The excess saline was expelled from the columns by centrifuging in an IEC Model CL benchtop centrifuge at speed settings of 3 and 5 for 120 ± 10 s each (800*g* and 1300*g*, respectively) for collection of eluate. After incubation for about 15 min, duplicate plasma samples were transferred to the Sephadex desalting columns. The columns were centrifuged so that the eluate was collected in standard counting tubes. The column was removed from the counting tube. The Sephadex gel was expelled by air into a separate counting tube. The eluate and the Sephadex gel were placed adjacently in the track of the γ-counter and their radioactivity assayed for 1 min or a σ error of 1%. The percent of bound plasma protein was calculated by the following formula:

$$\% \text{ bound protein} = \frac{\text{net cpm eluate}}{\text{net cpm (eluate + gel)}} \times 100$$

Acknowledgment. We express our appreciation to Messrs. Thomas Strubel, Steven J. Barco, and Gregory Cizek and Ms. Jo Ann Harris and Susan M. Wallach for their assistance with the experiments and to Dr. Randall Thummel (University of Houston) for making available trindan, *s*-indacene, and dodecahydrotriphenylene.

Synthesis and Evaluation of a Series of 3,5-Disubstituted Benzisoxazole-4,7-diones. Potent Radiosensitizers in Vitro

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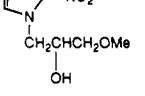
Received May 22, 1991

A series of 3,5-disubstituted-2,1-benzisoxazole-4,7-diones was synthesized and evaluated as radiosensitizers both in vitro and in vivo. These compounds were designed as non-nitro electron-affinic agents in an effort to alleviate some of the toxicities seen with the 2-nitroimidazole radiosensitizers evaluated in the clinic. Several compounds in this series were potent radiosensitizers in vitro, with sensitizer enhancement ratios of 2.0–2.3 at concentrations <0.5 mM. Compounds with potent in vitro activity were also evaluated in vivo. However, none of these compounds showed radiosensitizing activity in vivo. The reduction potentials of these compounds were determined by cyclic voltammetry and compared to other electron-affinic radiosensitizers. In general, the reduction potentials of this series of compounds was slightly more positive than the 2-nitroimidazoles, but they fell within the range postulated as acceptable to yield in vivo activity. The results suggest that factors other than reduction potential may be responsible for the lack of in vivo radiosensitizing activity observed for this class of radiosensitizers.

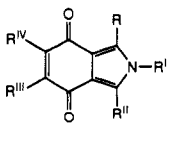
Introduction

In addition to the large number of chemical agents available for the treatment of human cancer, radiation therapy continues to be an important method for the local control of many types of tumors.[1,2] Several approaches for increasing the therapeutic benefit of radiation treatment have been examined.[3] One such approach has been the use of chemical agents which mimic the effects of oxygen.[1-3] Since oxygen mediates the lethal effects of radiation in cells, tumor cells in areas of diminished oxygen supply are radioresistant.[4] Therefore, "oxygen mimetics" have been investigated as a means to overcome this resistance.[5] Such compounds were designed to penetrate into tumors and react with radiation-produced free radicals to generate chemical species toxic to cells.[6,7] These so called "electron affinic agents" contain a reducible functionality and studies have shown that the ability of these compounds to act as radiosensitizers was related to their reduction potential.[8] If the reduction potential was too positive, the compound would be inactivated metabolically prior to reaching the tumor. If too negative, the compound would be unreactive and no sensitization would occur. It was demonstrated that nitroheterocycles with in vivo activity usually had one electron reduction potentials ($E^{1/7}$ values) in the range of −0.30 to −0.40 eV.[9]

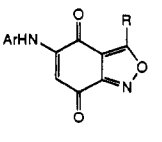
A variety of nitroheterocycles and quinones have been shown to sensitize cells to radiation in vitro by this mechanism. However, to date, only nitroimidazoles have displayed in vivo activity sufficient to conduct clinical trials.[1-3] One of the most widely studied nitroimidazoles has been misonidazole (1). Although effective as a ra-



1, Misonidazole



2, Isoindole-4,7-diones



3, Benzisoxazole-4,7-diones

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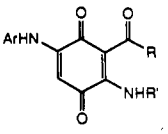
Table I. Physical Constants and Yields of the Substituted Quinones



