Soluble 2-Substituted Aminopyrido[2,3-*d*]pyrimidin-7-yl Ureas. Structure−Activity Relationships against Selected Tyrosine Kinases and Exploration of in Vitro and in Vivo Anticancer Activity

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In continuing our search for medicinal agents to treat proliferative diseases, we have discovered 2-substituted aminopyrido[2,3-*d*]pyrimidin-7-yl ureas as a novel class of soluble, potent, broadly active tyrosine kinase (TK) inhibitors. An efficient route was developed that enabled the synthesis of a wide variety of analogues with substitution on several positions of the template. From the lead structure **1**, several series of analogues were made that examined the C-6 aryl substituent, a variety of water solublizing substitutents at the C-2 position, and urea or other acyl functionality at the N-7 position. Compounds of this series were competitive with ATP and displayed submicromolar to low nanomolar potency against a panel of TKs, including receptor (platelet-derived growth factor, PDGFr; fibroblast growth factor, FGFr;) and nonreceptor (c-Src) classes. Several of the most potent compounds displayed submicromolar inhibition of PDGF-mediated receptor autophosphorylation in rat aortic vascular smooth muscle cells and low micromolar inhibition of cellular growth in five human tumor cell lines. One of the more thoroughly evaluated members, **32**, with IC_{50} values of 0.21 μM (PDGFr), 0.049 μM (bFGFr), and 0.018 μM (c-Src), was evaluated in in vivo studies against a panel of five human tumor xenografts, with known and/or inferred dependence on the EGFr, PDGFr, and c-Src TKs. Compound **32** produced a tumor growth delay of 14 days against the Colo-205 colon xenograft model.

Introduction

Signaling for a large number of cellular functions, including cell growth, differentiation, and migration,[1] is modulated by a variety of transmembrane growth factor receptor and cytoplasmic protein tyrosine kinases (TKs) that catalyze the specific phosphorylation of tyrosine residues on proteins.[2] Abnormal signaling via tyrosine kinases has been linked to a number of pathophysiological states including cancer, cardiovascular, and immunoinflammatory diseases. Since distinct TKs are implicated in such diverse conditions as angiogenesis,[3] restenosis,[4] atherosclerosis,[5] and tumor growth,[6] selective TK inhibitors are deemed highly desirable. Despite the large number of TKs that have been described and the structural homology between the TKs, especially in their ATP-binding regions,[7] several selective inhibitors have been identified including those of the EGFr,[8] FGFr,[9] PDGFr,[10] VEGFr,[11] and more recently c-Src TKs.[12]

While selective TK inhibitors should be less likely to affect normal cells and thus produce fewer side effects, broadly acting, nonselective inhibitors may be required to overcome redundancies in growth signaling pathways in order to arrest aggressively proliferating cells. Therefore, given the complex nature of signal transduction, i.e., redundancies and crosstalk between signaling pathways, absolute selectivity may not be desirable when the need arises to simultaneously inhibit multiple growth signals. Within this latter context, we have reported on a strategy for uncovering broadly acting, nonselective small molecule inhibitors of the PDGFr, FGFr, and c-Src TKs, which might serve to overcome these redundancies in growth signaling. Our initial efforts led us to the development of a class of ATP-competitive 6-arylpyrido[2,3-*d*]pyrimidine ureas that are active against a range of TKs, including EGFr, PDGFr, FGFr, and c-Src. We profiled the initial screening lead in this area, urea compound **1** (Figure 1), which possesses low micromolar potency toward inhibition of PDGFr, FGFr, and c-Src TKs.[13] Subsequent SAR studies led to the synthesis of analogues with improved potency, solubility, and bioavailability relative to the initial lead.[14] More recently, we have developed an extensive SAR around the related mass screen hit, 2-amino-6-(2,6-dichlorophenyl)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (**2**),[15] which shows a similar profile toward the above kinases, and have disclosed related work within the naphthyridine congeners **3**[16] and **4**,[17] respectively, of both series.

In this paper, we extend our earlier work within the urea series[14] and present the synthesis and structure−activity relationships (SAR) toward several TKs for

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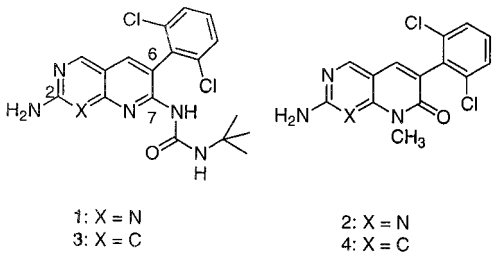


Figure 1.

analogues of **1** in which a wide range of solubilizing alkyl substituents have been introduced at the C-2 nitrogen in combination with selected variations on the urea moiety at the C-7 position. We also report on cellular effects and in vivo anticancer activity for selected compounds drawn from this series.[18]

Chemistry

Most of the compounds reported in this paper were prepared via the generalized route outlined in Scheme 1.[14,19] Aldehyde **5** was condensed with an arylacetonitrile under basic conditions to give the 2,7-diaminopyridopyrimidine, **6** (method A). Treating the diamine with 1 equiv of NaH followed by acylation with 1 equiv of an isocyanate gave mostly urea **7** with traces of bis-2,7-urea byproduct (method B). Alternatively, the N-2 amine of **6** could first be selectively displaced under acidic conditions with refluxing amine side chain as the solvent (method C). The resulting pyridopyrimidine, **8**, was treated with 1 equiv of NaH followed by quenching with 1 equiv of isocyante as described for method B above to give urea **9**. In cases where the isocyanate was unavailable, the urea was formed by refluxing **8** with 1,1′-carbonyldiimidazole in dry dioxane, followed by quenching with the desired amine (method D; **50**, **58** and **59**), or simply from reaction of the anion of **8** with an appropriate carbamoyl chloride (method E; **60**). While method C is extremely simple and efficient for introducing simple dialkyl aminoalkyl side chains at the N-2 position, it suffers from the disadvantage that the alkylamine must be used in large excess. The amine displacement also must be carried out at elevated temperatures under acidic conditions, which could pose problems for sensitive substrates or products. An alternate pathway that utilizes a methyl sulfoxide as the leaving group at C-2 has been developed and will be disclosed in future publications on compounds from this series.

In addition to urea moieties, we also incorporated amide, thiourea, guanidine, amidine, and sulfone functionality at N-7 for comparative purposes, as shown in Schemes 1 and 2. Preparation of amide **77**, by treating the anion of **8** with *tert*-butyl acetyl chloride (method E, Scheme 1), gave product in low yield (14%). In contrast, simply refluxing a suspension of **10** and the acyl chloride in dry dioxane gave the amides **71**, **76**, and **78** in good yield (58−64%; method F, Scheme 2). The amidine, **72**, was prepared by refluxing a solution of **10** and DMF dimethyl acetal in DMF (method G, Scheme 2), while thioureas **73**, **75**, **82**, and **83** were prepared in a manner similar to their oxygen counterparts by anion formation followed by quenching with the desired isothiocyanate (method H, Scheme 2).

Preparation of the disubstituted guanidine, **74**, with *N*,*N*′-bis(BOC)-*N*-ethyl-*S*-ethylisothiourea[20] gave the product, although in poor yield. Recently we reported that trisubstituted guanidines can be prepared via amine anion additions to disubstituted carbodiimides. Further, if one of the substituents of the carbodiimide is trityl, it can be removed under acidic conditions to give unsymmetrical disubstitued guanidines.[21] Using these conditions, the guanidines **80**[21] and **81** were prepared in good yields (66% and 81%, respectively; method I, Scheme 2). Last, the sulfonamide **79** was prepared in a manner similar to the amides by refluxing a suspension of **10** and phenylsulfonyl chloride in dioxane (method J, Scheme 2).

SAR vs Isolated Tyrosine Kinases

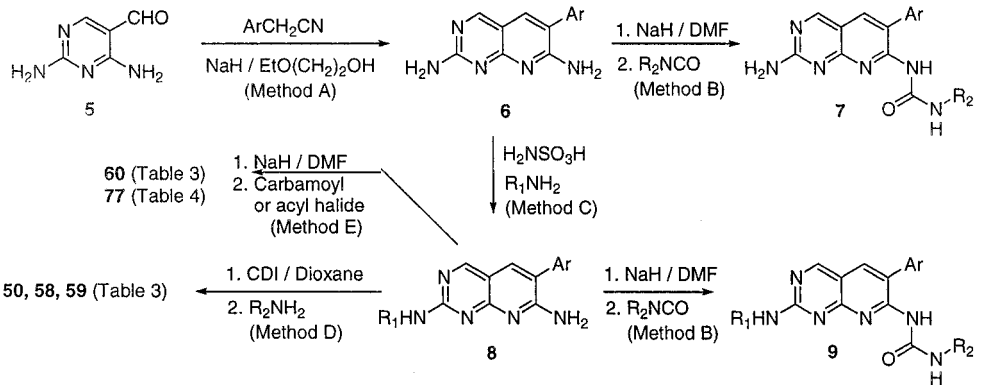
The structures of the 6-arylpyrido[2,3-*d*]pyrimidines (**1** and **11**−**83**) synthesized for this study are shown in Tables 1−4. They were evaluated for their ability to prevent phosphorylation of a synthetic glutamate-tyrosine polymer by isolated mouse PDGF-β, human FGF-1, and EGF receptor TKs, as well as the avian c-Src TK. At least two dose−response curves were determined for each compound, and averaged IC_{50} values are listed.

