Evolution of the Thienopyridine Class of Inhibitors of IκB Kinase-β: Part I: Hit-to-Lead Strategies

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Received October 28, 2005

High-throughput screening is routinely employed as a method for the identification of novel hit structures. Large numbers of active compounds are typically procured in this way and must undergo a rigorous validation process. This process is described in detail for a collection of screening hits identified as inhibitors of IκB kinase-β (IKKβ), a key regulatory enzyme in the nuclear factor-κB (NF-κB) pathway. From these studies, a promising hit series was selected. Subsequent lead generation activities included the development of a pharmacophore hypothesis and structure−activity relationship (SAR) for the hit series. This led to the exploration of related scaffolds offering additional opportunities, and the various structural classes were comparatively evaluated for enzyme inhibition, selectivity, and drug-like properties. A novel lead series of thienopyridines was thereby established, and this series advanced into lead optimization for further development.

Introduction

Lead discovery tactics in the pharmaceutical industry have undergone substantial changes and continue to evolve. The convergence of significant advancements in the areas of molecular biology, combinatorial chemistry, and assay automation has led to the utilization of high-throughput screening (HTS) as one mechanism for the identification of small molecule lead structures, and most pharmaceutical companies have implemented such a strategy as a component of their lead discovery efforts. Increasing numbers of molecular targets coupled with the rapid expansion of screening collections, often enriched with compounds focused toward core target areas, have amplified the potential for rapid identification of biologically active compounds, and the number of hits identified from a typical screening campaign can be sizable. Therefore, filtering out poor quality starting points for lead optimization (LO) has become an essential part of the discovery process. This has necessitated the establishment of hit-to-lead (HtL) or lead identification (LI) teams charged with the task of validating active compounds and evaluating hit classes on the basis of both target independent and target dependent criteria.[1] The goal is to identify the most promising lead candidates. The importance of selecting high quality lead structures cannot be overstated.[2] However, most drug discovery case histories do not detail the associated HtL activities.[1a] In this article, we describe our HtL efforts to identify a promising lead series of IKKβ inhibitors. The process is divided into two stages: a hit validation stage, engaging a filtering process and providing a group of validated hits, and a lead generation stage exemplified with a thiophenecarboxamide hit series. Various objectives, including pharmacophore identification, SAR studies, and the evaluation of related scaffolds, are discussed.

IKKβ has been identified as a potential target for the treatment of inflammatory and autoimmune diseases because of its critical role in the NF-κB signaling pathway.[3] Heterodimeric NF-κB transcription factors are composed of various combinations of monomers from the Rel family of DNA-binding proteins, with the p65:p50 heterodimer as the most abundant form in most cell types.[4] In unstimulated cells, NF-κB is retained in the cytosol as a complex with inhibitor of kappa B (IκB), of which there are several variants, the canonical family member being IκBα. Upon activation by pro-inflammatory stimuli, such as IL-1, TNF-α, or LPS, two specific serine residues (Ser 32/36) of IκBα are phosphorylated. This triggers ubiquitination and the subsequent degradation of IκBα, releasing NF-κB to translocate into the nucleus leading to the expression of a battery of pro-inflammatory genes, including chemokines such as IL-8, MCP-1, and RANTES, cytokines such as TNF-α, IL-1, and IL-6, and cell adhesion molecules such as ICAM, VCAM, and E-selectin. The expression of pro-inflammatory enzymes is also induced, including COX-2, iNOS, MMP-1, MMP-9, and MMP-13.[5] Phosphorylation of IκBα is accomplished by the IKK complex, which is made up of three main subunits: IKKα (IKK1), IKKβ (IKK2), and IKKγ (NEMO: NF-κB essential modulator).[6] IKKα and IKKβ are the two catalytic subunits, and both are capable of phosphorylating IκBα. A dominant role for the IKKβ unit has, however, been described.[7] By mutating the essential serine residues in the kinase activation loop of IKKα or IKKβ, it was found that in the case of catalytically incompetent IKKβ activation of the IKK complex was prevented. Activation was not, however, prevented when the IKKα subunit was rendered catalytically inactive. These results have been further substantiated by studies using embryonic fibroblasts from mice with targeted disruption of either the IKKα[8] or the IKKβ[9] gene. Therefore, IKKβ has been shown to be the critical target for inhibition of the pathway.

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10.1021/jm0510979 CCC: $33.50
Published on Web 04/20/2006

Table 1. Generic Criteria for Hit Validation

1. selectivity (counterscreen/secondary screen)
2. not a promiscuous inhibitor
3. structural identity confirmed
4. purity and stability established
5. structure free of reactive/undesirable chemotypes
6. activity confirmed on solids
7. structure reasonably drug-like
8. favorable intellectual property (ip) position
9. tractable synthetic route established
10. support for interaction with the molecular target
11. logical structure−activity relationship (SAR)

Results and Discussion

HTS Assay and Hit Validation Process. The corporate compound collection was screened in a scintillation proximity assay (SPA) against *N*-terminally Flag epitope-tagged IKKβ. An IκBα GST fusion protein served as the substrate. Anti-GST coated SPA beads were used to isolate the substrate, and the ^{33}P-labeled reaction products were quantified. Dose response data on hits showing reproducible activity from the screen was generated in a filtration assay using the same reagents, whereby the ^{33}P-labeled products were isolated by filtration and quantified.

Dose-responsive hits were then subjected to a hit-to-lead filtering and prioritization process. Table 1 provides generic criteria for the validation of the actives from the dose response. Counterscreen selectivity is a primary filter and is generally run when the dose response data is acquired. A counterscreen typically employs a related protein expected to have overlapping actives and potentially undesirable therapeutic contributions. The data provides biological refinement to the hit set. Secondary screens can also be employed during the dose response to prioritize hits. For the IKKβ project, no counterscreens were used for early filtering, but secondary screening using an in-house kinase panel was implemented. Historical data provided additional selectivity information as well as a perspective on potential promiscuity[10] of the inhibitor scaffolds, and examples that demonstrated activity against several target classes were eliminated. Hits were then structurally confirmed and evaluated for purity and stability by LCMS and NMR. Compounds with reactive functionality or other undesirable chemotypes were removed.[11] The remaining structures were clustered into related compound classes, and the activity of these inhibitor classes as well as singles was confirmed on selected samples of the available solids. Examples of actives from the combinatorial libraries were resynthesized for confirmation. Additional criteria included drug-like molecular properties,[12] novelty, and synthetic accessibility, preferably incorporating methods amenable to parallel synthesis.

An assessment of the mechanistic aspects of target−inhibitor interaction serves to eliminate false positives, such as reactive species, compounds that may perturb the assay, or nonspecific, property-based inhibitors. Initially, the goal is to identify a basic mechanism of inhibition (i.e., competition with an endogenous ligand) and establish reversibility. A variety of tools are available for early analysis. For kinase programs, competition experiments with ATP are typically utilized, and this was the case for the current project (Supporting Information). If covalent modification is suspected, these methods can be modified to incorporate the preincubation of the inhibitor and protein followed by an evaluation of the ability of ATP to reverse inhibition in a concentration-dependent manner. Impurities that can covalently modify the target must also be considered because their presence in very low concentration can provide significant inhibition.

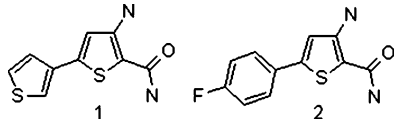
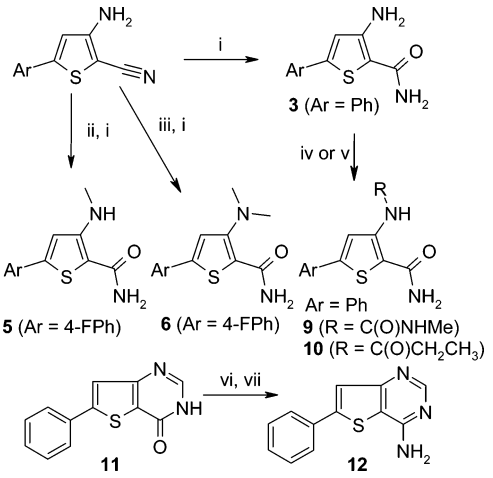


Figure 1. Screening data. (1) $IC_{50} = 4.5\ \mu M$; (2) $IC_{50} = 14.5\ \mu M$.

Scheme 1[a]



[a] (i) H_2SO_4 (concd); (ii) iodomethane, DMF, dichloromethane; (iii) iodomethane, DMF; (iv) methylisocyanate, THF; (v) propionyl chloride, THF; (vi) oxalyl chloride, DMF, dichloromethane; (vii) NH_3, dioxane.

Finally, hits should demonstrate a consistent SAR if related analogues are available. At the hit validation stage, this serves to corroborate structure-based activity. In the case of the IKK hit set, for compounds already associated with a series or a library, existing analogues were sufficient in this regard. For unique hits, a search of internal and external databases for related analogues was carried out. Alternative scaffolds with related functionality were also evaluated.

