Synthesis and Tyrosine Kinase Inhibitory Activity of a Series of 2-Amino-8*H*-pyrido[2,3-*d*]pyrimidines: Identification of Potent, Selective Platelet-Derived Growth Factor Receptor Tyrosine Kinase Inhibitors

Diane H. Boschelli,*,† Zhipei Wu,† Sylvester R. Klutchko,† H. D. Hollis Showalter,† James M. Hamby,† Gina H. Lu,‡ Terry C. Major,‡ Tawny K. Dahring,‡ Brian Batley,‡ Robert L. Panek,‡ Joan Keiser,‡ Brian G. Hartl,# Alan J. Kraker,# Wayne D. Klohs,# Bill J. Roberts,# Sandra Patmore,# William L. Elliott,# Randy Steinkampf,# Laura A. Bradford,§ Hussein Hallak,§ and Annette M. Doherty†

Departments of Medicinal Chemistry, Cancer Research, Vascular and Cardiac Diseases, and Pharmacokinetics and Drug Metabolism, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105

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Screening of a compound library led to the identification of 2-amino-6-(2,6-dichlorophenyl)-8-methylpyrido[2,3-*d*]pyrimidine (**1**) as a inhibitor of the platelet-derived growth factor receptor (PDGFr), fibroblast growth factor receptor (FGFr), and c-src tyrosine kinases (TKs). Replacement of the primary amino group at C-2 of **1** with a 4-(*N*,*N*-diethylaminoethoxy)phenylamino group yielded **2a**, which had greatly increased activity against all three TKs. In the present work, variation of the aromatic group at C-6 and of the alkyl group at N-8 of the pyrido[2,3-*d*]pyrimidine core provided several analogues that retained potency, including derivatives that were biased toward inhibition of the TK activity of PDGFr. Analogues of **2a** with a 3-thiophene or an unsubstituted phenyl group at C-6 were the most potent inhibitors. Compound **54**, which had IC_{50} values of 31, 88, and 31 nM against PDGFr, FGFr, and c-src TK activity, respectively, was active in a variety of PDGF-dependent cellular assays and blocked the in vivo growth of three PDGF-dependent tumor lines.

Introduction

The tyrosine kinases (TKs) are a key group of enzymes that catalyze the specific phosphorylation of tyrosine residues on proteins. The TKs can be divided into two classes, the cytoplasmic TKs such as c-src and the transmembrane growth factor receptor TKs (RTKs). When growth factors such as platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), or vascular endothelial growth factor (VEGF) bind their receptors, the receptors dimerize and become active TKs. The subsequent autophosphorylation of the receptors leads to a signal transduction cascade which triggers a wide variety of events including proliferation and migration.[1,2]

Since distinct TKs are implicated in such diverse conditions as angiogenesis,[3,4] restenosis,[5,6] atherosclerosis,[7] and tumor growth,[8] selective TK inhibitors are highly desirable. The obstacles to identifying selective inhibitors are the large number of TKs that have been described and the structural homology between the TKs, especially in their ATP-binding regions.[9] Despite these difficulties several selective TK inhibitors have been identified[10−38] including those of EGFr[27−35] and FGFr TK[36−38] activity reported by Parke-Davis. We were also interested in identifying compounds that could selectively modulate the activity of PDGFr TK due to the importance of PDGF and its receptors in cancer.[10−17] Several years ago it was found that PDGF was the human homologue of the simian oncoprotein v-sis.[39,40] Later studies showed that both PDGF and its receptor are expressed in several types of human gliomas.[41−44] Furthermore, a shortened form of the PDGF receptor inhibited the growth of C6 glioma cells.[45] Therefore a small molecule capable of suppressing PDGFr TK activity has potential utility as an anticancer therapeutic agent.

We previously reported that screening of a compound library led to the identification of 2-amino-6-(2,6-dichlorophenyl)-8-methyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (**1**) as an inhibitor of PDGFr, FGFr, and c-src TK activity.[46] Optimization of the various groups at C-2 of **1** determined that aromatic amino substituents were greatly preferred over primary or alkyl amino groups. This finding was exemplified by the broad-spectrum TK inhibitor **2a** which has a 4-(*N*,*N*-diethylaminoethoxy)-phenylamino group at C-2 and had IC_{50} values of 79, 43, and 9 nM against PDGFr, FGFr, and c-src TK activity, respectively.[47] While the initial focus was on variation of the C-2 amino group, it was also determined that replacement of the 8-methyl group of **2a** with an ethyl group provided **2b**, which was roughly equipotent to **2a** but more PDGFr-selective. It was considered that further modification at the N-8 position of the pyrido-[2,3-*d*]pyrimidin-7-one could possibly lead to more active and/or more selective congeners.

In the initial screen that identified **1** as a TK inhibitor, similar activity was found for the related pyrido-[2,3-*d*]pyrimidine **3a**.[48] This nonspecific kinase inhibi-

* Address for correspondence: Wyeth-Ayerst Research, 401 N. Middletown Rd., Pearl River, NY 10965. Phone: 914-732-3567. Fax: 914-732-5561. E-mail: bosched@war.wyeth.com.
† Department of Medicinal Chemistry.
‡ Department of Vascular and Cardiac Diseases.
# Department of Cancer Research.
§ Department of Pharmacokinetics and Drug Metabolism.

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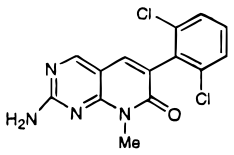

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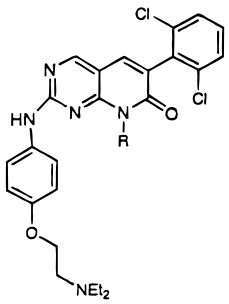
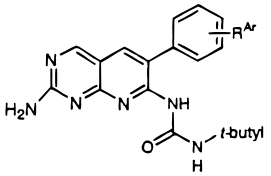


2a: R = Me
2b: R = Et



3a: R^{Ar} = 2,6-di-Cl
3b: R^{Ar} = 3,5-di-OMe

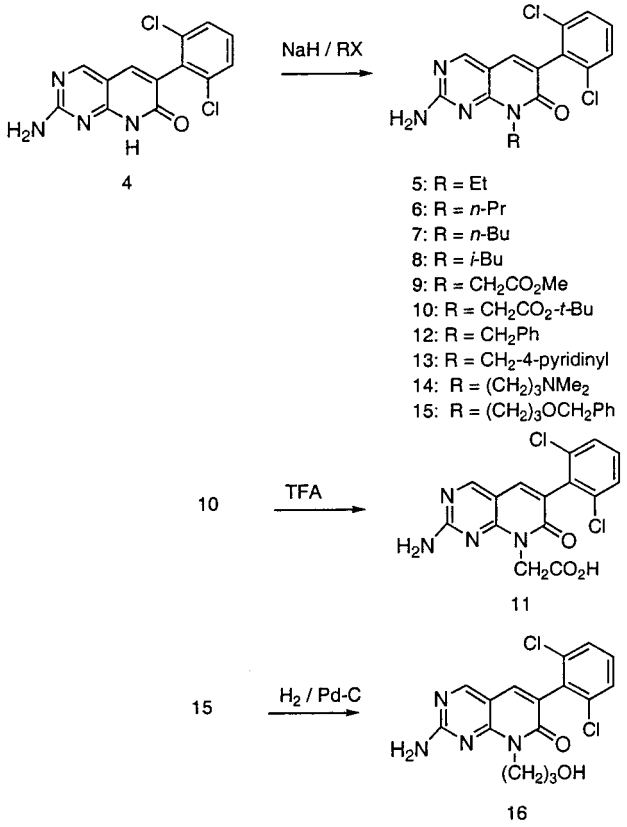
tor was converted into the selective FGFr TK inhibitor **3b** by replacement of the 2,6-dichlorophenyl group at C-6 of **3a** with a 3,5-dimethoxyphenyl group.[36,37] In addition, a model of the binding of these two classes of pyrido[2,3-*d*]pyrimidines to the kinase domains of TKs proposed that modification of the group at C-6 could confer selectivity.[49] All of these findings encouraged us to utilize **2a** as a template for the preparation of potential selective inhibitors, through variation of the C-6 and N-8 moieties.[50]

Chemistry

Additional N-8 alkyl derivatives of **1** were obtained by alkylation of **4**[46] with various halides employing sodium hydride as the base. The minor O-alkylation product was readily separated from the major product by chromatography. Compounds **5**−**10** and **12**−**15** were prepared by this route as shown in Scheme 1. Cleavage of the *tert*-butyl ester group of **10** with trifluoroacetic acid provided the acid derivative **11**. Catalytic hydrogenation of **15** removed the benzyl group to give **16**. We were not able to find conditions to convert **15** to **16** without some concomitant dechlorination.

Analogues of **2a** with various aromatic groups at C-6 of the pyrido[2,3-*d*]pyrimidin-7-one could be prepared as shown in Scheme 2. To this end, condensation of the 2-methyl sulfide derivative **17**[46] with phenylacetonitrile or 3-thiopheneacetonitrile in DMF in the presence of potassium carbonate gave the pyridopyrimidin-7-imines **18** and **19** that were then hydrolyzed to the 7-ones **20** and **21**. Oxidation of the methyl sulfide groups of **20** and **21** with *m*-chloroperbenzoic acid provided the sulfones **22** and **23**. The sulfone group was readily displaced by arylamines such as aniline, converting **22** and **23** to the 2-phenylamino derivatives **24** and **25**. In a similar fashion treatment of **22** and **23** with 4-aminopyridine provided **26** and **27**, while 4-(*N*,*N*-diethylaminoethoxy)aniline[51] provided **28** and **29**, respectively. While for arylamines it was necessary to employ the more labile sulfone group, in the case of the more nucleophilic alkylamines the sulfide group was sufficiently reactive. Therefore, treatment of **26** with

Scheme 1

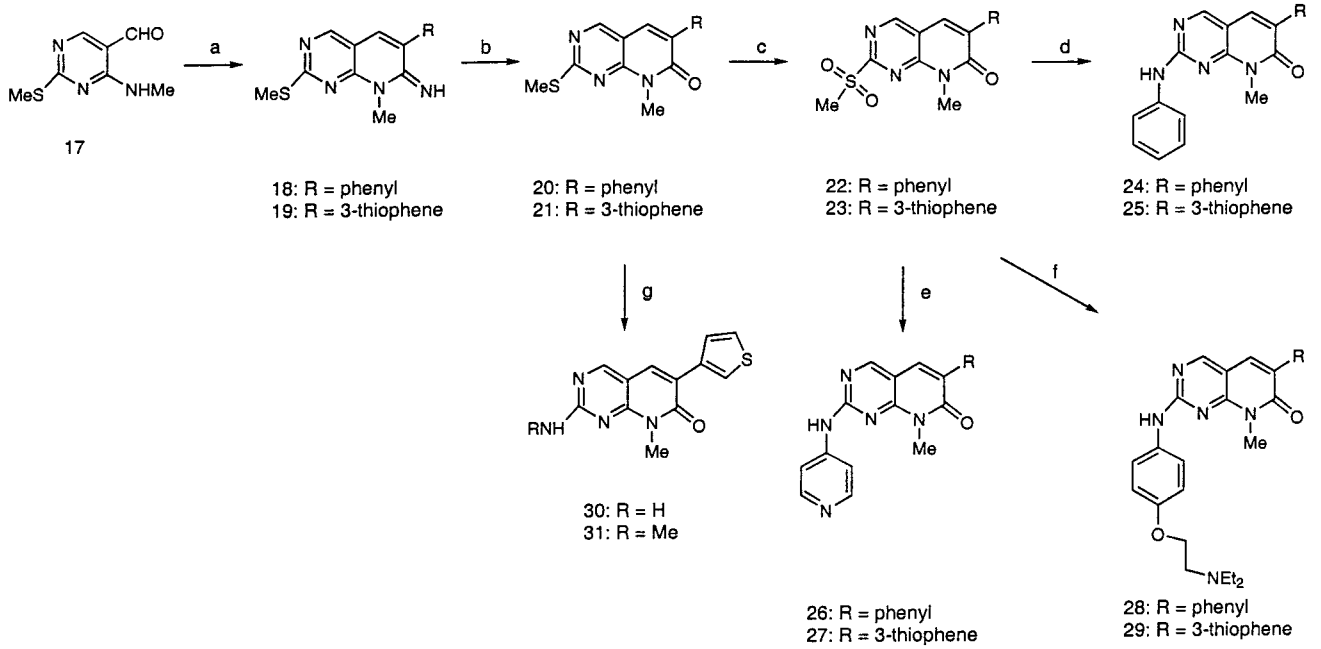


ammonia or methylamine in a sealed tube provided **30** and **31**, respectively (listed in Table 2).

