Antitumor Agents. 211. Fluorinated 2-Phenyl-4-quinolone Derivatives as Antimitotic Antitumor Agents[1]

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Fluorinated 2-phenyl-4-quinolone derivatives were synthesized and evaluated in National Cancer Institute's 60 human tumor cell line in vitro screen. From the results, the ketone moiety plays an essential role in activity. Among the compounds tested, 2′-fluoro-6-pyrrol-2-phenyl-4-quinolone (**13**) exhibited the most potent cytotoxic activities (log $GI_{50} < -8.00$) against renal and melanoma tumor cell lines. Compound **13** was also a potent inhibitor of tubulin polymerization ($IC_{50} = 0.46\ \mu M$) and of radiolabeled colchicine binding to tubulin, with activities comparable to those of the potent antimitotic natural products colchicine, podophyllotoxin, and combretastatin A-4.

Introduction

Microtubules are an important subcellular target for development of anticancer chemotherapeutic agents. Vinca alkaloids[2] and taxoids[3] are well-known examples of antimitotic agents that are widely used clinically to treat different cancers. Colchicine[4,5] (Figure 1) is another well-known agent that inhibits microtubule assembly. Although colchicine has limited utility for cancer therapy, the drug is 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.

Previously, we reported the synthesis and biological evaluation of a series of 2-phenyl-4-quinolones as a new class of antimitotic antitumor agents.[6−8] They were evaluated in the National Cancer Institute's (NCI's) 60 human tumor cell line (HTCL) in vitro screen and in a tubulin polymerization inhibition assay. Most compounds showed promising cytotoxicity in the HTCL assay with GI_{50} values in the low micromolar to nanomolar concentration range. In general, a good correlation was found between cytotoxicity and inhibition of tubulin polymerization. SAR studies led to the discovery of 2′-fluoro-6,7-methylenedioxy-2-phenyl-4-quinolone (**1**),[7] which showed potent cytotoxicity with an average log GI_{50} value of −6.47 (log of the concentration that reduced cell growth by 50%) in the HTCL screen. Compound **1** (Figure 1) was also a potent inhibitor of tubulin polymerization with an IC_{50} value of 0.85 μM. Most importantly, **1** demonstrated good in vivo activity against the OVCAR-3 ovarian cell line, prolonging the life span of mice bearing the tumor by 130%. Thus, because **1** was the only compound to show such good in vivo activity and that contained a fluorine atom at the 2′-position, synthesis of additional fluorinated quinolones and further characterizations of pharmacophores were warranted. It is well-known that fluorinated drugs often show unique pharmacological properties. Here, we describe the synthesis of enol ether (**2**−**5**), thioketone (**6**), and carbamate (**7**) derivatives of **1**.

Two functional moieties are present in **1**: a ketone and an amine. Ketone oxygen and amine hydrogen atoms are frequently involved in drug-receptor binding; however, the importance of these two functional moieties has not been well explored with the 2-phenyl-4-quinolone class. On the other hand, 2-phenyl-4-quinolones can tautomerize into a 4-hydroxy-2-phenylquinolone enol form, as seen by the absence of an infrared carbonyl stretching frequency in $CHCl_3$ solution. Although the keto−enol equilibrium usually lies far in favor of the keto form, the enol form can, under proper conditions, be trapped by alkylation of the hydroxyl group. The enol ether derivatives may undergo ready hydrolysis with liberation of the free enol in vivo, which then reverts to the ketone form. By appropriate selection of the alkyl group, enol ether derivatives can be obtained that have varying hydrolysis rates as well as varying lipophilicity/aqueous solubility ratios.[9] In addition, this modification can determine whether activity is maintained with both the ketone form and the enol form.

