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

Terence C. Jenkins,† Matthew A. Naylor, Peter O'Neill,* Michael D. Threadgill,‡ Shirley Cole, Ian J. Stratford, Gerald E. Adams, E. Martin Fielden, Mark J. Suto,§ and Michael A. Stier§

Medical Research Council Radiobiology Unit, Chilton, Didcot, Oxfordshire OX11 0RD, U.K., and Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105. *Received January 16, 1990*

α-[(1-Aziridinyl)methyl]-2-nitro-1*H*-imidazole-1-ethanols, of general formula $ImCH_2CH(OH)CH_2\overline{NCR^1R^2CR^3R^4}$, where Im = 2-nitroimidazole and R^1, R^2, R^3, R^4 = H, Me, are radiosensitizers and selective bioreductively activated cytotoxins toward hypoxic tumor cells in vitro and in vivo. Treatment of the aziridines with hydrogen halide in acetone or aqueous acetone gave the corresponding 2-haloethylamines of general formula $ImCH_2CH(OH)CH_2^+NH_2CR^1R^2CR^3R^4X\ X^-$, where R^1, R^2, R^3, R^4 = H, Me, and X = F, Cl, Br, I. These 2-haloethylamines were evaluated as prodrugs of the parent aziridines. The rates of ring closure in aqueous solution at pH ~6 were found to increase with increasing methyl substitution and to depend on the nature of the leaving group (I ~ Br > Cl ≫ F). A competing reaction of $ImCH_2CH(OH)CH_2^+NH_2CH_2CH_2X\ X^-$ (X = Cl, Br) with aqueous HCO_3^- ions gives 3-[2-hyroxy-3-(2-nitro-1*H*-imidazol-1-yl)propyl]-2-oxazolidinone. The activities of these prodrugs as radiosensitizers or as bioreductively activated cytotoxins were consistent with the proportion converted to the parent aziridine during the course of the experiment. α-[[(2-Bromoethyl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanol (RB 6145, **10**), the prodrug of α-[(1-aziridinyl)methyl]-2-nitro-1*H*-imidazole-1-ethanol (RSU-1069, **3**), is identified as the most useful compound in terms of biological activity and rate of ring closure under physiological conditions.

Introduction

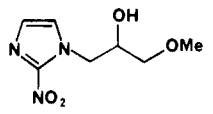
It is generally known that many solid tumors contain areas of diminished oxygen supply. While the underlying reasons for this hypoxia can vary depending upon the type and environment of the tumor, this phenomenon provides a potential basis for selectivity of cancer chemotherapeutic agents towards tumors. Compounds which are anaerobically reduced to a cytotoxin should be more cytotoxic to hypoxic tumor cells than to oxygenated normal tissues.[1] Many nitroheterocyclic compounds, such as the 2-nitroimidazoles, owe their selective cytotoxicity toward hypoxic cells within tumors to their bioreductive properties.[2] They are activated in vivo by anaerobic, enzymatic reduction to form metabolites which are considerably more cytotoxic than the parent compound from which they were derived.

Nitroimidazoles also act as hypoxic cell radiosensitizers, acting primarily by fast free-radical mechanisms.[3] The agents increase the sensitivity to radiation of the normally radiation-resistant hypoxic cells but have little or no effect on the response of well-oxygenated cells to radiation. The ability of these compounds to act as radiosensitizers[4] and hypoxic cell cytotoxins is related to their reduction potentials, although the mechanisms of the two effects are different.

Misonidazole (**1**)[5] has undergone extensive clinical evaluation as a radiosensitizer, but cumulative neurotoxicity severely limits the dosage which may be administered. An equally potent but less toxic analogue, etanidazole (**2**),[6] is in phase III trials. An alternative approach has been to develop compounds with greater potency than **1** or **2** (Chart I). An example is the dual functional agent α-[(1-aziridinyl)methyl]-2-nitro-1*H*-imidazole-1-ethanol (**3**), which combines the 2-nitroimidazole ring with an alkylating moiety, resulting in a powerful hypoxic cell radiosensitizer and bioreductively activated cytotoxic agent both in vitro and in vivo.[7-9] This aziridine **3** was selected from a series of compounds based upon its partition coefficient and activity in model tumors. The ability of **3** to sensitize hypoxic tumor cells to the lethal effects of radiation at 10-12 times less than the dose of **1** or **2** in experimental animal models led to its investigation clinically. Unfortunately, in early phase I trials, gastric toxicity (emesis) was observed.[10] The maximum tolerated dose achievable in humans was considered to be too low to achieve a usefully high degree of radiosensitization. This led to a search for equally active but less toxic analogues.

This study describes the synthesis, stability, and development of a series of [(haloethyl)amino]propyl-2-nitroimidazoles derived from **3** and its analogues. Preliminary biological studies of these compounds as radios-

Chart I. Structures of the Hypoxic Cell Radiosensitizers Misonidazole (1) and Etanidazole (2)



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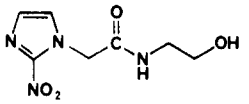

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† Present address: Cancer Research Campaign Biomolecular Structure Unit, Institute of Cancer Research, Sutton, Surrey SM2 5NG, UK.

‡ Present address: School of Pharmacy, University of Bath, Bath BA2 7AY, UK.

§ Warner-Lambert Co.

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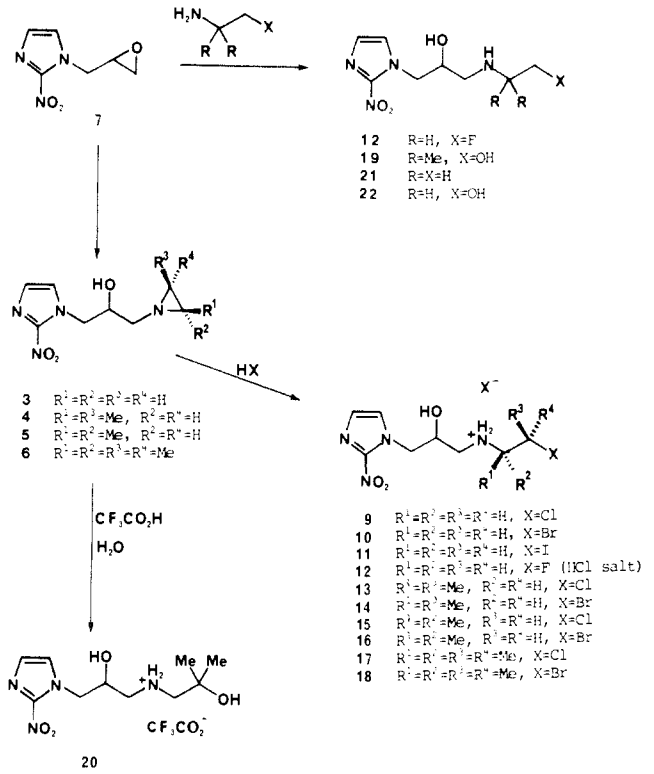
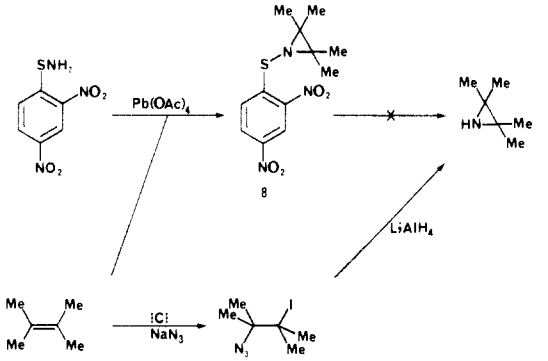
Scheme I. Synthesis of 2-Haloethylamines 9–18, 2-Hydroxyethylamines 19, 20, and 22, and Ethylamine 21



Scheme II. Synthetic Approaches to 2,2,3,3-Tetramethylaziridine



ensitizers are also provided.

