2-Substituted Aminopyrido[2,3-*d*]pyrimidin-7(8*H*)-ones. Structure−Activity Relationships Against Selected Tyrosine Kinases and in Vitro and in Vivo Anticancer Activity

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While engaged in therapeutic intervention against a number of proliferative diseases, we have discovered the 2-aminopyrido[2,3-*d*]pyrimidin-7(8*H*)-ones as a novel class of 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 **2**, a series of analogues bearing variable substituents at the C-2 position and methyl or ethyl at N-8 was made. 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; epidermal growth factor, EGFr) and nonreceptor (c-Src) classes. One of the more thoroughly evaluated members was **63** with IC_{50} values of 0.079 μM (PDGFr), 0.043 μM (bFGFr), 0.044 μM (EGFr), and 0.009 μM (c-Src). In cellular studies, **63** inhibited PDGF-mediated receptor autophosphorylation in a number of cell lines at IC_{50} values of 0.026−0.002 μM and proliferation of two PDGF-dependent lines at 0.3 μM. It also caused inhibition of soft agar colony formation in three cell lines that overexpress the c-Src TK, with IC_{50} values of 0.33−1.8 μM. In in vivo studies against a panel of seven xenograft tumor models with known and/or inferred dependence on the EGFr, PDGFr, and c-Src TKs, compound **63** produced a tumor growth delay of 10.6 days against the relatively refractory SK-OV-3 ovarian xenograft and also displayed activity against the HT-29 tumor. In rat oral bioavailability studies, compound **63** plasma concentrations declined in a biexponential manner, and systemic plasma clearance was high relative to liver blood flow. Finally, in rat metabolism studies, HPLC chromatography identified two metabolites of **63**, which were proved by mass spectrometry and synthesis to be the primary amine (**58**) and *N*-oxide (**66**). Because of the excellent potency of **63** against selected TKs, in vitro and in vivo studies are underway for this compound in additional tumor models dependent upon PDGFr, FGFr, and c-Src to assess its potential for advancement to clinical trials.

Introduction

It is well-established that multiple growth factors and their cognate receptor tyrosine kinases (TKs) play an important role in cellular processes. Signaling for a variety of cellular functions, including cell growth, is modulated by a variety of receptor and nonreceptor protein TKs and through their ability to phosphorylate tyrosine residues of specific proteins.[1] Abnormal signaling via a number of these has been linked to various pathophysiological states. For example, the platelet-derived growth factor receptor (PDGFr), fibroblast growth factor receptor (FGFr), and the epidermal growth factor receptor (EGFr) TKs have been implicated in the migratory and proliferative responses of vascular smooth muscle cells upon injury after balloon angioplasty.[2,3] Additionally, the PDGFr TK has been implicated in the mitogenesis and progression of a variety of tumor cell lines and types,[4−6] and the FGFr TK has been associated with neovascularization of several tumor types.[7] The nonreceptor TK c-Src and other members of this family participate in a number of signal transduction pathways that are important in cell proliferation and activation of cellular processes,[8] and its increased expression has been associated with transformed phenotypes in connection with other transforming genes.[9,10] Since c-Src is likely involved in cellular signaling and amplification of mitogenic signals initiated by other branches of signal transduction pathways, the inhibition of this and other Src kinase family members may provide opportunities for therapeutic intervention in cancer as well as a variety of other proliferative diseases.

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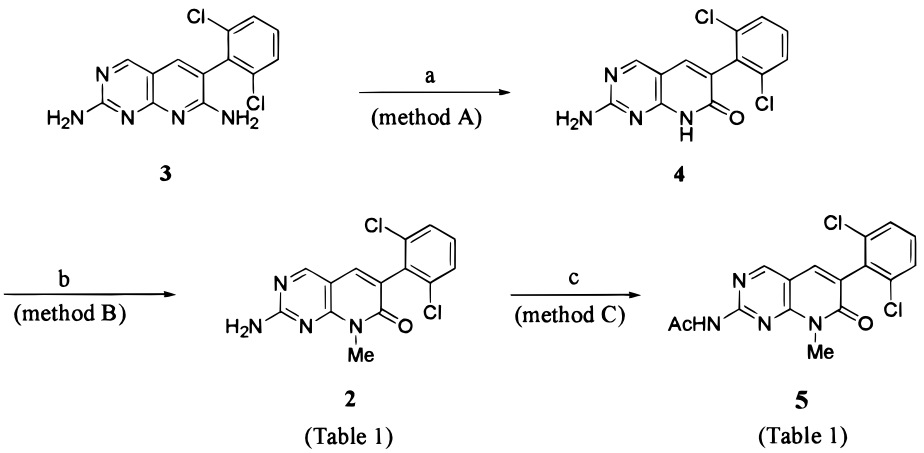
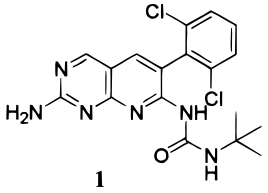
Scheme 1[a]



[a] (a) Concentrated HCl, reflux; (b) NaH, DMF; CH_3I, 60 °C; (c) Ac_2O, reflux.

The general paradigm for the suppression of specific kinases associated with a particular proliferative disease is to develop selective inhibitors toward them. In theory, selective TK inhibitors should be less likely to affect normal cells and thus produce fewer side effects. On the other hand, broadly acting, nonselective inhibitors may be required to overcome redundancies in growth signaling pathways in order to arrest aggressively proliferating cells. For example, it has been suggested that restenosis may, in part, result from a cascade of early events that triggers the early expression of cytokines and growth factors which, in turn, could stimulate multiple cell types via paracrine and autocrine mechanisms to regulate their own expression.[11] Thus, redundant signaling pathways could be involved that might circumvent any therapeutic agent aimed at blocking a specific growth factor or cytokine. Therefore, given the complex nature of signal transduction, i.e., redundancies and cross talk between signaling pathways, absolute selectivity may not be desirable when the need arises to simultaneously inhibit multiple growth signals. Thus, within this context, we have embarked on a two-pronged strategy for the potential treatment of restenosis and cancer by attempting to (a) uncover broadly acting, nonselective small molecule inhibitors of the PDGFr, FGFr, and c-Src TKs, which might serve to overcome these redundancies in growth signaling, and (b) design selective inhibitors of these kinases to dissect out their contribution toward the above signaling pathways and their role in the expression of the disease phenotype.

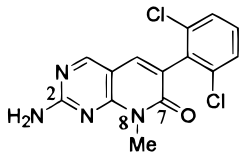
A number of inhibitors of protein TKs are now known with most of the work having been carried out toward suppression of the intracellular tyrosine phosphorylation of the EGFr TK. This work has been extensively covered in a number of recent reviews.[12−18] Most efforts to date from our laboratories draw from the EGFr inhibitor class and include highly potent and specific ATP-competitive inhibitors of the quinazoline[19] and pyrido[3,4-*d*]pyrimidine[20] classes. More recently, we have reported on the development of another ATP-competitive pyridopyrimidine class, the 6-arylpyrido[2,3-*d*]pyrimidines, that are active against a range of TKs, including EGFr, PDGFr, FGFr, and c-Src. We have profiled the initial screening lead in this area, urea compound **1**, which possesses low micromolar potency



1

toward inhibition of PDGFr, FGFr and c-Src TKs.[21] Subsequent SAR studies led to the synthesis of new analogues with improved potency, solubility, and bioavailability relative to the initial lead.[22]

A related structure, 2-amino-6-(2,6-dichlorophenyl)-pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (**2**), which was also derived from mass screening, showed a similar profile toward the above kinases with micromolar inhibitory activity. Although both **1** and **2** possess the same core



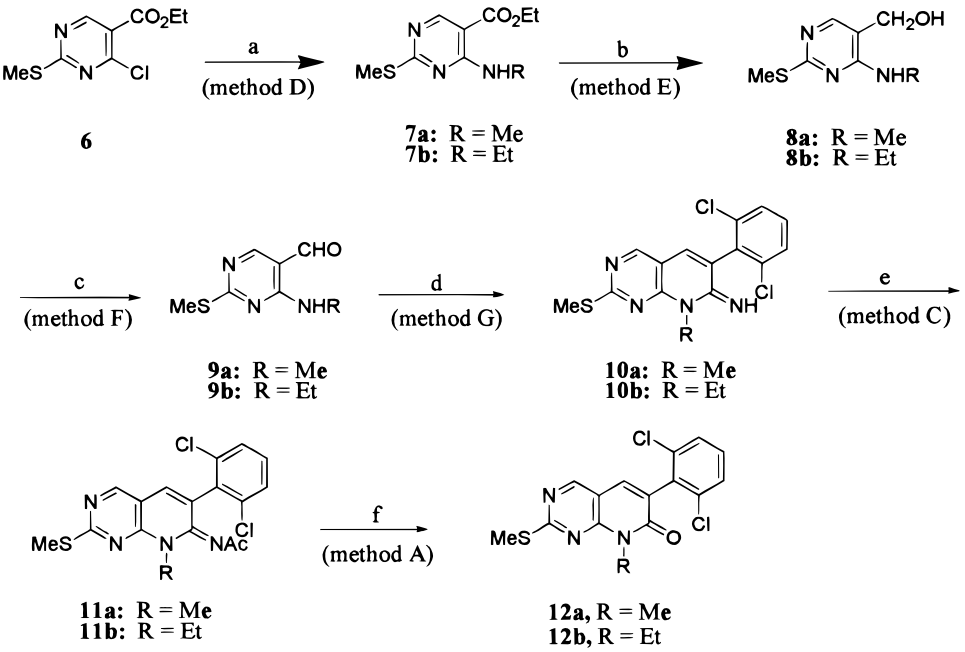
2

pyrido[2,3-*d*]pyrimidine ring system, we expected that a systematic SAR development of both leads would generate unique profiles with respect to inhibitory activity, bioavailability, metabolism, and selectivity.

In this paper, we present the synthesis and structure−activity relationships (SAR) toward several TKs for a series of analogues of **2** in which a wide range of alkyl and aryl substituents have been introduced at the C-2 nitrogen while keeping the substituent at the N-8 nitrogen either as methyl or ethyl. We also report on cellular effects, in vivo anticancer activity, animal bioavailability, and metabolism for selected compounds drawn from this series.[23]

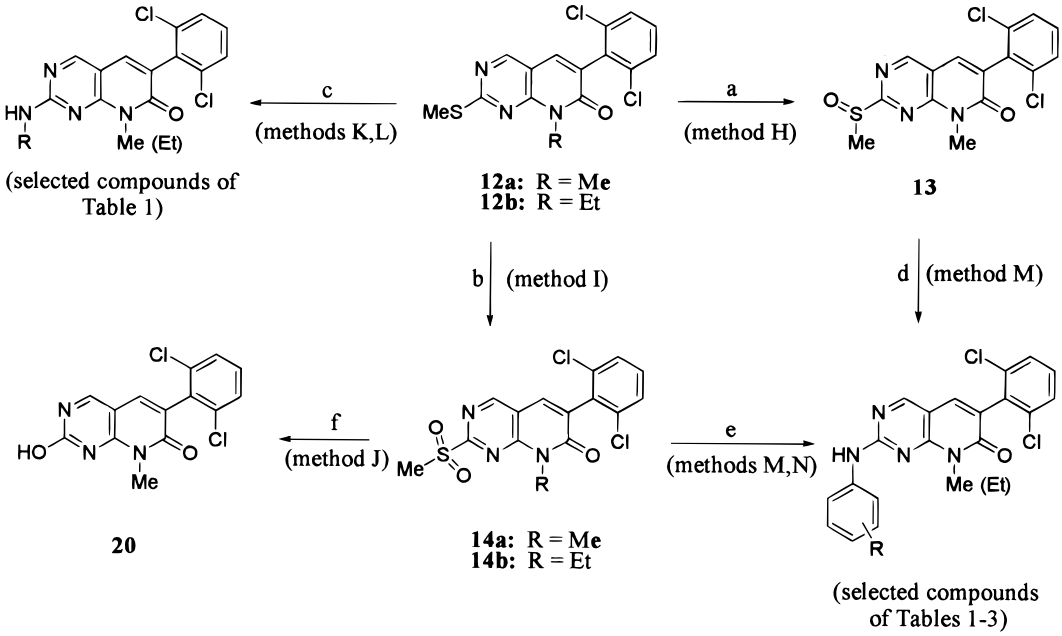
Chemistry. The synthesis of lead structure **2** and its C-2 acetamide derivative **5** is shown in Scheme 1. Hydrolysis of readily available 2,7-diaminopyridopyrimidine **3**[24] in refluxing concentrated HCl provided the pyrimidin-7-ol (**4**) in poor yield (19%) following recrystallization from DMF (method A). N-8 methylation to give **2** was achieved in 49% yield with NaH/iodomethane in DMF at 60−80 °C for 3 h (method B). Heating **2** in

Scheme 2[a]



[a] (a) Aqueous $MeNH_2$ or $EtNH_2$, NEt_3, THF; (b) LAH, THF; (c) MnO_2, $CHCl_3$; (d) (2,6-Cl_2)$PhCH_2CN$, K_2CO_3, DMF, 105 °C; (e) Ac_2O, reflux; (f) 6 N HCl, reflux.

Scheme 3[a]

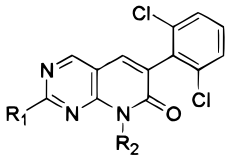


[a] (a) 3-Phenyl-2-(phenylsulfonyl)oxaziridine, $CHCl_3$, 25 °C; (b) *m*-CPBA, $CHCl_3$; (c) excess neat RNH_2, 135−210 °C; or excess RNH_2, DMF, 110−135 °C; (d) excess neat RNH_2, 135−250 °C; (e) excess neat RNH_2, 135−250 °C; or 1.8 equiv RNH_2, glacial HOAc, reflux; (f) aqueous HOAC, reflux.

refluxing acetic anhydride for 20 min (method C) then produced the C-2 acetamide **5** in 61% yield.

