

Pyrido[2,3-*d*]pyrimidin-7-one Inhibitors of Cyclin-Dependent Kinases

Mark Barvian,[§] Diane H. Boschelli,[§] Jennifer Cossrow,[§] Ellen Dobrusin,[§] Ali Fattaey,[†] Alex Fritsch,[†] David Fry,[‡] Patricia Harvey,[‡] Paul Keller,[‡] Michelle Garrett,[†] Frances La,[§] Wilbur Leopold,[‡] Dennis McNamara,[§] Maire Quin,[†] Susanne Trumpp-Kallmeyer,[§] Peter Toogood,^{*,§} Zhipei Wu,[§] and Erli Zhang[§]

Departments of Chemistry and Cancer Research, Parke-Davis Pharmaceutical Research, Division of Warner Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105, and Onyx Pharmaceuticals, 3031 Research Drive, Richmond, California 94806

Received June 22, 2000

The identification of 8-ethyl-2-phenylamino-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (**1**) as an inhibitor of Cdk4 led to the initiation of a program to evaluate related pyrido[2,3-*d*]pyrimidin-7-ones for inhibition of cyclin-dependent kinases (Cdks). Analysis of more than 60 analogues has identified some clear SAR trends that may be exploited in the design of more potent Cdk inhibitors. The most potent Cdk4 inhibitors reported in this study inhibit Cdk4 with $IC_{50} = 0.004 \mu M$ ([ATP] = $25 \mu M$). X-ray crystallographic analysis of representative compounds bound to the related kinase, Cdk2, reveals that they occupy the ATP binding site. Modest selectivity between Cdks is exhibited by some compounds, and Cdk4-selective inhibitors block pRb⁺ cells in the G₁-phase of the cell division cycle.

Introduction

Uncontrolled cell proliferation is a hallmark of tumorigenesis and cancer as well as other disorders such as restenosis. In normal tissues, eukaryotic cell proliferation and cell division are highly regulated. Cells traverse a series of coordinated events known collectively as the cell cycle. This cell cycle is generally regarded as comprising four primary phases known as G₁, S, G₂, and M. DNA replication occurs during S-phase, and mitosis (cell division) occurs in the subsequent M-phase. Transition between these four phases is controlled by the action of cyclin-dependent kinases (Cdks). These serine-threonine kinases are activated by regulatory units called cyclins. Cyclin levels fluctuate during the cell cycle in response to a variety of extracellular and intracellular signals, and Cdk activities are turned on and off in response to raised and lowered cyclin levels.¹

At least 9 Cdks and 16 cyclins have been described in the literature.² The essential enzymes for cell cycle control appear to be cyclin D/Cdk4, cyclin D/Cdk6, cyclin E/Cdk2, cyclin A/Cdk2, and cyclin B/Cdk1 (also known as Cdc2/cyclin B). Cyclin D/Cdk4, cyclin D/Cdk6, and cyclin E/Cdk2 control passage through the G₁-phase and the G₁- to S-phase transition by phosphorylation of the retinoblastoma phosphoprotein, pRb. Cyclin A/Cdk2 regulates passage through the S-phase, and cyclin B/Cdk1 controls the G₂ checkpoint and regulates entry into mitosis. The activation of G₁ cyclins such as cyclin D occurs in response to growth factors. Once cells exit the G₁-phase and enter S-phase they are committed to cell division. Thus, inhibition of cells in the G₁-phase would appear to be an important mechanism for pre-

venting cell proliferation.³ Indeed, the endogenous Cdk inhibitor proteins of the INK4A (e.g. p16) and CIP/KIP families (e.g. p27) inhibit G₁ cyclin/Cdk complexes and cause cells to accumulate in late G₁-phase.

Further support for the importance of G₁ cyclin/Cdk enzymes may be found by genetic analysis of human tumor cell lines. Greater than 90% of all human tumors analyzed exhibit aberrant up-regulation of the Cdk4/Rb pathway including: overexpression of cyclin D1, mutation of Cdk4, mutation of Rb, or deletion of p16.⁴ These observations argue strongly that restoration of cyclin D/Cdk activity to "normal" by use of a specific Cdk inhibitor might counteract the tumorigenic effect of misregulation in this pathway and provide a treatment for disorders characterized by cell proliferation.

Encouraged by the prior discovery of EGF receptor kinase-selective inhibitors,⁵ we and others set out to identify specific Cdk inhibitors that could be used to test the hypothesis that inhibition of cyclin D/Cdk4 will inhibit cell proliferation. Recently, attention has shifted more toward inhibitors of cyclin E/cdk2 as a result of observations that p16 may cause a G₁ cell cycle block because it displaces p27 from the cyclin D/Cdk4 complex, making it available to inhibit cyclin E/Cdk2.⁶ The protein p27 exhibits activity both as a Cdk inhibitor and as a catalyst to cyclin D/Cdk4 assembly. Despite reports of several potent Cdk inhibitors in the literature,^{7–10} the majority of these compounds display potent inhibition of Cdk1 and/or Cdk2. Consequently, the hypothesis that a selective inhibitor of cyclin D/Cdk4 will inhibit cell proliferation and ultimately tumor growth still has not been tested. Here we describe our efforts toward this goal with the development of selective Cdk4 inhibitors based on the pyrido[2,3-*d*]pyrimidine template.

Initial Lead Identification

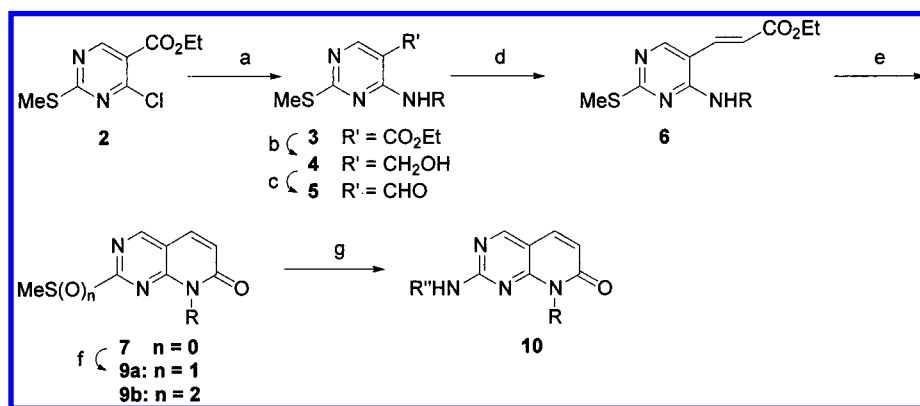
Recently, our laboratories have reported a class of highly potent tyrosine kinase inhibitors based on a 6-aryl-substituted pyrido[2,3-*d*]pyrimidin-7-one tem-

* Corresponding author, current address: Pfizer Global Research and Development, Ann Arbor Laboratories, 2800 Plymouth Rd., Ann Arbor, MI 48105. Tel: (734) 622-1335. Fax: (734) 622-1407. E-mail: peter.toogood2@pfizer.com.

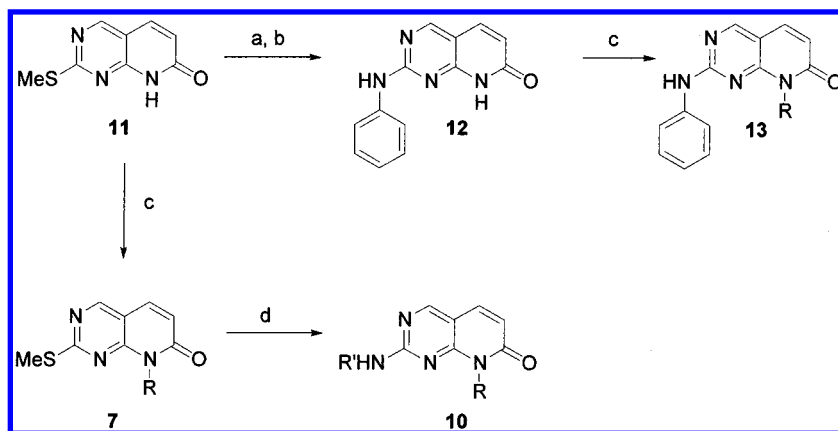
[§] Department of Chemistry, Parke-Davis Pharmaceutical Research.

[‡] Department of Cancer Research, Parke-Davis Pharmaceutical Research.

[†] Onyx Pharmaceuticals.

Scheme 1^a

^a (a) RNH_2 , Et_3N , THF; (b) $LiAlH_4$, THF; (c) MnO_2 , $CHCl_3$; (d) $Ph_3PC=CO_2Et$, THF, reflux; (e) iPr_2EtN , DBU, heat; (f) **8**, $CHCl_3$; (g) $R''-NH_2$, heat.

Scheme 2^a

^a (a) **8**, CH_2Cl_2 ; (b) aniline, heat; (c) NaH , $R-I$, DMF; (d) $R'-NH_2$, (DMSO), heat.

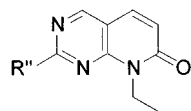
plate.^{11,12} These compounds are broadly active against several tyrosine kinases including EGFr, FGFr, PDGFr, and c-Src but are inactive against Cdk5. A model for the binding mode of these inhibitors bound to tyrosine kinases was proposed using homology-derived three-dimensional models and inhibitor SAR data.¹³ In this model, the pyrido[2,3-*d*]pyrimidin-7-one forms two hydrogen bonds to Met-341 of cSrc kinase and the 6-aryl substituent is located in a deep pocket adjacent to the ATP binding site. In EGFr, FGFr, PDGFr, and cSrc kinases this pocket is relatively large. In contrast, in Cdk5, this pocket is occluded by bulky amino acid side chains including the phenyl ring from Phe-80 (Cdk2 numbering). The proposed binding site model suggested that pyrido[2,3-*d*]pyrimidines lacking a 6-aryl substituent would be able to bind in the ATP binding site of Cdk5. This hypothesis was confirmed with the discovery that compound **1** (Table 1) inhibits cyclin D/Cdk4 with an $IC_{50} = 0.62 \mu M$. In addition, compound **1** exhibits some selectivity for Cdk5 vs other kinases including FGFr ($IC_{50} = 3.7 \mu M$) and cSrc ($IC_{50} = 21 \mu M$). With this encouraging result in hand, we embarked upon a full investigation of the SAR of the C6-hydrogen pyrido[2,3-*d*]pyrimidin-7-one template in order to identify more selective and more potent Cdk4 inhibitors.

Chemistry

The pyrido[2,3-*d*]pyrimidin-7-one analogues used in this study were prepared by following the general routes illustrated in Schemes 1 and 2. As shown in Scheme 1,

condensation of commercially available 4-chloro-2-methylthio-5-pyrimidinecarboxylic acid ethyl ester (**2**) with a primary alkylamine in THF containing triethylamine provided intermediates with structure **3**.¹¹ It was found that reduction of the ester using lithium aluminum hydride to alcohol **4**, followed by reoxidation with MnO_2 , provided aldehyde **5** more reliably than the corresponding one-step reduction. Reaction of the aldehyde with (carbethoxymethylene)triphenylphosphorane in THF under reflux gave the undesired (*E*)-acrylates **6**, which could be conveniently isomerized with ring closure to provide the pyrido[2,3-*d*]pyrimidine core molecules **7** by heating in the presence of DBU. Oxidation of the methyl sulfide in compounds **7** with (\pm)-*trans*-2-(phenylsulfonyl)-3-phenyloxaziridine (**8**)¹⁴ or *m*-chloroperbenzoic acid provided the corresponding sulfoxide **9a** or sulfone **9b**. Introduction of amines at the C2-position then was achieved by heating the sulfoxide or sulfone with at least 2 equiv of amine, in the presence or absence of solvent, at temperatures ranging from 100 to 175 °C, to provide the general structure **10**.

When the first step of this synthesis was performed with ammonium hydroxide instead of a primary alkylamine then the example of structure **7** obtained was compound **11**. This intermediate could be oxidized with oxaziridine **8** and reacted with aniline to produce analogue **12**, which in turn was alkylated at N8 by treatment with sodium hydride and alkyl halides to produce compounds with the structure **13**. Alternatively, compound **11** was alkylated at N8 to give structure **7**,

Table 1. Variation of the C2-Side Chain

Compound	R''	Cdk4/D IC ₅₀ (μM)	Compound	R''	Cdk4/D IC ₅₀ (μM)
1		0.620	27		4.083
14	EtNH—	11.967	28		1.825
15	ⁱ PrNH—	4.325	29		2.333
16	^t BuNH—	5.250	30		0.160
17		3.300	31		0.330
18		>40	32		2.750
19		>40	33		1.400
20		1.238	34		0.300
21		1.400	35		0.300
22		1.168	36		0.900
23		7.833	37		0.085
24		>40			
25		0.600			
26		0.585			

then oxidized and treated with amines to produce compounds **10**.

Specific examples of compounds prepared by all three of these routes are detailed in the Experimental Section.

Results and Discussion

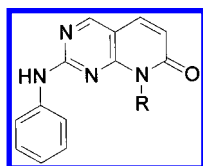
Structure–Activity Profile. Compound **1** possesses an aniline at the C2-position. To investigate whether an aromatic amine was important for potent inhibition of Cdk4, we prepared a small number of alkylamines (**14–18**) and measured their potency in purified enzyme assays. Strikingly, the cyclohexylamine derivative **17** displayed a 5-fold drop in potency with IC₅₀ = 3.3 μM. Other alkylamine derivatives fared no better with IC₅₀ values ranging from 4.3 μM for the isopropylamine **15** to > 40 μM for the benzylamine **18** (see Table 1). These results discouraged us from further probing alkylamine derivatives and allowed us to focus on C2-anilines.

