Hypoxia-Selective Antitumor Agents. 15. Modification of Rate of Nitroreduction and Extent of Lysosomal Uptake by Polysubstitution of 4-(Alkylamino)-5-nitroquinoline Bioreductive Drugs

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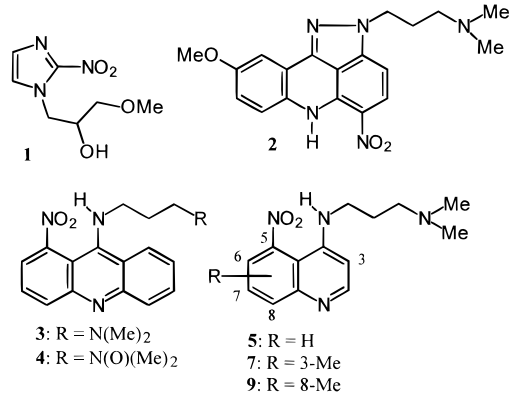
Studies have shown that 4-(alkylamino)-5-nitroquinolines possess high selectivity (20−60-fold) for hypoxic tumor cells *in vitro*, but are not active as hypoxia-selective cytotoxins (HSCs) *in vivo*. The compounds show inadequate rates of extravascular diffusion, likely due both to sequestration of the bisbasic compounds into lysosomes and rapid nitroreduction. A further series of analogues, designed to counteract these limitations, has been synthesized and evaluated. Analogues bearing one to three electron-donating substituents on the quinoline have one-electron reduction potentials up to 100 mV lower than that of the unsubstituted compound (**5**), but do not have improved biological activity. The relationship between hypoxic selectivity and rates of metabolic reduction suggests at least two mechanisms of cytotoxicity for this series of 5-nitroquinolines. Compounds with high rates of reduction are toxic via oxygen-sensitive net bioreduction, while compounds which are poor substrates for nitroreduction are toxic through an oxygen-insensitive non-bioreductive mechanism. As rates of metabolic reduction are lowered, the non-bioreductive mechanism of toxicity becomes dominant and hypoxic selectivity is lost. A small series of analogues bearing hydrophilic but neutral side chains were also prepared. Compounds with a dihydroxypropyl side chain retained cytotoxic potency and hypoxic cell selectivity in cell culture assays, and had lowered uptake into lysosomes, but none of three analogues evaluated against KHT tumors in mice showed activity as an HSC *in vivo*.

Hypoxic cells in tumors are resistant to ionizing radiation, and probably to some chemotherapeutic drugs, and are therefore important targets for drug development. [1−3] A number of different nitroheterocycles have been shown to act as hypoxia-selective cytotoxins (HSCs), possessing selective toxicity to hypoxic mammalian cells by virtue of their oxygen-inhibited bioreduction to reactive species. Examples include 2-nitroimidazoles such as misonidazole (**1**),[1] 4-nitropyrazoloacridines such as (**2**),[4] and 1-nitroacridines such as nitracrine (**3**).[5−7] The 1-nitroacridines are particularly interesting because of their high potency; **3** shows similar hypoxic selectivity to **1** toward AA8 cells in culture (ca. 10-fold) but is 100000-fold more potent.[8] However, **3** showed no activity against hypoxic cells in solid tumors *in vivo*.[5,9] Major factors contributing to this were considered to be limited diffusion due to a combination of intercalative DNA binding ($K_{DNA} = 2.2 \times 10^5$ M^{-1} at 0.01 ionic strength, pH 7, 22 °C)[10] and rapid reductive metabolism related to a high reduction potential (−303 mV).[5,7]

Various strategies to lower the reversible DNA binding affinity of the nitroacridine **3** while retaining potency and hypoxic selectivity have been explored. Masking the side chain cationic charge with an *N*-oxide group gave a compound (**4**) of lower binding affinity



($K_{DNA} = 1.5 \times 10^4$ M^{-1} at 0.01 ionic strength, pH 7, 22 °C) and greatly enhanced hypoxic selectivity (the *N*-oxide is also bioreducible).[10] In an alternative approach, involving a more substantial structural change, we explored structure−activity relationships for cytotoxic potency and hypoxic selectivity among analogous 5-nitroquinolines (e.g., **5**), possessing one less aromatic ring in the chromophore.[11−13] The nitroquinoline **5** has a lower DNA binding affinity than **3** ($K_{DNA} = 3.6 \times 10^3$ M^{-1} at 0.03 ionic strength, pH 7, 25 °C)[14] but comparable hypoxic selectivity in AA8 cell cultures (ca. 15-fold). It also showed superior activity to **3** toward EMT6 spheroids (suggesting improved extravascular diffusional properties).[12] However, neither **5** nor its 3- and 8-methyl analogues (**7** and **9**) were active as HSCs or as radiosensitizers of hypoxic cells in SCCVII tumors.[9,13]

The 5-nitroquinolines evaluated to date generally have reduction potentials from −280 to −320 mV, which

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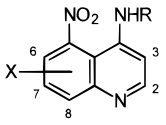
Table 1. Physicochemical and Biological Properties of 4-(Alkylamino)-5-nitroquinolines



					growth inhibition assays[c]		clonogenic assays (AA8)[d]		rate of reduction[e]
no.	X	R	pK_a[a]	$E(1)$[b]	AA8 (air) IC_{50} (μM)	AA8/UV4 ratio	CT_{10} (air) (μM h)	air/N_2 ratio	((nmol/min)/10^6 cells)
5[f]	H	$(CH_2)_3NMe_2$	6.39	−286	12.4	14	660	14	1.47
6[f]	2-Me	$(CH_2)_3NMe_2$	6.41	−274	6.6	17	770	22	1.75
7[f]	3-Me	$(CH_2)_3NMe_2$	6.01	−369	33	18	1870	47	0.28
8[f]	6-Me	$(CH_2)_3NMe_2$	6.15	−319	170	9	3300	16	0.16
9[f]	8-Me	$(CH_2)_3NMe_2$	6.84	−316	29	13	2100	60	0.96
10[f]	8-OMe	$(CH_2)_3NMe_2$	6.76	−310	6.8	12	3090	21	0.89
11[f]	8-NHMe	$(CH_2)_3NMe_2$	4.22	−520	98	2.3	2100	1.3	0.05
12	6-Et	$(CH_2)_3NMe_2$	(6.2)[g]	−294 ± 11[h]	108 ± 33	3.8 ± 0.6	3070 ± 410	4.8 ± 1.7	0.22
13	2,3-diMe	$(CH_2)_3NMe_2$	6.64	−320	47 ± 6	6.0 ± 2.3	2960 ± 260	33 ± 2	0.48
14	3,6-diMe	$(CH_2)_3NMe_2$	6.07	−367 ± 8	201 ± 20	1.4 ± 0.2	6630 ± 1890	1.1 ± 0.1	< 0.03
15	3,8-diMe	$(CH_2)_3NMe_2$	6.10	−334 ± 10	277 ± 28	33 ± 13	3790 ± 260	20 ± 8	0.18
16	6,8-diMe	$(CH_2)_3NMe_2$	6.37	−329 ± 12[h]	74 ± 8	17 ± 2	1780 ± 290	4.3 ± 1.3	0.16
17	2-Ph,8-Me	$(CH_2)_3NMe_2$	5.43	−342 ± 10	12 ± 2	2.6 ± 0.2	300	>1	
18	6,8-diOMe	$(CH_2)_3NMe_2$	6.25	−304 ± 10	186 ± 40	1.3 ± 0.3	3990	4.8	
19	2,3,8-triMe	$(CH_2)_3NMe_2$	6.67	−342	113 ± 13	3.0 ± 0.3	830 ± 350	3.7 ± 2.4	
20	2,3-diMe,8-OMe	$(CH_2)_3NMe_2$	6.64	−374 ± 10	166 ± 25	28 ± 6	10500	6.3	
21	H	$CH_2CH(OH)CH_2OH$	6.81	−276	33 ± 15	5.9 ± 1.2	950 ± 100	34 ± 10	0.71

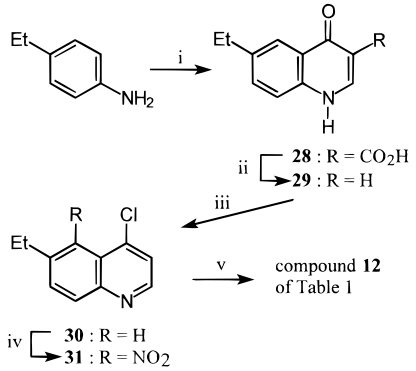
[a] Quinoline pK_a values were determined in aqueous solution at 25 °C by spectrophotometry; see ref 36. [b] $E(1)$ values for one-electron reduction were determined by pulse radiolysis; see refs 13 and 17. Values with standard errors are new determinations. [c] Growth inhibition assay described in the text. IC_{50} determined against aerobic AA8 or UV4 cells, using an exposure time of 18 h. AA8/UV4(air) ratio = hypersensitivity factor (HF) = IC_{50}(AA8)/IC_{50}(UV4), for an 18 h drug exposure under aerobic conditions. AA8(air/N_2) ratio = IC_{50}(AA8: air)/IC_{50}(AA8:N_2). [d] Clonogenic assay described in the text. CT_{10} is the product of the drug concentration (μM) and exposure time (hr) needed to reduce cell survival to 10% of controls, using AA8 cells at 10^6/mL in the clonogenic assay (see text). CT_{10} ratio = CT_{10}(air)/CT_{10}(nitrogen). [e] Rate of one electron reduction measured as oxygen consumption induced by 200 μM drug in cyanide-inhibited cells. Data from ref 20. [f] Data from ref 13. [g] Estimated pK_a. [h] Data from ref 20, corrected assuming the $E(1)$ for the redox indicator benzyl viologen to be −374 mV.[21]

is probably at the upper end of the desired range. In this paper we complete our examination of the class of 5-nitro-4-[[(dimethylamino)propyl]amino]nitroquinolines by reporting the synthesis and evaluation of a series of polysubstituted analogues (**13**−**20**). The activities of these compounds, designed to have lower reduction potentials, are compared with selected monosubstituted derivatives prepared previously. It has also recently been suggested that high lysosomal uptake of dibasic nitroquinolines may be a major factor limiting their ability to diffuse in tumor tissue.[14] The present study thus also evaluates the properties of several analogues (**21**−**25**) bearing hydrophilic but neutral side chains (Table 2). Two alternative basic side chain variants (**26** and **27**) were also investigated.