Therefore, we first converted the ketone moiety of **1** to various enol ethers (**2**−**5**) (Scheme 1). Different chain lengths should result in compounds with varying hy-

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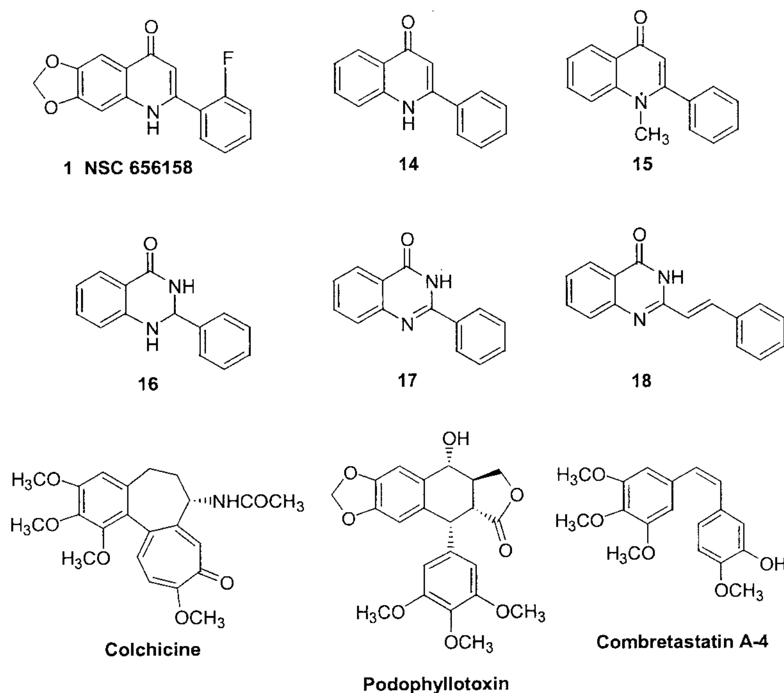
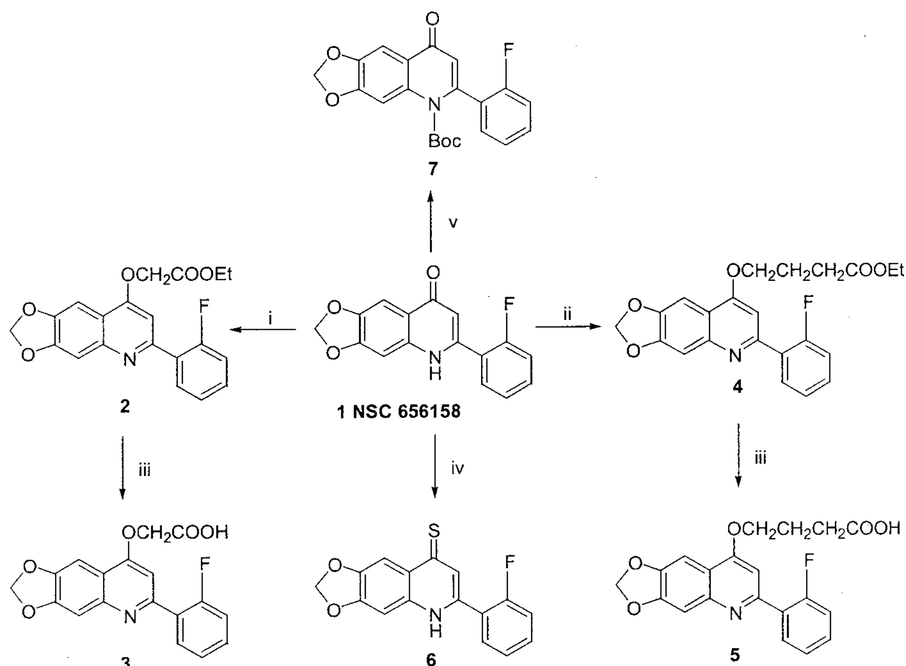


Figure 1.

