Peptide **10** was prepared by coupling unprotected 1,8-diaminooctane to the proline acid peptide **19** using DCC/HOBt in dimethylformamide. Each of the final peptide products were purified by preparative reverse phase HPLC on 5-μm ODS. Their physical properties are listed in Table II.

Pmp-D-Tyr(Et)-Phe-Val-Asn-Cys-Pro-NH-$(CH_2)_4$-NH-C(=NH)NH_2 (6). *O*-Methylisourea hydrogen sulfate (88 mg, 0.51 mmol) was dissolved in water (3 mL) and the pH of the solution was adjusted to 10 by using 3 N aqueous NaOH. The peptide **5** (8.3 mg, 0.0084 mmol) in water (2 mL) was added at 0 °C, the pH was again adjusted to 10, and the reaction mixture was kept at 0 °C until the reaction was shown to be complete by HPLC. The solution was then adjusted to pH 4.5 with 1% aqueous acetic acid and evaporated. The residue was purified by preparative reverse phase HPLC (5-μm ODS, 10 × 250 mm column, isocratic elution with 60:40 0.25% aqueous TFA/0.25% TFA in acetonitrile) to give 5.5 mg (64%) of pure **6**. The peptide **16** was prepared in an analogous fashion from the terminal amino peptide **15**.

Acknowledgment. We thank Robert Sanchez of Peptide Chemistry for amino acid analysis and Gerald Roberts and Walter Johnson of Physical and Structural Chemistry and Lewis Killmer of Analytical Chemistry for obtaining mass spectra. We also thank Professor M. Manning of the Medical College of Ohio for providing a sample of compound **13**.

Registry No. **1**, 80148-24-9; **2**, 90332-82-4; **3**, 98612-53-4; **4**, 117861-32-2; **5**, 99733-17-2; **6**, 99733-16-1; **7**, 98612-54-5; **8**, 117861-33-3; **9**, 99733-19-4; **10**, 117861-34-4; **11**, 94497-37-7; **12**, 98612-56-7; **13**, 117861-35-5; **14**, 117861-36-6; **15**, 98612-57-8; **16**, 117861-37-7; **17**, 108467-93-2; **18**, 108467-94-3; **19**, 98612-55-6; **20**, 98612-58-9; **21**, 68076-36-8; **22**, 51644-96-3; **23**, 51857-17-1; **24**, 99733-18-3; **25**, 108467-99-8; **26**, 108468-00-4; **27**, 57260-73-8; BOC-Cys(4-MeBzl)-OH, 61925-77-7; BOC-Val-OH, 13734-41-3; BOC-Phe-OH, 13734-34-4; BOC-Asn-OH, 7536-55-2; BOC-D-Tyr(Et)-OH, 76757-92-1; $H_2N(CH_2)_4NH_2$, 110-60-1; $H_2N(CH_2)_5NH_2$, 462-94-2; $H_2N(CH_2)_6NH_2$, 124-09-4; $H_2N(CH_2)_7NH_2$, 646-19-5; $H_2NCH_2C_6H_4$-*m*-CH_2NH_2, 1477-55-0; $H_2NCH_2C_6H_4$-*p*-CH_2NH_2, 539-48-0; $H_2NCH_2CH_2OH$, 141-43-5; BOC-$NHCH_2CH_2OH$, 26690-80-2; $H_2N(CH_2)_8NH_2$, 373-44-4; HN=C(OMe)-$NH_2 \cdot {}^1/_2H_2SO_4$, 52328-05-9; BOC-$NHCH_2CH_2N_3$, 117861-38-8.

Potential Antitumor Agents. 57. 2-Phenylquinoline-8-carboxamides as "Minimal" DNA-Intercalating Antitumor Agents with in Vivo Solid Tumor Activity

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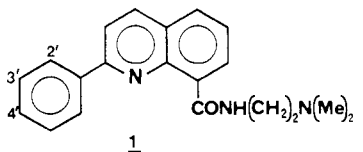
A series of phenyl-substituted derivatives of the "minimal" DNA-intercalating agent *N*-[2-(dimethylamino)ethyl]-2-phenylquinoline-8-carboxamide (**1**) have been synthesized and evaluated for in vivo antitumor activity, in a continuing search for active compounds of this class with the lowest possible DNA association constants. Substitution on the 2′-position of the phenyl ring gave compounds of lower DNA binding ability that did not intercalate DNA, indicating that it is necessary for the phenyl ring to be essentially coplanar with the quinoline for intercalative binding. An extensive series of 4′-substituted derivatives was evaluated, but there was no overall relationship between biological activity and substituent lipophilic or electronic properties. However, several compounds showed good solid tumor activity, with the 4′-aza derivative **18** being clearly superior to the parent compound, effecting about 50% cures in both leukemia and solid tumor models.

The evaluation of DNA-intercalating ligands as broad-spectrum agents with the ability to act against remotely sited solid tumors in vivo is an active area of anticancer drug development, and several new agents of this type are now in clinical trial.[1] Recent results from this laboratory[2-5] have shown that, while intercalative binding is an absolute requirement for antitumor activity, there may be additional advantages in compounds that interact with DNA in this fashion but have the lowest possible association constants. Such compounds, with a higher proportion of unbound drug available at equilibrium, which may permit better distribution in vivo, generally show a broader spectrum of activity than structurally related compounds with higher binding constants.[2]

An important goal of recent work in this laboratory has therefore been to delineate the minimum chromophore requirement for intercalative binding, in a search for compounds of this class with the lowest possible association constants. A systematic study[5] of the isomeric phenylquinoline-8-carboxamides showed that, while the mode of binding depended critically on the placement of the appended phenyl ring, several of the compounds did intercalate DNA and had in vivo antitumor activity. In particular, the 2-isomer *N*-[(2-dimethylamino)ethyl]-2-phenylquinoline-8-carboxamide (**1**) possesses excellent broad-spectrum activity, providing a high proportion of cures against the remotely sited Lewis lung carcinoma.[5]



1

In this paper we present structure–activity relationships for substitution of the phenyl ring of **1** at the three available positions with groups of varying steric, electronic, and lipophilic properties. One aim of this work was the identification of compounds with superior activity to that of the parent **1**. Additionally, the hypothesis[5] that the phenyl ring has to lie coplanar with the quinoline in order for the compounds to intercalate DNA was tested by studying the mode of binding of compounds substituted at the ortho position of the phenyl ring, which cannot easily assume such a coplanar conformation.