Lead Generation. From these efforts, a group of validated hits was identified, and the most promising chemotypes were chosen for further profiling. It is important to identify multiple candidates from diverse chemical classes such that at the conclusion of the HtL efforts at least two to three architecturally distinct lead series will be available for the LO team.[1b] One series which we pursued is represented by thiophenecarboxamides **1** and **2** (Figure 1).[13] The lead generation process involved synthetic efforts to rapidly develop SARs and explore the potential of this structural class as a lead series. We established key features of the pharmacophore and developed a pharmacophore hypothesis, which allowed us to expand to related series (scaffold hopping) providing additional opportunities. We also identified areas we felt should be exploitable to probe for additional binding or the incorporation of solublizing groups. An empirical assessment of drug-like properties as well as a more extensive evaluation of selectivity was carried out. Our goal was to provide the LO team with a reasonably potent series demonstrating a logical SAR consistent with structural information, showing good selectivity, and having appropriate physicochemical properties and an acceptable in vitro ADME profile. A clear IP position was also expected.

Thiophene Series. Chemistry. Aminothiophenecarboxamides, which were not commercially available, were prepared by hydrolysis of the available nitriles (Scheme 1). Methylated derivatives **5** and **6** were acquired by treatment of a thiophene nitrile with methyl iodide, followed by hydrolysis. Compounds **9** and **10** were derived from **3** by treatment with the corresponding isocyanate or acid chloride. Thienopyrimidine **12** was

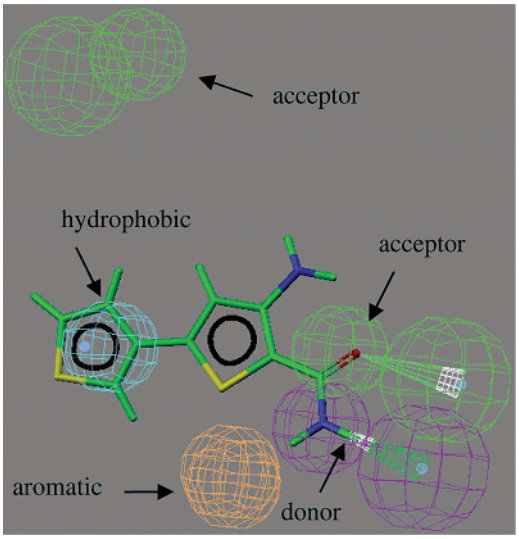


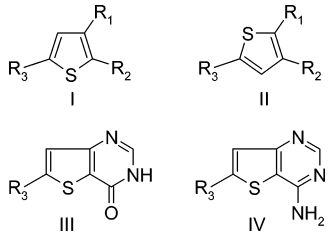
Figure 2. Pharmacophore model with inhibitor **1**.

synthesized from available thienopyrimidone **11** by the formation of the chloride and displacement with ammonia (Scheme 1).

Thiophene Series. Identification of the Pharmacophore and SAR Studies. Early efforts were directed toward the identification of the pharmacophore by synthesis of single point analogues and the generation of a pharmacophore hypothesis[14] using a subset of 16 hits from structural classes with known ATP-like binding characteristics.[15] Figure 2 depicts the pharmacophore hypothesis shown with inhibitor **1** fit to match three of the suggested binding interactions. As can be seen from the figure, the amide of inhibitor **1** was predicted to contribute to polar donor−acceptor interactions, whereas the additional thiophene ring attached to the core structure was expected to occupy a hydrophobic space. An additional hydrogen bond acceptor and an aromatic component were identified from the consensus hypothesis but were not utilized by the thiophene inhibitors.

Early SAR studies for this series were used to probe the suggested binding components readily accessible with this scaffold (Table 2). The importance of the amide was suggested by the observed loss of activity upon replacement with the ester (**4**). It was anticipated that the 3-amino substituent may be important in restricting the conformation of the adjacent amide through an intramolecular hydrogen bond with the carbonyl oxygen. Indeed, monomethylation of the primary amine (**5**) was not substantially detrimental, but the dimethylated derivative **6** showed no inhibition. Removal of the amine was also not acceptable (**7**). Monosubstitution with groups other than methyl, for example, ureas or amides, was also tolerated (**9**, **10**). To further establish the importance of the amide conformation and the proposed contribution of the amino group in maintaining this conformation, examples **11** and **12** were used to rigidify the polar groups in a six-membered heterocyclic ring, providing inhibitors with opposing donor−acceptor components. The potency of **12** in contrast to the lack of activity for **11** was consistent with the hypothesis. The replacement of the halogen on the phenyl ring with an electron donating methoxy group (**13**) afforded no significant effects on potency. Transposition of the amine and amide functionality provided isomeric **14**. This analogue showed the same level of inhibition as its regioisomer **3**, supporting the expectation that the core was not a critical component of the pharmacophore and suggesting that other scaffolds could be used for SAR studies.

Table 2. SAR for Thiophene Series



compd no.	core	R1	R2	R3	IC_{50} (μM)
1	I	NH_2	$CONH_2$	thiophen-3-yl	1.3
2	I	NH_2	$CONH_2$	4-FPh	2.6
3	I	NH_2	$CONH_2$	Ph	1.1
4	I	NH_2	CO_2CH_3	Ph	>30
5	I	$NHCH_3$	$CONH_2$	4-FPh	5.0
6	I	$N(CH_3)_2$	$CONH_2$	4-FPh	>23.5
7	I	H	$CONH_2$	4-ClPh	>50
8	I	NH_2	$CONH_2$	4-ClPh	6.6
9	I	$NHC(O)NHCH_3$	$CONH_2$	Ph	1.6
10	I	$NHC(O)CH_2CH_3$	$CONH_2$	Ph	10.3
11	III			Ph	>15.5
12	IV			Ph	0.97
13	I	NH_2	$CONH_2$	4-OCH_3Ph	2.9
14	II	NH_2	$CONH_2$	Ph	1.9

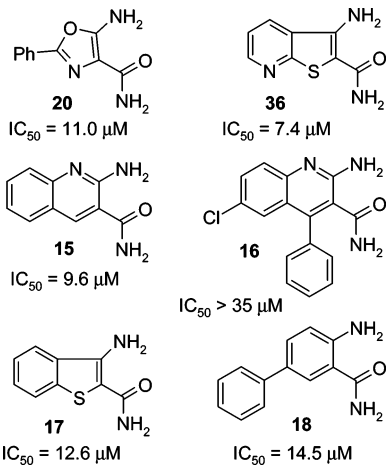
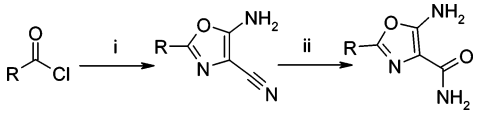


Figure 3. Investigation of additional scaffolds.

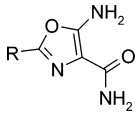
Related Structural Classes. Synthetic methods for the preparation of more advanced thiophene derivatives were somewhat challenging, and therefore, the scaffold was not amenable to rapid analogue generation and provided limited opportunity for expansion into unexplored space. Therefore, other more opportunistic scaffolds were examined (Figure 3). Extension of the initial hit class into alternative structural classes (scaffold hopping) offered various opportunities, including improved synthetic accessibility, the capacity to explore otherwise inaccessible areas, the potential improvement to physicochemical properties, and a more favorable IP position. We used a series of oxazoles, which were readily accessible synthetically (**20**), to rapidly explore the hydrophobic component of the proposed pharmacophore, and a series of thienopyridine inhibitors (**36**), also conveniently synthesized, provided access to additional space. Quinoline analogues **15** and **16** were acquired by direct hydrolysis of the commercially available nitriles and together were used to probe the additional aromatic interaction suggested by the pharmacophore hypothesis. A comparison of phenyl-substituted quinoline **16** with unsubstituted analogue **15** suggested that the aromatic component (or the halogen) had a negative impact on binding. Other core

Scheme 2[a]



[a] (i) aminomalonobisnitrile tosylate, *N*-methyylpyrrolidinone; (ii) H_2SO_4.

Table 3. SAR for Oxazole Series



compd no.	R	IC_{50} (μM)
19	thiophen-3-yl	4.7
20	Ph	11.0
21	4-ClPh	13.6
22	4-CH_3OPh	17.7
23	4-CH_3Ph	12.7
24	4-CF_3Ph	8.0
25	4-*t*-BuPh	6.3
26	4-$NH_2C(O)$Ph	25.8
27	2-ClPh	6.6
28	2-CH_3OPh	36.2
29	2-CH_3Ph	6.3
30	3-ClPh	10.6
31	3-CH_3OPh	13.9
32	2,4,6-triClPh	>39.5
33	cyclohexyl	>32.5
34	benzthiophen-2-yl	4.6
35	pyridin-3-yl	35.7

replacements included benzothiophene **17** and substituted anthranilamides from which **18** is an example.

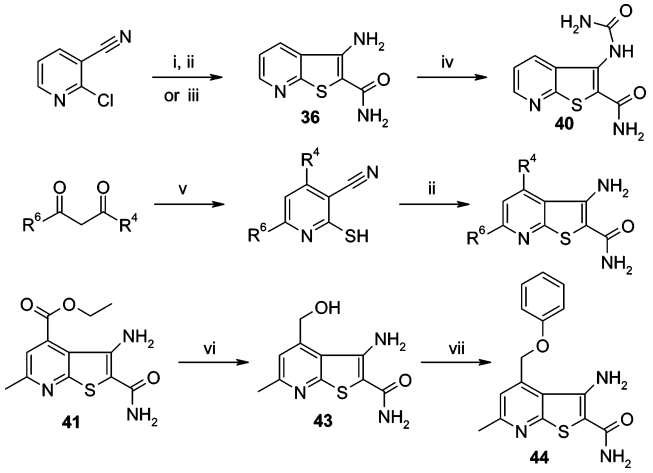
Oxazole Series. Chemistry. The synthesis of the oxazoles is shown in Scheme 2. These adducts were readily acquired from commercially available acid chlorides using a one-pot procedure that directly furnished the aminooxazolenitriles.[16] Facile hydrolysis provided the final products.