Chemistry

Compounds **3–6** were prepared as described previously by us[8,11,12] from oxirane **7** and the corresponding aziridine (Scheme I). The overall yield of **4** was increased considerably with stereochemically pure *cis*-2,3-dimethylaziridine prepared from *cis*-2,3-epoxybutane by the method of Dickey et al.[13] Alternative procedures for the synthesis of 2,2,3,3-tetramethylaziridine, used in the preparation of **6**, were explored, in order to avoid the use of the expensive, noxious, and unreliable nitrosyl chloride.[14,15]

The cyclization of 2-amino alcohols via the *O*-sulfates fails for the more substituted examples. The insertion of [(2,4-[dinitrophenyl)sulfenyl]nitrene, generated from 2,4-dinitro benzenesulfonamide and lead tetraacetate,[16] into the double bond of 2,3-dimethylbut-2-ene gave 1-[2,4-dinitrophenyl)thio]-2,2,3,3-tetramethylaziridine (**8**) in high yield (Scheme II). However, reductive cleavage of the S–N bond could not be achieved in preparatively useful procedures. Addition of iodine azide, formed in situ from sodium azide and iodine monochloride, across the double bond of 2,3-dimethylbut-2-ene, reduction of the resultant azide, and concomitant ring closure furnished the aziridine in acceptable yield[17] (Scheme I).

The 2-haloethylamines **9–18** were obtained from the corresponding aziridines **3–6** by acid-catalyzed nucleophilic ring opening with the appropriate hydrogen halide, as shown in Scheme II. The yields were generally greater if anhydrous conditions were employed, thus avoiding the possibility of nucleophilic attack by water leading to the corresponding 2-hydroxyethylamines.

Compounds **13** and **14** have the threo relative stereochemistry in the haloalkylamine moiety but were isolated as mixtures of diastereoisomers with respect to the chiral center adjacent to the hydroxy group. Proton NMR at 300 MHz and HPLC analysis revealed that **13** consisted of such diastereoisomers in the approximate 1:1 ratio, indicating that the electrophilic centers in protonated **4**, which are prochiral, are roughly equivalent in reactivity and/or steric crowding in the reacting conformation. HPLC analysis of **14** also indicated the presence of both the analogous diastereomers in similar quantities. Ring-closure of **13** and **14** in the presence of aqueous base gave exclusively *cis*-2,3-dimethylaziridine **4**, showing that both ring-opening and ring-closing reactions proceed with complete inversion at the reacting center.

The correct regioisomeric structures of **15** and **16** as 1,1-dimethyl-2-haloethylamines which are products of attack at CH_2, rather than isomeric 2-halo-2-methylpropylamines resulting from alternative attack at CMe_2, were also confirmed by two pieces of evidence from ^1H NMR spectra. Firstly, the CMe_2CH_2Cl protons of **15**, in solution in 10% deuterotrifluoroacetic acid in deuterium oxide, resonate as a sharp singlet at δ 3.90. In comparison, the spectrum of **19**, in the same solvent, shows a corresponding singlet at δ 3.80 for the CMe_2CH_2OD protons whereas that of isomeric alcohol **20**, prepared in situ from **3** by hydrolytic ring opening in the NMR solvent, contains a singlet for the $^+ND_2CH_2CMe_2OD$ protons at δ 3.40. Secondly, when the spectra were recorded of solutions of these materials in anhydrous protiotrifluoroacetic acid, coupling was evident between the $^+NH_2$ protons and the adjacent CH_2CMe_2 in **20** but was absent from the $^+NH_2CMe_2CH_2OH$ signal of **19**. The corresponding signal of **15** also showed no coupling to the $^+NH_2$ protons. These observed regioselectivities of acid-catalyzed ring opening of 2,2-dimethylaziridines with the weak nucleophilic water and the strong nucleophile chloride are consistent with the reported[18] corresponding reactions of simple *N*-unsubstituted aziridines. The attack of chloride proceeds via a S_N2-type mechanism involving the less hindered CH_2 center; whereas water can only trap the more stable tertiary carbonium ion in a S_N1-like process.

The diastereotropic nature of the $NC(CH_3)_2$ and $ClC(CH_3)_2$ groups of protons of **17** was revealed by proton NMR at 250 MHz in solution in $(CD_3)_2SO$. The $\Delta\delta$ for the pairs of methyl groups were 0.02 and 0.01 ppm, respec-

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Scheme III. "One-Pot" Conversion of 2-Hydroxyethylamines **22** and **19** to 2-Bromoethylamines **10** and **16**

22 ($R^1=R^2=H$)[a] **10** ($R^1=R^2=H$)[a]

19 ($R^1=R^2=Me$)[b] **16** ($R^1=R^2=Me$)[b]

[a]Reagents; Me_2SBr_2 [b]Reagents; NBS/Ph_3P

tively, indicating their remoteness from the *CHOH chiral center. Resolution of the corresponding NCH_2CH_2Cl prochiral methylene protons was not possible. However, in the 250- and 300-MHz spectra of **12**, **13**, and **17**, the $^+NH_2$ protons were found to be significantly inequivalent, with $\Delta\delta$ ranging from 0.14 to 0.13 ppm, possibly indicating conformations involving one, but not the other, NH proton in an intramolecular hydrogen bond.

Direct reaction of oxirane **7** with 2-fluoroethylamine or with ethylamine in ethanol provided compounds **12** and **21**, respectively, as shown in Scheme I. However, this method cannot be used to prepare the more reactive haloethylamino compounds **9**–**11** and **13**–**18**, since unavoidable cyclization and/or polymerization of the starting (substituted) 2-haloethylamine occurs.

Attempts to replace directly the primary hydroxyl groups of **19** and **22** by bromine, thus avoiding the relatively toxic and unstable aziridines **3** and **5**, met with some limited success (Scheme III). Treatment of diol **22** with bromodimethylsulfonium bromide by the general procedure of Furukawa et al.,[19] followed by chromatographic isolation and addition of hydrogen bromide, gave bromo compound **10** in 18% yield. The corresponding reaction of diol **19** with triphenylphosphine and *N*-bromosuccinimide according to the method of Ponpipom and Hanessian[20] similarly afforded **16** in 27% yield. However, in view of the extremely rapid cyclization of the free bases of **10** and **16** noted below, it is possible that the materials eluted from the chromatography columns were not these free bases. These "brominating agents" may have effected cyclization of the hydroxyethylamines to the corresponding aziridines **3** and **5** and treatment of these chromatographically purified intermediates with hydrogen bromide may have caused ring-opening as above. Triphenylphosphine dibromide[21] and triphenylphosphine/carbon tetrachloride[22] are known to effect such cyclizations.

