Antitumor Agents. 196.[†] Substituted 2-Thienyl-1,8-naphthyridin-4-ones: Their Synthesis, Cytotoxicity, and Inhibition of Tubulin Polymerization[1]

Shun-Xiang Zhang,[§] Kenneth F. Bastow,[§] Yoko Tachibana,[§] Sheng-Chu Kuo,[▽] Ernest Hamel,[‡] Anthony Mauger,[⊥] Ven L. Narayanan,[⊥] and Kuo-Hsiung Lee*[,§]

Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, Graduate Institute of Pharmaceutical Chemistry, China Medical College, Taichung 400, Taiwan, Republic of China, Laboratory of Drug Discovery Research and Development, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Frederick Cancer Research Center, Frederick, Maryland 21702, and Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

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As part of our continuing search for potential anticancer drug candidates in the 2-aryl-1,8-naphthyridin-4-one series, we have synthesized a series of substituted 2-thienyl-1,8-naphthyridin-4-ones. Most compounds showed significant cytotoxic effects (log $GI_{50} < -4.0$; log molar drug concentration required to cause 50% growth inhibition) against a variety of human tumor cell lines in the National Cancer Institute's in vitro screen, including cells derived from solid tumors such as non-small-cell lung, colon, central nervous system, melanoma, ovarian, prostate, and breast cancers. The most active compounds (**31**−**33**, **40**) demonstrated strong cytotoxic effects with ED_{50} values in the micromolar or submicromolar range in most of the tumor cell lines. The most cytotoxic compounds inhibited tubulin polymerization at concentrations substoichiometric to the tubulin concentration. The most potent inhibitors of polymerization (**40**, **42**, **43**) had effects comparable to those of the potent antimitotic natural products podophyllotoxin and combretastatin A-4 and to that of NSC 664171, a particularly potent, structurally related analogue. Only compound **40** was a potent inhibitor of the binding of radiolabeled colchicine to tubulin, and it was both the most cytotoxic agent and the most effective inhibitor of polymerization among the newly synthesized compounds.

Introduction

Microtubules are cylindrical organelles found in almost all cell types in eukaryotes. They are involved in many cellular processes, including mitosis, cell signaling, and motility,[2] and consequently are an important target for development of compounds potentially useful as anticancer chemotherapeutics. Microtubule dynamics play an important role in cell proliferation, and inhibition of microtubule dynamics now appears to be the mechanistic basis underlying the antitumor effects of most antimitotic compounds. Numerous chemically diverse antimitotic agents, many of which are derived from natural products, have been found to interact specifically with tubulin rather than with other components of microtubules or other proteins involved in mitosis.[3] Examples of clinically used antimitotic agents are the vinca alkaloids,[4] which inhibit microtubule polymerization, and the taxoids,[5] which promote microtubule assembly. Colchicine is another important antimitotic agent, and it plays a limited role in the therapy of selected inflammatory diseases. Colchicine has also been of major importance in studying the functions of microtubules.[6] Taxoids, vinca alkaloids, and colchicinoids appear to bind to three different binding domains of tubulin. In general, compounds binding to the colchicine domain are structurally much simpler than those binding to vinca or taxoid domains and include many natural products, such as cornigerine,[7] podophyllotoxin,[8] steganacin,[9] combretastatin A-4,[10,11] and some flavonones[12] (Figure 1). These compounds share "homology" with the A and C rings of colchicine, and this common feature has been described as a "biaryl" system connected by a hydrocarbon bridge of variable length.[13,14] The simplicity of these molecules provides for the discovery or rational design of mitotic inhibitors as antitumor agents.

In our continuing search for potent and selective cytotoxic antitumor agents, we synthesized two series of substituted heterocyclic ketones, 2-phenyl-4-quinolones[15−17] and 2-phenyl-1,8-naphthyridin-4-ones,[18,19] and discovered potent antimitotic agents in each series. Structure−activity relationship (SAR) studies of the phenylquinolone and arylnaphthyridinone classes led to the discovery of the particularly potent compounds NSC 664171 and NSC 679036, respectively. These agents inhibited the growth of the NCI tumor cell lines at nanomolar concentrations (log $GI_{50} < -8.0$). Both compounds acted as inhibitors of tubulin polymerization and colchicine binding to tubulin, and their potencies were comparable to those of natural products, such as combretastatin A-4 and 5,3′-dihydroxy-3,6,7,8,4′-pentamethoxyflavone. We have now extended this study to the synthesis and cytotoxic evaluation of a related series

[†] For Part 195, see ref 1.
* To whom correspondence should be addressed.
[§] University of North Carolina at Chapel Hill.
[▽] China Medical College.
[‡] Laboratory of Drug Discovery Research and Development, NCI.
[⊥] Drug Synthesis and Chemistry Branch, NCI.

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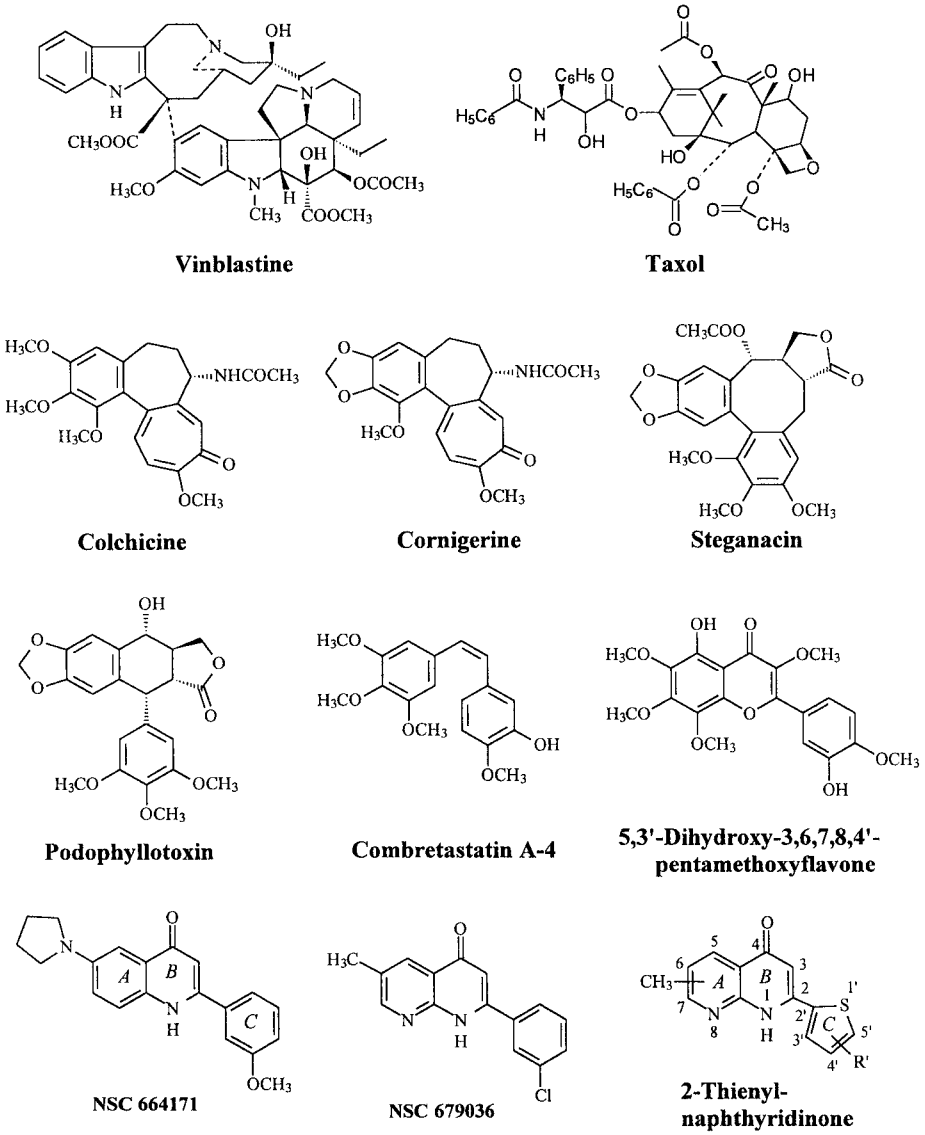


