H), 5.96–6.06 (br d, NH), 7.80–7.84 (br dd, $C_{4,5}$ H). Anal. ($C_{23}H_{30}N_3O_{14}\cdot H_2O$) C, H, N.

Biological Experiments. Asynchronous monolayer cultures of Chinese hamster cell line (V-79) were employed in all the experiments. The monolayers were derived from exponentially growing cultures. Methods of culturing and handling have been reported earlier.[7] The plated cultures in permanox petri dishes were rendered hypoxic in sealed containers capable of holding seven petri dishes, by purging with 95% nitrogen (oxygen-free grade) and 5% CO_2. The oxygen enhancement ratio (OER) under these conditions was 3.0 (D_0 for hypoxic cells 630 rad; for oxic cells, 210 rad). For toxicity tests, petri dishes containing approximately 200 cells per dish were exposed to a range of concentrations of each drug for 2 h at 37 °C in air or in hypoxia. Drug concentrations between 100 μM and 1 mM were employed. Irradiation was carried out with a cobalt-60 source at a dose rate of approximately 240 rad/min according to the procedure described previously.[7] Complete survival curves were obtained for each compound at the radiation doses of 400 to 2700 rad. The radiosensitization experiments were performed after exposing the V-79 cells to the maximum nontoxic concentration (1 mM or less) of each agent for 1 h at 37 °C under hypoxic conditions. Cell survival was estimated from unirradiated hypoxic cells exposed to the same drug concentration. Cultures were incubated for 6 days at 37 °C in an atmosphere of 5% CO_2; the resulting colonies were fixed in absolute ethanol and stained with Methylene blue and counted.

Acknowledgment. The authors are grateful to Dr. Joseph V. Schlosser at the Charity Hospital, New Orleans, LA, for his interest and encouragement of this project and for allowing the use of the cobalt-60 source to conduct the radiosensitization experiments. This investigation was supported by a grant (CA-21050) from the National Cancer Institute, NIH.

Potential Radiosensitizing Agents. 5. 2-Substituted Benzimidazole Derivatives

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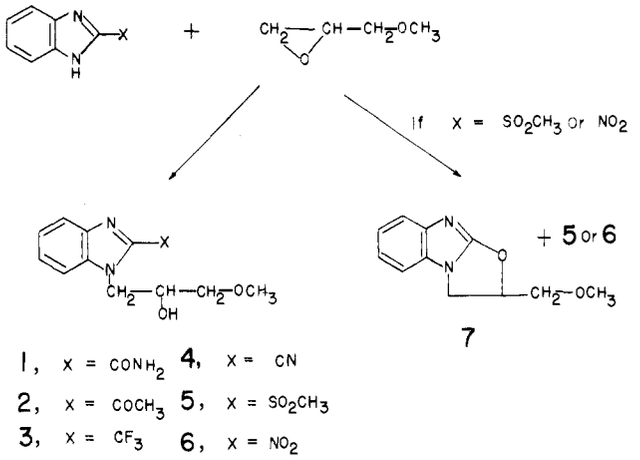
Received January 25, 1982

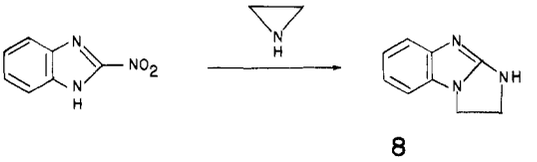
A series of 2-substituted benzimidazoles and their derivatives have been synthesized and tested for their ability to selectively sensitize hypoxic Chinese hamster cells (V-79) toward the lethal effect of ionizing radiation. These compounds were prepared by reacting the 2-substituted benzimidazoles with 1,2-epoxy-3-methoxypropane in the presence of potassium carbonate. Reaction of the 2-nitro and 2-methylsulfonyl analogue with the epoxide also yielded a cyclized material, which was confirmed to be a benzimidazo[2,1-*b*]oxazole. In an attempt to increase the electron affinity, 5- or 6-nitro-2-substituted-benzimidazoles were also synthesized and then reacted with the epoxide to yield the corresponding 1-substituted derivatives. The results of the biological tests for the radiosensitizing activity of these agents against Chinese hamster cells (V-79) in culture indicated that the 2-nitro-substituted analogues were the most effective sensitizers in this series.

Nitroaromatic compounds are known to differentially sensitize hypoxic tumor cells to the lethal effects of ionizing radiation.[1] Of these, the nitroimidazoles are particularly promising as radiosensitizers in view of their favorable pharmacological properties, i.e., low toxicity, free distribution in tissues, and relatively long metabolic half-life.[2] 1-(2-Hydroxy-3-methoxypropyl)-2-nitroimidazole (misonidazole) is a considerably more effective radiosensitizer than 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole (metronidazole), and this has been attributed to its higher electron affinity.[3] To achieve the high electron affinity, we previously synthesized a series of 1-substituted 2,4-dinitroimidazole analogues; these analogues were tested for their radiosensitizing ability for selectively sensitizing hypoxic mammalian cells to the lethal effect of radiation.[4] The effect of inserting other electron-affinic groups, such as the acetyl function, in the 2-nitroimidazole nucleus has also recently been studied.[5] In this report, we have attempted to study systematically the effect of a variety of electron-withdrawing groups on the biological activity by synthesizing and testing 2-substituted benzimidazoles. It was hypothesized that due to the extended conjugation of

Scheme I



Scheme II



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an additional aromatic ring fused to the imidazole nucleus, the benzimidazoles might be more electron affinic than the imidazole derivatives.