Stability of Compounds and Biological Studies "in Vitro"

The first half-lives for the reaction of a series of 2-haloethylamino compounds in phosphate-buffered saline solution (PBS) were determined from the dependence of their rate of loss with time and are shown in Table I for the conditions indicated. With increasing methyl-substitution of the analogues, it was necessary to investigate their degradation at a lower temperature, e.g. 4 °C. Even at this temperature, the tetramethyl analogues **17** and **18** are consumed within the time required for preparation of solutions. The compounds become more stable when the solution pH $\ll$ pK_a of the amino group, the pK_a of which is estimated to be ~7.2 for compounds **9**–**11**. The major product formed in PBS is the corresponding aziridine, the product of ring-closure, as shown in Table I. For compound **10**, it was confirmed that the rate of release of bromide is the same as that for the loss of **10**, indicating that the rate-determining step involves ring closure. These findings are consistent with the degradation occurring predominantly via the free base. From the half-lives given in Table I, it is apparent that the rate of ring closure is influenced by (i) the leaving-group ability of the halide and (ii) the pattern of methyl substitution. The aqueous stability of these agents is in agreement with previous observations with simple substituted haloethylamino compounds.[23]

Table I. Half-Life of the Agents in PBS Aqueous Solution at pH 5.5–6.0 Together with the Major Product Formed at 23 °C

substance	half-life/min	major product	% methanol for HPLC
9	$\gg$300 (~280)[a]	**3**	10
10	30	**3**	10
11	80	**3**	10
13	120	**4**	13.75
14	30[b]	**4**	10
15	76[b]	**5**	25
16	<5[b]	**5**	25
17	$\ll$2[b]	**6**	10
18	$\ll$2[b]	**6**	10

[a]At pH 7.0. [b]At 4 °C.

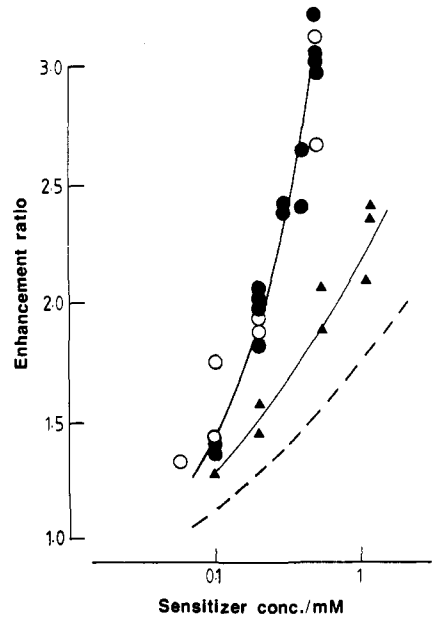

Figure 1. The dependence of hypoxic radiosensitization of V79 cells in vitro on the concentration of **3** (●), **9** (▲), and **10** (○). The dashed line shows the dependence of **1** for comparison.

From assessment of the dependence of hypoxic radiosensitization in vitro for **9** and **10** on concentration, as shown in Figure 1, it is apparent that the active agent in the biological response to **10** is ring-closed aziridine **3**. In contrast, the radiosensitizing efficiency of **9** is less than that of **10** and similar to that of **1**, a conventional 2-nitroimidazole radiosensitizer whose redox potential (E_7^1 = −389 mV)[24] is similar to that of **3**. These findings are consistent with the observation that, at pH 7, **10** but not **9** is cyclized to the aziridine **3** in PBS within a time considerably less than the preirradiation contact period used

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Table II. Concentration of Agent Required To Reduce Cell Survival to 1% following Incubation (3 h) with V79 Mammalian Cells under Aerobic (C_{air}) or Hypoxic (C_{N_2}) Conditions

substance	C_{air}/mM	C_{N_2}/mM	C_{air}/C_{N_2}
9	5	0.35	14.3
10	2.3	0.09	25.5
11	2.4	0.08	30.0
12	35	2.5	14.0
3	0.3	0.003	100.0
22	70	3.5	20.0

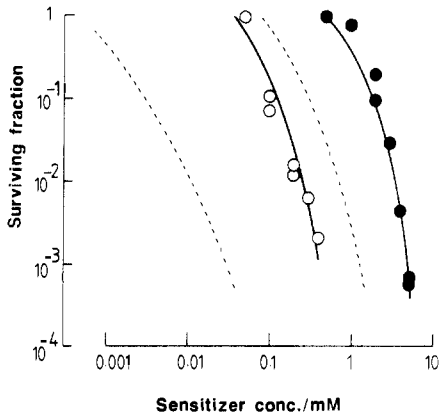


Figure 2. The in vitro cytotoxicity of **10** following exposure (3 h) under hypoxic (○) and aerobic (●) conditions. The dashed line represents the cytotoxicity exhibited by **3** under both gassing conditions.

in the radiosensitization studies in vitro. Further, the similarity of radiosensitizing efficiency of **9** and **1** is consistent with the determined stability of **9** under these conditions. The abilities of the other agents listed in Table I to act in vitro as radiosensitizers was not determined, owing to their rapid conversion in solution at pH 7 to the corresponding aziridine.

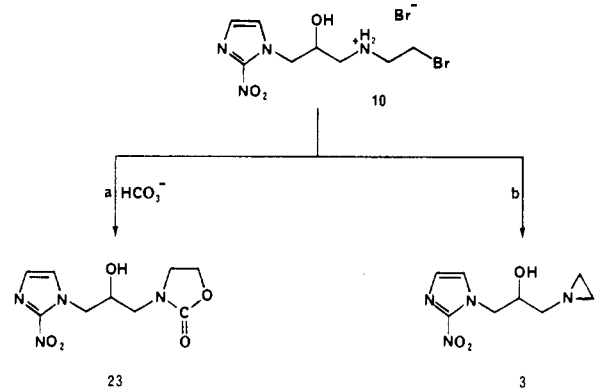
The cytotoxicity of these agents was determined in vitro in V79 cells held in cell culture growth medium at pH 7.4 instead of the PBS used for the radiosensitization studies. In contrast to the similarity of the radiosensitizing efficiency of **10** with that of the corresponding aziridine **3**, it is apparent that the haloethylamines tested (**9–12**) are far less cytotoxic, under both aerobic and hypoxic conditions, than the corresponding aziridine **3** as shown in Table II and in Figure 2. The cytotoxicity of the hydroxyethylamine **22** is shown in Table II for comparison. The other haloethylamines were not tested since their stabilities in solution at pH 7 will result in their conversion within a very short period compared with the contact period of 3 h. From these differences in biological response of **9–12** compared with that of **3**, it is inferred that the reactive haloethylamines undergo additional chemical transformations in cell-culture medium to yield not only **3** but also a compound which is less cytotoxic than **3**.

It was established, from testing several of the individual components of the medium, that **9–11** interact with hydrogen carbonate ion. With a concentration of hydrogen carbonate (24 mM) in PBS equivalent to that in cell-culture medium, it was found that an additional product is formed in competition with formation of the aziridine in the ratios shown in Table III. Increasing the concentration of hydrogen carbonate (24–107 mM) increases the importance of the pathway leading to the product as shown in Scheme IV. This product was identified, by coelution with authentic compound,[25] to be the corresponding oxazolidin-2-one **23**. Formation of oxazolidin-2-ones has previously been shown to occur upon interaction of hydrogen carbonate with simple 2-haloethylamines.[26] Scheme IV shows the various pathways with **10** as an example. For the methylated analogues **13–18**, the rates of cyclization to aziridines were so great as to preclude the formation of detectable quantities of the corresponding oxazolidinones. Thus, whereas methyl substitution enhances substantially the rate of formation of the 3-membered ring owing to the relief of steric crowding in the approach to the transition state, such steric relief is not available in the transition state for formation of the 5-membered cyclic analogue.

