Inhibitors of NF-κB and AP-1 Gene Expression: SAR Studies on the Pyrimidine Portion of 2-Chloro-4-trifluoromethylpyrimidine-5-[*N*-(3′,5′-bis(trifluoromethyl)-phenyl)carboxamide]

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Received April 7, 2000

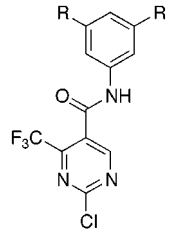
We investigated the structure−activity relationship studies of *N*-[3,5-bis(trifluoromethyl)phenyl]-[2-chloro-4-(trifluoromethyl)pyrimidin-5-yl]carboxamide (**1**), an inhibitor of transcription mediated by both NF-κB and AP-1 transcription factors, with the goal of improving its potential oral bioavailability. Compounds were examined for cell-based activity, were fit to Lipinski's rule of 5, and were examined for potential gastrointestinal permeability using the intestinal epithelial cell line, Caco-2. Selected groups were substituted at the 2-, 4-, and 5-positions of the pyrimidine ring using solution-phase combinatorial methodology. The introduction of a fluorine in the place of 2-chlorine of **1** resulted in a compound with comparable activity. However, other substitutions at the 2-position resulted in a loss of activity. The trifluoromethyl group at the 4-position could be replaced with a methyl, ethyl, chlorine, or phenyl without a substantial loss of activity. The carboxamide group at the 5-position is critical for activity. If it was moved to the 6-position, the activity was lost. The 2-methyl analogue of **1** (**81**) showed comparable in vitro activity and improved Caco-2 permeability compared to **1**.

Introduction

The production of proinflammatory cytokines, as well as a number of other cellular regulators, is controlled by a family of proteins known as transcription factors (TFs). These TFs, when activated, bind to a specific region of DNA and upregulate gene expression. The activation of these TFs is caused by a variety of external signals including physiological stress, infectious agents, and other bioregulatory molecules. Two transcription factors, nuclear factor-κB (NF-κB) and activator protein-1 (AP-1), play an important role in mediating inflammatory and immune responses.[2] AP-1 is important for the regulation of interleukin-2 (IL-2) production, T-cell activation, and production of matrix metalloproteinases, while NF-κB is essential for the regulation of interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-α (TNF-α).[3] Therefore, the inhibition or modulation of AP-1 and/or NF-κB activation represents an approach for the identification of novel immunoinflammatory agents.[4,5]

Previously, *N*-[3,5-bis(trifluoromethyl)phenyl][2-chloro-4-(trifluoromethyl)pyrimidin-5-yl]carboxamide (**1**; Chart 1) was reported as an inhibitor of AP-1 and NF-κB activation.[6,7] The compound was active in several animal models of inflammation and immunosuppression (ip, 10−20 mg/kg).[5] However, the compound exhibited poor oral activity, consistent with poor permeability in the gastrointestinal cell line, Caco-2 (apparent permeability coefficient (P_{app}) = $11 \pm 4 \times 10^{-7}$ cm/s; Table 4). Using Caco-2 cells as a measure of potential oral bioavailability,[8] and Jurkat T cells as an in vitro measure of efficacy, we sought to further define the key structure−activity parameters (SAR) for this class of compounds and identify analogues of **1** with comparable in vitro activity and improved Caco-2 permeability.

Chart 1



1, R = CF_3
2, R = Cl

Herein, we describe the preparation of a series of pyrimidines modified at the 2-, 4-, 5-, and 6-positions and the parallel synthesis of amide derivatives using a solution-phase parallel synthesis technique. In addition, we examined several related ring systems such as pyridazine, pyrazine, and benzene rings in the place of the pyrimidine ring. We identified compounds that retained inhibitory activity against AP-1- and NF-κB-mediated transcriptional activation while displaying improved Caco-2 permeability.

Chemistry

Libraries of pyrimidine-, pyridazine-, pyrazine-, and benzenecarboxamides were designed and prepared to explore SARs and to identify compounds with comparable in vitro activity and enhanced Caco-2 permeability. The preparation of the libraries involved two components: (1) synthesis of the appropriately substi-

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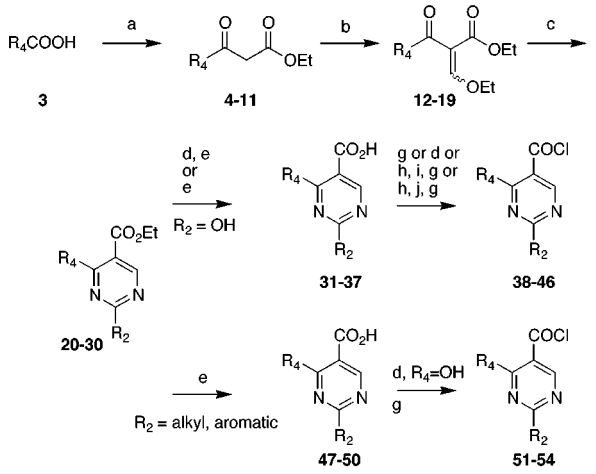
10.1021/jm0001626 CCC: $19.00
Published on Web 09/19/2000

Scheme 1[a]



No.	R_4
3	2'-Thienyl
4, 12	CF_3
5, 13	CF_2CF_3
6, 14	CH_3
7, 15	Et
8, 16	Pr
9, 17	Ph
10, 18	2'-Thienyl
11, 19	OEt

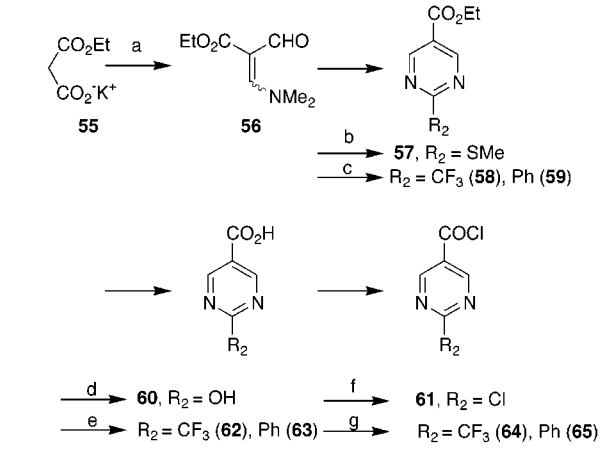
No.	R_2	R_4
20	OH	2'-Thienyl

No.	R_2	R_4
21	OH	CF_3
22	OH	CF_2CF_3
23	OH	CH_3
24	OH	Et
25	OH	Pr
26	OH	Ph
27	CF_3	OH
28	CF_3	CH_3
29	CH_3	CF_3
30	CF_3	CF_3
31, 38	OH, Cl	2'-Thienyl
32, 39	Cl	CF_3

No.	R_2	R_4
33, 40	OH, Cl	CF_2CF_3
34, 41	Cl	CH_3
35, 42	OH, Cl	Et
36, 43	OH, Cl	Pr
37, 44	Cl	Ph
45	F	CF_3
46	CN	CF_3
47	CF_3	OH
48, 52	CF_3	CH_3
49, 53	CH_3	CF_3
50, 54	CF_3	CF_3
51	CF_3	Cl

[a] Reagents: (a) $Mg(OCOCH_2CO_2Et)_2$, CDI, THF; (b) $CH(OEt)_3$; (c) urea or $NH_2(CR_2)$=NH, Δ, NaOEt; (d) $POCl_3$; (e) aq NaOH−THF; (g) $(COCl)_2$, DMF, CH_2Cl_2; (h) Me_3N, THF; (i) KF, DMF−H_2O; (j) KCN, DMF−H_2O.

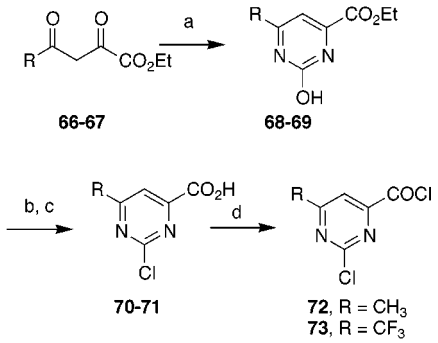
Scheme 2[a]



[a] Reagents: (a) DMF, $POCl_3$; (b) $NH_2C(SMe)$=NH; (c) $NH_2C(R_2)$=NH; (d) KOH; (e) NaOH, THF−H_2O; (f) $POCl_3$; (g) $(COCl)_2$, CH_2Cl_2.

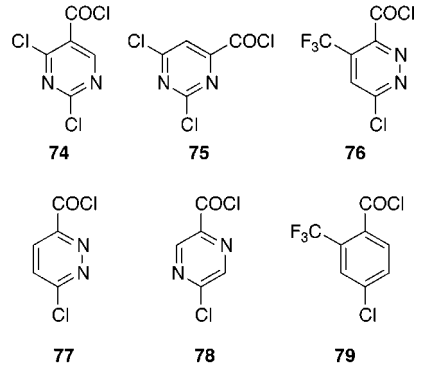
tuted aryl or heteroaryl acid chlorides and (2) reaction of the acid chlorides with various commercially available amines using solution-phase parallel synthesis techniques. For the pyrimidine libraries, a variety of 2,4,5-, 2,5-, and 2,4,6-substituted pyrimidine acid chlorides (Schemes 1−3) were prepared in order to determine the optimum pyrimidine substituents and substitution pattern. Various pyridazine, pyrazine, and benzene acid chlorides (Chart 2) were also synthesized to determine

Scheme 3[a]



[a] Reagents: (a) urea, MeOH, HCl; (b) $POCl_3$; (c) NaOH (for R = CH_3) or LiOH (for R = CF_3); (d) $(COCl)_2$, CH_2Cl_2, rt, 30 min.

