Structure-Guided Design of Aminopyrimidine Amides as Potent, Selective Inhibitors of Lymphocyte Specific Kinase: Synthesis, Structure–Activity Relationships, and Inhibition of in Vivo T Cell Activation

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The lymphocyte-specific kinase (Lck), a member of the Src family of cytoplasmic tyrosine kinases, is expressed in T cells and natural killer (NK) cells. Genetic evidence, including knockout mice and human mutations, demonstrates that Lck kinase activity is critical for normal T cell development, activation, and signaling. Selective inhibition of Lck is expected to offer a new therapy for the treatment of T-cell-mediated autoimmune and inflammatory disease. With the aid of X-ray structure-based analysis, aminopyrimidine amides **2** and **3** were designed from aminoquinazolines **1**, which had previously been demonstrated to exhibit potent inhibition of Lck and T cell proliferation. In this report, we describe the synthesis and structure–activity relationships of a series of novel aminopyrimidine amides **3** possessing improved cellular potency and selectivity profiles relative to their aminoquinazoline predecessors **1**. Orally bioavailable compound **13b** inhibited the anti-CD3-induced production of interleukin-2 (IL-2) in mice in a dose-dependent manner (ED_{50} = 9.4 mg/kg).

Introduction

Signal transduction pathways mediated by the T cell receptor (TCR[a]) have been extensively researched.[1] Novel immunosuppressive agents that target components of these pathways may be useful as therapies for T-cell-mediated inflammatory or autoimmune diseases such as rheumatoid arthritis, polyarthritis scleroderma, inflammatory bowel disease, type I diabetes, multiple sclerosis, ulcerative colitis, Crohn's disease, Sjogren's disease, polymyositis, dermatomyositis, vasculitis, myasthenia gravis, psoriasis, and lupus. Such therapies could also prove advantageous in transplant organ or graft rejection.[2]

The Src family of cytoplasmic protein tyrosine kinases includes Src, Lck, Fyn, Lyn, Hck, Fgr, Blk, and Yes.[3] Lck and Fyn have been shown to have important roles in TCR-mediated signal transduction.[4] TCR signals propagated by the stimulation of Lck ultimately lead to gene regulation events triggering cytokine release, proliferation, and survival of antigen specific T cells, thereby amplifying immune responses. Defects in T cell maturation and signaling, which affect expression and/or catalytic activity, have been observed in Lck knockout mice and humans with Lck mutations.[5] These findings suggest that a small-molecule inhibitor of Lck activity could prove useful as an immunosuppressive agent.

In recent years, we and others have disclosed several classes of small-molecule Lck inhibitors.[6] Some of these agents have demonstrated oral bioavailability and inhibitory activities in rodent models of T-cell-dependent immune responses.[1,6d–f,i–p] 2-Aminoquinazolines **1** (−L− = −CONH−, −NHCO−, or −NHCONH−) were identified via high-throughput screening of our kinase-preferred compound collection as potent, ATP-competitive inhibitors of Lck.[6p] Compounds with general structure **1** also exhibited potent inhibitory activity against T-cell proliferation and activation as measured by the human mixed lymphocyte reaction (MLR) and the T cell receptor/anti-CD3-induced production of interleukin-2 (IL-2). Extensive medicinal chemistry efforts were invested toward understanding the SAR of the 2-aminoquinazolines. These efforts led to the identification of orally bioavailable compounds with inhibitory activity in the anti-CD3-induced model of IL-2 production in mice and moderate to good selectivity profiles. Analysis of several X-ray cocrystal structures of aminoquinazolines **1** and Lck revealed key H-bonding interactions in the ATP-binding site, which are depicted in Figure 1. We sought to improve the selectivity of our aminoquinazoline series by taking advantage of the sequence differences among structurally related kinases in and around the ATP-binding pocket (Table 1). Specifically, we targeted structural modifications that would allow for an additional H-bonding interaction with the threonine gatekeeper residue (T316) in Lck. We hypothesized that such a new interaction would increase selectivity over kinases with non-threonine

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[a] Abbreviations: Lck, lymphocyte-specific kinase; TCR, T cell receptor; IL-2, interleukin 2; PTK, protein tyrosine kinase; MLR, human mixed lymphocyte reaction; DFG, aspartic acid−phenylalanine−glycine; MAPK, mitogen activated protein kinase; VEGF, vascular endothelial growth factor.

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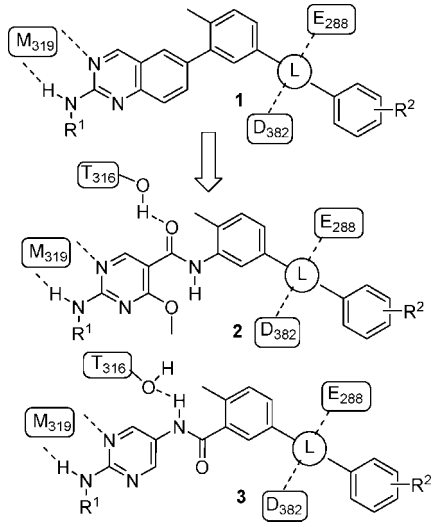


Figure 1. Design of aminopyrimidine amides **2** and **3** from aminoquinazolines **1**: −L− = −CONH−, −NHCO−, or −NHCONH−.

Table 1. Sequence Comparison at Gatekeeper Residue and Extended Hydrophobic Pocket

Lck	Thr316[a]	Met292	Leu295	Leu300	Ile355	Tyr360
Tie2	Ile	Leu	Leu	Ile	Leu	Phe
Jak3	Met	Leu	Leu	Ile	Leu	Cys
P38α	Thr	Leu	Met	Val	Ile	Ile
KDR	Val	Leu	Ile	Val	Leu	Cys

[a] Gatekeeper residue.

gatekeeper residues (namely, Tie-2, Jak3, and KDR).[7] Support for this hypothesis is provided by imatinib, which selectively inhibits a subset of kinases (BCR-Abl, cKit, PDGFR) through preferential binding to the inactive (DFG-out) conformation of the protein and hydrogen bonding to the threonine gatekeeper residue.[8] Furthermore, a single point mutation at the Abl gatekeeper residue (T315I) results in resistance to imatinib. Toward this end, we designed aminopyrimidine amides with general structures **2**[9] and **3**. Herein, we describe synthesis, X-ray structure, structure–activity relationships, and pharmacological characterization of aminopyrimidine amides **3** and the identification of an orally bioavailable Lck inhibitor (**13b**) with dose-dependent efficacy in a mouse model of T-cell receptor stimulated IL-2 production.

Chemistry

Aminopyrimidine amides (**3**, −L− = −CONH−, i.e., **10a**,**b**) were prepared as depicted in Scheme 1. 2,5-Diaminopyrimidines **6** were prepared from 2-amino-5-nitropyrimidines **5**, which were either commercially available (**5a**, R^1 = H) or derived from the S_NAr reaction of 2-chloro-5-nitropyrimidine (**4**) and anilines (i.e., **6b**, R^1 = 4-(4-methylpiperizino)aniline) or amines (i.e., **6c**,**d**). Commercially available 3-iodo-4-methylbenzoyl chloride (**7**) was condensed with 3-(trifluoromethyl)aniline in the presence of triethylamine to provide iodoamide **8**, which was then subjected to palladium-catalyzed carbonylative esterification and subsequent saponification to afford acid **9**. Condensation of **9** with 2,5-diaminopyrimidines **6a**,**b** provided the final compounds **10a**,**b**. The synthesis of aminopyrimidine reverse amides (**3**, −L− = −NHCO−, i.e., **13a**−**d**, **14**–**22**) was also accomplished in a convergent manner (Scheme 1). Nitrobenzamides **12**, prepared by the reaction of 2,5-diaminopyrimidines **6** and acid chlorides, were reduced to the corresponding anilines and selectively coupled to acids or acid chlorides to generate reverse amides. Similarly, aminopyrimidine amide ureas (**3**, −L− = −NHCONH−, i.e., **23a**,**b**) were prepared from **12** via reduction of the nitro group and reaction of the derived aniline with isocyanates. These synthetic routes proved to be quite general such that a variety of reverse amides and ureas could be prepared in moderate to good overall yields.

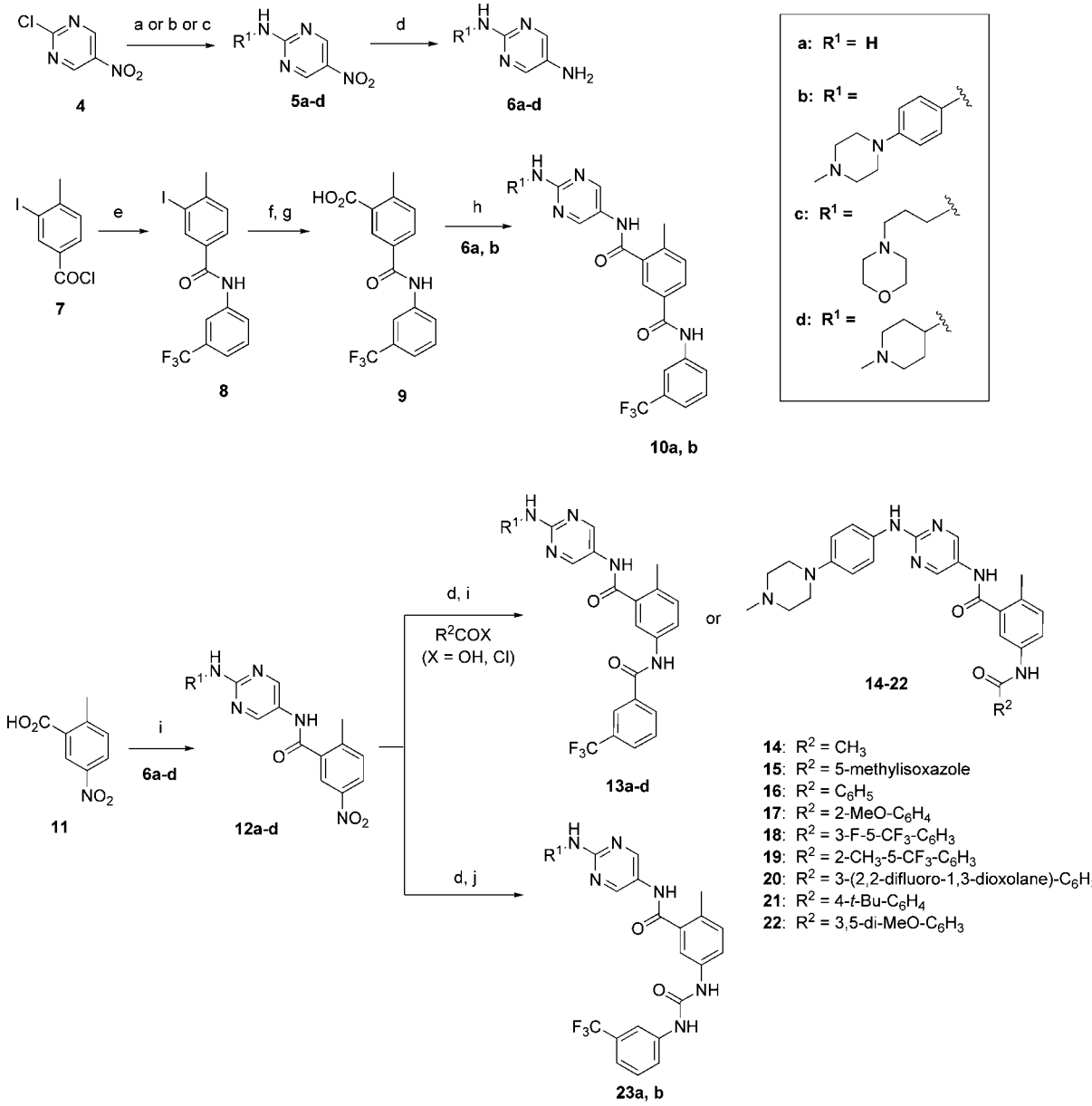
In some instances, unsubstituted 2-aminopyrimidine nitrobenzamide **12a** was converted to iodide **24** via a Sandmeyer reaction (Scheme 2).[10] S_NAr reactions of iodide **24** were accomplished under thermal, basic conditions with alkylamines (i.e., **13e**) or under thermal, acidic conditions with anilines. The nitrobenzamide products were then reduced to the corresponding anilines and coupled to acids or acid chlorides to generate the final compounds. Variations on the central aryl ring were typically introduced early in the synthetic route beginning with appropriately functionalized, commercially available benzoic acids (i.e., **25**, **26**, **30**). Final compounds were thus prepared following standard procedures, as depicted in Scheme 2.

