3-(3,5-Dimethoxyphenyl)-1,6-naphthyridine-2,7-diamines and Related 2-Urea Derivatives Are Potent and Selective Inhibitors of the FGF Receptor-1 Tyrosine Kinase

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A series of 3-aryl-1,6-naphthyridine-2,7-diamines and related 2-ureas were prepared and evaluated as inhibitors of the FGF receptor-1 tyrosine kinase. Condensation of 4,6-diaminonicotinaldehyde and substituted phenylacetonitriles gave intermediate naphthyridine-2,7-diamines, and direct reaction of the monoanion of these (NaH/DMF) with alkyl or aryl isocyanates selectively gave the 2-ureas in varying yields (23−93%). For the preparation of more soluble 7-alkylamino-2-ureas, a number of protecting groups for the 2-amine were evaluated (phthaloyl, 4-methoxybenzyl) following selective blocking of the 7-amine (trityl), but these were not superior to the (required) 2-*tert*-Bu-urea group itself. Direct alkylation of the anion of the (unprotected) 7-amino group with excess 4-(3-chloropropyl)morpholine in DMF gave low (10%) yields of the desired product, but alkylation of the 7-acetamido anion, followed by mild alkaline hydrolysis, raised this to 64%. 3-Phenyl analogues were nonspecific inhibitors of isolated c-Src, FGFR, and PDGFR tyrosine kinases, whereas 3-(2,6-dichlorophenyl) analogues were most effective against c-Src and FGFR, and 3-(3,5-dimethoxyphenyl) derivatives showed high selectivity for FGFR alone. A water-soluble (7-morpholinylpropylamino) analogue retained high FGFR potency (IC_{50} 31 nM) and selectivity. Pairwise comparison of the 1,6-naphthyridines and the corresponding known pyrido[2,3-*d*]pyrimidine analogues showed little differences in potency or patterns of selectivity, suggesting that the 1-aza atom of the latter is not important for activity. A 7-acetamide derivative inhibited the growth of FGFR-expressing tumor cell lines and was particularly potent against HUVECs (IC_{50} 4 nM). This compound was also a very potent inhibitor of HUVEC microcapillary formation (IC_{50} 0.01 nM) and Matrigel invasion (IC_{50} 7 nM) and showed significant in vivo antitumor effects in a highly vascularized mammary adenocarcinoma 16/c model at nontoxic doses. The compounds are worthy of further evaluation as antiangiogenesis agents.

Introduction

Angiogenesis is essential for the growth and survival of solid tumors.[1] Fibroblast growth factors (FGFs) are major angiogenic factors for some tumors.[2,3] The inhibition of FGF receptor (FGFR) tyrosine kinases may therefore be an effective strategy to prevent this inappropriate vascularisation.[4,5] Overexpression of FGFRs, their ligands, or other aberrant kinase function has been implicated in various diseases, including psoriasis, rheumatoid arthritis, atherosclerosis,[6] and restenosis,[7] as well as several human tumors[8,9] (e.g. breast, pancreatic, ovarian, and prostate cancers), and in some cases this correlates with poor survival.[10]

The FGF receptor-1 tyrosine kinase is the most predominant FGFR subtype in vascular cells,[11] and its abnormal expression has been shown to accelerate the tumorigenicity of prostate epithelial cells in vivo.[12] Crystal structures of this receptor alone or in complex with ATP or with inhibitors of the kinase have been published.[9,13] To date, few selective inhibitors of FGFR tyrosine kinases have been reported.[13,14] One of the members of the thioindole class prepared in our earlier studies, PD 145709 (**1**), specifically inhibited bFGF-mediated tyrosine phosphorylation with moderate potency (IC_{50} 4.5 μM) as well as FGFR autophosphorylation and was a potent inhibitor of protein synthesis.[15] Recently two compounds from the indolinone class were reported to have moderate activity against the FGF receptor-1 tyrosine kinase (IC_{50}s of 10−20 μM), but only one of these (**2**) displayed some selectivity against other kinases.[9] The Parke-Davis group has described[16] studies on the 1-oxo-3-aryl-1*H*-indene-2-carboxylic acid derivatives which are also selective inhibitors (e.g. **3**, IC_{50} 5.1 μM).

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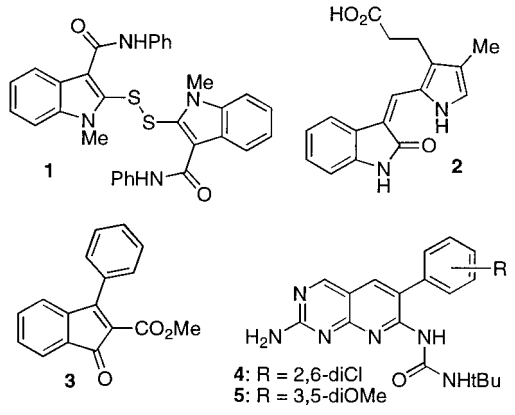


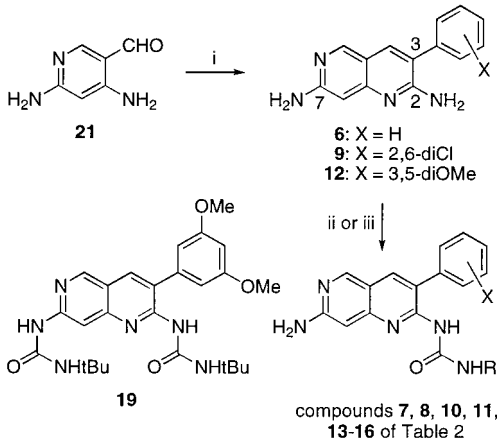
Recently the Parke-Davis group has also reported[17] the discovery (through compound library screening) of the novel pyrido[2,3-*d*]pyrimidine urea PD 089828 (**4**) as a potent broad-spectrum inhibitor of the PDGFR, FGFR, EGFR, and c-Src tyrosine kinases. Further structure−activity relationship (SAR) studies[18] resulted in the identification of PD 166866 (**5**), as a potent and very selective ATP competitive inhibitor of the FGF receptor-1 tyrosine kinase,[11] and developed compounds with improved potency, solubility, and bioavailability.[13,19,20] In studies designed to determine the importance of the pyrimidine ring aza atoms, we describe here the synthesis and biological evaluation of 3-aryl-1,6-naphthyridine-2,7-diamines and related 2-ureas (as 1-deaza analogues of the pyrido[2,3-*d*]pyrimidines) (**6**−**20**). Among these, the 7-acetamido derivative **17** shows potent in vivo activity. Also reported are synthetic strategies aimed at the preparation of more soluble derivatives (e.g., the 7-[(3-morpholinylpropyl)amino] derivative **20**).

Chemistry

The 3-aryl-1,6-naphthyridine-2,7-diamines and related 2-ureas were prepared by the general method shown in Scheme 1. To start, the known[21] 4,6-diaminonicotinaldehyde (**21**) (derived from Raney nickel reduction of the corresponding nitrile[22]) was condensed with substituted phenylacetonitriles in refluxing 2-ethoxyethanol under basic conditions (the alkoxide generated from addition of sodium to the solvent) as previously reported[21−23] to give the corresponding 3-aryl-1,6-naphthyridine-2,7-diamines **6**, **9**, and **12**.

Various conditions were then examined for the introduction of the urea substituents (Table 1). In general (method A), reaction of the diamines with sodium hydride in dry DMF, followed by a solution of the isocyanate in DMF at 20 °C for 1−24 h, selectively gave 2-mono-ureas in moderate yield, together with starting material and small amounts of bis-ureas. The latter were generally not isolated, but **19** was obtained (2% yield) from a larger-scale preparation of **15**. In the 3-(2,6-dichlorophenyl) series, the *tert*-butylurea **11** was formed in much lower yield (23%) than was the corresponding *tert*-butylurea **15** in the 3-(3,5-dimethoxyphenyl) series (51%), presumably due to the increased steric hindrance in the former case. The structure of phenylurea **16** was conclusively proved by 2-D NMR (HMQC, HMBC) experiments from long-range correlations between the NH_2 and C-8 resonances and between the acylated NH and both C-2 and C-3 resonances. Selectivity toward urea formation at the more hindered C-2 amine position was seen in all three 3-aryl-1,6-naphthyridine-2,7-diamines examined and has also been reported for the corresponding pyrido[2,3-*d*]pyrimidines,[19] suggesting the formation of a thermodynamically preferred anion.

Scheme 1[a]



[a] (i) $XPhCH_2CN$/Na/2-EtO$(CH_2)_2$OH/135 °C/30 min (ref 23); (ii) NaH/DMF, then RNCO/DMF or RNCO/0−20 °C/1−24 h; (iii) NaH/DMSO, then RNCO/DMSO/20 °C/24 h.

Table 1. Synthetic Yields for the Preparation of *N*-(7-Amino-3-aryl-1,6-naphthyridin-2-yl)-*N*′-(alkyl or aryl)ureas (Table 2) from Diamines **6**, **9**, and **12** by Various Methods

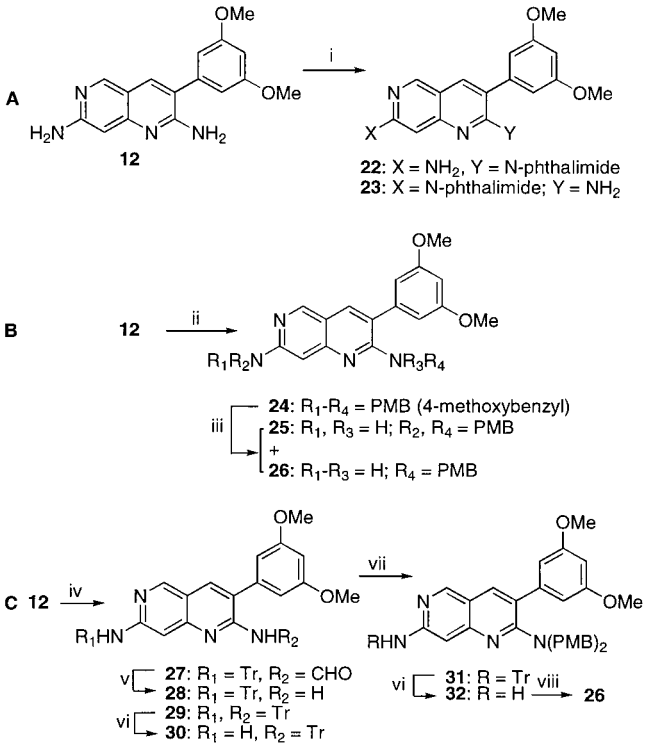
diamine	isocyanate	method[a]	scale (g)	time (h)	product	yield (%)
6	EtNCO	B	0.10	24	**7**	66
6	tBuNCO	A	0.10	4	**8**	66
9	EtNCO	B	0.11	24	**10**	<37[b]
9	EtNCO	C	0.13	24	**10**	50
9	tBuNCO	A	0.10	1	**11**	23
12	MeNCO	A[c]	0.11	1	**13**	47
12	EtNCO	B	0.10	20	**14**	56
12	tBuNCO	A	0.07	1	**15**	51
12	tBuNCO	A	0.25	18	**15**	70
12	tBuNCO	B	0.14	24	**15**	89
12	tBuNCO	B	5.0	24	**15** (+**19**)	93 (2)
12	PhNCO	A	0.10	1	**16**	45

[a] Method A: NaH (1.0−1.2 equiv)/DMF/20 °C/10 min, then RNCO (1.0−1.2 equiv)/DMF/20 °C/time. Method B: NaH (1.2−1.3 equiv)/DMF/20 °C/10−15 min, then RNCO (1.1−1.25 equiv)/0−20 °C/time. Method C: NaH (1.4 equiv)/DMSO/40−50 °C/5 min, then RNCO (1.1 equiv)/DMSO/20 °C/time. [b] Impure. [c] 0.6 equiv of MeNCO added, to minimize bis-urea formation.

Method B, similar to that described by Hamby,[19] employed the addition of the neat isocyanate at 0 °C to the anions generated with sodium hydride in DMF at 20 °C. This method usually gave higher yields (up to 93%) but failed for the 3-(2,6-diClPh)ethylurea **10**, giving a low yield (<37%) and an inseparable bis-urea impurity. A further modification (method C), forming the anion from excess sodium hydride in DMSO at higher temperature, then adding a solution of the isocyanate in DMSO at 20 °C, mostly overcame these difficulties, giving **10** in 50% purified yield.

