylmethyl)-2-nitro-1*H*-imidazole-1-ethanol (RSU 1069, 1) were synthesized and evaluated as selective hypoxic cell cytotoxic agents and as radiosensitizers. The aziridine moiety was replaced with a number of other potential alkylating groups including cycloalkylaziridines and azetidines. The data indicated that modification of the aziridine of 1 resulted in a substantial decrease in the ability of the compounds to selectively kill hypoxic cells. However, these modifications did not affect the compounds' in vitro radiosensitizing activity since many of the derivatives were as potent as 1. All of the compounds that were evaluated in vivo were less toxic than 1, and several members of this series had significant activity. The best compound was *trans*-α-[[(4-bromotetrahydro-2*H*-pyran-3-yl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanol (18), which, due to its activity and log *P* value, is a candidate for additional in vivo studies.

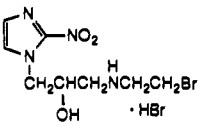
Introduction

Radiotherapy continues to play a major role in the treatment of many forms of human cancer.[2] Although successful in some cases, ca. 30% of patients treated with radiation eventually succumb to lack of control of the primary disease. The failure of radiotherapy has been attributed to many factors, one of which is the presence of radioresistant hypoxic cells found in many solid tumors.[3,4] Studies have demonstrated that tumors contain various subpopulations of cells and that the higher the percentage of hypoxic cells found within a tumor, the higher the dose of radiation needed to eradicate the tumor.[5] Since radiotherapy is limited by the total dose of X-rays a patient can receive without normal tissue damage, methods for overcoming the radioresistance of hypoxic cells have been investigated.[6,7] One means of achieving this goal is through the use of agents which mimic the sensitizing effects of oxygen. These are compounds that are able to penetrate into tumors and selectively sensitize hypoxic tumor cells to the lethal effects of radiation.

Historically, the class of compounds that has received the most study in this regard is the 2-nitroimidazoles.[6,7] Several of these compounds have proceeded into clinical trials, but most were dropped due to a variety of unmanageable side effects. One such compound is α-(1-aziridinylmethyl)-2-nitro-1*H*-imidazole-1-ethanol (RSU 1069, 1).[8] It was the most potent radiosensitizer ever studied in vitro and in vivo, and in addition it was selectively cytotoxic to hypoxic cells.[8] The improved activity seen with 1 was attributed to its bifunctional character;[9] that is, 1 contains an alkylating group (aziridine) in addition to the electron-affinic 2-nitroimidazole ring. This compound proceeded into Phase 1 clinical trials, but was quickly dropped due to severe emesis.[10] Analogues of 1 were subsequently synthesized and evaluated,[11] but none of these compounds warranted additional study. Recently, Jenkins et al.[12] reported the synthesis of a prodrug of 1, α-[[(2-bromoethyl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanol (2, RB 6145). This compound was, as expected, equally active compared to 1, both in vitro and in vivo, but since it was a prodrug of 1, there was concern that it might exhibit the same side effects previously associated with 1.



1
RSU 1069



2
RB 6145

Herein, we report the synthesis and evaluation of a series of bifunctional 2-nitroimidazoles related to 1. In these compounds the aziridine moiety of 1 has been replaced with a series of cycloalkylaziridines and related groups. The goal was to modify the reactivity of the alkylating moiety so as to limit its toxic side effects, but preserve

(1) Presented in part at the 198th American Chemical Society Meeting, Miami Beach, Florida, 1989.

(2) Diamond, J. J.; Hanks, G. H.; Kramer, S. The Structure of Radiation Oncology Practices in the Continental United States. *Int. J. Radiat. Oncol. Biol. Phys.* **1988**, *14*, 547-548.
(3) Fowler, J. F.; Adams, G. E.; Denekamp, J. Radiosensitizers of Hypoxic Cells in Solid Tumors. *Cancer Treat. Rev.* **1976**, *3*, 227-256.
(4) Peters, L. J.; Withers, H.; Thames, H. D., Jr.; Fletcher, G. H. The Problem: Tumor Radioresistance in Clinical Radiotherapy. *Int. J. Radiat. Oncol. Biol. Phys.* **1982**, *8*, 101-108.
(5) Russo, A.; Mitchell, J.; Kinsella, T.; Morstyn, G.; Glatstein, E. Determinants of Radiosensitivity. *Semin. Oncol.* **1985**, *12*, 332-349.
(6) Coleman, C. N.; Bump, E. A.; Kramer, R. A. Chemical Modifiers of Cancer Treatment. *J. Clin. Oncol.* **1988**, *6*, 709-733.
(7) Wasserman, T. H.; Klingerman, M. Chemical Modifiers of the Radiation Effects. In *Principles of Practice of Radiation Oncology*; Perez, C. A., Brady, L. W., Eds.; Lippincott: Philadelphia, PA, 1987; pp 360-376.
(8) Adams, G. E.; Ahmed, I.; Sheldon, P. W.; Stratford, I. J. Radiation Sensitization and Chemopotentiation: RSU 1069, A Compound More Efficient than Misonidazole in Vitro and in Vivo. *Br. J. Cancer* **1984**, *49*, 571-577.
(9) Stratford, I. J.; O'Neill, P.; Sheldon, P. W.; Silver, A. R. J.; Walling, J.; Adams, G. E. RSU 1069, A Nitroimidazole Containing an Aziridine Group. *Biochem. Pharm.* **1986**, *35*, 105-109.
(10) Horwich, A.; Holliday, S. B.; Deacon, J. M.; Peckham, M. J. A Toxicity and Pharmacokinetic Study in Man of the Hypoxic Cell Radiosensitizer RSU-1069. *Br. J. Rad.* **1986**, *59*, 1238-1240.
(11) O'Neill, P.; Jenkins, T. C.; Stratford, I. J.; Silver, A. R. J.; Ahmed, I.; McNeil, S. S.; Fielden, E. M.; Adams, G. E. Mechanism of Action of Some Bioreducible 2-Nitroimidazoles: Comparison of in Vitro Cytotoxicity and Ability to Induce DNA Strand Breakage. *Anticancer Drug Des.* **1987**, *1*, 271-280.
(12) Jenkins, T. C.; Naylor, M. A.; O'Neill, P.; Threadgill, M. D.; Cole, S.; Stratford, I. J.; Adams, G. E.; Fielden, E. M.; Suto, M. J.; Stier, M. A. Synthesis and Evaluation of α-[[(2-Haloethyl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanols as Prodrugs of α-[[(1-Aziridinyl)methyl]-2-nitro-1*H*-imidazole-1-ethanol (RSU 1069) and Its Analogues Which are Radiosensitizers and Bioreductively Activated Cytotoxins. *J. Med. Chem.* **1990**, *33*, 2603-2610.