As reported earlier, the initial SAR of **1**, wherein variation about the C-6 aryl and N-7 urea moieties was explored, showed that compounds with the 2,6-dichlorophenyl substituent (e.g., compound **1**, Table 1) at C-6 were the most potent inhibitors against a range of tested TKs while the 3,5-dimethoxyphenyl (e.g., compound **23**, Table 1) and 2,3,5,6-tetramethylphenyl (e.g., compound **25**, Table 1) substituents were found to impart FGFr selectivity.[14] Unfortunately, the poor solubility of **1** and its analogues precluded the possibility of further in vivo studies. Three positions of the template, namely the C-6 aryl, the N-7 urea, and the N-2 amino substituents, offered potential sites for structural changes to increase potency and solubility. In light of our previous studies, we decided to complete an initial survey around the C-6 position of **1** toward determining its effect on TK selectivity and then hold the optimal substituent constant while focusing on developing the SAR of the N-7 urea and N-2 amine positions.

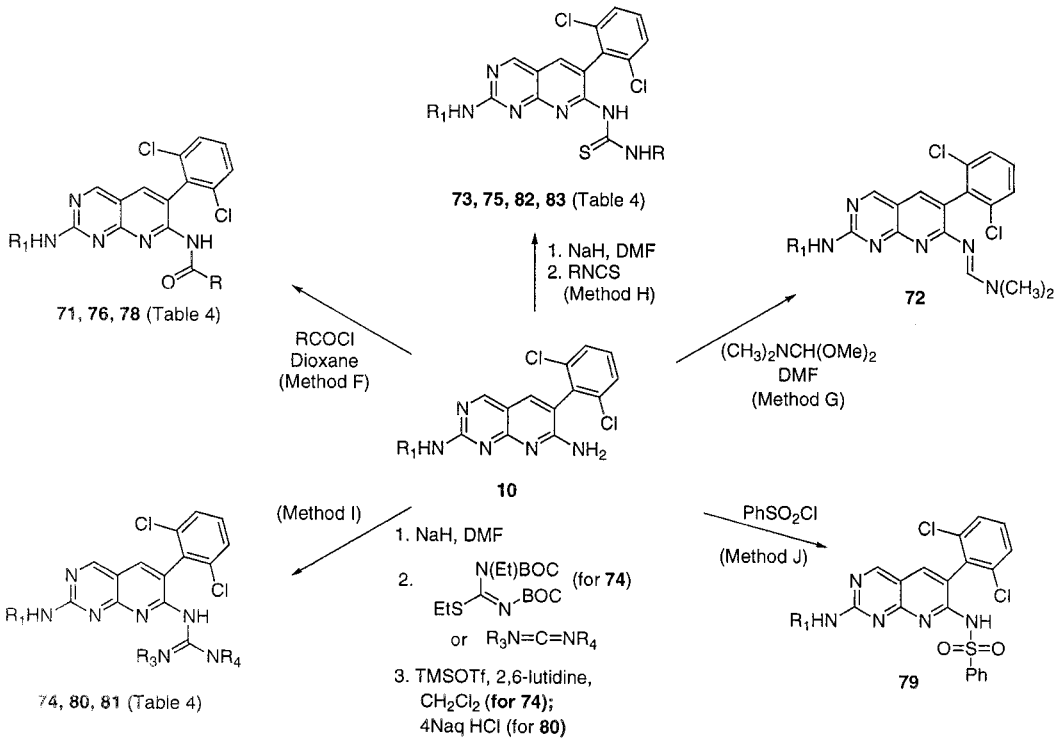
The initial SAR survey for compounds of this class, built upon the lead structure **1**, is shown in Table 1. Initially we examined the attributes of the *tert*-butyl urea at the N-7 position. The diamine, **11**, which shows poor aqueous solubility, is 10- and 20-fold less potent toward PDGFr and FGFr TK, respectively, than its urea congener **1**. Similar to **1**, the nonselective TK inhibitors **14** and **18** showed dramatic decreases in PDGFr and FGFr activity and moderate decreases in c-Src relative to their 7-ureas, **15** and **19**, respectively, while the FGFr selective inhibitors showed a substantial decrease in FGFr potency with removal of the *tert*-butyl urea (**23** vs **22** and **25** vs **24**). The necessity of the urea functionality for good enzyme potency was independent of the C-6 aryl substituent.

Recently the crystal structure of PD 173074, an FGFr selective inhibitor within the same series of ureas, was determined in complex with the tyrosine kinase domain of FGFr1.[22] Two significant features surrounding the urea functionality were observed. The first is that the hydrogen on the terminal nitrogen of the N-7 urea forms

Scheme 1



Scheme 2



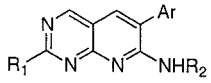
a six-membered ring by intramolecular hydrogen bonding to N-8 of the pyridopyrimidine template. Second, the carbonyl of the urea is bound to a water molecule. In the case of PD 173074, this bound water in turn is hydrogen bonded to Lys514 and Asp641, conserved residues for this kinase. These two aspects of the N-7 urea correlated well with small molecule crystal structures of other molecules within this series[14,23] as well as a previously reported binding model wherein the carbonyl oxygen of the urea forms an electrostatic hydrogen bond to Lys295 of c-Src.[23]

To improve the solubility of the 2,7-diamino series of compounds, we decided to install basic aliphatic side chains at C-2 of the pyridopyrimidine template. As was the case for pyridopyrimidinones, **2**,[15] the introduction of a single distal basic functionality on an aliphatic chain improved aqueous solubility (vide infra) but did not impart greater potency toward the TKs evaluated (**11** vs **12**, **14** vs **16**, and **18** vs **20**). However, reintroduction of the essential *tert*-butyl urea onto the N-7 amine (**12** vs **13**, **16** vs **17**, and **20** vs **21)** increased potency against all three kinases to a level similar to that of **1**, **15**, and **19**, respectively, and also provided for an increase in aqueous solubility. In summary, the SAR shown in Table 1 revealed the importance of the *tert*-butyl urea at N-7 for maintaining good tyrosine kinase potency. In addition, the data showed that it was indeed possible to introduce substituents at the C-2 position to increase aqueous solubility, while at the same time maintaining good enzyme activity. Out of those substituents examined, the data revealed that for nonselective inhibitors the 2,6-dichlorophenyl was optimal for the 6-position.

Having established the essential requirement of N-7 *tert*-butyl urea substitution, we next synthesized a series of analogues where the C-2 position was substituted with a number of aliphatic amine side chains to explore the SAR at this position. Within this series of compounds, an initial survey of smaller aliphatic ureas, similar to the *tert*-butyl urea of **1,** and one aromatic urea was carried out. These compounds are listed in Table 2.

Analogues incorporating ten side chains with the potential for increasing aqueous solubility were pre-

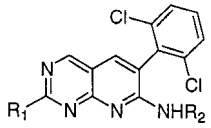
Table 1. Initial SAR Survey around the N-2, C-6, and N-7 Positions[a]



no.	Ar	R_1	R_2	method	IC_{50}, μM PDGFr	FGFr	c-Src
1	2,6-$(Cl)_2$Ph	$-NH_2$	*t*-BuNHCO	ref 14	1.2	0.14	0.22
11	2,6-$(Cl)_2$Ph	$-NH_2$	H	ref 14	16	3.0	0.21
12	2,6-$(Cl)_2$Ph	$-NH(CH_2)_3NEt_2$	H	ref 14	46	2.4	0.75
13	2,6-$(Cl)_2$Ph	$-NH(CH_2)_3NEt_2$	*t*-BuNHCO	ref 14	0.66	0.082	0.073
14	2,6-$(Me)_2$Ph	$-NH_2$	H	ref 14	29	13	0.43
15	2,6-$(Me)_2$Ph	$-NH_2$	*t*-BuNHCO	ref 14	0.34	0.40	0.11
16	2,6-$(Me)_2$Ph	$-NH(CH_2)_3NEt_2$	H	C	25	18	0.45
17	2,6-$(Me)_2$Ph	$-NH(CH_2)_3NEt_2$	*t*-BuNHCO	B	0.80	0.34	0.098
18	2,6-$(Br)_2$Ph	$-NH_2$	H	A	>50	13	1.6
19	2,6-$(Br)_2$Ph	$-NH_2$	*t*-BuNHCO	B	1.4	0.29	0.21
20	2,6-$(Br)_2$Ph	$-NH(CH_2)_3NEt_2$	H	C	>50	8.3	0.76
21	2,6-$(Br)_2$Ph	$-NH(CH_2)_3NEt_2$	*t*-BuNHCO	B	1.1	0.19	0.097
22	2,3,5,6-$(Me)_4$Ph	$-NH_2$	H	ref 14	>50	>50	>50[b]
23	2,3,5,6-$(Me)_4$Ph	$-NH_2$	*t*-BuNHCO	ref 14	>50	0.71	>50[b]
24	3,5-$(MeO)_2$Ph	$-NH_2$	H	ref 14	>50	0.23	>50
25	3,5-$(MeO)_2$Ph	$-NH_2$	*t*-BuNHCO	ref 14	>50	0.048	>50

[a] IC_{50} values reported for kinase inhibition represent the means of at least two separate determinations done in triplicate with typical variations of less than 30% between replicate values. [b] IC_{50} values represent a single determination.

