Potential Radiosensitizing Agents. 4. 2-Nitroimidazole Nucleosides

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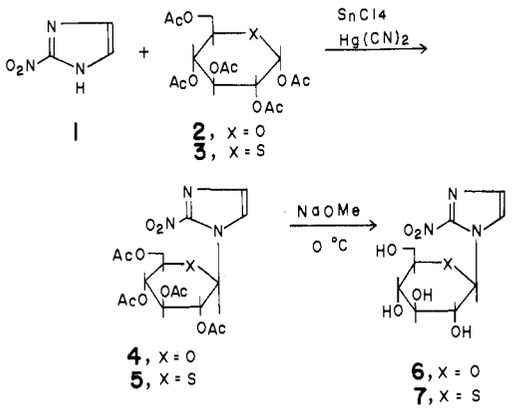
A series of 2-nitroimidazole nucleoside analogues has been synthesized as potential radiosensitizers in an effort to reduce neurotoxicity and increase therapeutic efficacy. The 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl and glucothiopyranosyl analogues of 2-nitroimidazole were synthesized by condensation in the presence of stannic chloride and mercuric cyanide. The deacetylation of these esters was carried out with sodium methoxide at 0 °C. Condensation of the trimethylsilyl derivative of 2-nitroimidazole with methyl 2-deoxy-2-chloro-4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-α-D-neuraminate was achieved in the presence of mercuric bromide. These agents were tested for cytotoxicity and radiosensitization in vitro against Chinese hamster (V-79) cells under oxic and hypoxic conditions. The thioglucose and sialic acid analogues were found to be active radiosensitizers.

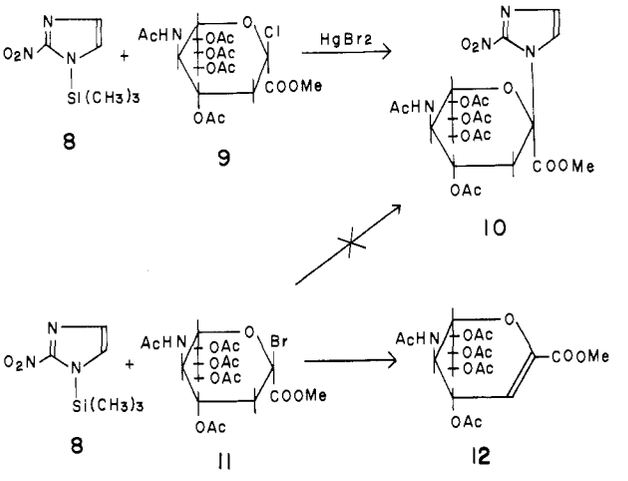
A considerable effort has been made recently for the development of effective regimens in radiotherapy that would allow the sensitizaion of the radioresistant hypoxic tumor cells.[1] Among the chemical sensitizers tested so far from a variety of available nitroheterocyclic compounds, metronidazole, a 5-nitroimidazole derivative, and misonidazole, a 2-nitroimidazole analogue, have been found to be particularly promising in view of their favorable pharmacological properties, i.e., relatively low toxicity, free distribution in tissues, and longer metabolic half-life.[2] Metronidazole is relatively less potent as a radiosensitizer than misonidazole in experimental studies.[3] Misonidazole has been shown to be an effective radiosensitizer in at least 16 different animal tumors and seemed to be of some benefit in clinical radiotherapy.[4] However, large doses of misonidazole, required for radiosensitization in vivo, seems to be a limiting factor because of resulting neurotoxicity. Convulsions and peripheral neuropathy were encountered in a relatively large number of patients.[5]

