Antitumor Agents. 181.† Synthesis and Biological Evaluation of 6,7,2′,3′,4′-Substituted-1,2,3,4-tetrahydro-2-phenyl-4-quinolones as a New Class of Antimitotic Antitumor Agents

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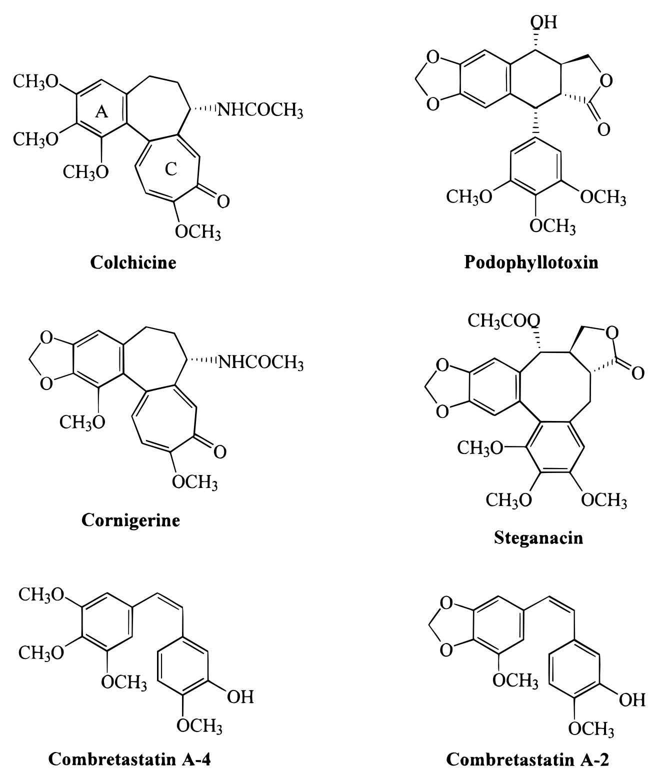
A novel series of 6,7,2′,3′,4′-substituted-1,2,3,4-tetrahydro-2-phenyl-4-quinolones were synthesized and evaluated for interactions with tubulin and for cytotoxic activity against a panel of human tumor cell lines, including ileocecal carcinoma (HCT-8), breast cancer (MCF-7), lung carcinoma (A-549), epidermoid carcinoma of the nasopharynx (KB), renal cancer (CAKI-1), and melanoma cancer (SKMEL-2). Most compounds (**18**, **20**, **22**−**27**) showed potent cytotoxic and antitubulin effects. The most active compounds (**23**, **26**, **27**) demonstrated strong cytotoxic effects with ED_{50} values in the nanomolar or subnanomolar range in almost all tumor cell lines. Three active racemates (**20**, **22**, **25**) were separated into the enantiomers, and generally, the optically pure (−)-isomers (**20a**, **22a**, **25a**) exhibited greater biological activity than the racemates or (+)-isomers. Cytotoxicity and antitubulin activity were closely correlated, with the most active compounds (**23**, **26**, **27**) having effects comparable to those of colchicine, podophyllotoxin, and combretastatin A-4.

Introduction

Microtubules are an important target for development of compounds potentially useful as anticancer chemotherapeutics. Examples of such drugs are the vinca alkaloids,[2] which inhibit microtubule polymerization, and taxoids,[3] which promote microtubule assembly. Colchicine[4,5] (Chart 1) is another well-known agent that inhibits microtubule assembly. Although too toxic to be useful for cancer therapy, colchicine has been an important tool in studies of microtubule structure and function. The vinca alkaloids, taxoids, and colchicine each interact with tubulin by a unique mechanism, probably involving distinct binding sites on the protein.

A large number of compounds act as antimitotic agents through interactions at the colchicine binding site on tubulin, including the natural products podophyllotoxin,[6] cornigerine,[7] steganacin,[8] and combretastatins A-2 and A-4[9,10] (Chart 1). In addition, a variety of heterocyclic ketones (Chart 2) are potent antimitotic agents that inhibit the tubulin−colchicine interaction and presumably bind in the same site on the protein. Over 2 decades ago, 2,3-dihydro-2-aryl-4(1*H*)-quinazolinone (DHQZ) derivatives were reported to display antitumor activities[11,12] and thus were reevaluated in the National Cancer Institute cancer cell line screen. Significant inhibition of tubulin assembly and of the binding of radiolabeled colchicine to tubulin[13] was demonstrated. 2-Styrylquinazolin-4(3*H*)-one (SQZ) derivatives and flavonols have also been found to inhibit tubulin polymerization, colchicine binding, and the growth of L1210 murine leukemia cells.[14−18]

Chart 1. Antimitotic Natural Products



In our continuing study aimed at the discovery and development of potential anticancer drug candidates, we synthesized two series of substituted heterocyclic ke-

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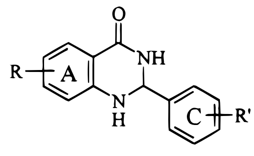
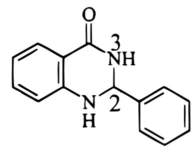
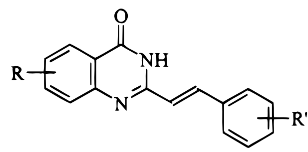
Chart 2. Antimitotic Heterocyclic Ketones



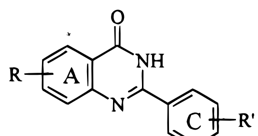
DHQZ



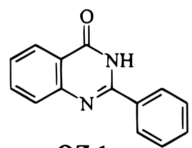
DHQZ 1



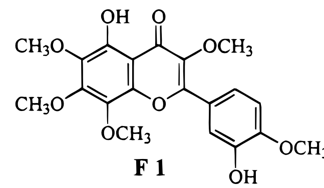
SQZ



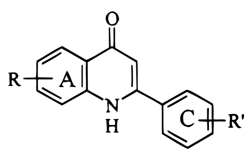
QZ



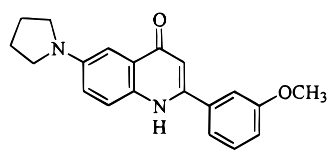
QZ 1



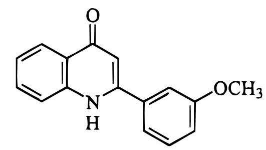
F 1



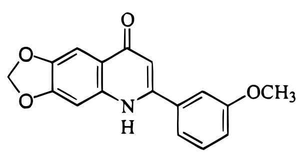
PQ



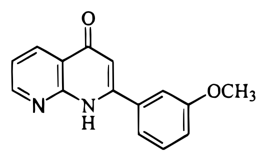
PQ 1



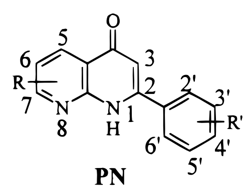
PQ 2



PQ 3



PN 1



PN

tones, 2-phenyl-4-quinolones (PQ)[19−21] and 2-phenyl-1,8-naphthyridin-4-ones (PN),[22,23] and identified potent cytotoxic antimitotic agents in each series. Structure−activity relationship (SAR) studies of the quinolone class led to the discovery of the particularly potent compound PQ1, which totally inhibited the growth of about half of the NCI tumor cell lines at subnanomolar concentrations (log TGI < −9.0). The activity of PQ1 as an inhibitor of tubulin polymerization and colchicine binding was comparable to those of colchicine, podophyllotoxin, and combretastatin A-4 in these assays.