Chemistry

Most of the required 4-chloroquinolines were known, and new ones were prepared by either the Gould−Jacobs[15] (Scheme 1) or Conrad−Limpach[16] procedures. Preparation of the alkyl-substituted compounds of Table 1 was by nitration of the requisite 4-chloroquinolines in fuming HNO_3/concentrated H_2SO_4, followed by displacement of the 4-chloro group with excess *N*,*N*-dimethyl-1,3-propanediamine at 100 °C.[13] Analogues **21**, **23**, and **24**, containing hydroxylated side chains, were prepared by a minor variation of this route, coupling in DMSO. As noted previously,[13] reaction of methoxy-containing 5-nitroquinolines under these conditions resulted in preferential displacement of the nitro group, and compounds **18**, **20**, and **25** were thus prepared by nitration of the preformed 4-[[(dimethylamino)propyl]amino]quinolines (Schemes 2 and 4). The 2-phenyl analogue (**17**) was prepared (Scheme 3) by

Scheme 1[a]

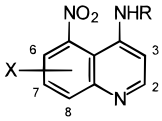


[a] (i) EtOCH=C(CO_2Et)$_2$/120 °C/1 h, then Dowtherm A/reflux, then OH^-; (ii) benzophenone/275 °C; (iii) $POCl_3$/reflux; (iv) concentrated H_2SO_4/fuming HNO_3/0−10 °C/20 min; (v) $H_2N(CH_2)_3NMe_2$/100 °C/3.5 h (N_2).

condensation of 2-methyl-5-nitroaniline with ethyl benzoylacetate, followed by thermal cyclization of the resulting acrylate (**35**) to give the quinolone (**36**), which was elaborated to **17** by standard methods.

One-electron reduction potentials $E(1)$ (in millivolts) for the nitroquinolines were determined by pulse radiolysis using published procedures,[13,17] by establishing a reversible equilibrium against suitable redox indicators. The equilibrium constant K for the reaction was determined spectrophotometrically for each compound, and the $E(1)$ for the $ArNO_2/ArNO_2^{\bullet-}$ redox couples calculated from this are recorded in Tables 1 and 2. The solubilities of the compounds were determined in both water and culture medium by UV spectrophotometry; all the compounds were soluble to concentrations of at least 45 mM.

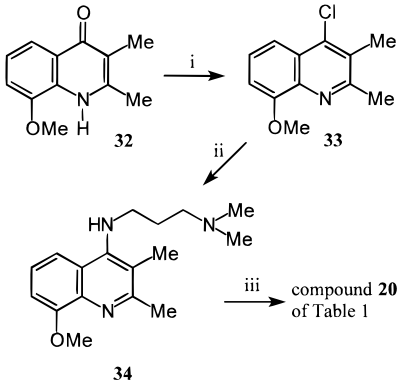
Table 2. Physicochemical and Biological Properties of 5-Nitroquinolines with Neutral or Alternative Basic Side Chains



					growth inhibition assays[c]		clonogenic assays (AA8)[d]	
no.	X	R	pK_a[a]	$E(1)$[b]	AA8 (air) IC_{50} (μM)	AA8/UV4 ratio	CT_{10} (air) (μM h)	air/N_2 ratio
21	H	$CH_2CH(OH)CH_2OH$	6.81	−276	33 ± 15	5.9 ± 1.2	950 ± 100	34 ± 10
22	H	$(CH_2)_3Me$	7.18	−294 ± 10	9.6 ± 0.4	>15.3	350 ± 120	20 ± 6
23	H	$(CH_2)_3OH$	7.06	−265 ± 10	26 ± 7	15 ± 2	1100	10
24	8-Me	$CH_2CH(OH)CH_2OH$	(7.2)[e]	−302 ± 10	110 ± 24	3.7 ± 0.2	2450 ± 350	7.4 ± 2.6
25	8-OMe	$CH_2CH(OH)CH_2OH$	(7.1)[e]	−355 ± 11	50 ± 8	11 ± 1	2370 ± 250	6.2 ± 1.5
26	H	$(CH_2)_2$morpholide	6.78	−278	18 ± 9	11 ± 4	860 ± 130	10 ± 1
27	H	$NHPhpCONH(CH_2)_2NMe_2$	5.08	−352 ± 10	13 ± 2	12 ± 3	440 ± 120	2.1 ± 0.1

[a] Quinoline pK_a values were determined in aqueous solution at 25 °C by spectrophotometry; see ref 36. [b] $E(1)$ values for one-electron reduction were determined by pulse radiolysis; see refs 13 and 17. [c] Growth inhibition assay described in the text. IC_{50} determined against aerobic AA8 or UV4 cells, using an exposure time of 18 h. AA8/UV4(air) ratio = hypersensitivity factor (HF) = IC_{50}(AA8)/IC_{50}(UV4) for an 18 h drug exposure under aerobic conditions. AA8(air/N_2) ratio = IC_{50}(AA8:air)/IC_{50}(AA8:N_2). [d] Clonogenic assay described in the text. CT_{10} is the product of the drug concentration (μM) and exposure time (h) needed to reduce cell survival to 10% of controls, using AA8 cells at 10^6/mL in the clonogenic assay (see text). CT_{10} ratio = CT_{10}(air)/CT_{10}(nitrogen). [e] Estimated pK_a.

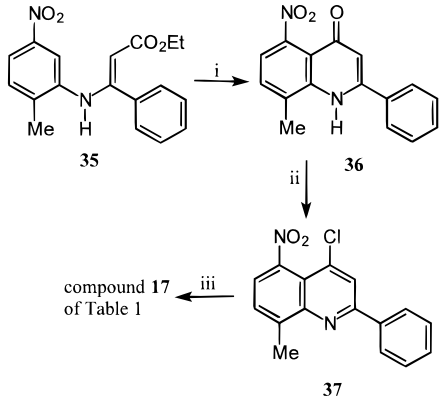
Scheme 2[a]



32 33 34

[a] (i) $POCl_3$/reflux; (ii) $H_2N(CH_2)_3NMe_2$/excess phenol/140 °C; (iii) concentrated H_2SO_4/fuming HNO_3/0−10 °C/5 min.

Scheme 3[a]



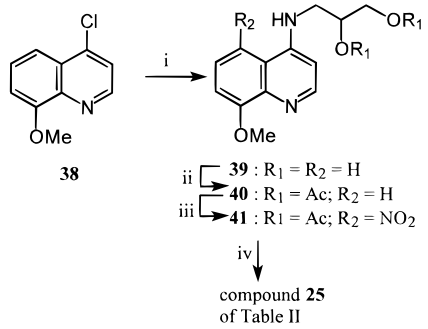
35 36 37

[a] (i) Dowtherm A/reflux; (ii) $POCl_3$/reflux; (iii) $H_2N(CH_2)_3NMe_2$/100 °C/3.5 h (N_2).

Biological Studies

Aerobic cytotoxicities were determined against both AA8 and UV4 cells using a growth inhibition assay[7] as IC_{50} values (the drug concentration required to reduce cell numbers to 50% of those in untreated controls on the same 96-well dish). The UV4 cell line is a repair-defective mutant, derived from AA8, that is ≥ 2-fold hypersensitive to agents whose cytotoxicity results from formation of bulky DNA adducts or crosslinks.[18] The ratio of IC_{50} values in the two cell lines can thus provide initial information on mechanisms of cytotoxicity.

Scheme 4[a]



38

[a] (i) $H_2NCH_2CHOHCH_2OH$/phenol/145 °C/1 h; (ii) Ac_2O/pyridine/90−100 °C/10 min; (iii) concentrated H_2SO_4/KNO_3/−5 °C/10 min; (iv) MeOH/2 N aqueous Na_2CO_3/reflux/5 min.

