Antitumor Agents. 150.† 2',3',4',5',5,6,7-Substituted 2-Phenyl-4-quinolones and Related Compounds: Their Synthesis, Cytotoxicity, and Inhibition of Tubulin Polymerization

Leping Li,‡ Hui-Kang Wang,‡ Sheng-Chu Kuo,§ Tian-Shung Wu,⊥ Dan Lednicer,∥ Chii M. Lin,▽ Ernest Hamel,▽ and Kuo-Hsiung Lee*,‡

Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, 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, and Drug Synthesis and Chemistry Branch and Laboratory of Molecular Pharmacology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

Received October 21, 1993•

As part of our continuing search for potential anticancer drug candidates in the 2-phenyl-4-quinolone series, we have synthesized a series of 6,7-methylenedioxy-substituted and unsubstituted 2-phenyl-4-quinolones, as well as related compounds. Their *in vitro* inhibition of human tumor cell lines and tubulin polymerization is reported. In general, a good correlation was found between cytotoxicity and inhibition of tubulin polymerization. Compounds **7**, **9**, **13**, **16**, **22**, **23**, **36**, and **37** showed potent inhibitory effects in both assays. All rigid analogs (**47**–**49**) and trimethoxy-substituted compounds showed little or no activity. Substitution at the 4'-position also resulted in compounds with little or no activity, except for hydroxyl or methyl groups at this position. Further investigation is underway to determine if substitution at the 3'-position will result in compounds with increased activity.

Introduction

Microtubules are among the most strategic subcellular targets of anticancer chemotherapeutics. Many structurally diverse compounds have been reported to attack microtubules through their major structural component, tubulin,[2,3] and cause mitotic arrest. This occurs whether microtubule assembly is inhibited or stimulated by the action of the drug. Interestingly, all clinically used antimitotic agents are natural products (e.g., taxol,[4-7] podophyllotoxin,[8,9] and the *Vinca* alkaloids[5]) of their semisynthetic derivatives. Although colchicine itself is too toxic to have chemotherapeutic potential, it is of major importance in studying the functions of microtubules.[10] Recently, combretastatin A-4 and related compounds have been isolated and found to inhibit *in vitro* tubulin polymerization.[11-13] Combretastatin A-4 is the strongest competitive inhibitor of colchicine binding to tubulin that has been reported to date. In a previous paper,[14] we reported a novel series of biaryl-containing antimitotic agents, the 1,6,7,8-substituted 2-(4'-substituted phenyl)-4-quinolones and related compounds. We also confirmed their classification as colchicine-site drugs. In this series, the most potent compound inhibited tubulin polymerization with an IC_{50} of 2.7 μM, which was comparable to that of colchicine (IC_{50} = 1.9 μM). Although many structurally diverse colchicine-site compounds have been documented and some have been studied extensively, the tubulin-binding site for colchicine remains unknown. Although an X-ray crystal structure of an inhibitor–tubulin complex would provide a clear picture of the binding site, tubulin's heterogeneity has made such data unobtainable thus far.

(Methylenedioxy)benzene is a common moiety in natural products. It also appears commonly in antimitotic agents, such as cornigerine,[15] podophyllotoxin,[8] steganacin,[16] and combretastatin A-2[12,17] (Figure 1). Cornigerine[15] differs from colchicine only in the substitutions of the 2- and 3-positions of the A ring. While cornigerine has a 2,3-methylenedioxy substitution, colchicine has a 2,3-dimethoxy substitution. Cornigerine is about 1.5 times more potent than colchicine in binding to tubulin, inhibiting cell growth, and inhibiting microtubule assembly. In contrast, methylenedioxy-substituted combretastatin A-2 is less potent than the corresponding dimethoxy-substituted combretastatin A-4 in both cytotoxicity and tubulin-binding assays.[12,17] In exploring the structure–activity relationships of the 2-phenyl-4-quinolone series, we synthesized several 6,7-methylenedioxy-substituted derivatives. Generally, these compounds had activity equivalent to 6-methoxy-substituted analogs and substantially greater activity than 6,7-dimethoxy-substituted analogs. This represents a third pattern among colchicine-site compounds for which a methylenedioxy-substituted derivative can be directly compared to equivalent methoxy-substituted derivatives. In addition, we explored the effects of a variety of substituents in the phenyl C ring of this class of compounds.

Chemistry

2-Phenyl-4-quinolones can be synthesized by several pathways; an anthranilic acid or its ester can be heated with the acetal of an alkyl aryl ketone, or, alternatively, an arylamine can be condensed with an ethyl arylacetate in the presence of PPA or sulfuric acid.[18,19] Compounds **32**–**35** and **42**–**45** (Tables 1 and 2) were prepared according to these previously reported methods.[14] However, these

† For part 149, see ref 1.
* To whom correspondence should be addressed.
‡ University of North Carolina.
§ China Medical College.
⊥ National Cheng-Kung University.
∥ Drug Synthesis and Chemistry Branch, NCI.
▽ Laboratory of Molecular Pharmacology, NCI.
• Abstract published in *Advance ACS Abstracts*, March 1, 1994.

0022-2623/94/1837-1126$04.50/0

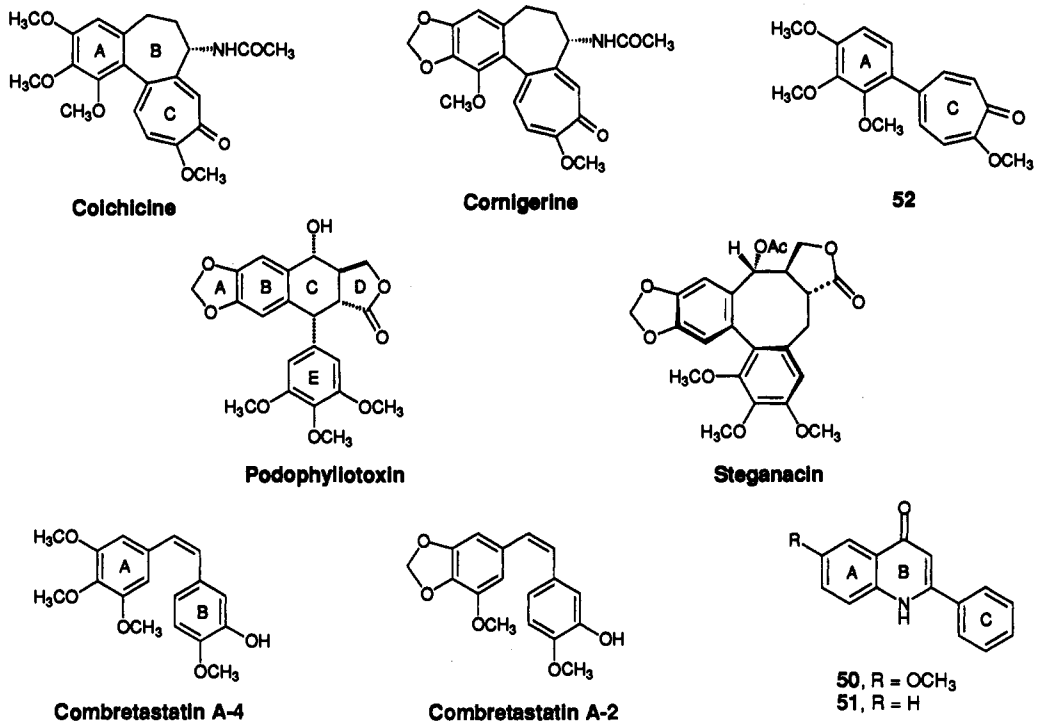


Figure 1.

methods are not suitable for the preparation of 2-phenyl-4-quinolones with multiple substituents on the phenyl rings. More convenient methods with higher yields have been developed.[20,21] One method (Scheme 1) is to condense substituted anthranilamides 1 with substituted acetophenones 2 to give the corresponding ketimines 3. The substituted anthranilamides 1 were prepared by the following steps: Jones oxidation of *ortho*-substituted nitrophenyl aldehydes, formation of an acid chloride using thionyl chloride, conversion to an *N,N*-diethylamide, and reduction of the *ortho* nitro group to the amine by hydrogenation. Lithium diisopropylamide (LDA)-mediated cyclization of 3 gave 2-phenyl-4-quinolones **7–21, 24–31, 36–41,** and **47–49** (Tables 1, 2, and 3) in good yield. However, LDA-mediated cyclization failed when an electron-withdrawing group (e.g., a nitro group) was present on the 2-phenyl ring. This may be due to enhanced stabilization of the anion intermediate by the electron-withdrawing group. A second method (Scheme 2) involves the formation of diarylamides 6 from substituted *ortho* amino acetophenones 4 and an appropriately substituted benzoyl chloride (5). Cyclization in the presence of *tert*-butoxide (*t*-BuOK) gave compounds **7, 9, 22, 23,** and **46** (Table 1). The starting materials, substituted *ortho* amino acetophenones 4, were prepared by nitration of the corresponding acetophenones followed by hydrogenation in the presence of 10% palladium on active carbon.[22]

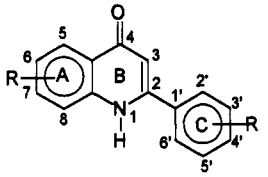
2-Phenyl-4-quinolones have low solubility in most organic solvents; however, they dissolve moderately in DMSO, DMF, and a mixture of $CHCl_3$ and MeOH. 2-Phenylquinolones can be purified either by chromatography or by recrystallization from DMF or a mixture of $CHCl_3$ and MeOH.

Results and Discussion

a. Evaluation of the Cytotoxicity of 2-Phenyl-4-quinolones. The 2-phenyl-4-quinolones and related compounds were tested in the National Cancer Institute's *in vitro* disease-oriented antitumor screen, which determines a test agent's effect on growth parameters against a panel of approximately 60 human tumor cell lines.[23,24] The cytotoxic effects of each compound are obtained as TGI or GI_{50} values, which represent the molar drug concentrations required to cause total growth inhibition and half growth inhibition, respectively. The results are expressed in Table 4 as log GI_{50} values. Among the compounds tested, **7, 9, 13, 16, 22, 23, 33, 36,** and **37** showed the strongest inhibitory effects against a variety of tumor cell lines, especially colon, CNS, ovarian, and small-cell lung cancer cell lines. This result is in agreement with our previous report on this series. In addition to leukemia, melanoma, non-small-cell lung, colon, CNS, ovarian, and renal cancer panels, compound **23** was also tested against breast and prostate cancer cell lines. It has low activity against prostate cancer cell lines; however, it showed strong cytotoxic effects against several breast cancer cell lines such as MCF-7/ADR-RES, MDA-MB-435, MDA-MB-231/ATCC, and MDA-N (with log GI_{50} values equal to −7.16, −5.77, −7.50, and −7.40 μM, respectively). Overall, compounds substituted at the 4′-position were inactive, except for compounds **14, 17, 19,** and **33.** All rigid analogs (2,3-fused quinolones) (**47–49**) and all 2-heteroaryl-4-quinolones (**38–46**) were inactive or less active than the 2-phenyl-4-quinolones. The 6,7-methylenedioxy-substituted compounds (**9** and **13**) were more potent than their corresponding 6,7-dimethoxy-substituted (**30** and **29**) or unsubstituted (**26** and **24**) compounds. Trimethoxy substitutions on the 2-phenyl ring resulted in loss of activity (compounds **20, 21,** and **27**). 2-Styryl-4-quinolone **46** was also inactive in the cytotoxicity assay.

b. Interactions of 2-Phenyl-4-quinolones with Tubulin. In the previous paper,[14] selected molecules of this class of compounds were reported to be antimitotic agents interacting at the colchicine site ot tubulin.[25–28] To further delineate structure–activity relationships, the newly synthesized compounds were evaluated as inhibitors of tubulin polymerization and compared with the classic antimitotic agents, colchicine, podophyllotoxin, and combretastatin