Oxazole Series. SAR Studies. Table 3 shows representative results from the oxazole series. Analogues **19**−**22** are direct comparisons to thiophenes **1**, **3**, **8**, and **13** and demonstrate a 2- to 10-fold reduction in potency. Substitution at the para position on the aromatic ring was generally tolerated including the large *tert*-butyl moiety (**25**). However, trisubstitution (**32**) or saturation of the phenyl group (**33**) induced a significant loss in potency. Monosubstitution at the ortho position with a methoxy group (**28**) also had a negative impact. These analogues define some constraints for this pocket and may suggest the importance of coplanarity for the two rings. The larger benzthiophene **34** was tolerated, but the corresponding pyridine **35** was less acceptable in the proposed hydrophobic space, as was *p*-benzamide **26**.

Thienopyridine Series. Chemistry. The methods for the preparation of the thienopyridines are shown in Scheme 3. Commercially available 2-chloro-3-cyanopyridines were treated with 2-mercaptoacetamide or thiourea followed by 2-bromoacetamide to give the desired products.[17] Alternatively, 2-mercapto-3-cyanopyridines could be generated from the appropriate 1,4 diketones and 2-cyanothioacetamide,[18] then carried forward in the same manner. Primary urea **40** was acquired by the reaction of **36** with trimethylsilylisocyanate. Alcohol **43** was prepared by the reduction of ester **41** with lithium borohydride and was subsequently utilized for the synthesis of **44**−**47** via Mitsunobu chemistry.

Thienopyridine Series. SAR Studies. The thienopyridine series was found to be a useful replacement for the arylthiophenes. Ease of synthesis and the opportunity to explore

Scheme 3[a]



[a] (i) Thiourea, ethanol; (ii) sodium methoxide/methanol or NaH/THF, $BrCH_2CONH_2$; (iii) sodium methoxide/methanol or NaH/DMF, $HSCH_2C(O)NH_2$; (iv) trimethylsilylisocyanate, dichloromethane/DMF; (v) triethylamine, $NCCH_2C(S)NH_2$, ethanol; (vi) $LiBH_4$, THF/methanol; (vii) diisopropyl azodicarboxylate, Ph_3P, PhOH, THF.

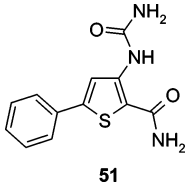
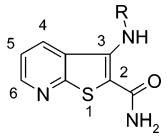


Figure 4. IKKβ inhibitor;[13a] IC_{50} 25 nM.

additional space, including the remaining hydrogen bonding component suggested by the pharmacophore hypothesis, were key advantages. The data in Table 4 shows that basic scaffold **36** could replace phenylthiophene **3**. Substitution at positions 4 and 6 was preferred compared to a 5-substituent (**37**, **38**), and trisubstitution (**39)** was not tolerated. Interestingly, monosubstitution of the primary amine with a primary urea was detrimental (**40**). This was clearly a point of divergence from the thiophene series, which has been shown to acquire a significant boost in potency from the incorporation of a primary urea.[13a,g] Our rationale for this unexpected result is derived from the quantum mechanical conformational analysis of structures **40** and **51** (Figure 4) using density functional theory (DFT). The results from this analysis indicated that although the urea moiety in **51** is planar with respect to the aromatic core, such is not the case for **40**, where the urea is moved out of plane by approximately 30°. It was expected that this potentially disrupts a key hydrogen bond between the urea and the protein reported for **51**.[13a] Amide **47**, generated to explore the additional hydrogen-bonding component of the pharmacophore hypothesis, showed a modest improvement over related analogues **44**−**46**, suggesting that a polar interaction at this position could be beneficial. Larger substituents at position 6 were acceptable (**48**−**50**), and notably, the large *tert*-butyl group provided nearly a 5-fold improvement in potency compared to that of the related methyl analogue (**50** vs **41**). Overall, the results indicate that both the 4 and 6 positions demonstrate tolerance to larger substituents, suggesting that these areas were amenable to exploration for additional binding interactions.

Profiling Data. Drug candidates must achieve acceptable pharmacokinetic and safety profiles in addition to pharmacological activity (potency and selectivity). Historical methods for lead identification typically produced structures with favorable drug-like properties. Current tactics, however, have resulted in

Table 4. SAR for Thienopyridine Series



compd no.	4	5	6	R	IC_{50} (μM)
36	H	H	H	H	7.4
37	H	CH_3	H	H	19.9
38	CH_3	H	CH_3	H	2.2
39	CH_3	Cl	CH_3	H	>27.5
40	H	H	H	$C(O)NH_2$	>50
41	$C(O)OCH_2CH_3$	H	CH_3	H	15.1
42	C(O)OH	H	CH_3	H	>49
43	CH_2OH	H	CH_3	H	12.7
44	CH_2OPh	H	CH_3	H	8.2
45	CH_2O(4-NCPh)	H	CH_3	H	5.6
46	CH_2O(4-CH_3OPh)	H	CH_3	H	13.0
47	CH_2O(4-$NH_2C(O)$Ph)	H	CH_3	H	1.4
48	CH_3	H	$CH_2CH(CH_3)_2$	H	4.1
49	CH_3	H	$CH_2CH_2CH_2CH_3$	H	2.5
50	$C(O)OCH_2CH_3$	H	$C(CH_3)_3$	H	3.4

Table 5. Compound Profiling

compd no.	IC_{50} (μM) IKKβ	IC_{50} (μM) IKKα	sol. pH 7.4 (μg/mL)	caco-2 A to B 10^{-6} (cm/s)	HLM $t_{1/2}$ (min)	CYP 1A2[a]	CYP 2C9	CYP 2C19	CYP 2D6	CYP 3A4	Alomar blue (% viable) at μM
2	2.6	24	7	19.3	12	12	7	4	>30	2	100% at 50
3	1.1	>30	10	9.3	22	5	26	>30	>30	3.5	100% at 50
20	11.0	>30	32	6.4	68	>30	>30	>30	>30	>30	100% at 50
24	8.0	>30	13	18.9	15	>30	>30	>30	>30	>30	68% at 50 100% at 10
36	7.4	21	32	12.8	146	>30	>30	>30	>30	>30	93% at 50
38	2.2	2.8	24	13.6	78	>30	>30	>30	>30	>30	68% at 50 100% at 10

[a] CYP data reported as IC_{50} (μM) values.

lead series that have suffered from high attrition rates, often due to poor pharmacokinetics or safety issues.[19] It has, therefore, become apparent that selecting for a balance of key properties in early drug discovery, especially during the lead-seeking stage, is highly desirable. Empirically derived profiling data for the three series that emerged from the foregoing hit-to-lead evaluation is shown in Table 5. Two of the inhibitor classes showed some selectivity for IKKβ over IKKα. Isoform selectivity was not, however, a requirement during the lead identification stage. Additional selectivity within the kinase family and against a group of nonkinase targets for at least one example from each series (Supporting Information) revealed that all three classes were generally selective. The compounds were evaluated in HeLa cells, and **2**, **3**, and **38** showed marginal cellular activity (Supporting Information). However, a potency shift between the molecular assay and the cell assay was anticipated for these ATP competitive inhibitors.[20] The oxazoles and thienopyridines exhibited acceptable solubility (>10 μg/mL),[21] and compounds from all three series showed good permeability in the caco-2 model (>7 $\times$ 10^{-6} cm/s).[22] The half-life in human liver microsomes[23] was used to compare metabolic stability, and the thiophenes demonstrated a poorer level of stability compared to that of the other series. None of the compounds were potent CYP inhibitors (IC_{50} < 1 μM),[24] but micromolar activity against at least four isozymes was observed for the thiophene class. Cell toxicity was assessed using the Alomar Blue assay.[25] The oxazoles and thienopyridines showed some effect on viability at high doses.