Results and Discussion

Structure–Activity Relationships (SAR). To test our hypothesis that the introduction of an H-bond donor moiety would confer enhanced selectivity over kinases that possess a gatekeeper residue other than threonine, we investigated a series of direct 2-aminopyrimidine amide analogues of our nonselective 2-aminoquinazoline leads (Table 2). Replacement of the 2-aminoquinazoline with a 2-aminopyrimidine amide leads to a 10- to 75-fold loss of potency on Lck, as evidenced by comparison of the in vitro profiles for representative amides (**34** vs **10a**), reverse amides (**35** vs **13a**), and ureas (**36** vs **23a**). The potency loss is counteracted by the introduction of a 4-(4-methylpiperazino)anilino substituent at the 2-position in the pyrimidine series (**10b**, **13b**, **23b**). We speculate that this gain in enzyme potency results from additional stabilizing van der Waals interactions between the aryl ring of the aniline substituent and the two lipophilic residues (Leu251 of the glycine-rich loop and Gly332) between which it is situated.[9] Interestingly, reverse amide **13b** demonstrated greater selectivity than amide **10b** and greater cellular potency than urea **23b**. Unfortunately, the arylpiperazine **13b** does not afford a substantial improvement in selectivity over KDR relative to aminoquinazoline **35** (40-fold vs 15-fold). In KDR, the valine gatekeeper residue is relatively small and can presumably tolerate a proximal hydrophilic amide moiety.

X-ray crystallography was used to confirm our hypothesis that a 2-aminopyrimidine amide binds to Lck in a manner similar to that observed for the 2-aminoquinazoline but also engages the threonine gatekeeper in an H-bonding interaction (between the amide NH and the oxygen of T316). The cocrystal structures of Lck and aminopyrimidine amide **10b** (Figure 2a) and Lck and aminopyrimidine reverse amide **19** (Figure 2b) were solved at 2.2 and 2.3 Å resolution, respectively. In both structures, the inhibitor occupies the ATP-binding site and induces the protein to assume a DFG-out conformation.[11] The aminopyrimidine ring makes two hydrogen bond contacts to the hinge region of the enzyme at M319 (**10b**, 3.04 and 2.78 Å; **19**, 2.99 and 2.89 Å). As hypothesized, the pyrimidine amide moiety engages the gatekeeper residue (T316) in an H-bond interaction (**10b**, 3.07 Å; **19**, 3.11 Å), acting as an H-bond donor to the threonine oxygen. The arylamide moiety makes hydrogen bond contacts with the backbone NH of D382 (**10b**, 2.89 Å; **19**, 2.97 Å) from the DFG sequence and E288 (**10b**, 2.80 Å; **19**, 2.99 Å) from the C-helix. The aryl ring of the second amide thus occupies the extended hydrophobic pocket, making van der

Scheme 1. Preparation of Aminopyrimidine Amides, Reverse Amides, and Ureas[a]



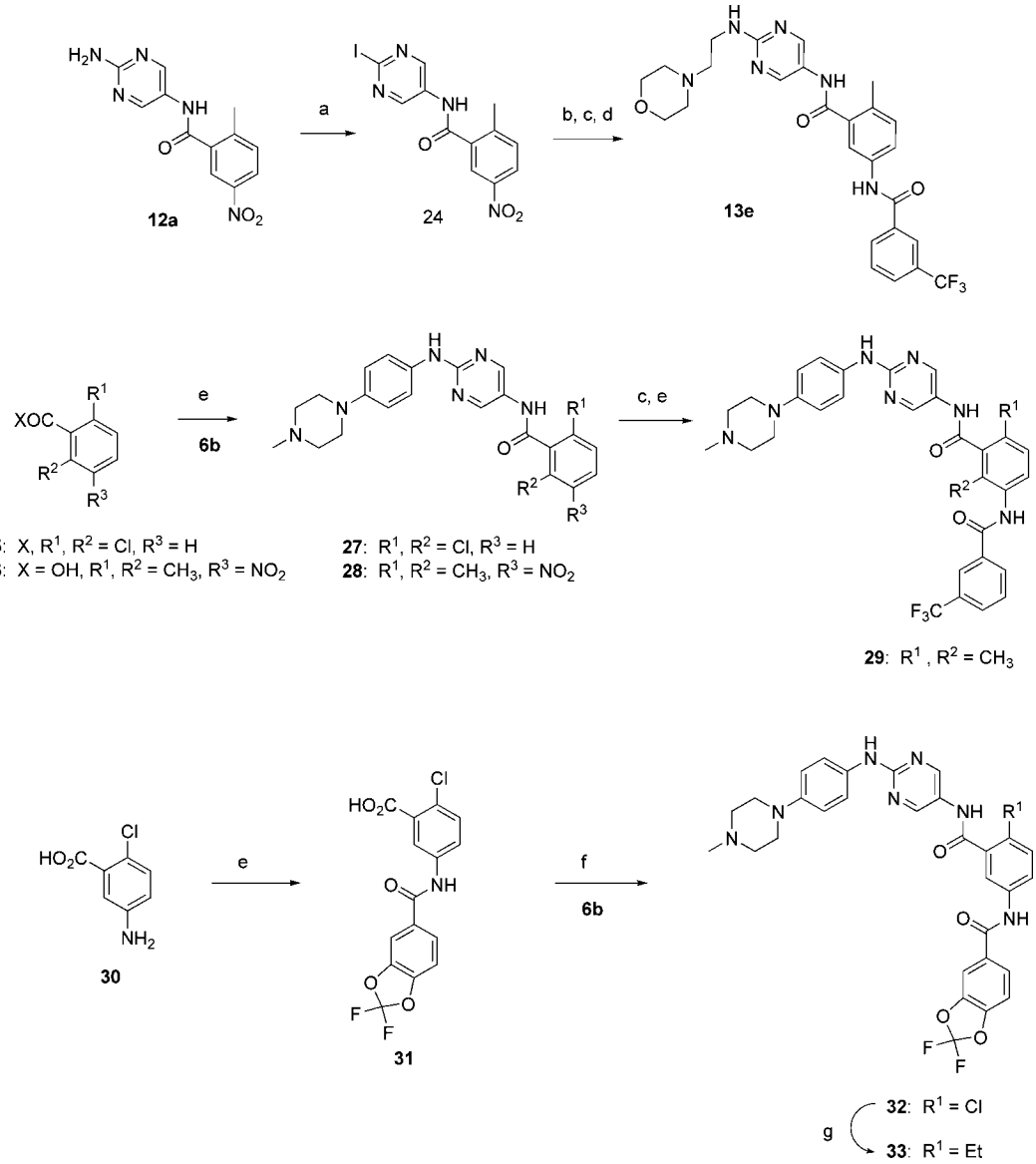
[a] (a) 4-(4-methylpiperazino)aniline, TFA, IPA, 80 °C (**5b**, 88%); (b) 3-morpholinopropan-1-amine, DIPEA, IPA, 70 °C (**5c**, 100%); (c) 1-methylpiperidin-4-amine, NaH, THF (**5d**, 100%); (d) 10% Pd/C, H_2, CH_3OH/EtOAc; (e) 3-(trifluoromethyl)aniline, NEt_3, CH_2Cl_2 (84%); (f) CO(g), CH_3OH, $Pd(OAc)_2$, dppp, NEt_3, 70−100 °C (85%); (g) LiOH, THF/H_2O (92%); (h) EDC−HCl, HATU, DIPEA, CH_2Cl_2 (**10a**, 65%; **10b**, 91%); (i) (i) acid, $SOCl_2$, 80 °C, (ii) aniline, NEt_3, CH_2Cl_2; (j) 1-isocyanato-3-(trifluoromethyl)benzene, benzene, 75 °C (**23a**, 20%; **23b**, 46%).

Waals contacts to several lipophilic residues. This type of DFG-out binding mode was previously observed for the parent 2-aminoquinazolines and is not observed for most other reported Src-family kinase inhibitors such as PP1 and PP2.[12] In view of the marked similarity in binding mode between amides and reverse amides with Lck, we are currently unable to offer a rational explanation for the superior selectivity of **13b** relative to **10b**. Similarities in the binding modes of aminopyrimidine amides and quinazolines are demonstrated by the overlay of two cocrystal structures with Lck (Figure 2c). As observed in the cocrystal structures, substituents on the 2-amino group of the pyrimidine can extend to the solvent front and may be engaged in additional binding interactions with residues lining the hinge region and the glycine-rich loop.[13,6s,9]

In light of the gain in potency observed for **13b** relative to **13a** and the potential for additional H-bonding[6s] or van der Waals[9] interactions with residues lining the hinge region, we examined the effect on potency and selectivity of smaller tertiary amine containing chains to the 2-position of the pyrimidine (Table 3, **13c**−**e**). Although these analogues proved quite selective over KDR, Tie-2, and Jak3, their Lck kinase and cellular potency was significantly less than **13b**. As anticipated, the H-bond donor to the hinge is important for potency; a substantial loss in potency was also observed with the unsubstituted pyrimidine **37**.

Selected SAR from variations on the arylamide is summarized in Table 4. Moderate potency and good selectivity (vs KDR, Tie-2, and Jak3) was observed with isoxazole **15**, benzamide **16**, and 2-methoxybenzamide **17**. These three analogues possessed kinase selectivity profiles comparable to that of lead **13b** but were less potent. Compounds lacking the southern aryl ring, such as acetamide **14**, were also significantly (>50-fold) less potent than **13b** on Lck. Exceptional enzyme and cellular potency, comparable to that of lead **13b**, was consistently observed for meta-

Scheme 2. Preparation of Aminopyrimidine Reverse Amides[a]



[a] (a) isoamyl nitrite, CuI, CH_2I_2, THF, 70 °C (21%); (b) 2-morpholinoethanamine, DIPEA, IPA, 70 °C (100%); (c) 10% Pd/C, H_2, CH_3OH/EtOAc; (d) 3-(trifluoromethyl)benzoyl chloride, NEt_3, CH_2Cl_2 (63%); (e) (i) acid, $SOCl_2$, 80 °C, (ii) aniline, NEt_3, CH_2Cl_2; (f) EDC−HCl, HATU, DIPEA, DMF (42%); (g) BEt_3, $Pd(OAc)_2$, S-Phos, K_3PO_4, THF, 60 °C (20%).

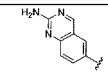
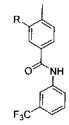
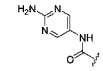
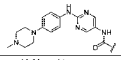
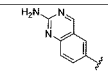
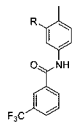
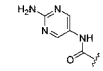
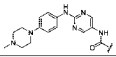
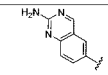
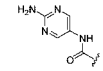
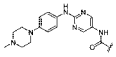
or para-substituted derivatives, such as **18**–**22**. Of these, **20** proved most selective over KDR (>200-fold).

An examination of the central aryl ring situated in the classical hydrophobic pocket led to compounds with significantly improved selectivity profiles (Table 5). On the basis of SAR from the parent aminoquinazoline series, we surmised that a small lipophilic substituent such as methyl or chloro at R^1 was important for cellular potency. We also discovered that the larger ethyl substituent was tolerated with only a moderate (2- to 5-fold) loss of Lck potency and afforded excellent selectivity over p38α (i.e., **33**). Small substituents at R^2, such as methyl or chloro, were also better tolerated by Lck than by p38α (**29** and **27**). Unfortunately, none of these more selective analogues demonstrated acceptable cellular potency. The narrow SAR is consistent with expectations, given the lack of space in this region of the binding site (see X-ray structures, Figure 2).[6p,14]

On account of their in vitro potencies and selectivity profiles, selected aminopyrimidine amides were advanced into our secondary cellular screening assay, which measures the compound's inhibitory effect on the anti-CD3 induced production of IL-2 in 50% human whole-blood (WB IL-2) (Table 6). Compounds **13b**, **18**−**20**, and **22** were very potent inhibitors of IL-2 production in human whole blood. On the basis of the general inhibitory activity in the p38α kinase assay (<10 nM) for this series, three representative compounds, **13b**, **18**, and **19**, were further counterscreened for inhibitory activity in the lipopolysaccharide-induced production of TNFα in 50% human whole blood (WB TNFα). The whole blood cellular selectivity factor (WB IL-2 IC_{50}/WB TNFα IC_{50}) was substantial for all three compounds (102-, 63-, and 685-fold, respectively). For several compounds, the protein binding profiles were examined in plasma from three species (Table 6).