Table 2. Further Exploration around the C-2 Amine and C-7 Urea Positions[a]



no.	R_1	R_2	method	IC_{50}, μM PDGFr	FGFr	c-Src
13	$-NH(CH_2)_3NEt_2$	*t*-BuNHCO	ref 14	0.66	0.082	0.073
26	$-NH(CH_2)_2NEt_2$	H	C	nt[b]	nt	nt
27	$-NH(CH_2)_2NEt_2$	EtNHCO	B	12	1.3	2.6
28	$-NH(CH_2)_2NEt_2$	*t*-BuNHCO	B	9.0	1.8	4.1
29	$-NH(CH_2)_3NEt_2$	EtNHCO	B	1.3	0.13	0.094
30	$-NH(CH_2)_3NEt_2$	*i*-PrNHCO	B	1.1	0.077	0.078
31	$-NH(CH_2)_4NEt_2$	H	ref 14	nt	nt	nt
32	$-NH(CH_2)_4NEt_2$	EtNHCO	B	0.21	0.049	0.018
33	$-NH(CH_2)_4NEt_2$	*t*-BuNHCO	ref 14	0.36	0.048	0.0074
34	$-NH(CH_2)_4NEt_2$	cyclohexylNHCO	B	0.33	0.043	0.012
35	$-NH(CH_2)_4NEt_2$	PhNHCO	B	0.45	0.11	0.0075
36	$-NH(CH_2)_3NMe_2$	H	C	nt	nt	nt
37	$-NH(CH_2)_3NMe_2$	*t*-BuNHCO	B	0.68	0.075	0.12
38	$-NMe(CH_2)_3NMe_2$	H	C	nt	nt	nt
39	$-NMe(CH_2)_3NMe_2$	*t*-BuNHCO	B	16	2.0	1.1
40	$-NHCH_2CMe_2CH_2NMe_2$	H	C	nt	nt	nt
41	$-NHCH_2CMe_2CH_2NMe_2$	*t*-BuNHCO	B	3.2	0.21	3.5
42	$-NH(CH_2)_3$(morpholin-1-yl)	H	C	nt	nt	nt
43	$-NH(CH_2)_3$(morpholin-1-yl)	*t*-BuNHCO	B	0.84	0.072	0.10
44	$-NH(CH_2)_3$(2-methylpiperidin-1-yl)	H	C	nt	nt	nt
45	$-NH(CH_2)_3$(2-methylpiperidin-1-yl)	*t*-BuNHCO	B	0.73	0.060	0.016
46	$-NH(CH_2)_3$(*N*-methylpiperazin-1-yl)	H	ref 14	9.6	0.45	0.18
47	$-NH(CH_2)_3$(*N*-methylpiperazin-1-yl)	*t*-BuNHCO	ref 14	0.47	0.051	0.032
48	$-NH(CH_2)_4$(*N*-methylpiperazin-1-yl)	H	C	nt	nt	nt
49	$-NH(CH_2)_4$(*N*-methylpiperazin-1-yl)	*t*-BuNHCO	B	0.28	0.035	0.010

[a] IC_{50} values reported for kinase inhibition represent the means of at least two separate determinations done in triplicate with typical variations of less than 30% between replicate values. [b] nt = not tested.

pared. With regard to the effect of side chain length, for a given urea, shortening the alkylamino chain length by one carbon (see **13** vs **28**; **29** vs **27**) resulted in a dramatic decrease in inhibition against all three TKs. In contrast, extension of the alkylamino side chain by one carbon resulted in a 2−10-fold increase in inhibition of PDGFr, FGFr, and c-Src kinases (see **13** vs **33**; **29** vs **32**; **47** vs **49**).

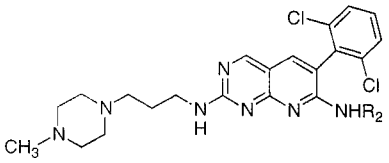
Having established the effect of side chain length, we looked next at modification of the vicinal nitrogen. Disubstitution of the C-2 amine (see **37** vs **39**) resulted in a 15−20-fold loss of enzyme potency. This result and others reported within the pyridopyrimidinone series[15] support the essential requirement of a 2-NHR substitution pattern, which is shown in the crystal structure to form a hydrogen bond to the carbonyl oxygen of Ala564 in the FGFr TK (or the corresponding amino acid in c-Src and PDGFr TKs).[23] Similarly, branching along the carbon backbone of the alkylamino side chain led to decreased activity (**37** vs **41**).

Our next focus was on the nature of the terminus of the amine side chain. To examine the steric constraints around the distal amine, we compared the *N*,*N*-diethylamino moiety in the side chain of **13** with the *N*-(morpholino) and *N*-(2-methylpiperidino) moieties. No change in potency was observed for either modification (**13** vs **43**; **13** vs **45**). Earlier work on the pyridopyrimidinones[15] showed that incorporating a second basic amine into the side chain is beneficial. Attempting to improve enzyme potency even further, we incorporated the *N*-(4-methylpiperazino) moiety in the side chain of **13** and **33**. In this case, we observed a moderate increase in TK inhibition (see **13** vs **47**; **33** vs **49**). In summary, the data in Table 2 show that the optimal alkylamino side chain at C-2 is four carbons in length and unbranched along the backbone and possesses a terminal *N*-(4-methylpiperazino) group. It should be noted, however, that while four carbons are preferred over three and *N*-(4-methylpiperazino) is preferred over *N*,*N*-diethylamino, combinations of three carbons terminating in *N*-(4-methylpiperazino) or four carbons terminating in *N*,*N*-diethylamino were essentially equipotent against the range of kinases evaluated.

A preliminary survey on the effect of N-7 urea substitution on enzyme potency is also contained in Table 2. Examination of the urea functionality when the substitution at C-2 was held constant showed that replacing the *tert*-butyl urea of **13** with other smaller alkyl ureas had no effect on potency (**28** vs **27**; **13** vs **29** and **30**; **33** vs **32** and **34**). Interestingly, even the aryl urea analogue **35** possessed activity comparable to its *tert*-butyl urea counterpart **33** in the inhibition of PDGFr and c-Src. Both of these compounds are the most potent inhibitors of c-Src (0.007 μM) for analogues listed in Table 2. In summary, the data in Table 2 show that aliphatic ureas comprising ≤6 carbons are comparable in potency to the *tert*-butyl urea congener of **33**.

Since a number of aliphatic substituents were well tolerated at N-7, and **35** with its aryl urea was one of our most potent compounds against the c-Src kinase, we decided to investigate the nature of the urea at N-7 in more detail. First, we held the C-2 position fixed using one of our better side chains, the *N*-(4-methylpiperazino)propylamine, and constructed a SAR with 22 different ureas at N-7. These compounds are shown in Table 3. We began our study by taking a closer look at the effect of the size of the alkyl urea. Replacing the *tert*-butyl substituent with hydrogen on the urea resulted in lowering PDGFr potency by 5-fold while FGFr and c-Src suffered a 2−3-fold decrease in potency (**47** vs **50**). As observed above, compounds with the smaller aliphatic ureas, (e.g., ethyl, allyl, isopropyl, and cyclohexyl) were essentially equipotent to **47** against the three kinases (**47** vs **51**, **52**, **53**, and **56**). Parenthetically, it should be noted that the data for **51** and **53** reinforce the observation made above regarding the attributes of the *N*-(4-methylpiperazino) group. In the case of the *tert*-butyl urea, only a marginal improvement in activity was observed when the *N*,*N*-diethylamino substituent was replaced with *N*-(4-methylpiperazino) (Table 2; **13** vs **47** and **33** vs **49**). Comparing the data in Table 2 and Table 3, a more pronounced effect is seen in the case of the ethyl and isopropyl ureas (**51** vs **29** and **53** vs **30**) where a 2-fold increase in PDGFr and FGFr and a 4-fold increase in c-Src TK potencies are obtained.

