Antitumor Agents. 174. 2′,3′,4′,5,6,7-Substituted 2-Phenyl-1,8-naphthyridin-4-ones: Their Synthesis, Cytotoxicity, and Inhibition of Tubulin Polymerization[1]

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Two series of 2′,3′,4′,5,6,7-substituted 2-phenyl-1,8-naphthyridin-4-ones and 2-phenylpyrido-[1,2-*a*]pyrimidin-4-ones have been synthesized and evaluated as cytotoxic compounds and as inhibitors of tubulin polymerization. Most 2-phenyl-1,8-naphthyridin-4-ones showed potent cytotoxic and antitubulin activities, whereas 2-phenylpyrido[1,2-*a*]pyrimidin-4-ones showed no activity in either assay. In general, a good correlation was found between cytotoxicity and inhibition of tubulin polymerization in the 2-phenyl-1,8-naphthyridin-4-one series. The 2-phenyl-1,8-naphthyridin-4-ones (**44**−**49**) with a methoxy group at the 3′-position showed potent cytotoxicity against most tumor cell lines with GI_{50} values in the low micromolar to nanomolar concentration range in the National Cancer Institute's 60 human tumor cell line *in vitro* screen. Introduction of substituents (e.g. F, Cl, CH_3, and OCH_3) at the 4′-position led to compounds with reduced or little activity and substitution at the 2′-position resulted in inactive compounds. The effects of various A-ring substitutions on activity depend on the substitution in ring C. Compounds **44**−**50** were potent inhibitors of tubulin polymerization, with activity nearly comparable to that of the potent antimitotic natural products colchicine, podophyllotoxin, and combretastatin A-4. Compounds **44**−**49** also inhibited the binding of radiolabeled colchicine to tubulin, but the inhibition was less potent than that obtained with the natural products. Further investigation is underway to determine if substitution at the 3′-position and multisubstitutions in ring C will result in compounds with increased activity.

Introduction

The microtubule system of eukaryotic cells is an attractive target for the development of compounds useful in anticancer chemotherapeutics. Microtubules show highly dynamic instability and play an important role in mitosis.[2] Chemicals that attack microtubules through their major structural component, tubulin, disrupt or suppress both microtubule structure and normal functions by inhibition or promotion of microtubule assembly, resulting in cell arrest in mitosis. Examples of clinically used antimitotic agents are the *vinca* alkaloids,[3] which inhibit microtubule polymerization, and the taxoids,[4] which promote microtubule assembly. Colchicine (Figure 1) is another important antimitotic agent. Although it has limited medicinal application due to its high toxicity, colchicine has played a fundamental role in elucidation of the properties and functions of tubulin and microtubules.[5] Many natural products, such as cornigerine,[6] podophyllotoxin,[7] steganacin,[8] and combretastatin,[9,10] bind to the colchicine site. Structurally, the compounds binding to this site are much simpler than those binding to *vinca* alkaloid or taxol domains. These compounds generally share "homology" with the A and C rings of colchicine; this common feature has been described as a "biaryl" system connected by a hydrocarbon bridge of variable length.[11,12] The simplicity of these molecules provides promise for the discovery or rational design of mitotic inhibitors as antitumor agents.

In the course of our search for novel plant-derived potent cytotoxic agents that are active against slow-growing solid tumors,[13] we have isolated several flavonols as antitumor principles from a phytochemically and biologically uninvestigated plant, *Polanisia dodecandra*.[14] Among these flavonols, 5,3′-dihydroxy-3,6,7,8,4′-pentamethoxyflavone showed remarkable cytotoxicity *in vitro* against panels of central nervous system, lung, ovarian, colon, and renal cancers and against melanoma and leukemia cell lines with GI_{50} values in the low micromolar to nanomolar concentration range. Flavonoids belong to the biaryl structural pattern and would be predicted to have antimitotic activity. Actually, this compound was found to be a strong inhibitor of tubulin polymerization with an IC_{50} value of 0.83 ± 0.2 μM and to be a potent inhibitor of radiolabeled colchicine binding to tubulin, showing 59% inhibition when present in an equimolar concentration with colchicine, and, accordingly, can be classified as a colchicine site drug.

In parallel with our studies of plant antitumor agents, we synthesized a large series of 2-arylquinolones, the amino analogs of cytotoxic antimitotic flavonoids, and found that many of these compounds were cytotoxic and inhibitors of tubulin polymerization and colchicine bind-

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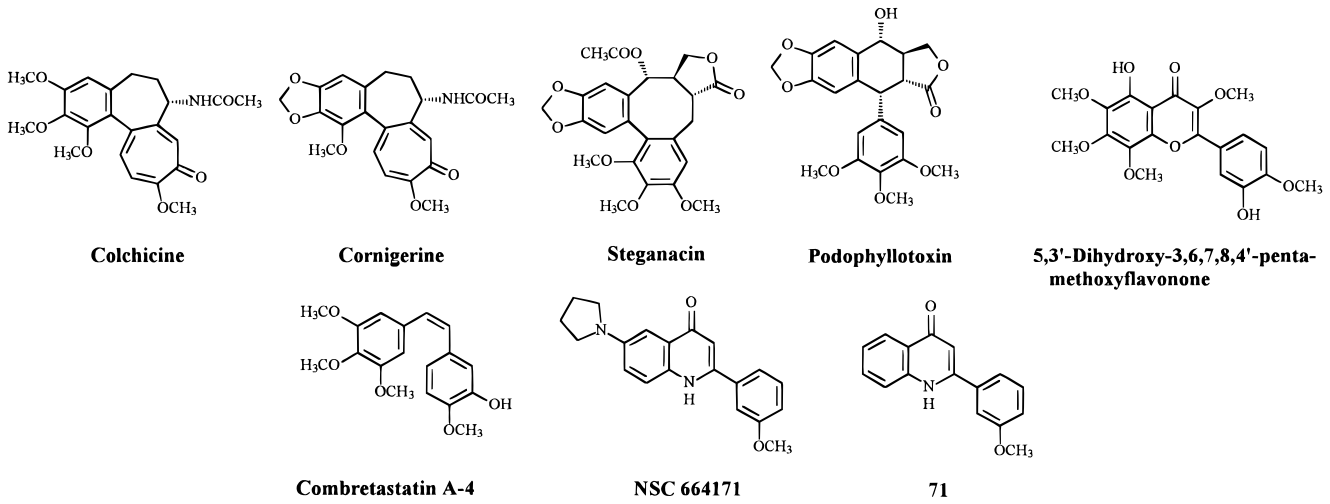


Figure 1.

ing to tubulin.[15−17] Structure−activity relationship studies of this class of antimitotic and antitumor agents led to the discovery of a particularly potent compound, NSC 664171, which showed inhibitory activity against tubulin polymerization and radiolabeled colchicine binding to tubulin comparable to that of the potent antimitotic natural products colchicine, podophyllotoxin, and combretastatin A-4. NSC 664171 also showed strong cytotoxic effects with GI_{50} values in the nanomolar or subnanomolar concentration range in most human tumor cell lines tested by the National Cancer Institute (NCI).

Encouraged by these promising results, we have extended our investigation of the 2-phenyl-4-quinolones to a novel, structurally related azo series, in which a nitrogen atom was introduced into the 4a-, 5-, 6-, 7-, or 8-position. A preliminary study showed that incorporation of the nitrogen atom at the 4a-, 5-, 6-, or 7-position failed to yield significantly cytotoxic compounds, while introduction of the nitrogen atom at the 8-position gave active agents, which equal or exceed the potency of the corresponding 2-phenyl-4-quinolones. In a direct comparison of the naphthyridinone and quinolone series, 2-(3′-methoxyphenyl)-1,8-naphthyridin-4-one (**44**) showed about 100-fold greater activity than the corresponding 2-(3′-methoxyphenyl)-4-quinolone (**71**) (Table 1), and **44** also showed potent cytotoxic effects against prostate and breast cancer cell lines (Table 2). Moreover, the most active members of the 2-phenyl-l,8-naphthyridin-4-one series are potent inhibitors of tubulin assembly (Table 1). On the basis of the initial results, a systematic investigation of substituted 2-phenyl-1,8-naphthyridin-4-ones as antitubulin and cytotoxic agents was initiated, as reported here.

Chemistry

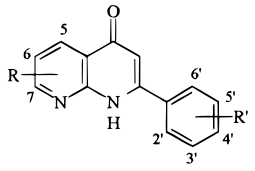
2-Phenyl-1,8-naphthyridin-4-ones were synthesized according to the two general methods shown in Scheme 1. Condensation of substituted 2-aminopyridines (**1**) with substituted ethyl benzoylacetates (**2**) in the presence of polyphosphoric acid (PPA) formed the corresponding pyridopyrimidinones (**7**−**38**). These kinetically favored products were then thermally converted at 350 °C in mineral oil to the target compounds (**39**−**70**). The second method involved the formation of a Schiff base (**4**) between substituted 2-aminopyridines (**1**) and substituted benzaldehydes (**3**). Cyclization of **4** with chloroacetyl chloride and triethylamine gave the corresponding pyridopyrimidinones, which were converted to the target compounds on heating.[18,19] The starting substituted ethyl benzoylacetates (**2**) were prepared according to a literature method.[20] Condensation of substituted acetophenones (**5**) with diethyl carbonate (**6**) in the presence of sodium hydride formed the required ethyl benzoylacetates. The structures and chemical features of the synthesized compounds are summarized in Table 1.

Results and Discussion

a. Evaluation of the Cytotoxicity of 2-Phenylnaphthyridin-4-ones. The 2-phenylpyrido[1,2-*a*]pyrimidin-4-ones were assayed at the School of Medicine, University of North Carolina at Chapel Hill, for their cytotoxicity *in vitro* against six tumor cell lines, including human epidermoid carcinoma of the nasopharynx (KB), lung carcinoma (A-549), ileocecal carcinoma (HCT-8), melanoma (PRMI-7951), and medulloblastoma (TE-671), as well as one murine leukemia cell line (P-388). The results (data not shown) demonstrated that essentially all 2-phenylpyrido[1,2-*a*]pyrimidin-4-ones were inactive (EC_{50} > 4 μg/mL); only a few compounds showed marginal activity.

