cooled, and filtered. The precipitate, which appeared to be a mixture of two isomeric components in a 2:1 ratio by ^{1}H NMR, was extracted in a Soxhlet apparatus with boiling MeOH, and the extracts were concentrated and cooled to give successively two crops of 0.39 and 0.29 g. The first crop was recrystallized from MeOH to yield 0.32 g of 2-[(5-amino-6,7-dihydro-7-oxo-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)methoxy]ethyl benzoate (**26**), mp 236–239 °C. Anal. ($C_{14}H_{14}N_6O_4$) C, H, N. This was identical with the second unrecrystallized crop by ^{1}H NMR except the former still contained 8% of the other isomeric component. ^{13}C NMR (Me_2SO-d_6) δ 165.46 (C=O), 155.81 (C^5), 155.54 (C^7), 151.8 (C^9), 133.24 (phenyl C^4), 129.39 (phenyl C^1), 128.94 and 128.56 (phenyl C-2, -3, -5 and -6), 124.16 (C^8), 74.05 (NCH_2O), 67.02 (CCH_2O), 63.38 (CH_2OC=O). The MeOH insolubles from the Soxhlet extraction step were recrystallized from 20 mL of hot Me_2SO to yield 0.457 g of a white solid, mp 263–267 °C. The ^{13}C NMR spectrum identified this as the N-1-substituted isomer 2-[(5-amino-6,7-dihydro-7-oxo-1*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-1-yl)methoxy]ethyl benzoate (**27**) and was identical with the minor (8%) component in the isomer described above. ^{13}C NMR (Me_2SO-d_6) δ 165.5 (C=O), 161.29 (C^9), 153.89 (C^5), 153.48 (C^7), 133.26 (phenyl C^4), 129.39 (phenyl C^1), 128.98 and 128.62 (phenyl C-2, -3, -5, and -6) 113.3 (C^8), 77.7 (NCH_2O), 66.96 (OCH_2C), 63.38 (CH_2OC=O). Anal. ($C_{14}H_{14}N_6O_4$) C, H, N. A second crop of **27** (0.245 g) was obtained from the mother liquor by dilution with EtOH. The overall yield of the two isomers was 32%.

A solution of 0.235 g (0.71 mmol) of **26** in 6 mL of 40% aqueous $MeNH_2$ was heated on a steam bath for 30 min. The solution was evaporated, and the residue was partitioned between Et_2O and H_2O. The aqueous layer was evaporated in vacuo and recrystallized from EtOH to give the title compound **28**, 0.128 g (80%), mp 239–241 °C. The ^{1}H NMR showed only a single component (Me_2SO-d_6) δ 7.00 (br s, 2 H, NH_2), 5.64 (s, 2 H, NCH_2O), 4.65 (br s, 1 H, OH), 3.53 (m, 4 H, OCH_2CH_2O). Anal. ($C_7H_{10}N_6O_3$) C, H, N.

Acknowledgment. We recognize the contributions of Dr. S. Hurlbert, R. Crouch, and A. Ragouzeos in obtaining and discussing the NMR spectra, Dr. D. Brent and R. Johnson for the mass spectral data, C. Jenkins and M. L. Russ for some of the elemental analyses, Dr. M. Tisdale and J. Selway for the antiviral screening (other than HSV), N. Cohn for tissue culture testing, and M. Bushby for antibacterial evaluation. We also thank A. Melton and R. Kimmins for excellent technical assistance and T. Cozart for typing the manuscript.

Registry No. **2**, 59278-00-1; **3**, 58305-05-8; **4**, 94-33-7; **6a**, 77856-29-2; **6a**·HCl, 96446-06-9; **6b**, 77856-27-0; **7**, 360-97-4; **8b**, 96445-86-2; **10**, 3641-10-9; 11, 96445-87-3; **12**, 96445-88-4; **13**, 96445-89-5; **14**, 96445-90-8; **15**, 96445-91-9; **16**, 108-53-2; **17**, 96445-92-0; **18**, 96445-93-1; **20**, 91897-92-6; **21**, 2537-04-4; **22** (isomer 1), 96445-94-2; **22** (isomer 2), 96445-95-3; **23**, 96445-96-4; **24**, 96445-97-5; **25**, 134-58-7; **26**, 96445-98-6; **27**, 96445-99-7; **28**, 81475-46-9; **29**, 7355-55-7; **30**, 84955-38-4; **31**, 62160-25-2; **32**, 96446-00-3; **33**, 96446-01-4; **34**, 96446-02-5; **35**, 60064-29-1; **36**, 96446-03-6; **37**, 96446-04-7; **38**, 96446-05-8; thymidine kinase, 9002-06-6.