Various routes were considered for the preparation of a soluble 7-alkylamino derivative of the FGFR-selective lead compound **15**. We have shown[23] that selective diazotization of diamines **6** and **9** in concentrated HCl or 50% HBF_4, followed by amine displace-

Scheme 2[a]



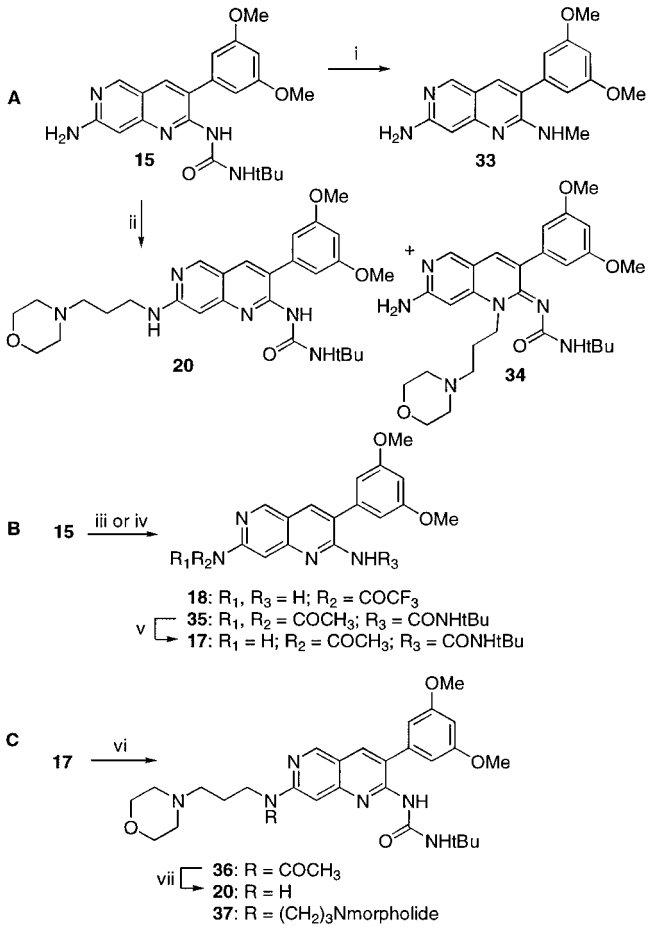
[a] (i) NaH/DMF, then phthaloyl dichloride/20 °C/8 h; (ii) NaH/DMF, then 4-OMePhCH$_2$Cl/20 °C/24 h; (iii) TFA/50 °C/5 min; (iv) NaH/DMF, then TrCl/20 °C/24 h or TrCl/Et$_3$N/THF/50−60 °C/3 days; (v) NaOH/MeOH/water/CH$_2$Cl$_2$/40 °C/2.5 h; (vi) silica gel/CH$_2$Cl$_2$/20 °C/3−4 days; (vii) NaH/DMF, then 4-OMePhCH$_2$Cl/20 °C/2 h; (viii) TFA/70 °C/8 h or HCO$_2$H/20 °C/20 h.

ment of the resulting 7-halides, is a useful synthetic route to 7-alkylamino derivatives. However, we encountered considerable difficulty with diazotization of the more electron-rich diamine **12**, due to competing nitrosation/oxidation reactions.[23] We therefore considered selective alkylation of the 7-amino group of **12**, by first selectively protecting the 2-amine, which forms an anion preferentially (see above). The various protecting group strategies examined are summarized in Scheme 2.

Phthaloylation of **12** under anionic conditions (NaH/DMF, then ca. 0.45 equiv of phthaloyl dichloride) was explored by analogy with the urea chemistry above. This gave a crude mixture in which the desired **22** was a major product, but there was much recovered starting material, and both the 2- and 7-phthalimides (**22** and **23**) were isolated in extremely low yield (5%). Furthermore, surprisingly, purified **22** was relatively unstable (on silica gel and on standing in solution), and this route was therefore not suitable.

A second strategy used 4-methoxybenzyl (PMB) protecting groups, seeking initial double protection of both amino groups, followed by selective deprotection at the less hindered 7-position. Alkylation of **12** with excess PMB chloride gave **24** in moderate yield (62%), but treatment of this with TFA gave nonselective cleavage at both positions to form **25** and **26** as the major products. Use of the bulky trityl group to selectively block the 7-position was then investigated. Tritylation of **12** under anionic conditions (TrCl/NaH/DMF) unexpectedly gave the DMF-derived 7-tritylamino-2-formamide derivative **27** as a significant byproduct along

Scheme 3[a]



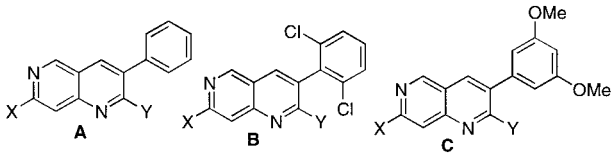
[a] (i) NaH/DMF, then MeI/DMF/20 °C/2.5 h; (ii) 4-(3-chloropropyl)morpholine·HCl/NaH/DMF/20 °C/7 days; (iii) TFAA/py/20 °C/16 h; (iv) AcCl/Et$_3$N/THF/20 °C/8 days or Ac$_2$O/py/20 °C/24 h; (v) NaOH/MeOH/water/20 °C/30 min; (vi) step ii/52 °C/26 h; (vii) step v/43 h.

with the desired **28** (but **27** was easily hydrolyzed to **28** in good yield). Column chromatography of these compounds on silica gel resulted in considerable detritylation, as reported for trityl ethers.[24] Tritylation under more typical conditions (e.g. TrCl/Et$_3$N/THF) followed by flash chromatography on silica gel gave **28** in good yield (78−80%), along with small amounts of the ditritylated derivative **29**. The structure of **28** was confirmed by comparison of its NMR data with that for the 2-tritylamino derivative **30**, obtained in low yield from the ditritylated derivative **29** by partial detritylation on silica gel.[24]

Alkylation of **28** with PMB chloride as above gave **31** in good yield (83%), and selective removal of the trityl group with silica gel[24] gave **32** (69% after two cycles). However, removal of the PMB groups unexpectedly proved impossible. Many reagents (TFA, AcOH, HCO$_2$H, CAN, H$_2$/10% Pd−C) removed one PMB group to give **26**, but only refluxing HCO$_2$H and hydrogenolysis gave the diamine **12** (in low yield as part of a complex mixture). Therefore this route was also abandoned in favor of a more direct approach, using the readily accessible 2-*t*-Bu-urea group as the only protection for the 2-amine (Scheme 3).

Methylation of **15** (NaH/DMF, then MeI/DMF) gave many products, the major one being **33** (36% yield),

Table 2. Structure and Kinase Inhibitory Activities of 1,6-Naphthyridines as FGFR Inhibitors



no.	Fm	X	Y	IC$_{50}$ (μM) FGF[a]	PDGF[a]	c-Src[a]
6	A	NH_2	NH_2	17	17	23
7	A	NH_2	NHCONHEt	1.3	7.7	5.1
8	A	NH_2	NHCONHtBu	1.9	2.6	4.4
9	B	NH_2	NH_2	2.8	35	0.68
10	B	NH_2	NHCONHEt	0.15	11	0.10
11	B	NH_2	NHCONHtBu	0.12	1.8	0.17
12	C	NH_2	NH_2	0.20	>50	>50
13	C	NH_2	NHCONHMe	0.095	>50	>50
14	C	NH_2	NHCONHEt	0.029	>50	>50
15	C	NH_2	NHCONHtBu	0.042	38	>50
16	C	NH_2	NHCONHPh	0.51	>50	>50
17	C	NHAc	NHCONHtBu	0.025	4.7	3.8
18	C	$NHCOCF_3$	NH_2	0.061	>50	>50
19	C	NHCONHtBu	NHCONHtBu	0.16	>50	>50
20	C	$NH(CH_2)_3$Nmorph[b]	NHCONHtBu	0.031	45	>50

[a] IC$_{50}$: concentration of drug (μM) to inhibit the phosphorylation of a random glutamate−tyrosine (4:1) copolymer by FGFR, PDGFR, or c-Src proteins. For active compounds, values are an average of two or more separate determinations; variation was generally ±30%. [b] *N*-Morpholinyl.

resulting from N^2-methylation and cleavage of the urea group. The structure of **33** was deduced from the similarity of its NMR to that of the 2-PMB-amino derivative **26** above and by HSQC and HMBC 2-D NMR (long-range correlations between the NH_2 and C-8 resonances, between the alkylated NH and both C-2 and C-3 resonances, and between the NCH_3 and C-2 resonances). Alkylation of **15** with excess 4-(3-chloropropyl)-morpholine hydrochloride/NaH/DMF gave a low yield of the desired **20** (10%), together with the N-1 alkylated product **34** (1.5%) and recovered **15** (48%). The structure of **34** is proposed from its distinctive UV spectrum and strong upfield shifts in the ^{1}H NMR, similar to those observed for 1,6-naphthyridin-2(1*H*)-ones[22] in comparison to the corresponding 2-*t*-Bu-ureas. This was further supported by the observation that strong base hydrolysis of **34** did not yield a 2-NH-alkyl derivative (TLC, NMR), whereas loss of the 2-urea to give **33** above was evidently very facile.

These alkylation results are consistent with those reported for reactions on 2-aminopyridine by Dome,[25] where mixtures of mono- and dibenzylated products were obtained in moderate yield (35% and 23%, respectively), and by Whitmore,[26] who prepared the 2-(morpholinylpropyl)amino derivative (18%) by heating the preformed anion with 0.5 equiv of the alkyl chloride in toluene. However, selective monoalkylation of 2-aminopyridine in better yield has been reported[27,28] via the corresponding 2-formamide or acetamide (whereas 2-sulfonamides reportedly[29] gave alkylation on the ring nitrogen).

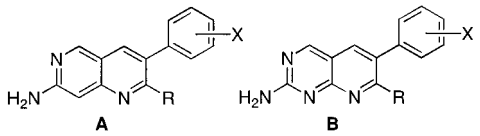
Trifluoroacetylation of **15** with TFAA/pyridine gave one major product, **18** (78%), which had lost the 2-*t*-Bu-urea functionality (possibly due to acylation, which could activate it toward hydrolysis). Because **18** was also somewhat unstable, we instead examined the more stable acetamide. Acetylation of **15** with AcCl/Et_3N/THF gave a mixture of the 7-acetamido and 7-diacetylamino derivatives **17** and **35** in good yield, and mild alkaline hydrolysis[30] converted the mixture cleanly to the 7-acetamide **17** (72% yield overall). Reactions involving Ac_2O and Et_3N or NaH gave undesired products, probably due to deprotonation of the 2-urea. However, Ac_2O/pyridine gave an excellent yield of the desired product **17** directly (92%).

Alkylation of **17** using excess 4-(3-chloropropyl)-morpholine hydrochloride/NaH/DMF gave a 1:1 mixture of the 7-NH- and 7-NAc-alkyl derivatives **20** and **36** in good yield (70%). This demonstrates high selectivity for alkylation of the assumed dianion (from deprotonation of the 2-urea and 7-acetamide) at the least hindered (7-N) position. Mild alkaline hydrolysis of this mixture selectively cleaved the N,N-disubstituted acetamide in the presence of the 2-urea to give **20** in 64% overall yield. However, alkylation of **17** appears to be very sensitive to traces of moisture, since repetition (on twice the scale) gave a much lower yield of **20** (16%) and no **36**, together with the 7,7-bis derivative **37** (14%), a mixture of **17** and the 7-amino derivative **15** (ca. 32%), and more polar components.

Results and Discussion

The compounds listed in Table 2 were evaluated for their ability to prevent phosphorylation of a model glutamate−tyrosine copolymer substrate by isolated avian c-Src, human FGF receptor-1 (FGFR), and mouse PDGF-β receptor (PDGFR) tyrosine kinase enzymes, using published methods.[11,17,31] The IC$_{50}$ values were defined as the concentration of inhibitor to reduce by 50% the level of ^{32}P (from added [^{32}P]ATP) incorporated into the copolymer substrate. The 3-phenyl compounds **6**−**8** were nonspecific inhibitors, with about equal activity in the three assays, similar to the pattern seen with the corresponding pyrido[2,3-*d*]pyrimidines.[19] Formation of the 2-urea increased potency but not selectivity. The corresponding 3-(2,6-dichlorophenyl) analogues **9**−**11** were significantly more potent than **6**−**8** for inhibition of the FGFR and c-Src kinases only but

Table 3. Comparison of the Kinase Inhibitory Activities of 1,6-Naphthyridines (A) and Pyrido[2,3-*d*]pyrimidines (B)



				IC_{50} (μM)		
no.	Fm	X	R	FGF[a]	PDGF[a]	c-Src[a]
8	A	H	NHCONHtBu	1.9	2.6	4.4
38[b]	B	H	NHCONHtBu	3.7	4.7	>50
9	A	2,6-diCl	NH_2	2.8	35	0.68
39[c]	B	2,6-diCl	NH_2	3.0	21	0.21
10	A	2,6-diCl	NHCONHEt	0.15	11	0.10
40[b]	B	2,6-diCl	NHCONHEt	0.13	1.3	0.08
11	A	2,6-diCl	NHCONHtBu	0.12	1.8	0.17
4[b]	B	2,6-diCl	NHCONHtBu	0.13	1.1	0.22
12	A	3,5-diOMe	NH_2	0.20	>50	>50
41[b]	B	3,5-diOMe	NH_2	0.23	>50	>50
15	A	3,5-diOMe	NHCONHtBu	0.042	38	>50
5[b]	B	3,5-diOMe	NHCONHtBu	0.060	>50	>50

[a] As for Table 2. [b] Ref 19. [c] Data from ref 21.

