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Synthesis and structure–activity relationships of novel IKK-β inhibitors. Part 2: Improvement of in vitro activity

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Abstract—A series of 2-amino-3-cyano-4-alkyl-6-(2-hydroxyphenyl)pyridine derivatives was synthesized and evaluated as IκB kinase β (IKK-β) inhibitors. Substitution of an aminoalkyl group for the aromatic group at the 4-position on the core pyridine ring resulted in a marked increase in both kinase enzyme and cellular potencies, and provided potent IKK-β inhibitors with IC_{50} values of below 100 nM.

Previously we described the identification of 2-amino-3-cyano-4-aryl-6-(2-hydroxyphenyl)pyridines as small molecule inhibitors of IκB kinase β (IKK-β).[1] IKK-β is a serine–threonine protein kinase, which is critically involved in the activation of the transcription factor Nuclear Factor kappa B (NF-κB) in response to various inflammatory stimuli.[2] In general, inhibition of NF-κB activation results in strong anti-inflammatory effects similar or superior to those brought upon by steroids. Therefore, such IKK-β inhibitors are of potential use in disease indications such as asthma, atopic dermatitis, allergic rhinitis, and rheumatoid arthritis.

The initial structure–activity relationships (SAR) study[1] of our first lead compound **1** indicated that various functional groups were tolerated on the 4-phenyl ring without losing activity whereas the *ortho*-phenol and the 2-aminopyridine were demonstrated to be essential moieties for activity. Hence, the emphasis of our initial synthetic efforts was focused on a modification of the substituents on the 4-phenyl ring. The optimized analog **2** shows potent inhibitory activity against IKK-β ($IC_{50} = 600$ nM) and excellent selectivity versus other kinases such as IKK-α ($IC_{50} = 20$ μM), Syk and MKK4 ($IC_{50} > 20$ μM). Furthermore, compound **2** demonstrates significant in vivo activity in an acute model of cytokine release (LPS-induced TNFα production model in mice). However, further improvement of the in vitro activity, especially cellular activity, could not be accomplished by modification of the substituents on the 4-phenyl ring. Thus, our synthetic strategy shifted to replacing the 4-phenyl ring itself. Conveniently, the improved and facile procedure for the pyridine core ring synthesis offers ample possibilities for significant structural modifications at the 4-position (Fig. 1).

Herein, we report the synthesis and SAR of a series of 2-amino-3-cyano-4-alkyl-6-(2-hydroxyphenyl)pyridine analogs.

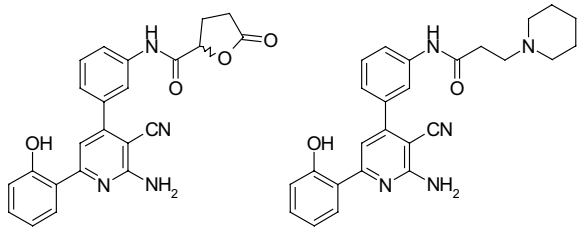

Figure 1. Initial lead compounds.

Keywords: IKK-β; NF-κB; Kinase inhibitor.
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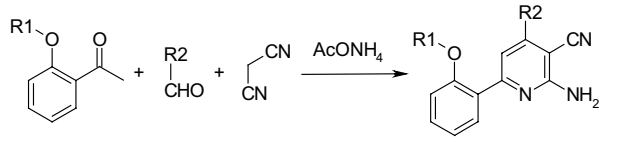
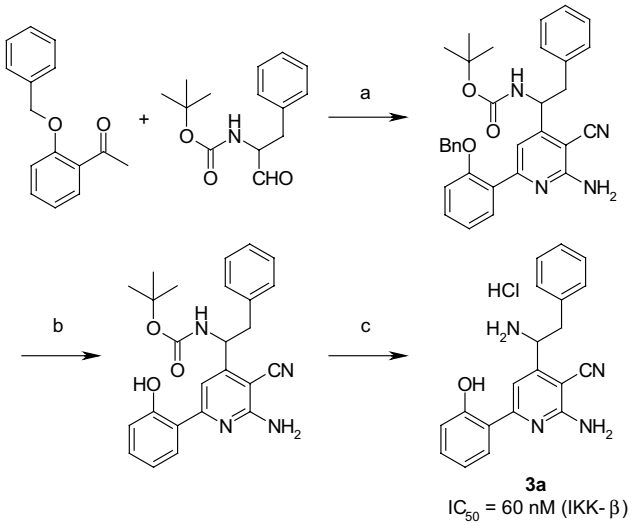
The 2-amino-3-cyano-4,6-diarylpyridine core structures can be simply constructed using a one-pot coupling reaction of four components, acetophenone, benzaldehyde, malononitrile, and ammonium acetate. Although only aromatic aldehydes were exemplified as the starting aldehyde in the reported procedure,[3] we found that various aliphatic aldehydes could also be used in the pyridine construction reaction using traditional and combinatorial chemistry approaches,[4] as shown in Scheme 1.