Chemistry. The 2-substituted benzimidazoles were initially synthesized by published procedures (Table I) and were then reacted with 1,2-epoxy-3-methoxypropane to

(1) (a) Flower, J. W.; Adams, G. E.; Denekamp, J. *Cancer Treat. Rev.* **1976**, *3*, 227. (b) Adams, G. E.; Denekamp, J.; Fowler, J. F. *Chemotherapy* **1976**, *7*, 187.
(2) Chapman, J. E.; Reuvers, A. P.; Borsa, J.; Henderson, J. S.; Migliore, R. D. *Cancer Chemother. Rep.* **1974**, *58*, 559.
(3) Asquith, J. C.; Watts, M. E.; Patel, K.; Smithen, C. E.; Adams, G. E. *Radiat. Res.* **1974**, *60*, 108.
(4) (a) Agrawal, K. C.; Bears, K. B.; Sehgal, R. K.; Brown, J. N.; Rist, P. E.; Rupp, W. D. *J. Med. Chem.* **1979**, *22*, 583. (b) Sehgal, R. K.; Webb, M. W.; Agrawal, K. C. *Ibid.* **1981**, *24*, 601.
(5) Sehgal, R. K.; Agrawal, K. C., *J. Pharm. Sci.*, in press.

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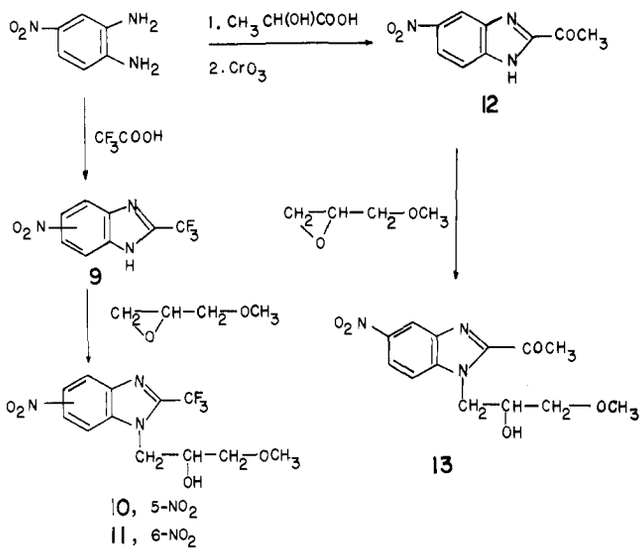
Table I. Reaction Conditions for the Synthesis of 2-Substituted Benzimidazoles

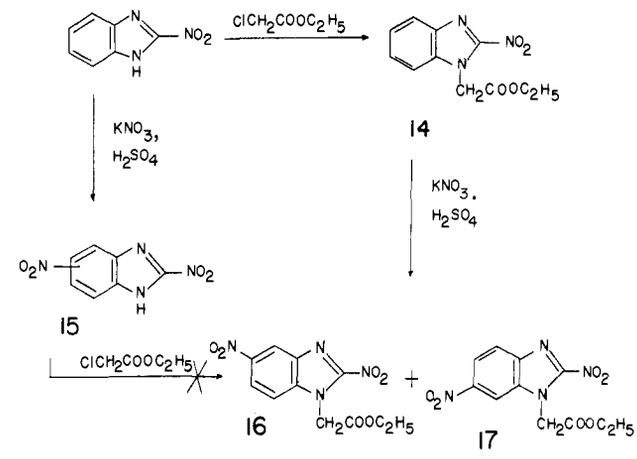
no.	2-substituted benzimidazole,[a] g	epoxide,[b] mL	potassium carbonate, g	temp of the reaction, °C	time of the reaction, h
1	0.3[c]	3	0.03	rt[d]	24
2	0.5[e]	5	0.05	70 °C	2
3	0.2[f]	2	0.02	rt	72
4	0.5[g]	5	0.05	rt	24
5	0.5[h]	5	0.05	rt	72
6	0.5[i]	5	0.05	rt	24

[a] 2-Substituted benzimidazoles were synthesized by the published procedures that are cited under each compound. [b] 1,2-Epoxy-3-methoxypropane. [c] Copeland, R. A. B.; Day, A. R. *J. Am. Chem. Soc.* **1943**, *65*, 1072. [d] Room temperature of about 25 °C. [e] Cheeseman, G. W. H. *J. Chem. Soc.* **1964**, 4645. [f] Lane, E. S. *J. Chem. Soc.* **1955**, 534. [g] Petrov, A. S.; Somin, I. N. *Geterotsikl. Soedin. Akad. Nauk. Latv. SSR* **1966**, *3*, 472. [h] Beaman, A. G.; Tautz, W.; Gabriel, T.; Keller, D.; Toume, V.; Duschinsky, R. *Antimicrob. Agents Chemother.* **1965**, 469. [i] Hoggarth, E. *J. Chem. Soc.* **1949**, 3311.