Potential Radiosensitizing Agents. 7. 4(5)-Iodo-5(4)-nitroimidazole Derivatives[1]

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A series of 4(5)-iodo-5(4)-nitro-1-substituted-imidazoles has been synthesized and tested for their ability to selectively radiosensitize hypoxic Chinese hamster cells (V-79) to the lethal effect of radiation. The reaction of 4(5)-iodo-5-(4)-nitroimidazole with 1,2-epoxy-3-methoxypropane and ethyl α-chloroacetate produced two isomeric products in each case, which were identified by their NMR spectra. The ethyl esters were further reacted with 3-picolylamine to produce corresponding amides. The 5-iodo-4-nitroimidazole-1-*N*-(3-picolyl)acetamide on further reaction with *m*-chloroperbenzoic acid produced the corresponding *N*-oxide. These compounds were generally more toxic to V-79 cells than the 2-nitroimidazole derivatives and were found to be more effective radiosensitizers in vitro. The 5-iodo-4-nitroimidazole derivatives were more efficient as sensitizers than the 4-iodo-5-nitroimidazole derivatives, and the sensitizing efficiency of this class of agents was found to have significant correlation with their partition coefficients.

In continuation of our efforts to develop potent and effective radiosensitizers to selectively sensitize the relatively resistant hypoxic tumor cells toward ionizing radiation, we have synthesized a series of 4(5)-iodo-5(4)-nitroimidazole derivatives.[1] It has been demonstrated that the radiosensitizing efficiency of a sensitizer is directly related to its electron affinity.[2] However, 5-chloro, 5-bromo, and 5-sulfonamido analogues of 1-methyl-4-nitroimidazole have been reported recently to sensitize the hypoxic cells at concentrations 50–100 times lower than misonidazole.[3-6] Since our preliminary communication of iodonitroimidazoles,[1] Stratford et al.[7] have reported the radiosensitizing properties of two isomeric compounds of 4(5)-iodo-5(4)-nitroimidazoles. These agents have been termed as anomalous radiosensitizing compounds since under in vitro conditions they sensitize the hypoxic cells much more effectively than that predicted from their one-electron reduction potential. Corresponding 4-substituted analogues of 1-methyl-5-nitroimidazole were found to be comparatively less active as sensitizers. Rapid mix experiments have demonstrated that the dissociation of the ortho-substituted leaving group to produce the radical anion was not responsible for the observed radiosensiti-

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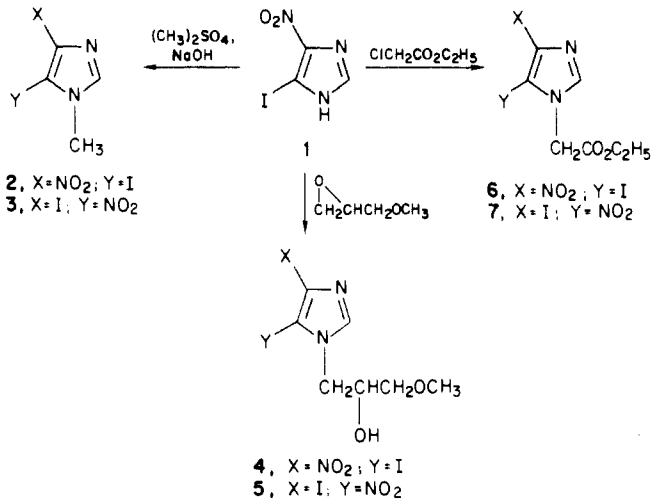
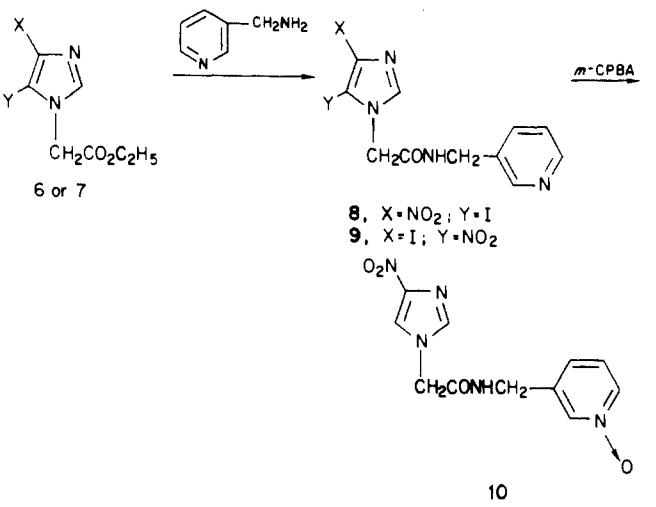
Scheme I