In our initial efforts to replace the substituted phenyl ring with an aliphatic group for the R2 moiety, compound **3**, which incorporates a primary amine was found to be 25-fold more potent than the lead compound **1** in the IKK-β biochemical assay. Compound **3** was synthesized starting from commercially available *N*-Boc-phenylalaninal, as shown in Scheme 2. Although optically pure *N*-Boc-phenylalaninal was also used as the starting material, the desired chiral analog was not obtained because racemization of the asymmetric center readily occurred during the pyridine ring synthesis. For the sake of simplicity, all the analogs were evaluated as racemic mixtures.



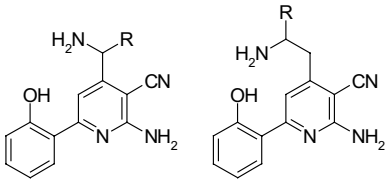
Scheme 1. Four component coupling reaction to construct the pyridine core ring. R1 = phenol protecting group (benzyl, *p*-methoxybenzyl, methoxyethoxy-methyl, *tert*-butyldimethylsilyl), or Wang resin. R2 = aryl or alkyl group.

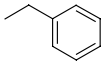
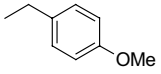
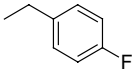
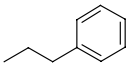


Scheme 2. Reagents and conditions: (a) malononitrile, ammonium acetate, toluene, 120 °C in a sealed vessel, 3 h, 49% yield; (b) 10% Pd–C, ethyl acetate, THF, H_2 at 3 atm, rt, 24 h, 89% yield; (c) 2.5 N HCl in 1,4-dioxane, rt, 3 h, quant.

We next focused our attention on the synthesis and SAR of the 4-aminoalkyl analogs prepared from *N*-Boc-protected glycinal and β-alaninal derivatives. Table 1 describes the SAR of these compounds. While compounds **3a**–**c** prepared from phenylalaninal derivatives exhibit potent IKK-β inhibitory activity with IC_{50} values below 100 nM, compounds **3e**–**g** derived from aliphatic amino acids also maintain moderate activity, suggesting that the aromatic ring is not so important for activity. Similar results are also observed with the 4-aminoethylpyridine analogs **4a**–**c**. The simple aminoethyl analog **4a** is more potent than the phenyl-substituted analog **4c**. The IKK-β inhibitory activity could be improved by introduction of the primary aminoalkyl moiety at the 4-position, and these analogs are more potent than the natural protein kinase inhibitor staurosporine[5] in our assay. Nevertheless, no significant improvement in cellular potency (TNFα-induced RANTES production) was achieved. We next sought to investigate the SAR of 4-aminoalkylpyridine analogs using not only amino acid moieties but also other aminoalkyl aldehydes as the starting material for the pyridine core synthesis.

Table 1. SAR of 4-aminoalkylpyridine analogs



Compds	–R	IKK-β[a] IC_{50} (nM)	RANTES[b] IC_{50} (nM)
1		1500	8000
2		600	7000
3a		60	8000
3b		80	5000
3c		40	5000
3d		730	15,000
3e	$-CH_3$	150	4000
3f	*n*-Butyl	480	15,000
3g	*iso*-Propyl	240	5000
4a	–H	70	800
4b	$-CH_3$	470	3000
4c		110	2000
Staurosporine		250	Not done

[a] Enzyme inhibition assay using recombinant human IKK-β.
[b] ELISA assay measuring TNFα-induced RANTES production in A549 cells.