Hypoxic selectivity was determined by clonogenic assay, where AA8 cells in the early plateau phase were exposed to drugs in continuously-gassed stirred suspension cultures, and survival was assessed at various times by determining plating efficiency as described previously.[6,8] Cytotoxicity under aerobic and hypoxic conditions was determined as the CT_{10} value (the drug concentration multiplied by the time required to reduce cell survival to 10% of controls), and the ratio of these values was used to assess hypoxic selectivity. Cellular uptake in aerobic cell cultures, both with and without the lysosomotropic agent ammonium chloride to raise lysosomal pH,[14] was determined by HPLC. The *in vivo* activity of compounds **21**, **24**, and **25** against the aerobic and hypoxic subpopulations of cells in KHT tumors was evaluated by clonogenic assay, using ionizing radiation to selectively kill the oxygenated tumor cells.[19]

Results and Discussion

The physicochemical and biological properties of the compounds are listed in Tables 1 and 2. The data show that substitution with multiple electron-donating groups generally results in a lowering of one-electron reduction potential (measured at pH 7). As has been shown

previously,[13,22] this does not always occur with aromatic weak bases like nitroquinolines, where the ability of electron-donating substituents to lower reduction potential by inductive effects can be counteracted by their concomitant ability to increase base strength, resulting in a higher proportion of the ionized form under physiological conditions. However, the majority of the compounds in Table 1 showed little increase in base strength; for example the 2,3,8-trimethyl derivative (**19**) has a pK_a little different to that of the 8-methyl compound (**9**). Nevertheless, the structure−activity relationships for reduction potential among the polysubstituted 5-nitroquinolines are not straightforward. Among the monomethyl analogues (**6**−**9**), the 3-derivative showed the lowest $E(1)$. This may relate both to the relatively low pK_a and (possibly) to steric crowding among the adjacent 3-, 4-, and 5-substituents. While the monosubstituted derivatives **6**−**11** showed widely varying IC_{50} values in the growth inhibition assay, all, with the exception of **11,** showed substantial hypoxic selectivity in the clonogenic assay (15−60-fold).[13] The 8-methylamino derivative (**11**) had the lowest $E(1)$ (−520 mV) of the compounds investigated in this study, and although it showed reasonable potency in the clonogenic assay, it lacked hypoxic selectivity.

The 6-Et analogue (**12**) was prepared to explore the effects of larger groups *ortho* to the nitro group, but no major differences were found between it and the 6-methyl analogue **8**. Both had relatively high $E(1)$s (around −300 mV) and moderate cytotoxic potency and hypoxic selectivity, although **12** was only half as selective under hypoxic conditions (ratio 6.5-fold). Derivatives with larger groups at the 6-position (iPr, tBu) were sought, but could not be prepared (nitration of the corresponding 4-chloro-6-alkylquinolines gave complex mixtures of products, presumably due to increased steric hindrance to nitration at the 5-position). The dimethyl derivatives **13**−**16** showed a range of $E(1)$ values (from −320 to −367 mV). The 2,3-dimethyl derivative (**13**) showed high selective toxicity under hypoxic conditions (34-fold), with the 6,8-dimethyl derivative (**16**) having hypoxic selectivity about 10-fold lower. The dimethyl analogue of lowest reduction potential (the 3,6-dimethyl derivative **14**) showed low potency and no hypoxic selectivity. It has previously been shown[20,23] that **14** is a poor substrate for nitroreduction. The 2,3,8-trimethyl analogue **19** also had a low reduction potential (−342 mV) and poor hypoxic selectivity (3.7-fold) although its cytotoxic potency was high.

Because the 8-methoxy derivative (**10**) showed a significant drop in reduction potential, relative to the unsubstituted compound (**5**), while retaining high potency and good hypoxic selectivity,[13] the related compounds **18** and **20** were evaluated. The 2,3-dimethyl-8-methoxy derivative **20** had the lowest $E(1)$ of the polysubstituted derivatives (−374 mV) and the lowest aerobic toxicity in the clonogenic assay. The 6,8-dimethoxy derivative (**18**) had similar potency to **10**, although it, and **20**, showed significantly lower hypoxic selectivity.

This series of 5-nitroquinolines, with a wide range in $E(1)$ and hypoxic selectivity, provides an opportunity to examine the relationship between reduction potential, rate of reduction, and hypoxic selectivity within a congeneric series. For bioreductive drugs, reduction potential is often considered a surrogate measure of the rate of metabolic reduction, and a correlation between cytotoxic potency and $E(1)$ can therefore be expected.[24] However, we have previously reported[20,23] for a subset of these nitroquinolines (**5**, **7**, **9**, **11**, **14**, and **15**) that $E(1)$ is not the sole determinant of rates of metabolic reduction as steric interactions have a larger effect on enzymatic reduction than on $E(1)$. We also showed that the rate of drug-stimulated oxygen consumption in cyanide-inhibited AA8 cell suspensions is equal to the rate of net nitroreduction under hypoxia, and that this is a useful measure of the rate of the initial (one-electron) enzymatic reduction in this series.[20,23] Consistent with this, if the rate of O_2 consumption (Table 1) is used as a measure of enzymatic nitroreduction, there is a significant positive correlation between rates of nitroreduction and hypoxic cytotoxic potency (Figure 1A):

$$\log_{10}(\text{hypoxic } CT_{10}) = (1.65 \pm 0.13) - (1.15 \pm 0.18)\log_{10}(\text{rate of reduction}) \quad (1)$$

$$r = 0.890, \quad p < 0.001, \quad F = 42$$

whereas the relationship between $\log_{10}$(hypoxic cytotoxic potency) and $E(1)$ is less strong ($r = 0.573$, $p = 0.04$, $F = 5.4$).

In contrast to hypoxic cytotoxicity, aerobic cytotoxic potency showed less dependence on rates of nitroreduction as measured by drug-induced O_2 consumption (Figure 1A), the linear regression of $\log_{10}$(aerobic CT_{10}) versus $\log_{10}$(rate of reduction) giving a lower gradient

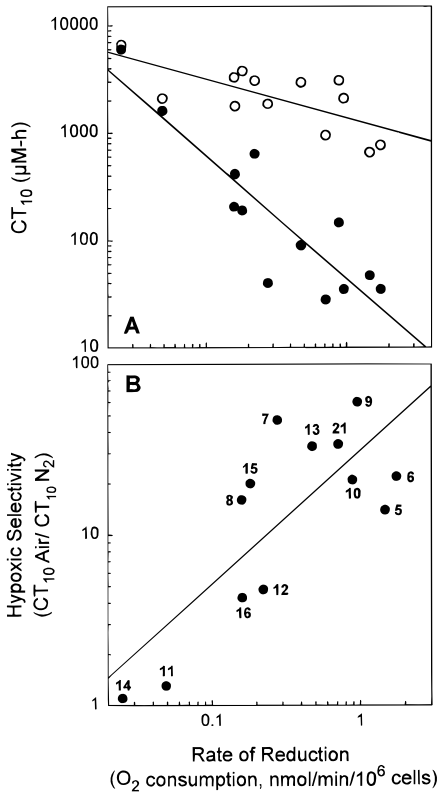


Figure 1. (A) Relationship between aerobic (○) and hypoxic (●) cytotoxicity and rates of metabolic reduction, measured as rates of drug-stimulated oxygen consumption in respiration-inhibited cells. (B) Relationship between hypoxic selectivity and rates of metabolic reduction. The solid lines are linear regressions.

(coefficient -0.36 ± 0.11; $r = 0.700$, $p = 0.008$, $F = 11$). If both hypoxic and aerobic potency are linearly related to $\log_{10}$(rate of reduction), their ratio should also be linearly related to the latter parameter. This is tested in Figure 1B, which shows a significant linear relationship:

$$\log_{10}(CT_{10}\ \text{air}/CT_{10}\ N_2) = (1.50 \pm 0.14) + (0.79 \pm 0.19)\log_{10}(\text{rate of reduction}) \quad (2)$$

$$r = 0.777, \quad p = 0.002, \quad F = 17$$

The explained variance in eq 2 is relatively low, but there is no reason to expect the relationship to be strictly linear. It can be inferred that there is a non-bioreductive mechanism of toxicity in this series which dominates when the rate of enzymatic reduction is low, resulting in loss of hypoxic selectivity. The data of Figure 1B could also be interpreted as showing an optimal rate of reduction of approximately (0.5 nmol/min)/10^6 cells and that hypoxic selectivity also decreases at higher rates (possibly because redox cycling is then rapid enough that reactive oxygen species contribute to aerobic cytotoxicity).

This analysis demonstrates that the lack of hypoxic selectivity of nitroquinolines which are poorly reduced (e.g. **11** and **14**) arises because of the contribution of a second mechanism of toxicity, not dependent on metabolic reduction. The existence of a second mechanism is also consistent with the lack of differential between AA8 and UV4 cells for compounds **11** and **14** (Table 1) indicating that they are not acting as DNA alkylating agents. The presence of a non-bioreductive mechanism of toxicity presents a fundamental dilemma in the nitroquinoline series in that improvement of metabolic stability (to facilitate extravascular diffusion) can be expected to result in loss of hypoxic selectivity.

Recent studies[14] of a series of 4-[(alkylamino)alkyl]-5-nitroquinolines, including some of the compounds described here, showed accumulation in cells to high concentrations in a manner which did not correlate with DNA binding affinity. Cellular uptake was inhibited by ammonium chloride, which raises intralysosomal pH, and it was suggested that high lysosomal uptake of these bisbasic nitroquinolines may contribute to their lack of activity as HSCs *in vivo*, by limiting their ability to diffuse to the hypoxic regions of tumors.[14] Restricted tissue diffusion of a related dibasic acridine as a result of lysosomal sequestration has been demonstrated recently.[25] This suggested that analogues bearing neutral but hydrophilic side chains would be of interest, and a small series was prepared and evaluated to see whether analogues with lowered cellular and lysosomal uptake could be developed (Table 2). Because no discernible benefit could be seen by the above variations in reduction potential, compounds **21**−**23** employed the 5-nitroquinoline nucleus. All three compounds showed significant hypoxic selectivity in the clonogenic assay, with the dihydroxypropyl derivative (**21**) being the best. The 8-methyl and 8-methoxy analogues (**24** and **25**) were therefore evaluated, but showed lower aerobic potencies and considerably lower hypoxic selectivity. Variants **26** and **27**, bearing other types of basic side chain, also did not show interesting levels of hypoxic selectivity.

