Mammalian Topoisomerase II Inhibitory Activity of 1-Cyclopropyl-6,8-difluoro-1,4-dihydro-7-(2,6-dimethyl-4-pyridinyl)-4-oxo-3-quinolinecarboxylic Acid and Related Derivatives[1]

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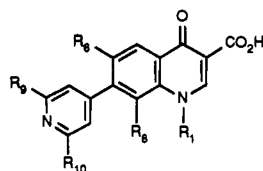
1-Cyclopropyl-6,8-difluoro-1,4-dihydro-7-(2,6-dimethyl-4-pyridinyl)-4-oxo-3-quinolinecarboxylic acid (1), a previously reported potent inhibitor of bacterial DNA gyrase, was found to be interactive with mammalian topoisomerase II (topo II). In a DNA-cleavage assay using topo II isolated from HeLa cells, 1 exhibited an EC_{50} value of 7.6 μM (VP-16; EC_{50} = 0.81 μM). A series of analogues modified at the 1-, 2-, 3-, 5-, and 7-positions of 1 were subsequently made and assessed for topo II inhibition. Compound 1 was considerably more potent than derivatives where the 1-substituent was alkyl, aryl, or H, or when N-*c*-C_3H_5 was replaced with S. The descarboxyl (i.e., 3-H) analogue had potency comparable to that of 1; when both these compounds were substituted at the 2-position with methyl or phenyl, an interesting relationship between activity and the conformation of the carboxyl group emerged. Upon replacement of the 5-H of 1 with NH_2 or F, sustained potency was seen. No enhancement of activity was evident upon replacing the 7-substituent of 1 with other pyridinyl groups, 4-methyl-1-piperazinyl, or pyrrolidinyl groups; however, the 7-(4-hydroxyphenyl) analogue (CP-115,953) was 6-fold more potent than 1. The topo II inhibitory properties of 1 translated to modest in vitro cytotoxicity and in vivo activity versus P388.

Introduction

The antibacterial properties of the quinolone class of compounds have been known for over 30 years.[2,3] The molecular target of these agents is DNA gyrase, an enzyme that catalyzes the introduction of negative supercoils into circular duplex DNA; this process is necessary for DNA replication to occur in bacteria.[4] A hallmark for antibacterial agents, including the clinically useful quinolones, is low eucaryotic toxicity. DNA gyrase, like many other bacterial molecular targets[5] (e.g., inhibition of enzymes involved in bacterial cell wall biosynthesis by β-lactams), is unique to bacteria, which accounts for the low mammalian toxicity generally observed for quinolones.[2]

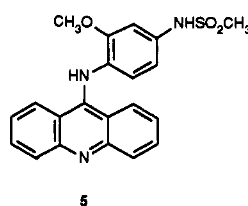
One subset of the quinolone class that has been of particular interest and importance to us are derivatives having a 7-(4-pyridinyl) appendage. Reported in 1978, rosoxacin (2) was the first of these agents to demonstrate



1; R_1 = *c*-C_3H_5, R_6 = R_8 = F, R_9 = R_{10} = CH_3
2; R_1 = CH_2CH_3, R_6 = R_8 = R_9 = R_{10} = H
3; R_1 = CH_2CH_3, R_6 = R_8 = H, R_9 = R_{10} = CH_3
4; R_1 = CH_2CH_3, R_6 = R_8 = F, R_9 = R_{10} = H

antibacterial activity and is particularly effective in treating gonococcal infections.[6,7] A closely related analogue, 3, has the 7-(2,6-dimethyl-4-pyridinyl) [DMP] group that imparts to the molecule very good Gram-positive antibacterial activity, which has historically been a deficiency of the quinolone class.[6,8] When the 7-(2,6-dimethyl-4-pyridinyl) group was incorporated into the latest generation fluoroquinolone nuclei, excellent in vitro and in vivo Gram-positive (including methicillin-resistant *Staphylococcus aureus*) and antianaerobe activity was observed.[9,10]

The development of one of these new compounds, 1, as a human anti-infective agent was precluded, however, due to an unacceptable in vitro toxicity profile. The observations that 1 was both clastogenic (human lymphocyte clastogenic assay) and mutagenic (CHO/HGPRT mutagenesis assay) were consistent with the agent being interactive with mammalian topoisomerase II (topo II). The topoisomerase II enzyme, like DNA gyrase, catalyzes the double-strand breakage of DNA to allow strand passage and thereby control the topology and conformation of DNA. Compound 1 was subsequently found to possess moderate topo II inhibitory activity. Examples of other quinolone structures, including several with 4-pyridinyl groups (e.g., CP-67,015, 4), having topo II activity have recently been reported.[11-21] For the study we are now reporting, analogues of the lead compound 1 modified at the 1-, 2-, 3-, 5- and 7-positions of the quinoline ring were prepared as probes to aid in identifying a relationship between structure and mammalian topo II inhibitory potency. Since there are many topo II inhibitors that demonstrate useful preclinical and/or human antitumor activity [e.g., m-AMSA (5) and VP-16 (6)],[22] another



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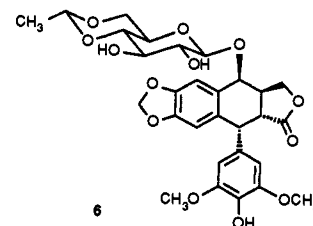

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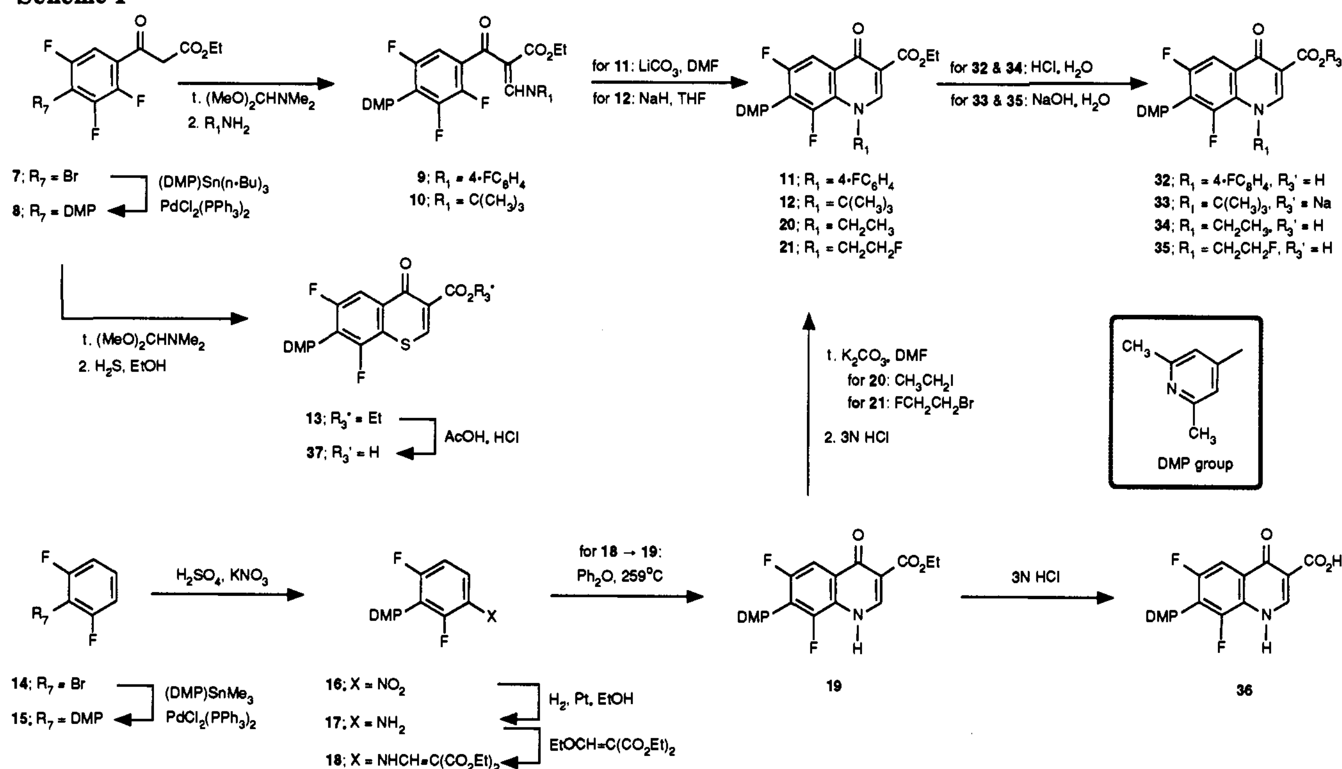
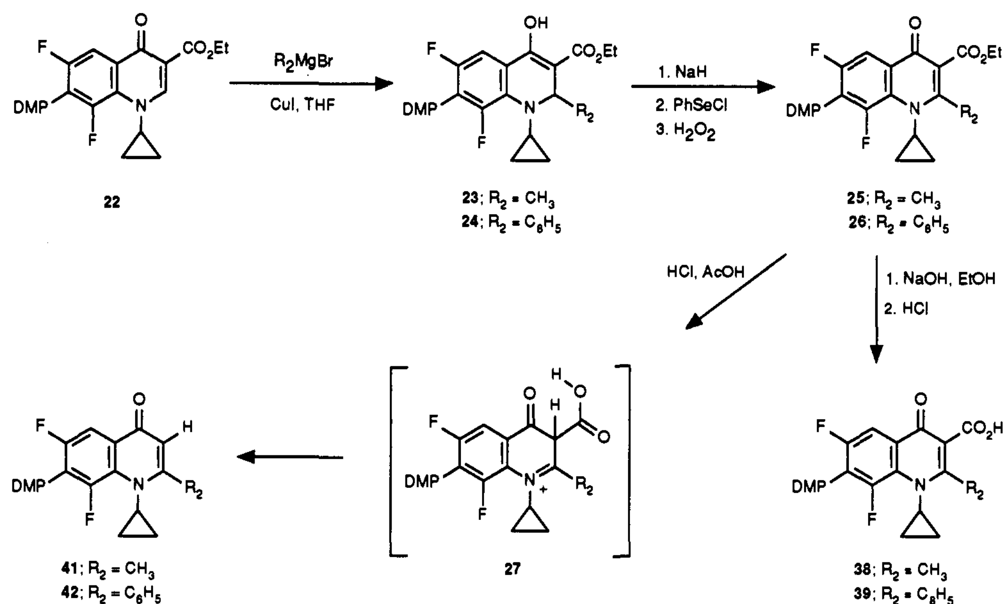
Scheme I



