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Imidazo[4,5-*d*]thiazolo[5,4-*b*]pyridine based inhibitors of IKK2: Synthesis, SAR, PK/PD and activity in a preclinical model of rheumatoid arthritis

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ABSTRACT

The synthesis, structure–activity relationships (SAR) and biological evaluation of thiazole based tricyclic inhibitors of IKK2 are described. Compound **9** was determined to be orally efficacious in a murine model of rheumatoid arthritis.

The NF-κB family of nuclear transcription factors are ubiquitously expressed proteins that regulate the transcription of a variety of genes relating to immune and inflammatory disorders, cancers and diabetes among others.[1] NF-κB is held in an inactive state in the cytoplasm of unstimulated cells by a family of inhibitor proteins known as IκBs, which mask the nuclear localization signals of NF-κB. In response to various stimuli, IκB is phosphorylated by IkB-kinase (IKK), leading to its ubiquitination and subsequent proteosomal degradation which allows NF-κB to translocate to the nucleus and activate gene transcription.[2a] IKK is a high molecular weight (700–900 kD) trimeric complex consisting of IKK1 (IKKα), IKK2 (IKKβ) and NEMO (NF-κB essential modulator). The IKK2 subunit is responsible for the phosphorylation of IκB in the canonical signaling pathway leading to NF-κB activation.[2b]

Efforts to modulate NF-κB activity through small molecule inhibition of IKK2 have been reported from several research groups.[3] We have previously disclosed several tricyclic based inhibitors of IKK2 (Fig. 1) including imidazoquinoxaline **1** (**BMS-345541**),[4a] pyrazolopurine (**2**),[4b] and more recently tricyclic imidazole (**3**), oxazole (**4**) and thiazole (**5**) based structures.[4c] Herein we disclose SAR studies within the imidazothiazolopyridine (**5**) series in addition to results from in vivo evaluation in models of acute and chronic inflammation.

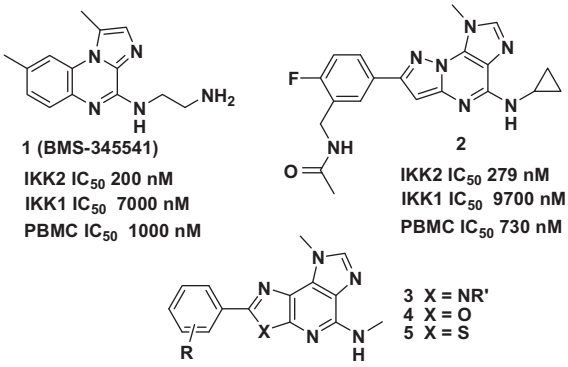

Figure 1. Tricyclic inhibitors of IKK2.

Preparation of the target compounds was accomplished following several routes that permitted the SAR evaluation at the *meta* position of the C2 aryl group as well as the substitution at C5 (Scheme 1). The previously described advanced intermediate **6** was key to facilitating this evaluation.[4c] Suzuki–Miyaura reaction of **6** with boronic acid **16** proceeded selectively at the C2-bromo position to provide chloro-tricyclic compound **7**. Displacement of the chlorine of **7** with a variety of amines was effected under microwave heating. Alternatively, **6** could be coupled with boronic ester **17** and then reacted with ethylamine to afford, after Boc deprotection, intermediate **13**. Acylation of **13** with a variety of

