# Antitumor Agents 155. Synthesis and Biological Evaluation of 3′,6,7-Substituted 2-Phenyl-4-quinolones as Antimicrotubule Agents

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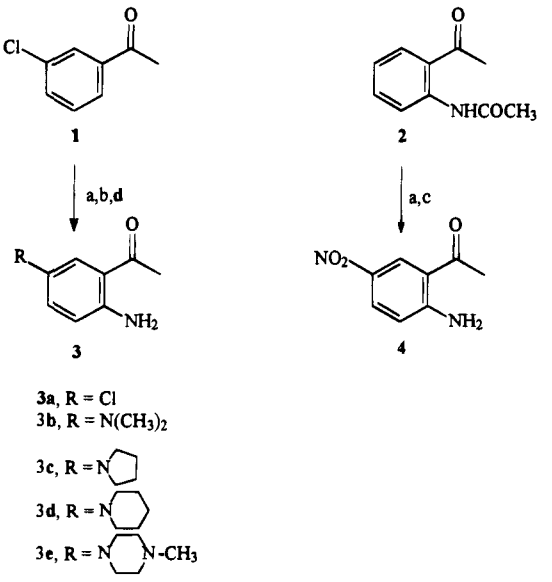
A series of 3′,6,7-substituted 2-phenyl-4-quinolones were designed and synthesized as antimitotic antitumor agents. All compounds showed cytotoxic effects (log $GI_{50} \leq -4.0$; log drug molar concentration required to cause 50% inhibition) against the growth of a variety of human tumor cell lines, including those derived from solid tumors such as non-small cell lung, colon, central nervous system, ovary, prostate, and breast cancers, when evaluated in the National Cancer Institute's 60 human tumor cell line *in vitro* screen. The most potent compound (**26**) demonstrated strong cytotoxic effects with $GI_{50}$ values in the nanomolar or subnanomolar range in almost all the tumor cell lines. Compound **26** was also a potent inhibitor of tubulin polymerization and radiolabeled colchicine binding to tubulin, with activity comparable to those of the potent antimitotic natural products colchicine, podophyllotoxin, and combretastatin A-4.

In our continuing search for antitumor agents, 2-phenyl-4-quinolones have been identified as antimitotic antitumor agents interacting with tubulin at the colchicine site.[2,3] Previously, we reported the cytotoxic effects of a series of 3′,4′,5′,5,6,7-substituted 2-phenyl-4-quinolones and their inhibition of *in vitro* tubulin polymerization. In this series,[2,3] several 2-phenyl-4-quinolones demonstrated strong inhibitory effects against a variety of human tumor cell lines, including those derived from solid tumors such as small cell lung, non-small cell lung, colon, central nervous system (CNS), and breast cancers, when evaluated in the National Cancer Institute's 60 human tumor cell line *in vitro* screen.[4,5] The most potent 2-phenyl-4-quinolones also inhibited tubulin polymerization with activity comparable to that of three potent antimitotic natural products colchicine,[6,7] podophyllotoxin,[8,9] and combretastatin A-4.[10–12] Investigation of the structure–activity relationships of the 2-phenyl-4-quinolones[3] showed that a methylenedioxy bridge at the 6,7-positions is equivalent to a methoxy group at the 6-position and that introduction of an additional methoxy at the 7-position led to a substantial loss of activity. A methoxy substituent at the *para* position of the 2-phenyl ring resulted in loss of activity, and a methoxy substituent at the *ortho* position of the 2-phenyl ring resulted in greatly reduced activity. However, compounds with methoxy groups at the *meta* position were highly active. Therefore, we synthesized a new series of 3′,6,7-substituted 2-phenyl-4-quinolones in order to investigate the steric and electronic effects of substituents at these positions with regard to their effects on cytotoxicity and inhibition of tubulin polymerization.

**Scheme 1**[a]




[a] Reagents: (a) $HNO_3$(f)/$H_2SO_4$; (b) $R_2NH$; (c) KOH/EtOH; (d) 10% Pd/C.

## Chemistry

The 3′,6,7-substituted 2-phenyl-4-quinolones were synthesized according to the previously reported method.[3] In brief, biaryl amides (**7**) were formed from an appropriately substituted *o*-aminoacetophenone (**3**, **4**, and **5**) and a 3′-substituted benzoyl chloride (**6**) in THF. Cyclization of **7** in the presence of *tert*-butoxide (*t*-BuOK) gave compounds **8**, **11**–**15**, and **18**–**29** (Scheme 2). The substituted *o*-aminoacetophenones (**3** and **4**) were prepared according to literature methods (Scheme 1).[13,14] Nitration of 3′-chloroacetophenone (**1**) gave 2′-nitro-5′-chloroacetophenone. Nucleophilic displacement of the

For part 154, see ref 1.
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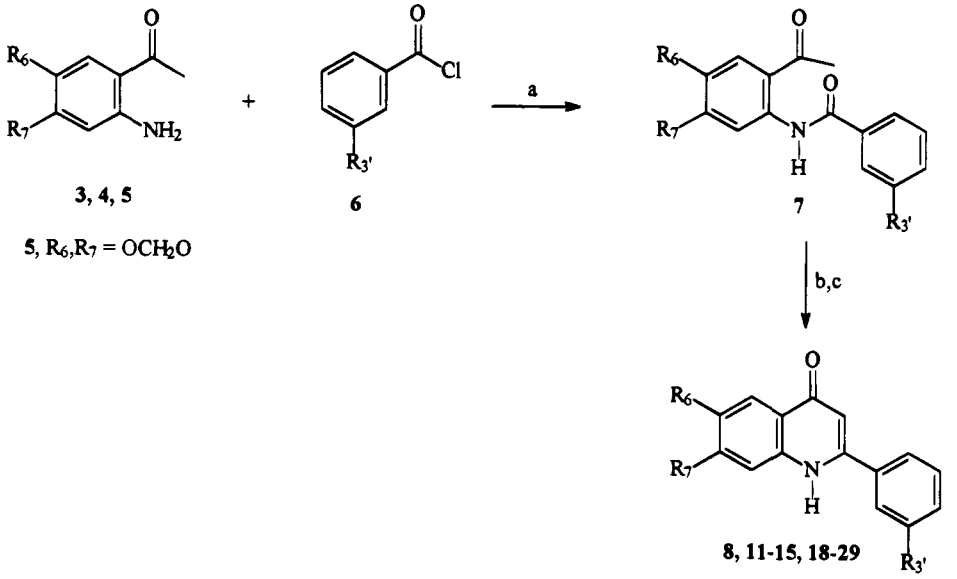
**Scheme 2**[a]




[a] Reagents: (a) $NEt_3$/THF; (b) 10% Pd/C ($R_6 = NO_2$); (c) *t*-BuOK/*t*-BuOH.

5′-chloro group by different secondary amines followed by hydrogenation gave **3b–e**. Nitration of 2′-acetamidoacetophenone (**2**) followed by hydrolysis with KOH/EtOH gave **4**. The preparation of 2′-amino-4′,5′-(methylenedioxy)acetophenone (**5**) was reported previously.[3] The structures and chemical features of the newly synthesized compounds are summarized in Table 1.

