Expedited Articles

Structure−Activity Relationships for a Novel Series of Pyrido[2,3-*d*]pyrimidine Tyrosine Kinase Inhibitors

James M. Hamby,*,† Cleo J. C. Connolly,† Mel C. Schroeder,† R. Thomas Winters,† H. D. Hollis Showalter,† Robert L. Panek,‡ Terry C. Major,‡ Bronislawa Olsewski,‡ Michael J. Ryan,‡ Tawny Dahring,‡ Gina H. Lu,‡ Joan Keiser,‡ Aneesa Amar,§ Cindy Shen,§ Alan J. Kraker,‡ Veronika Slintak,§ James M. Nelson,§ David W. Fry,§ Laura Bradford,|| Hussein Hallak,|| and Annette M. Doherty†

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 for inhibitors of the fibroblast growth factor (FGFr) and platelet-derived growth factor (PDGFr) receptor tyrosine kinases led to the development of a novel series of ATP competitive pyrido[2,3-*d*]pyrimidine tyrosine kinase inhibitors. The initial lead, 1-[2-amino-6-(2,6-dichlorophenyl)pyrido[2,3-*d*]pyrimidin-7-yl]-3-*tert*-butylurea (**4b**, PD-089828), was found to be a broadly active tyrosine kinase inhibitor. Compound **4b** inhibited the PDGFr, FGFr, EGFr, and c-src tyrosine kinases with IC_{50} values of 1.11, 0.13, 0.45, and 0.22 μM, respectively. Subsequent SAR studies led to the synthesis of new analogs with improved potency, solubility, and bioavailability relative to the initial lead. For example, the introduction of a [4-(diethylamino)butyl]amino side chain into the 2-position of **4b** afforded compound **6c** with enhanced potency and bioavailability. Compound **6c** inhibited PDGF-stimulated vascular smooth muscle cell proliferation with an IC_{50} of 0.3 μM. Furthermore, replacement of the 6-(2,6-dichlorophenyl) moiety of **4b** with a 6-(3′,5′-dimethoxyphenyl) functionality produced a highly selective FGFr tyrosine kinase inhibitor **4e**. Compound **4e** inhibited the FGFr tyrosine kinase with an IC_{50} of 0.060 μM, whereas IC_{50}s for the inhibiton of the PDGFr, FGFr, EGFr, c-src, and InsR tyrosine kinases for this compound (**4e**) were all greater than 50 μM.

Polypeptide growth factors such as the platelet-derived growth factor (PDGF),[1] fibroblast growth factor (FGF),[2] and epidermal growth factor (EGF),[3] play a critical role in the regulation of normal cellular growth and differentiation. However, strong evidence exists implicating the overexpression of these growth factors or their cognate receptors with the progression of proliferative disorders such as cancer,[3a,4] atherosclerosis,[5] transplant rejection,[6] and restenosis.[7] Consequently, the interruption of growth factor mediated signal transduction presents a potential opportunity for controlling pathological cellular growth. In particular, the inhibition of protein tyrosine kinases (PTKs) have attracted a lot of attention over the past few years as a strategy for impeding cellular proliferation.[8] Growth factor receptors themselves have tyrosine kinase activity associated with their cytoplasmic domains and are referred to as receptor tyrosine kinases (RTK). In addition to RTKs, cytoplasmic nonreceptor tyrosine kinases such as c-src[9] are also integral components of growth factor signaling pathways. For example, c-src has been reputed to participate in PDGFr,[10] FGFr,[11] and EGFr[12] mediated signal transduction pathways, and like the aforementioned RTKs, the abnormal regulation of c-src has also been associated with neoplastic growth.[13,14]

Protein tyrosine kinases constitute a large family of proteins with highly conserved topology for the ATP binding site.[15] ATP competitive inhibitors represent one of the largest mechanistic categories of PTK inhibitors reported in the literature.[16] However, the design of selective ATP competitive inhibitors specific for targeted PTKs still remains a difficult challenge. In theory, selective TKIs should be less likely to affect normal cells, producing fewer unwanted side effects. On the other hand, broadly acting nonselective inhibitors may be required to overcome redundancies in growth signaling pathways in order to arrest aggressively proliferating cells. The picture is further complicated by the fact that over 200 protein kinases are known and many more likely remain to be discovered, making it impossible to evaluate inhibitors against a complete panel of enzymes. Thus, given the complex nature of signal transduction, i.e., redundancies and cross talk between signal transduction pathways, selectivity may be better assessed at the cellular level. There is clearly a need for tyrosine kinase inhibitors possessing varying specificities for PTKs as tools to aid in the understanding of growth signaling in cells. Given the potential usefulness of TKIs as agents for treating proliferative diseases and/or as tools for understanding growth factor signal transduction mechanisms, we embarked on a project to develop inhibitors of growth factor receptor tyrosine kinases. Herein, we report the synthesis and biological

* To whom correspondence should be addressed: phone, 313-996-7736; fax, 313-996-3107; e-mail, HambyJ@aa.wl.com.
† Departments of Cardiovascular, Cancer and Peptide Chemistry.
‡ Department of Vascular and Cardiac Diseases.
§ Department of Cancer Research.
|| Department of Pharmacokinetics and Drug Metabolism.
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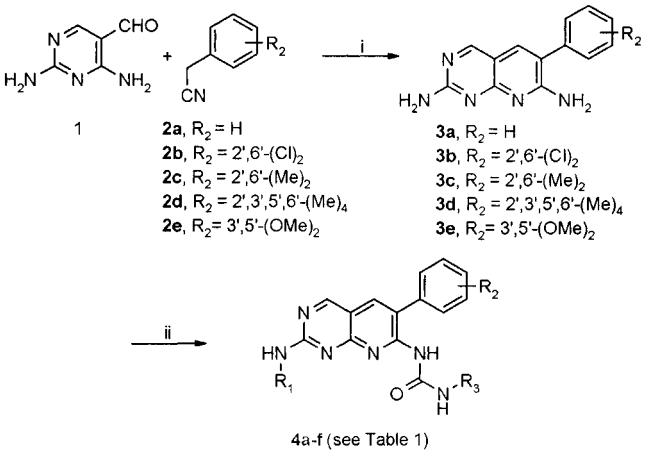
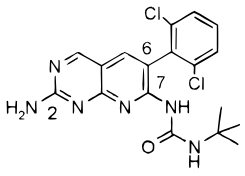
Scheme 1[a]



[a] (i) $EtOCH_2CH_2O^-Na^+$, reflux; (ii) (1) NaH, DMF, (2) $R_3N{=}C{=}O$, room temperature (For R_1 = H).

activity of a novel series of potent ATP competitive pyrido[2,3-*d*]pyrimidine tyrosine kinase inhibitors.