Pharmacokinetic Profile. Selected PK data for discrete intravenous (iv) and oral (po) dosing in male Sprague–Dawley rats are shown in Table 7. Clearance ranged from low (**22**, 0.29 L/h/kg) to greater than hepatic blood flow (**21**, 6.64 L/h/kg), and oral bioavailability was generally low (4–22%). Compound **13b** exhibited a moderate rate of clearance, volume of distribution, oral exposure, and bioavailability. In light of the rat pharmacokinetic profile and the exceptional in vitro potency,

Table 2. Aminoquinazolines vs Aminopyrimidine Amides[a]

Cmpd.	R	Ar^1-L-Ar^2	IC_{50} (nM) Lck	Tie-2	KDR	p38α	Jak3	MLR
34			0.2	2	2	6	20	9
10a			15	>5000	205	87	>25000	---
10b			0.5	123	5	10	438	15
35			0.4	21	6	1	156	43
13a			14	>25000	125	14	>25000	107
13b			0.6	9950	24	5	>25000	11
36			7	93	169	28	3040	205
23a			63	>25000	587	--	>25000	158
23b			0.6	>8300	41	2	>25000	87

[a] IC_{50} values are the mean of two or more separate determinations, in duplicate.

13b appeared suitable for assessment via oral dosing in our rodent model of T cell activation.

Selectivity Profile. Prior to in vivo testing, compound **13b** was screened for inhibitory activity against an extensive panel of kinases (Table 8).[15] Excellent selectivity (>1000-fold) was observed over a number of structurally diverse kinases. As expected, **13b** was not selective over other members of the Src family (Src, Fyn, Lyn), p38α, Abl, or cFMS, all of which possess a threonine gatekeeper residue. The Src-family kinases are broadly expressed in a variety of tissues with high levels in hematopoietic cells. The lack of intra-Src family selectivity remains a concern because of potential adverse effects from multikinase inhibition. In particular, Lyn knockout mice show defective BCR signaling and decreased peripheral B-cells.[3,16,17] The intra-Src family specificity required for safe clinical application and/or therapeutic treatment of autoimmune or inflammatory disease remains an unknown at this time. Despite numerous reports of assorted chemotypes with varying selectivity profiles,[6] a small-molecule inhibitor of Lck has yet to demonstrate successful clinical application in autoimmune, inflammatory disease, or transplant rejection. We believe that the overall kinase selectivity profile offered by this new aminopyrimidine amide class could pave the way to the identification of tool compounds to answer such questions. Compound **13b** was also tested for its ability to inhibit IL-2 production in Jurkat cells stimulated with phorbol 12-myritstyl 13-acetate (PMA) in the presence of ionophore. In this assay, the IC_{50} was >20 μM, which provides further evidence that the inhibition of kinases downstream of Lck is minimal.

Pharmacodynamic Profile. Compound **13b** was further evaluated as an inhibitor in the anti-CD3-induced IL-2 production model in female BALB/c mice (Figure 3). Upon oral administration at 10, 30, and 100 mg/kg, **13b** exhibited statistically significant dose-dependent inhibition of IL-2 production. In this study, terminal plasma levels (at 2.5 h) of **13b** were determined in order to estimate the levels of exposure at each dose. At the estimated ED_{50} (9.4 mg/kg) the mean terminal plasma concentration (EC_{50}) was ~175 nM. This concentration

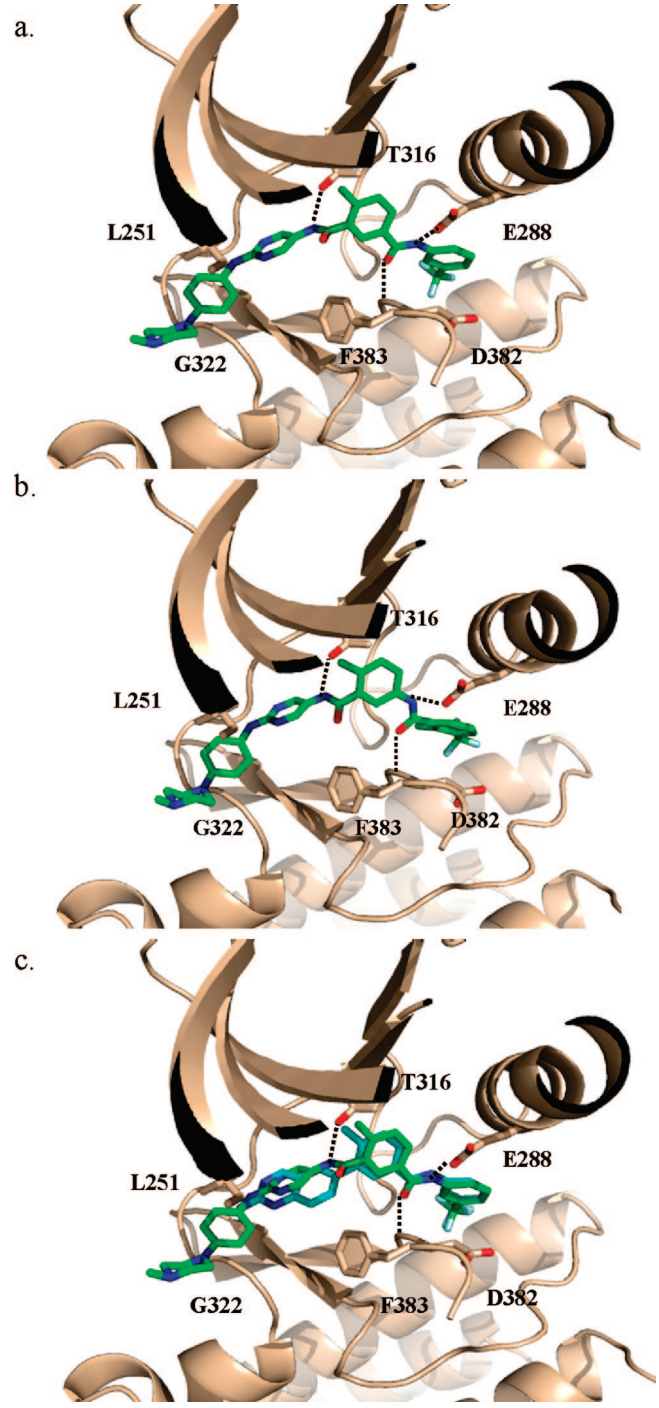


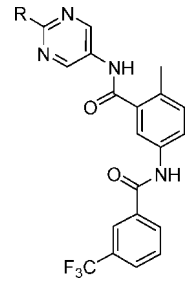
Figure 2. Cocrystal structures: (a) Lck and aminopyrimidine amide **10b**; (b) Lck and aminopyrimidine reverse amide **19**; (c) overlay of aminopyrimidine amide **10b** (green) with quinazoline **34** (blue) in Lck.

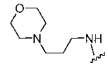
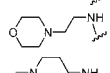
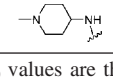
provides 25-fold coverage over the in vitro potency in the 50% human whole-blood IL-2 assay (IC_{50} = 7 nM) at 2.5 h. When adjusted for free fraction based on mouse protein binding (Table 6), the EC_{50} (fu) for **13b** is 4 nM,[18] which is consistent with the in vitro inhibitory activity in the absence of whole blood (IL-2 IC_{50} = 0.6 nM). **13b** demonstrates comparable in vivo efficacy to cyclosporine A (ED_{50} = 10 mg/kg) in this model.[6i]

Conclusions

Aminopyrimidine amides **3**, designed from aminoquinazolines **1**, were demonstrated to afford improved potency and selectivity

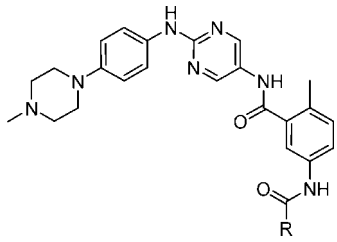
Table 3. SAR: Variations on the Pyrimidine Substituent[a]



Cmpd.	R	IC50 (nM) Lck	Tie-2	KDR	p38α	Jak3	MLR
37	H	239	>25000	>8300	199	>25000	---
13c		24	>25000	>8300	10	>25000	821
13e		72	>25000	>8300	6	>25000	202
13d		1310	>25000	>25000	5	>25000	---

[a] IC_{50} values are the mean of two or more separate determinations, in duplicate.

Table 4. SAR: Variations on the Arylamide[a]

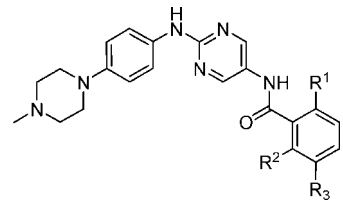


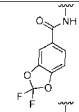
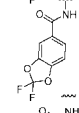
Cmpd.	R	IC50 (nM) Lck	Tie-2	KDR	p38α	Jak3	MLR
14	CH_3	48	>25000	>5000	63	>25000	---
15		14	>25000	3784	179	>25000	3920
16		5	>25000	947	0.9	>25000	187
17		5	>25000	1123	8	>25000	399
18		0.3	6078	17	3	>25000	21
19		0.8	>8300	54	12	>25000	60
20		1.3	>8300	272	10	>25000	58
21		1.5	>8300	32	22	>25000	10
22		1.4	>8300	120	4	>25000	77

[a] IC_{50} values are the mean of two or more separate determinations, in duplicate.

for the inhibition of Lck, T cell proliferation, and T cell activation. Our hypothesis that the introduction of an amide H-bond donor moiety would provide an additional site of interaction between the ligand and the gatekeeper residue (T316) of Lck was verified by X-ray crystallographic studies. This interaction presumably contributes to the enhanced selectivity afforded by these compounds relative to the parent amino-quinazolines. Extensive SAR investigations resulted in a diverse collection of novel aminopyrimidine amides, from which compound **13b** was identified as an orally active agent that exhibited dose-dependent inhibitory activity (ED_{50} = 9.4 mg/kg) in the anti-CD3 induced production of IL-2 in mice.

Table 5. SAR: Variations on the Central Aryl Ring[a]



Cmpd.	R1	R2	R3	IC50 (nM) Lck	Tie-2	KDR	p38α	Jak3	MLR
32	Cl	H		1.8	>5000	13	6	>5000	29
33	Et	H		6	>25000	>5000	233	>25000	928
29	Me	Me		6	>25000	2540	103	>25000	836
27	Cl	Cl	H	10	>25000	>25000	1070	>25000	555

[a] IC_{50} values are the mean of two or more separate determinations, in duplicate.

Table 6. Cellular Assay (WB IL-2) and Counterscreen (WB TNFα) in 50% Human Whole Blood and Protein Binding[a]

compd	IC50 (μM) WB IL-2	WB TNFα	protein binding (%) human	mouse	rat
13a	1.27	>10			
13b	0.007	0.718	96.7	97.8	99.4
18	0.016	1.01	96.4	98.2	97.7
19	0.002	1.37	98.1	98.4	98.6
20	0.013		95.8	98.1	98.9
22	0.043		96.8	98.8	98.3

[a] IC_{50} values are the mean of two or more separate determinations, in duplicate.

