Synthesis and SAR of [1,2,4]Triazolo[1,5-*a*]pyrimidines, a Class of Anticancer Agents with a Unique Mechanism of Tubulin Inhibition

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The synthesis and SAR of a series of triazolopyrimidines as anticancer agents are described. Treatment of 5-chloro-6-(trifluorophenyl)-*N*-fluoroalkyl [1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine with an alcohol, a thiol, or an alkylamine provided the corresponding final compounds. A clear SAR requirement has been established for optimal activity. A (1*S*)-2,2,2-trifluoro-1-methylethylamino group or an achiral 2,2,2-trifluoroethylamino group is required at the 5-position to achieve high potency. On the phenyl ring, both fluoro atoms, at the positions ortho to the triazolopyrimidine core, are needed for optimal activity. At the position para to the triazolopyrimidine core, on the phenyl ring, the best activity is achieved with an oxygen linkage followed by a three-methylene unit, and an alkylamino or a hydroxy group. The mechanism of action for this series of triazolopyrimidines was shown to be unique in that they promoted tubulin polymerization *in vitro*, but did not bind competitively with paclitaxel.[1] Instead, they inhibit the binding of *vincas* to tubulin. Selected compounds were studied further, and it was shown that these compounds were able to overcome resistance attributed to several multidrug resistance transporter proteins. Lead compounds were shown to inhibit tumor growth in several nude mouse xenograft models, with high potency and efficacy, when dosed either orally or intravenously.

Introduction

Microtubules are dynamic protein fibers formed by polymerization of α,β-tubulin heterodimers. They are vital components of the mitotic spindle and serve multiple functions, including maintenance of cell shape, vesicle and protein transport, and chromosome segregation during mitosis. Antimicrotubule drugs are a major category of anticancer agents.[2] These agents work by interfering with the microtubule dynamics and thus disrupting normal spindle function.[3] The most potent compounds and those of clinical relevance are natural products or semisynthetic derivatives of natural products.

Most antimicrotubule agents work by inhibiting polymerization of tubulin to microtubules.[4] These agents are categorized into two major classes depending on their distinctive binding sites on β-tubulin. The first class is the *vinca* alkaloid site binders, as represented by vinblastine and vincristine. The other class is the colchicine site binders, which have attracted considerable synthetic efforts. So far, no colchicine site binders have proven successful in clinical trials. Taxoids are the first class of antimicrotubules that were reported to promote tubulin polymerization and stabilize microtubules.[5] Since then, a number of natural products have been discovered that have taxoid-like activity, including epothilones,[6] discodermolide,[7] eleutherobin,[8] laulimalide,[9] and peloruside.[10] While epothilones, discodermolide, and eleutherobin bind to microtubules competitively with paclitaxel,[11−13] laulimalide and peloruside do not compete with paclitaxel and thus do not bind in the taxoid site.[14,15] The clinical success of the taxanes has encouraged efforts to develop other microtubule-stabilizing agents for cancer chemotherapy.[16]

Now, we report the synthesis and structure−activity relationship of a series of triazolopyrimidines as anticancer agents that interfere with the microtubule dynamics in a manner unique among all microtubule-active compounds. The initial lead compound **5**[17] came through selective screening of our Agricultural Research Division compound libraries based on evidence for antimicrotubule-like mechanism of action against fungi. Our subsequent study in human tumor cells showed that this compound inhibits tumor cell growth by interacting directly with tubulin with a novel mechanism of action. Additional analogues were then prepared to optimize potency and other pharmaceutical properties.

Chemistry. Compounds **5**−**30** and **32**−**39** were prepared as shown in Scheme 1. Coupling of 1-bromo-2,4,6-trifluorobenzene **1a** with diethyl malonate gave diethyl 2,4,6-trifluorophenylmalonate **2**.[18] Cyclization of **2** with 3-amino-1,2,4-triazole provided 5,7-dihydroxy-6-(2,4,6-trifluorophenyl)[1,2,4]triazolo[1,5-*a*]pyrimidine **3**[19] as a single regioisomer. Bis-chlorination was achieved by refluxing **3** in excess $POCl_3$, affording compound **4**.[19] Treatment with a fluoroalkylamine [$CF_3C(R_1R_2)NH_2$] in DMF replaced the 7-chloro exclusively,[17] yielding the desired 7-amino compounds **5**−**7**. Compounds **8**−**30** (Table 1) were prepared by further treatment of compounds **5**−**7** with the corresponding alcohols (for compounds **8**−**27**) or a thiol (for compound **28**), in the presence of sodium hydride in DMSO, or with neat amines in the presence of sodium hydride (for compounds **29** and **30**).

Compounds **32**−**39** (Tables 1 and 2) were prepared analogously to compounds **5**−**30**. Compound **32** was prepared by treating dihydroxy compound **3** with $POBr_3$ instead of $POCl_3$, followed by replacement of the 7-bromo with an amine. Subsequent treatment with 3-dimethylamino-1-propanol gave compound **33**. Compounds **34** and **36** were prepared in the same manner as compound **5**, starting from 1-bromo-2,4-difluorobenzene (**1b**) and 1-bromo-2,3,6-trifluorobenzene (**1c**), respectively. Reaction of compounds **34** and **36** with amino alcohols provided compounds **35** and **37**−**39**, respectively.

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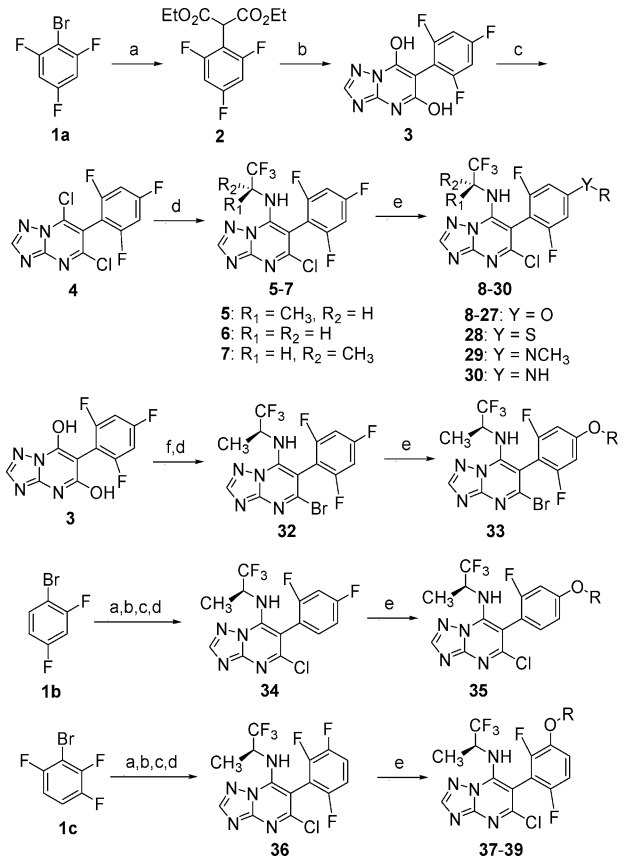
Scheme 1. Synthesis of **5**−**30** and **32**−**39**[a]



[a] (a)[18] Diethyl malonate, CuI, NaH, dioxane, 60 °C; (b)[19] 3-Amino-1,2,4-triazole, tributylamine, 160−180 °C; (c)[19] $POCl_3$, reflux; (d) $CF_3(CR_1R_2)NH_2$, *N*,*N*-diisopropylethylamine, DMF; (e) For **8**−**27**, **33**, **35**, **37**−**39**: ROH, NaH, DMSO; for **28**: RSH, NaH, DMSO; for **29**: $RN(CH_3)H$, NaH, 100 °C; for **30**: RNH_2, NaH, 100 °C; (f) $POBr_3$, 120 °C.

We found that the 4-fluoro group on the phenyl ring (in **5**−**7**, **32**, and **34**) could be replaced cleanly with no replacement of the 2-fluoro group detected. Replacement of the 3-fluoro group on the phenyl ring (in **36**), however, was less selective, with some replacement of the 2-fluoro group detected.

As depicted in Scheme 2, synthesis of compound **31** started from diethyl 2-(2,6-difluoro-4-methoxyphenyl)malonate **40**,[20] which was prepared from 1-bromo-2,6-difluoro-4-methoxybenzene **1d**. Demethylation followed by triflate formation set the stage for palladium-catalyzed coupling, to introduce the alkyl chain. Conversion of the hydroxy group of the resultant compound **43** to the dimethylamino group was accomplished by mesylation and subsequent nucleophilic displacement. Cyclization of **44** with 3-amino-1,2,4-triazole followed by chlorination in excess $POCl_3$ and replacement of the 7-chloro with (1*S*)-2,2,2-trifluoro-1-methylethylamine in DMF, as before, yielded the desired compound **31**.