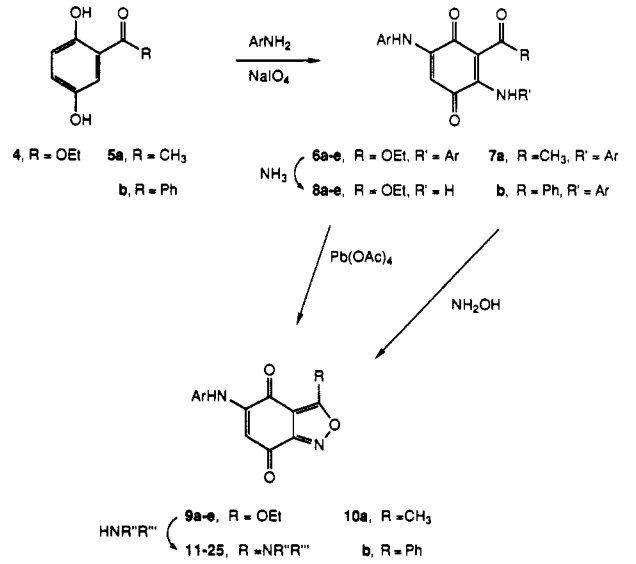
no.	R	R'	Ar	molecular formula	mp, °C	% yield	anal.
6a	OEt	Ph	Ph	$C_{21}H_{18}N_2O_4$	175 (d)	85	C, H, N
6b	OEt	4-FC_6H_4	4-FC_6H_4	$C_{21}H_{16}N_2O_4F_2 \cdot 0.5H_2O$	200–203	97	C, H, N
6c	OEt	4-ClC_6H_4	4-ClC_6H_4	$C_{21}H_{16}N_2O_4Cl_2 \cdot 0.5H_2O$	>300	91	C, H, N, Cl
6d	OEt	4-$CF_3C_6H_4$	4-$CF_3C_6H_4$	$C_{23}H_{16}N_2O_4F_6 \cdot 0.25H_2O$	195–197	83	C, H, N
6e	OEt	4-$CH_3C_6H_4$	4-$CH_3C_6H_4$	$C_{23}H_{22}N_2O_4$	206–207	88	C, H, N
7a	CH_3	Ph	Ph	$C_{20}H_{16}N_2O_3 \cdot 0.2H_2O$	176–179	82	C, H, N
7b	Ph	Ph	Ph	$C_{25}H_{18}N_2O_3 \cdot 0.05C_4H_8O_2$	206–208	24	C, H, N
8a	OEt	H	Ph	$C_{15}H_{14}N_2O_4 \cdot 0.5H_2O$	287–292	94	C, H, N
8b	OEt	H	4-FC_6H_4	$C_{15}H_{13}N_2O_4F \cdot 0.3H_2O$	294 (d)	85	C, H, N
8c	OEt	H	4-ClC_6H_4	$C_{15}H_{13}N_2O_4Cl \cdot 0.4H_2O$	285–297	88	C, H, N[a]
8d	OEt	H	4-$CF_3C_6H_4$	$C_{16}H_{13}N_2O_4F_3 \cdot 0.25H_2O$	287 (d)	93	C, H, N[b]
8e	OEt	H	4-$CH_3C_6H_4$	$C_{16}H_{16}N_2O_4 \cdot 0.25H_2O$	266–268	71	C, H, N

[a] Cl: calcd, 10.81; found, 12.07. [b] F: calcd, 15.89; found 16.35.

diosensitizer in animal models, the usefulness of this compound in the clinic has been limited due to its propensity to cause peripheral neuropathy.[10,11] Although this side effect has been attributed primarily to the log *P* of the compound,[12] some studies have shown that this toxicity may result ultimately from aerobic reduction of the nitro group.[13,14]