Figure 1. Structures of vinblastine, taxol, colchicine, cornigerine, podophyllotoxin, steganacin, combrestatin A-4, flavonol, quinolones, and naphthyridinone derivatives.

containing a thienyl ring: substituted 2-thienyl-1,8-naphthyridin-4-ones.

Chemistry

The syntheses of substituted 2-heterocyclic-1,8-naphthyridin-4-ones are outlined in Scheme 1. Substituted 2-aminopyridines **1** were condensed with substituted ethyl thiophenecarbonylacetates **2** in the presence of polyphosphoric acid (PPA) to form the corresponding pyridopyrimidinones (**8**−**19** and **21**−**25**). These kinetically favored products were then thermally converted at 350 °C in mineral oil to the target compounds **26**−**43**.[20−22] The starting substituted ethyl thiophenecarbonylacetates **2** were prepared according to a literature method: condensation of substituted acetylthiophene **4** with diethyl carbonate (**3**) in the presence of sodium hydride.[23] Compound **38** could be synthesized by the same method, but dimethyl α-benzo[*b*]thiophene-2-carbonylmalonate (**7**) was substituted for the ethyl thiophenecarbonylacetates. Compound **7** was prepared by condensation of α-benzo[*b*]thiophene-2-carbonyl chloride (**6**) with dimethyl malonate in the presence of sodium hydride.[24]

Results and Discussion

a. Evaluation of Cytotoxicity of 2-Thienylnaphthyridin-4-ones. In preliminary testing of 7-methyl-2-heterocyclic-1,8-naphthyridin-4-ones against seven human tumor cell lines (Table 1), several compounds were active at micromolar concentrations, in particular compounds with a thienyl substituent. Therefore, a series of substituted thienyl compounds were synthesized and submitted to the NCI for in vitro testing against human tumor cell lines derived from multiple tumor types. All submitted compounds were active against these cell lines (Table 2) with average log GI_{50} (M) values ranging from −4.3 (**38**) to −7.8 (**40**) (GI_{50} is the molar concentration causing 50% cell growth inhibition). Table 2 also presents log GI_{50} values for selected cell lines. Among compounds tested, **40** showed the strongest inhibitory effects against a variety of tumor cell lines, with values in the low micromolar to nanomolar concentration range. Notably, **40** showed highly selective effects on several cell lines from the leukemia, colon, and breast cancer panels.

Table 1. In Vitro Cytotoxic Activities of Substituted 2-Thienyl-1,8-naphthyridin-4-ones

	ED_{50}[a] (μM)						
compd	KB[b]	A-549	HCT-8	CAKI-1	MCF-7	SKMEL-2	KB-Vin^r
26	3	4.6	2.1	5.0	1.2	>10	2.5
27	>10	>10	>10	>10	>20	>10	ND[c]
28	6.3	>6.3	ND[c]	ND[c]	>1.6	ND[c]	9.5
29	6.3	>6.3	>3.1	ND[c]	>3.1	ND[c]	ND[c]
30	1.1	1.3	0.9	1.25	1.1	>2.5	1.1
31	1.3	2.5	1.0	>5	1.1	ND[c]	1.9
36	0.8	>3.1	>0.8	ND[c]	>0.8	>10	0.5
41	0.04	0.20	ND[c]	>0.25	0.25	ND[c]	ND[c]
42	0.027	0.056	0.057	0.134	0.125	0.135	ND[c]
43	0.12	0.30	ND[c]	>0.5	0.50	ND[c]	ND[c]

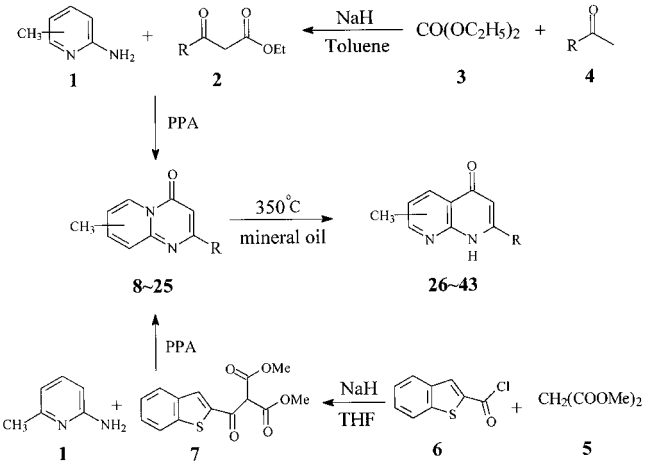
[a] Cytotoxicity as ED_{50} for each cell line, the concentration of compound that caused a 50% reduction in adsorbance at 562 nm relative to untreated cells using SRB assay. [b] KB, human epidermoid carcinoma of the nasopharynx; A-549, human lung carcinoma; HCT-8, human ileocecal carcinoma; CAKI-1,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