Results and Discussion

Compounds **5**−**39** were evaluated in the COLO 205 cytotoxicity assay for their ability to inhibit cancer cell proliferation. Comparable IC_{50} values were obtained when a given compound was tested either as a free base or as a hydrogen chloride salt. As shown in Table 1, the chirality in the 2,2,2-trifluoro-1-methylethylamino group is important for activity, as the *S*-enantiomers **5** and **8** were 3−4 fold more potent than their corresponding *R*-enantiomers **7** and **10**.[21] Removal of the chirality by removal of the 1-methyl group in the chiral amino group resulted in analogues that retained potency, as shown by comparison of **9** to **8** or **12** to **11** (also **6** to **5**). In contrast, removal of the chirality by introduction of an additional 1-methyl group to the 2,2,2-trifluoro-1-methylethylamino group (i.e., *gem*-1,1-dimethyl-2,2,2-trifluoroethylamino group) led to much diminished activity (data to be published separately), illustrating space limitations in the binding region.

Substitution of the 4-fluoro group on the phenyl ring in compounds **5**−**7** led to compounds **8**−**31**. A clear trend of SAR was manifested from activity shown by these compounds. High potency was achieved with an oxygen linkage and a three-methylene tether followed by an alkylamino group or a hydroxy group, as in compounds **8**, **9**, **11**−**16**, and **19**. Compound **15**, bearing a six-membered piperidine ring at the terminal, was less potent than compound **14**, with a five-membered pyrrolidine ring at the terminal. With a more hydrophilic morpholino group, compound **16** was comparable to compound **14** in potency. Surprisingly, the four-membered azetidine ring, as in compound **17**, led to much decreased potency. When the terminal amino group was unsubstituted, as in compound **18**, lower potency was observed. Lower potency was also observed when the terminal hydroxy group was capped by an alkyl group (compound **20**). Activity was further diminished when the terminal group was a straight alkyl group, as in compound **21**. These results suggest that the potency is at its best with a moderately hydrophilic group at the terminal position.

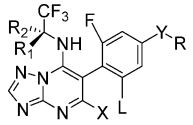
Compounds **22**−**24**, with a two-methylene tether instead of the three-methylene tether, showed much lower potency, suggesting strict spatial requirements for the functional groups at the binding site. This hypothesis is further supported by activity of compounds **25**−**27**. With a four- or a five-methylene tether, compounds **25**−**27** showed to be less potent than their three-methylene analogues, though twice as potent as their two-methylene analogues.

Replacement of the oxygen linkage with a sulfur linkage (compound **28**), an amino group (compounds **29** and **30**), or a methylene unit (compound **31**) invariably led to reduced activity. Among these analogues, compound **28** exhibited about 4-fold reduction in potency. Other analogues were even less potent. Compounds **32** and **33**, with a 5-bromo substituent instead of a 5-chloro group, were comparable with or slightly less potent than their 5-chloro analogues.

As shown in Table 2, compounds **37**−**39**, with the aminoalkoxy group moved from the 4- to the 3-position on the phenyl ring, proved to be less potent than their corresponding regioisomers **22**, **11**, and **25**, even though the 2,3,6-trifluorophenyl compound **36** showed potency comparable to that of the 2,4,6-trifluorophenyl analogue **5**. Interestingly, compound **39**, with a four-methylene tether, showed the highest activity among **37**−**39**. Modeling suggests that, from the 3-position on the phenyl ring, it takes a four-methylene tether (as in **39**) to reach the same space and putative interactions, as reached by a three-methylene tether from the 4-position (as in **11**).

Compounds **8**−**9**, **11**−**12**, **16**, and **33** were further studied in order to elucidate the mechanism of action for this series of compounds.[22] Tubulin polymerization studies using either MAP-rich tubulin or pure tubulin showed these compounds promoted tubulin polymerization *in vitro*, a phenomenon similar to the effect of paclitaxel. Competitive binding studies with [^{3}H]-vinblastine, [^{3}H]colchicine, and [^{3}H]paclitaxel showed that these compounds did not bind competitively with [^{3}H]colchicine or [^{3}H]paclitaxel. Instead, they prevented binding of [^{3}H]vinblastine to tubulin.[22] These results established the unique mechanism of action for these compounds, in that they promote tubulin polymerization like paclitaxel does, but through a binding site

Table 1. Inhibition of COLO 205 Cell Proliferation by Compounds **5**−**35**

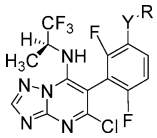


5-35

compd[a]	R_1	R_2	X	L	Y	R	IC_{50} (nM)[b]
5	CH_3	H	Cl	F	F		267 ± 73
6	H	H	Cl	F	F		372 ± 68
7	H	CH_3	Cl	F	F		705 ± 21
8[c]	CH_3	H	Cl	F	O	$(CH_2)_3NH(CH_3)$	31 ± 10
9[c]	H	H	Cl	F	O	$(CH_2)_3NH(CH_3)$	29 ± 7
10	H	CH_3	Cl	F	O	$(CH_2)_3NH(CH_3)$	126 ± 1
11[c]	CH_3	H	Cl	F	O	$(CH_2)_3N(CH_3)_2$	56 ± 9
12[c]	H	H	Cl	F	O	$(CH_2)_3N(CH_3)_2$	53 ± 16
13	CH_3	H	Cl	F	O	$(CH_2)_3N(CH_3)(CH_2CH_3)$	48 ± 6
14	CH_3	H	Cl	F	O	$(CH_2)_3$-pyrrolidine	44 ± 13
15	CH_3	H	Cl	F	O	$(CH_2)_3$-piperidine	113 ± 12
16[c]	CH_3	H	Cl	F	O	$(CH_2)_3$-morpholine	51 ± 30
17	CH_3	H	Cl	F	O	$(CH_2)_3$-azetidine	326 ± 116
18	CH_3	H	Cl	F	O	$(CH_2)_3NH_2$	452 ± 71
19	CH_3	H	Cl	F	O	$(CH_2)_3OH$	67 ± 12
20	CH_3	H	Cl	F	O	$(CH_2)_3OCH_2CH_3$	269 ± 45
21	CH_3	H	Cl	F	O	$(CH_2)_3CH_3$	1898 ± 258
22	CH_3	H	Cl	F	O	$(CH_2)_2N(CH_3)_2$	581 ± 157
23	CH_3	H	Cl	F	O	$(CH_2)_2$-morpholine	245 ± 118
24	CH_3	H	Cl	F	O	$(CH_2)_2OH$	422 ± 175
25	CH_3	H	Cl	F	O	$(CH_2)_4N(CH_3)_2$	276 ± 53
26	CH_3	H	Cl	F	O	$(CH_2)_4OH$	151 ± 19
27	CH_3	H	Cl	F	O	$(CH_2)_5OH$	214 ± 31
28	CH_3	H	Cl	F	S	$(CH_2)_3N(CH_3)_2$	192 ± 72
29	CH_3	H	Cl	F	$N(CH_3)$	$(CH_2)_3N(CH_3)_2$	1671 ± 34
30	CH_3	H	Cl	F	NH	$(CH_2)_3N(CH_3)_2$	719 ± 185
31	CH_3	H	Cl	F	CH_2	$(CH_2)_3N(CH_3)_2$	665 ± 103
32	CH_3	H	Br	F	F		232 ($n = 1$)
33	CH_3	H	Br	F	O	$(CH_2)_3N(CH_3)_2$	96 ± 21
34	CH_3	H	Cl	H	F		953 ± 54
35	CH_3	H	Cl	H	O	$(CH2)_3N(CH_3)_2$	262 ± 18
paclitaxel							3.3 ± 1
vincristine							2.6 ± 0.5

[a] Unless indicated otherwise, compounds were tested as the free base. [b] Concentration needed to inhibit cell growth by 50% as determined from the dose−response curve. Determinations were made at 10 concentrations, in triplicate, and repeat values agreed, on average, within 40%. [c] Compounds were tested as the HCl salts.

Table 2. Inhibition of COLO 205 Cell Proliferation by Compounds **36**−**39**



36-39

compd[a]	Y	R	IC_{50} (nM)[b]
36	F		183 ± 23
37	O	$(CH_2)_2N(CH_3)_2$	1568 ± 81
38	O	$(CH_2)_3N(CH_3)_2$	785 ± 64
39	O	$(CH_2)_4N(CH_3)_2$	433 ± 74

[a] Compounds were tested as the free base. [b] Concentration needed to inhibit cell growth by 50% as determined from the dose−response curve. Determinations were made at 10 concentrations, in triplicate, and repeat values agreed, on average, within 40%.