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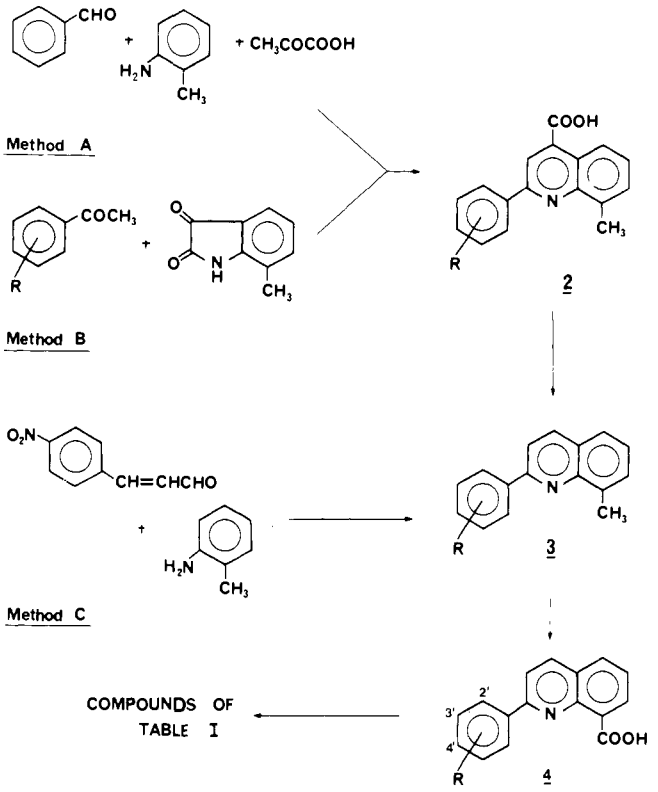
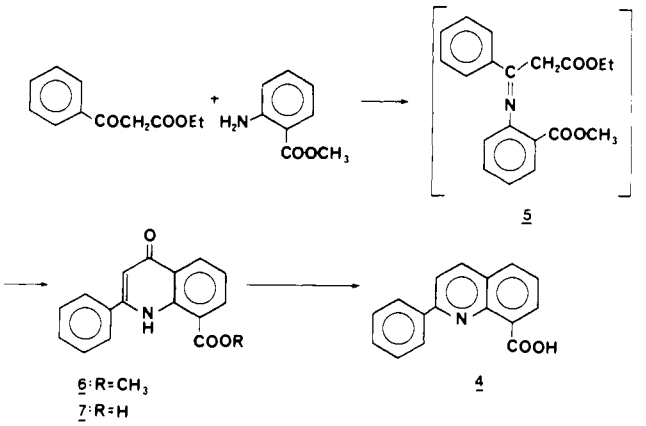
Scheme I



Chemistry

This work required the preparation of a series of 2-phenylquinoline-8-carboxylic acids. The unsubstituted parent compound **4** (R = H) was most efficiently prepared by the Doebner pyruvic acid synthesis[6] of 8-methyl-2-phenylquinoline-4-carboxylic acid (**2**: R = H) (method A, Scheme I), which was decarboxylated and then oxidized with SeO_2. However, most of the acids (**4**) were prepared by the related Pfitzinger reaction[7] of substituted acetophenones with 7-methylisatin (method B, Scheme I), followed by decarboxylation and oxidation. Neither of these two methods work well with nitro-substituted aldehydes and ketones,[8] and other routes to the 4′-nitro derivative **4** (R = 4′-NO_2) were sought. Reaction of 4-nitrocinnamaldehyde and 2-methylaniline in concentrated HCl (Doebner–Miller reaction,[9] method C, Scheme I) gave the corresponding 8-methyl-2-phenylquinoline (**3**: R = 4′-NO_2) directly, but the yield was very low. Low-temperature nitration of 2-phenylquinoline-8-carboxylic acid in fuming HNO_3 gave a mixture of mononitro products, from which the 4′-isomer could be isolated in moderate yield by repeated recrystallization. 4-(Methylthio)acetophenone for the synthesis of **28** was prepared by Friedel–Craft acylation of thioanisole, by using the method of Pines.[10] Condensation of this with 7-methylisatin followed by decarboxylation gave the 4′-methylthio derivative **3** (R = 4′-SCH_3). This was oxidized to the methyl sulfone **3** (R = 4′-SO_2CH_3). Both compounds showed a three-proton singlet at δ 2.88 in their NMR spectra, assigned to the 8-methyl group, with the similarity in chemical shift indicating that no N-oxidation had occurred. In contrast, the signal at δ 2.54 in the spectrum of the methylthio compound was shifted to δ 3.10 on oxidation, in complete agreement with the downfield shift seen between the methyl resonances of thioanisole (δ 2.47) and (methylsulfonyl)benzene (δ 3.04).

Scheme II



A severe limitation of all these methods is the necessity for oxidation of the methyl derivative **3** to give the carboxylic acid **4**, since many potential substituents R are not compatible with this procedure. An alternative route that begins at the carboxylic acid oxidation level was therefore evaluated (Scheme II). Reaction of ethyl benzoylacetate and methyl anthranilate gave the Schiff base **5**, which was thermally cyclized to the quinolone ester **6** in 35% overall yield. The corresponding acid **7** was then reduced with Al/Hg amalgam to 2-phenylquinoline-8-carboxylic acid (**4**), but the yield (15%) was disappointingly low compared to similar reactions of substituted acridone-4-carboxylic acid to form acridine-4-carboxylic acids.[2] However, this method remains a possible route to compounds bearing oxidation-sensitive R groups.

The carboxamides of Table I were prepared from the acids **4** by coupling with the appropriate (dialkylamino)alkylamine with use of 1,1′-carbonyldiimidazole.[11] Compounds **29–32** of Table I were prepared from the 4′-NO_2 derivative **26** by reduction with Fe/AcOH, followed by acylation.