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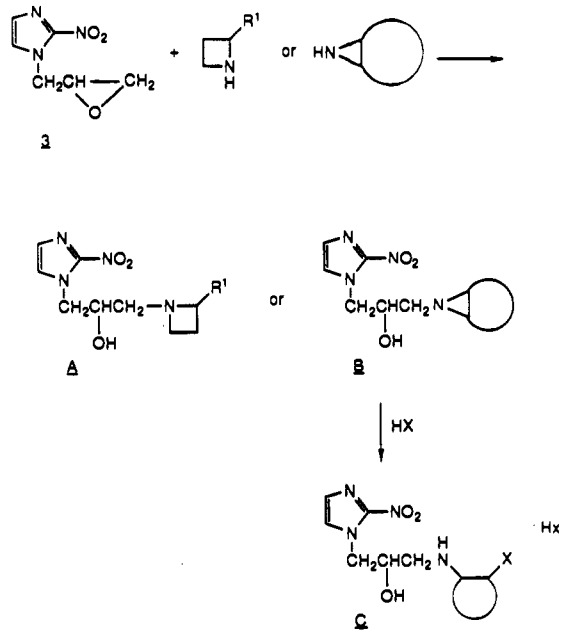
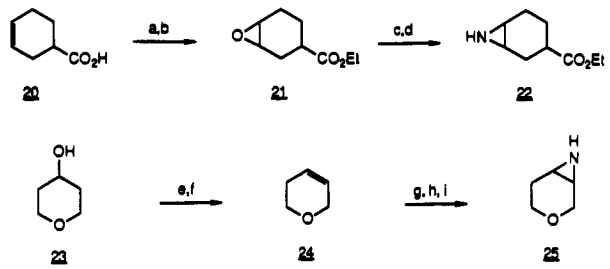
Scheme I



Scheme II[a]



[a] (a) 1. $(COCl)_2$, 2. EtOH; (b) MCPBA; (c) NaN_3; (d) Ph_3P; (e) $MsCl/Et_3N$; (f) DBU; (g) $AgNCO/I_2$; (h) MeOH; (i) 50% KOH/MeOH.

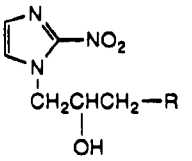
enough bifunctional character to retain potent radiosensitizing activity. The compounds synthesized were evaluated as radiosensitizers and as hypoxic cell cytotoxins in vitro. Selected compounds were also evaluated in vivo for their radiosensitizing activity.

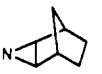
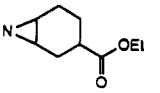
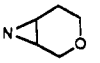
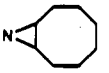
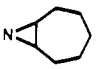
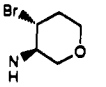
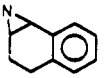
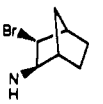
Chemistry

The compounds listed in Table I were prepared by the general procedure outlined in Scheme I. Treatment of the epoxide **3**,[13] with various azetidines or aziridines in methanol, provided compounds of general Formula A or B. Ring-opened analogues of Formula C were prepared from the corresponding aziridines by treatment with HBr in ethanol. Compounds **16–18** were obtained in good yields, but **19** was obtained in a yield of only 20%. The norbornyl aziridine was quite stable and significant decomposition of the starting material (**12**) occurred under prolonged heating with HBr.

The azetidines and aziridines used were commercially available or prepared by using literature procedures (see the Experimental Section). Scheme II illustrates the synthesis of the aziridines not previously reported. Compound **22** was prepared starting from 3-cyclohexenecarboxylic acid (**20**). Reacting the acid choride (prepared from **20** with oxalyl chloride) with ethanol gave the ester[14]

Table I



no.	R	log *P*	no.	R	log *P*
1		0.33	12		1.60
4		−1.51			
5		−2.02	13		0.37
6		−0.29	14		1.13
7		1.07	15		0.07
8		1.84	16		1.21
9		1.44	17		0.43
10		1.55	18		−0.17
11		2.15	19		1.36

which was epoxidized with MCPBA to provide **21**.[15] The azido alcohol was obtained with NaN_3, and aziridine **22** was obtained by reductive cyclization with Ph_3P. 3-Oxa-7-azabicyclo[4.1.0]heptane (**25**) was prepared starting from tetrahydro-2*H*-pyran-4-ol (**23**).[17] The alcohol was converted to the mesylate (methanesulfonyl chloride/Et_3N) and immediately dehydrated with DBU to provide **24**.[18] Treatment of **24** with $AgNCO/I_2$ and quenching with methanol gave the iodocarbamate, which was treated with 50% KOH/MeOH to provide **25**.