As a result, 4-(3-piperidinyl)pyridine analog **5a** was found to have a desirable in vitro biological profile (IKK-β $IC_{50} = 25$ nM). Figure 2 describes the SAR of the piperidine analogs. Change of the substitution position (**5b** and **5c**), aromatization (**5d**) or *N*-methylation (**5e**) of the piperidine nitrogen result in a decrease of activity, suggesting that the piperidine NH is necessary for high in vitro potency. However, the 4-piperidine analog **5b** shows cellular activity comparable to the 3-piperidine analog **5a**. Although the *N*-methylation of the 2-amino moiety (**5f**) leads to a drastic loss in activity, the *N*-acetylation (**5g**) maintains moderate activity. Removal of the phenolic hydroxide (**5h**) results in a complete loss of activity. The same result has been observed in initial SAR of the lead compound **1** and ascribed to the hydrogen-bonding interaction between the phenolic hydroxide and the pyridine nitrogen atom, which is hypothesized to retain an important conformation for potent IKK-β inhibitory activity. Compound **5i**, incorporating a fluorine atom at the 5-position of the pyridine core, maintains moderate activity, suggesting that the fluorine atom does not significantly interrupt the hydrogen bonding.

In this piperidine series, compound **5a** was found to be the most promising. Compound **5a** exhibits good selectivity versus other kinases such as IKK-α ($IC_{50} =$ 450 nM) and MKK4 ($IC_{50} > 20$ μM), and excellent activity in various cellular assays such as TNFα-induced RANTES production in A549 cell ($IC_{50} =$ 300 nM), anti-CD3/anti-CD28-induced IL-2 production in Jurkat T-cell ($IC_{50} = 600$ nM) and LPS-induced TNFα production in human PBMC ($IC_{50} =$ 400 nM).

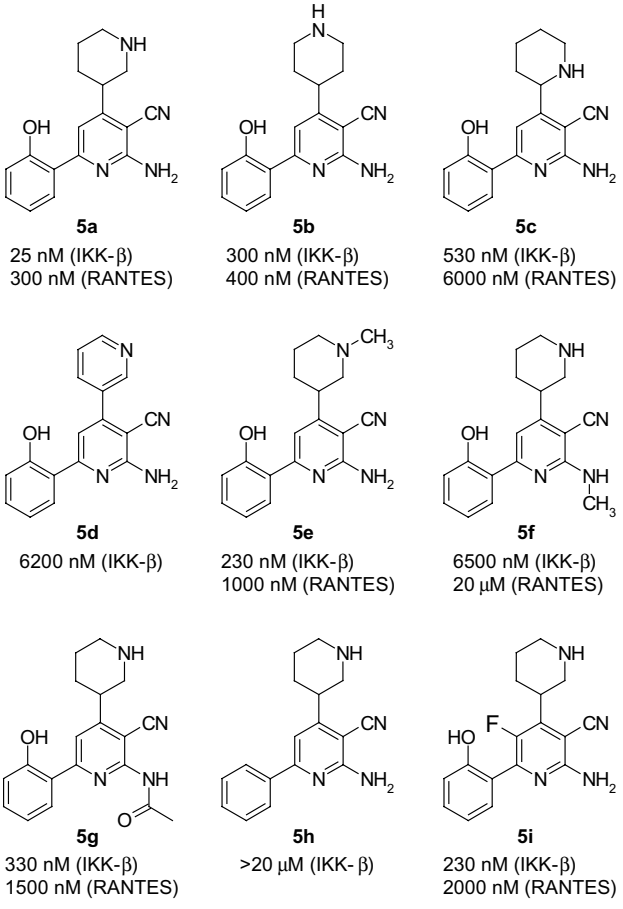
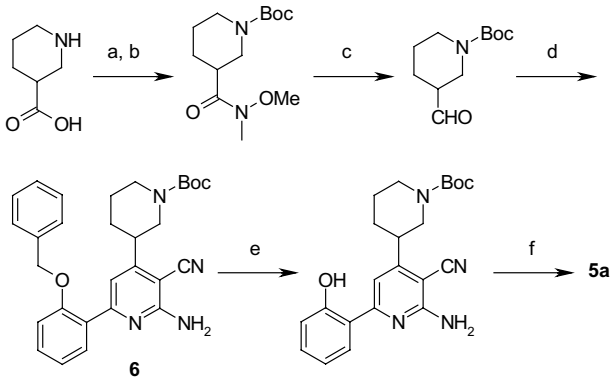