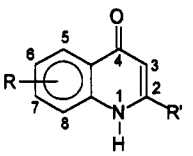
Table 1. Physical Properties and Antitubulin Effects of 2′,3′,4′,5′,6,7-Substituted 2-Phenyl-4-quinolones



compd	$R_{2'}$	$R_{3'}$	$R_{4'}$	$R_{5'}$	R_6	R_7	ITP[a] IC_{50} (μM) ± SD	ICB[b] (% inhibition)	formula[c]	mp (°C)	method	yield (%)
7	H	H	H	H	OCH_2O		0.63 ± 0.2	26 ± 10	$C_{18}H_{15}NO_3 \cdot 0.25H_2O$	>300	A or B	50 (A)[e]
8	H	H	OMe	H	OCH_2O		>40		$C_{17}H_{13}NO_4$	>300	A	53
9	H	OMe	H	H	OCH_2O		0.57 ± 0.1	39 ± 8	$C_{17}H_{13}NO_4 \cdot 1.5H_2O$	>300	A or B	56 (A)[e]
10	OMe	H	H	H	OCH_2O		14 ± 0.5		$C_{17}H_{13}NO_4$	285–287[d]	A	32
11	H	H	OPh	H	OCH_2O		>40		$C_{22}H_{15}NO_4$	>300	A	34
12	H	H	$N(Me)_2$	H	OCH_2O		>40		$C_{18}H_{16}N_2O_3 \cdot 3.25H_2O$	>300	A	41
13	H	$N(Me)_2$	H	H	OCH_2O		0.7 ± 0.03	29 ± 7	$C_{18}H_{16}N_2O_3$	>300	A	45
14	H	H	Me	H	OCH_2O		1.0 ± 0.2	12 ± 8	$C_{17}H_{13}NO_3 \cdot 0.33H_2O$	>300	A	51
15	H	OMe	OMe	H	OCH_2O		>40		$C_{18}H_{15}NO_5$	>300	A	44
16	H	OMe	H	OMe	OCH_2O		0.62 ± 0.2	31 ± 2	$C_{18}H_{15}NO_5 \cdot H_2O$	>300	A	47
17	OMe	H	OMe	H	OCH_2O		>40		$C_{18}H_{15}NO_5$	286–288[d]	A	33
18	OMe	H	H	OMe	OCH_2O		5.5 ± 0.8		$C_{18}H_{15}NO_5$	294–296[d]	A	28
19	H	OCH_2O		H	OCH_2O		1.1 ± 0.2		$C_{17}H_{131}NO_5 \cdot 0.5H_2O$	>300	A	56
20	H	OMe	OMe	OMe	OCH_2O		>40		$C_{19}H_{17}NO_6 \cdot 0.33H_2O$	285–287	A	42
21	OMe	OMe	OMe	H	OCH_2O		>40		$C_{19}H_{17}NO_6$	258–259	A	33
22	F	H	H	H	OCH_2O		0.85 ± 0.1	37 ± 3	$C_{16}H_{10}FNO_3 \cdot 0.25H_2O$	>300[d]	B	83
23	Cl	H	H	H	OCH_2O		0.89 ± 0.1	24 ± 4	$C_{16}H_{10}ClNO_3$	>300[d]	B	78
24	H	$N(Me)_2$	H	H	H	H	1.5 ± 0.3		$C_{17}H_{15}N_2 \cdot 0.33H_2O$	276–278	A	70
25	H	H	$N(Me)_2$	H	H	H	>40		$C_{17}H_{16}N_2O$	>300	A	67
26	H	OMe	H	H	H	H	1.4 ± 0.4		$C_{18}H_{13}NO_2$	267–268	A	75
27	H	OMe	OMe	OMe	H	H	>40		$C_{18}H_{17}N_2O_4$	257–258	A	66
28	H	H	H	H	OMe	OMe	18 ± 1		$C_{15}H_{17}NO_3$	274–275[d]	B	70
29	H	$N(Me)_2$	H	H	OMe	OMe	15 ± 2		$C_{19}H_{20}N_2O_3$	294–296[d]	A	77
30	H	OMe	H	H	OMe	OMe	4.3 ± 1		$C_{18}H_{17}NO_4 \cdot H_2O$	149–151	A	40
31	H	OMe	OMe	H	OMe	OMe	>40		$C_{19}H_{19}NO_5$	162–163	A	48
32	H	H	OMe	H	OMe	H	>40		$C_{17}H_{15}NO_3$	>300	C	42
33	H	H	OH	H	OMe	H	5.6 ± 1		$C_{18}H_{13}NO_3$	>260[d]	C	11
34	H	H	OMe	H	Cl	H	>40		$C_{18}H_{12}ClNO_2$	295–297	C	13
35	H	H	OMe	H	H	F	>40		$C_{18}H_{12}FNO_2 \cdot MeOH$	265–266	C	8
36	H	$N(Me)_2$	H	H	OMe	H	0.84 ± 0.2	18 ± 6	$C_{18}H_{16}N_2O_2$	>270[d]	A	32
37	H	OMe	H	H	OMe	H	0.74 ± 0.1	13 ± 2	$C_{18}H_{15}NO_3$	280–281	B	83
colchicine							0.80 ± 0.07					
podophyllotoxin							0.46 ± 0.02	86 ± 1				
combretastatin A-4							0.53 ± 0.05	94 ± 2				
dihydrocombretastatin A-4							0.63 ± 0.03	65 ± 4				
50							0.61 ± 0.05	11 ± 7				

[a] ITP = inhibition of tubulin polymerization. [b] ICB = inhibition of colchicine binding. [c] All compounds were analyzed for C, H, and N, and the results agreed to ±0.4% of the theoretical values. [d] Decomposed. [e] The yields were 82% and 78% for 7 and 9, respectively, when method B was used.

Table 2. Physical Properties and Antitubulin Effects of 5,6,7-Substituted 2-Heterocyclic 4-Quinolones and a Related Styryl Derivative

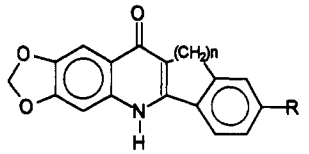


compd	R′	R_5	R_6	R_7	ITP[a] IC_{50} (μM) ± SD	formula[b]	mp (°C)	method	yield (%)
38	2′-pyrrolyl	H	OCH_2O		18 ± 1	$C_{14}H_{10}N_2O_3$	>300	A	20
39	2′-furyl	H	OCH_2O		23 ± 7	$C_{14}H_9NO_4$	>300	A	18
40	2′-thienyl	H	OCH_2O		2.2 ± 0.2	$C_{14}H_9NO_3S \cdot 0.33H_2O$	>300	A	24
41	3′-indolyl	H	OCH_2O		1.3 ± 0.4	$C_{18}H_{12}N_2O_3$	>300	A	22
42	2′-pyridyl	H	H	F	>40	$C_{14}H_9FN_2O$	274–275	C	17
43	2′-pyridyl	F	H	H	>40	$C_{14}H_9FN_2O$	267–268	C	16
44	2′-pyridyl	H	OMe	H	>40	$C_{15}H_{12}N_2O_2 \cdot 0.125H_2O$	236–237	C	18
45	2′-pyridyl	H	Cl	H	>40	$C_{14}H_9ClN_2O \cdot 0.5H_2O$	>276[c]	C	17
46	*trans*-styryl	H	OCH_2O		2.3 ± 0.6	$C_{18}H_{13}NO_3$	>300	B	61

[a] ITP = inhibition of tubulin polymerization. [b] All compounds were analyzed for C, H, and N except compound 36 which was also analyzed for S, and the results agreed to ±0.4% of the theoretical values. [c] Decomposed.

A-4, and with the most active of the previously prepared compounds (**50**) (Figure 1). The results are summarized in Tables 1–3. Overall, there was good correlation between cytotoxicity and inhibition of *in vitro* tubulin polymerization. Eight of nine strongly cytotoxic compounds (**7**, **9**, **13**, **16**, **22**, **23**, **36**, and **37**) demonstrated the most potent inhibitory effects (IC_{50} values less than 1.0 μM) on tubulin polymerization, and these were similar to the inhibitory effects of colchicine, podophyllotoxin, combretastatin A-4, and compound **50**. The equally cytotoxic compound **33**

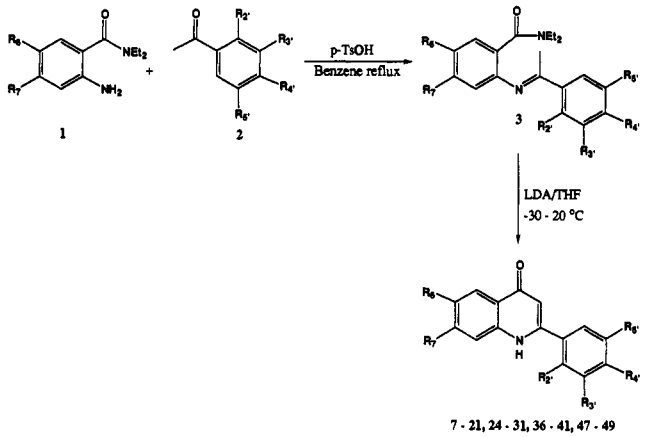
Table 3. Physical Properties and Antitubulin Effects of 2′,3′-Fused 6,7-(Methylenedioxy)-2-phenyl-4-quinolones



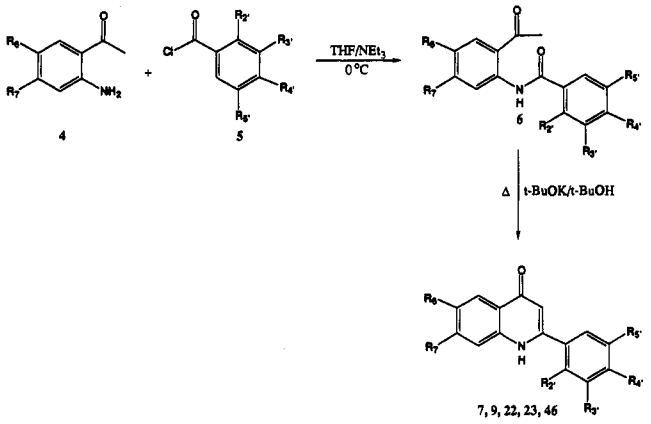
compd	*n*	R	ITP[a] IC_{50} (μM) ± SD	formula[b]	mp (°C)	method	yield (%)
47	2	H	>40	$C_{17}H_{13}NO_4 \cdot 0.25H_2O$	>300	A	12
48	2	OMe	>400	$C_{19}H_{15}NO_4$	202–204[c]	A	18
49	3	H	>40	$C_{19}H_{15}NO_3$	>300[c]	A	14

[a] ITP = inhibition of tubulin polymerization. [b] All compounds were analyzed for C, H, and N, and the results agreed to ±0.4% of the theoretical values. [c] Decomposed.

Scheme 1



Scheme 2



was almost 6–10-fold less active as an inhibitor of tubulin polymerization (IC_{50} value of 5.6 μM). Compounds **14**, **26**, **30**, and **40**, which were moderately cytotoxic, also exhibited strong inhibitory effects (IC_{50} values of 1.0–2.0 μM) in the polymerization assay. In contrast, the moderately cytotoxic compounds **18** and **29** showed reduced inhibitory effects, and compounds **19**, **24**, and **41**, which were weakly cytotoxic, demonstrated relatively strong inhibitory activity on tubulin polymerization (IC_{50} values of 1.0–2.0 μM). The strongest inhibitors of polymerization (IC_{50} values ≤1.0 μM) were also evaluated as inhibitors of the binding of radiolabeled colchicine to tubulin (Table 1). Previously[14] we had found compound **50** to be a weak inhibitor of this reaction, with significant inhibition only occurring when the phenylquinolone was present in large molar excess over colchicine. In the experiments summarized in Table 1, the potential inhibitor and colchicine were present in equimolar concentrations. Most of the potent inhibitors of polymerization were more active than compound **50** as inhibitors of colchicine binding, but none approached the potency of podophyllotoxin or combretastatin A-4. This could indicate a relatively slow binding and/or rapid dissociation reaction in the interaction of this group of agents with tubulin relative to the binding and dissociation reactions of colchicine with tubulin.