Additional information regarding the C2-amine function was gleaned from the *N*-methylaniline derivative **19**. The proposed binding model for compound **1** suggested that the aniline NH might form a hydrogen bond to the carbonyl of Leu-83 in Cdk2 (Val-96 in Cdk4).

Similarly, the pyrimidine N3 nitrogen could form a hydrogen bond to the NH of Leu-83. Based on this model it was anticipated that the *N*-methyl derivative **19** would be a significantly weaker inhibitor of Cdk4. Indeed, **19** failed to inhibit Cdk4 at all concentrations up to 40 μM.

Electron-poor anilines were generally found to be worse inhibitors of Cdk4 than the initial lead. For example, the *p*-fluoroaniline (**20**) and *m*-fluoroaniline (**21**) derivatives were both approximately 2-fold worse than compound **1**. The compound with a 4-aminopyridine side chain at C2 (**22**) was similarly worse than compound **1**, while a compound possessing a pentafluoroethoxy substituent on the aniline ring (**23**) lost over an order of magnitude of activity.

Mixed results were obtained with compounds containing electron-rich anilines at C2. The Cdk4 inhibitory activity of oxygen-substituted anilines was sensitive to the position and nature of the oxygen substituent. For example, the *o*-methoxyaniline **24** was inactive vs Cdk4. Indeed, substitution in this position was found generally to be detrimental to Cdk inhibitory activity. The *p*-methoxyaniline **25** was equivalent to compound **1**, while

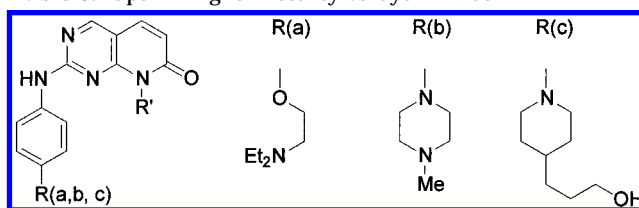
Table 2. Variation of the N8-Side Chain

compd	R	Cdk4/D IC ₅₀ (μM)
38	methyl	5.480
1	ethyl	0.620
39	isopropyl	0.145
40	<i>n</i> -propyl	0.553
41	<i>sec</i> -butyl	0.190
42	<i>n</i> -butyl	1.495
43	isobutyl	0.297
44	isopentyl	0.159
45	cyclopentyl	0.210
46	cyclohexyl	0.047
47	cycloheptyl	0.182
48	<i>exo</i> -1-bicyclo[2.2.1]hept-2-yl	0.038
49	phenyl	1.667
50	cyclohexylmethyl	13.500
51	CH ₂ Ph	0.940
52	methoxymethyl	4.150
53	2-methoxyethyl	2.860
54	2-ethoxyethyl	7.800
55	<i>C</i> -oxiranylmethyl	5.000
56	CH ₂ CO ₂ Me	31.000

the *p*-hydroxyaniline **26** was marginally better, possibly due to partial ionization of the phenol. This trend was reinforced by comparison of the 3,4-dimethoxyaniline **27** (IC₅₀ = 4.08 μM) and the 4-hydroxy-3-methoxyaniline **28** (IC₅₀ = 1.83 μM) which was twice as potent. Extension of the alkyl substituent appeared to be detrimental by comparison of **29** and **25**; however, the amine-containing alkyl chain in **30** provided a significant leap in potency with IC₅₀ = 0.16 μM.

Amine-substituted anilines in general proved to yield more potent Cdk4 inhibitors than oxygen-substituted anilines or halogenated anilines. For example, **31** was twice as potent as **25** and 4 times as potent as **20**. Examination of compounds **32–36** indicated that this effect was not solely electronic; constraint of the nitrogen substituents into a six-membered heterocycle (**34–36**), but not a five-membered aromatic ring (**32**), significantly improved activity against Cdk4 compared to the diethylamine derivative **33**. A further improvement in potency was exhibited by the *N*-methylpiperazine-substituted aniline **37**, which possessed an IC₅₀ = 0.085 μM, 7-fold better than compound **1** and 3.5-fold more potent than the morpholine analogue **34**. Similar to **30**, the tertiary nitrogen in **37** appeared to participate in a specific beneficial interaction with the Cdk protein, possibly by electrostatically countering the presumed negative charge on Asp-86 (Cdk2 numbering).

Having identified a C2-side chain that greatly improved the potency of these Cdk inhibitors, attention was turned to the N8-substituent. Clear trends rapidly became evident regarding the SAR at this position of the molecule (Tables 2 and 3). First, oxygenated substituents were typically poor choices for this position (see compounds **52–56**). [Note: This was true even for groups designed to crudely mimic the ribose ring of ATP that would occupy the same region of the Cdk active site (e.g. **63**, Table 3).] Second, it was obvious that a fairly large and predominantly hydrophobic space was available for occupation by the N8-substituent (e.g. norbornyl in **48**). In contrast to the C2-position, alkyl

Table 3. Optimizing for Potency vs Cyclin D/Cdk4

compd	R(a, b, or c)	R'	Cdk4/D IC ₅₀ (μM)
30	R(a)	ethyl	0.160
57	R(a)	isopropyl	0.045
58	R(a)	cyclopentyl	0.0007
59	R(a)	cyclohexyl	0.011
60	R(a)	cycloheptyl	0.004
61	R(a)	<i>exo</i> -1-bicyclo[2.2.1]hept-2-yl	0.450
62	R(b)	2-benzyloxyethyl	2.100
63	R(b)	CH ₂ (CHOH)CH ₂ OH	1.650
64	R(b)	phenyl	0.175
65	R(b)	cyclopropyl	0.140
37	R(b)	ethyl	0.085
66	R(b)	isopropyl	0.032
67	R(b)	isopentyl	0.013
68	R(b)	cyclopentyl	0.009
69	R(b)	<i>exo</i> -1-bicyclo[2.2.1]hept-2-yl	0.006
70	R(b)	cyclohexyl	0.004
36	R(c)	ethyl	0.900
71	R(c)	cyclopentyl	0.034
72	R(c)	<i>exo</i> -1-bicyclo[2.2.1]hept-2-yl	0.008

Table 4. Selectivity of Cdk Inhibitors

compd	IC ₅₀ (μM)				
	Cdk1/B	Cdk2/A	Cdk2/E	Cdk4/D	FGFr
1	1.015	0.129	0.41	0.620	3.295
23	0.091	0.238	1.200	7.833	44.200
40	0.299	0.050	0.112	0.553	6.600
42	2.000	0.037	0.058	1.495	5.290
66	0.675	0.058	0.270	0.032	0.070
70	0.079	0.015	0.020	0.004	0.051
72	>40	0.209	0.165	0.008	8.62

groups generally were preferred to aryl groups at N8, and among this set, cycloalkyl groups fared better than simple linear or branched chain alkyl groups (compare **39–44** to **45–48**). A definite trend in potency may be observed in Table 3. With either 4-(diethylaminoethoxy)-phenylamino or 4-(*N*-methylpiperazinyl)phenylamino at C2, potency improved in going from simple alkyl to cyclopentyl to cyclohexyl in a reasonably predictable fashion. Compounds **60** and **70** were among the most potent Cdk4 inhibitors prepared to date with IC₅₀ = 0.004 μM. These compounds represent a 150-fold improvement in potency when compared to the initial chemical lead (**1**).

Enzyme Selectivity. All new Cdk inhibitors were routinely screened against cyclin B/Cdk1, cyclin A/Cdk2, and cyclin E/Cdk4 as well as representative tyrosine kinases such as FGFr, c-Src, and PDGFr. In general, it was found that the potency of our inhibitors against all of these kinases tended to increase with increased potency against Cdk4. For example **70** is an 8-fold more effective Cdk4 inhibitor than **66**; however, it also is more potent against each of the other kinases examined (Table 4). Despite this trend, **70** is 3-fold more potent against cyclin D/Cdk4 than against cyclin A/Cdk2 and at least an order of magnitude less potent against FGFr. In contrast, **66** is only 2-fold more potent against Cdk4 than either cyclin A/Cdk2 or FGFr. One of the most interesting inhibitors of Cdk4 among the compounds

that we have described is compound **72**. This analogue displays greater than 15-fold selectivity for cyclin D/Cdk4 vs cyclin E/Cdk2 and is inactive vs cyclin B/Cdk1. It is more than 1000-fold selective for cyclin D/Cdk4 vs the tyrosine kinase FGFR ($IC_{50} = 8.62 \mu M$). In addition, **72** fails to inhibit another serine-threonine kinase, protein kinase C, at concentrations up to $50 \mu M$. Further details regarding the biochemical behavior of this compound are described in an accompanying manuscript.¹⁵

Distinguishing between Cdk4 and Cdk2 was typically the most difficult challenge for this class of Cdk inhibitors. Indeed, several analogues are of interest precisely because they are selective for Cdk2 vs other kinases. Of particular note, compounds **40** and **42** display quite respectable selectivity for Cdk2 vs Cdk1, Cdk4, or FGFR, and compound **42** in particular is 40-fold selective for cyclin A/Cdk2 vs cyclin D/Cdk4. A quite different selectivity was discovered for compound **23** which is unusual in inhibiting cyclin B/Cdk1 better than other Cdk2s or FGFR. In summary, compounds have been identified in the pyrido[2,3-*d*]pyrimidin-7-one class that are selective for cyclin B/Cdk1, cyclin A/Cdk2, or cyclin D/Cdk4 as a function of the choice of N8- and C2-substituents. Compounds with even marginal selectivity for cyclin D/Cdk4 *in vitro* were found to cause a specific G₁ block in an Rb-positive cell line (MDA MB453) consistent with Cdk4 inhibition in cells. Overall, however, higher levels of selectivity are likely to be necessary in order to draw conclusions regarding the effect of inhibiting a single Cdk *in vivo*.

Binding of Cdk Inhibitors to Cdk2. The initially proposed binding mode for pyrido[2,3-*d*]pyrimidin-7-ones binding to the Cdk catalytic subunit is supported by kinetic studies that indicate that **66** is competitive with ATP. In addition, crystal structure data confirm that **66** binds in the ATP site as predicted and that the C2-side chain projects out of the binding pocket toward solution (Figure 1). X-ray crystallographic analysis further revealed that **66** binds to Cdk2 with nitrogen N3 accepting a hydrogen bond from the amide nitrogen of Leu-83 in the protein and the C2-NH donating a hydrogen bond to the oxygen of Leu-83. Thus, although the pyrido[2,3-*d*]pyrimidin-7-one ring is positioned in a fashion similar to the purine ring of ATP bound to cdk2,^{16,17} its precise hydrogen-bonding pattern is different. In crystal structures of Cdk2-ATP, Cdk2-staurosporine, and other inhibitor-bound Cdk2 complexes,¹⁸⁻²⁰ the ATP or inhibitor donates a hydrogen bond to the main chain carbonyl oxygen of residue Glu-81, and no such H-bond is observed with compound **66**.

In conclusion, a class of pyrido[2,3-*d*]pyrimidin-7-one inhibitors of Cdk2 has been identified and some members of this class display modest selectivity for Cdk4 vs other kinases. In addition, representative compounds inhibit the proliferation of human tumor cells in tissue culture; for example, compound **72** inhibits the HCT116 human colon carcinoma cell line with $IC_{50} = 0.213 \mu M$. Such data suggests that **72** can enter cells and indicates the potential of this class of compounds for treating cancer *in vivo*. SAR analysis together with structural data are currently being employed to facilitate the design of even more selective and potent Cdk inhibitors.

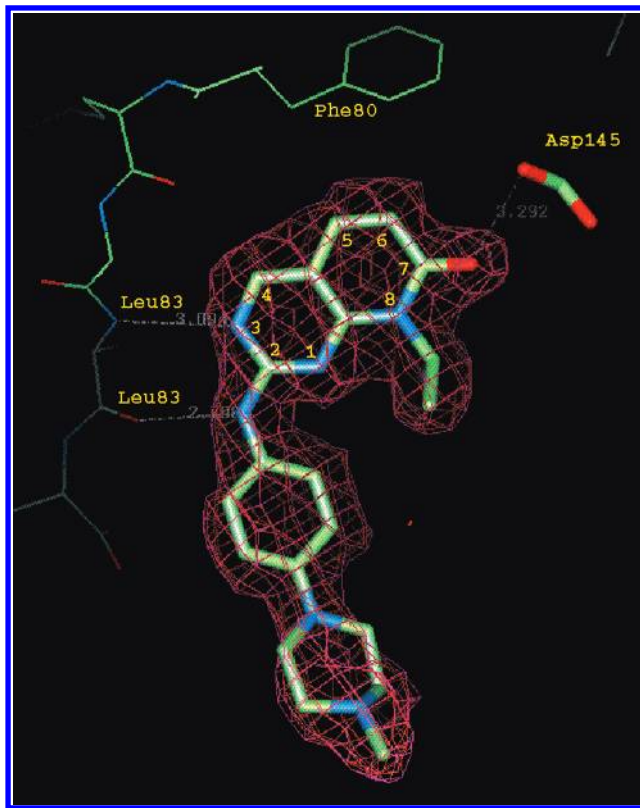


Figure 1. Electron density around compound **66** at 2.0 Å resolution in a complex of compound **66** bound to Cdk2. The $2f_o - f_c$ electron density map was produced using Quanta and displayed at 1.0σ .