In our efforts to develop effective radiosensitizers that would allow the selective sensitization of the radioresistant hypoxic tumor cells toward radiotherapy, 1-(2-hydroxyethyl)-2,4-dinitroimidazole was synthesized.[6] This derivative was found to be a significantly more potent radiosensitizer than misonidazole, at 0.1 mM concentration, against Chinese hamster cells in vitro.[7] Further modifications have been made to increase the electron affinity by synthesizing 2-nitro-4-acetylimidazole analogues.[8] We have now extended these investigations in an attempt to synthesize agents that may be relatively less neurotoxic than misonidazole. Accordingly, we have initiated the synthesis of a series of 2-nitroimidazole nucleoside analogues in an effort to limit their crossing of the blood–brain barrier, which may result in the reduction of the central neurotoxicity.[9] In this investigation, we report the synthesis and biological results of glucosyl, thioglucosyl and neuraminic acid derivatives of 2-nitroimidazole.

Scheme I



Scheme II



Chemistry. The chemical reactions for the synthesis of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl (**4**) and 2,3,4,6-tetra-*O*-acetyl-β-D-glucothiopyranosyl (**5**) analogues of 2-nitroimidazole (**1**) are shown in Scheme I. The condensation of **1** with the pentaacetylglucose (**2**) or pentaacetylthioglucose (**3**) was achieved in the presence of stannic chloride and mercuric cyanide according to the procedure of Prisbe et al.[10] The β-anomeric configurations of **4** and **5** were established by proton NMR because of a large trans diaxial coupling constant of 8.5 and 10.1 Hz, respectively, between $C_{1'}$ H and $C_{2'}$ H. The α-anomeric isomer was not produced during the glycosidation of **1** under these conditions.

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(3) Asquith, J. C.; Watts, M. E.; Patel, K.; Smithan, C. E.; Adams, G. E. *Radiat. Res.* **1974**, *60*, 108.
(4) Fowler, J. F.; Adams, G. E.; Denekamp, J. *Cancer Treat. Rev.* **1976**, *3*, 227.
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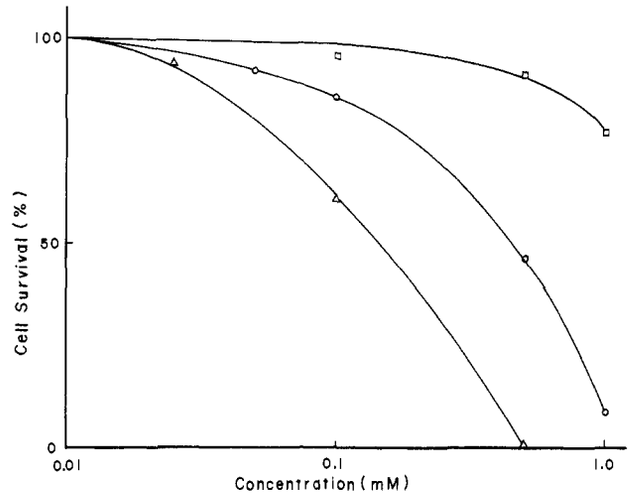

Figure 1. The effect of esters of 2-nitroimidazole nucleosides on the survival of Chinese hamster cells upon 2-h exposure at 37 °C as a function of drug concentration: (O–O) compound **4**; (Δ–Δ) compound **5**; (□–□) compound **10**.

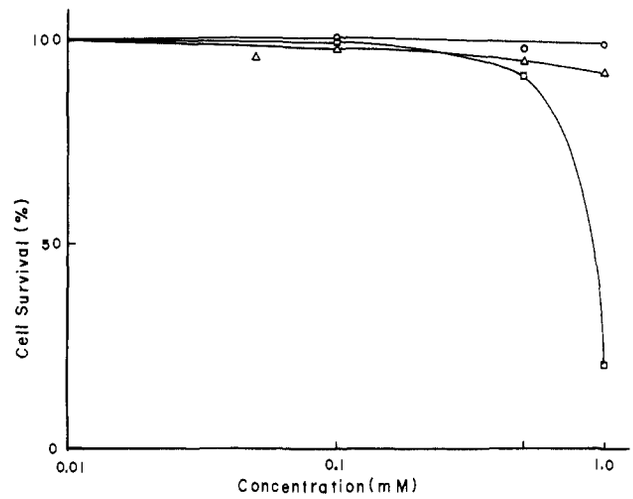

Figure 2. The effect of 2-nitroimidazole nucleosides on the survival of Chinese hamster cells upon 2-h exposure at 37 °C as a function of drug concentration: (O–O) compound **6**; (Δ–Δ) compound **7**; (□–□) compound **14**.