A more direct route to the preparation of analogues retaining the methyl group at N-8 and a phenylamino group at C-2 while varying the aromatic group at C-6 is shown in Scheme 3. Treatment of 4-chloro-5-cyano-2-methylsulfanyl-pyrimidine[52] with methylamine provided **32**. Displacement of the 2-methyl sulfide group of **32** with aniline in the presence of a catalytic amount of concentrated hydrochloric acid gave **33**. Reduction of the nitrile group of **33** with diisobutylaluminum hydride followed by hydrolysis gave the corresponding aldehyde **34**. It should be noted that while the desired product was obtained by this protocol, it was necessary to stop the reaction while some **33** remained since extended reaction time led to the formation of additional products. Condensation of **34** with 2-thiopheneacetonitrile or 4-biphenylacetonitrile in 2-ethoxyethanol and sodium hydride gave good yields of the pyridopyrimidin-7-imines **35** and **36**, respectively. Acetylation of the imines followed by acid hydrolysis provided the corresponding 7-ones **37** and **38**.

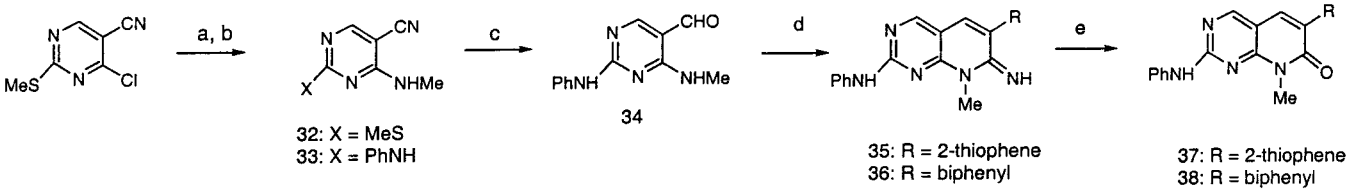
2-Phenylamino analogues with an ethyl group at N-8 group were prepared from **44**, the 4-ethylamino analogue of **34**. While a similar route to that used to prepare **34** could be adapted, a more reproducible route to **44** starting with commercially available **39** was employed (Scheme 4). Thus, the chloro group of **39** was displaced with aqueous ethylamine to give **40**. Oxidation of the 2-methyl sulfide group of **40** with *m*-chloroperbenzoic acid gave mixtures of products thought to arise due to the instability of the corresponding sulfone. It was found that use of (±)-*trans*-2-phenylsulfonyl-3-phenyloxaziridine[53] as the oxidant provided

Scheme 2[a]



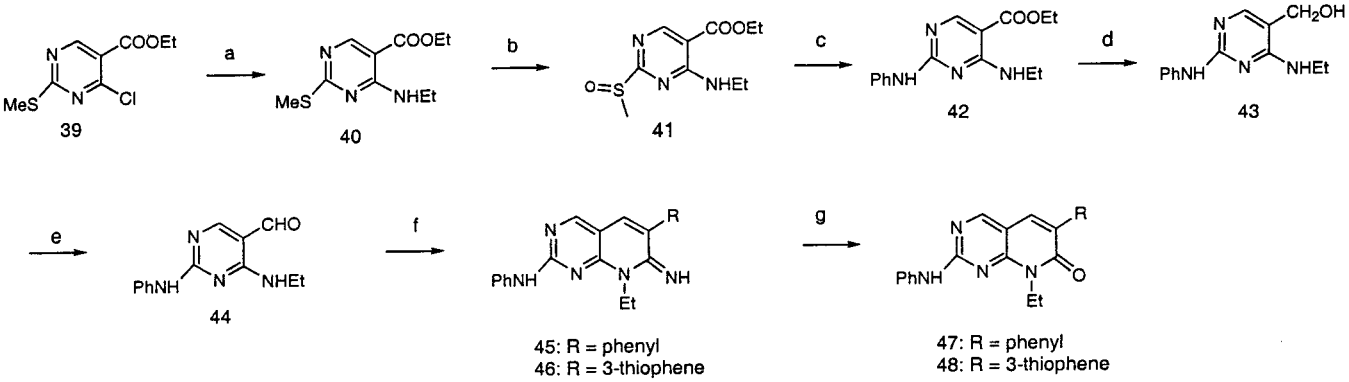
[a] (a) RCH_2CN, K_2CO_3, DMF; (b) (1) Ac_2O, reflux, (2) concd HCl; (c) *m*-CPBA, $CHCl_3$; (d) aniline, 150 °C; (e) 4-aminopyridine, 175 °C; (f) 4-(*N*,*N*′-diethylaminoethoxy)aniline, 175 °C; (g) RNH_2, DMF, high pressure.

Scheme 3[a]



[a] (a) Methylamine; (b) aniline, concd HCl, 170 °C; (c) DiBAL, THF, CH_2Cl_2; (d) RCH_2CN, NaH, $EtO(CH_2)_2OH$; (e) (1) Ac_2O, reflux; (2) concd HCl, reflux.

Scheme 4[a]

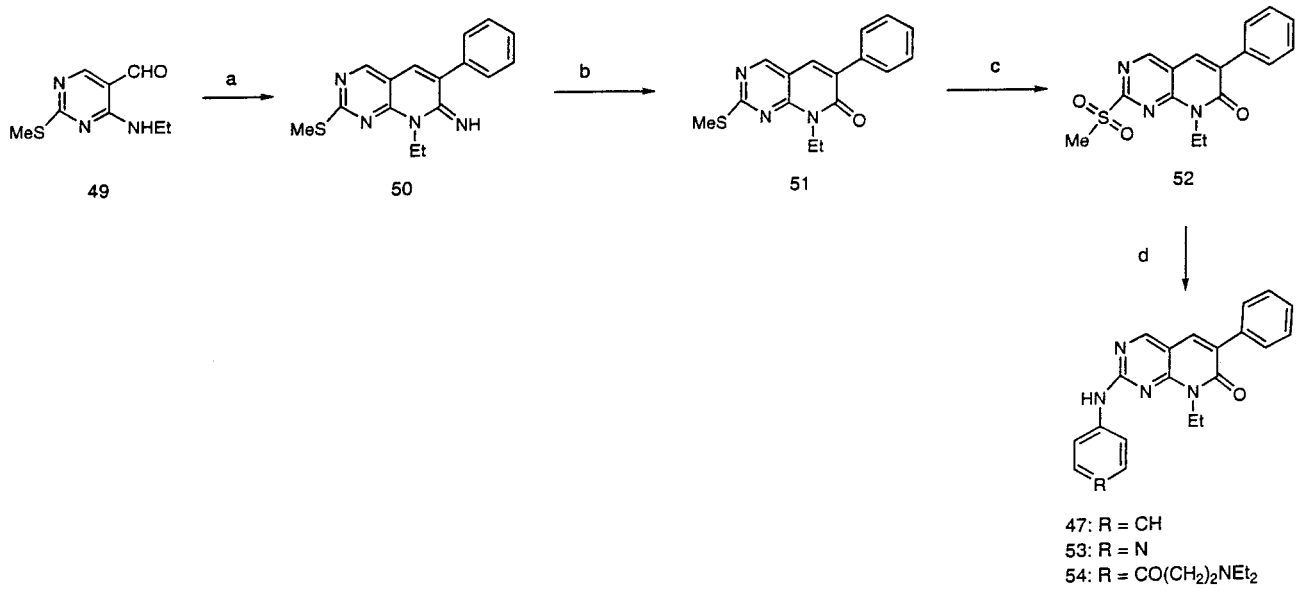


[a] (a) Aqueous ethylamine, triethylamine, THF; (b) *trans*-2-phenylsulfonyl-3-phenyloxaziridine, $CHCl_3$; (c) aniline, 130 °C; (d) $LiAlH_4$, THF; (e) MnO_2, $CHCl_3$; (f) RCH_2CN, NaH, $EtO(CH_2)_2OH$; (g) (1) Ac_2O, (2) concd HCl.

the corresponding sulfoxide **41** in good yield. Displacement of the sulfoxide group of **41** with aniline at elevated temperature gave the desired 2-phenylamino derivative **42**. The ester group at C-5 was sequentially reduced to the alcohol **43** with lithium aluminum hydride and then oxidized to the aldehyde **44** with manganese oxide. Condensation of **44** with phenylacetonitrile in the presence of sodium hydride in ethoxyethanol gave the pyrido[2,3-*d*]pyrimidin-7-imine **45** which was hydrolyzed to the 7-one derivative **47** as described previously. The 7-imine (**46**) and 7-one (**48**) derivatives containing a 3-thiophene group at C-6 were prepared in an analogous fashion from **44**.

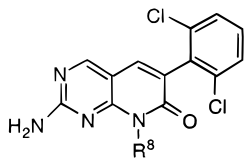
The more flexible route employing the 2-methyl sulfide group was then used to allow for variation of the amino group at C-2 as shown in Scheme 5. Aldehyde **49**[46] was condensed with phenylacetonitrile, and the resultant imine **50** was hydrolyzed to the 7-one **51**. Oxidation of the 2-methyl sulfide group of **51** with *m*-chloroperbenzoic acid gave sulfone **52** that was treated with aniline to provide **47** by an alternate route to that used initially (Scheme 5). The additional 2-ary-

Scheme 5[a]



[a] (a) $PhCH_2CN$, K_2CO_3, DMF; (b) (1) Ac_2O, reflux, (2) concd HCl; (c) *m*-CPBA, $CHCl_3$; (d) $ArNH_2$, 175 °C.

Table 1. Inhibition of Tyrosine Kinase Activity by 8-Alkyl-2-amino-6-(2,6-dichlorophenyl)pyridopyrimidin-7-ones[a]



		IC_{50} (μM)		
no.	R^8	PDGFr	FGFr	c-src
1	Me	3.8	1.3	0.26
5	Et	0.98	0.54	0.16
6	*n*-Pr	1.2	0.51	0.63
7	*n*-Bu	1.2	0.58	0.33
8	*i*-Bu	1.6	0.50	0.89
9	CH_2COOMe	5.0	1.7	0.34
10	CH_2COO-*t*-Bu	7.2	1.7	0.56
11	CH_2COOH	>50	7.9	>50
12	CH_2Ph	4.2	1.4	0.16
13	CH_2-4-pyridinyl	1.2	0.72	0.63
14	$(CH_2)_3NMe_2$	1.8	0.88	0.05
15	$(CH_2)_3OCH_2Ph$	6.8	3.2	1.4
16	$(CH_2)_3OH$	1.1	0.37	0.18

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

lamines **53** and **54** were obtained from sulfone **52** by the standard conditions.