Conclusion

In summary, a group of hits found to inhibit IKKβ was validated and prioritized to identify a promising series, and a subset of the hits was used to generate a binding hypothesis. The hit series was expanded to additional scaffolds, all of which were utilized for the evaluation of the pharmacophore, and several of the suggested interactions were supported by SAR studies. Profiling of the predominant scaffolds (thienopyridines, thiophenes, and oxazoles) with respect to potency, selectivity, and drug-like properties provided data to prioritize the series. From these efforts, the thienopyridine class of inhibitors was found to demonstrate acceptable metrics, and the overall profile was considered to be more favorable in terms of solubility, microsomal stability, and CYP inhibition (compared to that of the thiophenes), and molecular and cellular potency (with respect to the oxazoles). The synthetic accessibility was an improvement over the thiophene series, and inherent opportunities for expansion were seen as a definite advantage over both thiophenes and oxazoles. The series also had a clearer IP position. Therefore, this inhibitor class appeared to offer the greatest potential for further development and was recommended for LO. The series was later optimized into a potent class of IKKβ inhibitors, and these developments will be reported subsequently.[26]

Experimental Section

General Methods. Melting point determinations are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker UltraShield-400 MHz spectrometer operating at 400 or 100 MHz, respectively,

in solvents as noted. Mass spectra were obtained on Micromass Platform LCZ mass spectrometers using either electrospray positive/negative ionizations or chemical ionization. Elemental analysis was performed by Quantitative Technologies, Whitehouse, NJ. The HPLC system consisted of an Agilent 1100 system (binary pump, diode array detector, and autosampler) with a Zorbax Eclipse XDB-C18 column (4.6 × 150 mm, 5μm, part number 993967-902). Detection was carried out at 254 nm, and integration was performed using Agilent Chemstation software, version A 10.02. Two mobile phases were used with a flow rate of 1 mL/min in both cases. Mobile phase 1 was a gradient from 15:85 A/B to 5:95 A/B in 8 min and held, and mobile phase 2 was a gradient from 15:85 C/D to 5:95 C/D in 7.5 min and held. Solvent A was 0.1% TFA in acetonitrile, B was 0.1% TFA in water, C was methanol, and D was water. All solvents were HPLC grade or better. The reactions were followed by TLC on precoated Uniplate silica gel plates purchased from Analtech. The developed plates were visualized using 254 nm UV illumination or by PMA stain. Flash chromatography was done on Redi Sep prepacked disposable columns using the Isco Combiflash. Reactions were carried out under an atmosphere of N_2. THF and DMF solvents were anhydrous and purchased from Aldrich. Ethanol was purchased from Aaper Alcohol and Chemicals. All other solvents were purchased from EM Science. Screening compounds **1**, **4**, **7**, **8**, **11**, **13**, **14**, and **38** were purchased from one of the following suppliers: Maybridge, Scientific Exchange, Hans Knoell Institute, or Chembridge chemical suppliers.

General Procedure for Hydrolysis of Thiophene Amino Nitriles 2−3 and 5−6. A 2-cyano-3-amino-arylthiophene was added to concentrated H_2SO_4 (1 mmol/mL). The resulting mixture was stirred at room temperature for 0.5−3 h, poured over ice water, and then neutralized with saturated aqueous $NaHCO_3$. The product was isolated by filtration or extraction with EtOAc, in which case, the combined extracts were washed with water and brine, dried with Na_2SO_4, and evaporated. If necessary, the product was purified by recrystallization.

3-Amino-5-(4-fluoro-phenyl)-thiophene-2-carboxylic Acid Amide (2). The title compound was purified by recrystallization from EtOAc. Yield, 53%; mp 220−221 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.62 (2H, m), 7.26 (2H, m), 6.90 (1H, s), 6.93 (2H, br), 6.46 (2H, br). ESMS *m*/*z*: 237 (M + H). Anal. ($C_{11}H_9FN_2OS$) C, H, N.

3-Amino-5-phenyl-thiophene-2-carboxylic Acid Amide (3). The title compound was purified by recrystallization from EtOAc. Yield, 32%; mp 210−211 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.59 (2H, d, J = 7.5 Hz), 7.44 (2H, t, J = 7.5 Hz), 7.36 (1H, t, J = 7.5 Hz), 6.94 (1H, s), 6.91 (2H, br), 6.47 (2H, br). ESMS *m*/*z*: 219 (M + H). Anal. ($C_{11}H_{10}N_2OS$) C, H, N.

5-(4-Fluoro-phenyl)-3-methylamino-thiophene-2-carboxylic Acid Amide (5). Step 1. 2-Cyano-3-amino-5-(4-fluoro-phenyl)-thiophene (218 mg, 1 mmol) was dissolved in a solution of 1 mL of dichloromethane and 0.5 mL of DMF, and methyl iodide (0.2 mL, 3.2 mmol) was added. The reaction vessel was sealed, and the mixture was stirred overnight at 40 °C, after which time, it was diluted with saturated aqueous $NaHCO_3$ and extracted 2× with EtOAc. The combined extracts were washed with brine, dried with Na_2SO_4, and evaporated. The resulting product was purified by flash chromatography using 4:1 hexane/EtOAc providing 90 mg of pure product (38% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.54 (2H, m), 7.11 (2H, m), 6.70 (1H, s), 4.50 (1H, br), 3.06 (3H, s). ESMS *m*/*z*: 233 (M + H).

Step 2. The title compound was prepared according to the general procedure. It was purified by recrystallization from EtOAc/hexane. Yield, 68%; mp 198−200 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.71 (2H, m), 7.28 (2H, m), 7.23 (1H, q, J = 5.1 Hz), 7.19 (1H, s), 6.90 (2H, br), 2.90 (3H, d, J = 5.1 Hz). ESMS *m*/*z*: 251 (M + H). Anal. ($C_{12}H_{11}FN_2OS$) C, H, N.

3-Dimethylamino-5-(4-fluoro-phenyl)-thiophene-2-carboxylic Acid Amide (6). Step 1. 2-Cyano-3-amino-5-(4-fluoro-phenyl)-thiophene (100 mg, 0.46 mmol) was dissolved in 1 mL of DMF, and methyl iodide (0.2 mL, 3.2 mmol) was added. The reaction vessel was sealed, and the mixture was stirred at 40 °C for 5 days, after which time, it was diluted with saturated aqueous $NaHCO_3$ and extracted 2× with EtOAc. The combined extracts were washed with brine, dried with Na_2SO_4, and evaporated. The resulting product was purified by flash chromatography using 4:1 hexane/EtOAc providing 44 mg of pure product (39% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.52 (2H, m), 7.09 (2H, m), 6.69 (1H, s), 3.21 (6H, s). ESMS *m*/*z*: 247 (M + H).

Step 2. The title compound was prepared according to the general procedure. It was purified by trituration with diethyl ether. Yield 65%; mp 193−196 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.55 (1H, br), 7.58 (2H, m), 7.23 (1H, s), 7.10 (2H, m), 5.70 (1H, br), 2.77 (3H, s). ESMS *m*/*z*: 265 (M + H). Anal. ($C_{13}H_{13}FN_2OS$) C, H, N.

3-(3-Methyl-ureido)-5-phenyl-thiophene-2-carboxylic Acid Amide (9). Compound **3** (28 mg, 0.128 mmol) was dissolved in THF (0.5 mL), and excess methyl isocyanate (100 μL) was added. The reaction mixture was allowed to stir at room temperature for 4 h, after which time, it was concentrated. The resulting residue was purified by flash chromatography using 40−100% EtOAc in hexane to provide 18 mg of the title compound (51% yield). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.15 (1H, br), 8.25 (1H, s), 7.65 (2H, d, J = 8.4 Hz), 7.47−7.32 (6H, m), 2.62 (3H d, J = 4.4 Hz). ESMS *m*/*z*: 274 (M − H). HPLC: 1 (rt = 5.7 m, 94%); 2 (rt = 7.8 m, 94%).

5-Phenyl-3-propionylamino-thiophene-2-carboxylic Acid Amide (10). Compound **3** (10 mg, 0.045 mmol) was dissolved in THF (0.5 mL) and treated with excess propionyl chloride (0.25 mL), and the resulting mixture was stirred at room temperature for 3 days and then concentrated. The residue was purified by flash chromatography using 40−100% EtOAc in hexane to provide 4 mg of the title compound (32% yield). ^{1}H NMR (methanol-d_4, 400 MHz): δ 8.35 (1H, s), 7.70 (2H, d, J = 8.4 Hz), 7.50−7.35 (3H, m), 2.47 (2H, q, J = 7.5 Hz), 1.27 (3H, t, J = 7.5 Hz). ESMS *m*/*z*: 273 (M − H). HPLC: 1 (rt = 6.7 m, 98%); 2 (rt = 8.5 m, 98%).

6-Phenyl-thieno[3,2-*d*]pyrimidin-4-ylamine (12). Anhydrous DMF (0.58 mL, 7 mmol) was added dropwise at room temperature to a solution of oxalyl chloride (0.66 mL, 7 mmol) in dichloromethane (20 mL). The reaction mixture was stirred at room temperature for 1/2 h, after which time, **11** was added (684 mg, 3 mmol). The resultant mixture was heated at reflux for 1/2 h, cooled, and diluted with saturated aqueous Na_2SO_4. The organic layer was separated, and the aqueous was extracted with dichloromethane. The organic fractions were combined, washed with brine, and dried with $MgSO_4$, and the solvent was evaporated. The resulting solid was dissolved in a 0.5 M solution of ammonia in dioxane, and 7 mL of concentrated ammonium hydroxide was added. The reaction vial was sealed, and the reaction mixture was heated at 40−65 °C for a total of 92 h, after which time, it was diluted with water, and the product was filtered. Recrystallization from EtOAc/methanol provided 315 mg of the title compound (46% yield). Mp 283−284 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.35 (1H, s), 7.82 (2H, d, J = 7.1 Hz), 7.78 (1H, s), 7.52−7.44 (5H, m). ESMS *m*/*z*: 228 (M + H); Anal. ($C_{12}H_9N_3S$) C, H, N.

