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Thienopyrimidine Ureas as Novel and Potent Multitargeted Receptor Tyrosine Kinase Inhibitors

Yujia Dai,*,† Yan Guo,† Robin R. Frey,† Zhiqin Ji,† Michael L. Curtin,† Asma A. Ahmed,‡ Daniel H. Albert,† Lee Arnold,‡ Shannon S. Arries,† Teresa Barlozzari,‡ Joy L. Bauch,† Jennifer J. Bouska,† Peter F. Bousquet,‡ George A. Cunha,‡ Keith B. Glaser,† Jun Guo,† Junling Li,† Patrick A. Marcotte,† Kennan C. Marsh,† Maria D. Moskey,‡ Lori J. Pease,† Kent D. Stewart,† Vincent S. Stoll,† Paul Tapang,† Neil Wishart,‡ Steven K. Davidsen,† and Michael R. Michaelides†

Global Pharmaceutical Research and Development, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, Illinois 60064-6100, and Abbott Bioresearch Center, 100 Research Drive, Worcester, Massachusetts 01605-5314

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A series of novel thienopyrimidine-based receptor tyrosine kinase inhibitors has been discovered. Investigation of structure−activity relationships at the 5- and 6-positions of the thienopyrimidine nucleus led to a series of *N,N′*-diaryl ureas that potently inhibit all of the vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) receptor tyrosine kinases. A kinase insert domain-containing receptor (KDR) homology model suggests that these compounds bind to the “inactive conformation” of the enzyme with the urea portion extending into the back hydrophobic pocket adjacent to the adenosine 5′-triphosphate (ATP) binding site. A number of compounds have been identified as displaying excellent in vivo potency. In particular, compounds **28** and **76** possess favorable pharmacokinetic (PK) profiles and demonstrate potent antitumor efficacy against the HT1080 human fibrosarcoma xenograft tumor growth model (tumor growth inhibition (TGI) = 75% at 25 mg/kg·day, per os (po)).

Introduction

Receptor tyrosine kinases (RTKs) are a family of transmembrane proteins possessing extracellular ligand-binding domains and intracellular kinase domains. Mitogenic signaling, mediated by a variety of RTKs, represents a major type of signal transduction pathway in the communication among eukaryotic cells and plays a central role in the regulation of diverse biological processes.[1,2] Upon binding to their specific extracellular growth factors, receptor tyrosine kinases undergo dimerization and autophosphorylation, initiating a cascade of downstream signaling events. RTK activity in normal cells is tightly regulated. Abnormal activation of RTKs has been linked to the development and progression of a variety of human cancers.

A principal subfamily of RTKs is vascular endothelial growth factor receptor (VEGFR) tyrosine kinases which are specifically expressed in vascular endothelial cells and include FLT1 (Fms-like tyrosine kinase 1; VEGFR1), KDR (kinase insert domain-containing receptor tyrosine kinase; VEGFR2), and FLT4 (VEGFR3). Activation of VEGFR family RTKs and, in particular, of KDR by vascular endothelial growth factors (VEGFs) plays a primary role in tumor angiogenesis.[3,4] It has been shown that angiogenesis is a rate-limiting step in tumor development.[5] Without blood supply, a solid tumor is limited to a maximum size of 1−2 mm.[6,7] VEGF-mediated KDR signaling induces a series of endothelial responses such as proliferation, migration, and survival and ultimately leads to new vessel formation and stabilization. Similar to KDR, Tie2 is also an endothelium-specific receptor tyrosine kinase and promotes tumor angiogenesis through interaction with angiopoietin ligands.[8] Platelet-derived growth factor receptor (PDGFR) tyrosine kinases are another structurally related subfamily of RTKs, consisting of PDGFRβ, cKit, CSF1R (colony-stimulating factor 1 receptor), and FLT3. PDGFR kinases are believed to not only indirectly promote tumor angiogenesis but also contribute directly to tumor growth.[9−13] Due to the vital role of RTK signaling in tumor progression, inhibition of RTK signaling pathways has emerged as one of the most compelling targets for therapeutic intervention in cancer. Enormous effort has been allocated toward this area in the past two decades.[14−18] In particular, development of antiangiogenesis-based agents by the interruption of KDR signaling has been intensively pursued.[19−22] The recent approval by the Federal Drug Administration (FDA) of Avastin, a VEGF antibody for treating colorectal cancer, has promoted even greater interest in this field.

The first generation of therapeutic agents based on RTK signaling interruption primarily targeted selective RTK inhibition. However, the focus has shifted in recent years toward multitargeted inhibitors. While selective RTK inhibitors should be less likely to affect normal cells and, thus, should produce fewer side effects, broad-acting and multitargeted RTK inhibitors may be required to overcome redundancies in signaling pathways and, thus, effectively inhibit tumor growth.[23,24] This is evidenced by the significant preclinical antitumor efficacy of the RTK inhibitor SU11248 (Sutent)[25] and the RAF/RTK inhibitor BAY 43-9006 (Sorafenib).[26] Both

* To whom correspondence should be addressed at R47J/AP10, Cancer Research, 100 Abbott Park Road, Abbott Park, IL 60064. Tel (847) 937-8977; fax (847) 935-5165; e-mail yujia.dai@abbott.com.
† Abbott Laboratories.
‡ Abbott Bioresearch Center.

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Figure 1. Initial KDR screening hit (I) and the design of thienopyrimidine kinase inhibitors (II).

compounds are multitargeted kinase inhibitors currently in phase III clinical trials.

SU11248

BAY 43-9006

Part of our program toward counterscreening of compounds prepared as small molecule lymphoid T-cell protein-tyrosine kinase (LCK) inhibitors led to the pyrazolopyrimidine urea **I**, which was found to moderately inhibit KDR (IC_{50} = 0.26 μM).[27] On the basis of this result, we investigated the replacement of the pyrazolopyrimidine template with alternate five-membered heterocycle-fused pyrimidines. In this paper, we report the synthesis and characterization of a series of potent VEGFR and PDGFR kinase inhibitors based on a thienopyrimidine scaffold (**II**) (Figure 1).[28]

Chemistry

A general synthesis of the C5- and C6-substituted aminothienopyrimidines is exemplified by the synthesis of the 6-methyl-substituted analogues in Scheme 1. Knoevenagal condensation of ketone **2** with malononitrile gave rise to compound **3**, which, through treatment with elemental sulfur in the presence of an organic base such as diethylamine, was converted to thiophene analogue **4** (Gewald reaction). Heating of **4** with formamide at 155 °C generated thienopyrimidine **5**. Aniline **6**, obtained via reduction of **5**, served as a key intermediate for the preparation of various 6-methyl-substituted thienopyrimidine target compounds. For instance, coupling aniline **6** with *m*-toluic acid afforded amide **7**, whereas reaction of **6** with benzenesulfonyl chloride produced sulfonamide **8**. Ureas **9**−**32** were synthesized either by directly reacting **6** with commercially available isocyanates or in a stepwise fashion by first treating **6** with *p*-nitrophenyl chloroformate in the presence of triethylamine and then reacting with the corresponding amines.

By substituting various *p*-nitrophenyl ketones for *p*-nitropropiophenone (**2**) in Scheme 1, the alternatively C6-substituted thienopyrimidine analogues, including ureas **74**, **76**, **79**−**90**, and **93**, were synthesized in a similar fashion. The commercially unavailable *p*-nitrophenyl ketones **34a** and **b**, **36a** and **b**, **39**, and **41a**−**d** were prepared as illustrated in Scheme 2.

Aldol condensation of *p*-nitropropiophenone with 3- and 4-pyridinecarboxaldehyde yielded unsaturated ketones **33a** and **33b**, respectively, which underwent palladium-catalyzed conjugation reduction[29] to give rise to **34a** and **34b**, respectively. Ketone **36a** was synthesized using a palladium-catalyzed and Zn−Cu couple-mediated coupling reaction[30] between 4-nitrobenzoyl chloride and iodide **35a**. The same method was also used to prepare ketone **36b** from 4-nitrobenzoyl chloride and iodide **35b**. Ketone **36a** also served as the intermediate for preparation of ketone **39**, which was synthesized from **36a** via the sequences of the desilylation, mesylation, and reaction with dimethylamine. Ketones **41a**−**d** were conveniently prepared through the reaction of the corresponding acid chlorides **40a**−**d** with dimethyl malonate and subsequent decarboxylation of the intermediate 2-acylmalonates.

To avoid tedious preparation of the required *p*-nitrophenyl ketones in some cases, the synthesis outlined in Scheme 1 could be started with the corresponding phenyl ketones instead, and the required nitro group could be introduced later by nitration. This approach is represented by the synthesis of amide ureas **51**−**53** shown in Scheme 3. The nitro group in **49a**−**c** was incorporated through the nitration of **44** and **48a**−**b** using nitric acid in concentrated sulfuric acid. Interestingly, heating amino nitrile **44** with formamide at 155 °C not only led to the formation of a pyrimidine ring but also converted the carboxylic acid functionality into its amide (**45**).

An alternative method to introduce diversifying substituents to the 6-position of the thienopyrimidines is to functionalize the 6-methyl group in **5**. This is demonstrated by the synthesis of ureas **59**−**61** via bromide **55** shown in Scheme 4. Prior to bromination of the 6-methyl group, the amino group in **5** was protected with a *tert*-BOC group. Without protecting the amino group, direct bromination of **5** with 2,2′-azobisisobutyronitrile (AIBN) and *N*-bromosuccinimide (NBS) in benzene led to a complex mixture. However, bromination of **54** often failed to reach completion, and the separation of **55** from unreacted **54** was difficult. Thus, the bromination mixture was usually treated directly with the corresponding amines, and the unreacted **54** was then removed from **56a**−**c** using flash column chromatography. Removal of the *tert*-BOC group followed by reduction of the nitro group and reaction of the resulting anilines with *m*-tolyl isocyanate provided **59**−**61**.

As described in Scheme 5, amide **64** (cf. **7**) was prepared from bromide **62**, which was synthesized from *p*-bromopropiophenone following a sequence similar to that of the synthesis of **5** shown in Scheme 1. Acid **63** was then smoothly generated by the treatment of **62** with *n*-butyllithium, followed by the reaction with

Scheme 1. Synthesis of 6-Methyl Thienopyrimidine RTK Inhibitors[a]

[a] Conditions: (a) EtMgBr, $ZnCl_2$, $Pd(PPh_3)_4$, THF; (b) $NCCH_2CN$, NH_4OAc, benzene, reflux; (c) S_8, Et_2NH, EtOH; (d) $HCONH_2$, 155 °C; (e) Fe, NH_4Cl, EtOH, THF, H_2O; (f) *m*-toluic acid, HOBt, EDC, NMM, DMF, room temperature (rt); (g) $PhSO_2Cl$, pyridine, CH_2Cl_2, 0 °C → rt; (h) RNCO, CH_2Cl_2, THF, 0 °C → rt or (i) *p*-$O_2NC_6H_4OCOCl$, Et_3N, THF, 0 °C; (ii) RNH_2, rt.

Scheme 2. Syntheses of *p*-Nitrophenyl Ketones[a]

[a] Conditions: (a) NaOH, EtOH, H_2O, rt; (b) Bu_3SnH, $Pd(PPh_3)_4$, THF, rt; (c) Zn−Cu, $Pd(PPh_3)_4$, *p*-$NO_2C_6H_4COCl$, benzene, DMF; (d) TBAF, THF; (e) MsCl, Et_3N, CH_2Cl_2; (f) Me_2NH, DMF; (g) (i) dimethyl malonate, $MgCl_2$, Et_3N, toluene, rt; (ii) DMSO, H_2O, reflux.

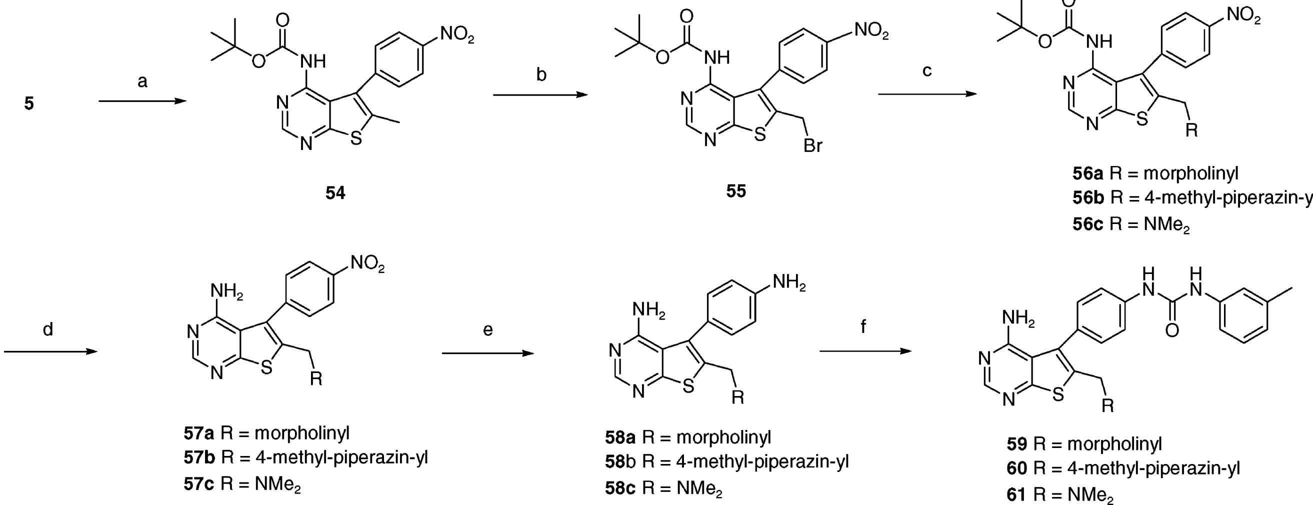
carbon dioxide. Finally, 1-hydroxybenzotriazole-mediated coupling of **63** with *m*-toluidine yielded amide **64**.

Aniline **6** was also used to prepare *N*-cyanoguanidine **67** and aminooxazole **69** as shown in Scheme 6. Heating aniline **6** with diphenyl cyanocarbonimidate in dimethylformamide (DMF) afforded **66**, which was then converted to **67** by reaction with *m*-toluidine under microwave conditions. Synthesis of **69** was achieved via the sequential treatment of **6** with 1,1-thiocarbonyldiimidazole, 2-aminophenol, and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC). The synthesis of compound **72**, a urea *N*-methylation derivative of **11**, is also described in Scheme 6. *N*-Methylaniline

Scheme 3. Synthesis of Thienopyrimidine Amide Ureas **51**−**53**[a]

[a] Conditions: (a) $NCCH_2CN$, NH_4OAc, benzene, reflux; (b) S_8, Et_2NH, EtOH, rt; (c) $HCONH_2$, 155 °C; (d) HNR_1R_2, HOBT, EDC, NMM, DMF; (e) concentrated HNO_3, concentrated H_2SO_4, 0 °C → rt; (f) Fe, NH_4Cl, EtOH, THF, H_2O, 80 °C; (g) *m*-tolyl isocyanate, DMF, 0 °C → rt.

Scheme 4. Synthesis of C6-Substituted Thienopyrimidines[a]



[a] Conditions: (a) NaH, DMF, $(BOC)_2O$, 0 °C; (b) AIBN, NBS, benzene, 80 °C; (c) amine, DMF, rt, overnight; (d) TFA, CH_2Cl_2, rt; (e) Fe, NH_4Cl, EtOH, THF, H_2O; (f) *m*-tolyl isocyanate, DMF, rt.

71 was obtained from an N-formylation and reduction sequence.

Results and Discussion

Our initial studies of structure−activity relationships (SARs) were focused on optimizing activity against KDR, as this kinase plays the primary role in tumor angiogenesis. Activity was measured in the presence of a high concentration of adenosine 5′-triphosphate (ATP) (1.0 mM, a physiologically relevant concentration[31]). Gratifyingly, thienopyrimidene-based urea **9** displayed potent inhibition of KDR (IC_{50} = 36 nM, see Table 1), representing a 9-fold boost in activity relative to that of the initial pyrazolopyrimidine hit (**I**). A further 6-fold boost was obtained upon introduction of an *m*-methyl group (**11**, IC_{50} = 6 nM). The urea moiety turned out to be optimal for KDR inhibition; all attempts to replace the urea link in this series were detrimental to the KDR

Scheme 5. Synthesis of Amide **64**[a]

[a] Conditions: (a) *n*-BuLi (2.5 equiv), THF, −78 °C; then CO_2, −78 °C → rt; (b) *m*-toluidine, HOBt, EDC, NMM, DMF, rt.

inhibitory activity. For instance, significant reduction in KDR activity was observed for sulfonamide **8**, thiourea **65**, cyanoguanidine **67**, 2-indole amide **68**, amide **7**, and its close analogue **64**. A less dramatic deterioration of KDR activity was seen with **69**, a cyclized version of urea. The much weaker activity shown by **70** and **73** in comparison to that of **11** shows the importance of the external urea NH toward KDR inhibition in this series.