One approach for overcoming the toxicities of these compounds, while their usefulness as radiosensitizers is maintained, has been the use of non-nitro electron-affinic agents.[15] Some of the first non-nitro radiosensitizers to be investigated were quinones and naphthoquinones. They were shown to sensitize cells in vitro to radiation, but were generally inactive in vivo. This lack of in vivo activity was attributed to the ease with which the quinones thus far evaluated were reduced metabolically in vivo and thus inactivated. Infante et al.[16] later reported the in vivo radiosensitizing activity of a series of isoindole-4,7-diones (**2**). The activity displayed by this series of compounds was attributed to their favorable reduction potentials which, unlike other quinones studied previously, were comparable to the 2-nitroimidazoles. Unfortunately, these compounds were very insoluble and efforts to synthesize more soluble analogues were only moderately successful.[17]

Scheme I



Herein, we report our work on a series of 3,5-disubstituted-2,1-benzisoxazole-4,7-diones (**3**). These compounds were selected for their similarity to **2**, and their synthetic accessibility. The compounds were evaluated in vitro as radiosensitizers and their reduction potentials were determined. Selected compounds were also evaluated in vivo.

Chemistry

The compounds required for this study were prepared as illustrated in Scheme I. Compounds **9a–e** were prepared starting from the ethyl ester of gentisic acid (**4**),[18] in three steps using the previously described procedures:[19] (1) oxidation with sodioum periodate in the presence of various anilines provided quinones **6a–e**, (2) selective displacement of the arylamine adjacent to the carbonyl

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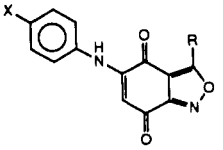
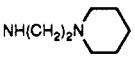
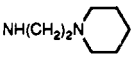
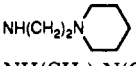
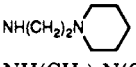
Table II. Physical Constants and Yields of 3,5-Disubstituted-1,2-benzisoxazole-4,7-diones



no.	X	R	molecular formula	mp, °C	% yield	recryst solvent	anal.
9a	H	OEt	$C_{15}H_{14}N_2O_4 \cdot 0.15H_2O$	123–124	66	*a*	C, H, N
9b	F	OEt	$C_{15}H_{11}N_2O_4F$	130–133	54	*a*	C, H, N, F
9c	Cl	OEt	$C_{15}H_{11}N_2O_4Cl \cdot 0.5H_2O$	117–120	55	*a*	C, H, N, Cl
9d	CF_3	OEt	$C_{16}H_{11}N_2O_4F_3 \cdot H_2O$	141–143	52	*a*	C, H, N, F
9e	CH_3	OEt	$C_{16}H_{14}N_2O_4 \cdot 0.25H_2O$	136–139	22	EtOAc/hex.	C, H, N
10a	H	CH_3	$C_{14}H_{10}N_2O_3$	196–200	59	*b*	C, H, N
10b	H	Ph	$C_{19}H_{12}N_2O_3$	215–218	53	*b*	C, H, N
11	H	$N(CH_2CH_3)_2$	$C_{17}H_{17}N_3O_3 \cdot 0.25H_2O$	175–176	65	*b*	C, H, N
12	H	$N(CH_2CH_2OH)_2$	$C_{17}H_{17}N_3O_5 \cdot H_2O$	178–182	67	*b*	C, H, N
13	H	$NHCH_2CH_3$	$C_{15}H_{13}N_3O_3$	146–149 (d)	69	*b*	C, H, N
14	H	$N(CH_3)(CH_2)_2N(CH_3)_2$	$C_{18}H_{20}N_4O_3 \cdot HCl \cdot 1.85H_2O$	156–158	65	*c*	C, H, N, Cl
15	H	$NH(CH_2)_2N(CH_3)_2$	$C_{17}H_{18}N_4O_3 \cdot HCl \cdot 0.5H_2O$	>300	55	*c*	C, H, N, Cl
16	H	$N(CH_3)(CH_2)_3N(CH_3)_2$	$C_{19}H_{22}N_4O_3$	154–156	40	*b*	C, H, N
17	H	$NH(CH_2)_3N(CH_3)_2$	$C_{18}H_{20}N_4O_3 \cdot HCl$	>300	61	*c*	C, H, N, Cl
18	H		$C_{20}H_{22}N_4O_3 \cdot HCl \cdot 0.5H_2O$	156	64	*c*	C, H, N, Cl
19	F		$C_{20}H_{21}N_4O_3F \cdot HCl \cdot 0.5H_2O$	174–175	61	*d*	C, H, N, F
20	F	$NH(CH_2)_3N(CH_3)_2$	$C_{18}H_{19}N_4O_3F \cdot 1.3HCl \cdot H_2O$	143–144	31	*d*	C, H, N, Cl, F
21	Cl		$C_{20}H_{21}N_4O_3Cl \cdot HCl \cdot 0.75H_2O$	175–176	38	*d*	C, H, N, Cl
22	Cl	$NH(CH_2)_3N(CH_3)_2$	$C_{18}H_{19}N_4O_3Cl \cdot HCl \cdot 1.4H_2O$	159–164	39	*d*	C, H, N, Cl
23	CF_3		$C_{21}H_{21}N_4O_3F_3 \cdot HCl \cdot 1.25H_2O$	175–177	61	*d*	C, H, N, F
24	CF_3	$NH(CH_2)_3N(CH_3)_2$	$C_{19}H_{19}N_4O_3F_3 \cdot HCl \cdot 1.5H_2O \cdot 0.56IPA$	145–147	49	*d*	C, H, N, F
25	CH_3	$NH(CH_2)_2N(CH_3)_2$	$C_{18}H_{20}N_4O_3 \cdot 0.25H_2O$	123–127 (d)	25	EtOH	C, H, N