Scheme 1[a]

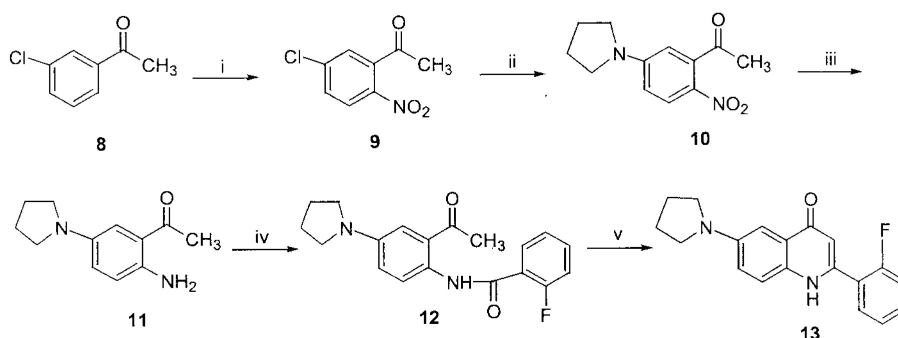


[a] (i) NaH/DMF, $ClCH_2COOEt$; (ii) NaH/DMF, $ClCH_2CH_2CH_2COOEt$; (iii) 10% NaOH; (iv) Lawessen reagent–toluene, reflux; (v) Boc–CH_2Cl_2, room temp.

drophobicity and steric hindrance. Hydrolysis of the esters (**2, 4**) to the carboxylic acids (**3, 5**) allowed the preparation of water-soluble salts. These compounds could circumvent the solubility problem in the 2-phenyl-4-quinolone class of compounds, which have only moderate solubility even in DMSO. We also synthesized the thioketone derivative (**6**) of **1**. Interchanging such groups in bioactive molecules often results in different solubility and bioavailability properties.[10] Second, we explored the importance of the amine hydrogen atom by replacing this atom with *tert*-butoxycarbonyl (Boc), a frequently used amino protecting group, in compound **7**. Compounds **2**–**7** were synthesized to explore which pharmacophores of the lead compound are essential for its antitumor activity as well as its interaction with tubulin.

Finally, in our previous studies of 2-phenyl-4-quinolones, compounds with a heterocyclic ring at the 6-position showed increased activity in in vitro cytotoxicity and tubulin-based assays.[8] These results prompted us to synthesize the fluorinated 6-heterocyclic quinolone **13** (Scheme 2).

Scheme 2[a]



[a] (i) HNO_3(f)$-H_2SO_4$; (ii) pyrrolidine; (iii) 10% Pd/C; (iv) 2′-fluorobenzoyl chloride$-Et_3N$, THF; (v) *t*-BuOK$-$*t*-BuOH.

Table 1. Inhibition of in Vitro Tumor Cell Growth[a] by Fluorinated Quinolone Derivatives

compd	cytotoxicity log GI_{50} (M)[b]								
	K562	NCI-H226	HCT116	OVCAR-3	RXF-393	SK-Mel5	SF-268	SF-295	mean log GI_{50}
1	*c*	−6.35	−7.22	−7.09	Nt	−7.68	−5.64	−7.26	−6.87
2	−4.42	>−4.00	−4.14	*c*	>−4.00	−4.14	>−4.00	>−4.00	−4.10
3	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00
4	−4.41	−4.31	>−4.00	−4.82	−4.60	−4.24	>−4.00	−4.21	−4.32
5	−5.68	−5.07	−5.40	−5.63	−5.11	−5.40	−4.79	−5.56	−5.33
6	−4.25	−4.78	−5.26	−4.67	−4.53	−5.39	−4.70	−4.93	−4.81
7	>−4.00	−7.60	−7.35	−7.49	>−4.00	*c*	*c*	−6.93	−6.23
13	−7.49	−7.51	−7.47	−7.35	<−8.00	<−8.00	−7.41	−7.73	−7.62

[a] Data obtained from NCI's in vitro disease-oriented human tumor cell screen. K-562, leukemia cell line; NCI-H226, non-small-cell lung cancer cell line; HCT-116, colon cancer cell line; OVCAR-3, ovarian cancer cell line; RXF-393, renal cancer cell line; RXF-393, renal cancer cell line; SK-Mel5, melanoma; SF-286 and SF-295, CNS tumor cell lines. [b] The log concentrations that reduced cell growth by 50%. [c] Not tested.

