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Synthesis and structure–activity relationships of novel IKK-β inhibitors. Part 3: Orally active anti-inflammatory agents

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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. Modification of a novel IKK-β inhibitor **1** (IKK-β IC_{50} = 1500 nM, Cell IC_{50} = 8000 nM) at the 4-phenyl ring and 6-phenol group on the pyridine core ring resulted in a marked increased in biological activities. An optimized compound, 2-amino-6-[2-(cyclopropylmethoxy)-6-hydroxyphenyl]-4-piperidin-4-yl nicotinonitrile, exhibited excellent in vitro profiles (IKK-β IC_{50} = 8.5 nM, Cell IC_{50} = 60 nM) and a strong oral efficacy in in vivo anti-inflammatory assays (significant effects at 1 mg/kg, po in arachidonic acid-induced ear edema model in mice).

1. Introduction

IκB kinase β (IKK-β) is a 756 amino acid-containing serine-threonine protein kinase.[1] As part of the IKK-complex, IKK-β is critically involved in the activation of the transcription factor Nuclear Factor kappa B (NF-κB) in response to various inflammatory stimuli.[2] NF-κB is an inducible transcription factor that is thought to be a pivotal target for drugs for cancer and chronic inflammatory diseases. Herein, we report the synthesis and the structure–activity relationship (SAR) study of a novel class of small molecule IKK-β inhibitors.

In the preceding communications,[3,4] we described the SAR study of a novel IKK-β inhibitor **1** identified via screening of the Bayer compound library (Fig. 1). First we found that various functional groups were tolerated on the 4-phenyl ring without losing activity.[3] Moreover, replacing the substituted phenyl ring at the 4-position of the pyridine ring with an aliphatic group incorporating a primary amine resulted in a marked increase of the

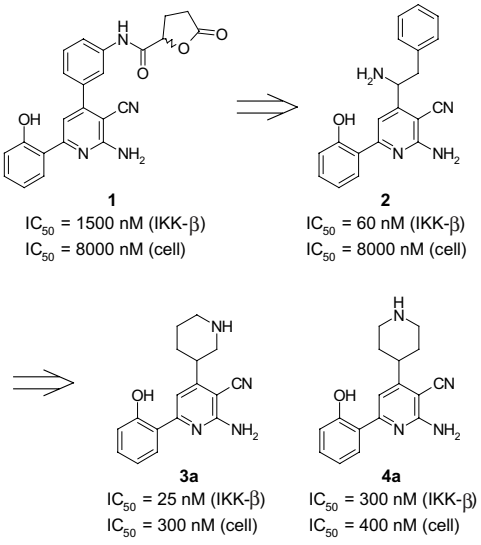

Figure 1. Initial lead compound **1** and optimized compounds.

Keywords: IKK-β; NF-κB; Kinase inhibitor.
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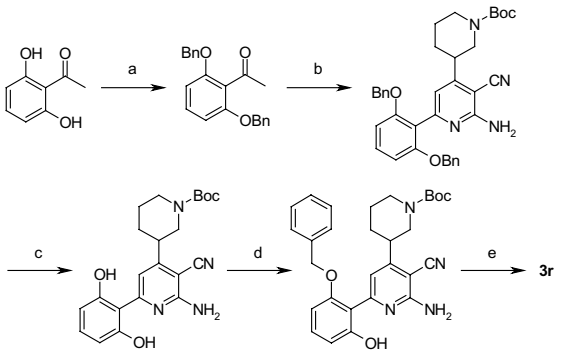
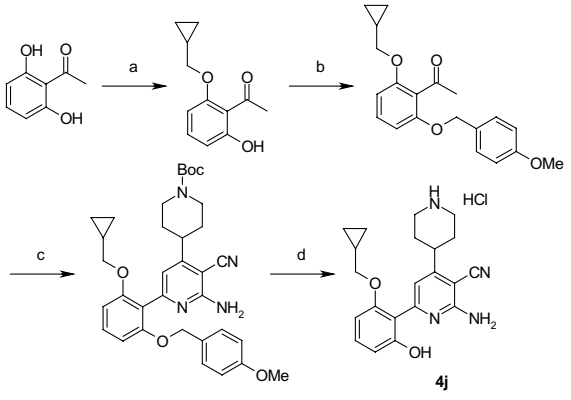
IKK-β inhibitory activity, albeit with no improvement in the cellular activity (TNFα-induced RANTES production in A549 cells), as exemplified by compound **2** derived from a phenylalanine derivative.[4] In order to improve the cellular activity, the 4-aminoalkyl group was further optimized, and 4-piperidin-3-ylpyridine analog **3a** was identified as a potent IKK-β inhibitor with excellent cellular activities.[4] However, the bioavailability of compound **3a** in mice and rats was less than satisfactory. Thus, we continued the modification of compound **3a** to discover highly potent and orally active IKK-β inhibitors with anti-inflammatory effect.