Experimental Section

General Methods. NaH refers to 60 wt % NaH in mineral oil. All solvents and reagents were used as obtained. Anhydrous solvents were obtained commercially and used without further drying. Melting points were determined with a Thomas-Hoover capillary melting point apparatus or a MEL-TEMP melting point apparatus and are uncorrected. ¹H NMR spectra were recorded using a Varian Unity 400-MHz spectrometer. Chemical shifts are in parts per million (δ) referenced to Me₄-Si (0.00 ppm) or CHCl₃ (7.26 ppm). The amount of solvent or water present in the molecular formula was determined by ¹H NMR and microanalysis. Chemical ionization mass spectra (CI) were recorded on a VG Trio 2 mass spectrometer instrument using a reagent gas of 1% NH₃ in CH₄. Atmospheric pressure chemical ionization mass spectra (APCIMS) were recorded using a VG Trio 2000 mass spectrometer in a matrix of MeOH/MeCN/DMSO. Combustion analyses (CHN) were determined by Robertson Microlit Laboratories, Inc., Madison, NJ.

4-Ethylamino-2-methanesulfanylpurimidine-5-carboxylic Acid Ethyl Ester (3; R = Et). To a room-temperature solution of 4-chloro-2-methanesulfanylpurimidine-5-carboxylic acid ethyl ester (**2**; 10.00 g, 43.10 mmol) in 150 mL of tetrahydrofuran was added triethylamine (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 *in vacuo* and partitioned between chloroform and saturated aqueous sodium bicarbonate. The organic layer was dried over magnesium sulfate, filtered, and concentrated to provide 9.32 g (90%) of 4-ethylamino-2-methanesulfanylpurimidine-5-carboxylic acid ethyl ester as an oil: ¹H NMR (DMSO-*d*₆) δ 1.70 (t, $J = 7$ Hz, 3H), 1.30 (t, $J = 7$ Hz, 3H), 2.48 (s, 3H), 3.51 (q, $J = 7$ 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. (C₁₀H₁₅N₃O₂S) C, H, N.

(4-Ethylamino-2-methanesulfanylpurimidin-5-yl)methanol (4; R = Et). A solution of 4-ethylamino-2-methanesulfa-

nylpyrimidine-5-carboxylic acid ethyl ester (8.93 g, 37.1 mmol) in 100 mL of tetrahydrofuran was added dropwise to a room temperature suspension of lithium aluminum hydride (2.30 g, 60.5 mmol) in 100 mL of tetrahydrofuran. After 10 min, the reaction was carefully quenched with 4.5 mL of water, 4.5 mL of 15% NaOH, and an additional 16 mL of water, and the mixture was stirred for 1.5 h. The white precipitate was removed by filtration, washing with ethyl acetate. The filtrate was concentrated in vacuo and 1:1 hexane:ethyl acetate was added. The solids were collected to give 6.77 g (92%) of (4-ethylamino-2-methanesulfanylpurimidine-5-yl)methanol: mp 152–156 °C; ¹H NMR (DMSO-*d*₆) δ 1.13 (t, *J* = 7 Hz, 3H), 2.41 (s, 3H), 3.39 (q, *J* = 6 Hz, 2H), 4.29 (d, *J* = 5 Hz, 2H), 5.09 (t, *J* = 5 Hz, 1H), 6.79 (t, *J* = 5 Hz, 1H), 7.82 (s, 1H); MS (CI) *m/z* 200 (*M* + 1). Anal. (C₈H₁₃N₃OS) C, H, N.

4-Ethylamino-2-methanesulfanylpurimidine-5-carboxaldehyde (5; R = Et). To (4-ethylamino-2-methanesulfanylpurimidine-5-yl)methanol (6.44 g, 32.4 mmol) in 600 mL of chloroform was added manganese oxide (21.0 g, 241 mmol). The suspension was stirred at room temperature for 2 h and an additional 5.5 g of manganese oxide was added. Stirring was continued for 4.5 h. The mixture was filtered through Celite, washing with chloroform. The filtrate was concentrated in vacuo to give 6.25 g (97%) of 4-ethylamino-2-methanesulfanylpurimidine-5-carboxaldehyde: mp 58–61 °C; ¹H NMR (DMSO-*d*₆) δ 1.17 (t, *J* = 7 Hz, 3H), 2.51 (s, 3H), 3.54 (q, *J* = 6 Hz, 2H), 8.52 (s, 1H), 8.69 (b, 1H), 9.74 (s, 1H); MS (CI) *m/z* 198 (*M* + 1). Anal. (C₈H₁₁N₃OS) C, H, N.

Ethyl 3-(4-ethylamino-2-methanesulfanylpurimidine-5-yl)acrylate (6; R = Et). To a room-temperature solution of 4-ethylamino-2-methanesulfanylpurimidine-5-carboxaldehyde (6.34 g, 32.14 mmol) in 100 mL of tetrahydrofuran was added (carbethoxymethylene)triphenylphosphorane (14.32 g, 41.14 mmol). The reaction mixture was heated at reflux for 70 min. The reaction mixture was concentrated in vacuo, and the residue was purified by flash chromatography, eluting with ethyl acetate, to provide 7.04 g (82%) of ethyl 3-(4-ethylamino-2-methanesulfanylpurimidine-5-yl)acrylate: mp 79–80 °C; ¹H NMR (DMSO-*d*₆) δ 1.15 (t, *J* = 7 Hz, 3H), 1.25 (t, *J* = 7 Hz, 3H), 2.45 (s, 3H), 3.42 (q, *J* = 6 Hz, 2H), 4.18 (q, *J* = 7 Hz, 2H), 6.50 (d, *J* = 6 Hz, 1H), 7.70 (d, *J* = 6 Hz, 1H), 7.78 (b, 1H), 8.37 (s, 1H); MS (CI) *m/z* 268 (*M* + 1). Anal. (C₁₂H₁₇N₃O₂S) C, H, N.

8-Ethyl-2-methanesulfanyl-8H-pyrido[2,3-*d*]pyrimidin-7-one (7; R = Et). To a room-temperature solution of ethyl 3-(4-ethylamino-2-methanesulfanylpurimidine-5-yl)acrylate (6.62 g, 24.78 mmol) in 30 mL of *N,N*-diisopropylethylamine was added 1,8-diazabicyclo[5.4.0]undec-7-ene (4.25 mL). The reaction mixture was heated at reflux overnight then cooled to room temperature. The resultant solid was collected by filtration and washed with 1:1 hexane:ethyl acetate to give 1.83 g (33%) of 8-ethyl-2-methanesulfanyl-8H-pyrido[2,3-*d*]pyrimidin-7-one. The filtrate was concentrated in vacuo and upon the addition of hexane, a solid formed that was collected, washed with hexane, and purified by flash chromatography eluting with ethyl acetate to provide an additional 2.22 g (40%) of title product: ¹H NMR (DMSO-*d*₆) δ 1.15 (t, *J* = 7 Hz, 3H), 2.53 (s, 3H), 4.25 (q, *J* = 6 Hz, 2H), 6.56 (d, *J* = 9 Hz, 1H), 7.86 (d, *J* = 9 Hz, 1H), 8.82 (s, 1H); MS (CI) *m/z* 222 (*M* + 1). Anal. (C₁₀H₁₁N₃OS) C, H, N.

8-Ethyl-2-methanesulfanyl-8H-pyrido[2,3-*d*]pyrimidin-7-one (9a; R = Et). To a room-temperature solution of 8-ethyl-2-methanesulfanyl-8H-pyrido[2,3-*d*]pyrimidin-7-one (2.22 g, 10.04 mmol) in 100 mL of chloroform was added (±)-*trans*-2-(phenylsulfonyl)-3-phenyloxaziridine (3.17 g, 12.15 mmol). The solution was stirred at room temperature overnight then concentrated in vacuo. The residue was treated with ethyl acetate to give a solid that was collected by filtration and washed with ethyl acetate to provide 2.21 g (93%) of 8-ethyl-2-methanesulfanyl-8H-pyrido[2,3-*d*]pyrimidin-7-one: mp 202–203 °C; ¹H NMR (DMSO-*d*₆) δ 1.17 (t, *J* = 7 Hz, 3H), 2.88 (s, 3H), 4.30 (q, *J* = 7 Hz, 2H), 6.80 (d, *J* = 9 Hz, 1H), 8.03 (d, *J* = 9 Hz, 1H), 9.17 (s, 1H); MS (CI) *m/z* 238 (*M* + 1). Anal. (C₁₀H₁₁N₃O₂S) C, H, N.

4-Amino-2-methanesulfanylpurimidine-5-carboxylic Acid Ethyl Ester (3; R = H). To a room-temperature solution of 4-chloro-2-methanesulfanylpurimidine-5-carboxylic acid ethyl ester (15.0 g, 65 mmol) in 200 mL of tetrahydrofuran was added 25 mL of triethylamine followed by 35 mL of aqueous ammonium hydroxide. After stirring at room temperature for 1.5 h, an additional 30 mL of aqueous ammonium hydroxide was added, and stirring was continued for 1 h. The reaction mixture was concentrated in vacuo and partitioned between ethyl acetate and saturated aqueous sodium bicarbonate. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo. Ethyl acetate and hexane were added, and the resultant solid was collected by filtration to provide 10.84 g (79%) of 4-amino-2-methanesulfanylpurimidine-5-carboxylic acid ethyl ester which was used directly without further purification.

(4-Amino-2-methanesulfanylpurimidine-5-yl)methanol (4; R = H). A solution of 4-amino-2-methanesulfanylpurimidine-5-carboxylic acid ethyl ester (13.36 g, 63 mmol) in 250 mL of tetrahydrofuran was added dropwise to a room-temperature suspension of lithium aluminum hydride (3.82 g, 100 mmol) in 250 mL of tetrahydrofuran. After 30 min, the reaction was cooled to 0 °C, and isopropyl alcohol was added until bubbling diminished. The reaction was quenched with 15 mL of water, 15 mL of 15% NaOH, and 50 mL of water, and the mixture was stirred for 1 h. The white precipitate was removed by filtration, washing with ethyl acetate. The filtrate was concentrated in vacuo and 3:1 hexane:ethyl acetate was added. The solids were collected, washed with 3:1 hexane:ethyl acetate, followed by hexane. The solid was dissolved in ethyl acetate, and the solution was dried over magnesium sulfate. Filtration followed by concentration in vacuo gave 8.14 g (76%) of (4-amino-2-methanesulfanylpurimidine-5-yl)methanol: ¹H NMR (DMSO-*d*₆) δ 2.33 (s, 3H), 4.23 (s, 2H), 5.05 (br s, 1H), 6.63 (br s, 2H), 7.83 (s, 1H); MS (CI) *m/z* 172 (*M* + 1). Anal. (C₆H₉N₃OS) C, H, N.

4-Amino-2-methanesulfanylpurimidine-5-carboxaldehyde (5; R = H). To (4-amino-2-methanesulfanylpurimidine-5-yl)methanol (8.14 g, 48 mmol) in 1 L of chloroform was added manganese oxide (33.13 g, 381 mmol). The suspension was stirred at room temperature overnight then filtered through Celite washing with 300 mL of chloroform. The filtrate was concentrated in vacuo to give 8.14 g (quantitative yield) of 4-amino-2-methanesulfanylpurimidine-5-carboxaldehyde: mp 185–187 °C (lit.²¹ mp 183–184 °C); ¹H NMR (DMSO-*d*₆) δ 2.43 (s, 3H), 7.97 (s, 1H), 8.24 (s, 1H), 8.51 (s, 1H), 9.71 (s, 1H); MS (CI) *m/z* 170 (*M* + 1). Anal. (C₆H₇N₃OS) C, H, N.

Ethyl 3-(4-amino-2-methanesulfanylpurimidine-5-yl)acrylate (6; R = H). To a room-temperature solution of 4-amino-2-methanesulfanylpurimidine-5-carboxaldehyde (4.08 g, 24.14 mmol) in 100 mL of tetrahydrofuran was added (carbethoxymethylene)triphenylphosphorane (10.80 g, 31 mmol). The reaction mixture was heated at reflux for 3 h then stirred at room temperature overnight. The reaction mixture was concentrated in vacuo, and the residue was purified by flash chromatography, eluting with 1:1 ethyl acetate:hexane, to provide 4.30 g (75%) of ethyl 3-(4-amino-2-methanesulfanylpurimidine-5-yl)acrylate: mp softens at 108 °C; ¹H NMR (DMSO-*d*₆) δ 1.25 (t, *J* = 7 Hz, 3H), 2.43 (s, 3H), 4.17 (q, *J* = 7 Hz, 2H), 6.51 (d, *J* = 6 Hz, 1H), 7.38 (s, 2H), 7.70 (d, *J* = 6 Hz, 1H), 8.44 (s, 1H); MS (CI) *m/z* 240 (*M* + 1). Anal. (C₁₀H₁₃N₃O₂S) C, H, N.

2-Methanesulfanyl-8H-pyrido[2,3-*d*]pyrimidin-7-one (7; R = H). To a room-temperature solution of ethyl 3-(4-amino-2-methanesulfanylpurimidine-5-yl)acrylate (368 mg, 1.53 mmol) in 3 mL of *N,N*-diisopropylethylamine was added 1,8-diazabicyclo[5.4.0]undec-7-ene (380 μL). The reaction mixture was heated at reflux for 3 h then cooled to room temperature and concentrated. The residue was purified by flash chromatography eluting with ethyl acetate. The fractions containing the product were partially concentrated in vacuo, and the solids were removed by filtration to provide 134 mg (45%) of 2-methanesulfanyl-8H-pyrido[2,3-*d*]pyrimidin-7-one: mp 269–271 °C; ¹H NMR (DMSO-*d*₆) δ 2.56 (s, 3H), 6.50 (d, *J* = 9 Hz,

1H), 7.90 (d, $J = 9$ Hz, 1H), 8.85 (s, 1H), 12.40 (s, 1H); MS (CI) m/z 194 ($M + 1$). Anal. ($C_8H_7N_3OS$) C, H, N.