While suitable for making some simple C-2 amino analogues for initial SAR studies, the route outlined in Scheme 1 was too inefficient for generating a wide range of more elaborate congeners. A more expedient route for synthesizing C-2 amino analogues of **2** was developed wherein the amine could be introduced in the last step by displacement of either a C-2 methyl sulfide, sulfoxide, or sulfone leaving group (Schemes 2 and 3). Thus, as shown in Scheme 2, room temperature condensation of commercially available 5-pyrimidinecarboxylic acid ester **6** with either methyl- or ethylamine in THF provided **7a,b** (method D), which was reduced under standard LAH reduction conditions (method E) to give benzylic alcohol **8a,b** in high overall isolated yield. Oxidation with MnO_2 proceeded cleanly to the 5-pyrimidinecarboxaldehyde **9a,b** (method F), which was then condensed with 2,6-dichlorophenylacetonitrile in DMF at 105 °C in the presence of anhydrous K_2CO_3 as base to provide the 7-imino-pyridopyrimidine product **10a,b** in 50% yield (method G). The use of more strongly basic conditions was avoided to keep the somewhat base-sensitive methylthio group intact. Direct hydrolysis of

Table 1. Initial SAR Survey[a]



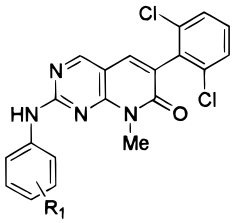
no.	R_1	R_2	method	IC_{50} (μM)[b] PDGFr	FGFr	c-Src	EGFr
2	NH_2	Me	B	4.9	1.3	0.26	5.6
5	NHAc	Me	C	5.2	5.8	4.2	nt[c]
19	Me	Me	*d*	>50	>50	>50	nt
20	OH	Me	J	>50	>50	>50	nt
21	NHMe	Me	L	6.2	2.5	0.75	7.5
22	NHEt	Me	L	4.0	0.96	0.59	4.5
23	NH-*i*-Pr	Me	L	3.2	0.70	1.6	>5
24	NH-*n*-Bu	Me	L	10.3	4.48	2.3	4.4
25	NH-*c*-Hex	Me	M	12.5	6.8	1.6	>50
26	$NH(CH_2)_2OH$	Me	L	6.5	1.66	0.33	>5
27	$NHCH_2Ph$	Me	L	23.8	14.0	10.4	>50
28	NMe_2	Me	K	>50	>50	>50	50
29	$NH(CH_2)_3NEt_2$	Me	A	8.9	13.9	0.96	1.4
30	$NH(CH_2)_3$Nmorph[e]	Me	L	6.8	1.41	0.53	>5
31	$NH(CH_2)_3$Npip(Me)[f]	Me	K	2.8	0.38	0.15	8.7
32	$NH(CH_2)_4$Npip(Me)[f]	Me	K	0.89	0.32	0.07	0.21
33	$NH(CH_2)_5$Npip(Me)[f]	Me	K	0.71	0.24	0.06	4.5
34	$NHCH_2$-2-pyridyl	Me	L	4.8	3.21	0.81	>5
35	$NHCH_2$-3-pyridyl	Me	L	6.6	3.15	3.2	>5
36	$NH(CH_2)_2$-2-pyridyl	Me	L	9.3	6.53	1.6	>5
37	$NH(CH_2)_5CO_2H$	Me	R	0.73	0.58	0.37	1.0
38	NHPh	Me	M	0.40	0.46	0.02	0.26
39	NHPh	Et	M	0.80	0.21	0.053	0.20
40	NH-3-pyridyl	Me	K	0.12	0.15	0.038	0.51
41	NH-3-pyridyl (6-OMe)	Me	M	0.40	0.54	0.077	1.3
42	NH-4-pyridyl	Me	M	0.10	0.19	0.010	0.91
43	NH-4-pyridyl	Et	M	0.10	0.11	0.012	0.49

[a] Inhibitory activity against PDGFr, FGFr, c-Src, and EGFr tyrosine kinases. [b] IC_{50} values for PDGFr, FGFr, c-Src, and EGFr tyrosine kinases were measured as previously described.[28] 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. [c] nt, not tested. [d] See ref 43. [e] Morpholin-1-yl derivative. [f] *N*-Methylpiperazin-1-yl derivative.

imine **10a,b** to the 7-oxo compound **12a,b** could not be achieved satisfactorily. However, when **10a,b** was first acetylated to the *N*-acetylimine **11a,b** (method C), then hydrolysis occurred rapidly in boiling 6 N hydrochloric acid (method A) or 10% sulfuric acid in dioxane to provide the key intermediate 2-methylthio-7-oxo-pyridopyrimidine derivative **12a,b**. As detailed in Scheme 3, oxidation of **12a,b** with 3-phenyl-2-(phenylsulfonyl)-oxaziridine[25] in chloroform provided the sulfoxide **13** in 70% yield (method H), whereas oxidation with 2 equiv of *m*-chloroperbenzoic acid under similar conditions gave the corresponding sulfone **14a,b** in 92% yield (method I). The sulfone **14a,b** was quite reactive and could readily be hydrolyzed to the 2-hydroxy compound **20** in refluxing aqueous acetic acid (method J).

The choice of intermediate **12**, **13**, or **14** for subsequent amine condensations was based on the relative reactivity of the leaving group and that of the amine to be introduced at the C-2 position. For example, condensations with more nucleophilic alkylamines were best carried out on sulfides **12 a,b**, whereas the less reactive arylamines were best condensed with the more reactive sulfoxide **13** or sulfone **14a,b**. Generally, the reaction conditions for amine displacement involved heating the sulfide, sulfoxide, or sulfone with a 20−100% excess of amine either neat (methods K, M) or diluted with a solvent (method L, DMF; method N, glacial acetic acid) at temperatures of 100−250 °C. Yields varied widely (17−86%) but generally ran in the 30−50% range. The 2-(alkylamino)- and 2-(arylamino)-pyrido[2,3-*d*]pyrimidin-7(8*H*)-ones that were synthesized utilizing these methods are listed in Tables 1−3.

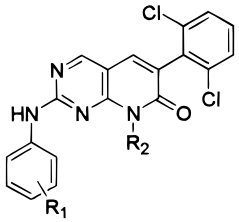
Table 2. Initial Exploration around Phenylamino at C-2[a]



no.	R_1	method	IC_{50} (μM) PDGFr	FGFr	c-Src	EGFr
38	H	M	0.40	0.46	0.02	0.26
44	4-Cl	M	2.6	3.0	0.14	>5
45	3-Br	M	3.1	2.4	0.06	0.67
46	4-Me	M	1.2	0.91	0.05	0.48
47	2-OMe	M	1.8	3.2	1.2	0.66
48	3-OMe	M	0.53	0.37	0.05	0.03
49	4-OMe	M	0.68	0.41	0.02	0.22
50	3-OH	M	0.41	0.18	0.02	0.15
51	4-OH	O	0.28	0.15	0.04	nt
52	3-CH_2OH	M	0.21	0.08	0.006	0.09
53	3-Me, 4-OMe	M	1.5	0.45	0.05	0.25
54	3,5-$(OMe)_2$	M	1.7	0.43	0.02	0.33
55	3-CO_2Et	M	1.7	1.5	0.014	nt
56	4-CO_2Et	M	21.4	33.6	0.08	>50
57	4-$(CH_2)_3CO_2Et$	M	4.2	2.6	0.30	0.70

[a] See footnotes in Table 1.

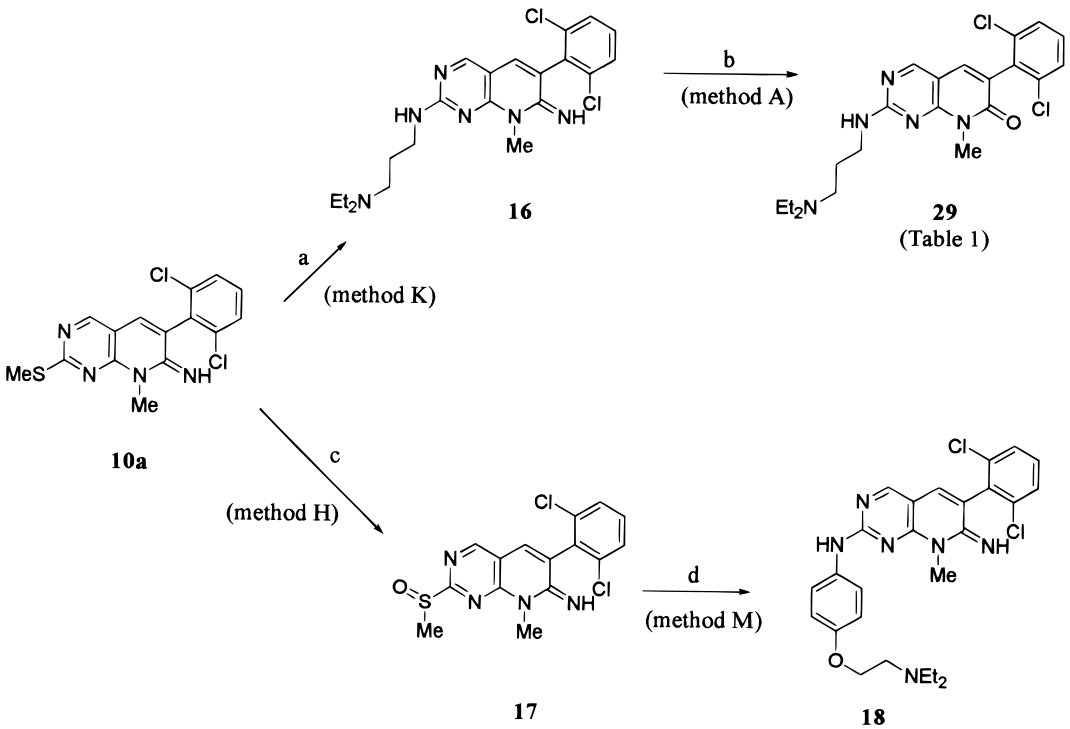
Table 3. 2-Phenylamino Compounds with Solubilizing Functionality at R_1[a]



				IC$_{50}$ (μM)			
no.	R_1	R_2	method	PDGFr	FGFr	c-Src	EGFr
58	4-O$(CH_2)_2NH_2$	Me	R	0.074	0.055	0.013	nt
59	4-O$(CH_2)_2NHEt$	Me	R	0.19	0.066	0.015	nt
60	4-O$(CH_2)_2NMe_2$	Me	Q	0.096	0.034	0.009	nt
61	4-O$(CH_2)_3NMe_2$	Me	Q	0.12	0.033	0.013	nt
62	3-O$(CH_2)_2NEt_2$	Me	P	0.103	0.032	0.009	nt
63	4-O$(CH_2)_2NEt_2$	Me	M,N	0.079	0.043	0.009	0.044
64	4-O$(CH_2)_2NEt_2$	Et	N	0.15	0.030	0.017	nt
65	4-O$(CH_2)_2N^+(Me)Et_2I^-$	Me	T	0.081	0.040	0.011	nt
66	4-O$(CH_2)_2N(O)Et_2$	Me	H	0.12	0.046	0.015	nt
67	4-O$(CH_2)_2$Npiper[b]	Me	Q	0.098	0.039	0.008	nt
68	4-Npip(Me)[c]	Me	M	0.11	0.028	0.010	nt
69	4-CH_2Npip(Me)[c]	Me	M	0.101	0.049	0.008	nt
70	3-CO_2H	Me	S	0.13	0.11	0.009	0.19
71	4-CH_2CO_2H	Me	S	0.072	0.061	0.010	0.22
72	4-$(CH_2)_3CO_2H$	Me	S	0.12	0.11	0.032	0.08

[a] See footnotes in Table 1. [b] Piperidin-1-yl derivative. [c] *N*-Methylpiperazin-1-yl derivative.

Scheme 4[a]

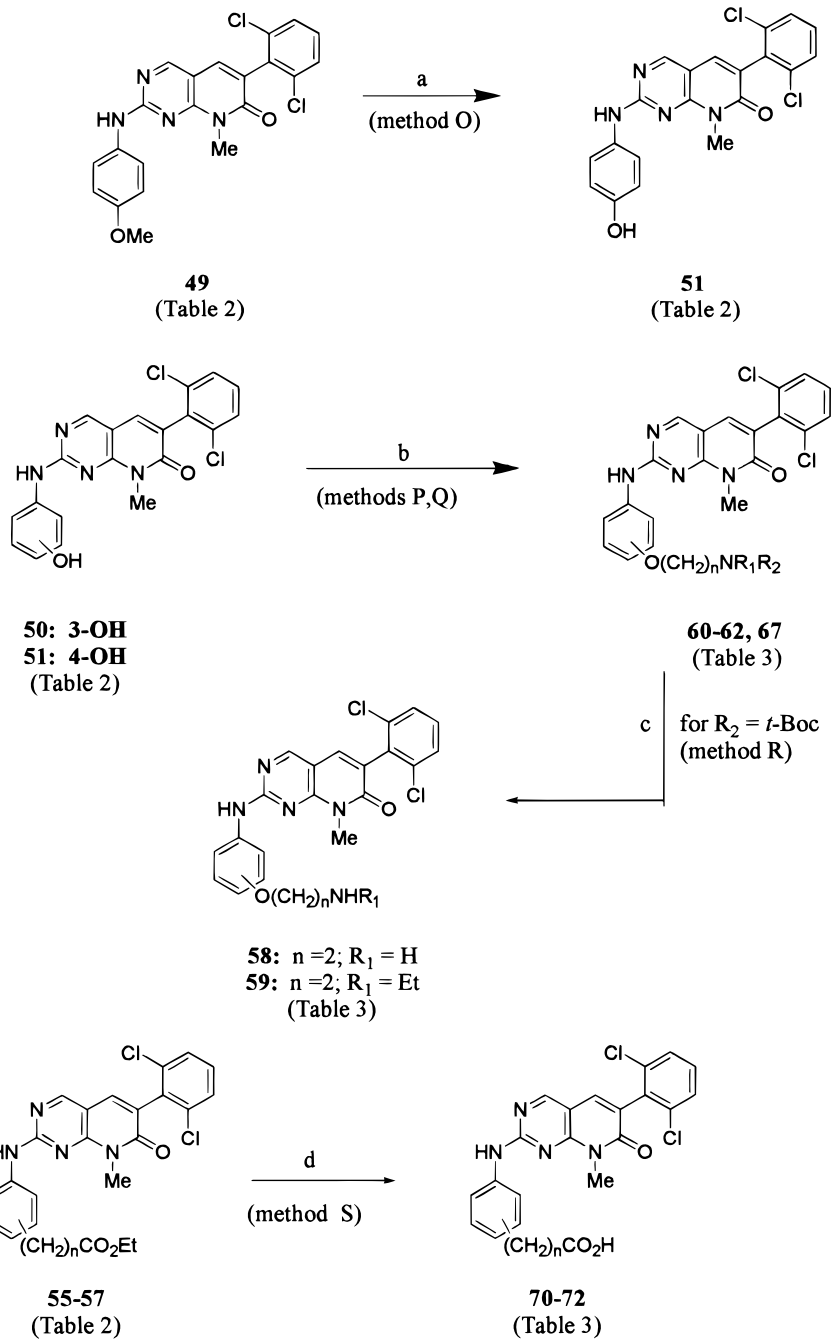


[a] (a) Excess neat $Et_2N(CH_2)_3NH_2$, 135 °C; (b) 6 N HCl, reflux; (c) 3-phenyl-2-(phenylsulfonyl)oxaziridine, $CHCl_3$, 25 °C; (d) excess neat 4-[$Et_2N(CH_2)_2O$]-Ph-NH_2, 160 °C.