Stability Studies

As reported,[12] β-bromoethylamino compounds, such as **2**, serve as prodrugs to the parent aziridine **1**. It was also shown that under physiological conditions (high bicarbonate concentrations) only a percentage of **2** is converted to aziridine **1**. The remainder reacts with HCO_3^- to form the corresponding oxazolidinone.[12]

Since compounds **16–18** are also β-bromoethylamino derivatives their stability under a variety of conditions was examined.[25] It was determined that compounds **16–18** do

(13) Beaman, A. G.; Tautz, W.; Duchinsky, R. Studies in the Nitroimidazole Series III. 2-Nitroimidazole Derivatives Substituted at the 1-Position. *Antimicrob. Agents Chemother.* **1968**, 520–530.

(14) Frostick, F. C.; Philips, B.; Starcher, P. S. Synthesis of Some Epoxy Vinyl Monomers by Epoxidation with Peracetic Acid. *J. Am. Chem. Soc.* **1959**, *81*, 3350–3356.

(15) Batog, A. E.; Stepko, O. P.; Nikonova, L. P. Kinetics of the Reaction of Alicyclic Monoepoxides with Carboxylic Acids. *Zh. Org. Khim.* **1980**, *16*, 1126–1131.

(16) Tanner, D.; Somfai, P. An Aziridine Route to Chiral β-Lactams. A Novel Entry to (+)-Thienamycin. *Tetrahedron Lett.* **1987**, *28*, 1211–1214.

(17) Aldrich Chemical Co., Milwaukee, WI.

(18) Paul, R.; Tschelitcheff, S. A Study Comparing Properties of 2 Isomeric Dihydropyrans. *C. R. Seances Acad. Sci.* **1947**, *224*, 1722–1723.

(19) Hassner, A.; Heathcock, C. Synthesis of Fused Ethyleneimines from Cyclic Olefins. *Tetrahedron* **1964**, *20*, 1037–1042.

Table II. In Vitro Radiosensitizing Activity[a]

no.	conc, mM	SER	$C_{1.6}$, mM	hypoxic cell cytotoxicity[b]
1	0.8	1.9	0.33	50
4	2.0	2.0		1.0
5	10.0	2.3		1.0
6	3.0	2.1		1.0
7	0.5	1.9	0.35	1.6
8	3.0	2.1	0.75	1.2
9	3.0	1.7	1.0	1.4
10	1.5	2.0	0.75	1.0
11	0.38	1.2		1.2
12	3.0	1.6	3.0	1.4
13	0.75	2.0	0.48	2.0
14	3.0	2.0	3.0	1.1
15	0.19	1.3		c
16	0.75	2.4	0.35	2.5
17	1.5	2.3	0.75	2.5
18	3.0	1.8	1.8	1.2
19	3.0	2.1	0.35	1.3

[a] Values represent the mean of replicate experiments agreeing within ±20%. [b] IC_{50}(oxic)/IC_{50}(hypoxic). [c] Compound was unstable under the test conditions.

not form the corresponding oxazolidiones and are converted, even in mouse plasma, to their respective aziridines 7, 13, and 15.

Biological Results

Table II contains data for in vitro radiosensitization and hypoxia selective cytotoxicity. Since sensitizer enhancement ratios (SER) were determined at the maximum nontoxic dose of each compound, the cell killing observed reflects only the radiosensitizing capability of the compounds. Compounds which yielded an SER of 1.8 or greater were further evaluated in a dose-response study ($C_{1.6}$ determination). The $C_{1.6}$ of a compound is a measure of its efficiency of radiosensitization and is defined as the concentration required to give an SER of 1.6. In vitro cytotoxicity data are also presented in Table II. The values were determined by exposing V79 cells to various concentrations of test compound at 37 °C, under hypoxic and oxic conditions. The values given represent the ratio IC_{50}(oxic)/IC_{50}(hypoxic). For compound 1, the value of 50 indicates that the compound is cytotoxic to hypoxic cells at a concentration 50 times lower than to oxic cells, indicating selective toxicity for hypoxic cells.

The results in Table II indicate that modification of the parent aziridine results in diminished hypoxic cell selective cytotoxicity. Only the five- and six-membered ring analogues (7, 13, 16, and 17) possessed some selectivity toward hypoxic cells (about 2-fold). However, modifying the aziridine did not greatly influence in vitro radiosensitizing activity. A number of the compounds yielded SER values of ≥1.8 and several had $C_{1.6}$ values comparable to that of

Table III. In Vivo Radiosensitizing Activity

no.	MTD,[a] mg/kg	fold reduction in survival[b]	no.	MTD,[a] mg/kg	fold reduction in survival[b]
1 (RSU 1069)	100	6.7	15	800	5.7
7	400	3.9	16	700	4.5
8	500	2.6	17	1000	4.3
10	500	2.1	18	1500	13.0
13	450	6.1			

[a] MTD = maximum tolerated dose. [b] Plating efficiency of drug-treated irradiated tumors relative to irradiated only group.

1. The azetidines (4-6) retained in vitro activity as radiosensitizers, but were much less cytotoxic, indicating that the azetidine moiety cannot be used as a replacement for the aziridine group.

