Hypoxia-Selective Antitumor Agents. 6. 4-(Alkylamino)nitroquinolines: A New Class of Hypoxia-Selective Cytotoxins

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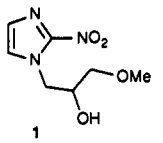
A series of isomeric 4-[[3-(dimethylamino)propyl]amino]nitroquinolines has been synthesized and evaluated as hypoxia-selective cytotoxins and as radiosensitizers of hypoxic cells. The compounds showed widely-differing hypersensitivity factors (ratios of cytotoxicity against wild-type and repair-deficient mammalian cells). Many compounds showed oxygen-sensitive bioreduction resulting in DNA alkylation, while others show oxygen-insensitive modes of action. Of the nitro isomers studied, the 5-nitro showed the greatest hypoxic selectivity. A series of ring-substituted analogues were then prepared, in an effort to lower its reduction potential of -286 mV. Structure-activity studies showed that the effects of substitution on reduction potential were complex, being mediated by electronic and steric effects on the nitro group, as well as by effects on quinoline pK_a. Two compounds of lower reduction potential, the 3- and 8-methyl analogues, showed improved selectivity (47- and 60-fold in a clonogenic assay). These two compounds also showed the highest "in vitro therapeutic indices" of the series as hypoxic cell radiosensitizers. Despite these favorable in vitro properties, neither compound had activity against hypoxic cells in SCCVII tumors when administered at 60% of the MTD.

One of the few consistently different characteristics of solid tumor tissue compared with normal tissue is the existence in the former of significant areas which are either transiently or chronically hypoxic.[1,2] While some such cells may be destined to die in untreated tumors, many remain viable for extended periods, and there is little doubt that the noncycling hypoxic cells in solid tumors represent a clinical problem for chemotherapy as well as radiotherapy.[3,4] Consequently, there is increasing interest in the development of hypoxia-selective cytotoxins (HSCs), including compounds capable of undergoing oxygen-inhibited reductive activation to DNA-reactive species.

Nitroheterocycles such as the 2-nitroimidazoles (e.g., misonidazole, 1) were originally developed as radiosensitizers,[5] but are also among the most intensively studied HSCs. Reduction of the nitro group generates a variety of reactive products, including species resulting from fragmentation of the unstable 2-hydroxylamine.[6] Cytotoxicity is enhanced under hypoxia because the first intermediate in the reduction pathway, the nitro radical anion, is efficiently reoxidized to the parent drug by molecular oxygen, thereby inhibiting net reduction. However, simple nitroheterocycles without other reactive functionality appear to lack the potency required for significant activity as HSCs in vivo.



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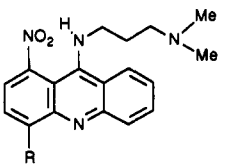

2 : R = H
3 : R = OMe

One approach to improving cytotoxic potency is the development of reductively-activated nitroheterocycles with DNA-binding affinity, and three classes of such HSCs have been reported: the 1-nitroacridines,[7-9] the 4-nitropyrazoloacridines,[10] and nitroimidazoles linked to DNA intercalators such as phenanthridine[11] or acridine[12] chromophores. Studies by us of the prototypical DNA-affinic

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(1) Moulder, J. E.; Rockwell, S. Hypoxic fractions of solid tumors: Experimental techniques, methods of analysis and a survey of existing data. *Int. J. Radiat. Biol. Oncol. Phys.* **1984**, *10*, 695-712.

(2) Chapman, J. D. Measurement of tumor hypoxia by invasive and non-invasive procedures: A review of recent clinical studies. *Radiother. Oncol.* **1991** (Suppl. 20), 13-19.

(3) Jain, R. K. Delivery of novel therapeutic agents in tumors: Physiological barriers and strategies. *J. Natl. Cancer Inst.* **1989**, *81*, 570-576.

(4) Durand, R. E. Distribution and activity of antineoplastic drugs in a tumor model. *J. Natl. Cancer Inst.* **1989**, *81*, 146-152.

(5) Adams, G. E.; Stratford, I. J. Hypoxia-mediated nitroheterocyclic drugs in the radio- and chemotherapy of cancer. *Biochem. Pharmacol.* **1986**, *35*, 71-76.

(6) McClelland, R. A.; Panicucci, R.; Rauth, A. M. Products of the reductions of 2-nitroimidazoles. *J. Am. Chem. Soc.* **1987**, *109*, 4308-4314.

(7) Wilson, W. R.; Denny, W. A.; Stewart, G. M.; Fenn, A.; Probert, J. C. Reductive metabolism and hypoxia-selective cytotoxicity of nitracrine. *Int. J. Radiat. Oncol. Biol. Phys.* **1986**, *12*, 1235-1238.

(8) Wilson, W. R.; Anderson, R. F.; Denny, W. A. Hypoxia-selective antitumor agents. 1. Relationships between structure, redox properties and hypoxia-selective cytotoxicity for 4-substituted derivatives of nitracrine. *J. Med. Chem.* **1989**, *32*, 23-30.

(9) Wilson, W. R.; Thompson, L. H.; Anderson, R. F.; Denny, W. A. Hypoxia-selective antitumor agents. 2. Electronic effects of 4-substituents on the mechanisms of cytotoxicity and metabolic stability of nitracrine analogues. *J. Med. Chem.* **1989**, *32*, 31-38.

(10) Sebolt, J. S.; Scavone, S. V.; Pinter, C. D.; Hamehehle, K. L.; Von Hoff, D. D.; Jackson, R. C. Pyrazoloacridines, a new class of anticancer agents with selectivity against solid tumors in vivo. *Cancer Res.* **1987**, *47*, 4299-4304.

(11) Panicucci, R.; Heal, R.; Laderoute, K.; Cowan, D. M. S.; McClelland, R. A.; Rauth, A. M. *Int. J. Radiat. Oncol. Biol. Phys.* **1989**, *16*, 1039-1043.

(12) Denny, W. A.; Roberts, P. B.; Anderson, R. F.; Brown, J. M.; Wilson, W. R. NLA-1- and 2-nitroimidazole radiosensitizer targeted to DNA by intercalation. *Int. J. Radiat. Oncol. Biol. Phys.* **1992**, *22*, 553-556.

0022-2623/92/1835-4832$03.00/0

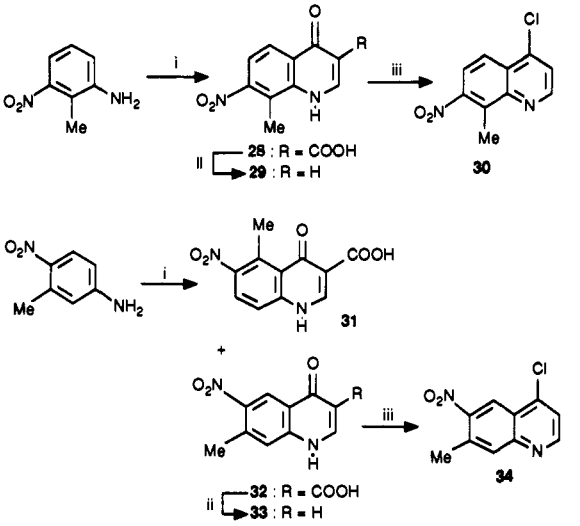
HSC 9-[[3-(dimethylamino)propyl]amino]-1-nitroacridine (nitracrine, **2**) have shown that it binds to DNA by intercalation and, while possessing hypoxia-selectivity similar to that of misonidazole as a cytotoxin in cell culture, is about 100 000-fold more potent.[13] However, nitracrine is not active against hypoxic cells in solid tumors in vivo,[7,14] probably because it does not reach them. Such limited ability to penetrate into either the hypoxic regions of tumors in vivo or the interior of tumor spheroids in vitro[7] probably reflects rapid metabolism due to a high reduction potential ($E(1) = -303$ mV)[8] and a low rate of extravascular diffusion due to tight DNA binding.[15]

Analogues of nitracrine with lower reduction potentials do have longer half-lives in cell culture,[8] and the 4-methoxy derivative **3** ($E(1) = -361$ mV)[8] showed selective activity against the hypoxic fraction of EMT-6 tumors in vivo, although this was observed (by clonogenic assay of excised tumors) only at doses eventually toxic to the mouse.[8] Such compounds are still likely to have low intrinsic rates of extravascular diffusion, due to tight DNA binding. We therefore recently investigated[14,16] the physicochemical and biological properties of the 5-nitroquinoline (**6**), which is structurally related to nitracrine but with one less aromatic ring in the chromophore. In comparison with nitracrine, this compound showed a lower DNA binding affinity,[14] superior activity in EMT6 spheroids suggesting improved extravascular diffusional properties,[16] hypoxic selectivity as a cytotoxin in single-cell suspensions which was at least as great as that of nitracrine,[14,16] and an ability to radiosensitize hypoxic tumor cells which was much superior to that of nitracrine.[16] This prompted us to carry out a broader structure–activity relationship study of the general class of nitroquinolines as potential hypoxia-selective cytotoxins and radiosensitizers, work that we report here.

Chemistry

Many of the 4-(chloromethyl)nitroquinolines required for this work had not been reported previously, and were prepared by nitration of the appropriate 4-(chloromethyl)-quinolines with fuming HNO_3 in H_2SO_4. Contrary to expectations and to literature reports,[17] the majority of these reactions gave only one nitro isomer. 4-Chloro-6-methylquinoline gave an 81% isolated yield of 4-chloro-6-methyl-5-nitroquinoline, while 4-chloro-8-methoxyquinoline gave 59% of 4-chloro-8-methoxy-5-nitroquinoline. 4-Chloro-7-methylquinoline gave 82% of 4-chloro-7-methyl-8-nitroquinoline, and 4-chloro-8-methylquinoline gave 91% of 4-chloro-8-methyl-5-nitroquinoline, and 4-chloro-3-methylquinoline gave a mixture of 4-chloro-3-methyl-8-nitroquinoline (32%) and 4-chloro-3-methyl-5-nitroquinoline (40%). Assignment of these structures was primarily by NMR chemical shift and coupling data. The C2 proton always resonated at lowest field (δ 8.78–8.92), followed by the C8 proton (δ 8.07–8.33) and the C5 proton (δ 7.98–8.23).

Scheme I[a]



[a] (i) EtOCH=C(COOEt)$_2$/Dowtherm A/260 °C, then OH$^-$; (ii) PhCOPh/270 °C; (iii) $POCl_3$.

4-Chloro-8-methyl-7-nitroquinoline for preparation of compound (**23**) and 4-chloro-7-methyl-6-nitroquinoline for compound (**22**) were made from 2-methyl-3-nitroaniline and 3-methyl-4-nitroaniline, respectively, via the standard diethyl(ethoxymethylene)malonate–quinolone-3-carboxylic acid synthesis[18] (Scheme I).

The majority of the 4-(alkylamino)nitroquinolines of Table I were prepared in moderate to good yields by coupling of the requisite 4-chloronitroquinolines with excess of the aliphatic amine at 95–100 °C, with monitoring of the reaction by TLC until starting material was consumed (Scheme II). Unlike comparable reactions with nitroacridines,[8] the use of phenol in the coupling reaction resulted in much lower yields. Both the yields and the optimum time for reaction varied markedly with the nature of the quinoline; for more reactive analogues the reaction was carried out at 30 °C. No attempts were made to optimize yields of particular compounds, but it is unlikely that higher temperatures would be beneficial; for example, the 5-nitro derivative (**6**) was prepared in 65% yield by reaction at 95 °C for 1.5 h, compared with a yield of 13% reported previously[19] after reaction at 120 °C for 1.5 h.

