3,5-Disubstituted-indole-7-carboxamides as IKK β Inhibitors: Optimization of Oral Activity via the C3 Substituent

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Supporting Information

ABSTRACT: I κ B kinase β (IKK β or IKK2) is a key regulator of nuclear factor kappa B (NF- κ B) and has received attention as a therapeutic target. Herein we report on the optimization of a series of 3,5-disubstituted-indole-7-carboxamides for oral activity. In doing so, we focused attention on potency, ligand efficiency (LE), and physicochemical properties and have identified compounds **24** and (R)-**28** as having robust in vivo activity.

KEYWORDS: I κ B Kinase β , IKK β , IKK2, NF- κ B, Indole carboxamide, Cyclic sulfone, LPS neutrophilia, LPS-TNF α , Dog segmental LPS challenge

I κ B kinase β (IKK β or IKK2) plays a central role in a signaling cascade that ultimately leads to translocation of

Compound	1	2
IKK β pIC ₅₀	5.8	7.6
LE	0.44	0.36
Solubility (μ M)	37	22
PAMPA (nm/s)	370	250

Figure 1. Comparison of potency, ligand efficiency (LE), solubility, and parallel artificial membrane permeability (PAMPA) of early lead compounds¹⁰

nuclear factor kappa B (NF- κ B) to the nucleus of the cell where it is involved in the up-regulation of pro-inflammatory mediators, cytokines, and adhesion proteins.¹ Targeting NF- κ B remains a key strategy in the development of nonsteroidal anti-inflammatory therapeutics.^{2–4} In response to extracellular

Figure 2. Model of **2** with human IKK β based on the X-ray structure of human IKK β ^{13,14}

mediators like tumor necrosis factor- α (TNF α) and lipopolysaccharide (LPS), IKK β phosphorylates Ser32 and Ser36 of the protein inhibitory kappa B α (I κ B α).^{5–7} As a result, I κ B α is ubiquitinated by an E3 ligase and subsequently

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Table 1. Structure–Activity Relationships for Substituted Piperidine Sulfonamides

Entry	R ₁	IKK β pIC ₅₀ ^a	LE	Aqueous Solubility (μ M) ^b	PAMPA (nm/s) ^c
3		7.8	0.31	173	200
4		8.0	0.31	185	160
5		8.1 (n=1)	0.31	162	240
6		7.5	0.29	36	318
7		8.2	0.30	93	290
8		7.7	0.29	85	140
9		8.1	0.37	33	3
10		8.0	0.34	8	270
11		8.1	0.32	41	310

^aIKK β pIC₅₀ values are the mean of $n \geq 2$ determinations unless noted. ^bKinetic aqueous solubility was measured via HPLC with UV detection. PAMPA values are $n = 1$ determinations.

degraded by the 26S proteasome, revealing the nuclear translocation sequence on NF- κ B.¹ To date, no IKK β inhibitors have been approved for clinical use. This may be due to increased secretion of the pro-inflammatory cytokine IL1 β resulting from chronic IKK β inhibition.^{8,9} During our work, it became clear that inhibiting IKK β may be problematic, however, the potential benefits warranted optimization of a chemical series to achieve in vivo efficacy to allow exploration of its potential beneficial and adverse effects. In this case, we advanced a series of indolecarboxamides. The optimization of this series is described herein.

In a previous report, a novel series of potent, selective, and ATP competitive inhibitors of IKK β , namely the 3,5-disubstituted indole-7-carboxamides, was described.¹⁰ In this, selectivity was assessed against more than 45 kinases and pIC₅₀ values were <5.5 against the entire panel. Figure 1 shows two compounds described in the earlier work. Key elements of the series important for high binding affinity include a C3 sulfonamide, a C5 aromatic, and C7 primary carboxamide. The binding hypothesis includes a tridentate interaction between Y98 (NH) and C99 (CO, NH) and the indole NH and primary carboxamide as well as a hydrogen bond between K106 (NH₃⁺) and/or D103 (NH) and the sulfonamide oxygens. The C5 aromatic occupies a hydrophobic region of the ATP site. We noted that while 2 achieved good binding potency, the ligand efficiency (LE)^{11,12} had decreased relative to the less potent compound 1 lacking the C3 sulfonamide (Figure 1). Examining the binding of compound 2 (Figure 2), the C3 substituent projects into the solvent front and thus appeared suitable for modulating physicochemical properties. We optimized the C3 substituent to increase ligand efficiency and to balance physicochemical properties to develop a therapy for chronic obstructive pulmonary disease (COPD).