Colchicine, podophyllotoxin, combretastatin A-4, and steganacin all contain a trimethoxybenzene ring, while cornigerine, combretastatin A-2, podophyllotoxin, and steganacin all contain a (methylenedioxy) benzene ring. While it has been proposed that the binding sites for podophyllotoxin and colchicine only partially overlap, with the podophyllotoxin E ring and the colchicine A ring sharing a binding domain,[29,30] the structural variety of the natural products now known to bind strongly in the colchicine site makes analogous features of active molecules uncertain. The known interaction of the phenylquinolone derivatives at the colchicine site,[14] their homology to styrylquinazolinone derivatives, another class of agents known to bind in the colchicine site,[31] and their possession of two aromatic domains, a common feature of colchicine-site drugs,[12,26] led us to prepare many of the compounds whose synthesis is described here. We hoped not only to obtain agents with promising antineoplastic activity but also to gain insight into features required for interaction at the colchicine site and to define structural homologies in this class of drug. Therefore, much of our effort was concentrated on molecules with different patterns of methoxy substitution.

Our earlier study[14] showed that the unsubstituted compound **51** (Figure 1) was 3-fold less active as an inhibitor of polymerization than compound **50** with a methoxy substituent at the 6-position. A methylenedioxy bridge spanning the 6,7-positions is probably equivalent to a methoxy group at the 6-position, as compounds **7** and **50**, **9** and **37**, and **13** and **36** were equipotent. In contrast, two methoxy groups at the 6,7-positions led to a substantial loss of activity (compounds **28** vs **7** and **50**, **29** vs **13** and **36**, and **30** vs **9** and **37**). This can also be seen by comparison of the 6,7-dimethoxy-substituted compounds (**29** and **30**) with the corresponding unsubstituted compounds (**24** and **26**). However, the 6,7-unsubstituted compounds (**24** and **26**) were about 2-fold less active than compounds with either a methoxy at the 6-position (compounds **36** and **37**) or a methylenedioxy at the 6,7-positions (compounds **13** and **9**). Three sets of compounds (**7**, **9**, and **13**; **28**, **29**, and **30**; and **36**, **37**, and **50**) showed that compounds with a methoxy or an *N*,*N*-dimethylamino substituent at the *meta* position of ring C were almost equipotent to the corresponding unsubstituted compounds, except compound **30**, which was about 4-fold more active as an inhibitor of polymerization than the corresponding unsubstituted **28**.

Compound **7** with no substituent in the C ring showed

Table 4. Inhibition of *in Vitro* Tumor Cell Growth[a] by 2′,3′,4′,5′,5,6,7-Substituted 2-Phenyl-4-quinolones and Related Compounds

	cytotoxicity log GI_{50} (μM)[b]								
compd	NCI-H226[c]	DMS114[c]	HCT-116[c]	KM20L2[c]	OVCAR-3[c]	RXF-393[c]	SK-Mel-5[c]	SF-268[c]	SF-295[c]
7	−6.50	−6.93	−7.15	−6.74	−6.79	−6.95	−7.45	−7.52	−6.72
8	−4.54	−[d]	–	–	–	−4.42	–	–	–
9	−6.70	−6.62	−6.76	−6.74	−7.34	−7.34	−7.43	−6.88	−7.04
10	−4.64	−4.60	−4.55	−4.73	−4.40	−4.82	−4.05	−4.19	−4.68
11	–	–	–	–	–	–	–	–	–
12	–	–	–	–	–	–	–	–	–
13	−6.50	−6.39	−6.43	−6.64	−6.61	−6.62	−6.71	−6.13	−6.62
14	−5.80	nt	−6.17	−5.85	−6.37	nt	−5.76	−5.66	−5.72
15	–	–	–	–	–	–	–	–	–
16	−6.66	−6.52	−6.50	−6.15	−6.71	−6.66	−6.51	−6.15	−6.59
17	−4.03	−4.58	−4.39	−4.37	–	−4.34	−4.37	−4.20	−4.34
18	−5.20	−5.30	−5.49	−5.16	−5.31	−5.52	−5.39	−5.08	−5.18
19	−4.40	−4.62	−4.42	−4.58	−4.71	−4.68	−4.60	−4.33	−4.53
20	–	−4.08	−4.13	–	–	−4.66	−4.03	−4.49	−4.49
21	–	4.53	–	–	–	–	–	–	−4.12
22	−6.35	−6.29	−7.22	nt	−7.09	nt	−7.68	−5.64	−7.26
23	−6.58	nt	−7.06	nt	−7.11	nt	−7.44	−6.16	nt
24	−4.53	−4.34	−4.23	−4.46	−4.38	−4.45	nt	−4.12	−4.55
25	–	–	–	–	–	–	nt	–	–
26	−5.55	−5.25	−5.24	−5.07	−5.26	−5.18	nt	−5.17	−5.64
27	–	–	–	–	–	–	nt	–	–
28[e]									
29	−5.73	−5.32	−5.30	−5.70	<−6.0	−5.65	nt	−5.28	−5.71
30	−4.67	−4.26	−4.23	−4.62	−4.47	−4.48	nt	−4.30	−4.39
31	–	nt	–	nt	–	−4.17	–	–	–
32	–	–	–	–	–	–	–	−4.44	nt
33	−6.68	−6.75	−6.68	−6.39	−6.52	−6.77	−6.54	−6.38	−6.61
34	–	–	–	–	–	–	–	–	–
35	–	–	–	–	–	–	–	–	–
36	−6.43	nt	−6.71	nt	−6.77	−6.92	−6.71	−6.22	−6.69
37	−6.46	nt	−6.81	nt	nt	−6.72	−6.93	−6.64	−6.82
38	−4.45	−4.49	−4.53	−4.70	−4.51	−4.80	−5.11	–	−4.47
39	−4.61	−4.43	−4.40	−4.67	−4.37	−4.60	−4.87	−4.38	−4.55
40	−5.20	−5.17	−5.32	−4.84	−5.10	−5.69	−5.87	−4.95	−5.49
41	–	–	–	–	–	–	–	–	−4.04
42	–	−4.28	−4.36	–	0	0	−4.35	−4.53	–
43	–	–	nt	–	–	–	−4.21	nt	–
44	−4.44	−4.65	−4.47	−4.45	–	−5.60	−4.55	−4.62	−4.29
45	−4.33	−4.86	−4.58	−4.40	−4.20	−4.47	−4.58	−4.63	−4.29
46	−5.54	nt	−5.55	nt	−5.65	−5.37	−5.66	−5.15	−5.52
47	–	–	–	–	–	–	–	–	–
48	–	–	–	–	–	–	–	–	–
49	–	nt	−4.55	nt	–	–	–	–	−4.71

[a] Data obtained from NCI's *in vitro* disease-oriented human tumor cells screen (see refs 23 and 24 for detail). [b] log concentrations which reduced cell growth to 50% of level at start of experiment. [c] NCI-H226, non-small-cell lung cancer cell line; DMS 114, small-cell lung cancer cell line; HCT-116 and KM20L2, colon cancer cell lines; OVCAR-3, ovarian cancer cell line; RXF-393, renal cancer cell line; SK-Mel-5, melonoma; SF-268 and SF-295, CNS tumor cell lines. [d] "–" means log GI_{50} is greater than −4. [e] Cytotoxicity assay is pending.

significant activity as an inhibitor of tubulin polymerization. In comparison, either one (compound **9**) or two (compound **16**) methoxy groups in the *meta* positions [C(3′) and C(5′)] yielded analogs with activity essentially identical with that of the unsubstituted **7**. Compound **10**, with an *ortho* methoxy substituent, had greatly reduced activity (IC_{50} value of 14 vs 0.6 μM), while compound **8**, with a *para* methoxy substituent, was inactive. The presence of this *para* methoxy group was routinely associated with inactivity, in compounds with either one (**15** and **17**) or two (**20** and **21**) additional methoxy groups in the C ring. This effect of the *para* methoxy group is probably steric in origin, for compounds with a 4′-methyl substituent (compound **14**) or a 3′,4′-methylenedioxy bridge (compound **19**) were effective inhibitors of polymerization, with only minor loss of activity relative to compound **7** (also compare the partially active compound **33**, with a 4′-hydroxy group, to the inactive **32**, with a 4′-methoxy group).

We can summarize our findings, based on these compounds prepared thus far, with methoxy substituents in the phenylquinolone class of agents as follows. In the A ring, a methoxy substituent at the 6-position or a 6,7-methylenedioxy bridge enhances activity, while an additional methoxy group at the 7-position reduces activity. In the C ring, *meta* methoxy substituent(s) have little effect, an *ortho* methoxy substituent reduces activity, and a *para* methoxy group eliminates activity. The latter effect is probably steric, at least in part, since derivatives with smaller substituents at the *para* position retain significant activity.

In contrast, in colchicine, reduction of activity occurs with demethylation of any of the three methoxy groups of the A ring, with the 1-methoxy group apparently the most important and the 3-methoxy substituent the least important.[32-34] In the C ring, reversal of the substituents at the 9- and 10-positions yields isocolchicine and results in nearly complete loss of activity. Similarly, while the phenyltropolone **52** (Figure 1) has inhibitory activity comparable to that of colchicine, removal of a single methoxy group leads to significant loss of activity (the 1-methoxy substituent most important, the 2-substituent least important).[35] With combretastatin A-4, its dihydro derivative, and analogous compounds, maximum activity also seems to require all three methoxy groups in the A ring and a *para* methoxy group in the B ring (reduced

activity with a *meta* methoxy, none with an *ortho* methoxy).[17,36,37]

The limited tolerance of the phenylquinolone for substitution on either the A ring or the C ring thus makes it hard to speculate with confidence on structural analogies to colchicine and combretastatin A-4. Perhaps, the active phenylquinolone derivatives, through either the A ring or the C ring, interact primarily with the region of the tubulin molecule that binds the C ring of colchicine and the B ring of combretastatin A-4. The active phenylquinolones may also have a limited interaction with the site on tubulin that interacts with the trimethoxybenzene ring of the natural products. Alternatively, the phenylquinolone derivatives may not interact in the region of tubulin that binds trimethoxybenzene rings. Either model would explain the weak inhibitory effects of phenylquinolone derivatives on the binding of radiolabeled colchicine to tubulin.

Like colchicine and cornigerine, the methylenedioxy-substituted phenylquinolones (**7** and **9**) are more potent than the corresponding dimethoxy-substituted compounds (**26** and **24**) in terms of cytotoxicity and inhibition of tubulin polymerization. The reason for this difference is unknown. A difference in either the size of the binding pocket for the two moieties or the desolvation energy of the two molecules could play a role. Compounds with two vicinal methoxy groups should be slightly more hydrophobic and, accordingly, less likely to stay in an aqueous solution than the corresponding methylenedioxy-substituted compounds. Therefore, dimethoxy-substituted compounds would require less desolvation energy than their corresponding methylenedioxy-substituted compounds when moving from the aqueous environment to the tubulin-binding site. Which factor is dominant is not clear.

