Synthesis and Cytotoxicity of 1,6,7,8-Substituted 2-(4'-Substituted phenyl)-4-quinolones and Related Compounds: Identification as Antimitotic Agents Interacting with Tubulin

Sheng-Chu Kuo,*,† Hong-Zin Lee,† Jung-Pin Juang,† Yih-Tyng Lin,† Tian-Shung Wu,‡ Jer-Jang Chang,§ Dan Lednicer,⊥ Kenneth D. Paull,∥ Chii M. Lin,# Ernest Hamel,# and Kuo-Hsiung Lee*,@

Graduate Institute of Pharmaceutical Chemistry, China Medical College, Taichung 400, Taiwan, Republic of China, Department of Chemistry, National Cheng-Kung University, Tainan, Taiwan, Republic of China, Department of Laboratory Animal Medicine, School of Medicine, and Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599, and Drug Synthesis and Chemistry Branch, Information Technology Branch, and Laboratory of Molecular Pharmacology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

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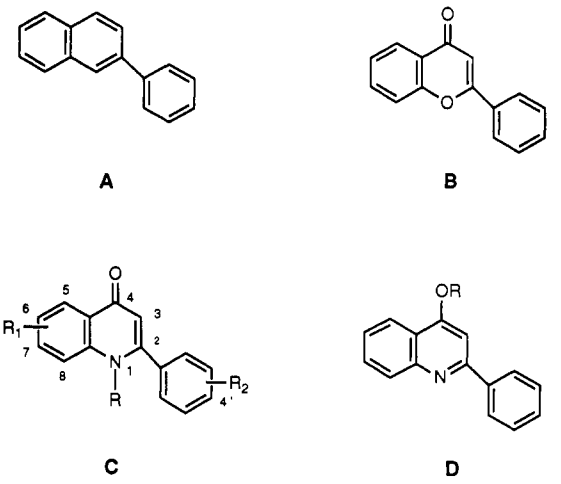
A series of 1,6,7,8-substituted 2-(4'-substituted phenyl)-4-quinolones and related compounds have been synthesized and evaluated as cytotoxic compounds and as antimitotic agents interacting with tubulin. The 2-phenyl-4-quinolones (**22–30**) with substituents (e.g. F, Cl, and OCH_3) at C-6, C-7, and C-8 show, in general, potent cytotoxicity against human lung carcinoma (A-549), ileocecal carcinoma (HCT-8), melanoma (RPMI-7951), and epidermoid carcinoma of the nasopharynx (KB) and two murine leukemia lines (P-388 and L1210). Introduction of alkyl groups at N-1 or C-4 oxygen led to inactive compounds (**35–43** and **50**). In addition, compounds **24**, **26**, and **27** were evaluated in the National Cancer Institute's 60 human tumor cell line in vitro screen. These compounds demonstrated the most marked effects in the screen on two colon carcinoma cell lines (COLO-205 and KM-20L2) and on a central nervous system tumor cell line (SF-539) with compound **26** the most potent of the three agents. Compounds **24**, **26**, and **27** were potent inhibitors of tubulin polymerization, with activity nearly comparable to that of the potent antimitotic natural products colchicine, podophyllotoxin, and combretastatin A-4. The three agents also inhibited the binding of radiolabeled colchicine to tubulin, but this inhibition was less potent than that obtained with the natural products.

Introduction

A tricyclic chemical structural pattern, consisting of a phenyl ring attached to the 2-position of a naphthalene nucleus, such as A, or composed of various heterocyclic units with similar structural arrangements, was observed among a large number of antineoplasic compounds by Cheng in 1986.[1] The flavonoids belonging to this common structural pattern A, such as B, have shown antimutagenic,[2] anticarcinogenic,[3] and antileukemic activities. The antileukemic activity of the flavonoids was seen in tricin and kaempferol 3-*O*-β-D-glycopyranoside isolated from *Wikstroemia indica*.[4] Aza analogs of B, such as the 2-phenyl-4-quinolone pattern C, should be of interest as potential antitumor agents even though no antitumor activity of this class of compounds has yet been reported. As a result of our continuing study aimed at the discovery and development of selective cytotoxic agents against slowly growing solid tumors,[5] the basic pattern-C compound, 2-phenyl-4-quinolone (**22**), was synthesized and found to demonstrate potent cytotoxicity against A-549 lung carcinoma and HCT-8 colon carcinoma cells with ED_{50} values of 0.50 and 0.80 μg/mL, respectively. Encouraged by this initial result, a systematic investigation on the 1,6,7,8-substituted 2-(4'-substituted phenyl)-4-quinolones (C) as well as their isomeric 4-alkoxy-2-phenylquinolines (D) as promising cytotoxic agents was initiated. We report herein on the synthesis and the cytotoxicity of these new compounds, their identification as antimitotic agents interacting with tubulin, and preliminary structure–activity findings.



Chemistry

As illustrated in Scheme I, 2-phenyl-4-quinolone (**22**)[6-9] was synthesized from aniline (**1**) by condensation with ethyl benzoylacetate (**11**) followed by a thermal cyclization in diphenyl ether at 240–250 °C. Similarly, the 6-substituted 2-phenyl-4-quinolones (**23–26**) and the 8-substituted 2-phenyl-4-quinolones (**30** and **31**) were prepared from the para-substituted anilines (**2–5**) and ortho-substituted anilines (**9–10**), respectively. However, ther-

* To whom correspondence should be addressed.
† China Medical College.
‡ National Cheng-Kung University.
§ School of Medicine, University of North Carolina.
⊥ Drug Synthesis and Chemistry Branch, NCI.
∥ Information Technology Branch, NCI.
Laboratory of Molecular Pharmacology, NCI.
@ School of Pharmacy, University of North Carolina.

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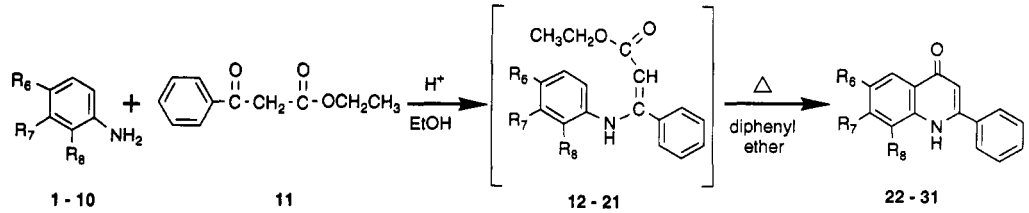
Scheme I



1, $R_6 = R_7 = R_8 = H$
2, $R_6 = F$, $R_7 = R_8 = H$
3, $R_6 = Cl$, $R_7 = R_8 = H$
4, $R_6 = CH_3$, $R_7 = R_8 = H$
5, $R_6 = OCH_3$, $R_7 = R_8 = H$
6, $R_6 = R_8 = H$, $R_7 = F$
7, $R_6 = R_8 = H$, $R_7 = Cl$
8, $R_6 = R_8 = H$, $R_7 = OCH_3$
9, $R_6 = R_7 = H$, $R_8 = F$
10, $R_6 = R_7 = H$, $R_8 = OCH_3$

12, $R_6 = R_7 = R_8 = H$
13, $R_6 = F$, $R_7 = R_8 = H$
14, $R_6 = Cl$, $R_7 = R_8 = H$
15, $R_6 = CH_3$, $R_7 = R_8 = H$
16, $R_6 = OCH_3$, $R_7 = R_8 = H$
17, $R_6 = R_8 = H$, $R_7 = F$
18, $R_6 = R_8 = H$, $R_7 = Cl$
19, $R_6 = R_8 = H$, $R_7 = OCH_3$
20, $R_6 = R_7 = H$, $R_8 = F$
21, $R_6 = R_7 = H$, $R_8 = OCH_3$

22, $R_6 = R_7 = R_8 = H$
23, $R_6 = F$, $R_7 = R_8 = H$
24, $R_6 = Cl$, $R_7 = R_8 = H$
25, $R_6 = CH_3$, $R_7 = R_8 = H$
26, $R_6 = OCH_3$, $R_7 = R_8 = H$
27, $R_6 = R_8 = H$, $R_7 = F$
28, $R_6 = R_8 = H$, $R_7 = Cl$
29, $R_6 = R_8 = H$, $R_7 = OCH_3$
30, $R_6 = R_7 = H$, $R_8 = F$
31, $R_6 = R_7 = H$, $R_8 = OCH_3$

mal cyclization of **17**, **18**, and **19**, obtained by acid condensation of **11** with their corresponding meta-substituted starting materials **6**, **7**, and **8**, yielded a small amount of their isomeric 5-fluoro(or chloro, or methoxy)-2-phenyl-4-quinolones in addition to the desired target compounds **27**, **28**,[10] and **29**[11] as the major products. The structures of **27–29** were confirmed by spectral analyses (Table I). For example, compound **27** showed a molecular formula of $C_{15}H_{10}FNO$ as established by elemental analysis and mass spectral measurement (m/z 239, M^+). Two one-proton signals at δ 6.38 (1 H, s) and 8.16 (1 H, dd, J = 6.3 and 8.8 Hz), which are due to protons at C-3 and C-5 of the 4-quinolone ring, indicated that **27** is 7-fluoro-2-phenyl-4-quinolone.