To improve the solubility, we designed analogues with amine substituents at the solvent front. As seen in Table 1, amines were introduced to either a 2- or 3-carbon alkyl sulfonamide. These gave similar potency to 2 but with no improvement in ligand efficiency. The basicity of the amine (see Supporting Information) did not appear to be important for potency (5 vs 6 and 10), supporting the idea that the C3 vector accesses the solvent front. Importantly, solubility was improved in the 3-carbon linked amines (3–8). In addition, we could modulate permeability as measured in our artificial membrane permeability assay (PAMPA).^{15,16} In this regard,

Table 2. Pharmacokinetic Profile of Lead Compounds^a

entry	monocyte IC ₅₀ (μ M) ^b	oral AUC (ng/mL·h)	Clp (mL/min/kg)	Vdss (L/kg)	T _{1/2} (min)	%F	PAMPA (nm/s)
2	0.19	1222 (1176, 1268)	16 (13, 18)	1 (1, 1)	69 (70, 68)	58 (47, 69)	250
4	0.05	998 (436, 1560)	4 (4, 4)	1 (1, 1)	276 (279, 272)	12 (6, 18)	160
6	0.20	2034 (2544, 1524)	8.9 (7.8, 9.9)	0.43 (0.37, 0.49)	71 (71, 71)	53 (60, 46)	318
9	0.14	48 $\pm$ 42	14 (15, 14)	3 $\pm$ 1	266 $\pm$ 43	3 $\pm$ 1	<3
10	0.50	2083 (2012, 2153)	7.43 (8.42, 6.44)	0.84 (1.06, 0.61)	105 (122, 88)	46 (51, 40)	270

^aPharmacokinetic profiling was done in male Sprague–Dawley rats ($n = 2$, with the exception of compound 9 where $n = 3$) using 5% DMSO and 20% Cavitron for the i.v. leg and 5% DMSO and 6% Cavitron for the oral leg. All compounds were dosed at 2 mg/kg for the oral leg with the exception of 10 which was dosed at 2.1 mg/kg. ^bValues are the mean of at least two determinations. Compound 10 had the greatest variability with IC₅₀'s of 0.88 and 0.12 μ M. See Supporting Information for assay details.

Table 3. Potency and LE for C3 Substituent Analogues

Entry	R1	IKK β pIC ₅₀ ^a	LE
12		6.9 (n=1)	0.41
13		7.2	0.42
14		7.1	0.41
15		7.4	0.42
16		6.3	0.35
17		6.7	0.35
18		7.7	0.41
19		6.3 (n=1)	0.35
20		7.1	0.39
21		7.0	0.34
22		7.4	0.36
23		7.1	0.35
24		8	0.42
25		6.8	0.37

^aIKK β pIC₅₀ values are the mean of $n \geq 2$ determinations unless noted. Compounds were tested as racemic mixtures.

less basic tertiary amines (**6**, **10**, **11**) gave higher permeability than either more basic (**3**, **4**), primary (**9**), or secondary amines (**8**). This permeability SAR was important for optimization of the series.

Next, we examined the cellular potency and pharmacokinetics (PK) of compounds **2**, **4**, **6**, **9**, and **10** (Table 2). The cell activity was determined by treating monocytes with

lipopolysaccharide (LPS) and measuring the inhibition of tumor necrosis factor alpha (TNF α), a product of NF- κ B transcription. Compound **4** was the most potent in cells. These leads possessed low clearance and low to moderate volumes of distribution in rat. The differences in bioavailability, however, were striking. We presumed the limiting factor was absorption given the low clearance and noted that compounds with <30% oral bioavailability and low oral AUC had low or moderate PAMPA values, while those with bioavailability >30% had high (>200) PAMPA values. This observation allowed us to use the PAMPA to prioritize compounds for PK evaluation. The permeability was not important for high cell potency (**9** vs **2**, **4**, **6**, **10**). While there were clear differences in solubility, this property did not appear to be important for obtaining good oral exposure a likely consequence of dosing the compounds as solutions.

To assess in vivo activity, we compared compound **6** to the previously reported **2**¹⁰ in an LPS induced neutrophilia model in rat. Both compounds were given orally. Compound **6** was administered at 50 μ mol/kg, while compound **2** was dosed at 100 μ mol/kg. While these compounds exhibited good in vitro potency and oral exposure, both were weakly active in vivo, giving approximately 30% inhibition of neutrophilia.