The synthesis of compound **29** in Table 1 exemplifies yet another sequence toward making selected compounds within this series. This is shown in Scheme 4 and involves an ordering of the reactions different from that shown in Schemes 2 and 3. Thus, condensation of the 7-imino derivative **10a** with 3-diethylaminopropylamine at 135 °C gave adduct **16** in 85% yield (method K). In contrast to that of **10a,b**, strong acid hydrolysis of **16** could be achieved to give the 7-oxo-pyridopyrimidine **29** in 41% yield. In another permutation, imino derivative **10a** was oxidized to sulfoxide **17** (method H), which was then condensed with neat 4-(2-diethylaminoethoxy)aniline[26] (method M) to provide in low yield the imino product **18** for direct biological comparison to its 7-oxo congener **63** (vide infra).

Toward expanding the SAR around certain C-2 arylamino analogues of Tables 2 and 3, standard reactions were performed on a number of derivatives with reactive functionality appended to the aryl moiety. These are outlined in Scheme 5. Reaction of anisidine adduct **49**

Scheme 5[a]

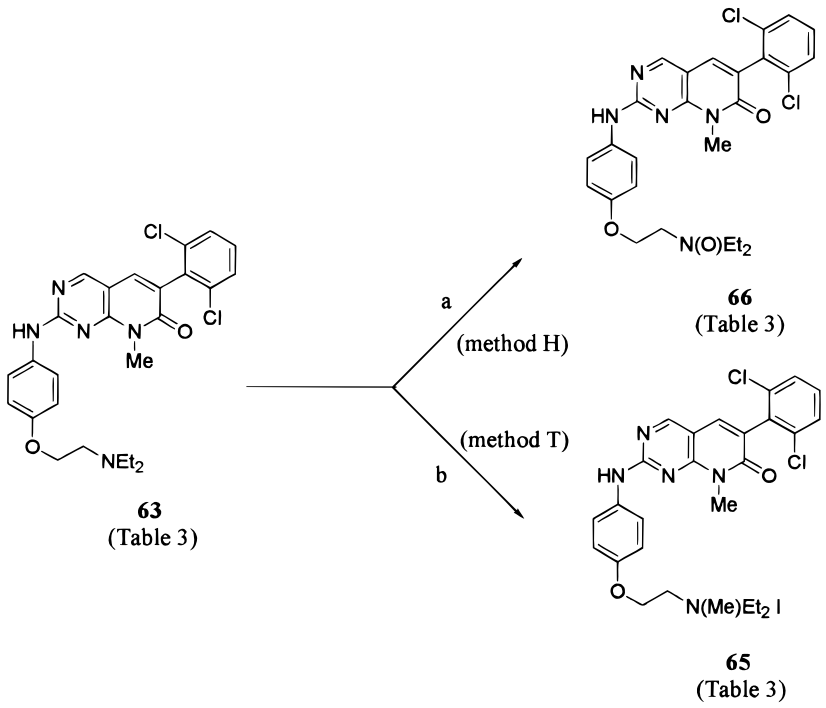


[a] (a) 48% HBr, propanoic acid, reflux; (b) $HO(CH_2)_nNR_1R_2$, PPh_3, DEAD, THF, 25 °C; or $Cl(CH_2)_nNR_1R_2$, K_2CO_3, DMF, reflux; (c) TFA/CH_2Cl_2 (1:4), 25 °C; (d) 2 N aqueous NaOH, reflux.

of Table 2 with 48% HBr in refluxing propanoic acid gave phenolic product **51** in 92% yield (method O). Homologation of phenolic derivatives **50** and **51** of Table 2 to the corresponding (dialkylamino)alkoxy derivatives **60**−**62** and **67** of Table 3 was achieved under Mitsunobu conditions from a precursor amino alcohol (method P) or by direct alkylation of the phenol with an appropriate aminoalkyl halide in DMF in the presence of powdered K_2CO_3 as the base (method Q). In the case where primary amino- or alkylaminoalkoxy chain extension was desired, installation was achieved under Mitsunobu conditions utilizing an *N*-*t*-Boc protected amino alcohol followed by standard TFA deprotection (method R). In this fashion, compounds **58** and **59** of Table 3 were synthesized. The TFA deprotection conditions were also utilized to generate compound **37** of Table 1 from its *tert*-butyl ester precursor. Last, the synthesis of C-2 arylamino derivatives substituted with terminal carboxylic acid moieties was achieved by standard alkaline hydrolysis of precursor esters (method S). Compounds **70**−**72** of Table 3 were made in this manner.

As part of our expanded evaluation of compound **63**, we were interested in synthesizing analogues with a fixed charge on the terminal basic amine moiety of the C-2 side chain. Two of these were synthesized as shown in Scheme 6, with the *N*-oxide **66** made to confirm its identity as a metabolite in rat studies (vide infra). The *N*-oxide **66** was made in 48% yield by oxidation of **63** with 3-phenyl-2-(phenylsulfonyl)oxaziridine[25] in chloroform (method H). The quaternary ammonium salt **65**

Scheme 6[a]



[a] (a) 3-Phenyl-2-(phenylsulfonyl)oxaziridine, $CHCl_3$, 25 °C; (b) CH_3I, CH_2Cl_2, 25 °C.

was derived in 87% yield from simple iodomethane alkylation of **63** in dichloromethane.

SAR vs Isolated Tyrosine Kinases. The structures of the C-2 substituted pyrido[2,3-*d*]pyrimidin-7-ones (**5**, **19**−**72**) synthesized for this study are shown in Tables 1−3. 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 by the avian c-Src TK. At least two dose−response curves were determined for each compound, and averaged IC_{50} values are listed.

Early work within this class of pyrido[2,3-*d*]pyrimidin-7(8*H*)-ones established that modifications at the C-2 position would be important toward generating compounds with good enzyme inhibitory activity. Therefore, almost all of the SAR studies reported herein involved variations of substituents at this position while maintaining the C-6 and N-8 substituents as 2,6-dichlorophenyl and methyl, respectively. This ultimately led to the discovery of compounds with medium to low nanomolar potency toward the PDGFr, FGFr, EGFr, and c-Src TKs. Further SAR development at the C-6 and N-8 positions has been extensive and ultimately lead to defined selectivity patterns amongst some of the TKs profiled herein. The results of these efforts will be the subject of future reports.

An initial SAR survey for compounds of this class built upon the lead structure **2** is shown in Table 1. Our plan was to optimize TK inhibitory activity by introducing various moieties at the C-2 position while retaining the methyl and, occasionally, the ethyl substituent at the N-8 position. Three types of C-2 substituent modifications were investigated for this initial survey, small neutral moieties (amide, **5**; alkyl, **19**; hydroxyl, **20**; nonbasic amines, **22**−**28**), aliphatic and alkaryl cationic moieties (polyamines, **29**−**33**; aminoalkylpyridyl, **34**−**36**), aliphatic anionic moieties (aminoalkylcarboxylic acid **37**), aromatic neutral moieties (anilines, **38**, and **39**), and weakly cationic moieties (aminopyridyl, **40**−**43**). The neutral substituents generally showed poorer potency against the TKs evaluated, especially for compounds without an amine substituent at C-2 (**19** and **20**), as well as for the tertiary amine analogue **28**, all of which were inactive. An evaluation of analogues derived from the addition of simple primary amines revealed that those with straight chain monoalkylamine substitution (**21**, **22, 24,** and **26**) or with branching chain substitution (**23**, **25**, and **27**) provided a potency greater than that of **19** and **20** toward the kinases of Table 1, but this was still generally inferior to that of lead structure **2**. These analogues also revealed the essential requirement of a 2-NHR substitution pattern, which is shown by molecular modeling to form a hydrogen bond to the carbonyl oxygen of Met341 in the c-Src TK (or the corresponding amino acid in FGFr, EGFr, and PDGFr TKs).[27] It should be pointed out that this nitrogen within this template is very weakly basic. Thus, analogues with the small, neutral substituents tended to be quite insoluble in water and would not form acid addition salts. The introduction of a single distal basic functionality onto the aliphatic chain (**29**, **30**) improved aqueous solubility but did not impart greater potency toward the TKs evaluated. However, the addition of a second basic center in the *N*-methylpiperazine analogues **31**−**33** improved potency toward each of the PDGFr, FGFr, and c-Src TKs, especially as the aliphatic chain length from the proximal to the distal nitrogen was increased, resulting in overall potencies in the submicromolar range. In contrast, distal functionality drawn from weakly basic pyridyl moieties (**34**−**36**) abolished this improvement, whereas introduction of a side chain aliphatic acid function (**37**) restored it.

A marked improvement in potency relative to the lead **2** and its aliphatic homologues discussed above was

realized in analogues incorporating arylamino moieties at the C-2 position. Thus, the simplest of *N*-aryl derivatives (**38**), derived from aniline, displayed a 3−10-fold enhancement in potency toward the PDGFr (IC_{50} 0.40 μM), FGFr (IC_{50} 0.46 μM), and c-Src (IC_{50} 0.02 μM) TKs relative to **2**, and also showed good potency (IC_{50} 0.26 μM) toward the EGFr TK. This same general pattern of enhanced activity was retained in the 3- and 4-pyridyl congeners **40**−**42**. Compounds **39** and **43**, each with an N-8 ethyl moiety, were synthesized to determine the effect on activity by a simple substituent change at this position of the bicyclic template. These compounds showed an essentially equivalent activity profile toward the four kinases as had been observed for their N-8 methyl congeners **38** and **42**, respectively.

In summary, the initial SAR survey shown in Table 1 revealed that while C-2 amino analogues with chain extended aliphatic dibasic moieties improved aqueous solubility and potency relative to lead structure **2**, the introduction of simple arylamino moieties effected potency much more dramatically. Since there appeared to be little, if any, increase in potency for N-8 ethyl vs methyl substitution, further SAR studies within this series were continued with methyl at this position.

Having established the essential requirement of C-2 arylamino substitution, we next synthesized a series of analogues with simple positional substituents around the anilino ring to determine the effect on the SAR. These compounds are listed in Table 2. Relative to **38**, monosubstitution of the anilino ring with simple electron donating or withdrawing substituents (**44**−**49**) generally decreased potency toward the PDGFr, FGFr, and EGFr TKs, whereas there was little effect on c-Src except for the 2′-OMe compound **47**, which showed much poorer activity toward all of these kinases. We have observed this trend for other anilino congeners substituted in the 2′-position (unpublished data). Disubstituted anilino compounds (**53** and **54**) and monosubstituted compounds with larger substituents in the 3′- or 4′-positions (**55**−**57**) showed the same general pattern of TK inhibition toward the PDGFr, FGFr, and c-Src kinases relative to **38**. On the other hand, analogues possessing either phenolic (**50** and **51**) or hydroxymethylene (**52**) functionality at these positions displayed potency generally equivalent to or better than **38** toward the kinases profiled, especially **52**, which showed modest c-Src selectivity with IC_{50} values of 0.21μM (PDGFr), 0.08 μM (FGFr), 0.09 μM (EGFr), and 0.006 μM (c-Src).

In summary, the initial SAR exploration for C-2 arylamino analogues with small functional groups in the anilino ring suggested that substitution was tolerated at the 3′- and 4′-positions. The marked enhancement in potency for **52**, possessing improved aqueous solubility over the parent *N*-aryl compound **38**, argued for additional SAR development around the 3′- and 4′-positions with analogues that would impart even greater aqueous solubility. Compounds incorporating various cationic (amine) or anionic (acid) moieties to test this concept are delineated in Table 3.

Compounds **58**-**63** were made to explore a homologous series of compounds with different aminoalkoxy moieties appended to the 3′- or 4′-position of the aniline ring. In general, each of these compounds displayed markedly enhanced potency toward the four TKs profiled, especially toward PDGFr and FGFr. The effect of increased basicity of the terminal amine moiety (compare NH_2 **58**, vs NHEt **59**, vs NEt_2 **63**) was minimal, although **63** displayed a better potency profile overall. The same could be said for compounds with terminal amine heads of comparable basicity but different size (compare NMe_2 **60**, vs NEt_2 **63**, vs piperidino **67**). The 3′-positional isomer (**62**) and the N-8 ethyl congener (**64**) of compound **63** displayed an essentially equivalent profile toward PDGFr, FGFr, and c-Src TKs. Similarly, the enzymatic profile of congeners of **63** with charged heads (*N*-methylammonium **65**, *N*-oxide **66**) was essentially identical to the parent compound. While not extensively explored, a comparison of **60** vs **61** suggests that there is tolerance for increasing chain length, which is consistent with predictions based on molecular modeling.[27] The substitution of the 3′-position with a nitrogen- or carbon-attached basic moiety (**68** and **69**, respectively) also provided analogues with a potency profile similar to the compounds discussed above. Last, for a series of 3′- or 4′-substituents terminating with a carboxylic acid moiety (**70**−**72**), excellent potency was displayed toward each of the four kinases profiled with the 4′-substituted acetic acid analogue **71** showing the best profile overall.