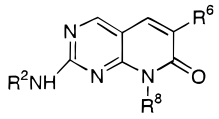
Results and Discussion

SAR against Tyrosine Kinases. Beginning with an examination of the effect of varying the N-8 alkyl group, it was determined that replacement of the methyl group at N-8 of **1** with ethyl, **5**, provided a 4-fold increase in potency against PDGFr and 2.5- and 1.5-fold increased inhibition of FGFr and c-src TK activity, respectively (Table 1). However, no substantive advantage was seen with the additional N-8 alkyl derivatives **6**−**8** when compared to **5**. The ester analogues **9** and **10** retained the c-src TK inhibitory activity of **1** but were much less potent inhibitors of PDGFr and FGFr TK activity. The acetic acid analogue **11** had greatly decreased activity against all three TKs. Although none of the additional N-8-substituted analogues (**12**−**16**) showed improved PDGFr TK inhibition, **14**, which contains a *N*,*N*-dimethylaminopropyl group, was the most potent inhibitor of c-src TK activity observed in this series (IC_{50} = 0.05 μM). In summary, for PDGFr TK inhibition the best group at N-8 was ethyl.

Certain analogues of **1** with a methyl group at N-8 and a phenylamino group at C-2, namely, **24**, **25**, and **37** where the group at C-6 was phenyl, 3-thiophene, and 2-thiophene, respectively, showed selectivity for PDGFr (Table 2). In contrast **38**, which contains a biphenyl group at C-6, was inactive against all three TKs, suggesting that size is critical at the C-6 position also. The 4-pyridinylamino analogues of **24** and **25**, **26** and **27**, respectively, had basically unchanged activity against PDGFr but were relatively more potent inhibitors of c-src. The direct analogues of **2a** with a phenyl or a 3-thiophene group at C-6, namely, **28** and **29**, were still very potent inhibitors of PDGFr with IC_{50} values of 42 and 54 nM, respectively. However these compounds were weaker inhibitors of both FGFr and c-src than was **2a**. The most dramatic difference was seen in the c-src activity which decreased from an IC_{50} of 9 nM for **2a** to IC_{50} values of 140 and 210 nM for **28** and **29**, respectively. As was seen in the 6-(2,6-dichlorophenyl) series, for compounds with a 6-(3-thiophene) substituent the corresponding 2-amino and 2-methylamino derivatives **30** and **31** were less active than the 2-phenylamino derivative **29**. The decrease in activity seen with the primary amino group at C-2, however, was much greater when the substituent at C-6 was 3-thiophene. Compared to **1** which had IC_{50} values of 3.8, 1.3, and 0.26 μM against PDGFr, FGFr, and c-src, the IC_{50} values for **30** were 24, >50, and >50 μM, respectively.

In the C-6 2,6-dichlorophenyl series, for compounds with a C-2 primary amino group, replacing the methyl group at N-8 with an ethyl resulted in increased inhibition (**1** vs **5**), while this same change at N-8 for compounds with a C-2 aromatic amino group did not alter the biological activity to such a large extent (**2a**

Table 2. Inhibition of Tyrosine Kinase Activity by 8-Alkylpyrido[2,3-*d*]pyrimidines[a]



no.	R^6	R^2	R^8	IC_{50} (μM) PDGFr	FGFr	c-src
2a	2,6-di-ClPh	Ph-4-O$(CH_2)_2NEt_2$	Me	0.079	0.043	0.009
24	phenyl	phenyl	Me	0.29	5.0	2.4
25	3-thiophene	phenyl	Me	0.80	>50	8.1
26	phenyl	4-pyridine	Me	0.32	4.7	0.47
27	3-thiophene	4-pyridine	Me	0.48	22.6	1.7
28	phenyl	Ph-4-O$(CH_2)_2NEt_2$	Me	0.042	0.35	0.14
29	3-thiophene	Ph-4-O$(CH_2)_2NEt_2$	Me	0.054	2.9	0.21
30	3-thiophene	H	Me	24.4	>50	>50
31	3-thiophene	Me	Me	>50	>50	>50
37	2-thiophene	phenyl	Me	0.95	>50	>50
38	biphenyl	phenyl	Me	>50	>50	>50
2b	2,6-di-ClPh	Ph-4-O$(CH_2)_2NEt_2$	Et	0.15	0.030	0.017
47	phenyl	phenyl	Et	0.18	1.5	1.8
48	3-thiophene	phenyl	Et	0.41	>50	41
53	phenyl	4-pyridine	Et	0.074	1.1	0.41
54	phenyl	Ph-4-O$(CH_2)_2NEt_2$	Et	0.031	0.088	0.031

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

vs **2b**). In comparing those compounds with a phenylamino group at C-2 and a 3-thiophene group at C-6, the N-8 ethyl analogue **48** was 2-fold more potent in inhibiting PDGFr than the N-8 methyl analogue **25**. While **48** was a 5-fold less potent c-src inhibitor than **25**, neither compound inhibited FGFr activity. In examining the effect of varying the N-8 group from methyl to ethyl for analogues with an unsubstituted phenyl group at C-6, the general trend was that the N-8 ethyl group resulted in increased activity against all three TKs. When compared to **2b**, the corresponding C-6 2,6-dichlorophenyl analogue **54** was 3 times more potent against PDGFr and 2−3-fold weaker against both FGFr and c-src TK activity. With an IC_{50} of 31 nM against PDGFr TK activity, **54** is the most potent inhibitor of this TK identified in this work. When tested against a panel of other kinases, **54** showed moderate activity against EGFr TK (IC_{50} = 0.34 μM) and much weaker activity against cdk4, MAPK, and the insulin receptor (IC_{50} values of 2.4, 10, and 50 μM).[54]

Cellular Studies. The best inhibitors of PDGFr TK activity in the in vitro assay were tested for their ability to inhibit PDGF-stimulated autophosphorylation of the PDGF receptor in cells. Rat aortic vascular smooth muscle cells (RAVSMCs) that express the receptor for PDGF were pretreated for 2 h with compound and then exposed to PDGF. The extent of autophosphorylation was measured by anti-phosphotyrosine immunoblotting as reported previously.[47] Compounds **29**, **47**, and **54**, which had in vitro IC_{50} values of 54, 180, and 31 nM, had IC_{50} values of 54, 4.6, and 1.5 nM, respectively, in this autophosphorylation assay (Table 3). In this assay the C-6 phenyl derivatives **47** and **54** were not substantially different from **2a**, which had an IC_{50} of 6.5 nM,[47] while **29**, the C-6 3-thiophene analogue of **54**, was much less active.

In addition to RAVSMCs, C6 rat glioma cells also express the PDGF receptor. In a similar PDGF autophosphorylation assay in these cells, **54** was again equipotent to **2a**, while **53**, the 2-(4-pyridinyl)amino analogue of **54**, was 1 log order less active (Table 3). Further studies showed that **2a**, **53**, and **54** strongly inhibited the proliferation of C6 cells in culture while **47** and **48** were much less active (IC_{50} > 25 nM). The three most active compounds were next examined in soft agar clonogenic assays. While **2a** and **53** had a decreased effect on cell growth when the compound was removed from the growth media as opposed to continuous exposure, no decrease in activity was seen with **54**.

Table 3. Effect of Tyrosine Kinase Inhibitors on PDGFr Autophosphorylation and C6 Proliferation

	inhibition of PDGFr autophos/IC_{50} (μM)			soft agar clonogenic IC_{50} (μM)	
no.	RAVSMCS[a]	C6 cells[c]	C6 proliferation[d] IC_{50} (μM)	contin exp[e]	drug removed[f]
2a	0.0065	0.005	0.34	0.12	1.80
29	0.054	NT[b]	NT[b]	NT[b]	NT[b]
47	0.0046	>0.025	NT[b]	NT[b]	NT[b]
48	NT[b]	>0.025	NT[b]	NT[b]	NT[b]
54	NT[b]	0.058	7.2	4.9	13.2
53	0.0015	0.003	1.2	0.12	0.15

[a] See ref 47 for assay conditions. [b] NT, not tested. [c] 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 PDGF for 5 min. Anti-phosphotyrosine Western blots were performed as described in the Experimental Section. [d] C6 cells were treated for 4 days with various concentrations of compound in 96-well plates, and the effect of each compound was determined using sulforhodamine B staining. [e] C6 cells were treated for 3 days with various concentrations of compound in 6-well tissue culture plates and cloned in soft agar in the continued presence of drug. [f] C6 cells were treated as in footnote e but were cloned in soft agar in the absence of drug.

In Vivo Studies. The TK inhibitor **54** was next evaluated for efficacy in various tumor model systems. The C6 glioma was chosen based on the importance of PDGF for the in vivo growth of this tumor.[45] The NIH 3T3 fibroblast line transfected with PDGF was included due to its high tumorigenic potential as compared to the parent fibroblast line implicating the potential for an autocrine loop via the ligand PDGF. In these studies the C6 glioma and PDGF-transfected NIH 3T3 cell lines

Table 4. In Vivo Activity of **54** against Tumor Xenografts in Nude Mice

tumor[a]	dose (mg/kg)	schedule[b]	weight change (g)	T/C (%) on last therapy day[c]	T − C (days)[d]	net cell kill (log_{10})[e]
C6 glioma	40	days 1−15	−0.7	0	9.6	−0.3
PDGF transfect	20	days 1−15	+	1	6.9	−1.2
DU-145	48	days 16−20, 23−27, 30−34	+	40	11.4	−0.4

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

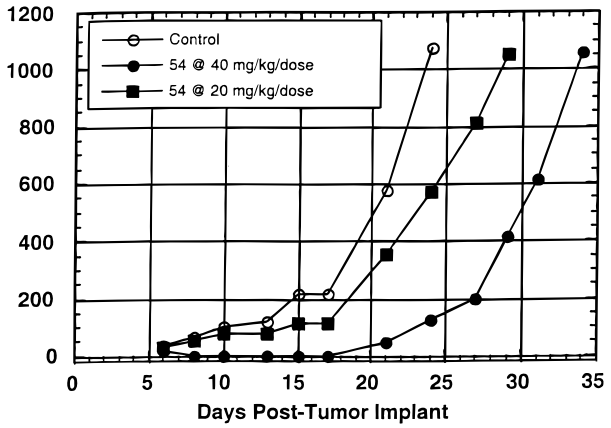


Figure 1. In vivo effectiveness of **54** against the C6 rat glioma. Tumor fragments were implanted sc on day 0, and po therapy with **54** was initiated on day 1 and continued through day 15.