8-Ethyl-2-methanesulfanyl-8H-pyrido[2,3-*d*]pyrimidin-7-one (7; R = Et) by Alkylation. To a suspension of NaH (80 mg of a 60% suspension of NaH in mineral oil) in 10 mL of dimethylformamide was added 2-methanesulfanyl-8H-pyrido[2,3-*d*]pyrimidin-7-one (262 mg, 1.35 mmol). The reaction mixture was heated to 50 °C resulting in a brown solution. The solution was cooled slightly and iodoethane (150 μ L, 1.88 mmol) was added. The reaction was heated to 50 °C for 10 min, then cooled to room temperature and partitioned between cold water and ethyl acetate. The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash chromatography, eluting with 1:1 ethyl acetate:hexane to 100% ethyl acetate, to provide the title compound 192 mg (64%).

8-Isopropyl-2-methanesulfanyl-8H-pyrido[2,3-*d*]pyrimidin-7-one (7; R = ⁱPr). To a suspension of NaH (48 mg of a 60% suspension of NaH in mineral oil) in 6 mL of dimethylformamide was added 2-methanesulfanyl-8H-pyrido[2,3-*d*]pyrimidin-7-one (158 mg, 0.82 mmol). The reaction mixture was heated to 50 °C resulting in a yellow solution. The solution was cooled slightly and 2-iodopropane (120 μ L, 1.20 mmol) was added. The reaction was heated at 50 °C for 30 min then cooled to room temperature and partitioned between water and ethyl acetate. The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash chromatography, eluting with a gradient of 1:3 ethyl acetate:hexane to 100% ethyl acetate, to provide 140 mg (69%) of 8-isopropyl-2-methanesulfanyl-8H-pyrido[2,3-*d*]pyrimidin-7-one: mp 101–102 °C; ¹H NMR (DMSO-*d*₆) δ 1.54 (d, $J = 7$ Hz, 6H), 2.60 (s, 3H), 5.69 (b, 1H), 6.57 (d, $J = 9$ Hz, 1H), 7.88 (d, $J = 9$ Hz, 1H), 8.86 (s, 1H); MS (CI) m/z 236 ($M + 1$). Anal. ($C_{11}H_{13}N_3OS$) C, H, N.

8-Isopropyl-2-methanesulfanyl-8H-pyrido[2,3-*d*]pyrimidin-7-one (9a; R = ⁱPr). To a room-temperature solution of 8-isopropyl-2-methanesulfanyl-8H-pyrido[2,3-*d*]pyrimidin-7-one (1.19 g, 5.08 mmol) in 50 mL of chloroform was added (\pm)-*trans*-2-(phenylsulfonyl)-3-phenyloxaziridine (1.76 g, 6.75 mmol). The solution was stirred at room temperature overnight then concentrated in vacuo. The residue was treated with ethyl acetate and hexane to give a solid which was collected by filtration and purified by flash chromatography, eluting with a gradient of ethyl acetate to 10% methanol in ethyl acetate, to provide 1.00 g (78%) of 8-isopropyl-2-methanesulfanyl-8H-pyrido[2,3-*d*]pyrimidin-7-one: mp 132–133 °C; ¹H NMR (DMSO-*d*₆) δ 1.57 (d, $J = 7$ Hz, 6H), 2.94 (s, 3H), 5.72 (m, 1H), 6.81 (d, $J = 9$ Hz, 1H), 8.04 (d, $J = 9$ Hz, 1H), 9.19 (s, 1H); MS (CI) m/z 252 ($M + 1$). Anal. ($C_{11}H_{13}N_3O_2S$) C, H, N.

2-Methanesulfanyl-8H-pyrido[2,3-*d*]pyrimidin-7-one (9a; R = H). To a room-temperature solution of 2-methanesulfanyl-8H-pyrido[2,3-*d*]pyrimidin-7-one (120 mg, 0.62 mmol) in 20 mL of chloroform was added (\pm)-*trans*-2-(phenylsulfonyl)-3-phenyloxaziridine (**8**; 200 mg, 0.77 mmol). The solution was stirred at room temperature overnight. The solid was collected by filtration and found to be 2-methylthio-8H-pyrido[2,3-*d*]pyrimidin-7-one. The filtrate was stirred at room temperature for 2 days then concentrated. Addition of ethyl acetate resulted in the formation of a solid that was collected by filtration to provide 64 mg (76% based on recovered starting material) of 2-methanesulfanyl-8H-pyrido[2,3-*d*]pyrimidin-7-one: mp 237–242 °C; ¹H NMR (DMSO-*d*₆) δ 2.90 (s, 3H), 6.73 (d, $J = 9$ Hz, 1H), 8.05 (d, $J = 9$ Hz, 1H), 9.16 (s, 1H), 12.96 (s, 1H); MS (CI) m/z 210 ($M + 1$). Anal. ($C_8H_7N_3O_2S \cdot 0.2H_2O$) C, H, N.

2-Phenylamino-8H-pyrido[2,3-*d*]pyrimidin-7-one (12). A suspension of 204 mg of the mixture of 2-methanesulfanyl-8H-pyrido[2,3-*d*]pyrimidin-7-one and 2-methanesulfanyl-8H-pyrido[2,3-*d*]pyrimidin-7-one in 1 mL of aniline was heated at reflux for 10 min resulting in a dark brown solution. Upon cooling to room temperature, a solid formed. Ethyl acetate was added, and the solid was collected by filtration, washed with ethyl acetate, then suspended in methanol and filtered, and washed with additional methanol to provide 175 mg of 2-phenylamino-8H-pyrido[2,3-*d*]pyrimidin-7-one: mp >350 °C; ¹H

NMR (DMSO-*d*₆) δ 6.12 (d, $J = 9$ Hz, 1H), 7.00 (t, $J = 7$ Hz, 1H), 7.79 (d, $J = 9$ Hz, 1H), 7.89 (d, $J = 7$ Hz, 1H), 8.73 (s, 1H), 9.95 (s, 1H), 12.09 (s, 1H); MS (CI) m/z 239 ($M + 1$). Anal. ($C_{13}H_{10}N_4O \cdot 0.15H_2O$) C, H, N.

General Procedure for the Preparation of 2-Amino-8-ethyl-8H-pyrido[2,3-*d*]pyrimidin-7-ones from 8-Ethyl-2-methanesulfanyl-8H-pyrido[2,3-*d*]pyrimidin-7-one (9a; R = Et). To 8-ethyl-2-methanesulfanyl-8H-pyrido[2,3-*d*]pyrimidin-7-one was added 1–10 equiv of an amine. In those cases where the amine was aniline, a substituted aniline, or a tertiary amine, the reaction mixture was heated at 100–175 °C for 10–60 min. In the case of primary or secondary alkylamines, the reaction was performed at room temperature for 10–60 min. A typical workup involved dilution of the cooled reaction mixture with ethyl acetate followed by an aqueous wash with sodium bicarbonate solution. The organic layer was dried over magnesium sulfate, filtered then evaporated to dryness. The products were purified by crystallization from ethyl acetate and hexanes, or by silica gel chromatography. Yields ranged from 40–75%.

8-Ethyl-2-phenylamino-8H-pyrido[2,3-*d*]pyrimidin-7-one (1): mp 203–204 °C; ¹H NMR (DMSO-*d*₆) δ 1.13 (t, $J = 7$ Hz, 3H), 4.25 (q, $J = 7$ Hz, 2H), 6.33 (d, $J = 9$ Hz, 1H), 6.97 (t, $J = 8$ Hz, 1H), 7.28 (t, $J = 8$ Hz, 2H), 7.75 (m, 3H), 8.72 (s, 1H), 10.05 (s, 1H); MS (CI) m/z 267 ($M + 1$). Anal. ($C_{15}H_{14}N_4O \cdot 0.05EtOAc$) C, H, N.

8-Ethyl-2-ethylamino-8H-pyrido[2,3-*d*]pyrimidin-7-one (14): mp 160–161 °C; ¹H NMR (DMSO-*d*₆) δ 1.19 (m, 6H), 3.39 (q, $J = 6$ Hz, 2H), 4.26 (q, $J = 7$ Hz, 2H), 6.23 (d, $J = 9$ Hz, 1H), 7.70 (d, $J = 9$ Hz, 1H), 7.87 (s, 1H), 8.56 (s, 1H); MS (CI) m/z 219 ($M + 1$). Anal. ($C_{11}H_{14}N_4O$) C, H, N.

8-Ethyl-2-isopropylamino-8H-pyrido[2,3-*d*]pyrimidin-7-one (15): mp 119–120 °C; ¹H NMR (DMSO-*d*₆) δ 1.20 (t, $J = 9$ Hz, 3H), 2.50 (d, 6H), 4.04–4.09 (m, 1H), 4.25 (q, $J = 9$ Hz, 2H), 6.23 (d, $J = 9$ Hz, 1H), 7.69 (d, $J = 9$ Hz, 1H), 7.68–7.88 (m, 1H), 8.57 (s, 1H); MS (CI) m/z 233 ($M + 1$). Anal. ($C_{12}H_{16}N_4O$) C, H, N.

2-*tert*-Butylamino-8-ethyl-8H-pyrido[2,3-*d*]pyrimidin-7-one (16): mp 103–104 °C; ¹H NMR (DMSO-*d*₆) δ 1.19 (t, $J = 7$ Hz, 3H), 1.44 (s, 9H), 4.26 (q, $J = 7$ Hz, 2H), 6.24 (d, $J = 9$ Hz, 1H), 7.52 (br s, 1H), 7.70 (d, $J = 9$ Hz, 1H), 8.58 (1H, s). Anal. ($C_{13}H_{18}N_4O \cdot 0.25H_2O$) C, H, N.

2-Cyclohexylamino-8-ethyl-8H-pyrido[2,3-*d*]pyrimidin-7-one (17): mp 135–136 °C; ¹H NMR (DMSO-*d*₆) δ 1.13 (t, $J = 7$ Hz, 3H), 1.24–1.93 (m, 10H), 3.67–3.77 (m, 1H), 4.18 (q, $J = 7$ Hz, 2H), 6.16 (d, $J = 9$ Hz, 1H), 7.63 (d, $J = 9$ Hz, 1H), 7.58–7.74 (m, 1H), 8.50 (s, 1H); MS (CI) m/z 273 ($M + 1$). Anal. ($C_{15}H_{20}N_4O$) C, H, N.

2-Benzylamino-8-ethyl-8H-pyrido[2,3-*d*]pyrimidin-7-one (18): mp 96–97 °C; ¹H NMR (DMSO-*d*₆) δ 1.00 (t, $J = 7$ Hz, 3H), 4.18 (q, $J = 7$ Hz, 2H), 4.55 (d, $J = 8$ Hz, 2H), 6.23 (d, $J = 9$ Hz, 1H), 7.19–7.39 (m, 5H), 7.70 (d, $J = 9$ Hz, 1H), 8.45 (b, 1H), 8.59 (s, 1H); MS (CI) m/z 281 ($M + 1$). Anal. ($C_{16}H_{16}N_4O$) C, H, N.

8-Ethyl-2-(methylphenylamino)-8H-pyrido[2,3-*d*]pyrimidin-7-one (19): mp 139–141 °C; ¹H NMR (DMSO-*d*₆) δ 1.11 (t, $J = 7$ Hz, 3H), 3.56 (s, 3H), 4.13 (q, $J = 7$ Hz, 2H), 6.32 (d, $J = 9$ Hz, 1H), 7.27–7.29 (m, 1H), 7.40–7.44 (m, 4H), 7.77 (d, $J = 9$ Hz, 1H), 8.66 (s, 1H); MS (CI) m/z 281 ($M + 1$). Anal. ($C_{16}H_{16}N_4O \cdot 0.75EtOAc$) C, H, N.

8-Ethyl-2-(4-fluorophenylamino)-8H-pyrido[2,3-*d*]pyrimidin-7-one (20): mp 215–217 °C; ¹H NMR (DMSO-*d*₆) δ 0.24 (t, $J = 7$ Hz, 3H), 4.29 (q, $J = 7$ Hz, 2H), 6.39 (d, $J = 9$ Hz, 1H), 7.20 (t, $J = 9$ Hz, 2H), 7.80 (d, $J = 9$ Hz, 1H), 7.81 (d, $J = 9$ Hz, 2H), 8.78 (s, 1H), 10.13 (s, 1H). Anal. ($C_{15}H_{13}N_4O \cdot F$) C, H, N.

8-Ethyl-2-(3-fluorophenylamino)-8H-pyrido[2,3-*d*]pyrimidin-7-one (21): mp 210–212 °C; ¹H NMR (DMSO-*d*₆) δ 1.27 (t, $J = 7$ Hz, 3H), 4.33 (q, $J = 7$ Hz, 2H), 6.43 (d, $J = 9$ Hz, 1H), 6.84 (dtr, $J = 3, 9$ Hz, 1H), 7.36 (q, $J = 7$ Hz, 1H), 7.54 (d, $J = 7$ Hz, 1H), 7.84 (d, $J = 9$ Hz, 1H), 7.89 (s, 1H), 8.83 (s, 1H), 10.33 (s, 1H). Anal. ($C_{15}H_{13}N_4O \cdot F \cdot 0.1EtOAc$) C, H, N.

8-Ethyl-2-(pyridin-4-ylamino)-8H-pyrido[2,3-d]pyrimidin-7-one (22): mp 259–260 °C; ^1H NMR (DMSO- d_6) δ 1.28 (t, J = 7 Hz, 3H), 4.35 (q, J = 7 Hz, 2H), 6.49 (d, J = 9 Hz, 1H), 7.81 (q, J = 2 Hz, 2H), 7.88 (d, J = 9 Hz, 1H), 8.43 (q, J = 2 Hz, 2H), 8.88 (s, 1H), 10.50 (s, 1H); MS (CI) m/z 268 (M + 1). Anal. ($\text{C}_{14}\text{H}_{13}\text{N}_5\text{O} \cdot 0.25\text{H}_2\text{O}$) C, H, N.