2. Chemistry[5]

The 2-amino-6-aryl-3-cyano-4-piperidinylpyridine core structures can be easily constructed using a one-pot coupling reaction of four components, acetophenone, *N*-Boc-formylpiperidine, malononitrile and ammonium acetate, as exemplified in Schemes 1 and 2.



Scheme 1. Reagents and conditions: (a) benzylbromide, K_2CO_3, NaI, acetone, reflux, 18 h, 46% yield; (b) *N*-Boc-3-formylpiperidine, malononitrile, ammonium acetate, 1,4-dioxane, 120 °C in a sealed vessel, 12 h, 37% yield; (c) 10% Pd–C, acetic acid, ethyl acetate, H_2 at 3 atm, rt, 2 days, 76% yield; (d) benzyl bromide, K_2CO_3, acetone, THF, rt, 2 days, 65% yield; (e) 2 N HCl in 1,4-dioxane, rt, 18 h, 88% yield.



Scheme 2. Reagents and conditions: (a) (bromomethyl)cyclopropane, K_2CO_3, acetone, 50 °C, 2 days, 83% yield; (b) 4-methoxybenzyl chloride, tetrabutylammonium iodide, acetone, reflux, 18 h, 89% yield; (c) *N*-Boc-4-formylpiperidine, malononitrile, ammonium acetate, 1,4-dioxane, 110 °C, 3 h, 42% yield; (d) 2 N HCl in 1,4-dioxane, rt, 10 h, quant.

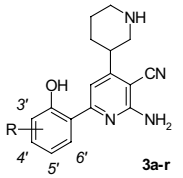
3. Results and discussion

In our initial SAR study, the in vitro activity was improved readily by the modification of the substituent at the 4-position on the pyridine core ring. In order to identify more potent and orally active IKK-β inhibitors, our synthetic strategy was next shifted to optimization of the *ortho*-phenol group of the compound **3a**, which would be readily implemented using commercially available substituted 2′-hydroxyacetophenone derivatives by the usual synthetic procedure. In addition, since the initial SAR study indicated that the phenol hydroxide was an essential moiety for activity, we focused on the optimization of the substituents on the *ortho*-phenol.

Table 1 describes the SAR of 4-piperidin-3ylpyridine analogs **3a**–**r** for optimization of the *ortho*-phenol group. Addition of a methyl substituent at the 3′- or 6′-position (**3b**,**e**) to the parent compound **3a** resulted in a drastic loss of activity. Although the addition of a methoxy substituent at the 3′- or 4′-position (**3c**–**d**) also resulted in a loss of activity, the IKK-β inhibitory and cellular activities were maintained when the methoxy substituent was introduced at the 6′-position (**3h**). However, the corresponding 6′-amino analog **3f** had no activity.

Thus, we further concentrated on the optimization of the alkoxy moiety at the 6′-position of the *ortho*-phenol group, as in **3g**–**r**. The 6′-hydroxy analog **3g** exhibited

Table 1. SAR of 4-piperidin-3-ylpyridine analogs **3a**–**r**



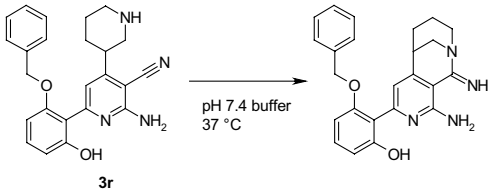
Compd	–R	IC_{50} (nM)	
		IKK-β[a]	Cell[b]
3a	–H	25	300
3b	3′-CH_3	1300	7000
3c	3′-OCH_3	20,000	Nd
3d	4′-OCH_3	560	2500
3e	6′-CH_3	2400	8000
3f	6′-NH_2	7800	>10,000
3g	6′-OH	15	200
3h	6′-OCH_3	34	300
3i	6′-OCH_2CH_3	14	150
3j	6′-$O(CH_2)_2CH_3$	5	80
3k	6′-$O(CH_2)_4CH_3$	6	300
3l	6′-$O(CH_2)_6CH_3$	14	1500
3m	6′-$OCH(CH_3)_2$	81	800
3n	6′-$OCH_2CH(CH_3)_2$	5	150
3o	6′-OCH_2-cyclopropyl	3	80
3p	6′-OCH_2-cyclobutyl	4	80
3q	6′-OCH_2-cyclohexyl	26	900
3r	6′-OCH_2-phenyl	9	300