In contrast to the manipulation of the external urea NH unit (**70** and **73**), introduction of a methyl group on the internal urea nitrogen is fairly well tolerated, as only a slight drop in activity was measured for **72**. The necessity of the terminal aryl group in retaining high potency is evidenced by the fact that substituting an aliphatic residue, including benzyl (**31**), cyclohexanylmethyl (**32**), cyclohexanyl (**75**), *n*-butyl (**77**), and isobutyl (**78**), led to a significant decrease of KDR activity.

To help understand the SARs observed at this point in the project and guide further SAR studies, a homology model of inhibitor **11** bound to the ATP binding site of KDR kinase was created using a recently published crystal structure of homologue Kit kinase (51% identity to KDR kinase within the catalytic domain) in the inactive conformation (Figure 2).[32]

In the modeling process, it was assumed that the amino thienopyrimidine core mimics the adenine of ATP in its interaction with the hinge region of KDR. Hydrogen bonds are formed between (1) the exocyclic amino group of **11** and the backbone carbonyl of Glu 917 and (2) the proximal ring nitrogen and the backbone N−H of Cys 919. Our earlier modeling exercise of an isoindolinone urea series[33] suggested that the urea unit of those KDR inhibitors accesses the back hydrophobic pocket adjacent to the ATP binding site that is unavailable when KDR is in an "active" kinase conformation. This also appears to be true for this series of compounds. The urea unit of **11** is nicely bound to the region with the carbonyl oxygen within H-bonding distance of the backbone N−H of Asp 1046, while both urea N−H groups of **11** project toward the side chain carboxylate of Glu 885, with the external N−H forming a more optimal H-bond interaction. This binding motif has also been reported by others as a general recognition mode for urea moieties.[28b,34] The importance of the interaction between the urea unit and the hydrophobic region is clearly reflected by the dramatic increase of the KDR inhibitory activity of **11** compared to that of **6**. This is also consistent with the deterioration of KDR activity when the urea link was replaced (Table 1). The suggestion that the external N−H of the urea has a better hydrogen bond interaction with the protein (the carboxylate side chain of Glu 885) is supported by the KDR potency of compound **72** versus **73**. While the methylation of the internal urea nitrogen (**72**) resulted in a 9-fold decrease in activity when compared to that of **11**, a 310-fold loss was observed for **73**, in which the terminal urea nitrogen was methylated.

The modeling also suggested that the terminal *m*-tolyl group in **11** projects into a hydrophobic region, which is comprised of the side chains of Ile 892, Ile 888, Leu 889, Val 898, and Leu 1019. This is consistent with the observed preference for an aromatic residue over an alkyl group in the urea terminus. The decrease of activity caused by the replacement of the terminal aryl group with alkyl residues is probably due to the combined effect of an inferior interaction of the alkyl groups with the hydrophobic region and the increased entropy of the flexible alkyl groups.

Concluding that an *N,N′*-diaryl urea moiety is optimal for achieving potent KDR activity, we examined the

Scheme 6. Synthesis of Compounds **67**, **69**, and **72**[a]

[a] Conditions: (a) $(PhO)_2CNCN$, DMF, 90 °C; (b) *m*-toluidine, DMF, microwave, 180 °C; (c) (i) 1,1-thiocarbonyldiimidazole, pyridine, 0 °C; (ii) 2-aminophenol, rt; (iii) EDC, 50 → 60 °C; (d) (i) CH_3CO_2CHO, THF, −15 °C; (ii) Red-Al, benzene, rt → reflux; (e) *m*-tolyl isocyanate, CH_2Cl_2, 0 °C → rt.

Table 1. KDR Inhibitory Activity of C5- and C6-Substituted Thienopyrimidines

compd	X	R	KDR (IC_{50}, nM)[a]	compd	X	R	KDR (IC_{50}, nM)[a]
6	Me	NH_2	4600	**70**	Me	NHCOCH$_2$-(3-CH$_3$-phenyl)	760
7	Me	NHCO-(3-CH$_3$-phenyl)	4600	**72**	Me	N(CH$_3$)CONH-(3-CH$_3$-phenyl)	55
8	Me	NHSO$_2$-phenyl	>50000	**73**	Me	NHCON(CH$_3$)-(3-CH$_3$-phenyl)	1900
9	Me	NHCONH-phenyl	36	**31**	Me	NHCONHCH$_2$-phenyl	330
11	Me	NHCONH-(3-CH$_3$-phenyl)	6	**32**	Me	NHCONHCH$_2$-cyclohexyl	790
64	Me	CONH-(3-CH$_3$-phenyl)	>50000	**74**	Et	NHCONH-(3-CH$_3$-phenyl)	26
65	Me	NHC(S)NH-(3-CH$_3$-phenyl)	2800	**75**	Et	NHCONH-cyclohexyl	170
67	Me	NHC(=N-CN)NH-(3-CH$_3$-phenyl)	1100	**76**	H	NHCONH-(3-CH$_3$-phenyl)	3
68	Me	NHCO-(indol-2-yl)	>50000	**77**	H	NHCONH-n-butyl	180
69	Me	NH-(benzoxazol-2-yl)	170	**78**	H	NHCONHCH$_2$CH(CH$_3$)$_2$	1700

[a] Each IC_{50} determination was performed with seven concentrations, and each assay point was determined in duplicate.

effect of substitution on the terminal phenyl (ring B; rings of the diaryl urea are labeled for clarity in the figure of Table 2) of the urea. The results are summarized in Table 2. To illustrate the inhibitory activity of this series of compounds against other subfamilies of RTKs, Table 2 also includes the potency of these compounds against cKit (PDGFR-family) and Tie2 (Tie-family). Substituting a small lipophilic group into the meta position of ring B is generally well tolerated. In fact, *m*-methyl substitution delivered the best KDR enzymatic potency. In contrast, substitution at the ortho position is unfavorable and resulted in diminished potency. Even compound **16**, which bears an *o*-fluoro group, is approximately 3-fold less potent than the parent compound **9**. Substitution at the para position is also detrimental to the activity in most cases, but the drop in activity is generally less striking than that with ortho substitution. Disubstitution of ring B with small lipophilic groups also appears to be tolerated. In addition to their potent activity against KDR, the compounds in Table 2 also displayed comparable potency against cKit with similar SAR trends but were generally less potent against Tie2 with the exception of inhibitor **28**, which exhibited an IC_{50} value of 17 nM against Tie2. A similar enhancement of Tie2 potency by the combination of a 2-F and a 5-CF_3 on the urea terminal phenyl was also reported recently for furanopyrimidine urea Tie2/VEGFR2 inhibitors.[28b]

Investigation of C6-substitution of the thienopyrimidines was also conducted (Table 3). The results are

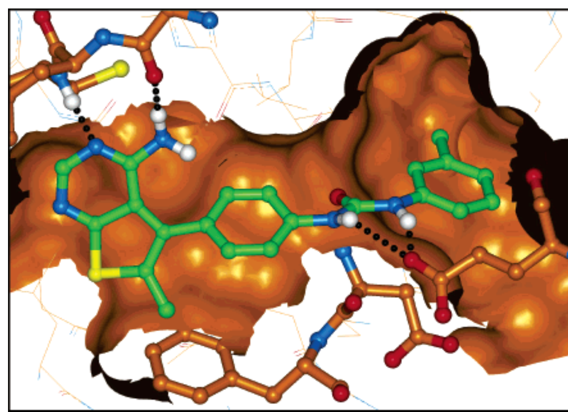


Figure 2. Model of **11** bound to KDR kinase. Hydrogen bonds in black are shown between the urea NHs and Glu 885 carboxylate, between the exocyclic amine and Glu 917 backbone carbonyl, and between the ring nitrogen and Cys 919 N−H. Also, in thick bonds are residues Asp 1046 and Phe 1047 of the DFG motif in the "inactive" conformation ("DFG-out").

Table 2. Substitutions at the Terminal Benzene Ring (Ring B) of *N*,*N*′-Diaryl Ureas

compd	R	KDR	cKit	Tie2
		IC_{50}[a] (nM)		
9	H	36	32	1200
10	*o*-CH_3	260	68	1300
11	*m*-CH_3	6	9	110
12	*p*-CH_3	53	83	890
13	*o*-CH_3O	110	50	920
14	*m*-CH_3O	34	19	360
15	*p*-CH_3O	69	15	1600
16	*o*-F	110		1000
17	*m*-F	54	27	400
18	*p*-F	52	33	850
19	*o*-Cl	340	110	900
20	*m*-Cl	35	22	210
21	*p*-Cl	96	29	1200
22	*m*-Br	46	50	130
23	*p*-Br	120	48	1100
24	*m*-CF_3	52	140	100
25	*m*-CO_2Et	290	43	510
26	3,5-di-CH_3	12	19	170
27	3,5-di-Cl	160	175	360
28	2-F-5-CF_3	45	182	17

[a] Each IC_{50} determination was performed with seven concentrations, and each assay point was determined in duplicate.

consistent with the modeling, which suggested that the methyl group at the 6-position of the thienopyrimidine nucleus of KDR-bound **11** lies near the hydrophobic Phe 1047 of the "DFG (Asp-Phe-Gly)" motif. Consequently, a sterically demanding group was predicted to be less favorable. Although the 6-methyl group in **11** has little impact on the KDR potency (**11** versus **76** in Table 3), introduction of larger groups at the 6-position of **76** led to deterioration of the potency, with the degree of loss correlating with the steric size of the introduced groups. For example, an 8-fold drop in potency was observed for both ethyl (**74**) and *n*-propyl (**79**) groups and a more than 40-fold drop for isopropyl (**80**). An even more severe drop in potency was recorded for 4-morpholinomethyl (**59**), 4-methylpiperazinomethyl (**60**), dimethylaminomethyl (**61**), and dimethylaminoethyl (**87**) groups. A large decrease in activity (about 70-fold compared to that of **76**) was also seen for benzyl groups (**81**); however, significantly better activity was measured for compounds with a 3- or 4-pyridylmethyl group (**82**, IC_{50} = 34 nm; **83**, IC_{50} = 53 nM). In contrast to a bulky residue, groups attached to the 6-position through a two- or three-carbon link appear to be well tolerated. Both compounds **84** and **86**, as well as the methyl ether analogue **85**, exhibited low double-digit nanomolar KDR inhibitory activity. The flexibility of the tethered groups in these molecules may help them avoid an unfavorable interaction with Phe 1047. The tolerance of some C6-substitutions may provide potential opportunities for tuning the physical properties and improving the pharmacokinetic profiles of this series of inhibitors. The large difference in potency between primary amide **51** and *N*-methyl analogue **52** or *N*-dimethyl amide **53** is also noteworthy. As for activity against cKit, substitutions at the 6-position of the thienopyrimidines result in effects very similar to those for KDR inhibitory activity. No significant selectivity for cKit versus KDR was observed for most compounds except **82** and **83**, which are 23- and 18-fold less potent against cKit than against KDR, respectively. In contrast to the inhibitory activity against KDR and cKit, the inhibitory activity against Tie2 is generally less susceptible to changes in C6-substitution, as all the compounds in Table 3 exhibit submicromolar potency against Tie2.

Table 3. Substitutions at the 6-Position of the Thienopyrimidines

compd	X	KDR	cKit	Tie2
		IC_{50}[a] (nM)		
11	Me	6	9	110
51	$(CH_2)_2CONH_2$	17	160	120
52	$(CH_2)_2CONHMe$	330	400	470
53	$(CH_2)_2CONMe_2$	210	930	140
59	[structure]	210	580	430
60	[structure]	590	800	630
61	CH_2NMe_2	180		
74	Et	26	28	340
76	H	3	6	720
79	*n*-Pr	26	82	290
80	*i*-Pr	130		580
81	Bn	280		880
82	[structure]	34	790	250
83	[structure]	53	960	270
84	CH_2CH_2OH	19	37	260
85	$(CH_2)_2OMe$	64	360	220
86	$(CH_2)_3OH$	15	26	110
87	$(CH_2)_2NMe_2$	230	810	190

[a] Each IC_{50} determination was performed with seven concentrations, and each assay point was determined in duplicate.

A brief SAR investigation on the substitution at the linking phenyl (ring A) was also conducted (Table 4). The modeling studies suggested that there is only limited space available for substitutions on ring A in

Table 4. Substitutions at the Linking Phenyl (Ring A) of Thienopyrimidine Ureas

compd	Y	IC_{50}[a] (nM) KDR	cKit	FLT3
76	H	3	6	3
88	1′-F	11	27	10
89	1′-Cl	100	200	59
90	1′-MeO	100	180	140
91	2′-F	19	38	67
92	2′-Cl	83	54	700
93	2′-MeO	13	28	130
94		150		36

[a] Each IC_{50} determination was performed with seven concentrations, and each assay point was determined in duplicate.

part due to the presence of the "gatekeeper" residue in its proximity (Val in both cKit and KDR, and Phe in FLT3). Given that the gatekeeper residue in FLT3 is a sterically more demanding group, it was predicted that the FLT3 inhibitory activity would be even more susceptible to substitution of this ring, especially at the 2′-position (see the numbering on the structure of Table 4). The results of SAR studies were consistent with these predictions (Table 4). The introduction of a methoxy or chloro group at either of the positions of ring A in **76** resulted in deterioration of inhibitory activity against KDR, cKit, and FLT3. However, the most dramatic drop in potency (over 230-fold) was seen for the FLT3 enzyme in the case of **92** (IC_{50} = 700 nM) bearing a 2′-chloro substituent. Compound **94**, an annulation analogue formed by connecting the 6-position of the thienopyrimidine nucleus of **76** and the 1′-position of ring A with an ethylene linker, displayed a much weaker activity (50-fold) against KDR than **76**. This may be due to sterics akin to the substitution at the 1′-position or due to significant reduction of the torsion angle between the thienopyrimidine ring system and ring A of the diaryl urea unit; this angle reduction could result in severe warping of the thienopyrimidine nucleus to accommodate the NH_2 group and ring A interaction and consequently lead to less optimized interactions of the aminopyrimidine moiety with the hinge region and of the urea unit with the back hydrophobic pocket. A torsion angle of approximately 68° was modeled for compound **11** bound to KDR; for compound **94**, however, the angle was calculated at only about 21°.

Compounds with significant KDR inhibitory activity (<100 nM) were further evaluated utilizing an enzyme-linked immunosorbent assay (ELISA) based screen for their inhibition of cellular KDR phosphorylation, which was induced by VEGF in 3T3 murine fibroblasts engineered to express human KDR (Table 5). Compounds with significant activity in the KDR cellular assay were then tested in an estradiol-induced mouse uterine edema model (UE) of VEGF activity. Increased vascular permeability is a hallmark of VEGF-induced responses. It has been shown that VEGF mRNA and protein are up-regulated in a temporal fashion following a bolus injection of estradiol in rat uterus, resulting in increased vascular permeability and edema.[35] The protocol was modified for use in mice. With tested compounds administered orally, the mouse uterine edema enabled rapid screening of KDR inhibitors' in vivo efficacy and systemic oral exposure. The cellular KDR potency and in vivo uterine edema activity of selected compounds are shown in Table 5. In general, compounds that possess a small lipophilic meta substituent such as *m*-Me, *m*-CF_3, and *m*-Cl on the urea terminal phenyl (ring B) and no substituent or a small alkyl group at the 6-position of the theinopyrimidine core delivered both potent KDR cellular activity and outstanding oral activity of uterine edema inhibition. For instance, outstanding oral activity was observed for compounds **24**, **28**, **30**, **76**, **95**, and **96**. In particular, an ED_{50} value of 5 mg/kg was recorded for both **28** and **76**, which are more potent than SU11248 (ED_{50} = 11 mg/kg) in the same assay.

Table 5. KDR Enzymatic and Cellular Inhibitory Activity and in Vivo Oral Uterine Edema Inhibitory Activity of Selected Thienopyrimidine Ureas

compd	X	Y	R	KDR (IC_{50}, nM)[a]	KDR (cell) (IC_{50}, nM)[b]	UE (ED_{50}, mg/kg)
9	Me	H	H	36	10	1% @ 50[c,d]
11	Me	H	*m*-Me	6	<10	43% @ 50[c,d]
20	Me	H	*m*-Cl	35	28	19% @ 60[c]
21	Me	H	p-Cl	96		15% @ 50[c]
24	Me	H	*m*-CF_3	52	10[e]	19
28	Me	H	2-F-5-CF_3	45	66	5
29	Me	H	*p*-CF_3	61	590	12% @ 100[c]
30	Me	H	2-F-5-Me	34	<10[e]	22
76	H	H	*m*-Me	3	1	5
91	H	2′-F	*m*-Me	19	2	44% @ 3[c]
95	Et	H	*m*-CF_3	50	23	19
96	H	H	*m*-Cl	3	9	19
SU11248				18	22	9

[a] Each IC_{50} determination was performed with seven concentrations, and each assay point was determined in duplicate. [b] Each cellular IC_{50} determination was performed with five concentrations, and each assay point was determined in duplicate. [c] Percent inhibition @ mg/kg. [d] Intraperitoneal dosing. [e] Determined by Western blot analysis.