Synthesis and Biological Assays

Synthesis of 2′-fluoro-6,7-methylenedioxy-2-phenyl-4-quinolone (**1**) was previously reported.[7] The syntheses of enol ether derivatives (**2**−**5**) are shown in Scheme 1. Treatment of **1** with NaH in DMF followed by alkylation with ethyl chloroacetate or ethyl 4-chlorobutyrate afforded **2** and **4**. Hydrolysis of **2** and **4** with 10% NaOH in MeOH gave carboxylic acids **3** and **5**, respectively. Treating **1** with Lawesson's reagent in toluene gave thioketone **6.** The nitrogen atom in **1** was converted to the carbamate (**7**) with *tert*-butoxycarbonyl (Boc) chloride in CH_2Cl_2 at room temperature.

Synthesis of **13** (Scheme 2) was based on a literature method.[7] Nitration of 3′-chloroacetophenone (**8**) gave 2′-nitro-5′-chloroacetophenone. Nucleophilic displacement of the 5′-chloro group by pyrroline followed by hydrogenation gave **11**. The biarylamide (**12**) was formed from condensation of **11** and 2-fluorobenzoyl chloride in THF. Cyclization of **12** in the presence of potassium *tert*-butoxide (*t*-BuOK) gave **13**.

Compounds **1**−**7** and **13** were tested in National Cancer Institute's HTCL screen.[11,12] This assay involves determination of a test agent's effect on growth parameters against a panel of approximately 60 human tumor cell lines, mostly derived from solid tumors. The cytotoxic effects of each compound were expressed as log GI_{50} values, which represents the log molar drug concentration required to cause 50% inhibition for selected tumor cell lines. These compounds were also assayed as inhibitors of tubulin polymerization, and the most active compounds were assayed as inhibitors of [^{3}H]colchicine binding to tubulin.

Table 2. Antitubulin Effects of Fluorinated Quinolone Derivatives

compd	ITP[a] (%) IC_{50} (μM) ± SD	ICB[b] (%) 5 μM[c]	1 ± μM[c]
1	0.68 ± 0.02	39 ± 2	
2	>40		
3	>20		
4	>40		
5	12 ± 3		
6	24 ± 2		
7	3.2 ± 1		
13	0.46 ± 0.003	93 ± 1	76 ± 5
14[d]	7.3 ± 1		
15[d]	>40		
16[e]	14 ± 0.9		
17[e]	>40		
18[e]	5.0 ± 0.6		
colchicine[f]	0.80 ± 0.07		
podophyllotoxin[f]	0.46 ± 0.02		
combretastatin A-4[f]	0.53 ± 0.05	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] Data from ref 6. [e] Data from ref 16. [f] Data from ref 15.

Results and Discussion

The cytotoxic activities of **1**−**7** and **13** are summarized in Table 1 and effects on tubulin-based assays in Table 2. The results showed that the cytotoxicity decreased about 100-fold when the ketone form of **1** was converted to an enol ether (**2**−**5**). Compounds **2**−**5** lack both functional moieties in the B ring (the amine H and the ketone O), and these compounds had little or no effect on tubulin polymerization. Reduced cytotoxic activity was also found with **6**, where a thioketone moiety replaced the ketone group. Thioketone **6,** like the enol

ethers, had minimal effect on tubulin polymerization. These observations suggest that the ketone moiety plays a crucial role in the interaction of 2-phenyl-4-quinolone derivatives with tubulin and in the inhibition of cell growth that results from this interaction. Although the exact reason is uncertain, possible factors are steric and electronic influences or reduced H-bonding ability between drug and target protein.

When the Boc protecting group replaced the amine hydrogen, the resulting compound (**7**) showed interesting cytotoxic data. Compound **7** was less active than **1** against the SF-295 CNS tumor cell line, equipotent with **1** against the HCT-116 colon, and OVCAR-3 ovarian cancer cell lines, and almost 20-fold more active than **1** against the NCI-H226 non-small-cell lung cancer. These cells are among those that are exceptionally sensitive to antitubulin agents,[13] and **7** retained moderate activity as an inhibitor of tubulin polymerization. It is also possible that **7** undergoes intracellular conversion to a more active compound. The reduced interaction of **7** relative to **1** with tubulin could be derived from either steric factors (bulky *tert*-butyl group) or loss of the amine hydrogen (altering H-bonding interactions with tubulin). The loss of antitubulin activities of *N*-methylquinolone (**15** vs **14**, Figure 1)[6] and of 2-phenylquinazolinone (**17** vs **16**)[16] supports the idea of a requirement for the N hydrogen. However, activity was substantially enhanced when the phenyl group of inactive compound **17** was replaced by a styryl group (compound **18**).[16] This result supports the hypothesis that steric factors account for the loss of activity in **7** vs **1**. However, it is also possible that the binding site for 2-phenyl-4-quinolones and quinazolinones (phenyl C ring directly attached to the B ring) does not completely overlap the binding site of 2-styrylquinazolin-4(3H)-one (**18**), which has a linker between the phenyl C ring and the B ring.