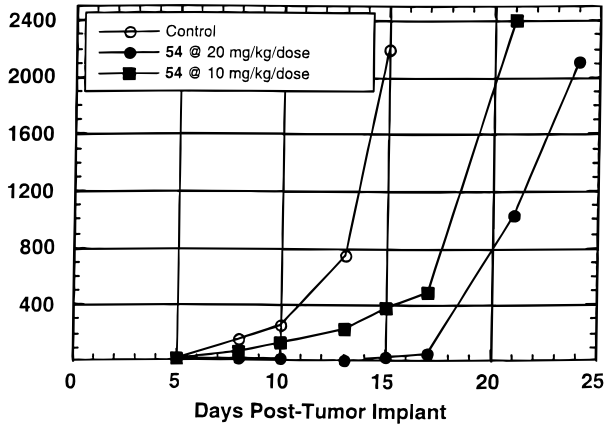


Figure 2. In vivo effectiveness of **54** against an NIH 3T3 fibroblast transfected with PDGF. Tumor fragments were implanted sc on day 0, and po therapy with **54** was initiated on day 1 and continued through day 15.

had tumor volume doubling times of 4.4 and 1.8 days, respectively. At tolerated doses **54** completely inhibited the progression of both tumor models as shown in the growth curves (Figures 1 and 2) and the very low zero or near zero T/C values on the last day of therapy (Table 4). Weight loss in these trials as a measure of drug toxicity was minimal, and no other gross toxicity was noted at the maximum tolerated dose. Tumor growth delays in the range of 7−9 days were measurable in both models but were more significant in the PDGF-transfected NIH 3T3 tumor model due to its short tumor volume doubling time and hence rapid growth.

Compound **54** was also effective against the DU-145 prostate epithelial cell line xenograft. The 11.4-day tumor growth delay observed in this study suggests that **54** may have the potential to produce activity against other human tumor xenografts (Table 4). As in the C6 and PDGF-transfected NIH 3T3 studies, there was no significant weight loss in the DU-145 tumor-bearing animals nor was any other gross toxicity noted at the maximum tolerated dosage level.

Table 5. Mean Oral Pharmacokinetic Parameter Values in Rat following a Single Oral Gavage Dose of **53** or **54**[a]

parameter	**54**	**53**
no. of animals	3	3
dose (mg/kg)	5	5
C_{max}	78.3	730
t_{max}	2.67	1.50
$t_{1/2}$	6.65	3.72
AUC(0−t_{ldc})	525	4764
AUC(0−∞)	828	5240

[a] C_{max}, maximum observed concentration (ng/mL); t_{max}, time to reach C_{max} (h); $t_{1/2}$, apparent terminal phase elimination half-life (h); AUC(0−t_{ldc}), area under concentration−time curve from time 0 to time of last detectable concentration (ng·h/mL); AUC(0−∞), area under concentration−time curve from time 0 to time infinity (ng·h/mL).

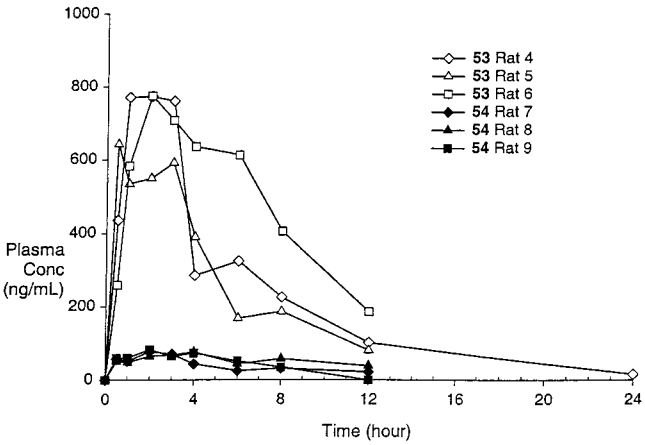


Figure 3. Individual animal plasma concentration−time profiles of **54** (closed symbols) and **53** (open symbols) following a single 5 mg/kg oral gavage dose.

Pharmacokinetics, Oral Bioavailability, and Dose Proportionality Studies in Rats. In an oral pharmacokinetic study, a single 5 mg/kg oral gavage dose of **53** or **54** was administered to three male Wistar rats. Both compounds were rapidly absorbed with a t_{max} value of less than 3 h (Table 5, Figure 3). Compound **53** achieved higher systemic exposure than **54** as expressed by C_{max} and AUC values. The differences are likely attributed to metabolism on the side chain of **54**. Previous studies with compound **2a**, which has the identical side chain as **54**, showed that the terminal dialkylamine is metabolized to the *N*-oxide; then further metabolism results in the formation of the terminal primary amine. The pyridine functionality of **53** is not susceptible to this metabolism.

Conclusion

We have demonstrated that a screening lead, **1**, could be converted into a potent inhibitor of the activity of PDGFr TK. By replacement of the 2-amino group with 4-(*N*,*N*-diethylaminoethoxy)phenylamino, the C-6 2,6-dichlorophenyl group with phenyl, and the N-8 methyl group with ethyl, **1** was converted into **54**. The IC_{50} values for the inhibition of the TK activity of PDGFr, FGFr, and c-src improved from 3.8, 1.3, and 0.26 μM for **1** to 0.031, 0.088, and 0.031 μM for **54**. This agent was active in several PDGF-dependent cellular assays including PDGFr autophosphorylation and inhibition of tumor cell growth. In addition, **54** exhibited meaningful in vivo activity against three tumor model systems. Studies to evaluate the potential use of **54** as an anticancer therapeutic agent are continuing.

Experimental Section

General Methods. Melting points were determined with a Thomas-Hoover capillary melting point apparatus or a MEL-TEMP melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded using a Varian Unity 400-MHz spectrometer. Chemical shifts are in parts per million (δ) referenced to Me_4Si. Chemical ionization mass spectra (CI) were recorded on a VG Trio 2 mass spectrometer instrument using a reagent gas of 1% NH_3 in CH_4. Atmospheric presssure chemical ionization (APCI) and electrospray mass (ES) spectra were recorded using a VG Trio 2000 mass spectrometer. Flash chromatography was performed with silica gel 60 (230−400 mesh; E. Merck Darmstad). Combustion analyses (CHN) were performed by the Parke-Davis Pharmaceutical Research Analytical Department or Robertson Microlit (Madison, NJ).

General Procedure for the Preparation of 8-*N*-Substituted-2-amino-6-(2,6-dichlorophenyl)-8*H*-pyrido[2,3-*d*]pyrimidin-7-ones from 4, Used To Prepare 5−10, 12, and 15. 2-Amino-6-(2,6-dichlorophenyl)-8-ethyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (5). To a suspension of NaH (60% in mineral oil, 27 mg) in 5 mL of DMF was added **4** (172 mg, 0.56 mmol). The mixture was heated at 50 °C for 1 h resulting in a clear solution. Ethyl iodine (60 μL, 0.75 mmol) was added, and the solution was stirred at 50 °C for 3.5 h, then cooled to room temperature, and poured onto 30 mL of ice water. The resulting precipitate was collected by filtration, washed with water, and purified by flash chromatography, eluting with 1:1 hexane/EtOAc to provide 104 mg (55%) of **6**: mp 207−209 °C; ^{1}H NMR (DMSO-d_6) δ 1.20 (t, J = 7 Hz, 3H), 4.30 (q, J = 7 Hz, 2H), 7.41−7.46 (m, 3H), 7.57 (d, J = 8 Hz, 2H), 7.70 (s, 1H), 8.65 (s, 1H); MS (CI) m/z 335 (M + 1). Anal. ($C_{15}H_{12}Cl_2N_4O$) C, H, N.

2-Amino-6-(2,6-dichlorophenyl)-8-propyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (6): yield 68%; mp 196−197 °C; ^{1}H NMR (DMSO-d_6) δ 0.89 (t, J = 7 Hz, 3H), 1.66 (m, 2H), 4.23 (t, J = 7 Hz, 2H), 7.41−7.46 (m, 3H), 7.57 (d, J = 8 Hz, 2H), 7.77 (s, 1H), 8.64 (s, 1H); MS (CI) m/z 351 (M + 1). Anal. ($C_{16}H_{14}Cl_2N_4O$) C, H, N.

2-Amino-8-butyl-6-(2,6-dichlorophenyl)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (7): yield 64%; mp 202−205 °C; ^{1}H NMR (DMSO-d_6) δ 0.91 (t, J = 7 Hz, 3H), 1.31 (m, 2H), 1.62 (m, 2H), 4.26 (t, J = 7 Hz, 2H), 7.39 (br s, 2H), 7.44 (t, J = 8 Hz, 1H), 7.56 (d, J = 8 Hz, 2H), 7.76 (s, 1H), 8.63 (s, 1H); MS (CI) m/z 365 (M + 1). Anal. ($C_{17}H_{16}Cl_2N_4O$·0.08EtOAc) C, H, N.

2-Amino-6-(2,6-dichlorophenyl)-8-isobutyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (8): yield 51%; mp 193−195 °C; ^{1}H NMR (DMSO-d_6) δ 0.87 (t, J = 6.5 Hz, 6H), 2.24 (m, 1H), 4.13 (d, J = 7.5 Hz, 2H), 7.37 (br s, 2H), 7.44 (t, J = 7.5 Hz, 1H), 7.57 (d, J = 7.5 Hz, 2H), 7.77 (s, 1H), 8.64 (s, 1H); MS (CI) m/z 365 (M + 1). Anal. ($C_{17}H_{16}Cl_2N_4O$) C, H, N.

[2-Amino-6-(2,6-dichlorophenyl)-7-oxo-7*H*-pyrido[2,3-*d*]pyrimidin-8-yl]acetic acid methyl ester (9): yield 61%; mp 188−190 °C; ^{1}H NMR (DMSO-d_6) δ 3.68 (s, 3H), 5.03 (s, 2H), 7.50 (br s, 2H), 7.45 (t, J = 8 Hz, 1H), 7.57 (d, J = 8 Hz, 2H), 7.87 (s, 1H), 8.69 (s, 1H); MS (CI) m/z 381 (M + 1). Anal. ($C_{16}H_{12}Cl_2N_4O_3$) C, H, N.

[2-Amino-6-(2,6-dichlorophenyl)-7-oxo-7*H*-pyrido[2,3-*d*]pyrimidin-8-yl]acetic acid *tert*-butyl ester (10): yield 30%; mp 171−173 °C; ^{1}H NMR (DMSO-d_6) δ 1.38 (s, 9H), 4.88 (s, 2H), 7.47 (br s, 2H), 7.45 (t, J = 8 Hz, 1H), 7.57 (d, J = 8 Hz, 2H), 7.85 (s, 1H), 8.68 (s, 1H); MS (CI) m/z 423 (M + 1). Anal. ($C_{19}H_{18}Cl_2N_4O_3$) C, H, N.

[2-Amino-6-(2,6-dichlorophenyl)-7-oxo-7*H*-pyrido[2,3-*d*]pyrimidin-8-yl]acetic Acid (11). To a solution of **10** (157 mg, 0.37 mmol) in 4 mL of CH_2Cl_2 was added 2 mL of trifluoroacetic acid. The solution was stirred at room temperature for 5 h and then concentrated. The resultant oil was partitioned between CH_2Cl_2 and brine. The aqueous layer was washed with EtOAc, and the organic layers were combined, dried over $MgSO_4$, filtered, and concentrated to give a gummy solid. Diethyl ether was added, and the resultant precipitate was collected. Hexane was added to the filtrate, and the resultant precipitate was collected. The solids were combined to give 71 mg (52%) of **11** that was >97% pure by HPLC: mp 297−300 °C dec; ^{1}H NMR (DMSO-d_6) δ 4.93 (s, 2H), 7.45 (t, J = 8 Hz, 1H), 7.48 (br s, 2H), 7.57 (d, J = 8 Hz, 2H), 7.85 (s, 1H), 8.68 (s, 1H); MS (CI) m/z 367 (M + 1). Anal. ($C_{15}H_{10}Cl_2N_4O_3$) Calcd: C, 49.34; H, 2.76; N, 15.34. Found: C, 46.01; H, 2.77; N, 13.28.