## Result and Discussion

**a. Evaluation of the Cytotoxicity of 3′,6,7-Substituted 2-Phenyl-4-quinolones.** The 2-phenyl-4-quinolones were evaluated in the National Cancer Institute's (NCI) in vitro disease-oriented antitumor screen, which determines a test agent's effect on growth against a panel of approximately 60 human tumor cell lines.[4,5] In the recently revised screen, prostate and breast cancer cell lines have been added. The cytotoxicity data (expressed as log $GI_{50}$ values, which represents the log molar drug concentration required to cause 50% inhibition) for selected tumor cell lines and the average log $GI_{50}$ values taken from all tumor cell lines tested are given in Table 2. All compounds had cytotoxic activity (log $GI_{50} \leq -4.0$) against a variety of human tumor cell lines, including leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancers. While compounds **8**, **9**, **11**, **12**, **15–17**, **21**, **28**, and **29** showed strong cytotoxic effects toward most of the tumor cell lines with $GI_{50}$ values in the micromolar to nanomolar range, compound **26** had exceptional activity. This highly cytotoxic agent had subnanomolar $GI_{50}$ values for most tumor cell lines (Table 2; Figure 2 presents the mean graph presentation of differential data for compound **26**). Moreover, in the panels of CNS, colon, and breast cancer, compound **26** totaly inhibited the growth of at least half the tumor cell lines at subnanomolar concentrations (log TGI < −9.00; see Figure 2). The cytotoxicity assays for compounds **22** and **27** are pending. We speculate that the $GI_{50}$ values for compounds **27** could also be in the nanomolar concentration range due to their structural similarity to compound **26**.

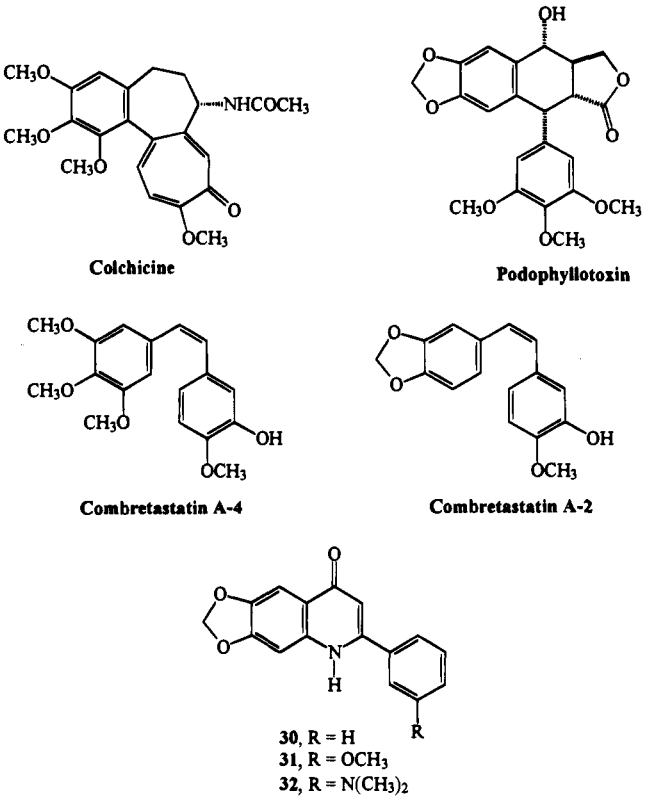
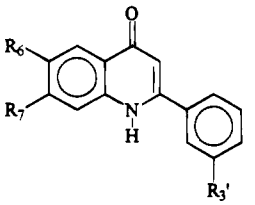
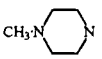



**Figure 1.**

**b. Interactions of 3′,6,7-Substituted 2-Phenyl-4-quinolones with Tubulin.** 2-Phenyl-4-quinolones have been found to be antimicrotubule agents, which interact with tubulin at the colchicine site.[2,3] Previously,[2,3] we had reported two series of 3′,4′,5′,5,6,7,8-substituted 2-phenyl-4-quinolones and related compounds. In order to further delineate structure–activity relationships, we synthesized a series of 3′,6,7-substituted compounds. The inhibition effects of these derivatives on tubulin polymerization and on the radiolabeled colchicine binding to tubulin are summarized in Table 1. Most compounds (**8–17**, **21**, **22**, and **26–28**) were potent inhibitors of polymerization with $IC_{50}$ values less than 1.0 $\mu$M. Their activity in this assay was thus

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



| compd | $R_6$ | $R_7$ | $R_3'$ | ITP[a] $IC_{50}$ ($\mu$M) ± SD | ICB[b] (% inhibition) | formula[c] | mp, °C | yield, % |
|---|---|---|---|---|---|---|---|---|
| **8** | $OCH_2O$ | | $NH_2$ | 0.73 ± 0.2 | 16 ± 7 | $C_{16}H_{12}N_2O_3 \cdot 0.5H_2O$ | >250[d] | 43 |
| **9** | $OCH_2O$ | | $NHCH_3$ | 0.65 ± 0.1 | 17 ± 11 | $C_{17}H_{14}N_2O_3 \cdot 0.2H_2O$ | 273–274[d] | 30 |
| **10** | $OCH_2O$ | | OH | 0.47 ± 0.1 | 26 ± 3 | $C_{16}H_{11}NO_4 \cdot H_2O$ | >300[d] | 27 |
| **11** | $OCH_2O$ | | F | 0.53 ± 0.1 | 51 ± 3 | $C_{16}H_{10}FNO_3 \cdot 0.25H_2O$ | >300[d] | 68 |
| **12** | $OCH_2O$ | | Cl | 0.37 ± 0.03 | 45 ± 7 | $C_{16}H_{10}ClNO_3$ | 190–190 | 70 |
| **13** | $OCH_2O$ | | Br | 0.53 ± 0.1 | 26 ± 3 | $C_{16}H_{10}BrNO_3$ | >300 | 69 |
| **14** | $OCH_2O$ | | $CF_3$ | 0.82 ± 0.2 | 5 ± 7 | $C_{17}H_{10}F_3NO_3$ | 281–184[d] | 72 |
| **15** | $OCH_2O$ | | $OCF_3$ | 0.50 ± 0.1 | 40 ± 5 | $C_{17}H_{10}F_3NO_4$ | >300 | 80 |
| **16** | $OCH_2O$ | | $OCH_2CH_3$ | 0.47 ± 0.1 | 40 ± 2 | $C_{18}H_{15}NO_4 \cdot 0.1H_2O$ | >280[d] | 56 |
| **17** | $OCH_2O$ | | OBz | 0.45 ± 0.03 | 26 ± 5 | $C_{23}H_{17}NO_4 \cdot 0.75H_2O$ | >300[d] | 60 |
| **18** | $NH_2$ | H | Cl | 6.1 ± 1 | | $C_{15}H_{11}ClN_2O$ | 261–263[d] | 60 |
| **19** | $NH_2$ | H | $CF_3$ | >40.0 | | $C_{16}H_{11}F_3N_2O$ | >280[d] | 68 |
| **20** | $NH_2$ | H | $OCH_3$ | 8.7 ± 3 | | $C_{16}H_{14}N_2O_2 \cdot 1.5H_2O$ | 124–126 | 64 |
| **21** | $N(CH_3)_2$ | H | $OCH_3$ | 0.38 ± 0.1 | 14 ± 8 | $C_{18}H_{18}N_2O_2 \cdot 0.2H_2O$ | 246–248[d] | 61 |
| **22** | Cl | H | $OCH_3$ | 0.70 ± 0.2 | 16 ± 5 | $C_{16}H_{12}ClNO_2$ | 283–285 | 65 |
| **23** | $NHCOCH_3$ | H | F | 1.5 ± 0.5 | | $C_{17}H_{13}FN_2O_2$ | >300 | 62 |
| **24** | $NHCOCH_3$ | H | Cl | 2.3 ± 0.7 | | $C_{17}H_{13}ClN_2O_2 \cdot 0.5H_2O$ | >300 | 64 |
| **25** | $NHCOCH_3$ | H | $OCH_3$ | 2.1 ± 0.5 | | $C_{18}H_{16}N_2O_3$ | 298–300 | 60 |
| **26** |  | H | $OCH_3$ | 0.44 ± 0.02 | 84 ± 6 | $C_{20}H_{20}N_2O_2$ | 276–278[d] | 68 |
| **27** |  | H | $OCF_3$ | 0.72 ± 0.2 | 58 ± 5 | $C_{20}H_{17}F_3N_2O_2$ | 280–282[d] | 66 |
| **28** |  | H | $OCH_3$ | 0.36 ± 0.1 | 43 ± 10 | $C_{20}H_{20}N_2O_3 \cdot 0.5H_2O$ | 280–282[d] | 73 |
| **29** |  | H | $OCH_3$ | 14 ± 2 | | $C_{21}H_{23}N_3O_2 \cdot 0.5H_2O$ | 273–275[d] | 69 |
| **30** | $OCH_2O$ | | H | 0.63 ± 0.2 | 26 ± 10 | | | |
| **31** | $OCH_2O$ | | $OCH_3$ | 0.57 ± 0.1 | 39 ± 8 | | | |
| **32** | $OCH_2O$ | | $N(CH_3)_2$ | 0.70 ± 0.03 | 29 ± 7 | | | |
| colchicine | | | | 0.80 ± 0.07 | | | | |
| podophyllotoxin | | | | 0.46 ± 0.02 | 84 ± 2 | | | |
| combretastatin A-4 | | | | 0.53 ± 0.05 | 94 ± 2 | | | |
| dihydrocombretastatin A-4 | | | | 0.63 ± 0.03 | 65 ± 4 | | | |