Results and Discussion

Physicochemical Properties. Table I provides data on the physicochemical and biological properties of the parent 2-phenylquinolinecarboxamide (**1**) and 25 analogues (**8–32**). Compounds **8–10** extend the side chain, while compounds **11** and **12** explore the use of different terminal cationic groups. Three different functional groups (aza, Cl, and OCH_3) of widely varying steric, electronic, and lipophilic properties were then used to establish preliminary structure–activity relationships for substitution on the phenyl ring at the three available positions (compounds **13–18**, **20**, and **23**).

The parent compound **1** binds unselectively to the two DNA homopolymers poly[d(A-T)] and poly[d(G-C)], as determined by the ethidium bromide displacement assay,[12] with association constants lower than those shown by the fused tricyclic carboxamides acridine-4-carboxamide[2] (log K(AT) = 6.12) and 9-aminoacridine-4-carboxamide[13] (log K(AT) = 7.08). However, **1** is appreciably more lipophilic

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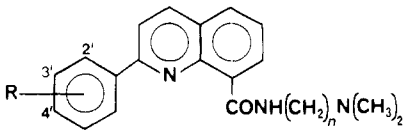
Table I. Physicochemical Data and Biological Activity of 2-Phenylquinoline-8-carboxamides



								log K^c			P388		LL	
no.	R	n	mp, °C	formula	anal.	R_m[a]	pK_a[b]	AT	GC	IC_{50}[d]	OD[e]	ILS_{max}[f]	OD	ILS_{max}
1	H	2				−0.01	2.75	5.98	5.92	1300	100	91	100	70 (4)[g]
8	H	3	214–216	$C_{21}H_{23}N_3O\cdot 2HCl$	C,H,N	0.06		5.90	5.62	1140	100	71	100	NA[h]
9	H	4	208–209	$C_{22}H_{25}N_3O\cdot HCl$	C,H,N,Cl	0.14		6.19	5.89	680	100	93	100	148 (2)
10	H	5	171–174	$C_{23}H_{27}N_3O\cdot HCl$	C,H,N,Cl	0.21		5.90	5.73	1300	150	NA	100	NA
11	H[i]	2	190–193	$C_{20}H_{21}N_3O_2\cdot 2HCl$	C,H,N,Cl	−0.05		6.22	6.31	36	65	NA	65	NA
12	H[j]	2	206–209	$C_{22}H_{23}N_3O_2\cdot 2HCl\text{-}H_2O$	C,H,N,Cl	−0.03		6.12	5.61	44	65	NA		NT[k]
13	2′-aza	2	168–170	$C_{19}H_{20}N_4O\cdot 2HCl$	C,H,N,Cl	−1.43		5.82	5.85	1640	65	73 (3)	65	84 (1)
14	2′-Cl	2	169–171	$C_{20}H_{20}ClN_3O\text{-}HCl$	C,H,N	0.15		5.32	5.12	8100	100	NA		NT
15	3′-aza	2	249–252	$C_{19}H_{20}N_4O\cdot 2HCl$	C,H,N,Cl	−0.58		5.81	6.09	840	100	58	100	55 (2)
16	3′-Cl	2	110–111	$C_{20}H_{20}ClN_3O$	C,H,N,Cl	0.05		6.05	6.47	1200	65	40	65	NA
17	3′-OCH_3	2	118–120	$C_{21}H_{23}N_3O_2\cdot 2HCl\text{-}H_2O$	C,H,N,Cl	−0.09		6.30	6.41	860	225	46		NT
18	4′-aza	2	235–237	$C_{19}H_{20}N_4O\cdot 2HCl$	C,H,N,Cl	−0.87		5.82	6.32	170	45	145 (2)	65	191 (3)
19	4′-F	2	133–136	$C_{20}H_{20}FN_3O\cdot 2HCl$	C,H,N,Cl	−0.04		6.74	6.66	730	100	24	100	NA
20	4′-Cl	2	250–251	$C_{20}H_{20}ClN_3O\cdot HCl$	C,H,N	0.05	2.42	6.08	5.99	1170	100	52	65	45 (2)
21	4′-Br	2	123–124	$C_{20}H_{20}BrN_3O$	C,H,N	0.15		6.63	6.68	600	100	72	100	NA
22	4′-I	2	118–119	$C_{20}H_{20}N_3IO$	C,H,N	0.13		6.80	6.87	400	100	48	100	NA
23	4′-OCH_3	2	200–201	$C_{21}H_{23}N_3O_2\cdot 2HCl$	C,H,N,Cl	−0.16	3.28	6.40	6.27	1290	150	42	100	NA
24	4′-OH	2	270–273	$C_{20}H_{21}N_3O_2\cdot 2HBr$	C,H,N,Br	−0.33		6.30	6.48	1280	65	22	100	NA
25	4′-Ph	2	180–183	$C_{26}H_{25}N_3O\cdot 2HCl$	C,H,N,Cl	0.13		6.88	6.96	1290	150	NA		NT
26	4′-NO_2	2	244–245	$C_{20}H_{20}N_4O_3\cdot HCl$	C,H,N,Cl	−0.18		6.28	6.13	290	65	58	65	85
27	4′-CF_3	2	218–219	$C_{21}H_{20}F_3N_3O\cdot HCl$	C,H,N,Cl	0.39		6.64	6.72	2100	100	NA	100	NA
28	4′-SO_2CH_3	2	117–119	$C_{21}H_{23}N_3O_3S\cdot 2HCl\cdot H_2O$	C,H,N,Cl	−0.74		5.68		5800	100	29	100	NA
29	4′-NH_2	2	116–120	$C_{20}H_{22}N_4O\cdot 2HCl$	C,H,N	−0.76		6.42	6.96	200	100	28	150	59 (2)
30	4′-$NHCOCH_3$	2	160–164	$C_{22}H_{24}N_4O_2\cdot 2HCl$	C,H,N,Cl	−0.46		6.49	7.07	600	65	NA		NT
31	4′-$NHSO_2CH_3$	2	274–275	$C_{21}H_{24}N_4O_3S\cdot 2HCl$	C,H,N,Cl	−0.38		6.04	6.22	1330	45	NA		NT
32	3′,4′-benz	2	199–202	$C_{24}H_{23}N_3O\cdot 2HCl$	C,H,N,Cl	−0.10		7.00	7.35	860	150	NA		NT