In summary, relative to parent *N*-aryl compound **38**, substitution of the anilino moiety with a variety of anionic or cationic aqueous solubilizing moieties markedly improved potency toward the four TKs profiled, especially toward PDGFr and FGFr.

Last, we looked at the TK inhibitory activity of a few selected 7-imino intermediates within this series. When assayed against the PDGFr and FGFr TKs, imine **16** (Scheme 4) displayed IC_{50} >50 μM and 22 μM, respectively, whereas the derived 7-keto congener **29** showed corresponding values of 8.9 μM and 13.9 μM. Similarly, the imine **18** (Scheme 4) showed inhibitory activity toward the PDGFr, FGFr, and c-Src TKs (IC_{50} values of 0.14 μM, 0.30 μM and 0.022 μM, respectively) weaker than that of the derived 7-keto congener **63**.

Within the series shown in Tables 1−3, **63** showed excellent activity against all TKs surveyed with IC_{50} values of 0.079 μM (PDGFr), 0.043 μM (FGFr), 0.044 μM (EGFr), and 0.009 μM (c-Src). This compound was isolated as a dihydrochloride salt, which rendered it water soluble (∼10 mg/mL) and was easily administered in in vivo studies. On the basis of its excellent potency toward the kinases profiled, as well as its physiochemical properties, it was selected for additional studies in cells and in animals. We also profiled other closely related congeners of **63** in selected cellular and in vivo studies.

Enzyme Kinetics. Biochemical characterization of kinase inhibition by selected compounds of this series shows them to be competitive with ATP binding. In a recent study,[28] Lineweaver−Burke plots have been published for the inhibition of PDGFr-β, FGFr-1, and EGFr TKs by compound **63**. A competitive mechanism of inhibition was shown against all three kinases, with inhibitory constants (K_i) of 139 nM, 54 nM, and 105 nM, respectively.

Cellular Studies. Selected compounds from this series were further profiled for inhibitory effects in a number of cell systems. Four compounds (**38** and **42** in

Table 4. Effect of 2-Aminopyrido[2,3-*d*]pyrimidin-7(8*H*)-one TK Inhibitors on C6 Proliferation and Phosphorylation of the PDGF Receptor

	IC_{50} (μM)			
no.	inhibition of PDGFr autophosphorylation[a]	C6 proliferation[b]	soft agar clonogenic contin. exposure[c]	soft agar clonogenic compd removed[d]
38	0.038	1.8	1.1	2.9
42	0.68	1.1	0.40	6.9
62	0.06	0.84	0.44	2.79
63	0.005	0.34	0.12	1.80
64	0.001	0.35	0.12	0.40

[a] C6 cells were serum-starved for 24 h, treated for 2 h with various concentrations of the indicated compound, and stimulated with 25 ng/mL of PDGF for 5 min. Antiphosphotyrosine Western blots were performed as described in the Experimental Section. [b] C6 cells were treated for 4 days with various concentrations of the specific compound in 96-well plates, and the effect of each compound was determined using sulforhodamine B staining.[37] Results are the mean of two experiments, each performed in triplicate. [c] C6 cells were treated for 3 days with various concentrations of the specific compound in 6-well tissue culture plates and cloned in soft agar in the continued presence of the compound. Results are the mean of two experiments, each performed in duplicate. [d] C6 cells were treated as above,[c] but were cloned in soft agar in the absence of compound. Results are the mean of two experiments, each performed in duplicate.

Table 2 and **63** and **65** in Table 3) were tested for their effect on PDGF-mediated receptor autophosphorylation in rat aortic vascular smooth muscle cells (RAVSMC). Stimulation of the PDGFr 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 kDa tyrosine phosphorylated receptor protein after Western blotting with an antiphosphotyrosine antibody. In this assay, three of these compounds potently inhibited autophosphorylation with IC_{50} values of 0.016 μM (**38**), 0.013 μM (**42**), and 0.026 μM (**63**). Not surprisingly, compound **65**, with a fixed charge on the quaternary ammonium salt moiety of the C-2 arylamino side chain, was inactive in this assay (6.5% inhibition at 0.30 μM), suggesting that it does not cross the cell membrane. The RAVSMC data do not correlate with inhibitory effects of these compounds on substrate phosphorylation by isolated PDGFr (Tables 1 and 3) where the rank order of potency is **63** $\approx$ **65** $\approx$ **42** > **38**.

Selected compounds possessing simple C-2 arylamino substituents (**38** and **42**) and arylamino substituents with solubilizing appendages (**62**−**64**) were also evaluated for their inhibitory effects on the C6 cell line, which is PDGF-dependent (Table 4), and the NIH3T3 and NIH3T3/PDGF-transfected (Table 5) cell lines. For the five compounds shown in Table 4, compounds **63** and **64** showed potent inhibition of PDGFr autophosphorylation in the rat C6 glioma cell line with IC_{50} values of 0.005 μM and 0.001 μM, respectively. These data do not correlate with the PDGFr enzyme inhibition for this series of compounds drawn from Tables 1 and 3. In contrast, the effect on cell proliferation against C6 cells more directly correlates with enzyme data with the greatest potency associated with **63** (IC_{50} 0.34 μM) and **64** (IC_{50} 0.35 μM). A similar pattern was observed for the inhibition of cell growth in soft agar, both with continuous inhibitor exposure and with a 3-day treatment followed by inhibitor removal.

Table 5. Effect of 2-Aminopyrido[2,3-*d*]pyrimidin-7(8*H*)-one TK Inhibitors on NIH3T3 and PDGF-Transfected NIH3T3 (NIH3T3/PDGF) Proliferation and Phosphorylation of the PDGF Receptor

	IC_{50} (μM)			
no.	inhibition of PDGFr autophosphorylation[a]	NIH3T3 proliferation[b]	soft agar clonogenic contin. exposure[c]	soft agar clonogenic compd removed[d]
38	0.020	0.105	0.37	0.377
42	nt[e]	0.104	0.306	0.350
62	nt	0.147	0.017	nt
63	0.0018	0.35	0.0011	0.01
64	0.0017	0.41	0.0015	0.017

[a] NIH3T3/PDGF cells were serum-starved for 24 h, treated for 2 h with various concentrations of the indicated compound, and stimulated with 25 ng/mL of PDGF for 5 min. Antiphosphotyrosine Western blots were performed as described in the Experimental Section. [b] NIH3T3 cells were treated for 4 days with various concentrations of the specific compound in 96-well plates, and the effect of each compound was determined using sulforhodamine B staining.[37] Results are the mean of two experiments, each performed in triplicate. [c] NIH3T3/PDGF cells were treated for 3 days with various concentrations of the specific compound in 6-well tissue culture plates and cloned in soft agar in the continued presence of the compound. Results are the mean of two experiments, each performed in duplicate. [d] NIH3T3/PDGF cells were treated as above,[c] but were cloned in soft agar in the absence of compound. Results are the mean of two experiments, each performed in duplicate. [e] nt, not tested.

Within the series tested against the NIH3T3/PDGF cell line (Table 5), compounds **63** and **64** were again the most potent toward inhibition of PDGFr autophosphorylation, each with an IC_{50} of ~0.002 μM. Surprisingly, both compounds, as well as positional isomer **62**, were less potent toward inhibiting cell proliferation of NIH3T3 cells than were the simple C-2 arylamino compounds **38** and **42**, each with an IC_{50} of ~0.10 μM. However, in soft agar, compounds **63** and **64** were again the most potent, both with continuous inhibitor exposure (IC_{50} of ~0.001 μM), and with a 3-day treatment followed by inhibitor removal (IC_{50} of ~0.01 μM).

In summary, for the data shown in Tables 4 and 5, compounds **63** and **64** behaved similarly and inhibited proliferation of both C6 and NIH3T3 cells with similar IC_{50} values. Also, in general, both compounds were the most potent compounds tested among this limited series of analogues. The NIH3T3 and the NIH3T3/PDGF-transfected cell lines were generally more sensitive than C6 cells to all of the compounds tested in cellular assays. However, no significant correlation exists between inhibition of PDGF receptor phosphorylation and inhibition of cell growth either in soft agar or on a plastic substratum.

We have also previously disclosed data for certain compounds of this series in a number of human colon adenocarcinoma cell lines that overexpress the c-Src TK.[29] For example, compound **63** caused growth delay on plastic with IC_{50} values of 1.8 μM (HT-29), 0.78 μM (HCT-8), and 0.33 μM (SW-620). We have also shown that **63** exhibits specificity for inhibition of cellular c-Src compared to EGFr kinase in a fibroblast cell line that overexpresses both of these kinases.[30] This is in marked contrast to enzyme data (Table 3) where both of these kinases are potently inhibited by **63** with IC_{50} values of 0.009 μM (c-Src) and 0.044 μM (EGFr).

In Vivo Studies. Within this series, four C-2 arylamino inhibitors were evaluated against a panel of

Table 6. Anticancer Activity of 2-Aminopyrido[2,3-*d*]pyrimidin-7(8*H*)-one TK Inhibitors against Human Tumor Xenografts, a Mouse Fibroblast Cell Line Transfected with PDGF, and the C6 Rat Glioma Cell Line[a]

tumor	no.	dose (mg/kg)	schedule	weight change (g)	T/C (%)[b]	T−C (days)[c]	net cell kill (log_{10})[d]
A431	**63**	20	po days 7−21	−3.1	62	3.7	−0.5
	64	5	po days 7−21	−2.7	96	3.3	−0.6
C6 glioma	**38**	100 hdt[e]	po days 1−15	+		−1.1	
	62	50 hdt	po days 1−15	+		−2.0	
	63	10	po days 1−15	+	83	0.4	−1.0
	63	5	po bid days 1−15	−0.1	76	0.3	−0.8
	64	10 hdt	po days 1−15	−1.2	56	3.0	−0.9
Colo 205	**38**	200 hdt	ip days 14−28	−2.0		−3.2	
	62	40 hdt	po days 9−23	+	67	6.8	−0.4
	63	10	po days 14−28	−4.0	86	0.6	−0.7
	64	8.9 hdt	po days 9−23	+	57	10.3	−0.2
HT-29	**38**	200 hdt	ip days 15−29	−2.0	86	1.1	−0.5
	62	44 hdt	po days 15−29	−2.0		−2.5	
	63	10.2	po days 15−29	0	56	9.6	−0.2
	64	8.3 hdt	po days 15−29	+		−4.8	
MCF-7	**62**	50 hdt	po days 1−15	+	89	0.8	−0.6
	63	12	po days 1−15	+	80	1.1	−0.6
	64	5	po days 1−15	+	51	18.3	+0.2
PDGF	**38**	100 hdt	ip days 1−15	+	25	1.7	−2.1
	62	50 hdt	po days 1−15	−1.6		−0.4	
	63	15	po days 1−15	−0.4	59	2.2	−1.8
	63	9.5	po bid days 1−15	−4.1	56	3.3	−1.6
	64	12	po days 1−15	−2.6	13	5.9	−1.2
SK−OV-3	**38**	200 hdt	ip days 20−34	−2.0		−0.4	
	62	40 hdt	po days 15−29	−2.0		−0.4	
	63	20.3	po days 20−34	−4.0	50	10.6	−0.1
	64	8.9 hdt	po days 15−29	+	106	−0.8	

[a] Tumor fragments were implanted sc into the right axilla of mice on day 0. The maximum tolerated dose ($^2LD_{10}$) from a complete dose response is shown unless otherwise indicated. [b] Ratio of median treated tumor mass (mg)/median control tumor mass × 100%. Value listed is for the last therapy day. [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. [e] hdt, highest dose tested.

seven xenograft tumor model systems in nude mice (Table 6). These models were selected on the basis of known and/or inferred dependence on the EGFr family, PDGFr receptor, and c-Src. The C-2 anilino analogue **38** (Table 1) and compound **62** (Table 3), each representative of congeners with a simple C-2 arylamino or arylamino substituted with a solubilizing amine side chain, were relatively ineffective against all the models tested as reflected by tumor growth delays of less than 6.5 days. In striking contrast, compound **63**, the 4′-positional isomer of **62**, produced a tumor growth delay of 10.6 days against the relatively refractory SK-OV-3 ovarian xenograft. The −0.1 net log tumor cell kill generated against this model indicates that the treated tumors grew very little during therapy. Similarly, Colo 205 and MCF-7 tumors grew very little when treated with compound **64**, the N-8 ethyl congener of **63**, as reflected in tumor growth delays of approximately 10 and 18 days, respectively, and net log tumor cell kills near zero. The total lack of substantial therapeutic effects with any of the compounds against the PDGF-transfected fibroblast line may indicate that this model, although producing PDGF, does not depend on it for growth in vivo.

Pharmacokinetics, Oral Bioavailability, and Dose Proportionality Studies in Rats. Studies were conducted in rats to characterize the pharmacokinetics, oral bioavailability, and dose proportionality following administration of **63**. In the first pharmacokinetic bioavailability study, a single 10 mg/kg IV bolus of compound **63** was administered to male Wistar rats. Following drug administration, plasma concentrations of **63** declined in a biexponential manner (Figure 2, Supporting Information). Compound **63** was extensively distributed in rats, and systemic plasma clearance was high relative to liver blood flow. The mean terminal elimination half-life was 2.91 h (Table 7; Supporting Information).

In a follow-up study, dose proportionality of **63** was assessed following a 10, 30, or 100 mg/kg single oral gavage dose in rats (Figure 2, Supporting Information). The C_{max} and AUC increased with increasing dose. The time for C_{max} to occur (t_{max}) was similar for all three doses with a mean of 3 to 4 h (Table 7, Supporting Information). Plasma elimination for the 30 and 100 mg/kg oral doses was longer ($t_{1/2}$ of 5.97 h and 7.69 h, respectively) than observed following the 10 mg/kg IV dose (2.91 h). Comparing the dose adjusted AUC (0−∞) for the 10 mg/kg IV dose in the first study to the dose adjusted AUC (0−∞) for the 30 mg/kg oral dose, one can estimate the absolute oral bioavailability of **63** to be approximately 67%.