Table 2. Inhibition of in Vitro Tumor Cell Growth by Substituted 2-Thienyl-1,8-naphthyridin-4-ones[a]

	cytotoxicity (log GI_{50}) (M)[b]									
compd	HL-60(TB)[c]	NCI-H23	SW-620	U251	SK-MEL-5	OVCAR-3	RXF-393	PC-3	MDA-MB-435	MDA-N
28	−5.57	−5.36	−5.53	−4.77	−5.22	−4.51	−4.95	−5.03	−5.70	−5.73
29	−5.66	−5.73	−6.00	−5.41	−5.59	−5.29	−5.49	−5.51	−6.31	−6.44
31	nt[d]	nt	−5.38	−5.04	−5.69	−5.42	−5.35	−5.27	−6.49	−6.17
32	−7.11	−6.86	−7.32	−6.49	−6.69	−6.59	−6.71	−6.69	−7.63	−7.64
33	−6.76	−6.84	−6.84	−6.33	−6.75	−6.18	−6.20	−6.61	−6.97	−7.11
34	−5.81	−5.91	−5.85	−5.46	−6.18	−5.55	−5.72	−5.65	−6.43	−6.20
35	−6.69	−6.00	nt	−5.92	−6.42	−5.69	−5.69	−5.80	−6.42	−6.73
37	−5.26	−5.22	−5.00	nt	−5.47	−4.67	−4.44	nt	−5.45	−5.41
38	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	>−4.00	−4.29	>−4.00	>−4.00	>4.00
39	−4.73	−4.71	−4.70	−4.41	−4.83	−4.71	−4.77	−4.48	−5.32	−4.77
40	<−8.00	−7.66	<−8.00	nt	<−8.00	<−8.00	<−8.00	nt	<−8.00	<−8.00

[a] Data obtained from NCI's in vitro disease-oriented human tumor cells screen (see refs 29 and 30 for details). [b] Log concentrations which reduced cell growth to 50% of level obtained in untested controls. [c] HL-60(TB), leukemia cell line; NCI-H23, non-small-cell lung cancer cell line; SW-620, colon cancer cell line; U251, CNS cancer cell line; SK-MEL-5, melanoma cell line; OVCAR-3, ovarian cancer cell line; RXF-393, renal cancer cell line; PC-3, prostate cancer cell line; MDA-MB-435, MDA-N, breast cancer cell lines. [d] nt, Not tested.

Scheme 1. General Synthetic Routes to 2-Thienyl-1,8-naphthyridines[a]

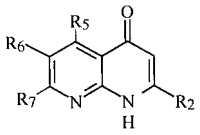


[a] For a specific R group, see Table 3.

In terms of average cytotoxicity, compounds without a methyl group in the C ring were substantially less active than those with a methyl group. Compounds with a methyl at the 5′-position (**30**, **32**) were more active than those substituted at the 3′-position (**31**, **33**) or at both the 2′- and 5′-positions (**34**). 5′-Chloro substitution (**35**) did not increase activity compared to 5′-methyl substitution. Compound **37** has a 5′-phenyl in place of the methyl group in **34** but was less active. Among the tested compounds with a 2-(α-thienyl) or 2-(β-thienyl) substituent, only minor differences in cytotoxicity were obtained. However, a fairly dramatic difference was observed between the 2-(β-benzo[*b*]thienyl) (**40**) and the 2-(α-benzo[*b*]thienyl) (**38**, **39**) substituted compounds. For **40**, low nanomolar GI_{50} values (log < −8.00) were observed in many cell lines. In contrast, marginal or no activity was obtained with **38** and **39**. Nevertheless, several less active compounds showed moderate cytotoxicity against two breast cancer cell lines, MDA-MB-435 and MDA-N.

b. Interactions of 2-Thienyl-1,8-naphthyridin-4-ones with Tubulin. In our previous studies, we demonstrated that potent cytotoxic 2-phenyl-1,8-naphthyridin-4-ones strongly interacted with tubulin at the colchicine site.[18,19] They inhibited the polymerization of 12 μM tubulin by 50% at concentrations below 1.0 μM, as did colchicine, combretastatin A-4, podophyllotoxin, and NSC 664171. In addition, they had moderate activity as inhibitors of the binding of [^{3}H]colchicine to tubulin, in contrast to the more potent inhibitory effects of combretastatin A-4, podophyllotoxin, and NSC 664171 on colchicine binding. Structurally, 2-thienyl-1,8-naphthyridin-4-ones can be considered isosteres of 2-phenyl-1,8-naphthyridin-4-ones, and thus, 2-thienyl-1,8-naphthyridin-4-ones could be predicted to have similar biological activity to 2-phenyl-1,8-naphthyridin-4-ones. COMPARE computations[25] were performed on the NCI screening data for the most active compound **40** and showed that this compound is an antimitotic agent, with Pearson correlation coefficients above 0.6 at all three levels against antimitotic agents in the NCI "Standard Agent" database. This postulate was confirmed when the series of substituted 2-thienyl-1,8-naphthyridin-4-ones were evaluated for their relative potencies as

Table 3. Antitubulin Effects of Compounds **26**−**43**



compd	R_2	R_5	R_6	R_7	average log GI_{50}	ITP[b] IC_{50} (μM) ± SD	ICB[c] (% inhibit)
26	3′-pyridyl	H	H	CH_3	not tested[e]	7.7 ± 2	
27	2′-furyl	H	H	CH_3	not tested[e]	14 ± 2	
28	2′-thienyl	H	H	CH_3	−4.83	6.0 ± 1	
29	3′-thienyl	H	H	CH_3	−5.40	7.0 ± 2	
30	2′-(5′-methylthienyl)	H	CH_3	H	not tested[e]	2.7 ± 0.4	
31	2′-(3′-methylthienyl)	H	CH_3	H	−5.13	3.8 ± 0.7	
32	2′-(5′-methylthienyl)	H	H	CH_3	−6.39	1.7 ± 0.01	
33	2′-(3′-methylthienyl)	H	H	CH_3	−6.26	2.8 ± 0.2	
34	3′-(2′,5′-dimethylthienyl)	H	H	CH_3	−5.43	4.8 ± 0.7	
35	2′-(5′-chlorothienyl)	H	H	CH_3	−5.81	1.7 ± 0.2	
36	2′-(5′-bromothienyl)	H	H	CH_3	not tested[e]	1.5 ± 0.2	19 ± 2
37	2′-(3′-methyl-5′-phenylthienyl)	H	H	CH_3	−4.79	14 ± 3	
38	2′-benzo[*b*]thienyl	H	H	CH_3	−4.25	>40	
39	2′-(3′-methylbenzo[*b*]thienyl)	H	H	CH_3	−4.7	13 ± 2	
40	3′-benzo[*b*]thienyl	H	H	CH_3	−7.78	0.37 ± 0.04	65 ± 1
41	3′-benzo[*b*]thienyl	CH_3	H	H	not tested[e]	1.2 ± 0.2	22 ± 5
42	3′-benzo[*b*]thienyl	H	CH_3	H	not tested[e]	0.91 ± 0.04	26 ± 0.9
43	3′-benzo[*b*]thienyl	CH_3	H	CH_3	not tested[e]	0.78 ± 0.1	26 ± 4
podophyllotoxin						0.59 ± 0.04	84 ± 0.6
combretastatin A-4						0.66 ± 0.1	98 ± 1
NSC 664171						0.49 ± 0.07	94 ± 0.5
NSC 679036[d]						0.72 ± 0.08	33 ± 2

[a] Data obtained from NCI's 49-human tumor cell line in vitro screen and calculated from all cell lines tested. [b] ITP, inhibition of tubulin polymerization. [c] ICB, inhibition of colchicine binding; evaluated only when polymerization $IC_{50} \leq 1.0$ μM; the concentration of both [^{3}H]colchicine and inhibitors was 5 μM. [d] Data from ref 19. [e] Cytotoxic data, see Table 1.

inhibitors of tubulin assembly. The results obtained are summarized in Table 3, together with those for the potent antimitotic natural products podophyllotoxin and combretastatin A-4 and the highly potent NSC 664171.