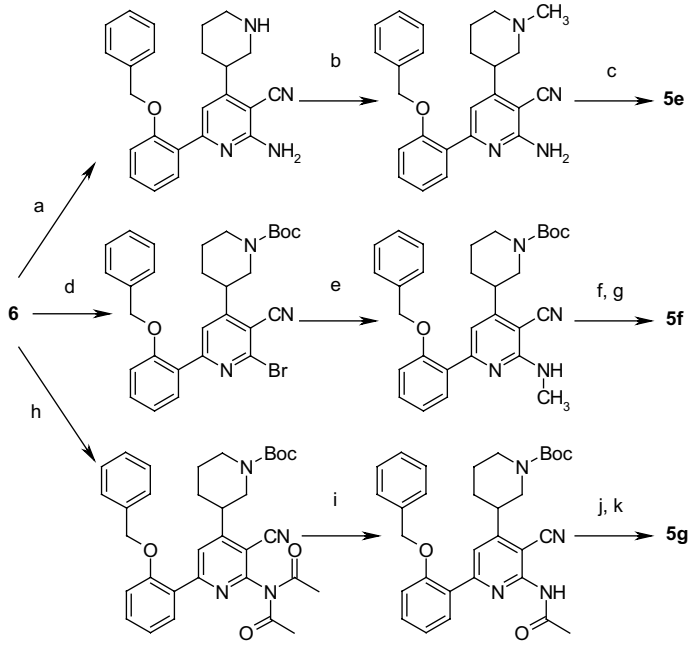
Figure 2. SAR of piperidine analogs (IC_{50} values).



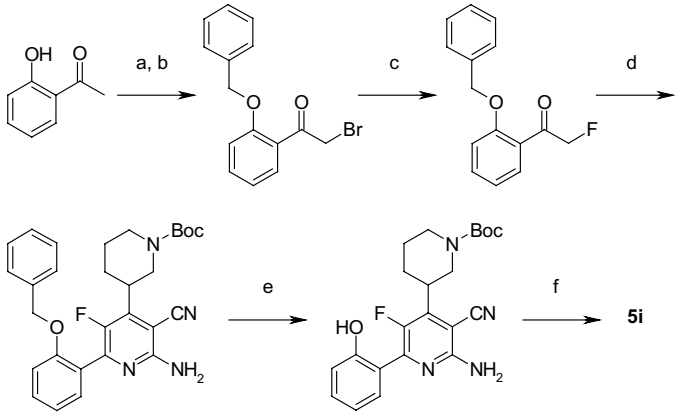
Scheme 3. Reagents and conditions: (a) di-*tert*-butyl dicarbonate, 2 N NaOH, 1,4-dioxane, rt, 12 h, 88% yield; (b) *N*,*O*-dimethylhydroxylamine hydrochloride, PyBOP, Et_3N, CH_2Cl_2, rt, 12 h, quant.; (c) $LiAlH_4$, THF, −15 °C, 30 min, then 1 N $KHSO_4$ aq, quant.; (d) 2′-benzyloxyacetophenone, malononitrile, ammonium acetate, toluene, 150 °C in a sealed vessel, 2 h, 34% yield; (e) 10% Pd–C, ethyl acetate, THF, H_2 at 3 atm, rt, 24 h, 89% yield; (f) 0.5 N HCl in 1,4-dioxane, rt, 12 h, 53% yield.