Finally, two peaks, which eluted before and after **63**, were observed in rat sample HPLC chromatograms. The peaks were collected, and their identities were confirmed by mass spectrometry and synthesis to be the *N*-oxide **66** (late eluting peak) and the primary amine **58** (early eluting peak). If one assumes that the UV absorptivity of the metabolites is similar to that of **63**, then plasma concentrations may be estimated. Such an analysis was conducted on the 30 mg/kg oral gavage dose of compound **63**, as shown in Figure 1. The *N*-oxide metabolite appeared to peak within 2 to 3 h and then to decline rapidly with an estimated $t_{1/2}$ of approximately 4.5 h. The amine metabolite formed slowly and displayed a long terminal elimination $t_{1/2}$. Plasma concentrations were still increasing at the last sample collected (24 h post dose). A metabolic pathway is

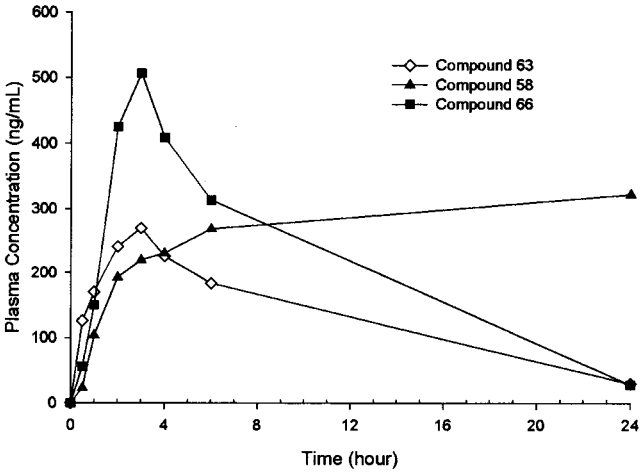


Figure 1. Mean plasma concentration−time profiles for compound **63** and major metabolites in rats following a single 30 mg/kg oral gavage dose.

proposed in which **63** initially converts to the *N*-oxide (**66**) then sequential metabolism results in the formation of the primary amine (**58**) as a final metabolite.

Conclusions

An efficient route has been developed that enables the synthesis of a wide variety of 2-substituted aminopyrido-[2,3-*d*]pyrimidin-7(8*H*)-ones and can be applied to analogues with variable substitution at the C-2 and N-8 positions of the template. The SAR results above show that compounds of this series of ATP-competitive inhibitors profiled herein display potent activity toward a range of TKs with modest selectivity toward c-Src. The overall SAR results in Tables 1−3 teach that (a) NH-aryl substitution is preferred to NH-aliphatic substitution at the C-2 position; (b) the installation of functionality in the 3′- or 4′-positions of the C-2 arylamino group has an enhancing effect on potency, especially for compounds with polar substituents or compounds with terminal moieties capable of holding a charge at physiological pH; and (c) for compounds with a constant moiety at C-2, substitution of the N-8 position with methyl or ethyl leads to essentially equivalent potency across the spectrum of kinases assayed.

Profiling selected C-2 arylamino compounds from this series for inhibitory effects in a number of functional assays shows them to have submicromolar to low nanomolar potency. This has been shown in cell lines (RAVSMC, C6 rat glioma cells, NIH3T3, and NIH3T3/PDGF-transfected cell lines) where processes are driven by the growth factor PDGF or its cognate receptor, as well as in cell lines that overexpress the nonreceptor TK c-Src (HT-29, HCT-8, and SW-620 colon adenocarcinoma). While there were variable levels of sensitivity toward these cell lines by the compounds tested, no significant correlation existed between inhibition of these processes and the corresponding isolated TKs. For several of the compounds evaluated in the cellular assays above, inhibition of PDGFr autophosphorylation occurs at concentrations lower than those for PDGFr TK inhibition. This raises a question of how these compounds actually suppress autophosphorylation. Is this mediated through PDGFr, or are these compounds simply toxic to cells? For example, the IC_{50} of **63** for inhibition of PDGFr autophosphorylation is 3- to 45-fold lower than that for inhibition of the PDGFr TK in the three cell lines profiled. The reasons for this disparity remain unclear at this time. However, we have previously demonstrated that RAVSMCs resume normal growth and remain viable after 3 days of treatment with **63** at a concentration exceeding 40 times the IC_{50} for inhibition of PDGFr autophosphorylation.[28] Therefore, it is unlikely that this enzyme vs cellular disparity is related to cellular toxicity. A more plausible explanation may involve the simultaneous inhibition of the PDGFr TK along with other kinases (e.g., FGFr, EGFr, and c-Src TKs as shown in Table 3) by this broadly active TK inhibitor. Moreover, it has recently been shown that phosphorylation of Tyr^{934} in the kinase domain of the PDGFr-β by c-Src modulates cellular activity.[31] Thus, the ability of **63** to block both PDGFr-β autophosphorylation and c-Src kinase phosphorylation of the PDGFr-β could possibly lead to enhanced cellular efficacy.

In in vivo testing, only compounds **63** and **64** displayed meaningful antitumor activity among the four compounds evaluated. In this context, meaningful activity is defined as no net tumor growth during therapy, i.e., net tumor cell kill values of −0.3 to +0.3. For the two active compounds, each was active only in selected models. Compound **63** was active against the SK-OV-3 and HT-29 tumors, while **64** was active against the Colo 205 and MCF-7 lines. Considering that this series of compounds is active against several TKs in vitro, it was somewhat surprising that each of the active compounds was effective only against a limited number of tumor models. This could be attributed to differences in their kinase inhibition profiles, or the data could indicate that the tumors in this panel are not particularly sensitive to this class of compounds. Furthermore, it is not known which growth factor or factors actually drive growth in these models, which complicates the mechanistic picture for tumor inhibition. There is also the possibility that these agents may be exerting most of their in vivo antitumor effects through some unknown mechanism unrelated to kinase inhibition. However, this is unlikely given the potent kinase inhibition of these compounds and their ability to potently inhibit growth factor receptor autophosphorylation in cells. Doses employed in our in vivo studies were tolerated with no overt signs of drug toxicity, and for all the test animals weight loss was <10% suggesting that antitumor effects are not the result of nonspecific drug toxicity. Inhibitor dosing and scheduling were based on the aforementioned pharmacokinetic data in rats (67% oral bioavailability; $t_{1/2}$ of 6 h for plasma elimination after a single 30 mg/kg/po dose) extrapolated to the mouse, which presumably afforded blood plasma levels well in excess of IC_{50}s for inhibition of targeted kinases. However, neither plasma nor tissue levels of the inhibitors were measured in these experiments. Despite these caveats, the data do demonstrate that the aqueous soluble, broadly active TK inhibitors of this class are active in vivo on a daily peroral administration schedule.

In summary, we have disclosed a novel series of ATP-competitive 2-substituted aminopyrido[2,3-*d*]pyrimidin-7(8*H*)-one inhibitors. Extensive SAR studies were

developed from the initial screen lead template (**2**), which have led to marked improvements in potency, solubility, and bioavailability relative to that of the parent compound. Further evaluation of selected members within this series have shown suppression of cellular processes in a number of cell lines mediated by PDGF or c-Src and significant tumor growth delays in in vivo studies against mouse xenografts with known and/or inferred dependence on the EGFr, PDGFr, and c-Src TKs. Additional in vivo studies in other appropriate tumor models and in animal models of restenosis and angiogenesis are in progress for **63** and related congeners to further assess the potential of a tyrosine kinase inhibitor within this series for advancement to clinical trials.

Experimental Section

Melting points were determined with a Thomas-Hoover capillary melting point apparatus or a MEL-TEMP melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were measured at 400 MHz using a Varian Unity 400 mHz spectrometer. Chemical shifts are reported as δ values (parts per million) downfield from internal Me_4Si. The following abbreviations are used to describe peak patterns when appropriate: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet. Chemical ionization mass spectra (CIMS) were recorded on a VG Trio 2 mass spectrometer instrument using a reagent gas of 1% NH_3 in CH_4. Atmospheric pressure chemical ionization mass spectra (APCIMS) were recorded using a VG Trio 2000 mass spectrometer in a matrix of MeOH/CH_3CN/DMSO. Column chromatography was carried out in the flash mode utilizing E. Merck 230−400 mesh silica gel. Analytical TLC was carried out on E. Merck silica gel 60 F_{254} plates with detection by UV light. Combustion analyses were determined by Robertson Microlit Laboratories, Inc., Madison, NJ. Fractional moles of water or organic solvents were sometimes retained in analytical samples after drying in vacuo (0.2 mmHg), and the presence of solvent in analytical samples was confirmed by ^{1}H NMR when possible. Following normal workup procedures, organic extracts were dried over anhydrous Na_2SO_4 or $MgSO_4$ prior to concentration.

Method A. 2-Amino-6-(2,6-dichlorophenyl)pyrido[2,3-*d*]pyrimidin-7-ol (4). A solution of 30.6 g (0.10 mol) of 2,7-diamino-6-(2,6-dichlorophenyl)pyrido[2,3-*d*]pyrimidine (**3**)[24] in 200 mL of concentrated HCl was heated at reflux for 24 h. The reaction mixture was cooled to room temperature, and the precipitated solids were collected, washed with H_2O, and dried to give 16.5 g of crude product. The filtrate was refluxed for another 24 h and upon cooling yielded an additional 8.8 g of product. The two crops were combined and recrystallized from DMF. The solids were washed with Et_2O and dried to afford 5.9 g (19%) of **4:** mp 410 °C dec; ^{1}H NMR (DMSO-d_6) δ 7.26 (br s, 2H), 7.43 (t, J = 8 Hz, 1H), 7.55 (d, J = 8 Hz, 2H), 7.74 (s, 1H), 8.60 (s, 1H); APCIMS m/z 307 (M^+). Anal. ($C_{13}H_8Cl_2N_4O$) C, H, N.

6-(2,6-Dichlorophenyl)-8-methyl-2-methylsulfanyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (12a). A solution of 49.0 g (0.125 mol) of iminoacetate **11a** in 325 mL of 6 N aqueous HCl was heated with stirring to the boiling point wherein a precipitate formed immediately. The thick mixture was heated an additional 2 min, cooled, and filtered. The cake was washed well with H_2O, 2-propanol, and Et_2O and then dried to give 42.2 g (96%) of **12a**: mp 249−251 °C; ^{1}H NMR (DMSO-d_6) δ 2.64 (s, 3H), 3.69 (s, 3H), 7.48 (t, J = 8 Hz, 1H), 7.61 (d, J = 8 Hz, 2H), 8.07 (s, 1H), 8.95 (s, 1H); CIMS m/z 352 (M^+). Anal. ($C_{15}H_{11}Cl_2N_3OS\cdot1.4H_2O$) C, N; H, calcd 3.57, found 3.12.

[6-(2,6-Dichlorophenyl)-8-methyl-7-oxo-7,8-dihydropyrido-[2,3-*d*]pyrimidin-2-yl]-(3-diethylaminopropyl)amine (29). A solution of 0.11 g (0.25 mmol) of imine **16** in 5 mL of 6 N aqueous HCl was refluxed for 6 d. The solution was concentrated and the residue was dissolved in 1 mL of H_2O. Aqueous 10% K_2CO_3 solution was added to precipitate a gum. The mixture was decanted and the gum was dissolved in CH_2Cl_2. The solution was dried (K_2CO_3) and concentrated to a gum that was dissolved in 0.5 mL of Et_2O. Crystallization afforded 0.045 g (41%) of **29**: mp 124−126 °C; ^{1}H NMR ($CDCl_3$) δ 1.08 (m, 6H), 1.81 (m, 2H), 2.58 (m, 6H), 3.60 (m, 2H), 3.72 (s, 3H), 6.98 (br s, 1H), 7.22 (m, 2H), 7.39 (m, 2H), 7.41 (s, 1H), 8.44 (s, 1H); CIMS m/z 434 (M^+). Anal. ($C_{21}H_{25}Cl_2N_5O$) C, H, N.

Method B. 2-Amino-6-(2,6-dichlorophenyl)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (2). To a mixture of 3.70 g (0.012 mol) of pyridopyrimidine **4** in 20 mL of DMF was added 0.64 g (0.013 mol) of 50% NaH−mineral oil. The resulting slurry was heated at 65 °C for 0.5 h at which time a solution formed. It was cooled to 50 °C and a solution of 2.0 g (0.014 mol) of iodomethane in 10 mL of DMF was added dropwise to the mixture. The mixture was maintained at 60−80 °C for 3 h, cooled to room temperature, and poured onto ice H_2O. The white precipitate was collected, washed with H_2O, and recrystallized from EtOH using charcoal clarification to give 1.90 g (49%) of **2**: mp 235−237 °C; ^{1}H NMR (DMSO-d_6) δ 3.56 (s, 2H), 7.42 (m, 3H), 7.55 (d, J = 8 Hz, 2H), 7.79 (s, 1H), 8.65 (s, 1H); APCIMS m/z 321 (M^+). Anal. ($C_{14}H_{10}Cl_2N_4O$) C, H, N.

Method C. *N*-[6-(2,6-Dichlorophenyl)-8-methyl-7-oxo-7,8-dihydropyrido[2,3-*d*]pyrimidin-2-yl]acetamide (5). A mixture of 64 mg (0.2 mmol) of lactam **2** and 1 mL of acetic anhydride was heated at reflux for 20 min and then concentrated at atmospheric pressure to about 0.3 mL. The solution was cooled to 25 °C and diluted with Et_2O (1 mL). The precipitate was collected and washed with Et_2O to provide 44 mg (61%) of **5**: mp 258−260 °C; ^{1}H NMR ($CDCl_3$) δ 2.67 (s, 3H), 3.81 (s, 3H), 7.30 (m, 1H), 7.41 (m, 2H), 7.62 (s, 1H), 8.32 (br s, 1H), 8.74 (s, 1H); CIMS m/z 363 ($M^+ + 1$). Anal. ($C_{16}H_{12}Cl_2N_4O_2$) C, H, N.