On the basis of their outstanding uterine edema activity, the pharmacokinetic profiles and the antitumor activity of compounds **28** and **76** were evaluated. As shown in Table 6, both **28** and **76** possess a reasonable mouse PK profile. A moderate volume of distribution (V_d) value was observed in CD-1 mouse for both compounds after intravenous dosing. With lower plasma clearance, **28** displayed a longer plasma elimination half-life than **76** while compound **76** had a much higher oral bioavailability (65%) than **28** (33%), presumably due to better absorption. Compound **76** was further studied in beagle dog and cynomolgus monkey for its pharmacokinetics. A similar profile was observed for **76** in both species, characterized by low plasma clearance

Table 6. Physical Properties and Pharmacokinetic Profiles of Thienopyrimidine Ureas **28** and **76**

compd	MW	$c \log P$	PSA (Å^2)	species	$T_{1/2}$ (h)	V_d (L/kg)	Cl (mL/min·kg)	F (%)	AUC (po, μg·h/mL)
28	461	5.1	93	mouse[a]	2.1	1.55	8.7	33	4.8
76	375	4.2	93	mouse[a]	0.7	1.30	20	65	4.9
				dog[b]	3.2	0.5	1.8	40	11.7
				monkey[b]	1.9	0.3	1.7	42	15.9

[a] Dosed intravenously at 3 mg/kg and orally at 10 mg/kg. [b] Dosed both intravenously and orally at 5 mg/kg.

Table 7. Kinase Inhibition Profiles of Thienopyrimidine Ureas **28** and **76**

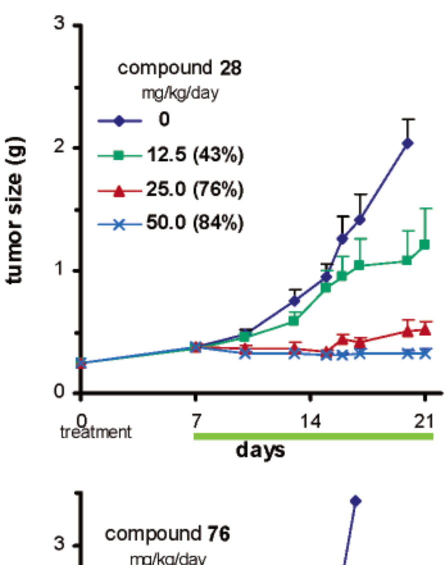
	IC$_{50}$ (nM)											
compd	KDR	FLT1	FLT4	FLT3	cKit	CSF1R	PDGFR[a]	Tie2	FGFR	EGFR	LCK	cMET
28	45	170	87	27	180	57	60	17	1500	>50 000	4 500	>50 000
76	3	2	13	2	6	3	48	730	4200	>50 000	20 000	>50 000

[a] IC$_{50}$ values of cellular phosphorylation by ELISA assay.

(Cl ≤ 1.8 mL/min·kg) and low volumes of distribution (V_d ≤ 0.5 L/kg). Consequently, compound **76** displayed a longer half-life in both dog and monkey than in mouse. The oral bioavailability in both species averaged at about 40%. While both **28** and **76** possess favorable animal PK profiles, poor aqueous solubility was measured for both compounds (0.02 μg/mL for **28** and 3.1 μg/mL for **76** in phosphate buffered saline (PBS)).

The antitumor activity of **28** and **76** was evaluated in an HT1080 fibrosarcoma mouse flank xenograft model. As shown in Figure 3, both **28** and **76** demonstrated excellent antitumor efficacy in a dose-dependent fashion. When orally dosed twice a day with a total daily dose of 50 mg/kg, both compounds inhibited tumor growth by at least 80% compared to the control, in which only the vehicle was administered. Approximately 75% inhibition was observed for both compounds at the 25 mg/kg daily dose. Both compounds compare favorably to SU11248 in the model, as a daily dose of 65 mg/kg was needed for SU11248 to achieve 75% inhibition in the same model.

The significant antitumor efficacy of **28** and **76** may be ascribed to their multitargeted kinase inhibiton. Table 7 shows the inhibitory potency of **28** and **76** against a panel of kinases. These data clearly demonstrate that **28** and **76** are multitargeted receptor tyrosine kinase inhibitors with potent activity against all the kinases of both the VEGFR and PDGFR families. However, they are much less active against those kinases structurally less homologous to KDR such as fibroblast growth factor receptor tyrosine kinase (FGFR), epidermal growth factor receptor tyrosine kinase (EGFR), LCK, and cMET.



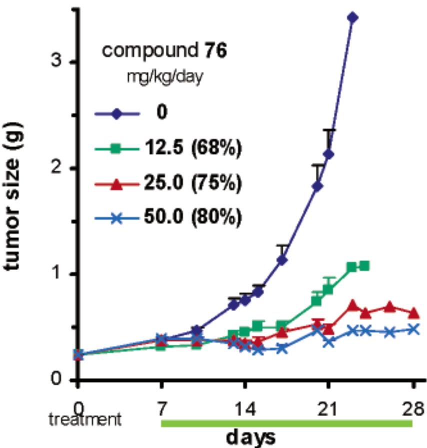


Figure 3. Effects of inhibitors **28** and **76** on the growth of HT1080 human xenografts. Dosing (BID) started on day seven. Tumor volumes are expressed as means ± SEM; n = 10 per group. Significant differences (p < 0.05 vs control) in mean tumor volume were observed for all treatment groups by day 20. Dose (mg/kg·day), and the percent inhibition of control (2 g) for each dosage, is indicated in the legend.

Conclusion

A series of thienopyrimidine-based receptor tyrosine kinase inhibitors has been discovered through a rational design based on an initial KDR screening hit. Extensive SAR studies led to the conclusion that an *N*,*N*′-diaryl urea moiety at the 5-position of the thienopyrimidine nucleus is optimal for KDR inhibition. A KDR homology model suggested that these compounds bind to the ATP binding site of KDR in an inactive conformation, with the urea portion interacting with the back hydrophobic pocket. These compounds are multitargeted receptor tyrosine kinase inhibitors, displaying potent activity against other structurally related kinases of the VEGFR and PDGFR families. Optimization of substituents at both the urea terminal phenyl ring and the 6-position of the thienopyrimidine nucleus generated a series of compounds with excellent enzymatic and cellular activity. Utilizing a mouse uterine edema model, a number of compounds have been identified to possess superior in vivo activity. In particular, compounds **28** and **76** have demonstrated significant oral efficacy in tumor growth inhibition and displayed favorable pharmacokinetic profiles.

Experimental Section

Chemistry. ^{1}H NMR spectra were recorded on a 300 MHz spectrometer (Nicolet QE-300 or General Electric GN-300) if

not otherwise indicated, and chemical shifts are reported in parts per million (ppm, δ) relative to tetramethylsilane as an internal standard. Mass spectra were obtained on a Finnigan MAT SSQ700 instrument. The above spectral data were obtained through the Department of Structural Chemistry, Abbott Laboratories. Elemental analysis was performed by Robertson Microlit Laboratories, Inc., Madison, NJ, and the results indicated by elemental symbols are within ±0.4% of theoretical values. Silica gel 60 (E. Merck, 230−400 mesh) was used for preparative column chromatography.

1-(4-Nitrophenyl)propan-1-one (2). A solution of 2 M ethylmagnesium chloride in THF (15 mL, 30 mmol) was added to a solution of 0.5 M $ZnCl_2$ in THF (60 mL, 30 mmol) at 0 °C. After being stirring first at 0 °C for 10 min and then at room temperature for 20 min, the reaction mixture was cooled to 0 °C and treated sequentially with $Pd(PPh_3)_4$ (1.73 g, 1.5 mmol) and a solution of 4-nitrobenzoyl chloride (6.12 g, 33 mmol) in THF (20 mL). The mixture was stirred at 0 °C for 40 min, diluted with water, adjusted to pH 1 with a 2 N aqueous HCl solution, and extracted with ethyl acetate three times. The combined extracts were washed sequentially with saturated aqueous Na_2CO_3, water, and brine, dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by flash column chromatography on silica gel eluting with 6:1 hexanes/ethyl acetate to provide the title compound (2.17 g, 40%) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 1.10 (t, *J* = 7.12 Hz, 3H), 3.14 (q, *J* = 7.12 Hz, 2H), 8.18 (d, *J* = 8.82 Hz, 2H), 8.34 (d, *J* = 8.82 Hz, 2H).

2-[1-(4-Nitrophenyl)propylidene]malononitrile (3). A mixture of **2** (3.40 g, 19 mmol), malononitrile (1.25 g, 19 mmol), ammonium acetate (1.40 g, 26 mmol), and acetic acid (2 mL) in benzene (50 mL) was heated to reflux in a round-bottomed flask fitted with a Dean−Stark trap for 14 h. Additional ammonium acetate (1.40 g, 26 mmol) and acetic acid (2 mL) were added. The reaction mixture was heated to reflux for an additional 4 h, cooled to room temperature, and partitioned between water and ethyl acetate. The aqueous layer was extracted with ethyl acetate two times, and the combined extracts were washed with water and brine, dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by flash column chromatography on silica gel eluting with 3:1 hexanes/ethyl acetate to provide the title compound (4.01 g, 93%) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 0.97 (t, *J* = 7.63 Hz, 3H), 3.00 (q, *J* = 7.57 Hz, 2H), 7.89 (d, *J* = 9.15 Hz, 2H), 8.40 (d, *J* = 9.15 Hz, 2H).

2-Amino-5-methyl-4-(4-nitrophenyl)thiophene-3-carbonitrile (4). Diethylamine (0.6 mL, 5.75 mmol) was added to a suspension of **3** (1.31 g, 5.75 mmol) and sulfur (0.18 g, 5.75 mmol) in ethanol (20 mL). The mixture was heated at 70 °C for 1.5 h, cooled to room temperature, and concentrated. The crude product was purified by flash column chromatography on silica gel eluting with 3:2 hexanes/ethyl acetate to provide the title compound (1.27 g, 85%). ^{1}H NMR (DMSO-d_6) δ 2.18 (s, 3 H), 7.63 (d, *J* = 8.82 Hz, 2H), 8.30 (d, *J* = 8.81 Hz, 2H); MS (CI) *m/z* 277 $(M + NH_4)^+$.

6-Methyl-5-(4-nitrophenyl)thieno[2,3-*d*]pyrimidin-4-amine (5). A suspension of **4** (4.03 g, 15.5 mmol) in formamide (60 mL) was stirred at 155 °C for 17 h, cooled to room temperature, diluted with water, and filtered. The filter cake was dried to provide the title compound (4.13 g, 93%). ^{1}H NMR (DMSO-d_6) δ 8.36 (d, *J* = 9.0 Hz, 2H), 8.30 (s, 1H), 7.68 (d, *J* = 9.0 Hz, 2H), 2.32 (s, 3H); MS (CI) *m/z* 287 $(M + H)^+$.

5-(4-Aminophenyl)-6-methylthieno[2,3-*d*]pyrimidin-4-amine (6). A suspension of **5** (1.01 g, 3.53 mmol) in ethanol (60 mL), THF (20 mL), and water (10 mL) was treated with ammonium chloride (0.19 g, 3.53 mmol) and iron powder (1.18 g, 21.2 mmol). After being stirred at 80 °C for 2 h, the mixture was diluted with ethanol (40 mL) and filtered through a pad of diatomaceous earth (Celite) while still hot. The pad was washed with ethanol, and the filtrate was concentrated. The concentrate was diluted with water and extracted with ethyl acetate three times. The combined extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated to provide the title compound (0.89 g, 98%). ^{1}H NMR (DMSO-d_6) δ 8.23 (s, 1H), 7.01 (d, *J* = 8.4 Hz, 2H), 6.70 (d, *J* = 8.4 Hz, 2H), 5.39 (s, 2H), 2.27 (s, 3H); MS (CI) *m/z* 257 $(M + H)^+$. Anal. ($C_{13}H_{12}N_4S$·0.2EtOAc·0.2H_2O) C, H, N.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-3-methylbenzamide (7).** A mixture of **6** (80 mg, 0.31 mmol), *m*-toluic acid (42 mg, 0.31 mmol), HOBT (46 mg, 0.34 mmol), EDC (66 mg, 0.34 mmol), and 4-methylmorpholine (NMM) (86 μL, 0.78 mmol) in DMF (3 mL) was stirred at room temperature for 14 h and then partitioned between water and ethyl acetate. The aqueous layer was extracted with ethyl acetate two times, and the combined extracts were washed with water and brine, dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by flash column chromatography on silica gel eluting with ethyl acetate to provide the title compound (39 mg, 35%). ^{1}H NMR (DMSO-d_6) δ 10.42 (s, 1H), 8.28 (s, 1H), 7.98 (d, *J* = 8.4 Hz, 2H), 7.80−7.75 (m, 2H), 7.45−7.36 (m, 4H), 2.42 (s, 3H), 2.31 (s, 3H); MS (CI) *m/z* 375 $(M + H)^+$. Anal. ($C_{12}H_{18}N_4OS$·0.2EtOAc) C, H, N.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]benzenesulfonamide (8).** A solution of **6** (100 mg, 0.39 mmol) in dichloromethane (4 mL) was treated at 0 °C with pyridine (38 μL, 0.47 mmol) and benzenesulfonyl chloride (50 μL, 0.40 mmol) and stirred at 0 °C for 1 h and then at room temperature overnight. The reaction mixture was diluted with water and extracted with ethyl acetate three times. The combined extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated. The concentrate was triturated from ethyl acetate/hexanes to provide the title compound (91 mg, 59%). ^{1}H NMR (DMSO-d_6) δ 10.49 (s, 1H), 8.25 (s, 1H), 7.77 (m, 2H), 7.65−7.55 (m, 3H), 7.24 (m, 4H), 1.99 (s, 3H); MS (ESI) *m/z* 397 $(M + H)^+$. Anal. ($C_{19}H_{16}N_4O_2S_2$·0.3EtOAc) C, H, N.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N*′-phenylurea (9).** A solution of **6** (80 mg, 0.3 mmol) in dichloromethane (4 mL) at 0 °C was treated with phenyl isocyanate (36 μL, 0.33 mmol) and stirred overnight as the temperature slowly rose to room temperature. To the resulting suspension, hexane was added to yield more precipitate. The solid material was collected by filtration to provide the title compound (103 mg, 87%). ^{1}H NMR (DMSO-d_6) δ 8.89 (s, 1H), 8.75 (s, 1H), 8.26 (s, 1H), 7.63 (d, *J* = 8.7 Hz, 2H), 7.47 (d, *J* = 8.7 Hz, 2H), 7.33−7.26 (m, 4H), 6.99 (t, *J* = 7.5 Hz, 1H), 2.30 (s, 3H); MS (CI) *m/z* 376 $(M + H)^+$. Anal. ($C_{20}H_{17}N_5OS$·0.1CH_2Cl_2) C, H, N.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N*′-(2-methylphenyl)urea (10).** Compound **10** was prepared using the same procedure as described for the synthesis of **9** by substituting 2-tolyl isocyanate for phenyl isocyanate. ^{1}H NMR (DMSO-d_6) δ 9.24 (s, 1H), 8.27 (s, 1H), 8.01 (s, 1H), 7.82 (d, *J* = 7.5 Hz, 1H), 7.64 (d, *J* = 8.1 Hz, 2H), 7.31 (d, *J* = 8.1 Hz, 2H), 7.21−7.13 (m, 2H), 6.97 (t, *J* = 7.5 Hz, 1H), 2.30 (s, 3H), 2.27 (s, 3H); MS (ESI) *m/z* 390 $(M + H)^+$. Anal. ($C_{21}H_{19}N_5OS$·0.7H_2O) C, H, N.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N*′-(3-methylphenyl)urea (11).** Compound **11** was prepared using the same procedure as described for the synthesis of **9** by substituting 3-tolyl isocyanate for phenyl isocyanate. ^{1}H NMR (DMSO-d_6) δ 8.87 (s, 1H), 8.67 (s, 1H), 8.26 (s, 1H), 7.63 (d, *J* = 8.1 Hz, 2H), 7.32−7.23 (m, 4H), 7.17 (t, *J* = 7.8 Hz, 1H), 6.81 (d, *J* = 7.8 Hz, 1H), 2.30 (s, 3H), 2.29 (s, 3H); MS (ESI) *m/z* 388 $(M - H)^-$. Anal. ($C_{21}H_{19}N_5OS$·0.5H_2O) C, H, N.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N*′-(4-methylphenyl)urea (12).** Compound **12** was prepared using the same procedure as described for the synthesis of **9** by substituting 4-tolyl isocyanate for phenyl isocyanate. ^{1}H NMR (DMSO-d_6) δ 8.84 (s, 1H), 8.63 (s, 1H), 8.26 (s, 1H), 7.62 (d, *J* = 8.4 Hz, 2H), 7.35 (d, *J* = 8.4 Hz, 2H), 7.30 (d, *J* = 8.4 Hz, 2H), 7.10 (d, *J* = 8.4 Hz, 2H), 2.29 (s, 3H), 2.25 (s, 3H); MS (ESI) *m/z* 390 $(M + H)^+$. Anal. ($C_{21}H_{19}N_5OS$·0.5H_2O·0.1C_6H_{12}) C, H, N.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N*′-(2-methoxyphenyl)urea (13).** Compound **13** was