8-Ethyl-2-[3-(1,1,2,2-tetrafluoroethoxy)phenylamino]-8H-pyrido[2,3-d]pyrimidin-7-one (23): mp 175–176 °C; ^1H NMR (DMSO- d_6) δ 1.19 (t, J = 7 Hz, 3H), 4.32 (q, J = 7 Hz, 2H), 6.44 (d, J = 9 Hz, 1H), 6.70–6.97 (m, 2H), 7.43 (t, J = 8 Hz, 1H), 7.62 (d, J = 8 Hz, 1H), 7.85 (d, J = 9 Hz, 1H), 8.08 (s, 1H), 8.83 (s, 1H), 10.39 (s, 1H); MS (CI) m/z 383 (M + 1). Anal. ($\text{C}_{17}\text{H}_{14}\text{N}_4\text{F}_4\text{O}_2$) C, H, N.

8-Ethyl-2-(2-methoxyphenylamino)-8H-pyrido[2,3-d]pyrimidin-7-one (24): mp 126–128 °C; ^1H NMR (DMSO- d_6) δ 1.19 (t, J = 7 Hz, 3H), 3.85 (s, 3H), 4.24 (q, J = 7 Hz, 2H), 6.38 (d, J = 9 Hz, 1H), 6.97–7.14 (m, 3H), 7.81 (d, J = 9 Hz, 1H), 8.03 (d, J = 8 Hz, 1H), 8.66 (s, 1H), 8.75 (s, 1H); MS (CI) m/z 297 (M + 1). Anal. ($\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}_2 \cdot 0.2\text{H}_2\text{O}$) C, H, N.

8-Ethyl-2-(4-methoxyphenylamino)-8H-pyrido[2,3-d]pyrimidin-7-one (25): mp 196–197 °C; ^1H NMR (DMSO- d_6) δ 1.23 (t, J = 7 Hz, 3H), 3.74 (s, 3H), 4.29 (q, J = 7 Hz, 2H), 6.35 (d, J = 9 Hz, 1H), 6.93 (d, J = 9 Hz, 2H), 7.70 (d, J = 9 Hz, 2H), 7.79 (d, J = 9 Hz, 1H), 6.73 (s, 1H), 9.95 (s, 1H); MS (CI) m/z 297 (M + 1). Anal. ($\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}_2 \cdot 0.5\text{H}_2\text{O}$) C, H, N.

8-Ethyl-2-(4-hydroxyphenylamino)-8H-pyrido[2,3-d]pyrimidin-7-one (26): A mixture of compound 25 (133 mg, 0.45 mmol) and 1 mL of 48% aqueous HBr in 10 mL of propionic acid was heated at reflux for 3 h. After cooling to room temperature, the reaction mixture was partitioned between ethyl acetate and saturated sodium bicarbonate. The aqueous layer was further extracted with ethyl acetate and the organic layers were combined and washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo. The resultant solid was purified by dissolving in ethyl acetate and passing the solution through silica gel to provide 26 (58 mg, 46%); mp 222–224 °C; ^1H NMR (DMSO- d_6) δ 1.21 (t, J = 7 Hz, 3H), 4.28 (q, J = 7 Hz, 2H), 6.33 (d, J = 9 Hz, 1H), 6.73 (d, J = 9 Hz, 2H), 7.54 (d, J = 8 Hz, 2H), 7.77 (d, J = 9 Hz, 1H), 8.70 (s, 1H), 9.19 (s, 1H), 9.83 (s, 1H); MS (CI) m/z 283 (M + 1). Anal. ($\text{C}_{15}\text{H}_{14}\text{N}_4\text{O}_2 \cdot 0.25\text{H}_2\text{O}$) C, H, N.

2-(3,4-Dimethoxyphenylamino)-8-ethyl-8H-pyrido[2,3-d]pyrimidin-7-one (27): ^1H NMR (DMSO) δ 1.17 (t, J = 7 Hz, 3H), 3.68 (s, sH), 3.72 (s, 3H), 4.27 (q, J = 7 Hz, 2H), 6.31 (d, J = 9 Hz, 1H), 6.88 (d, J = 9 Hz, 1H), 7.23 (dd, J = 2, 9 Hz, 1H), 7.48 (br s, 1H), 7.74 (d, J = 9 Hz, 1H), 8.69 (s, 1H), 9.89 (s, 1H). Anal. ($\text{C}_{17}\text{H}_{18}\text{N}_4\text{O}_3 \cdot 0.54\text{H}_2\text{O}$) C, H, N.

8-Ethyl-2-(4-hydroxy-3-methoxyphenylamino)-8H-pyrido[2,3-d]pyrimidin-7-one (28): mp 196–200 °C; ^1H NMR (DMSO) δ 1.16 (t, J = 8 Hz, 3H), 3.73 (s, 3H), 4.25 (q, J = 7 Hz, 2H), 6.29 (d, J = 9 Hz, 1H), 6.68 (d, J = 9 Hz, 1H), 7.05 (d, J = 8 Hz, 1H), 7.46 (br s, 1H), 7.73 (d, J = 9 Hz, 1H), 8.67 (d, J = 4 Hz, 1H), 9.80 (br s, 1H); MS (CI) m/z 313.1 (M + 1). Anal. ($\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}_3 \cdot 0.23\text{H}_2\text{O}$) C, H, N.

8-Ethyl-2-[4-(2-methoxyethoxy)phenylamino]-8H-pyrido[2,3-d]pyrimidin-7-one (29): Phenol 26 (0.099 g, 0.326 mmol), 2-bromoethyl methyl ether (0.055 g, 0.398 mmol) and potassium carbonate (0.36 g, 2.61 mmol) in DMF (5 mL) were heated to reflux for 5 min. The reaction mixture was allowed to cool then H_2O (40 mL) was added and a precipitate formed. This precipitate was collected by filtration then dissolved in ethyl acetate and dried over magnesium sulfate. Following removal of the drying agent and evaporation of the solvent, column chromatography provided the product as a yellow oil. Crystallization from hexanes–ethyl acetate gave a white solid (0.093 g, 83%); mp 169–171 °C; ^1H NMR (DMSO- d_6) δ 1.23 (t, J = 7 Hz, 3H), 3.31 (s, 3H), 3.65 (t, J = 5 Hz, 2H), 4.07 (t, J = 5 Hz, 2H), 4.29 (q, J = 7 Hz, 2H), 6.35 (d, J = 9 Hz, 1H), 6.94 (d, J = 9 Hz, 2H), 7.68 (d, J = 9 Hz, 2H), 7.79 (d, J = 9 Hz, 1H), 8.73 (2, 1H), 9.95 (s, 1H). Anal. ($\text{C}_{18}\text{H}_{20}\text{N}_4\text{O}_3 \cdot 0.25\text{H}_2\text{O}$) C, H, N.

2-[4-(2-Diethylaminoethoxy)phenylamino]-8-ethyl-8H-pyrido[2,3-d]pyrimidin-7-one (30): mp 128–129 °C; ^1H NMR (DMSO- d_6) δ 0.975 (t, J = 7 Hz, 6H), 1.23 (t, J = 7 Hz,

3H), 2.52 (q, J = 7 Hz, 4H), 2.76 (t, J = 6 Hz, 2H), 3.99 (t, J = 6 Hz, 2H), 4.28 (q, J = 7 Hz, 2H), 6.35 (d, J = 9 Hz, 1H), 6.92 (d, J = 9 Hz, 2H), 7.68 (d, J = 9 Hz, 2H), 7.78 (d, J = 9 Hz, 1H), 8.73 (s, 1H), 8.95 (s, 1H); MS (CI) m/z 382 (M + 1). Anal. ($\text{C}_{21}\text{H}_{27}\text{N}_5\text{O}_2 \cdot 0.5\text{H}_2\text{O}$) C, H, N.

2-(4-Dimethylaminophenylamino)-8-ethyl-8H-pyrido[2,3-d]pyrimidin-7-one (31): mp 189–191 °C; ^1H NMR (DMSO- d_6) δ 1.23 (t, J = 7 Hz, 3H), 2.87 (s, 6H), 4.28 (q, J = 7 Hz, 2H), 6.31 (d, J = 9 Hz, 1H), 6.74 (d, J = 9 Hz, 2H), 7.60 (br d, J = 7 Hz, 2H), 7.76 (d, J = 9 Hz, 1H), 8.69 (s, 1H), 9.81 (s, 1H). Anal. ($\text{C}_{17}\text{H}_{19}\text{N}_5\text{O}$) C, H, N.

8-Ethyl-2-(4-pyrrol-1-ylphenylamino)-8H-pyrido[2,3-d]pyrimidin-7-one (32): mp 220–222 °C; ^1H NMR (DMSO- d_6) δ 1.27 (t, J = 7 Hz, 3H), 4.33 (q, J = 7 Hz, 2H), 6.25 (t, J = 2 Hz, 2H), 6.40 (d, J = 9 Hz, 1H), 7.33 (t, J = 2 Hz, 2H), 7.56 (d, J = 9 Hz, 1H), 7.83 (d, J = 9 Hz, 1H), 7.89 (d, J = 9 Hz, 1H), 8.80 (s, 1H), 10.20 (s, 1H). Anal. ($\text{C}_{19}\text{H}_{17}\text{N}_5\text{O} \cdot 0.1\text{H}_2\text{O} \cdot 0.3\text{EtOAc}$) C, H, N.

2-(4-Diethylaminophenylamino)-8-ethyl-8H-pyrido[2,3-d]pyrimidin-7-one (33): mp 108–109 °C; ^1H NMR (DMSO- d_6) δ 1.08 (t, J = 7 Hz, 6H), 1.23 (t, J = 7 Hz, 3H), 3.30 (q, J = 7 Hz, 4H), 4.28 (q, J = 7 Hz, 2H), 6.30 (d, J = 9 Hz, 1H), 6.67 (d, J = 9 Hz, 2H), 7.55 (br s, 2H), 7.75 (d, J = 9 Hz, 1H), 8.68 (s, 1H), 9.77 (s, 1H). Anal. ($\text{C}_{19}\text{H}_{23}\text{N}_5\text{O}$) C, H, N.

8-Ethyl-2-(4-(morpholin-4-yl)phenylamino)-8H-pyrido[2,3-d]pyrimidin-7-one (34): mp 227–228 °C; ^1H NMR (DMSO- d_6) δ 1.18 (t, J = 7 Hz, 3H), 3.07 (t, J = 5 Hz, 4H), 3.74 (t, J = 5 Hz, 4H), 4.29 (q, J = 7 Hz, 2H), 6.34 (d, J = 9 Hz, 1H), 6.94 (d, J = 9 Hz, 2H), 7.66 (d, J = 9 Hz, 2H), 7.78 (d, J = 9 Hz, 1H), 8.72 (s, 1H), 9.91 (s, 1H). Anal. ($\text{C}_{19}\text{H}_{21}\text{N}_4\text{O}_2 \cdot 0.25\text{H}_2\text{O}$) C, H, N.

8-Ethyl-2-(4-piperidin-1-ylphenylamino)-8H-pyrido[2,3-d]pyrimidin-7-one (35): mp 188 °C; ^1H NMR (DMSO- d_6) δ 1.19 (t, J = 7 Hz, 3H), 1.47 (d, J = 4 Hz, 2H), 1.58 (s, 4H), 3.02 (d, J = 5 Hz, 4H), 4.24 (q, J = 7 Hz, 2H), 6.28 (d, J = 9 Hz, 1H), 6.87 (d, J = 9 Hz, 2H), 7.58 (d, J = 8 Hz), 7.73 (d, J = 9 Hz, 1H), 8.67 (s, 1H), 9.84 (s, 1H); MS (CI) m/z = 350.1 (M + 1). Anal. ($\text{C}_{20}\text{H}_{23}\text{N}_5\text{O}$) C, H, N.

8-Ethyl-2-[4-[4-(3-hydroxypropyl)piperazin-1-yl]phenylamino]-8H-pyrido[2,3-d]pyrimidin-7-one (36): ^1H NMR (DMSO- d_6) δ 1.13–1.28 (m, 7H), 1.38–1.44 (m, 2H), 1.70 (d, J = 12 Hz, 2H), 2.53 (quin., J = 11 Hz, 2H), 3.35 (q, J = 6 Hz, 2H), 3.57 (d, J = 12 Hz, 2H), 4.25 (q, J = 7 Hz, 2H), 4.35 (t, J = 5 Hz, 1H), 6.29 (d, J = 9 Hz, 1H), 6.88 (d, J = 9 Hz, 2H), 7.58 (d, J = 8 Hz, 2H), 7.73 (d, J = 9 Hz, 1H), 8.67 (s, 1H), 9.85 (br s, 1H).

8-Ethyl-2-[4-(4-methylpiperazin-1-yl)phenylamino]-8H-pyrido[2,3-d]pyrimidin-7-one (37): mp 185–186 °C; ^1H NMR (DMSO- d_6) δ 1.24 (t, J = 7 Hz, 3H), 2.22 (s, 3H), 2.45 (t, J = 5 Hz, 4H), 3.09 (t, J = 5 Hz, 4H), 4.29 (q, J = 7 Hz, 2H), 6.33 (d, J = 9 Hz, 1H), 6.93 (d, J = 9 Hz, 2H), 7.64 (d, J = 9 Hz, 2H), 7.77 (d, J = 9 Hz, 1H), 8.71 (s, 1H), 9.90 (s, 1H); MS (CI) m/z 365 (M + 1). Anal. ($\text{C}_{20}\text{H}_{24}\text{N}_6\text{O} \cdot 1.0\text{H}_2\text{O}$) C, H, N.