Scheme II



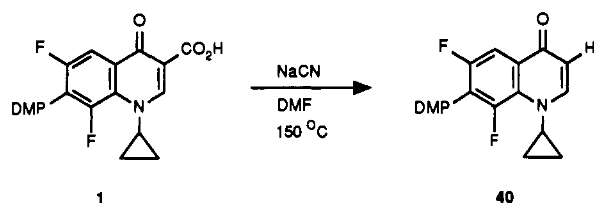
objective of this study was to determine if the topo II inhibitory activity observed for 1 would translate into useful in vitro and in vivo antitumor activity.

Chemistry

In the published synthesis of 1, the key step involved a novel Stille-type palladium-catalyzed cross coupling of (DMP)Sn(n-Bu)$_3$ with 7-bromoquinolone derivative 31 (Scheme V).[9,10] Compound 31 was prepared using the cycloaracylation process.[23] As shown in Scheme I, we used a slight modification of these procedures wherein the DMP group was introduced at an early stage in the sequence. The known β-keto ester 7[10] was coupled with (DMP)Sn(n-Bu)$_3$[10] to give 8, which was converted to the 1-(4-FC$_6$H$_4$) and 1-*tert*-butyl target compounds 32 and 33, respectively, using the standard cycloaracylation sequence. The instability of 33 to acidic conditions necessitated its isolation as the sodium salt, and it was made by hydrolysis of intermediate 12 in aqueous NaOH. Even conditions as mild as neutralization of the sodium salt promoted the loss of isobutylene from 33 to give the 1-H derivative 36. The novel 1-thia analogue 37 was also prepared using this general method.

A different approach, which is also shown in Scheme I, was used to prepare the 1-ethyl, 1-CH$_2$CH$_2$F, and 1-H derivatives, 34, 35, and 36, respectively. In this method, the classical quinolone synthetic method, the Gould–Jacobs reaction, was used.[24] Pyridinylation of 2,6-difluorobromobenzene (14) followed by nitration and reduction provided the aniline derivative 17. Treatment of 17 with

Scheme III



diethyl ethoxymethylenemalonate provided **18**, which was heated in diphenyl ether to afford **19**. Using standard conditions, **19** was alkylated and hydrolyzed to give targets **34** and **35**; direct hydrolysis of **19** gave target **36**.

Variation of the 2-substituent was accomplished using a known method[25] and is shown in Scheme II. Treatment of **22**[10] with CuI and methyl- or phenylmagnesium bromide in THF gave the 2-methyl and 2-phenyl dihydroquinolone derivatives **23** and **24**, respectively. Quenching the sodium salt of these analogues with PhSeCl followed by oxidation and elimination provided **25** and **26**. Using acidic conditions ($HOAc/H_2O/HCl$), commonly used to hydrolyze quinolinecarboxylic acid esters to the corresponding acids, **25** and **26** were cleanly and unexpectedly converted to the decarboxylated analogues **41** and **42**, respectively. These surprising results may be due to the greater stabilization of the requisite (for acid-induced decarboxylation of β-keto acids) iminium ion **27** by the 2-methyl or 2-phenyl substituents compared to the 2-H group.

In aqueous ethanolic sodium hydroxide, however, hydrolysis of **25** and **26** followed by careful neutralization gave the desired target acids, **38** and **39**, with no evidence of decarboxylation. The closely related derivative **40** was made from **1** and NaCN/DMF as shown in Scheme III.[26]

Two known[10] (**43** and **45** in Table I) and one new (**44**) 5-position variants of **1** were also evaluated for topo II inhibition. In the first step for the synthesis of **44** (Scheme IV), we used what seemingly was a straightforward procedure (NaN_3/DMF) for the introduction of nitrogen into the 5-position of quinolone **28**.[27] Instead of the expected 5-azido derivative **29**, we obtained the isoxazolo-fused quinolone **30** via an electrocyclic process where nitrogen was expelled from **29** at 100 °C. A significant upfield chemical shift was seen for the 2-H (δ 7.95, quinoline numbering) and cyclopropyl methine (δ 3.54) of **30** relative to the normal quinolones suggesting significant electronic differences between the two heterocyclic systems. For example, the corresponding resonances in the same NMR solvent ($CDCl_3$) for the same H's for several quinolone esters are δ 8.51 and 3.90 for **28** (R_5 = F), 8.63 and 3.93 for **22** (R_5 = H), and 8.49 and 3.85 for the ethyl ester of **44** (R_5 = NH_2). While this type of ring construction involving a quinolone nucleus is novel, the elimination of nitrogen from 2-azidoacetophenones and related compounds is well-documented to give benzisoxazoles.[28] Using varying temperatures and solvents, our attempts to isolate the 5-azido intermediate **29** were unsuccessful. Only at elevated temperatures was starting material consumed along the simultaneous formation of **30**, indicating that, at elevated temperatures, the rate of destruction of azide **29** (to **30**) was faster than its formation. Compound **30** was cleanly converted to the desired target **44** via hydrogenolysis and hydrolysis.

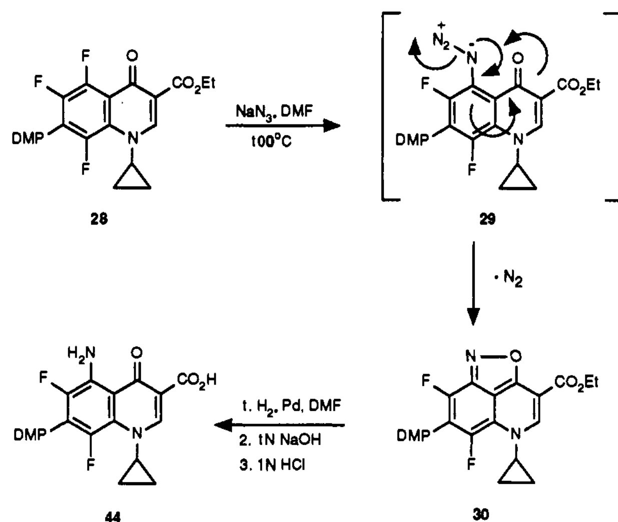
The syntheses of new 7-position variants (**46–49**) of **1** utilized the Pd-catalyzed coupling of various heteroaryl stannanes with the 7-bromoquinolone intermediate **31** as shown in Scheme V.