Screening of our compound library for small molecule inhibitors of the PDGFr-β[17] and FGFr-1[17] tyrosine kinases uncovered a novel pyrido[2,3-*d*]pyrimidine lead, PD-089828 (**4b**), with IC_{50} values for the inhibition of the PDGFr and FGFr tyrosine kinases of 1.11 and 0.13 μM, respectively. Upon further evaluation, **4b** was also found to inhibit the EGFr[18] and c-src[19] tyrosine kinases with IC_{50} values of 0.45 and 0.22 μM, respectively. The insulin receptor tyrosine kinase (InsR)[20] was not effected by **4b** at concentrations up to 50 μM. Moreover, enzyme kinetic studies found **4b** to be an ATP competitive inhibitor of the PDGFr, EGFr, and FGFr tyrosine kinases.[17] Interestingly, **4b** exhibited a noncompetitive kinetic profile with respect to the c-src tyrosine kinase and ATP.[17] Thus, our initial screening lead **4b** was, for the most part, an ATP competitive inhibitor with broad tyrosine kinase inhibitory activity relative to the panel of five tyrosine kinases profiled in this study.

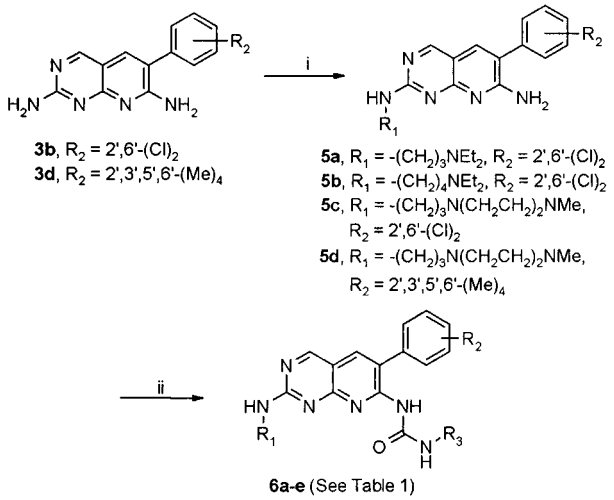


4b (PD-089828)

Compound **4b** was extremely insoluble in aqueous medium (<1 μg/mL in pH 7.4 buffer) and did not form soluble addition salts with strong acids. For these reasons, **4b** was unsuitable for iv administration in our animal models of proliferative diseases. Furthermore, *in vivo* studies found **4b** to be poorly available after oral or ip administration in rats.[21] Therefore, subsequent SAR studies based on **4b** focused not only on potency and selectivity but also on improving the bioavailability of this novel lead.

Scheme 1 shows the general synthetic route used to prepare **4b** and related analogs **4a,c**−**f**. As previously described,[22] the condensation of aldehyde **1** with an arylacetonitrile (**2a**−**e**) under basic conditions affords the corresponding 2,7-diamino-6-arylpyrido[2,3-*d*]pyrimidine intermediate (**3a**−**e**). Treatment of **3a**−**e** with

Scheme 2[a]



[a] (i) R_1NH_2, H_2NSO_3H, 140−180 °C; (ii) (1) NaH, DMF, (2) $R_3N{=}C{=}O$, room temperature.

sodium hydride in DMF followed by the addition of the designated isocyanate to the reaction mixture afforded the ureas **4a**−**f**. Under these conditions, acylation occurred predominately at the 7-amino position. However, in some cases a small amount of the bis-acylated product (usually <5%), where both the 2- and 7-amino groups were acylated, was also isolated as a byproduct from the reaction.[23]

Scheme 2 shows the synthetic route used to prepare analogs **6a**−**e**, which possess an aminoalkyl substituent tethered to the 2-amino group of the pyrido[2,3-*d*]-pyrimidine ring. The 2-amino moiety of the diamine intermediates **3b** and **3d** was directly displaced at high temperatures (140−180 °C) using the appropriate reacting amine as solvent and 2 equiv of sulfamic acid to afford compounds **5a**−**d**. Yields for this reaction were generally in the range 50−80%. Weak nucleophilic amines such as aniline did not react under these conditions. Elaboration of **5a**−**d** to the targeted analogs **6a**−**e** was accomplished as described above in Scheme 1 using NaH in DMF followed by the addition of the appropriate isocyanate.

Three regions of the parent molecule were targeted for initial SAR studies. Modifications made to the 2-, 6-, and 7-positions of the initial lead compound **4b** were explored. Accordingly, the effects of phenyl substitution on tyrosine kinase inhibition were investigated. It was found that disubstitution at the ortho positions of the phenyl ring by small groups such as 2′,6′-dichloro (**4b**) and 2′,6′-dimethyl (**4c**) resulted in a general increase in TKI activity relative to the unsubstituted compound **4a**. Compounds **4b** and **4c** were approximately 10-fold more potent at inhibiting the FGFr and EGFr tyrosine kinases compared to **4a**. PDGFr TKI activity was similar for **4a** and **4b**, while **4c** was approximately 10-fold more potent then **4a**. c-src activity was most sensitive to the phenyl 2′,6′-substitution pattern of **4b** and **4c**, producing at least a 100-fold increase in activity relative to the unsubstituted compound **4a** (Table 1). The insulin receptor tyrosine kinase (insR) was not inhibited by any of the compounds reported in this study. Larger groups in the ortho positions such as ethyl or methoxy resulted in decreased TKI activity across the panel of kinases tested (data not shown). Ortho substitution restricts the phenyl group to an

Table 1

compd no.	R_1	R_2	R_3	IC_{50} (μM) PDGFr TK	FGFr TK	EGFr TK	C-src TK	InsRT K	PDGF stim auto phos
4a	H	H	*t*-Bu	4.67	3.71	5.53	>50	>50	
4b	H	2′,6′-$(Cl)_2$	*t*-Bu	1.11	0.13	0.45	0.22	>50	0.63
4c	H	2′,6′-$(Me)_2$	*t*-Bu	0.34	0.4	0.61	0.11	>50	
4d	H	2′,3′,5′,6′-$(Me)_4$	*t*-Bu	>50	0.78	6.68	>50	>50	>50
4e	H	3′,5′-$(OMe)_2$	*t*-Bu	>50	0.060	>50	>50	>50	>50
4f	H	2′,6′-$(Cl)_2$	Et	1.3	0.13	1.36	0.077	>50	3.73
6a	$(CH_2)_3NEt_2$	2′,6′-$(Cl)_2$	*t*-Bu	0.66	0.082	6.20	0.073	>50	1.8
6b	$(CH_2)_3N(CH_2CH_2)_2NMe$	2′,6′-$(Cl)_2$	*t*-Bu	0.47	0.051	0.15	0.031	>50	0.27
6c	$(CH_2)_4NEt_2$	2′,6′-$(Cl)_2$	*t*-Bu	0.31	0.048	0.24	0.044	>50	0.45
6d	$(CH_2)_4NEt_2$	2′,6′-$(Cl)_2$	Et	0.19	0.033	1.26	0.023	>50	0.25
6e	$(CH_2)_3N(CH_2CH_2)_2NMe$	2′,3′,5′,6′-$(Me)_4$	*t*-Bu	>50	0.14	7.0	2.2	>50	4.30

[a] IC_{50} values for kinase inhibition and PDGF-stimulated autophosphorylation in RAVSMCs are means of at least two separate experiments with typical variation less than 30% between values.