Chart 2



if the pyrmidine ring was essential for the biological activity observed and if other ring systems would have improved potency and Caco-2 permeability. The use of parallel synthesis enabled rapid preparation and identification of optimum amide substituents for each aryl and heteroaryl investigated.

Illustrated in Scheme 1 is synthesis of the pyrimidine acid chlorides **38**−**46** and **51**−**54** used for the 2,4,5-substituted pyrimidine libraries. The 2,4-disubstituted pyrimidine-5-carbonyl chlorides were prepared from various β-keto esters **4**−**10** or from diethyl propane-1,3-dioate (**11**). The β-keto esters **4**−**9** and diethyl propane-1,3-dioate (**11**) were commercially available; however, thienoyl acetoacetate (**10**) required synthesis and was prepared by reaction of 2-thiophenecarboxylic acid (**3**) with carbonyldiimidazole and ethylmagnesium acetate.[9] Treatment of the esters **4**−**11** with triethyl orthoformate provided the corresponding ethoxymethylene intermediates **12**−**19**.[10] The pyrimidines **20**−**30** were subsequently prepared by treating the ethoxymethylene derivatives with either urea or the appropriately substituted amidines such as acetamidine or trifluoromethylacetamidine in the presence of NaOEt.[11,12]

Treatment of the 2-hydroxypyrimidines **21**, **23**, **25**, and **26** with $POCl_3$,[13] followed by hydrolysis of the esters with 1 N NaOH in THF provided the corresponding 2-chloro-5-carboxylic acids **32**, **34**, **36**, and **37**, respectively. In the case of **20**, **22**, and **24**, the compounds were hydrolyzed to the corresponding acids **31**, **33**, and **35** respectively, without $POCl_3$ treatment. Conversion of the acids **31**−**37** to the acid chlorides **38**−**44** was accomplished using either oxalyl chloride in CH_2Cl_2 (in the case of 2-chloro acids) or phosphorus oxychloride (in the case of 2-hydroxy acids). The 2-fluoro-5-carbonyl

chloride **45** and the 2-cyano-5-carbonyl chloride **46** were prepared from the corresponding 2-chloro-5-carboxylic acid **32** by reaction with Me_3N in THF, followed by KF or KCN in DMF/H_2O, respectively,[14] and finally with oxalyl chloride in CH_2Cl_2.

The 2-methyl- and 2-trifluoromethylpyrimidine acid chlorides **51**−**54** were prepared in a similar fashion. The pyrimidine esters **27**−**30** were first hydrolyzed to the corresponding carboxylic acids **47**−**50** using NaOH. The acids **48**−**50** were then converted to the acid chlorides **52**−**54** using oxalyl chloride. For the 4-chloropyrimidine acid chloride **51**, the 4-hydroxypyrimidine **47** was refluxed in $POCl_3$.

Scheme 2 illustrates the synthesis of 2-substituted pyrimidine-5-carbonyl chlorides **61**, **64**, and **65**. Reaction of potassium ethyl malonate with $POCl_3$ and DMF provided ethyl 3-(dimethylamino)-2-formylprop-2-enoate (**56**),[15] which was treated with *S*-methylthiopseudourea, trifluoroacetamidine, and benzamidine to give the pyrimidines **57**,[16] **58**,[17] and **59**,[18] respectively. The hydrolysis of 2-methylthiopyrimidine carboxylate **(57)** with NaOH was slow and resulted in incomplete reaction. Hydrolysis using KOH, however, was efficient and provided the pyrimidinecarboxylic acid **60**, which was then refluxed in $POCl_3$ to give the 2-chloropyrimidine acid chloride **61**. The 2-trifluoromethyl- and 2-phenylpyrimidine acid chlorides (**64** and **65**) were prepared from the corresponding pyrimidine esters (**58** and **59**, respectively) as described above.

The synthesis of two 2,4-disubstituted pyrimidine-6-carbonyl chlorides **72** and **73** is illustrated in Scheme 3. Methyl and trifluoromethyl β-keto esters (**66** and **67**) were treated with urea under acidic conditions to provide the 2-hydroxy-4-methylpyrimidine-6-carboxylate and the 2-hydroxy-4-trifluoromethylpyrimidine-6-carboxylate, respectively (**68** and **69**).[19] These compounds were hydrolyzed to the corresponding carboxylic acids **70** and **71**, treated with $POCl_3$, and then reacted with oxalyl chloride to provide 2-chloro-4-methylpyrimidine-5-carbonyl chloride (**72**) and 2-chloro-4-trifluoromethylpyrimidine-5-carbonyl chloride (**73**). Yields from this two-step procedure were higher than yields obtained from the one-pot procedure described earlier.

In addition to preparing a variety of 2,4,5-, 2,5-, and 2,4,6-substituted pyrimidines, various pyrimidine ring isosteres, such as pyridazine, pyrazine, and benzene, were also synthesized. 2,4-Dichloropyrimidine-5-carbonyl chloride (**74**),[10] 2,4-dichloropyrimidine-6-carbonyl chloride (**75**),[10] 3-chloro-5-trifluoromethylpyridazine-6-carbonyl chloride (**76**),[20] 3-chloropyridazine-6-carbonyl chloride (**77**),[21] 2-chloropyrazine-5-carbonyl chloride (**78**),[22] and 4-chloro-2-trifluoromethylbenzoyl chloride (**79**),[23] shown in Chart 2, were synthesized using literature procedures referenced herein.

The amide sublibraries were prepared by treating the aryl and heteroaryl acid chlorides (**38**−**46**, **51**−**54**, **61**, **64**, **65**, **72**−**79**) with a variety of amines and anilines using solution-phase parallel synthesis.[7,24,25] In a microtiter format, each acid chloride described above was reacted with 160 amines and anilines using two plates (80 compounds/plate). The synthesis involved adding 1.05 equiv of each acid chloride to 80 wells of a microtiter plate with each well containing one amine or aniline in EtOAc. Also present in each well was Amberlyst 21, which is a basic ion-exchange resin. The Amberlyst 21 functioned as a solid-phase basic scavenger and adsorbed any HCl produced in the reaction, allowing the limited amount of amine present to react. Sonication of the plates provided sufficient agitation of all the reactants. Following complete reaction of each amine, a small amount of water was added to the wells to tranform any unreacted acid chloride into the corresponding carboxylic acid. The Amberlyst 21 present would also adsorb the carboxylic acid onto the resin. The products were easily isolated by transferring the organic layer of each well into tared test tubes followed by concentration of the solutions.

Prior to testing, the purity of each compound within a library (80 compounds constituted one library) was determined using thin-layer chromatography (TLC). By TLC, all of the compounds were clean products with no residual starting materials present. Fifteen to twenty compounds from each library were randomly selected and analyzed by high-performance liquid chromatography (HPLC) and electron-impact mass spectroscopy (EIMS) for purity and structural verification, respectively. Molecular weights of the randomly selected compounds were confirmed, and all compounds had >90% purity.

Biology

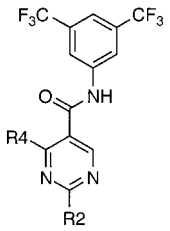
High-throughput screening and followup studies were performed using three distinct cell lines. Jurkat T-cells stably transfected with either an NF-κB-dependent, an AP-1-dependent, or the β-actin[26] promoter driving luciferase were pretreated for 0.5 h with compounds dissolved in 0.2% DMSO/H_2O. The 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 (Tables 1−3) where the IC_{50} value is defined as the concentration of compound required to reduce luciferase activity to 50% of control values.

Compounds that were active in the cell-based assay were studied for their Caco-2 permeability.[27] Compounds were dissolved in DMSO, then added to the apical layer, and monitored in the basolateral layer. The concentrations of the compound on the basolateral side were measured using HPLC equipped with a YMC C-18 column at 254 nm. The concentrations were calculated by comparing to standard curves. The results of the permeability coefficient of the compounds are summarized in Table 4.

Results and Discussion

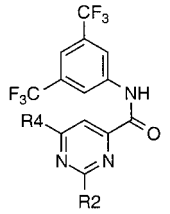
The goal of the current study was to further define the optimum substituents at the 2-, 4-, and 6-positions of the pyrimidine ring, as well as the importance of the pyrimidine ring itself, in an effort to identify analogues of **1** with comparable potency and improved Caco-2 permeability. Through the use of solution-phase parallel synthesis, we were able to determine the optimum amide functionality for each new pyrimidine synthesized. This study also resulted in the identification of the optimum positioning of the substituents on the pyrimidine ring (2, 4, 5 vs 2, 4, 6).

Table 1. Inhibition of AP-1- and NF-κB-Mediated Transcriptional Activation in Jurakat Cells



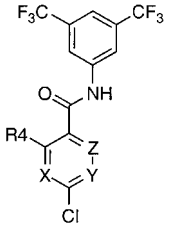
			IC$_{50}$, μM	
no.	R4	R2	AP-1	NF-kB
1	CF_3	Cl	0.05	0.05
80	H	Cl	0.3	0.3
81	CH_3	Cl	0.02	0.05
82	CH_3CH_2	Cl	0.3	0.4
83	$CH_3CH_2CH_2$	Cl	0.5	0.5
84	CF_3CF_2	Cl	0.6	0.8
85	Cl	Cl	0.2	0.6
86	Ph	Cl	0.4	0.4
87	2′-thienyl	Cl	8	9.8
88	CF_3	F	0.1	0.4
89	CF_3	CH_3	>10	>10
90	CF_3	CF_3	>10	>10
91	CF_3	CN	>10	>10
92	Cl	CF_3	>10	>10
93	H	CF_3	>10	>10
94	H	Ph	>10	>10
95	CH_3	CF_3	>10	>10
96	CF_3	CH_3	>10	>10

Table 2. Inhibition of AP-1- and NF-κB-Mediated Transcriptional Activation in Jurakat Cells



			IC$_{50}$, μM	
no.	R4	R2	AP-1	NF-kB
97	CF_3	Cl	>10	>10
98	CH_3	Cl	>10	>10
99	Cl	Cl	> 10	>10

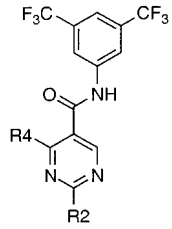
Table 3. Inhibition of AP-1- and NF-κB-Mediated Transcriptional Activation in Jurakat Cells



					IC$_{50}$, μM	
no.	R4	X	Y	Z	AP-1	NF-kB
100	CF_3	CH	CH	CH	>30	>30
101	CF_3	CH	N	N	10	10
102	H	CH	N	N	1.7	1.7
103	H	N	CH	N	4.0	4.0

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 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 against the β-actin control at the highest dose tested (3.3 μg/mL; data not shown).

