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Synthesis, initial SAR and biological evaluation of 1,6-dihydroimidazo[4,5-*d*]pyrrolo[2,3-*b*]pyridin-4-amine derived inhibitors of IκB kinase

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ABSTRACT

A new series of tricyclic-based inhibitors of IKK have been derived from an earlier lead compound. The synthesis and structure–activity relationships (SAR) are described. Compound **4k** inhibited TNF production in rats stimulated with LPS.

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The nuclear transcription factor NF-κB has a crucial role in the pathogenesis of several human disorders, particularly those with an inflammatory component.[1] In unstimulated cells, NF-κB is sequestered in the cytoplasm as an inactive complex with IκB inhibitory protein.[2] In the case of IκBα, the most extensively studied member of this protein family, the stimulation of pro-inflammatory receptors such as the TNF receptor activates the so-called 'canonical' pathway of NF-κB activation, in which a multi-subunit IκB kinase (IKK) complex catalyses the phosphorylation of IκBα at Ser32 and Ser36. This phosphorylation event is essential for signaling the subsequent ubiquitination and protosomal degradation of IκBα, thus leaving the NF-κB free to translocate to the nucleus and activate pro-inflammatory gene transcription.[3]

The IKK complex is comprised of two catalytic subunits, IKK1 and IKK2. Although both of these subunits can catalyse the phosphorylation of IκB protein, evidence has shown that the IKK2 subunit plays a major role in NF-κB activation arising from pro-inflammatory stimuli such as tumor necrosis factor TNF-α, IL1-β, IL-6, IL-17 and IL-23. Since IKK2 is vital for translating these pro-inflammatory stimuli into the activation of NF-κB, an inhibitor of IKK2 could be effective in the treatment of autoimmune and inflammatory disorders such as rheumatoid arthritis, inflammatory bowel disease, lupus and multiple sclerosis.[4] Interestingly, the activity of glucocorticoids, which are among the most effective anti-inflammatory agents available, is mediated primarily through the inhibition of NF-κB mediated transcription. This suggests that an IKK2 inhibitor would provide an opportunity to develop novel therapeutics which has the potential for glucocorticoid-like activity but without their use-limiting toxicities.[5]

The discovery of small molecule IKK inhibitors as orally active therapeutic agents for the treatment of inflammatory diseases has been the goal of numerous research groups, and many classes of IKK inhibitors have been reported.[6] We recently reported the identification of several tricyclic based inhibitors of IKK2 (Fig. 1), **1a**, **1b** and **1c**.[6h]

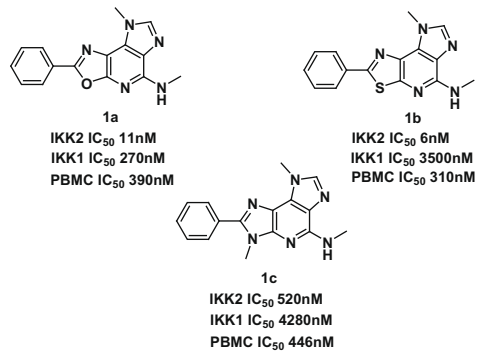

Figure 1. Tricyclic inhibitors of IKK2.

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The previously reported chemistry effort[6h] was greatly facilitated by using a common intermediate, **2,** which allowed our lab to build upon the SAR observations established across these, and other, earlier chemotypes. As part of a multi-chemotype strategy to isosteric inhibitors of IKK kinase, we sought to extend our earlier work and proposed an 'azaindole' core **4**. A synthetic route was devised that relied upon a 5-*endo*-dig cyclisation[7] from an appropriately substituted acetylene precursor **3** (Fig. 2). Since we had an abundant supply of **2** we devised a route to **3** which could take advantage of this advanced intermediate.

The synthetic pathway utilized in the preparation of the azaindole system is outlined in Scheme 1. The amine functionality in the common intermediate, **2**, was removed by diazotization followed by reduction with hypophosphorous acid to give the compound **5**, in quantitative yield. Regiospecific displacement of one of the chloro groups with methylamine followed by Boc-protection led to **7** in 68% yield over two steps. All attempts to displace the remaining chloropyridine in **7** with a variety of nitrogen nucleophiles failed, even under forcing microwave conditions. After some experimentation it was discovered that Buchwald amination conditions using benzophenone imine[8] as the nitrogen source in combination with $Pd_2(dba)_3$, Xanthphos and cesium carbonate in DMF led to an excellent 95% yield of **8**. Mild hydrolysis of the imine in **8** was achieved with 1 M aqueous HCl in THF to reveal the 2-amino pyridine **9**. This was then reacted with exactly 1 equiv of NIS in acetonitrile to give the late stage iodo-intermediate **10** in 80% yield. Sonagashira coupling of **10** with phenyl acetylene in the presence of a catalytic amount of $PdCl_2(PPh_3)_2$ and CuI using

DIPA as base provided **11a** (R = H) in 75% yield. Formation of the 'azaindole' ring was effected with potassium *tert*-butoxide[7] to provide the desired tricyclic system, **12a** (R = H). Final deprotection using 4 M HCl in dioxane produced the desired compound **4a**.