2-Amino-8-benzyl-6-(2,6-dichlorophenyl)-8*H*-pyrido-[2,3-*d*]pyrimidin-7-one (12): yield 43%; mp 220−222 °C; ^{1}H NMR (DMSO-d_6) δ 5.48 (s, 2H), 7.21−7.35 (complex m, 5H), 7.43−7.47 (complex m, 3H), 7.57 (d, J = 8 Hz, 2H), 7.84 (s, 1H), 8.67 (s, 1H); MS (CI) m/z 399 (M + 1). Anal. ($C_{20}H_{14}Cl_2N_4O$) C, H, N.

2-Amino-6-(2,6-dichlorophenyl)-8-(pyridin-4-ylmethyl)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (13). To a suspension of NaH (60% in mineral oil, 32 mg) in 6 mL of DMF was added **4** (200 mg, 0.65 mmol), and the mixture was heated to 70 °C. In a second flask containing Et_3N (220 μL, 1.59 mmol) in 4 mL of DMF was added 4-picolyl chloride hydrochloride (137 mg, 0.84 mmol). This dark red mixture was added to the solution of the sodium salt of **4**. The mixture was heated to 70 °C, then cooled to room temperature, and poured into 20 mL of ice water. The resulting precipitate was collected by filtration and washed with water followed by 10% MeOH in EtOAc to provide 96 mg of crude product. The organic filtrate was concentrated to provide an additional 77 mg of crude **13**. An analytical sample was obtained by flash chromatography, eluting with a gradient of EtOAc to 10% MeOH in EtOAc to provide **13:** mp 268−270 °C dec; ^{1}H NMR (DMSO-d_6) δ 5.49 (s, 2H), 7.22 (dd, J = 4.5, 1.5 Hz, 2H), 7.44 (t, J = 8 Hz, 1H), 7.48 (br s, 2H), 7.57 (d, J = 8 Hz, 2H), 7.90 (s, 1H), 8.49 (dd, J = 4.5, 1.5 Hz, 2H), 8.70 (s, 1H); MS (CI) m/z 400 (M + 1). Anal. ($C_{19}H_{13}Cl_2N_5O$) C, H, N.

2-Amino-6-(2,6-dichlorophenyl)-8-(3-dimethylaminopropyl)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (14). To a suspension of NaH (60% in mineral oil, 50 mg) in 8 mL of DMF was added **4** (319 mg, 1.04 mmol). The mixture was heated at 70 °C for 1.5 h resulting in a clear solution. In a second flask containing NaH (60% in mineral oil, 68 mg) in 6 mL of DMF was added 3-dimethylaminopropyl chloride hydrochloride (248 mg, 1.56 mmol). This suspension was stirred at room temperature for 30 min, then heated at 70 °C for 10 min, and added to the solution of the sodium salt of **4**. The resultant suspension was heated at 70 °C for 3 h, then cooled to room temperature, and filtered, washing with EtOAc. The filtrate was concentrated, and EtOAc and hexane were added. The resulting solid was collected by filtration to provide 216 mg (53%) of **14**: mp 136−141 °C; ^{1}H NMR (DMSO-d_6) δ 1.76 (pent, J = 7 Hz, 2H), 2.11 (s, 6H), 2.26 (t, J = 7 Hz, 2H), 4.29 (t, J = 7 Hz, 2H), 7.39 (br s, 2H), 7.44 (t, J = 8 Hz, 1H), 7.57 (d, J = 8 Hz, 2H), 7.77 (s, 1H), 8.64 (s, 1H); MS (CI) m/z 394 (M + 1). Anal. ($C_{18}H_{19}Cl_2N_5O$) C, H, N.

2-Amino-8-(3-benzyloxypropyl)-6-(2,6-dichlorophenyl)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (15): yield 71%; mp undefined (softens at 63 °C); ^{1}H NMR (DMSO-d_6) δ 1.94 (pent, J = 7 Hz, 2H), 3.50 (t, J = 7 Hz, 2H), 4.36 (t, J = 7 Hz, 2H),

4.42 (s, 2H), 7.23−7.34 (complex m, 5H), 7.40 (br s, 2H), 7.44 (t, J = 8 Hz, 1H), 7.56 (d, J = 8 Hz, 2H), 7.76 (s, 1H), 8.63 (s, 1H); MS (CI) m/z 457 (M + 1). Anal. ($C_{23}H_{20}Cl_2N_4O_2$·0.2EtOAc) Calcd: C, 60.44; H, 4.60; N, 11.85. Found: C, 60.90; H, 4.77; N, 11.91.

2-Amino-6-(2,6-dichlorophenyl)-8-(3-hydroxypropyl)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (16). A solution of **15** (379 mg, 0.83 mmol) in 75 mL of acetic acid was treated with increasing amounts of 5−20% palladium on carbon over 12 h. The catalyst was filtered off, and the filtrate was concentrated. The residue was partitioned between EtOAc and 1 N NaOH. The organic layer was dried over $MgSO_4$, filtered, and concentrated. Flash chromatography gave 94 mg (31%) of **16**: mp undefined (softens at 98 °C); ^{1}H NMR (DMSO-d_6) δ 1.78 (pent, J = 7 Hz, 2H), 3.45 (dt, J = 7, 5.5 Hz, 2H), 4.31 (t, J = 7 Hz, 2H), 4.46 (t, J = 5.5 Hz, 1H), 7.42 (br s, 2H), 7.44 (t, J = 8 Hz, 1H), 7.57 (d, J = 8 Hz, 2H), 7.78 (s, 1H), 8.65 (s, 1H); MS (CI) m/z 367 (M + 1). Anal. ($C_{16}H_{14}Cl_2N_4O_2$·0.2 H_2O) C, H, N.

8-Methyl-2-methylsulfanyl-6-phenyl-8*H*-pyrido[2,3-*d*]-pyrimidin-7-ylideneamine (18). To a solution of **17** (1.50 g, 8.18 mmol) and phenylacetonitrile (1.2 mL, 10.36 mmol) in 10 mL of DMF was added K_2CO_3 (6.60 g, 47.83 mmol). The mixture was heated at 125 °C for 1.5 h. The hot mixture was filtered, and the solid was washed with DMF. Water was added to the filtrate, and the black solid was removed by filtration. The crystals that formed in the filtrate were collected to give 449 mg (19%) of **18**: mp 150−151 °C; ^{1}H NMR (DMSO-d_6) δ 2.51 (s, 3H), 3.57 (s, 3H), 7.28 (s, 1H), 7.37−7.52 (m, 6H), 8.48 (s, 1H); MS (CI) m/z 283 (M + 1). Anal. ($C_{15}H_{14}N_4S$) C, H, N.

8-Methyl-2-methylsulfanyl-6-phenyl-8*H*-pyrido[2,3-*d*]-pyrimidin-7-one (20). A mixture of **18** (745 mg, 2.64 mmol) and 5.5 mL of Ac_2O was heated at reflux for 30 min. The cooled solution was mixed with diethyl ether and concentrated. The solid was washed with diethyl ether to provide 718 mg (84%) of the *N*-acetylimine derivative which was refluxed with concentrated HCl for 5 min. The resulting solid was collected and washed with water and 2-propanol to give 512 mg (87%) of **20** that was not purified.

8-Methyl-2-methylsulfonyl-6-thiophene-3-yl-8*H*-pyrido-[2,3-*d*]pyrimidin-7-one (22). To a solution of **20** (206 mg, 0.73 mmol) in 30 mL of $CHCl_3$ was added 50−60% *m*-chloroperbenzoic acid (600 mg, 1.74−2.09 mmol). The solution was stirred at room temperature for 2 h, then washed with saturated $NaHCO_3$, dried over $MgSO_4$, and concentrated. EtOAc was added to the residue followed by hexane. The solid was collected and washed with 4:1 hexane/EtOAc to give 139 mg (60%) of **22**: mp 191−193 °C; ^{1}H NMR (DMSO-d_6) δ 3.44 (s, 3H), 3.68 (s, 3H), 7.39−7.47 (m, 3H), 7.69 (d, J = 2 Hz, 2H), 8.26 (s, 1H), 9.26 (s, 1H); MS (CI) m/z 316 (M + 1). Anal. ($C_{15}H_{13}N_3O_3S$) C, H, N.

8-Methyl-6-phenyl-2-phenylamino-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (24). A mixture of **22** (174 mg, 0.55 mmol) and aniline (440 mg, 5.30 mmol) was heated at 175 °C for 10 min. The reaction was cooled to room temperature and directly purified by flash chromatography eluting with EtOAc to give 62 mg (34%) of **24**: mp 210−211 °C; ^{1}H NMR (DMSO-d_6) δ 3.68 (s, 3H), 7.04 (t, J = 7 Hz, 1H), 7.34−7.39 (m, 3H), 7.44 (d, J = 7 Hz, 2H), 7.68 (d, J = 7 Hz, 2H), 7.85 (d, J = 8 Hz, 2H), 8.03 (s, 1H), 8.85 (s, 1H), 10.16 (s, 1H); MS (CI) m/z 329 (M + 1). Anal. ($C_{20}H_{16}N_4O$·0.25H_2O) C, H, N.

8-Methyl-6-phenyl-2-(pyridin-4-ylamino)-8*H*-pyrido-[2,3-*d*]pyrimidin-7-one (26). A mixture of **22** (146 mg, 0.46 mmol) and 4-aminopyridine (262 mg, 2.78 mmol) was heated at 175 °C for 10 min. The reaction was cooled to room temperature and directly purified by flash chromatography eluting with EtOAc to give 77 mg (51%) of **26**: mp 244−245 °C; HPLC purity >99%, column VYDAC C_{18} 218TP54, mobile phase gradient 10−76% 0.1% TFA in acetonitrile to 0.1% TFA in water; ^{1}H NMR (DMSO-d_6) δ 3.67 (s, 3H), 7.31−7.42 (m, 3H), 7.63 (t, J = 7 Hz, 2H), 7.78 (d, J = 5 Hz, 2H), 8.03 (s, 1H), 8.38 (d, J = 5 Hz, 2H), 8.88 (s, 1H), 10.49 (s, 1H); MS (CI) m/z 330 (M + 1). Anal. ($C_{19}H_{15}N_5O$) Calcd: C, 69.29; H, 4.59; N, 21.26. Found: C, 66.79; H, 5.05; N, 19.04.

2-[4-(2-Diethylaminoethoxy)phenylamino]-8-methyl-6-phenyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (28). A mixture of **22** (197 mg, 0.63 mmol) and 4-(2-diethylaminoethoxy)aniline (260 mg, 1.25 mmol) was heated at 175 °C for 5 min. The reaction was cooled to room temperature, and the residue was purified by flash chromatography eluting with 5% MeOH in $CHCl_3$ to give 30 mg (11%) of **28**: mp 157−158 °C; ^{1}H NMR (DMSO-d_6) δ 0.98 (t, J = 7 Hz, 6H), 2.55 (q, J = 7 Hz, 4H), 2.77 (t, J = 6 Hz, 2H), 3.66 (s, 3H), 4.00 (t, J = 6 Hz, 2H), 6.94 (d, J = 9 Hz, 2H), 7.36−7.45 (m, 3H), 7.67−7.72 (m, 4H), 8.00 (s, 1H), 8.80 (s, 1H), 10.00 (s, 1H); MS (CI) m/z 444 (M + 1). Anal. ($C_{26}H_{29}N_5O_2$·0.5H_2O) C, H, N.