The deacetylation of **4** and **5** with sodium methoxide or methanolic ammonia at room temperature resulted in the production of a mixture of products that were not the desired compounds. The identification of these products is currently under investigation. It is probable that the nucleophilic displacement of the nitro function in the imidazole nucleus accompanied the hydrolysis, giving rise to the undesirable products. However, 1-β-D-glucopyranosyl (**6**) and 1-β-D-glucothiopyransolyl (**7**) analogues of **1** were obtained in approximately 30% yield after hydrolysis of the corresponding esters **4** and **5** with sodium methoxide at 0 °C.

The synthetic sequence for the preparation of the sialic acid analogue of **1** is shown in Scheme II. Initially, we attempted a direct condensation of methyl 2-deoxy-2-chloro-4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-α-D-neuraminate (**9**) with **1** under acid catalysis but were unable to isolate the desired compound. In an alternate approach, we condensed the trimethylsilyl derivative of **1** (**8**) with **9** in the presence of mercuric bromide in acetonitrile by stirring at room temperature for 2 days. Following chromatography (silica gel), a homogeneous foam of the sialic acid analogue of **1** (**10**) was obtained in 36% yield. This reaction was not stereospecific, since the foam was found to be an anomeric mixture of α and β isomers. Compound **10** was tested as an anomeric mixture for biological activity. Attempts to hydrolyze **10** with a base were unsuccessful and resulted in the production of a mixture of compounds that were not confirmed. Interestingly, if the condensation reaction of **8** was carried out with the 2-bromo analogue of sialic acid ester (**11**) in the presence of mercuric bromide, a 2,3-dehydro derivative of sialic acid (**12**) was obtained due to the elimination of HBr. We synthesized the 2-nitroimidazole riboside (**14**) according to the published procedure of Prisbe et al.[10] by hydrolyzing the corresponding tribenzoate ester (**13**) with methanolic ammonia.

Biological Results and Discussion

We initially tested the 2-nitroimidazole nucleoside analogues for cytotoxicity by employing the Chinese hamster cells (V-79) as the in vitro test system. The effects of the esters **4**, **5**, and **10** on the survival of V-79 cells are shown in Figure 1. These experiments were performed under both oxic and hypoxic conditions. There was no apparent differential toxicity to hypoxic cells during the drug–cell contact time of 2 h. Compound **5** was the most toxic of the three esters, since at 0.5 mM none of the cells survived as measured by colony formation, whereas 50% of the cells survived the 2-h exposure of **4** at an equivalent concentration. The sialic acid analogue **10** was the least toxic among the esters, allowing 90% of the cells to survive at a 0.5 mM concentration under similar conditions. The cytotoxicity data for the glucose and thioglucose analogues **6** and **7** and the 2-nitroimidazole riboside (**14**) are presented in Figure 2. Compounds **6** and **7** were relatively less toxic than their corresponding esters; more than 90% of the cells survived at a 1 mM concentration upon 2-h exposure. However, **14** was comparatively toxic to the V-79 cells, causing 80% inhibition of cell growth at 1 mM concentration under these conditions.