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

somewhat better activity than the parent compound **3a**. In a series of compounds with a straight-chain alkoxy moiety (**3h**–**l**), *n*-propoxy analog **3j** demonstrated the most potent activity. The longer alkoxy chain has a beneficial effect on the IKK-β inhibitory activity but a negative effect on cellular activity, as in **3k**–**l**. While introduction of a branch at the α-position on the ether moiety (**3m**) led to a decrease of activity, the β-branch compounds **3n**–**q** exhibited greatly potent activity. Among all such compounds, cyclopropyl and cyclobutyl analogs (**3o**,**p**) were the most potent inhibitors. We also found that various substituted benzylether moieties were tolerated to maintain the potent activity, as exemplified by compound **3r**.

The 4-piperidin-3-ylpyridine analogs with an alkoxy moiety at the 6′-position have potent in vitro activity, but do have poor pharmacokinetic profiles probably due to the intrinsic physicochemical instability. The 4-piperidin-3-ylpyridine analogs were prepared as the hydrochloric acid salts, which were stable in the solid state. However, the compounds were unstable under basic conditions and even in neutral buffer solution. For instance, compound **3r** was rapidly decomposed at 37 °C in a pH 7.4 isotonic buffer solution, and only 25% of the compound remained after 24 h. One of the major degradation products was a cyclized compound produced by a nucleophilic attack of the piperidine nitrogen onto the nitrile group, as exemplified in Scheme 3. Thus, we found that the combination of 4-piperidin-3-yl moiety with 3-nitrile moiety on the pyridine ring would cause physicochemical instability.

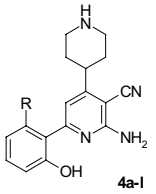
Next, we focused our attention on the synthesis and SAR of the 4-piperidin-4-ylpyridine analogs, because compound **4a** (Fig. 1) has only moderate IKK-β inhibitory activity but potent cellular activity comparable to the corresponding 4-piperidin-3-yl analog **3a**. In addition, no degradation of the 4-piperidine analogs was observed even under basic conditions because the reaction of the 4-piperidine nitrogen with the 3-nitrile moiety would be geometrically unfavourable. Table 2 describes the SAR of the 4-piperidin-4-ylpyridine analogs **4a**–**l**, which displays the similar tendency with that of the 4-piperidin-3-yl analogs **3a**–**r** shown in Table 1. Introduction of an alkoxy substituent at the 6′-position of the phenol (**4b**–**l**) resulted in an increase of activity relative to the parent compound **4a**. In this series, cyclopropyl analog **4j** and cyclobutyl analog **4k** exhibit the most potent IKK-β inhibitory and cellular activities.



3r

Scheme 3. Possible mechanism for degradation of 4-piperidin-3-ylpyridine analogs.

Table 2. SAR of 4-piperidin-4-ylpyridine analogs **4a**–**l**



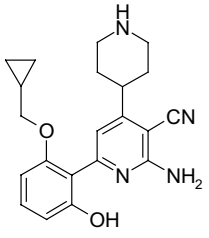
4a-l

Compd	–R	IC_{50} (nM)	
		IKK-β[a]	Cell[b]
4a	–H	300	400
4b	–OH	270	500
4c	$-OCH_2CH_3$	120	200
4d	$-O(CH_2)_2CH_3$	24	70
4e	$-O(CH_2)_3CH_3$	15	80
4f	$-O(CH_2)_4CH_3$	20	200
4g	$-O(CH_2)_5CH_3$	25	300
4h	$-O(CH_2)_6CH_3$	50	400
4i	$-OCH_2CH(CH_3)_2$	15	80
4j	$-OCH_2$-cyclopropyl	8.5	40
4k	$-OCH_2$-cyclobutyl	12	40
4l	$-OCH_2$-phenyl	110	1500

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

The cyclopropyl analog **4j** (IKK-β $IC_{50} = 8.5$ nM) moderately inhibits IKK-α with an IC_{50} of 250 nM but exhibits good selectivity towards other kinases, such as IKK3, Syk and MKK4 ($IC_{50} > 20{,}000$ nM). Moreover, compound **4j** demonstrates quite potent activity in various cellular assays, as shown in Table 3. Importantly, compound **4j** inhibited NF-κB-dependent reporter gene

Table 3. Inhibitory activities of compound **4j** in various kinase and cellular assays



4j

$IC_{50} = 8.5$ nM (IKK-β)
$IC_{50} = 250$ nM (IKK-α)
$IC_{50} > 20000$ nM (IKK3, Syk and MKK4)