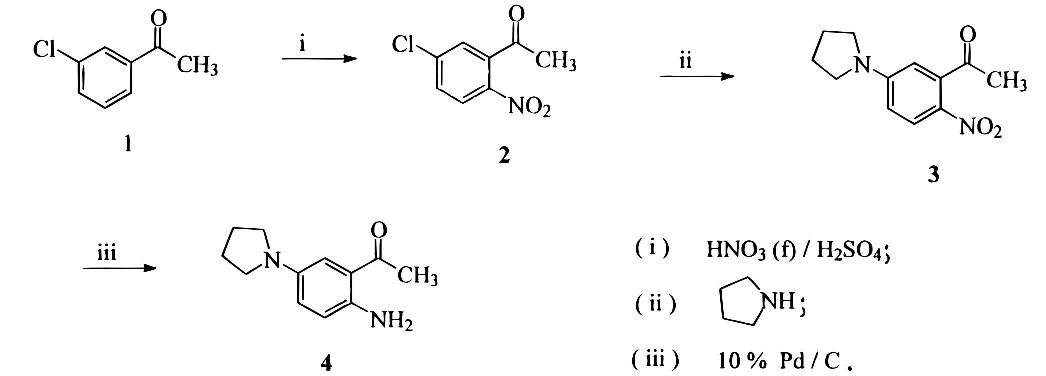
These antimitotic heterocyclic ketones share a common structural feature, a biaryl system composed of rings A and C, which are linked by an interposed B ring and sometimes a hydrocarbon bridge. Their biaryl system may be analogues to similar biaryl systems that occur in the natural products shown in Chart 1. The DHQZ, SQZ, PQ, and PN derivatives are aza compounds, in which one or more nitrogen atoms are present at the 1-, 3-, or 8-position, while the flavones have an oxygen atom at position 1. In particular, the DHQZ derivatives differ from the other classes in the oxidation status of the bond between C(2) and N(3). In a direct comparison, oxidation of the C(2)−N(3) bond converted the 2,3-dihydroquinazolinone ring (DHQZ1) to the quinazolinone ring (QZ1) and resulted in loss of activity in the tubulin-based biochemical assays.[13] This finding suggests that configurational and conformational changes in the biaryl system are important in the drug−tubulin interaction, as has been demonstrated with colchicinoids and allocolchicinoids.[1,4,5,24] Moreover, the DHQZ studies[11−13] were performed with racemic mixtures, and it was reasonable to anticipate differential activities in diastereoisomeric pairs, similar to that which occur in colchicinoids and allocolchicinoids.[1,4,5,24] These considerations prompted us to design, synthesize, and evaluate a series of 2,3-dihydro-2-phenyl-4(1*H*)-quinolone (DHPQ) derivatives, representing analogues of PQ derivatives with a reduced B ring double bond. We also resolve the racemates of several active compounds into the corresponding optically pure enantiomers. As expected, the two isomers differed in their biological activity, with the (−)-agents more active than the (+)-isomers.

Chemistry

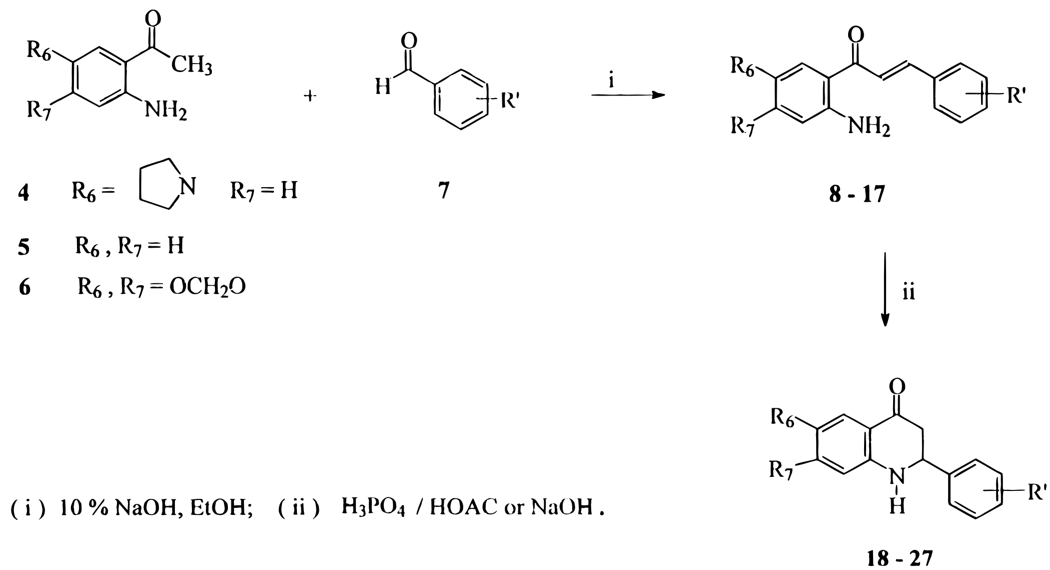
DHPQ derivatives were prepared from substituted 2′-aminoacetophenones (**3**−**5**). Scheme 1 shows the synthesis of 2′-amino-5′-pyrrolinylacetophenone (**4**) following the literature methods.[21,25] Nitration of 3′-chloroacetophenone (**1**) gave 2′-nitro-5′-chloroacetophenone. Nucleophilic displacement of the 5′-chloro group by pyrroline followed by hydrogenation gave compound **4**. Condensation of **3**−**5** with the appropriate benzaldehyde (**7**) followed by acid-or base-catalyzed cyclization gave the final products **18**−**27** [26−29] (Scheme 2).

The optically active quinolones were not obtained successfully by chromatography of the racemic mixtures

Scheme 1. Synthesis of 2′-Amino-5′-pyrrolinylacetophenone (4)



Scheme 2. General Synthetic Routes to DHPQ Derivatives



on a chiral column or by separation of amide diastereoisomers obtained with optically pure (1*S*)-(−)-camphanic chloride.