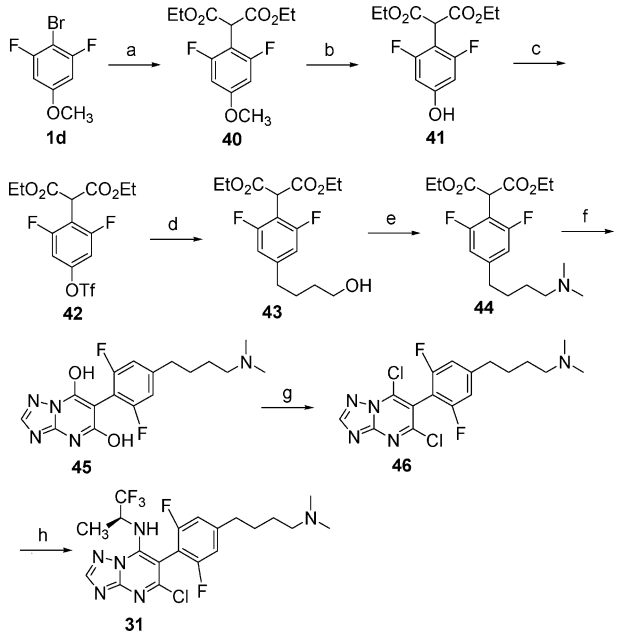
that may overlap with the binding site of agents which inhibit tubulin polymerization. Cell cycle analysis of compounds **8**, **9**, **11**, and **12** showed induction of apoptosis at low compound concentration and G2/M block at high compound concentration, a pattern that is similar to what was observed with paclitaxel.[23]

Compounds **8**, **9**, **11**, **12**, and **26** were shown to be poor substrates for multidrug resistance (MDR) transporter proteins. Their ability to overcome resistance due to P-glycoprotein (P-gp) is shown in Table 3. The KB lines[24] express different amounts of the P-gp (MDR1) membrane pump which causes resistance to the action of many cytotoxic compounds, including paclitaxel and vincristine, via enhanced efflux from the cell. The parental KB line expresses little P-gp, KB 8.5 expresses moderate, clinically relevant, levels of the protein, and KB-V1 expresses artificially high levels. The ability of P-gp to recognize and export a potential cytotoxic agent can be inferred from the change in IC_{50} values in these lines. Compared with paclitaxel and vincristine, compounds **8**, **9**, **11**, **12**, and **26** showed much lower levels of recognition by P-gp, as evidenced by the relatively low resistance ratios.

Selected lead compounds were evaluated in U87-MG human glioblastoma, using both intravenous and oral administration. Significant inhibition of tumor growth *in vivo* was observed with compounds **8**, **9**, **11**, **12**, **14**, and **16**. Representative testing results (compound **9** in U87-MG human glioblastoma and compound **8** in A549 non-small cell lung carcinoma) are shown in Figure 1 and Figure 2, respectively.

Compound **8** has excellent pharmaceutical properties. It is very stable metabolically and has high solubility in water and saline. It is minimally metabolized in female nude mouse, rat and human liver microsomes, with $t_{1/2}$ values at 433, 88, and 133 min, respectively. Its solubility in water is 0.89 mg/mL at pH 6.74 and 7.99 mg/mL at pH 5.48. Thus, no special

Scheme 2. Synthesis of **31**[a]



[a] (a) Diethyl malonate, CuI, NaH, dioxane, 60 °C; (b) BBr_3, CH_2Cl_2; (c) $(CF_3SO_2)_2O$, Et_3N, CH_2Cl_2; (d) (i) 9-BBN, 3-buten-1-ol, THF, (ii) **42**, $Pd(PPh_3)_4$, K_3PO_4, dioxane, 90 °C, (iii) trimethylamine-*N*-oxide, 180 °C; (e) (i) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0 °C, (ii) $(CH_3)_2NH$, THF; (f) 3-amino-1,2,4-triazole, tributylamine, 160−180 °C; (g) $POCl_3$, reflux; (h) (1*S*)-$CF_3CH(CH_3)NH_2$, *N*,*N*-diisopropylethylamine, DMF.

Table 3. Inhibition of KB, KB 8.5, and KBV1 Cell Proliferation by Compounds **8**, **9**, **11**, **12**, and **26**

	IC_{50} (nM)[b]			ratio[c]	
compd[a]	KB	KB 8.5	KB V1	KB8.5/KB	KB V1/KB
8	23 ± 1.4	67 ± 11	953 ± 480	2.9	41.5
9	19 ± 4.7	56 ± 17	2237 ± 880	3.0	118
11	18.7 ± 0.2	33 ± 13	114 ± 74	1.8	6.1
12	18.7 ± 0.9	38 ± 13	263 ± 133	2.0	14.0
26	42 ± 5.6	70 ± 6.4	67 ± 6.4	1.6	1.6
paclitaxel	2.45 ± 0.1	26 ± 0.1	2013 ± 108	11	822
vincristine	2.2 ± 0.6	58 ± 16	2035 ± 25	26	925

[a] Compounds **8**, **9**, **11**, and **12** were tested as the HCl salts; compound **26** was tested as a free base. [b] Concentration needed to inhibit cell growth by 50% as determined from the dose−response curve. Determinations were made at 10 concentrations, in duplicate, and repeat values agreed, on average, within 10%. [c] Ratio = IC_{50} on KB 8.5 or KB V1 cells/IC_{50} on KB cells. A ratio of about 1 indicates no resistance.

formulation is required. PK studies in rat showed high volume of distribution (14 L/kg), low clearance (14 mL/min/kg), high half-life (13 h), and good oral bioavailability (61%). Compound **8** showed comparable inhibition of tumor growth by either intravenous or oral administration in U87-MG human glioblastoma.[22] It also exhibited good tumor inhibition in other tumor models, e.g., in A549 non-small cell lung carcinoma (NSCLC, Figure 2). On the basis of its superb pharmaceutical properties, *in vivo* activity, and more favorable toxicity profile *in vivo*, compound **8** was chosen for further development.

Conclusion. A series of triazolopyrimidine analogues was systematically prepared and tested as anticancer agents. A clear SAR requirement was established for optimal activity. A (1*S*)-2,2,2-trifluoro-1-methylethylamino group or an achiral 2,2,2-trifluoroethylamino group was required at the 5-position to achieve high potency. Both fluoro atoms at the ortho positions on the phenyl ring were needed for optimal activity. On the para position on the phenyl ring, the best activity was achieved with an oxygen linkage followed by a three-methylene unit, and an alkylamino or a hydroxy terminal. The mechanism of action

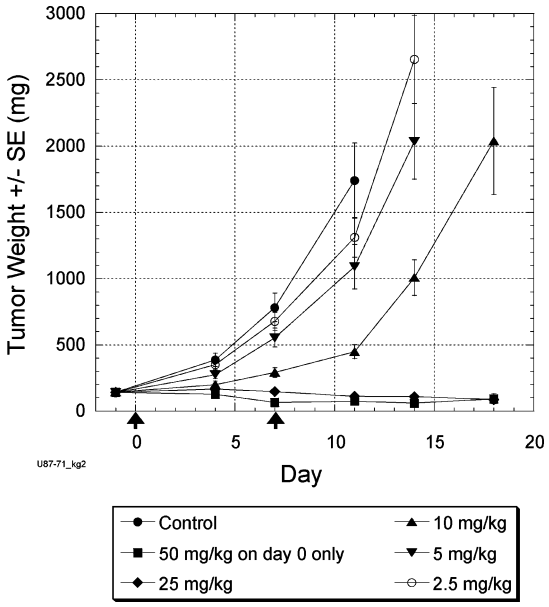


Figure 1. *In vivo* antitumor activity with compound **9** against U87-MG human glioblastoma. The compound was administered to tumor-bearing mice on days 0 and 7 after staging (indicated by arrows) by oral gavage. The control group received vehicle only (Klucel) by iv injection. Each group contained nine animals.

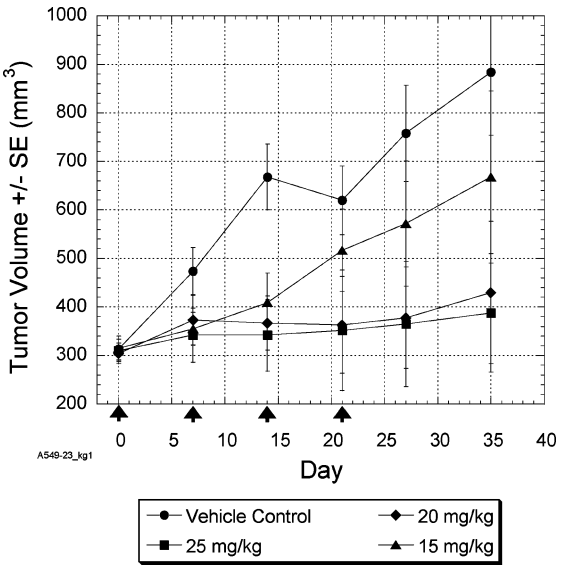


Figure 2. *In vivo* antitumor activity with compound **8** against A549 human non-small cell lung carcinoma (NSCLC). Tumors were initiated in the flanks of nude mice by subcutaneous injection of 10^7 cells per animal in 100% Matrigel and staged at about 300 mm^3. The compound was administered to tumor-bearing mice on days 0, 7, 14, and 21 after staging (indicated by arrows) by intravenous injection. Vehicle was 0.9% saline. Each group contained nine animals.

for this series of triazolopyrimidines was shown to be unique in that they promoted tubulin polymerization *in vitro*, but did not bind competitively with paclitaxel. As far as we know, these compounds represent the only series of small, synthetic molecules to promote tubulin polymerization. Selected compounds were shown to inhibit tumor growth in several nude mouse xenograph models with high potency and efficacy. Compound **8** (TTI-237) is currently in phase I clinical trials.