Cell	Stimulus	Read-out	IC_{50} (nM)[a]
A549	TNFα	RANTES	40
HUVECS	TNFα	VCAM-1	30
HEK293	TNFα	NF-κB transactivation	130
Jurkat T-cell	PMA/Ca	NF-κB transactivation	147
Jurkat T-cell	PMA/Ca	NF-AT transactivation	>10,000
MRC5	PMA	AP-1 transactivation	>10,000
huPBMCs	LPS	TNFα	50
huPBMCs	LPS	IL-1β	96[b]
huPBMCs	LPS	IL-6	45[b]
Mouse B-cells	LPS	Proliferation	46[b]

[a] Values are means of more than three experiments.
[b] Results were obtained from MDS pharmacology services (www.mdsps.com).

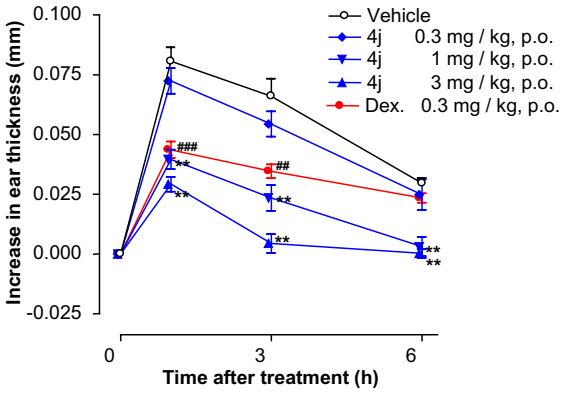


Figure 2. Oral efficacy of compound **4j** (blue) and dexamethasone (red) in arachidonic acid-induced mouse ear edema model:[7] Statistical differences between vehicle control and compound **4j** groups were analyzed using one-way ANOVA and Dunnett's method (**: $P < 0.01$, ***: $P < 0.001$). For dexamethasone, data were analyzed by Student's *t*-test (##, ###: $P < 0.01$, $P < 0.001$). Dex: dexamethasone.

activation in TNFα -activated HEK293 cells and PMA/calcium ionophore-activated Jurkat T cells. On the other hand, compound **4j** failed to inhibit PMA-induced AP-1 activation in MRC-5 cells and PMA/calcium ionophore-induced NF-AT dependent reporter gene transcription in Jurkat cells even at concentrations exceeding 10 μM. These results indicate that compound **4j** selectively interferes with the NF-κB signaling cascade by inhibition of IKK-β in living cells.

Compound **4j** has reasonable aqueous solubility (0.12 mg/mL in pH 7.4 isotonic buffer) and excellent Caco-2 permeability (P_{app} 62.3×10^{-7} cm/s), and demonstrates orally bioavailability in mice (BA: 16%) and rats (BA: 60%). The favourable bioavailability of compound **4j** in rats is likely due to its low clearance (0.33 L/h/kg).

Figure 2 describes oral efficacy of compound **4j** and dexamethasone in an acute inflammation model. The arachidonic acid-induced ear edema mechanistically depends on COX-2 expression, which is regulated by NF-κB activation.[6] Thus, this model is considered to be a suitable model of inflammation for the evaluation of IKK-β inhibitors. In this model, compound **4j** exhibited oral efficacy at 1 mg/kg in a dose-dependent manner. The effect was slightly stronger than that of dexamethasone (0.3 mg/kg).

In summary, we have investigated the SAR for IKK-β inhibition of 4-piperidinylnicotinonitrile analogs, **3a** and **4a**. Introduction of an alkoxy moiety at the 6′-position onto the *ortho*-phenol group resulted in a marked improvement of activity in IKK-β and cellular assays. The 4-piperidin-3-ylpyridine analogs **3** tend to be more potent than 4-piperidin-4-ylpyridine analogs **4** but have poor bioavailability due to the physicochemical instability. Thus, we have focused on the optimization of 4-piperidin-4-ylpyridine analogs **4**. As a result, compound **4j** has been identified as a highly potent inhibitor of IKK-β, which is orally bioavailable in mice and rats and demonstrates significant in vivo activity in anti-inflammatory models (arachidonic acid-induced mouse ear edema model).

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7. In vivo arachidonic acid-induced ear edema in mice: ear edema was induced by topical application of arachidonic acid (500 μg/ear). Compound **4j**, dexamethasone and vehicle (10% cremophor in saline) were given po 60 min before the arachidonic acid application. Ear thickness was measured at 0, 1, 3 and 6 h after the arachidonic acid application. Each column indicates the mean and SEM of 5 mice.