The radiosensitizing efficiency of these agents was assessed from the radiation survival curves obtained after exposure of V-79 cells at the maximum nontoxic drug concentrations (limited to 1 mM or less) under hypoxic conditions. The survival curves were obtained for each compound at the various radiation doses from 400 to 2700 rad, and 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 the cells irradiated in presence of the radiosensitizer under hypoxia. The SER values are presented in Table I. The SER for misonidazole, the prototypical drug, was 1.9 at 1 mM concentration under the conditions employed in these experiments.[8] The radiosensitization data in Table I, therefore, suggest that the nucleosides reported in this paper are relatively weak sensitizers. The thioglucose analogue **7** was the most active agent of this series and produced a SER of 1.7 at 1 mM concentration. The sialic acid analogue **10** was similar in radiosensitizing activity to **7** with a SER of 1.6. The radiosensitization produced by **7** or **10** was concentration dependent, both agents showing a progressive increase in sensitization with increasing concentration. The only other active agent of this series was the riboside **14** with a SER of 1.4 at 0.5 mM concentration. However, **14** is comparatively a much more toxic agent than either **7** or **10** (Figure 2). Compound **14** has also been reported to be a less effective radiosensitizer than misonidazole in the Lewis lung tumor regrowth delay assay.[11]

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Table I. Partition Coefficients and Sensitizer Enhancement Ratios of 2-Nitroimidazole Nucleoside Analogues

		radiosensitization	
no.	PC[a]	concn, mM	SER[b]
4	3.31	0.1	1.2
5	4.22	0.05	1.1
6	0.04	1.0	1.0
7	0.22	1.0	1.7
		0.5	1.4
		0.25	1.2
10	2.56	1.0	1.6
		0.5	1.4
		0.25	1.2
13[c]	15.66	0.01	1.0
14[d]	0.07	0.5	1.4

[a] Partition coefficients. [b] Sensitizer enhancement ratios were determined by dividing the D_0 values obtained from the radiation survival curves of the control hypoxic cells by the D_0 values obtained for the hypoxic cells irradiated in presence of the sensitizer. [c] 1-(2,3,5-Tribenzoyl-β-D-ribofuranosyl)-2-nitroimidazole.[9] [d] 1-β-D-Ribofuranosyl-2-nitroimidazole.[9]

The relationship of partition coefficients as a guide to the development of radiosensitizers has recently been described.[12] The tumor/plasma ratio of the drug concentration was suggested to be independent of partition coefficient over the range of 0.026 to 1.5. However, the most hydrophilic compound of this series (partition coefficient 0.014) was reported to be significantly a poorer sensitizer than misonidazole.[12] Moreover, Adams et al.[13] have concluded that partition coefficient is not an important factor for in vitro activity, whereas it is expected to have a much greater influence on in vivo biological response. We have, therefore, determined the partition coefficients of the 2-nitroimidazole nucleosides for comparison with in vitro radiosensitization (Table I). The data suggest that the partition coefficient does seem to play a role in in vitro activity, since compounds **6** and **7** differ significantly in radiosensitizing efficacy, although both will be expected to be similar in electron affinity. It is therefore probable that the lack of biological activity of **6** may be related to its low partition coefficient value of 0.04. The ester **4**, with a partition coefficient of 3.31, did demonstrate marginal activity; however, because of toxicity at higher concentrations, the radiosensitization experiments were limited to the maximum nontoxic dose of 0.1 mM.

Thus, the synthetic approach to develop 2-nitroimidazole nucleosides has provided active radiosensitizers, especially the thioglucose analogue **7** and the sialic acid derivative **10**. These findings therefore encourage further investigation in the development of new 2-nitroimidazole nucleosides.

Experimental Section

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Ultraviolet spectra were determined with a Beckman DB-G grating spectrophotometer. Infrared spectra were obtained in KBr 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. Preparative layer chromatography was performed on 20 × 20 cm glass plates coated with a 2-mm thick layer of silica gel PF_{154} (E. Merck AG, Darmstadt, Germany). The compounds were detected by visual examination under UV light at both long and short wave. The elemental analyses were performed by Integral Microanalytical Laboratories, Raleigh, NC, and are within ±0.4% of the calculated value.