Table 3. Cellular Uptake for 4-Alkylamino-5-nitroquinolines and Analogues with Neutral Dihydroxy Sidechains

	cellular uptake	
no.	C_i/C_e[a]	C_i/C_e + 50 mM NH_4Cl[b]
5	12 ± 1	1.4 ± 0.1
21	7.5 ± 0.7	6.6 ± 0.3
9	32 ± 1	3.2 ± 0.2
24	6.8 ± 0.1	4.7 ± 0.2
10	29 ± 1	2.1 ± 0.1
25	1.1 ± 0.1	1.1 ± 0.1

[a] Ratio of intracellular (C_i) to extracellular (C_e) drug concentrations determined after 60 min drug exposure (100 μM) in aerobic AA8 cell cultures (5×10^6 cells/mL). [b] Ratio of intracellular (C_i) to extracellular (C_e) drug concentrations determined in the presence of 50 mM ammonium chloride.

In order to assess whether a neutral side chain lowers intralysosomal and intracellular accumulation of the 5-nitroquinolines, ratios of intracellular (C_i) to extracellular (C_e) drug concentrations were determined for the unsubstituted parent compound (**5**), the 8-methyl derivative (**9**), the 8-methoxy derivative (**10**), and the corresponding analogues with dihydroxy side chains (**21**, **24**, and **25,** respectively) (Table 3). Cellular uptake of the compounds (100 μM, 1 h) with basic side chains (**5**, **9**, and **10**) was high, ranging from a C_i/C_e ratio of 32 for **9** to 12 for **5**. Addition of the lysomotropic agent ammonium chloride (50 mM) substantially inhibited cell uptake, lowering ratios of C_i/C_e to 3.2 for **9** and 1.4 for **5**. These results confirm the previous report[14] that these bisbasic compounds are accumulated inside cells to high concentrations and that this largely results from uptake into acidic lysosomal vesicles. Analogues with neutral side chains (**21**, **24**, and **25**) had lower cell uptake factors that were largely unaffected by the addition of ammonium chloride. Although ratios of C_i/C_e were greater than unity for **21** and **24**, the lack of effect of ammonium chloride on uptake suggests a different site of subcellular localization for these compounds. The results confirm that replacement of a basic with a neutral side chain lowers accumulation of the 5-nitroquinolines inside lysosomes.

Since compounds with lowered cell uptake are expected to have improved extravascular diffusion properties, the activity of the above neutral side chain compounds was evaluated against KHT tumors at 75% of the maximum tolerated dose (MTD) using single ip doses. The MTD of **21** was 562 μmol/kg, which was 7-fold higher than that for the corresponding [(*N*,*N*-dimethylamino)alkyl]amino compound (**5**, MTD 80 μmol/kg). Similarly, the MTD of **24** (316 μmol/kg) was greater than for **9** (100 μmol/kg), while the 8-methoxy compound **25** was the least toxic of the dihydroxy side chain compounds (MTD 1050 μmol/kg). However, none of the dihydroxy compounds (**21**, **24**, **and 25**) showed significant activity against the KHT tumor, either when administered alone or at various times before or after gamma irradiation (15 Gy, drug 2 h before to 5 min after radiation; data not shown), although the related drug nitracrine (**3**) does show activity against hypoxic cells in KHT tumors.[26] The lack of *in vivo* activity of the compounds with neutral dihydroxy side chains is comparable to results for the analogues (**5** and **9**) with basic [(dimethylamino)alkyl]amino side chains against SCCVII tumors,[9,13] indicating that a neutral side chain does not confer useful *in vivo* activity.

Conclusions

The above studies were carried out following indications that the inactivity of 4-[(alkylamino)alkyl]-5-nitroquinoline analogues as HSCs *in vivo*, despite good *in vitro* selectivities and potencies, resulted from inadequate rates of diffusion due to sequestration of the bisbasic compounds into lysosomes and rapid nitroreduction. Although polysubstitution substantially lowered reduction potentials relative to the unsubstituted compound (**5**), or the 8-methyl derivative (**9**), this did not result in improved biological activity or hypoxic selectivity. Hypoxic selectivity decreased at low rates of nitroreduction, suggesting at least two mechanisms of toxicity for these 5-nitroquinolines; oxygen-sensitive net bioreduction and an oxygen-insensitive non-bioreductive mechanism that is responsible for the cytotoxicity of compounds which are poor substrates for enzymatic reduction. The variable contribution of these two mechanisms may underlie the variations in hypoxic selectivity within this series of compounds.

The loss of hypoxic selectivity at low reduction potentials demonstrates a fundamental problem in attempting to improve extravascular transport by increasing metabolic stability. The alternative strategy would be to improve diffusion kinetics by suppressing entrapment of the drugs in cells, so that metabolic consumption would present less of a problem. Replacement of the cationic side chain of the parent compound (**5**) with a neutral dihydroxypropyl unit gave an analogue (**21**) which retained good hypoxic selectivity in cell culture. Cellular uptake and intralysosomal accumulation of (**21**) and the 8-methyl- (**24**) and 8-methoxy-substituted (**25**) analogues was substantially lower than for the corresponding analogues (**5**, **9**, and **10**) with a basic side chain. However, any possible gain in extravascular diffusion for the compounds with neutral side chains was not sufficient to provide useful *in vivo* activity. This study has identified methods for modifying hypoxic selectivity and cellular uptake in the 5-nitroquinolines, but the failure of these strategies to provide compounds active against hypoxic cells in tumors suggests that further development of 5-nitroquinolines as HSCs is not warranted.

Experimental Section

Analyses were performed by the Microchemical Laboratory, University of Otago, Dunedin. Melting points were determined on an Electrothermal Model 9200 digital melting point apparatus and are reported as read. NMR spectra were measured on Bruker AM-200 or AM-400 spectrometers and referenced to Me_4Si.

Preparation of 4-Chloro-6-ethylquinoline (30): Example of Gould−Jacobs Method (Scheme 1). Equimolar amounts of 4-ethylaniline and diethyl (ethoxymethylene)-malonate were heated together at 120 °C for 1 h and then heated under reflux in Dowtherm A to effect cyclization.[15] The resulting crude ester was saponified to give 6-ethyl-4(1*H*)-quinolone-3-carboxylic acid (**28**) (81% overall yield), mp (DMF) 257 °C dec. Anal. ($C_{12}H_{11}NO_3$) C, H, N. This acid was decarboxylated in benzophenone at 275 °C to give 6-ethyl-4(1*H*)-quinolone (**29**) (77%), mp (H_2O) 197.5−198.5 °C. Anal. ($C_{11}H_{11}NO$) C, H, N. The quinolone was treated with refluxing $POCl_3$ to give 4-chloro-6-ethylquinoline (**30**) (92%) as an oil which was used directly: ^{1}H NMR ($CDCl_3$) δ 8.71 (d, J = 4.7 Hz, 1 H, H-2), 8.05 (d, J = 8.7 Hz, 1 H, H-8), 8.00 (d, J = 1.1 Hz, 1 H, H-5), 7.64 (dd, J = 8.7, 1.1 Hz, 1 H, H-7), 7.46 (d, J = 4.7 Hz, 1 H, H-3), 2.89 (q, J = 7.6 Hz, 2 H, CH_2CH$_3$), 1.37 (t, J = 7.6 Hz, 3 H, CH$_2$CH_3).

Similar reaction of 2,4-dimethoxyaniline with diethyl (ethoxymethylene)malonate gave 6,8-dimethoxy-4(1*H*)-quinolone, mp (EtOH) 223−224 °C (lit.[27] mp 223−225 °C). Treatment of this with $POCl_3$ as above gave 4-chloro-6,8-dimethoxyquinoline, mp (petroleum ether) 115−115.5 °C; ^{1}H NMR ($CDCl_3$) δ 8.63 (d, J = 4.7 Hz, 1 H, H-2), 7.49 (d, J = 4.7 Hz, 1 H, H-3), 7.03 (d, J = 2.5 Hz, 1 H, ArH), 6.76 (d, J = 2.5 Hz, 1 H, ArH), 4.08 (s, 3 H, OCH$_3$), 3.95 (s, 3 H, OCH$_3$). Anal. ($C_{11}H_{10}ClNO_2$) C, H, N.

4-Chloro-6-ethyl-5-nitroquinoline (31). Example of Direct Nitration Method (Scheme 1). The above 4-chloroquinoline (**30**) (3.0 g, 15.7 mmol) was added slowly to stirred concentrated H_2SO_4 (13 mL), keeping the temperature below 10 °C. The solution was then cooled to -5 °C, and a mixture of fuming HNO_3 (1.09 g, 17.3 mmol) and concentrated H_2SO_4 (2 mL) was then added dropwise to the stirred solution at 0 °C. The reaction mixture was stirred for a further 20 min at 10 °C and then poured into ice/ammonia. The mixture was extracted with CH_2Cl_2, and evaporation gave a crystalline residue which was chromatographed on silica gel. Elution with CH_2Cl_2/MeOH (32:1) gave **31** (1.92 g, 52%): mp (petroleum ether) 54−55 °C; ^{1}H NMR ($CDCl_3$) δ 8.79 (d, J = 4.7 Hz, 1 H, H-2), 8.22 (d, J = 8.6 Hz, 1 H, H-8), 7.71 (d, J = 8.6 Hz, 1 H, H-7), 7.58 (d, J = 4.7 Hz, 1 H, H-3), 2.75 (q, J = 7.6 Hz, 2 H, CH_2CH$_3$), 1.35 (t, J = 7.6 Hz, 3 H, CH$_2$CH_3). Anal. ($C_{11}H_9ClN_2O_2$) C, H, N.