8-Methyl-2-methylsulfanyl-6-thiophene-3-yl-8*H*-pyrido-[2,3-*d*]pyrimidin-7-ylideneamine (19). To a solution of **17** (10.0 g, 54.6 mmol) and 3-thiopheneacetonitrile (13.52 g, 109.2 mmol) in 100 mL of DMF was added K_2CO_3 (37.7 g, 273.0 mmol). The mixture was heated at 125 °C for 1.5 h. The hot mixture was filtered, and the solid was washed with EtOAc. The solid that appeared in the filtrate was collected and washed with 10% EtOAc in hexane followed by acetone to give 10.87 g (69%) of **19**: mp 146−147 °C; ^{1}H NMR (DMSO-d_6) δ 2.57 (s, 3H), 3.64 (s, 3H), 7.27−7.28 (m, 1H), 7.41 (s, 1H), 7.71 (s, 1H), 7.76−7.78 (m, 2H), 8.53 (s, 1H); MS (CI) m/z 289 (M + 1). Anal. ($C_{13}H_{12}N_4S_2$) C, H, N.

8-Methyl-2-methylsulfanyl-6-thiophene-3-yl-8*H*-pyrido-[2,3-*d*]pyrimidin-7-one (21). A mixture of **19** (7.39 g, 25.7 mmol) and 50 mL of Ac_2O was heated at reflux for 15 min. The cooled solution was mixed with 200 mL of diethyl ether and concentrated. The solid was washed with diethyl ether to provide 6.27 g (74%) of the *N*-acetylimine derivative which was refluxed with concentrated HCl for 5 min. The resulting solid was collected, washed with water, and purified by flash chromatography eluting with CH_2Cl_2 to give 2.99 g (55%) of **21**: mp 176−177 °C; ^{1}H NMR (DMSO-d_6) δ 2.63 (s, 3H), 3.70 (s, 3H), 7.64−7.70 (m, 2H), 8.35−8.36 (m, 1H), 8.40 (s, 1H), 8.91 (s, 1H); MS (CI) m/z 290 (M + 1). Anal. ($C_{13}H_{11}N_3OS_2$) C, H, N.

8-Methyl-2-methylsulfonyl-6-thiophene-3-yl-8*H*-pyrido-[2,3-*d*]pyrimidin-7-one (23). To a solution of **21** (2.01 g, 6.95 mmol) in 150 mL of $CHCl_3$ was added 50−60% *m*-chloroperbenzoic acid (5.50 g, 16.0−19.2 mmol). The solution was stirred at room temperature for 1 h, then washed with saturated $NaHCO_3$, dried over $MgSO_4$, and concentrated. The solid was purified by flash chromatography eluting with CH_2Cl_2 to give 1.81 g (81%) of **23**: ^{1}H NMR (DMSO-d_6) δ 3.50 (s, 3H), 3.76 (s, 3H), 7.70−7.76 (m, 2H), 8.48−8.49 (m, 1H), 8.58 (s, 1H), 9.28 (s, 1H); MS (CI) m/z 322 (M + 1).

8-Methyl-2-phenylamino-6-thiophene-3-yl-8*H*-pyrido-[2,3-*d*]pyrimidin-7-one (25). A mixture of **23** (214 mg, 0.67 mmol) and aniline (400 mg, 4.82 mmol) was heated at 150 °C for 15 min. The reaction was cooled to room temperature and directly purified by flash chromatography eluting with 5% MeOH in $CHCl_3$ to give 67 mg (30%) of **25**: mp 237−238 °C; ^{1}H NMR (DMSO-d_6) δ 3.70 (s, 3H), 7.04 (t, J = 7 Hz, 1H), 7.36 (t, J = 8 Hz, 2H), 7.61−7.68 (m, 2H), 7.84 (d, J = 8 Hz, 2H), 8.27 (s, 1H), 8.30 (s, 1H), 8.83 (s, 1H), 10.15 (s, 1H); MS (CI) m/z 335 (M + 1). Anal. ($C_{18}H_{14}N_4OS$·0.25H_2O) C, H, N.

8-Methyl-2-(pyridin-4-ylamino)-6-thiophene-3-yl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (27). A mixture of **23** (190 mg, 0.59 mmol) and 4-aminopyridine (170 mg, 1.81 mmol) was heated at 175 °C for 5 min. The reaction was cooled to room temperature and directly purified by flash chromatography eluting with 5% MeOH in $CHCl_3$ to give 61 mg (31%) of **27**: mp 310−311 °C; ^{1}H NMR (DMSO-d_6) δ 3.74 (s, 3H), 6.69−7.63 (m, 2H), 7.84 (d, J = 6 Hz, 2H), 8.30−8.45 (m, 4H), 8.92 (s, 1H), 10.54 (s, 1H); MS (CI) m/z 336 (M + 1). Anal. ($C_{17}H_{13}N_5OS$·0.25H_2O) C, H, N.

2-[4-(2-Diethylaminoethoxy)phenylamino]-8-methyl-6-thiophene-3-yl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (29). A mixture of **23** (132 mg, 0.43 mmol) and 4-(2-diethylaminoethoxy)aniline (178 mg, 0.86 mmol) was heated at 175 °C for 5 min. The reaction was cooled to room temperature, and the residue was purified by flash chromatography eluting with 5% MeOH in $CHCl_3$ to give 40 mg (22%) of **29**: mp 190−192 °C;

^{1}H NMR (DMSO-d_6) δ 0.98 (t, J = 7 Hz, 6H), 2.56 (q, J = 7 Hz, 4H), 2.77 (t, J = 6 Hz, 2H), 3.67 (s, 3H), 4.00 (t, J = 6 Hz, 2H), 6.94 (d, J = 9 Hz, 2H), 7.60−7.72 (m, 4H), 8.25 (t, J = 1 Hz, 1H), 8.27 (s, 1H), 8.78 (s, 1H), 9.99 (s, 1H); MS (CI) m/z 450 (M + 1). Anal. ($C_{24}H_{27}N_5O_2S\cdot H_2O$) C, H, N.

2-Amino-8-methyl-6-thiophene-3-yl-8*H*-pyrido[2,3-*d*]-pyrimidin-7-one (30). A mixture of **21** (170 mg, 0.59 mmol), ammonia, and MeOH was sealed in a glass tube and heated at 100 °C for 86 h. The solution was concentrated and purified by flash chromatography eluting with EtOAc. Recrystallization from EtOAc and hexane gave 115 mg (76%) of **30**: mp 241−242 °C; ^{1}H NMR (DMSO-d_6) δ 3.59 (s, 3H), 7.33 (s, 2H), 7.58−7.64 (m, 2H), 8.21 (s, 1H), 8.22 (s, 1H), 8.63 (s, 1H); MS (CI) m/z 259 (M + 1). Anal. ($C_{12}H_{10}N_4OS$) C, H, N.

8-Methyl-2-methylamino-6-thiophene-3-yl-8*H*-pyrido-[2,3-*d*]pyrimidin-7-one (31). A mixture of **21** (125 mg, 0.43 mmol), methylamine, and DMF was sealed in a glass tube and heated at 110 °C for 40 h. The solution was concentrated and the solid washed with diethyl ether to provide 61 mg (52%) of **31**: mp 263−265 °C; ^{1}H NMR (DMSO-d_6) δ 2.92 (d, J = 4 Hz, 3H), 3.65 and 3.56 (two peaks, 3H), 7.58−7.65 (m, 2H), 7.83 (br s, 1H), 8.22 (s, 2H), 7.76 and 8.63 (two peaks, 1H); MS (CI) m/z 273 (M + 1). Anal. ($C_{13}H_{12}N_4OS\cdot 0.25H_2O$) C, H, N.

4-Methylamino-2-methylsulfanyl-pyrimidine-5-carbonitrile (32). Methylamine gas was bubbled through a 5 ºC solution of 4-chloro-2-methylsulfanyl-pyrimidine-5-carbonitrile (32.0 g, 170 mmol) in 700 mL of diethyl ether. The reaction mixture was stirred at room temperature overnight. Additional methylamine gas was added, and the mixture stirred at room temperature overnight. The solids were collected and suspended in water. The suspension was filtered to provide 25.8 g (85%) of product. Recrystallization from EtOAc gave an analytical sample of **32**: mp 189−190 °C; ^{1}H NMR ($CDCl_3$) δ 2.54 (s, 3H), 3.12 (d, J = 5 Hz, 3H), 5.50 (br s, 1H), 8.82 (s, 1H). Anal. ($C_7H_8N_4S$) C, H, N.

4-Methylamino-2-phenylamino-pyrimidine-5-carbonitrile (33). A suspension of **32** (10 g, 55.5 mmol) in 30 mL of aniline containing 4 drops of concentrated HCl was heated to 170 °C for 1 h. Upon removal of the heat source a solid rapidly formed. While the reaction mixture was still warm, 30 mL of MeOH was added. The thick mixture was cooled to room temperature and the solid collected washing with additional MeOH to provide 8.73 g (70%) of product. Recrystallization from EtOAc and MeOH gave an analytical sample of **33**: mp 275−277 °C; ^{1}H NMR (DMSO-d_6) δ 2.91 (d, J = 4 Hz, 3H), 6.99 (t, J = 7.5 Hz, 1H), 7.29 (t, J = 8 Hz, 2H), 7.71 (br s, 1H), 7.76 (d, J = 8 Hz, 2H), 8.33 (s, 1H), 9.81 (br s, 1H). Anal. ($C_{12}H_{11}N_5$) C, H, N.

4-Methylamino-2-phenylamino-pyrimidine-5-carbaldehyde (34). To a 0 °C solution of **33** (4.5 g, 20.00 mmol) in 100 mL of THF was added 20 mL of a 1 M solution of diisobutylaluminum hydride in CH_2Cl_2. The ice bath was removed, and an additional 20 mL of a 1 M solution of diisobutylaluminum hydride in CH_2Cl_2 was added. After 30 min an additional 5 mL of a 1 M solution of diisobutylaluminum hydride in CH_2Cl_2 was added. After 30 min the reaction was quenched by the dropwise addition of 30 mL of MeOH. This mixture was partitioned between 200 mL of EtOAc and 140 mL of 1 N HCl. The layers were separated, and the acid layer was treated with 150 mL of 1 N NaOH and extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated to give 2.20 g of a 3:1 mixture of **34**:**33** by NMR. Purification by flash chromatography eluting with 1:1 hexane/EtOAc gave an analytical sample of **34:** mp 210−212 °C; ^{1}H NMR (DMSO-d_6) δ 3.02 (d, J = 5 Hz, 3H), 7.01 (t, J = 7.5 Hz, 1H), 7.31 (t, J = 8 Hz, 2H), 7.83 (d, J = 8 Hz, 2H), 8.46 (s, 1H), 8.59 (br s, 1H), 9.55 (s, 1H), 9.97 (br s, 1H). Anal. ($C_{12}H_{13}N_4O$) C, H, N.

(7-Imino-8-methyl-6-thiophene-2-yl-7,8-dihydropyrido-[2,3-*d*]pyrimidin-2-yl)phenylamine (35). To a suspension of NaH (21 mg of a 60% suspension of NaH in mineral oil) in 5 mL of 2-ethoxyethanol were added 2-thiopheneacetonitrile (53 μL, 0.50 mmol) and **34** (101 mg, 0.44 mmol). The reaction mixture was heated at reflux for 17 h. The solution was cooled to room temperature, and the resulting precipitate was collected and washed with 1:1 hexane/EtOAc. The solid was purified by flash chromatography eluting with 10% MeOH in EtOAc to give 60 mg (41%) of **35**: mp 241−242 °C; ^{1}H NMR (DMSO-d_6) δ 3.66 (s, 3H), 7.00 (t, J = 7 Hz, 1H), 7.21 (two peaks, 1H), 7.34 (t, J = 7 Hz, 2H), 7.44 (two peaks, 1H), 7.73−7.79 (m, 4H), 7.81 (two peaks, 1H), 8.52 (s, 1H), 9.92 (s, 1H); MS (CI) m/z 334 (M + 1). Anal. ($C_{18}H_{15}N_5S$) C, H, N.