[a] R_m values were determined as detailed in ref 14, with 4′-(9-acridinylamino)methanesulfonanilide (AMSA) as a standard. [b] pK_a values were determined in aqueous solutions spectrophotometrically, as detailed in ref 19. [c] log K = binding constant to poly[d(A-T)] or poly[d(G-C)], determined by ethidium bromide displacement; see ref 12. [d] IC_{50} = concentration of drug in nM to inhibit growth of murine leukemia (L1210) cells in culture by 50%, following a 40-h exposure. See ref 20. [e] OD = optimal dose of drug in mg/kg per day, administered intraperitoneally as a solution in 0.1 mL of 30% v/v ethanol/water on days 1, 5, and 9 after intraperitoneal inoculation of 10^6 P388 leukemia cells, or on days 5, 9, and 13 after intravenous inoculation of 10^6 Lewis lung carcinoma cells. See ref 21. [f] ILS_{max} = the percentage increase in lifespan of drug-treated tumor-bearing animals when treated at the optimal dose; values above 20% for P388 and above 40% for Lewis lung are considered statistically significant. [g] Numbers in parentheses indicate the number of animals in a group of six which were long-term survivors (50 days for P388, 60 days for LL). [h] Compound inactive at all dose levels up to toxic ones. [i] Side chain 4-$CONH(CH_2)_2NH(CH_2)_2OH$. [j] Side chain 4-$CONH(CH_2)_2N(CH_2CH_2)_2O$. [k] Compound not tested in this assay.

(R_m = −0.01) than either of the above two compounds (R_m = −0.20 and −0.24, respectively), as measured by liquid–liquid chromatography.[14] The 2-phenylquinoline chromophore of 1 has a pK_a of 2.75 (determined by UV spectroscopy), which means the compound will exist as the monocation at physiological pH, since the side chain (dimethylamino)ethyl group has a pK_a of about 8.80. Although the aza group in the pyridyl derivatives (e.g. **18**) will have a significantly higher pK_a (ca. 5; see ref 15) than that of the quinoline, it is still sufficiently low for the chromophore to remain essentially uncharged under physiological conditions. Compound 1 has been shown to bind by intercalation[5] and by analogy with crystal structures of related compounds[16] is expected to be able to achieve coplanarity with little energy expenditure. Varying the side chain of 1 gave analogues **8–12** with the expected changes in lipophilicity, but with little alteration in DNA binding levels.

A number of derivatives of 1 substituted in the phenyl ring were then evaluated. These compounds showed the expected variation in overall lipophilicity with the π values of the substituent groups, as shown by eq 1 for the 4′-substituted compounds.

$$R_m = 0.39\pi - 0.16 \quad (1)$$
$$n = 15,\ r = 0.91$$

We were particularly interested in the effects that substitution of the phenyl ring would have upon DNA binding of the 2-phenylquinolines. In the related 6-phenylphenanthridines,[4] substitution of a Cl group had little effect on binding, even when it was placed at the 2′-position (where it should effectively prevent the phenyl ring from achieving coplanarity). This was interpreted to mean that the three-ring phenanthridine chromophore was a sufficient intercalating moiety, and that whether the appended phenyl ring lay coplanar or not had no effect on binding mode.

In the case of the quinolinecarboxamides, the two-ring quinoline chromophore is not sufficient for intercalative binding, and thus the coplanarity of the phenyl ring is of critical importance in determining the mode of binding (and thus the biological activity) of the compound.[5] While the 2′-aza compound **13** binds to DNA as tightly as the parent molecule 1, derivative **14**, bearing the more bulky 2′-Cl group, has a greatly lowered level of binding (Table I), and does not unwind covalently closed circular DNA. This effect is dependent on the positioning of the substituent, since the isomeric 3′-Cl derivative **16** binds as well

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as the parent and again shows the ability to unwind and rewind closed circular DNA, with an unwinding angle of 15°.

In Vitro Cytotoxicity. The parent compound 1 has moderate in vitro cytotoxic potency (IC_{50} = 1300 nM), and this was not significantly altered by simple homologation of the side chain. Replacement of the dimethylamino group with the less basic hydroxyethylamino and morpholino functions (compounds 11 and 12) improved cytotoxicity greatly, which is surprising, since earlier use of these functions in related series did not do so.[2,13] Substitution of the phenyl ring of 1 generally improved in vitro cytotoxicity, with the main exception being the nonintercalating 2′-substituted compound 14. For the 15 4′-substituted compounds (1, 18–32) there was no overall correlation between substituent properties and cytotoxicity. Although the relatively hydrophilic 4′-aza and 4′-OH compounds 18 and 24 were the most cytotoxic, the equally hydrophilic 4′-SO_2CH_3 derivative 28 was the least cytotoxic.

In Vivo Activity. As noted previously,[5] our interest in this class of compound was initially sparked by the combination of the relatively low level of DNA binding of the parent compound 1 and its excellent in vivo activity (Table I). The compound is active against both the P388 leukemia and the Lewis lung carcinoma but, unlike the majority of DNA-intercalating compounds studied, appears to be relatively more active against the solid tumor, showing a high proportion of cures at the optimal dose of 100 mg/kg (Table I). This in vivo activity was retained (at a lower level) as the side chain was lengthened to butyl (compound 9), but the hydroxyethylamino and morpholino compounds 10 and 11 were inactive in vivo, in spite of their greatly increased in vitro cytotoxicity.

The 2′-aza compound 13 retained activity in both tumor systems, but the nonintercalating 2′-Cl derivative 14 was inactive as expected, presumably due to its lack of intercalative binding to DNA. Aza, Cl, and OMe substituents in the 3′-position gave the moderately active compounds 15–17. Most of the 4′-substituted compounds 18–31 showed moderate P388 activity, but the 3′,4′-benz derivative 32 was inactive, in spite of much higher levels of DNA binding. Fewer of the analogues showed activity against the LL carcinoma, and only the 4′-aza derivative 18 proved significantly superior to the parent in both tumor systems, showing both improved potency and higher levels of activity (Table I).