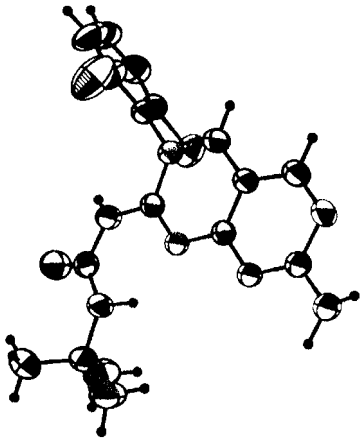


Figure 1. ORTEP diagram of **4b** shows an orthogonal relationship between the 2,6-dichlorophenyl moiety and the pyrido[2,3-*d*]pyrimidine ring.

orthogonal conformation with respect to the pyrido[2,3-*d*]pyrimidine ring. This relationship has been verified in the x-ray crystal structure of **4b** (Figure 1). Interestingly, the 2′,3′,5′,6′-tetramethyl- (**4d**) and 3′,5′-dimethoxy- (**4e**) substituted phenyl compounds showed good FGF selectivity relative to the other tyrosine kinases in Table 1. Overall, optimization of phenyl substituents in the 6-position led to only slight increases in TKI potency beyond that of the initial lead **4b**. However, phenyl substitution did have a significant impact on TKI selectivity. Compounds **4d** and **4e** with substitutions in the 3′- and 5′- positions afforded highly selective FGFr TKIs.

To improve the poor aqueous solubility of **4b**, several sites on the molecule were targeted for attaching aminoalkyl side chains. Unexpectedly, the 3-(diethylamino)propyl side chain of compound **6a** was found to afford enhanced TKI activity for the PDGFr, FGFr, and c-src tyrosine kinases as well as improved aqueous solubility relative to the lead compound **4b**. Further exploration of alkylamino side chains in this position culminated in the finding that 3-(4-methylpiperazinyl)-propyl and 4-(diethylamino)butyl alkylamino side chains provided some of the best enhancements in TKI activities (compounds **6b**−**e**). Bioavailability was also enhanced by this structural modification. Compound **6b** had a $t_{1/2}$ in rat plasma of 2.69 h after intravenous administration.[21] Interestingly, the FGFr tyrosine kinase selectivity seen with **4d** was similarly observed with **6e**, albeit with a slight increase in FGFr and c-src TKI potency. The FGFr TK selectivity imparted by the 2′,3′,5′,6′-tetramethylphenyl group of **4d** was not greatly altered by the addition of a 2-alkylamino side chain in **6e**, indicating the two modifications are compatible. Thus, the incorporation of an 2-alkylamino side chain generally resulted in enhanced TKI potency, aqueous solubility, and bioavailability relative to the parent compound **4b**.

SAR work focusing on understanding the contribution of the urea functionality of **4b** revealed the need for a mono N′-substituted alkylurea group in the 7-position of the pyrido[2,3-*d*]pyrimidine nucleus for good tyrosine kinase inhibitory activity. A variety of alkyl- or aryl ureas[22c] were well tolerated in this position. For example, only slight to moderate differences in activity were noted for the *tert*-butylurea **4b** vs the ethylurea **4f** for the PDGFr, FGFr, EGFr, and c-src tyrosine kinases. However, larger groups such as adamantylurea (data not shown) resulted in an overall decrease in activity. The urea functionality had little effect on the tyrosine kinase selectivity profile in this series, but was necessary for good TKI potency.

Table 1 shows IC_{50} values for PDGF-mediated receptor autophosphorylation for compounds **4b,d**−**f** and **6a**−**e** in rat aortic vascular smooth muscle cells (RAVSMCs). Stimulation of the PDGF receptor with ligand (PDGF) envokes phosphorylation of the intracellular cytoplasmic domain of the receptor. Drug inhibition is determined by lysing the cells and quantifying the level of the 190Kd tyrosine phosphorylated receptor protein after western blotting with an anti-phosphotyrosine antibody.[17]

The FGF selective inhibitors **4d** and **4e** did not inhibit PDGF-stimulated cellular autophosphorylation. This is not surprising, since these agents are selective for the FGF receptor tyrosine kinase. Interestingly, the FGF selective compound **6e** which possesses a 3-(4-methylpiperazinyl)propyl side chain, inhibited PDGF stimulated autophosphorylation with an IC_{50} of 4.3 μM. It is not clear by what mechanism this inhibition occurs. Although, **6e** lacks PDGFr TKI activity, it does inhibit to some extent the c-src (IC_{50} = 2.2 μM) and EGFr (IC_{50} = 7.0 μM) tyrosine kinases. Possible cross talk between signaling pathways of the c-src, EGFr, or other unknown tyrosine kinases with the PDGF receptor may be responsible for the effects seen with **6e**. All of the compounds (**4b,f** and **6a**−**d**) with PDGF receptor TKI activity inhibited PDGF-stimulated autophosphorylation, which corresponded well to their PDGFr tyrosine kinase inhibition. Compounds **6b**, **6c**, and **6d** which

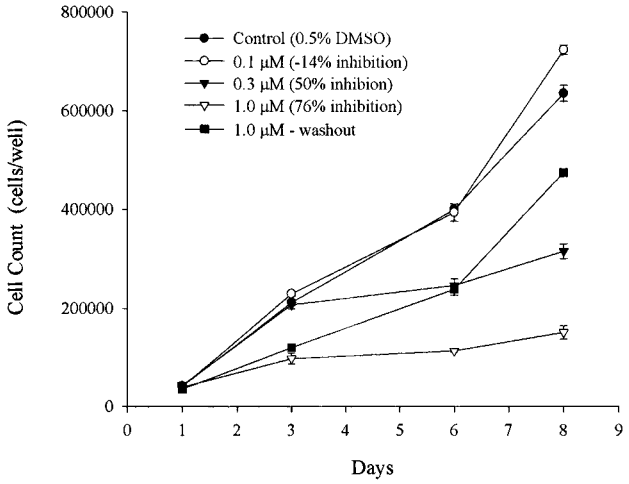


Figure 2. Growth delay assay showing inhibition of RAVSMC (p20) proliferation by compound **6c** dosed daily at three concentrations over 8 days. Values are mean ± SE of a single experiment performed in triplicate. The IC_{50} for growth inhibition at day 8 for **6c** was 0.3 μM.

possess either a 3-(4-methylpiperazinyl)propyl or 4-(diethylamino)butyl alkylamino side chain were the most potent inhibitors of PDGF-stimulated autophosphorylation in this series.

The aberrant proliferation of vascular smooth muscles cells (VSMCs) has been reputed to play a critical role in atherosclerosis and restenosis.[24] Accordingly, **6c** was evaluated in an 8 day RAVSMC growth delay assay to assess the ability of this compound to inhibit VSMC proliferation in cell culture (Figure 2).[25] Growth-arrested VSMCs were treated with either vehicle (control) or a concentration of **6c** (0.1, 0.3, or 1 μM) and stimulated to grow with serum. Drug was washed out and replaced with fresh drug daily. Cell number was monitored by counting the actual number of cells on days 1, 3, 6, and 8. The experimental results are shown in Figure 2. Compound **6c** inhibited VSMC proliferation in a dose dependent fashion with an IC_{50} of 0.3 μM at day 8. As an index of cytotoxicity (see 1μM washout in Figure 2), the treatment of RAVSMCs with **6c** (1 μM concentration) was discontinued after day 3. Normal cellular growth resumed, paralleling that of the control, indicating the compound was not cytotoxic. Thus, **6c** is a potent inhibitor of vascular smooth muscle cell proliferation *in vitro*.

