2-Chloro-4-(trifluoromethyl)pyrimidine-5-*N*-(3′,5′-bis(trifluoromethyl)phenyl)-carboxamide: A Potent Inhibitor of NF-κB- and AP-1-Mediated Gene Expression Identified Using Solution-Phase Combinatorial Chemistry

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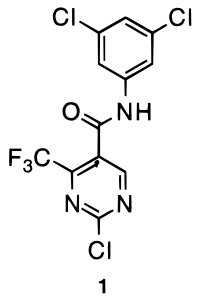
Described is the identification of a novel series of compounds that blocks the activation of two key transcription factors, AP-1 and NF-κB. These transcription factors regulate the expression of several critical proinflammatory proteins and cytokines and represent attractive targets for drug discovery. Through the use of high throughput screening and solution-phase parallel synthesis, inhibitors of both NF-κB and AP-1 were identified. In subsequent testing, these compounds were also shown to block both IL-2 and IL-8 levels in the same cells. One of the most potent compounds in this series, **28**, was active in several animal models of inflammation and immunosuppression, thus validating the importance of AP-1 and NF-κB as potential therapeutic targets. The synthesis and preliminary structure−activity relationships of these compounds is addressed.

Introduction

In certain autoimmune diseases and chronic inflammatory states, the continuous activation of T-cells leads to a self-perpetuating destruction of normal tissues or organs.[1] This activation initiates a cascade of events that results in the overproduction of certain transcription factors and proinflammatory cytokines.[2,3] Transcription factors are a family of proteins that act as molecular switches and regulate several cellular events, including gene expression, cytokine production, and the synthesis of additional cellular regulators.[4]

Two transcription factors in particular, nuclear factor-κ binding (NF-κB) and activator protein-1 (AP-1), control the production of many cytokines and related proteins elevated in immunoinflammatory diseases.[5−7] These include interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-α (TNFα). NF-κB plays a significant role in the regulation of IL-8 transcription during exposure of cells to external stimuli, while AP-1 regulates both IL-2 and TNF-α production during T-cell activation. Therefore, modulation of either one or both of these transcription factors should lead to suppression of cytokine levels and, thus, represent attractive targets for the prevention of immunoinflammatory diseases.[8]

To date, no known antiinflammatory or autoimmune drugs have been specifically developed clinically as inhibitors of NF-κB and/or AP-1. Herein, we report the identification of a series of novel inhibitors of both NF-κB and AP-1 transcriptional activation and the subsequent validation of these transcription factors as drug discovery targets. Using automated high-throughput assays with stably transfected human Jurkat T-cells, we identified a compound that inhibited both NF-κB and AP-1 transcriptional activation (**1**, IC_{50} = 0.3−0.5 μM).[9,10] In addition, compound **1** had a similar inhibitory effect on the production of IL-2 and IL-8 levels in stimulated cells and was active in an animal model of inflammation.[11] Through the use of solution-phase parallel chemistry and targeted synthesis, a 10-fold more potent derivative, **28**, was identified. This paper describes the use of these techniques and the resulting structure−activity relationships of this series of compounds.



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Chemistry

The compounds listed in Table 1 were prepared as illustrated in Schemes 1 and 2. The 2-amino derivatives (**2**−**8**) were synthesized by stirring **1** with an appropriate amine in THF. The 2-hydroxy and the 2-methoxy derivatives (**9** and **10**) were prepared by treatment of **1** with sodium hydroxide or sodium methoxide, respectively. Reduction of **1** with Pd/C/MgO in EtOH/H_2O (2/1) provided **11**. The *N*-methylamide **12** was prepared by alkylation of the sodium salt of **1** with MeI. Alkylation of **1** with benzyl bromide was unsuccessful; thus, the corresponding *N*-benzyl derivative **13** was prepared using the corresponding *N*-benzylaniline.[12]

The preparation of 160 amides of general structure **15** was done using solution-phase combinatorial techniques.[13] A series of 160 commercially available alkyl-

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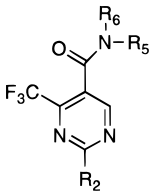
Table 1. In Vitro Evaluation of Substituted Pyrimidines



no.	R_2	R_5	R_6	mp (°C)	yield (%)	IC_{50} (μM) NF-κB	IC_{50} (μM) AP-1
1	Cl	H	A	183−184	92	0.50	0.50
2	$N(CH_3)_2$	H	A	163−164	80	>10	>10
3	NH_2	H	A	>250	60	>10	>10
4	nBuNH	H	A	162−163	41	>10	>10
5	aniline	H	A	228−229	91	>10	>10
6	benzylamine	H	A	202−203	87	>10	>10
7	cyclohexylamine	H	A	198−199	86	>10	>10
8	piperidyl	H	A	186−187	58	5.0	4.0
9	OH	H	A	dec 160	68	>10	>10
10	OCH_3	H	A	172−173	87	>10	>10
11	H	H	A	188−189	53	>10	>10
12	Cl	CH_3	A	124−125	20	2.30	3.0
13	Cl	Benzyl	A	102−104	25	2.70	2.70
19	Cl	H	B	96−97	61	>10	>10
20	Cl	H	C	180−181	62	>10	>10
21	Cl	H	D	248−249	27	>10	>10
22	Cl	H	E	172−173	85	0.38	0.49
23	Cl	H	F	135−136	55	1.20	1.60
24	Cl	H	G	190−191	35	0.80	0.50
25	Cl	H	H	141−143	35	2.30	2.80
26	Cl	H	I	170−171	75	4.0	3.80
27	Cl	H	J	200−201	40	0.9	0.9
28	Cl	H	K	165−166	75	0.05	0.05

[a] Key: A = 3,5-dichlorophenyl; B = butyl; C = phenyl; D = 2,6-dimethylphenyl; E = 4-(trifluoromethyl)phenyl; F = 3,5-dimethoxyphenyl; G = 2,6-dichloro-4-pyrimidine; H = 5-methyl-2-thiophene; I = 3-methyl-5-isoxazole; J = 3,4,5-trichlorophenyl; K = 3,5-bis(trifluoromethyl)phenyl.