Experimental Section

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Quinazolines **34**–**36** were prepared according to previously published procedures.[6p] Dry organic solvents (CH_2Cl_2, CH_3CN, DMF, etc.) were purchased from Aldrich packaged under nitrogen in Sure/Seal bottles. Reactions were monitored using Agilent 1100 series LCMS with UV detection at 254 nm and a low resonance electrospray mode (ESI). Medium pressure liquid chromatography (MPLC) was performed on a CombiFlash Companion (Teledyne Isco) with RediSep normal-phase silica gel (35 – 60 μm) columns and UV detection at 254 nm. Preparative reversed-phase HPLC was performed on a Gilson (215 liquid handler) YMC-Pack Pro C18, 150 mm × 30 mm i.d. column, eluting with a binary solvent system A and B using a gradient elution (A, H_2O with 0.1% TFA; B, CH_3CN with 0.1% TFA) with UV detection at 254 nm. Purity was measured using Agilent 1100 series high-performance liquid chromatography (HPLC) systems with UV detection at 254 nm (system A, Agilent Zorbax Eclipse XDB-C8 4.6 mm × 150 mm, 5 μm, 5−100% CH_3CN in H_2O with 0.1% TFA for 15 min at 1.5 mL/min; system B, Waters Xterra 4.6 mm × 150 mm, 3.5 μm, 5−95% CH_3CN in H_2O with 0.1% TFA for 15 min at 1.0 mL/min). ^1H NMR spectra were recorded on a Bruker AV-400 (400 MHz) spectrometer at ambient temperature or on a Varian 400 MHz or on a Varian 300 MHz spectrometer. Chemical shifts are reported in ppm from the solvent resonance (DMSO-d_6 2.49 ppm). Data

Table 7. Mean PK Parameters Following Intravenous Dose (iv) or Oral Dose (po) in male Sprague–Dawley Rats[a]

	iv[b]			po[c]			
compd	CL ((L/h)/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng· h/mL)	C_{max} (ng/mL)	T_{max} (h)	*F* (%)
13b	1.04	3.29	3.2	419	49	4.67	22
18	0.49	1.61	3.8	332	39	0.6	8
19	2.92	5.45	1.8	92	28	2.75	7
20	1.37	3.76	2.5	58	16	0.42	4
21	6.64	15.8	2.9				
22	0.29	0.79	2.5	528	191	0.33	8

[a] $n = 3$ animals per study. [b] Dosed iv at 0.5 mg/kg as a solution in DMSO. [c] Dosed po at 2 mg/kg as a suspension in 1% Tween-80, 2% HPMC, 97% water.

Table 8. Kinase Selectivity Profile of **13b**[a]

kinase	IC_{50} (μM)
Lck	0.0006
Src	0.003
Fyn	0.003
Lyn	0.0009
p38α	0.005
Abl	0.002
cFMS	0.040
KDR	0.024
Tie-2	9.95
JNK2	4.29
Syk	>5
TrkA	>5
IRK	>5
Itk	>25
CDK5	>25
PAK2	>25
RON	>25
Jak3	>25
Jak2	1.03
PIM1	>30
Aurora1	>8.3
Aurora2	>25
PKAα	>85
PKBα	>100
MSK1	>100
P70S6K	>100
ERK1	>100

[a] IC_{50} values are the mean of two or more separate determinations, in duplicate.

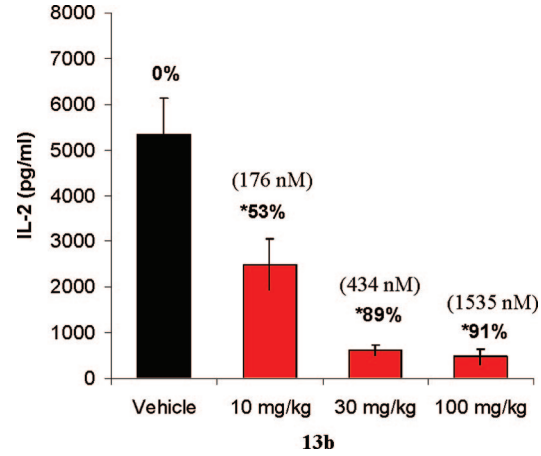


Figure 3. Effect of **13b** on Anti-CD3 induced IL-2 production in BALB/c mice. The 12 week old female BALB/c mice were pretreated (po) with **13b** at 10, 30, or 100 mg/kg. After 1.0 h the mice were challenged (iv) with anti-mouse CD3 monoclonal antibody (3 mg/mouse). After 90 min of anti-CD3 challenge, blood was collected via cardiac puncture. IL-2 was measured in serum using the BioSource ELISA kit. Data points represent the mean ± SE; $n = 5$ animals per group: (∗) $p \leq 0.05$ vs vehicle control by Mann–Whitney *U*-test. Mean plasma concentrations for each dose are expressed in parentheses.

are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants, and number of protons. Mass spectra were obtained on a high resonance electrospray time-of-flight mass spectrometer in positive ES ionization mode. Combustion analysis was performed by Galbraith Laboratories, Inc., Knoxville, TN. For final compounds that do not have elemental analysis data, the results of HPLC in two diverse chromatographic conditions can be found in the Supporting Information.

***N*2-(4-(4-Methylpiperazin-1-yl)phenyl)pyrimidine-2,5-diamine (6b).** To a mixture of 4-(4-methylpiperazino)aniline (57.7 g, 302 mmol) and **4** (48.1 g, 302 mmol) in IPA (500 mL) was added trifluoroacetic acid (46 mL, 603 mmol). The mixture was heated to 80 °C for 4 h, then cooled to room temperature. The solid was collected by suction filtration through a Buchner filtration apparatus equipped with a micromembrane filter and washed with IPA (80 mL) and CH_3OH (100 mL), then dried under vacuum to afford the trifluroacetic acid salt of **5b** as a pale-yellow amorphous solid (114 g, 266 mmol, 88%). MS (ESI, negative ion) *m/z*: 315 (MH^+). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.87 (br s, 1H), 10.73 (s, 1H), 9.18 (s, 2H), 7.63–7.61 (m, 2H), 7.02–7.04 (m, 2H), 3.75–3.81 (m, 2H), 3.47–3.50 (m, 2H), 3.05–3.16 (m, 4H), 2.81 (s, 3H). The TFA salt of **5b** (96 g, 224 mmol) was suspended in water (400 mL), and 2 N NaOH was added until the aqueous layer was pH 14. The solid was collected by suction filtration through a Buchner filtration apparatus equipped with a micromembrane filter, washed with water, and dried under vacuum overnight to afford **5b** (70 g, 100% yield) [MS (ESI, positive ion) *m/z*: 315 (MH^+)] as a red amorphous solid, which was used without further purification. **5b** (70 g, 224 mmol) was taken up in EtOH (500 mL), and Pd/C (10 g, 10% by weight wet) was added. The mixture was placed in a stirred pressure vessel under 50 psi of H_2 for 48 h. The catalyst was removed by suction filtration and washed with EtOH. The organics were concentrated under reduced pressure and dried under vacuum to afford the title compound (61.4 g, 96% yield) as a tan amorphous solid, which was used without purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.68 (s, 1H), 7.91 (s, 2H), 7.49–7.51 (m, 2H), 6.81–6.84 (m, 2H), 4.68 (br s, 2H), 3.0–3.02 (m, 4H), 2.43–2.51 (m, 4H), 2.21 (s, 3H). MS (ESI, positive ion) *m/z*: 285 (MH^+).

***N*3-(2-Aminopyrimidin-5-yl)-4-methyl-*N*1-(3-(trifluoromethyl)phenyl)isophthalamide (10a).** The title compound was prepared from **6a** (0.071 g, 0.371 mmol) and **9** (0.100 g, 0.309 mmol) using the procedure described for the synthesis of **10b**. Purification via column chromatography on silica gel (gradient elution with 2−10% CH_3OH/CH_2Cl_2) afforded the title compound (0.083 g, 65%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.71 (s, 1H), 10.39 (s, 1H), 8.67 (s, 2H), 8.38 (s, 1H), 8.28 (d, $J = 1.9$ Hz, 1H), 8.23 (d, $J = 8.5$ Hz, 1H), 8.15 (dd, $J = 7.9$, 1.9 Hz, 1H), 7.75 (t, $J = 7.9$ Hz, 1H), 7.64 (d, $J = 8.0$ Hz, 1H), 7.60 (d, $J = 8.0$ Hz, 1H), 6.70 (s, 2H), 2.61 (s, 3H). HRMS $(C_{20}H_{17}F_3N_5O_2)^+$: calcd, 416.132 89; found, 416.134 16. Anal. $(C_{20}H_{16}F_3N_5O_2 \cdot 0.5CH_3OH)$ C, H, N.

4-Methyl-*N*3-(2-(4-(4-methylpiperazin-1-yl)phenylamino)pyrimidin-5-yl)-*N*1-(3-(trifluoromethyl)phenyl)isophthalamide (10b). A solution of 3-(trifluoromethyl)aniline (0.63 g, 3.9 mmol) and triethylamine (0.55 mL, 3.9 mmol) in CH_2Cl_2 (10 mL) was cooled in an ice–water bath, and a solution of **7** (1.0 g, 3.6 mmol) in CH_2Cl_2 (15 mL) was added slowly. The mixture was warmed to room

temperature and stirred for 20 h. The reaction mixture was concentrated and purified via column chromatography on silica gel (gradient elution with 0−100% EtOAc/hexane) to afford **8** (1.219 g, 84% yield) [MS (ESI, positive ion) *m/z*: 404 (MH^+)]. A 45 mL stainless steel cylinder with glass liner was charged with **8** (1.22 g, 3.01 mmol) and CH_3OH (15 mL). Argon was bubbled through the mixture, and then bis(diphenylphosphino)propane (0.0683 g, 0.165 mmol), triethylamine (1.00 mL, 7.17 mmol), and palladium(II) acetate (0.0338 g, 0.150 mmol) were added. The cylinder was capped with a pressure gauge, charged with CO gas (200 psi), and heated to 70 °C for 20 h and then at 100 °C for 4 h. The reaction mixture was cooled to room temperature and concentrated. The residue was purified via column chromatography on silica gel (gradient elution with 0−100% EtOAc/hexane) to afford methyl 2-methyl-5-((3-(trifluoromethyl)phenyl)carbamoyl)benzoate (0.858 g, 85% yield) [MS (ESI, positive ion) *m/z*: 338 (MH^+)] as an orange oil. Lithium hydroxide (0.154 g, 6 mmol) was added to a solution of methyl 2-methyl-5-((3-(trifluoromethyl)phenyl)carbamoyl)benzoate (0.858 g, 2.54 mmol) in THF (10 mL) and water (2 mL). The mixture was stirred at room temperature for 20 h. The layers were separated, and the aqueous phase was acidified to pH 6 with 6 M HCl (aq) and extracted with EtOAc. The combined organic phases were dried over anhydrous Na_2SO_4, filtered, and concentrated to afford **9** (0.760 g, 92% yield) [MS (ESI, positive ion) *m/z*: 324 (MH^+)] as a light-purple solid. A solution of **9** (0.200 g, 0.619 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.125 g, 0.650 mmol), 1-hydroxy-7-azabenzotriazole (0.0842 g, 0.619 mmol), diisopropylethylamine (0.323 mL, 1.86 mmol), and **6b** (0.176 g, 0.619 mmol) in CH_2Cl_2 (4 mL) was stirred at room temperature for 20 h. The reaction mixture was concentrated and the residue was purified via column chromatography on silica gel (gradient elution with 0−100% 90:10:1, CH_2Cl_2/CH_3OH/NH_4OH−CH_2Cl_2) to afford the title compound (0.332 g, 91% yield) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.61 (s, 1H), 10.46 (s, 1H), 9.38 (s, 1H), 8.74 (s, 2H), 8.26 (s, 1H), 8.19 (d, J = 1.4 Hz, 1H), 8.10 (d, J = 8.1 Hz, 1H), 8.04 (dd, J = 8.0, 1.5 Hz, 1H), 7.62 (t, J = 8.2 Hz, 1H), 7.56 (d, J = 9.0 Hz, 2H), 7.52 (d, J = 8.1 Hz, 1H), 7.47 (d, J = 7.6 Hz, 1H), 6.89 (d, J = 9.0 Hz, 2H), 3.17 (d, J = 3.7 Hz, 3H), 3.09 - 3.01 (m, 4H), 2.48−2.42 (m, 4H), 2.22 (s, 3H). HRMS $(C_{31}H_{31}F_3N_7O_2)^+$: calcd, 590.248 58; found, 590.249 96. Anal. ($C_{31}H_{30}F_3N_7O_2 \cdot CH_3OH \cdot H_2O$) C, H, N.