The 2-phenyl-1,8-naphthyridin-4-ones were tested in the NCI's *in vitro* disease-oriented antitumor screen.[21,22] This assay involves determinations of a test agent's effect on growth parameters against a panel of approximately 60 human tumor cell lines, which consist largely of solid tumors and a few leukemia cell lines. The cytotoxic effects of each compound are obtained as GI_{50} or TGI values, which represent the molar drug concentrations required to cause half growth inhibition and total growth inhibition, respectively. The results are expressed in Table 2 as log GI_{50} values for selected cell lines and in Table 1 as average log GI_{50} values for the entire panel of cell lines. Among the compounds tested, **44**−**49** showed the strongest inhibitory effects against a variety of tumor cell lines, including leukemia, colon, CNS, melanoma, ovarian, renal, prostate, breast, and small cell lung cancer cell lines, with values in the low micromolar to nanomolar concentration range. It is notable that compounds **44, 46, 47,** and **49** show highly selective effects on several cell lines from the

Table 1. Physical Properties, Cytotoxicity, and Antimicrotubule Effects of Substituted 2-Phenyl-1,8-naphthyridin-4-ones



compd	R_5	R_6	R_7	R_2'	R_3'	R_4'	ITP[a] IC_{50} (μM) ± SD	ICB[b] (% inhibn)	av[c] log GI_{50}	formula[d]	mp, °C	yield, %[e]
39	H	H	H	OCH_3	H	H	> 40		−4.39	$C_{15}H_{12}N_2O_2$	196−197 [f]	27
40	CH_3	H	H	OCH_3	H	H	20 ± 1		−5.55	$C_{16}H_{14}N_2O_2$	199−200	23
41	H	CH_3	H	OCH_3	H	H	18 ± 1		−4.50	$C_{16}H_{14}N_2O_2$	202−203	38
42	H	H	CH_3	OCH_3	H	H	>40		−4.10	$C_{16}H_{14}N_2O_2$	198−199	48
43	CH_3	H	CH_3	OCH_3	H	H	>40		−4.24	$C_{17}H_{16}N_2O_2 \cdot 0.25H_2O$	203−204	38
44	H	H	H	H	OCH_3	H	0.96 ± 0.1	27 ± 1	−6.87	$C_{15}H_{12}N_2O_2$	183−184 [f]	42
45	CH_3	H	H	H	OCH_3	H	0.62 ± 0.1	28 ± 3	−7.23	$C_{16}H_{14}N_2O_2$	208−209	40
46	H	CH_3	H	H	OCH_3	H	0.80 ± 0.2	31 ± 4	−7.02	$C_{16}H_{14}N_2O_2$	215−216	39
47	H	H	CH_3	H	OCH_3	H	0.75 ± 0.2	29 ± 4	−7.24	$C_{16}H_{14}N_2O_2$	213−214	63
48	CH_3	H	CH_3	H	OCH_3	H	0.88 ± 0.08	22 ± 2	−6.19	$C_{17}H_{16}N_2O_2$	206−207	60
49	H	Cl	H	H	OCH_3	H	0.73 ± 0.2	35 ± 6	−7.01	$C_{15}H_{12}C_lN_2O_2 \cdot H_2O$	264−265	30
50	H	Br	H	H	OCH_3	H	1.5 ± 0.07		NT[g]	$C_{15}H_{12}BrN_2O_2 \cdot 0.5H_2O$	245−247 [f]	0.8 (B)
51	H	H	H	H	H	OCH_3	>40		−4.42	$C_{15}H_{12}N_2O_2$	217−218	33
52	CH_3	H	H	H	H	OCH_3	8.8 ± 3		−4.89	$C_{16}H_{14}N_2O_2$	196−197 [f]	35
53	H	CH_3	H	H	H	OCH_3	7.7 ± 0.7		−5.21	$C_{16}H_{14}N_2O_2$	261−262 [f]	36
54	H	H	CH_3	H	H	OCH_3	>40		−4.04	$C_{16}H_{14}N_2O_2$	255−256 [f]	59
55	CH_3	H	CH_3	H	H	OCH_3	>40		−4.48	$C_{17}H_{16}N_2O_2 \cdot 0.5H_2O$	213−214	56
56	H	H	H	H	H	F	>40		−4.34	$C_{14}H_9FN_2O \cdot 0.25H_2O$	200−202	33
57	CH_3	H	H	H	H	F	18 ± 2		−4.42	$C_{15}H_{11}FN_2O$	245−247 [f]	48
58	H	CH_3	H	H	H	F	20 ± 0.2		−4.58	$C_{15}H_{11}FN_2O$	286−288	29
59	H	H	CH_3	H	H	F	>40		−4.49	$C_{15}H_{11}FN_2O$	250−251	77
60	CH_3	H	CH_3	H	H	F	>40		−4.39	$C_{16}H_{13}FN_2O \cdot H_2O$	228−230 [f]	21
61	H	Cl	H	H	H	F	16 ± 3		−4.66	$C_{14}H_8ClFN_2O$	>300	31
62	H	H	H	H	H	Cl	22 ± 0.1		−4.41	$C_{14}H_9ClN_2O$	>300	32
63	CH_3	H	H	H	H	Cl	4.8 ± 1.4		−5.29	$C_{15}H_{11}ClN_2O$	261−262 [f]	44
64	H	CH_3	H	H	H	Cl	2.0 ± 0.01		−5.43	$C_{15}H_{11}ClN_2O$	290−292 [f]	64
65	H	H	CH_3	H	H	Cl	11 ± 0.7		−5.31	$C_{15}H_{11}ClN_2O$	263−265 [f]	72
66	CH_3	H	CH_3	H	H	Cl	32 ± 8		−4.71	$C_{16}H_{13}ClN_2O$	262−263 [f]	35
67	CH_3	H	H	H	H	CH_3	2.9 ± 0.7		NT[g]	$C_{16}H_{14}N_2O$	218−220	35
68	H	CH_3	H	H	H	CH_3	2.4 ± 0.8		NT[g]	$C_{16}H_{14}N_2O$	254−265	31
69	H	H	CH_3	H	H	CH_3	4.9 ± 0.2		NT[g]	$C_{16}H_{14}N_2O$	255−257	61
70	CH_3	H	CH_3	H	H	CH_3	9.8 ± 2		NT[g]	$C_{17}H_{16}N_2O$	236−237	63
71							1.4 ± 0.4		−5.21	$C_{17}H_{16}N_2O$	236−237	63
colchicine							0.80 ± 0.07[h]		−7.24			
podophyllotoxin							0.46 ± 0.02[h]	76 ± 5	−7.54			
combretastatin A-4							0.53 ± 0.05[h]	91 ± 2	−8.18			

[a] ITB = inhibition of tubulin polymerization. [b] ICB = inhibition of colchicine binding and evaluated only when polymerization $IC_{50} \leq 1\ \mu M$. [c] Data obtained from NCI's 60 human tumor cell line *in vitro* screen and calculated from all cell lines tested. [d] All compounds were analyzed for C, H, and N, and results agreed to ±0.4% of the theoretical values. [e] All yields were calculated from aminopyridines and obtained by method A unless indicated; see the Experimental Section. [f] Decomposed. [g] Not tested. [h] Previously obtained data, see ref 17.

leukemia, breast, and non-small lung cancer panels at the TGI level. The log TGI values for these four compounds are −7.19 to −7.40 in the HL-60 cell line, −6.16 to −7.12 in the NCI-H226 cell line, and −7.40 to −7.67 in MDA-MB-435 and MDA-N cell lines. Growth of cells from more sensitive cell lines was arrested at a concentration approximately log 2.5−3.7 concentration lower than less sensitive cell lines.

In terms of average cytotoxicity, compounds substituted at the 4′-position were substantially less active than those substituted at the 3′-position, while compounds with a methoxy group at the 2′-position were the least active. Among the tested compounds with a 4′-substituent, only minor differences in cytotoxicity were obtained, suggestive of a potency order of chloro > methoxy > fluoro. The effects of substitutions at the 5-, 6-, and/or 7-positions in ring A depend on the substitution in ring C. All 3′-substituted compounds with different substituents in ring A showed potent cytotoxicity, indicating that the substitution(s) can be present at different position(s) in ring A without substantially affecting cytotoxicity. For 2′- and 4′-substituted compounds, substitution at the 5- or 6-position generated slightly greater activity than no substitution or substitution at the 7-position or the 5- and 7-positions in ring A. It is notable that all less active compounds nevertheless showed moderate cytotoxicity on two breast cancer cell lines, MDA-MB-435 and MDA-N.

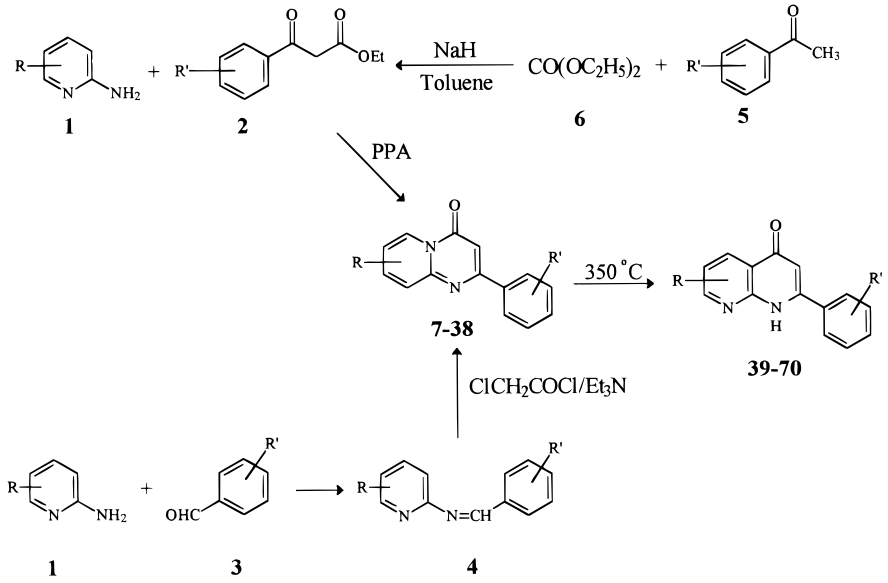
b. Interactions of 2-Phenyl-1,8-naphthyridin-4-ones with Tubulin. Previously, 2-phenyl-4-quinolones were found to be antimitotic agents that interact with tubulin at the colchicine site.[15−17] Structurally, 2-phenyl-1,8-naphthyridin-4-ones can be considered isosteres of 2-phenyl-4-quinolones. Since incorporation of an additional nitrogen atom into the quinolone at the 8-position does not change the basic 2-phenyl-4-quinolone skeleton, 2-phenyl-1,8-naphthyridin-4-ones could be predicted to have similar biological activity to 2-phenyl-4-quinolones. Thus, the series of substituted 2-phenyl-1,8-naphthyridin-4-ones were evaluated for