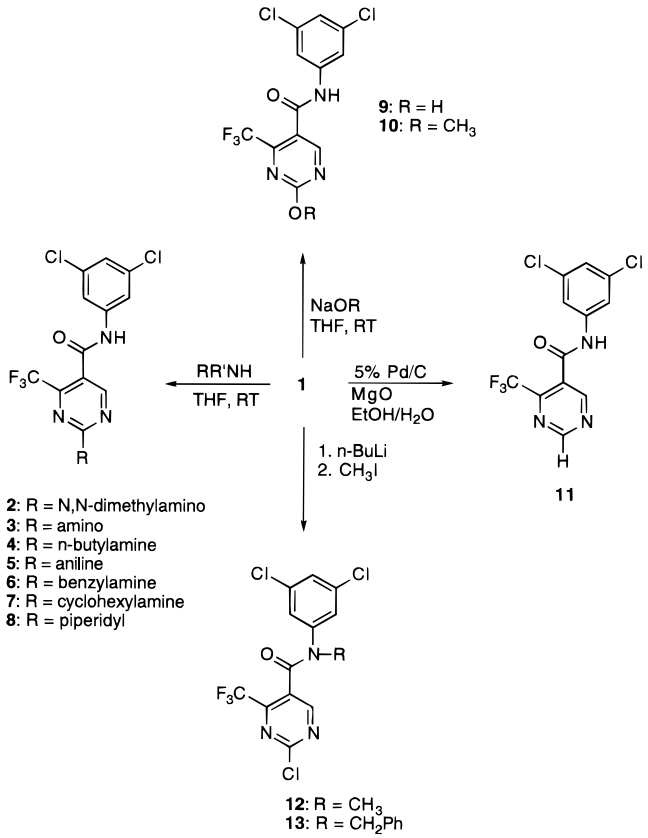
amines, anilines, and heterocyclic amines was selected for inclusion (see the Supporting Information) in this study. The compounds were prepared in a microtiter format 80 compounds at a time. The procedure involved sonicating EtOAc solutions of the amines and a slight excess of the pyrimidine acid chloride **14** in the presence of Amberlyst A-21 ion-exchange resin in a 96-well plate. A small amount of H_2O was added to each well to "quench" the excess acid chloride. The organic layer from each well was transferred to individually tared test tubes (Zymark BenchMate) and concentrated. The samples were then solvated (DMSO) for high throughput screening at a concentration of 5 mg/mL. TLC analysis was done on the entire plate, and HPLC analysis was performed on 15 random samples. All samples were run in a three-point dose response analysis in the cell-based transcription assays. Yields (based upon tared weight) ranged from 60 to 90%, and purities were greater than 85%.

The 3-(trifluoromethyl)-5-carbonylanilines were prepared starting with the commercially available 3-nitro-5-(trifluoromethyl)benzoic acid (**16**) as shown in Scheme 3. Treatment of **16** with oxalyl chloride followed by the appropriate alcohol or amine provided the desired ester or amide. Reduction with Pd/C and reaction with **14** provided the compounds listed in Table 2.

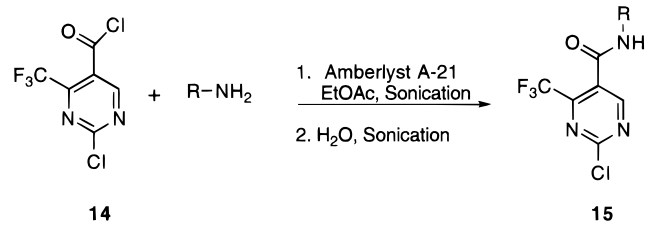
Biology

High throughput screening and follow-up studies were performed using three distinct cell lines. Jurkat

Scheme 1



Scheme 2



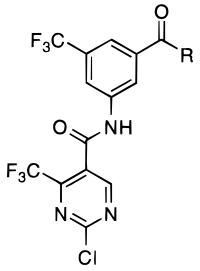
T-cells stably transfected with either an NF-κB binding site, an AP-1 binding site, or the β-actin promoter driving luciferase were pretreated for 0.5 h with compounds dissolved in 0.2% DMSO/H_2O. The cells were then stimulated with phorbol 12-myristate-13-acetate (PMA) and phytohemagglutin (PHA) and incubated for an additional 5 h. The cells were harvested by centrifugation for determination of luciferase activity. The results are expressed as IC_{50} values where the IC_{50} value is defined as the concentration of compound required to reduce luciferase activity to 50% of control values.

Cytokine determinations (IL-2 and IL-8) were performed by ELISA using commercially available kits.[14] The production IL-2 and IL-8 was determined in supernatants collected in the above luciferase studies. The results are plotted as percent of control (DMSO treated cells).

Results and Discussion

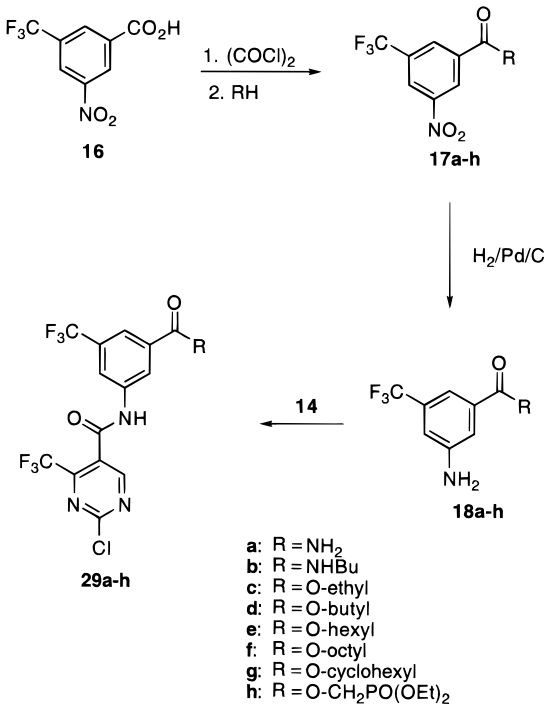
The compounds synthesized in library format were evaluated in a three-point dose response analysis (3.3, 0.3, and 0.03 μg/mL) in all three assays, and active derivatives (>50% at the 0.3 μg/mL dose) were then run

Table 2. In Vitro Evaluation of Substituted Pyrimidine Ester and Amides



no.	R	mp (°C)	yield (%)	IC_{50} (μM) NF-κB	IC_{50} (μM) AP-1
29a	NH_2	218−219	55	5.00	3.00
29b	NHBu	243−244	21	>10	>10
29c	OC_2H_5	67−71	68	0.28	0.15
29d	OC_4H_9	49−53	71	0.17	0.12
29e	OC_6H_{13}	103−105	72	0.62	0.66
29f	OC_8H_{17}	oil	35	1.80	1.20
29g	*O*-cyclohexyl	59−60	58	1.80	1.20
29h	$OCH_2PO(OEt)_2$	125−126	55	4.60	3.00

Scheme 3



in a six-point dose−response analysis. Compounds in Table 1 were run immediately in a six-point dose−response analysis. None of the compounds discussed had activity on the β-actin control at the highest dose tested (3.3 mg/mL, data not shown).