Table 4. Caco-2 Permeability of Active Compounds



no.	R4	R2	Caco-2 permeability, $\times 10^{-7}$ cm/s (n = 3; mean ± SD)	follows Lipinski's rule?
1	CF_3	Cl	11 ± 4	yes
80	H	Cl	61 ± 7	yes
81	CH_3	Cl	62 ± 6	yes
82	CH_3CH_2	Cl	58 ± 9	yes
83	$CH_3CH_2CH_2$	Cl	31 ± 6	yes
84	CF_3CF_2	Cl	9 ± 3	yes
85	Cl	Cl	31 ± 6	yes
86	Ph	Cl	9 ± 7	no
87	2′-thienyl	Cl	21 ± 3	yes
88	CF_3	F	19 ± 6	yes

Tables 1−3 contain the IC$_{50}$ values for a select group of 3′,5′-bis(trifluoromethyl)aniline pyrimidine derivatives. Originally, the compounds were prepared as part of the libraries. The library was composed of individual, pure compounds (>90%) in 5 ± 2 mg. This quantity of material is enough to test a compound at three different doses in three assays (AP-1, NF-κB, and β-actin assays). However, the individual, discrete compounds shown in Table 1 were also prepared in large quantities since each of the compounds listed in Table 1 was the most potent member of the sublibrary. The large-scale synthesis of the pure (>99%) potent compounds enabled us to fully characterize them using various analytical methods and evaluate them in various assays.

4-Position Derivatives. Examination of the various substituents in the 4-position in which the 2-position was held constant (chloro) indicated that small lipophilic groups were best tolerated (**80**−**87**). However, a substituent other than a hydrogen was needed as indicated by the decrease in activity as seen with the unsubstituted derivative (**1** vs **80**). Comparing the 4-chloro derivative (**85**) to the 4-CF_3 (**1**), we observed a loss in activity even though these substituents are of comparable size and have similar properties. An interesting observation was the difference in activity between the 4-phenyl (**86**) and 4-thienyl (**87**) derivatives. Thiophene had been reported as an isostere for a phenyl group. However, in this series the phenyl derivative was considerably more active.

2-Position Derivatives. Examination of the 2-position reveals that the chloro substituent is optimal and absolutely required. Reversing the 2- and 4-substituents (**1** vs **92**) resulted in a loss of activity. In addition, any modification to the 2-position (CH_3, CF_3, CN, Ph) resulted in inactive derivatives (**89**−**91**). Only the 2-fluoro compound had inhibitory activity (**1** vs **88**).

2,4,5- vs 2,4,6-Position Pyrimidines. To investigate the importance of the positioning of the carboxamide group with relation to the substituents at the 2- and 4-positions, we prepared the corresponding 2-chloro-4-substituted-6-carboxamide libraries as well as the individual 2-chloro-4-methyl, 4-trifluoromethyl, and 4-chloro derivatives **97**−**99**. The preparation of this library was critical since 2,4,6-substituted pyrimidines could have a different SAR profile than the 2,4,5-substituted compounds. Of interest was the benzyl derivatives prepared as part of the library, since the extended chain would in theory allow for a similar orientation to the parent carboxamide **1**. Upon evaluation of the compounds, it was found that none had potency in our assays. This was surprising due to the similarity in structures and indicated that a very critical interaction or binding was occurring.

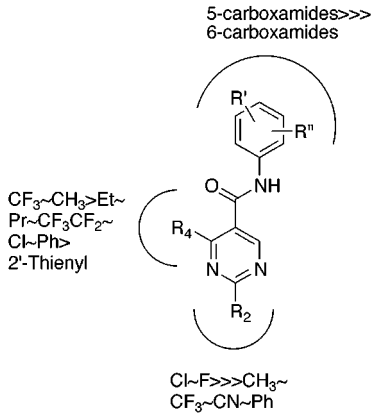
Other Heterocycles. Having established that only 2,4,5-substituted pyrimidines were active, we next turned our attention to the criticality of the pyrimidine ring. Table 3 contains several other ring systems with the various bis(trifluoromemthyl) derivatives examined (**100**−**103**). First, the 2-chloro-4-trifluorobenzamide **100** was prepared and found to be completely inactive. Next, several pyrimidine isomers were examined including the 1,2- and 1,4-positional isomers (pyridazines **101**, **102** and pyrazine **103**). The results for the pyridazine ring system were somewhat different in that the 5-hydrogen compound **102** was more potent than the 5-trifluoromethyl compound **101**. Compound **101** was 300-fold less potent than **1** (10 μM vs 30 nM). However, **102** was comparable in potency to the corresponding unsubstituted pyrimidine **80.** The 1,4-isomer **103** had comparable potency to **102**, (4 vs 1.7 μM), indicating that a heterocyclic ring is critical for activity.

Caco-2 Permeability. Compounds **1** and **80**−**88** were tested in the Caco-2 assay (gastrointestinal cell line). Compound **1** exhibited poor permeability in the Caco-2 (apparent permeability coefficient (P_{app}) = 11 ± 4 × 10^{-7} cm/s; Table 4). The corresponding nor-trifluoromethyl analogue **80** and 4-methyl analogue **81** showed increased Caco-2 permeability. The 4-ethyl analogue also exhibited increased permeability. The 4-pentafluoroethyl analogue **84**, 4-chloro analogue **85**, 4-phenyl analogue **86**, and 4-(2′-thienyl) analogue **87** all exhibited low Caco-2 permeability. The 2-fluoro-4-trifluoromethylpyrimidine analogue **88** also exhibited low Caco-2 permeability.

Conclusions

As described previously, compound **1** was a potent inhibitor of NF-κB and AP-1 activation. The compound was active in several animal models of inflammation but only when administered ip. The major focus of the work described here was the identification of the optimum combination of 2-, 4-, and 5-position substituents that would provide a compound with improved potential oral bioavailability. To this end, approximately 3200 analogues of **1** were prepared and evaluated in vitro. The results of the SAR studies are summarized in Chart 3. The most active derivatives all contained a chloro substituent at the 2-position of the pyrimidine ring. Examination of a variety of substituents at the 4-position indicated that this may provide a better opportunity for identifying a compound with the desired characteristics. The substitution of the trifluoromethyl group with a methyl or phenyl group still resulted in submicromolar inhibitors of NF-κB and AP-1 activation. The methyl analogue **81** was comparable in activity in the whole-cell assay.

Chart 3



The pyridazine analogue was less active than the pyrimidine analogue (**101** vs **1**). A similar trend was observed with the pyrazine analogues (**103** vs **1**). Several attempts to prepare the corresponding pyrazine analogue of **1** maintaining the trifluoromethyl substituent were unsuccessful. The introduction of a phenyl ring in place of the pyrimidine ring resulted in a loss of activity (**100** vs **1**). The orientation of groups on the pyrimidine ring was more crucial to activity, and the heterocyclic variation resulted in a less dramatic effect on activity. Among the compounds synthesized, **81** was comparable to **1** in activity in the in vitro assay. Table 4 shows the list of active compounds that were tested in the Caco-2 assay.[28] We also performed calculations on these compounds using Lipinski's rule of 5.[29] All the compounds except compound **86** followed Lipinski's rule. Compounds **80**−**82** showed superior Caco-2 permeability (Table 4) than **1**. Thus we identified a compound that is comparable in activity to **1** with superior Caco-2 permeability and potential oral bioavailability.

Experimental Section

Melting points (uncorrected) were obtained on a Mel-Temp-II using capillary tubes. Proton nuclear magnetic resonance spectra were obtained on a Varian Gemini 2000 spectrometer operating at 300 MHz for proton with tetramethylsilane as an internal standard, and chemical shifts are reported on the δ scale. Infrared spectra were obtained on a Nicolet Impact 400D spectrometer. Electron impact mass spectra (EIMS) were obtained on a Hewlett-Packard 5890 Series II plus gas chromatography coupled to a Hewlett-Packard 5972 mass selective detector. Electrospray ionization (ESMS) was obtained on a Hewlett-Packard 1100 MSD at Mass Consortium in San Diego, CA. Flash chromatography was performed using silica gel 60 (230−400 mesh). High-performance liquid chromatography was performed on a Rainin Dynamax system equipped with a YMC C-18 column using acetonitrile containing 35% water and 0.1% CF_3COOH. Elemental analyses were performed at Desert Analytics, Tucson, AZ, and were within 0.4% of the calculated values. All reactions requiring anhydrous conditions and/or an inert atmosphere were performed under positive nitrogen atmosphere. Tetrahydrofuran was distilled from sodium benzophenone ketyl. All other solvents and reagents were used as received from commercial suppliers.