[a] ITP = inhibition of tubulin polymerization. [b] ICB = inhibition of colchicine binding and evaluated only when polymerization $IC_{50} \leq 1.0$ $\mu$M. [c] All compounds were analyzed for C, H, and N, and results agreed to ±0.4% of the theoretical values. [d] Decomposed.

equivalent to that observed with the natural products colchicine, podophyllotoxin, and combretastatin A-4 (Table 1). Only compound **19** was inactive ($IC_{50}$ > 40 $\mu$M). A range of activity ($IC_{50}$ values from 1.5 to 14 $\mu$M) was observed with the remaining agents. Overall, highly cytotoxic agents showed strong inhibitory effects on polymerization.

A variety of substituents at the 3′-position of the 6,7-(methylenedioxy)-substituted compounds (**8**–**17**) were found to be well-tolerated ($IC_{50}$ < 1.0 $\mu$M). These substituents include both electron-donating groups such as $NH_2$, $NHCH_3$, OH, $OCF_3$, OEt, and OBz and electron-withdrawing groups such as F, Cl, and Br. The size of the substituents can be as small as H (**30**) and as large as OBz (**17**) without changing the activity significantly. Our previously reported 6,7-methylenedioxy substituted compounds **31** (3′-$OCH_3$) and **32** (3′-$N(CH_3)_2$) were also highly active ($IC_{50}$ values of 0.57 and 0.70 $\mu$M, respectively).[3]

Compounds with a heterocyclic ring at the 6-position (**26**–**28**) were highly active ($IC_{50}$ < 1.0 $\mu$M), except for compound **29** ($IC_{50}$ = 14 $\mu$M), which has a 1-methylpiperazinyl group at this position. The reason for the loss of activity in **29** is not clear. Although it could have resulted from formation of a salt on the second nitrogen, the relatively bulky size of this cyclic amine compared to other heterocyclic groups such as the pyrrolinyl might be a factor. The corresponding noncyclic 6-(*N*,*N*-dimethylamino)-substituted compound (**21**) was highly active ($IC_{50}$ = 0.38 $\mu$M).

The three 6-$NH_2$ substituted compounds were only moderately active (**18** and **20**) or inactive (**19**). However, as stated before, the dimethylamino derivative **21** and the acetamido-substituted compounds (**23**–**25**) showed strong to moderate inhibitory activity. The reason for the substantial loss of activity in the $NH_2$-substituted compounds is not clear. It could result from the relatively polar nature and/or small size of the amino group.

The most potent compounds ($IC_{50} \leq 1.0$ $\mu$M) were also evaluated for potential inhibition of radiolabeled colchicine binding to tubulin (Table 1). In this series of experiments, the potential inhibitors and radiolabeled colchicine were present in equimolar concentrations and in 5-fold molar excess over tubulin. The initial series[2] of phenylquinolone derivatives had feeble activity as

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

| | | cytotoxicity log $GI_{50}$ (M)[c] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| compd | average log $GI_{50}$[d] | K-562 | NCI-H226 | HCT116 | KM202L | SF-268 | SF-295 | SK-Mel-5 | OVCAR-3 | OVCAR-4 | RXF-393 | DU-145 | MDA-N |
| **8** | −5.79 | −6.05 | −5.66 | −6.32 | −6.15 | −6.12 | −5.62 | −6.03 | nt[e] | −4.41 | −6.22 | nt | nt |
| **9** | −6.29 | −6.40 | −6.27 | −6.61 | −6.48 | −5.26 | −6.49 | −6.35 | −6.47 | nt | −6.44 | nt | nt |
| **10** | −5.51 | −6.14 | nt | −5.74 | −5.48 | −5.64 | −5.26 | −5.83 | nt | nt | nt | nt | nt |
| **11** | −6.47 | −6.47 | −6.02 | −6.66 | nt | −6.18 | −6.85 | −6.57 | −6.54 | nt | nt | nt | nt |
| **12** | −6.30 | −6.51 | −5.74 | −6.47 | −6.57 | −6.21 | −6.65 | −6.68 | −6.74 | >−4.12 | −6.79 | nt | nt |
| **13** | −5.48 | −5.46 | −5.42 | −5.47 | −5.51 | −5.52 | −6.48 | −5.36 | −5.37 | −[f] | −5.62 | nt | nt |
| **14** | −5.56 | nt | −5.24 | −5.70 | −5.86 | −5.34 | −6.00 | −5.89 | −5.93 | −5.00 | −5.79 | −5.53 | −6.09 |
| **15** | −6.38 | −7.44 | −6.71 | −7.85 | −7.44 | nt | −7.59 | −6.77 | −7.20 | −7.59 | −7.39 | −7.34 | <−8.0 |
| **16** | −6.65 | −7.35 | −6.49 | −6.76 | −6.74 | −6.57 | −7.19 | −6.72 | −6.78 | nt | −6.56 | −6.60 | −7.75 |
| **17** | −6.17 | −6.44 | −6.64 | −6.41 | −6.65 | −4.89 | −6.35 | −6.55 | −6.61 | − | −6.39 | −5.96 | −6.84 |
| **18** | −5.26 | −5.66 | −5.27 | nt | −5.50 | −5.17 | −5.56 | nt | −5.54 | − | −5.74 | nt | nt |
| **19** | −4.40 | −4.45 | −4.41 | −4.28 | −4.46 | −4.23 | −4.55 | nt | −4.58 | −4.20 | −4.63 | −4.36 | −4.98 |
| **20** | −5.34 | −5.68 | −5.23 | −5.44 | −5.46 | −5.25 | −5.51 | nt | −5.56 | −4.08 | −5.72 | −5.41 | −6.12 |
| **21** | −7.52 | −8.05 | −7.35 | nt | −8.34 | −7.36 | −7.81 | −7.59 | −7.82 | −5.26 | −7.73 | −7.46 | −8.77 |
| **22**[g] | | | | | | | | | | | | | |
| **23** | −4.82 | −5.21 | −4.63 | −5.34 | −4.45 | −4.59 | −4.41 | −5.44 | −5.99 | − | − | − | −5.78 |
| **24** | −4.98 | −5.38 | −4.89 | −5.36 | −4.73 | −5.33 | −4.73 | nt | −5.47 | − | −5.09 | −4.56 | −5.71 |
| **25** | −4.70 | −5.34 | −4.27 | −4.95 | −4.49 | −5.26 | −4.35 | nt | −5.66 | −4.71 | −5.28 | −4.95 | −5.49 |
| **26** | −8.73 | <−9.00 | <−9.00 | <−9.00 | <−9.00 | <−9.00 | <−9.00 | <−9.00 | <−9.00 | −5.70 | <−9.00 | <−9.00 | <−9.00 |
| **27**[g] | | | | | | | | | | | | | |
| **28** | −7.25 | −7.61 | −7.47 | −7.57 | −7.47 | −7.27 | −7.31 | −7.45 | −7.70 | −5.20 | −7.91 | −7.36 | −8.08 |
| **29** | −5.81 | −6.27 | −5.82 | −6.36 | −5.82 | −5.73 | −5.74 | −6.04 | −6.11 | >−5.0 | −6.34 | −5.60 | −6.70 |