The results shown in Table 1 clearly indicate the importance of the chlorine atom at the 2-position of the pyrimidine ring. Any substitution at this position besides chlorine resulted in a complete loss of activity.[15] Derivatizations of the amide nitrogen as the *N*-methyl (**12**) or *N*-benzyl (**13**) analogues resulted in a 10-fold loss of activity, and these compounds were more active against the β-actin control.

The power of the combinatorial techniques described was demonstrated by the synthesis and evaluation of 160 analogues of **1** in a 2-week period. These results allowed for an immediate assessment of the key functional groups needed at the R_5 position. Dramatic changes in activity were seen with the different substituents attached to the amide nitrogen. To validate this approach to lead optimization, we also synthesized individually several key derivatives in the series and compared their activity to the analogues prepared in the library.[16] Clearly, a substituted aromatic group is essential since the *n*-butyl derivative **19** and the heterocyclic amides are less active (**24**−**26**).

The most active compounds were substituted aniline derivatives. A substituent on the ring is needed at either the 3-, 4-, or 5-position. The simple aniline derivative **20** was inactive. Compound **20** was synthesized as part of the library and then prepared individually to verify the unexpected lack of activity. Several other structure−activity relationships were observed. First, aniline derivatives substituted at the 3- or 4-position with small electron-withdrawing groups were the most potent (CF_3 > Cl > F > CH_3). Large bulky groups tended to decrease activity, and substituents at the 2- and/or 6-position resulted in a loss of activity (inactive at highest concentration tested). Increasing the distance between the amide nitrogen and the aromatic rings (benzyl derivatives) also resulted in inactive compounds. The results indicated that a small electron-withdrawing group was required at either the 3- or 4-position with the best activity found with substituents at both the 3- and 5-positions or the 3- and 4-positions. Trisubstituted compounds such as the 3′,4′,5′-trichloro derivative **27** offered no advantage over the 3,5-substituted compound (**27**, IC_{50} = 0.9 vs **1**, IC_{50} = 0.3 μM).

On the basis of the results obtained from the solution-phase libraries, we synthesized the 3,5-bis(trifluoromethyl)aniline derivative **28** (SP100030). This compound was the most potent inhibitor identified with an IC_{50} value of 50−100 nM in the cell-based assays (NF-κB and AP-1). Additional 3-(trifluoromethyl)-5-carbonyl derivatives were prepared in an effort to improve the solubility of these compounds and to further define the structure−activity relationships at the 5-position (Table 2). In summary, the ester derivatives were more active than the corresponding amides (**29b** vs **29d**), and the activity of the esters was optimal with a four carbon chain **29d**. However, all of the derivatives were less active than **28**.

The ability of compound **28** to block the production of IL-2 and IL-8 in Jurkat T-cells was also examined. As expected, both IL-2 and IL-8 were inhibited at the same concentrations as seen in the luciferase assay (IC_{50} $\approx$ 0.03 μM). In addition, the inhibitory activity seen with this compound was specific to T-cells. Subsequent studies were conducted examining the ability of **28** to block induced cytokine production in monocytes, epithelial cells, fibroblasts, osteoblasts, or endothelial cells. Surprisingly, this compound had no activity in the other cell lines examined, although studies indicated that the compound was able to cross the cell membranes.[17] In vivo studies were conducted with **28** and the compound was active i.p. in a dose-dependent manner in several animal models of inflammation and immunosuppression.[10]

Conclusions

Compound **28** (SP100030) represents one of the first inhibitors of NF-κB and AP-1 transcriptional activation specifically identified from high-throughput screening and solution-phase parallel synthesis. The exact mechanism of action of **28** is under investigation and will be reported shortly.[18] However, the compound has demonstrated activity in several animal models,[10] but its low aqueous solubility and high lipophilicity likely account for the lack of oral activity in the animal models tested. The activity of this series of compounds suggests that inhibitors of AP-1 and NF-κB may be useful as novel immunoinflammatory agents. Additional studies focused on further defining the structure−activity relationships of the pyrimidine ring as well as increasing the solubility of this novel class of compounds are in progress.

Experimental Section

Starting materials were obtained from commercial sources and used without purification. Silica gel (E. Merck, 60−230 mesh) was used for flash column chromatography, and silica gel plates (E. Merck) were used for thin-layer chromatography. All NMR spectra were recorded on a Varian Gemini 300 or a Bruker AM-500 spectrometer, and shifts are reported in parts per million relative to internal tetramethylsilane. Melting points were determined on a Mel Temp II and are uncorrected. IR spectra were recorded with a Nicolet Impact 400d spectrophotometer; mass spectra were obtained on a Hewlett-Packard 5890 Series II gas chromatogram with a Hewlett-Packard 5972 mass selective detector. Combustion elemental analyses were performed by Desert Analytics Laboratory, Tucson, AZ, and found values were within 0.4% of the theoretical values (unless otherwise indicated).

General Synthesis for the 2-Amino Derivatives (3−7). 2-(*N*,*N*-Dimethylamino)-4-(trifluoromethyl)-5-*N*-(3′,5′-dichlorophenyl)pyrimidinecarboxamide (2). To a solution of **1** (100 mg, 0.270 mmol) in THF was added gaseous *N*,*N*-dimethylamine. The mixture was stirred at room temperature under an atmosphere of N_2 for 3 h. The reaction was concentrated, and the resulting oil was purified by column chromatography (SiO_2, 12/1 hexanes/EtOAc), providing 82 mg (80%) of **2** as a white solid: mp 163−164 °C; ^{1}H NMR (500 MHz, acetone-d_6) δ 9.89 (bs, 1H), 8.82 (s, 1H), 7.80 (s, 2H), 7.24 (s, 1H), 3.27 (s, 6H); IR (KBr) 3383, 1652, 1585 cm^{-1}. Anal. ($C_{14}H_{11}Cl_2F_3N_4O$): C, H, N.