The synthesis of compound **5a** is depicted in Scheme 3. The key step of piperidine analogs **5a**–**i** is the one-pot coupling reaction of four components. The starting aldehyde, *N*-Boc-3-formylpiperidine, was prepared by a reduction of the corresponding Weinreb amide using $LiAlH_4$. The syntheses of the *N*-methylated compounds (**5e** and **5f**) and the *N*-acetyl compound **5g** were conducted starting from the key intermediate **6**,[6] as described in Scheme 4. The methyl group on the piperidine nitrogen was introduced by a reductive alkylation of the piperidine nitrogen using formalin and $NaBH_3CN$ to provide compound **5e**. The synthesis of the 2-alkylaminopyridine analog **5f** was achieved by a Sandmeyer reaction using $CuBr_2$ and *tert*-butyl nitrite followed by a nucleophilic attack of methylamine onto 2-bromopyridine. Reaction of compound **6** with acetyl chloride in pyridine gave the diacetylated analog, which was then treated with dilute ammonium hydroxide solution to cleave an acetyl bond to provide mono-acetyl analog **5g**. The fluoropyridine analog **5i** was synthesized from 2-fluoroacetophenone as the starting material for the pyridine synthesis, as shown in Scheme 5.

In summary, replacement of the substituted phenyl with an aminoalkyl moiety at the 4-position of the pyridine core ring resulted in a marked increase in in vitro activity. The optimized piperidine analog **5a** exhibited not only selective and potent inhibition of IKK-β kinase but also reasonably potent activity in a variety of relevant cellular assays.



Scheme 4. Reagents and conditions: (a) 2 N HCl in 1,4-dioxane, rt, 5 h, quant.; (b) formaldehyde, $NaBH_3CN$, water, methanol, rt, 2 h, 74% yield; (c) 10% Pd–C, ethyl acetate, THF, H_2 at 3 atm, rt, 24 h, 50% yield; (d) $CuBr_2$, *tert*-butyl nitrite, acetonitrile, 65 °C, 2 h, 45% yield; (e) methylamine hydrochloride, Et_3N, DMSO, 40 °C, 12 h, 58% yield; (f) 10% Pd–C, ethyl acetate, THF, H_2 at 3 atm, rt, 24 h, 82% yield; (g) 2 N HCl in 1,4-dioxane, rt, 5 h, quant; (h) acetyl chloride, pyridine, rt, 5 h; (i) 1 N ammonium hydroxide, THF, rt, 1 h, 80 % yield in two steps; (j) 10% Pd–C, ethyl acetate, THF, H_2 at 3 atm, rt, 12 h, 31% yield; (k) 2 N HCl in 1,4-dioxane, rt, 5 h, quant.



Scheme 5. Reagents and conditions: (a) benzylbromide, K_2CO_3, acetone, reflux, 18 h, 88% yield; (b) Br_2, ether, rt, 30 min, 92% yield; (c) tetrabutylammonium hydrogen difluoride, pyridine, THF, reflux, 22 h, 33% yield; (d) malononitrile, *N*-Boc-3-formylpiperidine, ammonium acetate, xylene, 120 °C, 3 h, 25% yield; (e) 10% Pd–C, ethyl acetate, THF, H_2 at 3 atm, rt, 12 h, 34% yield; (f) 2 N HCl in 1,4-dioxane, rt, 5 h, quant.

References and notes

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6. A typical procedure for the four component coupling to construct the pyridine analog (synthesis of compound **6**): A mixture of 2′-benzyloxyacetophenone (2.60 g, 11.5 mmol), *tert*-butyl 3-formyl-1-piperidine carboxylate (4.90 g, 23.0 mmol), malononitrile (1.52 g, 23.0 mmol), ammonium acetate (4.43 g, 57.5 mmol), and toluene (12 mL) in a sealed vessel was stirred at 150 °C for 2 h. After cooled to room temperature, the reaction mixture was partitioned between ethyl acetate and water. The organic phase was separated, washed with water and brine, dried over Na_2SO_4, filtered,

and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (hexane/ethyl acetate, 2/1) to give a yellow oil, which was then recrystallized from ethanol to give the desired product as a white solid (1.90 g, yield; 34% yield). For further details of the syntheses, see: Murata, T.; Umeda, M.; Sakakibara, S.; Yoshino, T.; Sato, H.; Masuda, T.; Koriyama, Y.; Shimada, M.; Shintani, T.; Kadono, H.; Ziegelbauer, K. B.; Fuchikami, K.; Komura, H.; Lowinger, T. B. WO 0224679, 2002; *Chem Abstr*. **2002**, *136*, 279345.