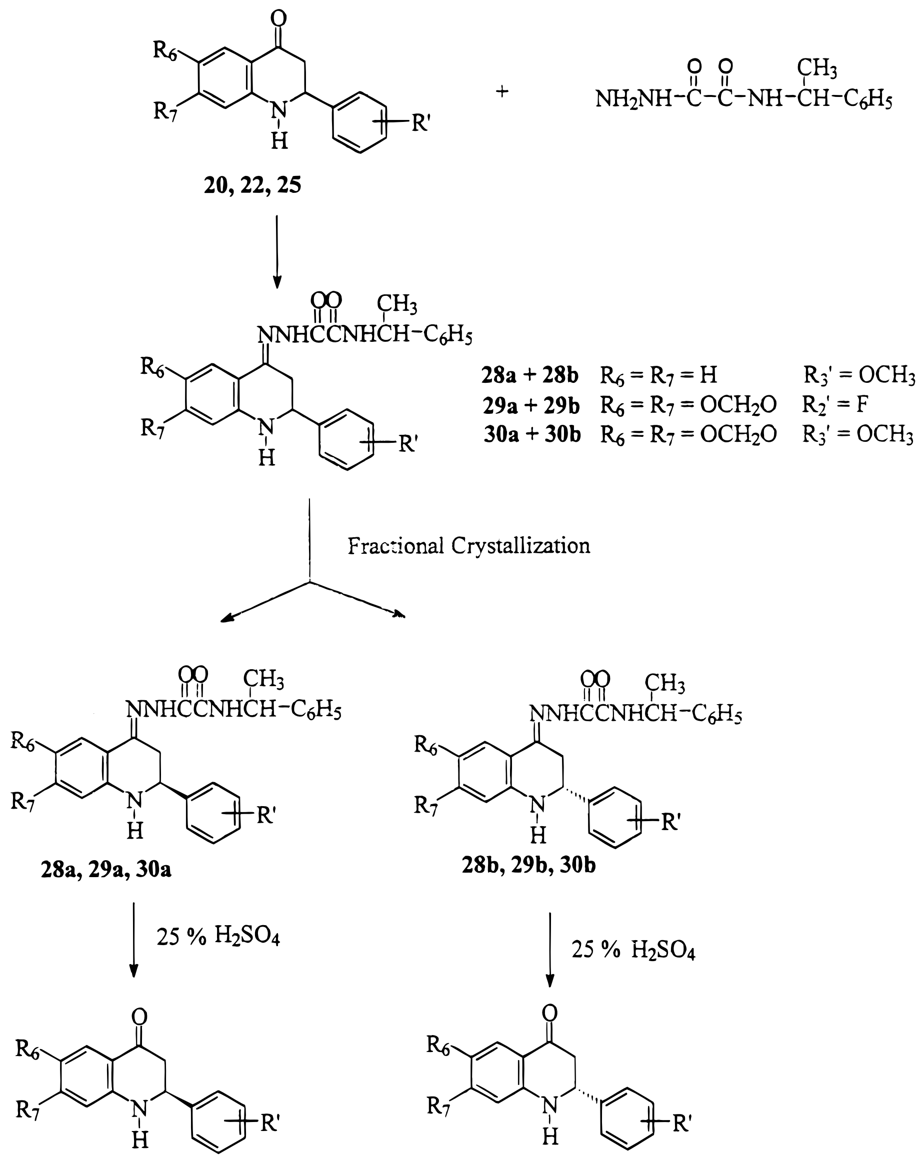
Because quinolones are the aza analogues of flavonoids which react smoothly with an oxo reagent to give the expected hydrazone, azine, and oxime derivatives,[30] **19**, **22**, and **25** were reacted with the optically active oxo reagent (−)-5-(α-phenethyl)semioxamazide, as shown in Scheme 3. Compounds **19**, **22**, and **25** were treated in MeOH with (−)-5-(α-phenethyl)semioxamazide to give diastereoisomeric mixtures (**28a**−**30a**, **28b**−**30b**). Separation of diastereomeric oxamazones was achieved by means of fractional crystallization, yielding crystalline, strongly levorotatory products (**28a**−**30a**) and less levorotatory products (**28b**−**30b**). Both diastereoisomeric oxamazones **28a**−**30a** and **28b**−**30b** were crystallized from methanol. Decomposition of the oxamazones with dilute sulfuric acid afforded the optically active enantiomers of **19**, **22**, and **25**. The enantiomers showed identical NMR spectra but opposite optical rotations.

Results and Discussion

a. Evaluation of Cytotoxicty of DHPQ Derivatives. The 6,7,2′,3′,4′-substituted DHPQ derivatives and related compounds (**18**−**30**) were assayed for their cytotoxicity in vitro against six human tumor cell lines, including ileocecal carcinoma (HCT-8), breast cancer (MCF-7), lung carcinoma (A-549), epidermoid carcinoma (KB), renal cancer (CAKI-1), and melanoma cancer (SKMEL-2). As shown in Table 1, compounds **18**, **20**, and **22**−**27** displayed significant activity, with ED_{50} values < 1.0 μg/mL in virtually all cases. In terms of SAR information, compounds substituted at the 4′-position (i.e., **21**, **24**) or 2′-position (i.e., **19**, **22**) were substantially less active than those substituted at the 3′-position (i.e., **20**, **23**, **25**), while compound **21** with a methoxy group at the 4′-position was the least active. (Methylenedioxy)benzene is a common moiety in many antimitotic agents, such as podophyllotoxin, steganacin, and combretastatin A-2 (Chart 1). However, the 6,7-(methylenedioxy)-substituted compounds (**22**−**25**) did not show any significant increase in activity compared to an unsubstituted compound (**18**). Compound **26**, with a heterocyclic ring at the 6-position, was the most potent compound with ED_{50} values in the nanomolar concentration range. The effects of substitutions at the 6- and/or 7-positions in ring A depend on the substitution in ring C.

The oxamazone derivatives **28**−**30** had minimal activity as inhibitors of cell growth (Table 1). Nevertheless, these compounds displayed the widest range in cytotoxic activity and may show tissue selectivity. In particular, compound **29a** showed highly selective effects on the ileocecal carcinoma line (HCT-8). Growth of HCT-8 cell was inhibited by a 10-fold lower concen-

Scheme 3. Resolution of (±)-DHPQ Derivatives **20**, **22**, and **25** with (−)-5-(α-Phenethyl)semioxamazide



tration than the concentration required with the less sensitive cell lines.

b. Interaction of DHPQ Derivatives with Tubulin. Previously, PQ derivatives were found to inhibit both tubulin polymerization and the binding of radiolabeled colchicine to tubulin.[19−21] The chief structural difference between the PQ agents and the new series of DHPQ derivatives is the oxidation status of the bond between C(2) and C(3) in the B ring. This modification results in configurational and conformational changes in the relative positions of the aromatic rings A and C. Many studies have suggested that the interaction between colchicine and tubulin is stereoselective and is highly dependent on the configuration and conformation of the biaryl system formed by the trimethoxyphenyl A ring and tropolonic C ring.[1,4,5,24,31] Thus, evaluation of the new agents for interactions with tubulin should provide additional insight into the mechanism of ligand binding at the colchicine site.