The lack of effect of methoxy substituents at the *meta* positions in the C ring of the phenylquinolone derivatives extended to the still bulkier dimethylamino group (compound **13**). The poor activity of compound **10**, with the *ortho* methoxy group, cannot be attributed solely to steric factors, for analogs with either a fluoro (compound **22**) or chloro (compound **23**) substituent at the 2′-position were highly active. Compounds **13**, **22**, and **23** all had activity as inhibitors of tubulin polymerization and colchicine binding comparable to that of compound **7**.

Generally, when a heterocyclic C ring replaced the phenyl C ring (compounds **38–45**), antitubulin activity was minimal. Exceptions were observed when the phenyl C ring of compound **7** (IC_{50} value of 0.63 μM) was replaced with either a thienyl or indolyl group (compounds **40** and **41**, with IC_{50} values of 2.2 and 1.3 μM, respectively). Three compounds in which the 2,3′-positions were linked by a 2- (compounds **47** and **48**) or 3-carbon (compound **49**) bridge were inactive.

Finally, we wished to gain insight into possible relationships between the styrylquinazolinone agents[31,38] and the phenylquinolone derivatives. The two classes differ by one atom in the B ring (the former class has an additional nitrogen at the 3-position) and by the attachment of the C ring at the 2-position (a styryl bridge in the former class; direct attachment in the latter). In the quinazolinone class with a chloro substituent at the 6-position, Jiang et al.[38] reported that derivatives with ethynyl and methylene bridges attaching the phenyl ring to the 2-position were inactive and that reduction of the styryl bridge resulted in a 3-fold loss in activity. They did not examine a compound in which the phenyl C ring was directly attached to the B ring.

Therefore, we prepared compound **46**, with a styryl bridge linking the phenyl C ring to the 2-position of the B ring of the quinolone moiety. Compound **46** had limited cytotoxicity, but it had significant activity as an inhibitor of tubulin polymerization (IC_{50} value of 2.3 μM) and was about 4-fold less active than compound **7**, with the C ring attached directly at the 2-position. This finding, together with the earlier results of Jiang et al.,[38] suggests that analogs with directly attached phenyl rings would have maximum activity in both series of compounds. Substituent patterns in different locations of either series could, however, alter this conclusion (cf. ref 26).

In summary, we have synthesized a series of novel 2′,3′,4′,5′,5,6,7-substituted 2-phenyl-4-quinolones and related compounds. Compounds **7**, **9**, and **13**, **14**, **16**, **22**, **23**, **33**, **36**, and **37** showed potent inhibitory effects on *in vitro* tubulin polymerization as well as on the growth of a variety of human tumor cell lines, especially colon, CNS, and small-cell lung cancer cell lines. Compound **23** also exhibited strong cytotoxicity against several breast cancer cell lines (the only compound of the 2-phenylquinolone series tested in this panel of cell lines). In general, there was good correlation between the results of the two assays. Within this data set, we found that the methylenedioxy-substituted compounds were more potent than their corresponding dimethoxy-substituted or unsubstituted compounds. Relatively bulky substitution at the 4′-position resulted in loss of activity. We speculate that this is due to disturbance of the local binding site by the 4′-substituent. Substituents at the 3′-position did not affect activity. On the basis of these preliminary structure–activity relationship studies, we speculate that substitution at the 3′-position could result in more potent compounds, particularly in terms of enhanced cytotoxicity, tumor-type specificity, or solubility. Studies on additional substitutions at this position are in progress.

Experimental Section

Chemistry. Melting points were determined on a Fisher-John melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlabs, Atlantic, GA; ^{1}H NMR spectra were measured at 300 MHz on a Bruker 300 spectrometer and recorded in $CDCl_3$, a mixture of $CDCl_3$ and CD_3OD, or DMSO-d_6. Chemical shifts are reported in δ (ppm) units relative to the internal reference Me_4Si. Infrared (IR) spectra were recorded on a Perkin-Elmer IR 400 spectrometer as KBr pellets. Mass spectra (MS) data were obtained on a TRIO 1000 mass spectrometer. Flash chromatography was performed on silica gel (mesh 25–150 μm) using a mixture of $CHCl_3$ and MeOH as eluant.

Procedure for Method A. Preparation of Ketimines 3. A solution of 2-amino-4,5-(methylenedioxy)-*N*,*N*-diethylbenzoylamide or 2-amino-*N*,*N*-diethylbenzoylamide (2.5 mmol), methyl aryl ketone (2.5 mmol), and *p*-toluenesulfonic acid (5 mg) was heated under reflux for 48–72 h with azeotropic removal of water. The mixture was concentrated on a rotary evaporator and the oily residue dissolved in 10 mL of THF and used directly in the next step.

Preparation of 2-Phenyl-4-quinolones 7-21, 24-31, 36–41, and 47–49. Lithium diisopropylamide (LDA) was used either as a commercially available 2.5 M solution in hexane (Aldrich) or prepared as follows. A solution of diisopropylamine (2.51 mmol) in 20 mL of THF was treated with a 2.5 M solution of *n*-butyllithium (2.5 mL, 2.5 mmol) in hexane at −60 °C, and the resulting mixture was stirred at −30 °C for 20 min. The ketimine solution was added dropwise, and the mixture was stirred at −30

°C for 30 min. The temperature of the reaction was raised to room temperature over 1 h, and the orange-colored mixture was stirred overnight. MeOH (10 mL) was added, the solvent was evaporated, and the residue was purified by flash chromatography using a mixture of $CHCl_3$ and MeOH (30:1–20:1) as eluant.

6,7-(Methylenedioxy)-2-phenyl-4-quinolone (7): obtained from 2-amino-4,5-(methylenedioxy)-*N,N*-diethylbenzoylamide and acetophenone; amorphous; ^{1}H NMR ($CDCl_3$ and CD_3OD) δ 6.13 (s, 2 H, OCH_2O), 6.57 (s, 1 H, H-3), 7.15 (s, 1 H, H-8), 7.59 (s, 1 H, H-5), 7.56 (m, 3 H, C ring Ar*H*), 7.74 (m, 2 H, C ring Ar*H*); IR (KBr) 3100, 1610 cm^{-1}; MS *m/z* 265 (M^+). Anal. C, H, N.

4′-Methoxy-6,7-(methylenedioxy)-2-phenyl-4-quinolone (8): obtained from 2-amino-4,5-(methylenedioxy)-*N,N*-diethylbenzoylamide and 4-methoxyacetophenone; amorphous; ^{1}H NMR (DMSO-d_6) δ 3.85 (s, 3 H, OCH_3), 6.15 (s, 2 H, OCH_2O), 6.23 (s, 1 H, H-3), 7.13 (d, *J* = 8.6 Hz, 2 H, H-3′, H-5′), 7.20 (s, 1 H, H-8), 7.39 (s, 1 H, H-5), 7.76 (d, *J* = 8.6 Hz, 2 H, H-2′, H-6′); IR (KBr) 3060, 1610 cm^{-1}. Anal. C, H, N.

3′-Methoxy-6,7-(methylenedioxy)-2-phenyl-4-quinolone (9): obtained from 2-amino-4,5-(methylenedioxy)-*N,N*-diethylbenzoylamide and 3-methoxyacetophenone; needles; ^{1}H NMR ($CDCl_3$ and CD_3OD) δ 3.87 (s, 3 H, OCH_3), 6.16 (s, 2 H, OCH_2O), 6.29 (s, 1 H, H-3), 7.13 (dd, *J* = 1.8, 8.0 Hz, 1 H, H-4′), 7.20 (s, 1 H, H-8), 7.32 (d, *J* = 1.8, 1 H, H-2′), 7.35 (d, *J* = 8.0 Hz, 1 H, H-6′), 7.40 (s, 1 H, H-5), 7.50 (t, *J* = 8.0 Hz, 1 H, H-5′); IR (KBr) 3100, 1610 cm^{-1}; MS *m/z* 295 (M^+). Anal. C. H, N.

2′-Methoxy-6,7-(methylenedioxy)-2-phenyl-4-quinolone (10): obtained from 2-amino-4,5-(methylenedioxy)-*N,N*-diethylbenzoylamide and 2-methoxyacetophenone; amorphous; ^{1}H NMR (DMSO-d_6) δ 3.82 (s, 3 H, OCH_3), 6.00 (d, *J* = 1.4 Hz, 1 H, H-3), 6.15 (s, 2 H, OCH_2O), 7.06 (s, 1 H, H-8), 7.11 (t, *J* = 7.5 Hz, 1 H, H-5′), 7.21 (d, *J* = 7.5 Hz, 1 H, H-3′), 7.40 (s, 1 H, H-5), 7.45 (d, *J* = 7.5 Hz, 1 H, H-6′), 7.53 (t, *J* = 7.5 Hz, 1 H, H-4′); IR (KBr) 3060, 1610 cm^{-1}. Anal. C, H, N.

4′-Phenoxy-6,7-(methylenedioxy)-2-phenyl-4-quinolone (11): obtained from 2-amino-4,5-(methylenedioxy)-*N,N*-diethylbenzoylamide and 4-phenoxyacetophenone; amorphous; ^{1}H NMR (DMSO-d_6) δ 6.16 (s, 2 H, OCH_2O), 6.25 (s, 1 H, H-3), 7.19 (s, H, H-8), 7.40 (s, H, H-5), 7.45 (d, *J* = 8.6 Hz, 2 H, H-3′, H-5′), 7.82 (d, *J* = 8.6 Hz, 2 H, H-2′, H-6′), 7.11–717 (m, 3 H, 4′-phenyl ring Ar*H*), 7.23, 7.47 (t, *J* = 7.5 Hz, 1 H each, 2 H, 4′-phenyl ring Ar*H*); IR (KBr) 3100, 1610 cm^{-1}. Anal. C, H, N.

4′-(*N,N*-Dimethylamino)-6,7-(methylenedioxy)-2-phenyl-4-quinolone (12): obtained from 2-amino-4,5-(methylenedioxy)-*N,N*-diethylbenzoylamide and 4-(*N,N*-dimethylamino)acetophenone; needles; ^{1}H NMR (DMSO-d_6) δ 2.50, 2.51 (both s, each 3 H, $N(CH_3)_2$), 6.14 (s, 2 H, OCH_2O), 6.20 (s, 1 H, H-3), 6.85 (d, *J* = 8.7 Hz, 2 H, H-3′, H-5′), 7.21 (s, 1 H, H-8), 7.37 (s, 1 H, H-5), 7.66 (d, *J* = 8.7 Hz, 2 H, H-2′, H-6′), 11.29 (s, 1 H, NH); IR (KBr) 3100, 1610, 1520 cm^{-1}; MS *m/z* 308 (M^+). Anal. C, H, N.

3′-(*N,N*-Dimethylamino)-6,7-(methylenedioxy)-2-phenyl-4-quinolone (13): obtained from 2-amino-4,5-(methylenedioxy)-*N,N*-diethylbenzoylamide and 3-(*N,N*-dimethylamino)acetophenone; needles; ^{1}H NMR ($CDCl_3$ and CD_3OD) δ 3.05 (s, 6 H, $N(CH_3)_2$), 6.12 (s, 2 H, OCH_2O), 6.55 (s, 1 H, H-3), 6.92 (dd, *J* = 1.6, 8.0 Hz, 1 H, H-4′), 7.00 (d, *J* = 1.6 Hz, 1 H, H-2′), 7.03 (d, *J* = 8.0 Hz, 1 H, H-6′), 7.15 (s, 1 H, H-8), 7.37 (t, *J* = 8.0 Hz, 1 H, H-5′), 7.58 (s, 1 H, H-5); IR (KBr) 3080, 1600, 1500 cm^{-1}. Anal. C, H, N.