Compounds displaying potent in vitro activity ($C_{1.6}$ < 1.0 mM) were evaluated in vivo (Table III). Several members of this series were found to possess significant radiosensitizing potential and five compounds resulted in a greater than 4-fold reduction in survival relative to radiation alone, at the optimum time point. These included cyclopentyl derivative 13 and its ring-opened analogue 17, the ring-opened six-membered compound 16, and also the tetrahydropyrano derivative 18. A correlation was observed between in vivo activity and the log P value of the compound. As shown in the tables, as the log P was changed, the in vitro radiosensitizing activity (Table II) was not greatly affected, but in vivo, compounds which were more hydrophilic were less toxic (higher MTD) and generally more active as radiosensitizers. Compound 18 (log P of −0.17) gave a MTD of 1500 mg/kg and a 13-fold increase in cell kill over radiation alone, which are comparable to those of compound 1, in our system. Additional in vivo studies with 18 are currently in progress.

Conclusions

In summary, we have shown that replacement of the aziridine ring of 1, with a number of cycloalkylaziridines varying in ring size, substituents, and log P, results in a series of compounds that are potent radiosensitizers in vitro. However, the high degree of hypoxia-selective cytotoxicity demonstrated by 1 was diminished in these compounds. Of the compounds evaluated in vivo, five compounds (13, 15–18) had significant activity and are candidates for further in vivo evaluation. The ring-opened analogues 16–18 functioned as prodrugs of the corresponding aziridines with little difference in potency or toxicity between the ring-opened compounds and their respective aziridines. The ring-opened analogues do offer the advantage that they are highly water soluble (25 mg/mL, pH 4.0 buffer) and stable. The results also indicate that hypoxic cell selective cytotoxicity was not a requirement for potent in vitro or in vivo radiosensitizing activity. Currently, we are examining other potential alkylating groups in conjunction with the 2-nitroimidazole nucleus, focusing not only on the "bifunctional character" but also examining more closely the physical properties displayed by these compounds.

Experimental Section

Chemical Synthesis. All melting points were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Associates EM-390, XL-200 or an IBM WP 100sy spectrometer using $CDCl_3$ or DMSO-d_6 as the internal reference standard. Purity was determined by microanalysis and by TLC (silica gel 60F 254, Merck). Silica gel chromatography utilized Kieselgel 60 (70–230 mesh or 230–400 for flash chromatography). IR spectra were recorded with a Nicolet FT-IR spectrophotometer and mass spectra were determined on a VG analytical 7070E/HF or Fin-

(20) Philips, B.; Cromwell, N. Azetidine-2-carboxylic Acid Derivatives. J. Heterocycl. Chem. 1973, 795–799.
(21) Hermes, M. E.; Marsh, F. D. N-Cyanoaziridines and 1-Alkylalkylidenecyanamides from Cyanogen Azide and Olefins. J. Org. Chem. 1972, 37, 2969–2979.
(22) (a) Fanta, P. E. Chemistry of Ethyleneimine. Part III. cyclo-Penteneimines or 6-Azabicyclo[3.1.0]hexane. J. Org. Chem. 1957, 22, 1441–1442. (b) Leighton, P. A.; Perkins, W. A.; Renquist, M. L. A Modification of Wenker's Method of Preparing Ethyleneimine. J. Am. Chem. Soc. 1947, 69, 1540.
(23) Nahum, A.; Horvath, C. Evaluation of Octanol–Water Partition Coefficients by Using High-Performance Liquid Chromatography. J. Chromatogr. 1980, 192, 315–322.
(24) Siemann, D. W. In Vivo Combination of Misonidazole and the Chemotherapeutic Agent CCNU. Br. J. Cancer 1981, 43, 367–377.
(25) Determined by HPLC, Alltech CN column (0.1 M KH_2PO_4/MeOH, 9:1, pH 3.4, 313 nm).

negan 4500 mass spectrometer. All compounds possessed analytical data consistent with the proposed structures.

General Procedure for the Synthesis of the Compounds in Table I. Preparation of α-[(2-Nitro-1*H*-imidazol-1-yl)methyl]-7-azabicyclo[4.1.0]heptane-7-ethanol (7). A solution of **3**[18] (1.3 g, 7.7 mmol) and 7-azabicyclo[4.1.0]heptane[20] in MeOH (30 mL) was heated at reflux for 3 h. The solution was concentrated to an oil which was recrystallized from EtOAc/hexane to provide **7** (1.65 g, 80%): mp 88–89 °C; ^{1}H NMR ($CDCl_3$) δ 1.24 (m, 3 H), 1.80 (m, 5 H), 2.28 (dd, 1 H), 2.44 (dd, 1 H), 3.62 (bs, 1 H), 4.10 (m, 2 H), 4.35 (m, 1 H), 4.71 (d, 1 H), 4.75 (d, 1 H), 7.13 (s, 1 H), 7.28 (s, 1 H); MS (CI$^+$) *m/e* 267. Anal. ($C_{12}H_{18}N_4O_3$) C, H, N.

α-(1-Azetidinylmethyl)-2-nitro-1*H*-imidazole-1-ethanol (4). This was prepared as described above with **3** and azetidine[17] in a yield of 52%: mp 87–90 °C; ^{1}H NMR ($CDCl_3$) δ 2.05 (m, 2 H), 2.43 (m, 2 H), 3.28 (m, 4 H), 3.73 (m, 2 H), 4.22 (d, 1 H), 4.75 (dd, 1 H), 7.08 (d, 1 H), 7.29 (d, 1 H); MS (FAB, M + 1) *m/e* 227. Anal. ($C_9H_{14}N_4O_3$) C, H, N.