Scheme III



Scheme IV



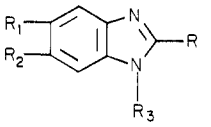
produce the corresponding 1-substituted carbinols **1–4** (Scheme I). The synthetic methodology indicating the amounts of reactants and reaction conditions are described in Table I. In the case of methylsulfonyl or nitro substituted benzimidazoles, in addition to **5** or **6**, respectively, a cyclized product (**7**) was also formed that was confirmed to be a benzimidazo[2,1-*b*]oxazole derivative (**7**). The yield of **7** depended upon reaction temperature and was found to increase at higher temperatures. Similar cyclized products have been reported with 2,4-dinitroimidazole and oxiranes from our laboratory.[6] Analogously, reaction of 2-nitrobenzimidazole with ethylenimine did not yield the corresponding aminoethyl derivative; instead, a 2,3-dihydrobenzimidazo[2,1-*b*]imidazole (**8**) was isolated from the reaction mixture in 93% yield (Scheme II). The structure of **8** was confirmed by the lack of an NO_2 function in the IR and the presence of a CH_2CH_2 group in the NMR. Formation of **8** may result due to the intramolecular cyclization with elimination of the NO_2 function after initial formation of the aminoethyl intermediate, a mechanism similar to the one proposed for the reaction of 2,4-dinitroimidazole with oxiranes.[4b]

In an attempt to increase the electron affinity of some of the 2-substituted benzimidazoles, we introduced an additional nitro function at the 5- or 6-position. The trifluoromethyl-substituted analogue was synthesized by condensing 4-nitro-*o*-phenylenediamine with trifluoroacetic acid to produce the 5(6)-nitro-2-(trifluoromethyl)benzimidazole (**9**; Scheme III). Compound **9** was also obtained in a reasonable yield by direct nitration of 2-(trifluoromethyl)benzimidazole. Reaction of **9** with 1,2-epoxy-3-methoxypropane produced a mixture of 5-nitro (**10**) and 6-nitro (**11**) substituted analogues. The structures of these isomeric compounds were confirmed by utilizing proton chemical shifts. The isomers **10** and **11** were differentiable by a significant greater downfield shift of the proton at the 4-position than of the proton at the 7-position caused by the electron-withdrawing effect of the nitro group at the 5- or 6-position, respectively.[4b]

The 2-acetyl-5-nitrobenzimidazole (**12**) was obtained by condensing the 4-nitro-*o*-phenylenediamine with lactic acid and subsequent oxidation of the carbinol with chromium trioxide (Scheme III). The synthesis of **12** was also attempted by direct nitration of 2-acetylbenzimidazole but resulted in production of only the tarry products. The reaction of **12** with 1,2-epoxy-3-methoxypropane yielded primarily one isomer, the 5-nitro analogue (**13**). It is intriguing that the introduction of the side chain at the 1-position in **12** resulted in loss of the carbonyl peak in the IR of **13** when run in the solid state in KBr, perhaps due to the intramolecular hydrogen bonding involving the carbonyl oxygen and the secondary alcohol. This was substantiated by the presence of a broad band at 3100 cm^{-1}. However, **13** in solution in chloroform did show a carbonyl peak in the IR at 1685 cm^{-1}, suggesting that a cyclic structure was not involved in loss of the carbonyl band in the solid state. Furthermore, the characteristic bands of the carbonyl function at 1690 cm^{-1} and of the OH at 3410 cm^{-1} were present in the IR spectrum of **2**, an analogue without the NO_2 functionality. Similarly, the amide bands at 1700, 3140, and 3280 cm^{-1} were also observed in compound **1**.

(6) Sehgal, R. K.; Agrawal, K. C. *J. Heterocycl. Chem.* **1979**, *16*, 1499.