Scheme II shows the synthesis of the hydroxylated compounds **32**, **33**,[12] and **34**. These compounds were prepared directly by demethylation of their corresponding methoxy precursors **26**, **29**, and **31**[11] with KOH in ethylene glycol.

The alkylation and sulfonation of **22** are shown in Scheme III. Treatment of **22** with NaH in DMF followed by methylation with MeI led to the formation of **35** (mp 145–147 °C) and **36** (mp 42–44 °C) in a ratio of 2:3. Elemental and mass spectral (m/z 235, M^+) data established the molecular formula of $C_{16}H_{13}NO$ for both compounds, suggesting that they are possibly the N- and O-methyl derivatives. The presence of an N-methyl group at δ 3.55, a one-proton singlet at δ 6.23 (H-3), a one-proton doublet at δ 8.46 (J = 8.0 Hz) (H-5), and eight aromatic protons at δ 7.24–7.81 (m) led to the assignment of **35** as N-methyl-2-phenyl-4-quinolone.[11,12] The assignment of **36** as 4-methoxy-2-phenylquinolone[6,15] was based on NMR spectral evidence, which includes the appearance of an O-methyl signal at δ 4.03, an atomic H-3 proton at δ 7.11 instead of at δ 6.10–6.60 (which is usually seen, as in the case of 2-phenyl-4-quinolones (**22–35**), and with nine aromatic protons (m).

However, when **22** was treated with ethyl halide or a higher alkyl halide in the same way, only one product, which was confirmed to be the O-alkylated compound (**37–43**) (Table II), was obtained. When compound **22** was treated with methanesulfonyl chloride or p-toluenesulfonyl chloride in pyridine, the mesylate (**44**) or the tosylate (**45**) (Table II) was produced.

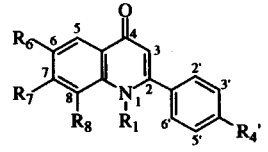
As shown in Scheme IV, the hydroxyl group of p-hydroxyacetophenone (**46**) was protected by treatment with methoxymethyl chloride in the presence of NaH to give rise to p-(methoxymethyl)acetophenone (**47**). Treatment of **47** with lithium diisopropylamine (LDA) and n-butyllithium at −30 °C generated the enol form of **47**, which was then reacted with N-methylisatoic anhydride (**48**) at −65 °C to yield 2-(4′-(methoxymethoxy)phenyl)-1-methyl-4-quinolone (**49**). The structure of **49** was established based on its ^{1}H-NMR, IR, and MS spectral data. Further treatment of **49** with concentrated HCl at room temperature, followed by neutralization with $NaHCO_3$, furnished the target compound 2-(4′-hydroxyphenyl)-1-methyl-4-quinolone (**50**). The identity of **50** with an authentic sample of reevesianine-A[16] was established by a direct spectral comparison.

Results and Discussion

a. Evaluation of Cytotoxicity of 2-Phenyl-4-quinolones. The 1,6,7,8-substituted 2-(4′-substituted phenyl)-4-quinolones and related compounds (**22–50**) were assayed for their cytotoxicity in vitro against six tumor cell lines (at the University of North Carolina), including human lung carcinoma (A-549), ileocecal carcinoma (HCT-8), melanoma (RPMI-7951), and epidermoid carcinoma of the nasopharynx (KB) and two murine leukemia lines (P-388 and L1210). As shown in Table III, compounds **22–30** and compound **33** demonstrated potent cytotoxicity, with EC_{50} values <1.0 μg/mL in virtually all cases. This indicates that many types of substituents can be present at positions 6 (F, Cl, CH_3, and OCH_3), 7 (F, Cl, OCH_3, and OH), and 8 (F) on the basis of 2-phenyl-4-quinolone molecule (i.e., **22**) without substantially affecting the general cytotoxicity obtained with this class of compound. Introduction of an OCH_3 group at C-8 led to a compound (**31**) with selective cytotoxicity (only cytotoxic to HCT-8 with EC_{50} = 0.55 μg/mL). The 6-OH (**32**) compound in contrast showed selectivity toward the KB and L1210 lines. Introduction of an OH group at C-8 (**34**) or the introduction of alkyl groups at N-1 (**35** and **50**) and at the C-4 oxygen (**36–43**) all led to inactive compounds. The C-4 O-mesylated compound (**44**) was also minimally cytotoxic.

Following initial identification of the cytotoxic properties of the 2-phenyl-4-quinolones, several of these compounds were submitted to the National Cancer Institute for testing in its new in vitro disease oriented antitumor screen.[17,18] This assay involves determination of a test agent's effect of growth parameters against a panel of approximately 60 cell lines derived from human cancers, which consists largely of solid tumors and a few leukemia lines. Assay results are reported as both dose–response curves and pictographically as "mean graphs".

Table I. Selected Physicochemical Data of 1,6,7,8-Substituted 2-(4′-Substituted phenyl)-4-quinolones