Table 4. Growth Delay and in Vitro Antiangiogenesis Activities of 1,6-Naphthyridines and Related Pyrido[2,3-*d*]pyrimidines

	IC_{50} (μM)[a]				
no.	C6[b]	A90[c]	HUVEC[d]	microcap[e]	invasion[e]
5	>25	15	0.58	0.1	1.1
12	8.9	17	0.82		
14	8.0	9.3	0.17		
15	8.5	1.2	0.0055	0.1	>10
17	0.65	12	0.004	0.00001	0.007
18	9.0	17	4.9		
19	13	11	0.017	0.01	
20	3.1	0.92	0.085	0.001	

[a] IC_{50}: concentration of drug (μM) to inhibit in vitro cell growth or HUVEC microcapillary formation or invasion. For active compounds, values are an average of two or more separate determinations. [b] PDGF-dependent rat glioma. [c] FGFR overexpressing human ovarian carcinoma. [d] FGF-dependent human umbilical vein endothelial cells. [e] Matrigel assay.

remained nonselective between these two kinases. In contrast, the 3-(3,5-dimethoxyphenyl) derivatives **12**−**20** showed both high potency and excellent selectivity for FGFR versus the other kinases. Both PDGFR and in particular c-Src activity was completely lost, resulting in c-Src/FGFR selectivities of >1000-fold for many of the compounds. The three alkyl ureas **13**−**15** did not differ markedly in potency, but the phenyl urea **16** was significantly less effective. This is consistent with results reported by Hamby et al.[19] for the related pyrido[2,3-*d*]pyrimidines. Acetamide **17** was the most potent FGFR inhibitor (IC_{50} 25 nM) but was less selective against PDGFR and c-Src. The bis-urea **19** was less potent, but the more soluble analogue **20** retained high potency and selectivity. Table 3 provides some pairwise comparisons of 1,6-naphthyridines and pyrido[2,3-*d*]pyrimidines. Overall no significant differences can be seen between the two chromophore series, either in absolute potency or in patterns of selectivity.

Certain of the FGFR-selective 3-(3,5-dimethoxyphenyl) derivatives were evaluated for growth inhibition in a variety of serum-stimulated cell lines, together with the pyrido[2,3-*d*]pyrimidine **5** (Table 4). A comparison of **5** and its direct 1,6-naphthyridine analogue **15** showed that the naphthyridine was considerably more potent in all the lines. Overall, the compounds displayed similar, modest potencies toward both the PDGF-dependent C6 rat glioma line[32] (expressing moderate FGFR levels[33]) and the FGFR-overexpressing human ovarian A90 line[34] but much higher potencies toward human umbilical vein endothelial cells (HUVECs), whose growth has been shown to be FGF-dependent.[35] The 7-acetamide **17** was particularly potent against HUVECs (IC_{50} 4 nM), and this compound was also an extremely effective inhibitor of HUVEC microcapillary formation and Matrigel invasion (Table 4).

One compound (**17**) was selected for in vivo evaluation against three murine tumor model systems: mammary adenocarcinoma 16/c, M5076 reticulum cell sarcoma, and Lewis lung carcinoma. These models were selected due to their high degree of vascularization and have been used by a number of other investigators in the analysis of angiogenesis inhibitors. While we expected that **17** would produce measurable antitumor effects in all three models, it was effective only against the mammary adenocarcinoma 16/c (Table 5). Although the drug had to be administered as a suspension, enough was apparently absorbed from the gastrointestinal tract following oral dosing to produce good antitumor activity. Similar results were obtained for both once and twice daily treatment schedules, and at the doses tested, there was no toxicity or significant clinical signs (animal weight loss was minimal). The reasons for the differences in the effectiveness of **17** among the three highly vascularized tumor models are not known. It could be due to redundancy in the tyrosine kinase receptor signaling pathways with respect to angiogenesis in these tumor models and is an area of current investigation.

Conclusions

The broadly similar SARs found above for the 1,6-naphthyridines and the previously reported[19] pyrido[2,3-*d*]pyrimidines suggest a very similar binding mode to the enzymes, with the 1-aza atom of the pyrido[2,3-*d*]pyrimidines not being required for this binding. In both series, substituents on the appended phenyl group have a major impact on the pattern of inhibition of different kinases, suggesting that the geometry of this ring is a critical factor in binding site selectivity.[19] Computer modeling studies[36] and crystal structure data[13] of the closely related 6-phenylpyrido[2,3-*d*]pyrimidine 7-ureas show the need for bifurcated H bonds between the N-2 exocyclic and N-3 endocyclic atoms and a residue (Ala564) on the extended coil stretch of the FGFR enzyme. The 7-NHR 1,6-naphthyridine-2-ureas also contain this required structural motif. The 3-(3,5-dimethoxyphenyl) compounds showed particular selectivity for FGFR over PDGFR and c-Src, presumably because the methoxy residues fit better into the larger hydrophobic pocket of FGFR.[13] The potent inhibition by **17** of HUVEC growth and microcapillary formation and invasion suggests that these compounds are worthy of further evaluation as antiangiogenesis agents. It is notable that the in vivo tumor growth delay values obtained for **17** against mammary 16/c were approximately equal to the duration of therapy (9 days), which suggests that this compound is cytostatic rather than cytotoxic under these test conditions. It remains to be

Table 5. In Vivo Activity of **17** against Murine Tumor Models

tumor[a]	dose (mg/kg)[b]	schedule[c]	wt change (g)	T/C (%) on last therapy day[d]	T−C (days)[e]
mammary 16/c	80 hdt	po, q12hx2, days 1−9	−0.7	0	10.8
mammary 16/c	200 hdt	po, days 1−9	+	0	11.3
M5076 sarcoma	200 hdt	po, days 8−16	+	78	1.0
Lewis lung	200 hdt	po, days 4−12	−0.4	109	0.0

[a] The indicated tumor fragments were implanted sc into the right axilla of mice on day 0. [b] hdt, highest dose tested. [c] Compound was administered orally on the indicated schedules. [d] Ratio of median treated tumor mass/median control tumor mass × 100%. [e] The difference in days for the treated (T) and control (C) tumors to reach 750 mg.

determined if more prolonged therapy would have cytotoxic effects in vivo, resulting in the regression of established tumors.

Experimental Section

Analyses were performed by the Microchemical Laboratory, University of Otago, Dunedin, NZ. Melting points were determined using an Electrothermal model 9200 digital melting point apparatus and are as read. NMR spectra were measured on a Bruker DRX-400 spectrometer and referenced to Me_4Si. Mass spectra were recorded on a Varian VG-70SE spectrometer at nominal 5000 resolution.

***N*-(7-Amino-3-phenyl-1,6-naphthyridin-2-yl)-*N′*-*tert*-butylurea (8): Example of General Method A**. A solution of 3-phenyl-1,6-naphthyridine-2,7-diamine[23] (**6**) (103 mg, 0.436 mmol) in dry DMF (5 mL) was treated with 60% NaH (20 mg, 0.50 mmol); then the reaction flask was immediately sealed with a rubber septum, degassed (water pump vacuum) and filled with dry N_2 (balloon), and the mixture stirred at 20 °C for 10 min. A solution of *tert*-butyl isocyanate (58 μL, 0.509 mmol) in dry DMF (1 mL, then 1 mL to rinse) was added (dropwise via syringe); then the mixture was stirred at 20 °C for 4 h. The resulting mixture was cooled in ice, then treated with ice/aqueous $NaHCO_3$ (50 mL) and extracted with EtOAc (5 × 50 mL). The extracts were evaporated to dryness and the residue was then chromatographed on silica gel. Elution with 25−30% EtOAc/light petroleum gave foreruns; then further elution with 33−40% EtOAc/light petroleum gave **8** (97 mg, 66%): mp (CH_2Cl_2/hexane) 174−175.5 °C; ^{1}H NMR [$(CD_3)_2$SO] δ 10.26 (br s, 1 H, NH), 8.67 (s, 1 H, H-5), 7.98 (s, 1 H, H-4), 7.58 (t, J = 7.2 Hz, 2 H, H-3′,5′), 7.51 (t, J = 7.3 Hz, 1 H, H-4′), 7.50 (d, J = 6.7 Hz, 2 H, H-2′,6′), 6.91 (br s, 1 H, NH), 6.49 (s, 1 H, H-8), 6.37 (br s, 2 H, NH_2), 1.41 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR δ 160.36 (s, C-7), 152.45, 151.99 (2 s, CONH, C-2), 151.49 (d, C-5), 149.42 (s, C-8a), 137.73 (d, C-4), 135.48 (s, C-1′), 129.42, 129.11 (2 d, 2 × 2 C, C-2′,3′,5′,6′), 128.52 (d, C-4′), 121.25 (s, C-3), 113.34 (s, C-4a), 95.73 (d, C-8), 49.93 (s, *C*$(CH_3)_3$), 28.64 (q, 3 C, C(*C*$H_3)_3$). Anal. ($C_{19}H_{21}N_5O$) C, H, N.

Further elution of the column with EtOAc gave a mixture; then further elution with 10% MeOH/CH_2Cl_2 gave crude recovered **6** (25 mg, 24%) as an oil.

***N*-[7-Amino-3-(2,6-dichlorophenyl)-1,6-naphthyridin-2-yl]-*N′*-*tert*-butylurea (11)**. Similar reaction of a stirred solution of 3-(2,6-dichlorophenyl)-1,6-naphthyridine-2,7-diamine[23] (**9**) (100 mg, 0.328 mmol) in dry DMF (5 mL) with 60% NaH (16 mg, 0.40 mmol) under N_2 at 20 °C for 10 min, then with a solution of *tert*-butyl isocyanate (45 μL, 0.395 mmol) in dry DMF (1 mL) at 20 °C for 1 h, followed by chromatography of the resulting product on silica gel (eluting with 0.25−0.5% MeOH/CH_2Cl_2) gave **11** (30 mg, 23%): mp (CH_2Cl_2/hexane) 165−167 °C; ^{1}H NMR [$(CD_3)_2$SO] δ 10.35 (br s, 1 H, NH), 8.65 (s, 1 H, H-5), 7.95 (s, 1 H, H-4), 7.65 (d, J = 8.4 Hz, 2 H, H-3′,5′), 7.53 (dd, J = 8.7, 7.4 Hz, 1 H, H-4′), 7.43 (br s, 1 H, NH), 6.48 (s, 1 H, H-8), 6.44 (br s, 2 H, NH_2), 1.40 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR δ 160.69 (s, C-7), 152.45, 152.37 (2 s, CONH, C-2), 151.60 (d, C-5), 149.80 (s, C-8a), 139.48 (d, C-4), 135.48 (s, 2 C, C-2′,6′), 132.56 (s, C-1′), 131.51 (d, C-4′), 128.77 (d, 2 C, C-3′,5′), 116.34 (s, C-3), 112.87 (s, C-4a), 95.57 (d, C-8), 49.91 (s, *C*$(CH_3)_3$), 28.64 (q, 3 C, C(*C*$H_3)_3$). Anal. ($C_{19}H_{19}Cl_2N_5O$) C, H, N.

Further elution of the column with 0.5−2% MeOH/CH_2Cl_2 gave a mixture; then further elution with 2−5% MeOH/CH_2Cl_2 gave crude recovered **9** (66 mg, 66%) as an oil.

***N*-[7-Amino-3-(3,5-dimethoxyphenyl)-1,6-naphthyridin-2-yl]-*N′*-methylurea (13)**. Similar reaction of a stirred solution of 3-(3,5-dimethoxyphenyl)-1,6-naphthyridine-2,7-diamine[23] (**12**) (106 mg, 0.358 mmol) in dry DMF (4 mL) with 60% NaH (14 mg, 0.35 mmol) under N_2 at 20 °C for 10 min, then with a solution of methyl isocyanate (12 μL, 0.204 mmol) in dry DMF (1 mL) at 20 °C for 1 h, followed by chromatography of the resulting product on silica gel (eluting with 1.5% MeOH/CH_2Cl_2) gave **13** (59 mg, 47%): mp (MeOH/CH_2Cl_2/hexane) 134−137 °C; ^{1}H NMR [$(CD_3)_2$SO] δ 9.73 (br q, J = 4.6 Hz, 1 H, N*H*CH_3), 8.66 (s, 1 H, H-5), 7.99 (s, 1 H, H-4), 7.24 (br s, 1 H, NH), 6.63 (s, 3 H, H-2′,4′,6′), 6.62 (s, 1 H, H-8), 6.31 (br s, 2 H, NH_2), 3.80 (s, 6 H, $2OCH_3$), 2.84 (d, J = 4.6 Hz, 3 H, NHC*H*$_3$); ^{13}C NMR δ 161.09 (s, 2 C, C-3′,5′), 160.23 (s, C-7), 154.12, 152.11 (2 s, CONH, C-2), 151.39 (d, C-5), 149.66 (s, C-8a), 137.51 (d+s, 2 C, C-4,1′), 121.14 (s, C-3), 113.36 (s, C-4a), 107.07 (d, 2 C, C-2′,6′), 100.16 (d, C-4′), 96.26 (d, C-8), 55.37 (q, 2 C, $2OCH_3$), 26.09 (q, CH_3). Anal. ($C_{18}H_{19}N_5O_3$·1.25H_2O) C, H, N.