1-[2-Hydroxy-3-(2-nitro-1*H*-imidazol-1-yl)propyl]-2-azetidinecarboxylic Acid (5). This was prepared as described above with **3** and 2-azetidinecarboxylic acid[17] in a yield of 32%: mp 182–184 °C; ^{1}H NMR (DMSO-d_6) δ 2.26 (m, 2 H), 2.82 (m, 2 H), 3.55 (m, 2 H), 4.10 (m, 1 H), 4.34 (m, 1 H), 4.52 (m, 1 H), 4.61 (m, 1 H), 7.16 (s, 1 H), 7.58 (s, 1 H); MS (FAB) *m/e* 271. Anal. ($C_{10}H_{14}N_4O_5 \cdot 0.25H_2O$) C, H, N.

Methyl 1-[2-Hydroxy-3-(2-nitro-1*H*-imidazol-1-yl)propyl]-2-azetidinecarboxylate (6). This was prepared as described above with **3** and methyl 2-azetidinecarboxylate[19] in a yield of 16%: mp 158–159 °C; ^{1}H NMR ($CDCl_3$) δ 2.35 (m, 3 H), 2.76 (dd, 1 H), 3.10 (m, 1 H), 3.51 (m, 1 H), 3.74 (s, 3 H), 3.96 (m, 3 H), 4.33 (m, 1 H), 4.64 (dd, 1 H), 7.13 (d, 1 H), 7.31 (d, 1 H); MS (FAB) *m/e* 284. Anal. ($C_{10}H_{16}N_4O_5$) C, H, N.

α-[(2-Nitro-1*H*-imidazol-1-yl)methyl]-9-azabicyclo[6.1.0]nonane-9-ethanol (8). This was prepared as described above with **3** and 9-azabicyclo[6.1.0]nonane[19] in a yield of 71%: mp 120–122 °C; ^{1}H NMR ($CDCl_3$) δ 1.42 (m, 12 H), 1.95 (d, 2 H), 2.36 (m, 1 H), 2.40 (m, 1 H), 3.85 (bs, 1 H), 4.26 (m, 1 H), 4.33 (m, 1 H), 4.71 (m, 1 H), 7.15 (s, 1 H), 7.27 (s, 1 H); MS (EI$^+$, M + 1) *m/e* 295. Anal. ($C_{14}H_{22}N_4O_3$) C, H, N.

α-[(2-Nitro-1*H*-imidazol-1-yl)methyl]-8-azabicyclo[5.1.0]octane-8-ethanol (9). This was prepared as described above with **3** and 8-azabicyclo[5.1.0]octane[19] in a yield of 67%: mp 91–91.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.55 (m, 10 H), 2.03 (bs, 2 H), 2.49 (m, 2 H), 4.10 (m, 1 H), 4.31 (m, 1 H), 4.70 (dd, 1 H), 7.15 (s, 1 H), 7.26 (s, 1 H); MS (CI$^+$, M + 1) *m/e* 281. Anal. ($C_{13}H_{20}N_4O_3$) C, H, N.

2-Methyl-α-[(2-nitro-1*H*-imidazol-1-yl)methyl]-7-azabicyclo[4.1.0]heptane-7-ethanol (10). This was prepared as described with **3** and 2-methyl-7-azabicyclo[4.1.0]heptane in a yield of 56%: mp 124.5–125 °C; ^{1}H NMR ($CDCl_3$) δ 0.77 (bs, 1 H), 1.05 (d, 3 H), 1.40–1.65 (m, 6 H), 1.82 (m, 2 H), 2.30 (dd, 1 H), 2.51 (dd, 1 H), 3.42 (s, 1 H), 4.05 (m, 1 H), 4.35 (dd, 1 H), 4.70 (dd, 1 H), 7.15 (s, 1 H), 7.28 (s, 1 H); MS (CI$^+$, M + 1) *m/e* 281. Anal. ($C_{13}H_{20}N_4O_3$) C, H, N.

1a,2,3,7b-Tetrahydro-α-[(2-nitro-1*H*-imidazol-1-yl)methyl]-1*H*-naphth[1,2-*b*]azirine-1-ethanol (11). This was prepared as described above with **3** and 1a,2,3,7b-tetrahydro-1*H*-naphth[1,2-*b*]azirine[19] in a yield of 30%: mp 144.5–146 °C; ^{1}H NMR ($CDCl_3$) δ 1.60 (m, 2 H), 2.36 (m, 2 H), 2.60 (m, 3 H), 3.01 (m, 2 H), 4.13 (m, 1 H), 4.35 (m, 1 H), 4.71 (m, 1 H), 7.05–7.34 (m, 6 H); MS (EI$^+$, M + 1) *m/e* 315. Anal. ($C_{16}H_{18}N_4O_3$) C, H, N.

α-[(2-Nitro-1*H*-imidazol-1-yl)methyl]-3-azatricyclo[3.2.1.0^{2,4}]octane-3-ethanol (12). This compound was prepared as described above with **3** and 3-azatricyclo[3.2.1.0^{2,4}]octane[21] in a yield of 50%: mp 92–92.5 °C; ^{1}H NMR ($CDCl_3$) δ 0.65 (d, 1 H), 1.18 (m, 2 H), 1.43 (d, 3 H), 1.61 (q, 2 H), 2.16 (dd, 1 H), 2.34 (m, 3 H), 3.4 (s, 1 H), 4.07 (m, 1 H), 4.33 (m, 1 H), 4.70 (dd, 1 H), 7.13 (s, 1 H), 7.27 (s, 1 H); MS (EI$^+$, M + 1) *m/e* 279. Anal. ($C_{13}H_{18}N_4O_3 \cdot 0.25H_2O$) C, H, N.