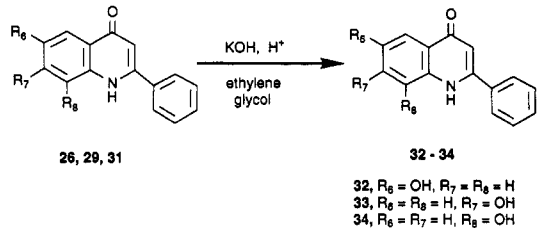


compd	R_1	R_6	R_7	R_8	$R_{4'}$	yield, %	mp, °C (solvents)	formula[a]	IR (KBr), cm^{-1}	^1H NMR (solvent) δ	MS m/z
22[b]	H	H	H	H	H	81	252–254 ($CHCl_3$–EtOH)	$C_{15}H_{11}NO$	3300 1630	(DMSO-d_6) 6.38 (1 H, s, H-3), 7.35 (1 H, dd, J = 8.0 and 8.7 Hz, H-6), 7.57–7.60 (3 H, m, H-3′, H-4′, and H-5′), 7.68 (1 H, dd, J = 8.0 and 8.4 Hz, H-7), 7.80 (1 H, d, J = 8.4 Hz, H-8), 7.83–7.86 (2 H, m, H-2′ and H-6′), 8.13 (1 H, d, J = 8.7 Hz, H-5), 11.80 (1 H, br, NH)	221 (M^+)
23	H	F	H	H	H	58	294–296 ($CHCl_3$–EtOH)	$C_{15}H_{10}FNO$	3200 1640	(DMSO-d_6) 6.35 (1 H, s, H-3), 7.22–8.00 (8 H, m, aromatic protons), 10.50 (1 H, br, NH)	239 (M^+)
24[c]	H	Cl	H	H	H	61	344–346 ($CHCl_3$–EtOH)	$C_{15}H_{10}ClNO$	3210 1630	(DMSO-d_6) 6.43 (1 H, s, H-3), 7.59–7.62 (3 H, m, H-3′, H-4′, and H-5′), 7.73 (1 H, dd, J = 2.4 and 9.0 Hz, H-7), 7.81 (1 H, d, J = 9.0 Hz, H-8), 7.83–7.87 (2 H, m, H-2′ and H-6′), 8.05 (1 H, J = 2.4 Hz, H-5), 11.50 (1 H, br, NH)	255 (M^+)
25[d]	H	CH_3	H	H	H	75	290–292 ($CHCl_3$–EtOH)	$C_{16}H_{12}NO$	3200 1630	(CF_3COOD) 2.16 (3 H, s, CH_3), 7.38–8.10 (8 H, m, H-3 and aromatic protons), 8.25 (1 H, d, J = 2.0 Hz, H-5)	235 (M^+)
26[e]	H	OCH_3	H	H	H	85	302–304 ($CHCl_3$–EtOH)	$C_{16}H_{13}NO_2$	3200 1630	(DMSO-d_6) 3.86 (3 H, s, CH_3), 6.33 (1 H, s, H-3), 7.34 (1 H, dd, J = 2.4 and 8.7 Hz, H-7), 7.53 (1 H, d, J = 2.4 Hz, H-5), 7.58–7.60 (3 H, m, H-3′, H-4′, and H-5′), 7.75 (1 H, d, J = 8.7 Hz, H-8), 7.82–7.84 (2 H, m, H-2′ and H-6′), 11.80 (1 H, br, NH)	251 (M^+)
27	H	H	F	H	H	60	298–300 ($CHCl_3$–EtOH	$C_{15}H_{10}FNO$	3210 1630	(DMSO-d_6) 6.38 (1 H, s, H-3), 7.22 (1 H, ddd, J = 2.4, 8.8, and 9.3 Hz, H-6), 7.50 (1 H, dd, J = 2.1 and 10.0 Hz, H-8), 7.59–7.62 (3 H, m, H-3′, H-4′, and H-5′), 7.82–7.85 (2 H, m, H-2′ and H-6′), 8.16 (1 H, dd, J = 6.3 and 8.8 Hz, H-5), 10.58 (1 H, br, NH)	239 (M^+)
28[f]	H	H	Cl	H	H	57	361–362 ($CHCl_3$–EtOH)	$C_{15}H_{10}ClNO$	3200 1630	(DMSO-d_6) 6.48 (1 H, s, H-3), 7.42 (1 H, dd, J = 2.4 and 8.7 Hz, H-6), 7.61–7.64 (3 H, m, H-3′, H-4′, and H-5′), 7.84–7.87 (3 H, m, H-8, H-2′, and H-6′), 8.13 (1 H, d, J = 8.7 Hz, H-5), 10.65 (1 H, br, NH)	255 (M^+)
29[g]	H	H	OCH_3	H	H	67	284–286 ($CHCl_3$–EtOH)	$C_{16}H_{13}NO_2$	3100 1615	(DMSO-d_6) 3.88 (3 H, s, CH_3), 6.29 (1 H, s, H-3), 6.96 (1 H, dd, J = 2.4 and 8.8 Hz, H-6), 7.22 (1 H, J = 2.0 Hz, H-8), 7.57–7.60 (3 H, m, H-3′, H-4′, and H-5′), 7.81–7.84 (2 H, m, H-2′ and H-6′), 8.02 (1 H, d, J = 8.8 Hz, H-5), 11.61 (1 H, br, NH)	251 (M^+)

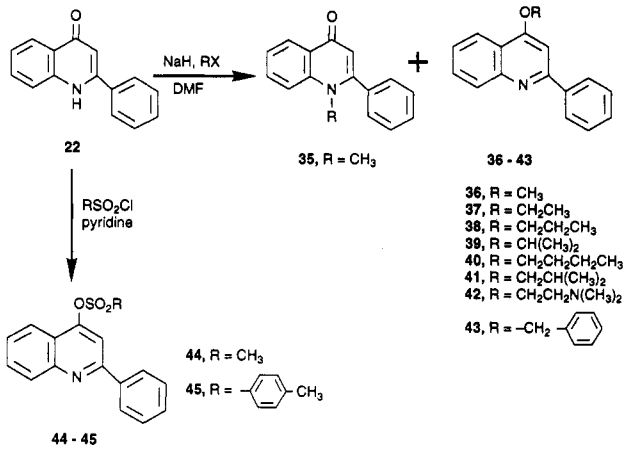
30	H	H	H	F	H	62	210–212 ($CHCl_3$–EtOH)	$C_{15}H_{10}FNO$	3100 1630	(DMSO-d_6) 6.63 (1 H, s, H-3), 7.20–8.10 (8 H, m, aromatic protons), 10.72 (1 H, br, NH)	239 (M^+)
31[h]	H	H	H	OCH_3	H	57	168–170 ($CHCl_3$–EtOH)	$C_{16}H_{13}NO_2$	3060 1630	(DMSO-d_6) 3.98 (3 H, s, CH_3), 6.38 (1 H, s, H-3), 7.25–7.33 (2 H, m, H-6 and H-7), 7.54–7.56 (3 H, m, H-3′, H-4′, and H-5′), 7.70 (1 H, dd, J = 2.4 and 8.4 Hz, H-5), 7.78–7.80 (2 H, m, H-2′ and H-6′), 10.80 (1 H, br, NH)	251 (M^+)
32	H	OH	H	H	H	60	168–170 (EtOH)	$C_{15}H_{11}NO_2$	3100 1610	(DMSO-d_6) 6.23 (1 H, s, H-3), 7.19 (1 H, dd, J = 2.5 and 8.8 Hz, H-7), 7.42 (1 H, d, J = 2.5 Hz, H-5), 7.56–7.60 (3 H, m, H-3′, H-4′, and H-5′), 7.66 (1 H, J = 8.8 Hz, H-8), 7.80–7.82 (2 H, br, H-2′ and H-6′), 9.71 (1 H, s, OH), 11.61 (1 H, s, NH)	237 (M^+)
33[i]	H	H	OH	H	H	61	324–326 (EtOH)	$C_{15}H_{11}NO_2$	3100 1620	(DMSO-d_6) 6.18 (1 H, s, H-3), 6.86 (1 H, dd, J = 2.1 and 8.7 Hz, H-6), 7.11 (1 H, d, J = 2.1 Hz, H-8), 7.58–7.60 (3 H, m, H-3′, H-4′, and H-5′), 7.80–7.83 (2 H, m, H-2′ and 6′), 7.98 (1 H, d, J = 8.7 Hz, H-5), 10.31 (1 H, s, OH), 11.42 (1 H, br, NH)	237 (M^+)
34	H	H	H	OH	H	62	262–264 (EtOH)	$C_{15}H_{11}NO_2$	3200 2900 1620	(DMSO-d_6) 6.43 (1 H, s, H-3), 7.12 (1 H, d, J = 7.8 Hz, H-7), 7.23 (1 H, dd, J = 7.8 and 8.1 Hz, H-6), 7.50–7.52 (3 H, m, H-3′, H-4′, and H-5′), 7.56 (1 H, d, J = 8.1 Hz, H-5), 7.73–7.75 (2 H, m, H-2′ and H-6′), 7.90 (1 H, br, OH), 10.80 (1 H, br, NH)	237 (M^+)
35[j]	CH_3	H	H	H	H	30	145–147 ($CHCl_3$–EtOH)	$C_{16}H_{13}NO$	1615	($CDCl_3$) 3.55 (3 H, s, NCH_3), 6.23 (1 H, s, H-3), 7.24–7.81 (8 H, m, aromatic protons), 8.46 (1 H, d, J = 8.0 Hz, H-5)	235 (M^+)
49	CH_3	H	H	H	OCH_2OCH_3	26	viscous liquid	$C_{18}H_{17}NO_3$[k]	1630	($CDCl_3$) 3.53 (3 H, s, NCH_3), 3.62 (3 H, s, OCH_3), 5.23 (2 H, s, OCH_2O), 6.23 (1 H, s, H-3), 7.02–7.63 (7 H, m, aromatic protins), 8.41 (1 H, d, J = 8.0 Hz, H-5)	295 (M^+)
50[l]	CH_3	H	H	H	OH	90	322–326 (EtOAc)	$C_{16}H_{13}NO_2$	3400 1610	($CDCl_3$ + DMSO-d_6) 3.66 (3 H, s, NCH_3), 6.07 (1 H, s, H-3), 6.90 (2 H, d, J = 9.0 Hz, H-3′ and H-5′), 7.38 (2 H, d, J = 9.0 Hz, H-2′ and H-6′), 7.37–7.83 (3 H, m, H-6, H-7, and H-8), 8.28 (1 H, d, J = 8.0 Hz, H-5), 9.72 (1 H, s, OH)	251 (M^+)

[a] All compounds were analyzed for C, H, and N, and results agreed to ±0.4% of the theoretical values except as noted. [b] See refs 6–9. [c] See refs 9 and 15. [d] See refs 9 and 15. [e] See refs 11 and 15. [f] See ref 10. [g] See ref 11. [h] See ref 11. [i] See ref 12. [j] See refs 13 and 14. [k] This was obtained by high-resolution mass spectral measurement: m/z 295.1206 (M^+) ($C_{18}H_{17}NO_3$ requires 295.1208). [l] See ref 16.