Further elution of the column with 2−4% MeOH/CH_2Cl_2 gave a mixture; then further elution with 4−10% MeOH/CH_2Cl_2 gave crude recovered **12** (57 mg, 54%) as an oil.

***N*-[7-Amino-3-(3,5-dimethoxyphenyl)-1,6-naphthyridin-2-yl]-*N′*-phenylurea (16)**. Similar reaction of a stirred solution of **12** (100 mg, 0.338 mmol) in dry DMF (5 mL) with 60% NaH (13 mg, 0.325 mmol) under N_2 at 20 °C for 10 min, then with a solution of phenyl isocyanate (35 μL, 0.322 mmol) in dry DMF (1 mL) at 20 °C for 1 h, followed by chromatography of the resulting product on silica gel (eluting with 0.25−1% MeOH/CH_2Cl_2) gave **16** (63 mg, 45%): mp (MeOH/CH_2Cl_2/hexane) 205−207 °C; ^{1}H NMR [$(CD_3)_2$SO] δ 12.68 (br s, 1 H, NH), 8.73 (s, 1 H, H-5), 8.10 (s, 1 H, H-4), 7.61 (d, J = 7.6 Hz, 2 H, H-2″,6″), 7.61 (br s, 1 H, NH), 7.39 (t, J = 7.9 Hz, 2 H, H-3″,5″), 7.12 (t, J = 7.4 Hz, 1 H, H-4″), 6.72 (s, 1 H, H-8), 6.69 (d, J = 2.2 Hz, 2 H, H-2′,6′), 6.64 (t, J = 2.2 Hz, 1 H, H-4′), 6.41 (br s, 2 H, NH_2), 3.82 (s, 6 H, $2OCH_3$); ^{13}C NMR δ 161.12 (s, 2 C, C-3′,5′), 160.50 (s, C-7), 152.09 (s, C-2), 151.75 (d, C-5), 151.08 (s, CONH), 149.14 (s, C-8a), 138.31 (s, C-1″), 138.19 (d, C-4), 137.26 (s, C-1′), 129.07 (d, 2 C, C-3″,5″), 123.28 (d, C-4″), 121.40 (s, C-3), 119.07 (d, 2 C, C-2″,6″), 113.53 (s, C-4a), 107.19 (d, 2 C, C-2′,6′), 100.25 (d, C-4′), 95.87 (d, C-8), 55.41 (q, 2 C, $2OCH_3$). Anal. ($C_{23}H_{21}N_5O_3$·0.5H_2O) C, H, N.

Further elution of the column with 2.5% MeOH/CH_2Cl_2 gave a mixture; then further elution with 5−8% MeOH/CH_2Cl_2 gave crude recovered **12** (55 mg, 55%) as an oil.

***N*-[7-Amino-3-(3,5-dimethoxyphenyl)-1,6-naphthyridin-2-yl]-*N′*-*tert*-butylurea (15) and *N*-(*tert*-Butyl)-*N′*-[7-(3-*tert*-butylureido)-3-(3,5-dimethoxyphenyl)-1,6-naphthyridin-2-yl]urea (19): Example of General Method B**. A solution of **12** (5.01 g, 16.9 mmol) in dry DMF (100 mL) was treated with 60% NaH (0.83 g, 20.8 mmol); then the mixture was sealed under N_2 (as above) and stirred at 20 °C for 15 min and then at 0 °C for 30 min. *tert*-Butyl isocyanate (2.40 mL, 21.0 mmol) was added (dropwise via syringe); then the mixture was stirred at 20 °C for 1 day. The resulting mixture was cooled in ice, then treated with ice/aqueous $NaHCO_3$ (500 mL) and extracted with EtOAc (10 × 400 mL). The extracts were evaporated to dryness and the residue was then chromatographed on silica gel. Elution with 33% EtOAc/light petroleum gave first *N*-(*tert*-butyl)-*N′*-[7-(3-*tert*-butylureido)-3-(3,5-dimethoxyphenyl)-1,6-naphthyridin-2-yl]urea (**19**) (186 mg, 2%): mp (EtOAc/light petroleum) 208−210 °C dec; ^{1}H NMR [$(CD_3)_2$SO] δ 10.10 (br s, 1 H, NH), 9.04 (br s, 1 H, NH),

8.87 (s, 1 H, H-5), 8.16 (s, 1 H, H-4), 7.83 (s, 1 H, H-8), 7.27 (br s, 1 H, NH), 7.15 (br s, 1 H, NH), 6.68 (d, J = 2.0 Hz, 2 H, H-2′,6′), 6.66 (t, J = 2.1 Hz, 1 H, H-4′), 3.81 (s, 6 H, 2OCH_3), 1.41 (s, 9 H, C(CH_3)$_3$), 1.34 (s, 9 H, C(CH_3)$_3$); ^{13}C NMR δ 161.15 (s, 2 C, C-3′,5′), 153.45, 153.07, 152.81, 151.77 (4 s, 2CONH, C-2,7), 150.39 (d, C-5), 149.09 (s, C-8a), 137.15 (d, C-4), 136.89 (s, C-1′), 124.13 (s, C-3), 115.72 (s, C-4a), 107.08 (d, 2 C, C-2′,6′), 102.04 (d, C-8), 100.51 (d, C-4′), 55.43 (q, 2 C, 2OCH_3), 50.03, 49.51 (2 s, 2C(CH_3)$_3$), 28.87, 28.64 (2 q, 2 × 3 C, 2C(CH_3)$_3$). Anal. ($C_{26}H_{34}N_6O_4$) H, N; C: calcd, 63.1; found, 63.6.

Further elution of the column with 40−50% EtOAc/light petroleum gave *N*-[7-amino-3-(3,5-dimethoxyphenyl)-1,6-naphthyridin-2-yl]-*N*′-*tert*-butylurea (**15**) (6.19 g, 93%): mp (CH_2Cl_2/hexane) 127−130 °C; ^{1}H NMR [(CD_3)$_2$SO] δ 10.24 (br s, 1 H, NH), 8.66 (s, 1 H, H-5), 7.99 (s, 1 H, H-4), 7.01 (br s, 1 H, NH), 6.63 (m, 3 H, H-2′,4′,6′), 6.47 (s, 1 H, H-8), 6.36 (br s, 2 H, NH_2), 3.81 (s, 6 H, 2OCH_3), 1.40 (s, 9 H, C(CH_3)$_3$); ^{13}C NMR δ 161.11 (s, 2 C, C-3′,5′), 160.35 (s, C-7), 152.34, 152.02 (2 s, CONH, C-2), 151.47 (d, C-5), 149.42 (s, C-8a), 137.41 (d, C-4), 137.37 (s, C-1′), 121.16 (s, C-3), 113.18 (s, C-4a), 107.11 (d, 2 C, C-2′,6′), 100.19 (d, C-4′), 95.74 (d, C-8), 55.39 (q, 2 C, 2OCH_3), 49.93 (s, C(CH_3)$_3$), 28.66 (q, 3 C, C(CH$_3$)$_3$). Anal. ($C_{21}H_{25}N_5O_3$·0.25H_2O) C, H, N.

***N*-(7-Amino-3-phenyl-1,6-naphthyridin-2-yl)-*N*′-ethylurea (7)**. Similar reaction of a stirred solution of **6** (102 mg, 0.432 mmol) in dry DMF (5 mL) with 60% NaH (22 mg, 0.55 mmol) under N_2 at 20 °C for 10 min, then (upon cooling to 0 °C) with ethyl isocyanate (43 μL, 0.544 mmol) at 0−20 °C for 1 day, followed by chromatography of the resulting product on silica gel (eluting with 0.9−1% MeOH/CH_2Cl_2), then further chromatography on silica gel (eluting with 50−75% EtOAc/light petroleum) gave **7** (87 mg, 66%): mp (CH_2Cl_2/hexane) 181−182.5 °C; ^{1}H NMR [(CD_3)$_2$SO] δ 9.94 (br m, 1 H, NHCH$_2$), 8.67 (s, 1 H, H-5), 7.99 (s, 1 H, H-4), 7.57 (t, J = 7.2 Hz, 2 H, H-3′,5′), 7.51 (m, 3 H, H-2′,4′,6′), 7.10 (br s, 1 H, NH), 6.59 (s, 1 H, H-8), 6.34 (br s, 2 H, NH_2), 3.30 (qd, J = 7.2, 5.9 Hz, 2 H, NHCH_2), 1.19 (t, J = 7.2 Hz, 3 H, CH_3); ^{13}C NMR δ 160.28 (s, C-7), 153.36, 152.32 (2 s, CONH, C-2), 151.45 (d, C-5), 149.62 (s, C-8a), 137.82 (d, C-4), 135.61 (s, C-1′), 129.41, 129.10 (2 d, 2 × 2 C, C-2′,3′,5′,6′), 128.48 (d, C-4′), 121.32 (s, C-3), 113.49 (s, C-4a), 96.13 (d, C-8), 34.10 (t, NCH_2),15.04 (q, CH_3). Anal. ($C_{17}H_{17}N_5O$·0.25H_2O) C, H, N.

***N*-[7-Amino-3-(3,5-dimethoxyphenyl)-1,6-naphthyridin-2-yl]-*N*′-ethylurea (14)**. Similar reaction of **12** (100 mg, 0.338 mmol) in dry DMF (5 mL) with 60% NaH (17 mg, 0.425 mmol) under N_2 at 20 °C for 10 min, and then (upon cooling to 0 °C) with ethyl isocyanate (30 μL, 0.379 mmol) at 0−20 °C for 20 h, followed by chromatography of the resulting product on silica gel (eluting with 1−1.25% MeOH/CH_2Cl_2) gave **14** (70 mg, 56%): mp (CH_2Cl_2/hexane) 149.5−151.5 °C; ^{1}H NMR [(CD_3)$_2$SO] δ 9.93 (br t, J = 5.3 Hz, 1 H, NHCH$_2$), 8.66 (s, 1 H, H-5), 8.00 (s, 1 H, H-4), 7.21 (br s, 1 H, NH), 6.63 (s, 3 H, H-2′,4′,6′), 6.58 (s, 1 H, H-8), 6.33 (br s, 2 H, NH_2), 3.81 (s, 6 H, 2OCH_3), 3.30 (qd, J = 7.2, 5.6 Hz, 2 H, NHCH_2), 1.19 (t, J = 7.2 Hz, 3 H, CH_3); ^{13}C NMR δ 161.10 (s, 2 C, C-3′,5′), 160.28 (s, C-7), 153.37, 152.21 (2 s, CONH, C-2), 151.45 (d, C-5), 149.60 (s, C-8a), 137.50 (d, C-4), 137.48 (s, C-1′), 121.19 (s, C-3), 113.33 (s, C-4a), 107.08 (d, 2 C, C-2′,6′), 100.17 (d, C-4′), 96.12 (d, C-8), 55.38 (q, 2 C, 2OCH_3), 34.09 (t, NCH_2), 15.04 (q, CH_3). Anal. ($C_{19}H_{21}N_5O_3$·H_2O) C, H, N.

***N*-[7-Amino-3-(2,6-dichlorophenyl)-1,6-naphthyridin-2-yl]-*N*′-ethylurea (10): Example of General Method C**. A solution of **9** (133 mg, 0.436 mmol) in dry DMSO (5 mL) was treated with 60% NaH (24 mg, 0.60 mmol); then the mixture was sealed under N_2 (as above) and stirred at 40−50 °C for 5 min and then at 20 °C for 90 min. A solution of ethyl isocyanate (38 μL, 0.481 mmol) in dry DMSO (1 mL, then 2 × 0.5 mL to rinse) was added (dropwise via syringe); then the mixture was stirred at 20 °C for 1 day. The resulting mixture was cooled in ice, then treated with ice/aqueous $NaHCO_3$ (50 mL) and extracted with EtOAc (5 × 50 mL). The extracts were evaporated to dryness and the residue was then chromatographed on silica gel. Elution with 0−0.5% MeOH/CH_2Cl_2 gave foreruns; then further elution with 0.5−1.25% MeOH/CH_2Cl_2 gave **10** (82 mg, 50%): mp (MeOH/CH_2Cl_2/hexane) 210−212 °C; ^{1}H NMR [(CD_3)$_2$SO] δ 10.06 (br t, J = 5.3 Hz, 1 H, NHCH$_2$), 8.66 (s, 1 H, H-5), 7.96 (s, 1 H, H-4), 7.81 (br s, 1 H, NH), 7.64 (d, J = 8.1 Hz, 2 H, H-3′,5′), 7.52 (dd, J = 8.8, 7.3 Hz, 1 H, H-4′), 6.58 (s, 1 H, H-8), 6.41 (br s, 2 H, NH_2), 3.29 (qd, J = 7.1, 5.6 Hz, 2 H, NHCH_2), 1.19 (t, J = 7.2 Hz, 3 H, CH_3); ^{13}C NMR δ 160.61 (s, C-7), 153.81, 152.44 (2 s, CONH, C-2), 151.56 (d, C-5), 149.99 (s, C-8a), 139.54 (d, C-4), 135.50 (s, 2 C, C-2′,6′), 132.74 (s, C-1′), 131.40 (d, C-4′), 128.73 (d, 2 C, C-3′,5′), 116.49 (s, C-3), 113.01 (s, C-4a), 95.95 (d, C-8), 34.07 (t, NCH_2), 14.98 (q, CH_3). Anal. ($C_{17}H_{15}Cl_2N_5O$) C, H, N.