Table I. Topoisomerase II Inhibitory Properties of 6,8-Difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acids and Related Derivatives

cmpd	R_1	R_2	R_3	R_5	R_7	topo II inhibition,[a] EC_{50}, μM
1	c-C_3H_5	H	CO_2H	H	DMP[b]	7.6
32	4-FC_6H_4	H	CO_2H	H	DMP	>430
33	$C(CH_3)_3$	H	CO_2Na	H	DMP	74
34	CH_2CH_3	H	CO_2H	H	DMP	110
35	CH_2CH_2F	H	CO_2H	H	DMP	>530
36	H	H	CO_2H	H	DMP	>300
37	$NR_1{=}S$	H	CO_2H	H	DMP	>140
38	c-C_3H_5	CH_3	CO_2H	H	DMP	>260
39	c-C_3H_5	C_6H_5	CO_2H	H	DMP	>220
40	c-C_3H_5	H	H	H	DMP	17
41	c-C_3H_5	CH_3	H	H	DMP	16
42	c-C_3H_5	C_6H_5	H	H	DMP	>240
43[c]	c-C_3H_5	H	CO_2H	F	DMP	13
44	c-C_3H_5	H	CO_2H	NH_2	DMP	6.2
45[c]	c-C_3H_5	H	CO_2H	SC_6H_5	DMP	>420
46	c-C_3H_5	H	CO_2H	H	2-CH_3-4-pyridinyl	16
47	c-C_3H_5	H	CO_2H	H	4-pyridinyl	17
48	c-C_3H_5	H	CO_2H	H	2,6-$(CH_3)_2$-3-pyridinyl	53
49	c-C_3H_5	H	CO_2H	H	3-pyridinyl	52
50[d]	c-C_3H_5	H	CO_2H	H	4-CH_3-1-piperazinyl	150
51[e]	c-C_3H_5	H	CO_2H	H	3-NH_2-1-pyrrolidinyl	340
52[f]	c-C_3H_5	H	CO_2H	H	4-HOC_6H_4	1.2
5 (m-AMSA)						0.72
6 (VP-16)						0.81

[a] See Experimental Section. [b] DMP = 2,6-dimethyl-4-pyridinyl. [c] See ref 10. [d] See ref 34. [e] PD-117,596; see ref 35. [f] CP-115,953; see ref 13.

Scheme IV



Topoisomerase II Inhibition Data

The topo II inhibitory potency for each target compound was assessed in a cleavage assay using enzyme isolated from HeLa cells and is described in the Experimental Section. The assay measures the amount of topo II covalently linked to pBR322 end-labeled DNA using the SDS–potassium precipitation method of Trask.[29] The EC_{50} value represents the concentration of drug that

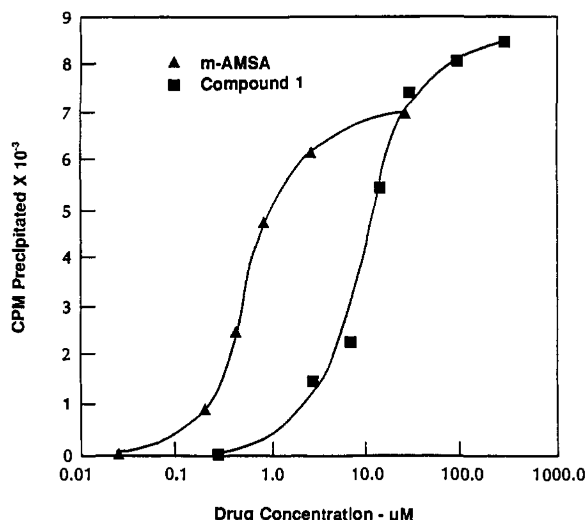
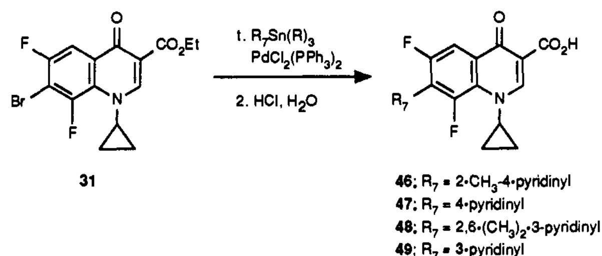


Figure 1. Inhibition of HeLa topoisomerase II by m-AMSA (5) and compound 1.

Scheme V



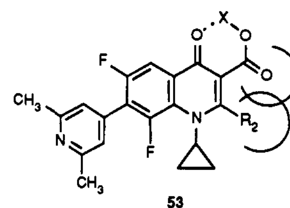
achieves 50% of the maximal effect of m-AMSA. The dose-response curves for compound 1 and m-AMSA (5) are shown in Figure 1. The variance of this assay was such that a 2-fold difference in EC_{50} values was not considered to be significantly different.

Results and Discussion

The structures and topo II inhibitory activity of the target quinolones compared to VP-16 are shown in Table I. The lead compound in this series, 1, had an EC_{50} value of 7.6 μM in the topo II assay; this potency was 9-fold less than that of VP-16. The 1-(4-fluorophenyl), 1-*tert*-butyl, 1-ethyl, and 1-fluoroethyl groups are common appendages on potent antibacterial quinolones, but when they replaced the cyclopropyl group of 1 to give 32, 33, 34, and 35, respectively, topo II inhibitory potency decreased from 10- to >70-fold. The 1-H analogue 36 was inactive and may be a consequence of an N to O proton tautomerization, a condition that is unique to this compound. The 1-thia derivative 37 was made to mimic the electron-releasing properties of the nitrogen compounds, but was found to be inactive. Of the seven compounds evaluated, topo II inhibitory potency does not correlate well to the size or the electron-releasing properties of the 1-substituent.

When the 2-H of 1 was replaced by methyl or phenyl to give 38 and 39, respectively, activity was abolished. Activity was sustained when the 3-CO_2H group of 1 was replaced by H to give 40. This unexpected result contrasts the SAR noted in quinolone antibacterials where the 3-CO_2H (or isosteric replacement) is required for gyrase inhibition.[19,30] Indeed, this dramatic divergence of SAR was corroborated by the observation that 40 was 193-fold less potent as a gyrase inhibitor than 1.[31]

When the 2-methyl and 2-phenyl groups were introduced into 40 to give 41 and 42, respectively, potency similar to that of 40 was observed for 41 while 42 was inactive. Within this subset of analogues, activity appears to be a sensitive function of the size of the 2-substituent. Clearly, the relatively large and bulky 2-phenyl group is detrimental to activity, irrespective of the nature of the 3-substituent. Spatially, the biochemical target appears to accommodate a 2-methyl group; however, activity is destroyed when it is accompanied by a 3-CO_2H group. A likely explanation for the inactivity of 38 rests in the interplay between the 2-methyl group and the 3-CO_2H group. The 3-CO_2H is not a prerequisite for activity; if present, however, these data indicate that it must be able to adopt a planar conformation with respect to the quinoline ring. For compound 1, the active intramolecular H-bonded (or metal chelated) planar conformation shown in 53 (R_2 = H) is of



low energy. For the corresponding 2-methyl analogue 38, the planar conformation (53, $R_2 = CH_3$) is less stable due to $A_{1,3}$ strain, which translates to lower affinity binding to the molecular target.

Among the several 5-position variants that were evaluated, potency was similar to that of 1 for the 5-F and 5-NH_2 derivatives 43 and 44, respectively. The 5-SPh derivative 45, which was used as a synthetic intermediate in an earlier study,[10] was devoid of activity. Within this small subseries, topo II inhibitory potency is not dependent on the electron-releasing/-withdrawing properties of the 5-substituent but may be dependent on its size. Regarding the effect on topo II inhibition upon varying the 7-substituent, activity was not appreciably effected by the degree of methyl substitution on the pyridine ring (46 and 47) but was reduced 7-fold when the quinoline ring was attached at the 3-position of a dimethyl-substituted (48) or unsubstituted (49) pyridine. When a methylpiperazine or aminopyrrolidine group (prototypic 7-substituents of bacterial DNA gyrase inhibitors) was appended to the 7-position to give 50 and 51, respectively, potency was reduced 20- and 45-fold, respectively. The only quinolone in this study to show greater potency than 1 was the 7-(4-HOC_6H_4) derivative CP-115,953 (52).[13] This recently reported analogue shown to have significant topo II inhibitory activity was 6-fold more potent than 1 in our hands.