Reaction of 4-chloro-8-methoxy-5-nitroquinoline (**25**) under these conditions resulted in preferential displacement of the methoxy group to give **26**, and the desired 8-methoxy-5-nitro derivative (**19**) was prepared by nitration of the preformed 8-methoxy compound (**27**) (Scheme III). The 8-(methylamino) compound (**20**) was then synthesized from this by displacement with methylamine.

(13) Wilson, W. R.; Denny, W. A.; Twigden, S. J.; Baguley, B. C.; Probert, J. C. Selective toxicity of nitracrine to hypoxic mammalian cells. *Br. J. Cancer* **1984**, *49*, 215–223.

(14) Wilson, W. R.; Siim, B. G.; Denny, W. A.; van Zijl, P. L.; Taylor, M. L.; Chambers, D. M.; Roberts, P. B. 5-Nitro-4-(N,N-dimethylaminopropylamino)quinoline (5-nitraquine), a new DNA-affinic hypoxic cell radiosensitizer: Comparison with nitracrine. *Radiat. Res.* **1992**, *131*, 257–265.

(15) Wilson, W. R.; Denny, W. A. DNA-Binding nitroheterocycles as hypoxia-selective cytotoxins. In *Radiation Research: A 20th-Century Perspective*; Dewey, W. C., Edington, M., Fry, R. J. M., Hall, E. J., and Whitmore, G. F., Eds.; Academic Press: New York, 1992; Vol. 2, pp 796–801.

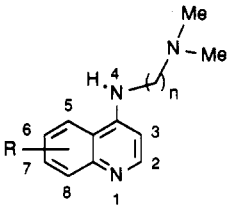
(16) Denny, W. A.; Wilson, W. R.; Atwell, G. J.; Boyd, M.; Pullen, S. M.; Anderson, R. F. Nitroacridines and nitroquinolines as DNA-affinic hypoxia-selective cytotoxins. In *Activation of Drugs by Redox Processes*; Adams, G. E., Ed.; NATO Advanced Study Series; 1991, pp 149–158.

(17) Buchmann, G.; Niess, R. Preparation and reactivity of chloronitro-7-methylquinolines. *J. Prakt. Chem.* **1962**, *16*, 207–219.

(18) Riegel, B.; Lappin, G. R.; Adelson, B. H.; Jackson, R. I.; Albisetti, C. J.; Dodson, R. M.; Baker, R. H. The synthesis of some 4-quinolinols and 4-chloroquinolines by the ethoxymethylenemalonic ester method. *J. Am. Chem. Soc.* **1946**, *68*, 1264–1266.

(19) Stefanska, B.; Zirra, J. A.; Peryt, J.; Kaminski, K.; Ledochowski, A. Research on tumour inhibiting compounds. Part LII. 5- and 8-nitro-4-aminoquinoline derivatives. *Rocz. Chem.* **1973**, *47*, 2339–2343.

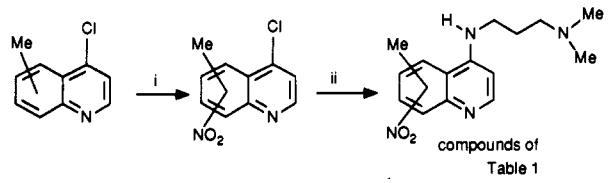
Table I. Physicochemical and Biological Properties of 4-[[(Dimethylamino)alkyl]amino]nitroquinolines



							growth inhibition assays[e]			clonogenic assays[f]	
no.	R	n	R_m[a]	pK_a[b]	C_{50}[c]	$E(1)$[d]	AA8 (air) IC_{50} (μM)	AA8/UV4 (air) ratio	AA8 (air/N_2) ratio	AA8 (air) CT_{10}(μM h)	AA8 (air/N_2) ratio
1,	misonidazole					-389	12080 ± 1100	1.4 ± 0.1	9.3 ± 0.9	$1.7 \times 10^5 \pm 3 \times 10^4$	14 ± 3
2,	nitracrine			6.21		-303	0.024 ± 0.001	16 ± 1	3.6 ± 0.2	0.38 ± 0.03	5.3 ± 0.8
4	H	3	-0.98	8.85	4.6		125 ± 5	1.20 ± 0.02	0.83 ± 0.06	1030 ± 80	2.4 ± 1.2
5	3-NO_2	3	-1.17	4.43	5.0	-475 ± 12	34 ± 1	1.06 ± 0.03	1.00 ± 0.02	730 ± 200	1.1 ± 1.2
6	5-NO_2	3	-1.157	6.39	1.1	-286 ± 11	12.4 ± 0.8	13.6 ± 0.9	5.9 ± 0.6	660 ± 60	14 ± 2
7	6-NO_2	3	-1.30	6.99	1.0	-392 ± 12	34 ± 5	1.87 ± 0.03	1.0 ± 0.1	680 ± 110	1.0 ± 0.1
8	7-NO_2	3	-1.23	6.77	0.9	-323 ± 11	46 ± 13	1.5 ± 0.3	3.6 ± 0.1	970 ± 140	8.9 ± 1
9	8-NO_2	3	-1.44	6.91	1.2	-268 ± 11	9.3 ± 0.8	1.9 ± 0.5	0.75 ± 0.02	240 ± 120	ca. 1[g]
10	5-NO_2	2	-1.17	5.77	1.5	-295 ± 11	20.9 ± 0.4	8.3 ± 0.4	2.7 ± 0.1	700 ± 300	3.9 ± 0.9
11	5-NO_2	4	-1.05	6.71	3.1	-278 ± 11	9.0 ± 0.1	13 ± 4	7.3 ± 0.9	400 ± 20	44 ± 7
12	5-NO_2, N^4-Me	2	-1.18	4.86	3.9	-363 ± 11	95 ± 5	1.2 ± 0.1	1.1 ± 0.1	4400	1.4
13	5-NO_2, N^1-Me	3	-1.36		3.6	-247 ± 10	5.8 ± 0.9	3.3 ± 0.9	1.0 ± 0.2	300 ± 30	0.78 ± 0.06
14	5-NO_2, 2-Me	3	-1.27	6.41	1.1	-274 ± 11	6.6 ± 1.2	17 ± 3	3.6 ± 0.9	770 ± 210	22 ± 6
15	5-NO_2, 3-Me	3	-0.98	6.01	1.0	-369 ± 10	33 ± 3	18 ± 2	7.2 ± 0.9	1870 ± 170	47 ± 11
16	5-NO_2, 3-aza	3	-0.86	4.05	4.3	-307 ± 11	82 ± 18	4.3 ± 0.5	2.2 ± 0.5	2300	1.4
17	5-NO_2, 6-Me	3	-1.06	6.15	3.1	-319 ± 12	170 ± 12	9 ± 1	16 ± 5	3300 ± 1000	16.7 ± 0.5
18	5-NO_2, 8-Me	3	-1.26	6.84	3.0	-316 ± 11	29 ± 5	13 ± 1	19.7 ± 4.4	2100 ± 380	60 ± 10
19	5-NO_2, 8-OMe	3	-1.24	6.76		-310 ± 10	6.8 ± 1.0	12 ± 2	4.3 ± 1.1	3090 ± 110	20.5 ± 0.5
20	5-NO_2, 8-NHMe	3	-0.70	4.22		-520 ± 12	98 ± 9	2.3 ± 0.3	1.3 ± 0.1	2100 ± 600	1.2 ± 0.1
21	3-NO_2, 2-Me	3	-0.63	5.35	8.6	-453 ± 7	226 ± 24	1.1 ± 0.3	1.3 ± 0.3	2600 ± 500	0.6
22	6-NO_2, 7-Me	3	-0.78	7.38	2.3	-453 ± 7	28 ± 3	1.4 ± 0.2	1.0 ± 0.1	450 ± 90	0.95 ± 0.05
23	7-NO_2, 8-Me	3	-0.76	6.71	2.9	-423 ± 8	21 ± 4	21 ± 3	3.7 ± 2.8	880 ± 200	4.5 ± 0.4
24	8-NO_2, 7-Me	3	-0.85	5.51	4.6	-456 ± 8	253 ± 54	2.1 ± 0.4	1.3 ± 0.1	4800 ± 1200	1.40 ± 0.4

[a] R_m = chromatographic measure of drug lipophilicity, measured as detailed in ref 44. [b] Quinoline pK_a values were determined in aqueous solution at 25 °C by spectrophotometry; see ref 45. [c] C_{50} = concentration of drug (μM) to reduce the fluorescence of DNA-bound ethidium bromide by 50%; a direct measure of DNA binding; see ref 45. [d] $E(1)$ values for one-electron reductions were determined by pulse radiolysis; see refs 8, 20, 21. [e] 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), using an 18-h drug exposure under aerobic conditions. AA8(air/N_2) ratio = IC_{50}[AA8(air)]/IC_{50}[AA8(N_2)]. [f] 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 control values using UV4 cells at 10^6/mL in the clonogenic assay (see text). CT_{10} ratio = CT_{10}(air)/CT_{10}(nitrogen). [g] Plots of plating efficiency versus time, with the same drug concentration, under aerobic and hypoxic conditions, cross at approximately 1 h.

Scheme II[a]



[a] (i) HNO_3/H_2SO_4/-5 °C; (ii) $H_2N(CH_2)_2NMe_2$/heat.

Nitro Group Reduction Potentials

One-electron reduction potentials ($E(1)$) for the nitro group were determined by pulse radiolysis, by establishing a reversible equilibrium against suitable redox indicators such as benzylviologen[20] (where the value of the redox couple $BV2^+/BV^{\bullet+}$ is -380 ± 10 mV) in pH 7 phosphate-buffered aqueous 2-propanol solutions (eq 1). The equi-

$$ArNO_2 + BV^{\bullet+} \overset{K}{\rightleftharpoons} ArNO_2^{\bullet-} + BV^{2+} \quad (1)$$

librium constant K for the reaction was determined spectrophotometrically for each compound, and the $E(1)$ values for the $ArNO_2/ArNO_2^{\bullet-}$) redox couples were calculated from this[21] and recorded in Table I.

Biological Studies

The cytotoxicity of the compounds was determined under both aerobic and hypoxic conditions against Chinese hamster ovary cells (CHO, subline AA8) in two assays, and the results are listed in Table I. In the growth inhibition microassay,[9] 0.05-mL cultures were grown and exposed to drugs for 18 h in 96-well tissue culture dishes under aerobic or hypoxic conditions, then grown aerobically without drugs for 3 days. The IC_{50} was defined as the drug concentration which reduced cell numbers to 50% of those in control cultures on the same 96-well dish. The UV4 cell line, a repair-defective mutant derived from AA8 which is hypersensitive to agents whose cytotoxicity is due to bulky DNA adducts or cross-links,[22] was also investigated in this assay to provide initial information on mechanisms of cytotoxicity.