Experimental Section

Chemistry. General. All reactions were conducted under nitrogen atmosphere with magnetic stirring. Chromatographic

purifications were performed using Baker 40-μm silical gel. Melting points were determined in open capillary tubes on a Meltemp melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a Bruker Avance 400 spectrometer at 400 MHz. Chemical shifts are quoted in parts per million from internal standard tetramethylsilane. Mass spectra were obtained using a Micromass LCT mass spectrometer operating at 20 eV.

5-Chloro-6-(2,4,6-trifluorophenyl)-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (5). A mixture of 5,7-dichloro-6-(2,4,6-trifluorophenyl)[1,2,4]triazolo[1,5-a]pyrimidine **4**[19] (3.0 g, 9.4 mmol), (1*S*)-2,2,2-trifluoro-1-methylethylamine hydrogen chloride[17] (4.2 g, 28.2 mmol), and *N*,*N*-diisopropylethylamine (4.9 mL, 28.2 mmol) in 100 mL of *N*,*N*-dimethylformamide was stirred at room temperature under nitrogen atmosphere for 13 h. The reaction mixture was diluted with ethyl acetate. The organic layer was washed with 1 N hydrochloric acid (2×) and saturated sodium chloride (2×), dried over magnesium sulfate, and concentrated. The residue was chromatographed over silica gel, eluting with 20% ethyl acetate in hexanes to provide 3.56 g (96%) of **5** as a light yellow solid: mp 124−126 °C; ^{1}H NMR (DMSO-d_6) δ 1.44 (d, J = 7 Hz, 3H), 6.17 (m, 1H), 7.48 (m, 2H), 8.03 (d, J = 8 Hz, 1H), 8.70 (s, 1H); MS (ES+): *m*/*z* 396.0 (M + 1). Anal. ($C_{14}H_8ClF_6N_5$) C, H, N.

Chiral HPLC showed that the compound had a 99.6% ee.

5-Chloro-6-(2,4,6-trifluorophenyl)-*N*-(2,2,2-trifluoroethyl)-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (6). According to the procedure used to prepare **5**, reaction of **4**[19] with 2,2,2-trifluoroethylamine provided **6** in 95% yield as a light yellow solid: mp 168−170 °C; ^{1}H NMR ($CDCl_3$) δ 4.19 (m, 2H), 6.17 (m, 1H), 6.93 (m, 2H), 8.42 (s, 1H); MS (ES+): *m*/*z* 382.0 (M + 1). Anal. ($C_{13}H_6ClF_6N_5$) C, H, N.

5-Chloro-6-(2,4,6-trifluorophenyl)-*N*-[(1*R*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (7). According to the procedure used to prepare **5**, reaction of **4**[19] with (1*R*)-2,2,2-trifluoro-1-methylethylamine hydrogen chloride[21] provided **7** in 83% yield as a light yellow solid: mp 98−101 °C; ^{1}H NMR ($CDCl_3$) δ 1.43 (d, J = 7 Hz, 3H), 4.69 (m, 1H), 5.87 (d, J = 10 Hz, 1H), 6.93 (m, 2H), 8.41 (s, 1H); MS (ES+): *m*/*z* 396.1 (M + 1). Anal. ($C_{14}H_8ClF_6N_5$) C, H, N.

5-Chloro-6-{2,6-difluoro-4-[3-(methylamino)propoxy]phenyl}-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (8). To a suspension of sodium hydride (60% in mineral oil, 2.3 g, 57.6 mmol) in 20 mL of dimethylsulfoxide at room temperature was added a solution of 3-(methylamino)propan-1-ol[25] (5.14 g, 57.6 mmol) in 10 mL of dimethyl sulfoxide. The solution was stirred at room temperature for 1 h, and **5** (5.7 g, 14.4 mmol) was added. The mixture was heated at 60 °C for 3 h and cooled to room temperature. The reaction mixture was diluted with ethyl acetate and washed with water and saturated sodium chloride. The organic layer was dried over magnesium sulfate, and concentrated. The residue was triturated with small amount of acetone and then hexanes and chromatographed over silica gel, eluting with a gradient of 100% ethyl acetate to 100% methyl alcohol to provide 2.7 g (40%) of **8** as a white solid. This product was dissolved in 150 mL of 10% methyl alcohol in methylene chloride and filtered. To the filtrate was bubbled hydrogen chloride gas. Concentration provided 2.92 g of hydrogen chloride salt of **8** as a light yellow solid: mp 42−44 °C; ^{1}H NMR (DMSO-d_6) δ 1.44 (d, J = 7 Hz, 3H), 2.14 (m, 2H), 2.57 (t, J = 6 Hz, 3H), 3.05 (m, 2H), 4.20 (t, J = 6 Hz, 2H), 5.90 (m, 1H), 6.99 (d, J = 9 Hz, 2H), 8.09 (d, J = 9 Hz, 1H), 9.01 (m, 2H); MS (ES+): *m*/*z* 465.2 (M + 1). Anal. ($C_{18}H_{18}ClF_5N_6O$·2HCl) C, H, N.

5-Chloro-6-{2,6-difluoro-4-[3-(methylamino)propoxy]phenyl}-*N*-(2,2,2-trifluoroethyl)[1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (9). According to the procedure used to prepare **8**, reaction of **6** with 3-(methylamino)propan-1-ol[25] provided hydrogen chloride salt of **9** in 50% yield as a yellow solid: mp 78−80 °C; ^{1}H NMR (DMSO-d_6) δ 2.11 (m, 2H), 2.58 (t, J = 5 Hz, 3H), 3.05 (m, 2H), 4.20 (t, J = 6 Hz, 2H), 4.72 (m, 2H), 7.00 (d, J = 9 Hz, 2H), 8.48 (t, J = 7 Hz, 1H), 8.68 (s, 1H), 8.37 (brs, 2H); MS (ES+): *m*/*z* 451.3 (M + 1). Anal. ($C_{17}H_{16}ClF_5N_6O$·1.3HCl) C, H, N.

5-Chloro-6-{2,6-difluoro-4-[3-(methylamino)propoxy]phenyl}-*N*-[(1*R*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (10). According to the procedure used to prepare **8**, reaction of **7** with 3-(methylamino)propan-1-ol[25] provided **10** in 51% yield as a light tan solid: mp 139−141 °C; ^{1}H NMR (DMSO-d_6) δ 1.18 (m, 3H), 2.03 (t, J = 7 Hz, 2H), 2.55 (s, 3H), 2.99 (t, J = 7 Hz, 2H), 4.10 (t, J = 6 Hz, 2H), 5.76 (m, 1H), 6.72 (brs, 2H), 8.06 (brs, 1H); MS (ES+): *m*/*z* 465.2 (M + 1). Anal. ($C_{18}H_{18}ClF_5N_6O$) C, H, N.

5-Chloro-6-{4-[3-(dimethylamino)propoxy]-2,6-difluorophenyl}-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]-pyrimidin-7-amine (11). According to the procedure used to prepare **8**, reaction of **5** with 3-dimethylamino-1-propanol provided hydrogen chloride salt of **11** in 84% yield as a white solid: mp 65−67 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (d, J = 7 Hz, 3H), 2.46 (m, 2H), 2.90 (d, J = 5 Hz, 6H), 3.28 (m, 2H), 4.23 (t, J = 6 Hz, 2H), 4.81 (brs, 1H), 5.91 (d, J = 10 Hz, 1H), 6.66 (d, J = 9 Hz, 2H), 8.39 (s, 1H); MS (ES+): *m*/*z* 479.1 (M + 1). Anal. ($C_{19}H_{20}ClF_5N_6O$·2HCl) C, H, N.

5-Chloro-6-{4-[3-(dimethylamino)propoxy]-2,6-difluorophenyl}-*N*-(2,2,2-trifluoroethyl)[1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (12). According to the procedure used to prepare **8**, reaction of **6** with 3-dimethylamino-1-propanol provided hydrogen chloride salt of **12** in 44% yield as a white solid: mp 130 °C (decomposed); ^{1}H NMR ($CDCl_3$) δ 2.47 (m, 2H), 2.87 (d, J = 5 Hz, 6H), 3.23 (m, 2H), 4.26 (t, J = 6 Hz, 2H), 4.42 (m, 2H), 6.47 (m, 1H), 6.66 (d, J = 8 Hz, 2H), 8.39 (s, 1H); MS (ES+): *m*/*z* 465.1 (M + 1). Anal. ($C_{18}H_{18}ClF_5N_6O$·1.95HCl) C, H, N.