Antitumor Activity and Conclusions

The topo II inhibitory potency observed for the lead compound 1 translated to modest in vitro cytotoxicity (IC_{50} = 29 μM, 1 h drug exposure) in a clonogenic assay vs P388 murine leukemia cells in culture (VP-16; IC_{50} = 0.30 μM).[32] At the maximum tolerated dose of 300 mg/kg, modest in vivo antitumor activity [% ILS (increased life span) = 90 with 0/6 long-term cures] was observed for 1 in a murine leukemia model in which P388 was implanted ip on day 0 and drug was administered ip (qd on days 1, 5, 9). For comparison, VP-16 at a well-tolerated dose of 180 mg/kg was significantly more potent and efficacious than 1 (% ILS

= 220 with 5/6 long-term cures). In vivo antitumor tests were conducted at Southern Research Institute by the standard NCI protocol.[33] While this study did not identify a candidate for advancement as a potential anti-tumor agent, the valuable knowledge regarding the effect of structure on enzyme inhibitory activity was used to design more potent derivatives. Results from these studies will be communicated in the future.

Experimental Section

General. Melting points were determined on a Thomas-Hoover melting point apparatus in open capillaries and are uncorrected. ^{1}H NMR (IBM AM-200, JEOL GSX-270 FT-NMR, or GE QE-300), chemical ionization mass spectra (Hewlett-Packard 5980A mass spectrometer), and infrared spectra (Nicolet 10DX FT-IR spectrophotometer) were consistent with the assigned structures. Complete ^{1}H NMR data are reported for all compounds except for tin reagents. ^{1}H NMR multiplicity data are denoted by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Coupling constants are in hertz. High-performance liquid chromatography was carried out on a Rainin HPXL system equipped with a Dynamax absorbance detector. Carbon, hydrogen, and nitrogen elemental analyses were performed by Galbraith Laboratories, Knoxville, TN, and were within ±0.4% of the theoretical values. Reactions were generally performed under a N_2 atmosphere.

Ethyl 3-[4-(2,6-Dimethyl-4-pyridinyl)-2,3,5-trifluorophenyl]-3-oxopropionate (8). To a solution of 7[10] (75 g, 0.23 mol) and tri-*n*-butyl-4-(2,6-dimethylpyridinyl)stannane[10] (99 g, 0.25 mol) in dioxane (750 mL) was added $PdCl_2(PPh_3)_2$ (8.7 g, 12.5 mmol). The mixture was heated at reflux for 16 h and concentrated. The residue was dissolved in Et_2O and extracted with 1 N HCl. The aqueous acid extracts were made basic with $KHCO_3$, and the resulting precipitate was collected and dried to afford 69.7 g (83.5%) of 8. An analytical sample was prepared by recrystallization from EtOAc: mp 99 °C; ^{1}H NMR ($CDCl_3$) δ 1.35 (t, 3H), 2.65 (s, 6H), 4.30 (q, 2H), 6.00 (s, 1H), 7.10 (s, 2H), 7.50 (m, 1H), 12.70 (s, 1H). Anal. ($C_{18}H_{16}F_3NO_3$) C, H, N.

Ethyl 3-[4-(2,6-Dimethyl-4-pyridinyl)-2,3,5-trifluorophenyl]-2-[[(4-fluorophenyl)amino]methylene]-3-oxopropionate (9). A solution of 8 (0.60 g, 1.71 mmol) and *N,N*-dimethylformamide dimethyl acetal (0.24 mL, 1.80 mmol) in THF (6 mL) was stirred overnight in a capped flask at 25 °C. 4-Fluoroaniline (0.18 mL, 1.85 mmol) was added dropwise via a syringe and stirring at 25 °C was continued for 3 d. The solution was concentrated to give crude enamine 9 (0.87 g, 108%). An analytically pure sample (viscous liquid, mixture of *E*/*Z* isomers) was prepared by silica gel chromatography (20% EtOAc–80% hexane): ^{1}H NMR ($CDCl_3$) δ 8.54 (pair of d, 1H), 7.31–7.00 (m, 7H), 4.16 (pair of q, 2H), 2.30 (s, 6H), 1.65 (br, 1H), 1.32 (br, H_2O), 1.17, 1.04 (2 t, 3H). Anal. ($C_{25}H_{20}F_4N_2O_3$·0.5H_2O) C, H, N.

Ethyl 6,8-Difluoro-1,4-dihydro-7-(2,6-dimethyl-4-pyridinyl)-1-(4-fluorophenyl)-4-oxo-3-quinolinecarboxylate (11). A mixture of 9 (0.52 g, 1.10 mmol), $LiCO_3$ (0.10 g, 1.375 mmol), and DMF (7 mL) was stirred and heated on a steam bath under N_2 for 4.5 h. The yellow suspension was poured into H_2O and the solid was collected and recrystallized from EtOH to give 11 as a tan solid (0.24 g, 47%): mp 272–273 °C dec; ^{1}H NMR ($CDCl_3$) δ 8.38 (s, 1H), 8.16 (dd, 1H), 7.44 (m, 2H), 7.27 (s, 1H), 7.22 (t, 2H), 6.94 (s, 1H), 4.40 (q, 2H), 2.55 (s, 6H), 1.40 (t, 3H). Anal. ($C_{25}H_{19}F_3N_2O_3$) C, H, N.

6,8-Difluoro-1,4-dihydro-7-(2,6-dimethyl-4-pyridinyl)-1-(4-fluorophenyl)-4-oxo-3-quinolinecarboxylic Acid Hydrochloride (32). A solution of 11 (0.050 g, 0.11 mmol) in 1 N HCl (0.65 mL, 0.65 mmol) was stirred and heated on a steam bath for 3 h. The colorless solution was concentrated and dried to afford 32 (0.51 g, 100%): mp 230–235 °C dec; ^{1}H NMR (DMSO-d_6) δ 8.66 (s, 1H), 8.21 (d, 1H), 7.89–7.70 (m, 4H), 7.46 (t, 2H), 2.73 (s, 6H). Anal. ($C_{23}H_{15}F_3N_2O_3$·HCl) C, H, N.

Ethyl 6,8-Difluoro-1,4-dihydro-7-(2,6-dimethyl-4-pyridinyl)-1-(1,1-dimethylethyl)-4-oxo-3-quinolinecarboxylate (12). To a solution of 2.2 g (5.11 mmol) of 10 (prepared from 8 and *tert*-butylamine using a procedure similar to that used for making 9) in 40 mL of THF was added 0.28 g of NaH (7.0 mmol; 60% oil dispersion washed with hexane). After stirring for 2 h at 25 °C, the mixture was treated with 2 drops of HOAc and concentrated. The residue was partitioned between H_2O and $CHCl_3$ and the organic layer was dried ($MgSO_4$) and concentrated giving an oil that upon tituration with Et_2O gave 1.67 g (79%) of 12. Analytically pure material was obtained by recrystallization from EtOH: mp 275–278 °C; ^{1}H NMR ($CHCl_3$) δ 8.96 (s, 1H), 8.16 (dd, 1H), 7.07 (s, 2H), 4.43 (q, 2H), 2.63 (s, 6H), 1.79 (s, 9H), 1.42 (t, 3H). Anal. ($C_{23}H_{24}F_2N_2O_3$·0.25H_2O) C, H, N.

Sodium 6,8-Difluoro-1,4-dihydro-7-(2,6-dimethyl-4-pyridinyl)-1-(1,1-dimethylethyl)-4-oxo-3-quinolinecarboxylate (33). A mixture of 12 (0.62 g, 1.50 mmol), MeOH (15 mL), and 0.1 M NaOH (14.5 mL, 1.45 mmol) was heated at 50 °C for 2.5 h. The resulting solution was concentrated and the residue was triturated with Et_2O to give 0.55 g (93%) of 33: mp >300 °C; ^{1}H NMR (D_2O) δ 8.90 (s, 1H), 7.72 (br d, 1H), 6.75 (s, 2H), 2.26 (s, 6H), 1.54 (s, 9H). Anal. ($C_{21}H_{19}F_2N_2O_3Na$·H_2O) C, H, N.

Ethyl 6,8-Difluoro-7-(2,4-dimethyl-4-pyridinyl)-4-oxo-4*H*-benzothiopyran-3-carboxylate (13). Compound 8 (1.53 g, 4.36 mmol) was dissolved in THF (100 mL) and *N,N*-dimethylformamide dimethyl acetal (6.00 mL, 42.30 mmol) was added in one portion. The solution was stirred at room temperature for 48 h and concentrated. EtOH (75 mL) was added and H_2S gas bubbled through the solution for 2 h, during which time a white solid precipitated. The crude product was filtered and recrystallized from EtOH to give 1.26 g (77%) of 13: mp 180 °C; ^{1}H NMR ($CDCl_3$) δ 8.72 (s, 1H), 8.22 (dd, *J* = 1.7, 10.2 Hz, 1H), 7.09 (s, 2H), 4.42 (q, *J* = 7.1, 2H), 2.61 (s, 6H), 1.41 (t, *J* = 7.1 Hz, 3H). Anal. ($C_{19}H_{15}F_2NO_3S$) C, H, N.