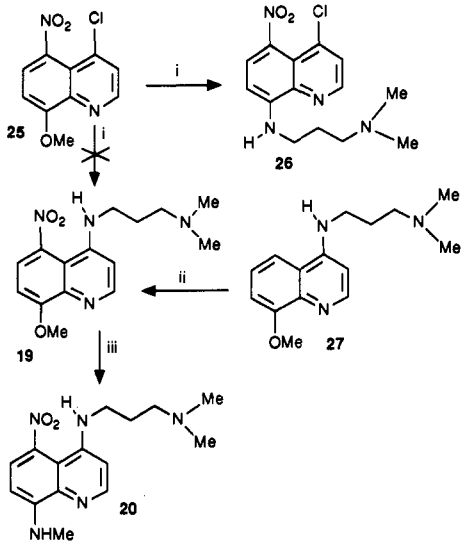
In the clonogenic assay, AA8 cells in early unfed plateau phase were exposed to drugs in continuously-gassed stirred

(20) Patel, K. B.; Willson, R. L. Semiquinone free radicals and oxygen pulse radiolysis study of one electron transfer equilibria. *J. Chem. Soc., Faraday Trans. 1* **1973**, *69*, 814–825.

(21) Van Leewen, J. W.; Van Dijk, C.; Veeger, C. A pulse radiolysis study of the reduction of flavodoxin from *Megasphaera elsdenii* by viologen radicals. A conformational change as a possible regulating mechanism. *Eur. J. Biochem.* **1983**, *135*, 601–607.

(22) Hoy, C. A.; Thompson, L. H.; Mooney, C. L.; Salazar, E. P. Defective DNA cross-link removal in Chinese hamster cell mutants hypersensitive to bifunctional alkylating agents. *Cancer Res.* **1985**, *45*, 1737–1743.

Scheme III[a]



[a] (i) $H_2N(CH_2)_2NMe_2$/heat; (ii) HNO_3/H_2SO_4/−5 °C; (iii) neat $MeNH_2$/heat.

suspension cultures, and survival was assessed at various times by determining plating efficiency as described previously.[8,13] The drug concentration multiplied by the time required to reduce cell survival to 10% of controls (the CT_{10}) was determined, under both aerobic and hypoxic conditions. This technique uses clonogenic potential as the end-point, has the advantage over the growth inhibition assay of providing essentially complete anoxia throughout the period of drug exposure, and generally provides higher values of hypoxic selectivity than the growth inhibition assay.[23]

Radiosensitization of AA8 cells was assessed under conditions identical to the (clonogenic) cytotoxicity assay by incubating cells with drug under hypoxic conditions at 37 °C for 30 min before and during irradiation with a ^{60}Co γ source. Radiation dose–response curves were generated at a range of drug concentrations to define the drug concentration required to increase hypoxic cell radiosensitivity (measured as the radiation dose giving a surviving fraction of 1%) by a factor of 1.3 ($C_{1.3}$).

The ability of drugs to kill or radiosensitize hypoxic cells in tumors was assessed using SCCVII squamous cell carcinomas growing subcutaneously in C_3H/HeN mice. Drugs were administered at 60% of the maximum tolerated dose (MTD) and cell survival determined by clonogenic assay 18 h after treatment.

Results and Discussion

Physicochemical Properties and in Vitro Cytotoxicities. Physicochemical data for the nitroquinolines are given in Table I. Compounds **6–9** are strictly analogous to (respectively) nitracrine (**2**) and the isomeric 2-, 3-, and 4-nitroacridines,[16,24] in which NMR studies[25] show that the free bases of both the 1- and 4-nitro compounds exist in the imino conformation, demonstrated both by nitro group twist angles and NOE interactions. However, similar studies with the 5-nitroquinoline (**6**) (corresponding to the 1-nitroacridine) showed no evidence of the imino conformation.[26] There was also little similarity between the two series in terms of the rank order of their nitro group reduction potentials $E(1)$ (Table I and ref 24). 5-Nitroquinoline (**6**) had the most different physicochemical properties from the other isomers. It had the lowest pK_a (6.39 vs 6.8–6.9), and was the most polar (R_m value −1.57, vs values of −1.2 to −1.4). However, all compounds showed similar levels of DNA binding as measured by the ethidium displacement assay. Shortening the (dimethylamino)alkyl side chain of **6** to give **10** lowered the pK_a due to coulombic effects.[23] Methylation at N4 (compound **12**) also lowered pK_a, due to loss of resonance interaction by out-of-plane twisting.[27] The 3-nitro derivative (**5**), which has no counterpart in the nitracrine series, had a low pK_a due to the electronic effects of the nitro group being in the same ring as the cationic center, and lesser resonance interactions due to steric interactions. Its low $E(1)$ and low DNA binding constant relative to the other nitro isomers is likewise due to the combination of this low pK_a and the severe steric interactions expected with the ortho side chain.

o-Methyl group substitution of these nitro isomers was carried out, in an attempt to lower the nitro group reduction potentials. The effects on $E(1)$ values of the resulting compounds (**17** and **21–24**) were found to be variable, and the resultant of three main factors. Lowering the pK_a lowers the reduction potential $E(1)$ at pH 7 by increasing the proportion of free base, while both electron donation from the methyl group and steric deconjugation of the nitro group should also act to lower $E(1)$. Previous ^{17}O studies[28] have shown that ortho substitution of a methyl group into nitrobenzene twists the nitro group out-of-plane by about 37°, with a resultant loss of conjugation.[28,29] Since the electronic effect of an ortho methyl group can be considered roughly constant, the main effects on $E(1)$ are via pK_a and steric effects.

Thus, while both 6- and 8-methyl substitution of **6** lower $E(1)$ by a similar amount (ca. 30 mV), in the case of the 6-methyl derivative (**17**) this is due largely to deconjugation, while for the 8-methyl compound (**18**) it is largely due to lowering of pK_a. For the other nitroquinolines, *o*-methyl substitution has much larger effects on the $E(1)$ values (ΔE = ca. 100–190 mV). The largest effect (ΔE = −188 mV) is seen with the 8-nitro-7-methyl compound (**24**), due to a substantial lowering of pK_a. The low $E(1)$ values of compounds **22** and **23** are more unexpected, since the pK_a values of the parent 6- and 7-nitro compounds are little affected by methyl substitution, and the steric effect of the *o*-methyl group cannot be significantly different to that with nitrobenzene.

Other monosubstituted analogues of the 5-nitro isomer (**6**) (compounds **14–16**, **19**, and **20**) show lesser but still

(23) Denny, W. A.; Atwell, G. J.; Anderson, R. F.; Wilson, W. R. Hypoxia-selective antitumor agents. 4. Relationships between structure, physicochemical properties and hypoxia-selective cytotoxicity for nitracrine analogues with varying side chains: The imidoacridan hypothesis". *J. Med. Chem.* **1990**, *33*, 1288–1295.

(24) Roberts, P. B.; Denny, W. A.; Wakelin, L. P. G.; Anderson, R. F.; Wilson, W. R. Radiosensitisation of mammalian cells in vitro by nitroacridines. *Radiat. Res.* **1990**, *123*, 153–164.

(25) Boyd, M.; Denny, W. A. NMR studies of configuration and tautomeric equilibria in nitroacridine antitumor agents. *J. Med. Chem.* **1990**, *33*, 2656–2659.

(26) Boyd, M.; Boyd, P. D. W.; Atwell, G. J.; Wilson, W. R.; Denny, W. A. Crystallographic and oxygen-17 NMR studies of nitro group torsion angles in a series of 4-alkylaminonitroquinolines designed as hypoxia-selective cytotoxins. *J. Chem. Soc., Perkin Trans. 2* **1992**, 579–584.

(27) Albert, A. *The Acridines*, 2nd ed.; Edward Arnold Ltd.: London, 1962; p 167.

(28) Balakrishnan, P.; Boykin, D. W. Relationship of nitro group torsion angles with ^{17}O chemical shift data. *J. Org. Chem.* **1985**, *50*, 3661–3663.

(29) Geske, D. H.; Ragle, J. L. Steric effects on nitrogen hyperfine coupling in methyl-substituted mono- and dinitrobenzene anion radicals. *J. Am. Chem. Soc.* **1961**, *83*, 3532–3533.

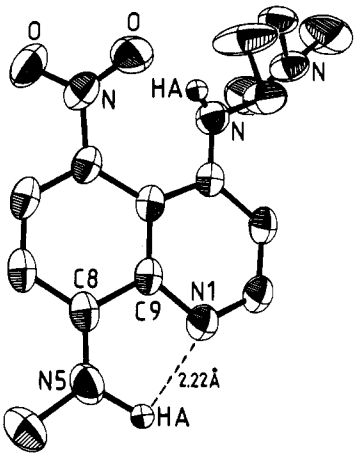


Figure 1. ORTEP diagram of 4-[[3-(dimethylamino)propyl]amino]-8-(methylamino)-5-nitroquinoline (compound 20), showing the planarity of the N1–C9–C8–N5–HA2 ring, and the short (2.22 Å) hydrogen bond between the methylamino NH and the quinoline nitrogen.

significant changes in critical parameters such as $E(1)$ and DNA binding. The very low pK_a of the 8-(methylamino) compound (20) is due largely to strong hydrogen-bond formation between the 8-NHMe group and the quinoline nitrogen, stabilizing the free base form. This H-bonding can clearly be seen in the crystal structure of 20 (Figure 1), where the five-membered ring N1–C9–C8–N5–HA2 is essentially planar, and the amine proton is only 2.22 Å from the quinoline nitrogen. The very low reduction potential of 20 is likely due to this low pK_a.

The cytotoxicities of the compounds against the Chinese hamster cell line AA8 were determined using both growth inhibition and clonogenicity assays, and are recorded in Table I. There was a significant ($\rho < 0.001$) correlation between the aerobic cytotoxic potencies of the drugs measured in both assays, as shown by eq 2, and as observed previously for nitracrine analogues.[23]

$$CT_{10}\ (\mu M\ h) = 14.9\ (\pm 3.4) IC_{50}\ (\mu M) + 850 \qquad (2)$$

$$n = 21 \qquad r = 0.71$$

The in vitro hypoxic selectivity of the compounds (defined as the ratios of their IC_{50} or CT_{10} values against AA8 under aerobic (20% O_2) versus hypoxic conditions[9,23]) are also given in Table I. Comparison of the hypoxic selectivities determined by the growth inhibition and clonogenic assays for the whole set of compounds (5–24) showed a significant ($\rho < 0.001$) linear correlation, with the clonogenic assay being the more sensitive of the two, giving ratios about 2.7-fold larger (eq 3). We have noted

$$CT_{10}[\text{ratio}] = 2.71\ (\pm 0.49) IC_{50}[\text{ratio}] + 0.88 \qquad (3)$$

$$n = 21 \qquad r = 0.78$$

a similar relationship previously with nitroacridine compounds, the lower sensitivity of the growth inhibition assay being due to slower deoxygenation kinetics in the hypoxic cultures.[23]

Although there was only a small range in aerobic cytotoxicities (4-fold) among the different nitro isomers (5–9), there was a wider range (150-fold) among the whole dataset. All but two of the nitro compounds were more cytotoxic than the parent desnitro derivative (4) in the growth inhibition assay. For the small homologous series of 5-nitro compounds (10, 6, 11) there was an increase in cytotoxicity with alkyl chain length, probably related to increasing drug lipophilicity.[23] Of the five nitro isomers studied, only the 5- and 7-nitro compounds (6 and 8) show significant selectivity, with the 5-nitro compound (6) being superior to nitracrine (4) in both the growth inhibition and clonogenic assays (Table I).