α-[(2-Nitro-1*H*-imidazol-1-yl)methyl]-6-azabicyclo[3.1.0]hexane-6-ethanol (13). This was prepared as described with **3** and 6-azabicyclo[3.1.0]hexane[22] in a yield of 75%: mp 83.5–84.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.45 (m, 4 H), 1.79 (m, 2 H), 2.08 (q, 2 H), 2.37 (dd, 1 H), 2.40 (dd, 1 H), 3.52 (bs, 1 H), 4.06 (m, 1 H), 4.33 (m, 1 H), 4.70 (dd, 1 H), 7.13 (s, 1 H), 7.27 (s, 1 H); MS (EI$^+$, M + 1) *m/e* 253. Anal. ($C_{11}H_{16}N_4O_3$) C, H, N.

Ethyl 7-[2-Hydroxy-3-(2-nitro-1*H*-imidazol-1-yl)propyl]-7-azabicyclo[4.1.0]heptane-3-carboxylate (14). This was prepared as described above with **3** and ethyl 7-azabicyclo[4.1.0]heptane-3-carboxylate **22** in a yield of 43%: mp 90.5–92 °C; ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3 H), 1.71 (m, 6 H), 2.18 (m, 3 H), 2.42 (m, 2 H), 3.33 (bs, 1 H), 4.14 (m, 3 H), 4.35 (m, 1 H), 4.72 (dd, 1 H), 7.14 (s, 1 H), 7.27 (s, 1 H); MS (FAB, M + 1) *m/e* 339. Anal. ($C_{15}H_{22}N_4O_5$) C, H, N.

α-[(2-Nitro-1*H*-imidazol-1-yl)methyl]-3-oxa-7-azabicyclo[4.1.0]heptane-7-ethanol (15). This was prepared as described above with **3** and **25** in a yield of 68%: mp 102–105 °C; ^{1}H NMR ($CDCl_3$) δ 1.86 (m, 4 H), 2.46 (m, 2 H), 3.36 (m, 2 H), 3.86 (bs, 3 H), 4.16 (m, 1 H), 4.31 (d, 1 H), 4.76 (d, 1 H), 7.10 (s, 1 H), 7.26 (s, 1 H); MS (FAB, M + 1) *m/e* 269. Anal. ($C_{11}H_{16}N_4O_4$) C, H, N.

Ethyl 7-Oxabicyclo[4.1.0]heptane-3-carboxylate (21). A solution of 3-cyclohexene-1-carboxylic acid (50 g, 0.4 mol) in toluene (200 mL) was cooled to 0 °C and treated dropwise with oxalyl chloride (55.3 g, 0.44 mol) in toluene (150 mL). The mixture was allowed to warm slowly to room temperature and stirred for 18 h. Then ethanol (100 mL) was added and stirring continued for an additional 18 h. The reaction was concentrated and the residue dissolved in EtOAc. The organic layer was washed with $NaHCO_3$ (saturated), brine, dried ($MgSO_4$), and concentrated to provide the ester (61.4 g, 100%); ^{1}H NMR ($CDCl_3$) δ 1.26 (t, 3 H), 1.5–2.3 (m, 6 H), 2.5 (m, 1 H), 4.09 (q, 2 H), 5.61 (s, 2 H). This material was used directly in the next step.

The ester (61.4 g, 0.4 mol) was dissolved in CH_2Cl_2 (400 mL), cooled to 0 °C, and treated with MCPBA (137 g, 0.8 mol) in CH_2Cl_2 (300 mL). The resulting suspension was allowed to warm to room temperature and stirred for 72 h. Water was added and the organic layer was separated, washed with saturated $NaHCO_3$, saturated Na_2SO_3, brine, and then dried ($MgSO_4$). The EtOAc was removed in vacuo to provide 46.9 g of material which was distilled to give **21** (34.2 g, 50%): bp 61–65 °C (0.2 mmHg); ^{1}H NMR ($CDCl_3$) δ 1.16 (t, 3 H), 1.61 (m, 1 H), 1.90 (m, 1 H), 1.96 (m, 2 H), 2.15 (m, 2 H), 2.61 (m, 1 H), 3.06 (bs, 2 H), 4.0 (q, 2 H); MS (EI$^+$, M + 1) *m/e* 171. Anal. ($C_9H_{14}O_3$) C, H.

Ethyl 7-Azabicyclo[4.1.0]heptane-3-carboxylate (22). A mixture of **21** (32.9 g, 0.19 mol) and NaN_3 (16.3 g, 0.25 mol) in EtOH (500 mL) was heated at reflux for 6 h. The solution was cooled, poured into H_2O (500 mL), and extracted with Et_2O. The combined Et_2O extracts were washed with saturated NaCl, dried ($MgSO_4$), and concentrated. The residue was distilled to provide the azido alcohol (37.9 g, 92%): bp 104.5–114 °C (0.2 mmHg); ^{1}H NMR ($CDCl_3$) δ 1.26 (t, 3 H), 1.61 (m, 3 H), 2.05 (m, 2 H), 2.33 (m, 1 H), 2.75 (m, 1 H), 2.89 (bs, 1 H), 3.60 (m, 2 H), 4.19 (q, 2 H); MS (EI$^+$, M + 1) *m/e* 214. Anal. ($C_9H_{15}N_3O_3$) C, H, N.