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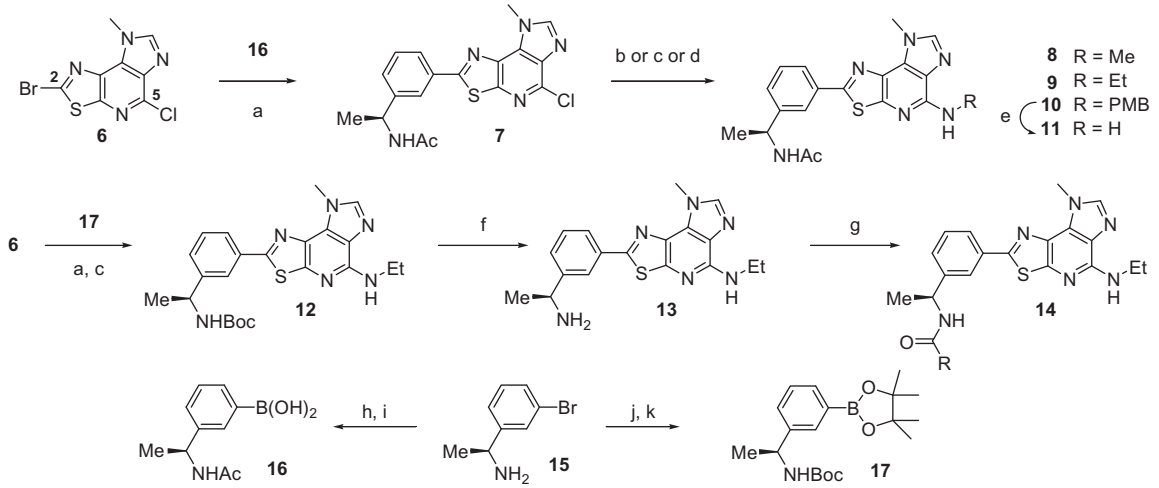
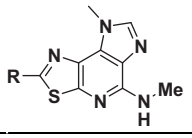
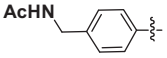
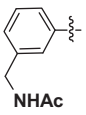
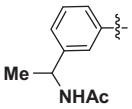


Scheme 1. Reagents and conditions: (a) $Pd(PPh_3)_4$, K_2CO_3 (aq), DME, 120 °C; (b) $MeNH_2$, EtOH, microwave 150 °C; (c) $EtNH_2$, EtOH, microwave 150 °C; (d) $PMBNH_2$, THF, DMF, microwave 200 °C; (e) TFA, CH_2Cl_2; (f) HCl (4 N in 1,4-dioxane); (g) R-COOH, EDCI, HOBt, DMF, 60 °C; (h) Ac_2O, pyr.; (i) MeLi, *t*BuLi, $(iPrO)_3B$, THF, −78 °C; (j) Boc_2O, THF; (k) $PdCl_2$(dppf), dppf, bis(pinacolato)diboron, KOAc, 1,4-dioxane, 80 °C.

Table 1
C2-Aryl SAR



Compd	R		IKK2[a] IC_{50}, μM	IKK1[a] IC_{50}, μM	hPBMC[a,b] IC_{50}, μM
18			0.006	3.50	0.310 ± 0.03 (2)
19			0.060	NT[c]	0.530
20			0.022	1.90	0.350
21		(±)	0.009 ± 0.005 (10)	0.430	0.170 ± 0.03 (43)
22		(*R*)	0.028 ± 0.02 (3)	NT	0.560
8		(*S*)	0.006 ± 0.004 (17)	0.230 ± 0.0 (2)	0.080

[a] Where values are derived from more than a single experiment, the standard deviation is noted followed by the number of test occasions in parenthesis.
[b] hPBMC = inhibition of LPS-induced TNF-α release from human peripheral blood mononuclear cells.
[c] NT = not tested.

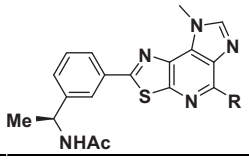
carboxylic acids provided the final compounds. The requisite chiral boronic acid/ester fragments (**16**/**17**) were prepared in two steps from the commercially available homochiral aryl bromide **15**.

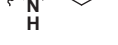
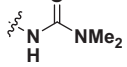
Final compounds were initially evaluated for inhibition of human IKK2 enzyme activity followed by inhibition of lipopolysaccharide (LPS) induced TNFα production in human PBMCs as a measure of cellular potency.[5,6] Counter screening of select compounds against human IKK1 provided early selectivity determination. Based on SAR from our earlier chemotypes[4b–d] we directed our efforts toward benzylamine substitution on the C-2 aryl group (Table 1).