In summary, we have disclosed a novel series of ATP competitive pyrido[2,3-*d*]pyrimidine tyrosine kinase inhibitors. Structure−activity relationship studies were developed from an initial lead PD-089828 (**4b**) which led to improvements in potency, solubility, and bioavailability over that of the parent compound. Structural modifications also led to the design of FGF selective compounds despite the fact that the ATP binding site is highly conserved in this large family of enzymes. Finally, a selected compound PD-161570 (**6c**) inhibited *in vitro* vascular smooth muscle cell proliferation, an important component of cardiovascular disease.

Experimental Section

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 pressure 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 Darmstadt). Preparative radial chromatography was carried out using a Harrison Research (Palo Alto, CA) chromatotron. Radial chromatography plates (4000 μm) were purchased from Analtech (Newark, DE). Medium-pressure liquid chromatography was carried out using silica gel 60 (230−400 mesh, E. Merck Darmstadt) on a ISCO Foxy 200 apparatus. Combustion analyses (CHN) were performed by the Parke-Davis Pharmaceutical Research Analytical Department or Robertson Microlit (Madison, NJ). Fractional moles of water or organic solvents were frequently retained in analytical samples after drying *in vacuo* (0.2 mmHg). The presence of solvent in analytical samples was confirmed by ^{1}H NMR when possible and purity analyzed by HPLC. High-pressure liquid chromatography (HPLC) was performed on a Waters HPLC system from Millipore Corp. equipped with a Model 600E system controller, a Model 600 solvent delivery system, a Model 490 variable-wavelength detector operating at 214 and 280 nm, and a Waters 717 autosampler. Reversed-phase HPLC was performed using a C_{18} Vydac analytical column (218TP54) (0.46 × 25.0 cm, 5 μm particle size) eluting with a linear gradient of 90:10 to 24:76 (0.1% aqueous TFA:0.1% TFA in AcCN) over 22 min at 1.5 mL/min (λ = 214 and 280 nm). Compounds with reported retention times (t_R) are greater than 98% pure unless otherwise indicated. The starting material 2,4-diamino-5-pyrimidinecarboxaldehyde was prepared according to ref 22b.

2,7-Diamino-6-(2,6-dichlorophenyl)pyrido[2,3-*d*]pyrimidine (3b). (Prepared by a modified method of Davoll.[22]) To 2-ethoxyethanol (60 mL) at 0 °C was added 60% sodium hydride (0.24 g, 6 mmol) cautiously in portions. The reaction mixture was allowed to warm to room temperature. To the solution was added **2b** (2.79 g, 15 mmol) followed by **1** (2.07 g, 15 mmol) and the mixture refluxed for 4 h. The reaction mixture was poured into water, and after being allowed to stand at room temperature for 1 h the insoluble product was filtered, washed with ether, and dried under high vacuum at 75 °C for 12 h to afford 3.20 g (70% yield) of **3b**. The product was used in subsequent steps without further purification. An analytical sample was obtained by recrystallizing from CH_3CO_2H/MeOH and drying at 130 °C under high vacuum (0.05 mmHg) for 1.5 h to give an off-white solid: mp 336−338 °C; ^{1}H NMR (DMSO-d_6) δ 8.64 (s, 1H, 4-H), 7.61−7.58 (m, 3H, 5-H and 3′,5′-H), 7.48 (d, 2H, 3′, 5′-ArH), 7.49−7.45 (m, 1H, 4′-ArH), 7.46 (app t, 1H, 4′-H), 6.74 (s, 2H, 2-NH_2), 6.60 (br s, 2H, 7-NH_2); MS (CI) *m*/*z* 306 (M^+). Anal. ($C_{13}H_9N_5Cl_2$) C, H, N.

2,7-Diamino-6-(2,6-dimethylphenyl)pyrido[2,3-*d*]pyrimidine (3c). Starting from **1** (1.5 g, 10.9 mmol) and **2c** (1.66 g, 11.4 mmol), **3c** was prepared as described above for **3b**. The product was purified by recrystallization from 20% aqueous EtOH to afford 0.91 g (32% yield) of **3c** as an off-white solid: mp >272 °C dec; ^{1}H NMR (DMSO-d_6) δ 8.61 (s, 1H, 4-H), 7.47 (s, 1H, 5-H), 7.24−7.15 (m, 3H, 3′, 4′, 5′-H), 6.64 (s, 2H, 2-NH_2), 2.02 (s, 6H, CH_3); MS (CI) *m*/*z* 266 (MH^+). Anal. ($C_{15}H_{15}N_5Cl_2 \cdot 0.7H_2O$) C, H, N.

2,7-Diamino-6-(2,3,5,6-tetramethylphenyl)pyrido[2,3-*d*]pyrimidine (3d). Starting from 60% NaH (0.18g, 4.41 mmol), **1** (1.52 g, 11.0 mmol), and **2d** (2.0 g, 11.5 mmol), **3d** was reacted as described above for **3b** to afford 2.69 g (83% yield) of the title compound. The crude reaction product after washing with ether (1.52 g,) was used in subsequent reactions without further purification: ^{1}H NMR (DMSO-d_6) δ 8.75 (s, 1H), 8.60 (s, 1H), 7.39 (s, 1H), 7.26 (br s, 2H, 2-NH_2), 7.03 (s, 1H), 6.63 (br s, 2H, 7-NH_2), 2.22 (s, 6H, 2′,6′-H), 1.86 (s, 6H, 3′,5′-H); HPLC t_R = 14.35 min (94% pure); MS (CI) *m*/*z* 294 (MH^+).

2,7-Diamino-6-(3,5-dimethoxyphenyl)pyrido[2,3-*d*]pyrimidine (3e). Starting from 60% NaH (0.086 g, 2.14 mmol), **1** (0.74 g, 5.35 mmol), and **2e** (1.0 g, 5.59 mmol), **3e** was prepared as described above for **3b.** An off-white product was obtained after washing with ether (1.52 g, 97% yield) and was

used in subsequent reactions without further purification: ^{1}H NMR (DMSO-d_6) δ 8.64 (s, 1H, 4-H), 7.66 (s, 1H, 5-H), 6.66 (br s, 2H, 2-NH_2), 6.66−6.58 (br s, 2H, 7-NH_2), 6.58 (s, 2H, 2′,6′-H), 6.58 (m, 1H, 4′-H), 3.34 (s, 6H, OCH_3); HPLC t_R = 11.10 min, MS (CI) m/z 298 (MH^+). Anal. ($C_{15}H_{15}N_5O_2\cdot 0.16Et_2O$) C, H, N.