The analogs reported in Table 1 were synthesized in a similar manner using either commercially available acetylenes or coupling partners synthesised according to a general two-step procedure as illustrated in Scheme 2 for 3-ethynyl-benzonitrile.

In the case of **15**, further elaboration was carried out as highlighted in Scheme 3 to afford final compound **18**. The nitrile in **17** was reduced under hydrogen using Ra-Ni as catalyst. This provided the benzylamine **19**, which then formed the basis for subse-

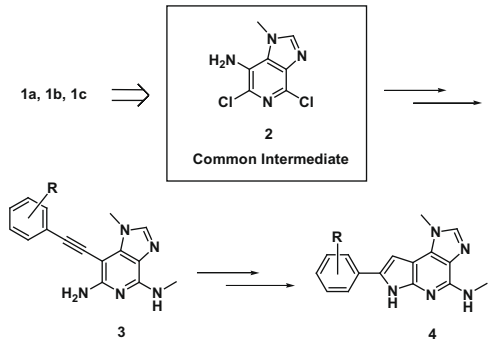
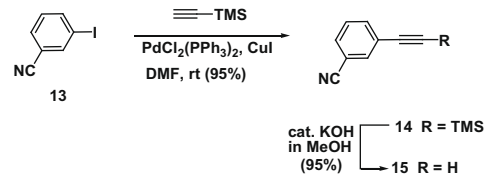
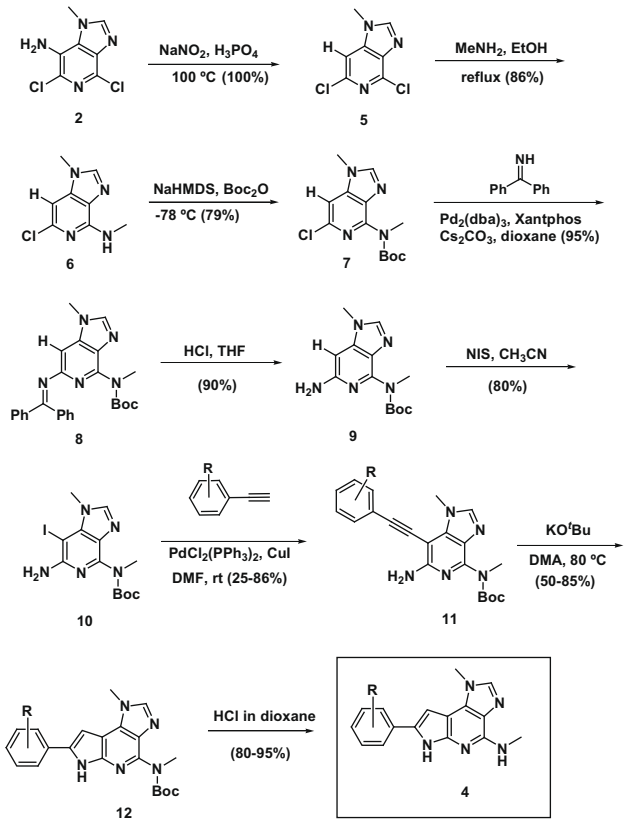


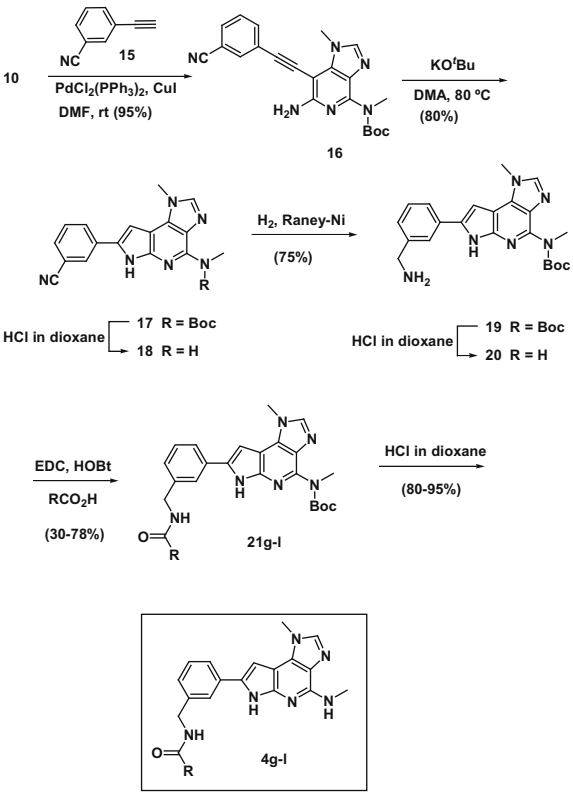
Figure 2. Proposed azaindole core from common intermediate, **2**.