The following compounds were prepared from 2 via a similar route to the one described for compound 1.

8-Methyl-2-phenylamino-8H-pyrido[2,3-d]pyrimidin-7-one (38): mp 244–247 °C; ^1H NMR (DMSO- d_6) δ 3.59 (s, 3H), 6.40 (d, J = 9.4 Hz, 1H), 7.03 (t, J = 7.2 Hz, 1H), 7.35 (t, J = 7.8 Hz, 2H), 7.82 (d, J = 9.2 Hz, 3H), 7.35 (t, J = 7.9 Hz, 2H), 7.82 (d, J = 9.2 Hz, 3H), 8.78 (s, 1H), 10.11 (s, 1H); MS (CI) m/z = 253 (M + 1). Anal. ($\text{C}_{14}\text{H}_{12}\text{N}_4\text{O} \cdot 0.20\text{H}_2\text{O}$) C, H, N.

8-Isopropyl-2-phenylamino-8H-pyrido[2,3-d]pyrimidin-7-one (39): mp 170–171 °C; ^1H NMR (DMSO- d_6) δ 1.53 (d, J = 7 Hz, 6H), 5.72 (br s, 1H), 6.31 (d, J = 9 Hz, 1H), 7.04 (t, J = 7 Hz, 1H), 7.34 (t, J = 7 Hz, 2H), 7.72–7.77 (m, 3H), 8.75 (s, 1H), 10.00 (s, 1H); MS (CI) m/z 281 (M + 1). Anal. ($\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}$) C, H, N.

8-Isopropyl-2-[4-(4-methylpiperazin-1-yl)phenylamino]-8H-pyrido[2,3-d]pyrimidin-7-one (40): mp 221–222 °C; ^1H NMR (DMSO- d_6) δ 1.51 (d, J = 7 Hz, 6H), 2.45 (t, J = 5 Hz, 4H), 3.09 (t, J = 5 Hz, 4H), 5.69 (b, 1H), 6.26 (d, J = 9 Hz, 1H), 6.92 (d, J = 9 Hz, 2H), 7.54 (d, J = 9 Hz, 2H), 7.71 (d, J = 9 Hz, 1H), 8.68 (s, 1H), 9.77 (s, 1H); MS (CI) m/z 379 (M + 1). Anal. ($\text{C}_{21}\text{H}_{26}\text{N}_6\text{O} \cdot 0.25\text{H}_2\text{O}$) C, H, N.

8-sec-Butyl-2-phenylamino-8H-pyrido[2,3-d]pyrimidin-7-one (41): mp 155–156 °C; ^1H NMR (DMSO- d_6) δ 0.78 (br s, 3H), 1.50 (d, J = 6.5 Hz, 3H), 2.15 (br s, 1H), 1.93 (dq, J = 6.7, 3.7 Hz, 1H), 5.54 (br s, 1H), 6.32 (d, J = 8.4 Hz, 1H), 7.04 (t, J = 7.2 Hz, 1H), 7.34 (t, J = 7.5 Hz, 2H), 7.73 (d, J = 8.0 Hz, 1H), 7.67 (d, J = 9.4 Hz, 2H), 8.76 (s, 1H), 10.02 (br s, 1H); MS (CI) m/z = 295 (M + 1). Anal. ($\text{C}_{17}\text{H}_{18}\text{N}_4\text{O}$) C, H, N.

8-Butyl-2-phenylamino-8H-pyrido[2,3-d]pyrimidin-7-one (42): mp 183–184 °C; ^1H NMR (DMSO- d_6) δ 0.94 (t, J = 7 Hz, 3H), 1.38–1.44 (m, 2H), 1.63–1.67 (m, 2H), 4.25 (t, J = 8 Hz, 2H), 6.39 (d, J = 9 Hz, 1H), 7.04 (t, J = 7 Hz, 1H), 7.33 (t, J = 7 Hz, 2H), 7.80–7.83 (m, 3H), 8.78 (s, 1H), 10.12 (s, 1H); MS (CI) m/z = 295 (M + 1). Anal. ($\text{C}_{17}\text{H}_{18}\text{N}_4\text{O} \cdot 0.25\text{H}_2\text{O}$) C, H, N.

8-Isobutyl-2-phenylamino-8H-pyrido[2,3-d]pyrimidin-7-one (43): mp 170–171 °C; ^1H NMR (DMSO- d_6) δ 0.89 (d, J = 7 Hz, 6H), 2.26–2.29 (m, 1H), 4.14 (d, J = 7 Hz, 2H), 6.39 (d, J = 9 Hz, 1H), 7.04 (t, J = 7 Hz, 1H), 7.34 (t, J = 7 Hz, 2H), 7.81–7.83 (m, 3H), 8.78 (s, 1H), 10.11 (s, 1H); MS (CI) m/z = 295 (M + 1). Anal. ($\text{C}_{17}\text{H}_{18}\text{N}_4\text{O} \cdot 0.10\text{EtOAc}$) C, H, N.

8-(1-Ethylpropyl)-2-phenylamino-8H-pyrido[2,3-d]pyrimidin-7-one (44): mp 116–118 °C; major conformer: ^1H NMR (DMSO- d_6) δ 0.76 (t, J = 7 Hz, 6H), 1.83–1.90 (m, 4H), 5.40–5.55 (m, 1H), 6.30 (d, J = 9 Hz, 1H), 7.04 (t, J = 7 Hz, 1H), 7.32–7.36 (m, 2H), 7.75–7.81 (m, 3H), 8.77 (s, 1H), 10.07 (s, 1H); MS (CI) m/z = 309 (M + 1). Anal. ($\text{C}_{18}\text{H}_{20}\text{N}_4\text{O} \cdot 0.2\text{H}_2\text{O}$) C, H, N.

8-Cyclopentyl-2-phenylamino-8H-pyrido[2,3-d]pyrimidin-7-one (45): mp 188–192 °C; ^1H NMR (DMSO- d_6) δ 1.59–1.64 (m, 2H), 1.73–1.78 (m, 2H), 1.81–1.92 (m, 2H), 2.22–2.30 (m, 2H), 5.81–6.03 (m, 1H), 6.33 (d, J = 9 Hz, 1H), 7.04 (t, J = 7 Hz, 1H), 7.34 (t, J = 7 Hz, 2H), 7.71 (d, J = 8 Hz, 2H), 7.77 (d, J = 9 Hz, 1H), 8.76 (s, 1H), 10.00 (s, 1H); MS (CI) m/z = 307.3 (M + 1). Anal. ($\text{C}_{18}\text{H}_{18}\text{N}_4\text{O} \cdot 0.28\text{H}_2\text{O}$) C, H, N.

8-Cyclohexyl-2-phenylamino-8H-pyrido[2,3-d]pyrimidin-7-one (46): mp 202–204 °C; ^1H NMR (DMSO- d_6) δ 1.21–1.39 (m, 3H), 1.57–1.99 (m, 5H), 2.49–2.60 (m, 2H), 5.35 (br s, 1H), 6.31 (d, J = 9 Hz, 1H), 7.06 (t, J = 7 Hz, 1H), 7.35 (t, J = 7 Hz, 2H), 7.59–7.77 (m, 3H), 8.75 (s, 1H), 10.04 (s, 1H); MS (CI) m/z = 321 (M + 1). Anal. ($\text{C}_{19}\text{H}_{20}\text{N}_4\text{O} \cdot 0.20\text{H}_2\text{O}$) C, H, N.

8-Cycloheptyl-2-phenylamino-8H-pyrido[2,3-d]pyrimidin-7-one (47): mp 156–158 °C; ^1H NMR (DMSO- d_6) δ 1.52–1.77 (m, 10H), 2.41–2.43 (m, 2H), 5.59 (br s, 1H), 6.31 (d, J = 9 Hz, 1H), 7.06 (t, J = 7 Hz, 1H), 7.34 (t, J = 7 Hz, 2H), 7.74–7.77 (m, 3H), 8.75 (s, 1H), 10.11 (s, 1H); MS (CI) m/z = 335 (M + 1). Anal. ($\text{C}_{20}\text{H}_{22}\text{N}_4\text{O}$) C, H, N.

8-Bicyclo[2.2.1]hept-2-yl-2-phenylamino-8H-pyrido[2,3-d]pyrimidin-7-one (48): mp 225–226 °C; ^1H NMR (DMSO- d_6) δ 1.16 (d, J = 9 Hz, 1H), 1.25–1.29 (m, 1H), 1.40 (t, J = 9 Hz, 1H), 1.51–1.62 (m, 2H), 1.69 (t, J = 10 Hz, 1H), 2.17–2.20 (m, 1H), 2.37 (br s, 1H), 2.41 (br s, 1H), 2.62 (d, J = 9 Hz, 1H), 5.32 (t, J = 8 Hz, 1H), 6.28 (d, J = 9 Hz, 1H), 7.05 (t, J = 7 Hz, 1H), 7.33 (t, J = 9 Hz, 2H), 7.73 (d, J = 9 Hz, 1H), 7.77 (d, J = 9 Hz, 2H), 8.74 (s, 1H), 10.06 (s, 1H). Anal. ($\text{C}_{20}\text{H}_{20}\text{N}_4\text{O} \cdot 0.2\text{H}_2\text{O}$) C, H, N.

8-Phenyl-2-phenylamino-8H-pyrido[2,3-d]pyrimidin-7-one (49): mp 300–302 °C; ^1H NMR (DMSO- d_6) δ 6.49 (d, J = 9 Hz, 1H), 6.84 (t, J = 7 Hz, 1H), 6.96 (t, J = 8 Hz, 2H), 7.29 (d, J = 8 Hz, 2H), 7.35 (dd, J = 1, 4 Hz, 2H), 7.55–7.63 (m, 3H), 7.94 (d, J = 9 Hz, 1H), 8.83 (s, 1H), 10.01 (s, 1H). Anal. ($\text{C}_{19}\text{H}_{14}\text{N}_4\text{O} \cdot 0.25\text{H}_2\text{O}$) C, H, N.

General Procedure for the Preparation of 8-Substituted-2-phenylamino-8H-pyrido[2,3-d]pyrimidin-7-ones from 2-Phenylamino-8H-pyrido[2,3-d]pyrimidin-7-one (7). To a suspension of NaH (1.0–1.5 equiv of a 60% suspension of NaH in mineral oil) in 5 mL of dimethylformamide was added 7 (1 equiv). The reaction mixture was heated to 50–60 °C resulting in a yellow solution. The solution was cooled slightly and the desired alkyl halide (1.1–2.0 equiv) was added. The reaction mixture was heated at 50 °C, for a time ranging from 5 min to 1 h, then cooled to room temperature and partitioned between water and ethyl acetate. In some cases, the organic layer was washed with additional water or

brine. The organic layer was dried over magnesium sulfate, filtered, and concentrated in vacuo.

8-Cyclohexylmethyl-2-phenylamino-8H-pyrido[2,3-d]pyrimidin-7-one (50): mp 230–231 °C; 1.00–1.20 (m, 5H), 1.57–1.60 (m, 3H), 1.67 (br s, 2H), 1.97 (br s, 1H), 1.45 (d, J = 7 Hz, 2H), 6.39 (d, J = 9 Hz, 1H), 7.04 (t, J = 7 Hz, 1H), 7.33 (t, J = 8 Hz, 2H), 7.80–7.84 (m, 3H), 8.78 (s, 1H), 10.11 (s, 1H); MS (CI) m/z = 335.2 (M + 1). Anal. ($\text{C}_{20}\text{H}_{22}\text{N}_4\text{O}$) C, H, N.

8-Benzyl-2-phenylamino-8H-pyrido[2,3-d]pyrimidin-7-one (51): mp 215–216 °C; ^1H NMR (DMSO- d_6) δ 5.49 (s, 2H), 6.48 (d, J = 9.4 Hz, 1H), 6.99 (t, J = 7.4 Hz, 1H), 7.20–7.31 (m, 7H), 7.60 (d, J = 8.0 Hz, 2H), 7.90 (d, J = 9.4 Hz, 1H), 8.82 (s, 1H), 10.09 (s, 1H); MS (CI) m/z = 329 (M + 1). Anal. ($\text{C}_{20}\text{H}_{16}\text{N}_4\text{O} \cdot 0.25\text{H}_2\text{O}$) C, H, N.

8-Methoxymethyl-2-phenylamino-8H-pyrido[2,3-d]pyrimidin-7-one (52): mp 173–174 °C; ^1H NMR (DMSO- d_6) δ 3.34 (s, 3H), 5.64 (s, 2H), 6.40 (d, J = 9 Hz, 1H), 7.03 (t, J = 7 Hz, 1H), 7.34 (t, J = 7 Hz, 2H), 7.81 (d, J = 7 Hz, 2H), 7.86 (d, J = 9 Hz, 1H), 8.80 (s, 1H), 8.83 (s, 1H), 10.14 (s, 1H); MS (CI) m/z = 283 (M + 1). Anal. ($\text{C}_{15}\text{H}_{14}\text{N}_4\text{O}_2$) C, H, N.

8-(2-Methoxyethyl)-2-phenylamino-8H-pyrido[2,3-d]pyrimidin-7-one (53): mp 179–180 °C; ^1H NMR (DMSO- d_6) δ 3.26 (s, 3H), 3.62 (t, J = 6.4 Hz, 2H), 4.47 (t, J = 6.4 Hz, 2H), 6.39 (d, J = 9.4 Hz, 2H), 7.03 (t, J = 7.3 Hz, 1H), 7.33 (t, J = 7.8 Hz, 2H), 7.80 (d, J = 6.8 Hz, 2H), 7.82 (d, J = 9.2 Hz, 1H), 8.78 (s, 1H), 10.11 (s, 1H); MS (CI) m/z = 297 (M + 1). Anal. ($\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}_2 \cdot 0.12\text{H}_2\text{O}$) C, H, N.