2-Amino-quinoline-3-carboxylic Acid Amide (15). 2-Amino-3-cyanoquinoline **(**30 mg, 0.178 mmol) was dissolved in 2 mL of concentrated H_2SO_4, and the resulting solution was stirred overnight at ambient temperature, after which time, it was poured into ice/water and neutralized with saturated aqueous $NaHCO_3$. The resulting mixture was extracted with EtOAc, washed with brine, dried, and evaporated. The product was triturated in hexane and filtered to provide 16 mg of the title compound (48% yield). Mp 238−239 °C. ^{1}H NMR (methanol-d_4, 400 MHz): δ 8.48 (1H, s), 7.72 (1H, d, J = 8 Hz), 7.62 (1H, t, J = 8 Hz), 7.54 (1H, d, J = 8 Hz), 7.27 (1H, t, J = 8 Hz). ESMS *m*/*z*: 188 (M + H).

2-Amino-6-chloro-4-phenyl-quinoline-3-carboxylic Acid Amide (16). 2-Amino-3-cyano-4-phenyl-6-chloroquinoline (50 mg, 0.18 mmol) was dissolved in 1 mL of concentrated sulfuric acid. The reaction mixture was stirred for a total of 1 week at room temperature to 50 °C, after which time, it was poured over ice, neutralized with $NaHCO_3$, and filtered. The resulting solid was purified by flash chromatography with 1:1 hexane/EtOAc to provide

50 mg of the title compound (94% yield). Mp 254−255 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.65 (1H, br), 7.56−7.46 (5H, m), 7.40 (1H, br), 7.35 (2H, m), 7.03 (1H, d, J = 2 Hz), 6.32 (2H, br). ESMS m/z: 298 (M + H). Anal. ($C_{16}H_{12}ClN_3O\cdot.1C_4H_8O_2\cdot.05C_6H_{14}$) C, H, N.

3-Amino-benzo[*b*]thiophene-2-carboxylic Acid Amide (17). NaH (44 mg, 1.6 mmol) was added to a solution of 2-mercaptoacetamide (132 mg, 1.45 mmol) in dry DMF (7 mL). The resulting solution was stirred at ambient temperature for 5 min, after which time, 2-chloro-benzonitrile (200 mg, 1.45 mmol) was added, and the reaction vessel was sealed and heated at 130 °C for 16 h. The reaction mixture was allowed to cool and was quenched by the addition of a saturated aqueous solution of ammonium chloride. The resulting mixture was diluted with EtOAc, then washed with water and brine, dried with Na_2SO_4, and concentrated. The crude product was triturated with 50% EtOAc in hexane and filtered to provide 101 mg of the title compound (36% yield). Mp 193−195 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.03 (1H, d, J = 8 Hz), 7.82 (1H, d, J = 8 Hz), 7.46 (1H, t, J = 8 Hz), 7.38 (1H, t, J = 8 Hz), 7.07 (4H, br). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.4, 148.0, 137.5, 132.7, 127.9, 124.1, 123.3, 122.9, 98.6. ESMS m/z: 193 (M + H).

4-Amino-biphenyl-3-carboxylic Acid Amide (18). 2-Nitro-5-phenylbenzonitrile (350 mg, 1.56 mmol) was dissolved in 50 mL of methanol and subjected to hydrogenation on a Parr apparatus at 60 psi for 5 h using a 10% Pd/C catalyst (40 mg). The catalyst was filtered, and the solvent was evaporated. The residue was treated directly with concentrated H_2SO_4 (1 mL), and the resulting reaction mixture was stirred overnight at room temperature. The reaction mixture was then poured over ice, neutralized with saturated aqueous $NaHCO_3$, and extracted with EtOAc. The organic extracts were combined, washed with brine, dried with Na_2SO_4, and evaporated. The residue was purified by flash chromatography using a gradient of 2:1−1:1 hexane/EtOAc to provide 62 mg of the title compound (19% yield). Mp 154−155 °C. ^{1}H NMR (methanol, 400 MHz): δ 7.80 (1H, d, J = 2.1 Hz), 7.58 (2H, d, J = 7.4 Hz), 7.50 (1H, dd, J = 8.5, 2.1 Hz), 7.37 (2H, dd, J = 7.4, 7.4 Hz), 7.23 (1H, t, J = 7.4 Hz) 6.82 (1H, d, J = 8.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 149.0, 140.5, 130.7, 128.8, 128.3, 126.6, 125.9, 125.7, 117.3, 114.6. ESMS m/z: 213 (M + H). Anal. ($C_{13}H_{12}N_2O\cdot 2C_4H_8O_2$) C, H, N.

General Procedure for the Synthesis of Oxazoles 19−32 and 34−35. Aminobismalononitrile tosylate and an acid chloride (10% molar excess) were dissolved in *N*-methylpyrrolidinone (4 mmol in 10 mL), and the resulting mixture was stirred overnight at room temperature. The product was isolated either by dilution with EtOAc followed by washing the resulting mixture with water and brine, drying with Na_2SO_4, and evaporating the solvent, or by dilution of the reaction mixture with water followed by filtration. The hydrolysis reaction was performed by dissolving the crude nitrile in concentrated sulfuric acid or a 1:1 mixture of concentrated sulfuric acid and acetic acid (0.5−1.5 mmol/mL) and stirring the resulting mixture for 1−16 h; after which time, the reaction mixture was poured over ice water and neutralized with aqueous $NaHCO_3$. The product was isolated by filtration or by extraction of the aqueous reaction mixture with EtOAc; in which case, the combined extracts were washed with brine, dried with Na_2SO_4, and evaporated. Alternatively, the product was collected by filtration prior to neutralization of the reaction mixture and then suspended between aqueous $NaHCO_3$ and EtOAc. The EtOAc was washed with brine, dried, and concentrated. If needed, the product was purified by recrystallization.

5-Amino-2-thiophen-3-yl-oxazole-4-carboxylic Acid Amide (19). Yield, 73%; mp 161−162 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.1 (1H, dd, J = 2.9, 1.4 Hz), 7.94 (1H, dd, J = 12.7, 2.9 Hz), 7.67 (1H, dd, J = 12.7, 1.4 Hz), 6.90 (4H, br). ESMS m/z: 210 (M + H).

5-Amino-2-phenyl-oxazole-4-carboxylic Acid Amide (20). Yield, 16%; mp 171−172 °C; ^{1}H NMR (DMSO d_6, 400 MHz): δ 7.80 (2H, d, J = 8.0 Hz), 7.35−7.50 (3H, m); 7.80−7.0 (2H, br m). Mass spectrum (ES$^+$): m/z 204 (M + H). Anal. ($C_{10}H_9N_3O_2$) C, H, N.

5-Amino-2-(4-chloro-phenyl)-oxazole-4-carboxylic Acid Amide (21). The title compound was purified by recrystallization from ethanol/water. Yield, 53%; mp 187−189 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.82 (2H, d, J = 8.5 Hz), 7.60 (2H, d, J = 8.5 Hz), 7.12 (2H, br), 7.04 (2H, br), ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.0, 157.4, 145.8, 132.9, 128.4, 125.5, 124.9, 105.7. ESMS m/z: 238 (M + H). Anal. ($C_{10}H_8ClN_3O_2\cdot.1H_2O$) C, H, N.

5-Amino-2-(4-methoxy-phenyl)-oxazole-4-carboxylic Acid Amide (22). Yield, 34%; mp 208−210 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.84 (2H, d, J = 9 Hz), 7.17 (2H, d, J = 9 Hz), 7.02 (4H, br), 3.92 (3H s). ESMS m/z: 234 (M + H). Anal. ($C_{11}H_{11}N_3O_3$) C, H, N

5-Amino-2-*p*-tolyl-oxazole-4-carboxylic Acid Amide (23). Yield, 34%; mp 195−197 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.67 (2H, d, J = 8.2 Hz), 7.28 (2H, d, J = 8.2 Hz), 6.93 (4H, br), 2.33 (3H, s). ESMS m/z: 218 (M + H). Anal. ($C_{11}H_{11}N_3O_2\cdot.2H_2O$) C, H, N.

5-Amino-2-(4-trifluoromethyl-phenyl)-oxazole-4-carboxylic Acid Amide (24). Yield, 52%; mp 139−140 °C. ^{1}H NMR (methanol d_4, 400 MHz): δ 8.03 (2H, d, J = 8.8 Hz,), 7.73 (2H, d, J = 8.8 Hz). ESMS m/z: 272 (M + H); Anal. ($C_{11}H_8F_3N_3O_2$) C, H, N.

5-Amino-2-(4-*tert*-butyl-phenyl)-oxazole-4-carboxylic Acid Amide (25). The title compound was recrystallized twice from hexanes. Yield, 18%; mp 180−182 °C. ^{1}H NMR (methanol d_4, 400 MHz): δ 7.82 (2H, d, J = 8.8 Hz,), 7.50 (2H, d, J = 8.8 Hz), 1.35 (9H s). ESMS m/z: 260 (M + H).

5-Amino-2-(4-carbamoyl-phenyl)-oxazole-4-carboxylic Acid Amide (26). Yield, 88%; mp 232−235 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.06 (1H br), 7.98 (2H, d J = 8.6 Hz), 7.84 (2H, d, J = 8.6 Hz), 7.46 (1H br), 7.13 (2H br), 7.02 (2H br); ESMS m/z: 247 (M + H). Anal. ($C_{11}H_{10}N_4O_3$) C, H, N.