Table 2 summarizes the effects of the DHPQ derivatives as inhibitors of tubulin polymerization and, for the most active compounds, on the binding of [^{3}H]colchicine to tubulin. Close structural analogues that had been studied previously (PQ1, PQ2, PQ3, and PN1; structures in Chart 2) were reevaluated. Table 2 also summarizes previous data, obtained under identical reaction conditions, for colchicine, podophyllotoxin, and combretastatin A-4. The inhibitory effects on tubulin activities were in excellent agreement with the cytotoxicity data. The cytotoxic compounds (**18**, **20**, **22−27)** all were substoichiometric inhibitors of tubulin polymerization, and the highly cytotoxic compounds (**23**, **26**, **27**) were also the most potent inhibitors of colchicine binding. These compounds had effects virtually identical to those of the three natural products included for comparison. Conversely, the least cytotoxic compounds (**19**, **21, 28a,b−30a,b**) had little or no inhibitory effect on tubulin polymerization (IC_{50} > 40 μM).

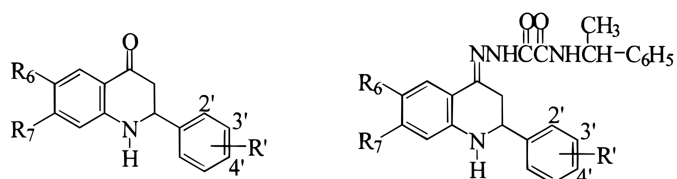
As with the PQ[21] and PN[22] derivatives, DHPQ compounds with 3′-substitution were more active than those with the same substituent at the 2′- or 4′-position. Compounds with an *o*-methoxy (**19**) or *p*-methoxy (**21**) substituent were inactive. This total loss of activity with the methoxy substituent is probably steric in

Table 1. In Vitro Cytotoxic Activities of 2′,3′,4′,6,7-Substituted DHPQ Derivatives

compd	ED_{50} (μg/mL)[a] KB[b]	A-549[b]	HCT-8[b]	CAKI-1[b]	MCF-7[b]	SKMEL-2[b]
18	0.07	0.013	0.013	0.013	0.13	0.13
19	1.8	6	6	25	6.5	12.5
20	0.11	0.23	0.12	0.32	0.32	0.2
20a	0.12	0.15	0.09	0.15	0.30	ND[c]
20b	0.50	0.50	0.50	3.8	0.60	ND
21	>25	>25	>25	ND[c]	>25	ND
22	0.06	0.08	0.13	1.0	0.06	0.125
22a	0.08	0.18	0.09	1.0	0.1	ND
22b	0.43	0.6	0.50	1.25	0.85	ND
23	0.012	0.012	0.016	0.016	0.032	0.016
24	0.3	1	0.4	0.8	0.4	0.2
25	0.02	0.04	0.04	0.06	0.04	0.06
25a	0.02	0.03	0.02	0.25	0.03	ND
25b	0.07	0.09	0.08	0.25	0.12	ND
26	0.008	0.01	0.016	0.008	0.11	0.016
27	0.05	0.09	0.09	0.95	0.31	0.08
28a	8	8	3.8	25	8	ND
28b	>25	>25	>25	>25	>25	ND
29a	18	13	2.1	>25	8.5	ND
29b	10	9	9	18	25	ND
30a	1.8	2.4	1.0	12.5	3.0	ND
30b	12	11.5	1.5	>25	6.3	ND
colchicine	0.002	0.002	0.016	0.4	>0.4	0.008

[a] Cytotoxicity as ED_{50} for each cell line, the concentration of compound that causes a 50% reduction in absorbance at 562 nm relative to untreated cells using the SRB assay.[32] [b]Human ileocecal carcinoma (HCT-8), human breast cancer (MCF-7), human lung carcinoma (A-549), human epidermoid carcinoma of the nasopharynx (KB), human renal cancer (CAKI-1), and human melanoma cancer (SKMEL-2). [c] ND, not determined.

Table 2. Antitubulin Effects of 2′,3′,4′,6,7-Substituted DHPQ Derivatives



18-27 **28-30**

compd	R_6	R_7	R_2'	R_3'	R_4'	ITP[a] IC_{50} (μM) ± SD	ICB[b] (% inhib ± SD) 5 μM[c]	1 μM[c]
18	H	H	H	H	H	2.7 ± 0.5		
19	H	H	OCH_3	H	H	>40		
20	H	H	H	OCH_3	H	3.3 ± 0.1		
20a[d]	H	H	H	OCH_3	H	1.2 ± 0.2		
20b[d]	H	H	H	OCH_3	H	7.2 ± 0.9		
21	H	H	H	H	OCH_3	>40		
22	OCH_2O		F	H	H	1.1 ± 0.2		
22a[d]	OCH_2O		F	H	H	0.56 ± 0.03	51 ± 2	
22b[d]	OCH_2O		F	H	H	12 ± 0.07		
23	OCH_2O		H	F	H	0.75 ± 0.04	75 ± 0.9	
24	OCH_2O		H	H	F	2.0 ± 0.3		
25	OCH_2O		H	OCH_3	H	0.82 ± 0.07	60 ± 3	
25a[d]	OCH_2O		H	OCH_3	H	0.69 ± 0.01	67 ± 0.5	
25b[d]	OCH_2O		H	OCH_3	H	2.3 ± 0.1		
26	pyrrolinyl	H	H	OCH_3	H	0.66 ± 0.03	91 ± 0.3	63 ± 5
27	pyrrolinyl	H	H	Cl	H	0.76 ± 0.09	89 ± 2	63 ± 4
28a	H	H	H	OCH_3	H	>40		
28b	H	H	H	OCH_3	H	>40		
29a	OCH_2O		F	H	H	>40		
29b	OCH_2O		F	H	H	>40		
30a	OCH_2O		H	OCH_3	H	>40		
30b	OCH_2O		H	OCH_3	H	>40		
PQ1[e]	pyrrolinyl	H	H	OCH_3	H	0.49 ± 0.07	91 ± 0.2	65 ± 8
PQ2[e]	H	H	H	OCH_3	H	1.5 ± 0.2		
PQ3[e]	OCH_2O		H	OCH_3	H	0.74 ± 0.09	33 ± 2	
PN1[e]	H	H	H	OCH_3	H	0.79 ± 0.03	24 ± 1	
colchicine						0.80 ± 0.07[f]		
podophyllotoxin						0.46 ± 0.02[f]		
combretastatin A-4						0.53 ± 0.05[f]	92 ± 3	88 ± 0.4