Similar nitration of 4-chloro-2,3-dimethylquinoline[28] gave a mixture which was chromatographed on silica gel. Elution with petroleum ether/EtOAc (3:1) gave 4-chloro-2,3-dimethyl-8-nitroquinoline (3.67 g, 37%): mp (petroleum ether) 114−114.5 °C; ^{1}H NMR δ 8.35 (dd, J = 8.5, 1.3 Hz, 1 H, H-7), 7.91 (dd, J = 7.5, 1.3 Hz, 1 H, H-5), 7.59 (dd, J = 7.5, 8.5 Hz, 1 H, H-6), 2.78 (s, 3 H, 2-Me), 2.61 (s, 3 H, 3-Me). Anal. ($C_{11}H_9ClN_2O_2$) C, H, N. Elution with petroleum ether/EtOAc (13:7) gave a mixture of the 5- and 6-nitro isomers. Recrystallization of this mixture once from petroleum ether and twice from MeOH gave pure 4-chloro-2,3-dimethyl-5-nitroquinoline (3.47 g, 35%): mp 142−142.5 °C; ^{1}H NMR ($CDCl_3$) δ 8.15 (m, 1 H, H-8), 7.66 (m, 2 H, H-6,7), 2.79 (s, 3 H, 2-Me), 2.58 (s, 3 H, 3-Me). Anal. ($C_{11}H_9ClN_2O_2$) C, H, N.

Similar nitration of 4-chloro-3,6-dimethylquinoline[29] gave 4-chloro-3,6-dimethyl-5-nitroquinoline (80%): mp (petroleum ether) 140−140.5 °C; ^{1}H NMR ($CDCl_3$) δ 8.75 (s, 1 H, H-2), 8.1 1 (d, J = 8.6 Hz, 1 H, H-8), 7.58 (d, J = 8.6 Hz, 1 H, H-7), 2.57 (s, 3 H, 6-CH$_3$), 2.48 (s, 3 H, 2-CH$_3$). Anal. ($C_{11}H_9ClN_2O_2$) C, H, N.

Similar nitration of 4-chloro-3,8-dimethylquinoline[30] gave 4-chloro-3,8-dimethyl-5-nitroquinoline (79%): mp (petroleum ether) 158−159 °C; ^{1}H NMR ($CDCl_3$) δ 8.83 (s, 1 H, H-2), 7.67 (d, J = 7.7 Hz, 1 H, H-6), 7.55 (dd, J = 7.7, 0.9 Hz, 1 H, H-7), 2.83 (d, J = 0.8 Hz, 3 H, 8-CH$_3$), 2.59 (s, 3 H, 2-CH$_3$). Anal. ($C_{11}H_9ClN_2O_2$) C, H, N.

Similar nitration of 4-chloro-6,8-dimethylquinoline[31] gave 4-chloro-6,8-dimethyl-5-nitroquinoline (88%): mp (petroleum ether) 113.5−114 °C; ^{1}H NMR ($CDCl_3$) δ 8.78 (d, J = 4.66 Hz, 1 H, H-2), 7.56 (d, J = 4.6 Hz, 1 H, H-3), 7.51 (br s, 1 H, H-7), 2.45 (s, 3 H, 8-CH$_3$), 2.79 (d, J = 0.8 Hz, 3 H, 6-CH$_3$). Anal. $C_{11}H_9ClN_2O_2$) C, H, N, Cl.

Similar nitration of 4-chloro-2,3,8-trimethylquinoline[32] gave 4-chloro-5-nitro-2,3,8-trimethylquinoline (87%): mp (petroleum ether) 110−110.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.59 (d, J = 7.7 Hz, 1 H, H-6), 7.49 (d, J = 7.7 Hz, 1 H, H-7), 2.82 (s, 3 H, 8-Me), 2.78 (s, 3 H, 2-Me), 2.57 (s, 3 H, 3-Me). Anal. ($C_{12}H_{11}ClNO_2$) C, H, N.

Synthesis of 4-[3-(Dimethylamino)propyl]amino]-6-ethyl-5-nitroquinoline (12). Example of the General Reaction. A mixture of 4-chloro-6-ethyl-5-nitroquinoline (**31**) (2.37 g, 10 mmol) and *N*,*N*-dimethyl-1,3-propanediamine (5.1 g, 50 mmol) was heated with stirring at 100 °C for 3.5 h under N_2 and then concentrated under reduced pressure below 70 °C. The residue was chromatographed on alumina, eluting with CH_2Cl_2/EtOAc (1:1), to give **12** (1.93 g, 64%): ^{1}H NMR (free base in $CDCl_3$) δ 8.54 (d, J = 5.4 Hz, 1 H, H-2), 8.02 (d, J = 8.7 Hz, 1 H, H-8), 7.51 (d, J = 8.7 Hz, 1 H, H-7), 6.57 (d, J = 5.4 Hz, 1 H, H-3), 5.67 (br s, 1 H, NH), 3.27 (q, J = 5.7 Hz, 2 H, NHCH_2), 2.66 (q, J = 7.6 Hz, 2 H, CH_2CH$_3$), 2.41, t,

J = 6.4 Hz, 2 H, CH_2N(CH_3)$_2$), 2.27 (s, 6 H, N(CH_3)$_2$), 1.84 (quintet, J = 6.4 Hz, 2 H, $CH_2CH_2CH_2$), 1.30 (t, J = 7.6 Hz, 3 H, CH_2CH_3); dihydrochloride salt mp (MeOH/EtOAc) 190−191 °C. Anal. ($C_{16}H_{22}N_4O_2$·2HCl·0.25H_2O) C, N; H: calcd 6.5, found 7.0.

Similarly prepared were the following.

2,3-Dimethyl-4-[[3-(dimethylamino)propyl]amino]-5-nitroquinoline (**13**) (reaction time 9 h, 52%): ^{1}H NMR (free base in $CDCl_3$) δ 8.06 (dd, J = 8.45, 1.0 Hz, 1 H, H-8), 7.66 (dd, J = 7.4, 1.1 Hz, 1 H, H-6), 7.52 (dd, J = 8.4, 7.4 Hz, 1 H, H-7), 5.38 (br t, 1 H, NH), 3.09 (q, J = 6.2 Hz, 2 H, NHCH_2), 2.66 (s, 3 H, 2-Me), 2.39 (t, J = 6.3 Hz, 2 H, CH_2NMe_2), 2.33 (s, 3 H, 3-Me), 2.24 (s, 6 H, NMe_2), 1.66 (quintet, J = 6.2 Hz, 2 H, $CH_2CH_2CH_2$); dihydrochloride salt mp (EtOAc/MeOH) 152−155 °C. Anal. ($C_{16}H_{22}N_4O_2$·2HCl) C, H, N, Cl.

3,6-Dimethyl-4-[[3-(dimethylamino)propyl]amino]-5-nitroquinoline (**14**) (gentle reflux, 8 h, 54%): ^{1}H NMR (free base in $CDCl_3$) δ 8.61 (s, 1 H, H-2), 8.05 (d, J = 8.6 Hz, 1 H, H-8), 7.55 (d, J = 8.6 Hz, 1 H, H-7), 4.49 (br t, J = 6.6 Hz, 1 H, NH), 3.10 (q, J = 6.6 Hz, 2 H, NHCH_2), 2.44 (s, 3 H, 6-CH_3), 2.41 (s, 3 H, 3-CH_3), 2.39 (t, J = 6.6 Hz, 2 H, CH_2NMe_2), 2.25 (s, 6 H, N(CH_3)$_2$), 1.75 (quintet, J = 6.5 Hz, 2 H, $CH_2CH_2CH_2$); dihydrochloride salt mp (MeOH/EtOAc) 219−222 °C. Anal. ($C_{16}H_{22}N_4O_2$·2HCl·H_2O) C, N, Cl; H: calcd 6.7, found 7.3.

3,8-Dimethyl-4-[[3-(dimethylamino)propyl]amino]-5-nitroquinoline (**15**) (reaction time 6 h, 59%): ^{1}H NMR (free base in $CDCl_3$) δ 8.57 (s, 1 H, H-2), 7.71 (d, J = 8.3 Hz, 1 H, H-6), 7.39 (d, J = 7.5 Hz, 1 H, H-7), 5.90 (s, 1 H, NH), 3.20 (q, J = 6.0 Hz, 2 H, NHCH_2), 2.78 (s, 3 H, 8-Me), 2.39 (t, J = 6.0 Hz, 2 H, CH_2N(CH_3)$_2$), 2.36 (s, 3 H, 3-CH_3), 2.25 (s, 6 H, N(CH_3)$_2$), 1.63 (quintet, J = 6.0 Hz, 2 H, $CH_2CH_2CH_2$); dihydrochloride salt mp (MeOH/EtOAc) 212−214 °C. Anal. ($C_{16}H_{22}N_4O_2$·2HCl·1.5H_2O) C, H, N, Cl.

6,8-Dimethyl-4-[[3-(dimethylamino)propyl]amino]-5-nitroquinoline (**16**) (reaction time 2 h, 74%): ^{1}H NMR (free base in $CDCl_3$) δ 8.58 (d, J = 5.4 Hz, 1 H, H-2), 7.27 (m, 1 H, H-7), 6.60 (d, J = 5.4 Hz, 1 H, H-3), 5.68 (s, 1 H, NH), 3.26 (q, J = 4.7 Hz, 2 H, NHCH_2), 2.74 (s, 3 H, 6-CH_3), 2.40 (t, J = 6.4 Hz, 2 H, CH_2N(CH_3)$_2$), 2.38 (s, 3 H, 8-CH_3), 2.23 (s, 6 H, N(CH_3)$_2$), 1.87 (m, 2 H, $CH_2CH_2CH_2$); dihydrochloride salt mp (MeOH/EtOAc) 214−215 °C dec. Anal. ($C_{16}H_{22}N_4O_2$.2HCl) C, H, N, Cl.