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

	cytotoxicity log GI_{50} (M)[b]										
compd	HL-60 (TB)[c]	NCI-H460	HCT-116	SF-295	U-251	SK-MEL-5	OVCAR-3	786-0	PC-3	MDA-MB-435	MDA-N
71	**−5.68**	**−5.40**	**−5.24**	**−5.64**	**−5.02**	**nt**[d]	**−5.26**	**−5.29**	**nt**	**nt**	**nt**
39	−4.41	−4.41	−4.56	−4.63	−4.17	−4.50	−4.32	−4.44	−4.53	−4.40	−4.70
40	−4.68	−4.62	−5.12	−4.63	−4.45	−4.65	−4.89	−4.22	−4.68	−5.19	−5.08
41	−5.02	−4.49	−4.37	−4.51	−4.56	−4.69	−4.71	−4.38	−4.57	−5.52	−5.35
42	−4.13	−4.01	−4.42	>−4	>−4	−4.29	>−4	>−4	>−4	−4.66	−4.70
43	−4.52	−4.15	−4.13	−4.27	>−4	−4.47	−4.19	>−4	−4.32	−4.49	−4.48
44	**−7.79**	**−7.04**	**−7.44**	**−7.28**	**−7.26**	**−7.27**	**−7.26**	**−7.21**	**−7.03**	**<−8.00**	**<−8.00**
45	−7.89	−7.32	−7.35	−7.54	−7.23	−7.65	−7.59	−7.29	−7.58	<−8.00	<−8.00
46	−7.72	−7.36	−7.65	−7.43	−7.35	−7.59	−7.38	−7.43	−7.37	<−8.00	<−8.00
47	−7.74	−7.36	−7.33	−7.52	−7.27	−7.47	−7.65	−7.27	−7.48	<−8.00	−7.96
48	−6.76	−6.35	−6.39	−6.38	−6.77	−6.54	−6.60	−6.36	−6.51	<−8.00	<−8.00
49	−7.57	−7.20	−6.92	−7.22	−6.70	−6.90	−6.31	−6.37	−6.86	−7.49	−7.50
51	−4.89	−4.58	−4.75	−4.19	−4.27	−4.46	−4.52	−4.25	−4.50	−4.70	−4.68
52	−5.36	−5.21	−5.19	−4.71	−5.20	−5.41	−4.86	−4.60	−4.74	−5.62	−5.68
53	−5.64	−5.39	−5.38	−5.28	−5.38	−5.43	−5.53	−5.29	−5.27	−5.74	−5.77
54	>−4	>−4	>−4	−4.05	>−4	−4.32	>−4	>−4	>−4	−4.37	−4.26
55	−4.809	−4.39	−4.44	−4.37	−4.04	−4.75	−4.49	−4.23	−4.62	−4.54	−4.48
56	−4.89	−4.46	−4.37	−4.30	−4.32	−4.52	−4.34	−4.35	−4.34	−4.92	−4.85
57	−5.32	−4.49	−4.79	−4.10	−4.44	−4.79	−4.25	−4.47	−4.29	−5.54	−5.45
58	−5.62	−4.47	−4.62	−4.53	−4.60	−4.66	−4.65	−4.49	−4.59	−5.58	−5.52
59	−4.83	−4.47	−4.47	−4.43	−4.43	−4.50	−4.49	−4.38	−4.68	−5.23	−4.93
60	−4.81	−4.29	−4.30	−4.30	−4.02	−4.58	−4.36	−4.10	−4.52	−4.73	−4.60
61	−5.41	−4.52	−4.77	−4.67	−4.53	−5.18	−4.76	−4.49	−4.66	−5.59	−5.53
62	−4.78	−4.42	−4.49	−4.39	−4.40	−4.54	−4.63	−4.47	−4.32	−5.23	−5.08
63	−5.75	−5.43	−5.43	−5.27	−5.34	−5.46	−5.50	−5.40	−5.54	−6.12	−5.88
64	−5.65	−5.41	−5.55	−5.62	−5.52	−5.66	−5.65	−5.20	−5.94	−6.22	−6.25
65	−5.53	−5.35	−5.48	−5.34	−5.32	−5.36	−5.59	−5.20	−5.78	−6.04	−6.00
66	−4.60	−4.65	−4.83	−4.79	−4.52	−4.85	−4.74	−4.67	−5.20	−5.39	−5.44

[a] Data obtained from NCI's *in vitro* disease-oriented human tumor cells screen (see refs 21 and 22 for details). [b] Log concentrations which reduced cell growth to 50% of level at start of experiment. [c] HL-60 (TB), leukemia cell line; NCI-H460, non-small-cell lung cancer cell line; HCT-116, colon cancer cell line; SF-295, U251, CNS cancer cell lines; SK-MEL-5, melanoma cell line; OVCAR-3, ovarian cancer cell line; 786−0, renal cancer cell line; PC-3, prostate cancer cell line; MDA-MB-435, MDA-N, breast cancer cell lines. [d] Not tested.

Scheme 1. General Synthetic Routes to 2-Phenyl-1,8-naphthyridin-4-ones



their relative potency as inhibitors of tubulin assembly to elucidate the pharmacophore requirements for binding to the protein and for comparison with the cytotoxicity data summarized above. The results are summarized in Table 1, with a comparison to previously obtained data[17] with the potent antimitotic natural products colchicine, podophyllotoxin, and combretastatin A-4 (the same tubulin preparation and reaction conditions were used in the study of ref 17 as in the current experiments).

With the most active compounds (**44**−**49**; no cytotoxicity data available for compound **50**), there was excellent correlation between cytotoxicity and inhibition of tubulin polymerization. These six strongly cytotoxic agents were the most potent inhibitors of assembly in the series. All had IC_{50} values below 1.0 μM, as did the three natural products. These six agents were also examined for inhibitory effects on the binding of [^{3}H]-colchicine to tubulin (1.0 μM tubulin), with inhibitor and drug at equimolar concentrations (5.0 μM). Significant and similar inhibition was observed with all six compounds, although they were substantially less potent than podophyllotoxin or combretastatin A-4 (Table 1).

Compounds **50**, **52**, **53**, **63**, **64**, and **67**−**70** showed

Table 3. COMPARE Correlations at GI_{50} and TGI Levels for Compounds **44-49**[a]

	colchicine		maytansine		vincristine		vinblastine		rhizoxin		paclitaxel	
seed	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI
44	0.66	0.55	0.65	0.64	0.58	0.45	0.47	0.67	0.60	0.60	0.36	0.63
45	0.69	0.47	0.73	0.37	0.61	0.41	0.60	0.53	0.58	0.40	0.30	0.47
46	0.73	0.53	0.69	0.62	0.58	0.44	0.56	0.62	0.54	0.58	0.40	0.53
47	0.79	0.47	0.74	0.45	0.71	0.43	0.64	0.56	0.61	0.45	0.41	0.55
48	0.71	0.55	0.71	0.51	0.62	0.48	0.65	0.64	0.65	0.51	0.46	0.61
49	0.70	0.58	0.63	0.69	0.62	0.55	0.51	0.77	0.55	0.62	0.31	0.65

[a] See refs 23 and 24 for details.

reduced inhibitory effects on tubulin polymerization, with IC_{50} values ranging from 1.5 to 9.8 μM. Slight inhibition was observed with compounds **40**, **41**, **57**, **58**, **61**, **62**, **65**, and **66** (IC_{50} values ranging from 11 to 32 μM), while the remaining compounds (**39**, **42**, **43**, **51**, **54-56**, **59**, and **60**) were inactive, with IC_{50} values greater than 40 μM. Although it is not dramatic among these less active compounds, those with greater antitubulin activity (IC_{50} values < 10 μM) tend to be more cytotoxic than those with little or no antitubulin activity.

From the tubulin data, the 3′-substitution of a methoxy group was well-tolerated, while the 4′- and 2′-methoxy substituents resulted in large, progressive losses of activity or total loss of activity. On the other hand, the series of 4′-substituents indicate that the methyl and chloride groups are better tolerated than the methoxy group and that the fluoride group is equivalent to the methoxy substituent at position 4′. This suggests that a strong electron-withdrawing or a large electron-donating group at the 4′-position is unfavorable for the drug−tubulin interaction.

The tubulin assay also yielded data of interest regarding A-ring substituents. No clear pattern was observed in the potent 3′-methoxy-substituted subseries (compounds **44**−**50**), for a variety of substituents at different position(s) of ring A were well-tolerated. These substituents include both an electron-donating group (CH_3) and electron-withdrawing groups (Cl and Br). The size of the substituents (as small as hydrogen, compound **44**, and as large as two methyl groups, compound **48**) and their positions (no substitution or different positions of both mono- and disubstitution) did not greatly affect relative compound activities.

However, a consistent pattern for A-ring substitution emerged in the other five subseries (2′-methoxy, 4′-methoxy, 4′-fluoride, 4′-chloride, and 4′-methyl). In all cases, C-5 and C-6 substituents (generally methyl groups) enhanced activity relative to the unsubstituted derivative and to those bearing a C-7 substituent or two substituent groups.

The most active 2-phenyl-1,8-naphthyridin-4-ones approach colchicine in their potency as inhibitors of tubulin polymerization. While these compounds appear to interact at the colchicine binding site of tubulin, they only weakly inhibit the binding of radiolabeled colchicine to tubulin. This apparent discrepancy probably arises from a relatively slow binding and/or rapid dissociation reaction of these agents with tubulin relative to the binding and dissociation reactions of colchicine with the protein. These properties are similar to those of a structurally related class of antimitotic agents, the 2-phenyl-4-quinolones, which were developed in our laboratory. The only structural difference between the 2-phenyl-1,8-naphthyridin-4-ones and 2-phenyl-4-quinolones is the atom at the 8-position: a N in the former series, and a C in the latter series. Both series have two aryl rings connected by a pyridinone ring. Thus, the 2-phenyl-1,8-naphthyridin-4-ones and 2-phenyl-4-quinolones together with other natural products, such as podophyllotoxin and combretastatin, fall into a group of colchicine site compounds that have two aryl ring systems connected by a hydrocarbon bridge of variable length.[11,12,14,15] In both classes of compounds, derivatives with a methoxy at the 3′-position were the most active compounds within their series and were much more active than isomers with the same substituent at the 2′- or 4′-position. However, unlike the 2-phenyl-4-quinolones, in which a 4′-methoxy group reduced activity more than a 2′-methoxy substituent, in the 2-phenyl-1,8-naphthyridin-4-ones, a 2′-methoxy group reduced activity more than did a 4′-methoxy substituent. Moreover, in the 2-phenyl-4-quinolone series, A-ring substituents were optimal at positions 6 and 7, whereas in the 2-phenyl-1,8-naphthyridin-4-one series, it appears that A-ring substituents are optimal at positions 5 and 6. These findings indicate that the binding site(s) for these two series might not completely overlap.

c. COMPARE Correlation. The COMPARE computer program uses the patterns of cellular responsiveness in the NCI 60 cell line screen to calculate Pearson correlation coefficients between data for seed compounds and those for past agents in the database.[23] The significance of these computations is that the observed correlation coefficients are greatest in pairs of compounds sharing common intracellular targets.[24] COMPARE computations for the six most potent compounds (**44**−**49**) against several known tubulin/microtubule binding agents are shown in Table 3 at both the GI_{50} and TGI levels. Correlation coefficients >0.6 at either level can be considered significant. These compounds display higher correlations with colchicine and maytansine than with the *vinca* alkaloids or rhizoxin, while correlations with paclitaxel (Taxol) are comparatively weak.

d. Summary We have synthesized a series of novel 2′,3′,4′,5,6,7-substituted 2-phenyl-1,8-naphthyridin-4-ones. Compounds **44**−**49** showed potent inhibitory effects *in vitro* on tubulin polymerization and appear to interact at the colchicine binding site of tubulin. Compounds **44**−**49** also showed potent inhibitory effects *in vitro* on the growth of a variety of human tumor cell lines with high selectivity for several cell lines from the leukemia, breast, and non-small-cell lung cancer panels. In general, there was good correlation between cytotoxicity and inhibition of *in vitro* tubulin polymerization. Compounds with a 3′-methoxy group had the highest potency in the two assays. Introduction of substituents (e.g. F, Cl, CH_3, and OCH_3) at the 4′-position led to compounds with reduced or little activity, while a

methoxy attached at the 2′-position resulted in inactive compounds. The effects of various substitutions in ring A on activity depend on the substitution in ring C. The 2-phenyl-1,8-naphthyridin-4-one series is more potent than the 2-phenyl-4-quinolone series. The discovery of a potent antimitotic and antitumor agent NSC 664171 in the 2-phenyl-4-quinolone series and promising results in the preliminary structure−activity relationship (SAR) studies of the 2-phenyl-1,8-naphthyridin-4-one series indicate that these compounds warrant further investigation.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. The solvents for crystallization are MeOH for 2-phenyl-1,8-naphthyridin-4-ones and Me_2CO for 2-phenylpyrido[1,2-*a*]pyrimidin-4-ones. Elemental analyses were performed on a Carlo Erba EA 1108 elemental analyzer. ^{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.