Among the new compounds, **13** (a fluorinated quinolone with a heterocyclic ring at the 6-position) was the most potent in all assays. It was more cytotoxic than **1** in virtually all cases, especially against RXF-393 renal and SK-Mel5 melanoma cancer cells with log GI_{50} values of less than −8.00. Over all 60 cell lines, **13** was about 6-fold more active than **1**, as shown by the mean log GI_{50} values. In keeping with its greater cytotoxicity **13** was more potent than **6** as an inhibitor of tubulin assembly, but the greater affinity of **13** relative to **6** was best demonstrated by its substantially greater activity as an inhibitor of the binding of [^{3}H]colchicine to tubulin. In the latter assay, **13** was nearly as active as the highly potent combretastatin A-4.[14]

Previously, 2-phenyl-4-quinolones were found to inhibit tubulin polymerization and the binding of radiolabeled colchicine to tubulin.[5−7] Numerous compounds with various substitutions on both A and C rings were studied. The "biaryl system", composed of rings A and C, is probably[15] analogous to the similar biaryl system occurring in many antimitotic natural products such as colchicine, podophyllotoxin, and combretastatin A-4[4] (Figure 1). However, the pharmacophores in the B ring of phenylquinolones have been relatively unexplored, and we show here that the ketone functional moiety is essential for a strong interaction with tubulin, providing additional insight into the mechanism of ligand binding at the colchicine site. The ketone oxygen and, most importantly, the ketone form of the B ring appear to be involved in the binding of this class of compound to tubulin. The amine hydrogen of the B ring also may be important for maximal antitubulin activity, suggested by the reduced activity of **7,** but steric factors remain to be excluded as the explanation for the reduced activity of this compound. Additional studies on the mechanism of action are ongoing, including the synthesis of additional analogues.

Experimental Section

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 an internal reference and $CDCl_3$ as the solvent. Flash chromatography was performed on silica gel (mesh 25−150 μm) using a mixture of hexanes−ethyl acetate as eluant.

2′-Fluoro-6,7-(methylenedioxy)-2-phenyl-4-quinolone (1).[7] 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 and MeOH. The solid was dried under vacuum and then suspended in 20 mL of *tert*-butyl alcohol. Potassium *tert*-butoxide (1.17 g, 10.5 mmol) was added, and the mixture was heated under N_2 at 70 °C for 24 h. The mixture was cooled and poured into 30 mL of aqueous NH_4Cl solution. The solid was collected and washed successively with water and a mixture of $CHCl_3$ and MeOH (10:1). The crude product was recrystallized from a mixture of $CHCl_3$ and MeOH (20:1). ^{1}H NMR (DMSO-d_6): δ 6.20 (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′). Anal. ($C_{16}H_{10}FNO_3$) C, H, N.

2′-Fluoro-6,7-(methylenedioxy)-2-phenyl-4-(*O*-ethyl acetate)quinoline (2). Compound **1** (283 mg, 1 mmol) was dissolved in dry DMF (12 mL), and NaH (60% in oil, 110 mg, 2.8 mmol) was added portionwise with stirring at 40 °C. Ethyl chloroacetate (500 mg, 4.08 mmol) was added, and the reaction mixture was stirred for 2 h at 60 °C. The reaction mixture was poured into ice−water and filtered. The precipitate obtained was washed with water and recrystallized from CH_2Cl_2−MeOH to afford 260 mg of **2**; yield 71.2%, mp 119−120 °C. ^{1}H NMR ($CDCl_3$): δ 1.31 (t, J = 3.7 Hz, 3 H, CH_3), 4.32 (q, J = 7.2 Hz, 2 H, CH_2CH_3), 4.88 (s, 2 H, OCH_2COO), 6.13 (s, 2 H, OCH_2O), 7.04 (s, 1 H, H-3), 7.17 (m, 1 H, H-3′), 7.30 (m, 1 H, H-5′), 7.40 (m, 1 H, H-4′), 7.42 (s, 1 H, H-8), 7.60 (s, 1 H, H-5), 8.06 (m, 1 H, H-6′). Anal. ($C_{20}H_{16}FNO_5$) C, H, N.