8-Methyl-2-phenylamino-6-thiophene-2-yl-8*H*-pyrido-[2,3-*d*]pyrimidin-7-one (37). A mixture of **35** (283 mg, 0.85 mmol) and 10 mL of Ac_2O was heated at reflux for 30 min. The solution was concentrated, and the resulting *N*-acetylimine derivative was dissolved in 10 mL of concentrated HCl and heated at reflux for 5 min. The reaction was cooled to room temperature, and the crude product was collected and purified by flash chromatography eluting with 5% MeOH in $CHCl_3$ to provide 74 mg (26%) of **37**: mp 235−236 °C; ^{1}H NMR (DMSO-d_6) δ 3.72 (s, 3H), 7.04 (t, J = 7 Hz, 1H), 7.16 (d, J = 5 Hz, 1H), 7.36 (t, J = 8 Hz, 2H), 7.59 (d, J = 5 Hz, 1H), 7.84 (d, J = 8 Hz, 2H), 8.45 (s, 1H), 8.87 (s, 1H), 10.19 (s, 1H); MS (CI) m/z 335 (M + 1). Anal. ($C_{18}H_{14}N_4OS\cdot 0.25H_2O$) C, H, N.

[6-(Biphenyl-4-yl)-7-imino-8-methyl-7,8-dihydropyrido-[2,3-*d*]pyrimidin-2-yl)phenylamine (36). To a suspension of NaH (110 mg of a 60% suspension of NaH in mineral oil) in 20 mL of 2-ethoxyethanol were added 4-biphenylacetonitrile (516 mg, 2.67 mmol) and **34** (538 mg, 2.36 mmol). The reaction mixture was heated at reflux for 17 h and then cooled to room temperature. The precipitate was collected and washed with 1:1 hexane/EtOAc to give 826 mg (87%) of **36**: mp 259−260 °C; ^{1}H NMR (DMSO-d_6) δ 3.69 (s, 3H), 7.00 (t, J = 7 Hz, 1H), 7.32−7.41 (m, 5H), 7.42−7.54 (m, 4H), 7.75 (d, J = 8 Hz, 2H), 7.82 (d, J = 8 Hz, 4H), 8.51 (s, 1H), 9.89 (s, 1H); MS (CI) m/z 404 (M + 1). Anal. ($C_{26}H_{21}N_5\cdot 0.3H_2O$) C, H, N.

6-(Biphenyl-4-yl)-8-methyl-2-phenylamino-8*H*-pyrido-[2,3-*d*]pyrimidin-7-one (38). A mixture of **36** (261 mg, 0.65 mmol) and 12 mL of Ac_2O was heated at reflux for 30 min. The solution was concentrated, and the resulting *N*-acetylimine derivative was dissolved in 10 mL of concentrated HCl and heated at reflux for 5 min. The reaction was cooled to room temperature, and the solid was collected and purified by flash chromatography eluting with 5% MeOH in $CHCl_3$ to provide 152 mg (58%) of **38**: mp 263−264 °C; ^{1}H NMR (DMSO-d_6) δ 3.70 (s, 3H), 7.04 (t, J = 8 Hz,1H), 7.35−7.41 (m, 3H), 7.49 (t, J = 8 Hz, 2H), 7.73−7.76 (m, 4 H), 7.80−7.86 (m, 4H), 8.11 (s, 1H), 8.87 (s, 1H), 10.17 (s, 1H); MS (CI) m/z 405 (M + 1). Anal. ($C_{26}H_{20}N_4O\cdot 0.80H_2O$) C, H, N.

4-Ethylamino-2-methylsulfanyl-5-pyrimidinecarboxylate Ethyl Ester (40). To a room-temperature solution of **39** (10.00 g, 43.10 mmol) in 150 mL of THF was added Et_3N (18.5 mL, 133 mmol) followed by 9 mL of a 70% aqueous solution of ethylamine. The solution was stirred for 30 min, then concentrated, and partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered, and concentrated to provide 9.32 g (90%) of **40** as an oil: ^{1}H NMR (DMSO-d_6) δ 1.17 (t, J = 7 Hz, 3H), 1.30 (t, J = 7 Hz, 3H), 2.48 (s, 3H), 3.51 (dq, J = 7, 5 Hz, 2H), 4.27 (q, J = 7 Hz, 2H), 8.31 (t, J = 5 Hz, 1H), 8.53 (s, 1H); MS (CI) m/z 242 (M + 1). Anal. ($C_{10}H_{15}N_3O_2S$) C, H, N.

4-Ethylamino-2-methylsulfinyl-pyrimidine-5-carboxylic Acid Ethyl Ester (41). To a room-temperature solution of **40** (2.011 g, 8.34 mmol) in 70 mL of $CHCl_3$ was added (±)-*trans*-2-phenylsulfonyl-3-phenyloxaziridine (2.70 g, 10.34 mmol). The solution was stirred at room temperature for 7 h and then concentrated. The residue was purified by flash chromatography eluting with a gradient of EtOAc to 3% MeOH in EtOAc to provide 2.07 g (97%) of **41**: mp 54−56 °C; ^{1}H NMR (DMSO-d_6) δ 1.18 (t, J = 7 Hz, 3H), 1.33 (t, J = 7 Hz, 3H), 2.84 (s, 3H), 3.55 (dq, J = 7, 5.5 Hz, 2H), 4.33 (q, J = 7 Hz, 2H), 8.59 (t, J = 5.5 Hz, 1H), 8.79 (s, 1H); MS (CI) m/z 258 (M + 1). Anal. ($C_{10}H_{15}N_3O_3S$) C, H, N.

4-Ethylamino-2-phenylamino-pyrimidine-5-carboxylic Acid Ethyl Ester (42). A solution of **41** (5.38 g, 20.9 mmol) in 4 mL of aniline was heated at 130 °C for 1 h. The solution was cooled to room temperature resulting in a solid; 20 mL of

1:1 hexane/EtOAc was added, and the white solid was collected by filtration providing 1.96 g (33%) of **42**. The filtrate was concentrated and purified by flash chromatography eluting with 3:1 hexane/EtOAc to give an additional 257 mg of **42**: mp 145−147 °C; ^{1}H NMR (DMSO-d_6) δ 1.21 (t, J = 7 Hz, 3H), 1.30 (t, J = 7 Hz, 3H), 3.52 (dq, J = 7, 5 Hz, 2H), 4.24 (q, J = 7 Hz, 2H), 6.98 (t, J = 7 Hz, 1H), 7.29 (t, J = 8 Hz, 2H), 7.79 (d, J = 8 Hz, 2H), 8.23 (t, J = 5 Hz, 1H), 8.56 (s, 1H), 9.77 (br s, 1H); MS (CI) m/z 287 (M + 1). Anal. ($C_{15}H_{18}N_4O_2$) C, H, N.

(4-Ethylamino-2-phenylamino-pyrimidin-5-yl)methanol (43). A solution of **42** (109 mg, 0.38 mmol) in 6 mL of THF was added dropwise to a room temperature suspension of LAH (35 mg, 0.92 mmol) in 5 mL of THF. After 25 min, an additional 30 mg of LAH was added and stirring was continued for 30 min. The reaction was carefully quenched with 120 μL of water, 200 μL of 15% NaOH, and 300 μL of water. After stirring for 1 h, the white precipitate was removed by filtration washing with EtOAc. The filtrate was concentrated and the residue purified by chromatography eluting with EtOAc to provide 36 mg (39%) of **43**: mp 174−176 °C; ^{1}H NMR (DMSO-d_6) δ 1.19 (t, J = 7 Hz, 3H), 3.44 (dq, J = 7, 5.5 Hz, 2H), 4.28 (d, J = 5.5 Hz, 2H), 4.93 (t, J = 5.5 Hz, 1H), 6.53 (t, J = 5.5 Hz, 1H), 6.84 (t, J = 7 Hz, 1H), 7.21 (t, J = 8 Hz, 2H), 7.74 (s, 1H), 7.77 (d, J = 8 Hz, 2H), 8.93 (s, 1H); MS (CI) m/z 245 (M + 1). Anal. ($C_{13}H_{16}N_4O$) C, H, N.

4-Ethylamino-2-phenylamino-pyrimidine-5-carbaldehyde (44). To a solution of **43** (173 mg, 0.71 mmol) in 15 mL of $CHCl_3$ was added MnO_2 (600 mg, 6.89 mmol). After stirring at room temperature overnight the mixture was filtered through a pad of Celite, washing with $CHCl_3$. The filtrate was concentrated to give 170 mg (99%) of **44**: mp 155−157 °C; ^{1}H NMR (DMSO-d_6) δ 1.21 (t, J = 7 Hz, 3H), 3.53 (m, 2H), 7.02 (t, J = 7 Hz, 1H), 7.31 (t, J = 8 Hz, 2H), 7.80 (d, J = 8 Hz, 2H), 8.47 (s, 1H), 8.65 (br s, 1H), 9.55 (s, 1H), 9.98 (br s, 1H); MS (CI) m/z 243 (M + 1). Anal. ($C_{13}H_{14}N_4O$) C, H, N.

(8-Ethyl-7-imino-6-phenyl-7,8-dihydropyrido[2,3-*d*]pyrimidin-2-yl)phenylamine (45). To a suspension of NaH (60% in mineral oil, 44 mg) in 5 mL of 2-ethoxyethanol was added phenylacetonitrile (120 μL, 1.08 mmol). After the mixture stirred for 5 min at room temperature, **44** (230 mg, 0.95 mmol) in 2 mL of 2-ethoxyethanol was added and the reaction mixture was heated at reflux for 5 h. The solution was cooled to room temperature, and the resulting precipitate was removed by filtration and washed with water to give 305 mg of product. Recrystallization from EtOAc provided 201 mg (62%) of **45**: mp 193−195 °C; ^{1}H NMR (DMSO-d_6) δ 1.29 (t, J = 7 Hz, 3H), 4.47 (q, J = 7 Hz, 2H), 7.00 (t, J = 7 Hz, 1H), 7.23−7.54 (complex m, 9H), 7.82 (d, J = 7.5 Hz, 2H), 8.48 (s, 1H), 9.86 (s, 1H); MS (CI) m/z 342 (M + 1). Anal. ($C_{21}H_{19}N$) C, H, N.

8-Ethyl-6-phenyl-2-phenylamino-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (47). 45 (182 mg, 0.53 mmol) was added to 2 mL of Ac_2O and heated at reflux for several min. The reaction was cooled to room temperature and concentrated. The residue was heated at reflux with 4 mL of concentrated HCl for several minutes. The reaction was cooled, and 10 mL of water was added resulting in the formation of a small amount of a precipitate that was removed by filtration and washed with water. The filtrate was made basic with 1 N NaOH, and the aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The residue was combined with the solid obtained previously. Purification by flash chromatography eluting with a gradient of 1:1 hexane/EtOAc to EtOAc gave 92 mg (51%) of **47**: mp 202−204 °C; ^{1}H NMR (DMSO-d_6) δ 1.30 (t, J = 7 Hz, 3H), 4.41 (q, J = 7 Hz, 2H), 7.04 (t, J = 7 Hz, 1H), 7.34−7.46 (complex m, 5H), 7.68 (d, J = 7.5 Hz, 2H), 7.85 (d, J = 7.5 Hz, 2H), 8.02 (s, 1H), 8.85 (s, 1H), 10.16 (s, 1H); MS (CI) m/z 343 (M + 1). Anal. ($C_{21}H_{18}N_4O$) C, H, N.