***N*-[6-(2,6-Dichlorophenyl)-8-methyl-2-methylsulfanyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-ylidene]acetamide (11a).** A mixture of 50.0 g (0.142 mol) of imine **10a** and 225 mL of acetic anhydride was heated at reflux. After 5 min, the solution was cooled. The resulting precipitate was filtered and the cake washed with 50 mL of acetic anhydride and then 50 mL of Et_2O to leave 49.2 g (88%) of **11a**: mp 229−231 °C; ^{1}H NMR (DMSO-d_6) δ 1.69 (s, 3H), 2.63 (s, 3H), 3.68 (s, 3H), 7.48 (t, J = 8 Hz, 1H), 7.56 (d, J = 8 Hz, 2H), 7.78 (s, 1H), 8.83 (s, 1H); CIMS m/z 393 (M^+). Anal. ($C_{17}H_{14}Cl_2N_4OS$) C, H, N.

Method D. 4-Methylamino-2-methylsulfanyl-5-pyrimidinecarboxylate Ethyl Ester (7a). To a solution of 18.7 g (0.08 mol) of commercially available 4-chloro-2-methylsulfanyl-5-pyrimidinecarboxylate ethyl ester (**6**) in 260 mL of THF was added 34 mL (0.244 mol) of triethylamine followed by 30 mL of 40% aqueous methylamine. The solution was stirred for 30 min at 25 °C and then concentrated and partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated to a white solid. The solid was triturated in hexane and collected to provide 14.7 g (81%) of **7a**: mp 91−93 °C; ^{1}H NMR (DMSO-d_6) δ 1.30 (t, J = 7 Hz, 3H), 2.49 (s, 3H), 2.97 (d, J = 5 Hz, 3H), 4.28 (q, J = 7 Hz, 2H), 8.26 (br s, 1H), 8.53 (s, 1H); CIMS m/z 228 ($M^+ + 1$). Anal. ($C_9H_{13}N_3O_2S$) C, H, N.

Method E. 4-Methylamino-2-methylsulfanyl-5-pyrimidinemethanol (8a). A solution of 4.36 g (19.3 mmol) of ester **7a** in 60 mL of THF was added dropwise to a room temperature suspension of 1.10 g (29.0 mmol) of lithium aluminum hydride in 40 mL of THF. After 10 min the reaction was carefully quenched with 2 mL of H_2O, 2 mL of 15% aqueous NaOH, and an additional 7 mL of H_2O. The mixture was stirred for 1 h, and the white precipitate was collected by filtration and washed with EtOAc. The filtrate was concentrated to a residue that was treated with 3:1 hexane−EtOAc. The precipitated solids were collected to give 3.0 g (84%) of **8a**: mp 155−157 °C; ^{1}H NMR (DMSO-d_6) δ 2.42 (s, 3H), 2.85 (m, 3H), 4.29 (s, 2H), 5.08 (m, 1H), 6.83 (br s, 1H), 7.81 (s, 1H); CIMS m/z 186 ($M^+ + 1$). Anal. ($C_7H_{11}N_3OS$) C, H, N.

Method F. 4-Methylamino-2-methylsulfanyl-pyrimidine-5-carboxaldehyde (9a). A mixture of 5.78 g (31.2

mmol) of alcohol **8a** and 25.0 g (286 mmol) of MnO_2 in 600 mL of $CHCl_3$ was stirred at room temperature for 6 h and filtered through Celite, washing with 300 mL of $CHCl_3$. The filtrate was concentrated to a residue that was treated with hexane. The precipitated solid was collected to leave 5.35 g (93%) of **9a**: mp 99−101 °C; ^{1}H NMR ($CDCl_3$) δ 2.57 (s, 3H), 3.12 (d, J = 5 Hz, 3H), 8.29 (s, 1H), 8.55 (br s, 1H), 9.70 (s, 1H); CIMS m/z 184 ($M^+ + 1$). Anal. ($C_7H_9N_3OS$) C, H, N.

Method G. 6-(2,6-Dichlorophenyl)-8-methyl-2-methylsulfanyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-ylideneamine (10a). To a solution of 34.1 g (0.186 mol) of aldehyde **9a** and 43 g (0.224 mol) of 2,6-dichlorophenylacetonitrile in 250 mL of DMF was added 110 g (0.80 mol) of powdered anhydrous K_2CO_3. The mixture was heated with stirring at 100−105 °C for 18 h, cooled to ca. 50 °C, and filtered. The cake was washed with 200 mL of DMF and the combined filtrates were cooled to 15 °C. Water (100 mL) was added until slight turbidity developed. The solids that separated were collected, washed successively with 150 mL of cold 3:1 DMF−H_2O and excess H_2O, and dried to give 24.5 g (38%) of **10a**. A second crop (5.3 g, 8%) was obtained by adding additional H_2O to the filtrate. The two crops were combined and used directly in the next step. An analytically pure sample was prepared by purification over SiO_2 chromatography, eluting with $CHCl_3$ and then 1:1 hexanes−EtOAc. Concentration of the product fractions left a solid that was dissolved in minimal CH_2Cl_2. After crystallization commenced, petroleum ether was added, and the crystals of pure imine **10a** were collected: mp 198−200 °C; ^{1}H NMR (DMSO-d_6) δ 2.60 (s, 3H), 3.65 (s, 3H), 7.00 (s, 1H), 7.41 (s, 1H), 7.55 (t, J = 8 Hz, 1H), 7.67 (d, J = 8 Hz, 2H), 8.58 (s, 1H); CIMS m/z 351 ($M^+ + 1$). Anal. ($C_{15}H_{12}Cl_2N_4S$) C, H, N.

Method H. 6-(2,6-Dichlorophenyl)-2-methanesulfinyl-8-methyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (13). A solution of 0.26 g (0.73 mmol) of sulfide **12a** in 15 mL of $CHCl_3$ was treated with 0.23 g (0.88 mmol) of 3-phenyl-2-(phenylsulfonyl)oxaziridine.[25] The solution was kept at 25 °C for 16 h, then added to a SiO_2 column packed with $CHCl_3$. The column was eluted with EtOAc and then with 20:1 $CHCl_3$−MeOH to obtain the fraction containing **13**. Concentration provided a solid that was triturated in 2 mL of Et_2O and collected to leave 0.19 g (70%) of **13**: mp 244−247 °C; ^{1}H NMR (DMSO-d_6) δ 2.98 (s, 3H), 3.74 (s, 3H), 7.52 (t, J = 7 Hz, 1H), 7.62 (d, J = 7 Hz, 2H), 8.26 (s, 1H), 9.28 (s, 1H); CIMS m/z 368 (M^+). Anal. ($C_{15}H_{11}Cl_2N_3O_2S$) H, N; C, calcd 48.93, found 48.42.

Alternatively, compound **13** was isolated in low yield as a byproduct in the *m*-chloroperbenzoic acid oxidation of **12a** in one run that was similar to that for the synthesis of **14a** except that the reaction time was shortened to 1 h; the TLC, ^{1}H NMR spectra, and mp on this compound were identical to **13** isolated in the 3-phenyl-2-(phenylsulfonyl)oxaziridine oxidation above.

6-(2,6-Dichlorophenyl)-2-[[4-[2-(diethylamino)ethoxy]-phenyl]amino]-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one, *N*-Oxide (66). To a stirred solution of 0.50 g (0.98 mmol) of **63** in 15 mL of $CHCl_3$ at room temperature was added dropwise a solution of 0.25 g (0.98 mmol) of 3-phenyl-2-(phenylsulfonyl)oxaziridine[25] in 10 mL of $CHCl_3$. The reaction mixture was stirred for 3 h and concentrated to a residue that was dissolved in minimum EtOAc−MeOH−NEt_3 (86:10:4), and the solution applied to a radial chromatography apparatus. The mixture was purified by a gradient elution of EtOAc−MeOH−NEt_3 (88:10:2) to EtOAc−MeOH−NEt_3 (86:10:4). The purified product was recrystallized from 2-propanol to give 0.25 g (48%) of **66**: mp 180−181 °C; ^{1}H NMR (DMSO-d_6) δ 1.18 (t, J = 7 Hz, 6H), 3.19−3.10 (m, 4H), 3.45−3.37 (m, 2H), 3.64 (s, 3H),), 4.47−4.45 (m, 2H), 7.01−6.98 (m, 2H), 7.48−7.44 (m, 1H), 7.60−7.58 (m, 2H), 7.76−7.74 (m, 2H), 7.88 (s, 1H), 8.80 (s, 1H), 10.15 (br s, 1H), APCIMS m/z 500 ($M^+ - 28$). Anal. ($C_{26}H_{27}N_5O_3Cl_2 \cdot 0.08H_2O$) C, H, N.

Method I. 6-(2,6-Dichlorophenyl)-2-methanesulfonyl-8-methyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (14a). To a stirred solution of 42.0 g (0.119 mol) of the methyl sulfide **12a** in 3.5 L of $CHCl_3$ at 25 °C was added 81.6 g (0.264 mol) of *m*-chloroperbenzoic acid (assumed 57% of active peracid). The solution was stirred overnight and then treated with 25 g (0.32 mol) of DMSO to decompose excess peracid. After 15 min, the solution was washed with 1.5 L of saturated aqueous $NaHCO_3$ and then several times with H_2O. The organic layer was dried (Na_2SO_4) and concentrated to ca. 200 mL, wherein crystallization occurred. The solids were collected and washed with $CHCl_3$ and then petroleum ether to leave 34.5 g (76%) of **14a**: mp >290 °C. Upon adding additional petroleum ether to the filtrate, 7.6 g (16%) of a second crop was obtained; ^{1}H NMR (DMSO-d_6) δ 3.50 (s, 3H), 3.75 (s, 3H), 7.52 (t, J = 7 Hz, 1H), 7.64 (d, J = 7 Hz, 2H), 8.30 (s, 1H), 9.36 (s, 1H); CIMS m/z 384 (M^+). Anal. ($C_{15}H_{11}Cl_2N_3O_3S$) C, H, N.

Method J. 6-(2,6-Dichlorophenyl)-2-hydroxy-8-methyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (20). A mixture of 0.15 g (0.39 mmol) of sulfone **14a** and 10 mL of 9:1 HOAc−H_2O was heated with stirring at reflux for 0.5 h. The solution was concentrated to 5 mL, and 20 mL of H_2O was added. The precipitated solids were collected, washed well with H_2O, and dried to leave 0.10 g (80%) of **20**: mp 180−200 °C; ^{1}H NMR (DMSO-d_6) δ 3.47 (s, 3H), 7.45 (t, J = 8 Hz, 1H), 7.56 (d, J = 8 Hz, 2H), 7.72 (s, 1H), 8.58 (s, 1H); APCIMS m/z 322 (M^+). Anal. ($C_{14}H_9Cl_2N_3O_2 \cdot 1.25H_2O$) C, H, N.

Method K. 6-(2,6-Dichlorophenyl)-8-methyl-2-[4-(4-methylpiperazin-1-yl)butylamino]-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (32). A mixture of 0.15 g (0.43 mmol) of **12a** and 0.50 g (2.90 mmol) of 1-(4-aminobutyl)-4-methylpiperazine[32] was heated with stirring at 170 °C. After 2 h the solution was cooled to room temperature, and the dark gum was partitioned between Et_2O and H_2O. The Et_2O phase was washed with H_2O (four times), dried (K_2CO_3), and concentrated to ca. 2 mL, wherein crystallization occurred. The solids were collected and washed with Et_2O to leave 0.06 g (31%) of **32**: mp 130−132 °C; ^{1}H NMR (DMSO-d_6) δ 1.45−1.65 (m, 4H), 2.12 (s, 3H), 2.20 and 2.40 (m, 8H), 3.40 (m, 2H), 3.55 and 3.59 (2s, 3H), 7.45 (t, J = 7 Hz, 1H), 7.55 (d, J = 7 Hz, 2H), 7.76 (s, 1H), 7.98 and 8.08 (2 m, 1H), 8.63 and 8.69 (2s, 1H); CIMS m/z 475 (M^+). Anal. ($C_{23}H_{28}Cl_2N_6O$) C, H, N.

6-(2,6-Dichlorophenyl)-8-methyl-2-(pyridin-3-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (40). A mixture of 0.17 g (0.47 mmol) of **12a**, 0.50 g (5.3 mmol) of 3-aminopyridine, and 0.07 g (0.50 mmol) of 3-aminopyridine hydrochloride was heated with stirring at 210 °C for 1 h. Water (5 mL) was added to the cooled reaction mixture to precipitate solids. The solids were collected, washed well with H_2O, and dried to leave 0.15 g of crude product that was purified by SiO_2 chromatography, eluting with $CHCl_3$ and then EtOAc. The EtOAc eluent containing the pure product was concentrated to 2 mL, wherein crystallization occurred. The solids were collected and washed successively with EtOAc and Et_2O to leave 0.081 g (43%) of **40**: mp 247−248 °C; ^{1}H NMR (DMSO-d_6) δ 3.68 (s, 3H), 7.41 (m, 1H), 7.49 (t, J = 7 Hz, 1H), 7.60 (d, J = 7 Hz, 2H), 7.95 (s, 1H), 8.28 (m, 2H), 8.89 (s, 1H), 8.90 (s, 1H), 10.43 (s, 1H); CIMS m/z 398 (M^+). Anal. ($C_{19}H_{13}Cl_2N_5O$) C, H, N.

Method L. 6-(2,6-Dichlorophenyl)-8-methyl-2-methylamino-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (21). A pressure vessel charged with 0.165 g (0.47 mmol) of **12a** was cooled to −78 °C. Methylamine (ca. 3 mL) was condensed in the vessel, followed by dilution with 1 mL of DMF. The vessel was sealed and heated at 110 °C for 20 h. The tube was cooled to −78 °C and opened, and the solution was concentrated to give a solid that was triturated in H_2O, collected, and dried to leave 0.13 g of crude **21.** Crystallization from EtOAc−petroleum ether gave 0.081 g (51%) of pure **21**; mp 243−244 °C; ^{1}H NMR (DMSO-d_6) δ 2.92 (m, 3H), 3.54 and 3.62 (2s, 3H), 7.42 (t, J = 8 Hz, 1H), 7.55 (d, J = 8 Hz, 2H), 7.80 (s, 1H), 7.82 and 7.95 (2m, 1H), 8.64 and 8.72 (2s, 1H); CIMS m/z 335 (M^+). Anal. ($C_{15}H_{12}Cl_2N_4O$) C, H, N.