2-Amino-4-(trifluoromethyl)-5-*N*-(3′,5′-dichlorophenyl)-pyrimidinecarboxamide (3): 58% yield; mp >250 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.85 (s, 1H), 8.74 (s, 1H), 7.75 (s, 2H), 7.72 (s, 2H), 7.60 (s, 1H); IR (KBr) 3378, 1661, 1590, 1419 cm^{-1}. Anal. ($C_{12}H_7Cl_2F_3N_4O$): C, H, N.

2-(*N*-Butylamino)-4-(trifluoromethyl)-5-*N*-(3′,5′-dichlorophenyl)pyrimidinecarboxamide (4): 41% yield; mp 162−163 °C; ^{1}H NMR (500 MHz, acetone-d_6) δ 9.87 (bs, 1H), 8.81 (s, 1H), 8.71 (s, 1H), 7.80 (s, 2H), 7.24 (s, 1H), 3.50 (m, 2H), 1.62 (m, 2H), 1.41 (m, 2H), 0.93 (m, 3H); IR (KBr) 3281, 1583, 1529, 1199 cm^{-1}. Anal. ($C_{16}H_{15}Cl_2F_3N_4O{\cdot}0.5H_2O$): C, H, N.

2-(*N*-Phenylamino)-4-(trifluoromethyl)-5-*N*-(3′,5′-dichlorophenyl)pyrimidinecarboxamide (5): 91% yield; mp 228−229 °C; ^{1}H NMR (500 MHz, acetone-d_6) δ 8.99 (s, 1H), 7.86 (s, 2H), 7.82 (s, 2H), 7.39 (m, 2H), 7.26 (s, 1H), 7.12 (m, 1H) 2.10 (bs, 2H); IR (KBr) 3237, 1579, 1517, 1145 cm^{-1}. Anal. ($C_{18}H_{11}Cl_2F_3N_4O$): C, H, N.

2-(*N*-Benzylamino)-4-(trifluoromethyl)-5-*N*-(3′,5′-dichlorophenyl)pyrimidinecarboxamide (6): 87% yield; mp 202−203 °C; ^{1}H NMR (500 MHz, acetone-d_6) δ 9.85 (bs, 1H), 8.90 (s, 1H), 7.95 (m, 1H), 7.79 (s, 2H), 7.40 (m, 2H), 7.32 (m, 2H) 7.24 (m, 2H), 4.73 (m, 2H); IR (KBr) 3272, 1583, 1145 cm^{-1}. Anal. ($C_{19}H_{13}Cl_2F_3N_4O$): C, H, N.

2-(*N*-Cyclohexylamino)-4-(trifluoromethyl)-5-*N*-(3′,5′-dichlorophenyl)pyrimidinecarboxamide (7): 86% yield; mp 198−199 °C; ^{1}H NMR (500 MHz, acetone-d_6) δ 9.82 (bs, 1H), 8.75 (s, 1H), 7.80 (s, 2H), 7.24 (s, 1H), 3.91 (bs, 1H), 2.81 (m, 2H), 1.80 (m, 2H), 1.65 (m, 6H); IR (KBr) 1583, 1523, 1306 cm^{-1}. Anal. ($C_{18}H_{16}Cl_2F_3N_4O$): C, H, N.

2-*N*-Piperidyl-4-(trifluoromethyl)-5-*N*-(3′,5′-dichlorophenyl)pyrimidinecarboxamide (8): 58% yield; mp 186−187 °C; ^{1}H NMR (500 MHz, acetone-d_6) δ 9.85 (bs, 1H), 8.80 (s, 1H), 7.80 (s, 2H), 7.24 (d, J = 1.5 Hz, 1H), 3.90 (s, 4H), 1.74 (m, 2H), 1.60 (m, 4H); IR (KBr) 3267, 1583, 1531, 1267 cm^{-1}. Anal. ($C_{17}H_{15}F_3Cl_2N_4O$): C, H, N.

2-Hydroxy-4-(trifluoromethyl)-5-*N*-(3′,5′-dichlorophenyl)pyrimidinecarboxamide (9). A mixture of **1** (370 mg, 1.0 mmol) in THF (10 mL) and aqueous NaOH (1 M, 10 mL, 10 mmol) was stirred for 16 h at room temperature. The mixture was acidified with HCl (1 N) and extracted with EtOAc (3 × 30 mL). The organic layers were combined, washed with brine, and dried over Na_2SO_4. The solvent was removed, and the resulting crude material was purified by column chromatography (SiO_2, 2/1 hexanes/EtOAc) to provide 240 mg (68%) of **9** as a white solid: mp dec >160 °C; ^{1}H NMR (500 MHz, acetone-d_6) δ 8.55 (s, 1H), 8.18 (bs, 1H), 7.77 (s, 1H), 7.67 (s, 2H), 2.82 (s, 1H); IR (KBr) 3390, 1611, 1508 1216 cm^{-1}. Anal. ($C_{12}H_6Cl_2F_3N_3O_2$): C, H, N.

2-Methoxy-4-(trifluoromethyl)-5-*N*-(3′,5′-dichlorophenyl)pyrimidinecarboxamide (10). A mixture of **1** (100 mg, 0.27 mmol) in MeOH (15 mL) and NaOMe (50 mg, 0.92 mmol) was stirred for 2.5 h under an atmosphere of N_2. The reaction was acidified with HCl (1 N) and extracted with EtOAc (3 × 30 mL). The organic layers were combined, washed with brine, and dried over Na_2SO_4. The organic layer was concentrated, and the resulting crude material was purified by column chromatography (SiO_2, 10/1 hexanes/EtOAc) to provide 84 mg (87%) of **10** as a white solid: mp 172−173 °C; ^{1}H NMR (500 MHz, acetone-d_6) δ 10.1 (bs, 1H), 9.17 (s, 1H), 7.79 (s, 2H), 7.29 (s, 1H), 4.10 (s, 3H); IR (KBr) 3242, 1655, 1494, 1391 cm^{-1}. Anal. ($C_{13}H_8Cl_2F_3N_3O_2$): C, H, N.