6,8-Difluoro-7-(2,4-dimethyl-4-pyridinyl)-4-oxo-4*H*-benzothiopyran-3-carboxylic Acid (37). A mixture of 13 (1.18 g, 3.20 mmol), HOAc (80 mL), H_2O (20 mL), and concentrated HCl (2 mL) was heated on a steam bath for 4 h. The reaction mixture was concentrated to yield a white solid which was recrystallized from EtOH to give 37 (1.07 g, 95%): mp 228–230 °C dec; ^{1}H NMR (CD_3OD) δ 9.66 (s, 1 H), 8.37 (dd, *J* = 1.7, 10.3 Hz, 1H), 7.67 (s, 2H), 2.75 (s, 6H). Anal. ($C_{17}H_{11}F_2NO_3S$·HCl·0.5H_2O) C, H, N.

4-(2,6-Difluorophenyl)-2,6-dimethylpyridine (15). A stirred mixture of 2,6-difluorobromobenzene (14, 35 g, 0.18 mol), 2,6-dimethyl-4-(trimethylstannyl)pyridine[36] (51.5 g, 0.19 mol), $PdCl_2(PPh_3)_2$ (8.8 g, 12.53 mmol), HMPA (18 mL), and 1,4-dioxane (300 mL) was heated at reflux for 24 h and concentrated. The oily residue was treated with H_2O (100 mL), whereupon a white solid precipitated. The product was collected, washed with H_2O, dried, and purified by silica gel chromatography (1:1 Et_2O–hexanes), to give 18.4 g (47%) of 15 as white needles: mp 92–93 °C (hexanes); ^{1}H NMR ($CDCl_3$) δ 7.43–6.91 (m, 5H), 2.61 (s, 6H). Anal. ($C_{13}H_{11}F_2N$) C, H, N.

4-(2,6-Difluoro-3-nitrophenyl)-2,6-dimethylpyridine (16). To stirred, concentrated H_2SO_4 (50 mL) cooled in ice was added 15 (8.2 g, 37 mmol) whereupon the temperature rose to 20 °C. The resulting mixture was stirred until most of the compound dissolved and the temperature dropped below 10 °C and then KNO_3 (3.74 g, 37 mmol) was added in one batch. The resulting mixture was stirred in an ice bath for 3 h and at room temperature for 1 h and then poured on ice. The resulting mixture was basified by adding concentrated NH_4OH. The product was extracted with $CHCl_3$ (200 mL). Removal of $CHCl_3$ gave a brown solid which was recrystallized from EtOAC–Et_2O to yield 7.9 g (81%) of 16: mp 156–158 °C; ^{1}H NMR ($CDCl_3$) δ 8.15 (m, 1H), 7.2 (m, 1H), 7.07 (s, 2H), 2.63 (s, 6H). Anal. ($C_{13}H_{10}F_2N_2O_2$) C, H, N.

2,4-Difluoro-3-(2,6-dimethyl-4-pyridinyl)benzenamine (17). A mixture of 16 (25 g, 94 mmol), PtO_2 (0.7 g), and EtOH (200 mL) was agitated under H_2 on a Parr hydrogenator until the required amount of H_2 was absorbed (0.28 mol, 2 h). Hot $CHCl_3$ was added and the mixture was filtered and concentrated. The resulting tan residue was stirred in Et_2O and a solid was collected to afford 19.8 g (90%) of 17: mp 176–178 °C; (DMSO-d_6) δ 7.10 (s, 2H), 6.85 (m, 2H), 5.10 (br s, 2H), 2.48 (s, 6H). Anal. ($C_{13}H_{12}F_2N_2$) C, N, H.

Diethyl [[[3-(2,6-Dimethyl-4-pyridinyl)-2,4-difluorophenyl]amino]methylene]propanedioate (18). A mixture of 17 (18.6 g, 80 mmol) and diethyl (ethoxymethylene)malonate (19.5

g, 90 mmol) was heated at 140–150 °C for 5 h. The resulting brown oil was cooled and crystallized from Et_2O to afford 28.9 (89%) of **18** as white needles: mp 130–131 °C; ^1H NMR ($CDCl_3$) δ 9.46 (d, J = 13 Hz, 1H), 7.41–6.97 (m, 5H), 4.31 (m, 4H), 2.62 (s, 6H), 1.40 (m, 6H). Anal. ($C_{21}H_{22}F_2N_2O_4$) C, H, N.

Ethyl 7-(2,6-Dimethyl-4-pyridinyl)-6,8-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate (19). To stirred Dowtherm (300 mL) heated to boiling point on a hot plate was added **18** (26.4 g, 65 mmol). The resulting mixture was boiled for 20 min and then cooled to 25 °C. The product which crystallized was collected, washed with Et_2O, and recrystallized from DMF to afford 16.4 g (70%) of **19** as a light pink solid: mp 304–305 °C dec; ^1H NMR (CF_3CO_2D) δ 9.50 (s, 1H), 8.3 (d, J = 9 Hz, 1H), 7.9 (s, 2H), 4.7 (q, J = 7 Hz, 2H), 2.95 (s, 6H), 1.55 (t, J = 7 Hz, 3H). Anal. ($C_{19}H_{16}F_2N_2O_3$) C, H, N.

6,8-Difluoro-1,4-dihydro-7-(2,6-dimethyl-4-pyridinyl)-4-oxo-3-quinolinecarboxylic Acid Hydrochloride (36). A mixture of **19** (1.3 g, 3.6 mmol) and 3 N HCl (50 mL) was heated on a steam bath for 5 h and concentrated. The resulting white solid was recrystallized from EtOH to give 0.92 g (69%) of **36** as white needles: mp 278–280 °C dec; ^1H NMR (CF_3CO_2D) δ 9.6 (s, 1H), 8.35 (d, J = 9 Hz, 1H), 8.00 (s, 2H), 3.00 (s, 6H). Anal. ($C_{17}H_{12}F_2N_2O_3 \cdot HCl$) C, H, N.

Ethyl 1-Ethyl-6,8-difluoro-1,4-dihydro-7-(2,6-dimethyl-4-pyridinyl)-4-oxo-3-quinolinecarboxylate (20). To a stirred mixture of **19** (9 g, 25 mmol), anhydrous K_2CO_3 (7 g, 50.7 mmol), and DMF (100 mL) heated on a steam bath was added ethyl iodide (4.5 mL, 56.2 mmol) over a period of 1 h. The resulting mixture was heated for 1 h and concentrated. The purple residue was partitioned between $CHCl_3$ and H_2O. The organic layer was washed with aqueous sodium thiosulfate solution and concentrated and the resulting residue was crystallized from i-PrOH to yield 7.2 g (74%) of **20**: mp 172–173 °C; ^1H NMR ($CDCl_3$) δ 8.45 (s, 1H), 8.15 (d, J = 9 Hz, 1H), 7.08 (s, 2H), 4.38 (m, 4H), 2.64 (s, 6H), 1.55 (t, J = 7 Hz, 3H), 1.44 (t, J = 7 Hz, 3H). Anal. ($C_{21}H_{20}F_2N_2O_3$) C, H, N.

1-Ethyl-6,8-difluoro-1,4-dihydro-7-(2,6-dimethyl-4-pyridinyl)-4-oxo-3-quinolinecarboxylic Acid Hydrochloride (34). A mixture of **20** (5.3 g, 13.7 mmol) and 3 N HCl (100 mL) was heated on a steam bath for 4 h and concentrated. The tan solid residue was recrystallized from EtOH to afford 4.8 g (89%) of **34** as white crystals; mp 278–280 °C; ^1H NMR (CF_3CO_2D) δ 9.58 (s, 1H), 8.48 (d, J = 9 Hz, 1H), 8.00 (s, 2H), 5.12 (m, 2H), 3.04 (s, 6H), 1.93 (t, J = 7 Hz, 3H). Anal. ($C_{19}H_{16}F_2N_2O_3 \cdot HCl$) C, H, N.