Acetamidomethyl substitution at the *para*-position (**19**) led to a 10-fold loss in enzymatic activity against IKK2 along with a reduction in cellular potency relative to the unsubstituted **18**. The isomeric analog **20** indicated that *meta* substitution was better tolerated. When combined with alkyl branching at the benzylic carbon (**21**), a significant improvement in potency was observed. Resolution of the enantiomers demonstrated a preference for the (*S*) isomer (**8**) relative to the (*R*) isomer (**22**), providing an increase in potency against IKK2 of approximately fivefold. The (*S*)-1-acetamidoethyl template was therefore selected for subsequent evaluation of the C5 SAR.

The SAR of the C5 position within a series of compounds bearing the preferred *meta*-(*S*)-1-acetamidoethyl substitution on the phenyl group is shown in Table 2. Optimal in vitro activity was achieved with amino (**11**) or small alkylamino (**8, 9**) groups. Fluorination or amination of the alkylamino group (**23**, **24**) resulted in

Table 2
C5-SAR with (*S*) 1-acetamidoethyl template



Compd	R	IKK2 IC_{50}, μM	IKK1 IC_{50}, μM	hPBMC IC_{50}, μM
8		0.006 ± 0.004 (17)	0.230 ± 0.10 (2)	0.080
9		0.019 ± 0.01 (34)	0.420	0.220 ± 0.08 (7)
11		0.013 ± 0.002 (3)	NT	0.290 ± 0.10 (3)
23[a]		0.057 ± 0.05 (2)	NT	1.36
24[a]		0.049	NT	4.89
25[b]		0.130	NT	NT
26[a]		0.100	NT	0.99
27[c]		>0.500	NT	>10

[a] Prepared in analogy to **8** by substituting with the appropriate amine.
[b] Prepared from **11** by reaction with *N*,*N*-dimethylcarbamoyl chloride, NaH, DMF.
[c] Prepared in analogy to **8** by heating **7** with MeOH, NaOH.

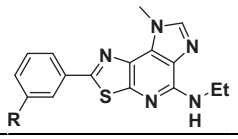
decreased potency, as did incorporation of the amine into a urea (**25**). An important role for the NH of the C5 substituent was suggested by the reduced potency for *N*,*N*-dimethylamino and methoxy derivatives (**26**, IKK2 IC_{50} = 0.10 μM; **27,** IKK2 IC_{50} >0.50 μM which was the highest compound concentration evaluated). Crystal structures have not been reported for IKK2 and this lack of structural information, coupled with the observation that compounds in this class may bind to a region outside of the traditional ATP binding site and allosterically modulate the enzyme,[4a] has prevented generation of a binding model. Thus, the exact role of the C5 NH is not understood.

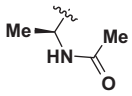
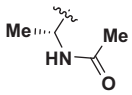
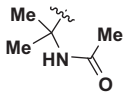
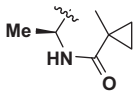
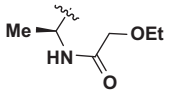
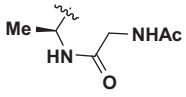
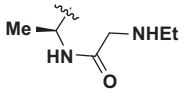
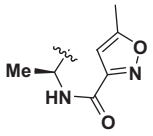
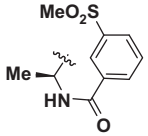
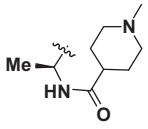
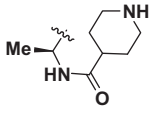
While the methylamino group at C5 afforded slightly improved in vitro potency over the ethylamino group, the latter generally conferred improved microsomal stability and reduced in vivo clearance (data not shown). Further optimization efforts around the *meta*-benzylamine side chain were therefore investigated using ethylamino substitution at C5. Extensive variations were explored for the acyl group of the benzylamine, with select examples presented in Table 3. In addition to in vitro potency, the pharmacokinetics (PK) in rats was evaluated for many compounds utilizing a cassette dosing strategy.[4c] Consistent with earlier SAR, the (*S*) enantiomer within this series continued to provide improved in vitro activity (**9** vs **28**) and was also found to be superior to the dimethyl analog (**29**). There was a high degree of tolerance for the nature of the acyl substituent in vitro, with alkyl, functionalized alkyl, aryl, heteroaryl and heterocyclic based acyl groups providing IKK2 inhibitors with reasonable enzyme and cellular activity. Differentiation was more pronounced on the basis of in vivo properties, with acyl group modifications leading to significantly improved rat PK properties in several examples. In comparison to acetamide **9**, the 1-methylcyclopropanecarboxamide (**30**), 5-methylisoxazole-3-carboxamide (**34**) and methylsulfonylbenzamide (**35**) analogs displayed reduced clearance (Cl = 7.5, 2.8, 5.1 mL/min/kg, respectively) with increased half life values ($t_{1/2}$ = 2.0, 2.8. 1.5 h, respectively). While 1-methylpiperidinecarboxamide **36** proved to be rapidly cleared (Cl = 130 mL/min/kg), the des-methyl analog (**37**) was significantly more stable in vivo (Cl = 3.0 mL/min/kg, $t_{1/2}$ = 2.9 h).