4′-Methyl-6,7-(methylenedioxy)-2-phenyl-4-quinolone (14): obtained from 2-amino-4,5-(methylenedioxy)-*N,N*-diethylbenzoylamide and 4-methylacetophenone; amorphous; ^{1}H NMR (DMSO-d_6) δ 2.40 (s, 3 H, CH_3), 6.15 (s, 2 H, OCH_2O), 6.24 (d, *J* = 1.7 Hz, 1 H, H-3), 7.20 (s, 1 H, H-8), 7.38 (d, *J* = 7.8 Hz, 2 H, H-3′, H-5′), 7.39 (s, 1 H, H-5), 7.69 (d, *J* = 7.8 Hz, 2 H, H-2′, H-6′); IR (KBr) 3060, 1610 cm^{-1}; MS *m/z* 279 (M^+). Anal. C, H, N.

3′,4′-Dimethoxy-6,7-(methylenedioxy)-2-phenyl-4-quinolone (15): obtained from 2-amino-4,5-(methylenedioxy)-*N,N*-diethylbenzoylamide and 3,4-dimethoxyacetophenone; amorphous; ^{1}H NMR(DMSO-d_6) δ 3.84, 3.88 (both s, 3 H each, OCH_3 × 2), 6.15 (s, 2 H, OCH_2O), 6.30 (s, 1 H, H-3), 7.14 (d, *J* = 8.3 Hz, 1 H, H-5′), 7.20 (s, 1 H, H-8), 7.32 (d, *J* = 1.8 Hz, 1 H, H-2′), 7.36 (dd, *J* = 1.8, 8.3 Hz, 1 H, H-6′), 7.40 (s, 1 H, H-5), 11.52 (br s, 1 H, -NH); IR (KBr) 3100, 1610 cm^{-1}. Anal. C, H, N.

3′,5′-Dimethoxy-6,7-(methylenedioxy)-2-phenyl-4-quinolone (16): obtained from 2-amino-4,5-(methylenedioxy)-*N,N*-diethylbenzoylamide and 3,5-dimethoxyacetophenone; amorphous; ^{1}H NMR (DMSO-d_6) δ 3.85 (s, 6 H, OCH_3 × 2), 6.16 (s, 2 H, OCH_2O), 6.31 (s, 1 H, H-3), 6.69 (s, 1 H, H-4′), 6.91 (s, 2 H, H-2′, H-6′), 7.20 (s, 1 H, H-8), 7.40 (s, 1 H, H-5); IR (KBr) 3100, 1590 cm^{-1}. Anal. C, H, N.

2′,4′-Dimethoxy-6,7-(methylenedioxy)-2-phenyl-4-quinolone (17): obtained from 2-amino-4,5-(methylenedioxy)-*N,N*-diethylbenzoylamide and 2,4-dimethoxyacetophenone; amorphous; ^{1}H NMR (DMSO-d_6) δ 3.80, 3.83 (both s, 3 H each, OCH_3 × 2), 5.97 (s, 1 H, H-3), 6.12, (s, 2 H, OCH_2O), 6.67 (dd, *J* = 1.5, 7.5 Hz, 1H, H-5′), 6.72 (d, *J* = 1.5 Hz, 1 H, H-3′), 7.05 (s, 1 H, H-8), 7.36 (s, 1 H, H-5), 7.38 (d, *J* = 7.5 Hz, 1 H, H-6′); IR (KBr) 3100, 1610 cm^{-1}. Anal. C, H, N.

2′,5′-Dimethoxy-6,7-(methylenedioxy)-2-phenyl-4-quinolone (18): obtained from 2-amino-4,5-(methylenedioxy)-*N,N*-diethylbenzoylamide and 2,5-dimethoxyaceteophenone; amorphous; ^{1}H NMR (DMSO-d_6) δ 3.77, 3.78 (both s, 3 H each, OCH_3 × 2), 6.02 (s, 1 H, H-3), 6.15 (s, 2 H, OCH_2O), 7.03, 7.09 (both d, *J* = 3.1 Hz, 1 H each, H-3′, H-4′), 7.06 (s, 1 H, H-8), 7.13 (br s, 1 H, H-6′), 7.40 (s, 1 H, H-5), 11.56 (br s, 1 H, NH); IR (KBr) 3100, 1600 cm^{-1}. Anal. C, H, N.

3′,4′:6,7-Bis(methylenedioxy)-2-phenyl-4-quinolone (19): obtained from 2-amino-4,5-(methylenedioxy)-*N,N*-diethylbenzoylamide and 3,4-(methylenedioxy)acetophenone; amorphous; ^{1}H NMR (DMSO-d_6) δ 6.14 (s, 4 H, OCH_2O × 2), 6.22 (s, 1 H, H-3), 7.11 (d, *J* = 8.1 Hz, 1 H, H-5′), 7.18 (s, 1 H, H-8), 7.32 (d, *J* = 8.1 Hz, 1 H, H-6′), 7.38 (s, 2 H, H-5, H-2′), 11.42 (br s, 1 H, NH); IR (KBr) 3160, 1610 cm^{-1}; MS *m/z* 309 (M^+). Anal. C, H, N.

3′,4′,5′-Trimethoxy-6,7-(methylenedioxy)-2-phenyl-4-quinolone (20): obtained from 2-amino-4,5-(methylenedioxy)-*N,N*-diethylbenzoylamide and 3,4,5-trimethoxyacetophenone; amorphous; ^{1}H NMR (DMSO-d_6) δ 3.73 (s, 3 H, 4′-OCH_3), 3.90 (s, 6 H, 3′,5′-OCH_3 × 2), 6.16 (s, 2 H, OCH_2O), 6.33 (s, 1 H, H-3), 7.05 (s, 2 H, H-2′, H-6′), 7.17 (s, 1 H, H-8), 7.40 (s, 1 H, H-5), 11.45 (br s, 1 H, NH); IR (KBr) 3040, 1610 cm^{-1}; MS *m/z* 355 (M^+). Anal. C, H, N.

2′,3′,4′-Trimethoxy-6,7-(methylenedioxy)-2-phenyl-4-quinolone (21): obtained from 2-amino-4,5-(methylenedioxy)-*N,N*-diethylbenzoylamide and 2,3,4-trimethoxyacetophenone; amorphous; ^{1}H NMR (DMSO-d_6) δ 3.70, 3.82, 3.87 (all s, each 3 H, OCH_3 × 3), 5.99 (s, 1 H, H-3), 6.14 (s, 2 H, OCH_2O), 6.96 (d, *J* = 8.7 Hz, 1 H, H-5′), 7.08 (s, 1 H, H-8), 7.19 (d, *J* = 8.7 Hz, 1 H, H-6′), 7.39 (s, 1 H, H-5); IR (KBr) 3080, 1610 cm^{-1}. Anal. C, H, N.

3′-(*N,N*-Dimethylamino)-2-phenyl-4-quinolone (24): obtained from 2-amino-*N,N*-diethylbenzoylamide and 4-(*N,N*-dimethylamino)acetophenone; needles; ^{1}H NMR (DMSO-d_6) δ 2.98 (s, 6 H, $N(CH_3)_2$), 6.34 (s, 1 H, H-3), 6.91 (dd, *J* = 2.0, 7.8 Hz, 1 H, H-4′), 7.02 (br s, 1 H, H-2′), 7.04 (d, *J* = 7.8 Hz, 1 H, H-6′), 7.35 (t, *J* = 3.5 Hz, 1 H, H-6), 7.37 (t, *J* = 7.8 Hz, 1 H, H-5′), 7.68 (t, *J* = 7.5 Hz, 1 H, H-7), 7.87 (d, *J* = 7.5 Hz, 1 H, H-8), 8.10 (d, *J* = 7.5 Hz, 1 H, H-5); IR (KBr) 3100, 1610 cm^{-1}; MS *m/z* 264 (M^+). Anal. C, H, N.

4′-(*N,N*-Dimethylamino)-2-phenyl-4-quinolone (25): obtained from 2-amino-*N,N*-diethylbenzoylamide and 4-(*N,N*-dimethylamino)acetophenone; needles; ^{1}H NMR (DMSO-d_6) δ 3.01 (s, 6 H, $N(CH_3)_2$), 6.29 (s, 1 H, H-3), 6.86 (d, *J* = 8.8 Hz, 2 H, H-3′, H-5′), 7.29 (t, *J* = 7.5 Hz, 1 H, H-6), 7.63 (t, *J* = 7.5 Hz, 1 H, H-7), 7.71 (d, *J* = 8.8 Hz, 2 H, H-2′, H-6′), 7.76 (d, *J* = 7.5 Hz, 1 H, H-8), 8.06 (d, *J* = 7.5 Hz, 1 H, H-5); IR (KBr) 3280, 3080, 1600 cm^{-1}. Anal. C, H, N.

3′-Methoxy-2-phenyl-4-quinolone (26): obtained from 2-amino-*N,N*-diethylbenzoylamide and 3-methoxyacetophenone; amorphous; ^{1}H NMR (DMSO-d_6) δ 3.87 (s, 3 H, OCH_3), 6.36 (d, *J* = 1.4 Hz, 1 H, H-3), 7.15 (dd, *J* = 1.8, 8.0 Hz, 1 H, H-4′), 7.34 (dt, *J* = 1.0, 8.0 Hz, 1 H, H-6), 7.37 (d, *J* = 1.8 Hz, 1 H, H-2′), 7.39 (d, *J* = 8.0 Hz, 1 H, H-6′), 7.51 (t, *J* = 8.0 Hz, 1 H, H-5′), 7.67 (dt, *J* = 1.0, 8.0 Hz, H-7), 7.76 (d, *J* = 8.0 Hz, 1 H, H-8), 8.10 (dd, *J* = 1.0, 8.0 Hz, 1 H, H-5); IR (KBr) 3080, 1620 cm^{-1}. Anal. C, H, N.

3′,4′,5′-Trimethoxy-2-phenyl-4-quinolone (27): obtained from 2-amino-*N,N*-diethylbenzoylamide and 3,4,5-trimethoxyacetophenone; amorphous; ^{1}H NMR (DMSO-d_6) δ 3.74 (s, 3 H,

OCH_3), 3.91 (s, 6 H, $OCH_3 \times 2$), 6.42 (s, 1 H, H-3), 7.11 (s, 2 H, H-2′, H-6′), 7.34 (t, J = 7.5 Hz, 1 H, H-6), 7.65 (d, J = 7.5 Hz, 1 H, H-8), 7.68 (t, J = 7.5 Hz, 1 H, H-7), 8.10 (d, J = 7.5 Hz, 1 H, H-5); IR (KBr) 3060, 1620 cm^{-1}. Anal. C, H, N.

6,7-Dimethoxy-2-phenyl-4-quinolone (28): obtained from 2-amino-4,5-dimethoxy-*N*,*N*-diethylbenzoylamide and benzoyl chloride; amorphous; ^1H NMR ($CDCl_3$) δ 3.54, 3.56 (s, 3 H each, $OCH_3 \times 2$), 6.05 (s, 1 H, H-3), 6.88 (s, 1 H, H-8), 7.11 (m, 3 H, C ring), 7.21 (s, 1 H, H-5), 7.34 (m, 2 H, C ring Ar*H*); IR (KBr) 3160, 1630 cm^{-1}. Anal. C, H, N.

3′-(*N*,*N*-Dimethylamino)-6,7-dimethoxy-2-phenyl-4-quinolone (29): obtained from 2-amino-4,5-dimethoxy-*N*,*N*-diethylbenzoylamide and 3-(*N*,*N*-dimethylamino)acetophenone; amorphous; ^1H NMR (DMSO-d_6) δ 3.38 (s, 6 H, $N(CH_3)_2$), 4.06, 4.07 (both s, 3 H each, $OCH_3 \times 2$), 7.47 (s, 1 H, H-3), 7.54 (s, 1 H, H-8), 7.57 (s, 1 H, H-5), 7.75 (br, d, J = 8.0 Hz, H-4′), 7.81 (t, J = 8.0 Hz, H-5′), 8.06 (br, d, J = 8.0 Hz, H-6′), 8.49 (br s, 1 H, H-2′); IR (KBr) 3080, 2800, 1580 cm^{-1}. Anal. C, H, N.