Conclusions

The parent 2-phenylquinoline-8-carboxamide (1) is the first member of the general class of tricyclic carboxamides not possessing a completely fused chromophore to show broad-spectrum anticancer activity. It shows a reduced level of DNA binding of compared to other members of the general class, and is an expression of our goal[5,17] of developing "minimal" DNA-intercalating agents as antitumor drugs with better distributive properties. Substitution on the 2′-position of the phenyl ring (except with the sterically undemanding aza group) inhibits intercalative binding, presumably by preventing chromophore coplanarity, leading to inactive compounds (e.g. 14). In the other two phenyl ring positions, sterically demanding groups are permitted. The most detailed study of substituent effects was carried out for 4′-substituted compounds, where 15 examples were available. However, in spite of the substituents covering a good range of lipophilic and electronic properties, there were no apparent correlations with in vitro cytotoxicity. Overall, compounds with powerful electron-withdrawing substituents (13, 15, 18, 26, and 28) generally showed the highest levels of in vivo solid tumor activity, although there were exceptions (e.g. the 4′-CF_3 derivative 27 and the 4′-SO_2CH_3 compound 28). In particular, the 4′-aza derivative 18 has clearly superior in vivo activity to that of the parent compound, showing about 50% cures in both the leukemia and solid tumor models. This compound is now under advanced evaluation.

Table II. Substituted 8-Methyl-2-phenylquinoline-4-carboxylic Acids 2

R	mp, °C	formula	analyses
2′-aza	319–320	$C_{16}H_{12}N_2O_2$	C,H,N
2′-Cl	230–231	$C_{17}H_{12}ClNO_2$	C,H,N
3′-aza	250–252	$C_{16}H_{12}N_2O_2 \cdot H_2O$	C,H,N
3′-Cl	272–274	$C_{17}H_{12}ClNO_2$	C,H,N,Cl
3′-OCH_3	210–211	$C_{18}H_{15}NO_3$	C,H,N
4′-aza	347–349	$C_{16}H_{12}N_2O_2$	C,H,N
4′-F	249–251	$C_{17}H_{12}FNO_2$	C,H,N,F
4′-Cl	253–255	$C_{17}H_{12}ClNO_2$	C,H,N
4′-Br	256–257	$C_{17}H_{12}BrNO_2$	C,H,N,Br
4′-I	276–278	$C_{17}H_{12}INO_2$	C,H,N,I
4′-Ph	237–239	$C_{23}H_{17}NO_2$	C,H,N
4′-OCH_3	242–244	$C_{18}H_{15}NO_3$	C,H,N
4′-CF_3	230–231	$C_{18}H_{12}F_3NO_2$	C,H,N,F
4′-SCH_3	227–229	$C_{18}H_{15}NO_2S$	C,H,N
3′,4′-benz	287–289	$C_{21}H_{15}NO_2$	C,H,N

Experimental Section

Where elemental analyses are indicated by the symbols of the elements, results obtained were within ±0.4% of the theoretical, and were carried out in the Microchemical Laboratory, University of Otago, Dunedin. Melting points were determined on an Electrothermal apparatus, using the supplied stem-corrected thermometer, and are as read. NMR spectra were measured on a Bruker AM-400 instrument (Me_4Si).

2-Phenylquinoline-8-carboxylic Acid (4: R = H). A solution of 2-methylaniline (28 g, 0.26 mol) in EtOH (50 mL) was added to a solution of pyruvic acid (33 g, 0.38 mol) and benzaldehyde (28 g, 0.26 mol) in EtOH (100 mL), and the mixture was heated under reflux for 3 h and allowed to cool overnight. The resulting solid was collected by filtration, washed sequentially with cold EtOH and benzene, and dried to give 8-methyl-2-phenylquinoline-4-carboxylic acid (2: R = H) (13.4 g, 20%), suitable for use in the next step. A sample crystallized from EtOH had mp 245–246 °C (lit.[6] mp 245 °C).

The above acid (9 g, 23 mmol) and Cu powder (0.7 g) were heated at 280–290 °C until gas evolution ceased. The cooled melt was extracted with boiling petroleum ether (bp 40–60 °C) in the presence of charcoal, and the resulting solution was filtered and concentrated to give crude 8-methyl-2-phenylquinoline (3: R = H) (7 g, 93%), suitable for the next step. A sample was crystallized from petroleum ether as plates, mp 49–50 °C (lit.[6] mp 50 °C).

The above compound (5 g, 23 mmol) and SeO_2 (5.5 g, 50 mmol) were mixed well and heated to 180–190 °C, when an exothermic reaction occurred, raising the temperature to 270–280 °C. The mixture was held at this temperature for 2 min and cooled, and the melt was extracted with hot $CHCl_3$. The resulting oil was extracted with boiling 2 N aqueous KOH, and the extract was filtered and acidified with AcOH. The precipitate was collected and crystallized from EtOH to give 2-phenylquinoline-8-carboxylic acid (4: R = H) (1.9 g, 34%), mp 159–161 °C (lit.[18] mp 158–159 °C).

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Table III. Substituted 8-Methyl-2-phenylquinolines 3

R	mp, °C	formula	analyses
2′-aza	83–84	$C_{15}H_{12}N_2$	C,H,N
2′-Cl	89–91	$C_{16}H_{12}ClN$	C,H,N,Cl
3′-aza	54–55	$C_{15}H_{12}N_2$	C,H,N
3′-Cl	45–46	$C_{16}H_{12}ClN$	C,H,N,Cl
3′-OCH_3	67.5–68	$C_{17}H_{15}NO$	C,H,N
4′-aza	77–78	$C_{15}H_{12}N_2$	C,H,N
4′-F	45–46	$C_{16}H_{12}FN$	C,H,N
4′-Cl	78–78.5	$C_{16}H_{12}ClN$	C,H,N,Cl
4′-Br	84–85	$C_{16}H_{12}BrN$	C,H,N,Br
4′-I	102–103	$C_{16}H_{12}IN$	C,H,N
4′-Ph	179–180	$C_{22}H_{17}N$	C,H,N
4′-OCH_3	85–85.5	$C_{17}H_{15}NO$	C,H,N
4′-CF_3	99–100	$C_{17}H_{12}F_3N$	C,H,N
4′-SCH_3	94–95	$C_{17}H_{15}NS$	C,H,N,S
4′-SO_2CH_3	139–141	$C_{17}H_{15}NO_2S$	C,H,N
3′,4′-benz	90–91	$C_{20}H_{15}N$	C,H,N