As a prototypical example of this series, compound **9** was selected for further profiling and in vivo evaluation in PK, pharmacodynamic, and efficacy models. Discrete PK properties for **9** (iv and po in mouse/rat and iv in dog) are shown in Table 4. The compound displayed good bioavailability in rodents and a half life of 0.6–1.9 h across the three species. The clearance was higher than expected based on previous rat cassette PK results, however the compound achieved reasonable C_{max} and AUC levels. A major metabolic pathway for **9** was found to be N-dealkylation. The extent of N-deethylation was examined across species, with the formation of the major metabolite (**11**) observed in an AUC ratio (metabolite/parent) ranging from 0.2 in dog to 1.1 in mouse (see Fig. 1).

Compounds **9** and **11** displayed similar potency in whole blood assays measuring inhibition of TNFα production in response to stimulation with LPS (human whole blood IC_{50} ~2 μM, mouse whole blood IC_{50} ~1.0 μM). General kinase selectivity was assessed through evaluation in a panel of more than 40 diverse kinases. At 10 μM concentration, activity of less than 10% of control was observed for only 4 kinases for **9** (DAPK2, GAK, JNK3, STK16) and 2 kinases for **11** (DAPK2, STK16). The compounds were subsequently

Table 3
m-Phenyl SAR



Compd	R	IKK2 IC_{50}, μM	hPBMC IC_{50}, μM	Rat cassette PK Cl (mL/ min/kg) $t_{1/2}$ (h) V_{ss} (L/kg)
9		0.019 ± 0.01 (34)	0.220 ± 0.08 (7)	Cl = 25 $t_{1/2}$ = 0.4 V_{ss} = 0.8
28		0.055 ± 0.01 (20)	0.700	NT
29[a]		0.036	0.400	Cl = 19 $t_{1/2}$ = 0.3 V_{ss} = 0.5
30		0.034 ± 0.02 (3)	0.440	Cl = 7.5 $t_{1/2}$ = 2.0 V_{ss} = 1.1
31		0.016 ± 0.01 (2)	0.600	Cl = 11 $t_{1/2}$ = 0.95 V_{ss} = 0.7
32		0.027	0.480	Cl = 19 $t_{1/2}$ = 0.7 V_{ss} = 0.7
33		0.039	0.440	Cl = 29 $t_{1/2}$ = 1.8 V_{ss} = 2.4
34		0.078 ± 0.02 (2)	0.940	Cl = 2.8 $t_{1/2}$ = 2.8 V_{ss} = 0.7
35		0.041	0.220	Cl = 5.1 $t_{1/2}$ = 1.5 V_{ss} = 0.6
36		0.010	0.560	Cl = 130 $t_{1/2}$ = 1.5 V_{ss} = 17
37		0.020	0.290	Cl = 3.0 $t_{1/2}$ = 2.9 V_{ss} = 5.6

[a] Prepared in analogy to **14** using the corresponding boronic ester.