[a] ITP, inhibition of tubulin polymerization. [b]ICB, inhibition of colchicine binding; evaluated only when polymerization $IC_{50} \leq 1.0$ μM. [c] In the colchicine binding experiments, these values refer to the inhibitor concentration used. The [^{3}H]colchicine concentration was 5 μM, and the tubulin concentration was 1 μM. [d] See Scheme 3 for details of chiral configuration at C(2). [e] See Chart 2 for structures. [f] Data from ref 20.

origin, for compounds with the small F atom at the 2′-position (**22**) or 4′-position (**24**) were still effective inhibitors of polymerization, although maximum activity was still observed with the F at the 3′-position (**23**). Methoxy, chloride, and fluoride groups at the 3′-position all appeared to be equivalent in activity (i.e., **23** with **25**, **26** with **27**), whether an electron-donating group (OCH_3) or an electron-withdrawing group (F, Cl). The size of the substituents can be as small as hydrogen or as large as OCH_3 without greatly affecting activity (i.e., **18**, **20**). Methylenedioxy substitution in the A ring (**25**) significantly (4-fold) increased activity (i.e., **25**, **20**), as with PQ[21] derivatives. Compounds with a pyrrolinyl ring at the 6-position (**26**, **27**) were exceptionally active, particularly as inhibitors of colchicine binding. The activity of the compounds with the 6-pyrrolinyl substituent approaches that of combretastatin A-4 as an inhibitor of colchicine binding. Compounds **26** and **27**, as well as PQ1, inhibited colchicine binding over 60% (as compared to 88% inhibition by combretastatin A-4) when present at 1 μM in a reaction mixture containing 5 μM [^{3}H]colchicine and 1 μM tubulin.

Compounds **18**−**27** were all racemic mixtures. Because protein−ligand interactions are almost always stereoselective, we obtained the pure enantiomers **(−)-20a**, **(+)-20b**, **(−)-22a**, **(+)-22b**, **(−)-25a**, and **(+)-25b**. Invariably, the racemic mixtures (**20**, **22**, **25**) were less active than the (−)-enantiomers (**20a**, **22a**, **25a**) and more active than the (+)-enantiomers (**20b, 22b, 25b**). The difference between optical isomers as inhibitors of tubulin assembly ranged from 4-fold between **25a** and **25b** to 20-fold between **22a** and **22b**. The apparent superiority of one enantiomer confirms the postulate that the binding interaction with tubulin is stereoselective and suggests that, for all racemates, it is the (−)-isomer that is primarily responsible for the inhibition of tubulin polymerization.

Our earlier study[13] suggested that the DHQZ derivatives were probably more active than the structurally similar QZ analogues. The data presented in Table 2 lead to an analogous conclusion for DHPQ derivatives versus their conjugate PQ derivatives. Thus, compound **20a** appears to be about 20% more active than PQ2, as an inhibitor of assembly, and **25a** is more active than PQ3, as judged from the colchicine inhibition data. Moreover, **26** has activities comparable to those of PQ1, suggesting that the (−)-enantiomer of **26** would be more active. Finally, PN1 appears to be more active than either **20a** or PQ2, suggesting that reduction of the C(2)−C(3) double bond in the PN derivatives would yield compounds with still greater potency.

Experimental Section

Chemistry. Melting points were determined on a Fisher-Johns melting point apparatus without correction. Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA. Optical rotations were determined with a DIP-1000 polarimeter. ^{1}H NMR spectra were measured on a Bruker AC-300 spectrometer with TMS as internal reference and $CDCl_3$ as solvent. Mass spectral (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 hexanes−EtOAc as eluant. Where not noted, compounds were recrystallized once from the same solvent given in the sample procedure.

General Procedure for the Synthesis of Compounds 8, 11, 15, and 17.[27] To a solution of 2′-aminoacetophenone (4.05 g, 30 mmol) and benzaldehyde (3.18 g, 30 mmol) in EtOH (50 mL) was added NaOH (9 mL, 15%). The solution was stirred at room temperature for 24 h and then filtered. The resulting yellow precipitate was crystallized once from MeOH to afford yellow needles of **8** (4.35 g, 65%): mp 71−72 °C; ^{1}H NMR ($CDCl_3$) δ 6.20 (brs, 2 H, NH_2), 6.57−6.91 (m, 2 H, 3′-H and 5′-H), 7.20−8.08 (m, 9 H); MS *m/z* 223 (M^+). Anal. ($C_{15}H_{13}NO$) C, H, N.

2′-Amino-4-methoxychalcone (11): obtained from 2′-aminoacetophenone and *p*-anisaldehyde; yield 86.0%, needles; mp 91−92 °C; ^{1}H NMR ($CDCl_3$) δ 3.76 (s, 3 H, OCH_3), 6.29 (brs, 2 H, NH_2), 6.67−6.72 (m, 2 H, H-3′ and H-5′), 6.93 (m, 2 H, H-3 and H-5), 7.29 (m, 1 H, H-4′), 7.50 (d, 1 H, H-α, J = 15.3 Hz), 7.57−7.61 (m, 2 H, H-2 and H-6), 7.72 (d, 1 H, H-β, J = 15.3 Hz), 7.75 (m, 1 H, H-6′); MS *m/z* 253 (M^+). Anal. ($C_{16}H_{15}NO_2$) C, H, N.

2′-Amino-4′,5′-(methylenedioxy)-3-methoxychalcone (15): obtained from 6′-amino-3′,4′-(methylenedioxy)acetophenone and *m*-anisaldehyde; yield 54.5%, needles; mp 92−94 °C; ^{1}H NMR ($CDCl_3$) δ 3.86 (s, 3 H, OCH_3), 5.94 (s, 2 H, OCH_2O), 6.20 (s, 1 H, H-3′), 6.60 (brs, 2 H, NH_2), 6.93 (m, 1 H, H-4), 7.13 (s, 1 H, H-6′), 7.20−7.35 (m, 3 H, H-2, H-5 and H-6), 7.46 (d, 1 H, H-α, J = 15.4 Hz), 7.67 (d, 1 H, H-β, J = 15.4 Hz); MS *m/z* 297 (M^+). Anal. ($C_{17}H_{15}NO_2$) C, H, N.