4-(Trifluoromethyl)-5-*N*-(3′,5′-dichlorophenyl)pyrimidinecarboxamide (11). To a solution of **1** (100 mg, 0.270 mmol) in EtOH/H_2O (2/1, 2.5 mL) were added Pd/C (5%, 10 mg) and MgO (24 mg, 0.59 mmol). The mixture was stirred under an atmosphere of H_2 for 2.5 h, filtered through Celite, and concentrated. The resulting crude material was purified by column chromatography (SiO_2, 10/1 hexanes/EtOAc), providing 48 mg (53%) of **11** as a white solid: mp 189−190 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.2 (s, 1H), 9.60 (s, 1H), 9.40 (s, 1H), 7.70 (s, 2H), 7.40 (s, 1H); IR (KBr) 3262, 1650, 1585, 1151 cm^{-1}. Anal. ($C_{12}H_6Cl_2F_3N_3O$): C, H, N.

2-Chloro-4-(trifluoromethyl)-5-*N*-methyl-*N*-(3′,5′-dichlorophenyl)pyrimidinecarboxamide (12). To a mixture of NaH (21 mg, 0.525 mmol) in DMF (20 mL) under N_2 was added a solution of **1** (86 mg, 0.23 mmol) in DMF (5 mL). This was stirred for 0.3 h, MeI (0.10 mL, 1.61 mmol) was added, and stirring continued for an additional for 2 h. The solution was acidified with 1 N HCl and concentrated. The resulting oil was dissolved in EtOAc, extracted with HCl (1 N, 2 × 20 mL), and washed with brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The resulting oil was purified by column chromatography (SiO_2, 8/2 hexanes/EtOAc) to provide a solid that was recrystallized from EtOH/H_2O to provide 20 mg (22%) of **12** as a white solid: mp 124−125 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.53 (s, 1H), 7.30 (s, 1H), 6.97 (s, 2H), 3.46 (s, 3H); IR (KBr) 1669, 1577, 1337 1171 cm^{-1}. Anal. ($C_{13}H_7Cl_3F_3N_3O$): C, H, N.

2-Chloro-4-(trifluoromethyl)-5-*N*-benzyl-*N*-(3′,5′-dichlorophenyl)pyrimidinecarboxamide (13). A mixture of benzaldehyde (1.04 g, 9.40 mmol), 3,5-dichloroaniline (1.71 g, 10.6 mmol), acetic acid (0.20 mL), and methanol (100 mL was cooled to 0 °C. A solution of $NaBH_3CN$ (1 M, 28.0 mL, 28.0 mmol) was added via a syringe pump over 0.3 h. The solution was stirred at 0 °C for 0.5 h and allowed to warm to room temperature, and stirring continued for 18 h. The mixture was acidified with HCl (1 N) and then concentrated. The

resulting oil was partitioned between EtOAc and H_2O and basified with NaOH (1 N) until pH 9. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude material was purified by column chromatography (SiO_2, 9/1 hexanes/EtOAc) to provide 1.61 g (64%) of *N*-(3,5-dichlorophenyl)benzylamine:[9] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (m, 5H), 6.66 (t, J = 1.8 Hz, 1H), 6.45 (d, J = 1.8 Hz, 2H), 4.24 (s, 1H), 4.13 (s, 1H).

To a mixture of Amberlyst A-21 resin (1 g) and *N*-(3,5-dichlorophenyl)benzylamine (0.204 g, 0.761 mmol) in EtOAc (15 mL) was added a solution of 2-chloro-4-(trifluoromethyl)-5-pyrimidine acid chloride (0.241 g, 0.984 mmol) in EtOAc (2.0 mL). The mixture was stirred for 1 h, H_2O (0.2 mL) was added, and stirring was continued for an additional 0.25 h. The organic layer was decanted and concentrated. The resulting oil was purified by column chromatography (SiO_2, 9/1 hexanes/EtOAc) to provide 52 mg (15%) of **13** as a clear oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.54 (s, 1H), 7.35 (m, 4H), 7.23 (m, 2H), 6.77 (m, 2H), 5.06 (s, 2H); IR (KBr) 3064, 1667, 1562 cm^{-1}. Anal. ($C_{19}H_{11}Cl_3F_3N_3O$): C, H, N.

General Procedure for the Synthesis of Compounds 17a−h. 3-Nitro-5-(trifluoromethyl)benzylamide (17a). To a solution of **16** (1.0 g, 4.2 mmol) in CH_2Cl_2 (50 mL) were added oxalyl chloride (1.45 mL, 18.8 mmol) and DMF (2 drops). The solution was stirred for 18 h under an atmosphere of N_2 and concentrated, and the resulting oil was dissolved in THF (20 mL). The solution was added to NH_4OH (2.2 mL) in THF (40 mL) and stirred for 18 h. The reaction was concentrated, and the residue was partitioned between EtOAc and H_2O. The organic layer was washed with H_2O (2×), extracted with $NaHCO_3$ (2 × 40 mL), washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude solid was purified by column chromatography (SiO_2, 1/1 hexanes/EtOAc) to provide 0.912 g (92% yield) of **17a** as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.81 (s, 1H), 8.63 (s, 1H), 8.42 (s, 1H) 6.20 (bs, 2H); GC−MS 10.33 min (*m/e* 234, 100).

***N*-Butyl-3-nitro-5-(trifluoromethyl)benzylamide (17b):** ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.71 (s, 1H), 8.62 (s, 1H), 8.42 (s, 1H), 6.34 (bs, 1H), 3.52 (m, 2H), 1.66 (m, 2H), 1.45 (m, 2H), 0.98 (t, J = 6.6 Hz, 3H); GC−MS 10.86 min (*m/e* 290, 99).

Ethyl 3-nitro-5-(trifluoromethyl)benzoate (17c): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.04 (s, 1H), 8.67 (s, 1H), 8.63 (s, 1H), 4.51 (q, J = 7.2 Hz, 2H), 1.46 (t, J = 6.9 Hz, 3H); GC−MS 8.41 min (*m/e* 263, 100).

Butyl 3-nitro-5-(trifluoromethyl)benzoate (17d): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.03 (s, 1H), 8.67 (s, 1H), 8.61 (s, 1H), 4.44 (t, J = 6.6 Hz, 2H), 1.82 (m, 2H), 1.50 (m, 2H), 1.01 (t, J = 7.2 Hz, 3H); GC−MS 9.08 min (*m/e* 292, 100).

Hexyl 3-nitro-5-(trifluoromethyl)benzoate (17e): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.04 (s, 1H), 8.68 (s, 1H), 8.62 (s, 1H), 4.43 (t, J = 6.9 Hz, 2H), 1.82 (m, 2H), 1.46 (m, 6H), 0.94 (t, J = 7.2 Hz, 3H); GC−MS 10.17 min (*m/e* 320, 98).