Table 4
Pharmacokinetic properties[a] of **9** in mice, rats and dogs

	Mouse	Rat	Dog (iv only)
% F_{po}	57%	88%	—
C_{max} (nM)	1330	1650	—
T_{max} (h)	0.50	2.00	—
$t_{1/2}$ (h)	1.9	0.6	0.7
MRT (h)	2.9	2.1	
Cl (mL/min/kg)	62	63	43
V_{ss} (L/kg)	1.5	2.3	3.1
AUC_{tot} (nM * h)	3000	4630	1980
11 AUC_{tot} (nM * h) (major metabolite produced after dosing with **9**)	3370	2400	400

[a] Vehicle: iv, PEG400:water = 80:20; po, PEG400 = 100%. Dose: iv, 2 mg/kg; po, 10 mg/kg.

examined in a murine model of acute inflammation for their ability to inhibit TNF-α production in response to an LPS challenge in vivo (Fig. 2).[4g] The compounds were administered orally at 30 and 100 mg/kg 5 h prior to LPS challenge and were found to dose-dependently inhibit the TNF-α response, with the 100 mg/kg dose of **9** affording the most robust response (75% inhibition). Drug exposures were obtained at 6.5 h post-dose and demonstrated that at the higher doses tested, the delivery of **11** from metabolism of **9** was more efficient than from direct dosing of compound **11**. As expected, the IKK2 activity of **11** (see Table 2) appeared to be helping drive the pharmacology resulting from administration of **9**.

Based on the in vitro profile, results in the murine LPS-TNF model and pharmacokinetic properties, compound **9** was examined for efficacy in a rodent model of arthritis (murine collagen-induced arthritis).[4g] Twice daily administration of **9** at 60 mg/kg was initiated on day 25, coinciding with the time of disease onset. In this pseudo-established mode, treatment with **9** provided significant inhibition in disease progression throughout the study, with 80–90% reduction in clinical scores (Fig. 3). Exposures obtained on day 40 indicated that the ratio of parent to metabolite (**9**:**11**) was nearly 1:1 and the combined drug levels remained above the mouse whole blood IC_{50} (>1 μM) for at least 6 h post-dose.

In conclusion, SAR investigation of C2 and C5-substituted imidazothiazolopyridines provided potent inhibitors of IKK2 with improved PK properties. Select compounds were shown to be active in a model of acute inflammation and compound **9** was found to be highly efficacious in a rodent model of chronic inflammation. Further studies on the evaluation of this chemotype will be reported in due course.

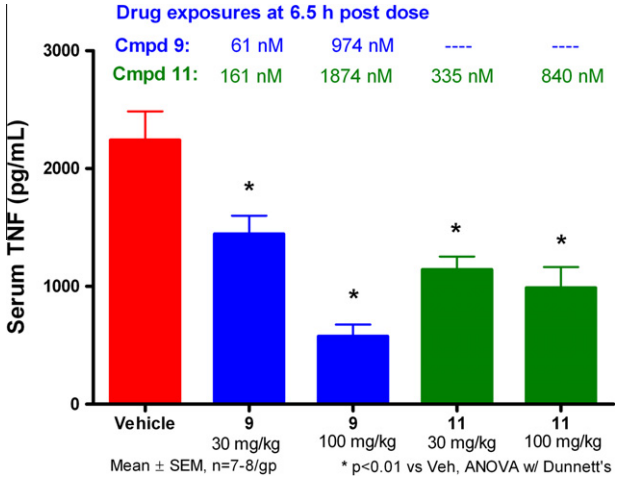


Figure 2. Inhibition of LPS-induced TNF-α release by **9** and **11** in mouse. BALB/c female mice (Harlan), 6–8 weeks of age, were used. Compounds were dosed in PEG400/HCl/Tween80/water (Vehicle) to mice (n = 7–8/treatment) by oral gavage in a volume of 0.1 mL. Control mice received vehicle alone. Five hours later, mice were injected intraperitoneally with 50 μg/kg lipopolysaccharide (LPS; *E. coli* O111:B4; Sigma). Blood samples were collected 90 min after LPS injection. Serum was separated and analyzed for the level of TNF-α by commercial ELISA assay (BioSource) according to the manufacturer's instructions. Data shown are mean ± standard error.