2-[7-Amino-3-(3,5-dimethoxyphenyl)-1,6-naphthyridin-2-yl]-1*H*-isoindole-1,3(2*H*)-dione (22) and 2-[2-Amino-3-(3,5-dimethoxyphenyl)-1,6-naphthyridin-7-yl]-1*H*-isoindole-1,3(2*H*)-dione (23). A solution of **12** (102 mg, 0.345 mmol) in dry DMF (6 mL) was treated with 60% NaH (15 mg, 0.375 mmol); then the mixture was sealed under N_2 (as above) and stirred at 20 °C for 10 min. Phthaloyl dichloride (25 μL of 'practical', ca. 0.156 mmol) was added directly (dropwise via syringe); then the mixture was stirred at 20 °C for 8 h. The resulting mixture was cooled in ice, then treated with ice/aqueous $NaHCO_3$ (50 mL) and extracted with EtOAc (4 × 50 mL). The extracts were evaporated to dryness and the residue was then chromatographed on silica gel. Elution with 0−1% MeOH/CH_2Cl_2 gave foreruns; then further elution with 1% MeOH/CH_2Cl_2 gave 2-[2-amino-3-(3,5-dimethoxyphenyl)-1,6-naphthyridin-7-yl]-1*H*-isoindole-1,3(2*H*)-dione (**23**) (7 mg, 5%): mp (DMSO/water) 270−271.5 °C; ^{1}H NMR [(CD_3)$_2$SO] δ 8.95 (s, 1 H, H-5), 8.07 (s, 1 H, H-4), 8.02, 7.95 (2 m, 2 × 2 H, H-3″,4″,5″,6″), 7.49 (s, 1 H, H-8), 6.90 (br s, 2 H, NH_2), 6.68 (d, J = 2.3 Hz, 2 H, H-2′,6′), 6.60 (t, J = 2.2 Hz, 1 H, H-4′), 3.82 (s, 6 H, 2OCH_3); ^{13}C NMR δ 166.66 (s, 2 C, 2C=O), 160.79 (s, 2 C, C-3′,5′), 159.04 (s, C-2), 151.83 (s, C-7), 150.71 (d, C-5), 143.98 (s, C-8a), 138.39 (s, C-1′), 135.36 (d, C-4), 134.90 (d, 2 C, C-4″,5″), 131.44 (s, 2 C, C-2a″,6a″), 126.54 (s, C-3), 123.59 (d, 2 C, C-3″,6″), 119.33 (s, C-4a), 116.94 (d, C-8), 106.52 (d, 2 C, C-2′,6′), 100.46 (d, C-4′), 55.27 (q, 2 C, 2OCH_3); HRFABMS calcd for $C_{24}H_{19}N_4O_4$ m/z (MH^+) 427.1406, found 427.1401. Anal. ($C_{24}H_{18}N_4O_4$·0.5H_2O) C, H, N.

Further elution of the column with 1−1.5% MeOH/CH_2Cl_2 gave 2-[7-amino-3-(3,5-dimethoxyphenyl)-1,6-naphthyridin-2-yl]-1*H*-isoindole-1,3(2*H*)-dione (**22**) (8 mg, 5%): mp (CH_2Cl_2/hexane) 213−214 °C; ^{1}H NMR [(CD_3)$_2$SO] δ 9.06 (s, 1 H, H-5), 8.48 (s, 1 H, H-4), 7.95 (m, 4 H, H-3″,4″,5″,6″), 6.72 (s, 1 H, H-8), 6.64 (br s, 2 H, NH_2), 6.43 (d, J = 2.4 Hz, 2 H, H-2′,6′), 6.40 (t, J = 2.3 Hz, 1 H, H-4′), 3.59 (s, 6 H, 2OCH_3); ^{13}C NMR δ 166.46 (s, 2 C, 2C=O), 160.26 (s, C-7), 160.22 (s, 2 C, C-3′,5′), 153.38 (d, C-5), 151.00, 147.35 (2 s, C-2,8a), 139.49 (d, C-4), 138.47 (s, C-1′), 135.42 (d, 2 C, C-4″,5″), 130.79 (s, 2 C, C-2a″,-6a″), 128.98 (s, C-3), 123.93 (d, 2 C, C-3″,6″), 116.55 (s, C-4a), 105.82 (d, 2 C, C-2′,6′), 99.79 (d, C-4′), 96.39 (d, C-8), 54.99 (q, 2 C, 2OCH_3). Anal. ($C_{24}H_{18}N_4O_4$) C, H, N.

Further elution of the column with 1.5−2.5% MeOH/CH_2Cl_2 gave a mixture; then further elution with 3.5−6% MeOH/CH_2Cl_2 gave crude recovered **12** (78 mg, 76%) as an oil.

3-(3,5-Dimethoxyphenyl)-N^2,N^2,N^7,N^7-tetrakis(4-methoxybenzyl)-1,6-naphthyridine-2,7-diamine (24). A solution of **12** (51 mg, 0.172 mmol) in dry DMF (5 mL) was treated with 60% NaH (60 mg, 1.50 mmol); then the mixture was sealed under N_2 (as above) and stirred at 20 °C for 5 min. 4-Methoxybenzyl chloride (0.183 mL, 1.35 mmol) was added; then the mixture was stirred at 20 °C for 1 day. The resulting solution was cooled in ice, then treated with ice/aqueous $NaHCO_3$ (50 mL) and extracted with EtOAc (6 × 50 mL). The extracts were evaporated to dryness and the residue was then chromatographed on silica gel. Elution with 50−75% CH_2Cl_2/light petroleum gave foreruns; then further elution with 80% CH_2Cl_2/light petroleum and CH_2Cl_2 gave **24** (83 mg, 62%): mp (CH_2Cl_2/hexane) 143.5−145.5 °C; ^{1}H NMR [(CD_3)$_2$SO] δ 8.74 (s, 1 H, H-5), 7.91 (s, 1 H, H-4), 7.20 (d, J = 8.7 Hz, 4 H, 2H-2″,6″), 7.02 (d, J = 8.6 Hz, 4 H, 2H-2‴,6‴), 6.88 (d, J = 8.7 Hz, 4 H, 2H-3″,5″), 6.78 (d, J = 8.7 Hz, 4 H, 2H-3‴,5‴), 6.68 (d, J = 2.2 Hz, 2 H, H-2′,6′), 6.46 (t, J = 2.3 Hz, 1 H, H-4′), 6.40 (s, 1 H, H-8), 4.79 (s, 4 H, N(CH_2)$_2$), 4.19 (s, 4 H, N(CH_2)$_2$),

3.74, 3.72, 3.69 (3 s, 3 × 6 H, 6OCH$_3$). Anal. ($C_{48}H_{48}N_4O_6$·0.5H_2O) C, H, N.

3-(3,5-Dimethoxyphenyl)-*N*^{2},*N*^{7}-bis(4-methoxybenzyl)-1,6-naphthyridine-2,7-diamine (25). A solution of **24** (0.8 mg, 1.03 μmol) in TFA (1 mL) was stirred at 50 °C for 5 min in a sealed vial. The solvent was removed by blowing with dry N_2; then the residue was treated with ice/aqueous Na_2CO_3 (30 mL) and extracted with EtOAc (3 × 30 mL). The extracts were evaporated to dryness and the residue was then purified by preparative silica gel TLC, developed twice in 1% MeOH/CH_2Cl_2, to give two bands, which were each eluted with 10% MeOH/CH_2Cl_2. The more polar component was identified as 3-(3,5-dimethoxyphenyl)-*N*^{2}-(4-methoxybenzyl)-1,6-naphthyridine-2,7-diamine (**26**) (0.1 mg, 23%) (see below), while the major, less polar component was 3-(3,5-dimethoxyphenyl)-*N*^{2},*N*^{7}-bis(4-methoxybenzyl)-1,6-naphthyridine-2,7-diamine (**25**) (0.4 mg, 72%), isolated as an oil: ^{1}H NMR [$(CD_3)_2SO$] δ 8.43 (s, 1 H, H-5), 7.61 (s, 1 H, H-4), 7.27 (d, *J* = 8.2 Hz, 4 H, H-2″,2‴,6″,6‴), 6.92 (br t, *J* = 6.2 Hz, 1 H, N*H*CH$_2$), 6.88 (d, *J* = 8.6 Hz, 2 H, H-3″,5″), 6.83 (d, *J* = 8.6 Hz, 2 H, H-3‴,5‴), 6.65 (br t, *J* = 6.0 Hz, 1 H, N*H*CH$_2$), 6.56 (d, *J* = 2.3 Hz, 2 H, H-2′,6′), 6.52 (t, *J* = 2.2 Hz, 1 H, H-4′), 6.22 (s, 1 H, H-8), 4.53 (d, *J* = 5.3 Hz, 2 H, NHC*H*$_2$), 4.41 (d, *J* = 4.6 Hz, 2 H, NHC*H*$_2$), 3.77 (s, 6 H, 2OCH$_3$), 3.72, 3.70 (2 s, 2 × 3 H, 2OCH$_3$); HRFABMS calcd for $C_{32}H_{33}N_4O_4$ *m*/*z* (MH$^+$) 537.2502, found 537.2500.

3-(3,5-Dimethoxyphenyl)-*N*^{7}-trityl-1,6-naphthyridine-2,7-diamine (28). Method A: A solution of **12** (51 mg, 0.172 mmol) in dry DMF (3 mL) was treated with 60% NaH (14 mg, 0.35 mmol); then the mixture was sealed under N_2 (as above) and stirred at 20 °C for 25 min. Trityl chloride (192 mg, 0.688 mmol) was added; then the mixture was stirred under N_2 at 20 °C for 1 day (TLC almost no starting material). The resulting mixture was cooled in ice, then treated with ice/aqueous Na_2CO_3 (50 mL) and extracted with EtOAc (3 × 50 mL). The combined extracts were evaporated to dryness and the residue was then chromatographed on silica gel. Elution with 67−80% CH_2Cl_2/light petroleum gave foreruns; then further elution with CH_2Cl_2 gave an oil (24 mg), which, upon crystallization from MeOH/CH_2Cl_2, gave 3-(3,5-dimethoxyphenyl)-7-(tritylamino)-1,6-naphthyridin-2-ylformamide (**27**) (13 mg, 13%): mp (MeOH/CH_2Cl_2) 201−203 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.54 (br d, *J* = 9.4 Hz, 1 H, N*H*CHO), 9.11 (d, *J* = 9.3 Hz, 1 H, NHC*H*O), 8.67 (s, 1 H, H-5), 8.04 (s, 1 H, H-4), 7.49 (br s, 1 H, NH), 7.38 (d, *J* = 8.1 Hz, 6 H, 3H-2″,6″), 7.31 (t, *J* = 7.7 Hz, 6 H, 3H-3″,5″), 7.22 (t, *J* = 7.2 Hz, 3 H, 3H-4″), 6.57 (d, *J* = 2.2 Hz, 2 H, H-2′,6′), 6.55 (t, *J* = 2.2 Hz, 1 H, H-4′), 6.26 (br s, 1 H, H-8), 3.76 (s, 6 H, 2OCH$_3$); ^{13}C NMR δ 162.65 (d, NCHO), 160.61 (s, 2 C, C-3′,5′), 157.67 (s, C-7), 151.19 (s, C-2), 150.74 (d, C-5), 149.40 (s, C-8a), 144.75 (s, 3 C, 3C-1″), 138.13 (d, C-4), 137.77 (s, C-1′), 128.67, 127.76 (2 d, 2 × 6 C, 3C-2″,3″,5″,6″), 126.56 (d, 3 C, 3C-4″), 123.09 (s, C-3), 115.43 (s, C-4a), 107.27 (d, 2 C, C-2′,6′), 100.35 (d, C-8), 99.71 (d, C-4′), 70.32 (s, *C*(Ph)$_3$), 55.22 (q, 2 C, 2OCH$_3$). Anal. ($C_{36}H_{30}N_4O_3$·0.25H_2O) C, H, N.

Further elution of the column with 0−1% MeOH/CH_2Cl_2 gave 3-(3,5-dimethoxyphenyl)-*N*^{7}-trityl-1,6-naphthyridine-2,7-diamine (**28**) (37 mg, 40%): mp (CH_2Cl_2/hexane) 166−168 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.38 (s, 1 H, H-5), 7.63 (s, 1 H, H-4), 7.37 (d, *J* = 7.5 Hz, 6 H, 3H-2″,6″), 7.31 (t, *J* = 7.7 Hz, 6 H, 3H-3″,5″), 7.21 (t, *J* = 7.2 Hz, 3 H, 3H-4″), 6.97 (br s, 1 H, NH), 6.52 (d, *J* = 2.0 Hz, 2 H, H-2′,6′), 6.50 (t, *J* = 2.2 Hz, 1 H, H-4′), 6.31 (br s, 2 H, NH$_2$), 5.86 (br s, 1 H, H-8), 3.76 (s, 6 H, 2OCH$_3$); ^{13}C NMR δ 160.62 (s, 2 C, C-3′,5′), 158.10 (s, C-2), 156.95 (s, C-7), 151.33 (s, C-8a), 149.44 (d, C-5), 144.87 (s, 3 C, 3C-1″), 139.20 (s, C-1′), 135.48 (d, C-4), 128.63, 127.74 (2 d, 2 × 6 C, 3C-2″,3″,5″,6″), 126.53 (d, 3 C, 3C-4″), 121.56 (s, C-3), 113.61 (s, C-4a), 106.49 (d, 2 C, C-2′,6′), 99.73 (d, C-4′), 99.42 (br d, C-8), 70.15 (s, *C*(Ph)$_3$), 55.14 (q, 2 C, 2OCH$_3$). Anal. ($C_{35}H_{30}N_4O_2$·0.5H_2O) C, H, N.