1-(2-Amino-6-phenylpyrido[2,3-*d*]pyrimidin-7-yl)-3-*tert*-butylurea (4a). The starting material for this reaction, **3a**, was prepared according to the method of Davoll.[22] To a stirred slurry of **3a** (0.25 g, 1.04 mmol) in DMF (5 mL) at room temperature was added 60% NaH (0.048 g, 1.2 mmol) in portions. After 1 h of stirring, *tert*-butyl isocyanate (0.11 g, 1.12 mmol) was added and the reaction mixture stirred at ambient temperature for 18 h. The reaction mixture was filtered, and the insoluble salts were washed with DMF. The filtrate was evaporated under high vacuum and the residue diluted with water. The insoluble product was collected by filtration, washed with water and then ether, and dried in air on the filter. Purification by medium-pressure liquid chromatography over silica gel, eluting with a solvent gradient of EtOAc:$CHCl_3$ (1:1) to EtOAc (100%) afforded 0.010 g (30% yield) of **4a** as a pale yellow solid: mp >250 °C dec; ^{1}H NMR (DMSO-d_6) δ 10.06 (s, 1H, NHCON*H*-*t*-Bu), 8.94 (s, 1H, 4-H), 7.99 (s, 1H, 5-H), 7.58−7.49 (m, 5H, Ph-*H*'s), 7.20 (br s, 2H, NH_2), 7.01 (br s, 1H, N*H*CONH-*t*-Bu), 1.4 (s, 9H, *t*-Bu H's); HPLC t_R = 13.87 min; MS (CI) m/z 337 (MH^+). Anal. ($C_{18}H_{20}N_6O\cdot 0.5CHCl_3\cdot 1.0EtOAc\cdot 2.25H_2O$) C, H, N.

1-[2-Amino-6-(2,6-dichlorophenyl)pyrido[2,3-*d*]pyrimidin-7-yl]-3-*tert*-butylurea (4b) and Diacylated Byproduct. To a slurry of **3b** (3.0 g, 9.8 mmol) from above in 45 mL of DMF was added 50% NaH (0.48 g, 10.0 mmol) in portions. The mixture was stirred for 1 h, *tert*-butyl isocyanate (1.0 g, 10.09 mmol) added, and the reaction mixture stirred at ambient temperature for 16 h. The reaction mixture was filtered to remove a small amount of insoluble material and the filtrate diluted with 500 mL of water. The insoluble product was collected by filtration, washed with water and then ether, and dried in air on the filter. The product was purified by silica gel chromatography, eluting with a gradient of 0−1% MeOH in $CHCl_3$ to afford, after crystallization from EtOH, 0.7 g (14% yield) of the bis-acylated byproduct 1-*tert*-butyl-3-[7-(3-*tert*-butylureido)-6-(dichlorophenyl)pyrido[2,3-*d*]-pyrimidin-2-yl]urea as a white solid: mp >200 °C dec; ^{1}H NMR (DMSO-d_6) δ 9.89 (s, 1H, N*H*), 9.41(s, 1H, N*H*), 9.20 (s, 1H, 4-H), 8.93 (s, 1H, N*H*), 8.23 (s, 1H, 5-H), 8.19 (s, 1H, N*H*), 7.68−7.65 (m, 2H, H-3′,5′), 7.60−7.52 (m, 1H, H-4′), 1.41(s, 9H, *t*-Bu H's), 1.39 (s, 9H, *t*-Bu H's); MS (APCI) m/z 506 (M^+). Anal. ($C_{23}H_{22}Cl_2N_7O_2\cdot 0.1H_2O$) C, H, N.

Continued elution afforded 1.5 g (38% yield) of the desired product (**4b**) as a white solid after crystallization from EtOH: mp 335 °C; ^{1}H NMR ($CDCl_3$) δ 10.06 (s, 1H, NHCON*H*-*t*-Bu), 8.81 (s, 1H, 4-H), 7.69 (s, 1H, 5-ArH), 7.49−7.47 (app d, 2H, H-3′,5′), 7.39−7.36 (app t, 1H, H-4′), 6.42 (br s, 1H, N*H*CONH-*t*-Bu), 5.56 (br s, 2H, NH_2), 1.5 (s, 9H, *t*-Bu H's); MS (CI) m/z 405 (M^+). Anal. ($C_{18}H_{18}Cl_2N_6O\cdot 0.5H_2O$) C, H, N.

1-[2-Amino-6-(2,6-dimethylphenyl)pyrido[2,3-*d*]pyrimidin-7-yl]-3-*tert*-butylurea (4c). Starting from **3c** (0.5 g, 1.88 mmol), 60% NaH (0.075 g, 1.88 mmol), and *tert*-butyl isocyanate (0.168 g, 1.88 mmol), **4c** was prepared as described above for **4a** with the following exceptions: After removal of the reaction solvent (DMF) under high vacuum, the residue was partitioned between EtOAc and water. The aqueous layer was extracted twice with EtOAc, and the organic layers were combined, dried ($MgSO_4$), and evaporated under reduced pressure. The crude product was purified by radial chromatography, eluting with a solvent gradient of 3−5% MeOH in $CHCl_3$ to afford 0.120 g (25% yield) of **4c** as an off-white solid: mp 203−205 °C; ^{1}H NMR ($CDCl_3$) δ 10.07 (s, 1H, NHCON*H*-*t*-Bu), 8.79 (s, 1H, 4-H), 7.60 (s, 1H, 5-H), 7.29−7.25 (app t, 1H, H-4′), 7.18−7.16 (app d, 2H, H-3′,5′), 6.57 (br s, 1H, N*H*CONH-*t*-Bu), 5.66 (br s, 2H, NH_2), 2.05 (s, 6H, 2′,6′-CH_3), 1.5 (s, 9H, *t*-Bu H's); MS (CI) m/z 365 (MH^+). Anal. ($C_{20}H_{24}N_6O\cdot 0.17H_2O$) C, H, N.

1-[2-Amino-6-(2,3,5,6-tetramethylphenyl)pyrido[2,3-*d*]-pyrimidin-7-yl]-3-*tert*-butylurea (4d). Starting from **3d** (0.3 g, 1.02 mmol), 60% NaH (0.047 g, 1.18 mmol), and *tert*-butyl isocyanate (0.108 g, 1.09 mmol), **4d** was prepared as described above for **4a**. The crude product was purified by medium-pressure liquid chromatography, eluting over silica gel with EtOAc:$CHCl_3$ (1:1) to afford 0.050 g (46% yield) of **4d** as a white solid: mp >300 °C; ^{1}H NMR ($CDCl_3$) δ 10.14 (s, 1H, NHCON*H*-*t*-Bu), 8.77 (s, 1H, 4-H), 7.55 (s, 1H, 5-H), 7.06 (s, 1H, H-4′), 6.57 (br s, 1H, N*H*CONH-*t*-Bu), 5.41 (br s, 2H, NH_2), 2.26 (s, 6H, 2′,6′-CH_3), 2.26 (s, 6H, 3′,5′-CH_3), 1.49 (s, 9H, *t*-Bu H's); HPLC t_R = 17.02 min; MS (CI) m/z 393 (MH^+). Anal. ($C_{22}H_{28}N_6O\cdot 0.41CHCl_3$) C, H, N.

1-[2-Amino-6-(3,5-dimethoxyphenyl)pyrido[2,3-*d*]pyrimidin-7-yl]-3-*tert*-butylurea (4e). Starting from **3e** (0.5 g, 1.68 mmol), 60% NaH (0.078 g, 1.94 mmol), and *tert*-butyl isocyanate (0.178 g, 1.80 mmol), **4e** was prepared as described above for **4a**. The crude product was purified by medium-pressure liquid pressure chromatography over silica gel, eluting with a solvent gradient of 1−2% MeOH in CH_2Cl_2 to afford 0.265 g (40% yield) of **4e** as an off-white solid: mp >250 °C dec; ^{1}H NMR (DMSO-d_6) δ 10.04 (s, 1H, NHCON*H*-*t*-Bu), 8.93 (s, 1H, 4-H), 8.00 (s, 1H, 5-H), 7.19 (s, 2H, H-2′,6′), 7.10 (s, 1H, H-4′), 6.64 (br s, 3H, NHCON*H*-*t*-Bu, NH_2), 3.80 (s, 6H, OCH_3), 1.38 (s, 9H, *t*-Bu H's); MS (CI) m/z 397 (MH^+). Anal. ($C_{20}H_{24}N_6O_3$) C, H, N.