2′-Amino-5′-pyrrolinyl-3-chlorochalcone (17): obtained from 2′-amino-5′-pyrrolinylacetophenone (**4**) and 3′-chlorobenzaldehyde; yield 69.4%, needles; mp 139−141 °C; ^{1}H NMR ($CDCl_3$) δ 2.03 (m, 4 H, *CH₂*CH₂NCH₂*CH₂*), 3.30 (m, 4 H, CH_2NCH_2), 5.70 (brs, 2 H, NH_2), 6.70 (d, 1 H, H-3′, J = 8.8 Hz), 6.81 (dd, 1 H, H-4′, J = 8.8, 2.6 Hz), 6.92 (d, 1 H, H-6′, J = 2.6 Hz), 7.34−7.37 (m, 2 H, H-4 and H-6), 7.48 (m, 1 H, H-5), 7.54 (d, 1 H, H-α, J = 15.6 Hz), 7.60 (s, 1 H, H-2), 7.66 (d, 1 H, H-β, J = 15.6 Hz); MS *m/z* 326 (M^+). Anal. ($C_{19}H_{19}ClN_2O$) C, H, N.

General Procedure for the Synthesis of Compounds 18, 21, 25, and 27. A mixture of 2′-amino-4-methoxychalcone (**11**) (760 mg, 3 mmol), HOAc (12.5 mL), and orthophosphoric acid (12.5 mL) was warmed at 100 °C for 20 min. After cooling, the mixture was poured into ice water. The product that precipitated was purified by column chromatography on silica gel using hexane−EtOAc (4:1) as eluant. Recrystallization from hexanes−EtOAc afforded yellow needles of **21** (540 mg, 71.1%): mp 131−132 °C; $[\alpha]^{25}_D$ 0° (*c* 0.40, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.72 (q, 1 H, H-3, J = −16.5, 3.9 Hz), 2.86 (q, 1 H, H-3, J = −16.5, 13.8 Hz), 3.82 (s, 3 H, OCH_3), 4.44 (brs, 1 H, NH), 4.70 (q, 1 H, H-2, J = 3.9, 13.8 Hz), 6.69 (d, 1 H, H-8, J = 8.2 Hz), 6.76−6.81 (m, 4 H, H-2′, H-3′, H-5′, and H-6′), 7.30−7.40 (m, 2 H, H-6 and H-7), 7.87 (d, 1 H, H-5, J = 7.8 Hz); MS *m/z* 253 (M^+). Anal. ($C_{16}H_{15}NO_2$) C, H, N.

1,2,3,4-Tetrahydro-2-phenyl-4-quinolone (18): obtained from **8**; yield 67%, yellow needles; mp 149−150 °C; $[\alpha]^{25}_D$ 0° (*c* 0.30, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.72 (q, 1 H, H-3, J = −16.4, 7.4 Hz), 2.90 (q, 1 H, H-3, J = −16.4, 10.3 Hz), 4.75 (q, 1 H, H-2, J = 10.6, 7.4 Hz), 4.75 (brs, 1 H, NH), 6.70−7.07 (m, 2 H, H-6 and H-8), 7.19−7.40 (m, 1 H, H-7), 7.45 (s, 5 H, C_6H_5), 7.93 (q, 1 H, H-5, J = 9.0, 1.5 Hz); MS *m/z* 223 (M^+). Anal. ($C_{15}H_{13}NO$) C, H, N.

3′-Methoxy-6,7-(methylenedioxy)-1,2,3,4-tetrahydro-2-phenyl-4-quinolone (25): obtained from **15**; yield 75.2%, yellow plates; mp 215−216 °C; $[\alpha]^{25}_D$ 0°(*c* 0.32, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.80 (q, 1 H, H-3, J = −16.8, 4.5 Hz), 2.75 (q, 1 H, H-3, J = −16.8, 13.4 Hz), 3.84 (s, 3 H, OCH_3), 4.39 (brs, 1 H, NH), 4.69 (q, 1 H, H-2, J = 4.5, 13.4 Hz), 5.95 (s, 2 H, OCH_2O), 6.19 (s, 1 H, H-8), 6.88 (m, 1 H, H-4′), 6.90 (s, 1 H, H-2′), 7.00 (m, 1 H, H-5), 7.27−7.35 (m, 2 H, H-5′ and H-6′); MS *m/z* 297 (M^+). Anal. ($C_{17}H_{15}NO_4$) C, H, N.

3′-Chloro-6-pyrrolinyl-1,2,3,4-tetrahydro-2-phenyl-4-quinolone (27): obtained from **17**; yield 71.4%, orange needles; mp 197−198 °C; $[\alpha]^{25}_D$ 0°(*c* 0.32, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.00 (m, 4 H, *CH₂*CH₂NCH₂*CH₂*), 2.70−2.87 (m, 2 H, H-3), 3.27 (m, 4 H, CH_2NCH_2), 4.10 (brs, 1 H, NH), 4.67 (q, 1 H, H-2, J = 12.9, 4.1 Hz), 6.70 (m, 1 H, H-8), 6.82 (m, 1 H, H-7), 7.03 (s, 1 H, H-5), 7.33 (m, 3 H, H-4′, H-5′, and H-6′), 7.51 (s, 1 H, H-2′); MS *m/z* 326 (M^+). Anal. ($C_{19}H_{19}ClN_2O$) C, H, N.

General Procedure for the Synthesis of Compounds 19, 20, 22−24, and 26. NaOH (0.8 mL, 15%) was added to a stirred solution of 2′-aminoacetophenone (2.7 g, 20 mmol) and *m*-anisaldehyde (4.08 g, 30 mmol) in EtOH (8 mL) at room temperature. After refluxing for 30 min, the mixture was evaporated to dryness. The residue was added with stirring in one portion to diphenyl ether (10 mL) at 180 °C. After 30 min, the mixture was cooled to room temperature and diluted with hexanes. The precipitate was collected and purified by chromatography on a silica gel column. Elution with hexanes−EtOAc (4:1) and recrystallization from hexanes−EtOAc afforded **20** (2.26 g, 44.7%): needles; mp 211−213 °C; $[\alpha]^{25}_D$ 0° (*c* 0.345, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.76 (q, 1 H, H-3, $J = -16.2$, 4.3 Hz), 2.87 (q, 1 H, H-3, $J = -16.2$, 13.3 Hz), 3.80 (s, 3 H, OCH_3), 4.50 (brs, 1 H, NH), 4.75 (q, 1 H, H-2, $J = 4.3$, 13.3 Hz), 6.73 (m, 1 H, H-8), 6.81 (m, 1 H, H-4′), 6.90−6.91 (m, 3 H, H-2′, H-5′, and H-6′), 7.30−7.38 (m, 2 H, H-6 and H-7), 7.87 (m, 1 H, H-5); MS *m/z* 253 (M^+). Anal. ($C_{16}H_{15}NO_2$) C, H, N.