Preparation of Substituted Ethyl Benzoylacetates. The substituted ethyl benzoylacetates (**2**) were prepared according to procedures described by Krapcho *et al.*[20] To a vigorously stirred suspension of NaH and $CO(OEt)_2$ in toluene was added dropwise a solution of substituted acetophenone (**5**) in toluene under reflux. The mixture was allowed to reflux and was stirred for 20 min after the addition was complete. When cooled to room temperature, the mixture was acidified with glacial AcOH. After ice-cold water was added, the mixture was extracted with toluene. Workup and distillation gave the corresponding substituted ethyl benzoylacetates.

Procedures for Method A.[18] A mixture of substituted 2-aminopyridine (**1**), substituted ethyl benzoylacetate (**2**), and PPA was heated at 125 °C with stirring. The reaction was monitored by TLC. After the reaction was complete, 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-phenylpyrido[1,2-*a*]pyrimidin-4-one (**7**−**38**). The 2-phenylpyrido[1,2-*a*]pyrimidin-4-one was then added to liquid paraffin at 350 °C with stirring. The oil was maintained at 350 °C for 2 h after the addition was complete. The cooled mixture was subjected to silica gel column chromatography. Elution with CH_2Cl_2−EtOAc gave the corresponding 2-phenyl-1,8-naphthyridin-4-one (**39**−**70**).

Procedures for Method B.[19] The substituted 2-aminopyridine (**1**) and substituted aldehyde (**3**) in equimolar concentration were heated in refluxing *m*-xylene until 90−95% of the theoretical amount of H_2O was collected in a Dean−Stark trap. After the *m*-xylene was evaporated at atmospheric pressure, the residue was distilled *in vacuo* to afford 2-(arylideneamino)-pyridine **4**. The 2-(arylideneamino)pyridine **4** and Et_3N were dissolved in anhydrous Et_2O; a solution of chloroacetyl chloride in anhydrous Et_2O was then added dropwise with stirring at −15 to −10 °C. After the addition was complete, the mixture was stirred for 1 h. The precipitated triethylamine chloride was filtered, and the filtrate was concentrated *in vacuo*. The residue was chromatographed on silica gel with CH_2Cl_2−EtOAc as eluant to give 2-phenylpyrido[1,2-*a*]pyrimidin-4-one, which was converted to the corresponding 2-phenyl-1,8-naphthyridin-4-one according to the procedures described above.

2-(2′-Methoxyphenyl)pyrido[1,2-*a*]pyrimidin-4-one (7): obtained from ethyl 2′-(methoxybenzoyl)acetate and 2-aminopyridine; needles; mp 148−149 °C; ^{1}H NMR ($CDCl_3$) δ 9.08 (d, J = 7.0 Hz, 1 H, H-6), 7.98 (dd, J = 7.5, 1.5 Hz, 1 H, H-6′), 7.78 (d, J = 7.0 Hz, 1 H, H-9), 7.74 (t, J = 7.0 Hz, 1 H, H-8), 7.44 (td, J = 7.5, 1.5 Hz, 1 H, H-4′), 7.15 (s, 1 H, H-3), 7.14 (t, J = 7.5 Hz, 1 H, H-5′), 7.12 (t, J = 7.0 Hz, 1 H, H-7), 7.04 (br d, J = 8.5 Hz, 1 H, H-3′), 3.92 (s, 3 H, OCH_3-2′); MS m/z 252 (M^+). Anal. C, H, N.

2-(2′-Methoxyphenyl)-8-methylpyrido[1,2-*a*]pyrimidin-4-one (8): obtained from ethyl (2′-methoxybenzoyl)acetate and 2-amino-4-picoline; prisms; mp 154−155 °C; ^{1}H NMR ($CDCl_3$) δ 8.96 (d, J = 7.0 Hz, 1 H, H-6), 7.95 (dd, J = 7.5, 1.5 Hz, 1 H, H-6′), 7.57 (br s, 1 H, H-9), 7.44 (td, J = 7.5, 1.5 Hz, 1 H, H-4′), 7.09 (t, J = 7.5 Hz, 1 H, H-5′), 7.05 (s, 1 H, H-3), 7.02 (br d, J = 8.0 Hz, 1 H, H-7), 6.97 (dd, J = 7.5, 1.5 Hz, 1 H, H-3′), 3.90 (s, 3 H, OCH_3-2′), 2.49 (s, 3 H, CH_3-8); MS m/z 266 (M^+). Anal. C, H, N.

2-(2′-Methoxyphenyl)-7-methylpyrido[1,2-*a*]pyrimidin-4-one (9): obtained from ethyl (2′-methoxybenzoyl)acetate and 2-amino-5-picoline; prisms; mp 148−149 °C; ^{1}H NMR ($CDCl_3$) δ 8.89 (s, 1 H, H-6), 7.96 (dd, J = 7.9, 1.9 Hz, 1 H, H-6′), 7.74 (d, J = 9.0 Hz, 1 H, H-9), 7.60 (dd, J = 9.0, 2.0 Hz, 1 H, H-8), 7.44 (td, J = 7.9, 1.9 Hz, 1 H, H-4′), 7.12 (s, 1 H, H-3), 7.10 (dt, J = 7.9 1.9 Hz, 1 H, H-5′), 7.03 (d, J = 7.9, Hz, 1 H, H-3′), 3.91 (s, 3 H, OCH_3-2′), 2.45 (s, 3 H, CH_3-7); MS m/z 266 (M^+). Anal. C, H, N.

2-(2′-Methoxyphenyl)-6-methylpyrido[1,2-*a*]pyrimidin-4-one (10): obtained from ethyl (2′-methoxybenzoyl)acetate and 2-amino-6-picoline; prisms; mp 153−154 °C; ^{1}H NMR ($CDCl_3$) δ 7.99 (dd, J = 7.5, 1.7 Hz, 1 H, H-6′), 7.52 (d, J = 9.0 Hz, 1 H, H-9), 7.42 (t, J = 9.0, Hz, 1 H, H-8), 7.42 (td, J = 7.5, 1.7 Hz, 1 H, H-4′), 7.09 (t, J = 7.5 Hz, 1 H, H-5′), 7.01 (d, J = 7.5, Hz, 1 H, H-3′), 6.97 (s, 1 H, H-3), 6.64 (d, J = 9.0 Hz, 1 H, H-7), 3.91 (s, 3 H, OCH_3-2′), 3.09 (s, 3 H, CH_3-6); MS m/z 266 (M^+). Anal. C, H, N.

2-(2′-Methoxyphenyl)-6,8-dimethylpyrido[1,2-*a*]pyrimidin-4-one (11): obtained from ethyl (2′-methoxybenzoyl)-acetate and 2-amino-4,6-dimethylpyridine; prisms, mp 170−171 °C; ^{1}H NMR ($CDCl_3$) δ 7.96 (dd, J = 7.5, 1.7 Hz, 1 H, H-6′), 7.41 (td, J = 7.5, 1.7 Hz, 1 H, H-4′), 7.33 (br s, 1 H, H-9), 7.08 (t, J = 7.5 Hz, 1 H, H-5′), 7.01 (d, J = 7.5, Hz, 1 H, H-3′), 6.88 (s, 1 H, H-3), 6.49 (br s, 1 H, H-7), 3.90 (s, 3 H, OCH_3-2′), 3.06 (s, 3 H, CH_3-6), 2.34 (s, 3 H, CH_3-8); MS m/z 280 (M^+). Anal. C, H, N.

2-(3′-Methoxyphenyl)pyrido[1,2-*a*]pyrimidin-4-one (12): obtained from ethyl (3′-methoxybenzoyl)acetate and 2-aminopyridine; prisms; mp 156−157 °C; ^{1}H NMR ($CDCl_3$) δ 9.08 (d, J = 7.0 Hz, 1 H, H-6), 7.76 (d, J = 7.0 Hz, 1 H, H-9), 7.75 (t, J = 7.0 Hz, 1 H, H-8), 7.67 (s, 1 H, H-2′), 7.66 (d, J = 7.5, Hz, 1 H, H-6′), 7.42 (t, J = 7.5 Hz, 1 H, H-5′), 7.15 (ddd, J = 7.0, 7.0, 2.0 Hz, 1 H, H-7), 7.05 (td, J = 7.5 Hz, 1 H, H-4′), 6.92 (s, 1 H, H-3), 3.92 (s, 3 H, OCH_3-3′); MS m/z 252 (M^+). Anal. C, H, N.

2-(3′-Methoxyphenyl)-8-methylpyrido[1,2-*a*]pyrimidin-4-one (13): obtained from ethyl (3′-methoxybenzoyl)acetate and 2-amino-4-picoline; plates; mp 136−137 °C; ^{1}H NMR ($CDCl_3$) δ 8.97 (d, J = 7.0 Hz, 1 H, H-6), 7.66 (br s, 1 H, H-2′), 7.64 (d, J = 7.5, 1 H, H-6′), 7.54 (s, 1 H, H-9), 7.41 (t, J = 7.5 Hz, 1 H, H-5′), 7.04 (dd, J = 7.5, 2.0 Hz, 1 H, H-4′), 6.98 (d, J = 7.0 Hz, 1 H, H-7), 6.85 (s, 1H, H-3), 3.91 (s, 3 H, OCH_3-3′), 2.51 (s, 3 H, CH_3-8); MS m/z 266 (M^+). Anal. C, H, N.

2-(3′-Methoxyphenyl)-7-methylpyrido[1,2-*a*]pyrimidin-4-one (14): obtained from ethyl (3′-methoxybenzoyl)acetate and 2-amino-5-picoline; prisms; mp 163−164 °C; ^{1}H NMR ($CDCl_3$) δ 8.90 (s, 1 H, H-6), 7.70 (d, J = 9.0 Hz, 1 H, H-9), 7.66 (d, J = 2.0 Hz, 1 H, H-2′), 7.65 (d, J = 7.8 Hz, 1 H, H-6′), 7.62 (dd, J = 9.0, 1.8 Hz, 1 H, H-8), 7.42 (t, J = 7.8, Hz, 1 H, H-5′), 7.04 (td, J = 7.8, 2.0 Hz, 1 H, H-4′), 6.90 (s, 1 H, H-3), 3.91 (s, 3 H, OCH_3-3′), 2.46 (s, 3 H, CH_3-7); MS m/z 266 (M^+). Anal. C, H, N.

2-(3′-Methoxyphenyl)-6-methylpyrido[1,2-*a*]pyrimidin-4-one (15): obtained from ethyl (3′-methoxybenzoyl)acetate and 2-amino-6-picoline; prisms; mp 115−116 °C; ^{1}H NMR ($CDCl_3$) δ 7.63 (s, 1 H, H-2′), 7.61 (d, J = 7.5 Hz, 1 H, H-6′), 7.49 (d, J = 8.5, Hz, 1 H, H-9), 7.45 (dd, J = 8.5, 6.3 Hz, 1 H, H-8), 7.39 (t, J = 7.5 Hz, 1 H, H-5′), 7.02 (td, J = 7.5, 2.0 Hz, 1 H, H-4′), 6.72 (s, 1 H, H-3), 6.64 (d, J = 6.3 Hz, 1 H, H-7), 3.90 (s, 3 H, OCH_3-3′), 3.08 (s, 3 H, CH_3-6); MS m/z 266 (M^+). Anal. C, H, N.