3′,6,7-Trimethoxy-2-phenyl-4-quinolone (30): obtained from 2-amino-4,5-dimethoxy-*N*,*N*-diethylbenzoylamide and 3-methoxyacetophenone; amorphous; ^1H NMR (DMSO-d_6) δ 3.85, 3.87, 3.88 (all s, 3 H each, $OCH_3 \times 3$), 6.31 (s, 1 H, H-3), 7.13 (br, d, J = 7.5 Hz, H-4′), 7.26 (s, 1 H, H-8), 7.35 (br, s, 1 H, H-2′), 7.37 (d, J = 7.5 Hz, H-6′), 7.45 (s, 1 H, H-5), 7.49 (t, J = 7.5 Hz, H-5′); IR (KBr) 3100, 1600 cm^{-1}. Anal. C, H, N.

3′,4′,6,7-Tetramethoxy-2-phenyl-4-quinolone (31): obtained from 2-amino-4,5-dimethoxy-*N*,*N*-diethylbenzoylamide and 3,4-dimethoxyacetophenone; amorphous; ^1H NMR (DMSO-d_6) δ 3.85, 3.89 (both s, 6 H each, $OCH_3 \times 4$), 6.31 (s, 1 H, H-3), 7.14 (d, J = 8.5 Hz, 1 H, H-5′), 7.25 (s, 1 H, H-8), 7.36 (br s, 1 H, H-2′), 7.39 (br d, J = 8.5 Hz, 1 H, H-6′), 7.49 (s, 1 H, H-5). Anal. C, H, N.

3′-(*N*,*N*-Dimethylamino)-6-methoxy-2-phenyl-4-quinolone (36): obtained from 2-amino-5-methoxy-*N*,*N*-diethylbenzoylamide and 3-(*N*,*N*-dimethylamino)acetophenone; amorphous; ^1H NMR (DMSO-d_6) δ 3.0 (s, 6 H, $N(CH_3)_2$), 3.85 (s, 3 H, OCH_3), 6.30 (s, 1 H, H-3), 6.91 (dd, J = 2.75 Hz, 1 H, H-4′), 7.03 (br s, 1 H, H-2′), 7.04 (br d, J = 7.5 Hz, 1 H, H-6′), 7.32 (dd, J = 3.9 Hz, 1 H, H-7), 7.37 (t, J = 7.5 Hz, 1 H, H-5′), 7.51 (d, J = 3 Hz, 1 H, H-5), 7.72 (d, J = 9 Hz, 1 H, H-8), 11.62 (br s, 1 H, NH); IR (KBr) 3100, 1600 cm^{-1}; MS m/z 294 (M^+). Anal. C, H, N.

3′,6-Dimethoxy-2-phenyl-4-quinolone (37): obtained from 2-acetyl-3-methoxyaniline and 3′-methoxybenzoyl chloride; amorphous; ^1H NMR (DMSO-d_6) δ 3.85, 3.87 (both s, 3 H, each, $OCH_3 \times 2$), 6.34 (d, J = 1.4 Hz, 1 H, H-3), 7.14 (dd, J = 2.0, 8.0, Hz, 1 H, H-4′), 7.32 (dd, J = 2.9, 9.0 Hz, 1 H, H-7), 7.35 (d, J = 2.0 Hz, 1 H, H-2′), 7.38 (br d, J = 8.0 Hz, 1 H, H-6′), 7.49 (t, J = 2.9 Hz, 1H, H-5), 7.50 (d, J = 2.9 Hz, 1 H, H-5), 7.73 (d, J = 9.0 Hz, 1 H, H-8); IR (KBr) 3100, 1600 cm^{-1}. Anal. C, H, N.

2-(2-Pyrrolyl)-6,7-(methylenedioxy)-2-phenyl-4-quinolone (38): obtained from 2-amino-4,5-(methylenedioxy)-*N*,*N*-diethylbenzoylamide and 2-acetylpyrrole; amorphous; ^1H NMR (DMSO-d_6) δ 6.13 (s, 2 H, OCH_2O), 6.26 (dd, J = 2.1, 3.5 Hz, 1 H, H-4′), 6.37 (s, 1 H, H-3), 6.94 (br s, 1 H, H-3′), 7.05 (br s, 1 H, H-5′), 7.15 (s, 1 H, H-8), 7.35 (s, 1 H, H-5), 11.09, 11.65 (both s, 1 H each NH $\times$ 2); IR (KBr) 3310, 3080, 1620 cm^{-1}. Anal. C, H, N.

2-(2-Furyl)-6,7-(methylenedioxy)-2-phenyl-4-quinolone (39): obtained from 2-amino-4,5-(methylenedioxy)-*N*,*N*-diethylbenzoylamide and 2-acetylfuran; amorphous; ^1H NMR ($CDCl_3$) δ 6.20 (s, 2 H, OCH_2O), 6.51 (br s, 1 H, H-4′), 6.96 (s, 1 H, H-3), 7.12 (s, 1 H, H-8), 7.13 (d, J = 3.5 Hz, 1 H, H-3′), 7.29 (s, 1 H, H-5), 7.54 (br s, 1 H, H-5′), 12.81 (br s, 1 H, NH); IR (KBr) 3100, 1620 cm^{-1}. Anal. C, H, N.

2-(2-Thienyl)-6,7-(methylenedioxy)-2-phenyl-4-quinolone (40): obtained from 2-amino-4,5-(methylenedioxy)-*N*,*N*-diethylbenzoylamide and 2-acetylthiophene; amorphous; ^1H NMR ($CDCl_3$ and CD_3OD) δ 6.12 (s, 2 H, OCH_2O), 6.60 (s, 1 H, H-3), 7.15 (s, 1 H, H-8), 7.21 (dd, J = 3.5, 5.0 Hz, 1 H, H-4′), 7.57 (s, 1 H, H-5), 7.59 (d, J = 5.0 Hz, 1 H, H-5′), 7.73 (d, J = 3.5 Hz, 1 H, H-3′); IR (KBr) 3060, 1600 cm^{-1}; MS m/z 271 (M^+). Anal. C, H, N, S.

2-(3-Indolyl)-6,7-(methylenedioxy)-2-phenyl-4-quinolone (41): obtained from 2-amino-4,5-(methylenedioxy)-*N*,*N*-diethylbenzoylamide and 3-acetylindole; amorphous; ^1H NMR (DMSO-d_6) δ 6.14 (s, 2 H, OCH_2O), 6.31 (s, 1 H, H-3), 7.17 (s, 1 H, H-8), 7.19, 7.24 (both t, J = 7.5 Hz, 1 H each, H-5′, H-6′), 7.41 (s, 1 H, H-5), 7.53 (d, J = 7.5 Hz, H-7′), 7.85 (d, J = 7.5 Hz, 1 H, H-4′), 8.01 (d, J = 1.5 Hz, 1 H, H-2′); IR (KBr) 3400, 3100, 1610 cm^{-1}. Anal. C, H, N.

6,7-(Methylenedioxy)-2-styryl-4-quinolone (46): obtained from 2-amino-4,5-(methylenedioxy)-*N*,*N*-diethylbenzoylamide and cinnamoyl chloride; amorphous; ^1H NMR (DMSO-d_6) δ 6.15 (s, 2 H, OCH_2O), 6.27 (s, 1 H, H-3), 7.08 (d, J = 20.0 Hz, 1 H, H-b of vinyl), 7.37 (s, 1 H, H-8), 7.41 (t, J = 7.2 Hz, 1 H, H-4′), 7.46 (t, J = 7.2 Hz, 2 H, H-3′, H-5′), 7.56 (d, J = 20.0 Hz, 1 H, H-a of vinyl), 7.65 (d, J = 7.2 Hz, 2 H, H-2′, H-6′). Anal. C, H, N.

2′,3-Ethano-6,7-(methylenedioxy)-2-phenyl-4-quinolone (47): obtained from 2-amino-4,5-(methylenedioxy)-*N*,*N*-diethylbenzoylamide and α-tetralone; amorphous; ^1H NMR ($CDCl_3$) δ 2.82 (m, 4 H, CH_2CH_2), 5.89 (s, 2 H, OCH_2O), 7.26 (s, 1 H, H-8), 7.30 (s, 1 H, H-5), 7.31 (d, J = 7.5, H-3′), 7.39, 7.50 (both t, J = 7.5 Hz, 1 H each, H-4′, H-5′), 7.95 (d, J = 7.5 Hz, H-6′), 12.58 (s, 1 H, NH); IR (KBr) 3140, 1610 cm^{-1}; MS m/z 291 (M^+). Anal. C, H, N.

2′,3-Ethano-6,7-(methylenedioxy)-4′-methoxy-2-phenyl-4-quinolone (48): obtained from 2-amino-4,5-(methylenedioxy)-*N*,*N*-diethylbenzoylamide and 6-methoxytetralone; amorphous; ^1H NMR (DMSO-d_6) δ 2.70, 2.80 (both m, 2 H each, CH_2CH_2), 3.84 (s, 3 H, OCH_3), 6.13 (s, 2 H, OCH_2O), 6.98 (d, J = 2.0 Hz, 1 H, H-3′), 7.01 (dd, J = 2.0, 8.5 Hz, 1 H, H-5′), 7.25 (s, 1 H, H-8), 7.40 (s, 1 H, H-5), 7.92 (d, J = 8.5 Hz, 1 H, H-6′), 11.25 (br s, 1 H, NH); IR (KBr) 3040, 1610 cm^{-1}. Anal. C, H, N.

2′,3-Propano-6,7-(methylenedioxy)-2-phenyl-4-quinolone (49): obtained from 2-amino-4,5-(methylenedioxy)-*N*,*N*-diethylbenzoylamide and benzosuberone; amorphous; ^1H NMR ($CDCl_3$) δ 2.22 (t, J = 6.9 Hz, 2 H, $CH_2CH_2CH_2$), 2.60 (t, J = 6.9 Hz, 2 H, $CH_2CH_2CH_2$), 3.37 (m, 2 H, $CH_2CH_2CH_2$), 6.13 (s, 1 H, OCH_2O), 7.35 (dd, J = 1.0, 7.5 Hz,-3′), 7.37 (s, 1 H, H-8), 7.45 (dt, J = 7.5 Hz, 2 H, H-4′, H-5′), 7.59 (dd, J = 1.0, 7.5 Hz, H-6′), 7.69 (s, 1 H, H-5); IR (KBr) 3140, 1610 cm^{-1}. Anal. C, H, N.

Method B. Preparation of 2′-Fluoro-6,7-(methylenedioxy)-2-phenyl-4-quinolone (22). 2-Acetyl-4,5-(methylenedioxy)aniline (3.0 mmol) was dissolved in 20 mL of THF and 10 mL of triethylamine. The mixture was cooled in an ice bath. A solution of 2-fluorobenzoyl chloride (3.0 mmol) was added dropwise. After 30 min at 0 °C, the mixture was stirred at room temperature overnight and poured onto 50 mL of ice water. The precipitate was collected and washed successively with water (several times) and MeOH. The solid was dried in a vacuum and then suspended in 20 mL of *tert*-butyl alcohol. Potassium *tert*-butoxide (1.17g, 10.5 mmol) was added, and the mixture was heated under argon at 70 °C for 10–24 h. The mixture was cooled and poured onto 30 mL of aqueous ammonium chloride solution. The solid was collected and washed successively with water and a mixture of $CHCl_3$ and MeOH (1:10). The crude product was recrystallized from a mixture of $CHCl_3$ and MeOH (20:1) or from DMF: ^1H NMR (DMSO-d_6) δ 6.11 (s, 1 H, H-3), 6.17 (s, 2 H, OCH_2O), 7.09 (s, 1 H, H-8), 7.43 (s, 1 H, H-5), 7.44 (m, 2 H, H-3′, H-6′), 7.62, 7.69 (both t, J = 7.5 Hz, 1 H each, H-4′, H-5′); IR (KBr) 3440, 1622 cm^{-1}; MS m/z 283 (M^+). Anal. C, H, N.