prepared using the same procedure as described for the synthesis of **9** by substituting 2-methoxyphenyl isocyanate for phenyl isocyanate. ^{1}H NMR (DMSO-d_6) δ 9.55 (s, 1H), 8.31 (s, 1H), 8.27 (s, 1H), 8.15 (dd, J = 7.8, 2.1 Hz, 1H), 7.63 (d, J = 8.7 Hz, 2H), 7.31 (d, J = 8.7 Hz, 2H), 7.04 (dd, J = 8.1, 1.8 Hz, 1H), 6.70–6.87 (m, 2H), 3.90 (s, 3H), 2.30 (s, 3H); MS (ESI) *m/z* 406 (M + H)$^+$. Anal. ($C_{21}H_{19}N_5O_2S{\cdot}0.25H_2O$) C, H, N.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N*′-(3-methoxyphenyl)urea (14).** Compound **14** was prepared using the same procedure as described for the synthesis **9** by substituting 3-methoxyphenyl isocyanate for phenyl isocyanate.^{1}H NMR (DMSO-d_6) δ 8.88 (s, 1H), 8.76 (s, 1H), 8.26 (s, 1H), 7.63 (d, J = 8.7 Hz, 2H), 7.31 (d, J = 8.7 Hz, 2H), 7.22–7.17 (m, 2H), 6.95 (m, 1H), 6.57 (m, 1H), 3.74 (s, 3H), 2.30 (s, 1H); MS (ESI) *m/z* 406 (M + H)$^+$. Anal. ($C_{21}H_{19}N_5O_2S{\cdot}0.3H_2O$) C, H, N.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N*′-(4-methoxyphenyl)urea (15).** Compound **15** was prepared using the same procedure as described for the synthesis of **9** by substituting 4-methoxyphenyl isocyanate for phenyl isocyanate. ^{1}H NMR (DMSO-d_6) δ 8.81 (s, 1H), 8.56 (s, 1H), 8.27 (s, 1H), 7.62 (d, J = 8.1 Hz, 2H), 7.38 (d, J = 9.0 Hz, 2H), 7.30 (d, J = 8.1 Hz, 2H), 6.89 (d, J = 9.0 Hz, 2H), 3.73 (s, 3H), 2.30 (s, 3H); MS (ESI) *m/z* 406 (M + H)$^+$. Anal. ($C_{21}H_{19}N_5O_2S{\cdot}0.2C_6H_{14}{\cdot}0.3H_2O$) C, H, N.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N*′-(2-fluorophenyl)urea (16).** Compound **16** was prepared using the same procedure as described for the synthesis of **9** by substituting 2-fluorophenyl isocyanate for phenyl isocyanate ^{1}H NMR (DMSO-d_6) δ 9.30 (s, 1H), 8.63 (d, J = 2.4 Hz, 1H), 8.27 (s, 1H), 8.17 (td, J = 8.1, 1.5 Hz, 1H), 7.64 (d, J = 8.4 Hz, 2H), 7.33 (d, J = 8.4 Hz, 2H), 7.26 (ddd, J = 12.0, 8.1, 1.2 Hz, 1H), 7.16 (t, J = 7.8 Hz, 1H), 7.05–6.99 (m, 1H), 2.30 (s, 3H); MS (ESI) *m/z* 394 (M + H)$^+$. Anal. ($C_{20}H_{16}FN_5OS{\cdot}0.2C_8H_{18}$) C, H, N.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N*′-(3-fluorophenyl)urea (17).** Compound **17** was prepared using the same procedure as described for the synthesis of **9** by substituting 3-fluorophenyl isocyanate for phenyl isocyanate. ^{1}H NMR (DMSO-d_6) δ 9.00 (s, 1H), 8.97 (s, 1H), 8.27 (s, 1H), 7.64 (d, J = 8.4 Hz, 2H), 7.51 (dt, J = 12.0, 2.1 Hz, 1H), 7.36–7.28 (m, 3H), 7.15 (d, J = 8.1 Hz, 1H), 6.80 (td, J = 8.1 Hz, 2.4 Hz), 2.30 (s, 3H); MS (ESI) *m/z* 394 (M + H)$^+$. Anal. ($C_{20}H_{16}FN_5OS{\cdot}0.3H_2O$) C, H, N.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N*′-(4-fluorophenyl)urea (18).** Compound **18** was prepared using the same procedure as described for the synthesis of **9** by substituting 4-fluorophenyl isocyanate for phenyl isocyanate. ^{1}H NMR (DMSO-d_6) δ 8.89 (s, 1H), 8.79 (s, 1H), 8.26 (s, 1H), 7.63 (d, J = 8.4 Hz, 2H), 7.51–7.46 (m, 2H), 7.31 (d, J = 8.4 Hz, 2H), 7.14 (t, J = 9.0 Hz, 2H), 2.29 (s, 3H); MS (ESI) *m/z* 394 (M + H)$^+$.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N*′-(2-chlorophenyl)urea (19).** Compound **19** was prepared using the same procedure as described for the synthesis of **9** by substituting 2-chlorophenyl isocyanate for phenyl isocyanate. ^{1}H NMR (DMSO-d_6) δ 9.64 (s, 1H), 8.40 (s, 1H), 8.27 (s, 1H), 8.17 (dd, J = 8.4, 1.8 Hz, 1H), 7.65 (d, J = 8.4 Hz, 2H), 7.48 (dd, J = 7.8, 1.8 Hz, 1H), 7.36–7.28 (m, 3H), 7.05 (td, J = 8.4, 1.8 Hz, 1H), 2.30 (s, 1H); MS (ESI) *m/z* 410, 412 (M + H)$^+$.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N*′-(3-chlorophenyl)urea (20).** Compound **20** was prepared using the same procedure as described for the synthesis of **9** by substituting 3-chlorophenyl isocyanate for phenyl isocyanate. ^{1}H NMR (DMSO-d_6) δ 8.97 (s, 2H), 8.27 (s, 1H), 7.73 (m, 1H), 7.64 (d, J = 8.7 Hz, 2H), 7.34–7.29 (m, 4H), 7.06–7.02 (m, 1H), 2.30 (s, 1H); MS (ESI) *m/z* 410 (M + H)$^+$. Anal. ($C_{20}H_{16}ClN_5OS{\cdot}0.2H_2O$) C, H, N.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N*′-(4-chlorophenyl)urea (21).** Compound **21** was prepared using the same procedure as described for the synthesis of **9** by substituting 4-chlorophenyl isocyanate for phenyl isocyanate. ^{1}H NMR (DMSO-d_6) δ 8.92 (s, 1H), 8.89 (s, 1H), 8.26 (s, 1H), 7.63 (d, J = 8.7 Hz, 2H), 7.51 (s, J = 9.0 Hz, 2H), 3.37–7.29 (m, 4H), 2.29 (s, 3H); MS (ESI) *m/z* 410 (M + H)$^+$.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N*′-(3-bromophenyl)urea (22).** Compound **22** was prepared using the same procedure as described for the synthesis of **9** by substituting 3-bromophenyl isocyanate for phenyl isocyanate. ^{1}H NMR (DMSO-d_6) δ 8.97 (s, 1H), 8.96 (s, 1H), 8.27 (s, 1H), 7.88 (t, J = 1.8 Hz, 1H), 7.64 (d, J = 9.0 Hz, 2H), 7.36–7.29 (m, 3H), 7.25 (t, J = 7.8 Hz, 1H), 7.19–7.14 (m, 1H), 2.30 (s, 3H); MS (ESI) *m/z* 454, 456 (M + H)$^+$. Anal. ($C_{20}H_{16}BrN_5OS$) C, H, N

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N*′-(4-bromophenyl)urea (23).** Compound **23** was prepared using the same procedure as described for the synthesis of **9** by substituting 4-bromophenyl isocyanate for phenyl isocyanate. ^{1}H NMR (DMSO-d_6) δ 8.93 (s, 1H), 8.90 (s, 1H), 8.26 (s, 1H), 7.63 (d, J = 8.7 Hz, 2H), 7.46 (s, 4H), 7.32 (d, J = 8.7 Hz, 2H), 2.29 (s, 3H); MS (ESI) *m/z* 454, 456 (M + H)$^+$. Anal. ($C_{20}H_{16}BrN_5OS{\cdot}0.4H_2O{\cdot}0.2C_8H_{18}$) C, H, N.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N*′-[3-(trifluoromethyl)phenyl]urea (24).** Compound **24** was prepared using the same procedure as described for the synthesis of **9** by substituting 3-trifluoromethylphenyl isocyanate for phenyl isocyanate. ^{1}H NMR (DMSO-d_6) δ 9.12 (s, 1H), 9.01 (s, 1H), 8.27 (s, 1H), 8.03 (s, 1H), 7.67–7.59 (m, 3H), 7.53 (t, J = 7.8 Hz, 1H), 7.33 (m, 3H), 2.30 (s, 1H); MS (ESI) *m/z* 444 (M + H)$^+$. Anal. ($C_{21}H_{16}F_3N_5OS$) C, H, N.

Methyl 3-[({[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)phenyl]amino}carbonyl)amino]benzoate (25). Compound **25** was prepared using the same procedure as described for the synthesis of **9** by substituting methyl 3-isocyanatobenzoate for phenyl isocyanate. ^{1}H NMR (DMSO-d_6) δ 9.04 (s, 1H), 8.94 (s, 1H), 8.27 (s, 1H), 8.22 (t, 1H), 7.65 (d, 3H), 7.59 (dt, 1H), 7.44 (t, 1H), 7.32 (d, 2H), 3.86 (s, 3H), 2.30 (s, 3H); MS (ESI) *m/z* 434 (M + H)$^+$. Anal. ($C_{22}H_{19}N_5O_3S{\cdot}0.5H_2O$) C, H, N.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N*′-(3,5-dimethylphenyl)urea (26).** Compound **26** was prepared using the same procedure as described for the synthesis of **9** by substituting 3,5-dimethylphenyl isocyanate for phenyl isocyanate. ^{1}H NMR (DMSO-d_6) δ 8.85 (s, 1H), 8.59 (s, 1H), 8.26 (s, 1H), 7.62 (d, J = 8.4 Hz, 2H), 7.30 (d, J = 8.4 Hz, 2H), 7.09 (s, 2H), 6.63 (s, 1H), 2.30 (s, 3H), 2.24 (s, 6H); MS (ESI) *m/z* 404 (M + H)$^+$. Anal. ($C_{22}H_{21}N_5OS$) C, H, N.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N*′-(3,5-dichlorophenyl)urea (27).** Compound **27** was prepared using the same procedure as described for the synthesis of **9** by substituting 3,5-dichlorophenyl isocyanate for phenyl isocyanate. ^{1}H NMR (DMSO-d_6) δ 9.14 (s, 1H), 9.11 (s, 1H), 8.27 (s, 1H), 7.64 (d, J = 8.4 Hz, 2H), 7.56 (d, J = 1.8 Hz, 2H), 7.33 (d, J = 8.4 Hz, 2H), 7.19 (t, J = 1.8 Hz, 1H), 2.29 (s, 3H); MS (ESI) *m/z* 444, 446 (M + H)$^+$.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N*′-[2-fluoro-5-(trifluoromethyl)phenyl]urea (28).** Compound **28** was prepared using the same procedure as described for the synthesis of **9** by substituting 2-fluoro-5-(trifluoromethyl)phenyl isocyanate for phenyl isocyanate. ^{1}H NMR (DMSO-d_6) δ 9.39 (s, 1H), 8.98 (d, J = 2.7 Hz, 1H), 8.64 (dd, J = 7.2, 1.8 Hz, 1H), 8.27 (s, 1H), 7.65 (d, J = 8.4 Hz, 2H), 7.52 (t, J = 9.0 Hz, 1H), 7.44–7.37 (m, 1H), 7.34 (d, J = 8.4 Hz, 2H), 2.30 (s, 3H); MS (ESI) *m/z* 462 (M + H)$^+$. Anal. ($C_{21}H_{15}F_4N_5OS$) C, H, N.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N*′-[4-(trifluoromethyl)phenyl]urea (29).** Compound **29** was prepared using the same procedure as described for the synthesis of **9** by substituting 4-(trifluoromethyl)phenyl isocyanate for phenyl isocyanate. ^{1}H NMR (DMSO-d_6) δ 9.29 (s, 1H), 9.02 (s, 1H), 8.27 (s, 1H), 7.71–7.63 (m, 6H), 7.33 (d, J = 8.7 Hz, 2H), 2.30 (s, 3H); MS (ESI) *m/z* 444 (M + H)$^+$.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N*′-(2-fluoro-5-methylphenyl)urea (30).** Compound **30** was prepared using the same procedure as described for the synthesis of **9** by substituting 2-fluoro-5-methylphenyl

isocyanate for phenyl isocyanate. ^{1}H NMR (DMSO-d_6) δ 9.28 (s, 1H), 8.56 (d, J = 2.7 Hz, 1H), 8.27 (s, 1H), 8.00 (dd, J = 8.1, 2.1 Hz, 1H), 7.63 (d, J = 9.0 Hz, 2H), 7.32 (d, J = 9.0 Hz, 2H), 7.12 (dd, J = 11.4, 8.1 Hz, 1H), 6.82 (m, 1H), 2.30 (s, 3H), 2.28 (s, 3H); MS (ESI) *m/z* 408 (M + H)$^+$.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N'*-benzylurea (31).** Compound **31** was prepared using the same procedure as described for the synthesis of **9** by substituting benzyl isocyanate for phenyl isocyanate. ^{1}H NMR (DMSO-d_6) δ 8.81 (s, 1H), 8.26 (s, 1H), 7.59 (d, J = 8.7 Hz, 2H), 8.38−7.28 (m, 4H), 7.27−7.22 (m, 3H), 6.71 (t, J = 6.0 Hz, 1H), 4.33 (d, J = 6.0 Hz, 2H), 2.28 (s, 3H); MS (ESI) *m/z* 390 (M + H)$^+$.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N'*-cyclohexylmethylurea (32).** To a solution of **6** (159 mg, 0.62 mmol) in THF (9 mL) at 0 °C was added triethylamine (95 μL, 0.68 mmol) and 4-nitrophenyl chloroformate (137 mg, 0.68 mmol). After being stirred at 0 °C for 1 h, cyclohexanylmethylamine (81 μL, 0.62 mmol) and triethylamine (95 μL, 0.68 mmol) were added. The reaction mixture was stirred overnight while the temperature slowly rose to room temperature. The reaction mixture was partitioned between ethyl acetate and water, extracted with ethyl acetate, dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by flash column chromatography on silica gel eluting with ethyl acetate to provide the title compound (24 mg, 10%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.85−0.97 (m, 2H), 1.07−1.27 (m, 3H), 1.36−1.46 (m, 1H), 1.63 (d, J = 11.29 Hz, 1H), 1.66−1.75 (m, 4H), 2.28 (s, 3H), 2.96 (t, J = 6.41 Hz, 2H), 6.25 (t, J = 5.80 Hz, 1H), 7.23 (d, J = 8.54 Hz, 2H), 7.55 (d, J = 8.54 Hz, 2H), 8.26 (s, 1H), 8.61 (s, 1H); MS (ESI) *m/z* 396 (M + H)$^+$. Anal. ($C_{21}H_{25}N_5OS$·0.2EtOAc) C, H, N.

1-(4-Nitrophenyl)-3-pyridin-3-ylpropenone (33a). A suspension of 3′-nitroacetophenone (5 g, 30.3 mmol) and 4-pyridinecarboxaldehyde (2.89 mL, 30.3 mmol) in water (45 mL) at room temperature was treated with 6% NaOH in H_2O/ethanol (2:1, 0.60 mL), stirred overnight, and filtered. The filter cake was washed with water and a small amount of ethanol and dried to provide the title compound (7.6 g, 98%). ^{1}H NMR (DMSO-d_6) δ 7.52 (dd, J = 7.80, 4.75 Hz, 1H), 7.84 (d, J = 15.93 Hz, 1H), 8.11 (d, J = 15.60 Hz, 1H), 8.36−8.44 (m, 5H), 8.65 (dd, J = 4.75, 1.36 Hz, 1H), 9.06 (d, J = 2.03 Hz, 1H); MS (ESI) *m/z* 255 (M + H)$^+$.

1-(4-Nitrophenyl)-3-pyridin-3-ylpropan-1-one (34a). Tributyltin hydride (0.36 mL, 1.34 mmol) was slowly added using a syringe pump at room temperature to a mixture of **33a** (200 mg, 0.78 mmol) and $Pd(PPh_3)_4$ (27 mg, 0.023 mmol) in THF (28 mL). After the addition was complete (1.3 h), the mixture was stirred at room temperature overnight, then diluted with water, and extracted with ethyl acetate three times. The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by flash column chromatography on silica gel eluting with 80% ethyl acetate/hexanes to provide the title compound (113 mg, 50%). ^{1}H NMR (DMSO-d_6) δ 2.98 (t, J = 7.46 Hz, 2H), 3.53 (t, J = 7.29 Hz, 2H), 7.31 (dd, J = 7.80, 4.75 Hz, 1H), 7.72 (dt, J = 7.80, 2.03 Hz, 1 H), 8.22 (d, J = 9.16 Hz, 2H), 8.34 (d, J = 8.82 Hz, 2H), 8.40 (dd, J = 4.75, 1.70 Hz, 1H), 8.53 (d, J = 2.37 Hz, 1H); MS (ESI) *m/z* 257 (M + H)$^+$.