5-Chloro-6-(4-{3-[ethyl(methyl)amino]propoxy}-2,6-difluorophenyl)-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo-[1,5-*a*]pyrimidin-7-amine (13). According to the procedure used to prepare **8**, reaction of **5** with 3-[ethyl(methyl)amino]propan-1-ol[26] provided **13** in 23% yield as colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.07 (t, J = 7 Hz, 3H), 1.40 (d, J = 7 Hz, 3H), 2.02 (m, 2H), 2.26 (s, 3H), 2.46 (q, J = 7 Hz, 2H), 2.54 (t, J = 7 Hz, 2H), 4.11 (t, J = 6 Hz, 2H), 4.76 (brs, 1H), 5.88 (brs, 1H), 6.66 (m, 2H), 8.39 (s, 1H); MS (ES+): *m*/*z* 493.1 (M + 1). Anal. ($C_{20}H_{22}ClF_5N_6O$) C, H, N.

5-Chloro-6-[2,6-difluoro-4-(3-pyrrolidin-1-ylpropoxy)phenyl]-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (14). According to the procedure used to prepare **8**, reaction of **5** with 3-pyrrolidin-1-ylpropan-1-ol[27] provided **14** in 60% yield as a tan solid: mp 35−38 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (d, J = 7 Hz, 3H), 1.83 (m, 4H), 2.08 (m, 2H), 2.59 (m, 4H), 2.69 (t, J = 7 Hz, 2H), 4.12 (t, J = 6 Hz, 2H), 4.78 (brs, 1H), 5.89 (brs, 1H), 6.67 (m, 2H), 8.39 (s, 1H); MS (ES+): *m*/*z* 505.2 (M + 1). Anal. ($C_{21}H_{22}ClF_5N_6O$·0.3EtOAc) C, H, N.

5-Chloro-6-[2,6-difluoro-4-(3-piperidin-1-ylpropoxy)phenyl]-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (15). According to the procedure used to prepare **8**, reaction of **5** with 3-piperidin-1-ylpropan-1-ol[27] provided **15** in 65% yield as a tan solid: mp 40−43 °C; ^{1}H NMR ($CDCl_3$) δ 1.40 (d, J = 7 Hz, 3H), 1.46 (m, 2H), 1.61 (m, 4H), 2.03 (m, 2H), 2.43 (m, 4H), 2.49 (t, J = 7 Hz, 2H), 4.10 (t, J = 6 Hz, 2H), 4.78 (brs, 1H), 5.87 (brs, 1H), 6.67 (m, 2H), 8.39 (s, 1H); MS (ES+): *m*/*z* 519.3 (M + 1). Anal. ($C_{22}H_{24}ClF_5N_6O$) C, H, N.

5-Chloro-6-[2,6-difluoro-4-(3-morpholin-4-ylpropoxy)phenyl]-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (16). According to the procedure used to prepare **8**, reaction of **5** with 3-morpholin-4-ylpropan-1-ol[27] provided hydrogen chloride salt of **16** in 65% yield as a white solid: mp 68−70 °C; ^{1}H NMR ($CDCl_3$) δ 1.43 (d, J = 7 Hz, 3H), 2.57 (m, 2H), 2.94 (m, 2H), 3.24 (m, 2H), 3.52 (d, J = 12 Hz, 2H), 4.02 (d, J = 10 Hz, 2H), 4.22 (t, J = 7 Hz, 2H), 4.35 (t, J = 12 Hz, 2H), 4.78 (brs, 1H), 5.90 (d, J = 10 Hz, 1H), 6.65 (d, J = 8 Hz, 2H), 8.39 (s, 1H); MS (ES+): *m*/*z* 521.1 (M + 1). Anal. ($C_{21}H_{22}ClF_5N_6O_2$·2HCl) C, H, N.

6-[4-(3-Azetidin-1-ylpropoxy)-2,6-difluorophenyl]-5-chloro-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (17). According to the procedure used to prepare **8**, reaction of **5** with 3-azetidin-1-ylpropan-1-ol[27] provided **17** in 45%

yield as a tan solid: mp 48−50 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (d, J = 7 Hz, 3H), 1.88 (m, 2H), 2.11 (m, 2H), 2.46 (m, 2H), 2.62 (t, J = 7 Hz, 2H), 3.25 (m, 2H), 4.08 (t, J = 6 Hz, 2H), 4.77 (brs, 1H), 5.88 (brs, 1H), 6.66 (m, 2H), 8.39 (s, 1H); MS (ES+): *m*/*z* 491.1 (M + 1). Anal. ($C_{20}H_{20}ClF_5N_6O\cdot 0.3EtOAc$) C, H, N.

6-[4-(3-Aminopropoxy)-2,6-difluorophenyl]-5-chloro-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (18). According to the procedure used to prepare **8**, reaction of **5** with 3-amino-1-propanol provided **18** in 42% yield as a yellow solid: mp 170−180 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (d, J = 7 Hz, 3H), 2.05 (m, 2H), 3.04 (t, J = 7 Hz, 2H), 4.16 (t, J = 6 Hz, 2H), 4.82 (m, 1H), 6.68 (m, 2H), 8.38 (s, 1H); MS (ES-): *m*/*z* 448.9 (M-1). Anal. ($C_{17}H_{16}ClF_5N_6O\cdot 1.0CH_3OH$) C, H, N.

3-[4-(5-Chloro-7-{[(1*S*)-2,2,2-trifluoro-1-methylethyl]amino}-[1,2,4]triazolo[1,5-*a*]pyrimidin-6-yl)-3,5-difluorophenoxy]propan-1-ol (19). According to the procedure used to prepare **8**, reaction of **5** with 1,3-propanediol provided **19** in 66% yield as a tan solid: mp 86−88 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (d, J = 7 Hz, 3H), 1.52 (t, J = 4 Hz, 1H), 2.11 (m, 2H), 3.90 (q, J = 4 Hz, 2H), 4.13 (t, J = 7 Hz, 2H), 4.77 (m, 1H), 5.89 (d, J = 6 Hz, 1H), 6.68 (m, 2H), 8.39 (s, 1H); MS (ES+): *m*/*z* 452.1 (M + 1). Anal. ($C_{17}H_{15}ClF_5N_5O_2\cdot 0.25EtOAc$) C, H, N.

5-Chloro-6-[4-(3-ethoxypropoxy)-2,6-difluorophenyl]-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (20). According to the procedure used to prepare **8**, reaction of **5** with 3-ethoxy-1-propanol provided **20** in 52% yield as a light yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.22 (t, J = 7 Hz, 3H), 1.41 (d, J = 7 Hz, 3H), 2.11 (m, 2H), 3.52 (q, J = 7 Hz, 2H), 3.61 (t, J = 6 Hz, 2H), 4.15 (t, J = 6 Hz, 2H), 4.76 (m, 1H), 5.89 (d, J = 11 Hz, 1H), 6.67 (m, 2H), 8.39 (s, 1H); MS (ES-): *m*/*z* 477.9 (M-1). Anal. ($C_{19}H_{19}ClF_5N_5O_2\cdot 0.2CH_3OH$) C, H, N.

6-(4-Butoxy-2,6-difluorophenyl)-5-chloro-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (21). According to the procedure used to prepare **8**, reaction of **5** with 1-butanol provided **21** in 34% yield as a light yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.01 (t, J = 6 Hz, 3H), 1.40 (d, J = 7 Hz, 3H), 1.52 (m, 2H), 1.84 (m, 2H), 4.03 (q, J = 6 Hz, 2H), 4.76 (m, 1H), 5.89 (d, J = 10 Hz, 1H), 6.65 (m, 2H), 8.39 (s, 1H); MS (ES-): *m*/*z* 447.9 (M-1). Anal. ($C_{18}H_{17}ClF_5N_5O\cdot 0.2EtOAc$) C, H, N.

5-Chloro-6-{4-[2-(dimethylamino)ethoxy]-2,6-difluorophenyl}-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (22). According to the procedure used to prepare **8**, reaction of **5** with *N*,*N*-dimethylethanolamine provided **22** in 46% yield as a light yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.41 (d, J = 7 Hz, 3H), 2.40 (s, 6H), 2.82 (t, J = 6 Hz, 2H), 4.15 (t, J = 6 Hz, 2H), 4.72 (m, 1H), 5.90 (d, J = 9 Hz, 1H), 6.69 (m, 2H), 8.39 (s, 1H); MS (ES+): *m*/*z* 465.1 (M + 1). Anal. ($C_{18}H_{18}ClF_5N_6O\cdot 1.0CH_3OH$) C, H, N.