[a] Compound crystallized from acetic acid and water. [b] Compound crystallized out of solution. [c] Compound was dissolved in EtOH or IPA; ethanolic HCl was added and the solid was collected. [d] Compound was dissolved in $CHCl_3$, HCl(g) was added, and the solid was collected.

group gave compounds **8a–e**, and (3) oxidative ring closure with $Pb(OAc)_4$ provided **9a–e**. Treatment of **9a–e** with various amines[20] provided compounds **11–25** in good yields. Compound **10a** was prepared from the corresponding ketone **5a** as was previously described.[21] Compound **10b** was prepared starting from **5b**,[22] which was first converted to **7b**.[23] Treatment with hydroxylamine provided the target compound **10b**.

Results

Table II shows the compounds synthesized for evaluation as radiosensitizers. The in vitro radiosensitizing activity (sensitizer enhancement ratio, SER) and reduction potentials (E^0) for these compounds are given in Table III. All of the compounds were evaluated for radiosensitizing activity at their highest noncytotoxic concentration as previously described.[24] This was done to maximize radiosensitization and to ensure that any enhancement of radiation-induced cell kill observed was a function of radiosensitization, not drug toxicity. Of the initial compounds synthesized (**9a, 10a,b, 12, 13**), most were too insoluble to evaluate as radiosensitizers. Only two compounds, **9a** and **12**, were soluble enough to test and both were active as radiosensitizers in vitro. Our efforts then

Table III. Biological Activity and Reduction Potentials of the 3,5-Disubstituted-1,2-benzisoxazole-4,7-diones

no.	SER[a]	concn,[b] mM	E^0,[d] V
9a	1.3	0.03	−0.72
9b	*c*	*c*	−0.68
9c	*c*	*c*	−0.63
9d	*c*	*c*	−0.65
9e	*c*	*c*	−0.71
10a	*c*	*c*	−0.67
10b	*c*	*c*	−0.63
11	*c*	*c*	−0.85
12	2.1	1.5	−0.82
13	*c*	*c*	−0.72
14	2.0	0.38	−0.81
15	1.5	0.19	−0.72
16	1.5	0.38	−0.86
17	1.7	0.19	−0.77
18	2.4	0.38	−0.78
19	1.6	0.29	−0.75
20	1.4	0.38	−0.72
21	1.9	0.19	−0.70
22	1.7	0.13	−0.69
23	1.6	0.13	−0.68
24	1.3	0.09	−0.70
25	1.2	0.05	−0.75

[a] Sensitizer enhancement ratio. [b] Concentration of highest nontoxic dose. [c] Compound was too insoluble to test. [d] Reduction potential (half-wave) determined by cyclic voltammetry.