Further elution of the column with 2−10% MeOH/CH_2Cl_2 gave crude **12** (28 mg) as an oil.

Method B: A solution of **12** (50 mg, 0.169 mmol) and Et_3N (0.5 mL, 3.59 mmol) in dry THF (5 mL) was treated with trityl chloride (301 mg, 1.08 mmol); then the mixture was sealed under N_2 (as above) and stirred at 50 °C for 20 h. Further Et_3N (1.0 mL, 7.19 mmol), trityl chloride (340 mg, 1.22 mmol) and dry THF (5 mL) were added; then the mixture was sealed under N_2 and stirred at 60 °C for 2 days. The resulting mixture was concentrated under reduced pressure (to ca. 1 mL) and then treated with ice/aqueous Na_2CO_3 (50 mL) and extracted with EtOAc (4 × 50 mL). The combined extracts were evaporated to dryness and the residue was then rapidly (total time ca. 40 min) flash chromatographed on silica gel. Elution with 0−0.5% MeOH/CH_2Cl_2 gave foreruns; then further elution with 1% MeOH/CH_2Cl_2 gave an oil (95 mg) which upon crystallization from CH_2Cl_2/hexane gave **28** (73 mg, 80%). The remaining liquors (22 mg) contained ditrityl derivative **29** (see below).

3-(3,5-Dimethoxyphenyl)-*N*^{2},*N*^{7}-ditrityl-1,6-naphthyridine-2,7-diamine (29) and 3-(3,5-Dimethoxyphenyl)-*N*^{2}-trityl-1,6-naphthyridine-2,7-diamine (30). The mother liquors from the crystallization of **28** above (method B) were combined with similar material from a repeat reaction (total 45 mg) and loaded onto a narrow column of silica gel (12 g) in CH_2Cl_2, then allowed to stand at 20 °C. After 1, 2 and 3 days the column was eluted with small amounts of CH_2Cl_2 (<10 mL); then, after 4 days, further elution with CH_2Cl_2 gave crude 3-(3,5-dimethoxyphenyl)-*N*^{2},*N*^{7}-ditrityl-1,6-naphthyridine-2,7-diamine (**29**) (4.5 mg) as an oil: ^{1}H NMR [$(CD_3)_2SO$] δ 8.27 (s, 1 H, H-5), 7.60 (s, 1 H, H-4), 7.4−7.1 (m, 30 H, 6H-2″,3″,4″,5″,6″), 6.97 (br s, 1 H, NH), 6.66 (d, *J* = 2.2 Hz, 2 H, H-2′,6′), 6.56 (t, *J* = 2.2 Hz, 1 H, H-4′), 6.43 (br s, 1 H, NH), 5.68 (br s, 1 H, H-8), 3.76 (s, 6 H, 2OCH$_3$); HRFABMS calcd for $C_{54}H_{45}N_4O_2$ *m*/*z* (MH$^+$) 781.3543, found 781.3547.

Further elution of the column with 1% MeOH/CH_2Cl_2 gave an oil (16 mg) which was further chromatographed on silica gel. Elution with 0−10% EtOAc/CH_2Cl_2 gave foreruns; then further elution with 10−15% EtOAc/CH_2Cl_2 gave 3-(3,5-dimethoxyphenyl)-*N*^{2}-trityl-1,6-naphthyridine-2,7-diamine (**30**) (6.5 mg): mp (DMSO/water) 115−118 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.38 (s, 1 H, H-5), 7.68 (s, 1 H, H-4), 7.26 (m, 12 H, 3H-2″,3″,5″,6″), 7.17 (tt, *J* = 6.8, 1.9 Hz, 3 H, 3H-4″), 6.73 (d, *J* = 2.1 Hz, 2 H, H-2′,6′), 6.60 (br s, 1 H, NH), 6.57 (t, *J* = 2.2 Hz, 1 H, H-4′), 5.84 (s, 1 H, H-8), 5.80 (br s, 2 H, NH$_2$), 3.77 (s, 6 H, 2OCH$_3$); ^{13}C NMR δ 161.03 (s, 2 C, C-3′,5′), 159.36 (s, C-7), 154.07 (s, C-2), 150.97 (s, C-8a), 150.31 (d, C-5), 145.13 (s, 3 C, 3C-1″), 139.07 (s, C-1′), 134.69 (d, C-4), 128.39, 127.44 (2 d, 2 × 6 C, 3C-2″,3″,5″,6″), 126.31 (d, 3 C, 3C-4″), 121.88 (s, C-3), 112.72 (s, C-4a), 106.55 (d, 2 C, C-2′,6′), 100.16 (d, C-4′), 96.58 (d, C-8), 70.44 (s, *C*(Ph)$_3$), 55.25 (q, 2 C, 2OCH$_3$); HRFABMS calcd for $C_{35}H_{31}N_4O_2$ *m*/*z* (MH$^+$) 539.2447, found 539.2460.

Hydrolysis of Formamide 27. A solution of **27** (12 mg, 21.2 μmol) in MeOH (4 mL) and CH_2Cl_2 (2 mL) was treated with NaOH (60 mg, 1.50 mmol) and water (0.5 mL); then the mixture was stirred at 40 °C for 2.5 h. A solution of aqueous $NaHCO_3$ (25 mL) was then added and the mixture extracted with EtOAc (4 × 20 mL). The combined extracts were evaporated and crystallized as above to give **28** (9 mg, 79%).

3-(3,5-Dimethoxyphenyl)-*N*^{2},*N*^{2}-bis(4-methoxybenzyl)-*N*^{7}-trityl-1,6-naphthyridine-2,7-diamine (31). A solution of **28** (144 mg, 0.268 mmol) in dry DMF (5 mL) was treated with 60% NaH (43 mg, 1.08 mmol); then the mixture was sealed under N_2 (as above) and stirred at 20 °C for 2 min. 4-Methoxybenzyl chloride (0.10 mL, 0.738 mmol) was added; then the mixture was stirred at 20 °C for 2 h. The resulting solution was cooled in ice, then treated with ice/aqueous $NaHCO_3$ to give a solid, which was isolated by filtration, washing with water and light petroleum. The filtrate was extracted with EtOAc (4 × 150 mL); then the extracts were combined with the solid above, evaporated to dryness and the residue was rapidly (total time ca. 5 min) flash chromatographed on silica gel. Elution with CH_2Cl_2 gave foreruns; then further elution with 1% MeOH/CH_2Cl_2 gave crude **31** (173 mg, 83%) as an oil, which was used directly. An analytical sample was obtained by crystallization: mp (MeOH/water) 136−140 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.47 (s, 1 H, H-5), 7.79 (s, 1 H, H-4),

7.38 (d, J = 7.6 Hz, 6 H, 3H-2‴,6‴), 7.31 (t, J = 7.6 Hz, 6 H, 3H-3‴,5‴), 7.22 (t, J = 7.1 Hz, 4 H, 3H-4‴, NH), 6.98 (d, J = 8.5 Hz, 4 H, 2H-2″,6″), 6.79 (d, J = 8.6 Hz, 4 H, 2H-3″,5″), 6.61 (d, J = 2.1 Hz, 2 H, H-2′,6′), 6.44 (t, J = 2.1 Hz, 1 H, H-4′), 6.18 (br s, 1 H, H-8), 4.13 (s, 4 H, $N(CH_2)_2$), 3.72, 3.71 (2 s, 2 × 6 H, $4OCH_3$); ^{13}C NMR δ 160.64 (s, 2 C, C-3′,5′), 159.47 (s, C-2), 158.13 (s, 2 C, 2C-4″), 157.37 (s, C-7), 149.85 (s, C-8a), 149.67 (d, C-5), 145.08 (s, 3 C, 3C-1‴), 142.07 (s, C-1′), 138.40 (d, C-4), 129.99 (s, 2 C, 2C-1″), 129.36 (d, 4 C, 2C-2″,6″), 128.67, 127.69 (2 d, 2 × 6 C, 3C-2‴,3‴,5‴,6‴), 126.42 (d, 3 C, 3C-4‴), 124.18 (s, C-3), 114.24 (s, C-4a), 113.50 (d, 4 C, 2C-3″,5″), 105.29 (d, 2 C, C-2′,6′), 100.13 (d, C-8), 99.38 (d, C-4′), 70.14 (s, $C(Ph)_3$), 55.13, 54.90 (2 q, 2 × 2 C, $4OCH_3$), 51.56 (t, 2 C, $N(CH_2)_2$). Anal. ($C_{51}H_{46}N_4O_4 \cdot 0.5H_2O$) C, H, N.

3-(3,5-Dimethoxyphenyl)-*N*^{2},*N*^{2}-bis(4-methoxybenzyl)-1,6-naphthyridine-2,7-diamine (32). Crude **31** (153 mg, 0.197 mmol) was loaded onto a narrow column of silica gel (12 g) in CH_2Cl_2 and allowed to stand at 20 °C. After 1 and 2 days, the column was eluted with small amounts of CH_2Cl_2 (<10 mL); then, after 3 days, elution with 10% MeOH/CH_2Cl_2 gave an oil (0.16 g) which was chromatographed on silica gel. Elution with 0−0.5% MeOH/CH_2Cl_2 gave foreruns; then further elution with 1% MeOH/CH_2Cl_2 gave recovered **31** (35 mg, 23%). Further elution with 1−1.5% MeOH/CH_2Cl_2 gave **32** (63 mg, 60%): mp (MeOH/water) 86−91 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.58 (s, 1 H, H-5), 7.87 (s, 1 H, H-4), 7.06 (dt, J = 8.6, 2.4 Hz, 4 H, 2H-2″,6″), 6.82 (dt, J = 8.7, 2.4 Hz, 4 H, 2H-3″,5″), 6.71 (d, J = 2.2 Hz, 2 H, H-2′,6′), 6.46 (t, J = 2.3 Hz, 1 H, H-4′), 6.41 (s, 1 H, H-8), 6.07 (br s, 2 H, NH_2), 4.22 (s, 4 H, $N(CH_2)_2$), 3.75, 3.70 (2 s, 2 × 6 H, $4OCH_3$); ^{13}C NMR δ 160.68 (s, 2 C, C-3′,5′), 159.84, 159.76 (2 s, C-2,7), 158.16 (s, 2 C, 2C-4″), 150.89 (d+s, C-5,8a), 142.24 (s, C-1′), 138.64 (d, C-4), 130.10 (s, 2 C, 2C-1″), 129.29 (d, 4 C, 2C-2″,6″), 123.32 (s, C-3), 113.86 (s, C-4a), 113.55 (d, 4 C, 2C-3″,5″), 105.24 (d, 2 C, C-2′,6′), 99.41 (d, C-4′), 96.50 (d, C-8), 55.14, 54.89 (2 q, 2 × 2 C, $4OCH_3$), 51.56 (t, 2 C, $N(CH_2)_2$); HRFABMS calcd for $C_{32}H_{33}N_4O_4$ m/z (MH^+) 537.2502, found 537.2486. Anal. ($C_{32}H_{32}N_4O_4 \cdot 0.5H_2O$) C, H, N.

Further elution of the column with 1.5% MeOH/CH_2Cl_2 gave crude 3-(3,5-dimethoxyphenyl)-*N*^{2}-(4-methoxybenzyl)-1,6-naphthyridine-2,7-diamine (**26**) (2.8 mg, 3%) as an oil (see below).

Further elution with 1.5−2.5% MeOH/CH_2Cl_2 gave a mixture; then elution with 10% MeOH/CH_2Cl_2 gave crude **12** (4.2 mg, 7%) as an oil.

Treatment of the recovered **31** (35 mg) on silica gel for 4 days, as above, followed by chromatography as above gave further **32** (9 mg, 9%).

3-(3,5-Dimethoxyphenyl)-*N*^{2}-(4-methoxybenzyl)-1,6-naphthyridine-2,7-diamine (26). Method A: A solution of **32** (1.3 mg, 2.43 μmol) in TFA (1 mL) was stirred at 70 °C for 8 h. The solvent was removed under a stream of dry N_2; then the residue was treated with aqueous Na_2CO_3 (25 mL) and extracted with EtOAc (3 × 30 mL). The extracts were evaporated to dryness and the residue was then purified by preparative silica gel TLC, developed first in 2% MeOH/CH_2Cl_2 and then in 1.3% MeOH/CH_2Cl_2. The major band was recovered and eluted with 7% MeOH/CH_2Cl_2 to give the crude product (0.8 mg), which was further purified by preparative silica gel TLC, developed in 90% EtOAc/light petroleum, to give **26** (0.6 mg, 59%) as an oil (see below).