Table 3. Extensive Exploration around the C-7 Urea Position[a]



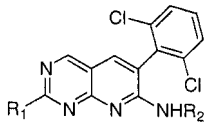
no.	R_2	method	IC_{50}, μM PDGFr	FGFr	c-Src
50	H_2NCO	D	2.4	0.14	0.061
51	EtNHCO	B	0.42	0.053	0.024
52	allylNHCO	B	0.76	0.035	0.022
53	*i*-PrNHCO	B	0.55	0.034	0.019
47	*t*-BuNHCO	ref 14	0.47	0.051	0.032
54	*n*-octylNHCO	B	6.2	0.42	0.12
55	benzylNHCO	B	2.5	0.062	0.030
56	cyclohexylNHCO	B	0.37	0.029	0.031
57	adamantylNHCO	B	2.8	0.12	0.12
58	$BOCNH(CH_2)_2NHCO$	D	4.2	0.067	0.15
59	$Me_2N(CH_2)_2NHCO$	D	2.4	0.075	0.020
60	Et_2NCO	E	>50	5.5	>50[b]
61	PhNHCO	B	0.57	0.084	0.015
62	4-ClPhNHCO	B	1.8	0.12	0.040
63	4-BrPhNHCO	B	1.5	0.11	0.033
64	$4\text{-}CF_3PhNHCO$	B	5.5	0.61	0.19
65	$3,4\text{-}(Cl)_2PhNHCO$	B	5.0	0.60	0.092
66	4-MePhNHCO	B	0.84	0.11	0.036
67	2-MeOPhNHCO	B	0.66	0.067	0.016
68	3-MeOPhNHCO	B	0.91	0.063	0.029
69	4-MeOPhNHCO	B	0.68	0.074	0.033
70	1-naphthylNHCO	B	2.6	0.30	0.13

[a] IC_{50} values reported for kinase inhibition represent the means of at least two separate determinations done in triplicate with typical variations of less than 30% between replicate values. [b] IC_{50} values represent a single determination.

Continuing our survey of ureas we observed that while smaller aliphatic ureas at N-7 gave compounds with consistently good activity, increasing their size or steric bulk tended to decrease inhibition toward the kinases evaluated. In particular, the compounds suffered from a 5−10-fold decrease in inhibition against PDGFr (**47** vs **54**, **55**, and **57**). We also incorporated urea moieties possessing a basic side chain off the distal nitrogen as another means of enhancing aqueous solubility. This, too, brought about a 5-fold decrease in PDGFr potency (**47** vs **59**). In completing our study of aliphatic ureas, we looked at the effect of disubstitution of the urea distal nitrogen, effectively eliminating any hydrogen bonding with N-8 of the pyridopyrimidine template. As reported above, this hydrogen bond has been observed in several small molecule crystal structures of these compounds[14,23] as well as in the cocrystal of the PD 173074/FGFr-1 complex.[22] Not surprisingly, the disubstituted urea was found to be inactive against the PDGFr and c-Src TKs, and its potency against FGFr decreased 2 orders of magnitude (**47** vs **60**), possibly due to an entropic effect and/or a repositioning of the urea alkyl substituent away from a small hydrophobic pocket of the enzyme.

Another area of SAR investigation was to look at the effect of aromatic ureas at N-7. As described in the initial example above (**33** vs **35**; Table 2) the phenyl urea, **61**, was equal in potency to the *tert*-butyl urea, **47**. A brief look at the effect of substitution of the phenyl group showed that analogues with electron-rich rings,

Table 4. Other C-7 Functionality[a]



no.	R_1	R_2	method	IC_{50}, μM PDGFr	FGFr	c-Src
13	$-NH(CH_2)_3NEt_2$	*t*-BuNHCO	ref 14	0.66	0.082	0.073
71	$-NH(CH_3)_3NEt_2$	*t*-BuCH_2CO	F	>50[b]	27	19
72	$-NH(CH_3)_3NEt_2$	Me_2NCH	G	>50	10	4.0
73	$-NH(CH_3)_3NEt_2$	EtNHCS	H	5.0	0.26	0.13
74	$-NH(CH_3)_3NEt_2$	EtNHCNH	I	>50	2.7	1.4
75	$-NH(CH_2)_4NEt_2$	(morpholin-1-yl)$(CH_2)_3$NHCS	H	1.1	0.13	0.022
76	$-NH(CH_2)_3$(*N*-methylpiperazin-1-yl)	EtCH_2CO	F	>50	3.7	2.7
77	$-NH(CH_2)_3$(*N*-methylpiperazin-1-yl)	*t*-BuCH_2CO	E	>50	8.0	8.4
47	$-NH(CH_2)_3$(*N*-methylpiperazin-1-yl)	*t*-BuNHCO	ref 14	0.47	0.051	0.032
78	$-NH(CH_2)_3$(*N*-methylpiperazin-1-yl)	PhCH_2CO	F	>50	6.8	3.3
79	$-NH(CH_2)_3$(*N*-methylpiperazin-1-yl)	PhSO_2	J	6.9	0.068	0.022
80	$-NH(CH_2)_3$(*N*-methylpiperazin-1-yl)	PhNHCNH	I (ref 21)	37	2.8	0.86
81	$-NH(CH_2)_3$(*N*-methylpiperazin-1-yl)	*i*-PrNHCN-*i*-Pr	I	>50	2.5	0.85
82	$-NH(CH_2)_3$(*N*-methylpiperazin-1-yl)	*t*-BuNHCS	H	1.9	0.10	0.22
83	$-NH(CH_2)_3$(*N*-methylpiperazin-1-yl)	PhNHCS	H	2.2	0.32	0.022

[a] IC_{50} values reported for kinase inhibition represent the means of at least two separate determinations done in triplicate with typical variations of less than 30% between replicate values. [b] IC_{50} values represent a single determination.

such as 2- or 4-methoxyphenyl substitution, were equipotent with the unsubstituted phenyl urea (**61** vs **67** and **69**, respectively). In contrast, analogues with electron-deficient rings, such as 4-(trifluoromethyl) or 3,4-dichloro substituents, or the bulky naphthyl urea resulted in a decreased potency when compared to the simple phenyl congener (**61** vs **64**, **65**; **61** vs **70**). Although the inhibitory activity patterns tended to follow the electronic effects of the phenyl substituents, substitution of the phenyl ring in general resulted in only minor to moderate changes in potency. In summary, the SAR data in Table 3 indicate that smaller alkyl or unsubstituted phenyl ureas are optimal, whereas larger, bulkier ureas lead to compounds with lower potency. Furthermore, the intramolecular hydrogen bond between the N-7 urea and N-8 of the pyridopyrimidine template appears essential toward maintaining good activity.

We wished to further explore the importance of this hydrogen bonding as it relates to the urea and other functional groups incorporated at the N-7 position. The compounds prepared for this purpose are shown in Table 4. As with the disubstituted urea described above, an amide functionality at N-7 still allows for hydrogen bonding between the carbonyl group of the amide and the kinase conserved lysine but vitiates the possibility for forming an intramolecular hydrogen bond with the N-8 of the pyridopyrimidine. Thus amide installation resulted in a total loss in PDGFr inhibitory activity, and FGFr and c-Src activity decreased 2 orders of magnitude (**13** vs **71**; **51** vs **76**; **47** vs **77**; **61** vs **78**; see also Table 3). In contrast to the bis-substituted urea, **60**, and the amide derivatives, both thiourea and guanidine analogues have the potential to form the apparent requisite hydrogen bond between the N-7 substituent and N-8 of the pyridopyrimidine nucleus. Comparison of urea to corresponding thiourea congeners showed a 4-fold decrease in PDGFr potency, and a 2−4-fold decrease in FGFr TK potency (**29** vs **73**; **47** vs **82**; **61** vs **83**; see also Tables 2 and 3). This decrease may be due to the fact that the sulfur atom of the thiourea is a poorer hydrogen bond acceptor relative to the oxygen of the urea.[24,25] This hydrogen bond has been shown to be important for enzyme binding in crystallogaphic[22] and binding model studies.[23] Alternatively, the decrease in in vitro enzyme potency may result from a repositioning of the thiourea due to different steric demands. Similarly, the guanidine congeners showed poor activity, being essentially inactive vs PDGFr and suffering greater than a 10-fold decrease in FGFr and c-Src potency (**29** vs **74**; **61** vs **80**; see also Tables 2 and 3). One possible explanation for this may be the guanidine's inability to offer a second hydrogen bonding possibility through the hydrogen of the imine nitrogen. To accomplish this, the ethyl group of **74** or the phenyl group of **80** must be rotated into a spatial position distal to that occupied by the same groups in the urea congeners, **29** and **61**, respectively. Supportive of this notion is the disubstituted guanidine, **81**, where the isopropyl group on the imine nitrogen is fixed into a space normally left vacant with urea substitution. The final replacement for the N-7 urea examined was the sulfonamide functionality. While **79** possessed good potency vs FGFr and c-Src, the activity against PDGFr was similar to that seen with the thioureas. In summary, the data in Table 4 show once again the importance of the intramolecular hydrogen bond between the hydrogen on the terminal amine of the urea and N-8 of the pyridopyrimidine ring. None of the analogues with alternate functional groups, including thioureas and guanidines, that are capable of forming this critical hydrogen bond increased enzyme potency over that found in the ureas.