The cytotoxicities of the series of substituted 5-nitro compounds (14–20) varied quite considerably (30-fold), but no relationships between aerobic cytotoxicity and physicochemical properties (including reduction potential) could be detected. In particular, the low potency of the 6-methyl derivative (17) was unexpected. It is not due to *o*-methyl substitution per se, since 22 and 23 are slightly more cytotoxic than their desmethyl analogues 7 and 8. However, it is notable that the three least cytotoxic nitro compounds (17, 21, and 24) are the only examples where the nitro group is flanked by either two ortho substituents (21) or is subject to both ortho and peri interactions (17, 24), where the nitro group is likely[28] to be twisted out of the aromatic ring plane by as much as 80–90°. In the case of nitro-substituted polycyclic hydrocarbons, derivatives with such highly twisted nitro groups are much less mutagenic than coplanar analogues.[30,31]

As shown previously with nitracrine analogues, the hypoxic selectivities of analogues of 6 were increased as the side chain was extended (compounds 10, 6, 11). However, alkylation of either of the quinoline nitrogens (compounds 12 and 13) resulted in loss of selectivity. All of the other ring-substituted 5-nitro compounds (14–20) retained selectivity with the exception of the 8-NHMe compound 20, but there was a considerable variation in magnitude. The best compounds were the 3- and 8-Me derivatives (15 and 18), with ratios in the clonogenic assay of 47- and 60-fold, respectively.

Mechanism of Cytotoxicity: Cell Line Studies. The extent to which cytotoxicity is due to DNA alkylation was assessed by determining the ratio of IC_{50} values (HF; hypersensitivity factor) of AA8 and UV4 cells in the growth inhibition assay. We have previously shown[9] that the UV4 cell line has pronounced hypersensitivity to nitracrine (2).

As expected, the desnitro compound (4) had an HF close to unity, suggesting that its mode of cytotoxicity does not involve the formation of DNA adducts. Of the five nitro isomers, only the 5-nitro (6) showed a significant HF (14-fold), consistent with the mechanism of cytotoxicity being the formation of bulky monoadducts or cross-links. Almost all of the 5-nitro analogues (10–20) also had high HF values although values close to unity were observed for compounds 12 and 20. It is noteworthy that these compounds were also unusual in lacking hypoxic selectivity. With the exception of 23, all the 6-, 7- and 8-nitro compounds (7–9, 22-24) had very low HF values, suggesting no involvement of DNA adducts. Again, with the exception of 23, these compounds showed no appreciable hypoxic selectivity. For the series overall, there was a positive correlation between HF values and the magnitude of the hypoxic selectivity [CT_{10}(air)/CT_{10}(N_2)

(30) Fu, P. P.; Chou, M. W.; Miller, D. W.; White, G. L.; Heflich, R. H.; Beland, F. A. The orientation of the nitro-substituent predicts the direct-acting bacterial mutagenicity of nitrated polycyclic aromatic hydrocarbons. *Mutat. Res.* 1985, *143*, 173–181.

(31) Vance, W. A.; Okamoto, H. S.; Wang, Y. Y. In *Carcinogenic and Mutagenic Responses to Aromatic Amines and Nitroarenes*; King, C. M., Romano, L. J., Schuetzle, D., Eds.; Elsevier: New York, 1988; p 291.

Table II. Hypoxic Cell Radiosensitization, in Vitro Therapeutic Index and Toxicity of 4-[[(Dimethylamino)propyl]amino]nitroquinolines in C_3H/HeN Mice

no.	$C_{1.3}$[a] (μM)	IVTI[b]	MTD[c] (μmol/kg)
1 (misonidazole)	300	570	5000
3 (nitracrine)	0.5*,[d]	0.76	0.3
5	400	1.8	110
6	4.5	150	100
7	7	100	700
8	30	30	800
9	17	20	600
13	>75[e]	<3	
14	6	130	400
15	8	230	300
17	55	60	200
18	6	350	100
19	9	39	
20	700	3	

[a] Concentration required to increase radiation sensitivity (measured as the radiation dose required to reduce surviving fraction to 1%) by a factor of 1.3 when AA8 cells are exposed to drug for 30 min before and during irradiation under hypoxic conditions. [b] In vitro therapeutic index; $CT_{10}(air)/C_{1.3}$. Calculated using values of CT_{10} for AA8 cells from Table I. Units are h^{-1}. [c] Maximum tolerated (nonlethal) dose in C_3H/HeN mice (single ip dose). [d] Data from ref 24. (Radiosensitivity determined at 0 °C to suppress cytotoxicity). [e] Cytotoxic at higher concentrations.

ratios] of the compounds, with the most hypoxic selective compounds also showing the highest HF values (eq 4).

$$CT_{10}[ratio] = 1.73\ (\pm 0.46)HF - 0.09 \qquad (4)$$

$$n = 21 \quad r = 0.66$$

Radiosensitization. The parent 5-nitroquinoline (6) has recently been shown to be a potent radiosensitizer in hypoxic AA8 cell cultures.[14] The much lower cytotoxic potency of 6 than nitracrine results in a correspondingly higher "in vitro therapeutic index" (IVTI, the ratio of hypoxic radiosensitizing potency to aerobic cytotoxicity) for the more weakly DNA binding quinoline (6) (IVTI = 150) than for nitracrine (3) (IVTI = 0.76). Comparison of the nitro positional isomers of 6 showed the latter to be the most potent radiosensitizer, while the compound with the lowest reduction potential (5) had a 100-fold lower radiosensitizing potency (Table II). This differential between the 3-NO_2 and 5-NO_2 compounds, which differ in reduction potential by 190 mV, is of the magnitude expected for a one-electron transfer process.[32] For the larger series of 4-alkylnitroquinolines studied (Table II), there was a moderately significant ($\rho = 0.025$) dependence of sensitizing potency on reduction potential (eq 5), although this was dominated by the two low-potential compounds 5 and 20.

$$\log (1/C_{1.3})(\mu M) = 5.55\ (\pm 2.13)E(1)\ (V) + 6.41 \qquad (5)$$

$$n = 12 \quad r = 0.63$$

Although DNA binding affinity itself was not a statistically significant variable in determining log $(1/C_{1.3})$, it is likely that physicochemical parameters other than $E(1)$ may be important in the case of DNA-affinic radiosensitizers. Differences in uptake by cells, and in the kinetics of dissociation of drug–DNA complexes, have been suggested to be important determinants of the radiosensitization potency of the nitroacridines,[24] and may also modify radiosensitizing potency in the nitroquinoline series. Thus none of the compounds showed IVTI values as high as that of the non-DNA-binding 2-nitroimidazole derivative misonidazole (1).

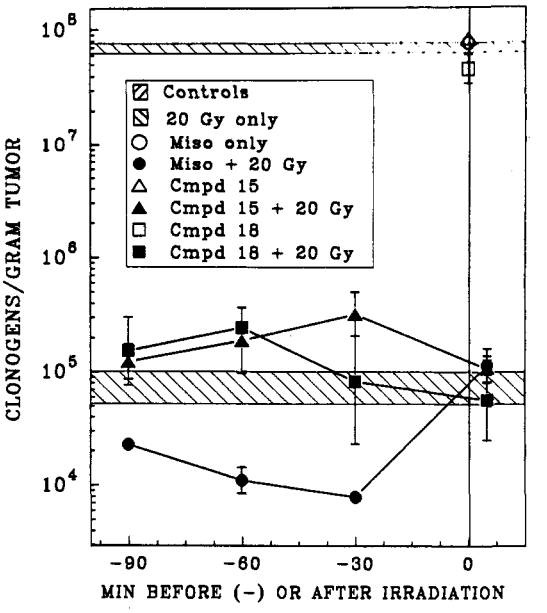


Figure 2. Effect of compounds 15, 18, and 1 (misonidazole) on the radiosensitivity of SCCVII tumors. Mice bearing subcutaneous tumors were treated ip with 1 (2500 μmol/kg), 15 (180 μmol/kg), or 18 (60 μmol/kg) before or after irradiation (20 Gy) and assayed 18 h later by dissociating tumors and plating in vitro. For each compound studied, each point is the geometric mean for a group of three mice, and error bars are SEM. Open symbols: drug only (no irradiation). Hatched bands are mean ± 1 SEM for untreated controls and for 20 Gy irradiation only over an extended series of experiments. The means for the controls and 20 Gy groups in the experiments with all three drugs fell within these bands.

In Vivo Activity against Hypoxic Cells. The above in vitro studies clearly identified the 3-Me and 8-Me analogues of 6 (compounds 15 and 18) as the most promising agents for evaluation in vivo. These compounds were not only superior to 6 as both hypoxia-selective cytotoxins and radiosensitizers, but also had the advantage of lower $E(1)$ values than the parent drug, and would thus be expected to have lower rates of nitro reduction than 6. For this compound, rapid metabolism in mice has been identified as a probable reason for its lack of activity as a hypoxic cell cytotoxin and/or radiosensitizer in vivo.[14] The toxicities of the compounds were determined in mice, using single intraperitoneal doses (Table II). Compounds 15 and 18 were administered at 60% of their MTDs either before or after irradiation of mice bearing SCCVII tumors, but neither induced additional tumor cell killing (Figure 2). These compounds therefore lack activity as hypoxic cell cytotoxins or hypoxic cell radiosensitizers in the SCCVII tumor in vivo. Compound 18 has also been tested against the KHT tumor and, again, found to be inactive as a radiosensitizer in vivo (W. R. Leopold, Warner-Lambert Co., personal communication).

Conclusions

The large differences in hypersensitivity of the UV4 cell line to the isomeric 4-[[3-(dimethylamino)propyl]amino]nitroquinolines suggest that quite different modes of action may exist. One (illustrated by compound

(32) Wardman, P. A. The use of nitroaromatic compounds as hypoxic cell radiosensitisers. *Curr. Topics Radiat. Res. Q.* **1977**, *11*, 347–398.

6) is a bioreductive mechanism inhibited by oxygen, resulting in formation of DNA-alkylating nitroreduction products. For other agents (including the cytotoxic desnitro 4-alkylquinoline (4)), some oxygen-insensitive process appears to be responsible for cell killing; this might explain the lack of dependence of cytotoxicity on $E(1)$ within the series. Despite a rather high reduction potential, the 5-nitro isomer (6) showed the greatest hypoxic selectivity, together with potent hypoxic cell radiosensitization and the best "in vitro therapeutic index". The effects of ring substitution in this compound proved complex, being the resultant of both the electronic and steric effects, as well as effects on chromophore pK_a, but did allow significant improvements in hypoxic selectivity. The 3-methyl and 8-methyl derivatives (15, 18) had reduction potentials lower than (6), suggesting improved metabolic stability, and showed exceptional hypoxic selectivities (CT_{10} ratios of 47 and 60, respectively, in the clonogenic assay), and the highest IVTIs as hypoxic cell radiosensitizers. However, despite these favorable in vitro properties, neither compound had activity against hypoxic cells in SCCVII tumors when administered at 60% of the MTD. While further exploration of this series may be appropriate, particularly the investigation of analogues with even lower reduction potentials, the question remains as to whether significant DNA-binding affinity precludes adequate distribution to hypoxic cells in tumors. At this time there is still no DNA-affinic nitroheterocycle which has been demonstrated to have activity as a hypoxic cell radiosensitizer or HSC in vivo.