***N*-(2-Aminopyrimidin-5-yl)-2-methyl-5-nitrobenzamide (12a).** **11** (0.66 g, 3.64 mmol) was heated to reflux in $SOCl_2$ (6 mL) for 1 h. After the mixture was cooled to room temperature and concentrated to dryness, the crude acid chloride was taken up in CH_2Cl_2 (25 mL). **6a** (0.40 g, 3.64 mmol) was added to the solution, and the mixture was allowed to stir at room temperature overnight. The mixture became a thick white suspension. Triethylamine (0.700 mL, 4.73 mmol) was added. After 2 h, a thick white suspension remained. The mixture was filtered through a Buchner apparatus equipped with a micromembrane filter, washed with CH_2Cl_2, and dried on high vacuum to afford the title compound (962 mg, 97%) [MS (ESI, positive ion) *m/z*: 273 (M^+)] as an off-white solid, which was used without further purification.

2-Methyl-*N*-(2-(4-(4-methylpiperazin-1-yl)phenylamino)pyrimidin-5-yl)-5-nitrobenzamide (12b). To a mixture of **11** (46.0 g, 181 mmol) in CH_2Cl_2 (2 L) was added EDC (61.0 g, 318 mmol). The mixture was allowed to stir for 30 min at room temperature. **6b** (60 g, 211 mmol) was added, and the mixture was allowed to stir for 70 h. Water (2 L) was added. Then 2N NaOH was added until the pH was 14. The organics were extracted into EtOAc (4 L). The aqueous layer was extracted with additional EtOAc, and the combined organics were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was taken up in CH_2Cl_2. The solid formed was collected by suction filtration and washed twice with CH_2Cl_2 (250 mL). The solid was dried under vacuum to obtain the title compound (47.6 g, 51%) as an orange amorphous solid, which was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.53 (s, 1H), 9.40 (s, 1H), 8.71 (s, 2H), 8.39 (s, 1H), 8.26–8.29 (m, 1H), 7.55–7.65 (m, 3H), 6.88–6.90 (m, 2H), 3.05–3.07 (m, 4H), 2.54 (s, 3H), 2.44–2.47 (m, 4H), 2.22 (s, 3H). MS (ESI, positive ion) *m/z*: 448 (MH^+).

***N*-(2-Amino-5-pyrimidinyl)-2-methyl-5-(((3-(trifluoromethyl)phenyl)carbonyl)amino)benzamide (13a).** Reduction of **12a** according to the procedure described in the preparation of **23a** afforded 5-amino-*N*-(2-aminopyrimidin-5-yl)-2-methylbenzamide. The title compound was prepared from 5-amino-*N*-(2-aminopyrimidin-5-yl)-2-methylbenzamide (0.086 g, 0.353 mmol) and 3-(trifluoromethyl)benzoyl chloride (0.052 mL, 0.353 mmol) using the procedure described for the synthesis of **27**. Purification via column chromatography on silica gel (gradient elution with 0−100% 90:10:1, CH_2Cl_2/CH_3OH/NH_4OH−CH_2Cl_2) afforded the title compound (0.060 g, 41% yield) as an off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.56 (s, 1H), 10.16 (s, 1H), 8.52 (s, 2H), 8.33 (s, 1H), 8.28 (d, J = 7.5 Hz, 1H), 7.99 (d, J = 6.6 Hz, 1H), 7.99 (d, J = 2.2 Hz, 1H), 7.85 - 7.77 (m, 2H), 7.32 (d, J = 8.5 Hz, 1H), 6.55 (s, 2H), 2.37 (s, 3H). HRMS $(C_{20}H_{17}F_3N_5O_2)^+$: calcd, 416.132 89; found, 416.133 27.

2-Methyl-*N*-(2-((4-(4-methyl-1-piperazinyl)phenyl)amino)-5-pyrimidinyl)-5-(((3-(trifluoromethyl)phenyl)carbonyl)amino)benzamide (13b). Reduction of **12a** in a manner analogous to the method described for the reduction of **12a** in the preparation of **23a** afforded amino-2-methyl-*N*-(2-(4-(4-methylpiperazin-1-yl)phenylamino)pyrimidin-5-yl)benzamide. The title compound was prepared from 5-amino-2-methyl-*N*-(2-(4-(4-methylpiperazin-1-yl)phenylamino)pyrimidin-5-yl)benzamide (0.113 g, 0.271 mmol) and 3-(trifluoromethyl)benzoyl chloride (0.044 mL, 0.298 mmol) using the procedure described for the synthesis of **27**. Trituration with CH_2Cl_2, filtration, washing with CH_2Cl_2, and drying on high vacuum overnight afforded the title compound (0.093 g, 58%) as a light-yellow amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.58 (s, 1H), 10.35 (s, 1H), 9.37 (s, 1H), 8.73 (s, 2H), 8.33 (s, 1H), 8.29 (d, J = 7.9 Hz, 1H), 7.99 (d, J = 6.9 Hz, 1H), 7.93 (d, J = 2.1 Hz, 1H), 7.85 (dd, J = 2.4, 8.2 Hz, 1H), 7.81 (t, J = 7.8 Hz, 1H), 7.56 (d, J = 9.2 Hz, 2H), 7.33 (d, J = 8.7 Hz, 1H), 6.89 (d, J = 9.2 Hz, 2H), 3.06 (m, 4H), 2.47 (m, 4H), 2.39 (s, 3H), 2.23 (s, 3H). HRMS $(C_{31}H_{31}F_3N_7O_2)^+$: calcd, 590.248 58; found, 590.248 12.

2-Methyl-*N*-(2-((3-(4-morpholinyl)propyl)amino)-5-pyrimidinyl)-5-(((3-(trifluoromethyl)phenyl)carbonyl)amino)benzamide (13c). **4** (0.500 g, 3.13 mmol), 3-morpholinopropan-1-amine (1.35 g, 9.39 mmol), and *N,N*-diisopropylethylamine (3.4 mL, 4.7 mmol) were taken up in IPA (20 mL) in a resealable Pyrex tube and heated at 70 °C for 20 h. After the mixture was cooled to room temperature, the product precipitated out of solution and was collected by filtration through a Buchner equipped with a micromembrane filter, washed with CH_3OH, and dried to afford **5c** [MS (ESI, positive ion) *m/z*: 268 (MH^+)] as a pale-yellow solid, which was used without further purification. Reduction of **5c** in a manner analogous to the method described for the reduction of **12a** in the preparation of **23a** afforded **6c** [MS (ESI, positive ion) *m/z*: 270 (M^+)] as a thick rust-colored oil, which was used without purification. The title compound was prepared from **6c** (0.074 g, 0.20 mmol) and 3-(trifluoromethyl)benzoyl chloride (0.030 mL, 0.20 mmol) using the procedure described for the synthesis of **27**. Trituration with CH_2Cl_2, filtration, washing with CH_2Cl_2, and drying under high vacuum overnight afforded the title compound (0.062 g, 58%) as a light-yellow amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.56 (s, 1H), 10.15 (s, 1H), 8.54 (s, 2H), 8.32 (s, 1H), 8.28 (d, J = 7.2 Hz, 1H), 7.99 (d, J = 8.2 Hz, 1H), 7.89 (s, 1H), 7.85−7.79 (m, 2H), 7.31 (d, J = 8.6 Hz, 1H), 7.13 (m, 1H), 3.57 (m, 4H), 3.31−3.26 (m, 2H), 2.36 (s, 3H), 2.35−2.30 (m, 6H), 1.68 (m, 2H). HRMS $(C_{27}H_{30}F_3N_6O_3)^+$: calcd, 543.232 60; found, 543.235 18.

2-Methyl-*N*-(2-((1-methyl-4-piperidinyl)amino)-5-pyrimidinyl)-5-(((3-(trifluoromethyl)phenyl)carbonyl)amino)benzamide (13d). 1-Methylpiperidin-4-amine (0.358 g, 3.13 mmol) and NaH (60% dispersion in mineral oil) (0.126 g, 3.13 mmol) were taken up in THF (16 mL) under an atmosphere of dry N_2. After the mixture was stirred at room temperature for 10 min, **4** (0.500 g, 3.13 mmol) was added. The mixture was allowed to stir at room temperature for 1 h, then quenched with 1 N HCl, extracted with CH_2Cl_2, and concentrated to afford **5d** [MS (ESI, positive ion) *m/z*: 239 (M^+)]

as a tan amorphous solid, which was used without further purification. Reduction of **5d** in a manner analogous to the method described for the reduction of **12a** in the preparation of **23a** afforded **6d** [MS (ESI, positive ion) *m*/*z*: 285 (MH^+)] as a tan solid, which was without further purification. The title compound was prepared from **6d** (0.063 g, 0.185 mmol) and 3-(trifluoromethyl)benzoyl chloride (0.027 mL, 0.185 mmol) using the procedure described for the synthesis of **27**. Trituration with CH_2Cl_2, filtration, washing with CH_2Cl_2, and drying under high vacuum overnight afforded the title compound as an off-white amorphous solid (0.065 g, 68%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.58 (s, 1H), 10.22 (s, 1H), 8.59 (s, 2H), 8.32 (s, 1H), 8.29 (d, J = 8.8 Hz, 1H), 7.99 (d, J = 8.2 Hz, 1H), 7.93 (d, J = 2.3 Hz, 1H), 7.83−7.79 (m, 2H), 7.33−7.30 (m, 2H), 3.88 (br s, 1H), 3.40 (br s, 2H), 3.03 (br s, 2H), 2.73 (br s, 3H), 2.36 (s, 3H), 2.06 (br s, 2H), 1.69 (br s, 2H). HRMS $(C_{26}H_{28}F_3N_6O_2)^+$: calcd, 513.222 04; found, 513.223 82.

2-Methyl-*N*-(2-((2-(4-morpholinyl)ethyl)amino)-5-pyrimidinyl)-5-(((3-(trifluoromethyl)phenyl)carbonyl)amino)benzamide (13e). **12a** (0.200 g, 0.732 mmol), CuI (0.140 g, 0.732 mmol), and CH_2I_2 (0.30 mL, 3.73 mmol) were taken up in THF (3.7 mL) in a 16 mm × 100 mm resealable Pyrex tube. The tube was sealed, and the mixture was heated at 70 °C for 2 h. After cooling to room temperature, the crude reaction mixture was taken up in 1:1 EtOAc/1 N HCl, and the layers were separated. The organics were washed with saturated aqueous NH_4Cl and then dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified via column chromatography on silica gel (elution with 100% EtOAc) to afford **24** (0.060 g, 21% yield) [MS (ESI, positive ion) *m*/*z*: 385 (MH^+)] as a light-yellow solid. **24** (0.060 g, 0.156 mmol), 2-morpholinoethanamine (0.200 g, 1.5 mmol), and *N,N*-diisopropylethylamine (0.041 mL, 0.234 mmol) were taken up in IPA (2.5 mL) in a 16 mm × 100 mm resealable Pyrex tube and heated at 70 °C for 4 h. The mixture was concentrated and purified by preparative thin layer chromatography on silica gel (elution with 2.5% CH_3OH/CH_2Cl_2) to afford 2-methyl-*N*-(2-(2-morpholinoethylamino)pyrimidin-5-yl)-5-nitrobenzamide (0.060 g, 100%) [MS (ESI, positive ion) *m*/*z*: 387 (M^+)] as yellow amorphous solid. Reduction of 2-methyl-*N*-(2-(2-morpholinoethylamino)pyrimidin-5-yl)-5-nitrobenzamide in a manner analogous to the method described for the reduction of **12a** in the preparation of **23a** afforded 5-amino-2-methyl-*N*-(2-(2-morpholinoethylamino)pyrimidin-5-yl)-benzamide [MS (ESI, positive ion) *m*/*z*: 357 (M^+)] as a light-yellow foam, which was used without further purification. The title compound was prepared from 5-amino-2-methyl-*N*-(2-(2-morpholinoethylamino)pyrimidin-5-yl)benzamide (0.060 g, 0.168 mmol) and 3-(trifluoromethyl)benzoyl chloride (0.025 mL, 0.168 mmol) using the procedure described for the synthesis of **27**. Preparative thin layer chromatography on silica gel (elution with 5% CH_3OH/CH_2Cl_2) afforded the title compound (0.056 g, 63%) as a light-yellow amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.58 (s, 1H), 10.17 (s, 1H), 8.55 (s, 2H), 8.33 (s, 1H), 8.29 (d, J = 8.1 Hz, 1H), 7.99 (d, J = 7.8 Hz, 1H), 7.90 (d, J = 2.0 Hz, 1H), 7.85−7.79 (m, 2H), 7.32 (d, J = 8.3 Hz, 1H), 6.93 (t, J = 5.8 Hz, 1H), 3.58 (m, 4H), 3.42−3.37 (m, 2H), 2.49−2.45 (m, 2H), 2.41 (m, 4H), 2.37 (s, 3H). HRMS $(C_{26}H_{28}F_3N_6O_3)^+$: calcd, 529.216 95; found, 529.220 00. Anal. ($C_{26}H_{27}F_3N_6O_3 \cdot 2CH_3OH$) H, C. N: calcd, 13.37; found, 14.28.