Scheme II



Scheme III



Phenylquinolones **24**, **26**, and **27** have their most notable effects in the screen on lines from the colon panel and on one line from the central nervous system (CNS) panel (Table V). In brief, all three compounds exhibit a modestly selective effect on close to half of the cell lines from the colon tumor panel at the TGI (total growth inhibition) level. Growth of cells from more sensitive lines is arrested at a concentration approximately 0.5–1 decade log concentration lower than less sensitive lines. This effect was especially noticeable when evaluation of the cytotoxicity data was extended to the LC_{50} (50% cell kill, corresponding to a 50% reduction in cell number relative to initial control values) level for two colon cancer cell lines (COLO-205, KM-20L2). Selectivity was also observed with one cell line from the CNS panel, SF-539. The quinolone **26** appears most potent of the three compounds based on the LC_{50} values obtained with the three cell lines.

A computer pattern recognition program, COMPARE, has been developed at the NCI which permits the cytotoxicity response pattern of cell lines to a new agent to be compared to standard agents used as seeds, or the pattern obtained with a new agent can be compared to all compounds in the data base.[19] The closeness of the match to each agent is expressed as a Pearson correlation coefficient. Results from COMPARE searches often provide insight into the mechanism of action of new agents[20,21] COMPARE analysis of the cytotoxicity data obtained with compound **24**, **26**, and **27** indicated these agents interacted with tubulin (Figure 1 shows the differential cytotoxicity patterns obtained with compounds **24**, **26**, and **27** in comparison with that obtained with colchicine). Correlation coefficients as high as 0.77 were obtained when known antimitotic drugs were used as seeds in a COMPARE search at the TGI level.[21] In late 1990, the time of the original evaluation of the COMPARE algorithm for its utility for the identification of new antitubulin compounds, cytotoxicity data from the NCI screen were available only for compounds **24**, **26**, and **27**. Subsequently, cytotoxicity data were also obtained for compound **28**, which was not available for tubulin studies (see below), and compound **33**. The pattern of differential cytotoxicity obtained with **28** was consistent with an antitubulin mechanism of action, while that obtained with **33** was not, by the criteria previously proposed.[21]

b. Interactions of 2-Phenyl-4-quinolones with Tubulin. The prediction obtained with the COMPARE algorithm that compounds **24**, **26**, and **27** were antimitotic agents that interacted with tubulin was confirmed.[21] These three compounds were all found to inhibit in vitro tubulin polymerization, and compound **26** was demonstrated to inhibit mitosis in HL-60 human leukemia cells at cytotoxic concentrations. In addition, **26** inhibited the binding of radiolabeled colchicine to tubulin, and as occurs with most colchicine site drugs,[22] stimulated tubulin-dependent GTP hydrolysis at concentrations that inhibit the polymerization reaction.

These findings led us to compare the effects of all available 2-phenyl-4-quinolones on tubulin polymerization to gain insight into structure–activity relationships among this class of compounds. Table VI presents our results, with a direct comparison performed contemporaneously with the potent antimitotic compounds colchicine, podophyllotoxin, and combretastatin A-4. These three natural products all bind at the colchicine site of tubulin.[22,23]

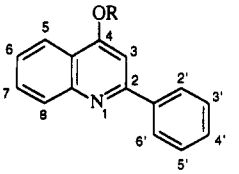
The unsubstituted **22** had significant inhibitory activity in the tubulin polymerization assay, with an IC_{50} value of 7.3 μM, measured for the extent of polymerization after 20 min. Among the available analogs, maximum enhancement of activity occurred with a substituent at position 6. Compared with the unsubstituted **22** there was a 2–3-fold increase in inhibition, in terms of IC_{50} values, whether the substituent group was methoxy (**26**), chloride (**24**), or fluoride (**23**). While the 6-methoxy substituent appeared to be slightly more inhibitory than the compounds with halides at position 6, the differences between the compounds were within the experimental error of the assay. A loss of activity, relative to **22**, occurred with a hydroxy substituent at position 6 (**32**, IC_{50} value, 16 μM).

A substituent at position 7 could also enhance activity about 2-fold relative to the unsubstituted **22**. Compounds **27** and **33**, with fluoride and hydroxyl groups, respectively, at position 7 had IC_{50} values similar to those of **23**, **24**, and **26**. Compound **29**, with a methoxy group at position 7, was slightly less active than the other compounds. With fluoride there was no significant difference in whether the substituent was at position 6 or position 7. With a methoxy group, greater activity was observed with the substituent at position 6 (IC_{50}, 2.7 ± 0.4 μM for compound **26**) than at position 7 (IC_{50}, 4.8 ± 0.5 μM for compound **29**). With the hydroxyl substituent, in contrast, position 7 was more favorable (IC_{50}, 3.4 ± 0.2 μM for compound **33** as compared with an IC_{50} value of 16 ± 2 μM for compound **32**).

Two compounds with a substituent at position 8 were available. Compound **30** with a fluorine atom at this position had relatively weak activity as an inhibitor of tubulin polymerization (IC_{50}, 11 ± 1 μM), and compound **31**, with a methoxy group at position 8, was inactive. A single agent (compound **35**) with a substituent at position 1 was evaluated, and it, too, was noninhibitory. Further modification of **35** by addition of a hydroxyl group at position 4′ (**50**) did not restore activity.

Compounds **24**, **26**, and **27**, three of the more potent inhibitors of polymerization, were also examined for effects on the binding of radiolabeled colchicine to tubulin (Table VI). In this assay, combretastatin A-4 nearly completely inhibits binding of colchicine to tubulin, and podophyl-

Table II. Selected Physicochemical Data of 4-Alkoxy-2-phenylquinolines (**36–43**), 2-Phenylquinolin-4-yl Mesylate (**44**), and 2-Phenylquinolin-4-yl Tosylate (**45**)