Ethyl 6,8-Difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(2,6-dimethyl-4-pyridinyl)-4-oxo-3-quinolinecarboxylate (21). A stirred mixture of **19** (10.5 g, 30 mmol), 2-bromo-1-fluoroethane (12 g, 94 mmol), anhydrous K_2CO_3 (14 g, 0.1 mmol), and DMF (100 mL) was heated on a steam bath for 24 h and concentrated. The residue was washed with H_2O and purified by silica gel chromatography (Et_2O–10% MeOH in Et_2O) to give 6.4 g of a pale yellow solid. Recrystallization from EtOAc gave 3.6 g (30%) of **21**: mp 185–186 °C; ^1H NMR ($CDCl_3$) δ 8.45 (s, 1H), 8.15 (d, J = 9 Hz, 1H), 7.05 (s, 2H), 4.95–4.58 (m, 4H), 4.42 (q, J = 7 Hz, 2H), 1.45 (t, J = 7 Hz, 3H). Anal. ($C_{21}H_{19}F_3N_2O_3$) C, H, N.

6,8-Difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(2,6-dimethyl-4-pyridinyl)-4-oxo-3-quinolinecarboxylic Acid (35). A mixture of **21** (2.1 g, 5.2 mmol) and 2% aqueous NaOH (25 mL) was heated on a steam bath for 30 min and neutralized with aqueous HCl. The white solid precipitate was collected, washed with H_2O, and recrystallized from EtOH to yield 0.9 g (46%) of **35** as white needles: mp 275–277 °C dec; ^1H NMR (CF_3CO_2D) δ 9.48 (s, 1H), 8.52 (d, J = 9 Hz, 1H), 7.96 (s, 2H), 5.42–4.9 (m, 4H), 3.00 (s, 6H). Anal. ($C_{19}H_{15}F_3N_2O_3$) C, H, N.

Ethyl 1-Cyclopropyl-6,8-difluoro-1,2,3,4-tetrahydro-2-methyl-7-(2,6-dimethyl-4-pyridinyl)-4-oxo-3-quinolinecarboxylate (23). Compound **22**[10] (4.05 g, 10.18 mmol) and cuprous iodide (0.62 g, 3.26 mmol) was added to THF (200 mL) under N_2 and cooled to −78 °C. Methylmagnesium bromide (2.8 M, 5.50 mL, 15.4 mmol) was added and the greenish yellow reaction mixture was stirred for 3 h at −78 °C. The reaction was quenched with saturated NH_4Cl (100 mL) and was allowed to warm to room temperature. The THF was removed in vacuo and the aqueous residue extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated to yield a yellow solid (4.09 g). The crude product was purified by silica gel chromatography (CH_2Cl_2) and the resulting product was recrystallized from EtOH to give **23** as yellow crystals (2.99 g, 71%): mp 111 °C; ^1H NMR ($CDCl_3$) δ 11.96 (s, 1H, exchanges with D_2O), 7.30 (dd, J = 1.7, 9.8 Hz, 1H), 7.12 (s, 2H), 4.45 (q, J = 6.4 Hz, 1H), 4.35 (q, J = 7.1 Hz, 2H), 3.01–2.94 (m, 1H), 2.65 (s, 6H), 1.37 (t, J = 7.1 Hz, 3H), 1.18 (d, J = 6.4 Hz, 3H), 0.80–0.64 (m, 4H). Anal. ($C_{23}H_{24}F_2N_2O_3$) C, H, N.

Ethyl 1-Cyclopropyl-6,8-difluoro-1,4-dihydro-2-methyl-7-(2,6-dimethyl-4-pyridinyl)-4-oxo-3-quinolinecarboxylate (25). Sodium hydride (0.19 g; 60% by weight, dispersion in oil, 4.75 mmol) was placed in the reaction flask and rinsed with THF and then suspended in dry THF (75 mL) and cooled to 0 °C. Compound **23** (1.10 g, 2.66 mmol) in THF (5 mL) was added over 20 min. Phenylselenyl chloride (0.67 g, 3.50 mmol) was added rapidly and the solution poured slowly into a stirred solution of Et_2O–pentane (1:1, 50 mL), saturated $NaHCO_3$ (20 mL), and ice (10g). The aqueous phase was separated and extracted with Et_2O–pentane. The combined organic phases were washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), filtered, and concentrated to yield a yellow solid (1.41 g, 94%). The crude selenide was dissolved in CH_2Cl_2 (25 mL) and 30% hydrogen peroxide (0.88 mL, 7.80 mmol) in H_2O (2 mL) was added slowly to keep the temperature between 25–30 °C; addition was completed in 20 min. The mixture was allowed to stir for 30 min, and then CH_2Cl_2 (25 mL) and $NaHCO_3$ (10%, 10 mL) were added. The aqueous layer was extracted with CH_2Cl_2, and the combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated to yield a yellow solid (1.40 g). The crude product was purified by silica gel flash chromatography (CH_2Cl_2–MeOH 20:1) and the product was recrystallized from EtOH–H_2O to give **25** as a white solid (0.90 g, 88%): mp 154–155 °C; ^1H NMR ($CDCl_3$) δ 7.90 (dd, J = 1.8, 9.4 Hz, 1H), 7.11 (s, 2H), 4.41 (q, J = 7.1 Hz, 2H), 3.64–3.57 (m, 1H), 2.66 (s, 3H), 2.65 (s, 6H), 1.39 (t, J = 7.1 Hz, 3H), 1.31–1.00 (m, 2H), 0.90–0.78 (m, 2H). Anal. ($C_{23}H_{22}F_2N_2O_3$) C, H, N.

1-Cyclopropyl-6,8-difluoro-1,4-dihydro-2-methyl-7-(2,6-dimethyl-4-pyridinyl)-4-oxo-3-quinolinecarboxylic Acid (38). Compound **25** (0.20 g, 0.49 mmol) was dissolved in EtOH (10 mL), and NaOH (1 M, 3 mL) was added. The mixture was heated on a steam bath for 5 h and concentrated. H_2O (5 mL) was added to the residue and the solution was acidified with concentrated HCl to pH 6 at which point a white solid precipitated. The product was filtered, washed with H_2O, and recrystallized from EtOH–H_2O to yield **38** as white crystals (0.17 g, 92%): mp 220 °C dec; ^1H NMR ($CDCl_3$) δ 15.51 (br s, 1H), 8.01 (dd, J = 1.9, 9.0 Hz, 1H), 7.07 (s, 2H), 3.86–3.75 (m, 1H), 3.25 (s, 3H), 2.63 (s, 3H), 1.45–1.33 (m, 2H), 0.88–0.80 (m, 2H). Anal. ($C_{21}H_{18}F_2N_2O_3$) C, H, N.

Ethyl 1-Cyclopropyl-6,8-difluoro-1,2,3,4-tetrahydro-7-(2,6-dimethyl-4-pyridinyl)-4-oxo-2-phenyl-3-quinolinecarboxylate (24). Compound **22** (2.1 g, 5.27 mmol), cuprous iodide (0.30 g, 1.56 mmol), and phenylmagnesium chloride (2.0 M, 4.00 mL, 8.00 mmol) in THF (100 mL) at −78 °C were employed following the procedure described for preparing **23**. The crude product was purified by silica gel flash chromatography (EtOAc-hexanes 3:1) to yield a yellow solid (1.50 g, 60%). Recrystallization from EtOH (95%) gave **24** as bright yellow crystals: mp 157–158 °C; ^1H NMR ($CDCl_3$) δ 12.21 (s, 1H, exchanges with D_2O), 7.34 (dd, J = 1.7, 9.7 Hz, 1H), 7.28–7.20 (m, 5H), 7.04 (s, 2H), 5.42 (s, 1H), 4.24 (q, J = 7.1 Hz, 2H), 3.00–2.89 (m, 1H), 2.59 (s, 6H), 1.24 (t, J = 7.1 Hz, 3H), 0.88–0.75 (m, 4H). Anal. ($C_{28}H_{26}F_2N_2O_3$) C, H, N.

Ethyl 1-Cyclopropyl-6,8-difluoro-1,4-dihydro-7-(2,6-dimethyl-4-pyridinyl)-4-oxo-2-phenyl-3-quinolinecarboxylate (26). Compound **24** (1.15 g, 2.31 mmol) was treated with sodium hydride (0.16 g, 3.93 mmol), phenylselenyl chloride (0.54 g, 2.82 mmol), and 30% hydrogen peroxide (0.70 mL, 6.17 mmol) in the same manner as described for **25**. The chromatographed material was recrystallized from EtOH–H_2O to give **26** as a white solid (0.73 g, 72%): mp 212 °C; ^1H NMR ($CDCl_3$) δ 7.98 (dd, J = 1.6, 9.4 Hz, 1H), 7.6–7.48 (m, 5H), 7.12 (s, 2H), 4.02 (q, J = 7.1 Hz, 2H), 3.56–3.43 (m, 1H), 2.65 (s, 6H), 0.94 (t, J = 7.1 Hz, 3H), 0.80–0.70 (m, 2H), 0.62–0.52 (m, 2H). Anal. ($C_{28}H_{24}F_2N_2O_3 \cdot 0.25H_2O$) C, H, N.