The azido alcohol (4 g, 19 mmol) was dissolved in toluene (80 mL) and Ph_3P (5.9 g, 23 mmol) was added. The solution was heated at reflux for 8 h, cooled, and washed with H_2O and saturated NaCl. The organic layer was dried ($MgSO_4$) and concentrated to provide an oil (9.3 g). Silica gel chromatography (1% MeOH/$CHCl_3$) provided 1.34 g of material which was distilled (bulb-to-bulb) to give **22** (0.9 g, 28%): bp 130–140 °C; ^{1}H NMR ($CDCl_3$) δ 0.65 (bs, 1 H), 1.23 (t, 3 H), 1.37 (m, 1 H), 1.65 (m, 2 H), 1.91 (m, 2 H), 2.18 (m, 3 H), 2.33 (m, 1 H), 4.12 (d, 2 H); MS (EI$^+$, M + 1) *m/e* 170. Anal. ($C_9H_{15}NO_2 \cdot 0.15H_2O$) C, H, N.

3,6-Dihydro-2*H*-pyran (24). To tetrahydro-2*H*-pyran-4-ol[17] (45 g, 0.44 mol) in CH_2Cl_2 (900 mL) was added Et_3N (53.9 g, 0.53 mol). The solution was cooled to 0 °C and treated dropwise with methanesulfonyl chloride (37.3 mL, 0.48 mol). The mixture was stirred for 1 h at 0 °C, and then 1.5 h at room temperature and diluted with H_2O. The organic layer was dried and concentrated to provide 77 g of the mesylate (NMR) which was not characterized further. The mesylate was transferred to a flask fitted for distillation and then DBU (75 mL) was added. The flask was heated slowly to 130 °C. The material boiling at 85–100 °C was collected to provide **24**[18] (31.3 g, 87%); ^{1}H NMR ($CDCl_3$) δ 2.08 (m, 2 H), 3.74 (m, 2 H), 4.08 (m, 2 H), 5.74 (m, 2 H).

3-Oxa-7-azabicyclo[4.1.0]heptane (25). To a solution of **24** (24.7 g, 0.29 mol) in Et_2O (300 mL) was added I_2 (77.4 g, 0.30 mol). The mixture was cooled to 0 °C and AgNCO (45.9 g, 0.30 mol)

was added. The dark mixture was stirred at 0 °C for 1 h and at room temperature for 18 h and then filtered. The filtrate was concentrated to a dark oil and MeOH (600 mL) was added. The solution was heated at reflux for 3 h, cooled, and concentrated to about one-third volume. The residue was partitioned between H_2O and CH_2Cl_2 and the organic layer was dried ($MgSO_4$) and concentrated to provide 70 g of the iodocarbamate. This was not characterized further but immediately treated with 50% KOH (135 mL) in MeOH (700 mL) and heated at reflux for 3 h. The MeOH was distilled off and the residue extracted with Et_2O for 48 h. The ether was carefully distilled to provide 21 g of crude aziridine. Bulb-to-bulb distillation provided **25** (17.3 g, 60%); ^1H NMR ($CDCl_3$) δ 1.55 (s, 1 H), 1.73 (m, 2 H), 2.00 (m, 1 H), 2.13 (m, 1 H), 3.16 (m, 1 H), 3.38 (m, 1 H), 3.73 (d, 2 H).

General Procedure for the Synthesis of Ring-Opened Analogues (Compounds 16–19). Preparation of *trans*-α-[[(2-Bromocyclohexyl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanol (16). A solution of **7** (3.5 g, 13.1 mmol) in EtOH (50 mL) was cooled to 0 °C and HBr/EtOH (13 mL, 2.2 M) was added. The mixture was allowed to warm to room temperature and heated at reflux until a solution was obtained. The reaction was cooled and the resulting solid was filtered and washed with EtOH and Et_2O to provide **16** (5.21, 93%); mp 182–184 °C; ^1H NMR (DMSO-d_6) δ 1.29 (m, 4 H), 1.51 (m, 2 H), 1.67 (m, 4 H), 2.08 (m, 1 H), 2.40 (m, 1 H), 3.88 (m, 1 H), 4.39 (m, 1 H), 4.76 (dd, 1 H), 5.07 (d, 1 H), 7.16 (d, 1 H), 7.57 (d, 1 H), 8.33 (m, 1 H), 8.91 (m, 1 H); MS (FAB, M + 1) *m/e* 348. Anal. ($C_{12}H_{19}N_4BrO_3$·HBr) C, H, N, Br.

***trans*-α-[[(2-Bromocyclopentyl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanol (17).** This was prepared as described from **13**, in a yield of 75%: mp 190–192 °C; ^1H NMR (DMSO-d_6) δ 1.75 (m, 3 H), 1.95 (m, 1 H), 2.2 (m, 1 H), 2.4 (m, 1 H), 3.05 (m, 1 H), 3.25 (m, 1 H), 3.85 (m, 1 H), 4.24 (m, 1 H), 4.37 (m, 1 H), 4.61 (m, 2 H), 5.94 (bs, 1 H), 7.23 (s, 1 H), 7.61 (s, 1 H), 8.91 (bs, 2 H); MS (EI^+, M + 1) *m/e* 334. Anal. ($C_{11}H_{17}N_4BrO_3$·HBr) C, H, N, Br.