Scheme II



zation.[3] It has recently been suggested that these anomalous compounds react spontaneously with cellular nonprotein thiols (NPSH) and that this may be partly responsible for their increased cytotoxicity.[8]

We have reported previously that the 4-nitro-5-chloroimidazole-1-ethanol was less effective than misonidazole in sensitizing hypoxic *Escherichia coli* cells to ionizing radiation.[9] This observation suggested that the increased sensitizing potency of 4-nitro-5-chloro-1-methylimidazole might have been influenced by enhanced lipid solubility due to the presence of the hydrophobic 1-methyl function. It was demonstrated earlier that the partition coefficient (PC) was unimportant for the radiosensitization of hypoxic Chinese hamster cells in vitro.[10] This conclusion was, however, based upon PCs of a large series of structurally unrelated nitroheterocyclic or nitroaromatic compounds. Employing a series of 2-nitroimidazoles, it was reported that although the efficiency of sensitization in hypoxic bacterial cells was similar for compounds with PC of <1.0, it was higher when the PC was >3.5.[11] Since cell membrane would be expected to play a role in diffusion of compounds into the cell, it seemed appropriate to examine the effect of PC on sensitizing efficiency within a series of structurally related compounds in a mammalian cell system. In this report we have examined a series of derivatives of 4-nitro-5-iodoimidazole containing a variety of groups in the side chain at the 1-position in an attempt to obtain compounds with a wide range of PCs. In addition, it was envisioned that if deemed appropriate the iodo function may also provide a means of labeling the desired analogues with ^{125}I or ^{131}I, which may then be utilized for labeling the hypoxic cell compartment of the tumor tissue.

Chemistry. Iodination of imidazole in alkaline medium led to the synthesis of 4,5-diiodoimidazole according to the procedure of Naidu and Bensusan.[12] Although earlier studies[13] had shown that the electrophilic substitution of imidazole produced 2,4-disubstituted derivatives, subsequent reports[12,14] demonstrated that dihalogenation of imidazole produced the 4,5-disubstituted analogues. Our NMR data on diiodoimidazole derivatives in this report have confirmed the later studies. Nitration of 4,5-diiodoimidazole according to the published procedure[13] produced the starting material 4(5)-iodo-5(4)-nitroimidazole (1) as reported by Dickens et al.[14] Methylation of 1 with dimethyl sulfate produced a mixture of 4-nitro-5-iodo-1-methylimidazole (2) and 4-iodo-5-nitro-1-methylimidazole (3) (Scheme I), which were separated by preparative TLC. Similarly, reaction of 1 with 1,2-epoxy-3-methoxypropane also yielded the corresponding two isomers 4 and 5 with a 1-methoxy-2-propanol side chain at the 1-position. In an attempt to increase the lipophilicity, 1 was reacted with ethyl α-chloroacetate to provide compounds 6 and 7.

Since neurotoxicity manifested by peripheral neuropathy and convulsions has been the major limitation in the clinical use of misonidazole,[15] an attempt was made to synthesize analogues of 1 that may be comparatively less neurotoxic. We have recently reported that the picolylamide derivative of ethyl 2-nitroimidazole-1-α-hydroxypropionate did not exhibit any pathological changes in the peripheral nerve fibers supplying the hind feet of mice upon administration of doses comparative to misonidazole.[16] We have, therefore, reacted 6 and 7 with 3-picolylamine to yield corresponding picolylamides 8 and 9 (Scheme II). In an attempt to further reduce the neurotoxicity, compound 8 was reacted with *m*-chloroperbenzoic acid to produce the pyridyl *N*-oxide derivative 10. The physicochemical properties of various iodonitroimidazoles are summarized in Table I.

The one-electron reduction potentials were determined by the pulse radiolytic method described previously.[17] Time profiles of optical absorption at 500 nm were followed in the absence and in the presence of various concentrations of 9,10-anthraquinone-2-sulfonate (AQS). In the absence of AQS, the anion radicals of the nitroimidazole produced by the radiolysis

$$N + (CH_3)_2\dot{C}OH \rightarrow N^{-\cdot} + (CH_3)_2CO + H^+$$

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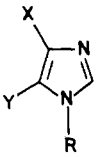
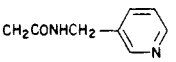
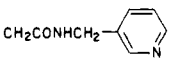
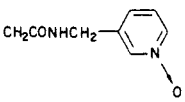
Table I. Physicochemical Properties of Iodonitroimidazoles