Table II. Physicochemical Properties of Substituted Benzimidazoles



no.	R	R_1	R_2	R_3	yield, %	mp, °C	recrystn solvent	formula	anal.
1	$CONH_2$	H	H	$CH_2CH(OH)CH_2OCH_3$	80	178-180	methanol-ether	$C_{12}H_{15}N_3O_2 \cdot HCl \cdot 0.25H_2O$	C, H, N
2	$COCH_3$	H	H	$CH_2CH(OH)CH_2OCH_3$	43	55-58	ether-hexane	$C_{13}H_{16}N_2O_3$	C, H, N
3	CF_3	H	H	$CH_2CH(OH)CH_2OCH_3$	68	67-68	ether-hexane	$C_{12}H_{13}F_3N_2O_2$	C, H, N
4	CN	H	H	$CH_2CH(OH)CH_2OCH_3$	51	102-103	ether-hexane	$C_{12}H_{13}N_3O_2$	C, H, N
5	SO_2CH_3	H	H	$CH_2CH(OH)CH_2OCH_3$	65	syrup		$C_{12}H_{16}N_2O_4 \cdot H_2O$	C, H, N
6	NO_2	H	H	$CH_2CH(OH)CH_2OCH_3$	45	105	ether-hexane	$C_{11}H_{13}N_3O_4$	C, H, N
10	CF_3	NO_2	H	$CH_2CH(OH)CH_2OCH_3$	32	67-68	ether-hexane	$C_{12}H_{12}F_3N_3O_4 \cdot H_2O$	C, H, N
11	CF_3	H	NO_2	$CH_2CH(OH)CH_2OCH_3$	29	102-104	ether-hexane	$C_{12}H_{12}F_3N_3O_4$	C, H, N
12	$COCH_3$	NO_2	H	H	81	217-219 dec	ethanol-water	$C_9H_7N_3O_3$	C, H, N
13	$COCH_3$	NO_2	H	$CH_2CH(OH)CH_2OCH_3$	71	106-108	ethyl acetate-hexane	$C_{13}H_{13}N_3O_5$	C, H, N
14	NO_2	H	H	$CH_2COOC_2H_5$	84	137-139	benzene-hexane	$C_{11}H_{11}N_3O_4$	C, H, N
15	NO_2	NO_2	H	H	77	227-228	methanol-water	$C_7H_4N_4O_4$	C, H, N
16	NO_2	H	NO_2	$CH_2COOC_2H_5$	27	154-155	ethyl acetate-hexane	$C_{11}H_{10}N_4O_6$	C, H, N
17	NO_2	NO_2	H	$CH_2COOC_2H_5$	46	170-171	ethyl acetate-hexane	$C_{11}H_{10}N_4O_6$	C, H, N

In order to further increase the electron affinity, we synthesized derivatives of 2,5-dinitrobenzimidazole (**15**). (Scheme IV). Initially, **15** was obtained by nitrating the 2-nitrobenzimidazole with potassium nitrate in sulfuric acid. Compound **15** could not be reacted either with 1,2-epoxy-3-methoxypropane or with ethyl α-chloroacetate even after refluxing it in acetonitrile for 24 h. However, the ethyl 2-(2-nitro-1-benzimidazolyl)acetate (**14**), obtained by refluxing 2-nitrobenzimidazole with ethyl α-chloroacetate in acetonitrile for 2.5 h, could be further nitrated with potassium nitrate in concentrated sulfuric acid to produce a mixture of 5-nitro (**16**) and 6-nitro (**17**) analogues, which were separated by preparative layer chromatography. We confirmed the structures of **16** and **17** by employing the proton chemical shifts. The proton at the 4-position in **16** absorbs downfield in comparison to the proton at the 7-position in **17**. Compounds **16** and **17** were extremely reactive to nucleophiles due to the presence of two powerful electron-withdrawing groups in these molecules. Attempts to synthesize the picolyl amide derivatives of these isomers upon reaction with picolylamine resulted in a mixture of products that were not identified. Presumably, intramolecular cyclization of the ester side chain with release of the 2-nitro group may have caused the production of a number of isomers. The physicochemical properties of various 2-substituted benzimidazoles are summarized in Table II.

Biological Results and Discussion

The newly synthesized compounds were tested for their radiosensitizing effectiveness against hypoxic Chinese hamster cells (V-79) in culture. The results are shown in Table III. Initially, these agents were tested to determine the ED_{50} concentration required to inhibit 50% of the growth of Chinese hamster cells in culture upon exposure for 2 h. The toxicity experiments were conducted primarily to determine the maximum nontoxic concentration that can be utilized in the radiosensitizing experiments. The toxicity experiments were carried out under both oxic and hypoxic conditions to determine if some of these agents are differentially more toxic to the hypoxic cells.

Table III. Toxicity and Radiosensitizing Activity of 2-Substituted Benzimidazole Derivatives against Chinese Hamster Cells (V-79)

		radiosensitization[b] (1400 rad)			
no.	ED_{50},[a] mM	concn, mM	survival, %	SER[d]	predicted SER[e]
	control[c]		25.8	1.0	0.9
1	>5.0	1.0	21.0	1.1	1.2
2	>5.0	1.0	23.0	1.0	1.1
3	>5.0	1.0	22.0	1.1	1.1
4	1.2	0.5	23.0	1.0	1.1
5	3.2	1.0	25.8	1.0	0.9
6	4.8	1.0	9.4	2.0	1.8
10	>5.0	1.0	13.8	1.4	1.6
11	3.0	1.0	17.6	1.2	1.4
13	>5.0	1.0	11.3	1.2	1.7
14	0.3	0.1	2.7	2.3	2.2
15	2.8	0.5	4.4	2.1	2.1
16	0.016	0.010	26.7	1.0	0.8
17	0.006	0.001	22.9	1.0	1.1

[a] Concentration required to inhibit 50% growth of Chinese hamster cells as measured by colony formation. [b] Radiosensitization of Chinese hamster cells in the presence of the sensitizer under nitrogen. [c] Control Chinese hamster cells under nitrogen. [d] Sensitizer enhancement ratios 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 of the sensitizers. [e] Calculated from regression analysis.