8-(2-Ethoxyethyl)-2-phenylamino-8H-pyrido[2,3-d]pyrimidin-7-one (54): mp 151–153 °C; ^1H NMR (DMSO- d_6) δ 0.99 (t, J = 7.0 Hz, 3H), 3.40 (q, J = 6.9 Hz, 2H), 3.58 (t, J = 6.7 Hz, 2H), 4.40 (t, J = 6.7 Hz, 2H), 6.34 (d, J = 9.3 Hz, 1H), 6.98 (t, J = 7.3 Hz, 1H), 7.27 (t, J = 7.8 Hz, 2H), 7.74–7.78 (m, 3H), 8.73 (s, 1H), 10.06 (s, 1H); MS (CI) m/z = 311.1 (M + 1). Anal. ($\text{C}_{17}\text{H}_{18}\text{N}_4\text{O}_2$) C, H, N.

8-Oxiranylmethyl-2-phenylamino-8H-pyrido[2,3-d]pyrimidin-7-one (55): mp 163–165 °C; ^1H NMR (DMSO- d_6) δ 2.61–2.62 (m, 1H), 2.73–2.75 (m, 1H), 3.30–3.32 (m, 1H), 4.34–4.39 (m, 1H), 4.52–4.57 (m, 1H), 6.42 (d, J = 9 Hz, 1H), 7.04 (t, J = 7 Hz, 1H), 7.35 (t, J = 7 Hz, 2H), 7.79 (d, J = 7 Hz, 2H), 7.86 (d, J = 9 Hz, 1H), 8.80 (s, 1H), 10.13 (s, 1H); MS (CI) m/z = 295 (M + 1). Anal. ($\text{C}_{16}\text{H}_{14}\text{N}_4\text{O}_2 \cdot 0.05\text{EtOAc}$) C, H, N.

(7-Oxo-2-phenylamino-7H-pyrido[2,3-d]pyrimidin-8-yl)acetic acid methyl ester (56): mp 232–233 °C; ^1H NMR (DMSO- d_6) δ 3.68 (s, 3H), 5.04 (s, 2H), 6.45 (d, J = 9 Hz, 1H), 7.04 (t, J = 7 Hz, 1H), 7.32 (t, J = 7 Hz, 2H), 7.69 (d, J = 7 Hz, 2H), 7.91 (d, J = 9 Hz, 1H), 8.83 (s, 1H), 10.18 (s, 1H); MS (CI) m/z = 311 (M + 1). Anal. ($\text{C}_{16}\text{H}_{14}\text{N}_4\text{O}_3$) C, H, N.

The following compounds were prepared from **2** via a similar route to the one described for compound **1**.

2-[4-(2-Diethylaminoethoxy)phenylamino]-8-isopropyl-8H-pyrido[2,3-d]pyrimidin-7-one (57): mp 84–85 °C; ^1H NMR (DMSO- d_6) δ 0.975 (t, J = 7 Hz, 6H), 1.50 (d, J = 6 Hz, 6H), 2.55 (q, J = 7 Hz, 4H), 2.76 (t, J = 6 Hz, 2H), 3.99 (t, J = 6 Hz, 2H), 5.45 (b, 1H), 6.27 (d, J = 9 Hz, 1H), 6.92 (d, J = 9 Hz, 2H), 7.58 (d, J = 9 Hz, 2H), 7.72 (d, J = 9 Hz, 1H), 8.70 (s, 1H), 9.82 (s, 1H); MS (CI) m/z = 396 (M + 1). Anal. ($\text{C}_{22}\text{H}_{29}\text{N}_5\text{O}_2$) C, H, N.

8-Cyclopentyl-2-[4-(2-diethylaminoethoxy)phenylamino]-8H-pyrido[2,3-d]pyrimidin-7-one (58): mp 105–107 °C; ^1H NMR (DMSO- d_6) δ 0.975 (t, J = 7 Hz, 6H), 1.56–1.87 (m, 6H), 2.21–2.28 (m, 2H), 2.50 (q, J = 7 Hz, 4H), 2.76 (t, J = 6 Hz, 2H), 3.99 (t, J = 6 Hz, 2H), 5.82 (br s, 1H), 6.29 (d, J = 9 Hz, 1H), 6.92 (d, J = 9 Hz, 2H), 7.55 (d, J = 9 Hz, 2H), 7.74 (d, J = 9 Hz, 1H), 8.70 (s, 1H), 9.81 (s, 1H); MS (CI) m/z = 422 (M + 1). Anal. ($\text{C}_{24}\text{H}_{31}\text{N}_5\text{O}_2 \cdot 0.25\text{H}_2\text{O}$) C, H, N.

8-Cyclohexyl-2-[4-(2-diethylaminoethoxy)phenylamino]-8H-pyrido[2,3-d]pyrimidin-7-one (59): mp 135–137 °C; ^1H NMR (DMSO- d_6) δ 0.975 (t, J = 7 Hz, 6H), 1.29–1.82 (m, 8H), 2.50 (q, J = 7 Hz, 4H), 2.76 (t, J = 6 Hz, 2H), 4.01 (t, J = 6 Hz, 2H), 5.35 (br s, 1H), 6.27 (d, J = 9 Hz, 1H), 6.92 (d, J = 9 Hz, 2H), 7.59 (br s, 2H), 7.72 (d, J = 9 Hz, 1H), 8.69 (s, 1H), 9.90 (s, 1H); MS (CI) m/z = 436 (M + 1). Anal. ($\text{C}_{25}\text{H}_{33}\text{N}_5\text{O}_2$) C, H, N.

8-Cycloheptyl-2-[4-(2-diethylaminoethoxy)phenylamino]-8H-pyrido[2,3-d]pyrimidin-7-one (60): mp 119–121 °C; ¹H NMR (DMSO-*d*₆) δ 0.99 (t, *J* = 7 Hz, 6H), 1.30–1.85 (m, 10H), 2.18 (s, 2H), 2.56 (q, *J* = 7 Hz, 4H), 2.80 (t, *J* = 6 Hz, 2H), 4.01 (t, *J* = 6 Hz, 2H), 5.38 (br s, 1H), 6.26 (d, *J* = 9 Hz, 1H), 6.91 (d, *J* = 9 Hz, 2H), 7.40 (br s, 2H), 7.71 (d, *J* = 9 Hz, 1H), 8.68 (s, 1H), 9.80 (s, 1H); MS (CI) *m/z* 450 (M + 1). Anal. (C₂₆H₃₅N₅O₂·0.15H₂O) C, H, N.

8-Bicyclo[2.2.1]hept-2-yl-2-[4-(2-diethylaminoethoxy)phenylamino]-8H-pyrido[2,3-d]pyrimidin-7-one (61): mp 133–134 °C; ¹H NMR (DMSO-*d*₆) δ 0.97 (t, *J* = 7 Hz, 6H), 1.13 (d, *J* = 9 Hz, 1H), 1.25 (br s, 1H), 1.35 (br s, 1H), 1.49–1.69 (m, 3H), 2.17 (br s, 1H), 2.37 (d, *J* = 11 Hz, 2H), 2.60 (d, *J* = 9 Hz, 1H), 2.75 (t, *J* = 6 Hz, 2H), 4.00 (t, *J* = 6 Hz, 2H), 5.27 (t, *J* = 7 Hz, 1H), 6.24 (d, *J* = 9 Hz, 1H), 6.90 (d, *J* = 9 Hz, 2H), 7.60 (d, *J* = 9 Hz, 2H), 7.69 (d, *J* = 9 Hz, 1H), 8.68 (s, 1H), 9.87 (s, 1H); MS (CI) *m/z* 484.4 (M + 1). Anal. (C₂₆H₃₃N₅O₂·0.4H₂O) C, H, N.

8-(2-Benzoyloxyethyl)-2-[4-(4-methylpiperazin-1-yl)phenylamino]-8H-pyrido[2,3-d]pyrimidin-7-one (62): mp 183–185 °C; ¹H NMR (DMSO-*d*₆) δ 2.21 (s, 3H), 2.42 (t, *J* = 5 Hz, 4H), 3.01 (br s, 4H), 3.72 (t, *J* = 7 Hz, 2H), 4.49–4.53 (m, 4H), 6.34 (d, *J* = 9 Hz, 1H), 6.86 (d, *J* = 9 Hz, 2H), 7.22–7.30 (m, 5H), 7.62 (d, *J* = 9 Hz, 2H), 7.79 (d, *J* = 9 Hz, 1H), 8.72 (s, 1H), 9.90 (s, 1H); MS (CI) *m/z* 470.9 (M + 1). Anal. (C₂₇H₃₈N₆O₂) C, H, N.

8-(2,3-Dihydroxypropyl)-2-[4-(4-methylpiperazin-1-yl)phenylamino]-8H-pyrido[2,3-d]pyrimidin-7-one (63): mp 188–195 °C; ¹H NMR (DMSO-*d*₆) δ 2.08 (s, 3H), 2.25–3.35 (m, 4H), 3.02–3.35 (m, 4H), 3.14–3.38 (m, 2H), 3.80–4.60 (m, 4H), 4.62 (d, *J* = 6 Hz, 1H), 6.27 (d, *J* = 9 Hz, 1H), 6.85 (d, *J* = 9 Hz, 2H), 7.65–7.68 (m, 2H), 7.71 (d, *J* = 9 Hz, 1H), 8.64 (s, 1H); MS (CI) *m/z* 411.2 (M + 1).

2-[4-(4-Methylpiperazin-1-yl)phenylamino]-8-phenyl-8H-pyrido[2,3-d]pyrimidin-7-one (64): mp 259–262 °C; ¹H NMR (DMSO-*d*₆) δ 2.21 (s, 3H), 2.43 (t, *J* = 5 Hz, 4H), 2.97 (br s, 4H), 6.43 (d, *J* = 9 Hz, 1H), 6.54 (br s, 2H), 7.13 (br s, 2H), 7.32 (dd, *J* = 1, 8 Hz, 2H), 7.56–7.62 (m, 3H), 7.89 (d, *J* = 9 Hz, 1H), 8.76 (s, 1H), 9.84 (s, 1H); MS (CI) *m/z* 413.1 (M + 1). Anal. (C₂₄H₂₄N₅O·0.3H₂O) C, H, N.

8-Cyclopropyl-2-[4-(4-methylpiperazin-1-yl)phenylamino]-8H-pyrido[2,3-d]pyrimidin-7-one (65): mp 210–211 °C; ¹H NMR (DMSO-*d*₆) δ 0.79–0.83 (m, 2H), 1.22–1.27 (m, 2H), 2.22 (s, 3H), 2.45 (t, *J* = 5 Hz, 4H), 2.83–2.86 (m, 1H), 3.08 (t, *J* = 5 Hz, 4H), 6.26 (d, *J* = 9 Hz, 1H), 6.94 (d, *J* = 9 Hz, 2H), 7.71 (d, *J* = 9 Hz, 1H), 7.79 (br s, 2H), 8.66 (s, 1H), 9.95 (br s, 1H); MS (CI) *m/z* 376.9 (M + 1). Anal. (C₂₁H₂₄N₅O) C, H, N.

8-Isopropyl-2-[4-(4-methylpiperazin-1-yl)phenylamino]-8H-pyrido[2,3-d]pyrimidin-7-one (66): mp 221–222 °C; ¹H NMR (DMSO-*d*₆) δ 1.51 (d, *J* = 7 Hz, 6H), 2.22 (s, 3H), 2.45 (t, *J* = 5 Hz, 4H), 3.09 (t, *J* = 5 Hz, 4H), 5.69 (br s, 1H), 6.26 (d, *J* = 9 Hz, 1H), 6.92 (d, *J* = 9 Hz, 2H), 7.54 (d, *J* = 9 Hz, 2H), 7.71 (d, *J* = 9 Hz, 1H), 8.68 (s, 1H), 9.77 (s, 1H); MS (CI) *m/z* 379 (M + 1). Anal. (C₂₁H₂₆N₆O·0.25H₂O) C, H, N.

8-(1-Ethylpropyl)-2-propyl-2-[4-(4-methylpiperazin-1-yl)phenylamino]-8H-pyrido[2,3-d]pyrimidin-7-one (67): mp 155–157 °C; major conformer: ¹H NMR (DMSO-*d*₆) δ 0.69 (t, *J* = 8 Hz, 6H), 1.75–1.82 (m, 4H), 2.16 (s, 3H), 2.39 (t, *J* = 5 Hz, 4H), 3.03 (t, *J* = 5 Hz, 4H), 5.40 (br s, 1H), 6.19 (d, *J* = 9 Hz, 1H), 6.86 (d, *J* = 9 Hz, 2H), 7.53 (d, *J* = 9 Hz, 2H), 7.70 (d, *J* = 9 Hz, 1H), 8.64 (s, 1H), 9.80 (br s, 1H); MS (CI) *m/z* 406 (M + 1). Anal. (C₂₃H₃₀N₆O·0.1H₂O) C, H, N.