1-(4-Nitrophenyl)-3-pyridin-3-ylpropan-1-one (34b). Compound **34b** was prepared using the same sequence as described for the synthesis of **34a** by substituting 4′-nitroacetophenone for 3′-nitroacetophenone. ^{1}H NMR (DMSO-d_6) δ 2.98 (t, J = 7.29 Hz, 2H), 3.54 (t, J = 7.46 Hz, 2H), 7.33 (d, J = 6.10 Hz, 2H), 8.22 (d, J = 8.82 Hz, 2H), 8.34 (d, J = 9.16 Hz, 2H), 8.46 (d, J = 6.10 Hz, 2H); MS (ESI) *m/z* 257 (M + H)$^+$.

4-{[*tert*-Butyl(dimethyl)silyl]oxy}-1-(4-nitrophenyl)-butan-1-one (36a). A mixture of Zn−Cu couple (2.68 g, 41.3 mmol) and *tert*-butyl(3-iodopropoxy)dimethylsilane (**35a**) (8.26 g, 27.5 mmol) in benzene (55 mL) and DMF (3.6 mL) was stirred vigorously at room temperature for 1 h and, then, heated at 60 °C for 4 h. After being cooled to room temperature, the reaction mixture was treated with $(Ph_3P)_4Pd$ (0.85 g, 0.73 mmol) and a solution of 4-nitrobenzoyl chloride (3.4 g, 18.3 mmol) in benzene (36 mL) and stirred at room temperature for 1 h. The mixture was filtered through diatomaceous earth (Celite) and partitioned between saturated aqueous ammonium chloride solution and ethyl acetate. The aqueous phase was extracted with ethyl acetate two times, and the combined extracts were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by flash column chromatography on silica gel eluting with 5% ethyl acetate/hexanes to provide the title compound (2.81 g, 47%). ^{1}H NMR (DMSO-d_6) δ 0.00 (s, 6H), 0.83 (s, 9H), 1.83 (m, 2H), 3.12 (t, J = 6.95 Hz, 2H), 3.64 (t, J = 6.27 Hz, 2H), 8.16 (d, J = 8.82 Hz, 2H), 8.33 (d, J = 9.16 Hz, 2H); MS (ESI) *m/z* 322 (M − H)$^-$.

5-(*tert*-Butyldimethylsilanyloxy)-1-(4-nitrophenyl)-pentan-1-one (36b). Compound **36b** was prepared using the same procedure as described for the synthesis of **36a** by substituting **35b** for **35a**. ^{1}H NMR (DMSO-d_6) δ 0.00 (s, 6H), 0.83 (s, 9H), 1.44−1.56 (m, 2H), 1.58−1.71 (m, 2H), 3.11 (t, J = 7.12 Hz, 2H), 3.59 (t, J = 6.27 Hz, 2H), 8.16 (d, J = 9.15 Hz, 2H), 8.31 (d, J = 8.82 Hz, 2H); MS (ESI) *m/z* 336 (M − H)$^-$.

4-Hydroxy-1-(4-nitrophenyl)butan-1-one (37). To a solution of **36a** (1.5 g, 4.60 mmol) in THF (20 mL) at 0 °C was dropwise added a 1.0 M tetrabutylammonium fluoride solution in THF (6.9 mL, 6.9 mmol). The reaction mixture was stirred overnight while the temperature rose slowly to room temperature, diluted with water, extracted with ethyl acetate, washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by flash column chromatography on silica gel with 80% ethyl acetate/hexanes to provide the title compound (0.66 g, 74%). ^{1}H NMR (DMSO-d_6) δ 1.72−1.85 (m, 2H), 3.13 (t, J = 7.29 Hz, 2H), 3.46 (m, 2H), 4.52 (t, 1H), 8.18 (d, J = 8.81 Hz, 2H), 8.34 (d, J = 9.15 Hz, 2H).

Methanesulfonic Acid 4-(4-Nitrophenyl)-4-oxobutyl Ester (38). A solution of **37** (5.68 g, 29.4 mmol) in dichloromethane (60 mL) at 0 °C was treated with triethylamine (4.9 mL, 35 mmol) and methylsulfonyl chloride (2.7 mL, 35 mmol). After being stirred at 0 °C for 3 h, the reaction mixture was poured into cold water and extracted with dichloromethane three times. The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel chromatography eluting with 50% ethyl acetate/hexanes to provide the title compound (6.42 g, 76%). ^{1}H NMR (DMSO-d_6) δ 1.95−2.16 (m, 2H), 3.18 (s, 3H), 3.25 (t, J = 6.95 Hz, 2H), 4.29 (t, J = 6.44 Hz, 2H), 8.20 (d, J = 8.81 Hz, 2H), 8.35 (d, J = 8.81 Hz, 2H).

4-(Dimethylamino)-1-(4-nitrophenyl)-1-butanone (39). A mixture of **38** (3.0 g, 10.5 mmol), a 2.0 M dimethylamine solution in THF (21 mL, 42.0 mmol), and triethylamine (2.9 mL, 21.0 mmol) in DMF (25 mL) was heated at 85−90 °C for 1.5 h and then cooled to room temperature, diluted with water, and extracted with ethyl acetate. The extract was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel chromatography eluting with 10% methanol/dichloromethane to provide the title compound (1.27 g, 51%). ^{1}H NMR (DMSO-d_6) δ 1.70−1.85 (m, 2H), 2.08 (s, 6H), 2.26 (t, J = 6.95 Hz, 2H), 3.08 (t, J = 6.95 Hz, 2H), 8.16 (d, J = 8.82 Hz, 2H), 8.33 (d, J = 8.82 Hz, 2H); MS (ESI) *m/z* 237 (M + H)$^+$.

1-(3-Methoxy-4-nitrophenyl)ethanone (41d). A suspension of $MgCl_2$ (932 mg, 9.8 mmol) in toluene (13 mL) was treated with triethylamine (4.7 mL, 33.4 mmol) and dimethyl malonate (1.9 mL, 16.6 mmol). After being stirred at room temperature for 1.5 h, the reaction mixture was treated portionwise over 30 min with 3-methoxy-4-nitrobenzoyl chloride (**40d**) (3 g, 13.9 mmol). The stirring was continued for an additional 45 min, and then, the mixture was treated with concentrated HCl (4 mL). The organic layer was separated, dried over Na_2SO_4, filtered, and concentrated. The residue was dissolved in DMSO (11.5 mL) and water (0.5 mL), heated to reflux overnight, and then cooled to room temperature and partitioned between water and ethyl acetate. The organic phase was washed sequentially with aqueous saturated $NaHCO_3$, water, and brine, dried over $MgSO_4$, filtered, and

concentrated to provide the title compound (1.63 g, 60%). ^{1}H NMR (DMSO-d_6) δ 2.66 (s, 3H), 4.00 (s, 3H), 7.65−7.72 (m, 1H), 7.75 (d, *J* = 1.70 Hz, 1H), 8.00 (d, *J* = 8.48 Hz, 1H); MS (ESI) *m/z* 194 (M − H)$^-$.

1-(2-Fluoro-4-nitrophenyl)ethanone (41a). Compound **41a** was prepared using the same procedure as described for the synthesis of **41d** by substituting **40a** for **40d**. ^{1}H NMR (DMSO-d_6) δ 2.65 (d, *J* = 3.73 Hz, 3H), 8.01−8.09 (m, 1H), 8.12−8.20 (m, 1H), 8.26 (dd, *J* = 10.51, 2.03 Hz, 1H); MS (ESI) *m/z* 182 (M − H)$^-$.

1-(2-Chloro-4-nitrophenyl)ethanone (41b). Compound **41b** was prepared using the same procedure as described for the synthesis of **41d** by substituting **40b** for **40d**. ^{1}H NMR (DMSO-d_6) δ 2.63 (s, 3H), 7.94 (d, *J* = 8.14 Hz, 1H), 8.28 (dd, *J* = 8.48, 2.03 Hz, 1H), 8.37 (d, *J* = 2.71 Hz, 1H); MS (ESI) *m/z* 197 (M − H)$^-$.

1-(2-Methoxy-4-nitrophenyl)ethanone (41c). Compound **41c** was prepared using the same procedure as described for the synthesis of **41d** by substituting **40c** for **40d**. ^{1}H NMR (DMSO-d_6) δ 2.57 (s, 3H), 4.01 (s, 3H), 7.74 (d, *J* = 7.80 Hz, 1H), 7.84−7.89 (m, 1H), 7.91 (d, *J* = 2.03 Hz, 1H); MS (ESI) *m/z* 194 (M − H)$^-$.

6,6-Dicyano-5-phenylhex-5-enoic Acid (43). Compound **43** was prepared using the same procedure as described for the synthesis of **3** by substituting 5-oxo-5-phenylvaleric acid (**42**) for 1-(4-nitrophenyl)propan-1-one (**2**). ^{1}H NMR (DMSO-d_6) δ 1.45−1.59 (m, 2H), 2.25 (t, *J* = 7.29 Hz, 2H), 3.03 (t, *J* = 9.00 Hz, 2H), 7.53−7.62 (m, 3H), 7.62−7.69 (m, 2H), 12.16 (s, br, 1H); MS (ESI) *m/z* 239 (M − H)$^-$.

3-(5-Amino-4-cyano-3-phenylthiophen-2-yl)propionic Acid (44). Compound **44** was prepared using the same procedure described for the synthesis of **4** by substituting **43** for **3** and using two molar equivalents of diethylamine. ^{1}H NMR (DMSO-d_6) δ 2.41 (t, *J* = 7.29 Hz, 2H), 2.72 (t, *J* = 7.46 Hz, 2H), 7.09 (s, br, 2H), 7.29−7.35 (m, 2H), 7.37−7.50 (m, 3H), 12.23 (s, br, 1H).

3-(4-Amino-5-phenylthieno[2,3-*d*]pyrimidin-6-yl)propionamide (45). Compound **45** was prepared using the same procedure as described for the synthesis of **5** by substituting **44** for **4**. In addition to the formation of the pyrimidine ring, the acid group in **44** was converted into the amide in the reaction. ^{1}H NMR (DMSO-d_6) δ 2.35 (t, *J* = 7.63 Hz, 2H), 2.82 (t, *J* = 7.63 Hz, 2H), 6.83 (s, br, 2H), 7.33 (s, br, 1H), 7.39−7.46 (m, 2H), 7.51−7.59 (m, 3H), 8.27 (s, 1H); MS (ESI) *m/z* 299 (M + H)$^+$.

3-[4-Amino-5-(4-nitrophenyl)thieno[2,3-*d*]pyrimidin-6-yl]propionamide (49a). To a suspension of **45** (2.50 g, 7.66 mmol) in concentrated sulfuric acid (25 mL) at 0 °C was added dropwise a solution of concentrated HNO_3 (0.53 mL) in concentrated sulfuric acid (8 mL). The mixture was stirred at 0 °C for 1 h and, then, at room temperature for 45 min, poured into ice, and neutralized with solid Na_2CO_3. The yellow solid was filtered, washed with water, dried over $MgSO_4$, and concentrated to provide the title compound (2.13 g, 81%). ^{1}H NMR (DMSO-d_6) δ 2.37 (t, *J* = 7.46 Hz, 2H), 2.84 (t, *J* = 7.46 Hz, 2H), 6.84 (s, 1H), 7.33 (s, br, 1H), 7.69 (d, *J* = 8.82 Hz, 2H), 8.30 (s, br, 1H), 8.36 (d, *J* = 8.48 Hz, 2H); MS (ESI) *m/z* 344 (M + H)$^+$.

3-[4-Amino-5-(4-aminophenyl)thieno[2,3-*d*]pyrimidin-6-yl]propionamide (50a). Compound **50a** was prepared using the same procedure as described for the synthesis of **6** by substituting **49a** for **5** and using *i*-PrOH/$CHCl_3$ (1:3) for the extraction instead of ethyl acetate during the aqueous workup. ^{1}H NMR (DMSO-d_6) δ 2.34 (dd, *J* = 8.48, 6.44 Hz, 2H), 2.82 (dd, *J* = 8.65, 6.61 Hz, 2H), 5.42 (s, br, 2H), 6.70 (d, *J* = 8.48 Hz, 2H), 6.83 (s, br, 1H), 7.01 (d, *J* = 8.48 Hz, 2H), 7.32 (s, br, 1H), 8.23 (s, 1H); MS (ESI) *m/z* 314 (M + H)$^+$.

3-{4-Amino-5-[4-({[(3-methylphenyl)amino]carbonyl}-amino)phenyl]thieno[2,3-*d*]pyrimidin-6-yl}propanamide (51). To a solution of **50a** (60 mg, 0.19 mmol) in DMF (3 mL) at 0 °C was added 3-tolyl isocyanate (24 μL, 0.19 mmol). The mixture was stirred overnight as the temperature rose slowly to room temperature. After DMF was removed, the crude material was purified by preparative high-performance liquid chromatography (HPLC) on a Waters Symmetry C8 column (25 mm × 100 mm, 7 μm particle size) using a gradient of 10−100% acetonitrile:0.1% aqueous trifluoroacetic acid (TFA) over 8 min (10 min run time) at a flow rate of 40 mL/min to provide the title compound as the trifluoroacetate salt (35 mg, 41%). ^{1}H NMR (DMSO-d_6) δ 2.29 (s, 3H), 2.36 (t, *J* = 7.63 Hz, 2H), 2.85 (t, *J* = 7.46 Hz, 2H), 6.81 (d, *J* = 7.46 Hz, 1H), 6.85 (s, br, 1H), 7.17 (t, *J* = 7.80 Hz, 1H), 7.26 (d, *J* = 9.00 Hz, 1H), 7.32 (m, 4H), 7.64 (d, *J* = 8.81 Hz, 2H), 8.33 (s, 1H), 8.71 (s, 1H), 8.93 (s, 1H); MS (ESI) *m/z* 447 (M + H)$^+$. Anal. ($C_{23}H_{22}N_6O_2S\cdot 1.0CF_3CO_2H$) C, H, N.

6,6-Dicyano-5-phenylhex-5-enoic Acid Methylamide (46a). A mixture of **43** (5.0 g, 20.81 mmol), methylamine hydrochloride (1.41 g, 20.81 mmol), HOBT (3.09 g, 22.89 mmol), EDC (4.39 g, 22.89 mmol), and NMM (5.7 mL, 52.03 mmol) in DMF was stirred at room temperature overnight and then partitioned between ethyl acetate and water. The aqueous phase was extracted with ethyl acetate, and the combined extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude material was purified by flash column chromatography on silica gel eluting with ethyl acetate to provide the title compound (3.94 g, 75%). ^{1}H NMR (DMSO-d_6) δ 1.42−1.60 (m, 2H), 2.05 (t, *J* = 7.46 Hz, 2H), 2.54 (d, *J* = 4.41 Hz, 3H), 2.98 (t, *J* = 7.50 Hz, 2H), 7.50−7.69 (m, 5H), 7.75 (s, 1H); MS (ESI) *m/z* 271 (M + NH_4)$^+$.

3-(5-Amino-4-cyano-3-phenylthiophen-2-yl)-*N*-methylpropionamide (47a). Compound **47a** was prepared using the same procedure as described for the synthesis of **4** by substituting **46a** for **3**. ^{1}H NMR (DMSO-d_6) δ 2.26 (t, *J* = 7.46 Hz, 2H), 2.54 (d, *J* = 4.41 Hz, 3H), 2.70 (t, *J* = 7.46 Hz, 2H), 7.07 (s, 2H), 7.28−7.51 (m, 5 H), 7.77 (s, br, 1H); MS (ESI) *m/z* 286 (M + H)$^+$.

3-(4-Amino-5-phenylthieno[2,3-*d*]pyrimidin-6-yl)-*N*-methylpropionamide (48a). Compound **48a** was prepared using the same procedure as described for the synthesis of **5** by substituting **47a** for **4**. ^{1}H NMR (DMSO-d_6) δ 2.36 (t, *J* = 7.63 Hz, 2H), 2.54 (d, *J* = 4.41 Hz, 3H), 2.83 (t, *J* = 7.46 Hz, 2H), 7.38−7.45 (m, 2H), 7.51−7.62 (m, 3H), 7.79 (s, br, 1H), 8.27 (s, 1H); MS (ESI) *m/z* 313 (M + H)$^+$.