(8-Ethyl-7-imino-6-thiophene-3-yl-7,8-dihydropyrido-[2,3-*d*]pyrimidin-2-yl)phenylamine (46). To a suspension of NaH (60% in mineral oil, 37 mg) in 5 mL of 2-ethoxyethanol was added 3-thiopheneacetonitrile (100 μL, 0.88 mmol) followed by **44** (180 mg, 0.74 mmol) in 2 mL of 2-ethoxyethanol. The reaction was heated at reflux for 5 h resulting in a dark brown solution. The solution was cooled to room temperature, and 3 mL of water was added. The resulting precipitate was removed by filtration and washed with water. Recrystallization from EtOAc and MeOH gave 129 mg (51%) of **46**: mp 223−225 °C; ^{1}H NMR (DMSO-d_6) δ 1.29 (t, J = 7 Hz, 3H), 4.47 (q, J = 7 Hz, 2H), 7.00 (t, J = 7 Hz, 1H), 7.26 (dd, J = 5, 1.5 Hz, 1H), 7.30−7.34 (m, 3H), 7.43 (s, 1H), 7.73−7.76 (m, 2H), 7.82 (d, J = 7.5 Hz, 2H), 8.47 (s, 1H), 9.87 (s, 1H); MS (CI) m/z 348 (M + 1). Anal. ($C_{19}H_{17}N_5S$) C, H, N.

8-Ethyl-2-phenylamino-6-thiophene-3-yl-8*H*-pyrido-[2,3-*d*]pyrimidin-7-one (48). Compound was prepared from **46** (173 mg, 0.50 mmol) following the procedure used to prepare **47**. The product was purified by flash chromatography, eluting with a gradient of 1:1 hexane/EtOAc to all EtOAc to give 47 mg (27%) of **48**: mp 225−227 °C; ^{1}H NMR (DMSO-d_6) δ 1.31 (t, J = 7 Hz, 3H), 4.43 (q, J = 7 Hz, 2H), 7.04 (t, J = 7 Hz, 1H), 7.36 (t, J = 7.5 Hz, 2H), 7.62 (dd, J = 5, 3 Hz, 1H), 7.66 (dd, J = 5, 1 Hz, 1H), 7.84 (d, J = 7.5 Hz, 2H), 8.27 (dd, J = 3, 1 Hz, 1H), 8.29 (s, 1H), 8.83 (s, 1H), 10.15 (s, 1H); MS (CI) m/z 349 (M + 1). Anal. ($C_{19}H_{16}N_4OS$) C, H, N.

8-Ethyl-2-methylsulfanyl-6-phenyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-ylideneamine (50). To a solution of **49** (570 mg, 2.89 mmol) and phenylacetonitrile (355 mg, 3.03 mmol) in 5 mL of DMF was added K_2CO_3 (1.94 g, 14.0 mmol). The mixture was heated at 125 °C for 17 h. The hot mixture was filtered and washed with EtOAc. The filtrate was concentrated and the resulting solid purified by flash chromatography eluting with a gradient of 1:1 hexane/EtOAc to EtOAc. Recrystallization from EtOAc and hexane gave 196 mg (23%) of **50** : mp 120−121 °C; ^{1}H NMR (DMSO-d_6) δ 1.24 (t, J = 7 Hz, 3H), 2.58 (s, 3H), 4.45 (q, J = 7 Hz, 2H), 7.35 (s, 1H), 7.43−7.55 (m, 6H), 8.56 (s, 1H); MS (CI) m/z 297 (M + 1). Anal. ($C_{16}H_{16}N_4S$) C, H, N.

8-Ethyl-2-methylsulfanyl-6-phenyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (51). A mixture of **50** (156 mg, 5.27 mmol) and 12 mL of Ac_2O was heated at reflux for 30 min. Diethyl ether was added, and the mixture was concentrated to give a solid that was washed with diethyl ether to provide 730 mg (41%) of the *N*-acetylimine derivative. This material was refluxed with concentrated HCl for 5 min. The resulting pale yellow solid was filtered off and washed with water and then diethyl ether to give 550 mg (92%) of **51**: mp 155−157 °C; ^{1}H NMR (DMSO-d_6) δ 1.27 (t, J = 7 Hz, 3H), 2.63 (s, 3H), 4.42 (q, J = 7 Hz, 2H), 7.40−7.48 (m, 3H), 7.68−7.71 (m, 2H), 8.13 (s, 1H), 8.94 (s, 1H); MS (CI) m/z 298 (M + 1).

8-Ethyl-2-methylsulfonyl-6-phenyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (52). To a solution of **51** (1.83 g, 6.16 mmol) in 150 mL of $CHCl_3$ was added 50−60% *m*-chloroperoxybenzoic acid (4.88 g, 14.2−17.0 mmol). The solution was stirred at room temperature for 2 h, then washed with saturated $NaHCO_3$, dried over $MgSO_4$, and concentrated. The solid was purified by flash chromatography eluting with a gradient of 1:1 hexane/EtOAc to EtOAc to give 1.07 g (53%) of **52**: mp 201−202 °C; ^{1}H NMR (DMSO-d_6) δ 1.30 (t, J = 7 Hz, 3H), 3.50 (s, 3H), 4.45 (q, J = 7 Hz, 2H), 7.46−7.53 (m, 3H), 7.73−7.75 (m, 2H), 8.31 (s, 1H), 9.32 (s, 1H); MS (CI) m/z 330 (M + 1). Anal. ($C_{16}H_{15}N_3O_3S$) C, H, N.

8-Ethyl-6-phenyl-2-phenylamino-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (47). A mixture of **52** (119 mg, 0.36 mmol) and aniline (301 mg, 3.63 mmol) was heated at 175 °C for 10 min. The reaction was cooled to room temperature and the residue purified by flash chromatography eluting with EtOAc to give 50 mg (40%) of **47**: mp 195−196 °C; ^{1}H NMR (DMSO-d_6) δ 1.31 (t, J = 7 Hz, 3H), 4.41 (q, J = 7 Hz, 2H), 7.04 (t, J = 7 Hz, 1H), 7.34−7.39 (m, 3H), 7.44 (t, J = 7 Hz, 2H), 7.68 (d, J = 7 Hz, 2H), 7.84 (d, J = 7 Hz, 2H), 8.02 (s, 1H), 8.85 (s, 1H), 10.16 (s, 1H); MS (CI) m/z 343 (M + 1). Anal. ($C_{21}H_{18}N_4O\cdot 0.25H_2O$) C, H, N.

8-Ethyl-6-phenyl-2-(pyridin-4-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (53). A mixture of **52** (150 mg, 0.46 mmol) and 4-aminopyridine (289 mg, 3.07 mmol) was heated at 175 °C for 1 h. The reaction was cooled to room temperature and the residue purified by flash chromatography eluting with

EtOAc to give 70 mg (45%) of **53**: mp 265−268 °C; ^{1}H NMR (DMSO-d_6) δ 1.27 (t, J = 7 Hz, 3H), 4.39 (q, J = 7 Hz, 2H), 7.31−7.41 (m, 3H), 7.64 (d, J = 8 Hz, 2H), 7.77 (d, J = 6 Hz, 2H), 8.02 (s, 1H), 8.38 (d, J = 6 Hz, 2H), 8.88 (s, 1H), 10.49 (s, 1H); MS (CI) m/z 344 (M + 1); HPLC purity 98%, column VYDAC C_{18} 218TP54, mobile phase gradient 10−76% 0.1% TFA in acetonitrile to 0.1% TFA in water.

2-[4-(2-Diethylaminoethoxy)phenylamino]-8-ethyl-6-phenyl-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (54). A mixture of **52** (129 mg, 0.39 mmol) and 4-(2-diethylaminoethoxy)aniline (163 mg, 0.78 mmol) was heated at 175 °C for 5 min. The reaction was cooled to room temperature and the residue purified by flash chromatography eluting with 5% MeOH in $CHCl_3$. The fractions containing product were collected, concentrated, and washed with 1:1 hexane/EtOAc. The filtrate was concentrated, and a minimum amount of EtOAc and a large amount of hexane were added to yield a solid which was collected to give 37 mg (21%) of **54**: mp 125−126 °C; ^{1}H NMR (DMSO-d_6) δ 0.98 (t, J = 7 Hz, 6H), 1.29 (t, J = 7 Hz, 3H), 2.56 (q, J = 7 Hz, 4H), 2.76 (t, J = 6 Hz, 2H), 4.01 (t, J = 6 Hz, 2H), 4.38 (q, J = 7 Hz, 2H), 6.94 (d, J = 9 Hz, 2H), 7.34−7.82 (m, 7H), 7.99 (s, 1H), 8.80 (s, 1H), 10.00 (s, 1H); MS (CI) m/z 458 (M + 1). Anal. ($C_{27}H_{31}N_5O_2 \cdot 0.5H_2O$) C, H, N.

Recombinant Tyrosine Kinases and Assays.[46] **1. 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 C6 rat glioma, the NIH 3T3 fibroblast line transfected with human PDGF ($\beta\beta$), and the DU-145 prostatic adenocarcinoma were maintained by serial passage in nude mice. Nude mice were also used as tumor hosts for test agent evaluations against these tumor models. In each experiment for anticancer activity evaluation, test mice (6−8/treatment group) weighing 18−22 g were randomized and implanted with tumor fragments in the region of the right axilla on day 0 (at least 6 mice/group and 12 in the control). Animals were treated with test compound on the basis of average cage weight on the days indicated in the table. Compound **54** was delivered as a solution in water. Compound dosing solution was prepared for 5 days at a time. Host body weight change data are reported as the maximum treatment-related weight loss in these studies and are included as a gross indicator of therapy-related toxicity. Calculation of tumor growth inhibition (% T/C), tumor growth delay (T − C), and net logs of tumor cell kill was performed as described previously.[55−57] 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.

2. Oral Pharmacokinetic Profiles in Rat. Oral pharmacokinetics were evaluated in three male Wistar rats following a single 5 mg/kg oral gavage dose of **53** and **54**. The doses were prepared in 17% PEG 400/83% methylcellulose (0.5%). Heparinized plasma samples were collected via a jugular vein cannula from 0 to 48 h. Calibration curves containing standards were prepared in methanol. Blank rat plasma (200 μL) was spiked with 20 μL of standards and extracted randomized with study samples. Following liquid−liquid extraction with ethyl ether, the organic layer was dried under nitrogen and reconstituted with 100 μL water/acetonitrile (50%/50% v/v). Samples were analyzed on an LC−MS/MS instrument with electrospray positive ion mode (VG Quattro II triple quadrupole). The assay lower limit of quantitation was 10 ng/mL. Pharmacokinetic parameter values were estimated by noncompartmental analysis of individual plasma concentration−time data. Maximum plasma concentrations (C_{max}) and times for these to occur (t_{max}) were recorded as observed. Apparent terminal elimination rate constants (λz) 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 0 to the time of last detectable concentration (t_{ldc}) using the trapezoidal rule and were extrapolated to infinity.

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