5-Amino-2-(2-chloro-phenyl)-oxazole-4-carboxylic Acid Amide (27). Yield, 29%; mp 176−178 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.81 (1H, m), 7.57 (1H, m), 7.44 (2H, m), 7.05 (2H, br), 6.94 (2H, br); ESMS m/z: 238 (M + H). Anal. ($C_{10}H_8ClN_3O_2$) C, H, N.

5-Amino-2-(2-methoxy-phenyl)-oxazole-4-carboxylic Acid Amide (28). The title compound was recrystallized from EtOAc. Yield, 17%; mp 117−118 °C. ^{1}H NMR (methanol d_4, 400 MHz): δ 7.85 (1H dd, J = 8.6, 1.8 Hz), 7.43 (1H, ddd, J = 8.4, 7.4, 1.8 Hz), 7.15 (1H, dd, J = 8.4, 1.0 Hz), 7.04 (1H ddd, J = 8.6, 7.4, 1.0 Hz), 3.95 (3H s). ESMS m/z: 234 (M + H). Anal. ($C_{11}H_{11}N_3O_3\cdot.1C_4H_8O_2$) C, H, N.

5-Amino-2-(2-methyl-phenyl)-oxazole-4-carboxylic Acid Amide (29). Yield, 47%; mp 115−117 °C. ^{1}H NMR (methanol d_4, 400 MHz): δ 7.80 (1H, d, J = 7.24 Hz), 7.27 (3H, m), 2.64 (3H, s). ESMS m/z: 218 (M + H). Anal. ($C_{11}H_{11}N_3O_2$) C, H, N. C 60.82, H 5.10, N 19.34; found, C 60.86, H 4.97, N 19.23.

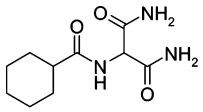
5-Amino-2-(3-chloro-phenyl)-oxazole-4-carboxylic Acid Amide (30). Yield, 40%; mp 198−200 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.75 (1H, t, J = 1.6), 7.69 (1H, dt, J = 7.4, 1.6 Hz), 7.48 (2H, m), 7.09 (2H, br), 7.00 (2H, br d). ESMS m/z: 238 (M + H). Anal. ($C_{10}H_8ClN_3O_2$) C, H, N.

5-Amino-2-(3-methoxy-phenyl)-oxazole-4-carboxylic Acid Amide (31). Yield 3%; mp 130−131 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.45−7.38 (2H, m), 7.29 (1H, d, J = 7.5 Hz), 7.10−6.95 (5H, br m), 3.79 (3H s). ESMS m/z: 234 (M + H).

5-Amino-2-(2,4,6-trichloro-phenyl)-oxazole-4-carboxylic Acid Amide (32). Compound **32** required an additional three days for the initial reaction to proceed to completion. The final product was purified by recrystallization from EtOAc/hexane. Yield, 10%. Mp 188 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.90 (2H s), 7.15−6.88 (4H br m); mass spectrum (ES$^+$): m/z 307 (M + H). Anal. ($C_{10}H_6\ Cl_3N_3O_2\cdot.1C_4H_8O_2$) C, H, N.

5-Amino-2-cyclohexyl-oxazole-4-carboxylic Acid Amide (33). Step 1. The reaction of aminobismalononitrile tosylate (1 g, 3.95 mmol) and cyclohexanecarbonyl chloride (0.636 g, 4.35 mmol) followed by the treatment of crude product with H_2SO_4 according

to the general procedure, and recrystallization of the isolate from MeOH provided 800 mg of **52** (89% yield), a product of the hydrolysis of an intermediate uncyclized bis-nitrile. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.65 (1H, d, J = 8 Hz), 7.30 (2H, s), 7.38 (2H, s), 4.73 (1H, d, J = 8 Hz), 2.31 (1H, m), 1.75−1.65 (4H, m), 1.62−1.50 (1H, m), 1.40−1.10 (5H, m). ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.8, 169.4, 57.7, 44.2, 29.8, 26.2, 25.9. ESMS *m*/*z*: 228 (M + H); Anal. ($C_{10}H_{17}N_3O_3$) C, H, N.



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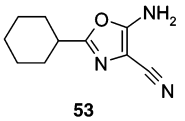

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Step 2. Compound **52** (100 mg, 0.44 mmol) was then treated with 3 mL of $POCl_3$ at 75 °C for 1 h. The reaction mixture was cautiously poured over ice with rapid stirring and subsequently neutralized with a saturated solution of $NaHCO_3$. The product was isolated by extraction with EtOAc. The combined extracts were washed with brine and dried with Na_2SO_4, and the solvent was evaporated giving the crude product, which was purified by flash chromatography using 4:1 hexane/EtOAc providing 50 mg of nitrile **53** (59% yield). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 6.53 (2H, s), 2.63−2.56 (1H, m), 1.88−1.85 (2H, m), 1.70−1.65 (2H, m), 1.60−1.57 (1H, m), 1.42−1.13 (5H, m). ESMS *m*/*z*: 192 (M + H); Anal. ($C_{10}H_{13}N_3O$) C, H, N.

Step 3. The title compound was obtained by hydrolysis of **53** with H_2SO_4 using the general procedure. It was purified by recrystallization from EtOAc/hexane. Yield 33%; mp 177 °C. Mp 177 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 6.72 (2H, br), 6.58 (2H, br), 2.64−2.55 (1H, m), 1.94−1.85 (2H, m), 1.74−1.66 (2H, m), 1.65−1.57 (1H, m), 1.48−1.13 (5H, m); ESMS *m*/*z*: 210 (M + H). Anal. ($C_{10}H_{15}N_3O_2$) C, H, N.

5-Amino-2-benzthiophene-2-yl-oxazole-4-carboxylic Acid Amide (34). Yield 18%; mp 231−233 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.00 (1H, dd, J = 5.2, 3.8 Hz), 7.93 (1H, ddd, J = 5.2, 4.3, 1.8 Hz), 7.71 (1H, s), 7.40 (2H, m), 7.19 (2H, br), 6.98 (2H, br); mass spectrum (ES$^+$): *m*/*z* 260 (M + H).

5-Amino-2-pyridin-3-yl-oxazole-4-carboxylic Acid Amide (35). Yield, 31%; mp 203−204 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.05 (1H, s), 8.67 (1H, d, J = 3.7 Hz), 8.16 (1H, dd, J = 6.4, 3.7 Hz), 7.60 (1H, d, J = 6.4 Hz), 7.1 (2H br), 7.0 (2H br). ESMS *m*/*z*: 205 (M + H). Anal. ($C_9H_8N_4O_2 \cdot 2H_2O$) C, H, N.

3-Amino-thieno[2,3-*b*]pyridine-2-carboxylic Acid Amide (36). A solution of 2-chloro-3-cyano-pyridine (277.2 mg, 2 mmol) in dry DMF (5 mL) was added to a solution of sodium methoxide (0.44 mL, 4.5M, 2 mmol) and 2-mercaptoacetamide (182.3 mg, 2 mmol) in dry DMF (5 mL) at room temperature. The reaction mixture was stirred for 1 h and then poured into ice water, and the product was isolated by filtration on a fritted funnel. The crude product was recrystallized from ethanol to provide 316 mg of the title compound as bright yellow needles (82% yield). Mp 268−272 °C, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.64 (1H, d, J = 7.4 Hz,), 8.37 (1H, d, J = 7.4 Hz,), 7.49 (1H, t, J = 7.4 Hz,), 5.04 (m, 4H). ESMS *m*/*z*: 194 (M + H). HPLC: 1 (rt = 5 m, 100%); 2 (rt = 3.3 m, 100%).

3-Amino-5-methyl-thieno[2,3-*b*]pyridine-2-carboxylic Acid Amide (37). A solution of 2-chloro-3-cyano-5-methyl-pyridine (305 mg, 2 mmol) in dry DMF (5 mL) was added to a solution of sodium methoxide (0.44 mL, 4.5M, 2 mmol) and 2-mercaptoacetamide (182.3 mg, 2 mmol) in dry DMF (5 mL) at room temperature. The reaction mixture was stirred for 1 h and then poured into ice water, and the product was isolated by filtration on a fritted funnel. The crude product was recrystallized from ethanol to provide 290 mg of the title compound as orange needles (70% yield). Mp 241−243 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.57 (1H, s), 8.16 (1H, s), 4.34 (4H, m), 2.32 (3H, s). ESMS *m*/*z*: 208 (M + H). HPLC: 1 (rt = 5.9 m, 100%); 2 (rt = 4.0 m, 100%).

3-Amino-5-chloro-4,6-dimethyl-thieno[2,3-*b*]pyridine-2-carboxylic Acid Amide (39). Step 1. Thiourea (378 mg, 4.97 mmol) was added to a solution of 2,5-dichloro-3-cyano-4,6-dimethylpyridine (500 mg, 2.49 mmol) in ethanol (20 mL) at room temperature. The mixture was heated to reflux for 24 h. The mixture was cooled to room temperature (crystallization began upon cooling). The solid (2-mercapto-3-cyano-4,6-dimethyl-5-chloro pyridine) was collected by filtration (145 mg, 30% yield) as a yellow-orange solid which was used directly.