Having optimized the N-7 position and C-2 positions, we selected four of our best compounds, based on their potency against our primary panel of kinases, to look at their selectivity against other kinases. Compounds **32**, **33**, **35**, and **47** (Table 2) were evaluated against the expanded panel shown in Table 5. While displaying good potency toward PDGFr, FGFr, and c-Src TKs, they were marginally active against the epidermal growth factor receptor (EGFr) and inactive against the insulin receptor (IR), two other tyrosine kinases. These compounds

Table 5. Expanded Panel of Kinase Enzyme Inhibition[a]

	IC_{50}, μM							
no.	PDGFr	FGFr	c-Src	EGFr	MEK/ ERK[b,c]	IR[b]	CDK4	PKC[b]
32	0.21	0.049	0.018	1.3[b]	>5	>50	>40	>50
33	0.36	0.048	0.0074	0.39	2.8	>50	>40	23
35	0.45	0.11	0.0075	1.0[b]	5.0	>50	>40	>50
47	0.49	0.051	0.032	0.15	7.0	>50	>40	22

[a] IC_{50} values reported for kinase inhibition represent the means of at least two separate determinations done in triplicate with typical variations of less than 30%. [b] IC_{50} values represent a single determination. [c] MEK/ERK was run as a cascade assay.

were also inactive toward the inhibition of cyclin dependent kinase (CDK4) and protein kinase C (PKC), both serine/threonine kinases, and possessed poor activity in a cascade assay for MEK1 (MAP kinase kinase) and ERK2. Thus, the data in Table 5 suggest that the compounds of this series are selective toward a range of tyrosine kinases, in particular PDGFr, FGFr, and c-Src.

While the aqueous solubility of **1** was insufficient for further in vivo studies (<1 μg/mL), optimization of the alkylamino substituent at N-2 and the urea substituent at N-7 resulted in compounds with a tremendous increase in aqueous solubility. In addition, we observed that compounds containing an ethyl urea, e.g., **32** (>5200 μg/mL) and **51** (>4500 μg/mL), were more soluble than the corresponding compounds bearing a *tert*-butyl urea, e.g., **33** (760 μg/mL) and **47** (210 μg/mL), or phenyl urea **35** (47 μg/mL). Within the series shown in Tables 1−4, **32** showed excellent potency toward all TKs surveyed with IC_{50} values of 0.21 μM (PDGFr), 0.049 μM (FGFr), and 0.018 μM (c-Src). On the basis of its excellent potency toward the kinases profiled, as well as its physiochemical properties, it was selected for additional functional, cellular, and animal studies. We also profiled other closely related congeners of **32** in selected in vitro studies.

Cellular Studies

Reputedly, the abnormal proliferation of vascular smooth muscle cells plays a critical role in some diseases such as restenosis and atherosclerosis.[4,5] We evaluated one of our better compounds, **47**, for its ability to inhibit PDGF-stimulated growth of rat aortic vascular smooth muscle cells (RAVSMCs) over an 5 day period. In this assay,[26] growth arrested RAVSMCs were treated with vehicle (control) or **47** (at concentrations of 0.1, 0.3, or 1.0 μM) and stimulated to grow in serum. The compound was washed out and replaced with fresh drug daily, and cells were counted on days 1−5. The results shown in Figure 2 indicate that **47** inhibits RAVSMC growth in a dose-dependent fashion with an IC_{50} of 0.35 μM on day 5. This result is similar to that reported earlier for **33** (IC_{50} 0.30 μM).[14] Additionally, when the treatment of RAVSMCs with **47** at 1 μM concentration was discontinued after 3 days, normal cellular growth resumed, paralleling that of the control (see 1 μM washout in Figure 2). These results indicate that **47** is not cytotoxic and is a potent inhibitor of vascular smooth muscle cell proliferation in vitro.

Looking further at the mechanism of inhibition, five compounds, **32**, **33**, **35**, **47**, and **61**, were tested for their effect on PDGF-mediated receptor autophosphorylation

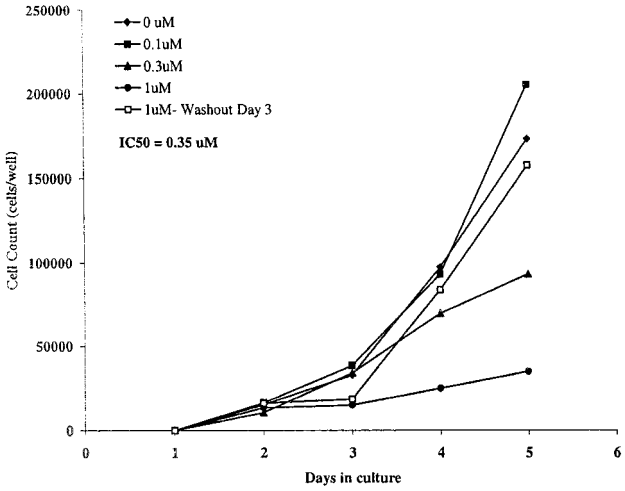


Figure 2. Growth delay assay for compound **47** using RAVSMC P6.

Table 6. PDGF Receptor Autophosphorylation and Growth Delay Effects on Human Colon Carcinoma Cell Lines, C-6 Rat Glioma, and NIH3T3/PDGF Mouse Transfected Fibroblasts

	IC_{50}, μM					
no.	PDGF autophos[a]	HCT-8[b]	SW-620[b]	HT-29[b]	C-6 rat glioma[b]	NIH3T3/ PDGF MTF[b]
32	0.24	4.02	2.76	1.17	8.8	1.01
33	0.45	16.3	>25	0.9	5.8	nt[d]
35	1.2	3.2	2.6	0.8	8.1	nt
47	0.27	>15.2	>25	11.1[c]	4.0	1.08
51	nt	9.3	2.5	0.9	6.7	nt
61	0.37	4.0	4.1	1.5	6.7	nt

[a] Results are the mean of at least two experiments. [b] Results are the mean of at least two experiments done in duplicate. [c] IC_{50} value represents a single determination. [d] nt, not tested.

in RAVSMCs[26] (Table 6). Stimulation of the PDGFr TK with its ligand (PDGF) results in phosphorylation of the intracellular cytoplasmic domain. Compound inhibition is determined by lysing the cells and quantifying the level of the 190 Kd tyrosine phosphorylated receptor protein after Western blotting with an anti-phosphotyrosine antibody. The IC_{50} values for the inhibition of autophosphorylation of the PDGF receptor found in RAVSMCs were similar to those found for the inhibition of substrate phosphorylation in isolated PDGFr. The IC_{50} for inhibition of PDGFr tyrosine kinase activity was 0.47 μM for compound **47**. This value correlated with both inhibition of vascular smooth muscle cell PDGFr autophosphorylation (IC_{50} 0.27 μM) and growth (IC_{50} 0.35 μM). These results indicate that the selected inhibitors permeate the cell membrane well and that RAVSMC growth was arrested due to inhibition of PDGFr phosphorylation.

In another set of cellular assays which served as a primary, front line screen, the same compounds plus **51** were evaluated in cell culture against five tumor cell lines, and the growth of these cells was monitored for 3 days (Table 6).[27] The first three cell lines, HCT-8, SW-620, and HT-29, are comprised of human colon carcinoma cells whose cellular growth is regulated by an unknown number of kinases. In the colon carcinoma cell lines, HCT-8, SW-620, and HT-29, compounds **32**, **35**, **51**, and **61** were more potent than **33** and **47**, possessing low micromolar activity. This potency is comparable to that observed for cisplatin in these cell lines. Although

Table 7. In Vivo Anticancer Activity of Compound **32** against Six Human Tumor Xenografts and a Mouse Fibroblast Line Transfected with PDGF[a]

tumor	tissue type	dose (mg/kg)	schedule	weight change (g)	*T*/*C* (%) on last therapy day[b]	*T* − *C* (days)[c]	net cell kill (log_{10})[d]
A431	epidermoid	30	days 7−21	−2.2	103	0.5	−0.8
C6	glioma	30	days 1−15	−1.5	76	0.0	
Colo-205	colon	26	days 9−23	−6.0	26	14	0.0
HT-29	colon	26	days 15−29	−4.0	127	0.0	
MCF-7	breast	30	days 1−15	−1.1	46	2.5	−0.5
SK-OV-3	ovarian	26	days 15−29	−3.0	85	0.0	
PDGF	fibroblast	40	days 1−15	−3.2	51	2.8	−1.8

[a] Tumor fragments (approximately 30 mg) were implanted sc into the right axilla of mice on day 0. All treatments were ip on the indicated schedules. The maximum tolerated dose ($\leq LD_{10}$) from a complete dose response is shown. [b] Ratio of median treated tumor mass (mg)/median control tumor mass × 100% on the last day of therapy. [c] The difference (in days) for the treated (*T*) and the control (*C*) tumors to reach 750 mg. [d] The net reduction in tumor burden (in logs) between the first and last treatments.