2-(3′-Methoxyphenyl)-6,8-dimethylpyrido[1,2-*a*]pyrimidin-4-one (16): obtained from ethyl (3′-methoxybenzoyl)-acetate and 2-amino-4,6-dimethylpyridine; prisms, mp 139−

140 °C; ^{1}H NMR ($CDCl_3$) δ 7.61 (br s, 1 H, H-2′), 7.60 (d, J = 7.5, Hz, 1 H, H-6′), 7.38 (t, J = 7.5 Hz, 1 H, H-5′), 7.28 (br s, 1 H, H-9), 7.01 (dd, J = 7.5, 2.0 Hz, 1 H, H-4′), 6.64 (s, 1 H, H-3), 6.49 (br s, 1 H, H-7), 3.89 (s, 3 H, OCH_3-3′), 3.05 (s, 3 H, CH_3-6), 2.34 (s, 3 H, CH_3-8); MS m/z 280 (M^+). Anal. C, H, N.

2-(3′-Methoxyphenyl)-7-chloropyrido[1,2-*a*]pyrimidin-4-one (17): obtained from ethyl (3′-methoxybenzoyl)acetate and 2-amino-5-chloropyridine; needles; mp 172−173 °C; ^{1}H NMR ($CDCl_3$) δ 9.08 (d, J = 1.5 Hz, 1 H, H-6), 7.67 (br d, 1 H, H-2′), 7.66 (d, J = 10.0 Hz, 1 H, H-8), 7.65 (d, J = 10.0 Hz, 1 H, H-9), 7.63 (d, J = 7.7 Hz, 1 H, H-4′), 7.41 (t, J = 7.7, Hz, 1 H, H-5′), 7.05 (dd, J = 7.7, 2.0 Hz, 1 H, H-6′), 6.92 (s, 1 H, H-3), 3.91 (s, 3 H, OCH_3-3′); MS m/z 286 (M^+). Anal. C, H, N.

2-(3′-Methoxyphenyl)-7-bromopyrido[1,2-*a*]pyrimidin-4-one (18): obtained from ethyl (3′-methoxybenzoyl)acetate and 2-amino-5-bromopyridine; needles; mp 197−198 °C; ^{1}H NMR ($CDCl_3$) δ 8.19 (d, J = 2.0 Hz, 1 H, H-6), 7.77 (dd, J = 9.5, 2.0 Hz, 1 H, H-8), 7.64 (br s, 1 H, H-2′), 7.63 (d, J = 7.7 Hz, 1 H, H-4′), 7.62 (dd, J = 9.0, 2.0 Hz, 1 H, H-9), 7.42 (t, J = 7.7, Hz, 1 H, H-5′), 7.05 (dd, J = 7.7, 2.0 Hz, 1 H, H-6′), 6.93 (s, 1 H, H-3), 3.91 (s, 3 H, OCH_3-3′); MS m/z 330 (M^+, 97), 332 (M^+ + 2, 92). Anal. C, H, N.

2-(4′-Methoxyphenyl)pyrido[1,2-*a*]pyrimidin-4-one (19): obtained from ethyl (4′-methoxybenzoyl)acetate and 2-aminopyridine; needles; mp 153−154 °C; ^{1}H NMR ($CDCl_3$) δ 9.07 (d, J = 7.2 Hz, 1 H, H-6), 8.09 (d, J = 9.0 Hz, 2 H, H_2-2′, 6′), 7.76 (d, J = 4.0 Hz, 2 H, H_2-8, 9), 7.12 (dd, J = 7.2, 4.0 Hz, 1 H, H-7), 7.04 (d, J = 9.0 Hz, 2 H, H_2-3′, 5′), 6.88 (s, 1 H, H-3), 3.90 (s, 3 H, OCH_3-4′); MS m/z 252 (M^+). Anal. C, H, N.

2-(4′-Methoxyphenyl)-8-methylpyrido[1,2-*a*]pyrimidin-4-one (20): obtained from ethyl (4′-methoxybenzoyl)acetate and 2-amino-4-picoline; needles; mp 167−168 °C; ^{1}H NMR ($CDCl_3$) δ 8.96 (d, J = 7.2 Hz, 1 H, H-6), 8.08 (d, J = 8.8 Hz, 2 H, H_2-2′,6′), 7.55 (br s, 1 H, H-9), 7.02 (d, J = 8.8 Hz, 2 H, H_2-3′,5′), 6.96 (dd, J = 7.2, 1.7 Hz, 1 H, H-7), 6.79 (s, 1 H, H-3), 3.89 (s, 3 H, OCH_3-4′), 2.51 (s, 3 H, CH_3-8); MS m/z 266 (M^+). Anal. C, H, N.

2-(4′-Methoxyphenyl)-7-methylpyrido[1,2-*a*]pyrimidin-4-one (21): obtained from ethyl (4′-methoxybenzoyl)acetate and 2-amino-5-picoline; prisms; mp 197−198 °C; ^{1}H NMR ($CDCl_3$) δ 8.87 (s, 1 H, H-6), 8.07 (d, J = 8.8 Hz, 2 H, H_2-2′,6′), 7.67 (d, J = 9.0 Hz, 1 H, H-9), 7.60 (dd, J = 9.0, 2.0 Hz, 1 H, H-8), 7.02 (d, J = 8.8 Hz, 2 H, H_2-3′,5′), 6.85 (s, 1 H, H-3), 3.89 (s, 3 H, OCH_3-4′), 2.44 (s, 3 H, CH_3-7); MS m/z 266 (M^+). Anal. C, H, N.

2-(4′-Methoxyphenyl)-6-methylpyrido[1,2-*a*]pyrimidin-4-one (22): obtained from ethyl (4′-methoxybenzoyl)acetate and 2-amino-6-picoline; prisms; mp 143−144 °C; ^{1}H NMR ($CDCl_3$) δ 8.05 (d, J = 9.0 Hz, 2 H, H_2-2′,6′), 7.48 (dd, J = 8.7, 1.4 Hz, 1 H, H-9), 7.42 (dd, J = 6.8, 8.7 Hz, 1 H, H-8), 7.00 (d, J = 9.0 Hz, 2 H, H_2-3′,5′), 6.67 (s, 1 H, H-3), 6.62 (d, J = 6.8 Hz, 1 H, H-7), 3.88 (s, 3 H, OCH_3-4′), 3.08 (s, 3 H, CH_3-6); MS m/z 266 (M^+). Anal. C, H, N.

2-(4′-Methoxyphenyl)-6,8-dimethylpyrido[1,2-*a*]pyrimidin-4-one (23): obtained from ethyl (3′-methoxybenzoyl)-acetate and 2-amino-4,6-dimethylpyridine; amorphous; mp 187−188 °C; ^{1}H NMR ($CDCl_3$) δ 8.03 (d, J = 9.0 Hz, 2 H, H_2-2′, 6′), 7.28 (br s, 1 H, H-9), 7.00 (d, J = 9.0 Hz, 2 H, H_2-3′,5′), 6.60 (s, 1 H, H-3), 6.48 (br s, 1 H, H-7), 3.88 (s, 3 H, OCH_3-4′), 3.06 (s, 3 H, CH_3-6), 2.35 (s, 3 H, CH_3-8); MS m/z 280 (M^+). Anal. C, H, N.

2-(4′-Fluorophenyl)pyrido[1,2-*a*]pyrimidin-4-one (24): obtained from ethyl (4′-fluorobenzoyl)acetate and 2-aminopyridine; needles; mp 193−195 °C; ^{1}H NMR ($CDCl_3$) δ 9.08 (d, J = 7.2 Hz, 1 H, H-6), 8.12 (dd, J = 8.8, 5.2 Hz, 2 H, H_2-2′,6′), 7.79 (dd, J = 9.0, 1.5 Hz, 1 H, H-9), 7.75 (dd, J = 9.0, 6.5 Hz, 1 H, H-8), 7.20 (t, J = 8.8 Hz, 2 H, H_2-3′,5′), 6.88 (s, 1 H, H-3); MS m/z 240 (M^+). Anal. C, H, N.

2-(4′-Fluorophenyl)-8-methylpyrido[1,2-*a*]pyrimidin-4-one (25): obtained from ethyl (4′-fluorobenzoyl)acetate and 2-amino-4-picoline; amorphous; mp 191−193 °C; ^{1}H NMR ($CDCl_3$) δ 8.97 (d, J = 7.3 Hz, 1 H, H-6), 8.10 (dd, J = 8.8, 5.7 Hz, 2 H, H_2-2′,6′), 7.53 (br s, 1 H, H-9), 7.19 (t, J = 8.8 Hz, 2 H, H_2-3′,5′), 6.99 (dd, J = 7.3, 2.0 Hz, 1 H, H-7), 6.80 (s, 1 H, H-3), 2.52 (s, 3 H, CH_3-8); MS m/z 254 (M^+). Anal. C, H, N.

2-(4′-Fluorophenyl)-7-methylpyrido[1,2-*a*]pyrimidin-4-one (26): obtained from ethyl (4′-fluorobenzoyl)acetate and 2-amino-5-picoline; amorphous; mp 174−175 °C; ^{1}H NMR ($CDCl_3$) δ 8.89 (s, 1 H, H-6), 8.10 (dd, J = 8.8, 5.7 Hz, 2 H, H_2-2′,6′), 7.67 (d, J = 9.0 Hz, 1 H, H-9), 7.63 (dd, J = 9.0, 2.0 Hz, 1 H, H-8), 7.19 (t, J = 8.8 Hz, 2 H, H_2-3′,5′), 6.85 (s, 1 H, H-3), 2.46 (s, 3 H, CH_3-7); MS m/z 254 (M^+). Anal. C, H, N.

2-(4′-Fluorophenyl)-6-methylpyrido[1,2-*a*]pyrimidin-4-one (27): obtained from ethyl (4′-fluorobenzoyl)acetate and 2-amino-6-picoline; prisms; mp 179−181 °C; ^{1}H NMR ($CDCl_3$) δ 8.07 (dd, J = 8.8, 5.2 Hz, 2 H, H_2-2′,6′), 7.48 (dd, J = 8.5, 2.0 Hz, 1 H, H-9), 7.44 (dd, J = 8.5, 6.5 Hz, 1 H, H-8), 7.17 (t, J = 8.8 Hz, 2 H, H_2-3′,5′), 6.67 (s, 1 H, H-3), 6.64 (dd, J = 6.5, 2.0 Hz, 1 H, H-7), 3.09 (s, 3 H, CH_3-6); MS m/z 254 (M^+). Anal. C, H, N.

2-(4′-Fluorophenyl)-6,8-dimethylpyrido[1,2-*a*]pyrimidin-4-one (28): obtained from ethyl (3′-fluorobenzoyl)acetate and 2-amino-4,6-dimethylpyridine; prisms; mp 199−201 °C; ^{1}H NMR ($CDCl_3$) δ 8.05 (dd, J = 8.7, 5.3 Hz, 2 H, H_2-2′, 6′), 7.27 (br s, 1 H, H-9), 7.16 (t, J = 8.7 Hz, 2 H, H_2-3′,5′), 6.61 (s, 1 H, H-3), 6.51 (br s, 1 H, H-7), 3.06 (s, 3 H, CH_3-6), 2.36 (s, 3 H, CH_3-8); MS m/z 268 (M^+). Anal. C, H, N.

2-(4′-Fluorophenyl)-7-chloropyrido[1,2-*a*]pyrimidin-4-one (29): obtained from ethyl (4′-fluorobenzoyl)acetate and 2-amino-5-chloropyridine; prisms; mp 187−189 °C; ^{1}H NMR ($CDCl_3$) δ 9.09 (s, 1 H, H-6), 8.10 (dd, J = 8.7, 5.6 Hz, 2 H, H_2-2′,6′), 7.70 (dd, J = 9.0, 2.0 Hz, 1 H, H-8), 7.67 (d, J = 9.0 Hz, 1 H, H-9), 7.20 (t, J = 8.7 Hz, 2 H, H_2-3′,5′), 6.89 (s, 1 H, H-3); MS m/z 274 (M^+). Anal. C, H, N.