Method B: A solution of **32** (54 mg, 0.101 mmol) in 99% HCO_2H (5 mL) was stirred at 20 °C for 20 h. The resulting solution was cooled in ice, then added slowly to a stirred mixture of ice and aqueous $NaHCO_3/Na_2CO_3$ (200 mL) and the resulting suspension was extracted with CH_2Cl_2 (4 × 100 mL). The extracts were evaporated to dryness and the residue was then chromatographed on silica gel. Elution with 0−1% MeOH/CH_2Cl_2 gave foreruns; then further elution with 1−1.5% MeOH/CH_2Cl_2 gave **26** (36 mg, 86%): mp (MeOH/water) 85−89.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.39 (s, 1 H, H-5), 7.61 (s, 1 H, H-4), 7.31 (d, J = 8.6 Hz, 2 H, H-2″,6″), 6.85 (d, J = 8.7 Hz, 2 H, H-3″,5″), 6.66 (br t, J = 6.1 Hz, 1 H, N*H*CH_2), 6.57 (d, J = 2.3 Hz, 2 H, H-2′,6′), 6.53 (t, J = 2.2 Hz, 1 H, H-4′), 6.32 (s, 1 H, H-8), 5.86 (br s, 2 H, NH_2), 4.55 (d, J = 5.6 Hz, 2 H, NHC*H*$_2$), 3.78 (s, 6 H, $2OCH_3$), 3.70 (s, 3 H, OCH_3); ^{13}C NMR δ 160.69 (s, 2 C, C-3′,5′), 159.64 (s, C-7), 157.90 (s, C-4″), 156.02 (s, C-2), 152.32 (s, C-8a), 150.07 (d, C-5), 139.00 (s, C-1′), 134.99 (d, C-4), 132.35 (s, C-1″), 128.72 (d, 2 C, C-2″,6″), 121.59 (s, C-3), 113.41 (d, 2 C, C-3″,5″), 112.92 (s, C-4a), 106.70 (d, 2 C, C-2′,6′), 99.82 (d, C-4′), 96.46 (d, C-8), 55.12 (q, 2 C, $2OCH_3$), 54.89 (q, OCH_3), 43.29 (t, $NHCH_2$); HRFABMS calcd for $C_{24}H_{25}N_4O_3$ m/z (MH^+) 417.1927, found 417.1923. Anal. ($C_{24}H_{24}N_4O_3$) C, H.

3-(3,5-Dimethoxyphenyl)-*N*^{2}-methyl-1,6-naphthyridine-2,7-diamine (33). A solution of **15** (50 mg, 0.127 mmol) in dry DMF (5 mL) was treated with 60% NaH (33 mg, 0.825 mmol); then the mixture was sealed under N_2 (as above) and stirred at 20 °C for 5 min. A solution of MeI (10 μL, 0.161 mmol) in dry DMF (1 mL, then 1 mL to rinse) was added (dropwise via syringe); then the mixture was stirred at 20 °C for 2.5 h. The resulting solution was cooled in ice, then treated with ice/aqueous $NaHCO_3$ (50 mL) and extracted with EtOAc (5 × 50 mL). The combined extracts were evaporated to dryness and the residue was then chromatographed on silica gel. Elution with 0−90% EtOAc/light petroleum gave minor mixtures; then further elution with 0−2.5% MeOH/EtOAc gave an oil (18 mg), which was further chromatographed on silica gel, eluting with 75% EtOAc/light petroleum and EtOAc, to give **33** (14 mg, 36%): mp (DMSO/water) 80−83 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 8.38 (s, 1 H, H-5), 7.57 (s, 1 H, H-4), 6.55 (d, J = 1.9 Hz, 2 H, H-2′,6′), 6.54 (t, J = 2.1 Hz, 1 H, H-4′), 6.36 (s, 1 H, H-8), 6.27 (br q, J = 4.5 Hz, 1 H, N*H*CH_3), 5.84 (br s, 2 H, NH_2), 3.79 (s, 6 H, $2OCH_3$), 2.87 (d, J = 4.5 Hz, 3 H, NHC*H*$_3$); ^{13}C NMR δ 160.65 (s, 2 C, C-3′,5′), 159.63 (s, C-7), 156.96 (s, C-2), 152.57 (s, C-8a), 150.01 (d, C-5), 139.07 (s, C-1′), 134.56 (d, C-4), 121.84 (s, C-3), 112.74 (s, C-4a), 106.80 (d, 2 C, C-2′,6′), 99.75 (d, C-4′), 96.53 (d, C-8), 55.15 (q, 2 C, $2OCH_3$), 28.33 (q, NCH_3); HREIMS calcd for $C_{17}H_{18}N_4O_2$ m/z (M^+) 310.1430, found 310.1425.

***N*-[2-Amino-3-(3,5-dimethoxyphenyl)-1,6-naphthyridin-7-yl]-2,2,2-trifluoroacetamide (18)**. A solution of **15** (27 mg, 0.068 mmol) in dry pyridine (3 mL) under N_2 was treated with a solution of trifluoroacetic anhydride (65 μL, 0.46 mmol) in pyridine (2 mL) under N_2; then the mixture was stirred at 20 °C for 16 h. The resulting solution was cooled in ice, then added slowly to a stirred mixture of ice and aqueous $NaHCO_3$. The resulting suspension was extracted with EtOAc (4 × 50 mL); then the combined extracts were evaporated to dryness and the residue chromatographed on silica gel. Elution with 25−33% EtOAc/light petroleum gave foreruns; then further elution with 33% EtOAc/light petroleum gave **18** (21 mg, 78%): mp (CH_2Cl_2/hexane) 221−222 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.97 (br s, 1 H, NH), 8.82 (s, 1 H, H-5), 7.98, 7.97 (2 s, 2 × 1 H, H-4,8), 6.80 (br s, 2 H, NH_2), 6.65 (d, J = 2.3 Hz, 2 H, H-2′,6′), 6.58 (t, J = 2.2 Hz, 1 H, H-4′), 3.81 (s, 6 H, $2OCH_3$); ^{13}C NMR δ 160.77 (s, 2 C, C-3′,5′), 159.12 (s, C-2), 155.01 (q, J_{C-F} = 39 Hz, C=O), 152.21 (s, C-7), 149.78 (d, C-5), 148.45 (s, C-8a), 138.53 (s, C-1′), 135.33 (d, C-4), 125.42 (s, C-3), 117.83 (s, C-4a), 115.65 (q, J_{C-F} = 289 Hz, CF_3), 107.63 (d, C-8), 106.53 (d, 2 C, C-2′,6′), 100.33 (d, C-4′), 55.25 (q, 2 C, $2OCH_3$). Anal. ($C_{18}H_{15}F_3N_4O_3$) C, H, N.

***N*-[2-[[(*tert*-Butylamino)carbonyl]amino]-3-(3,5-dimethoxyphenyl)-1,6-naphthyridin-7-yl]acetamide (17). Method A:** A solution of **15** (100 mg, 0.253 mmol) and Et_3N (0.15 mL, 1.08 mmol) in dry THF (5 mL) under N_2 was treated with AcCl (23 μL, 0.323 mmol); then the mixture was stirred at 20 °C for 3 days. Further Et_3N (0.5 mL, 3.59 mmol) and THF (7 mL) were added; then the resulting solution was cooled in ice and further AcCl (75 μL, 1.05 mmol) was added (dropwise); then the mixture was stirred at 20 °C for 5 days. The resulting mixture was then treated with ice/aqueous Na_2CO_3 (50 mL) and extracted with EtOAc (6 × 50 mL); then the combined extracts were evaporated to dryness to give an oil (0.16 g). A subsample (3.3 mg) was purified by preparative silica gel TLC (developed twice in 50% EtOAc/light petroleum) to give two components (each recovered by elution with 8% MeOH/CH_2Cl_2). The less polar compound was crude *N*-acetyl-*N*-[2-[[(*tert*-butylamino)carbonyl]amino]-3-(3,5-dimethoxyphen-

yl)-1,6-naphthyridin-7-yl]acetamide (**35**) (1.2 mg) as an oil: ^{1}H NMR [$(CD_3)_2SO$] δ 9.86 (br s, 1 H, NH), 9.19 (s, 1 H, H-5), 8.41 (s, 1 H, H-4), 7.81 (s, 1 H, H-8), 7.33 (br s, 1 H, NH), 6.72 (d, J = 2.2 Hz, 2 H, H-2′,6′), 6.70 (t, J = 2.2 Hz, 1 H, H-4′), 3.82 (s, 6 H, $2OCH_3$), 2.24 (s, 6 H, $2COCH_3$), 1.42 (s, 9 H, $C(CH_3)_3$).

The more polar component was *N*-[2-[[(*tert*-butylamino)-carbonyl]amino]-3-(3,5-dimethoxyphenyl)-1,6-naphthyridin-7-yl]acetamide (**17**) (1.4 mg) as an oil (see below).

The remaining product mixture (157 mg) in MeOH (45 mL) was treated with NaOH (0.20 g, 5.0 mmol) and water (5 mL, added dropwise); then the mixture was stirred at 20 °C for 30 min. The resulting solution was treated with excess aqueous $NaHCO_3$, concentrated under vacuum, then extracted with EtOAc (4 × 50 mL). The combined extracts were evaporated to dryness and the residue was then chromatographed on silica gel. Elution with CH_2Cl_2 gave foreruns; then elution with 1% MeOH/CH_2Cl_2 gave *N*-[2-[[(*tert*-butylamino)carbonyl]amino]-3-(3,5-dimethoxyphenyl)-1,6-naphthyridin-7-yl]acetamide (**17**) (80 mg, 72%): mp (CH_2Cl_2/hexane) 148−151 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.75 (br s, 1 H, NH), 10.13 (br s, 1 H, NH), 8.97 (s, 1 H, H-5), 8.33, 8.23 (2 s, 2 × 1 H, H-4,8), 7.21 (br s, 1 H, NH), 6.69 (d, J = 2.1 Hz, 2 H, H-2′,6′), 6.67 (t, J = 2.2 Hz, 1 H, H-4′), 3.82 (s, 6 H, $2OCH_3$), 2.16 (s, 3 H, $COCH_3$), 1.41 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR δ 169.43 (s, CONH), 161.16 (s, 2 C, C-3′,5′), 152.84, 151.75, 151.46 (3 s, CONH, C-2,7), 150.65 (d, C-5), 148.96 (s, C-8a), 136.99 (d, C-4), 136.77 (s, C-1′), 125.05 (s, C-3), 116.94 (s, C-4a), 107.08 (d, 2 C, C-2′,6′), 105.07 (d, C-8), 100.55 (d, C-4′), 55.43 (q, 2 C, $2OCH_3$), 50.06 (s, $C(CH_3)_3$), 28.57 (q, 3 C, $C(CH_3)_3$), 23.93 (q, CH_3). Anal. ($C_{23}H_{27}N_5O_4 \cdot 0.5H_2O$) C, H, N.

Method B: A solution of **15** (4.83 g, 12.2 mmol) in pyridine (100 mL) was treated (dropwise) with acetic anhydride (11.5 mL, 122 mmol); then the mixture was stirred at 20 °C for 1 day. The resulting solution was cooled in ice, then added slowly to a stirred mixture of ice and aqueous $NaHCO_3$, keeping the pH at 8 with excess $NaHCO_3$. The resulting suspension was extracted with CH_2Cl_2 (4 × 200 mL) and EtOAc (4 × 200 mL); then the combined extracts were evaporated to dryness and the residue crystallized directly (from warm CH_2Cl_2/light petroleum) to give **17** (4.91 g, 92%).