32 was slightly less potent than **47** in the growth delay of C-6 rat glioma cells, both compounds were equipotent (IC_{50} = 1 μM) toward the inhibition of the NIH3T3/PDGF transfected cell line. The growth delay data in Table 6 show that the selected inhibitors, which potently inhibit receptor and nonreceptor tyrosine kinases, are also capable of inhibiting normal and transfected cell proliferation, albeit at concentrations greater than those necessary for enzyme inhibition. Although the source of differing potency for isolated enzyme vs cellular inhibition was not characterized, these may reside in uptake and distribution mechanisms within cells. Given that these are broadly active TK inhibitors, it would also seem plausible that this could reflect different potencies for kinases that were not examined.

In Vivo Studies

Compound **32** was evaluated in vivo against six human tumor xenografts and a mouse fibroblast line transfected with PDGF (Table 7). The Colo-205, HT-29, and SK-OV-3 in vivo tumor models were selected on the basis of their endogenous c-Src activity.[28] Colo-205, HT-29, and SK-OV-3 are reported to have in vitro c-Src activities of 6, 11, and 19 pmol/min/mg protein with poly glu-tyr as substrate. These activity levels were not the highest nor the lowest reported for other cell lines in the NCI 60-cell line panel, but they were deemed to be representative of the cell lines in this panel. The A431 epidermoid xenograft and MCF-7 breast xenografts were selected based on the purported involvement of c-Src in the EGF signaling pathway and in breast tumors, respectively.[29] The C6 glioma was evaluated for sensitivity to **32** based on the importance of PDGF for the in vivo growth of this tumor model.[30] The fibroblast line transfected with PDGF was included due to its high tumorigenic potential as compared to the parent fibroblast line. Compound **32** produced tumor stasis against only the Colo-205 colon xenograft model as reflected in a tumor growth delay of 14 days for 15 days of therapy. Tumor growth resumed once therapy was terminated (Figure 3). The maximum tolerated dose of **32** produced varying degrees of animal weight loss from 4 to greater than 20%, with the greatest weight loss in the evaluation against the Colo-205 tumor model. There was no evidence of any antitumor effect against any of the other tumor models.

Summary

In conclusion, we have reported the synthesis and SAR of a number of soluble, potent tyrosine kinase inhibitors. These compounds inhibit selected kinases at the ATP binding site and have been shown to exert growth delay effects on rapidly proliferating cells, such as RAVSMCs and various tumor cell lines. One compound, **32**, demonstrated measurable in vivo activity against one of the murine models, the Colo-205 colon tumor xenograft. Due to their level of c-Src activity, we were surprised that none of the other tumor models responded. However, this could indicate that the inhibition of c-Src and/or the PDGF receptor in the nonresponding tumor models might not be sufficient to produce an antitumor effect due to the redundancy present in the signaling pathways in vivo. The growth delay observed for **32** against Colo-205 suggests that in sensitive tumors compounds of this type may produce tumor stasis in vivo and that prolonged treatment periods could maintain complete suppression of tumor growth.

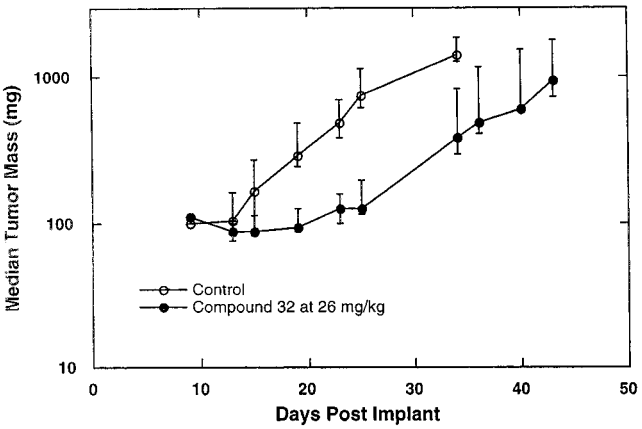


Figure 3. Effect of compound **32** on the growth of Colo-205 (colon adenocarcinoma xenograft) in nude mice. Tumors were implanted sc on day 0. Intraperitoneal treatments were administered on days 9−23. Tumors were approximately 100−120 mg on day 9 when the first treatment was administered. Data are the median tumor mass on each measurement day and the interquartile range.

Experimental Section

General Methods. See Supporting Information.

Method A. 6-(2,6-Dibromo-phenyl)-pyrido[2,3-*d*]pyrimidine-2,7-diamine (18). To cold (5 °C) 2-ethoxyethanol (10 mL) was cautiously added NaH (0.139 g, 5.80 mmol) in portions with stirring. The mixture was allowed to warm to room temperature. To the reaction was added 2,6-dibromophenylacetontrile (4.18 g, 15.2 mmol), followed by aldehyde **5**[14,19] (2.00 g, 14.48 mmol). The reaction was heated at reflux for 4 h, allowed to cool to room temperature, and poured into water.

After standing at room temperature for 1 h, the suspension was filtered and the precipitated crude product was washed sequentially with H_2O, CH_3CN, and Et_2O. Drying the solid in vacuo at 60 °C overnight gave **18** (3.62 g, 63% yield, mp 284 °C dec): ^{1}H NMR (DMSO-d_6) δ 6.58 (br s, 2H), 6.73 (br s, 2H), 7.29 (t, J = 8.2 Hz, 1H), 7.54 (s, 1H), 7.78 (d, J = 8.2 Hz, 2H), 8.64 (s, 1H); CIMS m/z 422 $(M + C_2H_5)^+$, 394 $(M + H)^+$. Anal. ($C_{13}H_9N_5Br_2$) C, H, N.

Method B. 1-[2-Amino-6-(2,6-dibromo-phenyl)-pyrido-[2,3-*d*]pyrimidin-7-yl]-3-*tert*-butyl-urea (19). A suspension of diamine **18** (0.25 g, 0.63 mmol) in DMF (5 mL) was treated with NaH (0.017 g, 0.73 mmol). After the mixture was stirred at room temperature for 1 h, *tert*-butyl isocyanate (0.077 mL, 0.68 mmol) was added, and the reaction was stirred at room temperature for 18 h. The insoluble salts were removed by filtration and rinsed with DMF. The filtrate was concentrated in vacuo and H_2O added to the wet residue to give a yellow precipitate. Purification of the precipitate by medium-pressure chromatography, eluting with a solution of EtOAc/$CHCl_3$ (1:1), gave **19** (0.18 g, 58% yield, mp > 300 °C dec): ^{1}H NMR (DMSO-d_6) δ 1.39 (s, 9H), 7.27 (br s, 2H), 7.33−7.44 (s, 1H), 7.48 (s, 1H), 7.79−7.90 (m, 2H), 7.93 (s, 1H), 8.94 (s, 1H), 10.17 (s, 1H); CIMS m/z 495 $(M + H)^+$. Anal. ($C_{18}H_{18}N_6Br_2O\cdot0.4H_2O$) C, H, N, Br.

1-*tert*-Butyl-3-[6-(2,6-dichloro-phenyl)-2-(2-diethylamino-ethylamino)-pyrido[2,3-*d*]pyrimidin-7-yl]-urea (28). To a solution of pyridopyrimidine **26** (1.00 g, 2.47 mmol) in DMF (10 mL) was added NaH (0.059 g, 2.47 mmol). After the mixture was stirred for 1 h at room temperature, *tert*-butyl isocyanate (0.244 g, 2.47 mmol) was added. After being stirred for 1 h, the reaction mixture was concentrated in vacuo and the residue partitioned between EtOAc and H_2O. The aqueous layer was extracted three times with EtOAc. The combined EtOAc layers were washed with a saturated solution of NaCl, dried ($MgSO_4$), and concentrated. Chromatography of the residue, eluting with a solution of EtOAc/EtOH/Et_3N (18:2:1), gave **28** (0.76 g, 61% yield, mp 94.5−96.5 °C): ^{1}H NMR (DMSO-d_6) δ 0.99 (t, J = 7.1 Hz, 6H), 1.39 (s, 9H), 2.49−2.58 (m, 4H), 2.58−2.68 (m, 2H), 3.43−3.53 (m, 2H), 7.50−7.58 (m, 1H), 7.62−7.69 (m, 2H), 7.69−7.82 (br m, 2H), 7.96 (s, 1H), 8.90 (s, 1H), 10.45 (s, 1H); CIMS m/z 504 $(M + H)^+$. Anal. ($C_{24}H_{31}N_7Cl_2O$) C, H, N.