2-(4′-Chlorophenyl)pyrido[1,2-*a*]pyrimidin-4-one (30): obtained from ethyl (4′-chlorobenzoyl)acetate and 2-aminopyridine; amorphous; mp 202−203 °C; ^{1}H NMR ($CDCl_3$) δ 9.10 (d, J = 7.0 Hz, 1 H, H-6), 8.07 (d, J = 8.5 Hz, 2 H, H_2-2′,6′), 7.90 (d, J = 9.0 Hz, 1 H, H-9), 7.83 (dd, J = 9.0, 6.5 Hz, 1 H, H-8), 7.50 (d, J = 8.5 Hz, 2 H, H_2-3′,5′), 7.21 (dd, J = 7.0, 6.5 Hz, 1 H, H-7), 6.88 (s, 1 H, H-3); MS m/z 256 (M^+). Anal. C, H, N.

2-(4′-Chlorophenyl)-8-methylpyrido[1,2-*a*]pyrimidin-4-one (31): obtained from ethyl (4′-chlorobenzoyl)acetate and 2-amino-4-picoline; needles; mp 211−213 °C; ^{1}H NMR ($CDCl_3$) δ 8.98 (d, J = 7.3 Hz, 1 H, H-6), 8.04 (d, J = 8.5 Hz, 2 H, H_2-2′,6′), 7.56 (br s, 1 H, H-9), 7.48 (d, J = 8.5 Hz, 2 H, H_2-3′,5′), 7.00 (dd, J = 7.3, 1.0 Hz, 1 H, H-7), 6.81 (s, 1 H, H-3), 2.53 (s, 3 H, CH_3-8); MS m/z 270 (M^+). Anal. C, H, N.

2-(4′-Chlorophenyl)-7-methylpyrido[1,2-*a*]pyrimidin-4-one (32): obtained from ethyl (4′-chlorobenzoyl)acetate and 2-amino-5-picoline; needles; mp 195−196 °C; ^{1}H NMR ($CDCl_3$) δ 8.90 (s, 1 H, H-6), 8.05 (d, J = 8.6 Hz, 2 H, H_2-2′,6′), 7.77 (d, J = 8.9 Hz, 1 H, H-9), 7.67 (dd, J = 8.9, 1.5 Hz, 1 H, H-8), 7.48 (d, J = 8.6 Hz, 2 H, H_2-3′,5′), 6.86 (s, 1 H, H-3), 2.47 (s, 3 H, CH_3-7); MS m/z 270 (M^+). Anal. C, H, N.

2-(4′-Chlorophenyl)-6-methylpyrido[1,2-*a*]pyrimidin-4-one (33): obtained from ethyl (4′-chlorobenzoyl)acetate and 2-amino-6-picoline; needles; mp 154−156 °C; ^{1}H NMR ($CDCl_3$) δ 8.02 (d, J = 8.5 Hz, 2 H, H_2-2′,6′), 7.51 (d, J = 8.5 Hz, 1 H, H-9), 7.47 (d, J = 8.5 Hz, 2 H, H_2-3′,5′), 7.46 (dd, J = 8.5, 6.3 Hz, 1 H, H-8), 6.69 (s, 1 H, H-3), 6.67 (d, J = 6.3 Hz, 1 H, H-7), 3.09 (s, 3 H, CH_3-6); MS m/z 266 (M^+). Anal. C, H, N.

2-(4′-Chlorophenyl)-6,8-dimethylpyrido[1,2-*a*]pyrimidin-4-one (34): obtained from ethyl (3′-chlorobenzoyl)acetate and 2-amino-4,6-dimethylpyridine; needles; mp 210−212 °C dec; ^{1}H NMR ($CDCl_3$) δ 8.01 (d, J = 8.5 Hz, 2 H, H_2-2′,6′), 7.46 (d, J = 8.5 Hz, 2 H, H_2-3′,5′), 7.27 (s, 1 H, H-9), 6.61 (s, 1 H, H-3), 6.57 (s, 1 H, H-7), 3.07 (s, 3 H, CH_3-6), 2.39 (s, 3 H, CH_3-8); MS m/z 284 (M^+). Anal. C, H, N.

2-(4′-Methylphenyl)-8-methylpyrido[1,2-*a*]pyrimidin-4-one (35): obtained from ethyl (4′-methylbenzoyl)acetate and 2-amino-4-picoline; prisms; mp 176−177 °C; ^{1}H NMR ($CDCl_3$) δ 8.96 (d, J = 7.3 Hz, 1 H, H-6), 7.99 (d, J = 8.2 Hz, 2 H, H_2-2′,6′), 7.54 (br s, 1 H, H-9), 7.31 (d, J = 8.2 Hz, 2 H, H_2-3′,5′), 6.96 (dd, J = 7.3, 1.5 Hz, 1 H, H-7), 6.83 (s, 1 H, H-3), 2.50 (s, 3 H, CH_3-8), 2.43 (s, 3 H, CH_3-4′); MS m/z 250 (M^+). Anal. C, H, N.

2-(4′-Methylphenyl)-7-methylpyrido[1,2-*a*]pyrimidin-4-one (36): obtained from ethyl (4′-methylbenzoyl)acetate and 2-amino-5-picoline; prisms; mp 171−172 °C; ^{1}H NMR ($CDCl_3$) δ 8.88 (s, 1 H, H-6), 7.99 (d, J = 8.2 Hz, 2 H, H_2-2′,6′), 7.68 (d, J = 9.0 Hz, 1 H, H-9), 7.60 (dd, J = 9.0, 2.0 Hz, 1 H, H-8), 7.31 (d, J = 8.2 Hz, 2 H, H_2-3′,5′), 6.88 (s, 1 H, H-3), 2.44 (s, 3 H, CH_3-7), 2.43 (s, 3 H, CH_3-4′); MS m/z 250 (M^+). Anal. C, H, N.

2-(4′-Methylphenyl)-6-methylpyrido[1,2-*a*]pyrimidin-4-one (37): obtained from ethyl (4′-methylbenzoyl)acetate and 2-amino-6-picoline; prisms; mp 140−141 °C; ^{1}H NMR ($CDCl_3$) δ 7.97 (d, J = 8.2 Hz, 2 H, H_2-2′,6′), 7.50 (d, J = 8.3 Hz, 1 H, H-9), 7.42 (dd, J = 8.3, 6.5 Hz, 1 H, H-8), 7.30 (d, J = 8.2 Hz, 2 H, H_2-3′,5′), 6.71 (s, 1 H, H-3), 6.64 (d, J = 6.5 Hz, 1 H, H-7), 3.08 (s, 3 H, CH_3-6), 2.42 (s, 3 H, CH_3-4′); MS m/z 250 (M^+). Anal. C, H, N.

2-(4′-Methylphenyl)-6,8-dimethylpyrido[1,2-*a*]pyrimidin-4-one (38): obtained from ethyl (3′-methylbenzoyl)acetate and 2-amino-4,6-dimethylpyridine; amorphous; mp 196−198 °C; ^{1}H NMR ($CDCl_3$) δ 7.95 (d, J = 8.2 Hz, 2 H, H_2-2′,6′), 7.30 (s, 1 H, H-9), 7.29 (d, J = 9.0 Hz, 2 H, H_2-3′,5′), 6.64 (s, 1 H, H-3), 6.49 (s, 1 H, H-7), 3.06 (s, 3 H, CH_3-6), 2.42 (s, 3 H, CH_3-4′), 2.35 (s, 3 H, CH_3-8); MS m/z 264 (M^+). Anal. C, H, N.

2-(2′-Methoxyphenyl)-1,8-naphthyridin-4-one (39): obtained from compound **7**; plates; ^{1}H NMR ($CDCl_3$) δ 8.65 (d, J = 8.0 Hz, 1 H, H-5), 8.62 (d, J = 4.5 Hz, 1 H, H-7), 7.64 (dd, J = 8.0, 1.7 Hz, 1 H, H-6′), 7.49 (td, J = 8.0, 1.7 Hz, 1 H, H-4′), 7.34 (dd, J = 8.0, 4.5 Hz, 1 H, H-6), 7.10 (t, J = 8.0 Hz, 1 H, H-5′), 7.06 (d, J = 8.0 Hz, 1 H, H-3′), 6.62 (s, 1 H, H-3), 3.94 (s, 3 H, OCH_3-2′); MS m/z 252 (M^+). Anal. C, H, N.

2-(2′-Methoxyphenyl)-5-methyl-1,8-naphthyridin-4-one (40): obtained from compound **8**; needles; ^{1}H NMR ($CDCl_3$) δ 10.45 (br s, 1 H, NH-1), 8.19 (d, J = 5.0 Hz, 1 H, H-7), 7.65 (dd, J = 7.5, 1.0 Hz, 1 H, H-6′), 7.50 (td, J = 7.5, 1.0 Hz, 1 H, H-4′), 7.13 (t, J = 7.5 Hz, 1 H, H-5′), 7.04 (d, J = 7.5 Hz, 1 H, H-3′), 6.98 (d, J = 5.0 Hz, 1 H, H-6), 6.56 (s, 1 H, H-3), 3.89 (s, 3 H, OCH_3-2′), 2.99 (s, 3 H, CH_3-5); MS m/z 266 (M^+). Anal. C, H, N.

2-(2′-Methoxyphenyl)-6-methyl-1,8-naphthyridin-4-one (41): obtained from compound **9**; needles; ^{1}H NMR ($CDCl_3$) δ 11.19 (br s, 1 H, NH-1), 8.48 (d, J = 1.8 Hz, 1 H, H-5), 8.01 (d, J = 1.8 Hz, 1 H, H-7), 7.62 (dd, J = 8.0, 1.7 Hz, 1 H, H-6′), 7.52 (td, J = 8.0, 1.7 Hz, 1 H, H-4′), 7.13 (t, J = 8.0 Hz, 1 H, H-5′), 7.01 (d, J = 8.0 Hz, 1 H, H-3′), 6.59 (s, 1 H, H-3), 3.78 (s, 3 H, OCH_3-2′), 2.40 (s, 3 H, CH_3-6); MS m/z 266 (M^+). Anal. C, H, N.

2-(2′-Methoxyphenyl)-7-methyl-1,8-naphthyridin-4-one (42): obtained from compound **10**; needles; ^{1}H NMR ($CDCl_3$) δ 10.66 (br s, 1 H, NH-1), 8.57 (d, J = 8.0 Hz, 1 H, H-5), 7.63 (dd, J = 7.5, 1.5 Hz, 1 H, H-6′), 7.49 (td, J = 7.5, 1.5 Hz, 1 H, H-4′), 7.15 (d, J = 8.0 Hz, 1 H, H-6), 7.12 (t, J = 7.5 Hz, 1 H, H-5′), 6.95 (d, J = 7.5 Hz, 1 H, H-3′), 6.59 (s, 1 H, H-3), 3.73 (s, 3 H, OCH_3-2′), 2.42 (s, 3 H, CH_3-7); MS m/z 266 (M^+). Anal. C, H, N.