1-[2-Amino-6-(2,6-dichlorophenyl)pyrido[2,3-*d*]pyrimidin-7-yl]-3-ethylurea (4f). Starting from **3b** (2.0 g, 6.5 mmol), 60% NaH (0.261 g, 6.5 mmol), and ethyl isocyanate (0.464 g, 6.5 mmol), **4f** was prepared as described above for **4a**. The crude product was purified by radial chromatography, eluting with a solvent gradient of EtOAc:$CHCl_3$ (70:30) to $CHCl_3$ (100%) to afford 1.8 g (71% yield) of **4f** as a white solid: mp 185−187 °C; ^{1}H NMR (DMSO-d_6) δ 10.12 (t, 1H, J = 5.67, 7.23 Hz, NHCON*H*Et), 8.94 (s, 1H, 4-H), 8.21 (s, 1H, N*H*CONHEt), 7.98 (s, 1H, 5-ArH), 7.64−7.62 (app d, 2H, 3′,5′-ArH), 7.54−7.52 (app t, 1H, 4′-ArH), 7.38 (br s, 2H, NH_2), 3.29 (dq, 2H, J = 5.67, 7.23 Hz, NHCH_2CH$_3$), 1.15 (t, 3H, J = 7.23 Hz, NHCH$_2$CH_3); MS (CI) m/z 377 (M^+). Anal. ($C_{16}H_{14}N_6Cl_2O\cdot 0.15EtOAc$) C, H, N.

6-(2,6-Dichlorophenyl)-N^2-[3-(diethylamino)propyl]pyrido[2,3-*d*]pyrimidine-2,7-diamine (5a). A mixture of **3b** (3.0 g, 9.8 mmol), sulfamic acid (0.66 g, 6.82 mmol), and 3-(diethylamino)propylamine (10 mL) was refluxed for 24 h. The warm reaction mixture was partitioned between water and hexane. The insoluble crude product was filtered, washed with water, and dried in air to afford 2.48 g of the intermediate **5a** as an off-white solid. The crude product was used in the next step without further purification. An analytical sample was obtained by trituating the crude compound in hot diisopropyl ether, filtering, and recrystallizing the insoluble material twice from EtOAc: mp 220−230 °C; ^{1}H NMR (DMSO-d_6) δ 8.62 (br s, 1H, 4-H), 7.61−7.58 (m, 3H, 5-H and 3′,5′-H), 7.47 (app t, 1H, 4′-H), 7.36 (br s, 1H, 2-N*H*), 6.58 (br s, 2H, 7-NH_2), 3.36 (br s, 4H, NHCH_2CH$_2$CH_2NEt$_2$), 2.45 (q, 4H, J = 7.23 Hz, NH(CH_2CH$_3$)$_2$), 1.70−1.66 (m, 2H, NHCH$_2$CH_2CH$_2$NEt$_2$), 0.95 (t, 6H, J = 7.23 Hz, NH(CH$_2$CH_3)$_2$); MS (APCI) m/z 419 (M^+). Anal. ($C_{20}H_{24}N_6Cl_2$) C, H, N.

6-(2,6-Dichlorophenyl)-N^2-[4-(diethylamino)butyl]pyrido[2,3-*d*]pyrimidine-2,7-diamine (5b). A mixture of **3b** (25.4 g, 0.13 mol), sulfamic acid (40 g, 0.26 mol), and 4-(diethylamino)butylamine (205 mL, 1.82 mol) was heated with stirring at 150 °C for 28 h. Excess amine was removed on a rotoevaporator at 95 °C under high vacuum (1.0 mmHg). After being cooled to 25 °C, the residue was suspended in water, and aqueous saturated $NaHCO_3$ solution was added to make the suspension alkaline. The suspension was extracted several times with CH_2Cl_2, and the combined organic layers were washed several times with saturated $NaHCO_3$ solution followed by several washings with a saturated solution of NaCl. The organic layer was dried over $MgSO_4$ and filtered, and the filtrate was evaporated under reduced pressure. The residue was washed several times with Et_2O and then crystallized from EtOAc. The product was further purified by column chromatography, eluting first with EtOAc:MeOH:Et_3N (85:14:1) followed by EtOAc:EtOH:Et_3N (9:2:1) to afford 36.2 g (64% yield) of the intermediate **5b** as a pale yellow solid: mp 228−232 °C; ^{1}H NMR ($CDCl_3$) δ 8.63 (s, 1H, 4-H), 7.53 (s, 1H, 5-H), 7.48 (d, 2H, H-3′, 5′), 7.49−7.45 (m, 1H, H-4′), 5.77 (br s, 1H,

NHCH$_2$), 4.97 (br s, 2H, ArNH$_2$), 3.63−3.58 (m, 2H, NHCH$_2$), 2.53 (q, 4H, J = 7.23 Hz, N(CH$_2$CH$_3$)$_2$), 2.47 (t, 2H, J = 7.35 Hz, CH$_2$CH$_2$NEt$_2$), 1.72−1.66 (m, 2H, NHCH$_2$CH$_2$CH$_2$CH$_2$NEt$_2$), 1.62−1.58 (m, 2H, NHCH$_2$CH$_2$CH$_2$CH$_2$NEt$_2$), 1.03 (t, 6H, J = 7.23 Hz, N(CH$_2$CH$_3$)$_2$); MS (CI) m/z 433 (M$^+$). Anal. (C$_{21}$H$_{26}$N$_6$Cl$_2$) C, H, N.

6-(2,6-Dichlorophenyl)-*N*^{2}-[3-(4-methylpiperazin-1-yl)-propyl]pyrido[2,3-*d*]pyrimidine-2,7-diamine (5c). A mixture of **3b** (50.0 g, 0.16 mol), sulfamic acid (32 g, 0.33 mol), and 3-(4-methylpiperazinyl)propylamine (228 mL) was heated with stirring at 151 °C for 24 h. A saturated aqueous solution of NaHCO$_3$ (600 mL) was added and the mixture extracted three times with CH$_2$Cl$_2$ (600 mL). The combined organic layers were dried over MgSO$_4$, and the filtrate was evaporated under reduced pressure. Excess amine was removed from the residue by heating at 65 °C under high vacuum (1.0 mmHg) for 2 h. The dried residue was partitioned between H$_2$O and EtOAc and heated at 75 °C with stirring to dissolve the gummy residue. Upon cooling a white precipitate formed. The solid was collected by filtration and dried under high vacuum at 65 °C to afford 61 g (84% yield) of the intermediate **5c** as a pale yellow solid: mp 208−211 °C; ^{1}H NMR (DMSO-d_6) δ 8.62 (s, 1H, 4-H), 7.61−7.57 (m, 3H, H-3′, 5′, H-5), 7.49−7.45 (m, 1H, H-4′), 7.34 (br s, 1H, NHCH$_2$), 6.58 (br s, 2H, NH$_2$), 3.34 (m, 2H, NHCH$_2$), 2.36−2.32 (m, 10H, CH$_2$N(CH$_2$CH$_2$)$_2$NCH$_3$), 2.14 (s, 3H, NCH$_3$), 1.72−1.70 (m, 2H, CH$_2$CH$_2$CH$_2$); MS (CI) 446 (M$^+$). Anal. (C$_{21}$H$_{25}$N$_7$Cl$_2$·0.25H$_2$O) C, H, N.