Method C. N^2-(3-Diethylamino-propyl)-6-(2,6-dimethyl-phenyl)-pyrido[2,3-*d*]pyrimidine-2,7-diamine (16). A mixture of diamine **14**[14] (3.00 g, 11.31 mmol), sulfamic acid (2.20 g, 22.61 mmol), and 3-(diethylamino)propylamine (30 mL) was heated at reflux for 18 h. The cooled reaction mixture was poured into 500 mL of ice H_2O. The resultant precipitate was filtered and washed with water. Crystallization of the crude product from EtOAc gave **16** (3.50 g, 80% yield, mp 216−219 °C): ^{1}H NMR (DMSO-d_6) δ 0.85−1.08 (t, 6H), 1.57−1.80 (m, 2H), 2.02 (s, 6H), 2.33−2.61 (m, 6H), 3.22−3.47 (m, 4H), 7.07−7.22 (m, 4H), 7.46 (s, 1H), 8.60 (br s, 1H); ESMS m/z 379.5 $(M + H)^+$. Anal. ($C_{22}H_{30}N_6\cdot0.2H_2O$) C, H, N.

Method D. {6-(2,6-Dichloro-phenyl)-2-[3-(4-methyl-piperazin-1-yl)-propylamino]-pyrido[2,3-*d*]pyrimidin-7-yl}-urea (50). Into a suspension of pyridopyrimidine **46**[14] (1.00 g, 2.24 mmol) in dry dioxane (6 mL) was added 1,1′-carbonyldiimidazole (0.44 g, 2.69 mmol). After heating at reflux overnight, the reaction was cooled to room temperature and poured into a saturated solution of NH_3 gas in dry dioxane. The reaction was concentrated to a residue that was partitioned between EtOAc and H_2O. The EtOAc layer was washed twice each with H_2O and brine, dried ($MgSO_4$), and concentrated. The crude product was purified by chromatography, eluting first with a solution of EtOAc/EtOH/Et_3N (9:2:1) then EtOAc/EtOH/Et_3N (9:3:1). Further purification of the product by crystallization from EtOAc/EtOH, followed by drying the product at 55 °C in vacuo, gave **50** (0.35 g, 32% yield, mp 166−168 °C): ^{1}H NMR (DMSO-d_6) δ 1.64−1.82 (m, 2H), 2.07−2.60 (m, 13H), 3.36−3.49 (m, 2H), 7.36 (br s, 1H), 7.49−7.57 (m, 1H), 7.60−7.68 (m, 2H), 7.85−7.93 (m, 1H), 7.96−8.11 (m, 2H), 8.91(s) + 9.00 (s) − total integration 1H, 9.42−9.61 (m, 1H); APCIMS m/z 489 $(M + H)^+$. Anal. ($C_{22}H_{26}N_8Cl_2O$) C, H, N, Cl, H_2O.

Method E. 3-{6-(2,6-Dichloro-phenyl)-2-[3-(4-methyl-piperazin-1-yl)-propylamino]-pyrido[2,3-*d*]pyrimidin-7-yl}-1,1-diethyl-urea (60). A solution of pyridopyrimidine **46**[14] (0.50 g, 1.12 mmol) in DMF (5 mL) was treated with NaH (0.059 g, 2.46 mmol). After being stirred at room temperature for 1 h, the suspension was cooled to 0 °C and diethylcarbamyl chloride (0.15 mL, 1.20 mmol) was added dropwise. The bath was removed, and the reaction mixture was stirred at room temperature for 18 h and then concentrated in vacuo. Purification of the residue by medium-pressure chromatography, eluting with a solution of EtOAc/MeOH/Et_3N (90:10:1), gave **60** (0.46 g, 75% yield, mp 66 °C dec): ^{1}H NMR ($CDCl_3$) δ 0.72−0.89 (m, 3H), 1.08−1.22 (m, 3H), 1.68−1.92 (m, 2H), 2.17−2.94 (m, 12H), 3.00−3.19 (m, 2H), 3.22−3.42 (m, 2H), 3.43−3.67 (m, 2H), 6.19−6.39 (m, 1H), 7.13−7.49 (m, 5H), 8.30−8.47 (m, 1H), 13.80−14.11 (m, 1H); CIMS m/z 504 $(M + H)^+$. Anal. ($C_{26}H_{34}N_8Cl_2O\cdot1.0H_2O\cdot0.3EtOAc$) C, H, N.

***N*-{6-(2,6-Dichloro-phenyl)-2-[3-(4-methyl-piperazin-1-yl)-propylamino]-pyrido[2,3-*d*]pyrimidin-7-yl}-3,3-dimethyl-butyramide (77).** Using the procedure described for the synthesis of **60**, reaction of the sodium salt of pyridopyrimidine **46**[14] and *tert*-butyl acetyl chloride for 48 h followed by medium-pressure chromatography, eluting with a solution of EtOAc/MeOH/Et_3N (9:2:1), gave **77** (0.17 g, 14% yield, mp 84−100 °C dec): ^{1}H NMR (DMSO-d_6) δ 0.78 (s, 9H), 1.72−1.80 (m, 2H), 2.08 (s, 2H), 2.15 (s, 3H), 2.21−2.54 (m, 8H), 3.19−3.50 (m, 3H), 7.19−7.40 (m, 1H), 7.49−7.54 (m, 2H), 7.90−7.99 (m, 1H), 8.11 (s, 1H), 9.09 (s, 1H), 10.17−10.29 (m, 2H); ESMS m/z 544.4 $(M + H)^+$. Anal. ($C_{27}H_{35}N_7Cl_2O\cdot0.4H_2O\cdot0.6EtOH$) C, H, N, Cl.

Method F. *N*-[6-(2,6-Dichloro-phenyl)-2-(3-diethylamino-propylamino)-pyrido[2,3-*d*]pyrimidin-7-yl]-3,3-dimethyl-butyramide (71). To a suspension of **12**[14] (42.0 mg, 0.20 mmol) in *p*-dioxane (0.5 mL) was added *tert*-butyl acetyl chloride (34.0 mg, 0.25 mmol). The reaction was heated at reflux for 1 h then concentrated in vacuo to a semisolid. This residue was dissolved in CH_2Cl_2, washed with cold 5% aqueous NaOH, dried ($MgSO_4$), and concentrated to a thick oil. Medium-pressure chromatography, eluting with a solution of EtOAc/MeOH/Et_3N (90:10:1), gave **71** (30.0 mg, 58% yield, mp 82 °C dec): ^{1}H NMR (DMSO-d_6) δ 0.58−1.13 (m, 15H), 1.60−1.83 (m, 2H), 1.97−2.13 (m, 2H), 2.29−2.71 (m, 4H), 3.14−3.56 (m, 4H), 7.28−7.42 (m, 1H), 7.46−7.60 (m, 2H), 7.90−8.03 (m, 1H), 8.11 (s, 1H), 9.08 (s, 1H), 10.17−10.30 (m, 1H); CIMS m/z 517 $(M + H)^+$. Anal. ($C_{26}H_{34}N_6Cl_2O\cdot0.6H_2O\cdot0.4EtOAc$) C, H, N.

Method G. *N*′-[6-(2,6-Dichlorophenyl)-2-{3-(diethylamino)propylamino}pyrido[2,3-*d*]pyrimidin-7-yl]-*N*,*N*-dimethylformamidine (72). To a suspension of pyridopyrimidine **12**[14] (210 mg, 1.00 mmol) in DMF (0.80 mL) was added DMF dimethyl acetal (0.80 mL). The mixture was stirred at room temperature for 5.5 h and then concentrated in vacuo. The residual oil was partitioned between CH_2Cl_2 and H_2O. The organic phase was dried ($MgSO_4$) and then concentrated to a glass that was crystallized from CH_3CN to give **72** (160 mg, 68% yield, mp 100−104 °C): ^{1}H NMR ($CDCl_3$) δ 1.04 (t, J = 7.0 Hz, 6 H), 1.81 (t, J = 6.4 Hz, 2 H), 2.60−2.52 (m, 6 H), 2.80, (s, 3 H), 3.08 (s, 3 H), 3.68 (br t, 1 H), 6.55 (br s, 1 H), 7.20 (t, J = 8 Hz, 1 H), 7.36 (d, J = 8 Hz, 2 H), 7.62 (s, 1 H), 8.73 (s, 1 H), 8.82 (s, 1 H); CIMS m/z 474 $(M + H)^+$. Anal. ($C_{23}H_{29}N_7Cl_2\cdot0.4H_2O$) C, H, N.