no.	X	Y	R	mp, °C	yield, %	recrystn solvent	formula[a]
1	NO_2	I	H	289–290[b]	83	$EtOH/H_2O$	$C_3H_2IN_3O_2$
2	NO_2	I	CH_3	239–240[c]	63	DMF	$C_4H_4IN_3O_2$
3	I	NO_2	CH_3	149–150[d]	10	DMF	$C_4H_4IN_3O_2$
4	NO_2	I	$CH_2CH(OH)CH_2OCH_3$	143–144	56	EtOAc	$C_7H_{10}IN_3O_4$
5	I	NO_2	$CH_2CH(OH)CH_2OCH_3$	121–122	20	EtOAc	$C_7H_{10}IN_3O_4$
6	NO_2	I	$CH_2COOC_2H_5$	144–145	78	EtOAc	$C_7H_8IN_3O_4$
7	I	NO_2	$CH_2COOC_2H_5$	83–84	11	EtOAc/hexane	$C_7H_8IN_3O_4$
8	NO_2	I		231–232	66	$MeOH/H_2O$	$C_{11}N_{10}IN_5O_3$
9	I	NO_2		202–203	57	MeOH	$C_{11}H_{10}IN_5O_3$
10	NO_2	I		221–223	90	$MeOH/H_2O$	$C_{11}H_{10}IN_5O_4$

[a] The compounds were analyzed for C, H, N. Analytical results were within 0.4% of the theoretical values. [b] Lit.[11] mp 281 °C. [c] Lit.[11] mp 240°C. [d] Lit.[11] mp 152 °C.

Table II. One-Electron Transfer Rates and Equilibria for 4(5)-Iodo-5(4)-nitroimidazoles

no.	E_7^1,[a] mV	K	k_f, M^{-1} s^{-1}	k_r, M^{-1} s^{-1}
1	503	119	3.0×10^8	2.0×10^6
2	505	133	6.6×10^8	
3	464	26	3.9×10^8	1.2×10^7
4	497	97	4.1×10^8	
5	441	10.7	5.0×10^8	
6	436	8.9	4.2×10^8	3.2×10^7
7	475	41	4.5×10^8	
8	insol			
9	448	14	4.0×10^8	1.3×10^7
10	465	28	3.0×10^8	

[a] One-electron reduction potentials were determined at pH 7.0 from the equilibrium constants with use of a redox potential of −380 mV for anthraquinone-2-sulfonate (AQS).

exhibited only very weak absorptions at 500 nm. However, upon addition of AQS the electron-transfer reaction

$$N^{-}\cdot + AQS \underset{k_r}{\overset{k_f}{\rightleftharpoons}} N + AQS^{-}\cdot$$

took place and an increase of absorption due to $AQS^{-}\cdot$ was observed. By monitoring the absorption at equilibrium with varying concentrations of AQS, the equilibrium constant $K = [N][AQS^{-}\cdot]/[AQS][N^{-}\cdot]$ was determined. From this value and use of $E_7^1 = -0.380$ V for AQS,[17] the redox potentials E_7^1 for the various nitroimidazoles were derived (Table II).

The kinetics traces allowed determination of k_f and K_r from a plot of $K_{obsd}/[N]$ vs. $[AQS]/[N]$ as indicated previously.[17] In four cases k_f/k_r were in reasonable agreement with the value of K determined from the absorption. In other cases determination of k_r was not sufficiently accurate so that only the values of k_f are shown in Table II. The redox potentials of various 4(5)-iodo-5(4)-nitroimidazoles are in the range of −436 to −505 mV; therefore, these agents are less electron affinic than misonidazole, which has a redox potential of −389 mV. There seems to be no distinct relationship between the substitution of the 1-position by a variety of chemical groups in 4(5)-iodo-5(4)-nitroimidazoles and their redox potentials. Within a set of pairs of 4-nitro and 5-nitro analogues such as compounds **2** and **3** and **4** and **5**, the 5-nitro isomers were more electron affinic as shown by their redox potentials (Table II). However, this general relationship did not hold true for compounds **6** and **7**; in this case the 4-nitro isomer (**6**) was more electron affinic (−436 mV) than the 5-nitro isomer (−475 mV). In fact, compound **6** was the most electron affinic compound of this series.

Table III. Toxicity and Radiosensitizing Activity of Various 4(5)-Iodo-5(4)-nitroimidazoles against Chinese Hamster Cells (V-79) in Vitro

			radiosensitization[c]		
no.	PC[a]	ED_{50},[d] mM	concn, mM	SER[d]	predicted SER[e]
1	1.9	>1.0	1.0	1.1	1.39
2	10.1	0.14	0.05	2.5	1.93
3	19.4	0.82	0.5	2.4	2.54
4	2.6	0.17	0.1	1.6	1.43
5	7.8	0.48	0.1	1.4	1.78
6	3.7	0.06	0.025	1.7	1.51
7	8.6	0.18	0.1	1.8	1.83
8	2.9	0.65	0.25	1.5	1.46
9	7.6	0.18	0.1	1.7	1.76
10	0.04	>1.0	1.0	1.2	1.27

[a] Partition coefficients. [b] Concentration required to kill 50% of Chinese hamster cells, as measured by colony formation, after exposure for 2 h. [c] Radiosensitization of Chinese hamster cells under hypoxic conditions. [d] Sensitizer enhancement ratios were determined by dividingthe D_0 value obtained from the control radiation survival curve by the K_0 value obtained from the radiation survival curve in the presence of each of the sensitizers. [e] Calculated from regression analysis.