5-(Acetylamino)-2-methyl-*N*-(2-((4-(4-methyl-1-piperazinyl)phenyl)amino)-5-pyrimidinyl)benzamide (14). The title compound was prepared from 5-amino-2-methyl-*N*-(2-(4-(4-methylpiperazin-1-yl)phenylamino)pyrimidin-5-yl)benzamide (0.100 g, 0.24 mmol) and acetyl chloride (0.019 mL, 0.26 mmol) using the procedure described for the synthesis of **27**. Trituration with CH_2Cl_2, filtration, washing with CH_2Cl_2, and drying under high vacuum overnight afforded the title compound (0.108 g, 96%) as an off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.32 (s, 1H), 10.07 (s, 1H), 9.45 (s, 1H), 8.73 (s, 2H), 7.75 (d, J = 1.6 Hz, 1H), 7.62 (d, J = 8.9 Hz, 2H), 7.56 (dd, J = 1.8, 8.1 Hz, 1H), 7.23 (d, J = 8.2 Hz, 1H), 6.96 (d, J = 8.8 Hz, 2H), 3.85−3.39 (br m, 4H), 3.25−2.91 (br m, 4H), 2.81 (br s, 3H), 2.33 (s, 3H), 2.05 (s, 3H). HRMS $(C_{25}H_{30}N_7O_2)^+$: calcd, 460.245 55; found, 460.244 07. Anal. ($C_{25}H_{29}N_7O_2 \cdot 3H_2O$) C, H, N.

5-Methyl-*N*-(4-methyl-3-((2-(4-(4-methylpiperazin-1-yl)phenylamino)pyrimidin-5-yl)carbamoyl)phenyl)isoxazole-4-carboxamide (15). The title compound was prepared from 5-amino-2-methyl-*N*-(2-(4-(4-methylpiperazin-1-yl)phenylamino)pyrimidin-5-yl)benzamide (0.100 g, 0.24 mmol) and 5-methylisoxazole-4-carbonyl chloride (0.038 g, 0.26 mmol) using the procedure described for the synthesis of **27**. Trituration with CH_2Cl_2, filtration, washing with CH_2Cl_2, and drying under high vacuum overnight afforded the title compound (0.112 g, 86% yield) as an off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.35 (s, 1H), 10.16 (s, 1H), 9.45 (s, 1H), 9.10 (s, 1H), 8.74 (s, 2H), 7.88 (d, J = 2.4 Hz, 1H), 7.71 (dd, J = 2.0, 8.5 Hz, 1H), 7.62 (d, J = 9.2 Hz, 2H), 7.31 (d, J = 8.2 Hz, 1H), 7.96 (d, J = 8.5 Hz, 2H), 2.70 (s, 3H), 2.37 (s, 3H). HRMS $(C_{28}H_{31}N_8O_3)^+$: calcd, 527.251 36; found, 527.251 78.

2-Methyl-*N*-(2-((4-(4-methyl-1-piperazinyl)phenyl)amino)-5-pyrimidinyl)-5-((phenylcarbonyl)amino)benzamide (16). The title compound was prepared from 5-amino-2-methyl-*N*-(2-(4-(4-methylpiperazin-1-yl)phenylamino)pyrimidin-5-yl)benzamide (0.080 g, 0.19 mmol) and benzoyl chloride (0.022 mL, 0.19 mmol) using the procedure described for the synthesis of **27**. Trituration with CH_2Cl_2, filtration, washing with CH_2Cl_2, and drying under high vacuum overnight afforded the title compound (0.053 g, 53% yield) as a light-yellow amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.37 (s, 1H), 10.34 (s, 1H), 9.37 (s, 1H), 8.73 (s, 2H), 7.98 (m, 3H), 7.63−7.53 (m, 6H), 7.31 (d, J = 8.3 Hz, 1H), 6.89 (d, J = 9.2 Hz, 2H), 3.05 (m, 4H), 2.45 (m, 4H), 2.37 (s, 3H), 2.22 (s, 3H). HRMS $(C_{30}H_{32}N_7O_2)^+$: calcd, 522.261 20; found, 522.260 61. Anal. ($C_{30}H_{31}N_7O_2 \cdot 1.5CH_3OH \cdot 0.5\ CH_3CN$) C, H, N.

2-Methyl-5-(((2-(methyloxy)phenyl)carbonyl)amino)-*N*-(2-((4-(4-methyl-1-piperazinyl)phenyl)amino)-5-pyrimidinyl)benzamide (17). The title compound was prepared from 5-amino-2-methyl-*N*-(2-(4-(4-methylpiperazin-1-yl)phenylamino)pyrimidin-5-yl)benzamide (0.100 g, 0.24 mmol) and 2-methoxybenzoyl chloride (0.037 mL, 0.24 mmol) using the procedure described for the synthesis of **27**. Purification via column chromatography on silica gel (gradient elution with 0−20% CH_3OH/CH_2Cl_2) afforded the title compound (0.092 g, 70% yield) as a yellow amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.34 (s, 1H), 10.22 (s, 1H), 9.36 (s, 1H), 8.73 (s, 2H), 7.92 (s, 1H), 7.75 (d, J = 8.3 Hz, 1H), 7.64 (d, J = 7.6 Hz, 1H), 7.57−7.50 (m, 3H), 7.28 (d, J = 8.3 Hz, 1H), 7.20 (d, J = 8.3 Hz, 1H), 7.08 (t, J = 7.5 Hz, 1H), 6.89 (d, J = 8.8 Hz, 2H), 3.91 (s, 3H), 3.05 (m, 4H), 2.46 (m, 4H), 2.36 (s, 3H), 2.22 (s, 3H). HRMS $(C_{31}H_{34}N_7O_3)^+$: calcd, 552.271 76; found, 552.271 44. Anal. ($C_{31}H_{34}N_7O_3$) H, N. C: calcd, 67.5; found, 66.7.

5-(((3-Fluoro-5-(trifluoromethyl)phenyl)carbonyl)amino)-2-methyl-*N*-(2-((4-(4-methyl-1-piperazinyl)phenyl)amino)-5-pyrimidinyl)benzamide (18). The title compound was prepared from 5-amino-2-methyl-*N*-(2-(4-(4-methylpiperazin-1-yl)phenylamino)pyrimidin-5-yl)benzamide (0.100 g, 0.24 mmol) and 3-fluoro-5-(trifluoromethyl)benzoyl chloride (0.040 mL, 0.26 mmol) using the procedure described for the synthesis of **27**. Trituration with CH_2Cl_2, filtration, washing with CH_2Cl_2, and drying under high vacuum overnight afforded the title compound (0.065 g, 44% yield) as a light-yellow amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.72 (s, 1H), 10.35 (s, 1H), 9.37 (s, 1H), 8.72 (s, 2H), 8.07 (m, 1H), 8.01 (m, 1H), 7.85 (s, 1H), 7.74 (dd, J = 1.8, 8.6 Hz, 1H), 7.64 (t, J = 8.9 Hz, 1H), 7.56 (d, J = 8.9 Hz, 2H), 7.33 (d, J = 8.5 Hz, 1H), 6.89 (d, J = 9.1 Hz, 2H), 3.05 (m, 4H), 2.46 (m, 4H), 2.38 (s, 3H), 2.22 (s, 3H). HRMS $(C_{31}H_{30}F_4N_7O_2)^+$: calcd, 608.239 16; found, 608.238 31. Anal. ($C_{31}H_{29}F_4N_7O_2$) C, N. H: calcd, 4.81; found, 5.23.

2-Methyl-*N*-(4-methyl-3-(((2-((4-(4-methyl-1-piperazinyl)phenyl)amino)-5-pyrimidinyl)amino)carbonyl)phenyl)-3-(trifluoromethyl)benzamide (19). The title compound was prepared from 5-amino-2-methyl-*N*-(2-(4-(4-methylpiperazin-1-yl)phenylamino)pyrimidin-5-yl)benzamide (0.080 g, 0.191 mmol) and 2-methyl-3-(trifluoromethyl)benzoyl chloride (0.047 g, 0.210 mmol) using the procedure described for the synthesis of **27**. Trituration with CH_2Cl_2,

filtration, washing with CH_2Cl_2, and drying under high vacuum overnight afforded the title compound (0.068 g, 59%) as an off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.62 (s, 1H), 10.34 (s, 1H), 9.36 (s, 1H), 8.71 (s, 2H), 7.90 (d, J = 1.9 Hz, 1H), 7.83 (d, J = 7.7 Hz, 1H), 7.71 (t, J = 7.3 Hz, 2H), 7.56−7.52 (m, 3H), 7.31 (d, J = 8.5 Hz, 1H), 6.88 (d, J = 8.8 Hz, 2H), 3.05 (m, 4H), 2.46 (m, 7H), 2.36 (s, 3H), 2.22 (s, 3H). HRMS $(C_{32}H_{33}F_3N_7O_2)^+$: calcd, 604.264 68; found, 604.264 68.

2,2-Difluoro-*N*-(4-methyl-3-(((2-((4-(4-methyl-1-piperazinyl)phenyl)amino)-5-pyrimidinyl)amino)carbonyl)phenyl)-1,3-benzodioxole-5-carboxamide (20). The title compound was prepared from 5-amino-2-methyl-*N*-(2-(4-(4-methylpiperazin-1-yl)phenylamino)pyrimidin-5-yl)benzamide (0.080 g, 0.191 mmol) and 2,2-difluorobenzo[*d*][1,3]dioxole-5-carbonyl chloride (0.046 g, 0.210 mmol) using the procedure described for the synthesis of **27**. Purification via column chromatography on silica gel (gradient elution with 0−100% 90:10:1, CH_2Cl_2/CH_3OH/NH_4OH−CH_2Cl_2) afforded the title compound (0.069 g, 60% yield) as a light-yellow amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.40 (s, 1H), 10.34 (s, 1H), 9.37 (s, 1H), 8.72 (s, 2H), 7.99 (s, 1H), 7.92 - 7.89 (m, 2H), 7.79 (dd, J = 1.6, 8.3 Hz, 1H), 7.60 (d, J = 8.3 Hz, 1H), 7.56 (d, J = 8.8 Hz, 2H), 731 (d, J = 8.3 Hz, 1H), 6.89 (d, J = 8.8 Hz, 2H), 3.08 (m, 4H), 2.55 (m, 4H), 2.37 (s, 3H), 2.29 (s, 3H). HRMS $(C_{31}H_{30}F_2N_7O_4)^+$: calcd, 602.232 19; found, 602.232 34.