Method H. 1-[6-(2,6-Dichloro-phenyl)-2-(3-diethylamino-propylamino)-pyrido[2,3-*d*]pyrimidin-7-yl]-3-ethyl-thiourea (73). To a solution of **12**[14] (420 mg, 1.00 mmol) in DMF (5 mL) was added NaH (0.034 g, 1.40 mmol). After the mixture was stirred at room temperature for 0.5 h, ethyl isothiocyanate (113 mg, 1.20 mmol) was added. The resulting mixture was stirred for 16 h, poured into a saturated aqueous solution of $NaHCO_3$, extracted three times with CH_2Cl_2, dried (Na_2SO_4), and concentrated. Chromatography of the residue, eluting with a solution of EtOAc/MeOH/Et_3N (85:15:2), gave **73** (362 mg, 77% yield, mp 172−173 °C): ^{1}H NMR ($CHCl_3/D_2O$) δ 0.99 (t,

J = 7.3 Hz, 3H), 1.02 (t, J = 6.0 Hz, 6 H), 1.18 (t, J = 5.8 Hz, 2H), 1.76−1.85 (m, 2H), 2.46−2.59 (m, 6H), 3.63 (q, J = 7.0 Hz, 2H), 3.75−3.81 (m, 2H), 7.43 (t, J = 8.9 Hz, 1H), 7.68 (d, J = 8.6 Hz, 2H) 7.56 (s, 1H), 8.70 (s, 1H); APCIMS m/z 506 $(M + H)^+$. Anal. ($C_{23}H_{29}N_7Cl_2S \cdot 0.3H_2O$) C, H, N.

Method I. *N*-[6-(2,6-Dichloro-phenyl)-2-(3-diethylamino-propylamino)-pyrido[2,3-*d*]pyrimidin-7-yl-*N*″-ethyl-guanidine (74). To a stirred solution of **12**[14] (419 mg, 1.00 mmol) in DMF (1 mL) was added NaH (52.0 mg, 1.30 mmol). After the mixture was stirred for 0.5 h at room temperature, *N*,*N*′-bis(*tert*-butoxycarbonyl)-*N*-(ethyl)-*S*-(ethyl)isothiourea[20] (360 mg, 1.10 mmol) was added in one portion. After being stirred for 18 h, the reaction mixture was diluted with CH_2Cl_2, washed twice with H_2O, dried (Na_2SO_4), and concentrated. The resulting oil was chromatographed, eluting with a solution of EtOAc/MeOH/Et_3N (85:15:2), to afford a 1:2 mixture of **12** and carbamic acid [[[6-(2,6-dichlorophenyl)-2-(3-diethylamino-propylamino)-pyrido[2,3-*d*]pyrimidin-7-yl]imino[[1,1-dimethyl-ethoxy)carbonyl]amino]methyl]ethylamino]-1,1-dimethylethyl ester. A portion of this mixture was dissolved in anhydrous CH_2Cl_2 (0.5 mL) containing 2,6-lutidine (65.0 mg, 0.60 mmol). Trimethylsilyl trifluomethanesulfonate (100 mg, 0.440 mmol) was added, and the mixture was stirred at room temperature for 30 h. The mixture was then poured into a saturated aqueous solution of $NaHCO_3$, extracted with CH_2Cl_2, dried (Na_2SO_4), and concentrated. Chromatography of the resulting oil by preparative HPLC, eluting with a linear gradient of 100/0 A/B to 63/37 A/B (where A is 0.1% aqueous TFA and B is 0.1% TFA in CH_3CN), gave the tris-TFA salt of **74** (26 mg, 4% yield): ^{1}H NMR ($CDCl_3/D_2O$) δ 1.27 (t, J = 7.3 Hz, 6H), 2.14−2.21 (m, 2H), 3.01−3.22 (m, 6H), 3.36−3.42 (m, 2H), 7.31 (t, J = 9.9 Hz, 1H), 7.85 (d, J = 10.1 Hz, 2H), 7.90 (s, 1H), 9.02 (s, 1H); APCIMS m/z 490 $(M + H)^+$; Anal. ($C_{23}H_{30}N_8Cl_2 \cdot 1.0H_2O \cdot 3.0$TFA) C, H, N.

***N*-{6-(2,6-Dichloro-phenyl)-2-[3-(4-methyl-piperazin-1-yl)-propylamino]-pyrido[2,3-*d*]pyrimidin-7-yl}-*N*′,*N*″-diisopropyl-guanidine (81).** To a solution of pyridopyrimidine **46**[14] (100 mg, 0.220 mmol) in DMF (2 mL) was added NaH (7.0 mg, 0.29 mmol) in one portion. After the mixture was stirred at room temperature for 0.5 h, *N*,*N*′-diisopropylcarbodiimide (43 mg, 0.34 mmol) was added, and the resulting mixture was stirred for 16 h. The reaction was poured into a saturated aqueous solution of $NaHCO_3$, extracted three times with CH_2Cl_2, dried (Na_2SO_4), and concentrated. Chromatography of the residue, eluting with a solution of EtOAc/MeOH/Et_3N (85:15:2), gave **81** (102 mg, 81%). An analytically pure sample was obtained as the tetra-HCl salt following crystallization from acidic 2-propanol (mp 229 °C dec): ^{1}H NMR ($CDCl_3/D_2O$) δ 1.06 (d, 3H), 1.09 (s, 3H), 2.22 (s, 3H) 2.31−2.58 (m, 10H), 3.50 (t, J = 7.1 Hz, 2H), 3.56 (q, J = 6.1 Hz, 2H), 7.16 (d, J = 8 Hz, 4H), 7.29 (d, J = 8 Hz, 4H) 7.42 (s, 1H), 8.53 (s, 1H); APCIMS m/z 572.2 $(M + H)^+$. Anal. ($C_{28}H_{39}N_9Cl_2 \cdot 1.0H_2O \cdot 4.0$HCl) C, H, N.

Method J. *N*-{6-(2,6-Dichloro-phenyl)-2-[3-(4-methyl-piperazin-1-yl)-propylamino]-pyrido[2,3-*d*]pyrimidin-7-yl}-benzenesulfonamide (79). A suspension of pyridopyrimidine **46**[14] (1.00 g, 2.24 mmol) in *p*-dioxane (5 mL) was treated with benzenesulfonyl chloride (0.36 mL, 2.80 mmol) and heated at reflux for 18 h. The reaction mixture was concentrated in vacuo. The resulting residue was dissolved in 9:1 CH_2Cl_2/MeOH, and the solution was washed with cold 5% aqueous NaOH, dried ($MgSO_4$), and concentrated to leave a dark foam. Purification by preparative HPLC, eluting with a linear gradient of 100/0 A/B to 60/40 A/B (where A is 0.1% aqueous TFA and B is 0.1% TFA in CH_3CN), followed by lyophilization gave the tris-TFA salt of **79** (0.38 g, 18% yield): ^{1}H NMR (DMSO-d_6) δ 1.75−2.04 (m, 2H), 2.17−4.44 (m, 15H), 7.28−7.58 (m, 8H), 7.99−8.18 (m, 1H), 8.30−8.60 (m, 1H), 8.76−8.96 (m, 1H), 11.54−12.10 (m, 1H); APCIMS m/z 586.4 $(M + H)^+$. Anal. ($C_{27}H_{29}N_7Cl_2O_2S \cdot 0.5H_2O \cdot 3.0$TFA) C, H, N.

Recombinant Tyrosine Kinases and Assays. The methods for production of the tyrosine kinases used in this study and assay conditions for each have been previously described (PDGFr-β,[26] FGFr-1,[26] EGFr,[26] c-Src,[26] MEK/ERK,[31] IR,[32] CDK4,[33] and PKC[34]).

PDGF Receptor Autophosphorylation. The assay in rat aortic vascular smooth muscle cells was carried out as previously described.[26]

Cell Culture. Previously established literature procedures were utilized to perform the growth delay assay using rat aortic smooth muscle cells[26] and the tumor cellular growth delay assay using the cell lines described in Table 6.[27] Briefly, for the tumor lines, cells were seeded into 96-well tissue culture plates and incubated for 24 h to allow for cell attachment. One hundred microliters of drug dilutions in culture media were added to the first well in duplicate rows followed by serial 2-fold dilution across the plate. Plates were incubated at 37 °C in 95% humidity and 5% CO_2 for 4 and 3 days for the SW-620, HCT-8, and HT-29 lines. Cell growth was determined by staining cells with sulforhodamine B and reading plates on a Molecular Devices Thermax microplate reader (Sunnyvale, CA).

In Vivo Chemotherapy. Immune-deficient 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.

The six in vivo tumor models listed in Table 7 were established from tissue culture cell lines and maintained by serial passage in nude mice (NCr nu/nu). Nude mice were also used as tumor hosts for anticancer agent evaluations against these tumor models.

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 with test compounds on the basis of average cage weight on the days indicated in the tables. The vehicle for the hydrochloride salt of **32** was sterile water for injection. Dosing solutions were 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), tumor growth delay ($T - C$), and net logs of tumor cell kill was performed as described previously.[35−38] A positive net cell kill indicates that the tumor burden at the end of therapy was less than at the beginning of therapy. A negative net log cell kill indicates that the tumor grew during treatment. Net cell kills near 0 indicate no tumor growth during therapy.

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Supporting Information Available: Experimental details and spectral data are available for compounds **17**, **20**, **21**, **26**, **27**, **29**, **30**, **32**, **34**−**45**, **48**, **49**, **51**−**59**, **61**−**70**, **75**, **76**, **78**, **82**, and **83**. This material is available free of charge via the Internet at http://pubs.acs.org.

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