3-{4-Amino-5-[4-({[(3-methylphenyl)amino]carbonyl}-amino)phenyl]thieno[2,3-*d*]pyrimidin-6-yl}-*N*-methylpropanamide (52). Compound **52** was prepared as the trifluoroacetate salt using the same sequence as described for the synthesis of **51** by substituting **48a** for **45**. ^{1}H NMR (DMSO-d_6) δ 2.29 (s, 3H), 2.36 (t, *J* = 7.46 Hz, 2H), 2.54 (d, *J* = 4.75 Hz, 3H), 2.86 (t, *J* = 7.29 Hz, 2H), 6.81 (d, *J* = 7.46 Hz, 1H), 7.17 (t, *J* = 7.80 Hz, 1H), 7.26 (m, 1H), 7.29−7.34 (m, 3H), 7.64 (d, *J* = 8.81 Hz, 2H), 7.80 (m, 1H), 8.33 (s, 1H), 8.71 (s, 1H), 8.93 (s, 1H); MS (ESI) *m/z* 461 (M + H)$^+$.

3-{4-Amino-5-[4-(3-*m*-tolylureido)phenyl]thieno[2,3-*d*]-pyrimidin-6-yl}-*N*,*N*-dimethylpropionamide (53). Compound **53** was prepared as the trifluoroacetate salt using the same sequence as described for the synthesis of **51** by substituting **48b** for **45**. ^{1}H NMR (DMSO-d_6) δ 2.29 (s, 3H), 2.60 (t, *J* = 7.12 Hz, 2H), 2.80 (s, 3H), 2.83−2.92 (m, 5H), 6.81 (d, *J* = 7.12 Hz, 1H), 7.17 (t, *J* = 7.50 Hz, 1H), 7.26 (d, *J* = 7.50 Hz, 1H), 7.29−7.37 (m, 3H), 7.64 (d, *J* = 8.48 Hz, 2H), 8.36 (s, 1H), 8.73 (s, 1H), 8.94 (s, 1H); MS (ESI) *m/z* 475 (M + H)$^+$.

[6-Methyl-5-(4-nitrophenyl)thieno[2,3-*d*]pyrimidin-4-yl]carbamic Acid *tert*-Butyl Ester (54). To a solution of **5** (1.50 g, 5.24 mmol) in THF (40 mL) at 0 °C was added sodium hydride (60% dispersion in mineral oil, 524 mg, 13.10 mmol) in portions. After the mixture was stirred at 0 °C for 10 min, a solution of di-*tert*-butyl dicarbonate (1.26 g, 5.76 mmol) in THF (10 mL) was dropwise added. The mixture was stirred at 0 °C for 2 h, quenched with saturated aqueous ammonium chloride solution, and extracted with ethyl acetate three times. The extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The solid residue was triturated with ethyl acetate to provide the title compound (1.02 g, 63%). ^{1}H NMR (DMSO-d_6) δ ppm 1.06 (s, 9H), 2.50 (s, 3H), 7.63 (d, *J* = 8.82 Hz, 2H), 8.27 (d, *J* = 8.82 Hz, 2H), 8.83 (s, 1H), 9.73 (s, 1H); MS (CI) *m/z* 387 (M + H)$^+$.

[6-Morpholin-4-ylmethyl-5-(4-nitrophenyl)thieno[2,3-*d*]pyrimidin-4-yl]carbamic Acid *tert*-Butyl Ester (56a). To a suspension of **54** (1.0 g, 2.59 mmol) in benzene (100 mL) was added *N*-bromosuccinimide (NBS) (0.51 g, 2.85 mmol) and 2,2′-azobisisobutyronitrile (AIBN) (100 mg). After being stirred at 80 °C for 3 h, the mixture was cooled, absorbed on silica gel, and purified by flash column chromatography eluting with hexanes/ethyl acetate (3:2) to provide a mixture of bromide **55** and the unreacted **54** (730 mg) with a ratio of 3:1.

The above mixture was dissolved in DMF (15 mL) and treated with morpholine (994 μL, 11.4 mmol) at room temperature. After being stirred at room temperature overnight, the reaction mixture was poured into a saturated sodium bicarbonate solution, extracted with ethyl acetate, washed with water and brine, dried with $MgSO_4$, filtered, and concentrated. The crude product was purified by flash column chromatography on silica gel eluting with ethyl acetate/hexanes (2:1) to provide the title compound (476 mg, 39% from **54**). MS (ESI) *m/z* 472 $(M + H)^+$.

[6-Morpholin-4-ylmethyl-5-(4-nitrophenyl)thieno[2,3-*d*]pyrimidin-4-yl]carbamic Acid *tert*-Butyl Ester (57a). To a solution of **56a** (220 mg, 0.47 mmol) in methylene chloride (4 mL) at 0 °C was added trifluoroacetic acid (4 mL). After being stirred at 0 °C for 1.5 h, the mixture was concentrated. The residue was neutralized with saturated aqueous sodium bicarbonate solution, extracted with chloroform/2-propanol (3:1), dried over $MgSO_4$, filtered, and concentrated to provide the title compound (170 mg, 98%). ^{1}H NMR (DMSO-d_6) δ 2.35 (t, *J* = 4.41 Hz, 4H), 3.51 (s, 2H), 3.55 (t, *J* = 4.41 Hz, 4H), 7.67 (d, *J* = 8.82 Hz, 2H), 8.31 (s, 1H), 8.36 (d, *J* = 8.81 Hz, 2H).

5-(4-Aminophenyl)-6-morpholin-4-ylmethylthieno[2,3-*d*]pyrimidin-4-ylamine (58a). Compound **58a** was prepared using the same procedure as described for the synthesis of **6** by substituting **57a** for **5**. ^{1}H NMR (DMSO-d_6) δ 2.35 (t, br, *J* = 3.73 Hz, 4H), 3.50 (s, 2H), 3.55 (t, *J* = 4.41 Hz, 4H), 5.41 (s, 2H), 6.68 (d, *J* = 8.48 Hz, 2H), 6.99 (d, *J* = 8.48 Hz, 2H), 8.24 (s, 1 H); MS (ESI) *m/z* 342 $(M + H)^+$.

***N*-{4-[4-Amino-6-(morpholin-4-ylmethyl)thieno[2,3-*d*]-pyrimidin-5-yl]phenyl}-*N*′-(3-methylphenyl)urea (59).** Compound **59** was prepared using the same procedure as described for the synthesis of **11** by substituting **58a** for **6**. ^{1}H NMR (DMSO-d_6) δ 8.90 (s, 1H), 8.69 (s, 1H), 8.28 (s, 1H), 7.63 (d, *J* = 8.7 Hz, 2H), 7.33−7.23 (m, 4H), 7.17 (t, *J* = 7.5 Hz, 1H), 6.81 (d, *J* = 7.5 Hz, 1H), 3.56 (s, br, 4H), 3.51 (s, 2H), 2.36 (s, br, 4H), 2.29 (s, 3H); MS (ESI) *m/z* 475 $(M + H)^+$. Anal. ($C_{25}H_{26}N_6O_2S$·$0.5H_2O$) C, H, N.

***N*-(4-{4-Amino-6-[(4-methylpiperazin-1-yl)methyl]-thieno[2,3-*d*]pyrimidin-5-yl}phenyl)-*N*′-(3-methylphenyl)urea (60).** Compound **60** was prepared using the same sequence as described for the synthesis of **59** by substituting *N*-methylpiperazine for morpholine in the case of **56a**. ^{1}H NMR (DMSO-d_6) δ 8.88 (s, 1H), 8.68 (s, 1H), 8.27 (s, 1H), 7.62 (d, *J* = 9.0 Hz, 2H), 7.32−7.23 (m, 4H), 7.17 (t, *J* = 7.5 Hz, 1H), 6.81 (d, *J* = 7.5 Hz, 1H), 3.50 (s, 2H), 2.45−2.20 (s, br, 8H), 2.29 (s, 3H), 2.14 (s, 3H).

***N*-(4-{4-Amino-6-[(dimethylamino)methyl]thieno[2,3-*d*]pyrimidin-5-yl}phenyl)-*N*′-(3-methylphenyl)urea (61).** Compound **61** was prepared using the same sequence as described for the synthesis of **59** by substituting dimethylamine for morpholine in the case of **56a**. ^{1}H NMR (DMSO-d_6) δ 8.92 (s, 1H), 8.71 (s, 1H), 8.28 (s, 1H), 7.32−7.22 (m, 4H), 7.17 (t, *J* = 7.8 Hz, 1H), 6.81 (d, *J* = 7.8 Hz, 1H), 3.45 (s, br, 2H), 2.29 (s, 3H), 2.17 (s, 6H); MS (ESI) *m/z* 433 $(M + H)^+$.

5-(4-Bromophenyl)-6-methylthieno[2,3-*d*]pyrimidin-4-amine (62). Compound **62** was prepared using the same sequence as described for the synthesis of **5** by substituting 4-bromophenyl ethyl ketone for **2**. ^{1}H NMR (DMSO-d_6) δ 8.27 (s, 1H), 7.74 (d, *J* = 8.1 Hz, 2H), 7.36 (d, *J* = 8.1 Hz, 2H), 2.28 (s, 3H); MS (ESI) *m/z* 320, 322 $(M + H)^+$.

4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)benzoic Acid (63). To a solution of **62** (1.5 g, 4.68 mmol) in THF (50 mL) at −78 °C was dropwise added 2.5 M *n*-butyllithium in hexanes (4.7 mL, 11.71 mmol). After being stirred at −78 °C for 30 min, the mixture was treated with an excess of dry ice and then stirred at −78 °C for an additional 30 min, warmed to room temperature, diluted with water, and adjusted to pH 3 with aqueous 2 N HCl. The solid material was filtered to provide the title compound (686 mg, 51%). ^{1}H NMR (DMSO-d_6) δ 13.13 (s, br, 1H), 8.29 (s, 1H), 8.09 (d, *J* = 8.4 Hz, 2H), 7.53 (d, *J* = 8.4 Hz, 2H), 2.28 (s, 3H); MS (CI) *m/z* 285 (M^+).

4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-*N*-phenylbenzamide (64). A suspension of **63** (89 mg, 0.31 mmol) and 1-hydroxybenzotriazole (46 mg, 0.35 mmol) in DMF (4 mL) at room temperature was treated with *m*-toluidine (29 μL, 0.31 mmmol), 4-methylmorpholine (86 μL, 0.78 mmol), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (66 mg, 0.34 mmol). After being stirred at room temperature overnight, the reaction mixture was partitioned between water and ethyl acetate. The aqueous phase was extracted with ethyl acetate three times, and the combined organic extracts were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was triturated with hexanes/ethyl acetate to provide the title compound (84 mg, 75%). ^{1}H NMR (DMSO-d_6) δ 10.40 (s, 1H), 8.30 (s, 1H), 8.12 (d, *J* = 8.4 Hz, 2H), 7.80 (d, *J* = 7.5 Hz, 2H), 7.58 (d, *J* = 8.4 Hz, 2H), 7.37 (t, *J* = 7.5 Hz, 2H), 7.12 (t, *J* = 7.5 Hz, 1H), 2.32 (s, 3H); MS (ESI) *m/z* 361 $(M + H)^+$. Anal. ($C_{20}H_{16}N_4OS$·0.1EtOAc) C, H, N.

***N*-[4-(4-Amino-6-methylthieno[2,3-d]pyrimidin-5-yl)-phenyl]-*N*′-(3-methylphenyl)thiourea (65).** Compound **65** was prepared using the same procedure as described for the synthesis of **11** by substituting 3-tolyl thioisocyanate for 3-tolyl isocyanate. ^{1}H NMR (DMSO-d_6) δ ppm 2.30 (s, 6H), 6.97 (d, *J* = 6.78 Hz, 1H), 7.17−7.30 (m, 3H), 7.34 (d, *J* = 8.14 Hz, 2H), 7.64 (d, *J* = 8.48 Hz, 2H), 8.27 (s, 1H), 9.84 (s, 1H), 9.95 (s, 1H); MS (ESI) *m/z* 406 $(M + H)^+$.

Phenyl *N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)phenyl]-*N*′-cyanoimidocarbamate (66). A mixture of **6** (0.40 g, 1.56 mmol) and diphenyl cyanocarbonimidate (0.37 g, 1.56 mmol) in DMF (10 mL) was heated at 90 °C for 2 days. After being cooled to room temperature, the reaction mixture was poured into water, and the solid material was filtered. The filter cake was suspended in hot ethanol and filtered. The filtrate was concentrated and purified by flash column chromatography on silica gel eluting with 5−8% methanol in dichloromethane to provide the title compound (150 mg, 24%). ^{1}H NMR (DMSO-d_6) δ 11.06 (s, 1H), 8.27 (s, 1H), 7.67 (d, *J* = 8.4 Hz, 2H), 7.50−7.43 (m, 4H), 7.36−7.29 (m, 3H), 2.29 (s, 3H); MS (ESI) *m/z* 401 $(M + H)^+$.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N*″-cyano-*N*′-(3-methylphenyl)guanidine (67).** A solution of **66** (40 mg, 0.01 mmol) and 3-methylaniline (12 μL, 0.01 mmol) in DMF (1 mL) was heated in a Smith Synthesizer microwave at 180 °C for 20 min. After being cooled, the reaction mixture was partitioned between water and ethyl acetate. The aqueous phase was extracted with ethyl acetate three times, and the combined extracts were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by flash column chromatography on silica gel eluting with 8% methanol in dichloromethane to provide the title compound (15 mg, 38%). ^{1}H NMR (DMSO-d_6) δ 9.65 (s, 1H), 9.48 (s, 1H), 8.26 (s, 1H), 7.45 (d, *J* = 8.4 Hz, 2H), 7.35 (d, *J* = 8.4 Hz, 2H), 7.23 (t, *J* = 7.8 Hz, 1H), 7.15−7.10 (m, 2H), 6.96 (d, *J* = 7.8 Hz, 1H), 2.29 (s, 6H); MS (ESI) *m/z* 414 $(M + H)^+$.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-1*H*-indole-2-carboxamide (68).** Compound **68** was prepared using the same procedure as described for the synthesis of **7** by substituting indole-2-carboxylic acid for *m*-toluic acid. ^{1}H NMR (DMSO-d_6) δ 11.80 (s, 1H), 10.40 (s, 1H), 8.28 (s, 1H), 8.01 (d, *J* = 8.7 Hz, 2H), 7.70 (d, *J* = 7.5 Hz, 1H), 7.48 (d, *J* = 7.5 Hz, 2H), 7.42 (d, *J* = 8.7 Hz, 2H), 7.24 (t, *J* = 7.5 Hz, 1H), 7.08 (t, *J* = 7.5 Hz, 1H), 2.32 (s, 3H); MS (ESI) *m/z* 400 $(M + H)^+$.

5-[4-(1,3-Benzoxazol-2-ylamino)phenyl]-6-methylthieno-[2,3-*d*]pyrimidin-4-amine (69). A solution of **6** (100 mg, 0.39 mmol) in pyridine (3 mL) was added dropwise over 5 min to a

solution of 1,1-thiocarbonyldiimidazole (77 mg, 0.39 mmol) in pyridine (3 mL) at 0 °C. After being stirred at 0 °C for 1.5 h, the mixture was treated with 2-aminophenol (43 mg, 0.39 mmol) and stirred at room temperature overnight. The reaction mixture was then treated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (90 mg, 0.47 mmol), heated at 50 °C for 20 h, and then concentrated. The residue was partitioned between ethyl acetate and water. The aqueous phase was extracted with ethyl acetate, and the combined extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by flash column chromatography on silica gel eluting with ethyl acetate to provide the title compound (28 mg, 20%). ^{1}H NMR (DMSO-d_6) δ 10.89 (s, 1H), 8.27 (s, 1H), 7.94 (d, J = 8.4 Hz, 2H), 7.51 (m, 2H), 7.42 (d, J = 8.4 Hz, 2H), 7.25 (td, J = 7.5, 1.5 Hz, 1H), 7.16 (td, J = 7.5, 1.5 Hz, 1H), 3.10 (s, 3H); MS (CI) *m/z* 374 (M + H)$^+$. Anal. ($C_{20}H_{15}N_5OS{\cdot}0.2EtOAc{\cdot}0.2H_2O$) C, H, N.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-2-(3-methylphenyl)acetamide (70).** Compound **70** was prepared using the same procedure as described for the synthesis of **7** by substituting *m*-tolylacetic acid for *m*-toluic acid. ^{1}H NMR (DMSO-d_6) δ 10.37 (s, 1H), 8.26 (s, 1H), 7.78 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 8.4 Hz, 2H), 7.26−7.13 (m, 3H), 7.07 (d, J = 7.5 Hz, 1H), 3.64 (s, 2H), 2.31 (s, 3H), 2.27 (s, 3H); MS (ESI) 389 (M + H)$^+$. Anal. ($C_{22}H_{20}N_4OS$) C, H, N.