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focused on increasing the solubility of these analogues. This was accomplished by substituting the 3-position with a variety of diamines (14–18). These compounds were highly soluble as their hydrochloride salts and displayed significant radiosensitizing activity. Two analogues, 14 and 18, were particularly potent, giving SER values of 2.0 and 2.4, respectively, at a concentration of 0.38 mM (misonidazole (1) in this assay gave an SER of 1.8 at 3.0 mM). We then set out to determine the effect substitution at the 5-position on the aromatic ring had on radiosensitizing activity. The *p*-fluoro, *p*-chloro, and *p*-(trifluoromethyl) analogues of the most potent unsubstituted analogue (18) were synthesized, as were a number of additional derivatives. All of the phenyl-substituted analogues were less active as radiosensitizers than the unsubstituted derivative 18. Those compounds containing the trifluoromethyl group (23, 24) were also more cytotoxic, as indicated by their reduced nontoxic concentrations. On the basis of these initial results, no additional substituted analogues were synthesized.

The half-wave reduction potentials of these compounds were determined using cyclic voltammetry[25,26] (see the Experimental Section) and were compared to both compounds 1 and 2. The reduction potentials (E^0) of the benzisoxazole-4,7-diones presented ranged from −0.63 to −0.86 V and were similar to those of the isoindole-4,7-diones examined (2, E^0 = −1.01 V, R = R^{IV} = CH_3, R^{II} = Ph, R^I = R^{III} = H). In general, they were somewhat less negative, but within acceptable levels.[27] Phenyl-substituted analogues 22–24 had reduction potentials that were somewhat more positive than the unsubstituted analogues and this may account for their diminished radiosensitizing activity and increased cytotoxicity in vitro.

Compounds 14, 18, and 22 were evaluated in vivo as radiosensitizers at their maximum tolerated doses (500, 125, and 100 mg/kg, respectively) by the previously described method.[24] None of these compounds resulted in any significant reduction in clonogenic cell survival.

Discussion

The results indicate that several of the 3,5-disubstituted-2,1-benzisoxazole-4,7-diones were potent radiosensitizers of hypoxic cells[28] in vitro, but unfortunately these compounds had no activity in vivo. A number of reasons could account for this lack of in vivo activity. The first is that the compounds may have been too toxic (in general) to the animals to be administered in quantities sufficient to achieve tumor levels needed for radiosensitization. This could be due to the reactivity of the quinone portion of these compounds. A second explanation for their inactivity could be that the compounds did not penetrate into the tumors. The third reason, and the one used to explain the lack of in vivo activity of other quinones previously evaluated, could be that the reduction potentials of the benzisoxazole-4,7-diones were still too positive and the compounds were inactivated metabolically in vivo. The reduction potentials of these compounds fall into the range thought to be acceptable for in vivo radiosensitizing activity, but this range was defined for nitroheterocycles. It may be that when evaluating this class of compounds, the parameters obtained for nitroheterocycles no longer apply. For example, the reversibility of the reduction/oxidation of quinones represents a major difference between these classes of compounds. The reduction of 2-nitroimidazoles, as measured by cyclic voltammetry, was not reversible. Additionally, quinones are reduced metabolically by a different group of reductases.[29] Therefore, it may be necessary to establish different parameters to predict in vivo activity of quinone radiosensitizers.

In summary, we have evaluated a series of 3,5-disubstituted-2,1-benzisoxazole-4,7-diones. These compounds were potent radiosensitizers in vitro, but were inactive in vivo. They do, however, represent a novel class of nonnitro radiosensitizers and provide some evidence that the parameters used to define in vivo activity for quinone type radiosensitizers may be different than those used for nitroheterocycles. Studies are now in progress using the data herein as a guide for further modification of these compounds with the aim of achieving radiosensitizing activity in vivo.

Experimental Section

Chemical Synthesis. All melting points were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Associates EM-390 or XL-200 or an IBM WP 100sy spectrometer using $CDCl_3$ or DMSO-d_6 as the internal reference standard. Purity was determined by microanalysis and by TLC (silica gel 60F 254, Merck). Silica gel chromatography utilized Kieselgel 60 (70–230 mesh or 230–400 mesh for flash chromatography). Mass spectra were determined on a VG analytical 7070E/HF or Finnegan 4500 mass spectrometer. The half-wave reduction potentials were performed on a CV-27 voltammetry unit from Bioanalytical Systems. The amines were commercially available for Aldrich Chemical Co. The DMF used in cyclic voltammetry studies was freshly distilled from CaH_2. All compounds possessed analytical data consistent with the proposed structures. Spectral data are provided as supplemental material.