Selective cytotoxicity to hypoxic cells was not observed under these conditions when cells were exposed to a limited time of 2 h. It is conceivable that these agents under longer incubation times may be differentially more cytotoxic to hypoxic cells as has been observed with various nitroimidazoles.[7] The dinitro-substituted benzimidazole esters **16** and **17** were found to be the most toxic to Chinese hamster cells and required a concentration of only 16 and 6 μM, respectively, to inhibit the cell growth by 50%.

(7) Hall, E. J.; Roizin-Towle, I. *Radiology* **1975**, *177*, 453.

However, the parent dinitrobenzimidazole **15** was relatively less toxic, perhaps due to the increased ionization, and required a 2.8 mM concentration to achieve a similar degree of inhibition of cell growth. The 2-nitrobenzimidazole ester **14** was comparatively toxic, requiring a 0.3 mM concentration to kill 50% of the exposed cells in a 2-h period. The other agents tested generally required a concentration greater than 1 mM to inhibit the cell growth.

The 2-substituted benzimidazole analogues were then tested for their radiosensitizing efficiency at a maximum nontoxic concentration (limited to 1 mM or less) against the Chinese hamster cells, which were made hypoxic by flushing with 95% N_2 and 5% CO_2 for 1 h. To facilitate the testing of a large number of compounds, complete radiation survival curves were not obtained initially for each agent individually. Instead, the compounds were tested at a fixed radiation dose of 1400 rad. Under these conditions, hypoxic control cells receiving only the radiation dose survived to the extent of 25.8%. However, in the presence of a sensitizer the percent of survival of these cells will be expected to be lowered according to the sensitizing ability of the agent. Thus, in general, the 2-substituted benzimidazoles (**1–5**) with electron-affinic groups other than the NO_2 function were not active sensitizers. Approximately 21 to 25.8% of the cells survived the exposure of 1400 rad in the presence of these agents. Compound **6**, however, was an active sensitizer, since only 9.4% of the cells survived with respect to 25.8% for the control. The 8 to 10% survival at 1400 rad is comparable to the effect of 1 mM concentration of misonidazole upon 2-h exposure under hypoxia. Introduction of an ester group at the 1-position in place of the secondary alcohol in **6** resulted in compound **14**, which was found to be the most active agent of this series, producing an enhanced sensitizing effect that resulted in only 2.7% of the cell survival at 0.1 mM concentration under these conditions.

Introduction of another electron-affinic group, i.e., NO_2, in **14** produced **16** or **17**, which were found to be highly toxic and at very low nontoxic concentrations of 10 or 1 μM, respectively, were found to be ineffective as sensitizers. However, the lack of an ester group in **15** did produce an agent that was an effective sensitizer and reduced the cell survival to 4.4% at 0.5 mM concentration. Similarly, insertion of an NO_2 function in **3** and **2** produced the analogues **10**, **11**, and **13**, respectively, which were better sensitizers than the parent compounds but comparatively were found to be weaker sensitizers than **6**, **14**, or **15**.

To substantiate our postulation that a rapid prescreening of a large number of compounds for radiosensitization activity can be carried out at a fixed radiation dose, such as 1400/rad, we have generated complete radiation survival curves for each of these agents. The survival characteristics of the cell line employed in these experiments were similar to the one described previously.[8] The sensitizer enhancement ratios (SER) were calculated by dividing the D_0 value (the radiation dose required to reduce survival by a factor of 0.37 in the exponential region of the curve) for the control hypoxic cells by the D_0 value obtained for cells irradiated in the presence of the radiosensitizer under hypoxia. The oxygen enhancement ratio under these conditions was 3.0.

It is obvious from the data presented in Table III that the active radiosensitizers can be preselected for detailed biological activity by screening at a fixed radiation dose in the presence of maximum nontoxic concentrations of each agent. A quantitative study has been carried out on the relationship of percent survival of cells at 1400 rad vs. SERs obtained from the radiation survival curves. The regression analysis on these two sets of data from Table III provided the following equation:

$$\mathrm{SER} = 2.34 - 0.056\ (\pm\ 0.004)\ \%\ S$$

$$n = 14,\ r = -0.97,\ s = 0.12,\ F = 188.9$$

where n is the number of data points, r is the correlation coefficient, s is the standard deviation of SER about the regression line with 12 degrees of freedom, the number within parentheses preceeding % S is the standard deviation of coefficient of percent survival, and F is the F ratio between the variances of calculated and experimentally observed enhancement ratios. There is a highly significant correlation between the percent survival at 1400 rad and SERs. The F value is significant at the 99% level [$F_{1,12(p=0.01)} = 9.33$].