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucothiopyranosyl)-2-nitroimidazole (5). To a suspenison of **3** (2.50 g, 6.16 mmol),[14] **1** (0.80 g, 7.08 mmol), and mercuric cyanide (2.98 g, 12 mmol) in anhydrous acetonitrile (170 mL) was added stannic chloride (1.4 mL, 12 mmol). The resulting solution was stirred for 1.5 h at 60–70 °C and then evaporated to dryness. A filtered solution of the residue in methylene chloride (250 mL) was washed successively with saturated aqueous sodium bicarbonate, 30% potassium iodide, and water. The organic phase was dried (anhydrous Na_2SO_4) and evaporated to give an amorphous material (1.50 g, 53.2%) consisting of mostly **5** mixed with **3**. This material was purified by column chromatography (silica gel), with chloroform–methanol (99:1) as eluent, to give 0.66 g (23.6%) of **5**, which was recrystallized from ether–hexanes: mp 145 °C; UV (EtOH) λ_{max} 225 nm (ε 3394), 313 (5018); IR (KBr) 1745 (OAc), 1475 and 1350 (NO_2) cm^{-1}; NMR (CD_3OD) δ 1.83, 2.00, 2.07, 2.10 (4 s, 12 H, 4 OAc), 3.41–3.70 (m, $C_{5'}$ H), 4.10–4.50 (m, 2 H, $C_{6'}$ H), 5.10–5.67 (m, 3 H, $C_{2'-4'}$ H), 6.47 (d, $C_{1'}$ H, $J_{1',2'}$ = 10.1 Hz), 7.19, 7.37 (d, 2 H, $C_{4,5}$ H). Anal. ($C_{17}H_{21}N_3O_{10}S$) C, H, N.

1-β-D-Glucopyranosyl-2-nitroimidazole (6). Compound **4**[9] (0.98 g, 2.2 mmol) was dissolved in 12 mL of absolute methanol, and 3.0 mL of 0.1 M methanolic sodium methoxide was added. The mixture was stirred at 0 °C for 30 min and then evaporated under vacuum at below 5 °C. The yellow gummy precipitate was dissolved in 3.0 mL of warm methanol and allowed to stand at 4 °C overnight to give white crystals (0.17 g, 31.7%): mp 113 °C; UV (EtOH) λ_{max} 222 nm (ε 2406), 312 (3600); IR (KBr) 3450 (OH), 1475 and 1350 (NO_2) cm^{-1}; NMR (Me_2SO-d_6) δ 2.82–3.87 (m, 6 H, $C_{2'-6'}$ H), 4.63–5.54 (br m, 4 H, $C_{2'-6'}$ OH), 5.89 (d, $C_{1'}$ H, $J_{1',2'}$ = 8.06 Hz), 7.24, 7.86 (d, 2 H, $C_{4,5}$ H). Anal. $C_9H_{13}N_3O_7 \cdot 2H_2O$) C, H, N.

1-β-D-Glucothiopyranosyl-2-nitroimidazole (7). Compound **5** (459 mg, 1.0 mmol) was dissolved in 6 mL of absolute methanol, and 2.0 mL of 0.1 M methanolic sodium methoxide was added. The mixture was stirred at 0 °C for 5 h and then neutralized with Dowex 50X8 (H^+) and filtered through Celite. The filtrate was evaporated to 1 mL of volume under vacuum at <30 °C, which on cooling gave 100 mg of **7** (recrystallized from 95% ethanol). The filtrate was purified by preparative TLC, with ethyl acetate–methanol (3:1) as eluant, and a further crop of 54 mg of **7** (total yield 54.8%) was obtained: mp 219 °C dec; UV (EtOH) λ_{max} 230 nm (ε 3917), 315 (5500); IR (KBr) 3450–3300 (OH), 1480 and 1350 (NO_2) cm^{-1}; NMR (Me_2SO-d_6) δ 2.83–3.98 (m, 6 H, $C_{2'-6'}$ H), 4.86–5.54 (br m, 4 H, $C_{2'-6'}$ OH), 5.95 (d, $C_{1'}$ H, $J_{1',2'}$ = 9.97 Hz), 7.25 and 7.86 (d, 2 H, $C_{4,5}$ H). Anal. ($C_9H_{13}N_3O_6S$) C, H, N.