Scheme 2. Synthesis of acetylene coupling partners.

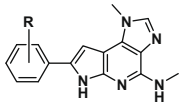


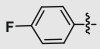
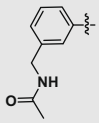
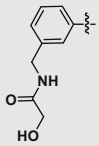
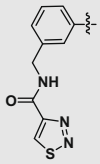
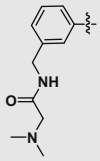
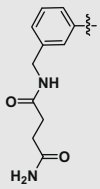
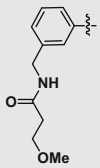
Scheme 1. General azaindole synthetic route.



Scheme 3. Azaindole scaffold amenable to further derivatization.

Table 1
SAR with respect to IKK and PBMC inhibition



Compd	R	IKK2 IC_{50} (nM)	IKK1 IC_{50} (nM)	PBMC IC_{50} (nM)
4a		40	1670	800
4b		44	NT[a]	931
4c		7	NT[a]	153
4d		43	1730	538
4e		8	NT[a]	320
18		7	NT[a]	86
20		11	NT[a]	340
4f		14	633	372
4g		25	NT[a]	478
4h		39	150	NT[a]
4i		79	NT[a]	2000
4j		22	NT[a]	1000
4k		12	2620	370

[a] NT = not tested.

quent coupling with a variety of carboxylic acids under standard carbodiimde mediated conditions. Final deprotection of the Boc protecting group with HCl in dioxane provided the compounds **4g–l**.

Compounds were evaluated for their ability to inhibit IKK1 or IKK2 catalyzed phosphorylation of GST-IκBα fusion protein[9] and a peripheral blood mononuclear cell assay (PBMC) was used to measure the ability of compounds to inhibit LPS-induced TNFα production in human primary cells.[10] The structure–activity relationships for the inhibition of IKK and PBMC activity are summarized in Table 1.

A direct comparison between the previous tricyclic systems (**1a–c**) and the first azaindole compound, **4a**, shows nearly a sevenfold loss in IKK2 activity against the most potent thiazole compound **1b**, with ~2-fold loss in PBMC activity compared to all three previous tricycles. Fluorine substitution (**4b**, **4c**, **4d**) gave essentially equipotent inhibitors to **4a** for *ortho*- and *para*-substitution. The *meta*-substituted analog, **4c**, however displayed almost a 6-fold increase in IKK2 and PBMC potency over **4a**. *Meta* methoxy compound **4c** possessed similar potency. The *meta*-nitrile compound **18** proved to be optimal with potent IKK2 inhibition and excellent translation into activity in cells. Unfortunately the physical characteristics of this compound precluded its evaluation in vivo (e.g., poor solubility <5 μg/mL). An attempt to improve the physicochemical properties began with the benzylamine analog, **20**, displaying potent IKK2 inhibition and modest PBMC activity. Derivatization of **20** provided a range of amide analogs containing alkyl (**4f**), alcohol (**4g**), heterocyclic (**4h**), amine (**4i**), amide (**4j**) and ether (**4k**) functionalities. Despite the wide tolerance of functionality by the enzyme, cell potency for **4i** and **4j** was significantly reduced compared to all other analogs studied. **4k** was the most potent IKK2 analog studied in the amide series. Based on its good IKK1 selectivity and acceptable PBMC activity, together with good physical characteristics imparted by the side chain (aqueous solubility at pH 1.0 = 1.823 mg/mL), it was decided to evaluate this compound further.

In a rat pharmacokinetic study (Table 2), **4k** was administered orally at 10 mg/kg and at 2 mg/kg intravenously. The compound had a favorable half-life ($t_{1/2}$) of 4.8 h, and a mean residence time (MRT) of 2.1 h. The measured oral bioavailability (F_{po}) in this experiment was acceptable at 31%. The steady-state volume of distribution (V_{ss}) was low as was the systemic clearance (Cl).

The in vivo efficacy of **4k** was next assessed in an acute rat model of TNFα inhibition.[11] Rats were dosed orally with **4k** at 3 mg/kg, 10 mg/kg and 30 mg/kg, 4 h prior to LPS administration. The TNFα levels were then measured 90 min later.