Table III. Ratio of Oxazolidinone to Aziridine Formed upon Treatment of the Agents with PBS Aqueous Solution Containing $NaHCO_3$ at pH 7.0–7.5 and 23 °C

substance	[oxazolidinone]/[aziridine]
9	>9.5:1
10	2.3:1
13	<0.05:1

Scheme IV. Competing Reactions of Prodrug **10** in Phosphate-Buffered Saline Solution Containing Hydrogen Carbonate Ions



Since the cytotoxicity experiments are performed at 37 °C, the effect of temperature (20–37 °C) upon the degradation of **10** in PBS containing hydrogen carbonate (24 mM) was determined. The formation of **23** is favored with increasing temperature (step a, Scheme IV). Assuming that **23** shows minimal cytotoxicity over the concentration range of **10** employed, the observed cytotoxicity of **10** in Table II is in reasonable agreement with that resulting from the yield of **3** formed under these biological conditions. Further, the formation of **23** may reduce the cytotoxicity of **3** under hypoxic conditions as has previously been shown for **3** coincubated with **1**.[27]

Biological Assessment of the Compounds in Mice

The maximum tolerated doses (MTD) determined for the compounds administered intraperitoneally (ip) to C3H/He mice are presented in Table IV. The dose-limiting factor for the agents is their toxicities (Table IV) with the exception of **14**, **16**, and **18**, which are limited by their solubilities. Since compounds **13–18** will be converted to the aziridines (see above) within the period required for preparation of the solution and prior to administration, the MTD values for those compounds limited by toxicity are consistent with those determined for the corresponding

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Table IV. MTD Determined for a Series of Haloethylamine Analogues in C3H Mice

substance	MTD/mmol kg^{-1}	limit to MTD
3	0.4	toxicity
4	3.0	toxicity
6	1.5[a]	toxicity
9	1.5	toxicity
10	1.0	toxicity
13	3.0	toxicity
14	1.0	solubility
15	0.8	toxicity
16	1.0	solubility
17	1.3	toxicity
18	0.7	solubility
21	3.9	toxicity

[a] Administered as a suspension in arachis oil.

aziridines **4–6** (Table IV). In contrast, the MTD values of **9** and **10**, which were administered as the haloethylamines, as verified with HPLC, are ~2.5 and 4 times greater than that of the corresponding aziridine **3** but less than the corresponding ethylamino compound **21**. The latter compound is stable to ring closure but has a pK_a value which is about 2 pK units greater than that of **9**, a property that has previously been shown to modify uptake of drugs of this type.[28] Such comparatively high values of MTD of compounds **9** and **10** are compatible with their partial conversion to a less toxic product as well as to **3** in vivo.

From the chemical studies, it is evident that only **9** and **10**, of the haloethylamines reported in Table IV, were sufficiently stable in aqueous solution for direct assessment of their ability to act as radiosensitizers of the KHT sarcoma. The properties in vivo of the other compounds are consistent with their conversion to the corresponding aziridine prior to administration.[29] The dependences of the surviving fraction of clonogenic tumor cells upon ip dose of **9** or **10** are shown in Figure 3 for administration 45 min prior to irradiation of the tumor with X-rays (10 Gy). This dose of radiation kills most of the oxic tumor cells and hence the responses reflect predominantly the modification of the survival of residual clonogenic, hypoxic cells. It is apparent that **10** is slightly less effective than **3** as a radiosensitizer but is about 1 order of magnitude more effective than **9** for an equal dose. From other studies, the effectiveness of **9** to act as a radiosensitizer has been shown to be similar to that of **21** for equivalent administered doses.[29] For compounds **3** and **10**, the cell survival falls to a constant value at the highest administered doses used. This value is equivalent to that corresponding to the maximum radiosensitizing effect of oxygen.[30] At the highest tolerated dose of **9**, the reduction of cell survival is about 1 order of magnitude less than that observed at the MTD of **3** and **10**.

The differences in the effectiveness of **9** and **10** as radiosensitizers could be explained if **23**, formed to a greater extent from **9** than from **10** in the presence of hydrogen carbonate (Table III), is a relatively ineffective radiosensitizer in vivo at these concentrations. The ability of **23** to act as a radiosensitizer of hypoxic tumor cells was investigated. Compound **23** was produced from **9** in PBS containing hydrogen carbonate; greater than 95% conversion to **23** was verified by HPLC prior to its administration. As shown in Figure 3, **23** produces little or no radiosensitization at the concentration used and is far less effective than **10** (or **3**).

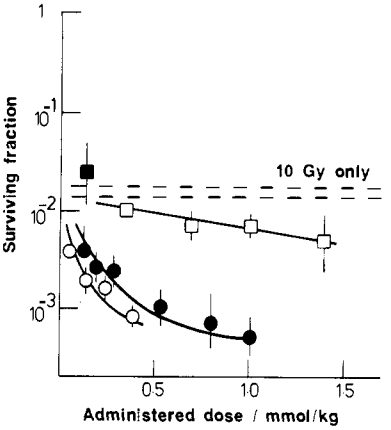

Figure 3. Comparison of the radiosensitization of the KHT sarcoma by various concentrations of **3** (○), **9** (□), **10** (●) and **23** (■) administered ip 45 min before a 10 Gy dose of X-rays.

Conclusions

Treatment of aziridines **3–6** with hydrogen halides under mild conditions provides an efficient route to 2-haloethylamine salts **9–18**. With the exception of fluoro compound **12**, the corresponding free bases revert to the parent aziridine at rates depending on the nature of the leaving halide and on substitution in the 2-haloethyl moiety. Compounds **9–11** and **13–18** can therefore be considered as prodrugs of **3–6**. As the conversions of **13–18** to the parent aziridines are extremely rapid in neutral or slightly acidic aqueous media, the soluble haloethylamine salts may be useful in the formulation of solutions of **4–6** for clinical use. However, the corresponding conversions of **9** and **10** to **3** are sufficiently slow at pH 5 to enable direct assessment of their biological activities in vivo. Studies with the murine KHT tumor demonstrate that the efficiency of **9** to act as a radiosensitizer is similar to that of **21**, reflecting its relative resistance to ring closure under the experimental conditions. In contrast, **10**, which converted in significant yield to **3** under the experimental conditions, shows an efficiency of radiosensitization comparable to that of **3** but is less toxic. Of the compounds tested for biological activity, **10**, acting as a prodrug of **3**, may show considerable promise as a radiosensitizer with an increased therapeutic ratio. The ability of this series of compounds to act as bioreductively activated cytotoxins in vivo is reported elsewhere.[29]

Experimental Section

Synthetic Chemistry. Unless otherwise stated, NMR spectra were obtained at 60 MHz with a JEOL PMX60SI spectrometer using $SiMe_4$ as internal standard. Other NMR spectra were obtained at 300 MHz (Bruker AC300) and at 250 MHz (Bruker AM250). A Philips PU9516 instrument furnished the IR spectra. Melting points are uncorrected. Elemental microanalyses were carried out by Butterworths Laboratories Ltd., Middlesex, UK, and Elemental Microanalysis Ltd., Okehampton, UK. *N,N*-Dimethylformamide (DMF) was distilled under reduced pressure from molecular sieves before use. Ether refers to diethyl ether. Solvents were evaporated under reduced pressure. α-[[(2-Hydroxyethyl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanol (**22**) was prepared as described previously.[31]

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α-[(*cis*-2,3-Dimethyl-1-aziridinyl)methyl]-2-nitro-1*H*-imidazole-1-ethanol (4). Oxirane **7** was treated with *cis*-2,3-dimethyl aziridine[13] in boiling ethanol by the general method of Adams et al.[8,11,12] to afford **4** (54%) as an off-white solid: mp 84–85 °C (lit.[12] mp 84–85 °C); NMR (300 MHz; $CDCl_3$) δ 1.10 (d, J = 5.5 Hz, 3 H, CH_3), 1.11 (d, J = 5.5 Hz, 3 H, CH_3), 1.53 (dq, J = 6.6 Hz, J = 5.5 Hz, 1 H, aziridine-H), 1.58 (dq, J = 6.6 Hz, J = 5.5 Hz, 1 H, aziridine-H), 1.8 (br, 1 H, OH), 2.32 (dd, J = 12.0 Hz, J = 4.1 Hz, 1 H), and 2.49 (dd, J = 12.0 Hz, J = 7.7 Hz, 1 H) (aziridine-CH_2), 4.06 (ddt, J = 4.1 Hz, J = 2.7 Hz, $J \approx 8$ Hz, 1 H, C*H*OH), 4.29 (dd, J = 13.8 Hz, J = 8.0 Hz, 1 H) and 4.67 (dd, J = 13.8 Hz, J = 2.7 Hz, 1 H) (imidazole-CH_2), and 7.12 (d, J = 1.0 Hz, 1 H) and 7.24 (d, J = 1.0 Hz, 1 H) (imidazole 4,5-H).