2′-Chloro-6,7-(methylenedioxy)-2-phenyl-4-quinolone (23): obtained from 2-amino-4,5-(methylenedioxy)acetophenone and 2-chlorobenzoyl chloride; ^1H NMR (DMSO-d_6) δ 5.95 (s, 1 H, H-3), 6.16 (s, 2 H, OCH_2O), 7.02 (s, 1 H, H-8), 7.42 (s, 1 H, H-5), 7.60 (m, 4 H, H-3′,4′,5′,6′); IR (KBr) 3440, 1615 cm^{-1}. Anal. C, H, N.

Method C. Preparation of Quinolones 32–35 and 42–45. The following compounds were prepared according to the method reported in a previous paper.[14]

4′,6-Dimethoxy-2-phenyl-4-quinolone (32): obtained from *p*-methoxyaniline and ethyl 4′-methoxybenzoyl acetate; amorphous; ^1H NMR ($CDCl_3$ and CD_3OD) δ 3.90, 3.93 (both s, 3 H each, $OCH_3 \times 2$), 6.57 (s, 1 H, H-3), 7.06 (d, J = 8.5 Hz, 2 H, H-3′, H-5′), 7.29 (dd, J = 2.5, 9.0 Hz, 1 H, H-7), 7.63 (d, J = 9.0 Hz, 1 H, H-8), 7.69 (d, J = 2.5 Hz, 1 H, H-5), 7.70 (d, J = 8.5 Hz, 2 H, H-2′, H-6′); IR (KBr) 3450, 1608 cm^{-1}. Anal. C, H, N.

4′-Hydroxy-6-methoxy-2-phenyl-4-quinolone (33): obtained as a byproduct from the synthesis of 31; needles; ^1H NMR ($CDCl_3$ and CD_3OD) δ 3.64 (s, 3 H, OCH_3), 6.27 (s, 1 H, H-3), 6.67 (d, J = 8.5 Hz, 2 H, H-3′, H-5′), 7.00 (dd, J = 2.0, 9.0 Hz, 1 H,

H-7), 7.34 (d, J = 8.5 Hz, 2 H, H-2′, H-6′), 7.36 (d, J = 9.0 Hz, 1 H, H-8), 7.38 (d, J = 2.0 Hz, 1 H, H-5); IR (KBr) 3440, 1620 cm^{-1}. Anal. C, H, N.

4′-Methoxy-6-chloro-2-phenyl-4-quinolone (34): obtained from *p*-chloroaniline and ethyl 4′-methoxybenzoyl acetate; amorphous; ^{1}H NMR (DMSO-d_6) δ 3.35 (s, 3 H, OCH_3), 6.90 (d, J = 8.5 Hz, 2 H, H-3′, H-5′), 7.70 (dd, J = 2.0, 9.0 Hz, 1 H, H-7), 7.96 (s, 1 H, H-3), 7.98 (d, J = 9.0 Hz, 1 H, H-8), 8.06 (d, J = 2.0 Hz, 1 H, H-5), 8.12 (d, J = 8.5 Hz, 2 H, H-2′, H-6′), 9.89 (br s, 1 H, NH); IR (KBr) 3450, 1600 cm^{-1}. Anal. C, H, N.

4′-Methoxy-7-fluoro-2-phenyl-4-quinolone (35): obtained from *m*-fluoroaniline and ethyl 4′-methoxybenzoyl acetate; amorphous; ^{1}H NMR (DMSO-d_6) δ 3.38 (s, 3 H, OCH_3), 6.90 (d, J = 8.0 Hz, 2 H, H-3′, H-5′), 7.44 (dt, J = 2.5, 10.0 Hz, 1 H, H-6), 7.67 (dd, J = 2.5, 10.0 Hz, 1 H, H-8), 7.89 (s, 3 H, H-3), 8.09 (dd, J = 6.0, 9.0 Hz, 1 H, H-5), 8.11 (d, J = 8.0 Hz, 2 H, H-2′, H-6′), 9.90 (s, 1 H, NH); IR (KBr) 3450, 1625 cm^{-1}. Anal. C, H, N.

7-Fluoro-2-(2-pyridyl)-4-quinolone (42): obtained from *m*-fluoroaniline and ethyl pyrid-2-yl acetate; needles; ^{1}H NMR ($CDCl_3$) δ 6.96 (s, 1 H, H-3), 7.13 (dt, J = 2.4, 8.8 Hz, 1 H, H-6), 7.39 (dd, J = 2.4, 9.2 Hz, 1 H, H-8), 7.50 (dd, J = 5.0, 7.8 Hz, 1 H, H-5′), 7.95 (dt, J = 1.6, 7.8 Hz, 1 H, H-4′), 8.07 (d, J = 7.8 Hz, 1 H, H-3′), 8.32 (dd, J = 2.4, 8.8 Hz, 1 H, H-5), 8.75 (br d, J = 5.0 Hz, 1 H, H-6′); IR (KBr) 3440, 1635 cm^{-1}. Anal. C, H, N.

5-Fluoro-2-(2-pyridyl)-4-quinolone (43): obtained from *m*-fluoroaniline and ethyl pyrid-2-yl acetate; amorphous; ^{1}H NMR ($CDCl_3$) δ 6.90 (s, 1 H, H-3), 6.98 (dd, J = 8.3, 11.5 Hz, 1 H, H-6), 7.46 (d, J = 8.3 Hz, 1 H, H-8), 7.49 (d, J = 5.0, 7.8 Hz, 1 H, H-5′), 7.59 (dt, J = 5.2, 8.3 Hz, 1 H, H-7), 7.94 (dt, J = 1.6, 7.8 Hz, 1 H, H-4′), 8.05 (d, J = 7.8 Hz, 1 H, H-3′), 8.75 (br d, J = 5.0 Hz, 1 H, H-6′); IR (KBr) 3440, 1635 cm^{-1}. Anal. C, H, N.

6-Methoxy-2-(2-pyridyl)-4-quinolone (44): obtained from *p*-methoxyaniline and ethyl pyrid-2-yl acetate; needles; ^{1}H NMR ($CDCl_3$) δ 6.95 (s, 1 H, H-3), 7.29 (dd, J = 2.8, 8.9 Hz, 1 H, H-7), 7.44 (dd, J = 5.0, 8.0 Hz, 1 H, H-5′), 7.47 (d, J = 8.9 Hz, 1 H, H-8), 7.77 (d, J = 2.8 Hz, 1 H, H-5), 7.89 (dt, J = 1.5, 8.0 Hz, 1 H, H-4′), 7.98 (d, J = 8.0 Hz, 1 H, H-3′), 8.71 (br d, J = 5.0 Hz, 1 H, H-6′), 10.41 (s, 1 H, NH); IR (KBr) 3460, 1605 cm^{-1}; MS m/z 252 (M$^+$). Anal. C, H, N.

6-Chloro-2-(2-pyridyl)-4-quinolone (45): obtained from *p*-chloroaniline and ethyl pyrid-2-yl acetate; needles; ^{1}H NMR ($CDCl_3$ and CD_3OD) δ 6.82 (s, 1 H, H-3), 7.35 (dd, J = 4.8, 7.8 Hz, 1 H, H-5′), 7.47 (dd, J = 2.0, 8.8 Hz, 1 H, H-7), 7.56 (d, J = 8.8 Hz, 1 H, H-8), 7.80 (dt, J = 1.6, 7.8 Hz, 1 H, H-4′), 7.92 (d, J = 7.8 Hz, 1 H, H-3′), 8.11 (d, J = 2.0 Hz, 1 H, H-5), 8.61 (br d, J = 4.8 Hz, 1 H, H-6′); IR (KBr) 3450, 1630 cm^{-1}; MS m/z 256 (M$^+$). Anal. C, H, N.

Biochemical Assays. Electrophoretically homogeneous bovine brain tubulin was purified as described previously.[39] Combretastatin A-4 was a generous gift of Dr. G. R. Pettit, Arizona State University. Dihydrocombretastatin A-4 was prepared as described previously.[26] [^{3}H]Colchicine was from DuPont, nonradiolabeled colchcine from Sigma, podophyllotoxin from Aldrich, and monosodium glutamate from USB. The binding of radiolabeled colchicine to tubulin was measured by the DEAE-cellulose filter technique, as described previously.[14] Reaction mixtures contained 1.0 μM tubulin, 5.0 μM [^{3}H]colchicine, and 5.0 μM potential inhibitor.

The tubulin-polymerization assay was performed as described previously, except for a modification in reaction conditions.[40] With an increasing number of colchicine-site compounds, we have observed atypical patterns of turbidity development, apparent persistence of polymerization reactions at high drug concentrations, and altered stability of polymer formed in the presence of drug[14,26,40] under reaction conditions we have used in the past (1.0 M monosodium glutamate, 1.0 mM $MgCl_2$, 37 °C). As a consequence, IC_{50} values for tubulin polymerization could not be obtained for a substantial number of agents without altering reaction conditions. Thus far, we have not had problems with the reaction conditions used here, but the modifications introduced have led to substantially lower IC_{50} values for all inhibitory compounds.[26,40]

In brief, tubulin at 1.2 mg/mL (12 μM) was preincubated for 15 min at 26 °C in a 0.24-mL volume of 0.8 M monosodium glutamate (pH 6.6 with NaOH in a 2 M stock solution) with varying drug concentrations. The drug stock solutions were in dimethyl sulfoxide, and the final solvent concentration was 4% (v/v). Atomic absorption spectroscopy indicated the Mg^{2+} concentration of the reaction mixtures was about 35 μM (26–27 μM from the glutamate, 8–9 μM from the tubulin), but no exogenous magnesium was added. All concentrations are in terms of the final reaction volume (0.25 mL). The reaction mixtures were chilled on ice, and 10 μL of 10 mM GTP was added to each reaction mixture. Samples were transferred to cuvettes held at 0 °C by an electronic temperature controller in Gilford spectrophotometers. Base lines were established at 350 nm, and polymerization was initiated by a temperature jump to 26 °C. The jump took about 50 s to complete. After 20 min, turbidity readings were recorded and the temperature controller was set to 0 °C. When depolymerization was complete, turbidity readings were again recorded. Generally, turbidity readings were about 90% cold-reversible, and the cold-reversible turbidity was taken to represent the extent of assembly for each reaction mixture. IC_{50} values were obtained graphically from inhibition of polymerization by different drug concentrations. Four spectrophotometers were used for each experimental sequence, with two control reactions (no drug) in each set. Generally the control reactions were within 5% of their average. A minimum of three independent IC_{50} values were obtained for each drug, except that inactive compounds were usually evaluated only two times. In most cases, IC_{50} values obtained with this polymerization assay are highly reproducible. Generally, standard deviations were within 20% of the mean values, but with some compounds, the standard deviations were 30–35% of the mean. Therefore, we can conservatively estimate that a 50% difference in IC_{50} values represents a difference in the relative activity of two agents.

Acknowledgment. This work was supported by Grant CA 17625 from the National Cancer Institute awarded to K.-H. Lee.