Experimental Section

Analyses were within ±0.4% of the theoretical value, where indicated by the symbols of the elements, and were performed by the Microchemical Laboratory, University of Otago, Dunedin. Melting points were determined using an Electrothermal Model 9200 digital melting point apparatus, and are as read. NMR spectra were measured on a Bruker AM-400 spectrometer (Me_4Si).

Synthesis of 4-Chloronitroquinolines. The following compounds were prepared by literature methods. 4-chloro-5-nitroquinoline:[33] mp 150 °C; ^1H NMR ($CDCl_3$) δ 8.88 (d, J = 4.71 Hz, 1 H, H-2), 8.33 (dd, J = 7.3, 2.5 Hz, 1 H, H-8), 7.8 (m, 2 H, H-6, H-7), 7.64 (d, J = 4.7 Hz, 1 H, H-3). 4-Chloro-8-nitroquinoline:[33] mp 128–129 °C; ^1H NMR ($CDCl_3$) δ 8.92 (d, J = 4.7 Hz, 1 H, H-2), 8.46 (dd, J = 8.55, 1.35 Hz, 1 H, H-7), 8.08 (dd, J = 7.5, 1.3 Hz, 1 H, H-5), 7.74 (dd, J = 8.4, 7.5 Hz, 1 H, H-6), 7.66 (d, J = 4.7 Hz, 1 H, H-3). 4-Chloro-2-methyl-3-nitroquinoline:[34] mp 91–93 °C; ^1H NMR ($CDCl_3$) δ 8.23 (dd, J = 8.4, 1.35 Hz, 1 H, H-8), 8.09 (dd, J = 8.5, 1.0 Hz, 1 H, H-5), 7.87 (ddd, J = 8.55, 8.4, 1.4 Hz, 1 H, H-7), 7.72 (ddd, J = 8.55, 8.4, 1.15 Hz, 1 H, H-6), 2.74 (s, 3 H, CH_3). 4-Chloro-2-methyl-5-nitroquinoline:[34] mp 111–112 °C; ^1H NMR ($CDCl_3$) δ 8.21 (dd, J = 8.3, 1.5 Hz, 1 H, H-8), 7.74 (dd, J = 8.25, 7.55 Hz, 1 H, H-7), 7.70 (dd, J = 7.5, 1.45 Hz, 1 H, H-6), 7.52 (s, 1 H, H-3), 2.75 (s, 3 H, CH_3). 4-Chloro-2-methyl-8-nitroquinoline:[34] mp 112–113 °C; ^1H NMR ($CDCl_3$) δ 8.38 (dd, J = 9.2, 1.3 Hz, 1 H, H-7), 7.99 (dd, J = 7.5, 1.35 Hz, 1 H, H-5), 7.64 (dd, J = 8.45, 7.55 Hz, 1 H, H-6), 7.53 (s, 1 H, H-3), 2.78 (s, 3 H, CH_3).

Nitration of 4-Chloro-3-methylquinoline (Example of General Preparation). 4-Chloro-3-methylquinoline[35] (14.2 g, 0.08 mol) was added slowly to concentrated H_2SO_4 (60 mL), the temperature being kept below 10 °C. The solution was then cooled to −5 °C, and a mixture of fuming HNO_3 (11.3 mL, 0.27 mol) and concentrated H_2SO_4 (11.3 mL) was added dropwise to the stirred solution, the temperature being kept below 0 °C. The reaction mixture was stirred for a further 5 min at 0 °C and then poured into ice-cold concentrated ammonia. The precipitated solid was collected, washed with water, dried, and extracted with boiling petroleum ether (bp 80–100 °C) to give a crude product (13.88 g) shown by TLC to contain two compounds. Chromatography on SiO_2 and elution with benzene/EtOAc (95:5) gave 4-chloro-3-methyl-8-nitroquinoline (5.64 g, 32%): mp (petroleum ether) 124–125 °C; ^1H NMR ($CDCl_3$) δ 8.88 (s, 1 H, H-2), 8.435 (dd, J = 8.55, 1.3 Hz, 1 H, H-7), 8.00 (dd, J = 7.5, 1.25 Hz, 1 H, H-5), 7.69 (dd, J = 8.55, 7.55 Hz, 1 H, H-6), 2.62 (s, 3 H, CH_3). Anal. ($C_{10}H_7ClN_2O_2$) C, H, N. Further elution with the same solvent mixture gave 4-chloro-3-methyl-5-nitroquinoline (7.05 g, 40%): mp (petroleum ether) 138–139 °C; ^1H NMR ($CDCl_3$) δ 8.84 (s, 1 H, H-2), 8.27 (dd, J = 7.9, 1.9 Hz, 1 H, H-8), 7.76 (dd, J = 7.55, 1.9 Hz, 1 H, H-6), 7.73 (m, 1 H, H-7), 2.50 (s, 3 H, CH_3). Anal. ($C_{10}H_7ClN_2O_2$) C, H, N.

Similar nitration of 4-chloro-6-methylquinoline[36] gave a crude product which contained only one compound by TLC. Crystallization of the crude product from petroleum ether gave an 81% yield of 4-chloro-6-methyl-5-nitroquinoline: mp 116.5–117 °C; ^1H NMR ($CDCl_3$) δ 8.76 (d, J = 4.7 Hz, 1 H, H-2), 8.12 (d, J = 8.7 Hz, 1 H, H-8), 7.64 (d, J = 8.7 Hz, 1 H, H-7), 7.55 (d, J = 4.7 Hz, 1 H, H-3), 2.50 (s, 3 H, CH_3). Anal. ($C_{10}H_7ClN_2O_2$) C, H, N.

Similar nitration of 4-chloro-7-methylquinoline[37] also gave a single compound. Crystallization from MeOH gave an 82% yield of 4-chloro-7-methyl-8-nitroquinoline: mp 147–148 °C; ^1H NMR ($CDCl_3$) δ 8.82 (d, J = 4.7 Hz, 1 H, H-2), 8.23 (d, J = 8.7 Hz, 1 H, H-5), 7.60 (d, J = 4.7 Hz, 1 H, H-3), 7.55 (d, J = 8.7 Hz, 1 H, H-5), 2.54 (s, 3 H, CH_3). Anal. ($C_{10}H_7ClN_2O_2$) C, H, N. Buchmann and Niess[17] report two products from the nitration of 4-chloro-7-methylquinoline under similar conditions. They identify the minor product (mp 165–167 °C) as the 8-nitro isomer, and the major product (mp 147–148 °C) as the 6-nitro compound. However, their major product has an identical melting point to the sole product found by us, and is almost certainly the 8-nitro isomer.

Similar nitration of 4-chloro-8-methylquinoline followed by crystallization of the crude product from MeOH gave a 91% yield of 4-chloro-8-methyl-5-nitroquinoline as the only product: mp 137–138 °C; ^1H NMR ($CDCl_3$) δ 8.87 (d, J = 4.7 Hz, 1 H, H-2), 7.70 (d, J = 7.7 Hz, 1 H, H-6), 7.63 (dd, J = 7.7, 0.9 Hz, 1 H, H-7), 7.62 (d, J = 4.65 Hz, 1 H, H-3), 2.85 (d, J = 0.87 Hz, CH_3). Anal. ($C_{10}H_7ClN_2O_2$) C, H, N.

Nitration of 4-chloro-8-methoxyquinoline was carried out using a modified procedure, employing KNO_3 (1.2 mol equiv) as nitrating agent. Crystallization of the crude product twice from benzene gave a 59% yield of 4-chloro-8-methoxy-5-nitroquinoline (25): mp 192–192.5 °C; ^1H NMR ($CDCl_3$) δ 8.80 (d, J = 4.7 Hz, 1 H, H-2), 7.79 (d, J = 8.55 Hz, 1 H, H-6), 7.61 (d, J = 4.65 Hz, 1 H, H-3), 6.99 (d, J = 8.55 Hz, 1 H, H-7), 4.09 (s, 3 H, OCH_3). Anal. ($C_{10}H_7ClN_2O_3$) C, H, N.

4-Chloro-8-methyl-7-nitroquinoline (30). Equimolar amounts of 2-methyl-3-nitroaniline and diethyl (ethoxymethylene)malonate were reacted together under standard conditions.[18] Cyclization of the resulting acrylate and saponification gave material which was extracted with warm 10% aqueous $KHCO_3$. Acidification of this clarified solution gave crude 8-methyl-7-nitro-4(1*H*)-quinolone-3-carboxylic acid (28) (48% overall yield) suitable for the next step. A sample crystallized from DMF/MeOH, mp >320 °C. Anal. ($C_{11}H_8N_2O_5$) C, H, N. Decarboxylation of this acid in benzophenone at 265 °C and crystallization of the crude product several times from small volumes of DMF gave 8-methyl-7-nitro-4(1*H*)-quinolone (29) (58% yield), mp >320 °C. Anal. ($C_{10}H_8N_2O_3$) C, H, N. Reaction

(33) Gouley, R. W.; Moersch, G. W.; Mosher, H. S. Heterocyclic basic compounds. X. 4,8-Diaminoquinoline and derivatives. *J. Am. Chem. Soc.* **1947**, *69*, 303–306.

(34) Halcrow, B. E.; Kermack, W. O. Nitration of 4-chloro- and 4-hydroxy-quinolines. *J. Chem. Soc.* **1945**, 415–417.

(35) Steck, E. A.; Hallock, L. L.; Holland, A. J. Quinolines. 1. The synthesis of 3-methyl-4-(1'-methyl-4'-diethylaminobutylamino)quinoline and some 6-substituted derivatives. *J. Am. Chem. Soc.* **1946**, *68*, 129–132.

(36) Burckhalter, J. H.; Tendick, F. H.; Jones, E. M.; Jones, P. A.; Holcomb, W. F.; Rawlins, A. L. Aminoalkylphenols as antimalarials. II. (Heterocyclic-amino)-α-amino-*o*-cresols. The synthesis of camoquin. *J. Am. Chem. Soc.* **1948**, *70*, 1363–1373.

(37) Breslow, D. S.; Bloom, M. S.; Shivers, J. C.; Adams, J. T.; Weiss, M. J.; Yost, R. S.; Hauser, C. R. Synthesis of antimalarials. V. The synthesis of certain 4-aminoquinoline derivatives. *J. Am. Chem. Soc.* **1946**, *68*, 1232–1238.

of this quinolone with $POCl_3$ as usual then gave 4-chloro-8-methyl-7-nitroquinoline (**30**): mp (petroleum ether) 126–127 °C; ^1H NMR ($CDCl_3$) δ 8.92 (d, J = 4.6 Hz, 1 H, H-2), 8.22 (d, J = 9.2 Hz, 1 H, H-6), 7.98 (d, J = 9.2 Hz, 1 H, H-5), 7.64 (d, J = 4.6 Hz, 1 H, H-3), 3.01 (s, 3 H, CH_3). Anal. ($C_{10}H_7ClN_2O_2$) C, H, N.