6-Methyl-5-[4-(methylamino)phenyl]thieno[2,3-*d*]pyrimidin-4-amine (71). A suspension of **6** (400 mg, 1.56 mmol) in dichloromethane (10 mL) and THF (10 mL) at −20 °C was treated with formic acetic anhydride (135 μL, 1.7 mmol). After being stirred at −20 °C for 1 h, the reaction mixture was concentrated. The concentrate was then suspended in benzene (50 mL), treated with 65% Red-Al in toluene (2.4 mL, 7.8 mmol), and stirred first at room temperature for 20 min and then at reflux for 6 h. The mixture was cooled to room temperature and partitioned between aqueous Rochelle's salt solution and ethyl acetate. The aqueous phase was extracted with ethyl acetate three times. The combined extracts were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by flash column chromatography on silica gel eluting with 7% methanol in dichloromethane to provide the title compound (89 mg, 21%). ^{1}H NMR (DMSO-d_6) δ 2.27 (s, 3H), 2.73 (d, J = 5.09 Hz, 3H), 5.99 (q, J = 4.97 Hz, 1H), 6.67 (d, J = 8.48 Hz, 2H), 7.08 (d, J = 8.48 Hz, 2H), 8.23 (s, 1H); MS (ESI) *m/z* 271 (M + H)$^+$.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N*-methyl-*N′*-(3-methylphenyl)urea (72).** Compound **72** was prepared using the same procedure as described for the synthesis of **9** by substituting **71** for **6**. ^{1}H NMR (DMSO-d_6) δ 8.32 (s, 1H), 8.28 (s, 1H), 7.47 (d, J = 8.4 Hz, 2H), 7.40 (d, J = 8.4 Hz, 2H), 7.29−7.23 (m, 2H), 7.12 (t, J = 7.8 Hz, 1H), 6.78 (d, J = 7.8 Hz, 1H), 3.33 (s, 3H), 2.33 (s, 3H), 2.25 (s, 3H); MS (ESI) *m/z* 404 (M + H)$^+$.

***N*-[4-(4-Amino-6-methylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N′*-methyl-*N′*-(3-methylphenyl)urea (73).** Compound **73** was prepared using the same procedure as described for the synthesis of **32** by substituting *N*-methyl-*m*-toluidine for cyclohexanylmethylamine. ^{1}H NMR (DMSO-d_6) δ 2.28 (s, 3H), 2.34 (s, 3H), 3.28 (s, 3H), 7.09 (d, J = 7.32 Hz, 1H), 7.14 (d, J = 7.93 Hz, 1H), 7.19 (s, 1H), 7.24 (d, J = 8.54 Hz, 2H), 7.31 (t, J = 7.78 Hz, 1H), 7.62 (d, J = 8.54 Hz, 2H), 8.26 (s, 1H), 8.30 (s, 1H); MS (ESI) *m/z* 404 (M + H)$^+$. Anal. ($C_{22}H_{21}N_5OS{\cdot}0.2EtOAc$) C, H, N.

***N*-[4-(4-Amino-6-ethylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N′*-(3-methylphenyl)urea (74).** Compound **74** was prepared following the same sequence as described for the synthesis of **11** by replacing **2** with 1-(4-nitrophenyl)butan-1-one, which was prepared using the same procedure as described for the synthesis of ketone **36a** by substituting *n*-propyl iodide for *tert*-butyl(3-iodopropoxy)dimethylsilane (**35a**). ^{1}H NMR (DMSO-d_6) δ 8.84 (s, 1H), 8.64 (s, 1H), 8.24 (s, 1H), 7.62 (d, J = 8.5 Hz, 2H), 7.10−7.35 (m, 5H), 6.80 (d, J = 7.2 Hz, 1H), 2.62 (q, J = 7.5 Hz, 2H), 2.24 (s, 3H), 1.18 (t, J = 7.5 Hz, 3H); MS (ESI) *m/z* 404 (M + H)$^+$. Anal. ($C_{22}H_{21}N_5OS{\cdot}0.15EtOAc$) C, H, N.

***N*-[4-(4-Amino-6-ethylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N′*-cyclohexylurea (75).** Compound **75** was prepared using the same procedure as described for the synthesis of **9** by substituting isocyanatocyclohexane for phenyl isocyanate. ^{1}H NMR (DMSO-d_6) δ 1.00−1.90 (m, 14H), 2.63 (q, J = 7.69 Hz, 2H), 6.17 (d, J = 7.80 Hz, 1H), 7.23 (d, J = 8.48 Hz, 2H), 7.54 (d, J = 8.48 Hz, 2H), 8.27 (s, 1H), 8.54 (s, 1H); MS (ESI) *m/z* 396 (M + H)$^+$. Anal. ($C_{21}H_{25}N_5OS{\cdot}0.3H_2O$) C, H, N.

***N*-[4-(4-Aminothieno[2,3-*d*]pyrimidin-5-yl)phenyl]-*N′*-(3-methylphenyl)urea (76).** Compound **76** was prepared following the same sequence as described for the synthesis of **11** by replacing **2** with 4′-nitroacetophenone. ^{1}H NMR (DMSO-d_6) δ 8.88 (s, 1H), 8.67 (s, 1H), 8.34 (s, 1H), 7.61 (d, J = 8.7 Hz, 2H), 7.43 (s, 1H), 7.39 (d, J = 8.7 Hz, 2H), 7.31 (s, 1H), 7.25 (d, J = 7.5 Hz, 1H), 7.17 (t, J = 7.5 Hz, 1H), 6.81 (d, J = 7.5 Hz, 1H); MS (ESI) *m/z* 376 (M + H)$^+$. Anal. ($C_{20}H_{17}N_5OS$) C, H, N.

***N*-[4-(4-Aminothieno[2,3-*d*]pyrimidin-5-yl)phenyl]-*N′*-butylurea (77).** Compound **77** was prepared following the same sequence as described for the synthesis of **32** by replacing **2** with 4′-nitroacetophenone in the case of **3** and *n*-butylamine for cyclohexanylamine in the case of **32**, respectively. ^{1}H NMR (DMSO-d_6) δ 0.90 (t, J = 7.1 Hz, 3H), 1.26−1.47 (m, 4H), 3.10 (q, J = 6.4 Hz, 2H), 6.20 (t, J = 5.3 Hz, 1H), 7.32 (d, J = 8.8 Hz, 2H), 7.43 (s, 1H), 7.54 (d, J = 8.8 Hz, 2H), 8.36 (s, 1H), 8.61 (s, 1H); MS (ESI) *m/z* 342 (M + H)$^+$. Anal. ($C_{17}H_{19}N_5OS{\cdot}0.5CF_3CO_2H$) C, H, N.

***N*-[4-(4-Aminothieno[2,3-*d*]pyrimidin-5-yl)phenyl]-*N′*-isobutylurea (78).** Compound **78** was prepared using the same procedure as described for the synthesis of **77** by substituting 2-methylpropylamine for *n*-butylamine. ^{1}H NMR (DMSO-d_6) δ 0.88 (d, J = 6.4 Hz, 6H), 1.66−1.75 (m, 1H), 2.94 (apparent t, J = 6.3 Hz, 2H), 6.26 (t, J = 5.9 Hz, 1H), 7.33 (d, J = 8.5 Hz, 2H), 7.44 (s, 1H), 7.54 (d, J = 8.5 Hz, 2H), 8.38 (s, 1H), 8.62 (s, 1H); MS (ESI) *m/z* 342 (M + H)$^+$. Anal. ($C_{17}H_{19}N_5OS{\cdot}0.6CF_3CO_2H$) C, H, N.

***N*-[4-(4-Amino-6-propylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N′*-(3-methylphenyl)urea (79).** Compound **79** was prepared following the same sequence as described for the synthesis of **11** by replacing **2** with 1-(4-nitrophenyl)pentan-1-one, which was prepared using the same procedure as described for the synthesis of ketone **36a** by substituting *n*-butyl iodide for **35a**. ^{1}H NMR (DMSO-d_6) δ 0.85 (t, J = 7.29 Hz, 3H), 1.50−1.68 (m, 2H), 2.29 (s, 3H), 2.61 (t, J = 7.63 Hz, 2H), 6.81 (d, J = 7.80 Hz, 1H), 7.24 (m, 5H), 7.62 (d, J = 8.48 Hz, 2H), 8.26 (s, 1H), 8.67 (s, 1H), 8.87 (s, 1H); MS (ESI) *m/z* 418 (M + H)$^+$. Anal. ($C_{23}H_{23}N_5OS{\cdot}0.2H_2O$) C, H, N.

***N*-[4-(4-Amino-6-isopropylthieno[2,3-*d*]pyrimidin-5-yl)phenyl]-*N′*-(3-methylphenyl)urea (80).** Compound **80** was prepared following the same sequence as described for the synthesis of **11** by replacing **2** with 1-(4-nitrophenyl)-3-methylbutan-1-one, which was prepared using the same procedure as described for the synthesis of ketone **36a** by substituting isobutyl iodide for **35a**. ^{1}H NMR (DMSO-d_6) δ 1.20−1.23 (d, J = 6.9 Hz, 6H), 2.29 (s, 3H), 3.00−3.09 (m, 1H), 6.79−6.82 (d, J = 7.8 Hz, 1H), 7.14−7.19 (t, J = 7.5 Hz, 1H), 7.24−7.27 (d, J = 8.1 Hz, 1H), 7.30−7.33 (m, 3H), 7.61−7.64 (d, J = 9.0 Hz, 2H), 8.26 (s, 1H), 8.67 (s, 1H), 8.88 (s, 1H); MS (ESI) *m/z* 418 (M + H)$^+$. Anal. ($C_{23}H_{23}N_5OS$) C, H, N.

***N*-[4-(4-Amino-6-benzylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N′*-(3-methylphenyl)urea (81).** Compound **81** was prepared following the same sequence as described for the synthesis of **11** by replacing **2** with 1-(4-nitrophenyl)-3-phenylpropan-1-one, which was prepared using the same procedure as described for the synthesis of ketone **36a** by substituting (2-iodoethyl)benzene for **35a**. ^{1}H NMR (DMSO-d_6) δ 2.29 (s, 3H), 3.99 (s, 2H), 6.79−6.82 (d, J = 7.5 Hz, 1H), 7.14−7.32 (m, 8H), 7.35−7.38 (d, J = 8.7 Hz, 2H), 7.63−7.66 (d, J = 8.4 Hz, 2H), 8.26 (s, 1H), 8.67 (s, 1H), 8.89 (s, 1H); MS (ESI) *m/z* 466 (M + H)$^+$. Anal. ($C_{27}H_{23}N_5OS{\cdot}0.75H_2O$) C, H, N.

***N*-{4-[4-Amino-6-(pyridin-3-ylmethyl)thieno[2,3-*d*]-pyrimidin-5-yl]phenyl}-*N′*-(3-methylphenyl)urea (82).**

Compound **82** was prepared following the same sequence as described for the synthesis of **11** by replacing **2** with **34a**. ^{1}H NMR (DMSO-d_6) δ 2.29 (s, 3H), 4.04 (s, 2H), 6.79−6.82 (d, *J* = 7.5 Hz, 1H), 7.14−7.19 (t, *J* = 7.8 Hz, 1H), 7.24−7.33 (m, 3H), 7.35−7.37 (d, *J* = 8.4 Hz, 2H), 7.53−7.56 (td, *J* = 2.1, 7.8 Hz, 1H), 7.63−7.66 (d, *J* = 8.4 Hz, 2H), 8.27 (s, 1H), 8.34−8.35 (d, *J* = 1.8 Hz, 1H), 8.42−8.44 (dd, *J* = 1.5, 4.8 Hz, 1H), 8.67 (s, 1H), 8.89 (s, 1H); MS (ESI) *m/z* 467 (M + H)$^+$. Anal. ($C_{26}H_{22}N_6OS{\cdot}0.2H_2O$) C, H, N.

***N*-{4-[4-Amino-6-(pyridin-4-ylmethyl)thieno[2,3-*d*]pyrimidin-5-yl]phenyl}-*N*′-(3-methylphenyl)urea (83).** Compound **83** was prepared following the same sequence as described for the synthesis of **11** by replacing **2** with **34b**. ^{1}H NMR (DMSO-d_6) δ 2.28 (s, 3H), 3.32 (s, 2H), 6.79−6.82 (d, *J* = 7.5 Hz, 1H), 7.13−7.30 (m, 5H), 7.32−7.35 (d, *J* = 8.4 Hz, 2H), 7.61−7.64 (d, *J* = 8.4 Hz, 2H), 8.28 (s, 1H), 8.45−8.47 (dd, *J* = 4.2, 1.5 Hz, 2H), 8.67 (s, 1H), 8.88 (s, 1H); MS (ESI) *m/z* 467 (M + H)$^+$; HRMS (FAB) Calcd for $C_{26}H_{23}N_6OS$ 467.1654, found 467.1649.

***N*-{4-[4-Amino-6-(2-hydroxyethyl)thieno[2,3-*d*]pyrimidin-5-yl]phenyl}-*N*′-(3-methylphenyl)urea (84).** Compound **84** was prepared following the same sequence as described for the synthesis of **11** by replacing **2** with **36a**, except that the reaction of the corresponding amino nitrile with formamide was conducted in a Smith Synthesizer microwave (70 min at 180 °C) to form the pyrimidine ring and that the *tert*-butyldimethylsilyl group was removed with tetrabutylammonium fluoride (TBAF) (in THF at room temperature) in the last step. ^{1}H NMR (DMSO-d_6) δ 2.29 (s, 3H), 2.75−2.80 (t, *J* = 6.6 Hz, 2H), 3.54−3.60 (m, 2H), 4.85−4.89 (t, *J* = 5.7 Hz, 1H), 6.79−6.82 (d, *J* = 7.5 Hz, 2H), 7.14−7.19 (t, *J* = 7.5 Hz, 1H), 7.24−7.32 (m, 4H), 7.61−7.63 (d, *J* = 8.4 Hz, 2H), 8.26 (s, 1H), 8.67 (s, 1H), 8.87 (s, 1H); MS (ESI) *m/z* 420 (M + H)$^+$. Anal. ($C_{22}H_{21}N_5O_2S{\cdot}0.4H_2O$) C, H, N.

***N*-{4-[4-Amino-6-(2-methoxyethyl)thieno[2,3-*d*]pyrimidin-5-yl]phenyl}-*N*′-(3-methylphenyl)urea (85).** Compound **85** was prepared following the same sequence as described for **11** by replacing **2** with 4-methoxy-1-(4-nitrophenyl)butan-1-one, which was prepared using the same procedure as described for the synthesis of ketone **36a** by substituting 3-methoxypropyl iodide for **35a**. ^{1}H NMR (DMSO-d_6) δ 2.29 (s, 3H), 2.83−2.87 (t, *J* = 6.6 Hz, 2H), 3.22 (s, 3H), 3.47−3.52 (t, *J* = 6.6 Hz, 2H), 6.79−6.82 (d, *J* = 7.5 Hz, 2H), 7.14−7.19 (t, *J* = 7.5 Hz, 1H), 7.24−7.32 (m, 4H), 7.61−7.64 (d, *J* = 9.0 Hz, 2H), 8.27 (s, 1H), 8.67 (s, 1H), 8.87 (s, 1H); MS (ESI) *m/z* 434 (M + H)$^+$.

***N*-{4-[4-Amino-6-(3-hydroxypropyl)thieno[2,3-*d*]pyrimidin-5-yl]phenyl}-*N*′-(3-methylphenyl)urea (86).** Compound **86** was prepared following the same sequence as described for the synthesis of **84** by substituting **36b** for **36a**. ^{1}H NMR (DMSO-d_6) δ 1.70 (m, 2H), 2.29 (s, 3H), 2.68 (m, *J* = 6.27 Hz, 2H), 3.37 (t, *J* = 6.27 Hz, 2H), 6.81 (d, *J* = 7.80 Hz, 1H), 7.17 (t, *J* = 7.63 Hz, 1H), 7.23−7.33 (m, 4H), 7.62 (d, *J* = 8.81 Hz, 2H), 8.27 (s, 1H), 8.69 (s, 1H), 8.90 (s, 1H); MS (ESI) *m/z* 434 (M + H)$^+$.

***N*-(4-{4-Amino-6-[2-(dimethylamino)ethyl]thieno[2,3-*d*]pyrimidin-5-yl}phenyl)-*N*′-(3-methylphenyl)urea (87).** Compound **87** was prepared following the same sequence as described for the synthesis of **11** by substituting **39** for **2**. ^{1}H NMR (DMSO-d_6) δ 2.11 (s, 6H), 2.29 (s, 3H), 2.42−2.46 (t, *J* = 7.2 Hz, 2H), 2.72−2.77 (t, *J* = 6.0 Hz, 2H), 6.79−6.82 (d, *J* = 7.5 Hz, 1H), 7.14−7.19 (t, *J* = 7.5 Hz, 1H), 7.24−7.32 (m, 4H), 7.61−7.64 (d, *J* = 6.6 Hz, 2H), 8.26 (s, 1H), 8.67 (s, 1H), 8.87 (s, 1H); MS (ESI) *m/z* 447 (M + H)$^+$.