1-Cyclopropyl-6,8-difluoro-1,4-dihydro-7-(2,6-dimethyl-4-pyridinyl)-4-oxo-2-phenyl-3-quinolinecarboxylic Acid (39). Compound **26** (0.23 g, 4.85 mmol) was hydrolyzed using the same conditions as described above for **38** to yield **39** as a white solid (0.18 g, 90%): mp 250 °C dec; ^{1}H NMR ($CDCl_3$) δ 15.40 (br s, 1H), 8.01 (dd, J = 1.5, 8.2 Hz, 1H), 7.58–7.45 (m, 5H), 7.12 (s, 2H), 3.60–3.46 (m, 1H), 2.66 (s, 6H), 0.84–0.75 (m, 2H), 0.63–0.54 (m, 2H). Anal. ($C_{26}H_{20}F_2N_2O_3{\cdot}H_2O$) C, H, N.

1-Cyclopropyl-6,8-difluoro-1,4-dihydro-2-methyl-7-(2,6-dimethyl-4-pyridinyl)quinolin-4-one (41). Compound **25** (0.10 g, 0.24 mmol) was dissolved in HOAc (8 mL), H_2O (2 mL), concentrated HCl (1 mL) and the mixture heated on a steam bath for 8 h. The solution was concentrated to yield a yellow oil which was purified by silica gel flash chromatography ($CHCl_3$–MeOH 20:1) to yield **41** as a white solid (0.065 g, 80%): mp 236 °C; ^{1}H NMR ($CDCl_3$) δ 7.91 (dd, J = 1.9, 9.5 Hz, 1H), 7.14 (s, 2H), 6.17 (s, 1H), 3.61–3.57 (m, 1H), 2.68 (s, 6H), 2.59 (s, 3H), 1.30–1.20 (m, 2H), 0.84–0.78 (m, 2H). Anal. ($C_{20}H_{16}F_2N_2O$) C, H, N.

1-Cyclopropyl-6,8-difluoro-1,4-dihydro-7-(2,6-dimethyl-4-pyridinyl)-2-phenylquinolin-4-one (42). Compound **26** (0.28 g, 0.59 mmol) was dissolved in HOAc (8 mL), H_2O (2 mL), and concentrated HCl (1 mL) and the mixture heated on a steam bath for 8 h. The solution was concentrated to yield a yellow oil which was purified by silica gel flash chromatography (EtOAc) to yield **42** as a white solid (0.14 g, 60%): mp 199 °C; ^{1}H NMR ($CDCl_3$) δ 7.97 (dd, J = 1.8, 9.5 Hz, 1H), 7.63–7.60 (m, 2H), 7.54–7.50 (m, 3H), 7.17 (s, 2H), 6.36 (s, 1H), 3.72–3.60 (m, 1H), 2.67 (s, 6H), 0.80–0.70 (m, 2H), 0.56–0.48 (m, 2H). Anal. ($C_{25}H_{20}F_2N_2O$) C, H, N.

1-Cyclopropyl-6,8-difluoro-1,4-dihydro-7-(2,6-dimethyl-4-pyridinyl)quinolin-4-one (40). A mixture of **1** (1.85 g, 5.0 mmol), NaCN (0.30 g, 6.0 mmol), and 10 mL of DMF was heated at 150 °C under argon for 0.5 h. The mixture was partitioned between EtOAc and H_2O and the EtOAc portion was filtered and concentrated to give 1.5 g of a crude solid. A portion (0.10 g) of this material was purified by preparative TLC (silica gel; EtOAc) and recrystallization (EtOH) to give **40**: mp 234–236 °C; ^{1}H NMR ($CDCl_3$) δ 8.03 (dd, 1H), 7.74 (d, J = 12 Hz, 1H), 7.11 (s, 2H), 6.15 (d, J = 12 Hz, 1H), 3.88 (m, 1H), 2.62 (s, 6H), 1.18 (m, 4H). Anal. ($C_{19}H_{16}F_2N_2O$) C, H, N.

Ethyl 5-Cyclopropyl-6,8-difluoro-7-(2,6-dimethyl-4-pyridinyl)-5*H*-isoxazolo[5,4,3-*de*]quinoline-3-carboxylate (30). A mixture of **28**[10] (0.22 g, 0.50 mmol), NaN_3 (0.39 g, 0.60 mmol), and 2 mL of DMF was heated at 100 °C for 2 h and concentrated. The residue was triturated with H_2O and the resulting solid was collected and purified by silica gel flash chromatography (EtOAc) to give 0.090 g (40%) of **30**: mp 220–223 °C dec; ^{1}H NMR ($CDCl_3$) δ 7.94 (s, 1H), 7.03 (s, 2H), 4.39 (q, J = 7.5 Hz, 2H), 3.55 (m, 1H), 2.60 (s, 6H), 1.42 (t, J = 7.5 Hz, 3H), 1.20 (d, 4H); IR (KBr) 1721 and 1674 cm^{-1}; CIMS m/z 411 (MH^+); UV (EtOH) λ_{max} 213 (ϵ 38 219), 227 (ϵ 39 610), 273 (ϵ 10 200), 412.5 nm (ϵ 11 154). Anal. ($C_{22}H_{19}F_2N_3O_3$) C, H, N.

5-Amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-(2,6-dimethyl-4-pyridinyl)-4-oxo-3-quinolinecarboxylic Acid (44). A mixture of **30** (0.38 g, 0.86 mmol), 10% Pd/C (0.05 g), and 20 mL of DMF was hydrogenated at 50 psi in a Parr shaker at 25 °C for 3 h. The mixture was filtered and the residue was concentrated and triturated with Et_2O to give 0.31 g (86%) of a 5-aminoquinolone ester intermediate. This material was heated at reflux for 14 h with 1.0 mL of 1N NaOH and 5 mL of THF. The mixture was concentrated and the residue was suspended in H_2O, acidified with 1 N HCl, and made neutral with aqueous $NaHCO_3$. The yellow solid that separated was collected, suspended in EtOH, and treated with excess methanesulfonic acid. Et_2O was added to the resulting solution and 0.27 g (76%) of **44** was collected: mp 264–265 °C; ^{1}H NMR (CF_3CO_2D) δ 11.7 (s, 0.5H), 9.3 (s, 1H), 8.0 (s, 2H), 4.4 (br s, 1H), 3.1 (s, 3H), 3.0 (s, 3H), 1.4–1.6 (m, 4H). Anal. ($C_{20}H_{17}F_2N_3O_3{\cdot}CH_3SO_3H{\cdot}1.5H_2O$) C, H, N.

1-Cyclopropyl-6,8-difluoro-1,4-dihydro-7-(2-methyl-4-pyridinyl)-4-oxo-3-quinolinecarboxylic Acid (46). A stirred solution of 3.15 g (0.183 mmol) of 4-bromo-2-methylpyridine[37] in 50 mL of Et_2O was cooled to −70 °C under N_2 and 2.5 M n-BuLi (7.3 mL, 0.0183 mol) was added dropwise in 20 min. Stirring at −70 °C was continued for 20 min and tri-n-butyltin chloride (5.2 mL, 0.0183 mol) was then added dropwise in 15 min. The reaction mixture was stirred at −70 °C for 1.5 h and then allowed stand overnight at 25 °C. The solution was filtered and concentrated to give 8.39 g (120%) of crude stannane. A portion of this stannane (1.07 g, 2.8 mmol), **31**[10] (1.00 g, 2.7 mmol), and $PdCl_2(PPh_3)_2$ (0.100 g, 0.14 mmol) in 2-methoxyethanol (40 mL) was heated under N_2 in an oil bath at 130–140 °C for 7 h. After treatment with Darco and filtration, the pale filtrate was concentrated and the residue was crystallized from iPrOAc to yield 0.480 g (43%) of a crude 2-methoxyethyl ester intermediate. This ester intermediate heated on a steam bath with 1 N HCl (6 mL) for 2 h. The precipitated solid was redissolved by dilution with 20 mL of warm H_2O and washed with $CHCl_3$. The aqueous phase was then carefully treated with aqueous NaOH and HOAc to pH 6.0. The precipitated solid was collected, washed with H_2O, and dried to give 0.220 g (56%) of **46**: mp 256–257 °C; ^{1}H NMR ($CDCl_3$) δ 14.41 (s, 1H), 8.92 (s, 1H), 8.73 (d, 1H), 8.15 (dd, 1H), 7.37 (s, 1H), 7.28 (m, 1H), 4.09 (m, 1H), 2.72 (s, 3H), 1.37 (m, 2H), 1.29 (m, 2H). Anal. ($C_{19}H_{14}F_2N_2O_3$) H, N, C: calcd, 64.04; found 63.49.