4-Chloro-7-methyl-6-nitroquinoline (34). Equimolar amounts of 3-methyl-4-nitroaniline and diethyl (ethoxymethylene)malonate were reacted together and processed under standard conditions[18] to give a mixture of ethyl 5- and 7-methyl-6-nitro-4(1*H*)-quinolone-3-carboxylates in 51% overall yield. This crude ester mixture (12.40 g, 0.045 mol) was heated under reflux in a mixture of 2 N aqueous NaOH (250 mL) and EtOH (250 mL) for 30 min. The solution was concentrated to 200 mL and cooled to 5 °C for 10 h, and the resulting deep orange precipitate of sodium salt was collected and washed with saturated aqueous NaCl. Dissolution in hot water, clarification, and acidification gave a solid which was recrystallized from DMF/EtOH/water and then from a small volume of DMF to afford pure 7-methyl-6-nitro-4(1*H*)-quinolone-3-carboxylic acid (**32**) (7.18 g), mp 279–280 °C. Anal. ($C_{11}H_8N_2O_5$) C, H, N. Acidification of the original mother liquors and recrystallization of the resulting precipitate in the same way gave pure 5-methyl-6-nitro-4(1*H*)-quinolone-3-carboxylic acid (**31**) (1.19 g), mp 281–284 °C. Anal. ($C_{11}H_8N_2O_5$) C, H, N.

Decarboxylation of **32** in benzophenone gave an 84% yield of 7-methyl-6-nitro-4(1*H*)-quinolone (**33**), mp (AcOH) 291–292 °C. Anal. ($C_{10}H_8N_2O_3$) C, H, N. Reaction of this with $POCl_3$ in the usual manner then gave 4-chloro-7-methyl-6-nitroquinoline (**34**), which crystallized from benzene/petroleum ether as colorless needles: mp 117–117.5 °C; ^1H NMR ($CDCl_3$) δ 8.89 (d, J = 4.7 Hz, 1 H, H-2), 8.84 (s, 1 H, H-5), 8.07 (br s, 1 H, H-8), 7.57 (d, J = 4.7 Hz, 1 H, H-3), 2.78 (d, J = 0.82 Hz, 3 H, CH_3). Anal. ($C_{10}H_7ClN_2O_2$) C, H, N. Buchmann and Niess[17] give mp 147–148 °C for this compound, from nitration of 4-chloro-7-methylquinoline, but the compound they describe is almost certainly 4-chloro-7-methyl-8-nitroquinoline, mp 147–148 °C (see above).

Preparation of 4-[[3-(Dimethylamino)propyl]amino]-5-nitroquinoline (6) (General Example). A mixture of 4-chloro-5-nitroquinoline (1.04 g, 5 mmol) and *N,N*-dimethylpropane-1,3-diamine (3.5 g, 35 mmol) was heated at 95–100 °C under N_2 with occasional agitation, until TLC indicated that the reaction was complete (1.5 h). The excess amine was removed at moderate temperature (<80 °C) under reduced pressure, and the residue was chromatographed on alumina (Brockmann, activity II–III). Elution with EtOAc gave 4-[[3-(dimethylamino)propyl]amino]-5-nitroquinoline (**6**): ^1H NMR ($CDCl_3$) δ 8.47 (d, J = 5.2 Hz, 1 H, H-2), 8.05 (dd, J = 8.4, 1.4 Hz, 1 H, H-6), 7.59 (dd, J = 7.4, 1.4 Hz, 1 H, H-8), 7.47 (dd, J = 7.5, 8.4 Hz, 1 H, H-7), 6.52 (br s, 1 H, NH), 6.47 (d, J = 5.5 Hz, 1 H, H-3), 3.20 (q, J = 6.1 Hz, 2 H, ArNHCH_2), 2.32 (t, J = 5.9 Hz, 2 H, CH_2N(CH_3)$_2$), 2.15 (s, 6 H, N(CH_3)$_2$), 1.77 (quintet, J = 6.1 Hz, 2 H, CH_2C$H_2$$CH_2$). Crystallization from EtOAc/MeOH/HCl gave the dihydrochloride salt (1.13 g, 65% yield), mp 211–213 °C (lit.[19] mp 180 °C dec). Anal. ($C_{14}H_{18}N_4O_2$·2HCl) C, H, N. Cl.

Most of the other compounds of Table I were prepared in similar fashion from the appropriate chloroquinoline and amine, using reaction times from 0.5 to 8 h, in yields varying between 44 and 82%. For the unsubstituted compound (**4**), 4-chloroquinoline and the amine were reacted in excess phenol at 140 °C. The more reactive 3-nitro-4-chloroquinolines for preparation of compounds (**5** and **21**) were reacted at 30 °C, and for 4-chloro-5-nitroquinazoline[38] the reaction was carried out at 30 °C in CH_2Cl_2 as a moderating solvent to give (**16**).

4-[[3-(Dimethylamino)propyl]amino]quinoline (4): ^1H NMR (free base in $CDCl_3$) δ 8.52 (d, J = 5.3, 1 H, H-2), 7.96 (dd, J = 8.4, 0.7 Hz, 1 H, H-8), 7.68 (dd, J = 8.4, 0.9 Hz, 1 H, H-5), 7.65 (s, 1 H, NH), 7.60 (m, 1 H, H-7), 7.40 (m, 1 H, H-6), 6.34 (d, J = 5.4 Hz, 1 H, H-3), 3.98 (q, J = 5.4 Hz, 2 H, NHCH_2), 2.56 (t, J = 5.5 Hz, 2 H, CH_2N(CH_3)$_2$), 2.38 (s, 6 H, N(CH_3)$_2$), 1.92 (quintet, J = 5.6 Hz, 2 H, CH_2C$H_2$$CH_2$). Dihydrochloride salt dihydrate, mp 74–76 °C. Anal. ($C_{14}H_{19}N_3$·2HCl·2H_2O) C, H, N. Cl.

4-[[3-(Dimethylamino)propyl]amino]-3-nitroquinoline (5): ^1H NMR (free base in $CDCl_3$) δ 10.05 (s, 1 H, NH), 9.32 (s, 1 H, H-2), 8.32 (d, J = 8.5 Hz, 1 H, H-8), 7.97 (d, J = 8.3 Hz, 1 H, H-5), 7.73 (t, J = 7.5 Hz, 1 H, H-7), 7.47 (t, J = 7.7 Hz, 1 H, H-6), 3.85 (q, J = 5.7 Hz, 2 H, NHCH_2), 2.57 (t, J = 5.5 Hz, 2 H, CH_2N(CH_3)$_2$), 2.31 (s, 6 H, N(CH_3)$_2$), 1.93 (quintet, J = 5.6 Hz, 2 H, CH_2C$H_2$$CH_2$). Dihydrochloride salt, mp 214–216 °C. Anal. ($C_{14}H_{18}N_4O_2$·2HCl) C, H, N, Cl.

4-[[3-(Dimethylamino)propyl]amino]-6-nitroquinoline (7): ^1H NMR (free base in $CDCl_3$) δ 9.32 (s, 1 H, NH), 8.77 (d, J = 2.3 Hz, 1 H, H-5), 8.58 (d, J = 5.4 Hz, 1 H, H-2), 8.33 (dd, J = 9.2, 2.4 Hz, 1 H, H-6), 7.97 (d, J = 9.3 Hz, 1 H, H-8), 6.36 (d, J = 5.5 Hz, 1 H, H-3), 3.44 (q, J = 4.0 Hz, 2 H, NHCH_2), 2.67 (t, J = 5.3 Hz, 2 H, CH_2N(CH_3)$_2$), 2.48 (s, 6 H, N(CH_3)$_2$), 1.96 (quintet, J = 5.5 Hz, 2 H, CH_2C$H_2$$CH_2$). Dihydrochloride salt, mp 250–252 °C. Anal. ($C_{14}H_{18}N_4O_2$·2HCl) C, H, N, Cl.

4-[[3-(Dimethylamino)propyl]amino]-7-nitroquinoline (8): ^1H NMR (free base in $CDCl_3$) δ 8.80 (d, J = 2.4 Hz, 1 H, H-8), 8.61 (d, J = 5.4 Hz, 1 H, H-2), 8.37 (s, 1 H, NH), 8.12 (dd, J = 9.2, 2.4 Hz, 1 H, H-7), 7.77 (d, J = 9.2 Hz, 1 H, H-6), 6.42 (d, J = 5.4 Hz, 1 H, H-3), 3.42 (q, J = 4.2 Hz, 2 H, NHCH_2), 2.62 (t, J = 5.4 Hz, 2 H, CH_2N(CH_3)$_2$), 2.40 (s, 6 H, N(CH_3)$_2$), 1.95 (quintet, J = 5.5 Hz, 2 H, CH_2C$H_2$$CH_2$). Dihydrochloride salt, mp 241–242 °C. Anal. ($C_{14}H_{18}N_4O_2$·2HCl·$^1/_2H_2O$) C, H, N, Cl.

4-[[3-(Dimethylamino)propyl]amino]-8-nitroquinoline (9): ^1H NMR (free base in $CDCl_3$) δ 8.59 (d, J = 5.4 Hz, 1 H, H-2), 8.35 (s, 1 H, NH), 7.84 (m, 2 H, H-4, H-5), 7.39 (t, J = 7.9 Hz, 1 H, H-6), 6.39 (d, J = 5.4 Hz, 1 H, H-3), 3.40 (q, J = 4.4 Hz, 2 H, NHCH_2), 2.60 (t, J = 5.5 Hz, 2 H, CH_2N(CH_3)$_2$), 2.38 (s, 6 H, N(CH_3)$_2$), 1.95 (quintet, J = 5.5 Hz, 2 H, CH_2C$H_2$$CH_2$). Dihydrochloride salt, mp 261–262 °C. Anal. ($C_{14}H_{18}N_4O_2$·2HCl·H_2O) C, H, N, Cl.

4-[[2-(Dimethylamino)ethyl]amino]-5-nitroquinoline (10): ^1H NMR (free base in $CDCl_3$) δ 8.59 (d, J = 5.4 Hz, 1 H, H-2), 8.16 (m, 1 H, H-8), 7.60 (m, 2 H, H-6, H-7), 6.59 (d, J = 5.4 Hz, 1 H, H-3), 5.90 (s, 1 H, NH), 3.22 (q, J = 5.7 Hz, 2 H, NHCH_2), 2.61 (t, J = 6.0 Hz, 2 H, CH_2N(CH_3)$_2$), 2.30 (s, 6 H, N(CH_3)$_2$). Dihydrochloride salt, mp 227–229 °C. Anal. ($C_{13}H_{16}N_4O_2$·2HCl·H_2O) C, H, N, Cl.

4-[[4-(Dimethylamino)butyl]amino]-5-nitroquinoline (11): ^1H NMR (free base in $CDCl_3$) δ 8.60 (d, J = 5.4 Hz, 1 H, H-2), 8.19 (m, 1 H, H-8), 7.65 (m, 2 H, H-6, H-7), 6.63 (d, J = 5.4 Hz, 1 H, H-3), 5.30 (s, 1 H, NH), 3.25 (q, J = 6.7 Hz, 2 H, NHCH_2), 2.31 (t, J = 7.0 Hz, 2 H, CH_2N(CH_3)$_2$), 2.22 (s, 6 H, N(CH_3)$_2$), 1.98 (quintet, J = 6.9 Hz, 2 H, C$H_2$$CH_2$N($CH_3$)$_2$), 1.95 (quintet, J = 1.62 Hz, 2 H, C$H_2$$CH_2$$CH_2$N($CH_3$)$_2$). Dihydrochloride salt, mp 221–223 °C. Anal. ($C_{15}H_{20}N_4O_2$·2HCl) C, H, N, Cl.