***N*-[4-(4-Aminothieno[2,3-*d*]pyrimidin-5-yl)-3-fluorophenyl]-*N*′-(3-methylphenyl)urea (88).** Compound **88** was prepared following the same sequence as described for the synthesis of **11** by substituting **41a** for **2**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.29 (s, 3H), 6.82 (d, *J* = 7.5 Hz, 1H), 7.18 (t, *J* = 7.8 Hz, 1H), 7.25 (d, *J* = 10.0 Hz, 1H), 7.27 (dd, *J* = 8.6, 2.0 Hz, 1H), 7.32 (s, 1H), 7.37 (t, *J* = 8.4 Hz, 1H), 7.52 (s, 1H), 7.66 (dd, *J* = 12.6, 2.0 Hz, 1H), 8.34 (s, 1H), 8.75 (s, 1H), 9.09 (s, 1H); MS (ESI) *m/z* 394.0 (M + H)$^+$. Anal. ($C_{20}H_{16}FN_5OS{\cdot}0.2H_2O$) C, H, N.

***N*-[4-(4-Aminothieno[2,3-*d*]pyrimidin-5-yl)-3-chlorophenyl]-*N*′-(3-methylphenyl)urea (89).** Compound **89** was prepared following the same sequence as described for the synthesis of **11** by substituting **41b** for **2**. ^{1}H NMR (DMSO-d_6) δ 2.29 (s, 3H), 6.82 (d, *J* = 7.5 Hz, 1H), 7.18 (t, *J* = 7.5 Hz, 1H), 7.23−7.26 (m, 1H), 7.31−7.34 (s, br, 1H), 7.41−7.42 (m, 2H), 7.49 (s, 1H), 7.91 (s, br, 1H), 8.33 (s, 1H), 8.75 (s, 1H), 9.05 (s, 1H); MS (ESI) *m/z* 409.9, 411.9 (M + H)$^+$. Anal. ($C_{20}H_{16}ClN_5O{\cdot}0.5H_2O$) C, H, N.

***N*-[4-(4-Aminothieno[2,3-*d*]pyrimidin-5-yl)-3-methoxyphenyl]-*N*′-(3-methylphenyl)urea (90).** Compound **90** was prepared following the same sequence as described for **11** by substituting **41c** for **2**. The crude material was purified by preparative HPLC using the conditions described for the purification of **51** to provide the title compound as the trifluoroacetate salt. ^{1}H NMR (DMSO-d_6) δ 2.29 (s, 3H), 3.72 (s, 3H), 6.81 (d, *J* = 7.1 Hz, 1H), 7.05 (dd, *J* = 8.1, 2.0 Hz, 1H), 7.14−7.25 (m, 3H), 7.34 (s, 1H), 7.38 (s, 1H), 7.50 (d, *J* = 2.0 Hz, 1H), 8.36 (s, 1H), 8.67 (s, 1H), 8.93 (s, 1H); MS (ESI) *m/z* 406.0 (M + H)$^+$. Anal. ($C_{21}H_{19}N_5O_2S{\cdot}1.0CF_3CO_2H{\cdot}0.2H_2O$) C, H, N.

***N*-[4-(4-Aminothieno[2,3-*d*]pyrimidin-5-yl)-2-fluorophenyl]-*N*′-(3-methylphenyl)urea (91).** Compound **91** was prepared following the same sequence as described for the synthesis of **51** by replacing **42** with 3′-fluoroacetophenone. ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3H), 6.83 (d, *J* = 7.1 Hz, 1H), 7.18 (t, *J* = 7.6 Hz, 1H), 7.23−7.27 (m, 2H), 7.32 (s, br, 1H), 7.39 (dd, *J* = 12.0, 1.9 Hz, 1H), 7.49 (s, 1H), 8.30 (d, *J* = 8.5 Hz, 1H), 8.34 (s, 1H), 8.70 (d, *J* = 2.7 Hz, 1H), 9.06 (s, 1H); MS (ESI) *m/z* 394 (M + H)$^+$. Anal. ($C_{20}H_{16}FN_5OS$) C, H, N.

***N*-[4-(4-Aminothieno[2,3-*d*]pyrimidin-5-yl)-2-chlorophenyl]-*N*′-(3-methylphenyl)urea (92).** Compound **92** was prepared following the same sequence as described for the synthesis of **51** by replacing **42** with 3′-chloroacetophenone. ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3H), 6.83 (d, *J* = 7.1 Hz, 1H), 7.19 (apparent t, *J* = 7.6 Hz, 1H), 7.26 (d, *J* = 8.1 Hz, 1H), 7.33 (s, 1H), 7.39 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.52 (s, 1H), 7.58 (d, *J* = 2.0 Hz, 1H), 8.34 (d, *J* = 8.8 Hz, 1H), 8.34 (s, 1H), 8.44 (s, 1H), 9.43 (s, 1H); MS (ESI) *m/z* 410 (M + H)$^+$. Anal. ($C_{20}H_{16}ClN_5OS{\cdot}0.7H_2O$) C, H, N.

***N*-[4-(4-Aminothieno[2,3-*d*]pyrimidin-5-yl)-2-methoxyphenyl]-*N*′-(3-methylphenyl)urea (93).** Compound **93** was prepared following the same sequence as described for the synthesis of **11** by substituting **41d** for **2**. ^{1}H NMR (DMSO-d_6) δ 2.29 (s, 3H), 3.94 (s, 3H), 6.80 (d, *J* = 7.5 Hz, 1H), 7.01 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.12 (d, *J* = 1.7 Hz, 1H), 7.17 (t, *J* = 7.8 Hz, 1H), 7.24 (d, *J* = 8.5 Hz, 1H), 7.32 (s, 1H), 7.46 (s, 1H), 8.29 (d, *J* = 8.5 Hz, 1H), 8.34 (s, 1H), 8.38 (s, 1H), 9.31 (s, 1H); MS (ESI) *m/z* 406 (M + H)$^+$. Anal. ($C_{21}H_{19}N_5O_2S{\cdot}0.7H_2O$) C, H, N.

***N*-(11-Amino-5,6-dihydro-7-thia-8,10-diazabenzo[*c*]fluoren-3-yl)-*N*′-(3-methylphenyl)urea (94).** Compound **94** was prepared following the same sequence as described for the synthesis of **51** by substituting 1-tetralone for **42**. ^{1}H NMR (DMSO-d_6) δ 2.28 (s, 3H), 2.91 (m, 4H), 6.6−7.0 (s, br, 2 H), 6.79 (d, *J* = 7.1 Hz, 1H), 7.16 (m, 1H), 7.23 (d, *J* = 8.1 Hz, 1H), 7.32 (s, 1H), 7.40 (m, 2H), 7.54 (d, *J* = 1.7 Hz, 1H), 8.30 (s, 1H), 8.61 (s, 1H), 8.72 (s, 1H); MS (ESI) *m/z* 402 (M + H)$^+$. Anal. ($C_{22}H_{19}N_5OS{\cdot}0.7H_2O$) C, H, N.

***N*-[4-(4-Amino-6-ethylthieno[2,3-*d*]pyrimidin-5-yl)-phenyl]-*N*′-[3-(trifluoromethyl)phenyl]urea (95).** Compound **95** was prepared using the same procedure as described for the synthesis of **74** by substituting 3-trifluoromethylphenyl isocyanate for *m*-tolyl isocyanate. ^{1}H NMR (DMSO-d_6) δ 9.18 (s, 1H), 9.06 (s, 1H), 8.32 (s, 1H), 8.02 (s, 1H), 7.50−7.70 (m, 4H), 7.32 (d, *J* = 8.4 Hz, 3H), 2.62 (q, *J* = 7.5 Hz, 2H), 1.98 (t, *J* = 7.5 Hz, 3H). Anal. ($C_{22}H_{18}F_3N_5OS{\cdot}0.9CH_2Cl_2$) C, H, N.

***N*-[4-(4-Aminothieno[2,3-*d*]pyrimidin-5-yl)phenyl]-*N*′-(3-chlorophenyl)urea (96).** Compound **96** was prepared using the same procedure as described for the synthesis of **76** by substituting 3-chlorophenyl isocyanate for *m*-tolyl isocyanate. ^{1}H NMR (DMSO-d_6) δ 8.96 (s, 1H), 8.34 (s, 1H), 7.73 (s, 1H), 7.61 (d, *J* = 8.4 Hz, 2H), 7.44 (s, 1H), 7.40 (d, *J* = 8.4 Hz,

2H), 7.32−7.28 (m, 2H), 7.03 (dt, $J = 2.1$ Hz, 1H); MS (ESI) m/z 396 (M + H)$^+$.

Computational Analysis. The homology model of KDR kinase in the inactive conformation was built (Insight II software, HOMOLOGY module, Accelrys, San Diego, CA) using the homologous Kit kinase (51% identity to KDR kinase within the catalytic domain, inactive conformation, PDB entry 1T46).[32] Manual docking of **11** into the homology model of KDR was accomplished by creating two canonical hinge hydrogen bonds between (1) the exocyclic amino group of **11** and the backbone carbonyl of Glu 917 and (2) the ring nitrogen and the backbone N−H of Cys 919. The torsions of the diaryl urea unit of **11** were then manually adjusted to follow the general path of the inhibitor STI-571, an inhibitor targeting the inactive conformations of Kit and c-Abl kinases, as observed in PDB entry 1T46.[32] Energy minimization using the CFF force field (25 iterations in a static active site, followed by 50 iterations of full ligand−protein minimization) provided the final model shown in Figure 2. No solvation was included.

Homogeneous Time-Resolved Fluorescence (HTRF) Assays of Receptor Tyrosine Kinases. Assays were performed in a total of 40 μL in 96-well Costar black half-volume plates using HTRF technology.[36] A peptide substrate (Biotin-Ahx-AEEEYFFLFA-amide) at 4 μM, 1 mM ATP, enzyme, and inhibitors were incubated for 1 h at ambient temperature in 50 mM Hepes/NaOH (pH 7.5), 10 mM $MgCl_2$, 2 mM $MnCl_2$, 2.5 mM DTT, 0.1 mM orthovanadate, and 0.01% bovine serum albumin (BSA). Inhibitors were added to the wells at a final concentration of 3.2 nM to 50 μM with 5% DMSO added as a cosolvent. The reactions were stopped with 10 μL/well of 0.5 M EDTA, and then, 75 μL of buffer containing streptavidin-allophycocyanin (Prozyme) (1.1 μg/mL) and PT66 antibody europium cryptate (Cis-Bio) (0.1 μg/mL) was added to each well. The plates were read from 1 to 4 h after addition of the detection reagents, and the time-resolved fluorescence (665:615 ratio) was measured using a Packard Discovery instrument. The amount of each tyrosine kinase added to the wells was calibrated to give a control (no inhibitor) to background (prequenched with ethylenediaminetetraacetic acid (EDTA)) ratio of 10−15, and it was shown to be in the low nanomolar concentration range for each kinase. The inhibition of each well was calculated using the control and background readings for that plate. KDR, CSF1R, cKit, FLT1, and FLT3 were assayed using the above protocol. Tie2 does not phosphorylate this peptide substrate; therefore, 10 ng/well of biotinylated poly(Glu-Tyr) (Cis-Bio) was used, and the assay was quenched with EDTA after 10 min. The HTRF detection was done in the same way as that for the other kinases. Each IC_{50} determination was performed with seven concentrations, and each assay point was determined in duplicate.

Cellular KDR Phosphorylation Assay. KDR Phosphorylation Determined by ELISA. NIH3T3 cells stably transfected with full length human KDR (VEGFR2) were maintained in a Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum and 500 μg/mL Geneticin. KDR cells were plated at 20 000 cells per well into duplicate 96-well tissue culture plates and cultured overnight in an incubator at 37 °C with 5% CO_2 and 80% humidity. The growth medium was replaced with a serum-free growth medium for 2 h prior to compound addition. Compounds in DMSO were diluted in the serum-free growth medium (final DMSO concentration 1%) and added to cells for 20 min prior to stimulation for 10 min with VEGF (50 ng/mL). Cells were lysed by addition of RIPA buffer (50 mM Tris-HCl (pH 7.4), 1% IGEPAL, 150 mM NaCl, 1 mM EDTA, and 0.25% sodium deoxycholate) containing protease inhibitors (Sigma cocktail), NaF (1 mM), and Na_3VO_4 (1 mM) and placed on a microtiter plate shaker for 10 min. The lysates from duplicate wells were combined, and 170 μL of the combined lysate was added to the KDR ELISA plate. The KDR ELISA plate was prepared by adding an anti-VEGFR2 antibody (1 μg/well, R&D Systems) to an unblocked plate and incubated overnight at 4 °C. The plate was then blocked for at least 1 h with 200 μL/well of 5% dry milk in PBS. The plate was washed two times with PBS containing 0.1% Tween 20 (PBST) before addition of the cell lysates. Cell lysates were incubated in the KDR ELISA plate with constant shaking on a microtiter plate shaker for 2 h at room temperature. The cell lysate was then removed, and the plate was washed five times with PBST. Detection of phospho-KDR was performed using a 1:2000 dilution of biotinylated 4G10 anti-phosphotyrosine (UBI, Lake Placid, NY). The wells were incubated with constant shaking for 1.5 h at room temperature and washed five times with PBST, and for detection, a 1:2000 dilution of streptavidin-HRP (UBI, Lake Placid, NY) was added and incubated with constant shaking for 1 h at room temperature. The wells were then washed five times with PBST, and K-Blue HRP ELISA substrate (Neogen) was added to each well. The development time was monitored at 650 nm in a SpectraMax Plus plate reader until 0.4−0.5 absorbance units were obtained (approximately 10 min) in the VEGF only wells. Phosphoric acid (1 M) was added to stop the reaction, and the plate was read at 450 nm. The percent inhibition was calculated using the VEGF only wells as 100% controls and wells containing 5 μM pan-kinase inhibitor as 0% controls (no VEGF wells were used to monitor the endogenous phosphorylation state of the cells). IC_{50} values were calculated by nonlinear regression analysis of the concentration response curve. Each IC_{50} determination was performed with five concentrations, and each assay point was determined in duplicate.

KDR Phosphorylation Determined by Western Blot Analysis. NIH3T3 cells stably transfected with full length human KDR (VEGFR2) were cultured in T75 or T150 flasks; compounds were added in the serum-free growth medium for 20 min prior to stimulation for 3 min with VEGF (50 ng/mL). The cells were washed one time with PBS and lysed with 1 mL/flask of RIPA buffer containing protease inhibitors, NaF, and Na_3VO_4 for 5 min on ice. Cells were scraped, and the lysate was incubated for an additional 20 min on ice with occasional mixing in a 2 mL microcentrifuge tube. The lysate was centrifuged at 20000g for 20 min at 4 °C, and the supernatant was used to immunoprecipitate KDR. The lysate, 500 μg, was precleared with Protein-G agarose beads (Calbiochem) for 30 min at 4 °C while rotating. The lysate was centrifuged (20000g for 1 min), and the supernatant was immunoprecipitated with 4 μg of an anti-VEGFR2 antibody (R&D Systems) overnight at 4 °C while rotating. After incubation, 50 μL of Protein-G agaraose was added and incubated for 1 h at 4 °C while rotating. Beads were washed five times with PBS containing Na_3VO_4 (1 mM) and protease inhibitors (Sigma). The washed beads were resuspended in 50 μL of sample buffer, electrophoresed on a 8−16% Tris-glycine gel (Invitrogen), transferred to a nitrocellulose membrane, blocked with 5% milk in PBST, and probed with an anti-KDR antibody (sc-315, Santa Cruz). KDR was visualized with enhanced chemoluminescence detection, and IC_{50} values were determined by comparison of the treated samples to the vehicle (DMSO) treated samples.

Estradiol-Induced Murine Uterine Edema Assay. Twelve week old balb/c female mice (Taconic, Germantown, NY) were pretreated with 10 units of pregnant mare's serum gonadotropin (PMSG) (Calbiochem) intraperitoneally (ip) administered 72 and 24 h prior to estradiol. Mice were randomized the day of the experiment. Test compounds were formulated in a variety of vehicles and administered po 30 min prior to stimulation with an intraperitoneal injection of water soluble 17β-estradiol (20−25 μg/mouse). Animals were sacrificed and uteri removed 2.5 h following estradiol stimulation by cutting just proximal to the cervix and at the fallopian tubes. After the removal of fat and connective tissue, uteri were weighed, squeezed between filter paper to remove fluid, and weighed again. The difference between wet and blotted weights represented the fluid content of the uterus. Compound treated groups were compared to vehicle treated groups of animals after subtracting the background water content of unstimulated uteri. The experimental group size was 5 or 6.

HT1080 Tumor Growth Inhibition Model. The 1080 human fibrosarcoma cells were obtained from the American Type Tissue Culture Collection and maintained in DMEM

supplemented with 10% fetal bovine serum and antibiotics. For tumor xenograft studies, cells were suspended in PBS, mixed with an equal volume of matrigel (phenol red free) to a final concentration of 2 million cells/mL, and inoculated (0.25 mL) into the flank of SCID-beige mice. One week after inoculation, tumor-bearing animals were divided into groups ($n = 10$), and administration of vehicle (2% EtOH, 5% Tween 80, 20% PEG 400, and 73% saline) or inhibitor at the indicted dose was initiated. Tumor growth was assessed every 2−3 days by measuring tumor size and calculating tumor volume using the formula [length × width^2]/2.

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