4-[[3-(Dimethylamino)propyl]amino]-5-nitro-2,3,8-trimethylquinoline (**19**) (reaction time 8 h, 55%): ^{1}H NMR (free base in $CDCl_3$) δ 7.58 (d, J = 7.6 Hz, 1 H, H-6), 7.36 (dd, J = 7.6, 0.6 Hz, 1 H, H-7), 5.20 (br s, 1 H, NH), 3.06 (q, J = 6.2 Hz, 2 H, NHCH_2), 2.77 (s, 3 H, 8-Me), 2.68 (s, 3 H, 2-Me), 2.38 (t, J = 6.3 Hz, 2 H, CH_2N(CH_3)$_2$), 2.32 (s, 3 H, 3-Me), 2.24 (s, 6 H, N(CH_3)$_2$), 1.65 (quintet, J = 6.3 Hz, 2 H, $CH_2CH_2CH_2$); dihydrochloride salt mp (EtOAc/MeOH) 248−251 °C. Anal. ($C_{17}H_{24}N_4O_2$·2HCl) C, H, N, Cl.

4-[[2-(4-Morpholyl)ethyl]amino]-5-nitroquinoline (26). A mixture of 4-chloro-5-nitroquinoline[33] (1.04 g, 5 mmol) and 4-(2-aminoethyl)morpholine (3.7 g, 28 mmol) was heated at 95 °C for 20 min, and the cooled mixture was partitioned between CH_2Cl_2 and aqueous Na_2CO_3. Workup of the organic layer and chromatography of the residue on alumina in EtOAc gave **26** (1.18 g, 75%): ^{1}H NMR (free base in $CDCl_3$) δ 8.60 (d, J = 5.4 Hz, 1 H, H-2), 8.18 (m, 1 H, H-8), 7.61 (m, 2 H, H-6,7), 6.56 (d, J = 5.4 Hz, 1 H, H-3), 6.03 (br s, 1 H, NH), 3.82 (t, J = 4.6 Hz, 4 H, N(CH_2CH_2)$_2$O), 3.25 (m, 2 H, ArNHCH_2CH_2), 2.71 (t, J = 5.0 Hz, 2 H, NHCH_2CH_2), 2.53 (t, J = 4.4 Hz, 4 H, N(CH_2CH_2)$_2$O); dihydrochloride salt mp (EtOAc/MeOH) 189−191 °C. Anal. ($C_{15}H_{18}N_4O_3$·2HCl) C, N; H: found 6.49, calcd 5.37.

2,3-Dimethyl-4-[[3-(dimethylamino)propyl]amino]-8-methoxy-5-nitroquinoline (20) (Scheme 2). Reaction of 2-methoxyaniline and ethyl 2-methylacetoacetate by the general Conrad−Limpach procedure[16] gave 2,3-dimethyl-8-methoxy-4(1*H*)-quinolone[34] (**32**), mp (MeOH) 295−296 °C. Anal. ($C_{12}H_{13}NO_2$) C, H, N. Reaction of this with $POCl_3$ as usual gave 4-chloro-2,3-dimethyl-8-methoxyquinoline[32] (**33**): mp (iPr_2O) 135 °C; ^{1}H NMR ($CDCl_3$) δ 7.74 (d, J = 8.5 Hz, 1 H, H-5), 7.47 (t, J = 8.2 Hz, 1 H, H-6), 7.03 (d, J = 7.8 Hz, 1 H, H-7), 4.20 (s, 3 H, OCH_3), 2.80 (s, 3 H, 2-CH_3), 2.55 (s, 3 H, 3-CH_3). Anal. ($C_{12}H_{12}ClNO$) C, H, N, Cl. A mixture of **33** (1 equiv) and *N*,*N*-dimethyl-1,3-propanediamine (1.2 equiv) was heated in excess phenol at 140 °C, and the crude product was chromatographed on alumina. Elution with EtOAc gave 2,3-dimethyl-4-[[3-(*N*,*N*-dimethylamino)propyl]amino]-8-methoxyquinoline (**34**) (64%), mp (petroleum ether) 67−69 °C; ^{1}H NMR ($CDCl_3$) δ 7.52 (d, J = 8.6 Hz, 1 H, H-5), 7.27 (t, J = 7.8 Hz, 1 H, H-6) 6.91 (d, J = 7.7 Hz, 1 H, H-7), 5.21 (br s, 1 H, NH), 4.04 (s, 3 H, OCH_3), 3.45 (t, J = 6.2 Hz, 2 H, NHCH_2), 2.70 (s, 3 H, 2-CH_3), 2.45 (t, J = 6.3 Hz, 2 H, CH_2N(CH_3)$_2$), 2.32 (s, 3 H, 3-CH_3), 2.28 (s, 6 H, N(CH_3)$_2$), 1.81 (quintet, J = 6.3 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{17}H_{25}N_3O$) C, H, N.

Nitration of **34** as described below for the preparation of **18**, followed by extraction of the crude product with hot petroleum ether, concentration of the extract and chromatography on alumina, eluting with EtOAc, gave 2,3-dimethyl-4-[[3-(dimethylamino)propyl]amino]-8-methoxy-5-nitroquinoline (**20**) as an oil (76%): dihydrochloride salt mp (MeOH/EtOAc) 196−197 °C dec; ^{1}H NMR [(CD_3)$_2$SO] δ 13.12 (s, 1 H, HCl), 11.12 (s, 1 H, HCl), 8.60 (s, 1 H, N*H*CH_2), 8.33 (d, J = 8.7 Hz, 1 H, H-6), 7.49 (d, J = 8.8 Hz, 1 H, H-7), 4.19 (s, 3 H, OCH_3), 3.35 (q, J = 6.0 Hz, 2 H, NHCH_2), 2.85 (m, 2 H, CH_2-NH^+Me_2), 2.74 (s, 3 H, 2-CH_3), 2.63 (d, J = 4.6 Hz, collapsing to singlet on D_2O exchange, 6 H, protonated N(CH_3)$_2$), 2.37 (s, 3 H, 3-CH_3), 2.02 (quintet, J = 7 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{17}H_{24}N_4O_3$·2HCl·0.5H_2O) C, H, N, Cl.

6,8-Dimethoxy-4-[[3-(dimethylamino)propyl]amino]-5-nitroquinoline (18). Similar condensation of 4-chloro-6,8-dimethoxyquinoline with *N*,*N*-dimethyl-1,3-propanediamine, as described above for the preparation of **20**, followed by chromatography of the crude product on alumina and elution with EtOAc/MeOH (10:1), gave 6,8-dimethoxy-4-[[3-(dimethylamino)propyl]amino]quinoline (51%): mp (EtOAc) 148−149 °C; ^{1}H NMR ($CDCl_3$) δ 8.44 (d, J = 5.2 Hz, 1 H, H-2), 7.47 (br s, 1 H, NH), 6.66 (d, J = 2.4 Hz, 1 H, ArH), 6.52 (d, J = 2.4 Hz, 1 H, ArH), 6.36 (d, J = 5.2 Hz, 1 H, H-3), 4.02 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 3.38 (q, J = 5.7 Hz, 2 H, NHCH_2), 2.57 (t, J = 5.6 Hz, 2 H, CH_2N(CH_3)$_2$), 2.36 (s, 6 H, N(CH_3)$_2$), 1.91 (quintet, J = 5.7 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{16}H_{23}N_3O_2$) C, H, N.

The above compound (2.60 g, 9 mmol) was added slowly to stirred concentrated H_2SO_4 (18 mL) below 10 °C. A mixture of fuming HNO_3 (0.4 2 mL, 10 mmol) and concentrated H_2SO_4 (3 mL) was added dropwise to this solution below 0 °C, and the mixture was stirred for a further 5 min at 0 °C and then poured into ice. The mixture was basified with excess 40% aqueous NaOH and extracted with CH_2Cl_2 (4 × 150 mL). The combined extracts were evaporated below 30 °C, and the residue was chromatograped on alumina (Brockmann activity II-III). Elution with EtOAc/MeOH (49:1) followed by crystallization of the fraction from EtOAc gave 6,8-dimethoxy-4-[[3-(dimethylamino)propyl]amino]-5-nitroquinoline (**18**) (38% yield). ^{1}H NMR (free base in $CDCl_3$) δ 8.50 (d, J = 5.4 Hz, 1 H, H-2), 6.76 (s, 1 H, H-7), 6.60 (d, J = 5.4 Hz, 1 H, H-3), 5.64 (br s, 1 H, NH), 4.11 & 4.01 (2xs, 2 × 3 H, 6- and 8-OMe), 3.25 (q, J = 4.5 Hz, 2 H, NHCH_2), 2.41 (t, J = 6.5 Hz, 2 H, CH_2N(CH_3)$_2$), 2.26 (s, 6 H, N(CH_3)$_2$), 1.83 (quintet, 6.4 Hz, 2 H, $CH_2CH_2CH_2$); dihydrochloride salt mp (EtOAc/MeOH) 184−185 °C. Anal. ($C_{16}H_{22}N_4O_4$·2HCl) C, N, Cl; H; calcd 5.9, found 6.6.