References

(1) Wang, K. H.; Liu, S. Y.; Hwang, K. M.; Lee, K. H. Antitumor Agents 149. Novel Water-soluble 7-(Acylhydrazono)-formyl Camptothecins as Potent Inhibitors of DNA Topoisomeriase I. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 579–583.
(2) Hamel, E. Interactions of Tubulin with Small Ligands. In *Microtubule Proteins*; Avila, J., Ed.; CRC Press: Boca Raton, FL, 1990; pp 89–191.
(3) Paull, K. D.; Lin, C. M.; Malspeis, L.; Hamel, E. Identification of Novel Antimitotic Agents Acting at the Tubulin Level by Computer-assisted Evaluation of Differential Cytotoxicity Data. *Cancer Res.* **1992**, *52*, 3892–3900.
(4) Rowinsky, E. K.; Cazenave, L. A.; Donehower, R. C. Taxol: A Novel Investigational Antimicrotubule Agent. *J. Natl. Cancer Inst.* **1990**, *82*, 1247–1259.
(5) Rowinsky, E. K.; Donehower, R. C. The Clinical Pharmacology and Use of Antimicrotubule Agents in Cancer Chemotherapeutics. *Pharamcol. Ther.* **1992**, *52*, 35–84.
(6) Rowinsky, E. K.; Onetto, N.; Canetta, R. M.; Arbuck, S. G. Taxol: the First of the Taxanes, an Important New Class of Antitumor Agents. *Semin. Oncol.* **1992**, *19*, 646–662.
(7) Runowicz, C. D.; Wiernik, P. H.; Einzig, A. I.; Goldberg, G. L.; Horwitz, S. B. Taxol in Ovarian Cancer. *Cancer* **1993**, *71*, 1591–1596.
(8) Kelly, M.; Hartwell, J. The Biological Effects and the Chemical Composition of Podophyllin. A Review. *J. Natl. Cancer Inst.* **1954**, *14*, 967–1010.
(9) Sullivan, M. Treatment of Cutaneous Carcinoma with Podophyllin. Preliminary Note. *Bull. John Hopkins Hosp.* **1949**, *85*, 200–203.
(10) Hastie, S. B. Interactions of Colchicine with Tubulin. *Pharmacol. Ther.* **1991**, *51*, 377–401.
(11) Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S.; Garcia-Kendall, D. Isolation and Structure of the Strong Cell Growth and Tubulin Inhibition Combretastatin A-4. *Experientia* **1989**, *45*, 209–211.
(12) Lin, C. M.; Ho, H. H.; Pettit, G. R.; Hamel, E. Antimitotic Natural Products Combretastatin A-4 and Combretastatin A-2; Studies on the Mechanism of Their Inhibition of the Binding of Colchicine to Tubulin. *Biochemistry* **1989**, *28*, 6984–6991.
(13) Zayat, A. A.; Degen, D.; Drabek, S.; Clark, G. M.; Pettit, G. R.; von Hoff, D. D. In Vitro Evaluation of the Antineoplastic Activity of Combretastatin A-4, A Natural Product from *Combretum Caffrum*. *Anticancer Drugs* **1993**, *4*, 19–25.

(14) Kuo, S. C.; Lee, H. Z.; Juang, J. P.; Lin, Y. T.; Wu, T. S.; Chang, J. J.; Lednicer, D.; Paull, K. D.; Lin, C. M.; Hamel, E.; Lee, K. H. 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. *J. Med. Chem.* **1993**, *36*, 1146–1156.
(15) Hamel, E.; Ho, H. H.; Kang, G.-J.; Lin, C. M. Cornigerine, A Potent Antimitotic *Colchicum* Alkaloid of Unusual Structure: Interactions with Tubulin. *Biochem. Pharmacol.* **1988**, *37*, 2445–2449.
(16) Wang, R. W.-J.; Rebhum, L. I.; Kupchan, S. M.; Antimitotic and antitubulin activity of the tumor inhibitor steganacin. *Cancer Res.* **1977**, *37*, 3071–3079.
(17) Lin, C. M.; Singh, S. B.; Chu, P. S.; Dempcy, R. O.; Schmidt, J. M.; Pettit, G. R.; Hamel, E. Interactions of Tubulin with Potent Natural and synthetic Analogs of the Antimitotic Agents Combretastatin, A Structure-activity Study. *Mol. Pharmacol.* **1988**, *34*, 200–208.
(18) Desai, K.; Desai, C. M. Direct Synthesis of 4-Hydroquinolines Using Polyphosphoric Acid. *Indian J. Chem.* **1967**, *5*, 170–171.
(19) Fuson, R. C.; Burness, D. M. A New Synthesis of 2-Aryl-4-hydroxyquinolines. *J. Chem. Soc.* **1946**, *68*, 1270–1273.
(20) Chen, B. C.; Huang, X.; Wang, J. A Versatile Synthesis of 2-Aryl-4-quinolones. *Synthesis* **1987**, 482–483.
(21) Chong, R. J.; Siddiqui, M. A.; Snieckus, V. Synthetic Connections to the Aromatic Metalation Reaction. A Modified von Niementowski Quinoline Synthesis from Anthranilamides. *Tetrahedron Lett.* **1982**, *27*, 5323–5326.
(22) Simpson, J. C. E.; Atkinson, C. M.; Schofield, K.; Stephenson, O. o-Amino Ketones of the Acetophenone and Benzophenone Type. *J. Chem. Soc.* **1945**, 646–657.
(23) Boyd, M. R. Status of the NCI Preclinical Antitumor Drug Discovery Screen. In *Cancer: Principles and Practice of Oncology Updates*; De Vita, V. T., Hellman, S.; Rosenberg, S. A., Eds.; J. B. Lippincott: Philadelphia, 1989; pp 1–12.
(24) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. Feasibility of a High-flux Anticancer Drug Screen Utilizing a Derive Panel of Human Tumor Cell Lines in Culture. *J. Natl. Cancer Inst.* **1991**, *83*, 757–766.
(25) Edwards, M. L.; Stemerick, D. M.; Sunkara, P. S. Chalcones: A New Class of Antimitotic Agents. *J. Med. Chem.* **1990**, *33*, 1948–1954.
(26) Getahun, Z.; Jurd, P. S.; Chu, P. S.; Lin, C. M.; Hamel, E. Synthesis of Alkoxy-Substituted Diaryl Compounds and Correlation of Ring Separation with Inhibition of Tubulin Polymerization: Differential Enhancement of Inhibitory Effects under Suboptimal Polymerization Reaction Conditions. *J. Med. Chem.* **1992**, *35*, 1058–1067.
(27) Sun, L.; McPhail, A. T.; Hamel, E.; Lin, C. M.; Hastie, S.; Chang, J. J.; Lee, K. H. Antitumor Agents 139. Synthesis and Biological Evaluation of Thiocolchicine Analogs 5,6-Dihydro-6(*S*)-(acyloxy)- and 5,6-Dihydro-6(*S*)-[(aroyloxy)methyl]-1,2,3-trimethoxy-9-(methylthio)-8*H*-cycloheptа[*a*]naphthalen-8-ones as Novel Cytotoxic and Antimitotic Agents. *J. Med. Chem.* **1993**, *36*, 544–551.
(28) Sun, L.; Hamel, E.; Lin, C. M.; Hastie, S. B.; Pyluck, A.; Lee, K. H. Antitumor Agents 141. Synthesis and Biological Evaluation of Novel Thiocolchicine Analogs: *N*-Acyl-, *N*-Aroyl-, and *N*-(Substituted Benzyl)deacetylthiocolchicines as Potent Cytotoxic and Antimitotic Compounds. *J. Med. Chem.* **1993**, *36*, 1474–1479.
(29) Cortes, F.; Bhattacharyya, B.; Wolff, J. Podophyllotoxin as a Probe for the Colchicine Binding Site of Tubulin. *J. Biol. Chem.* **1977**, *252*, 1134–1140.
(30) Andreu, J. M.; Timasheff, S. N. Conformational States of Tubulin Liganded to Colchicine, Tropolone Methyl Ester, and Podophyllotoxin. *Biochemistry* **1982**, *21*, 6465–6467.
(31) Lin, C. M.; Kang, J.; Roach, M. C.; Jiang, J. B.; Hesson, D. P.; Luduena, R. F.; Hamel, E. Investigation of the Mechanism of the Interaction of Tubulin with Derivatives of 2-Styrylquinazoline-4(3*H*)-one. *Mol. Pharmacol.* **1991**, *40*, 827–832.
(32) Rösner, M.; Capraro, H.-G. Jacobson, A. E.; Atwell, L.; Brossi, A.; Iorio, M. A.; Williams, T. H.; Sik, R. H.; Chignell, C. F. Biological Effects of Modified Colchicine: Improved Preparation of 2-Demethylcolchicine, 3-Demethylcolchicine, and (+)-Colchicine and Reassignment of the Position of the Double Bond in Dehydro-7-deacetamidocolchicines. *J. Med. Chem.* **1981**, *24*, 257–261.
(33) Brossi, A.; Yeh, H. J. C.; Chrzanowska, M.; Wolff, J.; Hamel, E.; Lin, C. M.; Quinn, F.; Suffness, M.; Silverton, J. Colchicine and Its Analogues: Recent Findings. *Med. Res. Rev.* **1988**, *8*, 77–94.
(34) Muzaffar, A.; Brossi, A.; Lin, C. M.; Hamel, E. Antitubulin Effects of Derivatives of 3-Demethylthiocolchicine, Methylthio Ethers of Natural Colchicinoids, and Thioketones Derived from Thiocolchicine. Comparison with Colchicinoids. *J. Med. Chem.* **1990**, *33*, 567–571.
(35) Banwell, M. G.; Cameron, J. M.; Collis, M. P.; Crisp, G. T.; Gable, R. W.; Hamel, E.; Lambert, J. N.; Mackay, M. E.; Scoble, J. A. The Palladium-mediated Cross Coupling of Bromotropolones with Organostannanes or Arylboronic Acids: Applications to the Synthesis of Natural Products and Natural Products Analogues. *Aust. J. Chem.* **1991**, *44*, 705–728.
(36) Cushman, M.; Nagarathnam, D.; Gopal, D.; Chakraborti, A. K.; Lin, C. M.; Hamel, E. Synthesis and Evaluation of Stilbene and Dihydrostilbene Derivatives as Potential Anticancer Agents that Inhibit Tubulin Polymerization. *J. Med. Chem.* **1991**, *34*, 2579–2588.
(37) Cushman, M.; Nagaratlınam, D.; Gopal, D.; He, H.-M.; Lin, C. M.; Hamel, E. Synthesis and Evaluation of Analogues of (*Z*)-1-(4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene as Potential Cytotoxic and Antimitotic Agents. *J. Med. Chem.* **1992**, *35*, 2293–2306.
(38) Jiang, J. B.; Hesson, D. P.; Dusak, B. A.; Dexter, D. L.; Kang, G. J.; Hamel, E. Synthesis and Biological Evaluation of 2-Styrylquinazolin-4(3*H*)-ones, a New Class of Antimitotic Anticancer Agents which Inhibit Tubulin Polymerization. *J. Med. Chem.* **1990**, *33*, 1721–1728.
(39) Hamel, E.; Lin, C. M. Separation of Active Tubulin and Microtubule-Associated Proteins by Ultracentrifugation and Isolation of a Component Causing the Formation of Microtubule Bundles. *Biochemistry* **1984**, *23*, 4173–4178.
(40) D'Amato, R. J.; Lin, C. M.; Flynn, E.; Folkman, J.; Hamel, E. 2-Methoxyestradiol, an Endogeneous Mammal Metabolite, Inhibits Tubulin Polymerization by Interacting at the Colchicine Site. *Proc. Natl. Acad. Sci. U.S.A.*, in press.