2-(2′-Methoxyphenyl)-5,7-dimethyl-1,8-naphthyridin-4-one (43): obtained from compound **11**; prisms; ^{1}H NMR ($CDCl_3$) δ 7.62 (dd, J = 7.5, 1.5 Hz, 1 H, H-6′), 7.47 (td, J = 7.5, 1.5 Hz, 1 H, H-4′), 7.10 (t, J = 7.5 Hz, 1 H, H-5′), 6.95 (d, J = 7.5 Hz, 1 H, H-3′), 6.84 (s, 1 H, H-6), 6.50 (s, 1 H, H-3), 3.77 (s, 3 H, OCH_3-2′), 2.95 (s, 3 H, CH_3-5), 2.35 (s, 3 H, CH_3-7); MS m/z 280 (M^+). Anal. C, H, N.

2-(3′-Methoxyphenyl)-1,8-naphthyridin-4-one (44): obtained from compound **12**; amorphous; ^{1}H NMR ($CDCl_3$) δ 11.10 (br s, 1 H, NH-1), 8.70 (dd, J = 8.0, 1.5 Hz, 1 H, H-5), 8.10 (dd, J = 4.5, 1.5 Hz, 1 H, H-7), 7.45 (t, J = 8.0 Hz, 1 H, H-5′), 7.31 (d, J = 8.0, Hz, 1 H, H-6′), 7.26 (br s, 1 H, H-2′), 7.24 (dd, J = 8.0, 4.5 Hz, 1 H, H-6), 7.10 (dd, J = 8.0, 2.0 Hz, 1 H, H-4′), 6.60 (s, 1 H, H-3), 3.85 (s, 3 H, OCH_3-3′); MS m/z 252 (M^+). Anal. C, H, N.

2-(3′-Methoxyphenyl)-5-methyl-1,8-naphthyridin-4-one (45): obtained from compound **13**; plates; ^{1}H NMR ($CDCl_3$) δ 10.74 (br s, 1 H, NH-1), 7.84 (d, J = 5.0 Hz, 1 H, H-7), 7.43 (t, J = 8.0 Hz, 1 H, H-5′), 7.29 (d, J = 8.0 Hz, 1 H, H-6′), 7.22 (t, J = 2.5 Hz, 1 H, H-2′), 7.09 (dd, J = 8.0, 2.5 Hz, 1 H, H-4′), 6.93 (d, J = 5.0 Hz, 1 H, H-6), 6.52 (s, 1 H, H-3), 3.85 (s, 3 H, OCH_3-3′), 2.97 (s, 3 H, CH_3-5); MS m/z 266 (M^+). Anal. C, H, N.

2-(3′-Methoxyphenyl)-6-methyl-1,8-naphthyridin-4-one (46): obtained from compound **14**; amorphous; ^{1}H NMR ($CDCl_3$) δ 10.75 (s, 1 H, NH-1), 8.49 (d, J = 2.0 Hz, 1 H, H-5), 8.00 (d, J = 2.0 Hz, 1 H, H-7), 7.46 (t, J = 8.0 Hz, 1 H, H-5′), 7.29 (d, J = 8.0 Hz, 1 H, H-6′), 7.23 (t, J = 1.8 Hz, 1 H, H-2′), 7.11 (dd, J = 8.0, 1.8 Hz, 1 H, H-4′), 6.58 (s, 1 H, H-3), 3.85 (s, 3 H, OCH_3-3′), 2.39 (s, 3 H, CH_3-6); MS m/z 266 (M^+). Anal. C, H, N.

2-(3′-Methoxyphenyl)-7-methyl-1,8-naphthyridin-4-one (47): obtained from compound **15**; amorphous; ^{1}H NMR ($CDCl_3$) δ 9.13 (br s, 1 H, NH-1), 8.57 (d, J = 8.0 Hz, 1 H, H-5), 7.45 (t, J = 8.0 Hz, 1 H, H-5′), 7.26 (d, J = 8.0 Hz, 1 H, H-6′), 7.21 (d, J = 8.0 Hz, 1 H, H-6), 7.20 (s, 1 H, H-2′), 7.08 (d, J = 8.0 Hz, 1 H, H-4′), 6.60 (s, 1 H, H-3), 3.88 (s, 3 H, OCH_3-3′), 2.60 (s, 3 H, CH_3-7); MS m/z 266 (M^+). Anal. C, H, N.

2-(3′-Methoxyphenyl)-5,7-dimethyl-1,8-naphthyridin-4-one (48): obtained from compound **16**; prisms; ^{1}H NMR ($CDCl_3$) δ 9.42 (br s, 1 H, NH-1), 7.40 (t, J = 8.0 Hz, 1 H, H-5′), 7.22 (d, J = 8.0 Hz, 1 H, H-6′), 7.15 (t, J = 2.0 Hz, 1 H, H-2′), 7.05 (dd, J = 8.0, 2.0 Hz, 1 H, H-4′), 6.88 (s, 1 H, H-6), 6.50 (s, 1 H, H-3), 3.83 (s, 3 H, OCH_3-3′), 2.94 (s, 3 H, CH_3-5), 2.40 (s, 3 H, CH_3-7); MS m/z 280 (M^+). Anal. C, H, N.

2-(3′-Methoxyphenyl)-6-chloro-1,8-naphthyridin-4-one (49): obtained from compound **17**; needles; ^{1}H NMR (DMSO-d_6) δ 12.57 (s, 1 H, NH-1), 8.85 (d, J = 2.7 Hz, 1 H, H-5), 8.44 (d, J = 2.0 Hz, 1 H, H-7), 7.47 (t, J = 8.0 Hz, 1 H, H-5′), 7.41 (d, J = 8.0 Hz, 1 H, H-6′), 7.41 (br s, 1 H, H-2′), 7.13 (br d, J = 8.0, Hz, 1 H, H-4′), 6.49 (s, 1 H, H-3), 3.87 (s, 3 H, OCH_3-3′); MS m/z 286 (M^+). Anal. C, H, N.

2-(3′-Methoxyphenyl)-6-bromo-1,8-naphthyridin-4-one (50): obtained from compound **18**; amorphous; ^{1}H NMR (DMSO-d_6) δ 12.54 (s, 1 H, NH-1), 8.90 (d, J = 2.3 Hz, 1 H, H-5), 8.55 (d, J = 2.3 Hz, 1 H, H-7), 7.46 (t, J = 7.8 Hz, 1 H, H-5′), 7.42 (d, J = 7.8 Hz, 1 H, H-6′), 7.41 (s, 1 H, H-2′), 7.12 (br d, J = 7.8 Hz, 1 H, H-4′), 6.49 (s, 1 H, H-3), 3.87 (s, 3 H, OCH_3-3′); MS m/z 330 (M^+, 100), 332 ($M^+ + 2$, 94). Anal. C, H, N.

2-(4′-Methoxyphenyl)-1,8-naphthyridin-4-one (51): obtained from compound **19**; plates; ^{1}H NMR ($CDCl_3$) δ 8.69 (dd, J = 8.0, 2.0 Hz, 1 H, H-5), 8.35 (dd, J = 5.2, 2.0 Hz, 1 H, H-7), 7.70 (d, J = 8.8 Hz, 2 H, H_2-2′,6′), 7.30 (dd, J = 8.0, 5.2 Hz, 1 H, H-6), 7.06 (d, J = 8.8 Hz, 2 H, H_2-3′,5′), 6.61 (s, 1 H, H-3), 3.91 (s, 3 H, OCH_3-4′); MS m/z 252 (M^+). Anal. C, H, N.

2-(4′-Methoxyphenyl)-5-methyl-1,8-naphthyridin-4-one (52): obtained from compound **20**; plates; ^{1}H NMR ($CDCl_3$) δ 8.09 (d, J = 4.8 Hz, 1 H, H-7), 7.67 (d, J = 8.8 Hz, 2 H, H_2-2′,6′), 7.05 (d, J = 8.8 Hz, 2 H, H_2-3′,5′), 6.98 (d, J = 4.8 Hz, 1 H, H-6), 6.51 (s, 1 H, H-3), 3.90 (s, 3 H, OCH_3-4′), 2.98 (s, 3 H, CH_3-5); MS m/z 266 (M^+). Anal. C, H, N.

2-(4′-Methoxyphenyl)-6-methyl-1,8-naphthyridin-4-one (53): obtained from compound **21**; amorphous; ^{1}H NMR ($CDCl_3$) δ 8.50 (s, 1 H, H-5), 8.30 (s, 1 H, H-7), 7.69 (d, J = 8.7 Hz, 2 H, H_2-2′, 6′), 7.07 (d, J = 8.7 Hz, 2 H, H_2-3′, 5′), 6.63 (s, 1 H, H-3), 3.90 (s, 3 H, OCH_3-4′), 2.45 (s, 3 H, CH_3-6); MS m/z 266 (M^+). Anal. C, H, N.

2-(4′-Methoxyphenyl)-7-methyl-1,8-naphthyridin-4-one (54): obtained from compound **22**; amorphous; ^{1}H NMR ($CDCl_3$) δ 8.60 (d, J = 8.0 Hz, 1 H, H-5), 7.68 (d, J = 8.8 Hz, 2 H, H_2-2′,6′), 7.23 (d, J = 8.0 Hz, 1 H, H-6), 7.07 (d, J = 8.8 Hz, 2 H, H_2-3′,5′), 6.61 (s, 1 H, H-3), 3.90 (s, 3 H, OCH_3-4′), 2.67 (s, 3 H, CH_3-7); MS m/z 266 (M^+). Anal. C, H, N.

2-(4′-Methoxyphenyl)-5,7-dimethyl-1,8-naphthyridin-4-one (55): obtained from compound **23**; amorphous; ^{1}H NMR ($CDCl_3$) δ 7.62 (d, J = 8.8 Hz, 2 H, H_2-2′,6′), 7.02 (d, J = 8.8 Hz, 2 H, H_2-3′,5′), 6.89 (s, 1 H, H-6), 6.48 (s, 1 H, H-3), 3.88 (s, 3 H, OCH_3-4′), 2.94 (s, 3 H, CH_3-5), 2.47 (s, 3 H, CH_3-7); MS m/z 280 (M^+). Anal. C, H, N.

2-(4′-Fluorophenyl)-1,8-naphthyridin-4-one (56): obtained from compound **24**; prisms; ^{1}H NMR ($CDCl_3$) δ 8.66 (dd, J = 7.9, 1.5 Hz, 1 H, H-5), 8.62 (dd, J = 4.5, 1.5 Hz, 1 H, H-7), 7.74 (dd, J = 8.5, 5.0 Hz, 2 H, H_2-2′,6′), 7.36 (dd, J = 8.0, 4.5 Hz, 1 H, H-6), 7.23 (d, J = 8.5 Hz, 2 H, H_2-3′,5′), 6.56 (s, 1 H, H-3); MS m/z 240 (M^+). Anal. C, H, N.

2-(4′-Fluorophenyl)-5-methyl-1,8-naphthyridin-4-one (57): obtained from compound **25**; needles; ^{1}H NMR ($CDCl_3$) δ 8.35 (d, J = 4.7 Hz, 1 H, H-7), 7.71 (dd, J = 8.5, 5.0 Hz, 2 H, H_2-2′,6′), 7.21 (t, J = 8.5 Hz, 2 H, H_2-3′,5′), 7.04 (d, J = 4.7 Hz, 1 H, H-6), 6.47 (s, 1 H, H-3), 2.93 (s, 3 H, CH_3-5); MS m/z 254 (M^+). Anal. C, H, N.