***trans*-α-[[(4-Bromotetrahydro-2*H*-pyran-3-yl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanol (18).** This was prepared as described from **15**, in a yield of 71%: mp 169–170 °C dec; ^1H NMR (DMSO-d_6) δ 1.95 (m, 1 H), 2.42 (m, 1 H), 3.05 (m, 1 H), 3.33 (m, 1 H), 3.51 (m, 6 H), 3.81 (m, 2 H), 4.27 (m, 1 H), 4.45 (m, 2 H), 4.62 (m, 1 H), 4.71 (m, 1 H), 5.91 (bs, 1 H), 7.21 (s, 1 H), 7.63 (s, 1 H), 8.83 (bs, 1 H); MS (EI^+, M + 1) *m/e* 302 (M − NO_2). Anal. ($C_{11}H_{17}N_4BrO_4$·HBr) C, H, N, Br.

***trans*-α-[[(2-Bromobicyclo[2.2.1]heptan-1-yl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanol (19).** This was prepared as described from **12**, in a yield of 20%: mp 226.5–228 °C dec; ^1H NMR (DMSO-d_6) δ 1.40 (m, 2 H), 1.73 (m, 3 H), 1.92 (d, 1 H), 2.92 (bs, 1 H), 3.17 (m, 2 H), 4.15 (m, 1 H), 4.36 (m, 2 H), 4.52 (dd, 1 H), 5.92 (d, 1 H), 7.23 (s, 1 H), 7.61 (s, 1 H), 8.82 (bs, 2 H); MS (FAB, M + 1) *m/e* 360. Anal. ($C_{13}H_{19}N_4BrO_3$·HBr) C, H, N, Br.

log *P* Determinations. log *P* values were obtained by using the shake-flask method of Nahum and Horvath.[23] The compound to be evaluated was partitioned between octanol and 0.05 M NH_4PO_4 (pH 7.4) containing nitrobenzene (10 mg/mL) as an internal standard. HPLC determinations were performed with an Alltech CN column (0.1 M KH_2PO_4/MeOH, 9:1, pH 3.4, 313 nm).

Stability Studies of Compounds 16–18. The stability of compounds **16–18** was determined in bicarbonate (22 mM, 23 °C) and mouse plasma (37 °C) as follows: Approximately 1–4 mg of each compound was added to bicarbonate (2.2 mL) at 23 °C. Aliquots (10 μL) were removed at 5, 10, 15, 30, 45, 60, and 120 min and analyzed by HPLC (Alltech CN column, 0.1 M KH_2PO_4/MeOH, 9:1 pH 3.4, 313 nm). Within 30 min all of the compounds tested were converted totally to their corresponding aziridines (**7**, **13**, **15**) which was verified by coinjection with authentic samples. Analysis of these samples at 24 h indicated that compounds **7** and **13** were stable under the assay conditions (>90%), but **15** progressively decomposed.

A sample of each compound (**16–18**, 1 mg) was added to mouse plasma (1 mL) and incubated at 37 °C. Aliquots were removed at 1, 5, 15, 45, and 60 min and quenched with 20% trichloroacetic acid. The samples were vortexed and analyzed as above. Compounds **16** and **17** were totally converted to their corresponding aziridines **7** and **13** within 15 min and the aziridines were stable for 24 h. None of compound **18** was present after 30 min, but only 52% of the material present corresponded to aziridine **15**. The remainder (>40%) consisted of a number of unidentified decomposition products.

Biological Studies. In Vitro. Chinese hamster V79-171b cells, maintained as monolayer cultures, were plated in 60-mm glass Petri dishes in RPMI 1640 media containing 10% fetal calf serum and allowed to incubate ca. 18 h at 37 °C. On the day of treatment, media was removed from dishes and replaced with fresh media. For radiosensitization studies, solutions of test compounds were freshly prepared and were added directly to the media in dishes.

The Petri dishes were placed into Plexiglas jigs outfitted with inlet and outlet valves for direct gassing. Hypoxia was induced by purging the jigs with 95% N_2/5% CO_2 for 1 h prior to irradiation at room temperature. Irradiations were performed with a Philips X-ray source operated at 320 kV and 10 mA with a 1.25-mm Thoraeus filter. The dose rate was measured with a standardized Victoreen electrometer (Victoreen Instruments) and was ca. 1.5 Gy/min. Immediately following irradiation, the drug-containing media was removed from dishes and replaced with fresh media for colony formation. After incubation for 6–7 days at 37 °C, colonies were stained with crystal violet and colonies of 50 or more cells were scored as survivors. Sensitizer enhancement ratios were determined from the ratio of the doses required to reduce cell survival to 25% of the control value obtained from hypoxic survival curves in the presence and absence of test compounds. $C_{1.6}$ values were determined by plotting SER values against concentration of compound tested as described above.

For cytotoxicity determinations, cells were plated as described above and exposed to various concentrations of test compounds in growth media for 1 h at 37 °C under either oxic or hypoxic conditions. Dishes were then rinsed free of drug-containing media and incubated for colony formation.

Biological Evaluation. In Vivo. The tumor excision assay used to evaluate these compounds was a modification of the procedure described by Siemann[24] and Jenkins.[12] Mice bearing KHT fibrosarcoma (200–500 mg), containing a hypoxic fraction of ca. 20%, were treated with vehicle or test compound at a dose previously determined to be the maximum tolerated dose (MTD). Initially, the optimum time of administration before irradiation for maximum radiosensitization was determined for each of the compounds by administering the MTD. Subsequently, compounds were administered ip at varying times (generally 30–120 min) prior to irradiation with a 15 Gy X-ray dose. Eighteen hours after X-ray treatment, animals were sacrificed by cervical dislocation, their tumors excised, and a single-cell suspension prepared. Clonogenic survival was determined by plating in soft agar in multiwell plates. After incubation for 12–16 days cells were stained and counted.

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