Octyl 3-nitro-5-(trifluoromethyl)benzoate (17f): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.04 (s, 1H), 8.68 (s, 1H), 8.62 (s, 1H), 4.43 (t, J = 6.9 Hz, 2H), 1.86 (m, 2H), 1.6−1.2 (m, 10H), 0.87 (m, 3H); GC−MS 11.24 min (*m/e* 348, 98).

Cyclohexyl 3-nitro-5-(trifluoromethyl)benzoate (17g): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.03 (s, 1H), 8.67 (m, 1H), 8.61 (m, 1H), 5.10 (m, 1H), 2.0−1.3 (m, 10 H); GC 10.50 min (98).

Diethyl phosphate methyl-3-nitro-5-(trifluoromethyl)-benzoate (17h): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.06 (s, 1H), 8.71 (s, 1H), 8.63 (s, 1H), 4.74 (d, J = 8.7 Hz, 2H), 4.26 (m, 4H), 1.38 (t, J = 6.9 Hz, 6H).

General Procedure for the Synthesis of Compounds 18a−h. 3-Amino-5-(trifluoromethyl)benzylamide (18a). To a solution of **17a** (0.550 g, 2.35 mmol) in EtOH (25 mL) was added 5% Pd/C (30 mg). The mixture was flushed with H_2 four times and then stirred under an atmosphere of H_2 for 18 h. The mixture was filtered through Celite and concentrated. The resulting crude material **18a** (0.425 g, 89% yield) was used without further purification: ^{1}H NMR (300 MHz, acetone-d_6) δ 7.51(bs, 1H), 7.45 (s, 1H), 7.40 (s, 1H), 7.10 (s, 1H), 6.70 (bs, 1H), 5.30 (bs, 2H); GC−MS 10.53 min (*m/e* 204, 98).

***N*-Butyl-3-amino-5-(trifluoromethyl)benzylamide (18b):** ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 (s, 1H), 7.23 (s, 1H), 6.99 (s, 1H), 6.10 (bs, 1H), 4.02 (bs, 2H), 3.46 (m, 2H), 1.61 (m, 2H), 1.43 (m, 2H), 0.96 (t, J = 7.2 Hz, 3H). GC−MS 11.42 min (*m/e* 260, 99).

Ethyl 3-amino-5-(trifluoromethyl)benzoate (18c): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (s, 1H), 7.49 (s, 1H), 7.05 (s, 1H), 4.39 (q, J = 7.2 Hz, 2H), 1.40 (t, J = 7.5 Hz, 3H); GC−MS 8.66 min (*m/e* 233, 96).

Butyl 3-amino-5-(trifluoromethyl)benzoate (18d): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (s, 1H), 7.48 (s, 1H), 7.05 (s, 1H), 4.32 (t, J = 6.6 Hz, 2H), 1.75 (m, 2H), 1.48 (m, 2H), 1.01 (t, J = 7.2 Hz, 3H); GC−MS 9.79 min (*m/e* 261, 100).

Hexyl 3-amino-5-(trifluoromethyl)benzoate (18e): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (s, 1H), 7.49 (s, 1H), 7.05 (s, 1H), 4.31 (t, J = 6.9 Hz, 2H), 2.0−1.2 (m, 8H), 0.91 (t, J = 6.6 Hz, 3H); GC−MS 10.97 min (*m/e* 289, 100).

Octyl 3-amino-5-(trifluoromethyl)benzoate (18f): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (s, 1H), 7.48 (s, 1H), 7.05 (s, 1H), 4.31 (t, J = 6.6 Hz, 2H), 4.00 (bs, 2H), 1.8−1.2 (m, 12H), 0.88 (t, J = 6.9 Hz, 3H); GC−MS 11.87 min (*m/e* 317, 99).

Cyclohexyl 3-amino-5-(trifluoromethyl)benzoate (18g): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (s, 1H), 7.50 (s, 1H), 7.08 (s, 1H), 5.00 (m, 1H), 2.0−1.3 (m, 10H); GC−MS 11.23 min (*m/e* 287, 98).

Diethyl phosphate methyl-3-amino-5-(trifluoromethyl)benzoate (18h): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.49 (s, 1H), 7.08 (s, 1H), 4.63 (d, J = 8.4 Hz, 2H), 4.23 (m, 6H), 1.36 (t, J = 7.2 Hz, 6H); GC−MS 12.6 min (*m/e* 355 M^+).

General Procedure for Synthesis of the Pyrimidine Amide Derivatives. 2-Chloro-4-(trifluoromethyl)-5-*N*-butylpyrimidinecarboxamide (19). To a 20 mL round-bottom flask were added Amberlyst A-21 ion-exchange resin (0.2 g), *n*-butylamine (0.068 g, 0.932 mmol), and EtOAc (10 mL). A solution of 2-chloro-4-(trifluoromethyl)-5-pyrimidine acid chloride (0.240 g, 0.981 mmol) in EtOAc (0.5 mL) was added to the round-bottom flask and the mixture stirred for 0.3 h. Water (0.2 mL) was added and stirring continued for 5 min. The reaction was filtered, dried over $MgSO_4$, and filtered and the solvent removed under reduced pressure. The resulting crude solid was recrystallized from EtOH/H_2O, providing 109 mg (61%) of **19** as a white solid: mp 96−97 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.90 (s, 1H), 5.90 (bs, 1H), 3.46 (q, J = 6.75 Hz, 2H), 1.58 (m, 2H), 1.42 (m, 2H), 0.97 (t, J = 7.2 Hz, 3H); IR (KBr) 3268, 1645, 1501 cm^{-1}. Anal. ($C_{10}H_{11}ClF_3N_3O$): C, H, N.

2-Chloro-4-(trifluoromethyl)-5-*N*-phenylpyrimidinecarboxamide (20): 62% yield; mp 180−181 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.04 (s, 1H), 7.56 (d, J = 7.5 Hz, 2H), 7.49 (bs, 1H), 7.41 (t, J = 7.5 Hz, 2H), 7.25 (t, J = 7.5 Hz, 1H); IR (KBr) 3185, 1650, 1326, 1172 cm^{-1}. Anal. ($C_{12}H_7ClF_3N_3O$): C, H, N.