Excellent correlation was found between cytotoxicity and inhibition of tubulin polymerization. The least cytotoxic compounds (**37**−**39**) had minimal inhibitory effects on tubulin polymerization. Conversely, all cytotoxic compounds were substoichiometric inhibitors of tubulin polymerization, and the highly cytotoxic compounds (**40**−**43**) were also the most potent inhibitors of tubulin polymerization, with IC_{50} values of 0.37−1.2 μM. Compound **40** was as good an inhibitor of assembly as podophyllotoxin, combretastatin A-4, and NSC 664171, but it was somewhat less effective as an inhibitor of the binding of [^{3}H]colchicine to tubulin. Further, although previously examined 2-phenyl-1,8-naphthyridin-4-ones[18,19] were not reexamined in the current studies, compound **40** appears to interact more strongly with tubulin than did these earlier compounds.

Considering structure−activity aspects based solely on inhibition of tubulin polymerization, the 5′-chloro, 5′-bromo, and 5′-methyl substituents (**30**, **32**, **33**, **35**, **36**) appeared to be essentially equivalent (IC_{50} values, 1.5−2.8 μM), and derivatives with methyl groups at different positions in the A ring had nearly comparable activity (cf. **30** with **32**; **31** with **33**; **40**−**43**). Adding a phenyl group (**37**) greatly reduced activity (cf. **37** with **33**). Although the 2-(α-thienyl) and 2-(*β*-thienyl) derivatives (**28**, **29**) had comparable activity, the 2-(*β*-benzo[*b*]-thienyl) (**40**) and 2-(α-benzo[*b*]thienyl) (**38**) compounds had totally different behavior. The former was the most active compound in the new series (IC_{50} = 0.37 μM), while the latter was nearly inert (IC_{50} > 40 μM). The conformations of both compounds were studied carefully, and our findings may explain the different effects of these compounds on tubulin polymerization. The lowest-energy conformation of compound **40** is similar to (*aR,7S*)-colchicine[31] (the active enantiomer), but that of compound **38** more closely resembles (*aS,7R*)-colchicine[31] (the inactive enantiomer) (Figure 2). With colchicinoids and allocolchicinoids, the conformation of the biaryl system is important in the drug−tubulin interaction.[6,26,27] Thus, in the 2-thienyl-1,8-naphthyridin-4-one class, the relationship between conformations in the biaryl system and tubulin binding warrants further investigation.

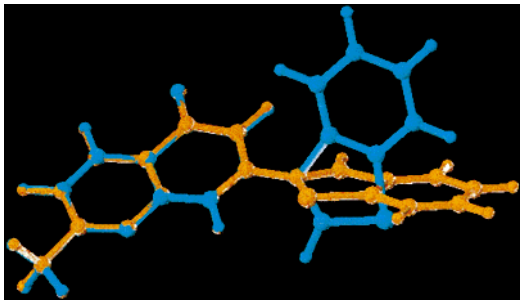


Figure 2. Stereoview of compounds **38** (orange) and **40** (cyan).

Experimental Section

A. Chemistry. a. General Experimental Procedures. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA. ^{1}H NMR spectra were measured on a Bruker AC-300 spectrometer with TMS as internal standard. Chemical shifts are reported in δ (ppm). Mass spectra (MS) were obtained on a TRIO 1000 mass spectrometer. Flash column chromatography was performed on silica gel (mesh 25−150 μm) using a mixture of CH_2Cl_2 and EtOAc as eluant. Precoated silica gel plates (Kieselgel 60 F_{254} 0.25 mm; Merck) were used for TLC analysis.

b. Preparation of Substituted Ethyl Thiophenecarbonylacetates. The substituted ethyl thiophenecarbonylacetates **2** were prepared according to procedures described by Krapcho et al.[23] To a vigorously stirred suspension of NaH and $CO(OEt)_2$ (**3**) in toluene was added dropwise a solution of substituted acetylthiophene **4** in toluene under reflux. The mixture was allowed to reflux and stirred for 20 min after the addition was completed. When cooled to room temperature, the mixture was acidified with glacial HOAc. After ice-cold water was added, the mixture was extracted with toluene. Workup and distillation gave the corresponding substituted ethyl thiophenecarbonylacetates.

c. Procedures for Method A.[20−22] A mixture of substituted 2-aminopyridine **1**, substituted ethyl thiophenecarbonylacetate **2**, and PPA was heated at 100−125 °C with stirring. The reaction was monitored by TLC. After the reaction was completed, the mixture was cooled to room temperature and neutralized with 4 M NaOH. After extraction with CH_2Cl_2, the extract was passed through a silica gel column to give 2-thienylpyrido[1,2-*a*]pyrimidin-4-one (**8**−**19**, **21**−**25**). This compound was then added to mineral oil at 300−350 °C with stirring. The oil was maintained at 350 °C for 2 h after the addition was completed. The cooled mixture was subjected to silica gel column chromatography. Elution with CH_2Cl_2-EtOAc gave the corresponding 2-thienyl-1,8-naphthyridin-4-one (**26**−**43**).

d. Procedures for Method B. This procedure is the same as method A but replaces ethyl thiophenecarbonylacetate with dimethyl benzo[*b*]thiophene-2-carbonylmalonate (**7**), which was prepared by condensation of benzo[*b*]thiophene-2-carbonyl chloride (**6**) with dimethyl malonate in the presence of sodium hydride to form the corresponding dimethyl benzo[*b*]thiophene-2-carbonylmalonate (**7**).[24]

2-(3′-Pyridinyl)-6-methylpyrido[1,2-*a*]pyrimidin-4-one (8): obtained from ethyl 3′-pyridinylacetate and 2-amino-6-picoline with yield 25%; needles, mp 159−160 °C; ^{1}H NMR ($CDCl_3$) δ 9.26 (s, 1 H, H-2′), 8.70 (d, J = 4.0 Hz, 1 H, H-9), 8.34 (d, J = 8.0 Hz, 1 H, H-6′), 7.45 (m, 3 H, H-7,8,5′), 6.69 (m, 2 H, H-3,4′), 3.09 (s, 3 H, CH_3-6); MS m/z 237 (M^+).