2′-Methoxy-1,2,3,4-tetrahydro-2-phenyl-4-quinolone (19): obtained from 2′-aminoacetophenone and *o*-anisaldehyde; yield 45.7%, yellow needles; mp 126−128 °C; $[\alpha]^{25}_D$ 0° (*c* 0.38, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.85 (q, 1 H, H-3, $J = -16.2$, 4.7 Hz), 2.95 (q, 1 H, H-3, $J = -16.2$, 11.4 Hz), 3.87 (s, 3 H, OCH_3), 4.62 (brs, 1 H, NH), 5.19 (q, 1 H, H-2, $J = 4.7$, 11.4 Hz), 6.70−6.80 (m, 2 H, H-8 and H-3′), 7.30−7.90 (m, 2 H, H-6 and H-7), 6.90−7.00 (m, 3 H, H-4′, H-5′, and H-6′), 7.88 (m, 1 H, H-5′); MS *m/z* 253 (M^+). Anal. ($C_{16}H_{15}NO_2$) C, H, N.

2′-Fluoro-6,7-(methylenedioxy)-1,2,3,4-tetrahydro-2-phenyl-4-quinolone (22): obtained from 6′-amino-3′,4′-(methylenedioxy)acetophenone and 2-fluorobenzaldehyde; yield 44.3%, amorphous; mp 229−231 °C; $[\alpha]^{25}_D$ 0° (*c* 0.32, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.84 (m, 2 H, H-3), 4.39 (brs, 1 H, NH), 5.10 (m, 1 H, H-2), 5.95 (s, 2 H, OCH_2O), 6.21 (s, 1 H, H-8), 7.07−7.59 (m, 5 H, aromatic); MS *m/z* 285 (M^+). Anal. ($C_{16}H_{12}FNO_3$) C, H, N.

3′-Fluoro-6,7-(methylenedioxy)-1,2,3,4-tetrahydro-2-phenyl-4-quinolone (23): obtained from 6′-amino-3′,4′-(methylenedioxy)acetophenone and 3-fluorobenzaldehyde; yield 48.1%, orange plates; mp 231−233 °C; $[\alpha]^{25}_D$ 0° (*c* 0.32, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.75 (q, 1 H, H-3, $J = -16.5$, 5.6 Hz), 2.80 (q, 1 H, H-3, $J = -16.5$, 12.1 Hz), 4.40 (brs, 1 H, NH), 4.72 (q, 1 H, H-2, $J = 5.6$, 12.1 Hz), 5.94 (s, 2 H, OCH_2O), 6.21 (s, 1 H, H-8), 7.02−7.42 (m, 5 H, aromatic); MS *m/z* 285 (M^+). Anal. ($C_{16}H_{12}FNO_3$) C, H, N.

4′-Fluoro-6,7-(methylenedioxy)-1,2,3,4-tetrahydro-2-phenyl-4-quinolone (24): obtained from 6′-amino-3′,4′-(methylenedioxy)acetophenone and 4-fluorobenzaldehyde; yield 44.8%, amorphous; mp 238−241 °C; $[\alpha]^{25}_D$ 0° (*c* 0.35, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.66−2.88 (m, 2 H, H-3), 4.36 (brs, 1 H, NH), 4.70 (m, 1 H, H-2), 5.95 (s, 2 H, OCH_2O), 6.20 (s, 1 H, H-8), 7.12 (s, 1 H, H-5), 7.30 (m, 2 H, H-2′ and H-6′), 7.45 (m, 2 H, H-3′ and H-5′); MS *m/z* 285 (M^+). Anal. ($C_{16}H_{12}FNO_3$) C, H, N.

3′-Methoxy-6-pyrrolinyl-1,2,3,4-tetrahydro-2-phenyl-4-quinolone (26): obtained from compound **4** and *m*-anisaldehyde; yield 42.2%, yellow needles; mp 194−196 °C; $[\alpha]^{25}_D$ 0° (*c* 0.32, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.03 (m, 4 H, $CH_2CH_2NCH_2CH_2$), 2.78 (q, 1 H, H-3, $J = -16.5$, 4.3 Hz), 2.87 (q, 1 H, H-3, $J = -16.5$, 13.4 Hz), 3.28 (m, 4 H, CH_2NCH_2), 3.84 (s, 3 H, OCH_3), 4.14 (brs, 1 H, NH), 4.67 (q, 1 H, H-2, $J = 4.3$, 13.4 Hz), 6.70−7.34 (m, 7 H, aromatic); MS *m/z* 322 (M^+). Anal. ($C_{20}H_{22}N_2O_2$) C, H, N.

Resolution of (±)-1,2,3,4-Tetrahydro-2-phenyl-4-quinolones 20, 22, and 25 with (−)-5-(α-Phenethyl)semioxamazide. Racemic **20** (506 mg, 2 mmol) was dissolved in boiling MeOH (30 mL), and (−)-5-(α-phenethyl)semioxamazide (520 mg, 2.5 mmol) was added gradually together with a piece of crystalline iodine. The mixture was refluxed for 3.5 h. After cooling to room temperature, the yellow crystalline product was separated by filtration. Recrystallization from MeOH afforded **28a** (310 mg, 35.1%): needles; mp 167−168 °C; $[\alpha]^{25}_D$ −176° (*c* 0.41, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.58 (d, 3 H, CH_3), 2.61 (1 H, B-part of ABX, H-3a, $J_{3e,3a} = 16.1$ Hz), 3.03 (1 H, A-part of ABX, H-3e), 3.83 (s, 3 H, OCH_3), 4.25 (s, 1 H, NH-1), 4.43 (dd, 1 H, H-2, $J_{2,3e} = 4.0$ Hz, $J_{2,3a} = 12.9$ Hz), 5.06 (m, 1 H, CH−Ph), 6.65 (d, 1 H, H-8), 6.80−7.27 (m, 11 H, aromatic), 7.76 (d, 1 H, CNH), 8.20 (d, 1 H, H-5), 10.06 (s, 1 H, NNH); MS *m/z* 442 (M^+). Anal. ($C_{26}H_{26}N_4O_3$) C, H, N.

Concentration of the mother liquors of **28a** and recrystallization from MeOH afforded **28b** (270 mg, 30.5%): needles; mp 180−182 °C; $[\alpha]^{25}_D$ −42.3° (*c* 0.40, $CHCl_3$); ^1H NMR spectrum of **28b** was essentially identical with the spectrum of compound **28a**; MS *m/z* 442 (M^+). Anal. ($C_{26}H_{26}N_4O_3$) C, H, N.