Ethyl Ethoxymethylene-4,4,4-trifluoroacetoacetate (12). A solution of 4,4,4-trifluoroacetoacetate (46 g, 0.25 mol),

triethyl orthoformate (74 g, 0.50 mol), and Ac_2O (77 g, 0.75 mol) was heated under N_2 at 120 °C for 2 h and 140 °C for 5 h. The solution was concentrated to an oil and distilled to obtain the title compound (58.6 g, 98%; bulb-to-bulb distillation, 80−90 °C, 1.5 mm/Hg): ^{1}H NMR ($CDCl_3$) δ 7.80 (d, 1H), 4.3 (m, 4H), 1.3 (m, 6H); IR (neat) 2992, 1741, 1623, 1590, 1202, 1019 cm^{-1}; EIMS *m/z* 240 (M^+).

Ethyl Ureidomethylenepentafluoropropionylacetate (13). A solution of ethyl pentafluoropropionylacetate (5 g, 21 mmol), urea (1.3 g, 21 mmol), and triethyl orthoformate (3.2 g, 21 mmol) was heated at reflux for 3 h. The reaction mixture was cooled, and the solid was collected by filteration. The solid was washed with water, ether, and dried to give the title compound (4.4 g, 68%) as a white solid: mp 160−162 °C; ^{1}H NMR (DMSO-d_6) δ 9.90 (s, 1H), 8.4 (s, 1H), 7.5 (d, 2H), 4.1 (q, J = 7.2 & 6.9, 2H), 1.19 (t, J = 7.2 Hz, 3H); IR (KBr) 3088, 2910, 1749, 1670, 1402, 1173, 1099 cm^{-1}; EIMS *m/z* 258 ((M − EtOH)$^+$).

Ethyl Ureidomethyleneacetoacetate (14). A mixture of ethyl acetoacetate (200 g, 1.54 mmol), urea (105 g, 1.54 mmol), and triethyl orthoformate (228 g, 1.54 mmol) was heated at 140 °C under N_2 for 22 h. The reaction mixture was cooled, and filtered. The solid was washed with Et_2O (3 × 100 mL), water (3 × 150 mL), and dried under vacuum to provide 156 g (51%) of the title compound: mp 173−174 °C; ^{1}H NMR (DMSO-d_6) δ 11.42 (d, J = 12.9 Hz, 1H), 8.46 (d, J = 12.6 Hz, 1H), 7.72 (s, 1H), 7.26 (s, 1H), 4.17 (q, J = 7.2 Hz, 2H), 2.42 (s, 3H), 1.27 (t, 3H); IR (KBr) 3418, 2995, 1709, 1655, 1558, 1466, 1391, 1234, 1062 cm^{-1}; EIMS *m/z* 200 (M^+).

Ethyl ureidomethylenepropionoylacetate (15): yield 37%; mp 148−150 °C; ^{1}H NMR ($CDCl_3$) δ 10.2 (dd, J = 12.6 Hz, 1H), 8.39 (d, J = 12.6 Hz, 1H), 7.67 (bs, 1H), 7.29 (s, 1H), 4.17 (m, 2H), 2.80 (q, J = 7.2 Hz, 2H), 1.25 (t, J = 7.2 Hz, 3H), 0.96 (t, J = 6.0 Hz, 3H); IR (KBr) 3386, 2995, 1713, 1643, 1552, 1230 cm^{-1}; EIMS *m/z* 214 (M^+).

Ethyl ureidomethylenebutonoylacetate (16): yield 42%; mp 153−154 °C; ^{1}H NMR ($CDCl_3$) δ 10.45 (d, J = 12 Hz, 1H), 8.56 (dd, J = 12 Hz, 1H), 4.27 (m, 2H), 2.96 (t, J = 7.2 Hz, 2H), 2.78 (t, J = 7.2 Hz, 2H0, 2.55 (s, 2H), 1.35 (m, 3H), 0.94 (q, J = 7.2 Hz, 3H); IR (neat) 3396, 3209, 2950, 1703, 1643, 1552, 1221, 1147, 766 cm^{-1}; EIMS *m/z* 228 (M^+).

Ethyl ureidomethylenebenzoylacetate (17): yield 55%; mp 124−126 °C; ^{1}H NMR ($CDCl_3$) δ 11.43 (d, J = 12.9 Hz, 1H), 8.44 (d, J = 12.7 Hz, 1H) 7.72 (s, 1H), 7.3 (m, 6H), 4.17 (q, J = 7.2 Hz, 2H), 1.27 (t, J = 7.1 Hz, 3H); IR (KBr) 3411, 2994, 1711, 1644, 1551, 1472 cm^{-1}; EIMS *m/z* 246 (M^+).

Ethyl Ureidomethylenethienoylacetate (18) and Diethyl Trifluoroacetamidomethylenemalonate (19). These compound were ring-closed to **20** and **27**, respectively, under the reaction conditions.

Ethyl 2-hydroxy-4-(2′-thienyl)pyrimidine-5-carboxylate (20): yield 51%; mp >220 °C; ^{1}H NMR ($CDCl_3$) δ 8.55 (s, 1H), 7.82 (dd, J = 1.2, 3.9 Hz, 1H), 7.59 (dd, 1H, 0.9, 5.1 Hz, 1H), 7.11 (m, 1H), 5.25 (bs, 1H), 4.31 (q, J = 7.2 Hz, 2H), 1.33 (t, J = 6.9 Hz, 3H), IR (neat) 2995, 1714, 1647, 1605, 1409, 1290 cm^{-1}; ESMS *m/z* 251 (M + H$^+$).

Ethyl 2-hydroxy-4-trifluoromethylpyrimidine-5-carboxylate (21): yield 45%; ^{1}H NMR (DMSO-d_6) δ 9.89 (s, 1H), 8.41 (bs, 1H), 4.0 (q, J = 7.2 Hz, 2H), 1.2 (t, J = 7.1 Hz, 3H); EIMS *m/z* 236 (M^+).

Ethyl 2-hydroxy-4-pentafluoroethylpyrimidine-5-carboxylate (22): yield 66%; mp 171 °C; ^{1}H NMR (DMSO-d_6) δ 8.56 (s, 1H), 4.15 (q, 2H), 1.23 (t, 3H); IR 2968, 1730, 1653, 1420, 1179, 1221 cm^{-1}; EIMS *m/z* 258 (M^+).

Ethyl 2-Hydroxy-4-methylpyrimidine-5-carboxylate (23). A solution of ethyl ureidomethylene acetoacetate (50 g, 250 mmol), NaOEt (22.1 g, 325 mmol) in EtOH (500 mL) was stirred at room temperature under N_2 for 3 days. The white precipitate was collected by filtration and washed with EtOH and dried under reduced pressure to yield 45 g (88%) of the title compound as the sodium salt: mp >220 °C dec; ^{1}H NMR (DMSO-d_6) δ 12.22 (bs, 1H), 8.75 (s, 1H), 4.25 (q, J = 7.2 & 6.7 Hz, 2H), 1.31 (t, J = 7.2 Hz, 3H); IR (KBr) 2646, 1755, 1711, 1583, 1470, 1375, 1277, 1215, 1107, 1022 cm^{-1}; EIMS *m/z* 182 (M^+).

Ethyl 4-ethyl-2-hydroxypyrimidine-5-carboxylate (24): yield 81%; mp 215−217 °C; ^{1}H NMR (DMSO-d_6) δ 8.47 (s, 1H), 8.30 (s, 1H), 4.09 (q, J = 7.2 Hz, 2H), 2.69 (q, J = 7.2 Hz, 2H), 1.22 (t, J = 7.2 Hz, 3H), 1.04 (t, J = 7.2 Hz, 3H); IR 2981, 1716, 1579, 1497, 1367, 1275, 1205, 800 cm^{-1}; EIMS *m/z* 196 (M^+).

Ethyl 2-hydroxy-4-propylpyrimidine-5-carboxylate (25): yield 72%; mp 185−186 °C; ^{1}H NMR (DMSO-d_6) δ 8.75 (bs, 1H), 8.04 (s, 1H), 4.21 (q, J = 6.8 Hz, 2H), 2.85 (t, J = 8.0 Hz, 2H), 1.59 (m, 2H), 1.26 (t, J = 6.8 Hz, 3H), 0.90 (t, J = 8.0 Hz, 3H); IR (neat) 2962, 1747, 1687, 1475, 1275 cm^{-1}; EIMS *m/z* 209 (M − H$^+$).

Ethyl 2-hydroxy-4-phenylpyrimidine-5-carboxylate (26): yield 65%; mp >260 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.00 (t, 3H, J = 7.2 Hz), 4.02 (q, 2H, J = 6.9 Hz), 7.46 (m, 5H), 8.05 (s, 1H), 10.30 (s, 1H); IR (KBr) 2981, 1713, 1587, 1547, 1261, 1105 cm^{-1}; EIMS *m/z* 243 (M − H$^+$).

Ethyl 4-Hydroxy-2-trifluoromethylpyrimidine-5-carboxylate (27). A solution of diethyl ethoxymethylenemalonate (35.0 g, 162 mmol), trifluoroacetamidine (18 g, 162 mmol) and NaOEt (11.0 g, 162 mmol) in EtOH (200 mL) was heated at reflux under N_2 for 6 h. The reaction mixture was concentrated under reduced pressure and treated with H_2O (48 mL). The solid was filtered and washed with Et_2O (300 mL) and H_2O (200 mL), and dried under vacuum to give the title compound (21 g, 50%): mp > 220 °C (turned brown); ^{1}H NMR (DMSO-d_6) δ 8.38 (s, 1H), 4.16 (q, J = 7.2 & 6.9 Hz, 2H), 1.25 (q, J = 7.2 Hz, 3H); IR (KBr) 3230, 2932, 1689, 1657, 1471, 1376, 1280, 1206,1062 cm^{-1}; EIMS *m/z* 236 (M^+).