8-Cyclopentyl-2-[4-(4-methylpiperazin-1-yl)phenylamino]-8H-pyrido[2,3-d]pyrimidin-7-one (68): yield 43%; mp 175–177 °C; ¹H NMR (DMSO-*d*₆) δ 1.56–1.87 (m, 6H), 2.22–2.26 (m, 5H), 2.45 (t, *J* = 5 Hz, 4H), 3.09 (t, *J* = 5 Hz, 4H), 5.82 (b, 1H), 6.27 (d, *J* = 9 Hz, 1H), 6.92 (d, *J* = 9 Hz, 2H), 7.50 (d, *J* = 9 Hz, 2H), 7.72 (d, *J* = 9 Hz, 1H), 8.69 (s, 1H), 9.75 (s, 1H); MS (CI) *m/z* 405 (M + 1). Anal. (C₂₃H₂₈N₆O) C, H, N.

8-Bicyclo[2.2.1]hept-2-yl-2-[4-(4-methylpiperazin-1-yl)phenylamino]-8H-pyrido[2,3-d]pyrimidin-7-one (69): mp 233–234 °C; ¹H NMR (DMSO-*d*₆) δ 1.17–1.71 (m, 6H), 2.20

(m, 1H), 2.22 (s, 3H), 2.37–2.41 (m, 2H), 2.45 (t, *J* = 5 Hz, 4H), 2.61–2.63 (m, 1H), 3.09 (t, *J* = 9 Hz, 4H), 5.32 (b, 1H), 6.28 (d, *J* = 9 Hz, 1H), 6.92 (d, *J* = 9 Hz, 2H), 7.54 (d, *J* = 9 Hz, 2H), 7.71 (d, *J* = 9 Hz, 1H), 8.67 (s, 1H), 9.82 (s, 1H); MS (CI) *m/z* 431 (M + 1). Anal. (C₂₅H₃₀N₆O·0.15H₂O) C, H, N.

8-Cyclohexyl-2-[4-(4-methylpiperazin-1-yl)phenylamino]-8H-pyrido[2,3-d]pyrimidin-7-one (70): mp 205–207 °C; ¹H NMR (DMSO-*d*₆) δ 1.30–1.82 (m, 8H), 2.22 (s, 3H), 2.52–2.56 (m, 2H), 2.45 (t, *J* = 5 Hz, 4H), 2.52–2.56 (m, 2H), 3.09 (t, *J* = 5 Hz, 4H), 5.35 (br s, 1H), 6.26 (d, *J* = 9 Hz, 1H), 6.92 (d, *J* = 9 Hz, 2H), 7.54 (d, *J* = 9 Hz, 2H), 7.71 (d, *J* = 9 Hz, 1H), 8.68 (s, 1H), 9.77 (s, 1H); MS (CI) *m/z* 419 (M + 1). Anal. (C₂₄H₃₀N₆O·0.1EtOAc) C, H, N.

8-Cyclopentyl-2-[4-[4-(3-hydroxypropyl)piperidin-1-yl]phenylamino]-8H-pyrido[2,3-d]pyrimidin-7-one (71): mp 223 °C; ¹H NMR (DMSO-*d*₆) δ 1.18–1.42 (m, 4H), 1.44–1.50 (m, 2H), 1.57 (br s, 2H), 1.74 (d, *J* = 11 Hz, 4H), 1.86 (br s, 2H), 2.23 (br s, 2H), 2.58 (t, *J* = 11 Hz, 2H), 3.40 (t, *J* = 6 Hz, 2H), 3.62 (d, *J* = 12 Hz, 2H), 4.39 (t, *J* = 5 Hz, 1H), 5.80 (br s, 1H), 6.27 (d, *J* = 9 Hz, 1H), 6.91 (d, *J* = 9 Hz, 2H), 7.47 (d, *J* = 9 Hz, 2H), 7.73 (d, *J* = 9 Hz, 1H), 8.68 (s, 1H), 9.73 (br s, 1H); MS (CI) *m/z* 448.3 (M + 1). Anal. (C₂₆H₃₃N₅O₂·0.55H₂O) C, H, N.

8-Bicyclo[2.2.1]hept-2-yl-2-[4-[4-(3-hydroxypropyl)piperidin-1-yl]phenylamino]-8H-pyrido[2,3-d]pyrimidin-7-one (72): mp 223 °C; ¹H NMR (DMSO-*d*₆) δ 1.12–1.75 (m, 16H), 2.17 (br s, 1H), 2.36 (d, *J* = 9 Hz, 2H), 2.58 (t, *J* = 12 Hz, 2H), 3.39 (q, *J* = 6 Hz, 2H), 3.60 (d, *J* = 12 Hz, 2H), 4.38 (t, *J* = 5 Hz, 1H), 5.28 (t, *J* = 8 Hz, 1H), 6.22 (d, *J* = 9 Hz, 1H), 6.89 (d, *J* = 9 Hz, 2H), 7.54 (d, *J* = 9 Hz, 2H), 7.68 (d, *J* = 9 Hz, 1H), 8.66 (s, 1H), 9.80 (br s, 1H); MS (CI) *m/z* 474.3 (M + 1). Anal. (C₂₈H₃₆N₅O₂·0.09H₂O) C, H, N.

Cdk Assays (Cdk4/cyclin D1, Cdk2/cyclin E, Cdk2 cyclin A, and Cdc2/cyclin B). All Cdk5 were human recombinant proteins expressed in insect cells through baculovirus infection. Enzyme assays for IC₅₀ determinations and kinetic evaluation were performed in 96-well filter plates (Millipore MADVN6550). The total volume was 0.1 mL containing a final concentration of 20 mM Tris (tris[hydroxymethyl]aminomethane), pH 7.4, 50 mM NaCl, 1 mM dithiothreitol, 10 mM MgCl₂, 25 μM ATP (for Cdk4) or 12 μM ATP (for Cdk2/E, Cdk2/A and Cdc2/B) containing 0.25 μCi of [³²P]ATP, 20 ng of enzyme, 1 μg of GST-retinoblastoma and appropriate dilutions of inhibitor. All components except the ATP were added to the wells and the plate was placed on a plate mixer for 2 min. The reaction was started by adding [³²P]ATP and the plate was incubated at 25 °C for 15 min. The reaction was terminated by addition of 0.1 mL of 20% trichloroacetic acid (TCA). The plate was kept at 4 °C for at least 1 h to allow the substrate to precipitate. The wells were then washed 5 times with 0.2 mL of 10% TCA and ³²P incorporation was determined with a beta plate counter (Wallac Inc., Gaithersburg, MD).

Tyrosine Kinase Assays. PDGF, FGF and SRC were obtained and assayed as previously described.²²

References

- (1) Sherr, C. J. Cancer Cell Cycles. *Science* **1996**, *274*, 1672–1677.
- (2) Johnson, D. G.; Walker, C. L. Cyclins and Cell Cycle Checkpoints. *Annu. Rev. Pharmacol. Toxicol.* **1999**, *39*, 295–312.
- (3) Rosania, G. P.; Chang, Y.-T. Targeting Hyperproliferative Disorders with Cyclin Dependent Kinase Inhibitors. *Exp. Opin. Ther. Patents* **2000**, *10*, 215–230.
- (4) Reviewed in Fry, D. W.; Garrett, M. D. Inhibitors of Cyclin-Dependent Kinases as Therapeutic Agents for the Treatment of Cancer. *Curr. Opin. Oncol. Endocrine Metab. Invest. Drugs* **2000**, *2*, 40–59.
- (5) Bridges, A. J. The Rationale and Strategy Used to Develop a Series of Highly Potent, Irreversible, Inhibitors of the Epidermal Growth Factor Receptor Family of Tyrosine Kinases. *Curr. Med. Chem.* **1999**, *5*, 825.
- (6) Cheng, M.; Olivier, P.; Diehl, J. A.; Fero, M.; Roussel, M. F.; Roberts, J. M.; Sherr, C. J. The p21^{Cip1} and p27^{Kip1} CDK Inhibitors are Essential Activators of Cyclin D-Dependent Kinases in Murine Fibroblasts. *EMBO J.* **1999**, *18*, 1571–1583.
- (7) Gray, N.; Detivaud, L.; Doerig, C.; Meijer, L. ATP-Site Directed Inhibitors of Cyclin-Dependent Kinases. *Curr. Med. Chem.* **1999**, *6*, 859–875.

- (8) Garrett, M. D.; Fattaey, A. CDK Inhibition and Cancer Therapy. *Curr. Opin. Genet. Dev.* **1999**, *9*, 104–111.
- (9) Sielecki, T. M.; Boylan, J. F.; Benfield, P. A.; Trainor, G. L. Cyclin Dependent Kinase Inhibitors: Useful Targets in Cell Cycle Regulation. *J. Med. Chem.* **2000**, *43*, 1–18.
- (10) Webster, K. R.; Kimball, S. D. Novel Drugs Targeting the Cell Cycle. *Emerging Drugs* **2000**, *5*, 45–49.
- (11) Klutchko, S. R.; Hamby, J. M.; Boschelli, D. H.; Wu, Z.; Kraker, A. J.; Amar, A. M.; Hartl, B. G.; Shen, C.; Klohs, W. D.; Steinkampf, R. W.; Driscoll, D. L.; Nelson, J. M.; Elliott, W. L.; Roberts, B. J.; Stoner, C. L.; Vincent, P. W.; Dykes, D. J.; Panek, R. L.; Lu, G. H.; Major, T. C.; Dahrting, T. K.; Hallak, H.; Bradford, L. A.; Showalter, H. D. S.; Doherty, A. 2-Substituted Aminopyrido[2,3-*d*]pyrimidine-(8*H*)-ones. Structure–Activity Relationships against Selected Tyrosine Kinases and in Vitro and in Vivo Anticancer Activity. *J. Med. Chem.* **1998**, *41*, 3276–3292.
- (12) Boschelli, D. H.; Wu, Z.; Klutchko, S. R.; Showalter, H. D. S.; Hamby, J. M.; Lu, G. H.; Major, T. C.; Dahrting, T. K.; Batley, B.; Panek, R. L.; Keiser, J.; Hartl, B. G.; Kraker, A. J.; Klohs, W. D.; Roberts, B. J.; Patmore, S.; Elliott, W. L.; Steinkampf, R.; Bradford, L. A.; Hallak, H.; Doherty, A. Synthesis and Tyrosine Kinase Inhibitory Activity of a Series of 2-Amino-8H-pyrido[2,3-*d*]pyrimidines: Identification of Potent, Selective Platelet-Derived Growth Factor Receptor Tyrosine Kinase Inhibitors. *J. Med. Chem.* **1998**, *41*, 4365–4377.
- (13) Trumpp-Kallmeyer, S.; Rubin, R. J.; Humblet, C.; Hamby, J.; Showalter, H. D. S. Development of a Binding Model to Protein Tyrosine Kinases for Substituted Pyrido[2,3-*d*]pyrimidine Inhibitors. *J. Med. Chem.* **1998**, *41*, 1752–1763.
- (14) Davis, F. A.; Stringer, O. D. Chemistry of Oxaziridines 2. Improved Synthesis of 2-Sulfonyloxaziridines. *J. Org. Chem.* **1982**, *47*, 1774–1775.
- (15) Fry, D. W.; Harvey, P. H.; Bedford, D.; Fritsch, A.; Keller, P. R.; Wu, Z.; Dobrusin, E.; Leopold, W. R.; Fattaey, A.; Garrett, M. D. Cell Cycle and Biochemical Effects of PD0183812; A Potent inhibitor of Cyclin-Dependent Kinase 4. *J. Biol. Chem.*, submitted.
- (16) Schulze-Gahmen, U.; De Bondt, H.; Kim, S.-H. High-Resolution Crystal Structures of Human Cyclin-Dependent Kinase 2 with and without ATP: Bound Waters and Natural Ligand as Guides for Inhibitor Design. *J. Med. Chem.* **1996**, *39*, 4540–4546.
- (17) Schulze-Gahmen, U.; Brandsen, J.; Jones, H. D.; Morgan, D. O.; Meijer, L.; Vesely, J.; Kim, S.-H. Multiple Modes of Ligand Recognition: Crystal Structures of Cyclin-Dependent Protein Kinase 2 in Complex with ATP and Two Inhibitors, Olomoucine and Isopentenyladenine. *Proteins: Struct. Funct. Genet.* **1995**, *22*, 378–391.
- (18) Lawrie, A. M.; Noble, M. E. M.; Tunnah, P.; Brown, N. R.; Johnson, L. N.; Endicott, J. A. Protein Kinase Inhibition by Staurosporine Revealed in Details of the Molecular Interaction with CDK2. *Nat. Struct. Biol.* **1997**, *4*, 796–800.
- (19) Filgueira de Azevedo, W., Jr.; Mueller-Diekmann, H.-J.; Schulze-Gahmen, U.; Worland, P. J.; Sausville, E.; Kim, S.-H. Structural Basis for Specificity and Potency of a Flavonoid Inhibitor of Human CDK2, a Cell Cycle Kinase. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 2735–2740.
- (20) Gray, N. A.; Wodicka, L.; Thunnissen, A.-M. W. H.; Norman, T. C.; Kwon, S.; Espinoza, F. H.; Morgan, D. O.; Barnes, G.; LeClerc, S.; Meijer, L.; Kim, S.-H.; Lockhart, D. J.; Schultz, P. G. Exploiting Chemical Libraries, Structure, and Genomics in the Search for Kinase Inhibitors. *Science* **1998**, *281*, 533–538.
- (21) Okuda, T.; Price, C. C. Some Analogues of Toxopyrimidine and Methioprim. *J. Org. Chem.* **1958**, *23*, 1738–1741.
- (22) Fry, D. W.; Kraker, A. J.; Connors, R. C.; Elliott, W. L.; Nelson, J. M.; Showalter, H. D. H.; Leopold, W. R. Strategies for the Discovery of Novel Tyrosine Kinase Inhibitors with Anticancer Activity. *Anticancer Drug Des.* **1994**, *9*, 331–351.

JM000271K