2-Nitro-α-[(2,2,3,3-tetramethyl-1-aziridinyl)methyl]-1*H*-imidazole-1-ethanol (6). 2,2,3,3-Tetramethylaziridine[14] [bp 102–103 °C (lit.[14] bp 104–104.5 °C); NMR ($CDCl_3$) δ −1.28 (s, 1 H, NH) and 1.25 (s, 12 H, 4 × CH_3)] was treated with **7**, by the method previously described,[12] to give **6** (64%) as a pale yellow solid: mp 131–132 °C (lit.[12] mp 131–132 °C); NMR [$CDCl_3$ + $(CD_3)_2SO$; 1:1] δ 1.05 (s, 12 H, 4 × CH_3), 2.30 (d, J = 5 Hz, 2 H, aziridine-CH_2), 3.8 (br, 1 H, OH), 4.1–5.1 (m, 3 H, imidazole-CH_2CH), and 7.05 (br s, 1 H) and 7.50 (br s, 1 H) (imidazole 4,5-H).

1-(Oxiranylmethyl)-2-nitroimidazole (7). Alkylation of 2-nitroimidazole with 1-chloro-2,3-epoxypropane, followed by base-catalyzed ring closure, generally according to the method of Beaman et al.,[32] gave **7** (96%) as white needles: mp 55.5–56 °C (lit.[32] mp 53.5–55 °C); NMR ($CDCl_3$) δ 2.55 (dd, J = 5 Hz, J = 3 Hz, 1 H) and 2.90 (t, J = 5 Hz, 1 H) (CH_2O), 3.45 (ddt, J = 6 Hz, J = 5 Hz, J = 3 Hz, 1 H, CHO), 4.30 (dd, J = 14 Hz, J = 6 Hz, 1 H) and 5.08 (dd, J = 14 Hz, J = 3 Hz, 1 H) (imidazole-CH_2), and 7.10 (s, 1 H) and 7.25 (s, 1 H) (imidazole 4,5-H).

1-[(2,4-Dinitrophenyl)thio]-2,2,3,3-tetramethylaziridine (8). $Pb(OAc)_4$ (85%; 1.0 g, ca. 2 mmol) was added during 10 min to 2,4-dinitrobenzenesulfenamide[16] [mp 118–119 °C (lit.[13] mp 119–120 °C)] (430 mg, 2 mmol) and 2,3-dimethylbut-2-ene (840 mg, 10 mmol) in CH_2Cl_2 (3 mL). After a further 20 min, the solution was diluted with CH_2Cl_2 (10 mL) and was washed with saturated aqueous $NaHCO_3$. The organic phase was dried (Na_2SO_4) and filtered and the solvent was evaporated to afford **8** (550 mg, 93%) as an orange solid: mp 148–149 °C; IR (Nujol) 1590, 1510, and 1300 cm^{-1}; NMR ($CDCl_3$) δ 1.40 (s, 12 H, 4 × CH_3), 8.35 (dd, J = 9 Hz, J = 2 Hz, 1 H, 5-H), 8.45 (dd, J = 9 Hz, J = 0.5 Hz, 1 H, 6-H), and 9.06 (dd, J = 2 Hz, J = 0.5 Hz, 1 H, 3-H). Anal. ($C_{12}H_{15}N_3O_4S$) C, H, N.

α-[[(2-Chloroethyl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanol Hydrochloride (9). Method A. A solution of α-(aziridinylmethyl)-2-nitro-1*H*-imidazole-1-ethanol[8,11,12] (**3**; 2.12 g, 10 mmol) in acetone (20 mL) at 40 °C was cooled rapidly to 5–10 °C and was treated with a saturated solution of HCl in acetone (20 mL). After 2 min, decolorizing charcoal (300 mg) was added and the mixture was filtered while still warm from the exothermic reaction. On cooling of the filtrate, there was obtained **9** (2.66 g, 93%) as very pale yellow crystals: mp 153–154 °C dec. An analytical sample was recrystallized from aqueous acetone to give pale yellow prisms: mp 153–154 °C dec; IR (KBr) 3450 (br), 3345 (br), 3146, 3090, 1593, 1542, 1509, and 1495 cm^{-1}; NMR [$(CD_3)_2SO$] δ 3.1 (m, 2 H, $CHCH_2{}^+NH_2$), 3.4 [m (becomes t, J = 7 Hz, on decoupling at δ 9.35), 2 H, $^+NH_2CH_2CH_2Cl$], 3.95 (t, J = 7 Hz, 2 H, CH_2Cl), 4.2–4.7 (m, 4 H, imidazole-CH_2CHOH), 7.15 (d, J = 1 Hz, 1 H) and 7.65 (d, J = 1 Hz, 1 H) (imidazole 4,5-H), and 9.35 (br, 1 H, $^+NH_2$). Anal. ($C_8H_{14}Cl_2N_4O_3$) C, H, Cl, N.

α-[[(2-Chloroethyl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanol Hydrochloride (9). Method B. Aqueous HCl (10.5 M; 2 mL, 21 mmol) was added in one portion to a fine suspension of **3** (2.12 g, 10 mmol) in acetone (20 mL) at 0–5 °C. The solution was stirred for 5 min then treated with decolorizing charcoal (300 mg) and filtered. The filtrate was chilled to afford **9** (1.88 g, 66%) as pale yellow microcrystals: mp 152.5–153.5 °C dec, identical (mixed melting point, IR, analysis) with the material prepared by method A above.

α-[[(2-Bromoethyl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanol Hydrobromide (10). Treatment of **3** with anhydrous HBr, according to method A for the preparation of **9** above, gave **10** (96%) as very pale yellow crystals: mp 150.5–151.5 °C dec. Alternatively, treatment of **3** with 2 equiv of aqueous HBr (5.9 M), according to method B for the preparation of **9** above, except that the charcoal was omitted and the recrystallization solvent was aqueous acetone, gave **10** (74%) as pale yellow prisms: mp 150–151 °C dec; IR (KBr) 3450 (br), 3345 (br), 3160, 3140, 3087, 1590, 1539, 1507, and 1493 cm^{-1}; NMR [250 MHz; $(CD_3)_2SO$] 2.94 [m (+D_2O becomes dd, J = 12.6 Hz, J = 9.8 Hz), 1 H] and 3.15 [m (+D_2O becomes dd, J = 12.6 Hz, J = 2.7 Hz), 1 H] ($CHCH_2{}^+NH_2$), 3.46 [m (+D_2O becomes t, J = 6.5 Hz, 2 H, $^+NH_2CH_2CH_2Br$], 3.68 (t, J = 6.5 Hz, 2 H, CH_2Br), 4.21 [m, (+D_2O becomes dddd, J = 9.8 Hz, J = 8.0 Hz, J = 3.9 Hz, J = 2.7 Hz), 1 H, C*H*OH], 4.31 (dd, J = 13.4 Hz, J = 8.0 Hz, 1 H) and 4.53 (dd, J = 13.4 Hz, J = 3.9 Hz, 1 H) (imidazole-CH_2), 5.95 (br d, $J \approx 5$ Hz, 1 H, OH), 7.22 (s, 1 H) and 7.60 (s, 1 H) (imidazole 4,5-H), and 8.72 (br, 2 H, NH_2). Anal. ($C_8H_{14}Br_2N_4O_3$). From some preparations of **10** according to this method, a crystalline monohydrate (mp 161–162 °C) was obtained.