4-[[3-(Dimethylamino)propyl]amino]-8-methyl-5-nitro-2-phenylquinoline (17) (Scheme 3). Reaction of 2-methyl-5-nitroaniline and ethyl benzoylacetate in benzene/1-methyl-2-pyrrolidinone under the conditions of the Conrad−Limpach procedure gave ethyl 3-phenyl-3-(2-methyl-5-nitroanilino)-acrylate (**35**) (36%): mp (EtOH/iPr_2O) 114−116 °C; ^{1}H NMR ($CDCl_3$) δ 8.89 (s, 1 H, NH), 7.67 (dd, J = 8.3, 2.3 Hz, 1 H, H-4), 7.3 (m, 5 H, phenyl), 7.26 (d, J = 8.3 Hz, 1 H, H-3), 7.09 (d, J = 2.3 Hz, 1 H, H-6), 5.21 (s, 1 H, C=C*H*), 4.24 (q, J = 7 Hz, 2 H, CH_2CH_3), 2.52 (s, 3 H, 2-CH_3), 1.35 (t, J = 7 Hz, 3 H, CH_2CH_3). Anal. ($C_{18}H_{18}N_2O_4$) C, H, N. Cyclization of **35** in refluxing Dowtherm A gave 8-methyl-5-nitro-2-phenyl-4(1*H*)-quinolone (**36**) (57%): mp (MeOH) 232−233 °C; ^{1}H NMR [(CD_3)$_2$SO] δ 10.82 (s, 1 H, NH), 7.32−8.34 (m, 7 H, PhH, H-6,7), 6.38 (s, 1 H, H-3), 2.70 (s, 3 H, CH_3). Anal. ($C_{16}H_{12}N_2O_3$) C, H, N. Treatment of **36** with $POCl_3$ gave 4-chloro-8-methyl-5-nitro-2-phenylquinoline (**37**) (74%): mp (petroleum ether)

123−123.5 °C; ^{1}H NMR ($CDCl_3$) d 8.22 (m, 2 H, phenyl), 8.08 (s, 1 H, H-3), 7.66 (d, J = 7.7 Hz, 1 H, H-6) 7.61 (dd, J = 7.7 Hz, 0.9 Hz, 1 H, H-7), 7.55 (m, 3 H, phenyl), 2.90 (s, 3 H, CH_3). Anal. ($C_{16}H_{11}ClN_2O_2$) C, H, N, Cl.

Coupling of **37** with *N*,*N*-dimethyl-1,3-propanediamine as for the preparation of **12** (reaction time 1 h) gave 4-[[3-(dimethylamino)propyl]amino]-8-methyl-5-nitro-2-phenylquinoline (**17**) (62%): ^{1}H NMR ($CDCl_3$) δ 8.15−8.23 (m, 2 H, phenyl H), 7.60 (d, J = 7.7 Hz, 1 H, H-6), 7.49 (dd, J = 5.9, 1.5 Hz, 1 H, H-7), 7.40−7.54 (m, 3 H, phenyl H), 7.06 (s, 1 H, H-3), 6.46 (t, J = 3.6 Hz, 1 H, NH), 3.40 (q, 2 H, NHCH_2), 2.86 (s, 3 H, 8-CH_3), 2.41 (t, 2 H, CH_2N(CH_3)$_2$), 2.26 (s, 6 H, N(CH_3)$_2$), 2.87 (quintet, 2 H, $CH_2CH_2CH_2$); dihydrochloride salt mp (MeOH/EtOAc) 165−168 °C. Anal. ($C_{21}H_{24}N_4O_2$·2HCl·H_2O) C, H, Cl; N: calcd 12.3, found 11.8.

4-[(3-Hydroxypropyl)amino]-5-nitroquinoline (23). A mixture of 4-chloro-5-nitroquinoline (1.04 g, 5 mmol), 3-aminopropanol (0.98 g, 13 mmol), and dry DMSO (4 mL) was heated at 95−100 °C for 45 min under N_2. The mixture was then cooled, diluted with 2 N aqueous Na_2CO_3, and extracted with CH_2Cl_2 (2 × 100 mL). The combined extracts were washed with saturated NaCl, dried, and evaporated, and the residue was chromatographed on alumina. Elution with EtOAc/MeOH (19:1) gave **23** (0.91 g, 74%): ^{1}H NMR (free base in $CDCl_3$) δ 8.55 (d, J = 5.5 Hz, 1 H, H-2), 8.16 (d, J = 7.9 Hz, 1 H, H-8), 7.54−7.64 (m, 2 H, H-6,7), 6.57 (d, J = 5.5 Hz, 1 H, H-3), 5.48 (s, 1 H, NH), 3.87 (t, J = 5.5 Hz, 2 H, CH_2OH), 3.37 (q, J = 5.7 Hz, 2 H, NHCH_2), 2.55 (br s, 1 H, OH), 1.97 (quintet, J = 5.9 Hz, 2 H, $CH_2CH_2CH_2$); hydrochloride salt mp (MeOH/EtOAc) 195−196 °C. Anal. ($C_{12}H_{13}N_3O_3$·HCl) C, H, N, Cl.

Similar reaction of 4-chloro-5-nitroquinoline with 3-amino-1,2-propanediol (95 °C, 1 h) and crystallization of the crude product from EtOAc gave 4-[(2,3-dihydroxypropyl)amino]-5-nitroquinoline (**21**) (66%): ^{1}H NMR (free base in $CDCl_3$) δ 8.59 (d, J = 5.4 Hz, 1 H, H-2), 8.12 (d, J = 8.4 Hz, 1 H, H-8), 7.94 (d, J = 7.5 Hz, 1 H, H-6), 7.75 (dd, J = 7.5, 8.4 Hz, 1 H, H-7), 6.82 (d, J = 5.4 Hz, 1 H, H-3), 5.67 (br s, 1 H, exchange with D_2O, NH), 5.14 (d, J = 4.4 Hz, 1 H, exchange with D_2O, CHOH), 4.75 (t, J = 5.2 Hz, 1 H, exchange with D_2O, CH_2OH), 3.70−3.80, 3.25−3.50 and 3.10−3.20 (3m, 5 H, CH_2CHOHCH_2-OH); hydrochloride salt mp (EtOAc/MeOH) 153−156 °C. Anal. ($C_{12}H_{13}N_3O_4$·HCl) C, H, N, Cl.

Similar reaction of 4-chloro-8-methyl-5-nitroquinoline with 3-amino-1,2-propanediol (95 °C, 2 h) gave 4-[(2,3-dihydroxypropyl)amino]-8-methyl-5-nitroquinoline (**24**) (71%): ^{1}H NMR [$(CD_3)_2SO$] δ 8.59 (d, J = 5.3 Hz, 1 H, H-2), 7.84 (d, J = 7.7 Hz, 1 H, H-6), 7.62 (d, J = 7.7 Hz, 1 H, H-7), 6.83 (d, J = 5.4 Hz, 1 H, H-3), 5.66 (t, J = 4.3 Hz, 1 H, NH), 5.10 (d, J = 4.3 Hz, 1 H, CHOH), 4.75 (t, J = 5.1 Hz, 1 H, CH_2OH), 3.69−3.78, 3.23−3.48, and 3.10−3.19 (3m, 5 H, CH_2CHOHCH_2OH), 2.70 (s, 3 H, 8-Me); hydrochloride salt mp (MeOH/EtOAc) 175 °C dec. Anal. ($C_{13}H_{15}N_3O_4$·HCl) C, H, N, Cl.

Similar reaction of 4-chloro-5-nitroquinoline with butylamine (100 °C, 1.5 h) gave 4-(butylamino)-5-nitroquinoline (**22**) (79%): ^{1}H NMR ($CDCl_3$) δ 8.61 (d, J = 5.4 Hz, 1 H, H-2), 8.18 (dd, J = 8.2 Hz, 1.5 Hz, 1 H, H-8), 7.57−7.67 (m, 2 H, H-6,7), 6.62 (d, J = 5.5Hz, 1 H, H-3), 5.09 (s, 1 H, NH), 3.22 (q, J = 6.2 Hz, 2 H, NHCH_2), 1.70 (quintet, J = 7.3 Hz, 2 H, $CH_2CH_2CH_2$), 1.47 (sextet, J = 7.4 Hz, 2 H, CH_2CH_3), 0.99 (t, J = 7.4 Hz, 3 H, CH_3); hydrochloride salt mp (MeOH/EtOAc) 214−215 °C. Anal. ($C_{13}H_{15}N_3O_2$·HCl) C, H, N, Cl.

4-[(2,3-Dihydroxypropyl)amino]-8-methoxy-5-nitroquinoline (25) (Scheme 4). A mixture of 4-chloro-8-methoxyquinoline (**38**) (1.94 g, 10 mmol), 3-amino-1,2-propanediol (1.09 g, 12 mmol), and dry phenol (6g) was heated at 145 °C for 1 h, and then excess phenol was removed under reduced pressure. The residue was dissolved in water, treated with charcoal, clarified by filtration, and basified with ammonia to provide 4-[(2,3-dihydroxypropyl)amino]-8-methoxyquinoline (**39**) (2.16 g, 87% yield: mp (MeOH) 255−256 °C; ^{1}H NMR [$(CD_3)_2$-SO] δ 8.33 (d, J = 4.8 Hz, 1 H, H-2), 7.69 (d, J = 8.2 Hz, 1 H, H-5), 7.31 (t, J = 7.9 Hz, 1 H, H-6), 7.06 (d, J = 7.6 Hz, H-7), 6.91 (s, 1 H, NH), 6.51 (d, J = 4.9 Hz, 1 H, H-3), 4.94 (br s, 1 H, CHOH), 4.71 (br s, 1 H, CH_2OH), 3.89 (s, 3 H, OCH_3), 3.13−3.84 (m, 5 H, CH_2CHOHCH_2OH). Anal. ($C_{13}H_{16}NO_3$) C, H, N.