2-(2′-Furyl)-6-methylpyrido[1,2-*a*]pyrimidin-4-one (9): obtained from ethyl 2′-furylacetate and 2-amino-6-picoline with yield 31%; needles, mp 179−180 °C; ^{1}H NMR ($CDCl_3$) δ 7.60 (s, 1 H, H-5′), 7.42 (d, J = 4.0 Hz, 2 H, H-9,7), 7.17 (d, J = 3.5 Hz, 1 H, H-3′), 7.15 (m, 1 H, H-4′), 6.62 (m, 1 H, H-8), 6.56 (s, 1 H, H-3), 3.06 (s, 3 H, CH_3-6); MS m/z 226 (M^+).

2-(2′-Thienyl)-6-methylpyrido[1,2-*a*]pyrimidin-4-one (10): obtained from ethyl thiophene-2-carbonylacetate and 2-amino-6-picoline with yield 25%; needles, mp 148−149 °C; ^{1}H NMR ($CDCl_3$) δ 7.70 (d, J = 3.5 Hz, 1 H, H-5′), 7.52 (d, J = 5.0 Hz, 1 H, H-9), 7.44 (m, 2 H, H-3′,7), 7.15 (m, 1 H, H-4′), 6.64 (m, 1 H, H-8), 6.62 (s, 1 H, H-3), 3.07 (s, 3 H, CH_3-6); MS m/z 242 (M^+).

2-(3′-Thienyl)-6-methylpyrido[1,2-*a*]pyrimidin-4-one (11): obtained from ethyl thiophene-2-carbonylacetate and 2-amino-6-picoline with yield 32%; needles, mp 133−134 °C; ^{1}H NMR ($CDCl_3$) δ 8.10 (dd, J = 1.5, 3.0 Hz, 1 H, H-9), 7.62 (dd, J = 1.5, 4.5 Hz, 1 H, H-5′), 7.45 (s, 1 H, H-2′), 7.41 (m, 2 H, H-4′,7), 6.64 (t, J = 4.5 Hz 1 H, H-8), 6.60 (s, 1 H, H-3), 3.08 (s, 3 H, CH_3-6); MS m/z 242 (M^+).

2-(5′-Methyl-2′-thienyl)-7-methylpyrido[1,2-*a*]pyrimidin-4-one (12): obtained from ethyl 5-methylthiophene-2-carbonylacetate and 2-amino-5-picoline with yield 28%; prisms, mp 187−188 °C; ^{1}H NMR ($CDCl_3$) δ 8.85 (s, 1 H, H-6), 7.59 (d, J = 1.5 Hz, 2 H, H-8,9), 7.55 (d, J = 3.5 Hz, 1 H, H-4′), 6.84 (d, J = 3.5 Hz, 1 H, H-3′), 6.72 (s, 1 H, H-3), 2.56 (s, 3 H, CH_3-7), 2.43 (s, 3 H, CH_3-5′); MS m/z 256 (M^+).

2-(3′-Methyl-2′-thienyl)-7-methylpyrido[1,2-*a*]pyrimidin-4-one (13): obtained from ethyl 3-methylthiophene-2-carbonylacetate and 2-amino-5-picoline with yield 25%; prisms, mp 145−146 °C; ^{1}H NMR ($CDCl_3$) δ 8.86 (s, 1 H, H-6), 7.61 (br s, 2 H, H-8,9), 7.41 (d, J = 5.0 Hz, 1 H, H-5′), 6.97 (d, J = 5.0 Hz, 1 H, H-4′), 6.75 (s, 1 H, H-3), 2.60 (s, 3 H, CH_3-7), 2.44 (s, 3 H, CH_3-3′); MS m/z 256 (M^+).

2-(5′-Methyl-2′-thienyl)-6-methylpyrido[1,2-*a*]pyrimidin-4-one (14): obtained from ethyl 5-methylthiophene-2-carbonylacetate and 2-amino-6-picoline with yield 41%; prisms, mp 181−182 °C; ^{1}H NMR ($CDCl_3$) δ 7.51 (d, J = 3.5 Hz, 1 H, H-3′), 7.40 (m, 2 H, H-7,9), 6.81 (d, J = 3.5 Hz, 1 H, H-4′), 6.61 (m, 1 H, H-8), 6.53 (s, 1 H, H-3), 3.06 (s, 3 H, CH_3-5′), 2.55 (s, 3 H, CH_3-6); MS m/z 256 (M^+).

2-(3′-Methyl-2′-thienyl)-6-methylpyrido[1,2-*a*]pyrimidin-4-one (15): obtained from ethyl 3-methylthiophene-2-carbonylacetate and 2-amino-6-picoline with yield 46%; needles, mp 113−114 °C; ^{1}H NMR ($CDCl_3$) δ 7.40 (m, 3 H, H-5′,7,9), 6.95 (d, J = 3.5 Hz, 1 H, H-4′), 6.62 (t, J = 4.0 Hz, 1 H, H-8), 6.55 (s, 1 H, H-3), 3.06 (s, 3 H, CH_3-3′), 2.57 (s, 3 H, CH_3-6); MS m/z 256 (M^+).

2-(2′,5′-Dimethyl-3′-thienyl)-6-methylpyrido[1,2-*a*]pyrimidin-4-one (16): obtained from ethyl 2,5-dimethylthiophene-3-carbonylacetate and 2-amino-6-picoline with yield 26%; cubic, mp 106−107 °C; ^{1}H NMR ($CDCl_3$) δ 7.42 (d, J = 4.5 Hz, 2 H, H-7,9), 7.04 (s, 1 H, H-4′), 6.63 (t, J = 4.5 Hz, 1 H, H-8), 6.41 (s, 1 H, H-3), 3.07 (s, 3 H, H-2′), 2.68 (s, 3 H, CH_3-5′), 2.44 (s, 3 H, CH_3-6); MS m/z 270 (M^+).

2-(5′-Chloro-2′-thienyl)-6-methylpyrido[1,2-*a*]pyrimidin-4-one (17): obtained from ethyl 5-chlorothiophene-2-carbonylacetate and 2-amino-6-picoline with yield 24%; prisms, mp 169−170 °C; ^{1}H NMR ($CDCl_3$) δ 7.70 (d, J = 3.5 Hz, 1 H, H-5′), 7.52 (d, J = 5.0 Hz, 1 H, H-9), 7.44 (m, 2 H, H-3′,7), 7.15 (m, 1 H, H-4′), 6.64 (m, 1 H, H-8), 6.62 (s, 1 H, H-3), 3.07 (s, 3 H, CH_3-6); MS m/z 276 (M^+).