Ethyl 4-methyl-2-trifluoromethylpyrimidine-5-carboxylate (28): yield 55%; ^{1}H NMR ($CDCl_3$) δ 9.26 (s, 1H), 4.51 (q, J = 6.9 Hz, 2H), 2.82 (s, 3H), 1.46 (t, J = 7.2 Hz, 3H); IR (KBr) 2863, 1748, 1652, 1593, 1487, 1238 cm^{-1}; EIMS *m/z* 234 (M^+).

Ethyl 2-methyl-4-trifluoromethylpyrimidine-5-carboxylate (29): yield 58%; ^{1}H NMR ($CDCl_3$) δ 9.13 (s, 1H), 4.46 (q, J = 7.2 Hz, 2H), 2.90 (s, 3H), 1.38 (t, J = 7.1 Hz, 3H); IR (KBr) 2932, 1758, 1668, 1620, 1418, 1381, 1275, 1179 cm^{-1}; EIMS *m/z* 234 (M^+).

Ethyl 2,4-Bis(trifluoromethyl)pyrimidine-5-carboxylate (30). A solution of ethyl ethoxymethylene-4,4,4-trifluoroacetoacetate (15 g, 62.5 mmol), and trifluoroacetamidine (12.6 g, 112.5 mmol) in EtOH (50 mL) was heated at reflux for 24 h under N_2. The reaction mixture was cooled to room temperature and concentrated to an oil under reduced pressure. Flash chromatography (SiO_2, 20% ethyl acetate in hexane) afforded the title compound as an oil (7.0 g, 39%): ^{1}H NMR ($CDCl_3$) δ 9.37 (s, 1H), 3.70 (q, J = 7.2 & 6.9 Hz, 2H), 1.27 (t, J = 7.2 Hz, 3H); IR (neat) 2978, 2864, 1732, 1216, 1159 cm^{-1}; EIMS *m/z* 287 (M^+).

2-Hydroxy-4-(2′-thienyl)pyrimidine-5-carboxylic acid (31): yield 89%; mp > 200 °C; ^{1}H NMR (DMSO-d_6) 8.37 (s, 1H), 7.81 (m. 2H), 7.15 (m, 1H); IR (KBr) 2994, 1630, 1605, 1420, 1400, 1215 cm^{-1}; ESMS *m/z* 221 (M − H$^+$). Anal. ($C_9H_6N_2O_3S$) C, H, N.

2-Chloro-4-trifluoromethylpyrimidine-5-carboxylic acid (32): mp 158 °C; ^{1}H NMR (DMSO-d_6) δ 12.4 (bs, 1H), 9.36 (s, 1H); ESMS *m/z* 225 (M − H$^+$).

2-Hydroxy-4-pentafluoroethylpyrimidine-5-carboxylic acid (33): yield 98%; mp >210 °C dec; ^{1}H NMR (DMSO-d_6) δ 9.9 (bs, 1H), 8.43 (s, 1H); IR (KBr) 3232; 2964, 1732, 1657, 1427, 1223, 1176 cm^{-1}; ESMS *m/z* 257 (M − H$^+$). Anal. ($C_7H_3F_5N_2O_3 \cdot 1.25H_2O$) C, H, N.

2-Chloro-4-methylpyrimidine-5-carboxylic Acid (34). A solution of ethyl 2-chloro-4-methylpyrimidine-5-carboxylate (1.0 g, 5 mmol), NaOH (0.24 g, 6 mmol), and H_2O (30 mL) was stirred at room temperature for 3 h. The solution was acidified to below pH = 1 with 6 N HCl and cooled to 0 °C. The white solid was filtered, and dried to give 0.67 g (78%) of the title compound: ^{1}H NMR (DMSO-d_6) δ 9.01 (s, 1H), 2.75 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 171.95, 165.33, 161.59, 161.47, 123.33,

23.80; IR (KBr) 3230, 2991, 1732, 1578, 1514, 1423, 1275, 1174 cm^{-1}; ESMS *m/z* 172 (M^+).

4-Ethyl-2-hydroxypyrimidine-5-carboxylic acid (35): yield 87%; mp >200 °C; ^1H NMR ($CDCl_3$) δ 9.11 (s, 1H), 8.12 (s, 1H), 3.24 (q, $J = 7.5$ Hz, 2H), 1.32 (t, $J = 7.5$ Hz, 3H); ESMS *m/z* 186 ($M - H^+$).

2-Chloro-4-propylpyrimidine-5-carboxylic Acid (36). Ethyl 2-chloro-4-propylpyrimidine-5-carboxylate (**25**) was hydrolyzed with NaOH to give the title compound (88%): mp 201 °C, ^1H NMR ($CDCl_3$) δ 9.18 (s, 1H), 3.19 (t, $J = 6.9$ Hz, 2H), 1.79 (m, 2H), 1.05 (t, $J = 7.0$ Hz, 3H); ESMS *m/z* 199 ($M - H^+$).

2-Chloro-4-phenylpyrimidine-5-carboxylic Acid (37). A solution of ethyl 2-hydroxy-4-phenylpyrimidine-5-carboxylate (2.0 g, 8.1 mmol) and $POCl_3$ was heated at reflux for 1 h under N_2 atmosphere. The reaction mixture was poured on crushed ice, and extracted with EtOAc (4 × 50 mL). The EtOAc layers were combined and concentrated. Flash chromatography of the residue afforded ethyl 2-chloro-4-phenylpyrimidine-5-carboxylate (1.5 g, 72%): mp 45−47 °C; ^1H NMR ($CDCl_3$) δ 8.96 (s, 1H), 7.6−7.4 (m, 5H), 4.25 (t, 2H, $J = 6.9$ Hz), 1.14 (t, 3H, $J = 7.2$ Hz); IR (KBr) 2998, 1703, 1645, 1560, 1213 cm^{-1}; EIMS *m/z* 262 (M^+). A solution of ethyl 2-chloro-4-phenylpyrimidine-5-carboxylate (1.5 g, 5.7 mmol) and NaOH (0.34 g, 8.58 mmol) in 20% THF in water (50 mL) was stirred at room temperature for 2 h. The reaction mixture was made acidic with 1 N HCl and EtOAc. The EtOAc layer was removed under reduced pressure to give the title compound (0.71 g, 53%): mp 108−110 °C; ^1H NMR (DMSO-d_6) δ 9.05 (s, 1H), 7.55 (m, 5H); IR (KBr) 2983, 1724, 1556, 1400, 1295, 1192 cm^{-1}; ESMS *m/z* 222 (M^+).

2-Chloro-4-trifluoromethylpyrimidine-5-carbonyl Chloride (39). The material was identical to the commercially obtained sample.[30]

2-Chloro-4-pentafluoroethylpyrimidine-5-carbonyl Chloride (40). The reaction mixture was concentrated under reduced pressure and distilled (bulb-to-bulb, 80−85 °C, 1 mm/Hg) to give the title compound (1.2 g, 35%): ^1H NMR (DMSO-d_6) δ 9.18 (s, 1H); IR (neat) 2895, 1794, 1566, 1227, 1005 cm^{-1}; EIMS *m/z* 294 (M^+).

2-Chloro-4-methylpyrimidine-5-carbonyl Chloride (41).[31] A solution of ethyl 2-hydroxy-4-methylpyrimidine-5-carboxylate (5 g, 27.5 mmol) and $POCl_3$ (84 g, 550 mmol) was heated at reflux under N_2 for 1 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The oil was dissolved in $CHCl_3$ (600 mL) and washed with H_2O (3 × 100 mL). The aqueous layers were combined and extracted with $CHCl_3$ (100 mL). The $CHCl_3$ layers were combined, dried (Na_2SO_4), filtered through a silica plug, concentrated under reduced pressure. The residue was distilled (bulb-to-bulb, 110−115 °C, 0.5 mm/Hg) to yield 1.5 g (27%) of ethyl 2-chloro-4-methylpyrimidine-5-carboxylate: ^1H NMR ($CDCl_3$) δ 9.04 (s, 1H), 4.42 (q, $J = 7.2$ & 6.9 Hz, 2H), 2.85 (s, 3H), 1.43 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 172.32, 163.84, 162.78, 161.17, 122.32, 62.03, 24.34, 14.17; IR (neat) 3001, 1743, 1570, 1541, 1448, 1362, 1284, 1132, 1072 cm^{-1}; EIMS *m/z* 200 (M^+). Ethyl 2-chloro-4-methylpyrimidine-5-carboxylate was hydrolyzed to an acid using aqueous 1 N NaOH and THF. The reaction mixture was acidified to give 2-chloro-4-methylpyrimidine-5-carboxylic acid. A solution of 2-chloro-4-methylpyrimidine-5-carboxylic acid (0.81 g, 4.7 mmol), oxalyl chloride (0.89 g, 7.05 mmol), and DMF (1 drop) in CH_2Cl_2 (23 mL) was stirred at room temperature under N_2 for 4 h. The reaction mixture was concentrated and distilled (bulb-to-bulb, 100 °C, 1.3 mm/Hg) to give 0.548 g (61%) of the title compound as a clear liquid: ^1H NMR ($CDCl_3$) δ, 9.02 (s, 1H), 2.74 (s, 3H); IR 2987, 1747, 1578, 1535, 1455, 1370, 1200 cm^{-1}; EIMS *m/z* 191 (M^+).

2-Chloro-4-phenylpyrimidine-5-carbonyl chloride (44): yield 71%; mp 42 °C; ^1H NMR ($CDCl_3$) δ 9.04 (s, 1H), 7.55 (m, 5H); IR (KBr) 2976, 1722, 1562, 1528, 1407, 1190 cm^{-1}; EIMS *m/z* 252 (M^+).