[a] Data obtained from NCI's in vitro disease-oriented human tumor cells screen (see refs 4 and 5 for detail). [b] K-562, leukemia cell line; NCI-H226, non-small cell lung cancer cell line; HCT-116 and KM20L2, colon cancer cell lines; OVCAR-3 and OVCAR-4, ovarian cancer cell lines; RXF-393, renal cancer cell line; SK-Mel-5, melanoma; SF-268 and SF-295, CNS tumor cell lines; DU-145, prostate cancer cell line; MDA-N, breast cancer cell line. [c] Log concentrations which reduced cell growth to 50% of level at start of experiment. [d] Average log $GI_{50}$ values calculated from all cell lines tested. [e] "nt" means not tested. [f] "−" means log $GI_{50}$ is greater than −4. [g] Cytotoxicity assay is pending.

inhibitors of colchicine binding; substantial inhibition could only be detected when they were in significant molar excess over colchicine.[2,3] In contrast, we have routinely observed 80−90% inhibition with equimolar podophyllotoxin and inhibition as high as 97% with equimolar combratastatin A-4. In the 6,7-methylenedioxy derivatives reported more recently,[3] three of seven potent inhibitors of polymerization (i.e., $IC_{50}$ values ≤ 1.0 μM) were more effective as inhibitors of colchicine binding. When equimolar with colchicine, these compounds inhibited binding of the radiolabeled ligand by 30−40%, but there was little correlation between inhibitory effects on tubulin polymerization and colchicine binding. Although this lack of correlation between the two tubulin-based assays is also observed in the new agents described here (Table 1), five compounds (**11**, **12**, **26**, **27**, and **28**) have greater inhibitory effects on colchicine binding than those previously reported.[2,3] In particular, the inhibitory activity of compound **26** was equal to that of podophyllotoxin, and since compound **26** is, thus far, the most cytotoxic agent in this entire series, it will be of interest to determine with future compounds whether strong inhibition of colchicine binding will be predictive of exceptional cytotoxicity.[15]

Besides compound **26**, two of the other relatively strong inhibitors of colchicine binding had heterocyclic substituents at position C(6). The second most active inhibitor, compound **27**, like **26**, has a pyrrolinyl group at C(6), but the methoxy group at C(3′) in **26** is replaced by a $OCF_3$ substituent in **27**.[16]

The remaining two agents with relatively strong inhibitory effects on colchicine binding were compounds **11** and **12**. These compounds contain a 6,7-methylenedioxy group and have F and Cl substituents, respectively, at C(3′). Compared with the unsubstituted **30**, which inhibited colchicine binding by 26%, a C(3′) Br (compound **13**) also resulted in 26% inhibition versus 45% inhibition with Cl and 51% inhibition with F. Thus, there was an apparent size and/or electron-withdrawing effect of halide substituents at C(3′).

In summary, based on this limited data set, we found that compounds with a $NH_2$ (**18**−**20**) or a $CH_3CONH$ (**23**−**25**) group at the 6-position were only moderately active or inactive either as inhibitors of tubulin polymerization or as cytotoxic agents. Compounds with a $OCH_2O$ bridge at the 6,7-positions (compounds **8**−**17**) or with a $N(CH_3)_2$ (compound **21**) or a $OCH_3$ (previously reported,[3] data not shown) group at the 6-position were highly active. However, compounds with a heterocyclic group such as a pyrrolinyl or a morpholinyl group (compounds **26**−**28**) at that position showed significantly increased activity. The reason for the differences of activity with these substituents is not clear. Further work will be done to elucidate the optimal substitution pattern for the 2-phenyl-4-quinolones.

## 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. $^1H$ 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.

**3′-Amino-6,7-(methylenedioxy)-2-phenyl-4-quinolone (8).** To a solution of 2-amino-4,5-(methylenedioxy)acetophenone (700 mg, 3.9 mmol) and $Et_3N$ (1.7 mL, 12.6 mmol) in THF (10 mL) at 0 °C was added dropwise 3′-nitrobenzoyl chloride (797 mg, 4.2 mol). After 30 min at 0 °C, the mixture