While these early data were disappointing, we sought to improve the in vivo activity by increasing the ligand efficiency as smaller molecular weight with equivalent potency might lead to better target exposure. As such, we aimed to increase the ligand efficiency by reducing molecular weight while retaining good potency, physicochemical properties, and pharmacokinetics. We explored several variations at C3 of the indole-7-carboxamide template. These are shown in Table 3. Initially, we incorporated a 2C linker to both a ketone (**12**) and methyl sulfone (**13**), resulting in moderate potency but improved ligand efficiency. Cyclization of the ketone to both the cyclopentanone (**14**) and γ -lactam (**15**) produced little potency improvement, and while the ligand efficiency for both these analogues was acceptable, the presumed poor metabolic stability of the ketone and moderate permeability of the lactam (PAMPA = 86 nm/s) precluded pharmacokinetic profiling. Methylation of the lactam (**16**) resulted in decreased potency. Interestingly, potency differentiation was obtained by incorporating a *gem*-dimethyl into the lactam system, with the 3,3-*gem*-dimethyl lactam (**18**) being 10-fold more potent than the corresponding 4,4-*gem*-dimethyl (**17**) system. Conformational search and docking revealed that the *gem*-dimethyl in **17** produces a very small perturbation to Arg20, and the loss of potency is driven by a polarity mismatch between the lipophilic *gem*-dimethyl and the polar Arg20 (see Supporting Information). The 4-cyclohexanone (**19**) showed moderate potency, while the 3-cyclohexanone (**20**) was similar in potency to the cyclopentanone (**14**) system. Like the cyclopentanone, the cyclic sulfone (**21**) also gave good potency and ligand efficiency. The sulfone is more likely to be metabolically stable than the ketone.

Expanding the cyclic sulfone to the [3.2.1] bicyclosulfone gave moderate potency with no gain in ligand efficiency, with both the *endo* (**22**) and *exo* (**23**) isomers. With the simplified six-membered ring with the sulfone in the 4-position (**24**), however, we achieved high in vitro potency. Importantly, **24** showed good ligand efficiency, cellular potency, lipophilicity (clogP = 1.6), and artificial membrane permeability (Table 4). The sulfone in the 3-position (**25**) gave similar potency

Table 4. In Vitro and Physicochemical Property Profile of Leads

	Entry	R1, R2, R3	IKK β pIC ₅₀ ^a	LE	Monocyte IC ₅₀ (μ M) ^a	Aqueous Solubility (μ M) ^b	PAMPA (nm/s) ^c
24	H, H, H	8.0	0.42	0.08	130	277
	26	F, H, H	7.9	0.4	0.05, 0.81	--	280
	27 (rac)	H, Me, Me	8.1	0.4	0.11 (n=1)	39	330
	(R)-28	F, Me, Me	8.1 (R)	0.38	0.13	32	283

^aValues are the mean of $n \geq 2$ determinations unless noted otherwise. ^bKinetic solubility was measured via HPLC using UV detection. For **27** and **(R)-28** chemiluminescent nitrogen detection (CLND) was used.¹⁸ ^cPAMPA values are $n = 1$ determinations except for **24** and **(R)-28** ($n = 3$).

Table 5. Pharmacokinetic Profile of Lead Compounds^a

entry	species	oral AUC (ng/mL·h)	Clp (mL/min/kg)	Vdss (L/kg)	T _{1/2} (min)	%F	PAMPA (nm/s)
24	rat	904 (866, 942)	35 (31, 39)	2.8 (2.6, 3)	94.8 (94.8, 94.2)	97 (104, 89)	277
	dog ^b	753 $\pm$ 219	23.6 $\pm$ 2.69	1.54 $\pm$ 0.22	0.949 $\pm$ 0.117	52.6 $\pm$ 11.8	
26	rat	1270 (1258, 1282)	23.5 (25.4, 21.6)	3.7 (3.2, 4.1)	132 (114, 150)	47.4 (51.2, 43.7)	280
	rat	1434 (1600, 1266)	51.1 (40.9, 61.3)	7.2 (7.4, 7)	140.4 (121.8, 159)	115 (104, 126)	330
(R)-28^c	rat	1961 (1543, 2379)	13.2 (14.9, 11.4)	4.7 (5.4, 4.0)	270 (282, 264)	94.1 (89.8, 99.7)	283
	dog ^b	8203 $\pm$ 153	1.86 $\pm$ 0.89	2.87 $\pm$ 0.06	20.9 $\pm$ 7.24	65.3 $\pm$ 15.0	