compd	R	yield, %	mp, °C (solvent)	formula[a]	IR (KBr), cm^{-1}	^1H NMR ($CDCl_3$) δ	MS *m/z*
36[b]	CH_3	45	42–43 (EtOH)	$C_{16}H_{13}NO$	1620	4.02 (3 H, s, OCH_3), 7.11 (1 H, s, H-3), 7.41–7.74 (5 H, m, aromatic protons), 8.06–8.20 (4 H, m, aromatic protons)	235 (M^+)
37	CH_2CH_3	78	99–100 (EtOH)	$C_{17}H_{15}NO$	1620	1.60 (3 H, t, J = 7.0 Hz, CH_3), 4.35 (2 H, q, J = 7.0 Hz, CH_2), 7.15 (1 H, s, H-3) 7.39–7.78 (5 H, m, aromatic protons), 8.04–8.27 (4 H, m, aromatic protons)	249 (M^+)
38	$CH_2CH_2CH_3$	81	66–68 (MeOH)	$C_{18}H_{17}NO$	1620	1.16 (3 H, t, J = 7.0 Hz, CH_3), 1.89–2.12 (2 H, m, CH_2CH_3), 4.24 (2 H, t, J = 7.0 Hz, OCH_2), 7.16 (1 H, s, H-3), 7.37–7.79 (5 H, m, aromatic protons), 8.02–8.28 (4 H, m, aromatic protons)	263 (M^+)
39	$CH(CH_3)_2$	77	93–95 (EtOH)	$C_{18}H_{17}NO$	1620	1.53 (6 H, d, J = 6.0 Hz, CH_3 × 2), 4.88–5.02 (1 H, m, –CH<), 7.16 (1 H, s, H-3), 7.35–7.78 (5 H, m, aromatic protons), 8.03–8.26 (4 H, m, aromatic protons)	263 (M^+)
40	$CH_2CH_2CH_2CH_3$	78	71–73 (MeOH)	$C_{19}H_{19}NO$	1615	1.01 (3 H, t, J = 6.0 Hz, CH_3), 1.27–1.97 (4 H, m, CH_2CH_2), 4.17 (2 H, t, J = 6.0 Hz, OCH_2), 7.05 (1 H, s, H-3), 7.33–7.73 (5 H, m, aromatic protons), 7.93–8.20 (4 H, m, aromatic protons)	277 (M^+)
41	$CH_2CH(CH_3)_2$	75	61–63 (MeOH)	$C_{19}H_{19}NO$	1610	1.12 (6 H, d, J = 7.0 Hz, CH_3 × 2), 1.91–2.68 (1 H, m, –CH<), 3.96 (2 H, d, J = 7.0 Hz, CH_2), 7.07 (1 H, s, H-3), 7.22–7.75 (5 H, m, aromatic protons), 7.98–8.25 (4 H, m, aromatic protons)	277 (M^+)
42	$CH_2CH_2N(CH_3)_2$	60	53–54 (MeOH)	$C_{19}H_{20}N_2O$	1610	2.44 (6 H, s, $N(CH_3)_2$), 2.94 (2 H, t, J = 6.0 Hz, $-CH_2N$<), 4.36 (2 H, t, J = 6.0 Hz, OCH_2), 7.13 (1 H, s, H-3), 7.22–7.77 (5 H, m, aromatic protons), 7.98–8.24 (4 H, m, aromatic protons)	292 (M^+)
43	$CH_2C_6H_5$	70	92–94 (EtOH)	$C_{22}H_{17}NO$		5.38 (2 H, s, CH_2), 7.18 (1 H, s, H-3), 7.22–8.30 (14 H, m, aromatic protons)	311 (M^+)
44	SO_2CH_3	88	132–134 (benzene–pet. benzin)	$C_{16}H_{13}NO_3S$	1615	3.28 (3 H, s, CH_3), 7.33–8.33 (10 H, m, H-3 and aromatic protons)	299 (M^+)
45	$SO_2CH_2C_6H_4CH_3$	91	126–128 (benzene–pet. benzin)	$C_{23}H_{17}NO_3S$	1610	2.46 (3 H, s, CH_3), 7.11–8.33 (14 H, m, H-3, aromatic protons, and SO_2CH_2)	389 (M^+)

[a] All compounds were analyzed for C, H, and N; analytical results were within ±0.4% of the theoretical values. [b] See refs 6 and 15.

lotoxin inhibits the reaction 85–90% when present in equimolar concentration with the radiolabeled colchicine.[23] In contrast, an equimolar concentration of the three 2-phenyl-4-quinolones had little effect. Only by increasing the concentration of the latter three compounds 10-fold was a significant effect on colchicine binding observed. Of the three compounds evaluated in this assay, the greatest inhibitory effect was observed with **26**.

Among the compounds available for evaluation both as cytotoxic agents and as inhibitors of tubulin polymerization there was good correlation in the two types of assay. With most cell lines the most effective inhibitors of polymerization (compounds **22–24**, **26**, **27**, **29**, and **33**) had good cytotoxicity, not differing greatly from the cytotoxicity of colchicine. Similarly, the noninhibitors of polymerization (compounds **31**, **35**, and **50**) had little or no cytotoxic properties in the cell lines we examined.

The most active 2-phenyl-4-quinolones approach colchicine in their potency as inhibitors of tubulin polymerization, and they appear to interact at the colchicine binding site of tubulin. Nevertheless, they only weakly inhibit the binding of radiolabeled colchicine to tubulin. These properties are similar to those of a structurally related class of antimitotic agent, derivatives of 2-styrylquinazolin-4(3*H*)-one.[24,25] In both classes of compounds, derivatives with halide and methoxy substituents at position 6 were more active than the isomer with the same substituents at position 7, and a hydroxyl group at position 6 in both classes resulted in a large loss of activity. In addition, the 6-methoxy derivative was more active than the 6-chloro and 6-fluoro derivatives in both series. In general, alterations in substituent pattern resulted in larger changes in relative activity in the styrylquinazolinone series than in the phenylquinolone series. Mechanistic studies with the styrylquinazolinone derivatives indicated that these agents bound to and dissociated rapidly from tubulin, accounting for their potent effect on polymerization mediated through the colchicine site despite feeble activity as inhibitors of colchicine binding.[25] A similar situation most likely holds for the 2-phenyl-4-quinolones.

The chief structural difference between the phenylquinolone and styrylquinazolinone derivatives is in the degree of separation of the phenyl ring from the heterocyclic ring system. In the former series the phenyl ring is directly attached to position 2 of the quinolone ring, while in the latter series attachment at position 2 occurs with an

Scheme IV

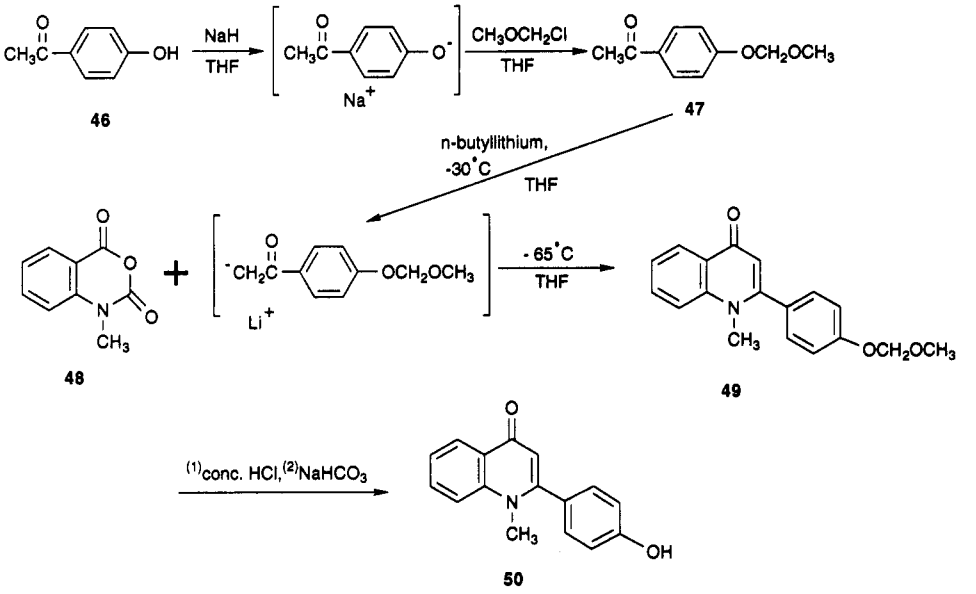


Table III. Cytotoxicity of 1,6,7,8-Substituted 2-(4'-Substituted phenyl)-4-quinolones

	EC_{50} (μg/mL) (N = 8)[a]					
compd	A-549[b]	HCT-8[b]	RPMI-7951[b]	KB[b]	P-388[b]	L-1210[b]
22	0.50	0.80	0.37	0.09	0.08	0.06
23	0.38	1.36	0.53	<0.01	0.08	<0.01
24	0.51	0.70	0.64	0.49	0.84	0.50
25	0.53	0.90	0.53	0.55	0.49	0.72
26	0.19	1.00	0.36	0.08	0.33	0.06
27	0.96	3.45	0.37	<0.1	0.29	0.16
28	2.89	0.45	<0.1	<0.1	<0.1	<0.1
29	0.73	4.86	0.43	0.53	0.67	0.57
30	0.61	0.04	0.38	0.53	0.78	0.67
31	6.77	0.55	>10.0	5.05	>10.0	7.68
32	8.14	7.75	3.92	0.41	2.86	0.83
33	0.81	>10.0	0.52	0.55	0.81	0.45
34	>10.0	>10.0	>10.0	>10.0	>10.0	>10.0
35	>10.0	>10.0	>10.0	>10.0	>10.0	>10.0
49					>10.0	>10.0
50	>10.0	5.71	>10.0	>10.0	>10.0	>10.0

[a] EC_{50} was the concentration of drug which affords 50% reduction in cell number after a 3-day incubation. For significant activity of the pure compound, an $EC_{50} \leq 4.0$ μg/mL is required. [b] Human lung carcinoma (A-549), human ileocecal carcinoma (HCT-8), human melanoma (RPMI-7951), human epidermoid carcinoma of the nasopharynx (KB), murine leukemia (P-388 and L-1210).