Biological Results and Discussion

The various analogues of iodo-substituted nitroimidazoles were tested for cytotoxicity and radiation sensitizing efficiency against hypoxic Chinese hamster (V-79) cells in culture. The results are shown in Table III. The cytotoxicity experiments were conducted under both oxic and hypoxic conditions; however, the exposure times were limited to 2 h. Within this period there was no differential cytotoxicity toward hypoxic cells at the various concentrations employed for determining ED_{50} values. Longer incubation times may be required to exploit the differences between oxic and hypoxic cytotoxicity.[18] The concen-

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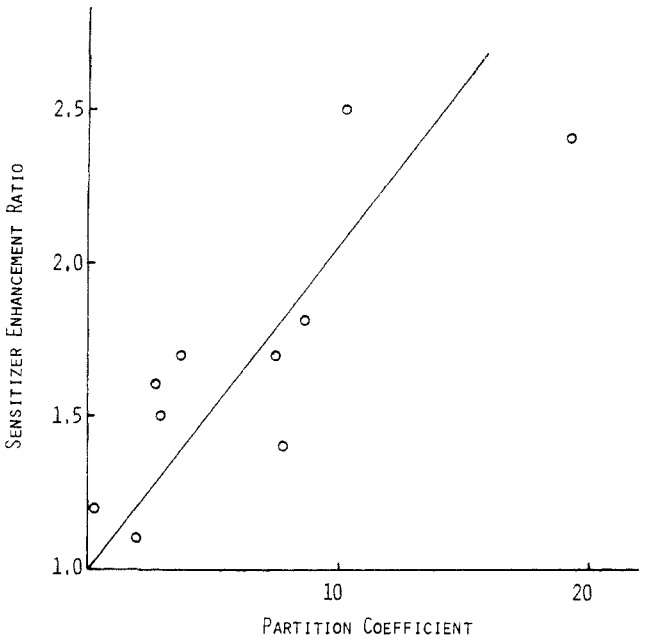

Figure 1. Correlation of partition coefficients and sensitizer enhancement ratios (SER) of various 1-substituted 4(5)-iodo-5-(4)-nitroimidazoles.

trations of each agent required to kill 50% of V-79 cells, as measured by colony formation, suggest that compound **6** was the most toxic compound and was also the most electron affinic in this series. However, there seems to be no definite simple relationship between electron affinity and cytotoxicity with these agents. Generally, the 4-nitro isomers (**2**, **4**, and **6**) were more cytotoxic than the corresponding 5-nitro isomers (**3**, **5**, and **7**). This may be related to their increased rate of reaction with SH groups.[7] The 4-nitro isomers with 5-substituted bromo, 5-chloro, or 5-sulfonamido groups have been shown to deplete intracellular nonprotein thiols more effectively than the corresponding 5-nitro analogues.[6-8] It is therefore, conceivable that the 4-nitro-5-iodoimidazoles would be expected to be more reactive toward SH groups than the 5-nitro-4-iodoimidazoles.

The radiation sensitizing efficiency of the 4(5)-iodo-5-(4)-nitroimidazoles is shown in Table III expressed as sensitizer enhancement ratios (SER), which were determined by dividing the D_0 value obtained from the control radiation survival curve by the D_0 value obtained from the radiation survival curve in the presence of each sensitizer as described previously.[19] The radiosensitizing effectiveness of each agent was determined at a maximum nontoxic concentration upon 2 h exposure up to a limit of 1 mM. The results suggest that the 4-nitro isomers **2**, **4**, and **6** were more potent sensitizers since they required much lower drug concentrations to produce a similar or higher SER. However, the picolyl-substituted analogue **8** did not fit in this generalization. Compound **2** was the most effective sensitizer with a SER of 2.5 at 0.05 mM concentration. This increased efficiency of sensitization has also been shown to be related to their rapid reaction with SH groups,[6-8] as suggested for cytotoxicity. However, our results with this series suggest that not all 4-nitro-5-iodoimidazoles were equally efficient sensitizers. In fact, compounds **1**, **8**, and **10** were much less effective sensitizers. We have therefore determined the PC of these agents in an effort to correlate the biological activity with lipophilicity. The relationship of PCs of this series of compounds with their SERs is shown in Figure 1. Most data points were along the straight line. A quantitative correlation of these two sets of data (PC and SER) was carried out by regression analysis. The following equation was obtained:

$$\text{SER} + 1.26 + 0.066\ (\pm 0.017)\ \text{PC}$$

$$n = 10,\ r = 0.811,\ s = 0.28,\ F = 15.3$$

where n is the number of data points with 10 different analogues of this series, r is the correlation coefficient, s is the standard deviation of SER about the regression line with 8 degrees of freedom, the number within parentheses preceding PC is the standard deviation of coefficient of the PC value, and F is the ratio between the variances of calculated and experimentally observed enhancement ratios. There is a significant correlation between the PC and SERs determined in vitro of this series of compounds. The F value is significant at the 99% level [$F_{1,8}(p = 0.01) = 11.26$]. These results, therefore, are in direct contrast to the earlier reports on the series of nitroheterocyclic compounds, suggesting that PC is unimportant for in vitro radiosensitization.[10] It is conceivable, therefore, that the nonprotein thiol depletion achieved by the exposure of V-79 cells to the anomalous radiosensitizers in vitro[20] may be directly related to the PCs of these agents.

Experimental Section

Infrared spectra were obtained with a Beckman IR-10 spectrophotometer. Proton nuclear magnetic resonance spectra were recorded at 90 MH_z on a Varian EM-390 spectrophotometer using tetramethylsilane as the internal reference. The elemental analyses were performed by Baron Consulting Co., Orange, CT. The preparative layer chromatography was performed on 20 × 20 cm glass plates coated with a 1.8-mm layer of silica gel PF_{254} (E. Merck, AG, Darmstadt, Germany). The compounds were detected by visual examination under light at 254 nM. Evaporation of solvents was done under reduced pressure with a rotary evaporator.

4(5)-Iodo-5(4)-nitroimidazole (1): prepared by the published procedure[13] in 83% yield; mp 289–290 °C (lit.[13] mp 281 °C); NMR (Me_2SO-d_6) δ 8.0 (s, C_2H).

1-Methyl-4-nitro-5-iodoimidazole (2). To a stirred solution of 0.717 g (3 mmol) of 1 in 10 mL of 1 N sodium hydroxide solution was added dropwise 0.31 mL (3.3 mmol) of dimethyl sulfate. The temperature of the reaction mixture was maintained at 60–75 °C. After the mixture was stirred at room temperature for 30 min, the product was filtered and dried. The isomeric mixture was then stirred with acetone (5 mL) to dissolve the 5-nitro derivative. The 4-nitro analogue was filtered and washed with small volume of acetone. Recrystallization from DMF yielded 0.5 g (66%) of **2** as yellow crystals: mp 239–240 °C (lit.[13] mp 240 °C); IR (KBr) 1520, 1370 cm^{-1} (NO_2); NMR (Me_2SO-d_6) δ 3.75 (s, NCH_3), 8.1 (s, C_2H).

1-Methyl-4-iodo-5-nitroimidazole (3). The acetone washings and the filtrate from the above reaction were collected, and the 5-nitro isomer was purified by preparative TLC with chloroform/ethyl acetate (5:2) as the eluent. The two major bands were extracted with ethyl acetate. The extracts from the lower band yielded a yellow solid, which was recrystallized to give **2**. The extracts from the upper band also yielded a yellow solid, which was recrystallized from 50% aqueous ethanol to yield 80 mg (11%) of **3**: mp 148–150 °C; IR (KBr) 1530, 1350 cm^{-1} (NO_2); NMR (Me_2SO-d_6) δ 3.9 (s, NCH_3), 8.0 (s, C_2H).

Reaction of 4(5)-Iodo-5(4)-nitroimidazole (1) with 1,2-Epoxy-3-methoxypropane. A suspension of **1** (3 g, 12.5 mmol) in 20 mL of 1,2-epoxy-3-methoxypropane and potassium carbonate (0.32 g, 10%) was stirred at room temperature for 48 h. Compound **1** went into solution after 28 h, and the reaction was followed by

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TLC (EtOAc). The excess of oxirane was removed under reduced pressure and the residual oil was purified by column chromatography (silica gel), employing ethyl acetate as the eluent. The UV-absorbing fractions were collected.

The first fraction was obtained as an oil, which was crystallized (ethyl acetate/hexane) to yield 500 mg (12.5%) of **4**: mp 121–122 °C; IR (KBr) 3260 (OH), 1520, 1360 cm^{-1} (NO_2); NMR (Me_2SO-d_6) δ 7.98 (s, C_2H), 5.22 (s, COH), 4.5 (d, NCH_2), 4.21 (d, CH_2O), 3.8 (m, CHO), 3.3 (s, OCH_3).