2-(5′-Bromo-2′-thienyl)-6-methylpyrido[1,2-*a*]pyrimidin-4-one (18): obtained from ethyl 5-bromothiophene-2-carbonylacetate and 2-amino-6-picoline with yield 27%; prisms, mp 163−164 °C; ^{1}H NMR ($CDCl_3$) δ 7.44 (m, 3 H, H-4′,8,9), 7.11 (d, J = 4.0 Hz, 1 H, H-3′), 6.65 (d, J = 6.0 Hz, H-7), 6.52 (s, 1 H, H-3), 3.07 (s, 3 H, CH_3-6); MS m/z 321 (M^+).

2-(2′-Methyl-5′-phenyl-3′-thienyl)-6-methylpyrido[1,2-*a*]pyrimidin-4-one (19): obtained from ethyl 2-methyl-5-phenylthiophene-3-carbonylacetate and 2-amino-6-picoline with yield 28%; prisms, mp 119−120 °C; ^{1}H NMR ($CDCl_3$) δ 7.61 (m, 3 H, H-2″,6″,9), 7.46 (m, 2 H, H-3″,5″), 7.40 (m, 2 H, H-4″,7), 7.29 (s, 1 H, H-4′), 6.67 (m, 1 H, H-8), 6.50 (s, 1 H, H-3), 3.10 (s, 3 H, CH_3-2′), 2.78 (s, 3 H, CH_3-6); MS m/z 332 (M^+).

2-(α-Benzo[*b*]thienyl)-6-methylpyrido[1,2-*a*]pyrimidin-4-one (20): obtained from dimethyl benzo[*b*]thiophene-2-carbonylmalonate and 2-amino-6-picoline with yield 31%; prisms, mp 168−170 °C; ^{1}H NMR ($CDCl_3$) δ 7.99 (s, 1 H, H-3′), 7.90 (m, 2 H, H-9,7′), 7.48 (m, 2 H, H-4′,7′), 7.40 (m, 2 H, H-5′,6′), 6.74 (s, 1 H, H-3), 6.67 (m, 1 H, H-8), 3.09 (s, 3 H, CH_3-6); MS m/z 292 (M^+).

2-(3′-Methyl-2′-Benzo[*b*]thienyl)-6-methylpyrido[1,2-*a*]-pyrimidin-4-one (21): obtained from dimethyl 3-methylbenzo[*b*]thiophene-2-carbonylmalonate and 2-amino-6-picoline with yield 26%; prisms, mp 176−177 °C; ^{1}H NMR ($CDCl_3$) δ 7.85 (m, 2 H, H-9,7′), 7.43 (m, 4 H, H-7,4′,5′,6′), 6.68 (m, 2 H, H-3.8), 3.10 (s, 3 H, CH_3-3′), 2.76 (s, 3 H, CH_3-6); MS m/z 306 (M^+).

2-(β-Benzo[*b*]thienyl)-5-methylpyrido[1,2-*a*]pyrimidin-4-one (22): obtained from methyl benzo[*b*]thiophene-3-carbonylacetate and 2-amino-6-picoline with yield 48%; prisms, mp 152−153 °C; ^{1}H NMR ($CDCl_3$) δ 8.55 (d, J = 8.0 Hz, 1 H, H-9), 8.06 (s, 1 H, H-2′), 7.92 (d, J = 8.0 Hz, 1 H, H-7), 7.46 (m, 4 H, H-4′,5′,6′,7′), 6.71 (m, 2 H, H-3,8), 3.13 (s, 3 H, CH_3-5); MS m/z 292 (M^+).

2-(β-Benzo[*b*]thienyl)-7-methylpyrido[1,2-*a*]pyrimidin-4-one (23): obtained from methyl benzo[*b*]thiophene-3-carbonylacetate and 2-amino-4-picoline with yield 27%; prisms, mp 178−180 °C; ^{1}H NMR ($CDCl_3$) δ 9.02 (d, J = 7.0 Hz, 1 H, H-5), 8.59 (d, J = 7.0 Hz, 1 H, H-6), 8.06 (s, 1 H, H-2′), 7.93 (d, J = 7.5 Hz, 1 H, H-7′), 7.58 (s, 1 H, H-8), 7.49−7.43 (m, 2 H, H-4′,5′), 7.02 (d, J = 6.0 Hz, 1 H, H-6′), 6.83 (s, 1 H, H-3), 2.53 (s, 3 H, CH_3-7); MS m/z 292 (M^+).

2-(β-Benzo[*b*]thienyl)-6-methylpyrido[1,2-*a*]pyrimidin-4-one (24): obtained from methyl benzo[*b*]thiophene-3-carbonylacetate and 2-amino-5-picoline with yield 71%; prisms, mp 155−157 °C; ^{1}H NMR ($CDCl_3$) δ 8.95 (s, 1 H, H-5), 8.59 (d,

J = 7.5 Hz, 1 H, H-8), 8.07 (s, 1 H, H-2′), 7.93 (d, J = 8.0 Hz, 1 H, H-7), 7.75−7.65 (m, 2 H, H-4′,7′), 7.52−7.41 (m, 2 H, H-5′, 6′), 6.89 (s, 1 H, H-3), 2.48 (s, 3 H, CH_3-6); MS m/z 292 (M^+).

2-(β-Benzo[*b*]thienyl)-6,8-dimethylpyrido[1,2-*a*]pyrimidin-4-one (25): obtained from methyl benzo[*b*]thiophene-3-carbonylacetate and 2-amino-4,6-dimethylpyridine with yield 30%; prisms, mp 170−172 °C; ^{1}H NMR ($CDCl_3$) δ 8.55 (d, J = 1.5 Hz, 1 H, H-5), 8.02 (s, 1 H, H-2′), 7.91 (d, J = 1.5 Hz, 1 H, H-7), 7.47−7.32 (m, 3 H, H-5′,6′,7′), 6.63 (s, 1 H, H-4′), 6.54 (s, 1 H, H-3), 3.08 (s, 3 H, H-8), 2.37 (s, 3 H, CH_3-6); MS m/z 306 (M^+).

7-Methyl-2-(3′-thienyl)-1,8-naphthyridin-4-one (26): obtained from compound **8** with yield 54%; prisms, mp 263−264 °C; ^{1}H NMR ($CDCl_3$ + MeOD-d_4) δ 8.96 (s, 1 H, H-2′), 8.73 (d, J = 4.5 Hz, 1 H, H-6′), 8.54 (7.90, d, J = 8.0 Hz, 1 H, H-5), 8.06 (d, J = 8.0 Hz, 1 H, H-6), 7.51 (m, 1 H, H-5′), 7.23 (d, J = 8.0 Hz, 1 H, H-4′), 6.56 (s, 1 H, H-3), 2.65 (s, 3 H, CH_3-7); MS m/z 237 (M^+). Anal. C, H, N.