As shown in Figure 3, oral dosing of **4k** inhibited TNFα production in a dose-dependent manner. Statistically significant inhibition of TNF production (85%) was observed at an oral dose of 30 mg/kg. Given the rat whole blood IC_{50} value of 8 μM for **4k**, the exposure observed at the 30 mg/kg dose (17 μM) is consistent with this significant inhibition of TNF. In contrast, the 10 mg/kg dose was not effective, consistent with an exposure (3 μM) below the rat whole blood IC_{50}.[12]

Table 2
Rat PK[b] data for **4k** following iv and po dosing

Dose[a] (mg/kg)	AUC_{tot} (nM h)	$t_{1/2}$ (h)	MRT (h)	Cl (mL/min/kg)	V_{ss} (L/kg)	F_{po} (%)
iv, 2	46923	4.8	2.1	1.8	0.2	
po, 10	71747	11.0	14.7			31

[a] Vehicle: iv, propylene glycol/water (80/20); po 100% propylene glycol.
[b] n = 3 animals.

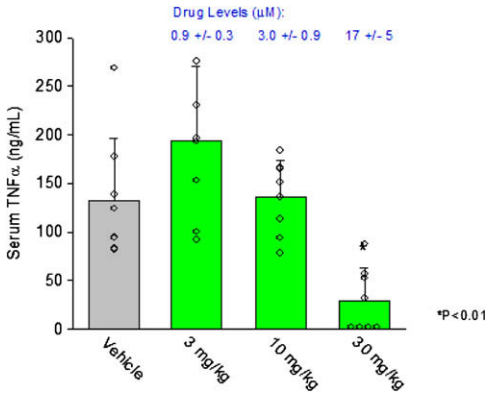


Figure 3. Inhibition of LPS-induced serum TNFα in rats ($n = 8$/group, mean ± SD).

In summary, we have reported the generation of a novel tricyclic scaffold utilizing the same common intermediate from previous efforts. This chemotype displays efficacy in an acute model of inflammation and current efforts to improve the whole blood potency of this series continues and will be reported at a later time.

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9. 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-NH_2) as measured using fluorescence polarization.
10. Human PBMCs were isolated from from whole blood collected from healthy donors. Blood was diluted into RPMI 1640 (Life Technologies) containing 2.5 mM EDTA (Life Technologies), 10 μg/mL polymyxin (Sigma), and then underlaid with ficoll (Accurate Scientific Co.) and centrifuged at 600*g* for 25 min. The interface was collected, and cells were washed twice and resuspended in RPMI, 10% FBS. Cells are then distributed (200 mL/well) into 96-well tissue culture treated plates (Falcon) at 1×10^6 cells/mL in RPMI, 10% FBS. Test compounds were added to appropriate wells and incubated with cells for 30 min. Cells were then stimulated by the addition of lipopolysaccharide (LPS, BioWhittaker), with a final concentration of 25 ng/mL, and incubated for 6 h at 37 °C, 5% CO_2. The cell supernatants were removed and assayed for TNF-α by ELISA (R&D Systems).
11. LPS-induced Serum TNF-α in Rats: Male Lewis rats (175–200 g, Harlan) were dosed by oral gavage with vehicle (50% PEG400, 50% 0.02 N HCl) or **4k** in vehicle. Four hours later, rats were injected intraperitoneally with 1 mL per rat of 100 μg lipopolysaccharide (LPS, *Escherichia coli* strain 0111:B4, Sigma-Aldrich, St. Louis, MO) suspended in phosphate buffered saline. Ninety minutes after LPS challenge, blood was collected from the retro-orbital sinus under isoflurane anesthesia. Serum was separated from clotted samples by centrifugation (5 min, 5000*g*×, room temperature) and analyzed for the levels of TNF-α by ELISA according to the manufacturer's instructions (BioSource, Camarillo, CA). Results are shown as mean ± SD of $n = 8$ rats per treatment group. Unless otherwise noted, statistical significance was determined using a one-way analysis of variance (ANOVA) with a Dunnett's post-test. Values of $p < 0.05$ were considered significant.
12. For rat whole blood experiments, blood was collected by cardiac puncture in collection tubes containing ACD-a as an anticoagulant. 300 μL of pooled whole blood was pipetted into deep-well 96 well assay plates (Costar), and inhibitor in phosphate buffered saline containing 16% (v/v) DMSO was then added to give a final DMSO concentration of 0.2% (v/v). After incubating 45 min at 37 °C, lipopolysaccahride (LPS, S.Typhosa, Sigma) was added to a final concentration of 5 μg /mL. After a 4 h incubation at 37 °C, the plates were centrifuged for 10 min at 14,000 rpm and plasma removed for TNFα measurement by ELISA (Biosource).