α-[[(2-Bromoethyl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanol Hydrobromide (10). Method C. In a modification of the general procedure of Furukawa et al.,[19] **22**[31] (2.30 g, 10 mmol) and dimethylbromosulfonium bromide[19] (2.22 g, 10 mmol) were stirred at 50 °C for 12 h in dry DMF (20 mL) before the solvent was evaporated. The residue, in ethanol (10 mL), was chromatographed (silica gel; $CHCl_3$, MeOH; 9:1). Treatment of the appropriate fraction of eluate with ethereal HBr gave **10** (680 mg, 18%) as a yellow solid: mp 148.5–150.5 °C dec, identical (mixed melting point, IR, analysis) with the material described above.

α-[[(2-Iodoethyl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanol Hydriodide (11). Treatment of **3** with 2 equiv of aqueous HI, according to method B for the preparation of **9** above, except that the recrystallization solvent was H_2O, gave **11** (51%) as yellow crystals: mp 172–173 °C dec; IR (KBr) 3460 (br), 3310 (br), 3135, 3106, 3092, 1539, 1509, and 1492 cm^{-1}; NMR [$(CD_3)_2SO$] δ 3.12 (m, 2 H, $CHCH_2{}^+NH_2$), 3.36 (s, 4 H), NCH_2CH_2I), 4.1–4.6 (m, 3 H, imidazole-CH_2CH), 5.8 (br, 1 H, OH), 7.16 (d, J = 1 Hz, 1 H) and 7.59 (d, J = 1 Hz, 1 H) (imidazole 4,5-H), and 8.6 (br, 2 H, $^+NH_2$). Anal. ($C_8H_{14}I_2N_4O_3$) H, N; C: calcd, 20.53; found 21.02; I: calcd, 54.23; found 53.76.

α-[[(2-Fluoroethyl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanol Hydrochloride (12). According to method B for the preparation of **9** above, **3** was treated with 2 equiv of aqueous HF (24 M; **CAUTION**). The reaction mixture was then stirred with ethanolic NaOH (5% w/w; 1.0 mol equiv) and decolorizing charcoal for 15 min. Treatment of the filtrate with a small excess of ethereal HCl gave **12** (41%) as a pale yellow solid: mp 177–179 °C dec, identical with the material described below.

α-[[(2-Fluoroethyl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanol Hydrochloride (12). Method D. Oxirane **7** (4.0 g, 23.7 mmol), (2-fluoroethyl)ammonium chloride (5.0 g, 50.2 mmol), and NaOH (2.0 g, 50 mmol) were stirred at 15 °C for 30 min in ethanol (60 mL) and heated to reflux for 3 h. The mixture was then treated with decolorizing charcoal (500 mg) and filtered. The evaporation residue, in ethanol (20 mL), was treated with a small excess of ethereal HCl to furnish, after recrystallization from aqueous ethanol, **12** (4.38 g, 69%) as pale yellow crystals: mp 178–179 °C dec; IR (KBr) 3450 (br), 3280 (br), 3140, 3090, 1580, 1531, and 1487 cm^{-1}; NMR [250 MHz; $(CD_3)_2SO$] δ 2.92 (m, (+D_2O becomes dd, J = 12.5 Hz, J = 9.9 Hz), 1 H] and 3.13 [m (+D_2O becomes dd, J = 12.5 Hz, J = 2 Hz), 1 H] ($CHCH_2{}^+NH_2$), 3.33 [br d, J = 27.9 Hz (+D_2O becomes dt, J = 27.9 Hz, J = 6.5 Hz), 2 H, $^+NH_2CH_2CH_2F$], 4.25 (m, 1 H, C*H*OH), 4.39 (dd, J = 13.6 Hz, J = 7.8 Hz, 1 H) and 4.58 (dd, J = 13.4 Hz, J = 4.0 Hz, 1 H) (imidazole-CH_2), 4.76 (dt, J = 47.1 Hz, J = 4.4 Hz, 2 H, CH_2F), 6.04 (br, 1 H, OH), 7.20 (s, 1 H) and 7.66 (s, 1 H) (imidazole 4,5-H), and 9.14 (br, 1 H) and 9.28 (br 1 H) NH_2. Anal. ($C_8H_{14}ClFN_4O_3$) C, H, Cl, F, N.

α-[[(*threo*-2-Chloro-1-methylpropyl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanol Hydrochloride (13). Compound **4** was treated with aqueous HCl generally according to method B for the preparation of **9** above, except that the charcoal was omitted and the recrystallization solvent was ethanol, to give **13** (88%) as a very pale yellow solid: mp 138.5–139 °C dec; NMR (300 MHz; $(CD_3)_2SO$) δ 1.30 (d, J = 6.6 Hz, 1.5 H, $NCHCH_3$ of one diastereoisomer), 1.31 (d, J = 6.7 Hz, 1.5 H, $NCHCH_3$ of other

(32) Beaman, A. G.; Tautz, W.; Duschinsky, R. *Antimicrob. Agents Chemother.* **1986**, 520.

diastereoisomer), 1.52 (d, J = 6.7 Hz, 3 H, ClCHCH_3), 2.96 (m, 1 H) and 3.16 (m, 1 H) and 3.52 (m, 0.5 H) and 3.52 (m, 0.5 H) (C$H_2$$^+$$NH_2CH$), 4.27 (m, 1 H) and 4.39 (m, 1 H) and 4.57 (m, 3 H) (imidazole-CH_2CHOH and ClCH), 7.19 (br s, 1 H) and 7.65 (br s, 1 H) (imidazole 4,5-H), and 8.70 (br, 0.5 H) and 8.84 (br, 0.5 H) and 9.02 (br, 0.5 H) and 9.38 (br, 0.5 H) ($^+$$NH_2$). Anal. ($C_{10}H_{18}Cl_2N_4O_3$) C, H, Cl, N.

α-[[(*threo*-2-Bromo-1-methylpropyl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanol Hydrobromide (14). Aziridine **4** was treated with aqueous HBr generally according to method B for the preparation of **9** above, except that the charcoal was omitted, to give **14** (83%) as a pale yellow powder: mp 165–166 °C dec; NMR [D_2O + CF_3CO_2D (8:1)] δ 1.50 (d, J = 7 Hz, (becomes s on decoupling at δ 3.65), 3 H, NCHCH_3], 1.85 [d, J = 6 Hz, (becomes s on decoupling at δ 4.4), 3 H, BrCHCH_3], 3.5 (m, 2 H, C$H_2$$^+$$ND_2$), 3.65 (m, 1 H, NC$HCH_3$), 4.3–4.8 (m, 4 H, imidazole-CH_2CH and BrCH), and 7.30 (br s, 1 H) and 7.55 (br s, 1 H) (imidazole 4,5-H). Anal. ($C_{10}H_{18}Br_2N_4O_3$) C, H, N.

α-[[(2-Chloro-1,1-dimethylethyl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanol Hydrochloride (15). α-[(2,2-Dimethyl-1-aziridine)methyl]-2-nitro-1*H*-imidazole-1-ethanol[8,11,12] (**5**) was treated with aqueous HCl, generally according to method B for the preparation of **9** above, except that the reaction was carried out at 40 °C and the recrystallization solvent was aqueous acetone containing a trace of HCl, to yield **15** (78%) as colorless prisms: mp 196.5–198 °C dec; NMR [D_2O + CF_3CO_2D (8:1)] δ 1.60 (s, 6 H, 2 × CH_3), 3.35 (dd, J = 13 Hz, J = 2 Hz, 1 H) and 3.42 (dd, J = 13 Hz, J = 4 Hz, 1 H) ($CH_2$$^+$$ND_2$), 3.92 (s, 2 H, CH_2Cl), 4.4–5.0 (m, 3 H, imidazole-CH_2CH), and 7.30 (br s, 1 H) and 7.53 (br s, 1 H) (imidazole 4,5-H). Anal. ($C_{10}H_{18}Cl_2N_4O_3$) C, H, Cl, N.