1-Cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxo-7-(4-pyridinyl)-3-quinolinecarboxylic Acid (47). A stirred mixture of **31**[10] (2.90 g, 7.8 mmol), 4-(tri-n-butylstannyl)pyridine[38] (3.36 g, 9.1 mmol), $PdCl_2(PPh_3)_2$ (0.19 g, 0.27 mmol), and DMF (2 mL) under N_2 was placed in a cold oil bath and the temperature was raised to 165 °C in 20 min and then maintained at 140–145 °C for 1 h. The cooled reaction mixture was stirred with $CHCl_3$ and H_2O, filtered, and the layers were separated. After drying ($MgSO_4$), the $CHCl_3$ layer was concentrated to give a crude ethyl ester intermediate as a brown oil (6.62 g). Digestion with hexane and Et_2O afforded a tan solid which was partially purified by extraction with EtOAc. The material obtained on evaporation of the EtOAc was further separated by treatment with cold 2 N HCl into an insoluble fraction (discarded) and a soluble fraction which on basification with K_2CO_3 gave product 0.220 g (7.6%) of ester intermediate. This material was heated in 1 N HCl (3 mL) on a steam bath for 2 h, washed with $CHCl_3$, and the pH of the solution was adjusted to 6.0 with 35% NaOH and HOAc. The precipitated white solid was collected, washed with H_2O, and dried to give **47** (0.120 g, 5%): mp 280–281 °C dec; ^{1}H NMR ($CDCl_3$) δ 14.22 (s, 1H), 8.92 (s, 1H), 8.89 (m, 2H), 8.19 (dd, 1H), 7.49 (m, 2H), 4.10 (m, 1H), 1.38 (m, 2H), 1.26 (m, 2H). Anal. ($C_{18}H_{12}F_2N_2O_3$) C, H, N.

1-Cyclopropyl-6,8-difluoro-1,4-dihydro-7-(2,6-dimethyl-3-pyridinyl)-4-oxo-3-quinolinecarboxylic Acid (48). A stirred solution of 2,6-dimethyl-3-(trimethylstannyl)pyridine (prepared from 3-bromo-2,6-dimethylpyridine[39] using a procedure similar to that described in the section for **46**) (1.51 g, 5.6 mmol), **31**[10] (1.90 g, 5.1 mmol), 2-methoxyethanol (80 mL), and $PdCl_2(PPh_3)_2$ (0.200 mg, 0.28 mmol) was heated under N_2 at 135–140 °C for 24 h. The resulting mixture was treated with Darco, filtered, and concentrated to give a crude product that was purified by silica gel chromatography (Et_2O–i$PrNH_2$ 96:4) to give a 2-ethoxyethyl ester intermediate as a white solid (0.530 g, 24%). This ester was treated with 1 N HCl (6 mL) on a steam bath for 2 h. Purification, effected through reprecipitation using NaOH and HOAc, afforded **48** (0.330 g, 74%): mp 213–221 °C; ^{1}H NMR ($CDCl_3$) δ 14.30 (s, 1H), 8.91 (s, 1H), 8.12 (dd, 1H), 7.52, 7.20 (dd_{AB}, 2H), 4.03 (m, 1H), 2.63 (s, 3H), 2.43 (s, 3H), 1.28 (m, 4H). Anal. ($C_{20}H_{16}F_2N_2O_3$) H, N; C: calcd, 64.86; found, 64.40.

1-Cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxo-7-(3-pyridinyl)-3-quinolinecarboxylic Acid (49). A stirred solution of 3-(trimethylstannyl)pyridine[40] (1.88 g, 7.8 mmol), **31**[10] (2.64 g, 7.1 mmol), $PdCl_2(PPh_3)_2$ (0.200 g, 0.28 mmol), and 2-methoxyethanol (80 mL) was heated at 140 °C for 20 h. Darco was added to the black suspension and it was filtered and concentrated to give a viscous liquid. The crude product was purified by silica gel chromatography (Et_2O–hexane–i$PrNH_2$ 66:30:4) to afford a 2-methoxyethyl ester intermediate (0.720 g, 25%). A mixture of this intermediate, 1 N HCl (10 mL), and EtOH (10 mL) was stirred at reflux on a steam bath for 3 h. The turbid solution was concentrated and the residue warmed with dilute NaOH for 15 min. After adjustment to pH 6 with HOAc, the white solid was collected and recrystallized from EtOH to afford **49** (0.270 g, 44%): mp 233–235 °C; ^{1}H NMR ($CDCl_3$) δ 14.28 (s, 1H), 8.92 (s, 1H), 8.78 (br s, 2H), 8.18 (dd, 1H), 7.90 (d, 1H), 7.53 (m, 1H), 4.08 (m, 1H), 1.30 (m, 4H). Anal. ($C_{18}H_{12}F_2N_2O_3$) C, H, N.

Mammalian Topoisomerase II Inhibition Assay Procedure. The inhibition of human topoisomerase II was quantitated using a known procedure.[29] Topo II was purified from late log phase suspension cultures of HeLa cells by minor modification of a known method.[41] One unit of topo II was defined as the amount of enzyme which forms a precipitable complex with 1 ng of pBR322 DNA under standard assay conditions in the presence of 10 μg/mL. Assays were assembled at 4 °C. A 5-μL sample of test compound in either 0.1 N NaOH or 0.04 N HCl or reference agent in 5% DMSO was added to 25 μL of assay mix (50 mM Tris-Cl, pH 7.9, 44 mM NaCl, 10 mM $MgCl_2$, 0.6 mM DTT, 0.5 mM EDTA, 30 μg/mL BSA, 0.5 mM ATP, 5.5% (w/v) glycerol, 4 ng 3'-[^{32}P]-pBR322 DNA (10^7 dpm/μg), 10 units topo II). Assays were incubated for 20 min at 37 °C and terminated by the addition of 3 μL of 10% SDS followed by the addition of 266 μL of 10 mM Tris-Cl, pH 7.5, 20 μg/mL calf thymus DNA, 1% SDS. A SDS/protein precipitate was formed by the addition of KCl to a final concentration of 0.2 M and chilling on ice for a minimum of 10 min. The precipitate was collected on GFB glass fiber filter membranes with a Brandell cell harvester and washed sequentially seven times with 10 mM Tris-Cl, pH 7.5, 100 mM KCl, one time with 95% EtOH, and one time with 70% EtOH. After drying, cpm was determined by liquid scintillation counting with 5 mL of Biofluor (NEN Research Products) or Readisafe (Beckman Instruments Inc.) cocktail.

Preparation of Test Compound. A stock solution (6 mg/mL) of test compound was prepared in either 0.1 N NaOH or 0.2 N HCl. This solution then was diluted 1:5 into H_2O and serially thereafter in either 0.02 N NaOH or 0.04 N HCl, respectively. The stock solution and serial dilution of the test compound was stored at −20 °C prior to testing. Reference agents (m-AMSA, VP-16) were prepared in 100% DMSO, diluted with H_2O to 5% DMSO, and serially thereafter in 0.05% DMSO.

Controls. A solvent control, which indicates the base level of topo II–DNA complex formed in the absence of the test compound, was included in each test. A control, in which topo II was omitted, was included for each test compound at the highest drug concentration tested.

Reference Agent. A dose–response curve with m-AMSA at 0.01, 0.08, 0.16, 0.32, 1.0, and 10 μg/mL was included in each test.

Data Reduction. The EC_{50} (effective concentration at which 50% of the maximal DNA–topo II complex is formed) of a test compound is defined to be the concentration with activity equal to the EC_{50} of the reference agent, mAMSA (EC_{50} = 0.72 μM). The maximal DNA–topo II complex formed is taken being equal to that formed at the nearly saturating dose of mAMSA (10 μg/mL).

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