Compounds **29a,b** and **30a,b** were prepared from **22** and **25**, respectively, using the same procedure described above for **28a,b**. ^1H NMR and MS data of the enantiomers were comparable.

Diastereoisomeric (−)-5-(α-phenethyl)oxamazones of 2′-fluoro-6,7-methylenedioxy-2-phenyl-1,2,3,4-tetrahydro-4-quinolone (29a, 29b): yield 20%, needles; mp 239−241 °C; $[\alpha]^{25}_D$ −17.4° (*c* 0.31, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.58 (d, 3 H, CH_3), 2.59 (1 H, B-part of ABX, H-3a, $J_{3e,3a} = 15.9$ Hz), 3.03 (1 H, A-part of ABX, H-3e), 4.06 (s, 1 H, NH-1), 4.82 (dd, 1 H, H-2, $J_{2,3e} = 3.8$ Hz, $J_{2,3a} = 12.0$ Hz), 5.05 (m, 1 H, CH−Ph), 5.92 (s, 2 H, OCH_2O), 6.18 (s, 1 H, H-8), 7.06−7.56 (m, 9 H, aromatic), 7.65 (s, 1 H, H-5), 7.78 (d, 1 H, CNH), 10.00 (s, 1 H, NNH); MS *m/z* 474 (M^+). Anal. ($C_{26}H_{23}FN_4O_4$) C, H, N.

29b: yield 29.4%, needles; mp 257−259 °C; $[\alpha]^{25}_D$ +100.5° (*c* 0.39, $CHCl_3$).

Diastereoisomeric (−)-5-(α-phenethyl)oxamazones of 3′-methoxy-6,7-(methylenedioxy)-2-phenyl-1,2,3,4-tetrahydro-4-quinolone (30a,b): yield 26%, needles; mp 198−200 °C; $[\alpha]^{25}_D$ −143.6° (*c* 0.33, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.57 (d, 3 H, CH_3), 2.56 (1 H, B-part of ABX, H-3a, $J_{3e,3a} = 15.7$ Hz), 3.00 (1 H, A-part of ABX, H-3e), 3.83 (s, 3 H, OCH_3), 4.12 (s, 1 H, NH-1), 4.38 (dd, 1 H, H-2, $J_{2,3e} = 7.15$ Hz, $J_{2,3a} = 14.6$ Hz), 5.06 (m, 1 H, CH−Ph), 5.92 (s, 2 H, OCH_2O), 6.17 (s, 1 H, H-8), 6.89−7.38 (m, 9 H, aromatic), 7.65 (s, 1 H, H-5), 7.78 (d, 1 H, CNH), 9.99 (s, 1 H, NNH); MS *m/z* 486 (M^+). Anal. ($C_{27}H_{26}N_4O_5$) C, H, N.

30b: yield 31.2%, needles; mp 218−220 °C; $[\alpha]^{25}_D$ −14.9° (*c* 0.35, $CHCl_3$).

(−)-3′-Methoxy-2-phenyl-1,2,3,4-tetrahydro-4-quinolone (20a). H_2SO_4 (25%) (8 mL) was added to a solution of **28a** (200 mg, 0.45 mmol) in EtOH (8 mL). After the mixture refluxed for 1 h, the EtOH was evaporated, and the reaction mixture was cooled, made neutral with $NaHCO_3$, and extracted with $CHCl_3$. The $CHCl_3$ phase was washed with water, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by column chromatography using hexane−EtOAc (4:1) as eluant to afford **20a** (70 mg, 61.1%): needles; mp 129−131 °C; $[\alpha]^{25}_D$ −19.5° (*c* 0.40, $CHCl_3$); ^1H NMR spectrum of **20a** was identical with the spectrum of racemic **20**.

Compounds **20b, 22a, 22b, 25a,** and **25b** were prepared in the same manner from **28b, 29a, 29b, 30a,** and **30b**, respectively. ^1H NMR spectra were identical with those of the racemic compounds.

(+)-3′-Methoxy-2-phenyl-1,2,3,4-tetrahydro-4-quinolone (20b): yield 72.1%, needles; mp 129−131 °C; $[\alpha]^{25}_D$ +19.5° (*c* 0.40, $CHCl_3$).

(−)-2′-Fluoro-6,7-(methylenedioxy)-2-phenyl-1,2,3,4-tetrahydro-4-quinolone (22a): yield 61.1%, needles; mp 214−215 °C; $[\alpha]^{25}_D$ −139° (*c* 0.31, $CHCl_3$).

(+)-2′-Fluoro-6,7-(methylenedioxy)-2-phenyl-1,2,3,4-tetrahydro-4-quinolone (22b): yield 68.2%, needles; mp 214−216 °C; $[\alpha]^{25}_D$ +143° (*c* 0.34, $CHCl_3$).

(−)-2′-Methoxy-6,7-(methylenedioxy)-2-phenyl-1,2,3,4-tetrahydro-4-quinolone (25a): yield 65.5%, needles; mp 230−232 °C; $[\alpha]^{25}_D$ −48° (*c* 0.30, $CHCl_3$).

(+)-2′-Methoxy-6,7-(methylenedioxy)-2-phenyl-1,2,3,4-tetrahydro-4-quinolone (25b): yield 81.8%, needles; mp 229−232 °C; $[\alpha]^{25}_D$ +50° (*c* 0.29, $CHCl_3$).

Biological Assays. The in vitro cytotoxicity assay was carried out according to procedures described in Rubinstein et al.[32] Drug stock solutions were prepared in DMSO, and the final solvent concentration was ≤2% DMSO (v/v), a concentration without effect on cell replication. The human tumor cell line panel constituted of epiderimoid carcinoma of the nasopharynx (KB), lung carcinoma (A-549), ileocecal carcinoma (HCT-8), renal cancer (CAKI-1), breast cancer (MCF-7), and melanoma cancer (SKMEL-2). Cells were cultured at 37 °C in RPMI-1640 with 100 μg/mL kanamycin and 10% (v/v) fetal bovine serum in a humidified atmosphere containing 5% CO_2. Initial seeding densities varied among the cell lines to ensure a final absorbance reading in control cultures in the range 1−2.5 A_{562} units. Drug exposure was for 3 days, and the ED_{50} value, the drug concentration that reduced the absorbance by 50%, was interpolated from dose−response data. Each test was performed in triplicate, and absorbance readings varied no more than 5%. ED_{50} values determined in independent tests varied no more than 30%.

The tubulin polymerization and [^{3}H]colchicine binding assays were performed as described previously.[21] For the polymerization assay a 25% difference in IC_{50} values represents a reproducible difference in the relative activity of two agents, while in the colchicine binding assay a 10% difference is usually reproducible.

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