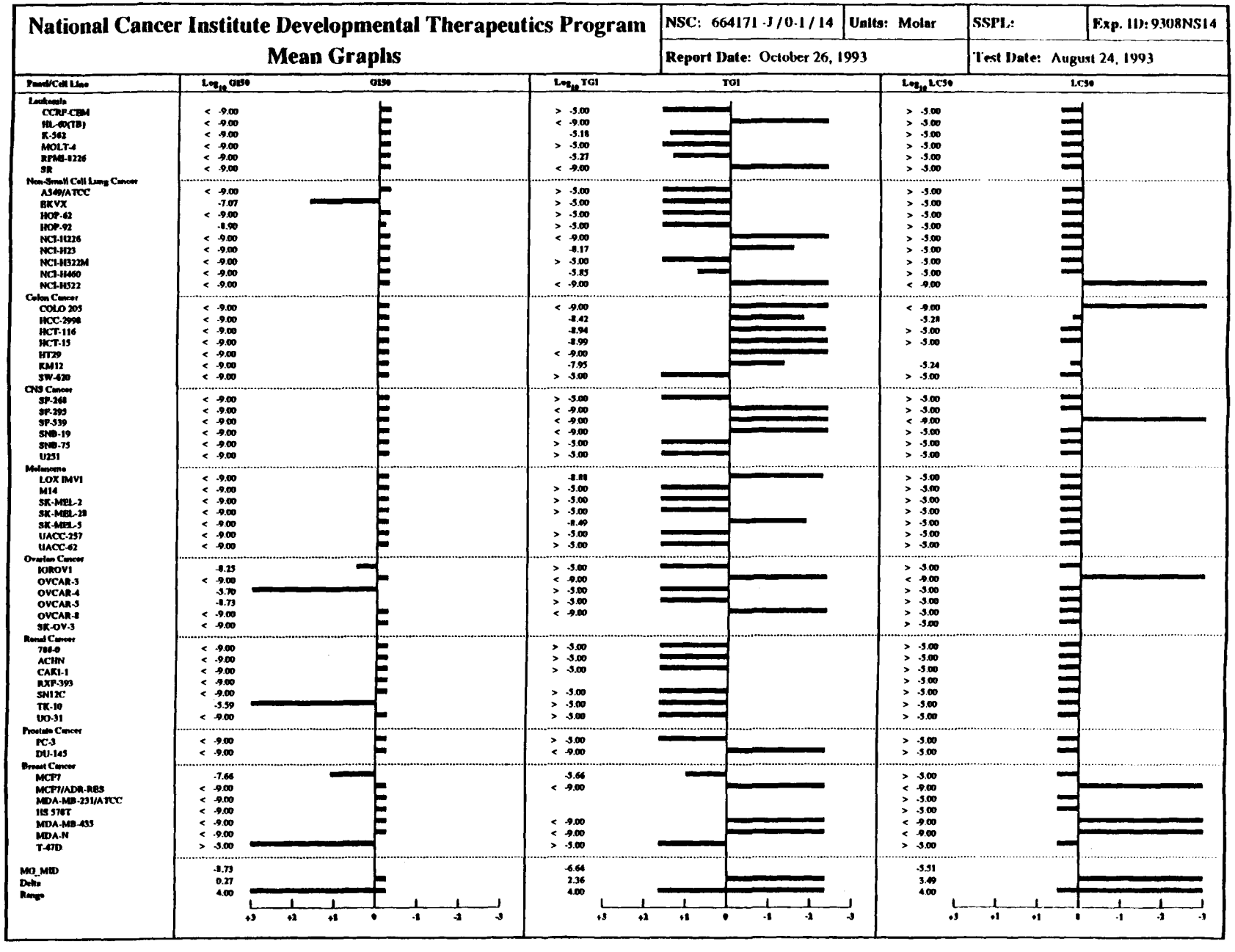



**Figure 2.** Mean graph presentation of differential data for compound **26**.

was stirred at room temperature for 2 h and poured into 20 mL of ice water. The precipitate was collected and washed with water and MeOH. The solid (1.18 g, 92%) was dissolved in 200 mL of a mixture of EtOAc and MeOH (1:1) and hydrogenated over 10% Pd/C for 4 h. The catalyst was removed by filtration, and the solution was dried by evaporation. The solid residue was dried in vacuo, and suspended in 20 mL of *t*-BuOH. Potassium *tert*-butoxide (1.55 g, 13.8 mmol) was added, and the mixture was heated at 70 °C under $N_2$ for 20 h. The mixture was cooled to room temperature and poured onto 30 mL of aqueous $NH_4Cl$ solution. The solid was collected and washed with distilled water (several times) and with a mixture of $CHCl_3$ and MeOH (1:10); amorphous; $^1$H NMR (DMSO-$d_6$) $\delta$ 5.38 (br s, 2 H, $NH_2$), 6.11 (s, 1 H, H-3), 6.14 (s, 2 H, $OCH_2O$), 6.72 (dd, $J$ = 1.6, 7.5 Hz, 1 H, H-4′), 6.86 (d, $J$ = 7.5 Hz, 1 H, H-6′), 6.89 (d, $J$ = 1.6 Hz, 1 H, H-2′), 7.18 (s, 1 H, H-8), 7.19 (t, $J$ = 7.5 Hz, 1 H, H-5′), 7.38 (s, 1 H, H-5), 11.51 (br s, 1 H, NH); IR (KBr) 3440, 3330, 3090, 1640 cm$^{-1}$; MS ($M^+$) 280. Anal. ($C_{16}H_{12}N_2O_3$·0.5$H_2O$) C, H, N.

**3′-(*N*-Methylamino)-6,7-(methylenedioxy)-2-phenyl-4-quinolone (9):** obtained from 3′-(*N*-methylamino)acetophenone and 2-amino-4,5-(methylenedioxy)-*N*,*N*-diethylbenzamide by the procedure reported previously;[3] amorphous; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.75 (d, $J$ = 4.5 Hz, 3 H, NHC$H_3$), 5.96 (q, $J$ = 4.5 Hz, 1 H, N$H$CH$_3$), 6.15 (s, 2 H, $OCH_2O$), 6.17 (s, 1 H, H-3), 6.71 (dd, $J$ = 1.8, 8.1 Hz, 1 H, H-4′), 6.83 (d, $J$ = 1.8 Hz, 1 H, H-2′), 6.89 (br d, $J$ = 8.1 Hz, 1H, H-6′), 7.20 (s, 1 H, H-8), 7.27 (t, $J$ = 8.1 Hz, 1 H, H-5′), 7.39 (s, 1 H, H-5), 11.51 (br s, 1 H, NH); IR (KBr) 3340, 3240, 3090, 1630 (sh) cm$^{-1}$; MS ($M^+$) 294. Anal. ($C_{17}H_{14}N_2O_3$·0.2$H_2O$) C, H, N.

**3′-Hydroxy-6,7-(methylenedioxy)-2-phenyl-4-quinolone (10):** obtained from 3′-hydroxyacetophenone and 2-amino-4,5-(methylenedioxy)-*N*,*N*-diethylbenzamide by the procedure reported previously;[3] amorphous; $^1$H NMR (DMSO-$d_6$) $\delta$ 6.15 (s, 2 H, $OCH_2O$), 6.17 (s, 1 H, H-3), 6.95 (dd, $J$ = 1.8, 8.0 Hz, 1 H, H-4′), 7.12 (br s, 1 H, H-2′), 7.18 (br d, $J$ = 8.0 Hz, 1 H, H-6′), 7.20 (s, 1 H, H-8), 7.37 (t, $J$ = 8.0 Hz, 1 H, H-5′), 7.39 (s, 1 H, H-5), 9.85 (s, 1 H, OH), 11.53 (br s, 1 H, NH); IR (KBr) 3200, 3090, 1610 (sh) cm$^{-1}$; MS ($M^+$) 281. Anal. ($C_{16}H_{11}NO_4$·$H_2O$) C, H, N.

**3′-Fluoro-6,7-(methylenedioxy)-2-phenyl-4-quinolone (11):** obtained from 3′-fluorobenzoyl chloride and 2-amino-4,5-(methylenedioxy)acetophenone;[3] amorphous; $^1$H NMR (DMSO-$d_6$) $\delta$ 6.17 (s, 2 H, $OCH_2O$), 6.33 (s, 1 H, H-3), 7.19 (s, 1 H, H-8), 7.40 (s, 1 H, H-5), 7.41 (m, 1 H, Ar$H$ of ring C), 7.65 (m, 3 H, Ar$H_3$ of C ring); IR (KBr) 3440, 1635 cm$^{-1}$; MS ($M^+$) 283. Anal. ($C_{16}H_{10}FNO_3$·0.25$H_2O$) C, H, N.