General Procedure for the Synthesis of 3,5-Disubstituted-2,1-benzisoxazole-4,7-diones (Compounds 11–25, Table II). Synthesis of 3-(Diethylamino)-5-(phenylamino)-2,1-benzisoxazole-4,7-dione (11). A mixture of **9a**[19] (0.86 g, 3.4 mmol) and diethylamine (2 mL, 19 mmol) in ethanol (75 mL) was stirred for 3 h at room temperature. The mixture was concentrated and the resulting solid was triturated with hexanes to provide 11 (0.70 g, 65%); mp 175–176 °C; ^{1}H NMR ($CDCl_3$) δ 1.35 (t, 6 H), 3.92 (q, 4 H), 6.23 (s, 1 H), 7.21–7.50 (m, 5 H), 8.02 (bs, 1 H); MS (EI$^+$) *m/e* 311.

General Procedure for the Synthesis of 3-Ethoxy-5-[(substituted-phenyl)amino]-2,1-benzisoxazole-4,7-diones (ref 19). Synthesis of 3-Ethoxy-5-[(4-fluorophenyl)amino]-2,1-benzisoxazole-4,7-dione (9b). 2,5-Bis[(4-fluorophenyl)amino]-3,6-dioxo-1,4-cyclohexadiene-1-carboxylic Acid, Ethyl Ester (6b, Table I). To a solution of 4 (20 g, 110 mmol) in methanol (750 mL) and H_2O (1 L), cooled to 0 °C, was added *p*-fluoroaniline (22.8 mL, 241 mmol) and sodium metaperiodate (28 g, 132 mmol). The resulting dark brown reaction was stirred at 0 °C for 1 h and then at room temperature for 18 h. The mixture was filtered and the solid was washed with 2-PrOH and dried to provide **6b** (42.3 g, 97%): mp 200–203 °C dec; ^{1}H NMR ($CDCl_3$) δ 1.07 (t, 3 H), 3.65 (q, 2 H), 5.95 (s, 1 H), 7.05–7.25 (m, 8 H), 8.09 (s, 1 H), 8.63 (bs, 1 H); MS (DEI) *m/e* 398. Anal. C, H, N.

(25) Mori, A.; Kusaba, T.; Isamaya, Y.; and Takeshita, H. Cyclic Voltammetry of *p*-Tropoquinones. *Chem. Lett.* **1986**, 155–156.

(26) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods*; Rossiter, B. W., Hamilton, J. F., Eds.; Wiley: New York, 1980; pp 230–231.

(27) The acceptable range for radiosensitization, as determined by cyclic voltammetry, was −0.80 to −1.20 V. This was derived by selecting a group of compounds (six nitroimidazoles and two quinones) whose one electron reduction potentials had been previously published and determining their reduction potentials in our system. An equation was derived $E^0 = (E^{1/7} - 0.088)/0.48$. Then using the estimated range of one electron reduction potentials required for in vivo activity, the values of −0.80 to −1.20 V were derived.

(28) No oxic cell sensitization was observed with any of these compounds.

(29) Smith, D.; Martin, L. F.; Wallin, R. Human DT-diaphorase, A Potential Cancer Protecting Enzyme. Its Purification Form Abdominal Adipose Tissue. *Cancer Lett.* **1988**, *42*, 103–112.

2-Amino-5-[(4-fluorophenyl)amino]-3,6-dioxo-1,4-cyclohexadiene-1-carboxylic Acid, Ethyl Ester (8b). To a solution of **6b** (40 g, 0.1 mol) in $CHCl_3$ (900 mL) was added a solution of NH_3 in MeOH (3.0 M, 138 mL). The mixture was stirred at room temperature for 18 h and the resulting red solid was filtered and washed with $CHCl_3$ and dried to provide **8b** (25.9 g, 85%): mp 294 °C dec; ^1H NMR ($CDCl_3$) δ 1.28 (t, 3 H), 4.23 (q, 2 H), 5.71 (s, 1 H), 7.18–7.42 (m, 4 H), 8.82 (s, 1 H), 9.21 (bs, 1 H), 9.63 (bs, 1 H); MS (DEI) *m/e* 304. Anal. C, H, N.