α-[[(2-Bromo-1,1-dimethylethyl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanol Hydrobromide (16). Aziridine **5**[8,11,12] was treated with aqueous HBr generally according to method B for the preparation of **9** above, except that the reaction was carried out at 40 °C, to yield **16** (82%) as very pale yellow prisms: mp 186–197 °C dec; NMR [$(CD_3)_2SO$] δ 1.42 (s, 6 H, 2 × CH_3), 3.1 (m, 2 H, C$H_2$$^+$$NH_2$), 3.80 (s, 2 H, CH_2Br), 4.1–4.6 (m, 3 H, imidazole-CH_2CH), 5.5 (br, 1 H, OH), 7.12 (d, J = 1 Hz, 1 H) and 7.53 (d, J = 1 Hz, 1 H) (imidazole 4,5-H), and 8.6 (br, 2 H, $^+$$NH_2$). Anal. ($C_{10}H_{18}Br_2N_4O_3$) C, H, N; Br: calcd, 39.75; found 39.23.

α-[[(2-Bromo-1,1-dimethylethyl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanol Hydrobromide (16). Method E. In a modification of the method of Ponpipom and Hanessian,[20] **19** (2.58 g, 10 mmol), *N*-bromosuccinimide (NBS; 2.67 g, 15 mmol), and triphenylphosphine (3.93 g, 15 mmol) were stirred in dry DMF (50 mL) at 50 °C for 1.5 h. The solvent was evaporated and the residue was extracted with ethanol (20 mL). This solution was filtered. Column chromatography (silica gel; $CHCl_3$, MeOH; 9:1) and treatment of the appropriate fraction of eluate with a small excess of ethereal HBr gave **16** (1.09 g, 27%) as a yellow solid: mp 181–184 °C dec. Anal. ($C_{10}H_{18}Br_2N_4O_3$) C, H, N; Br: calcd 39.75; found 38.91. TLC analysis indicated the presence of a very slight trace of triphenylphosphine oxide.

α-[[(2-Chloro-1,2,2-trimethylpropyl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanol Hydrochloride (17). Aziridine **6** was treated with aqueous HCl generally according to method B for the preparation of **9** above, except that the charcoal was omitted and the reaction was carried out at 20 °C, to give **17** (88%) as colorless microcrystals: mp 183–184 °C dec; NMR [250 MHz; $(CD_3)_2SO$] δ 1.42 (s, 3 H) and 1.44 (s, 3 H) (NC$(CH_3)_2$), 1.73 (s, 3 H) and 1.74 (s, 3 H) [C$(CH_3)_2$Cl], 3.11 [m, ($^+$$D_2O$ becomes dd, J = 12.5 Hz, J = 9.9 Hz), 1 H] and 3.33 [m, ($^+$$D_2O$ becomes brd, $J \approx$ 12 Hz), 1 H] ($^+$$NH_2CH_2$), 4.32 [m, ($^+$$D_2O$ becomes ddd, J = 9.9 Hz, J = 8.1 Hz, J = 3.7 Hz), 1 H CHOH], 4.42 (dd, J = 13.3 Hz, J = 8.1 Hz, 1 H) and 4.61 (dd, J = 13.3 Hz, J = 3.7 Hz, 1 H) (imidazole-CH_2), 6.25 (br, 1 H, OH), 7.21 (d, J = 0.8 Hz, 1 H) and 7.66 (d, J = 0.8 Hz, 1 H) (imidazole 4,5-H), and 7.97 (br quintet, $J \approx$ 7 Hz, 1 H) and 9.10 (br, 1 H) ($^+$$NH_2$). Anal. ($C_{12}H_{22}Cl_2N_4O_3$) C, H, N.

α-[[(2-Bromo-1,2,2-trimethylpropyl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanol Hydrobromide (18). Aziridine **6** (810 mg, 3 mmol), in acetone (10 mL), was treated with aqueous HBr (6 M; 1.2 mL, 7.2 mmol) at reflux for 10 min before being cooled. Recrystallization of the evaporation residue from aqueous acetone afforded **18** (950 mg, 73%) as pale yellow crystals: mp 163–164 °C dec; NMR ($(CD_3)_2SO$) δ 1.45 (s, 6 H, 2 × CH_3), 1.50 (s, 6 H, 2 × CH_3), 3.2 (m, 2 H, $^+$$NH_2CH_2$), 4.2–4.7 (m, 3 H, imidazole-CH_2CHOH), 5.2 (br, 1 H, OH), 7.15 (br s, 1 H) and 7.65 (br s, 1 H) (imidazole 4,5-H), and 8.1 (br, 1 H) and 8.7 (br, 1 H) ($^+$$NH_2$). Anal. ($C_{12}H_{22}Br_2N_4O_3$) C, H, Br, N.

α-[[(2-Hydroxy-1,1-dimethylethyl)amino]methyl]-2-nitro-1*H*-imidazole-1-ethanol (19). Oxirane **7** (4.0 g, 23.7 mmol) and 2-amino-2-methylpropanol (10.0 g, 112 mmol) were boiled under reflux in ethanol (100 mL) for 45 min. The solvent was evaporated and the residue was extracted with hot ether (75 mL). The extract was cooled to 0 °C and the precipitated solid was recrystallized from ethanol to furnish **19** (4.94 g, 81%) as almost colorless prisms: mp 114.5–115.5 °C; NMR [D_2O + CF_3CO_2D (8:1)] δ 1.55 (s, 6 H, 2 × CH_3), 3.2 (dd, J = 13 Hz, J = 2 Hz, 1 H) and 3.55 (dd, J = 13 Hz, J = 4 Hz, 1 H) ($^+$$ND_2CH_2$), 3.85 (s, 2 H, CH_2O), 4.3–5.0 (m, 3 H, imidazole-CH_2CH), and 7.40 (d, J = 1 Hz, 1 H) and 7.65 (d, J = 1 Hz, 1 H), (imidazole 4,5-H). Anal. ($C_{10}H_{18}N_4O_4$) C, H, N.

α-[(Ethylamino)methyl]-2-nitro-1*H*-imidazole-1-ethanol Hydrochloride (21). Oxirane **7** (507 mg, 3 mmol) was boiled under reflux with 70% aqueous $EtNH_2$ (2 mL) in ethanol (8 mL) for 1 h. The solvents and excess reagent were evaporated. Dry ethanol (20 mL) was added and was subsequently evaporated. The residue, in dry ethanol (4 mL), was treated with ethereal HCl (1 M, 4.5 mL, 4.5 mmol). The precipitate recrystallized from ethanol to furnish **21** (650 mg, 87%) as pale yellow crystals: mp 196–197 °C; IR (Nujol) 3300 (br), 1540, 1505, and 1495 cm^{-1}; NMR ($(CD_3)_2SO$) δ 1.20 [t, J = 7 Hz (becomes s on decoupling at δ 3.00), 3 H, CH_3], 3.7–3.1 (m, 4 H, C$H_2$$^+$$NH_2CH_2$), 4.2–4.6 (m, 3 H, imidazole-CH_2CH), 5.9 (br, 1 H, OH), 7.10 (d, J = 1 Hz, 1 H) and 7.58 (d, J = 1 Hz, 1 H) (imidazole 4,5-H), and 8.8 (br, 2 H, $^+$$NH_2$). Anal. ($C_8H_{15}ClN_4O_3$) C, H, Cl, N.