**3′-Chloro-6,7-(methylenedioxy)-2-phenyl-4-quinolone (12):** obtained from 3′-chlorobenzoyl chloride and 2-amino-4,5-(methylenedioxy)acetophenone; amorphous; $^1$H NMR (DMSO-$d_6$) $\delta$ 6.17 (s, 2 H, $OCH_2O$), 6.31 (s, 1 H, H-3), 7.18 (s, 1 H, H-8), 7.40 (s, 1 H, H-5), 7.60 (t, $J$ = 8.0 Hz, 1 H, H-5′), 7.64 (br dd, $J$ = 2.0, 8.0 Hz, 1 H, H-6′), 7.77 (br dd, $J$ = 2.0, 8.0 Hz, 1 H, H-4′), 7.89 (br s, 1 H, H-2′), 11.63 (br s, 1 H, NH); IR (KBr) 3090, 1640 cm$^{-1}$. Anal. ($C_{16}H_{10}ClNO_3$) C, H, N.

**3′-Bromo-6,7-(methylenedioxy)-2-phenyl-4-quinolone (13):** obtained from 3′-bromobenzoyl chloride and 2-amino-4,5-(methylenedioxy)acetophenone; amorphous; $^1$H NMR (DMSO-$d_6$) $\delta$ 6.17 (s, 2 H, $OCH_2O$), 6.30 (s, 1 H, H-3), 7.18 (s, 1 H, H-8), 7.40 (s, 1 H, H-5), 7.53 (t, $J$ = 8.0 Hz, 1 H, H-5′), 7.76 (dd, $J$ = 2.0, 8.0 Hz, 1 H, H-6′), 7.77 (dd, $J$ = 2.0, 8.0 Hz, 1 H, H-4′), 8.01 (br s, 1 H, H-2′); IR (KBr) 3080, 1620 cm$^{-1}$. Anal. ($C_{16}H_{10}BrNO_3$) C, H, N.

**3′-(Trifluoromethyl)-6,7-(methylenedioxy)-2-phenyl-4-quinolone (14):** obtained from 3′-(trifluoromethyl)benzoyl chloride and 2-amino-4,5-(methylenedioxy)acetophenone; amorphous; $^1$H NMR (DMSO-$d_6$) $\delta$ 6.17 (s, 2 H, $OCH_2O$), 6.35 (d, $J$ = 1.5 Hz, 1 H, H-3), 7.18 (s, 1 H, H-8), 7.41 (s, 1 H, H-5), 7.81 (br t, $J$ = 7.9 Hz, 1 H, H-5′), 7.94 (br d, $J$ = 7.9Hz, 1 H, H-6′), 8.11 (br d, $J$ = 7.9 Hz, 1 H, H-4′), 8.14 (br s, 1 H, H-2′), 11.71 (br s, 1 H, NH); IR (KBr) 3090, 1620 cm$^{-1}$. Anal. ($C_{17}H_{10}F_3NO_3$) C, H, N.

**3′-(Trifluoromethoxy)-6,7-(methylenedioxy)-2-phenyl-4-quinolone (15):** obtained from 3′-(trifluoromethoxy)benzoyl chloride and 2-amino-4,5-(methylenedioxy)acetophenone; amorphous; $^1$H NMR (DMSO-$d_6$) $\delta$ 6.15 (s, 2 H, $OCH_2O$), 6.61 (s, 1 H, H-3), 7.17 (s, 1 H, H-8), 7.44 (br d, $J$ = 8.0 Hz, 1 H, H-4′), 7.53 (s, 1 H, H-5), 7.61 (br s, 1 H, H-2′), 7.63 (t, $J$ = 8.0 Hz, 1 H, H-5′), 7.72 (br d, $J$ = 8.0 Hz, 1 H, H-6′); IR (KBr) 3090, 1610 cm$^{-1}$. Anal. ($C_{17}H_{10}F_3NO_4$) C, H, N.

**3′-Ethoxy-6,7-(methylenedioxy)-2-phenyl-4-quinolone (16):** obtained from 3′-ethoxyacetophenone and 2-amino-4,5-(methylenedioxy)-*N*,*N*-diethylbenzamide by the procedure reported previously;[3] amorphous; $^1$H NMR (DMSO-$d_6$) $\delta$ 1.37 (t, $J$ = 7.0 Hz, 3 H, $OCH_2CH_3$), 4.14 (q, $J$ = 7.0 Hz, 2 H, $OCH_2$-$CH_3$), 6.16 (s, 2 H, $OCH_2O$), 6.28 (s, 1 H, H-3), 7.12 (br d, $J$ = 8.0 Hz, 1 H, H-4′), 7.20 (s, 1 H, H-8), 7.31 (br s, 1 H, H-2′), 7.33 (br d, $J$ = 8.0 Hz, 1 H, H-6′), 7.39 (s, 1 H, H-5), 7.47 (t, $J$ = 8.0 Hz, 1 H, H-5′); IR (KBr) 3080, 1620 cm$^{-1}$; MS ($M^+$) 309. Anal. ($C_{18}H_{15}NO_4$·0.1$H_2O$) C, H, N.

**3′-(Benzyloxy)-6,7-(methylenedioxy)-2-phenyl-4-quinolone (17):** obtained from 3′-(benzyloxy)acetophenone and 2-amino-4,5-(methylenedioxy)-*N*,*N*-diethylbenzamide by the procedure reported previously;[3] amorphous; $^1$H NMR (DMSO-$d_6$) $\delta$ 5.22 (s, 2 H, $OCH_2$Ph), 6.15 (s, 2 H, $OCH_2O$), 6.29 (s, 1 H, H-3), 7.20–7.50 (m, 10 H, Ar$H$); IR (KBr) 3090, 1620 cm$^{-1}$; MS ($M^+$) 371. Anal. ($C_{23}H_{17}NO_4$·0.75$H_2O$) C, H, N.

**3′-Chloro-6-amino-2-phenyl-4-quinolone (18):** obtained from 3′-chlorobenzoyl chloride and 2-amino-5-nitroacetophenone[13] as described as in the preparation of **8**; amorphous; $^1$H NMR (DMSO-$d_6$) $\delta$ 6.19 (s, 1 H, H-3), 7.02 (dd, $J$ = 2.0, 8.5 Hz, 1 H, H-4′), 7.20 (d, $J$ = 2.0 Hz, 1 H, H-2′), 7.50 (br d, $J$ = 8.5 Hz, 1 H, H-6′), 7.58 (t, $J$ = 8.5 Hz, 1 H, H-5′), 7.59 (d, $J$ = 9.0 Hz, 1 H, H-8), 7.75 (br d, $J$ = 9.0 Hz, 1 H, H-7), 7.87 (br s, 1 H, H-5), 11.49 (br s, 1 H, NH); IR (KBr) 3340, 3360, 3080, 1630 cm$^{-1}$. Anal. ($C_{15}H_{11}ClN_2O$) C, H, N.