Methyl 2-Deoxy-2-(2-nitroimidazol-1-yl)-4,7,8,9-tetra-*O*-acetyl-*N*-acetyl-D-neuraminate (10). A mixture of **1** (0.50 g, 4.4 mmol), hexamethyldisilazane (24 mL), and ammonium sulfate (60 mg) was heated under reflux in pyridine (6.0 mL) for 1.5 h. The solvents were removed in vacuo at room temperature, leaving a solid residue of crude 1-(trimethylsilyl)-2-nitroimidazole (**8**).[9] To a clear solution of **8** in anhydrous acetonitrile (35 mL) was added a solution of 1.63 g (3.2 mmol) of **9**[15] in anhydrous acetonitrile (25 mL) and mercuric bromide (1.15 g, 3.2 mmol). The mixture was stirred for 2 days at room temperature and then evaporated to dryness. The residue was dissolved in ethanol (100 mL), and the solution was evaporated to dryness. A filtered solution of the residue in chloroform (300 mL) was washed successively with saturated aqueous sodium bicardonate (150 mL), 20% potassium iodide solution (150 mL), and water (150 mL). The organic phase was dried (anhydrous Na_2SO_4) and evaporated to dryness. The residual syrup was chromatographed on a column of silica gel G (100 g), with benzene–ethyl acetate (9:1 to 0:1) as eluant, to give 0.68 g (36.2%) of pure **10** as a foam: UV (EtOH) λ_{max} 212 nm (ε 2630), 313 (3180); IR (film) 1740, 1730 and 1720 (OAc), 1350 and 1470 (NO_2) cm^{-1}; NMR (CD_3OD) δ 1.90–2.20 (s, Ac) 2.43 (m, $C_{3'}$ H), 3.76–3.87 (s, $COOCH_3$), 4.03–5.31 (m, $C_{4'-9'}$

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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.

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Potential Radiosensitizing Agents. 5. 2-Substituted Benzimidazole Derivatives

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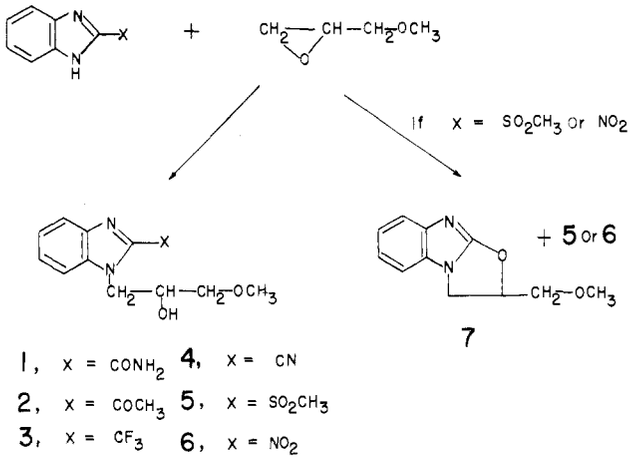
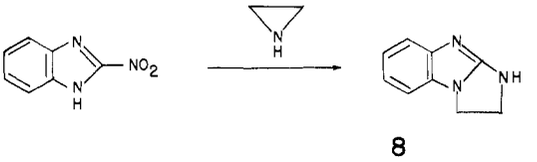
A series of 2-substituted benzimidazoles and their derivatives have been synthesized and tested for their ability to selectively sensitize hypoxic Chinese hamster cells (V-79) toward the lethal effect of ionizing radiation. These compounds were prepared by reacting the 2-substituted benzimidazoles with 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



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.
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(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.
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