HPLC Analysis for Stability of Drugs. The stability of compounds **9–11** and **13–18** was determined at 4 °C and at 23 °C by incubation of the agents (0.2–0.5 mM) in aqueous solution containing phosphate-buffered saline (PBS, Oxoid) and in the presence or absence of sodium hydrogen carbonate (24–107 mM) at pH 5–8. In all cases, the pH of the solutions was adjusted immediately following their preparation. At given times, aliquots were assayed for degradation of the compounds with HPLC. A Waters HPLC system with a UV–vis detector set at 313 nm (Model 440) was used to perform isocratic elutions with a CN column (Waters Resolve CN, 10 μm or a Nova-Pak CNHP, 4 μm). The mobile phase (flow rate 2 mL min^{-1}) contained 10–25% methanol in aqueous KH_2PO_4 (10 mM) at pH 3.0 (see Table I). The rate of release of Br^- from **10** in PBS solution was determined with a bromide ion selective electrode (Radiometer F1022Br) in conjunction with a pH meter (Radiometer PHM64).

Biological Studies. In Vitro. Chinese hamster V79-379A cells, maintained in suspension culture, were plated onto 6 cm diameter glass Petri dishes in MEM (modified essential medium) containing 10% fetal calf serum and allowed to attach for 2 h. For radiosensitization studies, the medium was then replaced with 2 mL of a freshly prepared solution of the agent in PBS at pH 7.4, and the Petri dishes were placed into gas-tight dural vessels. Hypoxia was induced by purging the vessels with N_2 (<10 ppm O_2) for 1 h prior to irradiation at 22 °C with ^{60}Co γ-rays at a dose rate of 8 Gy min^{-1}. The solution of drug was removed after irradiation and replaced with fresh medium. The cells were incubated at 37 °C for 7 days before assaying for formation of colonies. Sensitizer enhancement ratios were determined from the ratio of slopes of survival curves for hypoxic conditions in the presence and absence of the agent. For cytotoxicity assays, cells were plated into 100-mL glass bottles (1.5 × 10^6 cells/bottle), allowed to grow to confluency over 48 h and exposed to various concentrations of the agents for 3 h in growth medium under either hypoxic or aerobic conditions at 37 °C. Cultures were then trypsinized, diluted, and plated to assay formation of colonies.

Biological Studies. In Vivo. C3H/He mice were bred at the Radiobiology Unit to provide U.K. specified pathogen free, category IV, 8–12 week old female mice for experiments. The KHT sarcoma[33] was provided by Dr. P. Twentyman, MRC

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Cambridge, U.K., in 1983 and maintained by intramuscular inoculation of a tumor brei for up to 12 consecutive passages and then reestablished from frozen stocks. For experimentation, 2-4 $\times 10^5$ viable tumor cells were injected subcutaneously in the middorsal pelvic region of the mice. Treatments were initiated 10-14 days later when tumors reached a maximum diameter of 6-8 mm.

Prior to injection, compounds were usually dissolved at 20 °C in PBS at pH 7.0 by means of sonication for up to 5 min. Compounds **9** and **10** were dissolved in phosphate buffer at pH 5 to minimize cyclization to **3**. All solutions were administered within 10 min of preparation. Solutions were administered by the intraperitoneal route in a volume of 0.5 mL per 25 g of mouse body weight, with the exception of **14**, **16**, and **18**, which were administered in a volume of 1 mL per 25 g owing to their dose-limiting solubility. Escalating doses of each of the test compounds were administered to groups of two or three tumor-bearing mice to establish the maximum tolerated dose (MTD). The MTD is defined as the highest single dose which does not produce severe or persistent clinical signs or death of the animals within 24 h.

The responses of KHT sarcomas to therapy administered in vivo were measured by means of a soft agar clonogenic assay in vitro,[34] as described recently.[35] Tumors were excised 18-24 h after treatment and each was assayed individually. Surviving fractions were calculated as the ratios of the numbers of colonies scored to the number of viable cells plated, relative to the plating efficiency of a control tumor processed at the same time. The mean plating efficiency of cells from 66 untreated tumors was 49.5 ± 1.8% (mean ± SE) and the yield of cells ranged between 8.9×10^6 and 7.6×10^7 cells g^{-1} of tumor tissue. A Pantac X-ray set was used to produce 250 kV X-rays (15 mA) at a dose rate of 3.8 Gy min^{-1} with a half-value layer (HVL) equivalent to 1.33 mm Cu. Radiation doses were monitored with an air chamber corrected for ambient temperature and pressure. Unanesthetized mice were restrained in polyvinyl jigs with Pb shielding and a cutaway section to allow local irradiation of tumor by the unilateral beam.[36] A dose of 10 Gy of X-rays was used to test the efficiency of each compound as a hypoxic cell radiosensitizer. Each experiment included mice exposed to a 10 Gy dose of X-rays without drug and mice treated with drug without radiation. There is a minimum of three tumors in each group; most points were determined from the geometric mean of surviving fractions from at least four to six tumors from two separate experiments. Initially, the optimum time of administration before irradiation for maximum radiosensitization was determined for each of the compounds by administering the MTD. Subsequently a range of single doses of the compounds were administered at the optimum time (45-60 min in each case) before exposure to X-rays to obtain a drug dose/response curve for radiosensitization. Where appropriate, direct comparisons of radiosensitization by equimolar doses of chemically related compounds were made in a single experiment.

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Antimalarial Activity of New Water-Soluble Dihydroartemisinin Derivatives. 3.[1,2] Aromatic Amine Analogues

Ai J. Lin,*,† Liang-quan Li,† Daniel L. Klayman,† Clifford F. George,‡ and Judith L. Flippen-Anderson‡

Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100, and the Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D.C. 20375-5000. *Received February 12, 1990*

A series of artemisinin (**1**) derivatives containing bromo and heterocyclic or aromatic amine functions was prepared in the search for analogues with good water solubility and high antimalarial activity. Treatment of dihydroartemisinin (**2a**) with boron trifluoride etherate at room temperature gave the key intermediate, 9,10-dehydrodihydroartemisinin (**3**), which, on reaction with bromine, gave the dibromide **4**. The latter was condensed with amines in anhydrous CH_2Cl_2 at <-10 °C to give the desired products in 25-55% yield. The new derivatives, tested in vitro against *Plasmodium falciparum*, were found to be more effective against W-2 than D-6 clones and were not cross-resistant with existing antimalarials. Compound **6b**, 3-fluoroaniline derivative, was the most active of the series, with the $IC_{50} \leq 0.16$ ng/mL, making it several fold more potent than **1**. However, no significant in vivo antimalarial activity against *Plasmodium berghei* was observed in any of the new compounds tested.

Artemisinin (qinghaosu, arteannuin, **1**), an antimalarial agent isolated from the plant *Artemisia annua*, is an endoperoxide-containing sesquiterpene lactone.[3-7] The unusual chemical structure of artemisinin coupled with its low toxicity and proven antimalarial efficacy have attracted attention from both chemists and parasitologists since its discovery in 1972. The practical use of artemisinin as an antimalarial agent, however, is impaired by (a) its insolubility in both water and oil,[8] (b) its poor efficacy by oral administration,[9] and (c) the rate of recrudescence in treated patients.[9]

The lactol form of **1**, dihydroartemisinin (**2a**), prepared by the sodium borohydride reduction of the parent com-

* Author to whom correspondence should be addressed.
† Walter Reed Army Institute of Research.
‡ Naval Research Laboratory.

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