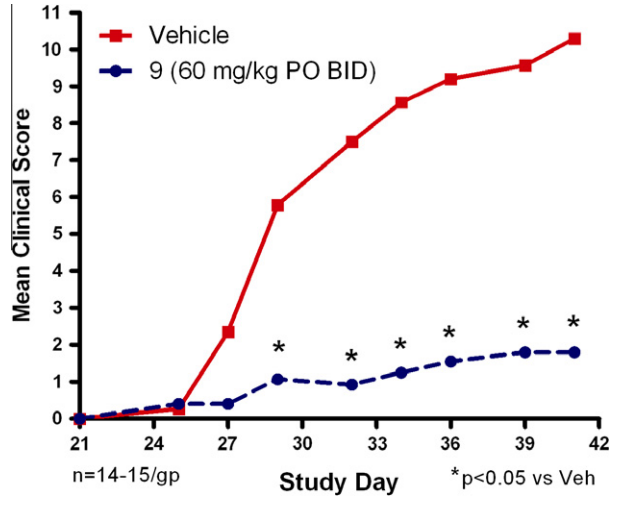


Figure 3. Efficacy of **9** (blue circles) in the murine model of collagen-induced arthritis. Mice (n = 14–15/group) were primed with collagen injection on day 1 and again on day 25. Compound **9** at 60 mg/kg or vehicle control (red squares) was administered by oral gavage twice daily beginning at the time of the second collagen injection on day 25. Average gross clinical score for all mice within each treatment group.