Compounds **1–5**, **16**, and **17** were essentially found to be inactive as radiosensitizers. Compounds **14** and **15** produced SERs of 2.3 and 2.1 at 0.1 and 0.5 mM, respectively. The sensitizing potency of these two agents compared very favorably with misonidazole, which required a concentration of 1 mM to produce an enhancement ratio of 2.0. Thus, this work has demonstrated that certain benzimidazole derivatives, such as **14** and **15**, are potent radiosensitizers and that further studies of these agents with regard to in vivo toxicity and radiosensitizing activity are warranted.

Experimental Section

Infrared spectra were obtained with a Beckman IR-10 spectrophotometer. Proton nuclear magnetic resonance spectra were recorded at 60 MHz on a Varian A-60 spectrometer with tetramethylsilane as the internal reference. The elemental analyses were performed by Integral Microanalytical Laboratories, Raleigh, NC. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Analytical thin-layer chromatography was performed on glass plates coated with a 0.25-mm layer of silica gel GF_{254}; preparative layer chromatography was performed on 20 × 20 cm glass plates coated with a 2-mm layer of silica gel PF_{254} (E. Merck, AG, Darmstadt, Germany). The compounds were detected by visual examination under UV light. Evaporation of solvents was done under reduced pressure with a rotary evaporator.

1-(2-Hydroxy-3-methoxypropyl)-2-substituted-benzimidazoles. Compounds 1 to **6** were prepared from 2-substituted benzimidazoles by utilizing the following procedure described for **6**. The reaction conditions and the amounts of reactants used are described in Table I.

1-(2-Hydroxy-3-methoxypropyl)-2-nitrobenzimidazole (6). A suspension of 2-nitrobenzimidazole[9] (0.5 g, 3.06 mmol) in 5 mL of 1,2-epoxy-3-methoxypropane with potassium carbonate (50 mg, 10%) was stirred at room temperature for 72 h and monitored by TLC ($CHCl_3$/EtOAc, 5:2). Excess oxirane was removed under vacuum, and the residual oil was purified by preparative TLC with chloroform and ethyl acetate (5:2) as eluant. The two major bands were extracted with ethyl acetate, and the spectral analysis (IR, UV) indicated the upper fraction as **6** and the lower fraction as **7**. Compound **6** was obtained as an oil, which was crystallized (ethyl ether/hexane) to yield 350 mg (45%): mp 105 °C; IR (KBr) 3360 (OH), 1540 and 1330 (NO_2) cm^{-1}; NMR (Me_2SO-d_6) δ 7.8 (m, Ar H), 5.4 (d, OH), 4.8 (d, NCH_2), 4.64 (m, CHO), 4.05 (t, CH_2O), 3.34 (s, OCH_3).

Compound **7** was also obtained as an oil after evaporating the solvent (ethyl acetate) under vacuum and was crystallized (ethyl ether/hexane) to yield 120 mg (20%): mp 101 °C; NMR ($CDCl_3$) δ 7.3 (m, Ar H), 5.4 (m, CHO), 4.21 (dd, NCH_2), 3.78 (d, CH_2O), 3.45 (s, OCH_3). Anal. ($C_{11}H_{12}N_2O_2$) C, H, N.

2,3-Dihydrobenzimidazo[2,1-*b*]imidazole (8). Ethylenimine (4 mL) was cooled in an ice bath, and 2-nitrobenzimidazole (0.4 g, 2.4 mmol) was added to it in one portion. The resulting solution was refluxed for 30 min. Excess ethylenimine was then removed under vacuum. The residual oil was dissolved in water and extracted with chloroform (25 × 7 mL). The chloroform extracts were dried ($MgSO_4$) and evaporated to yield a solid (365 mg, 93%),

which was crystallized from $CHCl_3$/hexane: mp 198–200 °C; NMR ($CDCl_3$) δ 7.3 (m, Ar H), 4.13 (s, NCH_2CH_2N). Anal. ($C_9H_9N_3 \cdot 0.5H_2O$) C, H, N.

Reaction of 2-(Trifluoromethyl)-5(6)-nitrobenzimidazole (9) with 1,2-Epoxy-3-methoxypropane. A suspension of **9** (1 g, 4.3 mmol) in 10 mL of 1,2-epoxy-3-methoxypropane and potassium carbonate (0.1 g, 10%) was stirred at room temperature for 72 h. After the reaction was completed, excess oxirane was removed under vacuum, and the residual oil was purified by preparative TLC with benzene–ethyl ether (1:1) as eluant.