The second fraction was also obtained as an oil, which showed a mixture of two components; yield 1.3 g (32%). This was subjected to preparative TLC, using ethyl acetate as the eluent. The two major bands were separated and extracted with ethyl acetate. The spectral analysis (IR, NMR) indicated that the upper band yielded **4** and the lower band produced **5**. Compound **4** was obtained as an oil, which was crystallized (ethyl acetate/hexane) to yield 325 mg (8%): mp 121–122 °C. Compound **5** was obtained as a crystalline solid to yield 850 mg (21%). This was pooled with the third fraction obtained from column chromatography, making a total amount of 2.28 g of **5** (56%): mp 143–144 °C; IR (KBr) 3280 (OH), 1520, 1370 cm^{-1} (NO_2); NMR (Me_2SO-d_6) δ 8.03 (s, C_2H), 5.32 (d, COH), 4.08 (d, NCH_2), 4.05 (m, CHO), 3.38 (d, CH_2O), 3.32 (s, OCH_2).

Reaction of 4(5)-Iodo-5(4)-nitroimidazole (1) with Ethyl α-Chloroacetate. A suspension of **1** (4.78 g, 20 mmol), potassium carbonate (2.76 g, 20 mmol), and ethyl α-chloroacetate (3 mL) in acetonitrite (100 mL) was stirred and refluxed for 2.6 h and monitored by TLC ($CHCl_3$/EtOAc, 5:2). After completion of the reaction, the solvent was removed under vacuum to leave a residual oil, which was crystallized from ethyl acetate (50 mL) to yield 3.5 g (54%) of **6**: mp 144–145 °C; IR (KBr) 1730 (COO), 1525, 1330 cm^{-1} (NO_2); NMR (Me_2SO-d_6) δ 8.15 (s, C_2H), 5.13 (s, NCH_2), 4.23 (q, CH_2CH_3), 1.27 (t, CH_2CH_3). The filtrate was concentrated under vacuum and was separated on a silica gel column (200 g) by elution with a mixture of chloroform and ethyl acetate (5:2). Three fractions as monitored by TLC were collected.

The first fraction gave an oil, which was crystallized (ethyl acetate/hexane) to yield 0.45 g (7%) of **7**: mp 83–84 °C; IR (KBr) 1730 (COO), 1510, 1350 cm^{-1} (NO_2); NMR (Me_2SO-d_6) δ 8.09 (s, C_2H), 5.28 (s, NCH_2), 4.18 (q, CH_2CH_3), 1.22 (t, CH_2CH_3). Anal. ($C_7H_8IN_3O_4$) C, H, N.

The second fraction was obtained as a mixture of **6** and **7** to yield 1.1 g (17%). The mixture was subjected to preparative TLC using ($CHCl_3$/EtoAc, 5:2) as the eluent. The faster moving band was identified as **7** and was crystallized (EtOAc/hexane) to yield 0.3 g (5%). The slower moving band was identified as **6** and was crystallized (EtOAc) to yield 0.7 g (10.7%). The third fraction obtained from column chromatgraphy yielded another 0.85 g of **6**. The three fractions were pooled to produce a total yield of 5.05 g (78%) of **6**.

5-Iodo-4-nitroimidazole-1-*N*-(3-picolyl)acetamide (8). A solution of **6** (1 g, 3.01 mmol) in methanol (10 mL) and 3-picolylamine (1.5 mL) was stirred at room temperature for 48 h. The separated solid was filtered and recrystallized ($MeOH/H_2O$) to yield 0.9 g (66%): mp 231–232 °C; IR (KBr) 1660 (CO), 1550, 1365 cm^{-1} (NO_2); NMR (Me_2SO-d_6) δ 8.78 (br s, CONH), 8.54–7.4 (m, pyridine), 8.15 (s, C_2H), 4.82 (s, NCH_2), 4.37 (t, CH pyridyl). Anal. ($C_{11}H_{10}IN_5O_3$) C, H, N.

4-Iodo-5-nitroimidazole-1-*N*-(3-picolyl)acetamide (9). A solution of **7** (0.4 g, 1.2 mmol) in methanol (10 mL) and 3-picolylamine (0.5 mL) was stirred at room temperature for 96 h. The separated solid was filtered and recrystallized ($MeOH/H_2O$) to yield 0.27 g (57%): mp 202–203 °C; IR (KBr) 1650 (CO), 1550, 1350 cm^{-1} (NO_2); NMR (Me_2SO-d_6) δ 8.82 (s, CONH), 8.48–7.6 (m, pyridine), 5.12 (s, NCH_2), 4.32 (t, CH_2 pyridyl). Anal. ($C_{11}H_{10}IN_5O_3$) C, H, N.