**3′-(Trifluoromethyl)-6-amino-2-phenyl-4-quinolone (19):** obtained from 3′-(trifluoromethyl)benzoyl chloride and 2-amino-5-nitroacetophenone as described in the preparation of **8**; amorphous; $^1$H NMR (DMSO-$d_6$) $\delta$ 5.33 (br s, 2 H, $NH_2$), 6.23 (s, 1 H, H-3), 7.03 (dd, $J$ = 2.0, 8.8 Hz, 1 H, H-7), 7.20 (d, $J$ = 2.0 Hz, 1 H, H-5), 7.50 (d, $J$ = 8.8 Hz, 1 H, H-8), 7.79 (br t, $J$ = 7.8 Hz, 1 H, H-5′), 7.91 (br d, $J$ = 7.8 Hz, 1 H, H-6′), 8.09 (br d, $J$ = 7.8 Hz, 1 H, H-4′), 8.12 (br s, 1 H, H-2′), 11.56 (br s, 1 H, NH); IR (KBr) 3390, 3280, 3090, 1620 cm$^{-1}$. Anal. ($C_{16}H_{11}F_3N_2O$) C, H, N.

**3′-Methoxy-6-amino-2-phenyl-4-quinolone (20):** obtained from 3′-methoxybenzoyl chloride and 2-amino-5-nitroacetophenone as described in the preparation of **8**; amorphous; $^1$H NMR (DMSO-$d_6$) $\delta$ 3.86 (s, 3 H, $OCH_3$), 5.29 (br s, 1 H, $NH_2$), 6.17 (s, 1 H, H-3), 7.00 (dd, $J$ = 2.1, 8.7 Hz, 1 H, H-7), 7.11 (br dd, $J$ = 1.7, 7.8 Hz, 1 H, H-4′), 7.19 (d, $J$ = 2.1 Hz, 1 H, H-5), 7.30 (br d, $J$ = 1.7 Hz, 1 H, H-2′), 7.33 (br d, $J$ = 7.8 Hz, 1 H, H-6′), 7.47 (t, $J$ = 7.8 Hz, 1 H, H-5′), 7.51 (d, $J$ = 8.0 Hz, 1 H, H-8), 11.40 (br s, 1 H, NH); IR (KBr) 3340, 3240, 3090, 1600 cm$^{-1}$; MS ($M^+$) 266. Anal. ($C_{16}H_{14}N_2O_2$·1.5$H_2O$) C, H, N.

**3′-Methoxy-6-(*N*,*N*-dimethylamino)-2-phenyl-4-quinolone (21):** obtained from 3′-methoxybenzoyl chloride and 2-amino-5-(*N*,*N*-dimethylamino)acetophenone;[14] amorphous; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.98 (s, 6 H, $N(CH_3)_2$), 3.87 (s, 3 H, $OCH_3$), 6.27 (s, 1 H, H-3), 7.12 (br dd, $J$ = 1.9, 7.9 Hz, 1 H, H-4′), 7.23 (d, $J$ = 2.0, 1 H, H-5), 7.30 (dd, $J$ = 2.0, 9.0 Hz, 1 H, H-7), 7.34 (br d, $J$ = 1.9 Hz, 1 H, H-2′), 7.37 (br dd, $J$ = 1.9, 7.9 Hz, 1 H, H-6′), 7.49 (t, $J$ = 7.9 Hz, 1 H, H-5′), 7.67 (d, $J$ = 9.0 Hz, 1 H, H-8), 11.50 (br s, 1 H, NH); IR (KBr) 3250, 3150, 3080, 1600 cm$^{-1}$; MS ($M^+$) 294. Anal. ($C_{18}H_{18}N_2O_2$·0.2$H_2O$) C, H, N.

**3′-Methoxy-6-chloro-2-phenyl-4-quinolone (22):** obtained from 3′-methoxybenzoyl chloride and 2-amino-5-chloroacetophenone;[14] amorphous; $^1$H NMR (DMSO-$d_6$) $\delta$ 3.87 (s, 3 H, $OCH_3$), 6.42 (s, 1 H, H-3), 7.17 (br dd, $J$ = 1.9, 7.9 Hz, 1 H, H-4′), 7.34 (br d, $J$ = 1.9 Hz, 1 H, H-2′), 7.39 (br d, $J$ = 7.9 Hz, 1 H, H-6′), 7.51 (t, $J$ = 7.9 Hz, 1 H, H-5′), 7.72 (dd, $J$ = 1.9, 8.9 Hz, 1 H, H-7), 7.81 (d, $J$ = 8.9 Hz, 1 H, H-8), 8.03 (d, $J$ = 1.9 Hz, 1 H, H-5); IR (KBr) 3090, 1630 cm$^{-1}$. Anal. ($C_{16}H_{12}ClNO_2$) C, H, N.

**3′-Fluoro-6-acetamido-2-phenyl-4-quinolone (23):** obtained from 3′-fluorobenzoyl chloride and 2-amino-5-acetamidoacetophenone;[13] amorphous; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.08 (s, 3 H, $COCH_3$), 6.34 (s, 1 H, H-3), 7.43 (dt, $J$ = 2.0, 7.0 Hz, H-5′), 7.59–7.73 (m, 5 H, H-5, 8, 2′, 4′, 6′), 7.89 (dd, $J$ = 2.5, 9.0 Hz,

H-7); IR (KBr) 3300, 3180, 3100, 1660, 1640 $cm^{-1}$. Anal. ($C_{17}H_{13}FN_2O_2$) C, H, N.

**3'-Chloro-6-acetamido-2-phenyl-4-quinolone (24):** obtained from 3'-chlorobenzoyl chloride and 2-amino-5-acetamidoacetophenone; amorphous; $^1H$ NMR (DMSO-$d_6$) $\delta$ 2.08 (s, 3 H, $COCH_3$), 6.32 (d, $J$ = 1.4 Hz, 1 H, H-3), 7.60 (br t, $J$ = 7.8 Hz, 1 H, H-5), 7.64 (br dd, $J$ = 1.9, 7.8 Hz, 1 H, H-6'), 7.70 (d, $J$ = 8.9 Hz, 1 H, H-8), 7.79 (br dd, $J$ = 1.9, 7.8 Hz, 1 H, H-4'), 7.90 (dd, $J$ = 2.2, 8.9 Hz, 1 H, H-7), 7.92 (br s, 1 H, H-2'), 8.32 (d, $J$ = 2.2 Hz, 1 H, H-5), 10.15 (br s, 1 H, NH), 11.75 (br s, 1 H, NH); IR (KBr) 3300, 3180, 3100, 1660, 1630 $cm^{-1}$; MS ($M^+$) 270. Anal. ($C_{17}H_{13}ClN_2O_2 \cdot 0.5H_2O$) C, H, N.