Step 2. Sodium methoxide (0.41 mL, 4.5 M, 1.86 mmol) was added to a suspension of 2-mercapto-3-cyano-4,6-dimethyl-5-chloropyridine (124 mg, 0.62 mmol) and 2-bromo acetamide (90 mg, 0.66 mmol) in 10 mL of methanol. The resulting mixture was heated to reflux and maintained at this temperature for 1/2 h. The mixture was cooled, and the product was filtered. The crude product was recrystallized from methanol giving 120 mg of the title compound (75% yield). Mp 266−267. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.25 (2H, s), 6.88 (1H, s), 6.84 (1H, s), 2.80 (3H, s); 2.61 (3H, s). ESMS *m*/*z*: 256 (M + H). Anal. ($C_{10}H_{10}ClN_3OS \cdot .8H_2O$) C, H, N. HPLC: 1 (rt = 8.0 m, 100%); 2 (rt = 5.9 m, 100%). Anal. ($C_{10}H_{10}ClN_3OS \cdot H_2O$), C, H, N.

3-Ureido-thieno[2,3-*b*]pyridine-2-carboxylic Acid Amide (40). To a suspension of **36** (660 mg, 3.4 mmoL) in dichloromethane (4 mL) and DMF (1 mL) was added excess trimethylsilylisocyanate (5 mL). The reaction mixture was stirred at 70 °C for 20 h, diluted with dichloromethane, and filtered. The filtrate was concentrated and purified by silica gel chromatography (Combiflash, 10 g column, 20 mL/min, flow rate, 1−10% MeOH/CH_2Cl_2) followed by preparative TLC (10% MeOH/CH_2Cl_2) to afford 2 mg of the title compound. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.9 (1H, br), 9.3 (1H, br), 8.64 (1H, d, J = 5 Hz), 8.21 (1H, d, J = 8 Hz), 7.46 (1H, dd, J = 5, 8 Hz), 6.2 (1H, br), 5.5 (1H, br), 5.1 (1H, br). ESMS *m*/*z*: 237 (M + H). HPLC: 1 (rt = 4.1 m, 95%); 2 (rt = 2.2 m, 95%).

3-Amino-2-carbamoyl-6-methyl-thieno[2,3-*b*]pyridine-4-carboxylic Acid Ethyl Ester (41). Step 1. Thiourea (339 mg, 4.46 mmol) was added to a solution of 2-chloro-3-cyano-6-methyl-isonicotinic acid ethyl ester (500 mg, 2.23 mmol) in ethanol (25 mL) at room temperature. The mixture was heated to reflux for 24 h. The mixture was cooled to room temperature (crystallization began upon cooling). The solid was collected by filtration giving 3-cyano-2-mercapto-6-methyl-isonicotinic acid ethyl ester (250 mg, 50% yield) as a yellow-orange solid, which was used directly.

Step 2. NaH (39 mg, 0.95 mmol) was added to a solution of the above ester (210 mg, 0.95 mmol) in THF (15 mL) at room temperature. After stirring for 5 min, 2-bromoacetamide (134 mg, 0.97 mmol) and *n*-Bu_4NI (10 mg) were added. The mixture was stirred at room temperature for 1 h, then NaH (39 mg, 0.95 mmol) was added, and the mixture was stirred for an additional 0.5 h. The reaction was quenched by the addition of saturated aqueous NH_4Cl and diluted with EtOAc, washed with brine, dried over Na_2SO_4, and concentrated giving an orange solid. The crude residue was recrystallized from methanol giving 160 mg of the title compound (60% yield). Mp 205−208. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.68 (1H, s), 7.30 (2H, br), 7.14 (1H br), 7.11 (1H, br), 4.43 (2H, q, J = 7 Hz), 2.64 (3H, s), 1.36 (3H, t J = 7 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.8, 166.4, 159.7, 158.9, 145.3, 134.8, 120.4, 119.2, 98.0, 62.7, 23.8, 13.7. ESMS *m*/*z*: 280 (M + H). HPLC: 1 (rt = 7.8 m, 100%); 2 (rt = 5.9 m, 100%).

3-Amino-2-carbamoyl-6-methyl-thieno[2,3-*b*]pyridine-4-carboxylic Acid (42). A mixture of **41** (0.94 g. 3.4 mmol) and lithium hydroxide (0.11 g, 4.7 mmol) in methanol/H_2O (3:1, 100 mL) was stirred for 2 h at room temperature. The reaction was neutralized with 2 M HCl and concentrated in vacuo to afford 0.78 g of the title compound as an orange solid (91% yield). Mp >250 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.73 (2H, br), 7.60 (1H, s), 7.12 (2H br), 2.75 (3H, s). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168, 159.8, 158.6, 148.8, 147.9, 120.6, 120.56, 24.4. ESMS *m*/*z*: 252 (M + H). HPLC: 1 (rt > 10 m); 2 (rt = 3.7 m, 97%).

3-Amino-4-hydroxymethyl-6-methyl-thieno[2,3-*b*]pyridine-2-carboxylic Acid Amide (43). $LiBH_4$ (62 mg, 2.04 mmol) was added

to a solution of 3-amino-2-carbamoyl-6-methyl-thieno[2,3-*b*]pyridine-4-carboxylic acid ethyl ester (**41**) (205 mg, 0.73 mmol) in 10:1 THF/methanol (15 mL) at room temperature. After stirring for 2 h, the reaction was quenched by the addition of 1 M HCl. The mixture was buffered to a pH ≈ 7, diluted with EtOAc, washed sequentially with H_2O and brine, dried over Na_2SO_4, concentrated, and triturated with 50% EtOAc/hexane giving 123 mg of the title compound as a yellow solid (71% yield). Mp >210 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.19 (1H, s), 7.16 (2H br), 7.13 (2H br), 6.22 (1H, t, J = 5 Hz), 4.81 (2H, d, J = 5 Hz), 2.53 (3H, s). ESMS *m*/*z*: 238 (M + H). HPLC: 1 (rt = 5.6 m, 96%); 2 (rt = 3.3 m, 95%).

General Procedure for the Synthesis of 44−47. Diisopropyl azodicarboxylate (DIAD) (5% molar excess) was added to a solution of 3-amino-4-hydroxymethyl-6-methyl-thieno[2,3-*b*]pyridine-2-carboxylic acid amide (**43**), a phenol (5% molar excess), and Ph_3P (5% molar excess) in THF (0.05 mmol **43**/mL) at 0 °C. The mixture was warmed to room temperature and stirred for 24 h. The reaction mixture was concentrated, and the product was purified.

3-Amino-6-methyl-4-phenoxymethyl-thieno[2,3-*b*]pyridine-2-carboxylic Acid Amide (44). The title compound was purified by preparative TLC (10% methanol/dichloromethane). Yield, 61%; mp 194−196 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.39 (1H, s), 7.32 (2H, dd, J = 8.5, 7.5 Hz), 7.23 (2H, dd, J = 8.5, 1 Hz), 7.12 (2H, dd, J = 1, 7.5), 6.98 (1H, tt, J = 7.5, 1 Hz), 6.83 (2H, bs), 5.60 (2H, s), 2.55 (3H, s). ESMS *m*/*z*: 314 (M + H). HPLC: 1 (rt = 8.4 m, 95%); 2 (rt = 6.4 m, 95%).

4-(4-Cyano-phenoxymethyl)-6-methyl-thieno[2,3-*b*]pyridine-2-carboxylic Acid Amide (45). The title compound was purified by preparative TLC (10% methanol/dichloromethane). Yield, 34%, mp 228−230 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.82 (2H, d, J = 8.5 Hz), 7.39 (1H, s), 7.32 (2H, d, J = 8.5 Hz), 7.24 (2H, br), 6.79 (2H, br), 5.73 (2H, s), 2.55 (3H, s). ESMS *m*/*z*: 339 (M + H).

4-(4-Methoxy-phenoxymethyl)-6-methyl-thieno[2,3-*b*]pyridine-2-carboxylic Acid Amide (46). The title compound was purified by preparative TLC (10% methanol/dichloromethane). Yield, 23%; mp >200 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.36 (1H, s), 7.22 (2H, br), 7.04 (2H, d, J = 9 Hz), 6.87 (4H, m), 6.86 (2H, br), 3.68 (3H, s), 2.54 (3H, s). ESMS *m*/*z*: 344 (M + H).

Amino-4-(4-carbamoyl-phenoxymethyl)-6-methyl-thieno[2,3-*b*]pyridine-2-carboxylic Acid Amide (47). The title compound was purified by trituration with 2:1 EtOAc/methanol. Yield, 24%; mp >250 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.86 (2H, d, J = 8.5 Hz), 7.85 (1H, br), 7.40−7.25 (5H, m), 6.82 (2H, br), 5.68 (2H, s), 2.55 (3H, s). ESMS *m*/*z*: 357 (M + H). HPLC: 1 (rt = 6.6 m, 98%); 2 (rt = 4.4 m, 98%).

3-Amino-6-isobutyl-4-methyl-thieno[2,3-*b*]pyridine-2-carboxylic Acid Amide (48). Step 1. To a stirred solution of 6-methyl-2,4-heptanedione (3.128 g, 22 mmol) and 2-cyanothioacetamide (2.203 g, 22 mmol) in ethanol (40 mL) was added triethylamine (0.4 mL, 2.87 mmol), and the resulting reaction mixture was heated at 50 °C for 1 h. The reaction mixture was cooled to room temperature, and the precipitates that formed were isolated by filtration, washed with ethanol, and dried to afford 2.8 g of 2-mercapto-3-cyano-4-methyl-6-isobutylpyridine (61.7% yield), which was directly used in the next step.