7-Methyl-2-(2′-thienyl)-1,8-naphthyridin-4-one (27): obtained from compound **9** with yield 80%; prisms, mp 283−284 °C; ^{1}H NMR ($CDCl_3$ + MeOD-d_4) δ 8.49 (d, 1 H, J = 8.0 Hz, H-5), 7.65 (1 H, s, H-5′), 7.19 (d, J = 8.0 Hz, 1 H, H-6), 7.10 (d, J = 3.5 Hz, 1 H, H-3′), 6.62 (m, 2 H, H-3, 4′), 2.63 (s, 3 H, CH_3-7); MS m/z 226 (M^+). Anal. C, H, N.

7-Methyl-2-(2′-thienyl)-1,8-naphthyridin-4-one (28): obtained from compound **10** with yield 51%; prisms, mp 274−275 °C; ^{1}H NMR ($CDCl_3$) δ 8.98 (s, 1 H, H-1), 8.53 (d, J = 8.0 Hz, 1 H, H-5), 7.56 (m, 2 H, H-3′,5′), 7.21 (m, 2 H, H-6,4′), 6.63 (s, 1 H, H-3), 2.64 (s, 3 H, CH_3-7); MS m/z 242 (M^+). Anal. C, H, N.

7-Methyl-2-(3′-thienyl)-1,8-naphthyridin-4-one (29): obtained from compound **11** with yield 58%; prisms, mp 283−284 °C; ^{1}H NMR ($CDCl_3$ + MeOD-d_4) δ 8.51 (d, J = 8.0 Hz, 1 H, H-5), 7.90 (s, 1 H, H-2′), 7.49 (m, 2 H, H-4′,5′), 7.19 (d, J = 8.0 Hz, 1 H, H-6), 6.61 (s, 1 H, H-3), 2.63 (s, 3 H, CH_3-7); MS m/z 242 (M^+). Anal. C, H, N.

6-Methyl-2-(5′-methyl-2′-thienyl)-1,8-naphthyridin-4-one (30): obtained from compound **12** with yield 56%; prisms, mp 263−264 °C; ^{1}H NMR ($CDCl_3$) δ 9.13 (s, 1 H, H-1), 8.45 (s, 2 H, H-5,7), 7.38 (d, J = 3.5 Hz, 1 H, H-4′), 6.87 (d, J = 3.5 Hz, 1 H, H-3′), 6.56 (s, 1 H, H-3), 2.58 (s, 3 H, CH_3-6), 2.46 (s, 3 H, CH_3-5′); MS m/z 256 (M^+). Anal. C, H, N.

6-Methyl-2-(3′-methyl-2′-thienyl)-1,8-naphthyridin-4-one (31): obtained from compound **13** with yield 54%; prisms, mp 222−223 °C; ^{1}H NMR ($CDCl_3$) δ 9.87 (s, 1 H, H-1), 8.48 (d, 1 H, J = 2.0 Hz, H-5), 8.13 (d, J = 2.0 Hz, 1 H, H-7), 7.46 (d, J = 5.0 Hz, 1 H, H-5′), 7.04 (d, J = 5.0 Hz, 1 H, H-4′), 2.45 (s, 3 H, CH_3-6), 2.44 (s, 3 H, CH_3-3′); MS m/z 256 (M^+). Anal. C, H, N.

7-Methyl-2-(5′-methyl-2′-thienyl)-1,8-naphthyridin-4-one (32): obtained from compound **14** with yield 45%; prisms, mp 252−253 °C; ^{1}H NMR ($CDCl_3$) δ 8.83 (s, 1 H, H-1), 8.51 (d, J = 8.0 Hz, 1 H, H-5), 7.36 (d, J = 3.5 Hz, 1 H, H-3′), 7.18 (d, J = 8.0 Hz, 1 H, H-6), 6.86 (d, J = 3.5 Hz, 1 H, H-4′), 6.54 (s, 1 H, H-3), 2.63 (s, 3 H, CH_3-5′), 2.57 (s, 3 H, CH_3-7); MS m/z 256 (M^+). Anal. C, H, N.

7-Methyl-2-(3′-methyl-2′-thienyl)-1,8-naphthyridin-4-one (33): obtained from compound **15** with yield 41%; prisms, mp 214−215 °C; ^{1}H NMR ($CDCl_3$) δ 9.03 (s, 1 H, H-1), 8.55 (d, J = 8.0 Hz, 1 H, H-5), 7.42 (d, J = 5.0 Hz, 1 H, H-3′), 7.19 (d, J = 8.0 Hz, 1 H, H-6), 6.99 (d, J = 5.0 Hz, 1 H, H-4′), 6.45 (s, 1 H, H-3), 2.58 (s, 3 H, CH_3-3′), 2.45 (s, 3 H, CH_3-7); MS m/z 256 (M^+). Anal. C, H, N.

7-Methyl-2-(2′,5′-dimethyl-3′-thienyl)-1,8-naphthyridin-4-one (34): obtained from compound **16** with yield 51%; prisms, mp 253−254 °C; ^{1}H NMR ($CDCl_3$) δ 8.82 (s, 1 H, H-1), 8.55 (d, J = 8.0 Hz, 1 H, H-5), 7.19 (d, J = 8.0 Hz, 1 H, H-6), 6.79 (s, 1 H, H-4′), 6.33 (s, 1 H, H-3), 2.59 (s, 3 H, CH_3-2′), 2.56 (s, 3 H, CH_3-7), 2.45 (s, 3 H, CH_3-5′); MS m/z 270 (M^+). Anal. C, H, N.

7-Methyl-2-(5′-chloro-2′-thienyl)-1,8-naphthyridin-4-one (35): obtained from compound **17** with yield 56%; prisms, mp 279−280 °C; ^{1}H NMR ($CDCl_3$ + MeOD-d_4) δ 8.51 (d, J = 8.0 Hz, 1 H, H-5), 7.42 (d, J = 4.0 Hz, 1 H, H-4′), 7.20 (d, J = 8.0 Hz, 1 H, H-6), 7.03 (d, J = 4.0 Hz, 1 H, H-3′), 6.49 (s, 1 H, H-3), 2.64 (s, 3 H, CH_3-7); MS m/z 276 (M^+). Anal. C, H, N.

7-Methyl-2-(5′-bromo-2′-thienyl)-1,8-naphthyridin-4-one (36): obtained from compound **18** with yield 61%; prisms, mp 274−275 °C; ^{1}H NMR ($CDCl_3$ + MeOD-d_4) δ 8.46 (d, J = 8.0 Hz, 1 H, H-5), 7.40 (d, J = 4.0 Hz, 1 H, H-4′), 7.18 (d, J = 8.0 Hz, 1 H, H-6), 7.13 (d, J = 4.0 Hz, 1 H, H-3′), 6.47 (s, 1 H, H-3), 2.63 (s, 3 H, CH_3-7); MS m/z 321 (M^+). Anal. C, H, N.