Table IV. Cytotoxicity of 4-Alkoxy-2-phenylquinolines (36–43) and 2-Phenylquinolin-4-yl Mesylate (44)

	EC_{50} (μg/mL) (N = 8)[a]				
compd	A-549[b]	HCT[c]	KB[d]	P-388[e]	L 1210[e]
36	>10.0	>10.0	>10.0	4.5	>10.0
37	>10.0	>10.0	6.7	6.0	>10.0
38	>10.0	>10.0	4.5	6.9	>10.0
39	>10.0	>10.0	4.2	>10.0	>10.0
40	>10.0	>10.0	3.8	>10.0	>10.0
41	>10.0	>10.0	>4.0	>10.0	>10.0
42	>10.0	>10.0	>4.0	>10.0	>10.0
43	>10.0	>10.0	1.6	>10.0	>10.0
44	6.21	5.6	2.8	>10.0	6.04

[a] EC_{50} was the concentration of drug which affords 50% reduction in cell number after a 3-day incubation. For significant activity of the pure compound, an $EC_{50} \leq 4.0$ μg/mL is required. [b] Human lung carcinoma (ATCC, Rockville, MD). [c] Human ileocecal carcinoma (ATCC, Rockville, MD). [d] Human epidermoid carcinoma of nasopharynx (ATCC, Rockville, MD). [e] Murine leukemia (ATCC, Rockville, MD).

Table V. In Vitro Tumor Cell Growth Inhibition[a] Log Concentration (M)

	response level for 24, 26, 27					
	24		26		27	
cell line	TGI[b]	LC_{50}[c]	TGI[b]	LC_{50}[c]	TGI[b]	LC_{50}[c]
colon						
COLO-205	-5.6	-4+[d]	-6.1	-4.4	-6.1	-4+[d]
HCC-2998[e]	-5.0	>-5	-6.2	>-4	-5.6	>-4
HT-29	-4.9	>-4	-6.1	>-4	-5.6	>-4
KM-20L2	-5.4	-4+[d]	-5.9	-4.6	-6.3	-5.6
CNS						
SF-539	-5.4	>-4	-6.2	-4.4	-6.0	-4+[d]

[a] Data from the NCI in vitro disease oriented antitumor screen (see refs 15 and 16 for details); values represent averages from 2 assays. [b] Concentration which reduces cell growth to level at start of experiment. [c] Concentration which reduces cell growth to 50% of level at start of experiment. [d] <-4 in one experiment, >-4 in other experiment. [e] Data represent single assay.

unsaturated 2-carbon bridge with the rings oriented trans relative to each other. We have not yet prepared analogs with a hydrocarbon bridge linking the phenyl ring with the quinolone system, while no analog with the phenyl ring directly attached to the quinazolinone system was prepared. Thus no specific data are available to suggest which heterocyclic ring system would yield the more active agent. Both the phenylquinolone and the styrylquinazolinone derivatives fall into a group of colchicine site compounds that can be described as consisting of two aryl ring systems connected by a hydrocarbon bridge of variable length.[23,26,27] Thus far no pattern has emerged for optimal bridge length or configuration in such series.[27] At this

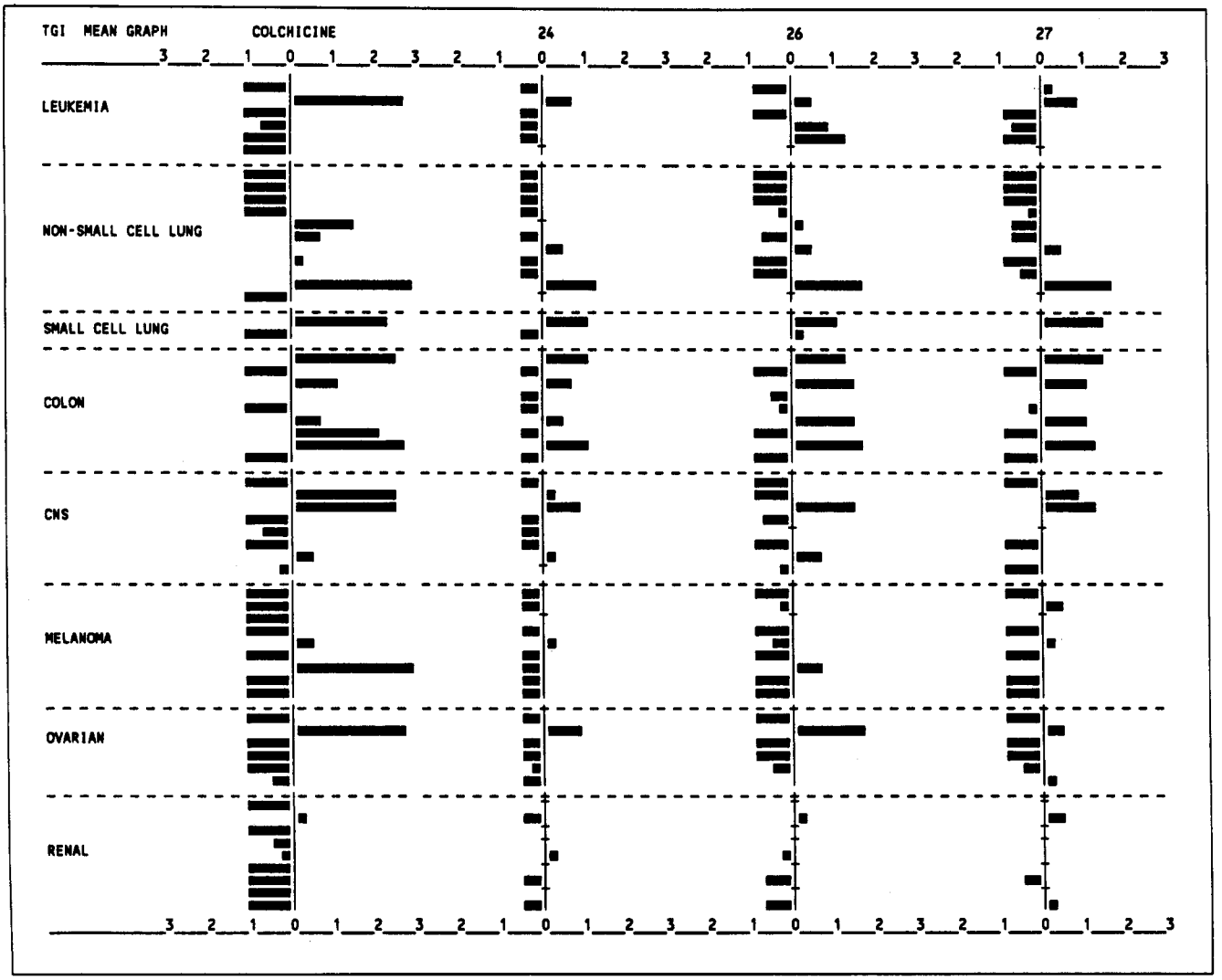


Figure 1. Patterns of differential cytotoxicity toward human tumor cell lines; comparison of colchicine with the 2-phenyl-4-quinolones **24**, **26**, and **27**. Drugs entered into the new National Cancer Institute screen are evaluated against approximately 60 different human tumor cell lines. Their cytotoxic effects are evaluated and entered into a database on a VAX9000 computer. This process is largely automated. For each cell line a value for each agent termed the "TGI" is obtained, representing the molar drug concentration which totally inhibits cell growth. For each agent a "mean TGI log" is then determined, defined as the mean of the $\log_{10}$'s of the individual TGI values. For each agent the difference between the $\log_{10}$ of each cell line and the mean TGI log for that agent is determined (as indicated at the top of the figure) to yield positive values for cell lines more sensitive than average (bars projecting to the right in the figure) and negative values for cell lines less sensitive than average (bars projecting to the left). The COMPARE algorithm permits these values to be compared for all agents in the database, and similarities in pattern are expressed in terms of a correlation coefficient (a value of 1.0 being obtained for identical patterns). The correlation coefficient of colchicine with compound **24** is 0.77, with compound **26**, 0.65, and with compound **27**, 0.77. In the figure, a small dash on an ordinate indicates that a cell line was not successfully tested with the agent. The cell lines used in the NCI screen (order as in the figure): leukemia lines CCRF-CEM, HL-60 (TB), K-562, MOLT-4, RPMI-8226, and SR; non-small cell lung carcinoma lines A549/ATCC, EKVX, HOP-18, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-H460, NCI-H522, and LXFL-529L; small cell lung carcinoma lines DMS 114 and DMS 273; colon carcinoma lines COLO 205, DLD-1, HCC-2998, HCT-116, HCT-15, HT29, KM12, KM20L2, and SW-620; central nervous system cancer lines SF-268, SF-295, SF-539, SNB-19, SNB-75, SNB-78, U251, and XF 498; melanoma lines LOX IMVI, MALME-3M, M14, M19-MEL, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-257, and UACC-62; ovarian carcinoma lines IGROVI, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, and SK-OV-3; and renal carcinoma lines 786-0, A498, ACHN, CAKI-1, RXF-393, RXF-631, SN12C, TK-10, and UO-31.

point we are unable to state whether the activity of the phenylquinolone derivatives would be enhanced by separating the phenyl ring from the quinolone ring system.