Step 2. To a stirred solution of the intermediate (1.00 g, 4.847 mmol) in DMF (20 mL) was added 2-bromoacetamide (0.669 g, 4.847 mmol) and sodium ethoxide (0.681 g, 10.00 mmol). The reaction mixture was heated at 70 °C for 1 h, diluted with water, cooled to room temperature, and the precipitates that formed were isolated by filtration, washed with water, and dried in vacuo to afford 0.50 g of the title compound (39.2% yield). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.15 (2H, br), 6.97 (1H, s), 6.77 (2H, br), 2.70 (3H, s), 2.63 (2H, d, J = 7.30 Hz), 2.10 (1H, m), 0.85 (6H, d, J = 6.62 Hz). Regio-chemistry was confirmed by NOE between 3-NH_2 and 4-CH_3. CIMS *m*/*z*: 264 (M + H). HPLC: 1 (rt = 8.2 m, 95%); 2 (rt = 5.6 m, 96%).

3-Amino-6-butyl-4-methyl-thieno[2,3-*b*]pyridine-2-carboxylic Acid Amide (49). Step 1. To a stirred solution of 2,4-octanedione (3.128 g, 22 mmol) and 2-cyanothioacetamide (2.203 g, 22 mmol) in ethanol (40 mL) was added triethylamine (0.4 mL, 2.87 mmol), and the reaction mixture was heated at 50 °C for 1 h. The reaction mixture was cooled to room temperature, and the precipitates that formed were isolated by filtration and washed with ethanol. The product was dried to afford 2.2 g of the intermediate (48% yield). ^{1}H NMR (DMSO-d_6): δ 13.81 (1H, s), 6.72 (1H, s), 2.56−2.63 (2H, m), 2.34 (3H, s), 1.47−1.59 (2H, m), 1.21−1.31 (2H, m), 0.86 (3H, t, J = 7.3 Hz). CIMS *m*/*z*: 207 (MH^+).

Step 2. Sodium methoxide (1.62 mL, 4.5M, 7.28 mmol) was added to a suspension of the intermediate (1.50 g, 7.28 mmol) and 2-chloroacetamide (0.68 g, 7.28 mmol) in 40 mL methanol. The resulting mixture was heated to reflux and maintained at this temperature for 3 h. The reaction mixture was cooled to room temperature, and the product was filtered. The crude product was recrystallized from methanol giving 780 mg of the title compound (41% yield). Mp 174−175 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.10 (2H, s), 7.00 (1H, s), 6.78 (2H, s), 2.74 (2H, m), 2.69 (3H, s), 1.63(2H, m), 1.31(2H, m), 0.88(3H, m). ESMS *m*/*z*: 264 (M + H). HPLC: 1 (rt = 8.4 m, 100%); 2 (rt = 5.8 m, 99%).

3-Amino-6-*tert*-butyl-2-carbamoyl-thieno[2,3-*b*]pyridine-4-carboxylic Acid Ethyl Ester (50). 2-Chloro-3-cyano-4-methyl-6-*tert*-butylpyridine (418 mg, 1.57 mmol) was added to a solution of sodium hydride (66 mg, 1.65 mmol) and 2-mercaptoacetamide (150 mg, 1.65 mmol) in dry DMF (5 mL) and dry THF (14 mL) at room temperature. The reaction mixture was stirred for 4 h, after which time, sodium hexamethyldisilazide (1.65 mL, 1 M solution in THF, 1.65 mmol) was added, and the resulting mixture was stirred an additional hour. The reaction mixture was quenched by the addition of saturated ammonium chloride and then diluted with EtOAc. The organic fraction was washed with brine, dried, and concentrated. The crude product was purified by flash chromatography (50−75% EtOAc/hexane) and further purified by preparative TLC (75% EtOAc/hexane) to provide 50 mg of the title compound (10% yield). Mp 149−151 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.78 (1H, s), 5.43 (2H br), 4.51 (2H, q, J = 7 Hz), 1.47 (3H, t, J = 7 Hz), 1.45 (9H, s). ESMS *m*/*z*: 322 (M + H). HPLC: 1 (rt = 9.3 m, 100%); 2 (rt = 8.2 m, 100%). Anal. ($C_{15}H_{19}N_3O_3S\cdot 1H_2O$) C, H, N.

Screening Method. The screen was performed in buffer (20 mM HEPES at pH 7.5, with 10 mM $MgCl_2$, 2 mM $MnCl_2$, 100 mM NaCl, 100 μM Na_3VO_4, 20 mM beta-glycerophosphate), 0.01% CHAPS, 1 mM DTT and 50 μg/mL of BSA. Test samples in DMSO were diluted into buffer, and aliquots were added to Packard Optiplate 96-well assay plates (final concentration of 10 μg/mL). IKKβ enzyme (80 ng) in 20 μL of buffer was added to each well. The kinase reaction was initiated by adding 30 μL of GST-IκBα (AA 1-54) substrate (1 μM) and ^{33}P-ATP cocktail at 10 Ci/mmol. After 1 h, the stop solution (50 μL Amersham anti-mouse PVT SPA beads coated with PharMingen anti-GST antibody (1 μg/mg) in buffer plus 200 μM ATP, 5 mM EDTA, and 0.1% TritonX-100) was added, and the solution was incubated for 2.5 h. CsCl (150 μL, 5 M) was added, and the plates were equilibrated for 24 h. The radioactive decay signal was read on a Packard Top Count scintillation counter.

Dose Response Method. The dose response assay associated with the screen utilized the same reagents described above in a standard filtration assay. DMSO stock solutions of compounds were diluted into buffer, then serially diluted to six final concentrations (range: 25−0.1 μg/mL) in Costar 96-well microtiter plates. IKKβ enzyme (25 μL) (912 ng/mL) was added to each well followed by 5 min of preincubation. The kinase reaction was initiated as described above. Plates were incubated for 1 h at room temperature and then quenched with 150 μL of TCA/pyrophosphate. The precipitated product was harvested onto Packard Unifilter-96 GF/B with a Packard micromate harvester. A scintillant was added and the radioactivity was read on a Packard Top Count scintillation counter.

Dose Response Method for Hit-to-Lead Analogues (IKKβ). Data for hit-to-lead analogues was generated using the standard filtration assay. The reaction mixtures (60 μL) contained 20 mM HEPES at pH 7.5, 10 mM $MgCl_2$, 2 mM $MnCl_2$, 100 mM NaCl,

100 μM Na_3VO_4, 20 mM β-glycerophosphate, 1 mM DTT, 2% DMSO, 250 nM ATP, 0.4 nM [^{33}P]ATP (specific activity, 3000 Ci/mmol), 53 μg/mL of IκBα substrate, 0.09 μg/mL of IKKβ enzyme, and the test compound. The reactions were initiated by adding a solution of IκBα substrate and ATP to polypropylene plates containing the IKKβ enzyme that was preincubated for 5 min with the test compound. Then, the reaction mixtures were incubated for 1 h at 25 °C and quenched by the addition of 150 μL of 10% trichloroacetic acid and 5% disodium pyrophosphate. After mixing, the entire contents of the quenched reaction mixtures were transferred to Packard Unifilter filtration plates, aspirated, and washed six times with 250 μL of doubly distilled H_2O using the Packard Filtermate Harvester. The filtration plates were left to air-dry, and then 40 μL of microscint 20 scintillation fluid was added to each well. The plates were briefly shaken, and the ^{33}P-labeled reaction products were quantitated using the Packard TopCount scintillation counter. The compounds were tested in duplicate, starting at a top concentration of 50 μM. The assay was run in conjunction with a concentration determination: DMSO stock solutions at 5 mg/mL were diluted 3-fold with the reaction buffer, and the samples were centrifuged for 10 min. The supernatants were used in the assay, and the concentration of the supernatants was determined using HPLC by comparison to a reference solution. The IC_{50} data reflects the corrected concentrations, and the values are an average of at least two data points.

IKKα Dose Response Method. The same method described above for IKKβ was used with the following exceptions: 245 μg/mL of IκBα substrate and 3.6 μg/mL of IKKα enzyme were used. The compound solutions were used directly with no centrifugation and no concentration determinations.

Solubility in HBSS. Approximately 1 mg of compound was combined with 10 mL of Hank's balanced salt solution (HBSS) in a brown 20.0 mL bottle. The mixture was stirred overnight. The mixture was then filtered using a 0.45 μm PVDF Whatman filter. The recovered solution was then analyzed by HPLC. Solubility was determined using a standard curve.

Acknowledgment. We thank Ed Pack for providing HPLC data, Kathy Last-Barney and Tin Htut for providing cellular data, Ming-Hong Hao for conformational analyses, and John Proudfoot, Eugene Hickey, and Roman Fleck for discussions and evaluation of the manuscript.

Supporting Information Available: Additional selectivity data for compounds **3**, **20**, **24**, and **38**, ATP competition data for compounds **2**, **3**, **20**, **24**, **36**, and **38**, cell data for compounds **2**, **3**, and **38**, and combustion analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM0510979