2-(4′-Fluorophenyl)-6-methyl-1,8-naphthyridin-4-one (58): obtained from compound **26**; needles; ^{1}H NMR ($CDCl_3$) δ 8.47 (d, J = 2.0 Hz, 1 H, H-5), 8.45 (br s, 1 H, H-7), 7.73 (dd, J = 8.8, 5.2 Hz, 2 H, H_2-2′,6′), 7.22 (t, J = 8.8 Hz, 2 H, H_2-3′,5′), 6.55 (s, 1 H, H-3), 2.46 (s, 3 H, CH_3-6); MS m/z 254 (M^+). Anal. C, H, N.

2-(4′-Fluorophenyl)-7-methyl-1,8-naphthyridin-4-one (59): obtained from compound **27**; prisms; ^{1}H NMR ($CDCl_3$) δ 9.04 (br s, 1 H, NH-1), 8.58 (d, J = 8.0 Hz, 1 H, H-5), 7.71 (dd, J = 8.5, 5.0 Hz, 2 H, H_2-2′,6′), 7.26 (t, J = 8.5 Hz, 2 H, H_2-3′,5′), 7.23 (d, J = 8.0 Hz, 1 H, H-6), 6.55 (s, 1 H, H-3), 2.64 (s, 3 H, CH_3-7); MS m/z 254 (M^+). Anal. C, H, N.

2-(4′-Fluorophenyl)-5,7-dimethyl-1,8-naphthyridin-4-one (60): obtained from compound **28**; prisms; ^{1}H NMR ($CDCl_3$) δ 8.88 (br s, 1 H, NH-1), 7.68 (dd, J = 8.7, 5.1 Hz, 2 H, H_2-2′,6′), 7.24 (t, J = 8.7 Hz, 2 H, H_2-3′,5′), 6.92 (s, 1 H, H-6), 6.45 (s, 1 H, H-3), 2.94 (s, 3 H, CH_3-5), 2.53 (s, 3 H, CH_3-7); MS m/z 268 (M^+). Anal. C, H, N.

2-(4′-Fluorophenyl)-6-chloro-1,8-naphthyridin-4-one (61): obtained from compound **29**; prisms; ^{1}H NMR (DMSO-d_6) δ 12.58 (br s, 1 H, NH-1), 8.85 (d, J = 2.4 Hz, 1 H, H-5), 8.44 (d, J = 2.4 Hz, 1 H, H-7), 7.92 (dd, J = 8.5, 5.5 Hz, 2 H, H_2-2′,6′), 7.40 (t, J = 8.5 Hz, 2 H, H_2-3′,5′), 6.45 (s, 1 H, H-3); MS m/z 274 (M^+). Anal. C, H, N.

2-**(4′-Chlorophenyl)-1,8-naphthyridin-4-one (62)**: obtained from compound **30**; plates; ^{1}H NMR ($CDCl_3$) δ 8.67 (dd, J = 4.5, 1.5 Hz, 1 H, H-7), 8.63 (dd, J = 8.0, 1.5 Hz, 1 H, H-5), 7.68 (d, J = 8.7 Hz, 2 H, H_2-2′,6′), 7.49 (d, J = 8.8 Hz, 2 H, H_2-3′,5′), 7.37 (dd, J = 8.0, 4.5 Hz, 1 H, H-6), 6.64 (s, 1 H, H-3); MS m/z 256 (M^+). Anal. C, H, N.

2-(4′-Chlorophenyl)-5-methyl-1,8-naphthyridin-4-one (63): obtained from compound **31**; amorphous; ^{1}H NMR ($CDCl_3$) δ 8.36 (d, J = 5.0 Hz, 1 H, H-7), 7.68 (d, J = 8.5 Hz, 2 H, H_2-2′,6′), 7.49 (d, J = 8.5 Hz, 2 H, H_2-3′,5′), 7.06 (d, J = 5.0 Hz, 1 H, H-6), 6.57 (s, 1 H, H-3), 2.95 (s, 3 H, CH_3-5); MS m/z 270 (M^+). Anal. C, H, N.

2-(4′-Chlorophenyl)-6-methyl-1,8-naphthyridin-4-one (64): obtained from compound **32**; needles; ^{1}H NMR ($CDCl_3$) δ 8.49 (s, 1 H, H-5), 8.41 (s, 1 H, H-7), 7.66 (d, J = 8.5 Hz, 2 H, H_2-2′,6′), 7.47 (d, J = 8.5 Hz, 2 H, H_2-3′,5′), 6.60 (s, 1 H, H-3), 2.44 (s, 3 H, CH_3-6); MS m/z 270 (M^+). Anal. C, H, N.

2-(4′-Chlorophenyl)-7-methyl-1,8-naphthyridin-4-one (65): obtained from compound **33**; needles; ^{1}H NMR ($CDCl_3$) δ 8.52 (d, J = 8.0 Hz, 1 H, H-5), 7.67 (d, J = 8.5 Hz, 2 H, H_2-2′,6′), 7.49 (d, J = 8.5 Hz, 2 H, H_2-3′,5′), 7.22 (d, J = 8.0 Hz, 1 H, H-6), 6.60 (s, 1 H, H-3), 2.64 (s, 3 H, CH_3-7); MS m/z 266 (M^+). Anal. C, H, N.

2-(4′-Chlorophenyl)-5,7-dimethyl-1,8-naphthyridin-4-one (66): obtained from compound **34**; prisms; ^{1}H NMR ($CDCl_3$) δ 7.66 (d, J = 8.5 Hz, 2 H, H_2-2′,6′), 7.52 (d, J = 8.5 Hz, 2 H, H_2-3′,5′), 6.95 (s, 1 H, H-6), 6.51 (s, 1 H, H-3), 2.96 (s, 3 H, CH_3-5), 2.56 (s, 3 H, CH_3-7); MS m/z 284 (M^+). Anal. C, H, N.

2-(4′-Methylphenyl)-5-methyl-1,8-naphthyridin-4-one (67): obtained from compound **35**; needles; ^{1}H NMR ($CDCl_3$) δ 7.97 (d, J = 4.9 Hz, 1 H, H-7), 7.61 (d, J = 8.2 Hz, 2 H, H_2-2′,6′), 7.34 (d, J = 8.2 Hz, 2 H, H_2-3′,5′), 6.95 (d, J = 4.9 Hz, 1 H, H-6), 6.52 (s, 1 H, H-3), 2.98 (s, 3 H, CH_3-5), 2.46 (s, 3 H, CH_3-4′); MS m/z 250 (M^+). Anal. C, H, N.

2-(4′-Methylphenyl)-6-methyl-1,8-naphthyridin-4-one (68): obtained from compound **36**; needles; ^{1}H NMR ($CDCl_3$) δ 10.61 (br s, 1H, NH-1), 8.49 (d, J = 1.5 Hz, 1 H, H-5), 8.00 (d, J = 1.5 Hz, 1 H, H-7), 7.62 (d, J = 8.1 Hz, 2 H, H_2-2′,6′), 7.36 (d, J = 8.1 Hz, 2 H, H_2-3′,5′), 6.59 (s, 1 H, H-3), 2.47 (s, 3 H, CH_3-6), 2.39 (s, 3 H, CH_3-4′); MS m/z 250 (M^+). Anal. C, H, N.

2-(4′-Methylphenyl)-7-methyl-1,8-naphthyridin-4-one (69): obtained from compound **37**; needles; ^{1}H NMR ($CDCl_3$) δ 8.57 (d, J = 8.0 Hz, 1 H, H-5), 7.59 (d, J = 8.2 Hz, 2 H, H_2-2′,6′), 7.33 (d, J = 8.8 Hz, 2 H, H_2-3′,5′), 7.19 (d, J = 8.0 Hz, 1 H, H-6), 6.59 (s, 1 H, H-3), 2.57 (s, 3 H, CH_3-7), 2.44 (s, 3 H, CH_3-4′); MS m/z 250 (M^+). Anal. C, H, N.

2-(4′-Methylphenyl)-5,7-dimethyl-1,8-naphthyridin-4-one (70): obtained from compound **38**; prisms; ^{1}H NMR ($CDCl_3$) δ 7.57 (d, J = 8.1 Hz, 2 H, H_2-2′,6′), 7.31 (d, J = 8.1 Hz, 2 H, H_2-3′,5′), 6.89 (s, 1 H, H-6), 6.50 (s, 1 H, H-3), 2.93 (s, 3 H, CH_3-5), 2.48 (s, 3 H, CH_3-7), 2.44 (s, 3 H, CH_3-4′); MS m/z 264 (M^+). Anal. C, H, N.

Cytotoxicity Assays. Compounds **7**−**38** were assayed for *in vitro* cytotoxicity with a panel of human and murine tumor cell lines at the School of Medicine, University of North Carolina at Chapel Hill, according to procedures described previously.[25] The cell lines include human epidermoid carcinoma of the nasopharynx (KB), lung carcinoma (A-549), ileocecal carcinoma (HCT-8), melanoma (PRMI-7951), and medulloblastoma (TE-671), as well as one murine leukemia cell line (P-388). Compounds **39**−**49** and **51**−**66** 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 60 human tumor cell lines.[21,22] 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 represent the molar drug concentrations required to cause 50% inhibition or total growth inhibition, respectively.

Materials for Tubulin Bioassays. Electrophoretically homogeneous bovine brain tubulin was purified as described previously.[26] 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, podophyllotoxin from Aldrich, and monosodium glutamate from USB.

Tubulin Polymerization Assay. The tubulin polymerization assay was performed as described previously.[15−17] In brief, tubulin at 1.2 mg/mL (12 μM) was preincubated for 15 min at 26 °C in a 0.24-mL volume of 0.8 M monosodium glutamate (pH 6.6 with NaOH in a 2 M stock solution) with varying drug concentrations. The drug stock solutions were in DMSO, and the final solvent concentration was 4% (v/v). All concentrations are in terms of the final reaction volume (0.25 mL). The reaction mixtures were chilled on ice, and 10 μL of 10 mM GTP was added to each reaction mixture. Samples were transferred to cuvettes held at 0 °C by an electronic temperature controller in Gilford spectrophotometers. Baselines were established at 350 nm, and polymerization was initiated by a temperature jump to 26 °C. The jump took about 50 s to complete. After 20 min, turbidity readings were recorded, and the temperature controller was set to 0 °C. When depolymerization was complete, turbidity readings were again recorded. Generally, turbidity readings were about 90% cold-reversible, and the cold-reversible turbidity was taken to represent the extent of assembly for each reaction mixture. IC_{50} values were obtained graphically from inhibition of polymerization by different drug concentrations. Four spectrophotometers were used for each experimental sequence, with two control reactions (no drug) in each set. Generally, the control reactions were within 5% of their average, and IC_{50} values obtained with this polymerization assay are usually highly reproducible. Generally, standard deviations were within 20% of the mean values, but in some cases, the standard deviations were 30−35% of the mean. Therefore, we can conservatively estimate that a 50% difference in IC_{50} values represents a difference in the relative activity of two agents.

Colchicine Binding Assay. The binding of radiolabeled colchicine to tubulin was measured by the DEAE−cellulose filter technique, as described previously.[15] In brief, each 0.1-mL reaction mixture contained 0.1 mg (1.0 μM) of tubulin, 1.0 M commercial monosodium glutamate (pH 6.6 with HCl), 1 mM $MgCl_2$, 0.1 mM GTP, 5.0 μM [^{3}H]colchicine, 5% (v/v) DMSO, and 5.0 μM inhibitor. Incubation was for 20 min at 37 °C. Each reaction mixture was filtered under reduced vacuum through a stack of two DEAE−cellulose paper filters, washed with water, and radioactivity quantitated in a liquid scintillation counter.

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