5-Iodo-4-nitroimidazole-1-*N*-(3-picolyl)acetamide *N*-Oxide (10). To a solution of **8** (0.7 g, 1.8 mmol) in triethyl phosphate (100 mL) was added *m*-chloroperbenzoic acid (0.4 g, 2.5 mmol) and the reaction was stirred at room temperature overnight. At the end of completion of the reaction as shown by TLC, diethyl ether (500 mL) was added. A white solid separated, which was recrystallized from methanol to yield 0.68 g (90%) of **10**: mp 221–223 °C; IR (KBr) 1550, 1360 cm^{-1} (NO_2); NMR (Me_2SO-d_6) δ 8.9 (s, CONH), 8.12–7.3 (m, pyridine), 8.1 (s, C_2H), 4.9 (s, NCH_2), 4.32 (t, CH_2 pyridyl).

Partition Coefficients. The partition coefficients were determined by employing the procedure of Fujita et al.[21] The test compounds were equilibrated in an equal volume of octanol and phosphate buffer (0.1 M, pH 7.4) by stirring at room temperature for 1 h. The concentration of the nitroimidazoles in each phase was determined spectrophotometrically.

Redox Potentials. One-electron reduction potentials were determined by the pulse radiolytic method described previously.[17] Aqueous solutions containing 2–3 mM nitroimidazole derivative, 3 M 2-propanol, and 2 mM phosphate buffer (pH 7.0) were saturated with N_2O and then irradiated with pulses of electrons from a linear accelerator (ARCO LP-7, 10-ns pulses of 8-MeV electrons, dose/pulse ~ 500 rad). Time profiles of optical absorption at 500 nm were followed in the absence and in the presence of various concentrations of 9,10-anthraquinone-2-sulfonate (AQS). By monitoring the adsorption at equilibrium with varying concentrations of AQS the equilibrium constant $K = [N][AQS^{\cdot-}]/[AQS][N^{\cdot-}]$ was determined. From this value and use of $E_7^1 = 380$ mV for AQS,[17] the redox potentials E_7^1 for the various nitroimidazoles were derived.

Radiosensitization Studies. The radiosensitization studies were carried out by employing asynchronous monolayer cultures of Chinese hamster cells (V-79). The techniques used for culturing and handling this cell line have been reported earlier.[22] The cells were grown as monlayers in 25-cm^2 plastic culture flasks (Falcon) in Eagle's minimum essential medium (MEM) with 15% fetal calf serum.

For toxicity tests approximately 200 cells were placed in permanox petri dishes (60 × 15 mm, Lux Scientific Corp.) containing 3 mL of media and were allowed to attach for 2 h. The medium was then removed by aspiration and replaced by 3 mL of medium containing the nitro compound under study. The cells were exposed to a range of concentrations of each drug for 2 h at 37 °C in air or in hypoxia. The plated cultures were rendered hypoxic in sealed containers, capable of holding seven petri dishes, by purging with 95% nitrogen/5% CO_2 for 90 min. At the end of a 2-h period, the medium containing the drug was removed and replaced by 3 mL of fresh medium. Cultures were incubated for 6 days at 37 °C in an atmosphere of 95% air/5% CO_2; the resulting colonies were fixed in absolute ethanol, stained with methylene blue, and counted. The ED_{50} values were calculated from the concentration vs. percent survival graphs and represent the concentration required to inhibit 50% of the cell growth.

Irradiation was carried out at room temperature with a linear accelerator at a dose rate of 180 rad/min. The petri dishes in the sealed containers were directly irradiated under aerobic and hypoxic conditions. Complete survival curves were obtained for each compound at the radiation doses of 400–2400 rad. The D_0 value was obtained for each compound and the ratio of the D_0 value for the hypoxic control cells to the D_0 value of hypoxic drug-treated cells provided the sensitizer enhancement ratio of the corresponding agent.

Acknowledgment. This investigation was supported by Grant R01 CA21050, awarded by the National Cancer Institute, NIH (to K.C.A.). The Radiation Laboratory is supported by the Office of Basic Energy Sciences of the Department of Energy; this is Document No. NDRL-2727.

Registry No. **1**, 76529-48-1; **2**, 35681-63-1; **3**, 76529-47-0; **4**, 96258-78-5; **5**, 96258-79-6; **6**, 96258-80-9; **7**, 96258-81-0; **8**, 96258-82-1; **9**, 96258-83-2; **10**, 96258-84-3; $\overline{OCH_2CHCH_2OMe}$, 930-37-0; $ClCH_2CO_2Et$, 105-39-5; 3-picolylamine, 3731-52-0.

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