6-(2,6-Dichlorophenyl)-8-methyl-2-(3-morpholin-4-ylpropylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (30). A mixture of 0.165 g (0.47 mmol) of **12a**, 1.0 g (6.9 mmol) of *N*-(3-aminopropyl)morpholine and 0.5 mL of DMF was heated with stirring at 125 °C for 1.5 h. The solution was concentrated, and the residue was triturated in 5 mL of H_2O. The gum was dissolved in EtOAc, and the solution was washed with H_2O

(two times), dried (K_2CO_3), and concentrated. Crystallization of the residue from Et_2O gave 0.10 g (47%) of **30**: mp 140−142 °C; ^{1}H NMR (DMSO-d_6) δ 1.75 (m, 2H), 2.38 (m, 6H), 3.42 (m, 2H), 3.59 (m, 7H), 7.42 (t, J = 8 Hz, 1H), 7.58 (d, J = 8 Hz, 2H), 7.78 (s, 1H), 7.95 and 8.05 (2m, 1H), 8.64 and 8.70 (2 s, 1H); CIMS m/z 448 (M^+). Anal. ($C_{21}H_{23}Cl_2N_4O_2$) C, H, N.

Method M. [6-(2,6-Dichlorophenyl)-7-imino-8-methyl-7,8-dihydropyrido[2,3-*d*]pyrimidin-2-yl]-[4-(2-diethylaminoethoxy)phenyl]amine (18). According to Method H, a solution of 0.50 g (1.9 mmol) of 3-phenyl-2-(phenylsulfonyl)-oxaziridine[25] and 0.54 g (1.5 mmol) of imine **10a** in 15 mL of $CHCl_3$ was kept at room temperature overnight. The solution was purified by SiO_2 column chromatography with successive elution with $CHCl_3$, EtOAc, and finally with 1:20 MeOH−$CHCl_3$ to obtain the fractions containing sulfoxide intermediate **17**. This sulfoxide (0.4 g; 73%) was used directly in the next step. A mixture of 0.21 g (0.6 mmol) of sulfoxide **17** and 0.25 g (1.1 mmol) of 4-(2-diethylaminoethoxy)aniline[26] was heated at 160 °C for 5 min. The cooled mixture was dissolved in 1 mL of hot EtOAc. After this mixture was cooled, the precipitated solids were collected and washed with EtOAc and Et_2O to leave 0.05 g (17%) of **18**: mp 145−147 °C; ^{1}H NMR (DMSO-d_6) δ 0.98 (t, J = 7 Hz, 6H), 2.55 (m, 2H), 2.75 (t, J = 6 Hz, 2H), 3.63 (s, 3H), 4.00 (t, J = 7 Hz, 2H), 6.62 (br s, 1H), 6.92 (d, J = 9 Hz, 2H), 7.27 (s, 1H), 7.55 (m, 1H), 7.65 (m, 2H), 8.46 (s, 1H), 9.79 (br s, 1H); CIMS m/z 511 (M^+). Anal. ($C_{26}H_{28}Cl_2N_6O\cdot0.5H_2O$) C, H, N.

2-Cyclohexylamino-6-(2,6-dichlorophenyl)-8-methyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (25). A mixture of 0.10 g (0.26 mmol) of sulfone **14a** and 0.30 g (3.0 mmol) of cyclohexylamine was heated at reflux for 2 min and then concentrated at reduced pressure. The remaining gum was crystallized from 1:1 EtOAc−petroleum ether to leave 0.09 g (86%) of crude **25**. Recrystallization from EtOAc gave 0.048 g of pure product **25**: mp 242−244 °C; ^{1}H NMR (DMSO-d_6) δ 1.10−1.40 (m, 5H), 1.55−1.99 (m, 5H), 3.55 and 3.58 (2s, 3H), 3.85 (br m, 1H), 7.45 (t, J = 8 Hz, 1H), 7.55 (d, J = 8 Hz, 2H), 7.77 (s, 1H), 7.82 and 7.95 (2 d, J = 10 Hz, 1H), 8.64 and 8.66 (2s, 1H); CIMS m/z 403 (M^+). Anal. ($C_{20}H_{20}Cl_2N_4O$) C, H, N.

6-(2,6-Dichlorophenyl)-8-methyl-2-phenylamino-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (38). (a) From Sulfoxide 13. A solution of 0.11 g (0.3 mmol) of sulfoxide **13** and 0.25 g (2.7 mmol) of aniline was heated at 184 °C for 3 min. Most of the excess aniline was evaporated at reduced pressure, and the remaining gum was dissolved in 1 mL of hot EtOAc. After the mixture was cooled, the precipitated solids were collected and washed successively with EtOAc and Et_2O to leave 0.07 g (57%) of **38**: mp 247−249 °C; ^{1}H NMR (DMSO-d_6) δ 3.68 (s, 3H), 7.05 (t, J = 7 Hz, 1H), 7.35 (t, J = 8 Hz, 2H), 7.45 (t, J = 7 Hz, 1H), 7.59 (d, J = 8 Hz, 2H), 7.85 (d, J = 8 Hz, 2H), 7.91 (s, 1H), 8.85 (s, 1H), 10.26 (br s, 1H); CIMS m/z 397 (M^+). Anal. ($C_{20}H_{14}Cl_2N_4O$) C, H, N.

(b) From Sulfone 14a. A solution of 0.11 g (0.29 mmol) of the sulfone **14a** and 1.0 g (10.7 mmol) of aniline was refluxed for 3 min. Further workup as described above gave a crude solid that was purified by SiO_2 chromatography, eluting with $CHCl_3$ and then 50% hexane−EtOAc to afford 0.046 g (40%) of **38**: mp 247 °C; The TLC and NMR for this compound were identical to those of **3** isolated from the sulfoxide method above.

6-(2,6-Dichlorophenyl)-2-(4-methoxyphenylamino)-8-methyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (49). A stirred mixture of 1.13 g (2.9 mmol) of **14a** and 3.0 g (24.4 mmol) of 4-methoxyaniline was heated at 160−165 °C for 10 min and then cooled and treated with glacial HOAc to dissolve the gum. The solution was diluted with 4 mL of H_2O to precipitate solids that were collected and then washed successively with H_2O, 2-propanol, and ether to leave 1.2 g (97%) of crude product. Recrystallization from EtOAc gave a pure sample of **49**: mp 221−223 °C; ^{1}H NMR (DMSO-d_6) δ 3.64 (s, 3H), 3.75 (s, 3H), 6.95 (d, J = 9 Hz, 2H), 7.47 (t, J = 8 Hz, 1H), 7.59 (d, J = 8 Hz, 2H), 7.72 (d, 2H), 7.88 (s, 1H), 8.80 (s, 1H), 10.11 (s, 1H); CIMS m/z 427 (M^+). Anal. ($C_{21}H_{16}Cl_2N_4O_2$) C, H, N.

6-(2,6-Dichlorophenyl)-2-[4-(2-diethylaminoethoxy)-phenylamino]-8-methyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (63). A stirred mixture of 0.155 g (0.40 mmol) of sulfone **14a**, 0.167 g (0.80 mmol) of 4-(2-diethylaminoethoxy)aniline,[26] and 1 mL of (2-methoxyethyl)ether was heated at 150 °C for 20 min. The resultant solution was cooled to 100 °C and then treated dropwise with H_2O until it was slightly turbid. After the mixture was allowed to stand, crystallization occurred. The solids were collected, washed successively with Et_2O and H_2O, and dried to leave 0.11 g of crude product that was purified over SiO_2 chromatography eluting with $CHCl_3$, EtOAc, and finally 10% MeOH−$CHCl_3$. Pure product fractions were concentrated to give an amorphous solid that was dissolved in EtOAc. After the mixture was allowed to stand, the precipitated solids were collected and washed sparingly with EtOAc and Et_2O to leave 0.042 g (21%) of **63**: mp 141−143 °C; ^{1}H NMR (DMSO-d_6) δ 0.98 (t, J = 7 Hz, 6H), 2.55 (m, 4H), 2.76 (m, 2H), 3.64 (s, 3H), 4.00 (m, 2H), 6.94 (d, J = 9 Hz, 2H), 7.45 (t, J = 8 Hz, 1H), 7.58 (d, J = 8 Hz, 2H), 7.71 (d, J = 8 Hz, 2H), 7.88 (s, 1H), 8.80 (s, 1H), 10.11 (s, 1H); CIMS m/z 512 (M^+). Anal. ($C_{26}H_{27}Cl_2N_5O_2$) C, H, N.

Method N. 6-(2,6-Dichlorophenyl)-2-[4-(2-diethylaminoethoxy)phenylamino]-8-methyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one, Dihydrochloride (63). A stirred mixture of 25.6 g (0.067 mol) of sulfone **14a**, 24.5 g (0.118 mol) of 4-(2-diethylaminoethoxy)aniline,[26] and 125 mL of glacial HOAc was heated at reflux for 15 min, and then the resultant solution was concentrated to ca. $^1/_2$ volume. The viscous residue was diluted with 500 mL of H_2O, and then the solution was treated carefully with excess saturated aqueous $NaHCO_3$ to precipitate a tacky yellow solid. The suspension was diluted with EtOAc (150 mL), and then the organic phase was seeded with **63** base to induce crystallization. The biphasic suspension was filtered, and the cake was washed well with H_2O and then petroleum ether. The damp cake was stirred in 500 mL of CH_2Cl_2, and the resultant suspension was filtered to collect the 2-hydroxy impurity. The filtrate was dried (K_2CO_3) and concentrated to give a gum that was dissolved in 2 L of boiling 2-propanol. The solution was diluted with 700 mL of saturated 2-propanolic HCl. Voluminous yellow crystals of **63** dihydrochloride slowly separated. The mixture was cooled, and the solids were collected and washed successively with 200 mL of 2-propanol and then 100 mL of Et_2O to leave 21.2 g (54%) of **63** as the dihydrochloride salt: mp 240−245 °C; ^{1}H NMR (DMSO-d_6) δ 1.25 (m, 6H), 3.15−3.25 (m, 4H), 3.50 (m, 2H), 3.65 (s, 3H), 4.35 (m, 2H), 7.05 (d, J = 9 Hz, 2H), 7.45 (t, 1H), 7.59 (d, J = 8 Hz, 2H), 7.76 (d, J = 8 Hz, 2H), 7.89 (s, 1H), 8.82 (s, 1H), 10.20 (br d, 2H); CIMS m/z 512 (M^+). Anal. ($C_{26}H_{27}Cl_2N_5O_2\cdot2HCl\cdot0.5H_2O$) C, H, N.

Method O. 6-(2,6-Dichlorophenyl)-8−2-(4-hydroxyphenylamino)-8-methyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (51). A stirred mixture of 0.50 g (1.17 mmol) of **49**, 5 mL of concentrated (48%) HBr, and 10 mL of propanoic acid was heated at reflux for 3 h. After the mixture was cooled, crystals formed, and these were collected to give 0.51 g of the hydrobromide salt. The salt was dissolved in 40 mL of MeOH, and the solution was neutralized with 3 mL of saturated aqueous $NaHCO_3$ and then treated with H_2O until it was slightly turbid. After the mixture was allowed to stand, a precipitate formed that was collected and washed well with H_2O to leave 0.38 g (92%) of the product. Dissolution of this in 30% MeOH−CH_2Cl_2 and concentration until crystallization commenced gave pure **51**: mp 289−291 °C; ^{1}H NMR (DMSO-d_6) δ 3.62 (s, 3H), 6.75 (d, J = 9 Hz, 2H), 7.45 (t, J = 8 Hz, 1H), 7.59 (d, J = 8 Hz, 4H), 7.86 (s, 1H), 8.77 (s, 1H), 9.23 (s, 1H), 9.99 (br s, 1H); CIMS m/z 413 (M^+). Anal. ($C_{20}H_{14}Cl_2N_4O_2$) C, H, N.

Method P. 6-(2,6-Dichlorophenyl)-2-[3-(2-diethylaminoethoxy)phenylamino]-8-methyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one, Dihydrochloride (62). To a mixture of 5.60 g (13.6 mmol) of **50**, 1.90 g (16.3 mmol) of *N*,*N*-diethylethanolamine, and 4.26 g (16.3 mmol) of triphenylphosphine in 200 mL of THF at 5 °C was added dropwise 2.83 g (16.3 mmol) of diethyl azodicarboxylate. The reaction mixture was stirred for 30 min at 5 °C and then warmed to room temperature and stirred overnight. The mixture was diluted with 95:5 CH_2Cl_2−MeOH and adsorbed onto 60 g of SiO_2. The solvent was

removed, and the SiO_2 charge was introduced onto a SiO_2 column. The column was eluted with 95:5 CH_2Cl_2-MeOH. Pure product fractions were dissolved in EtOAc, and the solution was treated with 8 mL of 4 N HCl in dioxane. The precipitated solids were collected and dried to afford 5.50 g (79%) of **62** as the dihydrochloride salt: mp 238−240 °C; ^{1}H NMR (DMSO-d_6) δ 1.28 (t, J = 7.23 Hz, 6H); 3.28−3.20 (m, 4H), 3.53−3.50 (m, 2H), 3.70 (s, 3H), 4.42−4.40 (m, 2H), 6.71−6.68 (m, 1H), 7.37−7.28 (m, 2H), 7.50−7.46 (m, 1H), 7.61−7.59 (m, 2H), 7.77 (br s, 1H), 7.94 (s, 1H), 8.88 (s, 1H), 10.34 (br s, 1H), 10.61 (br s, 1H); CIMS m/z 512 (M^+). Anal. ($C_{26}H_{27}N_5O_2Cl_2$·2HCl·1.9H_2O·0.01EtOAc) C, H, N.