2-Fluoro-4-trifluoromethylpyrimidine-5-carbonyl Chloride (45). A solution of 2-chloro-4-trifluoromethylpyrimidine-5-carboxylic acid (2.80 g, 12.4 mmol) in dry THF (50 mL) at 0 °C was stirred with Me_3N (gas, 5 min). A preceipitate was immediately formed. The solid was filtered (3.40 g, 97%), and washed with dry THF to give 2-trimethylammonium 4-trifluoromethylpyrimidine-5-carboxylic acid chloride: mp 120−121 °C dec; ^1H NMR (DMSO-d_6) δ 10.82 (bs, 1H), 9.20 (2, 1H), 3.62 (s, 9H); IR (KBr) 3510, 2994, 1705, 1507, 1196, 990 cm^{-1}; ESMS *m/z* 285 (M^+). A solution of 2-trimethylammonium chloride 4-trifluoromethylpyrimidine-5-carboxylic acid (75 mg, 0.26 mmol) and potassium fluoride (20 mg, 0.34 mmol) in DMF−water (50−50, 3 mL) was stirred for 0.5 h. The reaction mixture was acidified with 1 mL of 1 N HCl and extracted with EtOAc. The organic layer was concentrated to give 2-fluoro-4-trifluoromethylpyrimidine-5-carboxylic acid (40 mg, 73%): ^1H NMR (DMSO-d_6) δ 9.41 (s, 1H); IR (KBr) 3448, 2995, 1757, 1656, 1455, 1172, 995 cm^{-1}; ESMS *m/z* 210 (M^+). A solution of 2-fluoro-4-trifluoromethylpyrimidine-5-carboxylic acid (40 mg, 0.19 mmol), oxalyl chloride (0.2 mL), and DMF (1 drop) was stirred for 1 h at room temperature. The reaction mixture was concentrated to give the title compound (40 mg, 93%): ^1H NMR ($CDCl_3$) δ 9.42 (s, 1H); IR (neat) 2945, 1786, 1581, 1450, 1412, 1169, 945 cm^{-1}; EIMS *m/z* 228 (M^+).

2-Cyano-4-trifluoromethylpyrimidine-5-carbonyl Chloride (46). A solution of 2-trimethylammonium chloride-4-trifluoromethylpyrimidine-5-carboxylic acid (3.62 g, 12.7 mmol) and KCN (0.988 g, 15.2 mmol) in DMF (36.5 mL) and H_2O (18.3 mL) was stirred at room temperature under nitrogen for 0.25 h. The reaction mixture was concentrated under reduced pressure and dissolved in EtOAc (400 mL). The EtOAc layer was washed with H_2O (4 × 100 mL), brine (100 mL), and dried (Na_2SO_4). The EtOAc layer was filtered, and concentrated under reduce pressure to yield the title compound (2.03 g, 74%): mp 148−149 °C dec; ^1H NMR (DMSO-d_6) δ 9.56 (s); IR (KBr) 3331, 2996, 2226, 1705, 1663, 1588, 1296, 1142 cm^{-1}; ESMS *m/z* 216 ($M - 1^+$). A solution of 2-cyano-4-trifluoromethylpyrimidine-5-carboxylic acid (2.0 g, 9.2 mmol), oxalyl chloride (1.4 g, 11 mmol) and DMF (4 drops) in CH_2Cl_2 (46 mL) was stirred at room temperature under N_2 for 40 min. The reaction mixture was concentrated under reduced pressure, and distilled (bulb-to-bulb, 100 °C, 1.5 mm/Hg) to give the title compound as a clear liquid (1.8 g, 82%): ^1H NMR ($CDCl_3$) δ 9.49 (s); ^{13}C NMR ($CDCl_3$) δ 162.25, 160.07, 153.25 (q, $J = 39$ Hz), 145.88, 129.48, 118.72 (q, $J = 276$ Hz), 113.78; IR (neat) 3066, 2985, 2254, 1786, 1564, 1435, 1379, 1178, 908 cm^{-1}; EIMS *m/z* 235 (M^+).

4-Hydroxy-2-trifluoromethylpyrimidine-5-carboxylic Acid (47). A solution of ethyl 2-trifluoromethyl-4-hydroxypyrimidine-5-carboxylate (5.00 g, 19.4 mmol), NaOH (0.932 g, 23.3 mmol), and H_2O (20 mL) was stirred at 60 °C for 15 h. The reaction mixture was acidified to pH = 1 using 6 N HCl and concentrated under reduced pressure to one-half its volume. The solid was filtered and dried under reduced pressure to give the title compound (2.1 g, 53%): mp >198 °C dec; ^1H NMR (DMSO-d_6) δ 8.83; ^{13}C NMR (DMSO-d_6) δ 171.43, 16613, 159.19, 157.25 (q, $J = 6.4$ Hz,), 119.36 (q, $J = 275$ Hz), 113.15 IR 3336, 2922, 1716, 1567, 1344, 1291, 1222, 1163 cm^{-1}; ESMS *m/z* 207 ($M - 1^+$).

4-Methyl-2-trifluoromethylpyrimidine-5-carboxylic acid (48): yield 85%; mp >210 °C dec; ^1H NMR (DMSO-d_6) δ 9.27 (s, 1H), 2.81 (s, 3H), IR (KBr) 3375, 3065, 2863, 1748, 1652, 1487, 1238, 1153 cm^{-1}; FABMS *m/z* 207 ($(M + 1)^+$).

2-Methyl-4-trifluoromethylpyrimidine-5-carboxylic acid (49): yield 82%; mp >211 °C dec; ^1H NMR (DMSO-d_6) δ 9.22 (s, 1H), 2.79 (s, 3H); IR (KBr) 3416, 2980, 1721, 1578, 1546, 1285, 1099 cm^{-1}; FABMS *m/z* 207 ($(M + 1)^+$).

2,4-Bis(trifluoromethyl)pyrimidine-5-carboxylic Acid (50). A solution of ethyl 2,4-bis(trifluoromethyl)pyrimidine-5-carboxylate (5.0 g, 17 mmol) and NaOH (0.72 g, 18 mmol) in EtOH (20 mL) and H_2O (50 mL) was stirred at room temperature for 1 h. The solution was acidified at °C to pH = 1 using concentrated HCl. The resulting solid was filtered and dried under reduced pressure to give the title compound (1.5 g, 25%): mp 59 °C; ^1H NMR (DMSO-d_6) δ 9.62; IR (KBr) 2968, 1734, 1455, 1250, 1157 cm^{-1}; EIMS *m/z* 260 (M^+).

2-Trifluoromethyl-4-chloropyrimidine-5-carbonyl Chloride (51). A solution of 2-trifluoromethyl-4-hydroxypyrimidine-5-carboxylic acid (2.2 g, 10.6 mmol), $POCl_3$ (32 g, 212 mmol) and $SOCl_2$ (25 g, 212 mmol) was heated at reflux for 4 days. The reaction mixture was concentrated under reduced pressure and distilled (bulb-to-bulb, 90−95 °C, 1.5 mm/Hg) to provide the title compound (2.1 g, 81%): ^{1}H NMR ($CDCl_3$) δ 9.45 (s); IR (neat) 2998, 1774, 1645, 1566, 1535, 1443, 1362, 1163, 897 cm^{-1}; EIMS *m*/*z* 244 (M^+).

4-Methyl-2-trifluoromethylpyrimidine-5-carbonyl chloride (52): acid chloride was distilled (70−72 °C at 0.5 mm/Hg; yield 89%); ^{1}H NMR ($CDCl_3$) δ 9.53 (s, 1H), 2.93 (s, 3H); IR (neat) 2937, 1780, 1726, 1581, 1398, 1209, 1151, 930 cm^{-1}; EIMS *m*/*z* 224 (M^+).

2-Methyl-4-trifluoromethylpyrimidine-5-carbonyl chloride (53): yield 66%; bp 90 °C at 1.5 mm/Hg; ^{1}H NMR ($CDCl_3$) δ 9.32 (s, 1H), 2.93 (s, 3H); IR 2989, 1788, 1680, 1576, 1211, 843 cm^{-1}; EIMS *m*/*z* 224 (M^+).

2,4-Bis(trifluoromethyl)pyrimidine-5-carbonyl chloride (54): yield 89%; bp 105 °C at 1.5 mm/Hg; ^{1}H NMR ($CDCl_3$) δ 9.12; IR (neat) 2981, 2889, 1738, 1325, 1161, 1052 cm^{-1}; EIMS *m*/*z* 278 (M^+).

Ethyl 2-Trifluoromethylpyrimidine-5-carboxylate (58). A solution of α-carboethoxy-β-dimethylaminoacraldehyde[16] (10 g, 58 mmol) and trifluoromethylacetamidine (16 g, 146 mmol) in EtOH (200 mL) was heated at reflux for 3 h. The reaction mixture was cooled and concentrated under reduced pressure to an oil. Flash chromatography (SiO_2, 20% EtOAc−hexanes) afforded 5.4 g (44%) of the title compound as a white solid: mp 65 °C; ^{1}H NMR ($CDCl_3$) δ 9.43 (s, 2H), 4.51 (q, 2H, J = 6.9 Hz), 1.46 (t, 3H, J = 7.2 Hz); IR (KBr) 3022, 1722, 1566, 1352, 1196, 1151 cm^{-1}; EIMS *m*/*z* 220 (M^+).