The first fraction was obtained as an oil, which was crystallized (ethyl ether/hexane) to yield 400 mg (29%) of **11**: mp 102–104 °C; IR (KBr) 3320 (OH), 1510, 1340 (NO_2) cm^{-1}; NMR ($CDCl_3$) δ 8.64 (d, H_4, J = 2.0 Hz), 8.25 (dd, H_5, J = 8.0 and 2.0 Hz), 7.85 (d, H_7, J = 9.0 Hz), 4.5 (d, NCH_2, J = 7.0 Hz), 4.2 (m, CHO), 3.53 (d, CH_2O, J = 5.0 Hz), 3.46 (s, OCH_3).

The second fraction was also obtained as an oil, which was crystallized from (ethyl ether/hexane) to yield 430 mg (32%) of **10**: mp 67–68 °C; IR (KBr) 3330 (OH), 1510, 1340 (NO_2) cm^{-1}; NMR ($CDCl_3$) δ 8.72 (d, H_4, J = 2.0 Hz), 8.32 (dd, H_6, J = 10.0 and 2.0 Hz), 7.74 (d, H_7, J = 10.0 Hz), 4.46 (d, NCH_2, J = 7.0 Hz), 4.22 (m, CHO), 3.53 (d, CH_2O, J = 4.0 Hz), 3.45 (s, OCH_3).

2-Acetyl-5-nitrobenzimidazole (12). A mixture of 4-nitro-*o*-phenylenediamine (15 g, 0.1 mol), lactic acid (16 g, 0.2 mol), and 100 mL of 4 N hydrochloric acid was refluxed for 24 h. The reaction mixture was cooled, neutralized with ammonium hydroxide, and filtered, and the precipitate was recrystallized from absolute ethanol to yield 10 g (50%) of 2-(1-hydroxyethyl)-5-nitrobenzimidazole: mp 210–211 °C; IR (KBr) 3100 (NH), 1420 and 1340 (NO_2) cm^{-1}; NMR (Me_2SO-d_6) δ 8.64 (d, H_4, J = 2.0 Hz), 8.28 (dd, H_6, J = 9.0 and 2.0 Hz), 7.9 (d, H_7, J = 9.0 Hz), 6.18 (br s, H_1), 5.18 (q, CH), 1.6 (d, CH_3).

A solution of chromium trioxide (2.25 g, 0.025 mol) in 7.5 mL of water was added dropwise to a solution of 2-(1-hydroxyethyl)-5-nitrobenzimidazole (6.2 g, 0.03 mol) in 22.5 mL of glacial acetic acid at 90 °C. The reaction mixture was heated at 100 °C for a further 5 min and then poured into water (200 mL). The mixture was filtered, the filtrate was extracted with chloroform, and the combined extracts were dried ($MgSO_4$) and evaporated. Crystallization of the residue from aqueous ethanol yielded 5 g (81%) of **12**: mp 217–219 °C dec; IR (KBr) 3260 (NH), 1670 (C=O), 1520 and 1335 (NO_2) cm^{-1}; NMR (Me_2SO-d_6) δ 8.82 (d, H_4, J = 2.0 Hz), 8.42 (dd, H_6, J = 9.0 and 2.0 Hz), 8.01 (d, H_7, J = 9.0 Hz), 2.78 (s, $COCH_3$).

1-(2-Hydroxy-3-methoxypropyl)-2-acetyl-5-nitrobenzimidazole (13). A suspension of **12** (0.5 g, 2.4 mmol) in 5 mL of 1,2-epoxy-3-methoxypropane and potassium carbonate (0.05 g, 10% by weight) was stirred at room temperature for 24 h. After the reaction was completed, excess oxirane was removed under vacuum, and the residual oil was purified by preparative TLC with benzene and ethyl ether (1:1) as eluant. Compound **13** was crystallized (ethyl acetate/hexane) to yield 0.512 g (71%): mp 106–108 °C; IR (KBr): 3100 (br, OH), 1520 and 1340 (NO_2) cm^{-1}; IR ($CHCl_3$) 3500–3300 (br, OH), 1685 (CO), 1520 and 1340 (NO_2) cm^{-1}; NMR ($CDCl_3$) δ 8.27 (m, Ar H), 4.73 (d, NCH_2), 4.21 (m, CHO), 3.45 (d, CH_2O), 3.42 (s, OCH_3), 2.86 (s, $COCH_3$).

Ethyl 2-(2-Nitro-1-benzimidazolyl)acetate (14). A suspension of 2-nitrobenzimidazole (0.326 g, 2 mmol) in 5 mL of acetonitrile and 0.5 mL of ethyl α-chloroacetate with potassium carbonate (0.276 g, 2 mmol) was refluxed for 2.5 h, and the reaction was monitored by TLC (benzene/ethyl acetate, 3:1). After the reaction was complete, the excess solution was removed under vacuum, and the residue was chromatographed on a silica gel column (60 g) and eluted initially with benzene (500 mL), followed by a mixture of benzene/ethyl acetate (3:1). The UV-absorbing fraction was collected and crystallized from benzene/hexane to yield 0.5 g (84%): mp 137–139 °C; IR (KBr) 1730 (ester C=O), 1530 and 1320 (NO_2) cm^{-1}; NMR ($CDCl_3$) δ 7.5 (m, Ar H), 5.3 (s, NCH_2), 4.22 (q, CH_2C), 1.3 (t, CCH_3). Anal. ($C_{11}H_{11}N_3O_4$) C, H, N.