**3'-Methoxy-6-acetamido-2-phenyl-4-quinolone (25):** obtained from 3'-methoxybenzoyl chloride and 2-amino-5-acetamidoacetophenone; amorphous; $^1H$ NMR (DMSO-$d_6$) $\delta$ 2.08 (s, 3 H, $COCH_3$), 3.87 (s, 3 H, $OCH_3$), 6.30 (s, 1 H, H-3), 7.14 (dd, $J$ = 1.5, 8.5 Hz, 1 H, H-4'), 7.35 (br s, 1 H, H-2'), 7.38 (br d, $J$ = 8.5 Hz, 1 H, H-6'), 7.50 (t, $J$ = 8.5 Hz, 1 H, H-5'), 7.71 (d, $J$ = 9.0 Hz, H-8), 7.88 (dd, $J$ = 1.5, 9.0 Hz, 1 H, H-7), 8.32 (d, $J$ = 1.5 Hz, 1 H, H-5); IR (KBr) 3280, 3170, 3090, 1680, 1640 $cm^{-1}$. Anal. ($C_{18}H_{16}N_2O_3$) C, H, N.

**3'-Methoxy-6-pyrrolinyl-2-phenyl-4-quinolone (26):** obtained from 3'-methoxybenzoyl chloride and 2-amino-5-pyrrolinylacetophenone;[14] amorphous; $^1H$ NMR (DMSO-$d_6$) $\delta$ 2.00 (m, 4 H, $CH_2CH_2NCH_2CH_2$), 2.50 (m, 4 H, $CH_2NCH_2$), 6.26 (s, 1 H, H-3), 7.03 (br s, 1 H, H-5), 7.11 (dd, $J$ = 1.5, 8.5 Hz, 2 H, H-4', H-7), 7.34 (br s, 1 H, H-2'), 7.37 (br d, $J$ = 8.5 Hz, 1 H, H-6'), 7.48 (t, $J$ = 8.5 Hz, 1 H, H-5'), 7.67 (d, $J$ = 9.1 Hz, 1 H, H-8), 11.48 (br s, 1 H, NH); IR (KBr) 3240, 3090, 1600 $cm^{-1}$. Anal. ($C_{20}H_{20}N_2O_2$) C, H, N.

**3'-(Trifluoromethoxy)-6-pyrrolinyl-2-phenyl-4-quinolone (27):** obtained from 3'-(trifluoromethoxy)benzoyl chloride and 2-amino-5-pyrrolinylacetophenone; amorphous; $^1H$ NMR (DMSO-$d_6$) $\delta$ 2.09 (m, 4 H, $CH_2CH_2NCH_2CH_2$), 3.41 (m, 4 H, $CH_2NCH_2$), 6.56 (s, 1 H, H-3), 7.13 (dd, $J$ = 2.7, 9.0 Hz, 1 H, H-7), 7.22 (d, $J$ = 2.7 Hz, 1 H, H-5), 7.39 (br d, $J$ = 7.9 Hz, 1 H, H-4'), 7.60 (t, $J$ = 7.9 Hz, 1 H, H-5'), 7.63 (br d, $J$ = 1.9 Hz, H-2'), 7.65 (d, $J$ = 9.0 Hz, 1 H, H-8), 7.72 (br d, $J$ = 7.9 Hz, 1 H, H-6'); IR (KBr) 3260, 3100, 1600 $cm^{-1}$. Anal. ($C_{20}H_{17}F_3N_2O_2$) C, H, N.

**3'-Methoxy-6-morpholinyl-2-phenyl-4-quinolone (28):** obtained from 3'-methoxybenzoyl chloride and 2-amino-5-morpholinylacetophenone;[14] amorphous; $^1H$ NMR (DMSO-$d_6$) $\delta$ 3.16 (t, $J$ = 4.7 Hz, 4 H, $CH_2NCH_2$), 3.78 (t, $J$ = 4.7 Hz, 4 H, $CH_2OCH_2$), 3.87 (s, 3 H, $OCH_3$), 6.30 (d, $J$ = 1.5 Hz, 1 H, H-3), 7.13 (dd, $J$ = 3.0, 8.0 Hz, 1 H, H-4'), 7.34 (d, $J$ = 3.0 Hz, 1 H, H-2'), 7.36 (br d, $J$ = 8.0 Hz, 1 H, H-6'), 7.44 (d, $J$ = 2.5 Hz, 1 H, H-5), 7.49 (t, $J$ = 9.0 Hz, 1 H, H-5'), 7.50 (dd, $J$ = 2.5, 9.0 Hz, 1 H, H-7), 7.69 (d, $J$ = 9.0 1 H, H-8); IR (KBr) 3250, 3090, 1600 $cm^{-1}$; MS ($M^+$) 349. Anal. ($C_{20}H_{20}N_2O_3 \cdot 0.5H_2O$) C, H, N.

**3'-Methoxy-6-(4-methylpiperazinyl)-2-phenyl-4-quinolone (29):** obtained from 3'-methoxybenzoyl chloride and 2-amino-5-(4-methylpiperazinyl)acetophenone;[14] amorphous; $^1H$ NMR (DMSO-$d_6$) $\delta$ 2.25 (s, 3 H, N-$CH_3$), 3.18, 3.31 (both m, 4 H each, ($NCH_2CH_2N$) × 2), 3.87 (s, 3 H, $OCH_3$), 6.29 (d, $J$ = 1.5 Hz, 1 H, H-3), 7.13 (dd, $J$ = 2.0, 8.0 Hz, 1 H, H-4'), 7.34 (dd, $J$ = 2.0, 2.0 Hz, 1 H, H-2'), 7.37 (br d, $J$ = 8.0 Hz, 1 H, H-6'), 7.42 (d, $J$ = 2.5 Hz, 1 H, H-5), 7.48 (dd, $J$ = 2.5, 9.0 Hz, 1 H, H-7), 7.49 (t, $J$ = 8.0 Hz, 1 H, H-5'), 7.67 (d, $J$ = 9.0 Hz, 1 H, H-8); IR (KBr) 3280, 3160, 3090, 1670, 1650 $cm^{-1}$; MS ($M^+$) 349. Anal. ($C_{21}H_{23}N_3O_2 \cdot 0.5H_2O$) C, H, N.

**Biochemical Assays.** Electrophoretically homogeneous bovine brain tubulin was purified as described previously.[17] Combretastatin A-4 was a generous gift of Dr. G. R. Pettit, Arizona State University. Dihydrocombretastatin A-4 was prepared as described previously.[18] [$^3H$]Colchicine was obtained from Dupont, nonradiolabeled colchicine 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.[2] Reaction mixtures contained 1.0 $\mu$M tubulin, 5.0 $\mu$M [$^3H$]colchicine, and 5.0 $\mu$M potential inhibitor.

The tubulin polymerization assay was performed as described previously.[3] In brief, tubulin at 1.2 mg/mL (12 $\mu$M) was preincubated for 15 min at 26 °C in a 0.24 mL volume in 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 $\mu$M (26–27 $\mu$M from the glutamate, 8–9 $\mu$M 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 $\mu$L 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. Baselines 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 at least 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 was 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 in some cases the standard deviations were 30–35% from 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.

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(15) The average log $GI_{50}$ for compound **26** is −8.7. For comparison, the NCI cell screen has yielded average log $GI_{50}$ values of −7.4 for colchicine, −7.6 for podophyllotoxin, and −8.7 for combretastatin A-4.

(16) With the 6,7-methylenedioxy substituent, compound 31 with a C(3′)-$OCH_3$ group and compound **15** with a C(3′)-$OCF_3$ group had identical activities as inhibitors of colchicine binding. In contrast, although it was a potent inhibitor of polymerization, compound **14**, with a C(3′)-$CF_3$ group, had minimal effect on colchicine binding (when present in equimolar with colchicine).

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