Ethyl 2-Phenylpyrimidine-5-carboxylate (59). A solution of ethyl 3-*N*,*N*-dimethylamino-2-formylacrylate (4.0 g, 23 mmol), benzamidine hydrochloride (4.0 g, 26 mmol) and Na (0.65 g, 28 mmol) in EtOH (40 mL) was heated at reflux under N_2 for 1 h. The solution was filtered and concentrated. The residue was dissolved in EtOAc, washed with dilute HCl and H_2O. The EtOAc layer was dried (Na_2SO_4), filtered, and concentrated to give the title compound (4.0 g, 75%): mp >220 °C dec; ^{1}H NMR (DMSO-d_6) δ 9.28 (s, 2H), 8.46 (m, 2H), 7.56 (m, 2H), 4.37 (q, J = 7.1 Hz, 2H), 1.35 (t, J = 7.2 Hz, 3H); IR (KBr) 2987, 1724, 1657, 1431, 1181, 1219 cm^{-1}; EIMS *m*/*z* 228 (M^+).

2-Chloropyrimidine-5-carbonyl Chloride (61). The pure product was obtained from the reaction mixture by bulb-to-bulb distillation under reduced pressure (100 °C, 1.5 mm/Hg; yield 69%): ^{1}H NMR ($CDCl_3$) δ 9.25 (s); IR (neat) 2992, 1757, 1583, 1408, 1239, 1070, 586 cm^{-1}; EIMS *m*/*z* 177 (M^+).

2-Trifluoromethylpyrimidine-5-carboxylic acid (62): yield 87%; mp 169−170 °C; ^{1}H NMR ($CDCl_3$) δ 9.42; IR (KBr) 3514, 2897, 1724, 1586, 1342, 1203, 1105 cm^{-1}; EIMS *m*/*z* 192 (M^+). Anal. ($C_6H_3F_3N_2O_2$) C, H, N.

2-Phenylpyrimidine-5-carboxylic Acid (63). The title compound was prepared (350 mg, 80%) from ethyl 2-phenylpyrimidine-5-carboxylate in a manner similar to the synthesis of 2,4-bis(trifluoromethyl)pyrimidine-5-carboxylic acid: mp > 220 °C dec; ^{1}H NMR (DMSO-d_6) δ 9.29 (s, 2H), 8.47 (m, 2H), 7.58 (m, 3H); IR (KBr) 3400, 2968, 1693, 1580, 1410, 1294 cm^{-1}; ESMS *m*/*z* 199 ($M - H^+$).

2-Trifluoromethylpyrimidine-5-carbonyl chloride (64): yield 66%; mp 37−39 °C; ^{1}H NMR ($CDCl_3$) δ 9.4; IR 2897, 1724, 1586, 1342, 1203, 1105 cm^{-1}; EIMS *m*/*z* 210 (M^+).

2-Phenylpyrimidine-5-carbonyl Chloride (65). The title compound was prepared from 2-phenylpyrimidine-5-carboxylic acid in a manner similar to the synthesis of 2-chloro-4-methylpyrimidine-5-carbonyl chloride: yield 91%; mp 135 °C; ^{1}H NMR ($CDCl_3$) δ 9.39 (s, 1H), 8.57 (m, 2H), 7.56 (m, 3H); IR (neat) 2875, 1682, 1585, 1410, 1298 cm^{-1}; EIMS *m*/*z* 218 (M^+).

2-Chloro-4-methylpyrimidine-6-carboxylic Acid (70). Starting from ethyl 2-hydroxypyrimidine-6-carboxylate−urea complex (25.0 g, 103 mmol) and $POCl_3$ (238 g, 1.5 mol), ethyl 2-chloro-4-methylpyrimidine-6-carboxylate was obtained as a white solid (5.1 g, 25%, flash chromatography, SiO_2, 15% EtOAc in hexanes): mp 53−55 °C; ^{1}H NMR ($CDCl_3$) δ 7.81 (s, 1H), 4.49 (q, 2H), 2.65 (S, 3H), 1.44 (t, 3H); IR (KBr) 2987, 1743, 1574, 1529, 1263, 905 cm^{-1}; EIMS *m*/*z* 200 (M^+). Starting from ethyl 2-chloro-4-methylpyrimidine-6-carboxylate (5.1 g, 25 mmol) and NaOH (1.0 g, 25 mmol), in 20% THF in water (125 mL) 2-chloro-4-methylpyrimidine-6-carboxylic acid was obtained as a white solid (1.7 g, 84% yield): mp 172−173 °C; ^{1}H NMR (DMSO-d_6) δ 7.95 (s, 1H), 2.58 (s, 3H); IR (KBr) 3230, 2991, 1732, 1577, 1514, 1423, 1275, 1174, 1083 cm^{-1}; EIMS *m*/*z* 173 (M^+).

2-Chloropyrimidine-5-*N*-[3′,5′-bis(trifluoromethyl)-phenyl]carboxamide (80). This compound was prepared in 96% yield (HPLC) pure in a combinatorial well and screened in the whole-cell assay for IC_{50} values.

2-Chloro-4-methylpyrimidine-5-*N*-[3′,5′-bis(trifluoromethyl)phenyl]pyrimidine-5-carboxamide (81). A solution of 2-chloro-4-methylpyrimidine-5-carbonyl chloride (100 mg, 0.53 mmol), 3,5-bis(trifluoromethyl)aniline (121 mg, 0.53 mmol) and Amberlyst A-21 (100 mg) in EtOAc (5.3 mL) was stirred at room temperature for 1 h. The solution was filtered, concentrated, and purified by preparative centrifugal thin-layer chromatography (4 mm SiO_2, 10% EtOAc in hexane) to afford the title compound (170 mg, 84%): mp 156−157 °C; ^{1}H NMR (acetone-d_6) δ 10.34 (bs, 1H), 8.92 (s, 1H), 8.39 (s 2H), 7.23 (s, 1H), 2.72 (s 3H); IR (KBr) 3275, 3080, 1660, 1570, 1535, 1381, 1176, 1136, 893 cm^{-1}; EIMS *m*/*z* 383 (M^+). Anal. ($C_{14}H_8ClF_6N_3O$) C, H, N.

2-Chloro-4-ethylpyrimidine-5-*N*-[3′,5′-bis(trifluoromethyl)phenyl]carboxamide (82): mp 160−161 °C; ^{1}H NMR ($CDCl_3$) δ 8.70 (s, 1H), 8.15 (s, 2H), 7.19 (s, 1H), 3.04 (t, 2H), 1.38 (q, 3H); IR (KBr) 3296, 2984, 1702, 1566, 1380, 1189, 1274, 887; EIMS *m*/*z* 397 (M^+). Anal. ($C_{15}H_{10}ClF_6N_3O$) C, H, N.

2-Chloro-4-*n*-propylpyrimidine-5-*N*-[3′,5′-bis(trifluoromethyl)phenyl]carboxamide (83): mp 152−153 °C; ^{1}H NMR ($CDCl_3$) δ 8.71 (s, 1H), 8.15 (s, 2H), 7.93 (s, 1H), 7.73 (s, 1H), 2.98 (m, 2H), 1.83 (m, 2H), 1.0 (m, 3H); IR (KBr) 3279, 2969, 1662, 1569, 1529, 1380, 1275, 1179. 1135 cm^{-1}; EIMS *m*/*z* 411 (M^+). Anal. ($C_{16}H_{12}ClF_6N_3O$) C, H, N.

2-Chloro-4-pentafluoroethylpyrimidine-5-*N*-[3′,5′-bis-(trifluoromethyl)phenyl]carboxamide (84). This compound was prepared in 92% yield (HPLC) pure in a combinatorial well and screened in the whole-cell assay for IC_{50} values: EIMS *m*/*z* 487 (M^+).

2,4-Dichloropyrimidine-5-*N*-[3′,5′-bis(trifluoromethyl)-phenyl]carboxamide (85): mp 104−105 °C; ^{1}H NMR (acetone-d_6) δ 10.47 (bs, 1H), 9.01 (s 1H), 8.36 (s, 2H), 7.74 (s 1H); IR (KBr) 3101, 1672, 1562, 1381, 1279, 1134, 887 cm^{-1}; EIMS *m*/*z* 403 (M^+). Anal. ($C_{13}H_5Cl_2F_9N_3O$) C, H, N.

2-Chloro-4-phenylpyrimidine-5-*N*-[3′,5′-bis(trifluoromethyl)phenyl]carboxamide (86): mp 154−155 °C; ^{1}H NMR (DMSO-d_6) δ 11.40 (s, 1H), 9.11 (s, 1H), 8.21 (s, 2H), 7.5−7.9 (m, 5H); EIMS *m*/*z* 445 (M^+). Anal. ($C_{19}H_8ClF_6N_3O$) C, H, N.

2-Fluoro-4-trifluoromethylpyrimidine-5-*N*-[3′,5′-bis-(trifluoromethyl)phenyl]carboxamide (88): mp 133−135 °C; ^{1}H NMR ($CDCl_3$) δ 11.58 (s, 1H), 9.54 (s, 1H), 8.31 (s, 2H), 7.94 (s, 1H); IR (KBr) 3296, 2995, 1674, 1585, 1281, 1130, 950 cm^{-1}; EIMS *m*/*z* 421 (M^+). Anal. ($C_{14}H_5F_{10}N_3O$) C, H, N.

2-Methyl-4-trifluoromethylpyrimidine-5-*N*-[3′,5′-bis-(trifluoromethyl)phenyl]carboxamide (89): mp 147−148 °C; ^{1}H NMR ($CDCl_3$) δ 8.94 (s, 1H), 7.85 (s, 1H), 7.82 (s, 2H), 6.40 (bs, 1H); IR (KBr) 3267, 3082, 1655, 1560, 1284, 1136, 899 cm^{-1}. Anal. ($C_{15}H_8F_6N_3O$) C, H, N.