We are presently attempting to synthesize analogs of 2-phenyl-4-quinolones to address these questions.

Experimental Section

General Experimental Procedures. Melting points were determined in open-ended capillary tubes on a Thomas-Hoover apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Shimadzu IR 440 spectrometer in KBr. Nuclear magnetic resonance (NMR) spectra were taken at 90 MHz on a JEOL FX 90Q and Varian VXR-300 with tetramethylsilane (TMS) as an internal standard. Splitting patterns are designated as follows: s, singlet; br, broad; d, doublet; t, triplet; q, quartet; m, multiplet. Mass spectra (MS) were measured with an Hp 5995 GC-MS instrument and a JEOL JMS-D-30 mass spectrometer. Elemental analyses were performed by the Chung Shan Institute of Science Technology and National Cheng-Kang University, Taiwan, Republic of China.

2-Phenyl-4-quinolone (22).[6-9] Aniline (1) (1.86 g, 0.02 mol) and ethyl benzoylacetate (11) (3.84 g, 0.02 mol) were dissolved in EtOH (150 mL). Acetic acid (5 drops) was added at 50 °C. After 24 h the mixture was evaporated to dryness. The residue was added with stirring in one portion of diphenyl ether (50 mL) at 240 °C. The temperature was raised to 250 °C. After 10 min, the mixture was cooled to room temperature and diluted with *n*-hexane (80 mL). The precipitate was collected, washed with $CHCl_3$, and purified by chromatography on a silica gel column. Elution with $CHCl_3$–EtOH and recrystallization from $CHCl_3$–EtOH afforded **22** (Table I).

Table VI. Inhibition of Tubulin Polymerization and of [^{3}H]Colchicine Binding to Tubulin by Derivatives of 2-Phenyl-4-quinolone[a]

compd	inhibition of tubulin polymerization IC_{50} (μM) ± SD	% inhibition of colchicine binding: inhibitor:colchicine 1:1	10:1
22	7.3 ± 1		
23	3.3 ± 0.3		
24	3.2 ± 0.3	6	21
26	2.7 ± 0.4	8	53
27	3.7 ± 0.3	7	34
29	4.8 ± 0.5		
30	11 ± 1		
31	>40		
32	16 ± 2		
33	3.4 ± 0.2		
35	>40		
50	>40		
colchicine	1.9 ± 0.2		
podophyllotoxin	1.3 ± 0.06	88	
combretastatin A-4	1.0 ± 0.06	97	

[a] Reaction conditions for the assays in which inhibition of tubulin polymerization and of colchicine binding to tubulin were measured were described in the text. SD, standard deviation.

6-Substituted 2-Phenyl-4-quinolones (23–26). Para-substituted anilines (**2–5**) (0.02 mol) and ethyl benzoylacetate (**11**) (3.84 g, 0.02 mol) were allowed to react as in the preparation of **22**, to afford **23–26** (Table I).

7-Fluoro-2-phenyl-4-quinolone (27). *m*-Fluoroaniline (**6**) (2.22 g, 0.02 mol) and ethyl benzoylacetate (**10**) (3.84 g, 0.02 mol) were dissolved in EtOH (150 mL). Acetic acid (5 drops) was added at 50 °C. The reaction mixture was stirred at 50 °C for 24 h and then evaporated to dryness. The residue was washed with H_2O and purified by chromatography on a silica gel column. Elution with $CHCl_3$ yielded ethyl β-((*m*-fluorophenyl)amino)-cinnamate (**17**) (3.6 g, 63%), mp 54–56: IR (KBr, cm^{-1}) 1700 (C=O), 1600 (C=C); ^1H NMR ($CDCl_3$) δ 1.36 (3 H, t, J = 7.0 Hz, CH_3), 4.20 (2 H, q, J = 7.0 Hz, OCH_2), 5.01 (1 H, s, >C=CHCOO), 6.20–7.40 (9 H, m, aromatic protons), 10.30 (1 H, br, NH); high-resolution MS m/z M^+ calculated for $C_{17}H_{16}FNO_2$ 285.1165, found 285.1163.

Compound **17** (3.0 g, 0.01 mol) as a fine powder was added with stirring in one portion to diphenyl ether (50 mL) maintained at 240 °C. The temperature was raised to 250 °C. After 10 min the mixture was cooled to room temperature and diluted with *n*-hexane (80 mL). The precipitate was collected and washed with $CHCl_3$ and purified by chromatography on silica gel. Elution with $CHCl_3$–EtOH and recrystallization from $CHCl_3$–EtOH afforded **27** (Table I).

7-Chloro-2-phenyl-4-quinolone (28).[10] *m*-Chloroanaline (**7**) (2.55 g, 0.02 mol) and ethyl benzoylacetate (**11**) (3.84 g, 0.02 mol) were allowed to react as in the preparation of **22** to afford **28** (Table I).

7-Methoxy-2-phenyl-4-quinoline (29).[11] *m*-Methoxyaniline (**8**) (2.46 g, 0.02 mol) and ethyl benzoylacetate (**11**) (3.84 g, 0.02 mol) were allowed to react as in the preparation of **22** to afford **29** (Table I).

8-Substituted 2-Phenyl-4-quinolones (30 and 31). Ortho-substituted anilines (**9, 10**) (0.02 mol) and ethyl benzoylacetate (**11**) (3.84 g, 0.02 mol) were allowed to react as in the preparation of **22** to afford **30** and **31** (Table I).

6-Hydroxy-2-phenyl-4-quinolone (32). A mixture of **26** (100 mg, 0.0004 mol), KOH (1 g, 0.018 mol), and ethylene glycol (10 mL) was boiled under reflux for 24 h. The reaction mixture was poured into ice–water and filtered. The filtrate was neutralized with dilute HCl, and the precipitate was collected. The crude product was purified by chromatography on silica gel. Elution with $CHCl_3$–EtOH yielded **32** (Table I).

7-Hydroxy-2-phenyl-4-quinolone (33).[12] Compound **29** (100 mg, 0.0004 mol) was reacted with KOH (1 g, 0.018 mol) as described for the preparation of **32** to afford **33** (Table I).

8-Hydroxy-2-phenyl-4-quinolone (34). Compound **31** (100 mg, 0.0004 mol) was reacted with KOH (1 g, 0.018 mol) as described for the preparation of **32** to afford **34** (Table I).

Alkylation of Compound 22. Compound **22** (2.21 g, 0.01 mol) was dissolved in dry DMF (50 mL), and NaH (80% in oil, 0.3 g, 0.01 mol) was added portionwise with stirring for 30 min at room temperature. Alkyl halides (0.01 mol) were then added dropwise at 30–40 °C. Stirring was continued for an additional 30 min, and the reaction mixture was poured into ice–water and extracted with $CHCl_3$. The organic layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was purified by chromatography on silica gel. Elution with benzene yielded **35**[13,14] (Table I) and **36–43** (Table II).