7-Methyl-2-(2′-methyl-5′-phenyl-3′-thienyl)-1,8-naphthyridin-4-one (37): obtained from compound **19** with yield 48%; prisms, mp 275−276 °C; ^{1}H NMR (MeOD-d_4) δ 8.47 (d, J = 8.0 Hz, 1 H, H-5), 7.40 (d, J = 7.5 Hz, 2 H, H-2″,6″), 7.50 (s, 1 H, H-4′), 7.35 (m, 4 H, H-6,3″,4″,5″), 6.39 (s, 1 H, H-3), 2.62 (s, 3 H, CH_3-7), 2.66 (s, 3 H, CH_3-2′); MS m/z 332 (M^+). Anal. C, H, N.

7-Methyl-2-(2′-benzo[*b*]thienyl)-1,8-naphthyridin-4-one (38): obtained from compound **20** with yield 61%; prisms, mp >300 °C dec; ^{1}H NMR ($CDCl_3$) δ 8.98 (s, 1 H, H-1), 8.55 (d, J = 8.0 Hz, 1 H, H-5), 7.90 (m, 2 H, H-4′,7′), 7.80 (s, 1 H, H-3′), 7.47 (m, 2 H, H-5′,6′), 7.22 (d, J = 8.0 Hz, 1 H, H-6), 6.72 (s, 1 H, H-3), 2.67 (s, 3 H, CH_3-7); MS m/z 292 (M^+). Anal. C, H, N.

7-Methyl-2-(3′-methyl-2′-benzo[*b*]thienyl)-1,8-naphthyridin-4-one (39): obtained from compound **21** with yield 40%; prisms, mp 233 °C; ^{1}H NMR ($CDCl_3$) δ 8.90 (s, 1 H, H-1), 8.57 (d, J = 8.0 Hz, 1 H, H-5), 7.85 (m, 2 H, H-4′,7′), 7.51 (m, 2 H, H-5′,6′), 7.23 (d, J = 8.0 Hz, 1 H, H-6), 6.55 (s, 1 H, H-3), 2.64 (s, 6 H, CH_3-7,3′); MS m/z 306 (M^+). Anal. C, H, N.

7-Methyl-2-(3′-benzo[*b*]thienyl)-1,8-naphthyridin-4-one (40): obtained from compound **22** with yield 43%; prisms, mp 264 °C; ^{1}H NMR ($CDCl_3$) δ 9.18 (s, 1 H, H-1), 8.59 (d, J = 8.0 Hz, 1 H, H-5), 8.08 (m, 1 H, H-4′), 7.98 (m, 1 H, H-7′), 7.85 (s, 1 H, H-2′), 7.49 (m, 2 H, H-5′,6′), 7.22 (d, J = 8.0 Hz, 1 H, H-6), 6.64 (s, 1 H, H-3), 2.62 (s, 3 H, CH_3-7); MS m/z 292 (M^+). Anal. C, H, N.

5-Methyl-2-(3′-benzo[*b*]thienyl)-1,8-naphthyridin-4-one (41): obtained from compound **23** with yield 54%; prisms, mp 270 °C dec; ^{1}H NMR ($CDCl_3$) δ 9.52 (m, 1 H, H-1), 8.20 (d, J = 4.5 Hz, 1 H, H-7), 8.08 (m, 1 H, H-7′), 7.98 (m, 1 H, H-4′), 7.85 (s, 1 H, H-2′), 7.49 (m, 2 H, H-5′,6′), 7.02 (d, 1 H, J = 4.5 Hz, H-6), 6.58 (s, 1 H, H-3), 3.00 (s, 3 H, CH_3-5); MS m/z 292 (M^+). Anal. C, H, N.

6-Methyl-2-(3′-benzo[*b*]thienyl)-1,8-naphthyridin-4-one (42): obtained from compound **24** with yield 38%; prisms, mp 275 °C; ^{1}H NMR ($CDCl_3$ + MeOD-d_4) δ 8.51 (d, J = 1.0 Hz, 1 H, H-5), 8.44 (d, J = 1.0 Hz, 1 H, H-7), 8.06 (m, 1 H, H-4′), 7.95 (m, 1 H, H-7′), 7.90 (s, 1 H, H-2′), 7.48 (m, 2 H, H-5′,6′), 6.64 (s, 1 H, H-3), 2.48 (s, 3 H, CH_3-7); MS m/z 292 (M^+). Anal. C, H, N.

5,7-Methyl-2-(3′-benzo[*b*]thienyl)-1,8-naphthyridin-4-one (43): obtained from compound **25** with yield 50%; prisms, mp 273−275 °C; ^{1}H NMR ($CDCl_3$) δ 9.17 (m, 1 H, H-1), 8.07 (m, 1 H, H-6), 7.97 (m, 1 H, H-7′), 7.82 (s, 1 H, H-4′), 7.47 (m, 2 H, H-5′,6′), 6.91 (s, 1 H, H-2′), 6.55 (s, 1 H, H-3), 2.96 (s, 3 H, CH_3-5), 2.48 (s, 3 H, CH_3-7); MS m/z 306 (M^+). Anal. C, H, N.

B. Biological Assays. a. Cytotoxicity Assays. Compounds **26**−**31**, **36**, and **41**−**43** were assayed for in vitro cytotoxicity in a panel of human and murine tumor cell lines at the School of Pharmacy, University of North Carolina at Chapel Hill, according to procedures described previously.[28] The cell lines include human epidermoid carcinoma of the nasopharynx (KB), lung carcinoma (A-549), ileocecal carcinoma (HCT-8), human renal cancer (CAKI-1), human breast cancer (MCF-7), and human melanoma cancer (SKMEL-2). Compounds **28**, **29**, **31**−**35**, and **37**−**40** were submitted to NCI and assayed in the NCI's in vitro disease-oriented antitumor screen, which involves determination of a test compound's effects on the growth of approximately 49 human tumor cell lines.[29,30] These lines include leukemia, non-small-cell lung, colon, central nervous system (CNS), melanoma, ovarian, renal, prostate, and breast cancers. The cytotoxic effects of each compound were obtained as GI_{50} or TGI values, which repre-

sent the molar drug concentrations required to cause 50% inhibition or total growth inhibition, respectively.

b. Tubulin Assays. Electrophoretically homogeneous bovine brain tubulin was purified as described in ref 10. Combretastatin A-4 was a generous gift of Dr. G. R. Pettit, Arizona State University. [^{3}H]Colchicine was obtained from DuPont, nonradiolabeled colchicine from Sigma, and podophyllotoxin from Aldrich. The tubulin polymerization and colchicine binding assays were performed as described previously.[17] In the polymerization assays, the tubulin concentration was 12 μM. In the colchicine binding assays, the tubulin concentration was 1 μM, and the concentration of both [^{3}H]-colchicine and inhibitors was 5 μM.

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