2-Cyano-4-trifluoromethylpyrimidine-5-*N*-[3′,5′-bis(trifluoromethyl)phenyl]carboxamide (91): mp 146−147 °C; ^{1}H NMR (acetone-d_6) δ 10.69 (bs, 1H), 9.71 (s, 1H), 8.37 (s, 2H), 7.89 (2, 1H); IR (KBr) 3271, 2997, 1672, 1572, 1471, 1140, 893 cm^{-1}; EIMS *m*/*z* 428 (M^+). Anal. ($C_{15}H_5F_9N_4O$) C, H, N.

4-Chloro-2-trifluoromethylpyrimidine-5-*N*-[3′,5′-bis-(trifluoromethyl)phenyl]carboxamide (92): mp 165−166 °C; ^{1}H NMR ($CDCl_3$) δ 10.53 (s, 1H), 9.37 (s, 1H), 8.41 (s, 2H), 7.85 (s, 1H); EIMS *m*/*z* 437 (M^+). Anal. ($C_{14}H_5ClF_9N_3O$) C, H, N.

2-Trifluoromethylpyrimidine-5-*N*-[3′,5′-bis(trifluoromethyl)phenyl]carboxamide (93): mp 154 °C; ^{1}H NMR ($CDCl_3$) δ 9.41 (s, 2H), 8.41 (2, 1H), 8.21 (s, 2H), 7.76 (s, 1H); 2981, 1655, 1592, 1301, 1211, 1011 cm^{-1}. Anal. ($C_{13}H_6F_9N_3O$) C, H, N.

2-Phenylpyrimidine-5-*N*-[3′,5′-bis(trifluoromethyl)phenyl]carboxamide (94): mp 248−250 °C; ^{1}H NMR (DMSO-d_6) δ 11.16 (s, 1H), 9.41 (s, 2H), 8.5 (m, 4H), 7.89 (s, 1H), 7.61 (m, 3H); EIMS *m*/*z* 411 (M^+). Anal. ($C_{19}H_{11}F_6N_3O$) C, H, N.

4-Methyl-2-trifluoromethylpyrimidine-5-*N*-[3′,5′-bis(trifluoromethyl)phenyl]carboxamide (95): mp 152−153 °C; ^{1}H NMR ($CDCl_3$) δ 9.39 (s, 2H), 8.99 (s, 1H), 8.34 (bs, 1H), 8.18 (s, 1H), 2.96 (s, 3H); IR (KBr) 3256, 3055, 1705, 1660, 1290, 1153, 1122 cm^{-1}; EIMS *m*/*z* 417 (M^+). Anal. ($C_{15}H_8F_6N_3O$) C, H, N.

2-Methyl-4-trifluoromethylpyrimidine-5-*N*-[3′,5′-bis(trifluoromethyl)phenyl]carboxamide (96): mp 147−148 °C; ^{1}H NMR ($CDCl_3$) δ 9.38 (s, 2H), 8.98 (s, 1H), 8.34 (bs, 1H), 8.17 (s, 1H), 2.94 (s, 3H); IR (KBr) 3244, 3021, 1701, 1665, 1294, 1153, 1123 cm^{-1}; EIMS *m*/*z* 417 (M^+). Anal. ($C_{15}H_8F_6N_3O$) C, H, N.

2-Chloro-4-trifluoromethylpyrimidine-6-*N*-[3′,5′-bis(trifluoromethyl)phenyl]carboxamide (97). This compound was prepared in 88% pure (HPLC) in a combinatorial well and screened in the whole-cell assay for IC_{50} values: ESMS *m*/*z* 437 (M^+).

2-Chloro-4-methylpyrimidine-6-*N*-[3′,5′-bis(trifluoromethyl)phenyl]carboxamide (98). This compound was prepared in 85% pure (HPLC) in a combinatorial well and screened in the whole-cell assay for IC_{50} values: ESMS *m*/*z* 384 (M^+).

2,4-Dichloropyrimidine-6-*N*-[3′,5′-bis(trifluoromethyl)phenyl]carboxamide (99): mp 168−169 °C; ^{1}H NMR ($CDCl_3$) δ 10.78 (s, 1H), 8.49 (s, 1H), 7.99 (s, 2H), 7.60 (s, 1H); IR (KBr) 3001, 2867, 1735, 1535, 1279, 1134 cm^{-1}; EIMS *m*/*z* 403 (M^+). Anal. ($C_{13}H_5Cl_2F_6N_3O$) C, H, N.

3-Chloro-5-trifluoromethylpyridazine-6-*N*-[3′,5′-bis(trifluoromethyl)phenyl]carboxamide (101): mp 122−123 °C; ^{1}H NMR ($CDCl_3$) δ 10.12 (s, 1H), 8.28 (s, 2H), 8.07 (s, 1H), 7.73 (s, 1H); IR (KBr) 2995, 1702, 1601, 1450, 1280, 1120 cm^{-1}; EIMS *m*/*z* 437 (M^+). Anal. ($C_{14}H_5ClF_9N_3O$) C, H, N.

3-Chloropyridazine-6-*N*-[3′,5′-bis(trifluoromethyl)phenyl]carboxamide (102): mp 116−117 °C; ^{1}H NMR ($CDCl_3$) δ 10.19 (s, 1H), 8.40 (d, J = 9 Hz, 1H), 8.30 (s, 2H), 7.82 (d, J = 9 Hz, 1H), 7.72 (s, 1H); IR (KBr) 2999, 1711, 1611, 1440, 1289, 1123 cm^{-1}; EIMS *m*/*z* 369 (M^+). Anal. ($C_{13}H_5ClF_6N_3O$) C, H, N.

3-Chloropyrazine-6-*N*-[3′,5′-bis(trifluoromethyl)phenyl]carboxamide (103): mp 101−102 °C; ^{1}H NMR ($CDCl_3$) δ 9.76 (s, 1H), 9.30 (s, 1H), 8.62 (s, 1H), 8.28 (s, 2H), 7.70 (s, 1H); IR (KBr) 3011, 1691, 1621, 1430, 1279, 1103 cm^{-1}; EIMS *m*/*z* 369 (M^+). Anal. ($C_{13}H_5ClF_6N_3O$) C, H, N.

Synthesis of a Library. The synthesis involved adding 1.05 equiv of each acid chloride in ethyl acetate (100 μL) to 80 wells of a microtiter plate with each well containing 0.02 mmol of one amine or aniline in EtOAc (100 μL) and Amberlyst 21 (100 mg), which is a basic ion-exchange resin. The plates were covered and sonicated for 20 min. Water (50 μL) was added to the wells and sonicated for an additional 10 min. Ethyl acetate (1 mL) was added to each well and sonicated for 5 min. The products were easily isolated by transferring the organic layer (1 mL) of each well into tared test tubes followed by concentration of the solutions. The residue in each test tube was dried under vacuum. The purity of each compound within a library (80 compounds constituted one library) was determined using TLC. By TLC, all of the compounds were clean with no residual starting materials present. Fifteen to twenty compounds from each library were randomly selected and analyzed by HPLC and EIMS for purity and structural verification, respectively. Molecular weights of the randomly selected compounds were confirmed, and all compounds had >90% purity by HPLC.

NF-κB Assay. Human Jurkat T-cells stably transfected with an NF-kB binding site (from the MHC promoter) fused to a minimal SV-40 promoter driving luciferase were used in these experiments.[32] 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/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 mg/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 phorbol 12-myristate-13-acetate (PMA) and 1 μg/mL 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 the 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-kB 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.[29] In addition, the concentrations of PMA and PHA were 5 ng/mL and 1 μg/mL, respectively.

Caco-2 Permeability Assay. Caco-2 cells, strain HTB-37, were acquired from ATCC and cultured in MEM media with L-glutamine and Earles balanced salts (Hyclone, Logan, UT).[33] Additional supplements included 20% heat-inactivated fetal bovine serum (Hyclone, Logan, UT), 1 mM sodium pyruvate, 0.1 mM nonessential amino acids (GIBCO, Grand Island, NY), and 50 U/mL penicillin/ 50 μg/mL streptomycin (Cellgro). Cells were routinely passaged when 80% confluent as a 1:5 split using 0.05% trypsin (Hyclone, Logan, UT). The 3-day Caco-2 culture was performed using the Biocoat Intestinal Epithelium Differentiation Environment Kit (#30057 from Becton Dickinson, Bedford, MA) adhering to the manufacturers' protocol. Briefly, cells were passaged and then resuspended at a concentration of 0.4×10^6 cells/mL in basal seeding media containing MITO-serum extender. Cells (0.5 mL) were added to the precoated chamber inserts (200 000 cells/insert) and cultured for 24 h. The media was then replaced with Entero-Stim media containing MITO-serum extender and cultured for an additional 48 h. The compound permeability assay was performed at ambient room temperature and atmosphere. Inserts were washed twice with PBS (+Mg, +Ca) by transferring the chamber insert to a well (basal chamber) containing fresh PBS and washing the insert (apical chamber) with PBS. The insert was placed in a well containing 1 mL of fresh PBS, 280 μL of compound solution (100 μM in PBS, 0.5% DMSO) was added to the insert (apical chamber), and the plate was incubated on a shaking table (90 rpm). After 45 min, the insert was removed and the basal chamber PBS retained for analysis. Assay viability was confirmed in parallel using [^{3}H]PEG4000, [^{3}H]mannitol and [^{14}C]caffeine (NEN, Boston MA). Typical values for radiolabeled controls were 20×10^{-7}, 55×10^{-7}, and 320×10^{-7} cm/s, respectively. The compounds were detected using HPLC on a Rainin Dynamax system equipped with a YMC C-18 column using acetonitrile containing 35% water and 0.1% CF_3COOH

Acknowledgment. We thank Mr. Mark Rosen and Ms. Palka Patel for synthesis of some of the intermediates and analysis libraries using HPLC, respectively.

Supporting Information Available: List of amines that were used in the combinatorial library. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM0001626