5-(((4-(1,1-Dimethylethyl)phenyl)carbonyl)amino)-2-methyl-*N*-(2-((4-(4-methyl-1-piperazinyl)phenyl)amino)-5-pyrimidinyl)-benzamide (21). The title compound was prepared from 5-amino-2-methyl-*N*-(2-(4-(4-methylpiperazin-1-yl)phenylamino)pyrimidin-5-yl)benzamide (0.080 g, 0.191 mmol) and 4-*tert*-butylbenzoyl chloride (0.042 mL, 0.210 mmol) using the procedure described for the synthesis of **27**. Trituration with CH_2Cl_2, filtration, washing with CH_2Cl_2, and drying under high vacuum overnight afforded the title compound (0.058 g, 53%) as a white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.33 (s, 1H), 10.28 (s, 1H), 9.36 (s, 1H), 8.72 (s, 2H), 7.95 (d, J = 1.8 Hz, 1H), 7.91 (d, J = 8.5 Hz, 2H), 7.81 (dd, J = 2.0, 8.6 Hz, 1H), 7.56 (d, J = 8.5 Hz, 4H), 7.29 (d, J = 8.5 Hz, 1H), 6.89 (d, J = 9.0 Hz, 2H), 3.05 (m, 4H), 2.47 (m, 4H), 2.36 (s, 3H), 2.23 (s, 3H), 1.32 (s, 9H). HRMS $(C_{34}H_{40}N_7O_2)^+$: calcd, 578.323 80; found, 578.324 08.

5-(((3,5-Bis(methyloxy)phenyl)carbonyl)amino)-2-methyl-*N*-(2-((4-(4-methyl-1-piperazinyl)phenyl)amino)-5-pyrimidinyl)-benzamide (22). The title compound was prepared from 5-amino-2-methyl-*N*-(2-(4-(4-methylpiperazin-1-yl)phenylamino)pyrimidin-5-yl)benzamide (0.080 g, 0.191 mmol) and 3,5-dimethoxybenzoyl chloride (0.042 g, 0.210 mmol) using the procedure described for the synthesis of **27**. Trituration with CH_2Cl_2, filtration, washing with CH_2Cl_2, and drying under high vacuum overnight afforded the title compound (0.030 g, 27%) as an off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.32 (s, 1H), 10.28 (s, 1H), 9.36 (s, 1H), 8.72 (s, 2H), 7.92 (d, J = 1.9 Hz, 1H), 7.82 (dd, J = 2.0, 8.5 Hz, 1H), 7.55 (d, J = 8.8 Hz, 2H), 7.30 (d, J = 8.1 Hz, 1H), 7.12 (d, J = 2.2 Hz, 2H), 6.88 (d, J = 9.2, 2H), 6.72 (t, J = 2.0 Hz, 1H), 3.82 (s, 6H), 3.05 (m, 4H), 2.46 (m, 4H), 2.37 (s, 3H), 2.22 (s, 3H). HRMS $(C_{32}H_{36}N_7O_4)^+$: calcd, 582.282 33; found, 582.282 62.

***N*-(2-Amino-5-pyrimidinyl)-2-methyl-5-((((3-(trifluoromethyl)phenyl)amino)carbonyl)amino)benzamide (23a). 12a** (0.300 g, 1.1 mmol) and 10% Pd/C (∼0.150 g) were combined in EtOAc (24 mL) and CH_3OH (6 mL). The mixture was purged with H_2, then allowed to stir under 1 atm of H_2 for 24 h. The mixture was filtered through a pad of Celite, and washing with CH_3OH and CH_2Cl_2 and drying afforded 5-amino-*N*-(2-aminopyrimidin-5-yl)-2-methylbenzamide [MS (ESI, positive ion) *m*/*z*: 244 (MH^+)] as a pale-yellow amorphous solid, which was used without purification. 5-Amino-*N*-(2-aminopyrimidin-5-yl)-2-methylbenzamide (0.108 g, 0.444 mmol) was taken up in benzene (8 mL), and 1-isocyanato-3-(trifluoromethyl)benzene (0.062 mL, 0.444 mmol) was added. The mixture was allowed to stir at room temperature overnight. The reaction mixture was concentrated and the residue was purified via column chromatography on silica gel (gradient elution with 0−5% CH_3OH/CH_2Cl_2) to afford the title compound (0.040 g, 20% yield) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.11 (s, 1H), 9.09 (s, 1H), 8.89 (s, 1H), 8.52 (s, 2H), 8.03 (s, 1H), 7.62 (d, J = 1.9 Hz, 1H), 7.58 (d, J = 7.8 Hz, 1H), 7.52 (t, J = 7.8 Hz, 1H), 7.45 (dd, J = 8.3, 2.1 Hz, 1H), 7.32 (d, J = 7.3 Hz, 1H), 7.22 (d, J = 8.2 Hz, 1H), 6.54 (s, 2H), 2.32 (s, 3H). HRMS $(C_{20}H_{18}F_3N_6O_2)^+$: calcd, 431.143 78; found, 431.143 01.

2-Methyl-*N*-(2-((4-(4-methyl-1-piperazinyl)phenyl)amino)-5-pyrimidinyl)-5-((((3-(trifluoromethyl)phenyl)amino)carbonyl)amino)benzamide (23b). In a 2 L stainless steel pressure vessel with overhead stirring was charged a mixture of **12b** (25 g, 55.9 mmol), EtOH (300 mL), EtOAc (200 mL), and Pd/C (15 g, 10% by weight wet). The reactor was charged with 65 psi of H_2 for 72 h. The catalyst was removed by suction filtration and washed with EtOH. The organics were concentrated under reduced pressure and dried under vacuum to obtain 5-amino-2-methyl-*N*-(2-(4-(4-methylpiperazin-1-yl)phenylamino)pyrimidin-5-yl)benzamide [MS (ESI, positive ion) *m*/*z*: 418 (MH^+)], which was used without purification. The title compound was prepared from 5-amino-2-methyl-*N*-(2-(4-(4-methylpiperazin-1-yl)phenylamino)pyrimidin-5-yl)benzamide (0.071 g, 0.170 mmol) and 1-isocyanato-3-(trifluoromethyl)benzene (0.026 mL, 0.187 mmol) using the procedure described for the synthesis of **23a**. Purification via column chromatography on silica gel (gradient elution with 0−100% 90:10:1, CH_2Cl_2/CH_3OH/NH_4OH−CH_2Cl_2) afforded the title compound as a light-yellow solid (0.047 g, 46%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.29 (s, 1H), 9.35 (s, 1H), 9.08 (s, 1H), 8.89 (s, 1H), 8.71 (s, 2H), 8.03 (s, 1H), 7.64 (s, 1H), 7.58−7.44 (m, 5H), 7.31 (d, J = 7.6 Hz, 1H), 7.23 (d, J = 8.3 Hz, 1H), 6.89 (d, J = 8.7 Hz, 2H), 3.05 (br s, 4H), 2.46 (br s, 4H), 2.33 (s, 3H), 2.22 (s, 3H). HRMS $(C_{31}H_{32}F_3N_8O_2)^+$: calcd, 605.259 48; found, 605.260 56.

2,6-Dichloro-*N*-(2-((4-(4-methyl-1-piperazinyl)phenyl)amino)-5-pyrimidinyl)benzamide (27). 2,6-Dichlorobenzoyl chloride (0.061 mL, 0.43 mmol) was added to a suspension of *N*2-(4-(4-methylpiperazin-1-yl)phenyl)pyrimidine-2,5-diamine (0.11 g, 0.39 mmol) and CH_2Cl_2 (2 mL). The solution was stirred at room temperature for 10 min, and then triethylamine (0.070 mL, 0.50 mmol) was added. The suspension was stirred at room temperature overnight. The reaction mixture was filtered through a Buchner apparatus equipped with a micromembrane filter, washed with CH_2Cl_2, and dried under vacuum overnight to afford the title compound (0.141 g, 79%) as a pale-yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.78 (s, 1H), 9.44 (s, 1H), 8.68 (s, 2H), 7.93−7.51 (m, 5H), 6.89 (d, J = 8.8 Hz, 2H), 3.06 (m, 4H), 2.45 (m, 4H), 2.22 (s, 3H). HRMS $(C_{22}H_{23}C_{l2}N_6O)^+$: calcd, 457.130 49; found, 457.130 83. Anal. ($C_{22}H_{22}C_{l2}N_6O$) C, H, N.

2,6-Dimethyl-*N*-(2-((4-(4-methyl-1-piperazinyl)phenyl)amino)-5-pyrimidinyl)-3-(((3-(trifluoromethyl)phenyl)carbonyl)amino)benzamide (29). 28 was prepared from **26** (0.300 g, 1.5 mmol) and **6b** (0.48 g, 1.7 mmol) using the procedure described for the synthesis of **12a**. Purification via column chromatography on silica gel (gradient elution with 3−10% CH_3OH/CH_2Cl_2) afforded **28** (0.73 g, 90%) [MS (ESI, positive ion) *m*/*z*: 462 (MH^+)] as a yellow solid. Reduction of **28** in a manner analogous to the method described for the reduction of **12a** in the preparation of **23a** afforded crude 3-aniline, which was purified via preparative reverse-phase HPLC (eluting with 10–90% (0.1% trifluoroacetic acid in CH_3CN)−water over 15 min). Clean fractions were combined and partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. The aqueous phase was separated and extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated to afford 3-amino-2,6-dimethyl-*N*-(2-(4-(4-methylpiperazin-1-yl)phenylamino)pyrimidin-5-yl)benzamide (0.550 g, 80%) [MS (ESI, positive ion) *m*/*z*: 432.1 (MH^+)] as a light-yellow solid. The title compound was prepared from 3-amino-2,6-dimethyl-*N*-(2-(4-(4-methylpiperazin-1-yl)phenylamino)pyrimidin-5-yl)benzamide (80 mg, 0.2 mmol) and 3-(trifluoromethyl)benzoyl chloride (0.04 mL, 0.2 mmol) using the procedure described for the synthesis of **27** and purified via preparative reverse-phase HPLC (eluting with 10–90% (0.1% trifluoroacetic acid in CH_3CN)−water over 15 min). Clean fractions were combined and partitioned between CH_2Cl_2 and saturated aqueous

$NaHCO_3$. The aqueous phase was separated and extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated to afford the title compound (0.023 g, 20%) as a light-yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.55 (s, 1H), 10.29 (s, 1H), 9.44 (s, 1H), 8.78 (s, 2H), 8.37 (m, 2H), 8.05 (d, J = 8.8 Hz, 1H), 7.86 (t, J = 7.8 Hz, 1H), 7.62 (d, J = 8.8 Hz, 2H), 7.37 (d, J = 8.2 Hz, 1H), 7.24 (d, J = 8.1 Hz, 1H), 6.94 (d, J = 9.0 Hz, 2H), 3.12 (m, 4H), 2.53 (m, 4H), 2.38 (s, 3H), 2.29 (s, 3H), 2.26 (s, 3H). HRMS $(C_{32}H_{33}F_3N_7O_2)^+$: calcd, 604.264 23; found, 604.265 65.

***N*-(4-Chloro-3-(((2-((4-(4-methyl-1-piperazinyl)phenyl)amino)-5-pyrimidinyl)amino)carbonyl)phenyl)-2,2-difluoro-1,3-benzodioxole-5-carboxamide (32). 30** (0.150 g, 0.874 mmol) was added to a solution of 2,2-difluoro-1,3-benzodioxole-5-carbonyl chloride (0.202 g, 0.918 mmol) in CH_2Cl_2 (9.0 mL). The mixture was stirred at room temperature for 2.5 h to afford a white suspension, and triethylamine (0.16 mL, 1.14 mmol) was added. The resulting yellow solution was stirred at room temperature for 16 h. The reaction mixture was concentrated to afford an off-white solid. This solid was partitioned between CH_2Cl_2 and water. The aqueous phase was separated and extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated to afford **31** (0.193 g, 62%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.43 (s, 1H), 8.01 (d, J = 1.4 Hz, 1H), 7.97 (s, 1H), 7.90 (dd, J = 8.3, 1.4 Hz, 1H), 7.77−7.84 (m, 1H), 7.58 (d, J = 8.5 Hz, 1H), 7.36 (d, J = 8.5 Hz, 1H). MS (ESI, positive ion) m/z: 355.9 (MH^+). A resealable tube was charged with **6b** (0.100 g, 0.35 mmol), **31** (0.16 g, 0.46 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.10 g, 0.53 mmol), 1-hydroxy-7-azabenzotriazole (0.048 g, 0.35 mmol), and DMF (2.5 mL). *N*,*N*-Diisopropylethylamine (0.214 mL, 1.2 mmol) was added, and the system was flushed with argon. The tube was sealed and the mixture stirred at room temperature for 17 h. The reaction mixture was concentrated to afford an orange-brown oil. This oil was partitioned between CH_2Cl_2 and water, and saturated aqueous $NaHCO_3$ was added. The aqueous layer was separated and extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated to afford an orange-brown oil. This material was purified via column chromatography on silica gel (gradient elution with 0−100% 90:10:1, CH_2Cl_2/CH_3OH/NH_4OH−CH_2Cl_2) to afford an orange solid. This material was dissolved in 1:1 (DMSO/CH_3OH) and further purified via preparative reverse-phase HPLC (eluting with 10−90% (0.1% trifluoroacetic acid in CH_3CN−water over 15 min). Clean fractions were combined and partitioned between EtOAc and saturated aqueous $NaHCO_3$. The aqueous phase was separated and extracted with EtOAc. The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated to afford the title compound (0.092 g, 42%) as a yellow solid. MS (ESI, positive ion) m/z: 622 (MH^+). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.56 (s, 2H), 9.40 (s, 1H), 8.70 (s, 2H), 8.02 (d, J = 12.8 Hz, 2H), 7.90 (d, J = 8.6 Hz, 2H), 7.45−7.72 (m, 4H), 6.88 (d, J = 8.5 Hz, 2H), 3.05 (s, 4H), 2.41−2.48 (m, 4H), 2.22 (s, 3H). HRMS $(C_{30}H_{27}ClF_2N_7O_4)^+$: calcd, 622.177 56; found, 622.176 69.