***N*^{1}-Methyl-4-[[2-(dimethylamino)ethyl]amino]-5-nitroquinoline (12):** ^1H NMR (free base in $CDCl_3$) δ 8.75 (d, J = 5.7 Hz, 1 H, H-2), 8.19 (dd, J = 8.4, 1.4 Hz, 1 H, H-8), 7.73 (dd, J = 7.5, 1.3 Hz, 1 H, H-6), 7.64 (dd, J = 8.4, 7.4 Hz, 1 H, H-7), 7.08 (d, J = 5.2 Hz, 1 H, H-3), 3.25 (br s, 1 H, N(CH_3)CH_2), 2.85 (s, 3 H, NCH_3), 2.44 (t, J = 7 Hz, 2 H, CH_2N(CH_3)$_2$), 2.18 (s, 6 H, N(CH_3)$_2$). Dihydrochloride salt, mp 218–219 °C. Anal. ($C_{14}H_{18}N_4O_2$·2HCl·H_2O) C, H, N, Cl.

4-[[3-(Dimethylamino)propyl]amino]-1-methyl-5-nitroquinolinium chloride (13): ^1H NMR (hydrochloride chloride disalt in CD_3SOCD_3) δ 11.30 (br s, 1 H, HCl), 9.12 (s, 1 H, NH), 8.79 (d, J = 7.6 Hz, 1 H, H-2), 8.40 (m, 2 H, H-8, H-6), 8.16 (t, J = 8.2 Hz, 1 H, H-7), 7.35 (d, J = 7.6 Hz, 1 H, H-3), 4.20 (s, 3 H, N^+CH_3), 3.68 (t, J = 7 Hz, 2 H, NHCH_2), 3.16 (br t, 2 H, CH_2N(CH_3)$_2$), 2.76 (s, 6 H, N(CH_3)$_2$), 2.08 (quintet, J = 7 Hz, 2 H, CH_2C$H_2$$CH_2$); mp 106–108 °C. Anal. ($C_{15}H_{21}ClN_4O_2$·HCl) C, H, N, Cl.

4-[[3-(Dimethylamino)propyl]amino]-2-methyl-5-nitroquinoline (14): ^1H NMR (free base in $CDCl_3$) δ 8.51 (dd, J = 8.3, 1.4 Hz, 1 H, H-8), 7.62 (dd, J = 7.5, 1.3 Hz, 1 H, H-6), 7.56 (dd, J = 8.2, 7.4 Hz, 1 H, H-7), 6.50 (s, 1 H, H-3), 6.46 (s, 1 H, NH), 3.31 (q, J = 5.5 Hz, 2 H, NHCH_2), 2.62 (s, 3 H, 2-CH_3), 2.42 (t, J = 5.4 Hz, 2 H, CH_2N(CH_3)$_2$), 2.26 (s, 6 H, N(CH_3)$_2$), 1.85 (quintet, J = 5.5 Hz, 2 H, CH_2C$H_2$$CH_2$). Dihydrochloride salt, mp 210–211 °C. Anal. ($C_{15}H_{20}N_4O_2$·2HCl) C, H, N, Cl.

(38) Osborn, A. R.; Schofield, K.; Short, L. N. Studies of the amino-isoquinolines, -cinnolines and -quinazolines. (A) The basic strengths and ultraviolet absorption spectra. (B) The infrared spectra. *J. Chem. Soc.* 1956, 4191–4206.

4-[[3-(Dimethylamino)propyl]amino]-3-methyl-5-nitroquinoline (15): ^{1}H NMR (free base in $CDCl_3$) δ 8.55 (s, 1 H, H-2), 8.12 (dd, J = 8.5, 1.2 Hz, 1 H, H-8), 7.78 (dd, J = 7.4, 1.1 Hz, 1 H, H-6), 7.53 (dd, J = 8.5, 7.6 Hz, 1 H, H-7), 5.99 (s, 1 H, NH), 3.21 (q, J = 6.0 Hz, 2 H, $NHCH_2$), 2.41 (t, J = 6.0 Hz, $CH_2N(CH_3)_2$), 2.36 (s, 3 H, 2-CH_3), 2.25 (s, 6 H, $N(CH_3)_2$), 1.65 (quintet, J = 6.0 Hz, 2 H, $CH_2CH_2CH_2$). Dihydrochloride salt, mp 261–265 °C. Anal. ($C_{15}H_{20}N_4O_2 \cdot 2HCl \cdot {}^1/_2H_2O$) C, H, N, Cl.

4-[[3-(Dimethylamino)propyl]amino]-5-nitroquinazoline (16): ^{1}H NMR (free base in $CDCl_3$) δ 8.68 (s, 1 H, H-2), 8.14 (s, 1 H, NH), 8.00 (dd, J = 8.0, 1.7 Hz, 1 H, H-8), 7.74 (dd, J = 7.6, 1.7 Hz, 1 H, H-6), 7.70 (dd, J = 8.0, 7.6 Hz, 1 H, H-7), 3.72 (q, J = 4.5 Hz, 2 H, $NHCH_2$), 2.46 (t, J = 5.0 Hz, 2 H, $CH_2N(CH_3)_2$), 2.26 (s, 6 H, $N(CH_3)_2$), 1.81 (quintet, J = 5.1 Hz, 2 H, $CH_2CH_2CH_2$). Dihydrochloride salt, mp 197–198 °C. Anal. ($C_{13}H_{17}N_5O_2 \cdot 2HCl$) C, H, N, Cl.

4-[[3-(Dimethylamino)propyl]amino]-6-methyl-5-nitroquinoline (17): ^{1}H NMR (free base in $CDCl_3$) δ 8.47 (d, J = 5.4 Hz, 1 H, H-2), 7.98 (d, J = 8.6 Hz, 1 H, H-8), 7.43 (d, J = 8.6 Hz, 1 H, H-7), 6.58 (d, J = 5.4 Hz, 1 H, H-3), 5.75 (s, 1 H, NH), 3.27 (q, J = 4.6 Hz, 2 H, $NHCH_2$), 2.4 (m, 5 H, 6-Me and $CH_2N(CH_3)_2$), 2.27 (s, 6 H, $N(CH_3)_2$), 1.84 (quintet, J = 6.3 Hz, 2 H, $CH_2CH_2CH_2$). Dihydrochloride salt, mp 214–215 °C. Anal. ($C_{15}H_{20}N_4O_2 \cdot 2HCl \cdot H_2O$) C, H, N.

4-[[3-(Dimethylamino)propyl]amino]-8-methyl-5-nitroquinoline (18): ^{1}H NMR (free base in $CDCl_3$) δ 8.61 (d, J = 5.5 Hz, 1 H, H-2), 7.64 (d, J = 7.7 Hz, 1 H, H-6), 7.45 (d, J = 7.7 Hz, 1 H, H-7), 6.61 (d, J = 5.5 Hz, 1 H, H-3), 6.72 (s, 1 H, NH), 3.32 (q, J = 4.5 Hz, 2 H, $NHCH_2$), 2.79 (s, 3 H, 8-CH_3), 2.42 (t, J = 5.5 Hz, 2 H, $CH_2N(CH_3)_2$), 2.25 (s, 6 H, $N(CH_3)_2$), 1.85 (quintet, J = 5.5 Hz, 2 H, $CH_2CH_2CH_2$). Dihydrochloride salt, mp 145–148 °C. Anal. ($C_{15}H_{20}N_4O_2 \cdot 2HCl$) C, H, N, Cl.

4-[[3-(Dimethylamino)propyl]amino]-2-methyl-3-nitroquinoline (21): ^{1}H NMR (free base in $CDCl_3$) δ 9.10 (s, 1 H, NH), 7.88 (dd, J = 8.4, 0.7 Hz, 1 H, H-8), 7.80 (dd, J = 8.4, 0.7 Hz, 1 H, H-6), 7.65 and 7.4 (2 m, 2 H, H-6 and H-7), 3.40 (q, J = 5.5 Hz, 2 H, $NHCH_2$), 2.62 (m, 5 H, 2-Me and $CH_2N(CH_3)_2$), 2.39 (s, 6 H, $N(CH_3)_2$), 1.83 (quintet, J = 6.0 Hz, 2 H, $CH_2CH_2CH_2$). Dihydrochloride salt, mp 248–249 °C. Anal. ($C_{15}H_{20}N_4O_2 \cdot 2HCl$) C, H, N, Cl.

4-[[3-(Dimethylamino)propyl]amino]-7-methyl-6-nitroquinoline (22): ^{1}H NMR (free base in $CDCl_3$) δ 9.10 (s, 1 H, NH), 8.58 (s, 1 H, H-8), 8.54 (d, J = 5.4 Hz, 1 H, H-2), 7.79 (s, 1 H, H-5), 6.29 (d, J = 5.4 Hz, 1 H, H-3), 6.46, 3.41 (q, J = 4.2 Hz, 2 H, $NHCH_2$), 2.76 (s, 3 H, 7-CH_3), 2.63 (t, J = 5.1 Hz, 2 H, $CH_2N(CH_3)_2$), 2.45 (s, 6 H, $N(CH_3)_2$), 1.70 (quintet, J = 5.0 Hz, 2 H, $CH_2CH_2CH_2$). Dihydrochloride salt, mp 267–268 °C. Anal. ($C_{15}H_{20}N_4O_2 \cdot 2HCl$) C, H, N, Cl.

4-[[3-(Dimethylamino)propyl]amino]-8-methyl-7-nitroquinoline (23): ^{1}H NMR (free base in $CDCl_3$) δ 8.62 (d, J = 5.3 Hz, 1 H, H-2), 8.20 (s, 1 H, NH), 7.75 (d, J = 9.2 Hz, 1 H, H-6), 7.59 (d, J = 9.2 Hz, 1 H, H-5), 6.42 (d, J = 5.3 Hz, 1 H, H-3), 3.39 (q, J = 4.4 Hz, 2 H, $NHCH_2$), 2.94 (s, 3 H, 8-Me), 2.60 (t, J = 5.5 Hz, 2 H, $CH_2N(CH_3)_2$), 2.37 (s, 6 H, $N(CH_3)_2$), 1.93 (quintet, J = 5.5 Hz, 2 H, $CH_2CH_2CH_2$). Dihydrochloride salt, mp 231–233 °C. Anal. ($C_{15}H_{20}N_4O_2 \cdot 2HCl \cdot H_2O$) C, H, N, Cl.

4-[[3-(Dimethylamino)propyl]amino]-7-methyl-8-nitroquinoline (24): ^{1}H NMR (free base in $CDCl_3$) δ 8.51 (d, J = 5.4 Hz, 1 H, H-2), 8.12 (s, 1 H, NH), 7.62 (d, J = 8.6 Hz, 1 H, H-5), 7.23 (d, J = 8.6 Hz, 1 H, H-6), 6.34 (d, J = 5.4 Hz, 1 H, H-3), 3.39 (q, J = 4.6 Hz, 2 H, $NHCH_2$), 2.58 (t, J = 5.4 Hz, 2 H, $CH_2N(CH_3)_2$), 2.45 (s, 3 H, 7-CH_3), 2.37 (s, 6 H, $N(CH_3)_2$), 1.92 (quintet, J = 5.2 Hz, 2 H, $CH_2CH_2CH_2$). Dihydrochloride salt, mp 153–156 °C. Anal. ($C_{15}H_{20}N_4O_2 \cdot 2HCl$) C, H, N, Cl.