Method Q. 6-(2,6-Dichlorophenyl)-2-[4-(2-dimethylaminoethoxy)phenylamino]-8-methyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one, Dihydrochloride (60). A stirred mixture of 0.18 g (0.44 mmol) of the phenol **51**, 0.53 g (0.54 mmol) of *N*,*N*-dimethylaminoethyl chloride hydrochloride, 3 mL of DMF, and 1.0 g (7.2 mmol) of anhydrous K_2CO_3 powder was heated at reflux for 5 min. Water (20 mL) was added to the cooled mixture to precipitate a solid. The solid was collected, washed well with H_2O, and dried to leave 0.18 g of a residue that was dissolved in CH_2Cl_2. The solution was dried (K_2CO_3) and concentrated to a gum that was dissolved in warm 2-propanol. The solution was treated with excess HCl gas, and solids formed after the solution was cooled. The solids were collected and washed sequentially with 2-propanol and Et_2O. Recrystallization from MeOH−Et_2O afforded 0.12 g (45%) of pure **60** as the dihydrochloride salt: mp 246−248 °C; ^{1}H NMR (DMSO-d_6) δ 2.86 (m, 6H), 3.52 (m, 2H), 3.65 (s, 3H), 4.35 (m, 2H), 7.04 (d, J = 9 Hz, 2H), 7.48 (t, J = 8 Hz, 1H), 7.59 (d, J = 8 Hz, 2H), 7.78 (d, J = 8 Hz, 2H), 7.90 (s, 1H), 8.82 (s, 1H), 10.07 (br s, 1H), 10.18 (br s, 1H); CIMS m/z 484 (M^+). Anal. ($C_{24}H_{23}Cl_2N_5O_2$·2HCl·1.25H_2O) C, H, N.

Method R. 6-[6-(2,6-Dichlorophenyl)-8-methyl-7-oxo-7,8-dihydropyrido[2,3-*d*]pyrimidin-2-ylamino]hexanoic acid, Trifluoroacetic Acid Salt (37). According to Method M, a stirred mixture of 0.15 g (0.40 mmol) of **14a** and 0.75 g (4.0 mmol) of 6-aminohexanoic acid, *t*-butyl ester,[33] was heated at 120 °C for 10 min. The solution was cooled to room temperature, and 20 mL of ice-cold 10% aqueous $KHSO_4$ solution was added. The separated gum was partitioned between Et_2O and H_2O, and then the organic phase was further washed with H_2O (three times), dried ($MgSO_4$), and concentrated to 2 mL where crystallization occurred. The solids were collected and washed with Et_2O to afford 0.15 g (77%) of 6-[6-(2,6-dichlorophenyl)-8-methyl-7-oxo-7,8-dihydropyrido[2,3-*d*]pyrimidin-2-ylamino]hexanoic acid, *tert*-butyl ester: mp 70−75 °C; ^{1}H NMR (DMSO-d_6) δ 1.36 (s, 11H, $C(CH_3)_3$ overlapping CH_2), 1.50−1.65 (m, 4H), 2.19 (m, 2H), 3.39 (m, 2H), 3.54 and 3.59 (2s, 3H), 7.45 (t, J = 8 Hz, 1H), 7.55 (d, J = 8 Hz, 2H), 7.78 (s, 1H), 7.92 and 8.05 (2 m, 1H), 8.63 and 8.69 (2s, 1H); CIMS m/z 491 (M^+). Anal. ($C_{24}H_{28}Cl_2N_4O_3$·0.2H_2O).

A solution of 0.095 g (0.19 mmol) of the *tert*-butyl ester in 0.75 mL of TFA was kept at room temperature for 1 h and then concentrated. The remaining gum was triturated in 2 mL of H_2O, and the suspension was decanted. The residue was then taken up in MeOH, and the suspension was diluted with an equal volume of H_2O. The precipitated solids were collected and dried to leave 0.10 g (91%) of **37** as a trifluoroacetic acid salt: mp 240−242 °C; ^{1}H NMR (DMSO-d_6) δ 1.34 (m, 2H), 1.49−2.66 (m, 4H), 2.21 (m, 2H), 3.39 (m, 3H), 3.54 and 3.60 (2s, 3H), 7.43 (t, J = 8 Hz, 1H), 7.58 (d, J = 8 Hz, 2H), 7.78 (s, 1H), 7.96 and 8.08 (2 br m, 1H), 8.64 and 8.69 (2s, 1H); CIMS m/z 435 (M^+). Anal. ($C_{20}H_{20}Cl_2N_4O_3$·CF_3CO_2H·0.75CH_3OH) C, H, N.

6-(2,6-Dichlorophenyl)-2-[4-(2-ethylaminoethoxy)phenylamino]-8-methyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one, Dihydrochloride (59). By utilizing Method P, to a mixture of 0.40 g (0.97 mmol) of the phenol **51**, 0.20 g (1.10 mmol) of *tert*-butyl *N*-ethyl-*N*-(2-hydroxyethyl)carbamate,[34] and 0.28 g (1.10 mmol) of triphenylphosphine in THF (5 mL) at 5 °C was added dropwise 0.19 g (1.10 mmol) of diethyl azodicarboxylate. The mixture was stirred for 30 min at 5 °C and then at room temperature overnight. The mixture was concentrated, and the residue was purified by radial chromatography, eluting with a gradient of 1:1 petroleum ether−EtOAc to pure EtOAc to give the *N*-*t*-Boc-protected intermediate, which was used directly in the next step.

The *N*-*t*-Boc protected intermediate was stirred in a mixture of 1:4 TFA−CH_2Cl_2 at ambient temperature for 2 h and then concentrated to a residue that was dissolved in EtOAc. The solution was filtered and then treated dropwise with 4 N HCL in dioxane until precipitation ceased. The precipitated solids were collected, washed with EtOAc, and dried to afford 0.10 g (19%) of **59** as the dihydrochloride salt: mp >175 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.24 (t, J = 7 Hz, 3H), 3.06−3.01 (m, 2H), 3.34−3.31 (m, 2H), 3.65 (s, 3H), 4.27 (t, J = 5 Hz, 2H), 7.04−7.02 (m, 2H), 7.48−7.44 (m, 1H), 7.60−7.58 (m, 2H), 7.78−7.76 (m, 2H), 7.90 (s, 1H), 8.82 (s, 1H), 9.03 (br s, 1H), 10.19 (br s, 1H); CIMS m/z 484 (M^+). Anal. ($C_{24}H_{23}Cl_2N_5O_2$·2HCl·1.67H_2O) C, H, N.

Method S. 3-[6-(2,6-Dichlorophenyl)-8-methyl-7-oxo-7,8-dihydropyrido[2,3-*d*]pyrimidin-2-ylamino]benzoic Acid (70). To a solution of 0.065 g (0.14 mmol) of **55** in 75 mL of MeOH was added 2 mL of 2 N aqueous NaOH, and the mixture was refluxed for 2 h. The solution was concentrated to ca. 15 mL, and the turbid suspension was filtered while hot to remove traces of solid. The filtrate was concentrated to ca. 4 mL, diluted with 5 mL of H_2O, and then treated with 1 mL of glacial HOAc to precipitate a flocculent solid. The solids were collected and washed with H_2O. After the solids were dissolved in 4 mL of warm DMF and 20 mL of Et_2O was added, crystallization slowly occurred. The solids were collected and washed successively with Et_2O and H_2O to give 0.025 g (41%) of **70**: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 3.70 (s, 3H), 7.48 (m, 2H), 7.58−7.62 (m, 3H), 7.93 (s, 1H), 8.00 (d, J = 8 Hz, 1H), 8.65 (br s, 1H), 8.89 (s, 1H), 10.46 (s, 1H), 12.95 (br s, 1H); CIMS m/z 441 (M^+). Anal. ($C_{21}H_{14}Cl_2N_4O_3$) C, H, N.

Method T. (2-{4-[6-(2,6-Dichloro-phenyl)-8-methyl-7-oxo-7,8-dihydro-pyrido[2,3-*d*]pyrimidin-2-ylamino]phenoxy}ethyl)diethylmethylammonium Iodide (65). A solution of 0.15 g (0.29 mmol) of **63** and 0.17 g (1.20 mmol) of iodomethane in 8.0 mL of CH_2Cl_2 was stirred at room temperature for 1 h and then concentrated. The residual gum was triturated in 1 mL of 2-propanol. The precipitated solids were collected and washed successively with 2-propanol and Et_2O to give 0.17 g (87%) of **65**: mp 256−258 °C dec (MeOH − Et_2O); ^{1}H NMR (DMSO-d_6) δ 1.28 (t, 6H), 3.04 (s, 3H), 3.41 (m, 4H), 3.65 (s, 3H), 3.72 (m, 2H), 4.42 (m, 2H), 7.02 (d, J = 9 Hz, 2H), 7.48 (t, J = 8 Hz, 1H), 7.59 (d, J = 8 Hz, 2H), 7.78 (d, J = 8 Hz, 2H), 7.90 (s, 1H), 8.82 (s, 1H), 10.17 (s, 1H). Anal. ($C_{27}H_{30}Cl_2IN_5O_2$) C, H, N.

Recombinant Tyrosine Kinases and Assays. The methods for production of the tyrosine kinases used in this study (PDGFr-β, FGFr-1, EGFr, c-Src) and assay conditions for each have been previously described.[28]

PDGF Receptor Autophosphorylation. (a) In rat aortic vascular smooth muscle cells (RAVSMC), the assay was carried out as previously described.[28] (b) In C6 or NIH3T3/PDGF cells, inhibition of C6 or NIH3T3/PDGF PDGF-receptor phosphorylation was carried out as described.[28,35] Serum-starved cells were incubated for 2 h with the indicated concentration of compound prior to stimulation with 25 ng/mL PDGF-BB (UBI, Lake Placid, NY). Cell lysates or immunoprecipitates were analyzed by Western blotting using antiphosphotyrosine antibody (UBI, Lake Placid, NY). Bound antibodies were detected using the ECL Western blotting system from Amersham.

Cell Culture. C6 rat glioma cells and NIH3T3 cells were obtained from the American Type Culture Collection (Rockville, MD). C6 cells were cultured in F10 Ham's media supplemented with 15% horse serum and 2.5% fetal calf serum (Sigma Chemical Co., St. Louis, MO). NIH3T3 cells were cultured in RPMI 1640 media supplemented with 10% fetal calf serum. NIH3T3 cells transfected with the gene for PDGF-B (NIH3T3/PDGF cells) were kindly provided by Dr Stuart Aaronson (NCI, Bethesda, MD).[36] The effect of kinase

inhibitors on C6 or NIH3T3 proliferation was determined as previously described.[37] Briefly, 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 d. Cell growth was determined by staining cells with sulforhodamine B[37] and reading plates on a Molecular Devices Thermax microplate reader (Sunnyvale, CA). Soft agar clonogenic assays were carried out as previously described.[38] C6 or NIH3T3/PDGF cells were exposed to various concentrations of compounds for 3 days and plated in soft agar in the presence of the corresponding concentration of compound. Alternatively, cells were exposed for 3 days to compounds as above but washed free of inhibitor and plated in drug-free soft agar. Colonies were stained and counted after two weeks.

In Vivo Chemotherapy. Immunologically deficient nude mice (Charles River or Taconic) 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 seven in vivo tumor models, A431 epidermoid carcinoma, NIH 3T3 fibroblast transfected with the human PDGF-BB receptor, estrogen-dependent MCF-7 breast, C6 rat glioma, Colo 205 and HT-29 colon, DU-145 prostate adenocarcinoma, and the SK-OV-3 ovarian carcinoma cells were maintained by serial passage of tumor fragments 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, 6−8 per group, 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 all compounds except compound **38** and cisplatin was 50 mM sodium lactate buffer, pH 4.0. Compound **38** was administered as a suspension in 0.5% methyl cellulose in water. Compound dosing solutions were prepared for 5 days at a time. Cisplatin was prepared fresh for each injection and dissolved in saline. In each study, compounds were tested over a range of doses ranging from toxic to therapeutically ineffective levels, so that the maximum tolerated dose could be determined. 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.[39−42] 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 net tumor growth during therapy.

Pharmacokinetics, Oral Bioavailability, and Dose Proportionality Studies. In the first study, the pharmacokinetics and oral bioavailability of compound **63** were evaluated in rats following a single 10 mg/kg IV bolus or 100 mg/kg oral gavage doses ($n = 3$ per route). The IV dose was prepared in lactic acid vehicle, and the oral dose was prepared in methacel. Serial heparinized blood samples were collected up to 24 h post dose. Plasma was harvested and stored at −20 °C until analysis.

In a follow-up study, dose proportionality of compound **63** was evaluated in rats following single 10, 30, or 100 mg/kg oral gavage doses. The oral dose was prepared in methacel. Serial heparinized blood samples were collected up to 24 h post dose. Plasma was harvested and stored at −20 °C until analysis.

Compound **63** was isolated from rat plasma by protein precipitation with acetonitrile. Liquid chromatographic separation was achieved on a reverse phase C8 Varian Bondesil (4.6 × 100 mm, 5μ particle size) column. The mobile phase consisted of 30% acetonitrile/70% ammonium phosphate buffer (0.1 M, pH 3.5) pumped with a flow rate of 1 mL/min. Column temperature was set at 50 °C. Column effluent was monitored with an ultraviolet detector at a wavelength of 364 nm. The assay lower limit of quantitation for compound **63** was 25 ng/mL.

Pharmacokinetic parameter values were estimated by noncompartmental analysis of individual plasma concentration−time data. The maximum plasma concentrations (C_{max}) for compound **63** and times for these to occur (t_{max}) were recorded as observed. Apparent terminal elimination-rate constants (λ_z) were estimated as the absolute value of the slope of the least-squares linear regression of the log-linear terminal phase of concentration−time profiles. Apparent terminal elimination half-life values ($t_{1/2}$) were calculated from elimination-rate constants as $t_{1/2} = 0.693/\lambda z$. Area under plasma concentration−time curve AUC(0−∞) values were calculated from time zero to the time of last detectable concentration (t_{ldc}) using the trapezoidal rule and were extrapolated to infinity. Absolute oral bioavailability $F\% = [(\text{dose}_{IV} \times \text{AUC}(0-\infty)_{PO})/(\text{dose}_{PO} \times \text{AUC}(0-\infty)_{IV})] \times 100$.

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Supporting Information Available: Experimental details and spectral data for compounds **7b**−**9b**, **12b**, **14b**, **16**, **22**−**24**, **26**−**28**, **31**, **33**−**36**, **39**, **41**−**48**, **50**, **52**−**58**, **61**, **64**, **67**−**69**, **71**, and **72**; mean pharmacokinetic parameter values and mean concentration−time profiles in rats for **63** (Table 7 and Figure 2, respectively) (16 pages). Ordering information is given on any current masthead page.

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