***N*-(4-Ethyl-3-(((2-((4-(4-methyl-1-piperazinyl)phenyl)amino)-5-pyrimidinyl)amino)carbonyl)phenyl)-2,2-difluoro-1,3-benzodioxole-5-carboxamide (33).** A resealable tube was charged with **32** (0.050 g, 0.080 mmol), palladium(II) acetate (0.0018 g, 0.0080 mmol), S-Phos (0.0066 g, 0.016 mmol), potassium phosphate (0.068 g, 0.32 mmol), and THF (1.0 mL). A solution of triethylborane (1 M in THF, 0.12 mL, 0.12 mmol) was added, and the system was flushed with argon. The tube was sealed and the mixture stirred at 60 °C for 18 h. The THF was removed in vacuo, and DMF (2.0 mL) was added. Palladium(II) acetate (0.0018 g, 0.0080 mmol), S-Phos (0.0066 g, 0.016 mmol), potassium phosphate (0.068 g, 0.32 mmol), and triethylborane (0.12 mL, 0.12 mmol) were added, the system was evacuated and purged with argon, and the tube was sealed. The mixture stirred at 100 °C for 6 h. The reaction mixture was filtered through a pad of Celite and concentrated to afford a yellow-green solid. This material was purified via preparative thin layer chromatography TLC (eluting three times with 95:5:0.5, CH_2Cl_2/CH_3OH/NH_4OH-CH_2Cl_2) to afford the title compound (0.010 g, 0.016 mmol, 20%) as an off-white solid. MS (ESI, positive ion) m/z: 616 (MH^+). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.42 (s, 1H), 10.37 (s, 1H), 9.36 (s, 1H), 8.71 (s, 2H), 7.99 (s, 1H), 7.87−7.94 (m, 2H), 7.82 (d, J = 9.3 Hz, 1H), 7.60 (d, J = 8.3 Hz, 1H), 7.55 (d, J = 8.2 Hz, 2H), 7.35 (d, J = 8.0 Hz, 1H), 6.88 (d, J = 8.5 Hz, 2H), 3.06 (s, 4H), 2.67−2.78 (m, 2H), 2.42−2.50 (m, 4H), 2.24 (s, 3H), 1.17 (t, J = 7.4 Hz, 3H). HRMS $(C_{32}H_{32}F_2N_7O_4)^+$: calcd, 616.247 84; found, 616.248 29.

2-Methyl-*N*-(5-pyrimidinyl)-5-(((3-(trifluoromethyl)phenyl)-carbonyl)amino)benzamide (37). 5-Amino-2-methyl-5-nitro-*N*-(pyrimidin-5-yl)benzamide was prepared from **26** and pyrimidin-5-amine according to the procedure used for the synthesis of **12b**. Reduction of 5-amino-2-methyl-5-nitro-*N*-(pyrimidin-5-yl)benzamide in a manner analogous to the method described for the reduction of **12a** in the preparation of **23a** afforded 5-amino-2-methyl-*N*-(pyrimidin-5-yl)benzamide [MS (ESI, positive ion) m/z: 228 (M^+)], which was used without further purification. The title compound was prepared from 5-amino-2-methyl-*N*-(pyrimidin-5-yl)benzamide (0.087 g, 0.38 mmol) and 3-(trifluoromethyl)benzoyl chloride (0.088 g, 0.42 mmol) according to the procedure described for the synthesis of **27**. Purification via column chromatography on silica gel (gradient elution with 0−10% CH_3OH/CH_2Cl_2) afforded the title compound (0.083 g, 55% yield) as an off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.82 (s, 1H), 10.61 (s, 1H), 9.16 (s, 2H), 8.95 (s, 1H), 8.31 (m, 2H), 7.99 (s, 2H), 7.86 (s, 1H), 7.81 (s, 1H), 7.37 (s, 1H), 2.40 (s, 3H). HRMS $(C_{20}H_{16}F_3N_4O_2)^+$: calcd, 401.121 99; found, 401.123 99.

Biological Materials and Methods. Lck Kinase Assay. The Lck HTRF kinase assay involves ATP-dependent phosphorylation of a biotinylated substrate peptide of gastrin in the presence or absence of inhibitor compound. The final concentration of gastrin was 1.2 μM. The final concentration of ATP was 0.5 μM ($K_{m(app)}$ = 0.6 ± 0.1 μM), and the final concentration of Lck (a GST-kinase domain fusion (AA 225–509)) was 250 pM. Buffer conditions were as follows: 50 mM HEPES, pH 7.5, 50 mM NaCl, 20 mM MgCl, 5 mM MnCl, 2 mM DTT, 0.05% BSA. The assay was quenched and stopped with 160 μL of detection reagent. Detection reagents were as follows: buffer made of 50 mM Tris, pH 7.5, 100 mM NaCl, 3 mM EDTA, 0.05% BSA, 0.1% Tween-20. Prior to reading, steptavidin allophycocyanin (SA-APC) was added at a final concentration in the assay of 0.0004 mg/mL, along with europilated antiphosphotyrosine Ab (Eu-anti-PY) at a final concentration of 0.025 nM. The assay plate was read in a Discovery fluorescence plate reader with excitation at 320 nm and emission at 615 and 655 nm. Reported IC_{50} values are the mean of two or more separate determinations run in duplicate.

Assays for other kinases were done in a similar way as described above, varying the concentrations of enzyme, peptide substrate, and ATP added to the reaction, depending on the specific activity of the kinase and measured K_m values for the substrates.

Human Mixed Lymphocyte Reaction (MLR). The purpose of this assay is to test the potency of T cell activation inhibitors in an in vitro model of allogeneic T cell stimulation. Human peripheral blood lymphocytes (hPBL, 2 × 10^5/well) were incubated with mitomycin C treated B lymphoblastoid cells (JY cell line (ATCC, Rockville, MD), 1 × 10^5/well) as allogeneic stimulators in the presence or absence of dilutions of potential inhibitor compound in 96-well round-bottom tissue culture plates. These cultures were incubated at 37 °C in 5% CO_2 for 6 days total. The proliferative response of the hPBL was measured by ^{3}H-thymidine incorporation overnight between days 5 and 6 after initiation of culture. Cells were harvested onto glass fiber filters, and ^{3}H-thymidine incorporation into DNA was analyzed by liquid scintillation counter.

Anti-CD3/CD28-Induced T Cell IL-2 Secretion and Proliferation Assay (IL-2). The purpose of this assay was to test the potency of T cell receptor (TCR, CD3) and CD28 signaling pathway inhibitors in human T cells. T cells were purified from human peripheral blood lymphocytes (hPBL) and preincubated with or without compound prior to stimulation with a combination of an

anti-CD3 and an anti-CD28 antibody in 96-well tissue culture plates (1×10^5 T cells/well). Cells were cultured for ~20 h at 37 °C in 5% CO_2, then secreted IL-2 in the supernatants was quantified by cytokine ELISA (Pierce/Endogen, St. Louis, MO). The cells remaining in the wells were then pulsed with ^{3}H-thymidine overnight to assess the T cell proliferative response. Cells were harvested onto glass fiber filters, and ^{3}H-thymidine incorporation into DNA was analyzed by a liquid scintillation counter. For comparison purposes, phorbol myristic acid (PMA) and calcium ionophore can be used in combination to induce IL-2 secretion from purified T cells. Potential inhibitor compounds can be tested for inhibition of this response as described above for anti-CD3 and -CD28 antibodies.

Human whole-blood anti-CD3/CD28-induced IL-2 secretion assays were run in a similar fashion as described above using whole blood from normal volunteers diluted 50% in tissue culture medium prior to stimulation.

Human Whole-Blood LPS-Induced TNFα. Compounds were preincubated with heparinized human whole blood diluted in RPMI 1640 with L-glutamine (GIBCO) supplemented with 10% v/v human serum AB (Gemini BioSciences) and 1% pen/strep at a final whole-blood dilution of 50% in 96-well flat-bottom plates (Falcon) for 1 h at 37 °C. LPS (List Biological Laboratories, 0.1 μg/mL Final) was subsequently added. Plates were then incubated overnight (18 h) at 37 °C. Secreted cytokines were measured by ELISA.

Pharmacokinetic Studies. Male Sprague–Dawley rats were administered compound, iv as a solution in DMSO or po as a suspension in 2% hydroxypropyl methylcellulose with 1% Tween-80, at the indicated doses. Samples were taken at various times after dosing and were analyzed for parent compound by the LC−MS method.

Anti-CD3 Induced IL-2 Production in Mice. Twelve week old (20 g) BALB/c mice were dosed 1 h prior to challenge with compound po (8 per group) at the indicated doses in 2% hydroxypropyl methylcellulose with 1% Tween-80. Mice were then challenged iv with antimouse CD3 monoclonal antibody (145.2C11, BD PharMingen, San Diego, CA; 3 μg/mouse) diluted in PBS. At 90 min after anti-CD3 challenge, blood was collected via cardiac puncture. IL-2 levels were measured in serum by ELISA (BioSource, Camarillo, CA). Data points represent the mean IL-2 levels/group ± the standard error. The *p* values were determined vs vehicle control by the Mann–Whitney *U*-test.

Protein Binding Assay. An aliquot of test article (DMSO solution) was added to plasma in a well of a 96-deep well plate (Nunc, Nalge Nunc International, Rochester, NY) to make a 10 μM plasma sample. The DMSO content was 0.1%. The plate was then incubated at 37 °C for 45 min. After incubation aliquots of samples (two aliquots per sample) were transferred to a Millipore MultiScreen Filter Plate incorporating Ultracel-10 membrane with a 10 000 molecular weight cutoff (Millipore Co., Billerica, MA). The filter plate was centrifuged at 3000 rpm for 45 min at 37 °C by Swinging-Bucket Rotor (Allegra 25R, refrigerated benchtop centrifuge, Beckman Coulter, Fullerton, CA). After centrifugation, an aliquot of each filtrate was transferred and mixed with one volume of acetonitrile/water (25:75, v/v, containing 0.1% formic acid and internal standard) to create unbound samples. Another plate was created by spiking the test article (DMSO solution) to plasma filtrate to make 10 μM reference samples. Each well of the reference sample was mixed with one volume of acetonitrile/water (25:75, v/v, containing 0.1% formic acid and internal standard). Reference samples and unbound samples were analyzed by LC−MS. The peak ratio was determined from the MS response of the test article versus the MS response of the internal standard. The percent of binding was calculated as % protein binding = [1 − (mean peak ratios of unbound)/(peak ratio of reference)] × 100.

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Supporting Information Available: Elemental analysis and HPLC data, X-ray methods and data, exposures for **13b** in the mouse IL-2, and additional references. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) EC_{50} (fu) = (EC_{50})(mouse fu) = (175 nM)(0.022) = 4 nM.

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