Reaction of 4-chloro-8-methoxy-5-nitroquinoline (**25**) gave, as major product, 8-[[3-(dimethylamino)propyl]amino]-4-chloro-5-nitroquinoline (**26**): mp (EtOAc/MeOH) 236–237 °C; ^{1}H NMR ($CDCl_3$) δ 8.59 (d, J = 4.7 Hz, 1 H, H-6), 7.98 (d, J = 8.7 Hz, 1 H, H-2), 7.60 (d, J = 4.7 Hz, 1 H, H-7), 7.51 (br s, 1 H, NH), 6.50 (d, J = 8.7 Hz, 1 H, H-3), 3.49 (q, J = 6.7 Hz, 2 H, $ArNHCH_2$), 2.45 (t, J = 6.7 Hz, 2 H, $CH_2CH_2NMe_2$), 2.28 (s, 6 H, $N(CH_3)_2$), 1.93 (quintet, J = 6.7 Hz, 2 H, $CH_2CH_2CH_2$). Anal. ($C_{14}H_{17}ClN_4O_2 \cdot HCl$) C, H, N, Cl.

Preparation of Compound 19 of Table I. Reaction of 4-chloro-8-methoxyquinoline and *N,N*-dimethylpropane-1,3-diamine in excess phenol at 140 °C gave a 57% yield of 4-[[3-(dimethylamino)propyl]amino]-8-methoxyquinoline (**27**): mp (petroleum ether) 131–132 °C; ^{1}H NMR ($CDCl_3$) δ 8.54 (d, J = 5.3 Hz, 1 H, H-2), 7.59 (br s, 1 H, NH), 7.30 (m, 1 H, H-6), 7.23 (dd, J = 8.4, 1.0 Hz, 1 H, H-5), 6.97 (d, J = 7.6 Hz, 1 H, H-7), 6.37 (d, J = 5.3 Hz, 1 H, H-3), 4.04 (s, 3 H, OCH_3), 2.35 (s, 6 H, $N(CH_3)_2$). Anal. ($C_{15}H_{21}N_3O$) C, H, N. This compound (1.81 g, 7 mmol) was dissolved in concentrated H_2SO_4 (15 mL), the temperature being kept below 10 °C. The stirred solution was then treated portionwise with KNO_3 (0.82 g, 8 mmol) at below 0 °C, stirred for a further 15 min at 0 °C, poured onto ice, basified strongly with aqueous NaOH, and extracted with CH_2Cl_2 (3 × 50 mL). The combined extracts were washed with water and evaporated, and the resulting residue was extracted with hot petroleum ether (bp 80–100 °C). These extracts were decolorized (charcoal) and evaporated to give the free base of 4-[[3-(dimethylamino)propyl]amino]-8-methoxy-5-nitroquinoline (**19**) (1.25 g, 59%): ^{1}H NMR ($CDCl_3$) δ 8.59 (d, J = 5.5 Hz, 1 H, H-2), 7.88 (d, J = 8.6 Hz, 1 H, H-6), 6.88 (d, J = 8.6 Hz, 1 H, H-7), 6.76 (br s, 1 H, NH), 6.63 (d, J = 5.5 Hz, 1 H, H-3), 4.11 (s, 3 H, OCH_3), 2.25 (s, 6 H, $N(CH_3)_2$). Dihydrochloride salt, mp (EtOAc/MeOH) 183–184 °C. Anal. ($C_{15}H_{20}N_4O_3 \cdot 2HCl \cdot {}^1/_2H_2O$) C, H, N, Cl.

Preparation of Compound 20 of Table I. The free base of **19** (1.22 g, 4 mmol) was dissolved in dry DMA (5 mL). The solution was held at 95 °C, and a stream of dry methylamine was bubbled in for ca. 15 min, when TLC indicated the reaction was complete. Solvent was removed under reduced pressure, and the residue was partitioned between CH_2Cl_2 and water containing methylamine. The organic layer was washed once with water, dried, and evaporated, and the resulting solid was extracted with boiling petroleum ether (bp 40–60 °C). The extracts were decolorized (charcoal) and evaporated to give the free base of 4-[[3-(dimethylamino)propyl]amino]-8-(methylamino)-5-nitroquinoline (**20**) (0.77 g, 64%): ^{1}H NMR (free base in $CDCl_3$) δ 8.34 (d, J = 5.35 Hz, 1 H, H-2), 8.15 (d, J = 8.8 Hz, 1 H, H-7), 7.35 (br s, 1 H, $NHCH_3$), 6.63 (d, J = 5.35 Hz, 1 H, H-6), 6.59 (s, 1 H, NH), 6.38 (d, J = 8.8 Hz, 1 H, H-3), 3.34 (q, J = 4.5 Hz, 2 H, $NHCH_2$), 3.08 (d, J = 5.4 Hz, 3 H, $NHCH_3$), 2.40 (t, J = 6.35 Hz, 2 H, $CH_2N(CH_3)_2$), 2.25 (s, 6 H, $N(CH_3)_2$), 1.87 (quintet, J = 6.4 Hz, 2 H, $CH_2CH_2CH_2$). Dihydrochloride salt, mp (MeOH/EtOAc) 222–223 °C. Anal. ($C_{15}H_{21}N_5O_2 \cdot 2HCl \cdot H_2O$) C, H, N, Cl.

Crystallographic Determination of 20. The free base crystallized from petroleum ether bp 40–60 °C) as orange-needles, mp 98 °C; space group $P2_1$; cell constants a = 13.038 (3) Å, b = 7.516 (2) Å, c = 8.028 (2) Å, β = 96.43 (3)°, z = 2; V = 781.7 (4) Å^3. Lattice constants and intensity data were measured using graphite-monochromated Mo Kα radiation, λ = 0.71069 Å, on a Nonius CAD-4 diffractometer. The data set consisted of 1883 unique reflections, of which 1769 were considered observed ($I > 3\sigma(I)$). The structure was solved by two independent methods. One method used SHELXTL PC for solution and refinement, and the other used PATSEE[39] for solution and SHELX-76[40] for refinement. In both refinements, amine and ring hydrogens were found and the other hydrogens placed in calculated positions. The first method was halted at R and R_w of 0.0903 and 0.0918, respectively. The second gave R and R_w of 0.0793 and 0.0930 under the same conditions. When the ring and amine hydrogens were allowed to refine, these dropped to 0.0781 and 0.0918, respectively. There was obviously some disorder in the chain, as a peak of intensity 0.95 e Å^{-3} could be observed between C12 and N3. A carbon atom at this position of occupancy 0.29 combined with a reduction in the occupancy of C13 to 0.71 reduced R and R_w to 0.0634 and 0.0697, respectively. Further efforts to correct the disorder were not undertaken, as this did not significantly affect the geometry of the rest of the molecule. The largest shift/esd values during the final refinement were less than 0.5, and maximum and minimum peaks in the final difference map were 0.24 and 0.36 e Å^{-3}, respectively.

Reduction Potentials. Pulse radiolysis experiments were carried out on a 1.8 MV Linac, delivering ca. 3 Gy in 0.2 μs to a 2-cm pathlength cell. The dose was determined by measurement of the optical density at 472 nm in aerated KSCN (10 mM),

(39) Egert, E. PATSEE, Institut fur Anorganische Chemie, Universitat Gottingen, Germany, 1985.
(40) Sheldrick, G. M. SHELX-76; University Chemical Laboratory, Cambridge, UK, 1976.

assuming[41] an extinction coefficient E of 7580 L mol^{-1} cm^{-1} and a radiation chemical yield G of 0.29 μmol J^{-1}. Transients were recorded on a Tektronic 7621D digitizer interfaced to a PDP 11/34 computer for data analysis. Drug solutions were 3 mM in phosphate buffer at pH 7, using either 2-propanol (typically 0.2 M) or 2-propanol/acetone mixtures as cosolvent. The redox indicators used were methylviologen (1,1′-dimethyl-4,4′-bipyridinium dichloride) for compounds **5**, **21**, **23**, and **24**, triquat (7,8-dihydro-6*H*-dipyrido[1,2-*a*:2′,1′-*c*][1,4]diazepinediium dibromide) for compounds **20** and **22**, or benzylviologen (1,1′-dibenzyl-4,4′-bipyridinium dichloride) for all other compounds. Potentials of −447, −548, and −380 mV, respectively, were assumed for the appropriate one-electron redox couples of these indicators. Measured reduction potentials were corrected for ionic strength effects using the pK_a values of Table I.

Cell Line Studies. AA8 and UV4 cells were maintained in logarithmic-phase growth in 25-mL 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. Cultures were tested for mycoplasma contamination frequently, using a cytochemical staining method.[42] 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,[8,43] 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.[13] Both cell suspensions and drug solutions in growth medium were pre-equilibrated under the appropriate gas phase for 45 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 x 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 this, comparisons between aerobic and hypoxic cytotoxicity were based on CT_{10} values at concentrations which gave similar rates of cell killing (usually 1 log in about 1 h).

In Vivo Studies. Drugs were dissolved in water and administered intraperitoneally in a volume of 0.01 mL/g body weight at 1.5-fold dose increments to determine the maximum tolerated dose (MTD). The latter was defined as the highest dose causing no deaths in at least six mice, with an observation time of 40 days. Drug deaths usually occurred 1–5 days after treatment. Therapeutic activity was assessed by administering drugs at 60% of the MTD at various times before or after whole-body irradiation (^{60}Co, 20 Gy, ca. 1 Gy/min) of unrestrained, unanesthetized mice bearing 0.4–0.8-g subcutaneous SCCVII tumors. Cell survival was determined 18 h later by clonogenic assay as described.[14]

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Supplementary Material Available: X-ray crystallographic data for 4-[[3-(dimethylamino)propyl]amino]-8-(methylamino)-5-nitroquinoline (**20**) (5 pages); structure factors for **20** (13 pages). Ordering information is given on any current masthead page.

(41) Schuler, R. H.; Patterson, L. K.; Janata, E. Yield for the scavenging of OH radicals in the radiolysis of N_2O-sturated aqueous solutions. *J. Phys. Chem.* 1980, *84*, 2088–2089.

(42) Chen, T. R. In situ detection of *Mycoplasma* contamination in cell cultures by fluorescent Hoechst 33258 stain. *Exp. Cell Res.* 1977, *104*, 255–262.

(43) Finlay, G. J.; Baguley, B. C.; Wilson, W. R. A semiautomated microculture method for investigating growth inhibitory effects of cytotoxic compounds on exponentially growing carcinoma cells. *Anal. Biochem.* 1984, *139*, 272–277.

(44) Cain, B. F.; Atwell, G. J.; Denny, W. A. Potential Antitumor Agents. Part 16. 4′-(Acridin-9-ylamino)methane-sulfonanilides. *J. Med. Chem.* 1975, *18*, 1110–1117.

(45) Baguley, B. C.; Falkenhaug, E-M. (1978) The interaction of ethidium with synthetic double-stranded polynucleotides at low ionic strength. *Nucleic Acids Res.* 1978, *5*, 161–171.