***N*^{2}-[3-(4-Methylpiperazin-1-yl)propyl]-6-(2,3,5,6-tetramethylphenyl)pyrido[2,3-d]pyrimidine-2,7-diamine (5d).** A mixture of **3d** (1.0 g, 3.40 mmol), sulfamic acid (0.66 g, 6.82 mmol), and (4-methyl-1-piperazinyl)propylamine (10 mL) was refluxed for 24 h. Excess amine was removed by distillation under atmospheric pressure at an oil bath temperature of 115−125 °C. The reaction mixture was allowed to cool to room temperature and then diluted with CH$_2$Cl$_2$ (40 mL) followed by the addition of 25 mL of a half-saturated aqueous solution of NaHCO$_3$. The layers were separated, and the aqueous layer was washed three times with CH$_2$Cl$_2$ (25 mL). The combined organic layers were back-washed with brine, dried (MgSO$_4$), and evaporated. The residue was purified by medium-pressure chromatography, eluting with a solvent mixture of EtOAc/MeOH/NEt$_3$ (90:10:1) to give 0.5 g (34% yield) of the intermediate **5d** as a pale yellow solid: mp 218−223 °C; ^{1}H NMR (CDCl$_3$) δ 8.59 (s, 1H, 4-H), 7.37 (s, 1H, H-5), 7.04 (s, 1H, H-4′), 5.98 (br s, 1H, NHCH$_2$), 4.95 (br s, 1H, NH$_2$), 2.65−2.45 (m, 8H, piperazine CH$_2$), 2.31 (s, 3H, NCH$_3$), 2.27 (s, 6H, 2′,6′-CH$_3$), 1.95 (s, 6H, 3′,5′-CH$_3$), 1.86 (t, 2H, J = 6.75 Hz, CH$_2$N), 1.72−1.81 (m, 2H, NHCH$_2$CH$_2$CH$_2$); MS (APCI) m/z 434.6 (MH$^+$); HPLC t_R = 11.88 min. Anal. (C$_{25}$H$_{35}$N$_7$·0.3EtOAc) C, H, N.

1-*tert*-Butyl-3-[6-(2,6-dichlorophenyl)-2-[[(3-(diethylamino)propyl]amino]pyrido[2,3-*d*]pyrimidin-7-yl]urea (6a). To suspension of the crude product from above **5a** (2.48 g, 5.91 mmol) in DMF (25 mL) was added 60% NaH (0.26 g, 6.50 mmol) in portions. After the mixture was stirred at room temperature for 1 h, *tert*-butyl isocyanate was added and the reaction mixture stirred at ambient temperature for 18 h. The insoluble salts were removed by filtration and washed with DMF. The filtrate was evaporated under high vacuum and the residue diluted with water. Upon the mixture being allowed to stand and the sides of the vessel being scratched, a yellow precipitate formed which was collected and dried in air. Purification by medium-pressure liquid chromatography eluting with a solvent mixture of EtOAc/MeOH/NEt$_3$ (90:10:1) afforded 2.00 g (65% yield) of the target compound **6a** as a pale yellow solid: mp 82−90 °C; ^{1}H NMR (CDCl$_3$) δ 10.35 (br s, 1H, NHCONH-*t*-Bu) 8.72 (br s, 1H, 4-H), 7.62 (s, 1H, 5-H), 7.48−7.46 (app d, 2H, H-3′,5′), 7.38−7.36 (app t, 1H, H-4′), 6.98 (br t, 1H, J = 4.94 Hz, NHCH$_3$), 6.36 (s, 1H, NHCONH-*t*-Bu), 3.67−3.62 (m, 2H, NHCH$_2$), 2.62−2.52 (m, 6H, CH$_2$NH-(CH$_2$CH$_3$)$_2$), 1.84−1.79 (m, 2H, CH$_2$CH$_2$CH$_2$), 1.49 (s, 9H, *t*-Bu H's), 1.06 (t, 6H, J = 7.23 Hz, N(CH$_2$CH$_3$)$_2$); MS (CI) m/z 518 (M$^+$). Anal. (C$_{25}$H$_{33}$N$_7$O$_1$Cl$_2$·0.25H$_2$O) C, H, N.

1-*tert*-Butyl-3-[6-(2,6-dichlorophenyl)-2-[[3-(4-methylpiperazin-1-yl)propyl]amino]pyrido[2,3-*d*]pyrimidin-7-yl]-urea (6b). To a solution of the above intermediate **5c** (24g, 54 mmol) in DMF (360 mL) at 5 °C was added 60% NaH (2.15 g, 54 mmol) in portions. The reaction mixture was stirred at room temperature for 1 h, and then *tert*-butyl isocyanate (5.49 g, 54 mmol) was added dropwise. The reaction mixture was stirred at ambient temperature for 24 h and the solvent removed under high vacuum. The residue was partitioned between H$_2$O and EtOAc, the layers were separated, and the aqueous layer was washed with EtOAc. The combined organic layers were washed with brine, dried (MgSO$_4$), and evaporated. The residue was chromatographed over silica gel, eluting with a solvent mixture of EtOAc/MeOH/NEt$_3$ (9/2/1). The chromatographed material was further purified by dissolving in a minimum amount of hot acetonitrile and filtering. Upon the mixture being left to stand at room temperature overnight, crystals formed. The mixture was then stored again overnight at 0 °C to afford 18 g (61% yield) of **6b** as a pale yellow solid: mp >141 °C dec; ^{1}H NMR (CDCl$_3$) δ 10.35 (br s, 1H, NHCONH(CH$_3$)$_3$), 8.72 (s, 1H, 4-H), 7.62 (s, 1H, 5-H), 7.47 (app d, 2H, H-3′, 5′), 7.38−7.34 (m, 1H, H-4′), 6.55 (s, 1H, NHCONH-*t*-Bu), 6.36 (br s, 1H, NHCH$_2$), 3.67−3.63 (m, 2H, NHCH$_2$), 2.63−2.40 (m, 10H, CH$_2$N(CH$_2$CH$_2$)$_2$NCH$_3$), 2.32 (s, 3H, NCH$_3$), 1.92−1.80 (m, 2H, CH$_2$CH$_2$CH$_2$), 1.50 (s, 9H, *t*-Bu H); MS (ES) m/z 545 (M$^+$). Anal. (C$_{26}$H$_{34}$N$_8$Cl$_2$O) C, H, N.