5-Chloro-6-[2,6-difluoro-4-(2-morpholin-4-ylethoxy)phenyl]-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (23). According to the procedure used to prepare **8**, reaction of **5** with 4-(2-hydroxyethyl)morpholine provided **23** in 64% yield as a light yellow solid: mp 36−38 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (d, J = 7 Hz, 3H), 2.61 (t, J = 5 Hz, 4H), 2.86 (t, J = 6 Hz, 2H), 3.76 (t, J = 5 Hz, 4H), 4.17 (t, J = 6 Hz, 2H), 4.75 (brs, 1H), 5.89 (d, J = 10 Hz, 1H), 6.68 (m, 2H), 8.39 (s, 1H); MS (ES-): *m*/*z* 504.9 (M-1). Anal. ($C_{20}H_{20}ClF_5N_6O_2$) C, H, N.

2-[4-(5-Chloro-7-{[(1*S*)-2,2,2-trifluoro-1-methylethyl]amino}-[1,2,4]triazolo[1,5-*a*]pyrimidin-6-yl)-3,5-difluorophenoxy]ethanol (24). According to the procedure used to prepare **8**, reaction of **5** with ethylene glycol provided **24** in 16% yield as a light tan solid: mp 39−41 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (d, J = 7 Hz, 3H), 4.05 (t, J = 5 Hz, 2H), 4.16 (t, J = 5 Hz, 2H), 4.73 (m, 1H), 5.90 (d, J = 6 Hz, 1H), 6.71 (m, 2H), 8.39 (s, 1H); MS (ES+): *m*/*z* 438.1 (M + 1). Anal. ($C_{16}H_{13}ClF_5N_5O_2$) C, H, N.

5-Chloro-6-{4-[4-(dimethylamino)butoxy]-2,6-difluorophenyl}-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (25). According to the procedure used to prepare **8**, reaction of **5** with 4-dimethylamino-1-butanol provided **25** in 56% yield as a tan solid: mp 91−92 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (d, J = 7 Hz, 3H), 1.67 (m, 2H), 1.88 (m, 2H), 2.25 (s, 6H), 2.35 (t, J = 7 Hz, 2H), 4.06 (t, J = 6 Hz, 2H), 4.77 (m, 1H), 5.90 (brs, 1H), 6.65 (m, 2H), 8.39 (s, 1H); MS (ES+): *m*/*z* 493.1 (M + 1). Anal. ($C_{20}H_{22}ClF_5N_6O\cdot 0.6\ H_2O$) C, H, N.

4-[4-(5-Chloro-7-{[(1*S*)-2,2,2-trifluoro-1-methylethyl]amino}-[1,2,4]triazolo[1,5-*a*]pyrimidin-6-yl)-3,5-difluorophenoxy]butan-1-ol (26). According to the procedure used to prepare **8**, reaction of **5** with 1,4-butanediol provided **26** in 23% yield as a light yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.37 (t, J = 5 Hz, 1H), 1.41 (d, J = 7 Hz, 3H), 1.79 (m, 2H), 1.96 (m, 2H), 3.77 (q, J = 7 Hz, 2H), 4.09 (t, J = 6 Hz, 2H), 4.77 (brs, 1H), 5.88 (d, J = 10 Hz, 1H), 6.65 (m, 2H), 8.39 (s, 1H); MS (ES-): *m*/*z* 463.9 (M-1). Anal. ($C_{18}H_{17}ClF_5N_5O_2\cdot 0.4EtOAc$) C, H, N.

5-[4-(5-Chloro-7-{[(1*S*)-2,2,2-trifluoro-1-methylethyl]amino}-[1,2,4]triazolo[1,5-*a*]pyrimidin-6-yl)-3,5-difluorophenoxy]pentan-1-ol (27). According to the procedure used to prepare **8**, reaction of **5** with 1,5-pentanediol provided **27** in 63% yield as a light yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.32 (t, J = 5 Hz, 1H), 1.41 (d, J = 7 Hz, 3H), 1.60 (m, 2H), 1.67 (m, 2H), 1.89 (m, 2H), 3.71 (q, J = 6 Hz, 2H), 4.04 (t, J = 6 Hz, 2H), 4.76 (brs, 1H), 5.89 (d, J = 10 Hz, 1H), 6.65 (m, 2H), 8.39 (s, 1H); MS (ES-): *m*/*z* 477.9 (M-1). Anal. ($C_{19}H_{19}ClF_5N_5O_2\cdot 0.3EtOAc$) C, H, N.

5-Chloro-6-(4-{[3-(dimethylamino)propyl]thio}-2,6-difluorophenyl)-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo-[1,5-*a*]pyrimidin-7-amine (28). According to the procedure used to prepare **8**, reaction of **5** with 3-(dimethylamino)propan-1-thiol[28] provided **28** in 48% yield as a white solid: mp 38−40 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (d, J = 7 Hz, 3H), 1.90 (m, 2H), 2.26 (s, 6H), 2.44 (t, J = 7 Hz, 2H), 3.09 (t, J = 7 Hz, 2H), 4.80 (brs, 1H), 5.86 (d, J = 10 Hz, 1H), 7.03 (m, 2H), 8.40 (s, 1H); MS (ES+): *m*/*z* 495.2 (M + 1). Anal. ($C_{19}H_{20}ClF_5N_6S$) C, H, N.

***N*^{1}-[4-(5-Chloro-7-{[(1*S*)-2,2,2-trifluoro-1-methylethyl]amino}-[1,2,4]triazolo[1,5-*a*]pyrimidin-6-yl)-3,5-difluorophenyl]-*N*^{1},*N*^{3},*N*^{3}-trimethylpropane-1,3-diamine (29)**. To a mixture of **5** (200 mg, 0.51 mmol) in *N*,*N*,*N*′-trimethyl-1,3-propanediamine (3.0 g, 25.8 mmol) was added sodium hydride (100 mg, 2.5 mmol). The resulting mixture was heated at 100 °C for 16 h. The reaction was then quenched with water and extracted with ethyl acetate (×2). The combined organic extracts were washed with saturated sodium chloride, dried over magnesium sulfate, and concentrated. The residue was chromatographed over silica gel, eluting with a gradient of 100% ethyl acetate to 50% methyl alcohol in ethyl acetate to 100% methyl alcohol to provide 20 mg (8%) of **29** as a light yellow solid: mp 50−55 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (d, J = 7 Hz, 3H), 1.78 (m, 2H), 2.24 (s, 6H), 2.29 (t, J = 6 Hz, 2H), 3.02 (s, 3H), 3.43 (t, J = 7 Hz, 2H), 4.90 (brs, 1H), 5.86 (brs, 1H), 6.38 (m, 2H), 8.37 (s, 1H); MS (ES+): *m*/*z* 492.1 (M + 1). Anal. ($C_{20}H_{23}ClF_5N_7$) C, H, N.

***N*^{1}-[4-(5-Chloro-7-{[(1*S*)-2,2,2-trifluoro-1-methylethyl]amino}-[1,2,4]triazol o[1,5-*a*]pyrimidin-6-yl)-3,5-difluorophenyl]-*N*^{3},*N*^{3}-dimethylpropane-1,3-diamine (30)**. According to the procedure used to prepare **29**, reaction of **5** with 3-(dimethylamino)propylamine provided **30** in 17% yield as yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.41 (d, J = 6 Hz, 3H), 1.81 (m, 2H), 2.27 (s, 6H), 2.46 (t, J = 6 Hz, 2H), 3.24 (m, 2H), 4.91 (brs, 1H), 5.94 (brs, 2H), 6.25 (m, 2H), 8.36 (s, 1H); MS (ES+): *m*/*z* 478.2 (M + 1). Anal. ($C_{19}H_{21}ClF_5N_7\cdot 0.5CH_3OH$) C, H, N.

5-Chloro-6-{4-[4-(dimethylamino)butyl]-2,6-difluorophenyl}-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (31). To a solution of **40**[20] (2.11 g, 7.0 mmol) in 60 mL of methylene chloride at −78 °C was added boron tribromide (2.65 mL, 28 mmol) dropwise. The mixture was then stirred at −78 °C for 10 min, warmed to 0 °C, and stirred at 0 °C for 1 h. A 5% aqueous solution of sodium bicarbonate was added slowly to quench the reaction. The product was extracted with ethyl acetate. The combined organic extracts were washed with saturated sodium chloride, dried over magnesium sulfate, and concentrated. The residue was chromatographed over silica gel, eluting with a gradient of 10% ethyl acetate in hexanes to 30% ethyl acetate in hexanes, to provide 1.91 g (95%) of **41** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.23 (t, J = 7 Hz, 6H), 4.26 (q, J = 7 Hz, 4H), 4.86 (s, 1H), 6.01 (brs, 1H), 6.34 (d, J = 9 Hz, 2H); MS (ES-): *m*/*z* 287.2 (M-1).