***N*-(*tert*-Butyl)-*N*′-[3-(3,5-dimethoxyphenyl)-7-[[3-(4-morpholinyl)propyl]amino]-1,6-naphthyridin-2-yl]urea (20). Method A:** A solution of **15** (50 mg, 0.127 mmol) in dry DMF (5 mL) was treated with 4-(3-chloropropyl)morpholine hydrochloride (30 mg, 0.15 mmol) and 60% NaH (32 mg, 0.80 mmol); then the mixture was sealed under N_2 (as above) and stirred at 20 °C for 2 days. Further 4-(3-chloropropyl)morpholine hydrochloride (192 mg, 0.96 mmol) and 60% NaH (100 mg, 2.5 mmol) were added and the mixture sealed under N_2 and stirred at 20 °C for 5 days. The resulting solution was cooled in ice, then treated with ice/aqueous $NaHCO_3$ (50 mL) and extracted with EtOAc (5 × 50 mL). The combined extracts were evaporated to dryness and the residue was then chromatographed on silica gel. Elution with 0.5% MeOH/CH_2Cl_2 gave recovered **15** (24 mg, 48%). Further elution with 3−10% MeOH/CH_2Cl_2 gave a crude oil (33 mg), which was further chromatographed on silica gel. Elution with EtOAc gave foreruns; then further elution with 2.5% MeOH/EtOAc gave an oil (2.2 mg), which was further purified by preparative silica gel TLC, developed in 0.75% MeOH/EtOAc. Elution of the major band with 10% MeOH/CH_2Cl_2 gave *N*-[7-amino-3-(3,5-dimethoxyphenyl)-1-[3-(4-morpholinyl)propyl]-1,6-naphthyridin-2(1*H*)-ylidene]-*N*′-*tert*-butylurea (**34**) (1 mg, 1.5%) as an oil: ^{1}H NMR [$(CD_3)_2SO$] δ 11.34 (br s, 1 H, NH), 8.57 (s, 1 H, H-5), 7.90 (s, 1 H, H-4), 6.71 (d, J = 2.4 Hz, 2 H, H-2′,6′), 6.45 (t, J = 2.3 Hz, 1 H, H-4′), 6.41 (s, 1 H, H-8), 6.09 (br s, 2 H, NH_2), 4.18 (t, J = 6.6 Hz, 2 H, NCH_2), 3.76 (s, 6 H, $2OCH_3$), 3.53 (t, J = 4.6 Hz, 4 H, $O(CH_2)_2$), 2.28 (m, 4 H, $N(CH_2)_2$), 2.26 (t, J = 7.1 Hz, 2 H, NCH_2), 1.72 (pentet, J = 6.9 Hz, 2 H, CH_2), 1.42 (s, 9 H, $C(CH_3)_3$); HRFABMS calcd for $C_{28}H_{39}N_6O_4$ m/z (MH^+) 523.3033, found 523.3022.

Further elution of the second column above with 5% MeOH/EtOAc gave material which was treated with aqueous Na_2CO_3 (50 mL) and extracted with CH_2Cl_2 (4 × 50 mL) to give *N*-(*tert*-butyl)-*N*′-[3-(3,5-dimethoxyphenyl)-7-[[3-(4-morpholinyl)-propyl]amino]-1,6-naphthyridin-2-yl]urea (**20**) (6.4 mg, 10%) as an oil (see below).

Method B: (1) A solution of **17** (63 mg, 0.144 mmol) in dry DMF (5 mL) was treated with 4-(3-chloropropyl)morpholine hydrochloride (64 mg, 0.32 mmol) and 60% NaH (85 mg, 2.13 mmol); then the mixture was sealed under N_2 (as above) and stirred at 20 °C for 5 min and then at 52 °C for 26 h. The resulting solution was cooled in ice, then treated with ice/aqueous $NaHCO_3$ (50 mL), and extracted with EtOAc (5 × 50 mL). The combined extracts were evaporated to dryness and the residue was then chromatographed on silica gel. Elution with 0−2.5% MeOH/CH_2Cl_2 gave foreruns; then further elution with 4−6% MeOH/CH_2Cl_2 gave an oil (55 mg) (a mixture of **20** and **36**). This oil was dissolved in MeOH (18 mL), cooled to 0 °C and treated with NaOH (0.76 g, 19.0 mmol) and water (2 mL, added dropwise); then the mixture was stirred at 0 °C for 1 h, and then at 20 °C for 43 h. The resulting solution was treated with excess $NaHCO_3$ in ice−water (100 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The combined extracts were evaporated to dryness; then crystallization of the residue from CH_2Cl_2/hexane gave **20** (31 mg, 41%): mp (CH_2Cl_2/hexane) 120−121.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.21 (br s, 1 H, NH), 8.69 (s, 1 H, H-5), 7.98 (s, 1 H, H-4), 7.03 (br s, 1 H, NH), 6.93 (br t, J = 5.6 Hz, 1 H, N*H*CH_2), 6.63 (t, J = 2.1 Hz, 1 H, H-4′), 6.62 (d, J = 1.8 Hz, 2 H, H-2′,6′), 6.39 (s, 1 H, H-8), 3.80 (s, 6 H, $2OCH_3$), 3.58 (t, J = 4.6 Hz, 4 H, $O(CH_2)_2$), 3.32 (m, 2 H, $NHCH_2$), 2.38 (t, J = 7.1 Hz, 2 H, NCH_2), 2.36 (m, 4 H, $N(CH_2)_2$), 1.73 (pentet, J = 7.0 Hz, 2 H, CH_2), 1.40 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR δ 161.11 (s, 2 C, C-3′,5′), 159.54 (s, C-7), 152.38, 152.00 (2 s, CONH, C-2), 151.35 (d, C-5), 149.34 (s, C-8a), 137.43 (s+d, 2 C, C-4,1′), 121.06 (s, C-3), 113.11 (s, C-4a), 107.07 (d, 2 C, C-2′,6′), 100.17 (d, C-4′), 94.88 (br d, C-8), 66.15 (t, 2 C, $O(CH_2)_2$), 55.99 (t, NCH_2), 55.39 (q, 2 C, $2OCH_3$), 53.33 (t, 2 C, $N(CH_2)_2$), 49.92 (s, $C(CH_3)_3$), 39.57 (t, NCH_2), 28.64 (q, 3 C, $C(CH_3)_3$), 25.68 (t, CH_2). Anal. ($C_{28}H_{38}N_6O_4$) C, H, N.

The mother liquors were further purified by chromatography on silica gel. Elution with 0−2% MeOH/CH_2Cl_2 gave foreruns; then further elution with 3% MeOH/CH_2Cl_2 gave material which was treated with aqueous Na_2CO_3 (50 mL) and extracted with CH_2Cl_2 (5 × 50 mL) to give further **20** (17 mg, 23%).

(2) A solution of **17** (126 mg, 0.288 mmol) in dry DMF (10 mL) was treated with 4-(3-chloropropyl)morpholine hydrochloride (133 mg, 0.665 mmol) and 60% NaH (173 mg, 4.33 mmol); then the mixture was sealed under N_2 (as above) and stirred at 20 °C for 5 min and then at 54 °C for 25 h. The resulting solution was cooled in ice, then treated with ice/aqueous $NaHCO_3$ (100 mL), and extracted with EtOAc (5 × 100 mL). The combined extracts were evaporated to dryness and the residue was then chromatographed on silica gel. Elution with 0−3% MeOH/CH_2Cl_2 gave foreruns; then further elution with 4% MeOH/CH_2Cl_2 gave material which was treated with aqueous Na_2CO_3 (50 mL) and extracted with CH_2Cl_2 (4 × 50 mL). The combined extracts were evaporated to dryness; then crystallization of the residue from CH_2Cl_2/hexane gave **20** (24 mg, 16%).

Further elution with 5−6% MeOH/CH_2Cl_2 gave *N*-[7-[bis-[3-(4-morpholinyl)propyl]amino]-3-(3,5-dimethoxyphenyl)-1,6-naphthyridin-2-yl]-*N*′-*tert*-butylurea (**37**) (26 mg, 14%) as an oil: ^{1}H NMR [$(CD_3)_2SO$] δ 10.21 (br s, 1 H, NH), 8.75 (s, 1 H, H-5), 8.00 (s, 1 H, H-4), 7.06 (br s, 1 H, NH), 6.64 (t, J = 2.1 Hz, 1 H, H-4′), 6.62 (d, J = 2.1 Hz, 2 H, H-2′,6′), 6.43 (s, 1 H, H-8), 3.81 (s, 6 H, $2OCH_3$), 3.59 (t, J = 4.4 Hz, 8 H, $2O(CH_2)_2$), 3.59 (m, 4 H, $N(CH_2)_2$), 2.34 (m, 8 H, $2N(CH_2)_2$), 2.32 (t, J = 7.1 Hz, 4 H, $2NCH_2$), 1.76 (pentet, J = 7.0 Hz, 4 H, $2CH_2$), 1.40 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR δ 161.12 (s, 2 C, C-3′,5′), 157.80 (s, C-7), 152.42, 151.99 (2 s, CONH, C-2), 150.95 (d, C-5), 149.54 (s, C-8a), 137.37 (s, C-1′), 137.25 (d, C-4), 121.29 (s, C-3), 112.55 (s, C-4a), 107.05 (d, 2 C, C-2′,6′), 100.13 (d, C-4′), 94.16 (d, C-8), 66.14 (t, 4 C, $2O(CH_2)_2$), 55.55 (t, 2 C, $2NCH_2$), 55.38 (q, 2 C, $2OCH_3$), 53.28 (t, 4 C, $2N(CH_2)_2$), 49.93 (s, $C(CH_3)_3$), 46.35 (t, 2 C, $N(CH_2)_2$), 28.60 (q, 3 C, $C(CH_3)_3$), 23.90

(t, 2 C, $2CH_2$); HRFABMS calcd for $C_{35}H_{52}N_7O_5$ *m/z* (MH^+) 650.4030, found 650.4036.

HUVEC, C6, and A90 Cellular Proliferation Assays. Tissue culture plates (96 well) were seeded with 100 μL of cells in rows A−G, with row H remaining empty as a blank. HUVECs (Clonetics) were grown in EGM media (Clonetics) containing 2% fetal bovine serum. The cell seed density for HUVECs was 20 000/mL. C6 cells (ATCC) were seeded at 6000/mL in F10 medium supplemented with 15% horse serum, 2.5% fetal bovine serum, and 6.0 mL of 200 mM glutamine per 600 mL of medium. A90 cells (Dr. Kent Crickard, SUNY/AB Medical School) were also seeded at 6000/mL but grown in RPMI1640 plus 10% fetal bovine serum. Unless noted otherwise, tissue culture media and components were from GIBCO. Cells were allowed to incubate at 37 °C, 5% CO_2, and 100% relative humidity for 16−24 h.

Stock 5 mM solutions of compounds in DMSO were diluted to 50 μM in EGM medium and serially diluted in duplicate wells of the previously prepared cell plates, which were then incubated as above for an additional 4 days. Media were then removed and the cells were fixed using 10% trichloroacetic acid for 30 min at 4 °C. The plates were then washed with distilled water (5×), and the wells were treated with sulforhodamine B (100 μL of 0.75% in 1% AcOH). Following staining, excess stain was removed, the plates were washed with 1% AcOH (4×) and air-dried, and bound dye was solubilized with unbuffered TRIS base (100 μL of 10 mM per well). Absorbance was measured on a 96-well plate reader at 540 nm, using a reference filter wavelength of 630 nm. The concentration of compound needed to suppress 50% of cell proliferation (IC_{50}) was determined from the absorbance measurements.

HUVEC Microcapillary Assay. Matrigel (Becton Dickenson) was used to coat 24-well cluster plates (Costar) (0.3 mL/well). After polymerization of the Matrigel at 37 °C for 3 h, the compounds were added in 0.5 mL of 10% EGM media (Clonetics) at a 2× concentration. The HUVECs (Clonetics; 10^5-cells/mL) were then added suspended in 0.5 mL of 10% EGM media. After 18 h in a 37 °C 5% CO_2 incubator, 200 μL of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, thiazolyblue) was added, and incubation continued for 1 h at 37 °C. Images were captured with Image Analysis, and IC_{50} values were determined by minimum dose effect.

HUVEC Invasion Assay. Polycarbonate filters (Costar; 8-μm pore size) were coated with Matrigel (10 μg/insert; Beckton Dickenson) and placed in a culture hood to dry for 18−20 h. The inserts were rehydrated with serum-free EBM media (Clonetics) for 2 h at room temperature. HUVECs were harvested and washed twice with serum-free EBM media containing 0.2% heat-inactivated fetal bovine serum and adjusted to 3×10^5 cells/mL. Cells (100 μL) were added to the top of each insert; then 10% EGM media (0.5 mL) was added as attractant to the bottom of each well. Compounds were made up as a 200× stock solution in DMSO and added to the cells and the attractant. The plates were then placed in a 37 °C 5% CO_2 incubator for 18−20 h. After incubation, the top of each insert was wiped with a cotton swab and the bottom of each well was aspirated, then rinsed once with Hank's balanced salt solution. Calcein (Am Molecular Probes; 25 μM) was added to the bottom of each well and the plates were then incubated in the dark for 45 min. The number of cells that invaded the other side of the insert was counted using a fluorescence microscope and image analysis.

In Vivo Chemotherapy. Mice were housed in microisolator cages within a barrier facility on a 12-h light/dark cycle and received food and water ad libitum. Animal housing was in accord with AAALAC guidelines. All experimental protocols involving animals were approved by the institutional animal care and use committee. Tumors were maintained and anticancer efficacy determined in the inbred strain of tumor origin: C3H for mammary adenocarcinoma 16/c, C57BL/6 for Lewis lung carcinoma, and M5076 reticulum cell sarcoma.

In each experiment for anticancer activity evaluation, test mice weighing 18−22 g were randomized and implanted with tumor fragments in the region of the right axilla on day 0. Animals were treated orally with test compound **17** on the basis of average cage weight (6 mice/dose group). Treatment was initiated on the day indicated in Table 5 and was continued for 9 consecutive days. Compound **17** was suspended in 0.5% methylcellulose in water due to its low aqueous solubility, with dosing suspensions being prepared for 5 days at a time. Host body weight change data are reported as the maximum treatment related weight loss in these studies. Calculation of tumor growth inhibition (% T/C) and tumor growth delay (T−C) was performed as described previously.[37−39]

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