2′-Fluoro-6,7-(methylenedioxy)-2-phenyl-4-(*O*-acetic acid)quinoline (3). Compound **2** (160 mg, 0.44 mmol) was treated with aqueous NaOH (10%, 15 mL). The reaction mixture was refluxed for 2 h and cooled to room temperature. Aqueous HCl (10%) was added until the pH was 1−2. The resulting precipitate was harvested by filtration and recrystallized from MeOH−$CHCl_3$ to give a light-yellow solid, **3**; 130 mg, yield 87.0%, mp >300 °C. ^{1}H NMR (DMSO-d_6): δ 5.18 (s, 2 H, OCH_2COO), 6.33 (s, 2 H, OCH_2O), 7.42 (s, 1 H, H-3), 7.45 (s, 1 H, H-8), 7.48 (m, 2 H, H-3′ and H-5′), 7.54 (s, 1 H, H-5), 7.65 (m, 1 H, H-4′), 7.91 (m, 1 H, H-6′). Anal. ($C_{18}H_{12}FNO_5 \cdot 0.25\ H_2O$) C, H, N.

2′-Fluoro-6,7-(methylenedioxy)-2-phenyl-4-(*O*-ethyl 4′-butyrate)quinoline (4). **4** was obtained from **1** and ethyl 4-chlorobutyrate; yield 77.6%, mp 93−94 °C. ^{1}H NMR ($CDCl_3$): δ 1.27 (t, 3 H, CH_3, J = 7.0 Hz), 2.29 (m, 2 H, $CH_2CH_2CH_3$), 2.62 (t, J = 7.29 Hz, 2 H, H-3′), 4.18 (q, J = 7.0 Hz, 2 H, OCH_2CH_3), 4.28 (q, J = 6.0 Hz, 2 H, OCH_2CH_2,), 6.12 (s, 2 H, OCH_2O), 7.13 (s, 1 H, H-3), 7.15−7.30 (m, 3 H, H-3′, H-4′, H-5′), 7.41 (s, 1 H, H-8), 7.46 (s, 1 H, H-5), 8.02 (m, 1 H, H-6′). Anal. ($C_{22}H_{20}FNO_5$) C, H, N.

2′-Fluoro-6,7-(methylenedioxy)-2-phenyl-4-(*O*-ethyl 4′-butylacetic acid)quinoline (5). **5** was obtained by hydrolysis of **4** with aqueous NaOH, using the same synthetic procedure as for **3**; yield 82.5%, mp >300 °C. ^{1}H NMR (DMSO-d_6): δ 2.10 (m, 2 H, CH_2*CH_2*CH_2), 2.49 (t, 2 H, CH_2COO), 4.29 (t, 2 H, OCH_2), 6.22 (s, 2 H, OCH_2O), 7.23 (s, 1 H, H-3), 7.33 (m, 1 H, H-3′), 7.35 (m, 1 H, H-5′), 7.38 (s, 1 H, H-8), 7.44 (s, 1 H, H-5), 7.52 (m, 1 H, H-4′), 7.95 (m, 1 H, H-6′). Anal. ($C_{20}H_{16}FNO_5$) C, H, N.