To a solution of **41** (288 mg, 1.0 mmol) and triethylamine (505 mg, 5.0 mmol) in 5 mL of methylene chloride at room temperature was added trifluoromethanesulfonic anhydride (1.41 g, 5.0 mmol). The mixture was stirred at room temperature for 10 min. A 5% aqueous solution of sodium bicarbonate was added slowly to quench the reaction. The product was extracted with ethyl acetate. The combined organic extracts were washed with saturated sodium chloride, dried over magnesium sulfate, and concentrated. The residue was chromatographed over silica gel, eluting with a gradient of hexanes to 15% ethyl acetate in hexanes, to provide 361 mg (86%) of **42** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.29 (t, $J = 7$ Hz, 6H), 4.28 (q, $J = 7$ Hz, 4H), 4.94 (s, 1H), 6.95 (d, $J = 7$ Hz, 2H); MS (ES-): *m*/*z* 419.2 (M-1).

To a 0.5 M solution of 9-borabicyclo[3.3.1]nonane (9-BBN) in tetrahydrofuran (95 mL, 47.6 mmol) was added dropwise 3-buten-1-ol (4.1 mL, 47.6 mmol), and the mixture was stirred under nitrogen atmosphere at room temperature for 6 h. The resulting solution was then transferred by a double-ended needle into a mixture of **42** (10.0 g, 23.8 mmol), potassium phosphate (10.1 g, 47.6 mmol), and tetrakis(triphenylphosphine)palladium(0) (825 mg, 0.714 mmol) in 40 mL of dioxane under nitrogen pressure. The mixture was then heated at 90 °C for 8 h. The reaction was cooled to room temperature, and trimethylamine-*N*-oxide (3.57 g, 47.6 mmol) was added. The reaction was heated at 80 °C for 1 h and cooled to room temperature. Ethyl acetate was added to dilute the mixture. The organic phase was washed with saturated sodium chloride (×2), dried over magnesium sulfate, and concentrated. The residue was chromatographed over silica gel, eluting with a gradient of 10% ethyl acetate in hexanes to 50% ethyl acetate in hexanes, to provide 3.5 g (43%) of **43** as a brown oil: ^{1}H NMR ($CDCl_3$) δ 1.28 (t, $J = 7$ Hz, 6H), 1.60 (m, 2H), 1.69 (m, 2H), 2.63 (t, $J = 8$ Hz, 2H), 3.66 (t, $J = 7$ Hz, 2H), 4.25 (q, $J = 7$ Hz, 4H), 4.91 (s, 1H), 6.76 (d, $J = 9$ Hz, 2H); MS (ES+): *m*/*z* 345.2 (M + 1).

To a solution of **43** (2.0 g, 5.8 mmol) and triethylamine (2.43 mL, 17.4 mmol) in 15 mL of methylene chloride at 0 °C was added methanesulfonyl chloride (0.898 mL, 11.6 mmol). The resulting mixture was allowed to warm to room temperature in 1.5 h. The mixture was washed with 10% hydrochloric acid, saturated sodium bicarbonate, and saturated sodium chloride. The organic layer was dried over magnesium sulfate and concentrated. The yellow oil thus obtained was stirred with 2.0 M diethylamine in tetrahydrofuran (56 mL, 112 mmol) at room temperature for 16 h, followed by concentration. The residue was diluted with ethyl acetate. The organic layer was washed with water and saturated sodium chloride, dried over magnesium sulfate, and concentrated. The residue was chromatographed over silica gel, eluting with a gradient of 100% ethyl acetate to 50% methyl alcohol in ethyl acetate to 100% methyl alcohol, to provide 1.2 g (56%) of **44** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.27 (t, $J = 7$ Hz, 6H), 1.51 (m, 2H), 1.62 (m, 2H), 2.20 (s, 6H), 2.26 (t, $J = 7$ Hz, 2H), 2.61 (t, $J = 8$ Hz, 2H), 4.25 (q, $J = 7$ Hz, 4H), 4.91 (s, 1H), 6.75 (d, $J = 9$ Hz, 2H); MS (ES+): *m*/*z* 372.2 (M + 1).

A mixture of **44** (1.0 g, 2.7 mmol), 3-amino-1,2,4-triazole (250 mg, 3.0 mmol), and tributylamine (0.71 mL, 3.0 mmol) was stirred under nitrogen atmosphere at 160 °C for 16 h and cooled to room temperature. The mixture was stirred with 20 mL of hexanes. The precipitates were collected by filtration, washed with hexanes, to give 795 mg (81%) of **45** as a white solid: MS (ES-): *m*/*z* 362.1 (M-1).

A mixture of **45** (795 mg, 2.19 mmol) in 4 mL of phosphorus oxychloride was heated at 115 °C for 4 h. The excess phosphorus oxychloride was removed in vacuo, and the resulting residue was dried further under high vacuum to give 1.13 g of **46** as a yellow solid, which was used without further purification. A mixture of **46** (300 mg, 0.75 mmol), (1*S*)-2,2,2-trifluoro-1-methylethylamine hydrogen chloride (675 mg, 4.51 mmol), and *N*,*N*-diisopropylethylamine (0.787 mL, 4.51 mmol) in 4 mL of *N*,*N*-dimethylformamide was stirred at room temperature for 18 h. The reaction mixture was diluted with ethyl acetate. The organic layer was washed with saturated sodium bicarbonate and saturated sodium chloride, dried over magnesium sulfate, and concentrated. The residue was chromatographed over silica gel, eluting with a gradient of 100% ethyl acetate to 50% methyl alcohol in ethyl acetate to 100% methyl alcohol, to provide 44 mg of **31** as a light yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.39 (d, $J = 6$ Hz, 3H), 1.52 (m, 2H), 1.73 (m, 2H), 2.22 (s, 6H), 2.31 (t, $J = 7$ Hz, 2H), 2.77 (t, $J = 8$ Hz, 2H), 4.66 (brs, 1H), 5.89 (brs, 1H), 6.95 (m, 2H), 8.39 (s, 1H); MS (ES+): *m*/*z* 477.2 (M + 1). Anal. ($C_{20}H_{22}ClF_5N_6 \cdot 0.7\ CH_3OH$) C, H, N.

5-Bromo-6-(2,4,6-trifluorophenyl)-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (32). A mixture of 5,7-dihydroxy-6-(2,4,6-trifluorophenyl)[1,2,4]triazolo[1,5-*a*]pyrimidine[19] (282 mg, 1.0 mmol) and phosphorus oxybromide (2.0 g, 7.0 mmol) was heated at 120 °C for 4 h. Excess phosphorus oxybromide was then removed in vacuo. The residue was dissolved in methylene cholride and washed with water and saturated sodium chloride (×3). The organic layer was dried over magnesium sulfate, filtered through hydrous magnesium silicate, and concentrated to provide 380 mg (93%) of crude 5,7-dibromo-6-(2,4,6-trifluorophenyl)-[1,2,4]triazolo[1,5-*a*]pyrimidine as a tan semisolid. MS (ES+): *m*/*z* 408.9 (M + 1). The material was used directly in the next step without further purification.

A mixture of 5,7-dibromo-6-(2,4,6-trifluorophenyl)[1,2,4]triazolo-[1,5-*a*]pyrimidine (320 mg, 0.78 mmol), (1S)-2,2,2-trifluoro-1-methylethylamine hydrogen chloride[17] (235 mg, 1.57 mmol), and diisopropylethylamine (260 mg, 2.0 mmol) in 5 mL of *N*,*N*-dimethylformamide was stirred at room temperature for 18 h. Water was added to quench the reaction, and the product was extracted with ethyl acetate. The combined organic extracts were washed with saturated sodium chloride (×3), dried over magnesium sulfate, and concentrated. The residue was chromatographed over silica gel, eluting with a gradient of 9:1 hexanes/ethyl acetate to 2:1 hexanes/ethyl acetate, to provide 60 mg (17%) of **32** as a light tan solid: mp 95−97 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (d, $J = 7$ Hz, 3H), 4.71 (brs, 1H), 5.79 (d, $J = 10$ Hz, 1H), 6.94 (m, 2H), 8.40 (s, 1H); MS (ES+): *m*/*z* 440.0, 442.0 (M + 1). Anal. ($C_{14}H_8BrF_6N_5 \cdot 0.22EtOAc$) C, H, N.