A suspension of **39** (1.49 g, 6 mmol) in pyridine (20 mL) containing acetic anhydride (2.0 mL, 21 mmol) was heated at 90−100 °C until homogeneous and for a further 10 min. Volatiles were removed under reduced pressure, and the residue was dissolved in water and basified with ammonia. Recrystallization of the resulting precipitate from benzene/petroleum ether gave 4-[(2,3-diacetoxypropyl)amino]-8-methoxyquinoline (**40**) (1.56 g, 78% yield): mp 176−177 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.37 (d, J = 5.3 Hz, 1 H, H-2), 7.69 (d, J = 8.6 Hz, 1 H, H-5), 7.33 (t, J = 8.1 Hz, 1 H, H-6), 7.17 (t, J = 5.9 Hz, 1 H, NH), 7.07 (d, J = 7.7 Hz, 1 H, H-7), 6.64 (d, J = 5.3 Hz, 1 H, H-3), 5.25 (m, 1 H, CH_2CHCH_2), 4.1−4.4 (m, 2 H, CH_2O), 3.89 (s, 3 H, OCH_3), 3.53 (m, 2 H, NHCH_2), 2.03 (s, 3 H, CH_3), 2.00 (s, 3 H, CH_3). Anal. ($C_{17}H_{20}N_2O_5$) C, H, N.

The diacetate **40** (2.99 g, 9 mmol) was added to stirred concentrated H_2SO_4 (20 mL) while the temperature was kept below −5 °C, and the resulting solution was treated portionwise with KNO_3 (0.96 g, 9.5 mmol). The reaction mixture was stirred for a further 10 min at −5 °C, poured into excess ice/ammonia, and extracted with EtOAc (3 × 150 mL). Evaporation of the combined organic layers under reduced pressure gave the crude nitrodiacetate (**41**), which was heated under reflux in a mixture of MeOH (12 mL) and 2 N aqueous Na_2-CO_3 (20 mL) for 5 min. The mixture was concentrated to small volume and cooled, and the resulting precipitate was collected, washed with water, and dried. Recrystallization from MeOH/EtOAc gave 4-[(2,3-dihydroxypropyl)amino]-8-methoxy-5-nitroquinoline (**25**) (1.18 g, 45% yield): ^{1}H NMR [$(CD_3)_2SO$] δ 8.50 (d, J = 5.4 Hz, 1 H, H-2), 8.04 (d, J = 8.7 Hz, 1 H, H-6), 7.15 (d, J = 8.7 Hz, 1 H, H-7), 6.86 (d, J = 5.4 Hz, 1 H, H-3), 5.77 (m, 1 H, exchange with D_2O, NH), 5.09 (d, J = 3.5 Hz, 1 H, exchange with D_2O, CHOH), 4.77 (m, 1 H, exchange with D_2O, CH_2OH), 4.02 (s, 3 H, OMe), 3.68−3.79, 3.25−3.50, 3.10−3.20 (3m, 5 H, CH_2CHOHCH_2OH); hydrochloride salt mp (MeOH/EtOAc) 171.5−172 °C. Anal. ($C_{13}H_{15}N_3O_5$·HCl) C, H, N, Cl.

4-[[4-[*N*-[2-(Dimethylamino)ethyl]carbamoyl]phenyl]-amino]-5-nitroquinoline (27). A solution of 4-chloro-5-nitroquinoline (0.65 g, 3.1 mmol) and *N*-[2-(dimethylamino)-ethyl]-4-aminobenzamide (0.71 g, 3.4 mmol) in MeOH (30 mL) was treated with concentrated HCl (0.6 mL) and heated under reflux for 2 h. The mixture was diluted with EtOAc, and the resulting precipitate was recrystallized from MeOH/EtOAc to give **27** as the dihydrochloride salt (1.01 g, 72%): mp 181−183 °C; ^{1}H NMR (free base in $CDCl_3$) δ 8.72 (d, J = 4.6 Hz, 1 H, H-2), 8.31 (d, J = 8.3 Hz, 1 H, H-8), 7.82 (d, J = 7.6 Hz, 2 H, H-3′,5′), 7.82 (br d, 1 H, H-6), 7.72 (t, J = 7.8 Hz, 1 H, H-7), 7.36 (d, J = 4.6 Hz, 1 H, H-3), 7.16 (d, J = 7.9 Hz, 2 H, H-2′,6′), 6.92 (s, 1 H, NH), 6.84 (s, 1 H, NH), 3.54 (d, J = 4.7 Hz, 2 H, NHCH_2), 2.53 (s, 2 H, CH_2N(CH_3)$_2$), 2.28 (s, 6 H, N(CH_3)$_2$). Anal. ($C_{20}H_{21}N_5O_3$·2HCl·H_2O) C, H, N, Cl.

Reduction Potentials. Pulse radiolysis experiments were carried out as described previously,[13] using 3 mM drug solutions in phosphate buffer at pH 7, using either 2-propanol (typically 0.2 M) or 2-propanol/acetone mixtures as cosolvent. Redox indicators used were methylviologen (1,1′-dimethyl-4,4′-bipyridinium dichloride; $E(1)$ = -447 mV), triquat (7,8-dihydro-6*H*-dipyrido[1,2-*a*:2′,1′-*c*][1,4]diazepinediium dibromide; $E(1)$ = −548 mV), or benzylviologen (1,1′-dibenzyl-4,4′-bipyridinium dichloride; $E(1)$ = −374 mV), as appropriate. Measured reduction potentials were corrected for ionic strength effects using the pK_a values of Tables 1 and 2.

Cell Culture Assays. AA8 and UV4 cells were maintained in logarithmic-phase growth in 25 cm^3 tissue culture flasks with subculture twice weekly by trypsinization. The growth medium was antibiotic-free Alpha MEM with 10% v/v heat-inactivated (56 °C, 40 min) fetal calf serum. Doubling times were approximately 14 h for AA8 and 15 h for UV4 cells. Bulk cultures of AA8 cells were prepared in spinner flasks, using the above growth medium plus penicillin (100 IU/mL) and streptomycin (100 μg/mL). Growth inhibition studies were performed as described in detail elsewhere,[7,34] using 200 viable AA8 or 300 viable UV4 cells plus 5000 lethally-irradiated AA8 feeder cells per well in 96-well tissue culture dishes. The IC_{50} was determined as the drug concentration needed to reduce the cell mass (protein content, measured after 72−78 h by staining with methylene blue and measuring absorbance in a

microplate photometer) to 50% of the mean value for eight control cultures on the same 96-well plate.

Clonogenic assays with magnetically-stirred 10 mL suspension cultures (plateau-phase AA8 cells, 10^6/mL) were performed by removing samples periodically during continuous gassing with 5% CO_2 in air or N_2 as detailed elsewhere.[8] Both cell suspensions and drug solutions in growth medium were pre-equilibrated under the appropriate gas phase for 60 min prior to mixing, to ensure essentially complete anoxia throughout the period of drug contact in hypoxic cultures. Several drug concentrations were investigated for each agent, and the concentration × time required to reduce the surviving cell fraction to 10% (CT_{10}) was determined at each concentration. CT_{10} values were not strictly constant, with a trend toward lower values at higher drug concentrations. To minimize errors due to this, comparisons between aerobic and hypoxic cytotoxicity were based on CT_{10} values at concentrations which gave similar rates of cell killing (usually one log in about 1 h).

Cell Uptake Studies. Cellular uptake was determined in aerobic cultures of AA8 cells (5×10^6 cells/mL) equilibrated with or without 50 mM ammonium chloride at 37 °C. Intracellular and extracellular drug concentrations were determined 60 min after addition of 100 μM drug by HPLC following rapid centrifugation of triplicate 2 mL samples. Intracellular samples were prepared for HPLC analysis as described previously,[23] while aliquots of extracellular medium were injected directly. The HPLC system has been described in detail elsewhere.[23] The mobile phase consisted of formate buffer, pH 4.5 (0.44 M ammonium formate, 68 mM formic acid), and 80% MeCN. Compounds were eluted isocratically with the following mobile phase composition: 90:10 formate buffer/80% MeCN (v/v) for **5**, **9**, **21**, and **24**; 88:12 formate buffer/80% MeCN (v/v) for **10** and **25**. Quantitation was based on peak areas using absorbance at the following wavelength ranges (signal (nm), bandwidth (nm)) referenced to absorbance at 550 nm: (370, 10) for **5** and **21**; (260, 4) for **9** and **24**; (242, 4) for **10** and **25**. Intracellular drug concentrations were corrected for measured recovery efficiency determined as described previously.[23]

Antitumor Activity. MTD values were determined in groups of six male C_3H/HeN mice with single ip doses of drug in water (0.01 mL/g of body weight). The dose was escalated at 1.33-fold dose intervals, with an observation time of 30 days. Groups of two mice bearing bilateral KHT tumors in the gastrocnemius muscle were treated with ip drug at 0.75 of the MTD either alone, or at various times before or after whole body irradiation (cobalt 60, 15 Gy, ca. 2.5 Gy/min) of unanesthesized, unrestrained mice. Three or four tumors within the size range 0.5−1.0 mL at treatment were removed from each group 18 h later, pooled, and assayed for clonogenicity in agar essentially as described,[19] except that tumors were dissociated using an enzyme cocktail (0.5 mg/mL pronase, 0.2 mg/mL collagenase, 0.2 mg/mL DNAase I) for 40 min, and total nucleated cells were counted with an electronic particle counter.

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