2-Chloro-4-(trifluoromethyl)-5-*N*-(2′,6′-dimethylphenyl)-pyrimidinecarboxamide (21): 27% yield; mp 248−249 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.99 (s, 1H), 9.06 (s, 1H), 7.13 (s, 3H), 2.31 (s, 6H); IR (KBr) 3235, 1661, 1353, 1155 cm^{-1}. Anal. ($C_{14}H_{11}ClF_3N_3O$): C, H, N.

2-Chloro-4-(trifluoromethyl)-5-*N*-[4′-(trifluoromethyl)-phenyl]pyrimidinecarboxamide (22): 74% yield; mp 172−173 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.05 (s, 1H), 9.27 (s, 1H), 7.84 (d, J = 8.6 Hz, 2H), 7.64 (d, J = 8.6 Hz, 2H); IR (KBR) 3290, 1667, 1535, 1337 cm^{-1}. Anal. ($C_{13}H_6ClF_6N_3O\cdot 0.5H_2O$): C, H, N.

2-Chloro-4-(trifluoromethyl)-5-*N*-(3′,5′-dimethoxyphenyl)pyrimidinecarboxamide (23): 55% yield; mp 135−136 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.01 (s, 1H), 7.56 (s, 1H), 6.77 (s, 1H), 6.76 (s, 1H), 6.34 (t, J = 1.5 Hz, 1H), 3.81 (s, 6H); IR (KBR) 3290, 1661, 1606, 1568 cm^{-1}. Anal. ($C_{14}H_{11}ClF_3N_3O_3$): C, H, N.

2-Chloro-4-(trifluoromethyl)-5-*N*-[4-(2′,6′-dichloropyridino)]pyrimidinecarboxamide (24): 40% yield; mp 189−190 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.02 (s, 1H), 8.10 (s, 1H), 7.59 (s, 2H); IR (KBr) 1711, 1579, 1210, 1167 cm^{-1}. Anal. ($C_{11}H_4Cl_3F_3N_4O$): C, H, N.

2-Chloro-4-(trifluoromethyl)-5-*N*-[2′-(5″-methylthiopheneyl)]pyrimidinecarboxamide (25): 21% yield; mp 142−143 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.02 (s, 1H), 8.22 (s, 1H), 6.63 (m, 1H), 6.56 (m, 1H), 2.46 (s, 3H); IR (KBr) 3246, 1651, 1563, 1326 cm^{-1}. Anal. ($C_{11}H_7ClF_3N_3OS$): C, H, N.

2-Chloro-4-(trifluoromethyl)-5-*N*-[5′-(3″-methylisoxazolyl)]pyrimidinecarboxamide (26): 75% yield; mp 170−171 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 9.42 (s, 1H), 6.74 (s, 1H), 2.27 (s, 3H); IR (KBr) 1704, 1562, 1209 cm^{-1}. Anal. ($C_{10}H_6ClF_3N_4O_2$): C, H, N.

2-Chloro-4-(trifluoromethyl)-5-*N*-(3′,4′,5′-trichlorophenyl)pyrimidinecarboxamide (27): 40% yield; mp 200−201 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.48 (bs, 1H), 8.91 (s, 1H), 7.85 (s, 2H), 7.59 (s, 2H); IR (KBr) 1706, 1561, 1203 cm^{-1}. Anal. ($C_{12}H_4Cl_4F_3N_3O$): C, H, N.

2-Chloro-4-(trifluoromethyl)-5-*N*-[3′,5′-bis(trifluoromethyl)phenyl]pyrimidinecarboxamide (28): 75% yield; mp 166−167 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.05 (s, 1H), 8.10 (s, 2H), 7.86 (s, 1H), 7.76 (s, 1H); ^{13}C NMR (300 MHz, acetone-d_6) δ 162.9, 141.2, 133.4, 132.9, 128.2, 126.3, 122.8, 122.7, 121.0, 118.7; IR (KBr) 3292, 1668, 1517, 1380 cm^{-1}. Anal. ($C_{14}H_5ClF_9N_3O$): C, H, N.

2-Chloro-4-(trifluoromethyl)-5-*N*-[3′-(trifluoromethyl)-5′-(aminocarbonyl)phenyl]pyrimidinecarboxamide (29a): 55% yield; mp 218−219 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 10.41 (bs, 1H), 9.45 (s, 1H), 8.42 (s, 1H), 8.38 (s,1H), 8.07 (s, 1H), 7.84 (bs, 1H), 6.67 (bs, 1H); IR (KBr) 1677, 1559, 1339, 1211 cm^{-1}. Anal. ($C_{14}H_7ClF_6N_4O_2$): C, H, N.

2-Chloro-4-(trifluoromethyl)-5-*N*-[3′-(trifluoromethyl)-5′-[(*N*-butylamino)carbonyl]phenyl]pyrimidinecarboxamide (29b): 21% yield; mp 243−244 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 10.38 (bs, 1H), 9.44 (s, 1H), 8.37 (s, 1H), 8.34 (s, 1H), 8.09 (bs, 1H), 7.99 (s, 1H), 3.42 (q, J = 6.6 Hz, 2H), 1.60 (m, 2H), 1.40 (m, 2H), 0.93 (t, J = 7.2 Hz, 3H); IR (KBr) 1652, 1586, 1360, 1211 cm^{-1}. Anal. ($C_{18}H_{15}ClF_6N_4O_2$): C, H, N.

2-Chloro-4-(trifluoromethyl)-5-*N*-[3′-(trifluoromethyl)-5′-(ethoxycarbonyl)phenyl]pyrimidinecarboxamide (29c): 30% yield; mp 69−71 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.06 (s, 1H), 8.30 (s, 1H), 8.25 (s, 1H), 8.17 (s, 1H), 7.94 (bs, 1H), 4.43 (q, J = 6.9 Hz, 2H), 1.43 (t, J = 6.9 Hz, 3H); IR (KBr) 1694, 1575, 1355, 1262 cm^{-1}. Anal. ($C_{16}H_{10}ClF_6N_3O_3$): C, H, N.

2-Chloro-4-(trifluoromethyl)-5-*N*-[3′-(trifluoromethyl)-5′-(butoxycarbonyl)phenyl]pyrimidinecarboxamide (29d): 71% yield; mp 49−53 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.12 (bs, 1H), 9.04 (s, 1H), 8.29 (m, 2H), 8.08 (s, 1H), 4.29 (t, J = 6.6 Hz, 2H), 1.76 (m, 2H), 1.46 (m, 2H), 0.98 (t, J = 7.2 Hz, 3H); IR (KBr) 1708, 1574, 1267 cm^{-1}. Anal. ($C_{18}H_{14}ClF_6N_3O_3 \cdot 0.5H_2O$): C, H, N.