2,5-Dinitrobenzimidazole (15). To an ice-cold solution of 2-nitrobenzimidazole (1 g, 6.12 mmol) in 5 mL of concentrated sulfuric acid was added dropwise 3 mL of fuming nitric acid during 15 min. The reaction mixture was stirred at room temperature for 2 h and poured into ice–water. The resulting precipitate was separated by filtration and recrystallized from aqueous methanol to yield 0.8 g (77%): mp 227–228 °C; NMR (Me_2SO-d_6) δ 8.85 (d, H_4, J = 2.0 Hz), 8.44 (dd, H_6, J = 9.0 and 2.0 Hz), 8.04 (d, H_7, J = 9.0 Hz), 7.52 (br s, H_1).

Nitration of Ethyl 2-(2-Nitro-1-benzimidazolyl)acetate (14). To an ice-cold solution of **14** (1 g, 4 mmol) in 5 mL of concentrated sulfuric acid was added dropwise a solution of potassium nitrate (0.419, 4 mmol) in 5 mL of sulfuric acid during 10 min. The reaction mixture was stirred at room temperature for 5 h and then poured into ice–water (ca. 50 mL) slowly. The aqueous solution was extracted with ethyl acetate, dried ($MgSO_4$), and evaporated under vacuum. The residue was purified by preparative TLC, with benzene/ethyl acetate (4:1) as the eluant.

The upper band was extracted with ethyl acetate and recrystallized from ethyl acetate/hexane to yield 320 mg (27%) of **16**: mp 154–155 °C; IR (KBr) 1745 (ester CO), 1520 and 1330 (NO_2) cm^{-1}; NMR ($CDCl_3$) δ 8.5 (s, H_4), 8.3 (dd, J = 10.0 and 2.0 Hz), 8.1 (s, H_7), 5.38 (s, NCH_2), 4.3 (q, CH_2C), 1.36 (t, CCH_3).

The lower band was then extracted with ethyl acetate and recrystallized from (ethyl acetate/hexane) to yield 550 mg (46%) of **17**: mp 170–171 °C; IR (KBr) 1740 (ester CO), 1500, 1340 (NO_2) cm^{-1}; NMR ($CDCl_3$) δ 8.87 (d, H_4, J = 2.0 Hz), 8.5 (dd, J = 10.0 and 2.0 Hz), 7.58 (d, H_7, J = 10.0 Hz), 5.33 (s, NCH_2), 4.27 (q, CH_2C), 1.32 (t, CCH_3).

Biological Studies. The toxicity and the radiosensitization studies were carried out with asynchronous monolayer cultures of Chinese hamster cells (V-79). The techniques used for culturing and handling this cell line have been reported earlier.[8] The cells were grown as monolayers in 25-cm^2 plastic culture flasks (Falcon) in Eagle's minimum essential medium (MEM) with 15% fetal calf serum.

To determine the cytotoxicity of each agent, we initially placed approximately 200 cells in permanox petri dishes (60 × 15 mm, Lux Scientific Corp.) containing 3 mL of media and they were allowed to attach for 2 h. The medium was then removed by aspiration and replaced by the medium containing the test 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. We rendered the plated cultures hypoxic in sealed containers capable of holding seven petri dishes, by purging with 95% nitrogen/5% CO_2 for 1 h. At the end of the 2-h period, the medium containing the drug was removed and replaced with 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. ED_{50} values were calculated from the graphs and represent the concentration required to inhibit 50% of the cell growth.

Irradiation experiments were carried out at room temperature with a cobalt-60 source at a dose rate of approximately 230 rad/min. The petri dishes in the sealed containers were directly irradiated with 1400 rad under hypoxic conditions in the presence of the maximum nontoxic concentration (1 mM or less) of each compound. The drug exposure time was limited to 2 h at 37 °C. After irradiation, the medium was replaced with fresh medium, and the cultures were incubated for colony formation. Complete survival curves were also obtained for each agent at the radiation doses of 400 to 3000 rad. The D_0 value was calculated for each compound, and the ratio of the D_0 value of the hypoxic control cells to the D_0 value of hypoxic drug-treated cells provided the sensitizer enhancement ratio (SER) of the corresponding agent. The oxygen enhancement ratio under these conditions was 3.0 (D_0 of control hypoxic cells was 630 rad and D_0 of oxic cells was 210 rad).

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(8) Agrawal, K. C.; Millar, B. C.; Neta, P. *Radiat. Res.* **1979**, *78*, 532.

(9) Beaman, A. G.; Tautz, W.; Gabriel, T.; Keller, D.; Toume, V.; Duschinsky, R. *Antimicrob. Agents Chemother.* **1965**, 469.