Table IV. Substituted 2-Phenylquinoline-8-carboxylic Acids (4)

R	mp, °C	formula	analyses
2′-aza	199–200	$C_{15}H_{10}N_2O_2$	C,H,N
2′-Cl	251–252	$C_{16}H_{10}ClNO_2$	C,H,N,Cl
3′-aza	224–226	$C_{15}H_{10}N_2O_2$	C,H,N
3′-Cl	233–235	$C_{16}H_{10}ClNO_2$	C,H,N,Cl
3′-OCH_3	137–138	$C_{17}H_{13}NO_3$	C,H,N
4′-aza	255–257	$C_{15}H_{10}N_2O_2$	C,H,N
4′-F	214–216	$C_{16}H_{10}FNO_2$	C,H,N,F
4′-Cl	209–210	$C_{16}H_{10}ClNO_2$	C,H,N,Cl
4′-Br	226–227	$C_{16}H_{10}BrNO_2$	C,H,N,Br
4′-I	236–238	$C_{16}H_{10}INO_2$	C,H,N
4′-Ph	200–201	$C_{22}H_{15}NO_2$	C,H,N
4′-OCH_3	172–173	$C_{17}H_{13}NO_3$	C,H,N
4′-CF_3	181–182	$C_{17}H_{10}F_3NO_2$	C,H,N,F
4′-SO_2CH_3	293–294	$C_{17}H_{13}NO_4S$	C,H,N
3′,4′-benz	203–205	$C_{20}H_{13}NO_2$	C,H,N

2-(2-Pyridyl)quinoline-8-carboxylic Acid (4: R = 2′-Aza). A mixture of 2-acetylpyridine (6.05 g, 50 mmol) and 7-methylisatin (8.52 g, 53 mmol) in 50% aqueous EtOH (65 mL) containing KOH (13 g) was heated under reflux for 2 h and then diluted with 50% aqueous EtOH to obtain a homogeneous mixture. This was filtered and acidified with AcOH, and the precipitate was collected, washed with 30% aqueous EtOH, and recrystallized from DMF to give 8-methyl-2-(2-pyridyl)quinoline-4-carboxylic acid (2: R = 2′-aza) (9.4 g, 67%), mp 319–320 °C. Anal. Table II.

The above quinoline acid (7.0 g, 26 mmol) was decarboxylated as detailed previously, giving 8-methyl-2-(2-pyridyl)quinoline (3: R = 2′-aza) (5.2 g, 89%), mp (petroleum ether) 83–84 °C. Anal. Table III.

The above methylquinoline (3.5 g, 16 mmol) was oxidized with SeO_2 as detailed previously, to give 2-(2-pyridyl)quinoline-8-carboxylic acid (4: R = 2′-aza) (1.44 g, 36%), mp (EtOH) 199–201 °C. Anal. Table IV.

2-[4-(Methylsulfonyl)phenyl]quinoline-8-carboxylic Acid (4: R = 4′-SO_2CH_3). Condensation of 4-(methylthio)acetophenone[10] with 7-methylisatin under the above conditions gave 8-methyl-2-[4-(methylthio)phenyl]quinoline-4-carboxylic acid (68% yield), mp (EtOH) 227–229 °C. Anal. Table III. This was decarboxylated by the above method to give 8-methyl-2-[4-(methylthio)phenyl]quinoline (51% yield), mp (MeOH) 94–95 °C. Anal. Table III.

A stirred solution of this compound (3.18 g, 12 mmol) in CH_2Cl_2 (160 mL) was treated portionwise at 20 °C with 3-chloroperoxybenzoic acid (80%; 5.6 g, 26 mmol). The mixture was stirred for a further 8 h at room temperature and was then evaporated under reduced pressure. The residue was shaken with aqueous Na_2CO_3, and the solid was collected, washed with water, and crystallized from petroleum ether to give 8-methyl-2-[4-(methylsulfonyl)phenyl]quinoline as needles, mp 139–141 °C. Anal. Table III.

A stirred solution of the above methylquinoline (2.3 g, 10.2 mmol) in concentrated H_2SO_4 (25 mL) and water (40 mL) was heated to 90 °C and treated portionwise with CrO_3 (10 g, 100 mmol) at such a rate as to maintain the temperature below 105 °C. After completion of the reaction, the mixture was diluted with water and the resulting precipitate was collected, washed with water, dissolved in hot 2 N aqueous KOH, and filtered. Neutralization with dilute aqueous AcOH to ca. pH 7 precipitated impurities, which were removed by filtration, and addition of excess AcOH then gave the desired 2-[4-(methylsulfonyl)-phenyl]quinoline-8-carboxylic acid (4: R = 4′-SO_2CH_3) (1.14 g, 33% yield), mp (AcOH) 293–294 °C. Anal. Table IV.

Repetition of the above sequence beginning with other substituted acetophenones gave the corresponding substituted 2-phenylquinoline-8-carboxylic acids listed in Table IV, via the respective intermediates listed in Tables II and III.

2-(4-Nitrophenyl)quinoline-8-carboxylic Acid (4: R = 4′-NO_2). A. By the Doebner–Miller Condensation. A mixture of 4-nitrocinnamaldehyde (71 g, 0.4 mol), 2-methylaniline (48 g, 0.45 mol), and concentrated HCl (150 mL) was stirred and heated at 140–150 °C for 5 h. The hot acidic solution was decanted, and the remaining tar was extracted with hot concentrated HCl (150 mL). The combined acid fractions were concentrated under reduced pressure and basified with NH_4OH, and the resulting oil was extracted with $CHCl_3$ to give a crude product. This was crystallized, first as the methanesulfonate salt from boiling aqueous methanesulfonic acid and then as the free base from petroleum ether and finally from EtOAc to give pure 8-methyl-2-(4-nitrophenyl)quinoline (3: R = 4′-NO_2) as pale yellow needles (8.1 g, 8%), mp 117–117.5 °C. Anal. ($C_{16}H_{12}N_2O_2$) C, H, N.

The above compound (2.3 g, 8.7 mmol) was then oxidized with CrO_3 in H_2SO_4 as detailed above to give 2-(4-nitrophenyl)-quinoline-8-carboxylic acid, which was crystallized from AcOH/MeOH as pale yellow needles (4: R = 4′-NO_2) (1.7 g, 64%), mp 272–274 °C. Anal. ($C_{16}H_{10}N_2O_4$) C, H, N.