2-Phenylquinolin-4-yl Mesylate (44). Compound **22** (1.10 g, 0.005 mol) was dissolved in dry pyridine (10 mL). Methanesulfonyl chloride (1.15 g, 0.01 mol) was added dropwise at 0 °C. The reaction mixture was stirred for 24 h and poured into ice–water (40 mL). The precipitated solid was extracted with ether, and the extract washed with H_2O and dried over $MgSO_4$. The solvent was evaporated, and the residue was purified by chromatography on silica gel. Elution with benzene yielded **44** (Table II).

2-Phenylquinolin-4-yl Tosylate (45). Compound **22** (1.10 g, 0.005 mol) was reacted with *p*-toluenesulfonyl chloride (1.90 g, 0.01 mol) as described for the preparation of **44** to afford **45** (Table II).

***N*-Methyl-2-(4′-hydroxyphenyl)-4-quinolone (50).**[16] *p*-Hydroxyacetophenone (**46**) (13.6 g, 0.1 mol) was dissolved in dry tetrahydrofuran (THF) (200 mL), and NaH (80% in oil, 3.0 g, 0.1 mol) was added. Chloromethyl methyl ether (8.1 g, 0.1 mol) in dry THF (20 mL) was added dropwise at 50 °C. The reaction mixture was stirred at 50 °C for an additional 1 h and then evaporated to dryness. The residue was purified by chromatography on a silica gel column. Elution with benzene yielded *p*-(methoxymethoxy)acetophenone (**47**) (12 g, 67%) as a viscous liquid: IR (Nujol, cm^{-1}) 1685 (C=O); ^1H NMR ($CDCl_3$) δ 2.57 (3 H, s, $COCH_3$), 3.48 (3 H, s, OCH_3), 5.22 (2 H, s, OCH_2O), 7.03 (2 H, d, J = 9.0 Hz, H-3 and H-5), 7.88 (2 H, d, J = 9.0 Hz, H-2 and H-6); high-resolution MS m/z M^+ calcd for $C_{10}H_{12}O_3$ 180.0786, found 180.0789. Diisopropylamine (2.0 g, 0.02 mol) was dissolved in dry THF (75 mL), and *n*-butyllithium (1.6 M in hexane, 1.28 g, 0.02 mol) was added at −30 °C. After cooling to −65 °C, a solution of **47** (3.60 g, 0.02 mol) in THF (10 mL) was added dropwise, and the mixture was kept at this temperature for 1 h. A solution of *N*-methylisatoic anhydride (**48**) (1.8 g, 0.01 mol) in THF (40 mL) was added dropwise at −65 °C, and the resulting suspension was stirred for 5 h. The reaction mixture was quenched with saturated ammonium chloride solution and extracted with methylene chloride. The organic phase was combined and dried over $MgSO_4$. The solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel. Elution with $CHCl_3$–EtOH yielded 2-(4′-(methoxymethoxy)phenyl)-1-methyl-4-quinolone (**49**) (Table I). Compound **49** (1.48 g, 0.05 mol) was suspended in concentrated HCl (10 mL) and stirred at 30 °C for 30 min. The reaction mixture was neutralized with saturated $NaHCO_3$, and the precipitate was collected. The crude product was washed with $CHCl_3$ and recrystallized from EtOAc to afford **50** (Table I).

Cytotoxicity Assay. Candidate compounds (**22–50**) were assayed for in vitro cytotoxicity with a panel of human and murine tumor cell lines at the School of Medicine, UNC-CH. These cell lines include lung carcinoma (A-549), ileocecal carcinoma (HCT-8), epidermoid carcinoma of the nasopharynx (KB), melanoma (RPMI-7951), and murine leukemia (P-388 and L-1210). All cell lines were obtained from the American Type Culture Collection, Rockeville, MD, and were adapted to grow in antibiotic-free RPMI-1640 medium supplemented with 10% fetal calf serum. The assay principles followed those described by Monk et al.[18] except that tetrazolium was used to estimate live cell number in each well of tissue culture plate before and after addition of chemicals (Mosmann).[29] The EC_{50} value for each compound was calculated mathematically from two data points across 50% inhibition in cell growth. Compounds with high efficacy to these human and murine tumor cell lines were further examined and new derivatives synthesized. Compounds with promising potential against solid tumors were then submitted to NCI for further in vitro human tumor cell line assay and for further studies of molecular mechanism of action.

Cytotoxicity studies in human and murine tumor cell lines were performed in a 96-well format. Each well was deposited with ca. 1×10^5 cells, and after overnight stabilization, testing chemicals, in appropriate dilution, were added to each of the wells (each concentration performed in quadruplicate). Control wells received medium alone. After 3–4 days cultivation, the remaining cell number in each well was estimated by the formazan pigment formation assay. MTT was added to each well, and the plates were incubated overnight. The pigment formed was dissolved in DMSO, and the plates were analyzed on a Dynatech 360 ELISA reader at 570 nm. Actinomycin (Sigma Chemical Co., St. Louis, MO) was used as a positive control. The ED_{50} of actinomycin for these cell lines is usually less than 0.01 μg/mL.

Materials for Tubulin Polymerization Bioassay. Tubulin was purified from bovine brain as described previously.[29] Nonradiolabeled colchicine was obtained from Sigma, podophyllotoxin from Aldrich, and [^{3}H]colchicine from Du Pont. Combretastatin A-4 was a generous gift of Dr. G. R. Pettit of Arizona State University. Commercial monosodium glutamate, from Sigma, was repurified by acid precipitation and reneutralized (to pH 6.6, in a 2 M stock solution) with ultrapure NaOH obtained from Alfa.[30] Minimal residual contamination by Mg^{2+} and several other cations was confirmed by atomic absorption spectroscopy. Stock solutions of the compounds used in the tubulin assays were prepared with dimethyl sulfoxide, and an equivalent amount of the solvent was present in control reaction mixtures.

Tubulin Polymerization Assay. Each reaction mixture contained in a 0.24-mL volume of 1.0 mg/mL (10 μM) tubulin, 1.0 M monosodium glutamate, 1.0 mM $MgCl_2$, 4% (v/v) dimethyl sulfoxide, and varying concentrations of drugs (all concentrations, however, refer to final reaction volume of 0.25 mL). Reaction mixtures were preincubated at 37 °C for 15 min and chilled on ice, and 10 μL of 10 mM GTP (required for polymerization) was added to each mixture. Reaction mixtures were transferred to cuvettes in Gilford spectrophotometers held at 0 °C by electronic temperature controllers. Baseline absorbances at 350 nm were estabished, and the reaction was initiated by a temperature jump to 37 °C (the temperature rose at a rate of about 0.5 °C/s). The reactions were followed for 20 min, and IC_{50} values, defined as the drug concentration required to inhibit the extent of polymerization by 50% after a 20-min incubation, were determined graphically. At least three independent experiments were performed with each drug, except that inactive compounds (defined as IC_{50} value greater than 40 μM) were generally evaluated only twice. Four spectrophotometers (16 samples) were used in each experiment. Each experiment had two control reaction mixtures, with the turbidity readings generally within 5% of each other.

Repurified glutamate was used in the studies with the 2-phenyl-4-quinolones because several of these agents caused aberrant turbidity readings when commercial glutamate was used, as described previously[21,27] with other colchicine site drugs. Although we initially believed[27] this phenomenon was caused by Mg^{2+} contamination in the commercial glutamate, we were unable to reproduce the phenomenon by adding $MgCl_2$ to the repurified glutamate. Besides eliminating spurious high turbidity readings with several of the phenylquinolone derivatives, use of the repurified glutamate resulted in our obtaining significantly lower IC_{50} values with colchicine, podophyllotoxin, and combretastatin A-4 than we previously obtained with commercial glutamate.[23,31] The presumptive contaminant(s) in commercial glutamate is-(are) unknown.

Inhibition of the Binding of Radiolabeled Colchicine to Tubulin. Each 0.1-mL reaction mixture contained 0.1 mg (1.0 μM) of tubulin, 1.0 M commercial monosodium glutamate (pH 6.6 with HCl), 1 mM $MgCl_2$, 0.1 mM GTP, 5.0 μM [^{3}H]colchicine, 5% (v/v) dimethyl sulfoxide, and inhibitor as indicated. Incubation was for 20 min at 37 °C. Each reaction mixture was filtered under reduced vacuum through a stack of two DEAE-cellulose paper filters, washed with water, and radioactivity quantitated in a liquid scintillation counter.

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