References and notes

1. (a) Ghosh, S.; Hayden, M. S. *Nat. Rev. Immunol.* **2008**, 837; (b) Strnad, J.; Burke, J. R. *Trends Pharmacol. Sci.* **2007**, *28*, 142; (c) Yang, J.; Amiri, K. I.; Burke, J. R.; Schmid, J. A.; Richmond, A. *Clin. Cancer Res.* **2006**, 950; (d) Ammann, J. U.; Haag, C.; Kasperczyk, H.; Debatin, K. M.; Fulda, S. *Int. J. Cancer* **2009**, 1301; (e) Cai, D.; Yuan, M.; Frantz, D. F.; Melendez, P. A.; Hansen, L.; Lee, J.; Shoelson, S. E. *Nat. Med.* **2004**, 183.
2. (a) Ghosh, S.; Karin, M. *Cell* **2002**, *109*, S81; (b) Sun, S.-C.; Ley, S. C. *Trends Immunol.* **2008**, 469.
3. (a) Pitts, W. J.; Kempson, J. *Annu. Rep. Med. Chem.* **2008**, *43*, 155; (b) Bamborough, P.; Callahan, J. F.; Christoper, J. A.; Kerns, J. K.; Liddle, J.; Miller, D. D.; Morse, M. A.; Rumsey, W. L.; Williamson, R. *Curr. Top. Med. Chem.* **2009**, 623.
4. (a) Burke, J. R.; Pattoli, M. A.; Gregor, K. R.; Brassil, P. J.; MacMaster, J. F.; McIntyre, K. W.; Yang, X.; Iotzova, V. S.; Clarke, W.; Strnad, J.; Qiu, Y.; Zusi, F. C. *J. Biol. Chem.* **2003**, *278*, 1450; (b) Beaulieu, F.; Ouellet, C.; Ruediger, E. H.; Belema, M.; Qiu, Y.; Yang, X.; Banville, J.; Burke, J. R.; Gregor, K. R.; MacMaster, J. F.; Martel, A.; McIntyre, K. W.; Pattoli, M. A.; Zusi, F. C.; Vyas, D. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1233; (c) Kempson, J.; Spergel, S. H.; Guo, J.; Quesnelle, C.; Gill, P.; Belanger, D.; Dyckman, A. J.; Li, T.; Watterson, S. H.; Langevine, C. M.; Das, J.; Moquin, R. V.; Furch, J. A.; Marinier, A.; Dodier, M.; Martel, A.; Nirschl, D.; Van Kirk, K.; Burke, J. R.; Pattoli, M. A.; Gillooly, K. M.; McIntyre, K. M.; Chen, L.; Yang, Z.; Marathe, P. H.; Wang-Iverson, D.; Dodd, J. H.; McKinnon, M.; Barrish, J. C.; Pitts, W. J. *J. Med. Chem.* **2009**, *52*, 1994; (d) Belema, M.; Bunker, A.; Nguyen, V. N.; Beaulieu, F.; Ouellet, C.; Qiu, Y.; Zhang, Y.; Martel, A.; Burke, J. R.; McIntyre, K. W.; Pattoli, M. A.; Daloisio, C.; Gillooly, K. M.; Clarke, W. J.; Brassil, P. J.; Zusi, F. C.; Vyas, D. M. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4284; (e) Kempson, J.; Guo, J.; Das, J.; Moquin, R. V.; Spergel, S. H.; Watterson, S. H.; Langevine, C. M.; Dyckman, A. J.; Pattoli, M.; Burke, J. R.; Yang, X.; Gillooly, K. M.; McIntyre, K. W.; Chen, L.; Dodd, J. H.; McKinnon, M.; Barrish, J. C.; Pitts, W. J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2646; (f) Spergel, S. H.; Kempson, J.; Guo, J.; Dyckman, A.; Li, T.; Watterson, S. H.; Langevine, C. M.; Das, J; Moquin, R. V.; Nirschl, D.; Van Kirk, K.; Burke, J. R.; Quesnelle, C.; Gill, P.; Belanger, D.; Marinier, A.; Dodier, M.; Martel, A.; Gilloly, K.; McIntyre, K. W.; Chen, L.; Darienzo, C. J.; Yang, Z.; Marathe, P.; Wang-Iverson, D.; Dodd, J.; McKinnon, M.; Barrish, J.; Pitts, W. J. 238th ACS National Meeting, Washington, DC, United States, August 16–20, 2009; MEDI-454.; (g) Gillooly, K. M.; Pattoli, M. A.; Taylor, T. L.; Chen, L.; Cheng, L.; Gregor, K. R.; Whitney, G. S.; Susulic, V.; Watterson, S. H.; Kempson, J.; Pitts, W. J.; Booth-Lute, H.; Yang, G.; Davies, P.; Kukral, D. W.; Strnad, J.; McIntyre, K. W.; Darienzo, C. J.; Salter-Cid, L.; Yang, Z.; Wang-Iverson, D. B.; Burke, J. R. *J. Pharmacol. Exp. Ther.* **2009**, 349.
5. IKK enzymatic activity assay. Assays measuring the enzyme-catalyzed phosphorylation of GST-IκBα were performed by adding enzyme (IKK-2, typically to a final concentration of 3 μg/ml) at room temperature to solutions of 50 μg/mL GST-IκBα and 20 μM ATP in 25 mM Tris·HCl, pH 7.5, containing 7.5 mM $MgCl_2$, 34 mM sodium phosphate, 3 mM NaCl, 0.6 mM potassium phosphate, 1 mM KCl, 1 mM dithiothreitol, 5% (w/v) glycerol, and 470 μg/mL bovine serum albumin. After 60 min, the kinase reactions were stopped by the addition of EDTA to 33 mM. IκBα phosphorylation was quantitated by competition for binding to an anti-phospho-IκBα antibody (SantaCruz Biotechnology #sc-8404) with fluorescein-labeled phospho-peptide ([FL]-Asp-Asp-Arg-His-Asp-[p]Ser-Gly-Leu-Asp-Ser-Met-Lys-NH2) as measured using fluorescence polarization.
6. Experimental procedure for PBMC assay: Heparinized human whole blood was obtained from healthy volunteers. Peripheral blood mononuclear cells (PBMCs) were purified from human whole blood by Ficoll-Hypaque density gradient centrifugation and re-suspended at a concentration of 5×10^6/mL in assay medium (RPMI medium containing 10% fetal bovine serum). Fifty microliters of cell suspension was incubated with 50 μl of test compound (4× concentration in assay medium containing 0.2% DMSO) in 96-well tissue culture plates for 5 min at room temperature. 100 μl of LPS (200 ng/mL stock) was then added to the cell suspension and the plate was incubated for 6 h at 37 °C. Following incubation, the culture medium was collected and stored at −20 °C. TNF-α concentration in the medium was quantified using a standard ELISA kit (Pharmingen-San Diego, CA). Concentrations of TNF- and IC_{50} values for test compounds (concentration of compound that inhibited LPS-stimulated TNF-α production by 50%) were calculated by linear regression analysis.