B. By Nitration of 2-Phenylquinoline-8-carboxylic Acid (4: R = H). 2-Phenylquinoline-8-carboxylic acid (4 g, 16 mmol) was added portionwise to well-stirred fuming HNO_3 (*d* 1.50, 40 mL) at −10 °C. After a further 2 h at −5 °C, the mixture was poured on to ice, and the resulting precipitate was collected and washed well with water. Repeated recrystallization of this solid from AcOH/MeOH gave pure 2-(4-nitrophenyl)quinoline-8-carboxylic acid (4: R = 4′-NO_2) (1.6 g, 35%), mp and mixture mp 272–274 °C, identical by TLC with an authentic sample.

2-Phenyl-4(1*H*)-quinolone-8-carboxylic Acid (7). A mixture of methyl anthranilate (75.6 g, 0.50 mol) and ethyl benzoylacetate (96 g, 0.50 mol) in benzene (400 mL) containing methanesulfonic acid (0.5 mL) was heated under reflux for 36 h under a water entrainment head. The mixture was concentrated to half-volume and diluted with petroleum ether to precipitate the crude Schiff base 5 (64 g, 39%). This was collected and added over 15 min to refluxing Dowtherm A at 255 °C. The cooled mixture was diluted with benzene/petroleum ether and dried to give crude 8-(methoxycarbonyl)-2-phenyl-4(1*H*)-quinolone (6) (45.8 g, 35% overall), suitable for the next step. A sample was crystallized from benzene as colorless prisms, mp 216–217 °C. Anal. ($C_{17}H_{13}NO_3$) C, H, N.

The above ester (40 g, 0.145 mol) was suspended in 50% aqueous EtOH (500 mL) containing KOH (27 g), and the mixture was heated under reflux for 2 h. Enough 30% aqueous EtOH was added to render the hot mixture homogeneous, and the solution was filtered and slowly acidified with excess HCl in EtOH to give a granular precipitate. The material was collected and washed well with 30% aqueous EtOH followed by benzene to provide pure 2-phenyl-4(1*H*)-quinolone-8-carboxylic acid (7) (34.9 g, 98%). A sample was crystallized from DMF/EtOH/H_2O as prisms, mp 304–306 °C. Anal. ($C_{16}H_{11}NO_3$) C, H, N.

Reduction of 2-Phenyl-4(1*H*)-quinolone-8-carboxylic Acid (7). The above quinolone (5.5 g, 20.8 mmol) was dissolved in 50% aqueous EtOH containing KOH (1.4 g, 25 mmol), and the stirred, boiling solution was treated with portions of Al foil (total 3 g) which had been pretreated by immersion in a 9% solution of $HgCl_2$ in EtOH. After completion of the reaction, the mixture was filtered and acidified with HCl. The acid solution was treated with $FeCl_3$ (5 g) and heated under reflux for 30 min. Neutralization of the cooled mixture with aqueous KOAc gave a precipitate, which was collected and extracted with hot 2 N aqueous KOH. The extract was filtered and acidified, and the resulting precipitate was recrystallized twice from benzene/petroleum ether to give 2-phenylquinoline-8-carboxylic acid (4: R = H) (0.78 g,

15%), mp and mixture mp 159–161 °C.

***N*-[2-(Dimethylamino)ethyl]-2-(2-pyridyl)quinoline-8-carboxamide (13): General Example.** 2-(2-Pyridyl)-quinoline-8-carboxylic acid (1 equiv) was suspended in dry DMF (10 mL/g) and treated with 1,1′-carbonyldiimidazole (1.5 equiv) at 20–40 °C for 20 min. The homogeneous mixture was then cooled to 5 °C and treated with *N,N*-dimethylethylenediamine (2.5 equiv at 20 °C for 15 min). The solvent was removed under reduced pressure, and the residue was partitioned between CH_2Cl_2 and dilute aqueous $NaHCO_3$. The organic layer yielded the pure base, which was dissolved in MeOH. The pH was adjusted to ca. 1 with concentrated HCl and EtOAc was added to the hot solution until turbidity. On cooling, the dihydrochloride salt **13** crystallized as needles, mp 168–170 °C. Anal. Table I.

***N*-[2-(Dimethylamino)ethyl]-2-(4-aminophenyl)-quinoline-8-carboxamide (29).** A solution of the free base of the 4-nitrophenyl carboxamide **26** (1 g, 2.7 mmol) in hot 65% aqueous EtOH (100 mL) was treated with Fe powder (3 g) and AcOH (2 mL). The mixture was stirred vigorously under reflux for 1 h and then treated with excess NH_4OH and heated for a further 5 min. The hot mixture was filtered and the filter was washed well with hot EtOH. The combined filtrates were concentrated to small volume, and the precipitated solid was collected and washed well with water to give the crude free base. This was recrystallized as above to give the pure dihydrochloride salt **29** (0.8 g, 72%), mp 116–120 °C. Anal. (Table I).

The acetate and methanesulfonate derivatives **30** and **31** were prepared by acylation of the free base of the amino compound **29** in pyridine.