2′-Fluoro-6,7-(methylenedioxy)-2-phenylquino-4-thione (6). Compound **1** (500 mg, 1.77 mmol) in 30 mL of dry toluene was stirred for a few minutes at room temperature, and Lawessen reagent (1.07 g, 2.65 mmol) was added with continued stirring. The mixture was stirred at 110−120 °C for 24 h and became clear with a deep-orange color. The mixture was cooled to room temperature, poured into water, and extracted with CH_2Cl_2. The organic layer was dried over sodium sulfate and concentrated. Chromatography using CH_2Cl_2/CH_3OH as eluant afforded 430.6 mg of **6**; yield 81.5%, mp 226−228 °C. ^{1}H NMR (DMSO-d_6): δ 6.24 (s, 2 H, OCH_2O), 7.18 (s, 1 H, H-3), 7.33 (s, 1 H, H-8), 7.50 (m, 2 H, H-3′, H-5′), 7.72 (m, 1 H, H-4′), 7.77 (m, 1 H, H-6′), 8.08 (s, 1 H, H-5), 12.93 (s, 1 H, NH). Anal. ($C_{16}H_{10}FNO_2S \cdot 1.05\ H_2O$) C, H, N.

***N*-Boc-2′-fluoro-6,7-(methylenedioxy)-2-phenyl-4-quinolone (7).** To a solution of **1** (283 mg, 1 mmol) in 6 mL of methylene chloride were added triethylamine (0.15 mL, 1 mmol), di-*tert*-butyl dicarbonate (436 mg, 2 mmol), and 4-(dimethylamino)pyridine (61.25 mg, 1 mmol). The solution was stirred for 24 h at room temperature under N_2. The mixture was poured into water, extracted with CH_2Cl_2, and washed with water. The organic layer was dried over sodium sulfate and concentrated. Chromatography using EtOAc−hexane as eluant afforded **7**; yield 86.8%, mp 118−120 °C. ^{1}H NMR ($CDCl_3$): δ 1.61 (s, 9 H, 3 × CH_3), 6.14 (s, 2 H, OCH_2O), 7.14 (m, 1 H, H-3′), 7.20 (s, 1 H, H-3), 7.29 (s, 1 H, H-5), 7.40 (m, 1 H, H-5′), 7.46 (s, 1 H, H-8), 7.71 (m, 1 H, H-4′), 8.07 (m, 1 H, H-6′). Anal. ($C_{21}H_{18}FNO_5$) C, H, N.

2′-Fluoro-6-pyrroyl-2-phenyl-4-quinolone (13). 2-Amino-5-pyrroylacetophenone (**11**, 1 g, 4.9 mmol), prepared from commercially available 3′-chloroacetophenone according to literature procedures,[8,17] was dissolved in 10 mL of THF and 2 mL of triethylamine. The mixture was cooled in an ice bath. A solution of 2-fluorobenzoyl chloride (855 mg, 5.39 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 and MeOH. The solid (**12**) was dried under vacuum and suspended in 20 mL of *tert*-butyl alcohol. Potassium *tert*-butoxide (1.65 g, 14.7 mmol) was added, and the mixture was heated under N_2 at 70 °C for 16 h. The mixture was cooled and poured into 30 mL of ice−water. Aqueous 10% HCl was added to attain pH = 6. The solid was collected and washed several times with water. The crude product was recrystallized from a mixture of CH_2Cl_2 and MeOH to afford **13**; yield 59.3%. ^{1}H NMR (DMSO-d_6): δ 2.01 (m, 4 H, CH_2*CH_2CH_2*CH_2), 3.33 (m, 4 H, *CH_2*CH_2CH_2*CH_2*), 6.04 (s, 1 H, H-3), 7.04 (d, $J = 2.5$ Hz, 1 H, H-8), 7.10 (dd, $J = 2.5$, 9.1 Hz, 1 H, H-7), 7.39 (d, $J = 9.0$ Hz, 1 H, H-5), 7.43−7.71 (m, 4 H, H-3′, H-4′, H-5′, H-6′). Anal. ($C_{19}H_{17}FN_2O \cdot 0.25\ H_2O$) C, H, N.

Biological Assays. The tubulin polymerization and [^{3}H]colchicine binding assays were performed as described previously.[7] In the polymerization assay, reaction mixtures contained 10 μM tubulin, and in the colchicine binding assay, the reaction mixtures contained 1.0 μM tubulin and 5.0 μM [^{3}H]colchicine.

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