3-Ethoxy-5-[(4-fluorophenyl)amino]-2,1-benzisoxazole-4,7-dione (9b). To a suspension of **8b** (22.9 g, 75.4 mmol) in $CHCl_3$ (1600 mL) and THF (900 mL) was added $Pb(OAc)_4$ (67.75 g, 0.15 mol). The mixture was stirred for 1.5 h and concentrated and the residue dissolved in acetic acid. The solution was poured into H_2O (6 L) and the yellow precipitate was filtered, washed with H_2O and 2-PrOH, and dried to provide **9b** (11.6 g, 54%): mp 130–133 °C; ^1H NMR ($CDCl_3$) δ 1.61 (s, 3 H), 4.85 (q, 2 H), 6.14 (s, 1 H), 7.10–7.29 (m, 4 H), 7.69 (bs, 1 H); MS (DEI) *m/e* 302. Anal. C, H, N, F.

3-Phenyl-5-(phenylamino)-2,1-benzisoxazole-4,7-dione (10b). A mixture of hydroxylamine hydrochloride (1.23 g, 17.8 mmol) and anhydrous sodium acetate (1.76 g, 22.2 mmol) were suspended in methanol (80 mL) and stirred at room temperature for 1 h. The mixture was filtered and the filtrate was added to a solution of **7b** (3.5 g, 8.9 mmol) in methanol (35 mL). This was heated under reflux for 1.5 h, cooled and evaporated. The resulting solid was triturated with ethyl acetate to provide **10b** (1.5 g, 53%): mp 215–218 °C; ^1H NMR (DMSO-d_6) δ 6.02 (s, 1 H), 7.27–7.45 (m, 5 H), 7.63–7.78 (m, 3 H), 8.45 (d, 2 H), 9.67 (bs, 1 H); MS (EI^+, M + 1) *m/e* 316. Anal. C, H, N.

Cyclic Voltammetry. The half-wave reduction potentials were determined using the following general procedure: The compounds (5×10^{-5} mol) were dissolved in 0.1 N $(nBu)_4NBF_4$/DMF[25] (12 mL) and degassed with argon. The compounds were placed under a blanket of argon. The scans were run at room temperature using a glassy carbon working electrode, platinum wire counter electrode and silver/silver chloride reference electrode at 100 mV/scan. The values given in Table II (E^0) were calculated directly from the scans according to the procedure of Bard and Faulkner.[26]

Biological Studies. In Vitro. Chinese hamster V79-171b cells, maintained as monolayer cultures, were plated in 60-mm glass Petri dishes in RPMI 1640 medium containing 10% fetal calf serum and allowed to incubate for approximately 18 h at 37 °C. On the day of treatment, the medium was removed from dishes and replaced with fresh medium. For radiosensitization studies, solutions of these compounds were freshly prepared and were added directly to the medium in dishes. The highest nontoxic dose of each compound was used and is defined as the concentration of compound producing no cytotoxicity to either oxic or hypoxic cells during a 1-h incubation at room temperature.

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

Biological Evaluation. In Vivo. The tumor excision assay used to evaluate these compounds was described previously.[24] Mice bearing KHT fibrosarcoma (200–500 mg), containing a hypoxic fraction of ca. 20%, were treated with vehicle or test compound at a dose previously determined to be the maximum tolerated dose (MTD). The compounds were administered at varying times (30, 60, 90, and 120 min) prior to irradiation with a 15 Gy X-ray dose. Eighteen hours after X-ray treatment, animals were sacrificed by cervical dislocation, their tumors were excised, and a single-cell suspension was prepared. Clonogenic survival was determined by plating in soft agar in multiwell plates. After incubation for 12–16 days cells were stained and counted.

Acknowledgment. We thank Dr. F. MacKellar, Dr. G. McClusky, and their staff for their spectroscopic and analytical support and K. Hamelehle, M. Havlick, and P. Vincent for their technical assistance in performing the in vivo assays.

Supplementary Material Available: ^1H NMR data for **6a–e**, **7a,b**, **8a–e**, **9a–e**, **10a,b**, and **11–25** (4 pages). Ordering information is given on any current masthead page.