5-Bromo-6-{4-[3-(dimethylamino)propoxy]-2,6-difluorophenyl}-*N*-[(1S)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]-pyrimidin-7-amine (33). To a solution of **32** (44 mg, 0.1 mmol) and 3-dimethylamino-1-propanol (51 m g, 0.5 mmol) in 1 mL of dimethylsulfoxide at room temperature was added sodium hydride (60% in mineral oil, 20 mg, 0.5 mmol). The mixture was heated at 60 °C for 2h, and cooled to room temperature. Water is added to quench the reaction, and the product is extracted with ethyl acetate. The organic layer is washed with saturated sodium chloride (×3), dried over magnesium sulfate, and concentrated. The residue is chromatographed over silica gel, eluting with a gradient of ethyl acetate to 30% methyl alcohol in ethyl acetate, to provide 41 mg (78%) of **33** as a light tan solid: mp 40−42 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (d, $J = 7$ Hz, 3H), 2.03 (m, 2H), 2.31 (s, 6H), 2.52 (brs, 2H), 4.11 (t, $J = 6$ Hz, 2H), 4.81 (brs, 1H), 5.84 (brs 1H), 6.66 (d, $J = 9$ Hz, 2H), 8.38 (s, 1H); MS (ES+): *m*/*z* 523.1, 525.1 (M + 1). Anal. ($C_{19}H_{20}BrF_5N_6O \cdot 0.25EtOAc$) C, H, N.

5-Chloro-6-(2,4-difluorophenyl)-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (34). According to the procedure used to prepare **5**, reaction of 5,7-dichloro-6-(2,4-difluorophenyl)[1,2,4]triazolo[1,5-*a*]pyrimidine[17] with (1*S*)-2,2,2-trifluoro-1-methylethylamine hydrogen chloride[17] provided **34** in 86% yield as a white solid: mp 35−37 °C; ^{1}H NMR ($CDCl_3$) δ 1.37 (d, $J = 7$ Hz, 3H), 4.51−4.95 (m, 1H), 5.52−5.89 (m, 1H), 7.12 (m, 2H), 7.32 (m, 1H), 8.40 (s, 1H); MS (ES+): *m*/*z* 378.2 (M + 1). Anal. ($C_{14}H_9ClF_5N_5$) C, H, N.

5-Chloro-6-{4-[3-(dimethylamino)propoxy]-2-fluorophenyl}-*N*-[(1S)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (35). According to the procedure used to prepare **8**, reaction of **34** with 3-dimethylamino-1-propanol provided **35** in 52% yield as a light tan solid: mp 36−38 °C; ^{1}H NMR ($CDCl_3$) δ 1.36 (d, $J = 7$ Hz, 3H), 2.04 (m, 2H), 2.30 (s, 6H), 2.52 (t, $J = 7$ Hz, 2H), 4.11 (t, $J = 6$ Hz, 2H), 4.59 (brs, 0.5H), 5.02 (brs 0.5H), 5.56 (brs 0.5H), 5.92(brs 0.5H), 6.86 (m, 2H), 7.18 (m, 1H), 8.38 (s, 1H); MS (ES+): *m*/*z* 461.2 (M + 1). Anal. ($C_{19}H_{21}ClF_4N_6O$) C, H, N.

5-Chloro-6-(2,3,6-trifluorophenyl)-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (36). According to the procedure used to prepare **5**, reaction of 5,7-dichloro-6-(2,3,6-trifluorophenyl)[1,2,4]triazolo[1,5-*a*]pyrimidine[17] with (1*S*)-2,2,2-trifluoro-1-methylethylamine hydrogen chloride[17] provided **36** in 85% yield as a white solid: mp 145−150 °C; ^{1}H NMR ($CDCl_3$) δ 1.44 (d, J = 7 Hz, 3H), 4.70 (m, 1H), 5.97 (d, J = 8 Hz, 1H), 7.10 (m, 1H), 7.42 (m, 1H), 8.41 (s, 1H); MS (ES+): *m*/*z* 396.2 (M + 1). Anal. ($C_{14}H_8ClF_6N_5$) C, H, N.

5-Chloro-6-{3-[2-(dimethylamino)ethoxy]-2,6-difluorophenyl}-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (37). According to the procedure used to prepare **8**, reaction of **36** with *N*,*N*-dimethylethanolamine provided **37** in 16% yield as a white solid: mp 48−58 °C; ^{1}H NMR ($CDCl_3$) δ 1.39 (m, 3H), 2.36 (s, 6H), 2.78 (t, J = 6 Hz, 2H), 4.15 (t, J = 6 Hz, 2H), 4.64 (brs, 0.5H), 4.74 (brs, 0.5H), 5.91 (brs, 1H), 7.04 (m, 1H), 7.21 (m, 1H), 8.40 (s, 1H); MS (ES+): *m*/*z* 465.1 (M + 1). Anal. ($C_{18}H_{18}ClF_5N_6O$) C, H, N.

5-Chloro-6-{3-[3-(dimethylamino)propoxy]-2,6-difluorophenyl}-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]-pyrimidin-7-amine (38). According to the procedure used to prepare **8**, reaction of **36** with 3-dimethylamino-1-propanol provided **38** in 10% yield as a white solid: mp 45−50 °C; ^{1}H NMR ($CDCl_3$) δ 1.39 (m, 3H), 2.05 (m, 2H), 2.26 (s, 6H), 2.47 (m, 2H), 4.16 (m, 2H), 5.91 (brs, 1H), 7.02 (m, 1H), 7.20 (m, 1H), 8.41 (s, 1H); MS (ES+): *m*/*z* 479.1 (M + 1). Anal. ($C_{19}H_{20}ClF_5N_6O\cdot0.3EtOAc$) C, H, N.

5-Chloro-6-{3-[4-(dimethylamino)butoxy]-2,6-difluorophenyl}-*N*-[(1*S*)-2,2,2-trifluoro-1-methylethyl][1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (39). According to the procedure used to prepare **8**, reaction of **36** with 4-dimethylamino-1-butanol provided **37** in 15% yield as a white solid: mp 55−60 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (m, 3H), 1.69 (m, 2H), 1.81 (m, 2H), 2.23 (s, 6H), 2.33 (t, J = 7 Hz, 2H), 4.09 (m, 2H), 4.70 (brs, 1H), 5.90 (brs, 1H), 7.03 (m, 1H), 7.16 (m, 1H), 8.40 (s, 1H); MS (ES+): *m*/*z* 493.1 (M + 1). Anal. ($C_{20}H_{22}ClF_5N_6O\cdot0.3EtOAc$) C, H, N.

Biological Evaluation and Tumor Xenograft. **Cytotoxicity Assay**. The MTS cytotoxicity assay, which is sold in kit form by Promega (Madison, WI; CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay), is based on the conversion by viable cells, but not by dead cells, of the tetrazolium salt, MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt), into a water-soluble colored formazan which is detected by spectrophotometry. For the test procedure, cells were harvested by trypsinization, washed, counted and distributed to wells of 96-well flat-bottom microtiter plates at 1000 cells per well in 200 μL of medium. In addition, one row of wells on a separate plate received cells as above ("time 0" plate). All plates were incubated at 37 °C in humidified 5% CO_2 in air for about 24 h.

On day 2, compounds for test were diluted and added to wells. Compounds were dissolved in DMSO at 10−20 mM. For each compound, nine serial 2-fold dilutions were prepared in DMSO. Ten microliters of each dilution was transferred to 100 μL of medium, mixed well, and then 5 μL of this dilution was transferred in triplicate or quadruplicate to wells containing cells. The final high concentration of each compound was typically 5 μM. Plates were returned to the incubator for 3 days.

At the time of drug addition to the experimental plates, the MTS assay was run on the "time 0" plate. This produced the "time 0 MTS value" which was related to the number of viable cells per well at the time of drug addition.

After 3 days of culture with test compounds (day 5 overall), the MTS assay was done on all wells of the experimental plates. The absorbance values of the replicate sample wells were averaged and divided by the average of the "time 0" values. The average of control wells without drug, divided by the average "time 0" value, gave the maximal relative increase in MTS color yield due to cell growth during the final 3 days of culture. The average of control wells with high drug concentration, divided by the "time 0" value, gave the minimal relative color yield for cells that were completely killed. The nine values for each compound were plotted against concentration, and the concentration that produced a relative color yield half way between the maximum and minimum was taken as the IC_{50} value.

Tumor Xenograft Experiments. Athymic nu/nu female mice were implanted subcutaneously in the flank with either 1×10^6 U87-MG human glioblastoma cells or 1×10^7 A549 human non-small cell lung carcinoma (NSCLC) cells. Cells were suspended in culture medium for injection. When tumors attained a mass of between 80 and 120 mg for U87-MG or about 300 mg for NSCLC (day 0), animals were randomized into treatment groups each containing 5−10 animals. After staging, animals were treated intravenously with the compound formulated in Klucel or 0.9% saline according to the schedules given in the figure legend, or with vehicle alone. Tumor volumes ([length × width^2]/2) were determined at regular intervals. The data were analyzed by a one-sided Student's *t*-test. A p-value $\leq$0.05 indicated a statistically significant reduction in tumor growth of the treated group compared to that of the vehicle control group. The protocols for KB, KB8.5, and KB V1 cellular assays have been reported.[24]

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Supporting Information Available: Elemental analysis data for compounds **5**−**39** and description of the mechanism study results. This material is available free of charge via the Internet at http://pubs.acs.org.

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