1-*tert*-Butyl-3-[6-(2,6-dichlorophenyl)-2-[[4-(diethylamino)butyl]amino]pyrido[2,3-*d*]pyrimidin-7-yl]urea (6c). To a solution of the above intermediate **5b** (25 g, 57.68 mmol) in DMF (300 mL) at 5 °C was added 60% NaH (2.31 g, 57.68 mmol) in portions. The reaction mixture was stirred at room temperature for 1 h, and then *tert*-butyl isocyanate (5.72 g, 57.68 mmol) was added dropwise to the reaction mixture. After 24 h of stirring at room temperature, the solvent is removed under high vacuum and the residue partitioned between H$_2$O and CH$_2$Cl$_2$. The layers were separated, and the aqueous layer was washed with CH$_2$Cl$_2$. The combined organic layers were dried (MgSO$_4$), filtered, and evaporated. The residue was chromatographed over silica gel, eluting with a solvent gradient of EtOAc/EtOH/NEt$_3$ (90/10/1 to 90/20/1). The chromatographed material was dissolved in hot methyl *tert*-butyl ether and filtered. The filtrate was concentrated to a volume of ∼75 mL under reduced pressure. The crystallized product was collected by filtration and dried *in vacuo* at 55 °C for 24 h to afford 21.6 g (70% yield) of **6c** as a pale yellow solid: mp 157 °C; ^{1}H NMR (CDCl$_3$) δ 10.37 (br s, 1H, NHCONH-*t*-Bu), 8.71 (s, 1H, 4-H), 7.62 (s, 1H, 5-H), 7.48 (app d, 2H, H-3′,5′), 7.46−7.35 (m, 1H, H-4′), 6.35 (s, 1H, NHCONH-*t*-Bu), 6.18 (br s, 1H, NHCH$_2$), 3.60−3.55 (m, 2H, NHCH$_2$), 2.55 (q, 4H, J = 7.23 Hz, N(CH$_2$CH$_3$)$_2$), 2.48 (t, 2H, J = 7.35 Hz, CH$_2$CH$_2$NEt$_2$), 1.76−1.64 (m, 2H, NHCH$_2$CH$_2$CH$_2$CH$_2$NEt$_2$), 1.63−1.59 (m, 2H, NHCH$_2$CH$_2$CH$_2$CH$_2$NEt$_2$), 1.50 (s, 9H, *t*-Bu H), 1.05 (t, 6H, J = 7.23 Hz, N(CH$_2$CH$_3$)$_2$); MS (ES) m/z 532 (M$^+$). Anal. (C$_{26}$H$_{35}$N$_7$Cl$_2$O) C, H, N.

1-[6-(2,6-Dichlorophenyl)-2-[[4-(diethylamino)butyl]-amino]pyrido[2,3-*d*]pyrimidin-7-yl]-3-ethylurea (6d). To a solution of the intermediate **5b** (0.61g, 1.41 mmol) from above in THF (6 mL) was added KHMDS (0.31 g, 1.55 mmol) in portions. The solution was stirred at room temperature for 30 min, and then ethyl isocyanate (0.11 g, 1.55 mmol) was added dropwise to the reaction mixture. After 18 h of stirring at room temperature, the reaction mixture was poured into 200 mL of 0.25 N HCl. The resulting mixture was filtered and the filtrate made basic with 50% NaOH. The aqueous suspension was extracted twice with EtOAc, and the combined organic layers were dried over MgSO$_4$. The filtrate was evaporated under reduced pressure and the residue purified by radial chromatography, eluting with a solvent mixture of EtOAc/MeOH/NEt$_3$ (90/10/1) to give 178 mg (69% yield) of **6d** as a pale yellow solid: mp >67 °C dec; ^{1}H NMR (CDCl$_3$) δ 10.06 (br s, 1H, NHCONHEt), 8.73 (br s, 1H, 4-H), 7.64 (s, 1H, H-5), 7.49−7.47 (app d, 2H, H-3′,5′), 7.39−7.35 (m, 1H, H-4′), 6.56 (s, 1H, NHCONHEt), 6.32 (s, 1H, NHCH$_2$), 3.63−3.58 (m, 2H, NHCH$_2$CH$_3$), 3.49−3.43 (m, 2H, NHCH$_2$CH$_2$), 2.56 (q, 4H, J = 7.23 Hz, N(CH$_2$CH$_3$)$_2$), 2.49 (t, 2H, J = 7.23 Hz, CH$_2$NEt$_2$), 1.78−1.70 (m, 2H, NHCH$_2$CH$_2$CH$_2$), 1.65−1.60 (m, 2H, NHCH$_2$CH$_2$CH$_2$), 1.31 (t, 3H, J = 7.23 Hz, NHCH$_2$CH$_3$), 1.05 (t, 6H, J = 7.23 Hz, N(CH$_2$CH$_3$)$_2$); MS (CI) m/z 504 (M$^+$). Anal. (C$_{24}$H$_{31}$N$_7$Cl$_2$O·0.87H$_2$O) C, H, N.

1-*tert*-Butyl-3-[2-[[3-(4-methylpiperazin-1-yl)propyl]-amino]-6-(2,3,5,6-tetramethylphenyl)pyrido[2,3-*d*]pyrimidin-7-yl]urea (6e). To a solution of the above intermediate **5d** (0.41 g, 0.95 mmol) in DMF (5 mL) was added 60% NaH (0.046 g, 1.03 mmol) in portions. The reaction mixture was stirred at room temperature for 1 h, and then *tert*-butyl isocyanate (0.104 g, 1.03 mmol) was added and the reaction mixture stirred at room temperature for 18 h. The solvent was removed under high vacuum and the residue diluted with water. The insoluble crude product was filtered and dried in air on the filter. The product was purified by medium-pressure chromatography, eluting with a solvent mixture of EtOAc/MeOH/NEt_3 (90:10:1) to afford 0.175 g (35% yield) of **6e** as a pale yellow solid: mp 185−198 °C; ^{1}H NMR ($CDCl_3$) δ 10.39 (br s, 1H, NHCON*H*-*t*-Bu), 8.70 (br s, 1H, 4-H), 7.49 (s, 1H, H-5), 7.05 (s, 1H, H-4′), 6.52 (s, 1H, N*H*CH_2), 6.35 (br s, 1H, *N*HCONH-*t*-Bu), 3.67−3.62 (m, 2H, NHCH_2), 2.56−2.48 (m, 8H, piperazine CH_2), 2.31 (s, 3H, NCH_3), 2.25 (s, 6H, 2′,6′-CH_3), 1.88 (s, 6H, 3′,5′-CH_3), 1.64 (app s, 4 H, $NHCH_2CH_2CH_2N$), 1.49 (s, 9H, *t*-Bu H); MS (ES) *m*/*z* 533.7 (MH^+); HPLC t_R = 14.21 min. Anal. ($C_{30}H_{44}N_8O$) C, H, N.

Abbreviations

PDGF, platelet-derived growth factor; FGF, fibroblast growth factor; EGF, epidermal growth factor; PDGFr, platelet-derived growth factor receptor; FGFr, fibroblast growth factor receptor; EGFr, epidermal growth factor receptor; InsR, insulin receptor; TK, tyrosine kinase; TKI, tyrosine kinase inhibitor; PTK, protein tyrosine kinase; RTK, receptor tyrosine kinase; VSMCs, vascular smooth muscle cells; RAVSMCs, rat aortic vascular smooth muscle cells.

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Supporting Information Available: Details of X-ray crystallographic study and tables of relevant crystal structure data for compound **4b** (11 pages). Ordering information is given on any current masthead page.

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