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Registry No. **1** (R = H, *n* = 2), 107027-12-3; **1** (R = H, *n* = 3), 107026-87-9; **1** (R = H, *n* = 3) free base, 107027-17-8; **1** (R = H, *n* = 4), 107026-88-0; **1** (R = H, *n* = 4) free base, 107027-18-9; **1** (R = H, *n* = 5), 107026-89-1; **1** (R = H, *n* = 5) free base, 107027-19-0; **1** (R = H, (side chain 4-$CONH(CH_2)_2NH(CH_2)_2OH$)), 117874-39-2; **1** (R = H, (side chain 4-$CONH(CH_2)_2NH(CH_2)_2OH$)) free base, 117874-40-5; **1** (R = H, (side chain 4-$CONH(CH_2)_2N(CH_2CH_2)_2O$)), 117874-41-6; **1** (R = H, (side chain 4-$CONH(CH_2)_2N(CH_2CH_2)_2O$)) free base, 117874-42-7; **1** (R = 2′-Cl, *n* = 2), 107026-93-7; **1** (R = 2′-Cl, *n* = 2) free base, 107027-22-5; **1** (R = 3′-aza, *n* = 2), 107026-94-8; **1** (R = 3′-aza, *n* = 2) free base, 107027-14-5; **1** (R = 3′-Cl, *n* = 2), 107026-95-9; **1** (R = 3′-OCH_3, *n* = 2), 107026-96-0; **1** (R = 3′-OCH_3, *n* = 2) free base, 117874-43-8; **1** (R = 4′-aza, *n* = 2), 107026-97-1; **1** (R = 4′-aza, *n* = 2) free base, 107027-15-6; **1** (R = 4′-F, *n* = 2), 107026-98-2; **1** (R = 4′-F, *n* = 2) free base, 107027-23-6; **1** (R = 4′-Cl, *n* = 2), 107026-99-3; **1** (R = 4′-Cl, *n* = 2) free base, 107027-16-7; **1** (R = 4′-Br, *n* = 2), 107027-00-9; **1** (R = 4′-I, *n* = 2), 107027-01-0; **1** (R = 4′-OCH_3, *n* = 2), 107027-03-2; **1** (R = 4′-OCH_3, *n* = 2) free base, 107027-25-8; **1** (R = 4′-OH, *n* = 2), 107027-04-3; **1** (R = 4′-OH, *n* = 2) free base, 107027-26-9; **1** (R = 4′-Ph, *n* = 2), 107027-02-1; **1** (R = 4′-Ph, *n* = 2) free base, 107027-24-7; **1** (R = 4′-CF_3, *n* = 2), 117874-44-9; **1** (R = 4′-CF_3, *n* = 2) free base, 117874-45-0; **1** (R = 4′-SO_2CH_3, *n* = 2), 117874-46-1; **1** (R = 4′-SO_2CH_3, *n* = 2) free base, 117874-47-2; **1** (R = 3′,4′-benz, *n* = 2), 117874-48-3; **1** (R = 3′,4′-benz, *n* = 2) free base, 117874-49-4; **2** (R = H), 107027-34-9; **2** (R = 2′-aza), 107027-35-0; **2** (R = 2′-Cl), 107027-38-3; **2** (R = 3′-aza), 107027-39-4; **2** (R = 3′-Cl), 107027-40-7; **2** (R = 3′-OCH_3), 107027-41-8; **2** (R = 4′-aza), 107027-42-9; **2** (R = 4′-F), 18060-45-2; **2** (R = 4′-Cl), 107027-43-0; **2** (R = 4′-Br), 107027-44-1; **2** (R = 4′-I), 107027-45-2; **2** (R = 4′-Ph), 107027-46-3; **2** (R = 4′-OCH_3), 107027-47-4; **2** (R = 4′-CF_3), 117874-50-7; **2** (R = 4′-SCH_3), 117874-51-8; **2** (R = 3′,4′-benz), 117874-52-9; **3** (R = H), 5353-90-2; **3** (R = 2′-aza), 107027-36-1; **3** (R = 2′-Cl), 107027-50-9; **3** (R = 3′-aza), 107027-51-0; **3** (R = 3′-Cl), 107027-52-1; **3** (R = 3′-OCH_3), 107027-53-2; **3** (R = 4′-aza), 107027-54-3; **3** (R = 4′-F), 107027-55-4; **3** (R = 4′-Cl), 107027-56-5; **3** (R = 4′-Br), 107027-57-6; **3** (R = 4′-I), 107027-58-7; **3** (R = 4′-Ph), 107027-59-8; **3** (R = 4′-OCH_3), 107027-60-1; **3** (R = 4′-CF_3), 117874-53-0; **3** (R = 4′-SCH_3), 117874-54-1; **3** (R = 4′-SO_2CH_3), 117874-55-2; **3** (R = 3′,4′-benz), 117874-56-3; **3** (R = 4′-NO_2), 107027-75-8; **4** (R = H), 5093-81-2; **4** (R = 2′-aza), 107027-37-2; **4** (R = 2′-Cl), 107027-63-4; **4** (R = 3′-aza), 107027-64-5; **4** (R = 3′-Cl), 107027-65-6; **4** (R = 3′-OCH_3), 107027-66-7; **4** (R = 4′-aza), 107027-67-8; **4** (R = 4′-F), 107027-68-9; **4** (R = 4′-Cl), 28670-72-6; **4** (R = 4′-Br), 107027-69-0; **4** (R = 4′-I), 107027-70-3; **4** (R = 4′-OCH_3), 107027-72-5; **4** (R = 4′-NO_2), 107027-77-0; **4** (R = 4′-Ph), 107027-71-4; **4** (R = 4′-CF_3), 117874-57-4; **4** (R = 4′-SO_2CH_3), 117874-58-5; **4** (R = 3′,4′-benz), 117874-59-6; **5**, 117874-60-9; **6**, 107027-76-9; **7**, 90034-64-3; **13**, 107026-92-6; **13** (free base), 107027-13-4; **26**, 107027-05-4; **26** (free base), 107027-27-0; **29**, 107027-06-5; **29** (free base), 107027-28-1; **30**, 107027-07-6; **31**, 107027-08-7; 2-methylaniline, 95-53-4; pyruvic acid, 127-17-3; benzaldehyde, 100-52-7; 2-acetylpyridine, 1122-62-9; 7-methylisatin, 1127-59-9; 4′-(methylthio)acetophenone, 1778-09-2; 4-nitrocinnamaldehyde, 1734-79-8; methyl anthranilate, 134-20-3; ethyl benzoylacetate, 94-02-0; 2-phenyl-4-quinolinecarboxylic acid, 132-60-5; 2′-chloroacetophenone, 2142-68-9; 3-acetylpyridine, 350-03-8; 3′-chloroacetophenone, 99-02-5; 3′-methoxyacetophenone, 586-37-8; 4-acetylpyridine, 1122-54-9; 4′-fluoroacetophenone, 403-42-9; 4′-chloroacetophenone, 99-91-2; 4′-bromoacetophenone, 99-90-1; 4′-iodoacetophenone, 13329-40-3; 4′-phenylacetophenone, 92-91-1; 4′-methoxyacetophenone, 100-06-1; 4′-(trifluoromethyl)acetophenone, 709-63-7; acetylnaphthaline, 93-08-3.