2-Chloro-4-(trifluoromethyl)-5-*N*-[3′-(trifluoromethyl)-5′-[(hexyloxy)carbonyl]phenyl]pyrimidinecarboxamide (29e): 72% yield; mp 103−105 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.07 (s, 1H), 8.31 (s, 1H), 8.15 (s, 1H), 7.86 (bs, 1H), 4.38 (t, J = 7.2 Hz, 2H), 1.8−0.8 (m, 11H); IR (KBr) 1704, 1560, 1350, 1206 cm^{-1}. Anal. ($C_{20}H_{18}ClF_6N_3O_3$): C, H, N.

2-Chloro-4-(trifluoromethyl)-5-*N*-[3′-(trifluoromethyl)-5′-[(octyloxy)carbonyl]phenyl]pyrimidinecarboxamide (29f): 35% yield; oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.05 (s, 1H), 8.73 (s, 1H), 8.30 (s, 1H), 8.21 (s, 1H), 8.07 (s, 1H), 4.22 (t, J = 6.6 Hz, 2H), 1.76 (m, 2H), 1.31 (m, 10H), 0.86 (m, 3H); IR (KBr) 3306, 1689, 1562, 1359 cm^{-1}; HRMS calcd for $C_{22}H_{22}ClF_6N_3O_3$ 526.1332, found 526.1339 (MH^+).

2-Chloro-4-(trifluoromethyl)-5-*N*-[3′-(trifluoromethyl)-5′-[(cyclohexyloxy)carbonyl]phenyl]pyrimidinecarboxamide (29g): 58% yield; mp 59−60 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.06 (s, 1H), 8.31 (s, 1H), 8.21 (s, 1H), 8.13 (m, 2H), 5.02 (m, 1H), 2.04−1.23 (m, 10H); IR (KBr) 3295, 1689, 1568, 1348 cm^{-1}. Anal. ($C_{20}H_{16}ClF_6N_3O_3$): C, H, N.

2-Chloro-4-(trifluoromethyl)-5-*N*-[3′-(trifluoromethyl)-5′-[[(dimethyl phosphate)methoxy]carbonyl]phenyl]pyrimidinecarboxamide (29h): 55% yield; mp 125−126 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.89 (s, 1H), 9.35 (s, 1H), 8.65 (s, 1H), 7.71 (s, 1H), 7.58 (s, 1H), 4.54 (d, J = 8.1 Hz), 4.08 (m, 4H), 1.37 (t, J = 7.2 Hz, 6); IR (KBr) 1739, 1573, 1365 cm^{-1}. Anal. ($C_{19}H_{17}ClF_6N_3O_6P$): C, H, N.

Solution-Phase Library Preparation. Amberlyst A-21 ion-exchange resin (5−10 beads) was placed into 80 wells of a 1 mL deep well microtiter plate (rows 1 and 12 open). The 80 individual amines as shown in Table 2 (100 μL, 22.4 μmol in EtOAc) were added to the individual wells and diluted with additional EtOAc (0.2 mL). Then to each well was added a solution of 2-chloro-4-(trifluoromethyl)-5-pyrimidine acid chloride (100 μL, 24.6 μmol) in EtOAc. The wells were capped, and the plate was sonicated in a desk top sonicator (Branson 1210) for 0.25 h. To each well was added H_2O (30 μL), and the plate was sonicated for an additional 0.1 h. The organic layer (280 μL) of each well was transferred into a tared test tube and concentrated to provide 80 individual compounds. Each compound was analyzed by TLC (1/1 hexanes/EtOAc), and 15 random samples were analyzed by HPLC. The test tubes were then solvated at 5 mg/mL and submitted for biological testing.

NF-κB Assay. Human Jurkat T-Cells stably transfected with an NF-κB binding site (from the MHC promoter) fused to a minimal SV-40 promoter driving luciferase were used in these experiments.[19] Cells were counted, resuspended in fresh medium containing 10% Serum-Plus at a density of 1×10^6 cells/mL, and plated in 96-well round-bottom plates (200 μL per well) 18 h prior to running the assays.

Compounds dissolved in 0.2% DMSO/H_2O at the appropriate concentrations (3.3, 0.33, and 0.03 μg/mL for initial evaluation of libraries) were then added to the microtiter plates containing the cells, and the plates were incubated at 37 °C for 0.5 h. To induce transcriptional activation, 50 ng/mL of phorbol 12-myristate-13-acetate (PMA) and 1 μg/mL of phytohemagglutin (PHA) were added to each well, and the cells were incubated for an additional 5 h at 37 °C. The plates were centrifuged at 2200 rpm for 1 min at room temperature followed by removal of the media; 60 μL of cell lysis buffer was added to each well, and cells were lysed 0.25 h; 40 μL of each cell lysate was transferred to a black 96-well plate, and 50 μL of luciferase substrate buffer was added. Luminescence was immediately measured using a Packard TopCount. The results are expressed as IC_{50} values where the IC_{50} value is defined as the concentration of compound required to reduce luciferase activity to 50% of control values.

AP-1 Assay. The AP-1 assay was run as described above for NF-κB except that the Jurkat T-Cells were stably transfected with a plasmid that contained an AP-1 binding site from the collagenase promoter driving luciferase expression.[19] In addition, the concentrations of PMA and PHA were 5 ng/mL and 1 μg/mL, respectively.

β-Actin Assay. The β-actin assay was run as described above for NF-κB except that the Jurkat T-Cells were stably transfected with a plasmid that contained the β-actin promoter driving luciferase and the cells were not induced with PMA and PHA.

Inhibition of Cytokines. After centrifugation, supernatants from each well in the above luciferase experiments were collected and stored at −20 °C until assay. Approximately 20−50 μL aliquots were removed and cytokine levels determined by ELISA (Biosource International, IL-2 (AP-1) and IL-8 (NF-κB)). The results are expressed as IC_{50} values where the IC_{50} value is defined as the concentration of compound required to reduce cytokine levels to 50% of control values.

Supporting Information Available: Amine, aniline, and heterocyclic amine structures for library and HTS data for combinatorial plates (19 pages). Ordering information is given on any current masthead page.

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