^aPharmacokinetic profiling was done in male Sprague–Dawley rats ($n = 2$) or male beagle dogs ($n = 3$) using 5% DMSO and 20% Cavitrone for the iv leg and 5% DMSO and 6% Cavitrone for the oral leg. All compounds were dosed at 1 and 2 mg/kg for the iv and oral legs, respectively. ^bThe dose for compound **24** was 1.11 and 2.03 mg/kg for the iv and oral legs, respectively. ^cThe average dose for compound **(R)-28** was 0.77 and 2.40 mg/kg for the iv and oral legs, respectively.

Figure 3. Comparison of inhibition of TNF alpha in rats of compounds **24** and **(R)-28**.¹⁷

compared to the five-membered system but with lower ligand efficiency.

Because **24** possessed favorable potency, ligand efficiency, lipophilicity, and permeability, we determined its pharmacokinetic profile in the rat. As seen in Table 5, **24** has moderate in vivo clearance with high oral bioavailability. Indeed, the clearance of **24** was higher than any of the previously discussed analogues. To address the moderate clearance (35 ng/mL·h), we planned several modifications including: introducing a fluorine to the para position of the distal biaryl, substituting alpha to the sulfone, and the combination of both modifications.

Figure 4. Comparison of compounds **24** and **(R)-28** in inhibition of neutrophilia in male beagle dogs.¹⁷

Scheme 1. Synthesis of **24** and **26**

These substitutions were well tolerated as the compounds maintained potency, ligand efficiency, cell potency, and

Scheme 2. Synthesis of 27 and 28

permeability although the solubility had decreased (Table 4). Compound (*R*)-28 was obtained via resolution of the racemate and was $\sim 100\times$ more potent than the distomer (*S*)-28. Modeling (see Supporting Information) of (*R*)-28 and (*S*)-28 revealed that the loss in potency arises from a steric clash between the *gem*-dimethyl in (*S*)-28 and the Lys106 side chain. This drives a rotation of the ring with subsequent loss of the H-bond to Lys106. The pharmacokinetic profile of these compounds (24, 26, 27, (*R*)-28) in rat is shown in Table 5. The *para*-F analogue 26 was favorable in terms of reducing the plasma clearance, while the dimethyl analogue 27 showed increased plasma clearance on average. Compound (*R*)-28, possessing both the *para*-F and *gem*-dimethyl substituent, however, reduced the clearance by 2-fold while maintaining high oral bioavailability (94%).

Given these data, compounds 24 and (*R*)-28 were profiled in a rat LPS-TNF α model of inflammation (see Supporting Information). This offered greater throughput than the LPS-neutrophilia model. In this model, compound 24 had an ED_{50} of 7.6 $\mu\text{mol/kg}$ po (2.8 mg/kg), whereas compound (*R*)-28 demonstrated an ED_{50} of 21.7 $\mu\text{mol/kg}$ po (9 mg/kg) (Figure 3).

Encouraged by these results, compounds 24 and (*R*)-28 were advanced to a dog pharmacokinetic experiment. These results are shown in Table 5. Of the two, compound (*R*)-28 demonstrated superior pharmacokinetics with low clearance (1.86 mL/min/kg) and high oral bioavailability (65%).

To demonstrate *in vivo* efficacy in a second species, we used a dog segmental LPS challenge model. In this model, one lobe received localized delivery of saline as control while the contralateral lobe received localized delivery of LPS solution (see Supporting Information). These data are shown in Figure 4. In this model, compound 24 had an ED_{50} of 2.9 $\mu\text{mol/kg}$ po (1.1 mg/kg), whereas compound (*R*)-28 demonstrated an ED_{50} of 1.8 $\mu\text{mol/kg}$ po (0.74 mg/kg).

Preparation of the sulfones 24 and 26 was completed in five steps as shown in Scheme 1. Condensation of ester 29 with ketone 30 using TMSOTf and Et_3SiH produced the intermediate cyclic sulfides. Oxidation to the sulfone was accomplished using either oxone or trifluoroacetic anhydride/urea-hydrogen peroxide. Completion of the target compounds was achieved via ester hydrolysis and amide formation followed by Suzuki–Miyaura cross-coupling. The preparation of dimethyl analogues 27 and 28 is shown in Scheme 2 and began with condensation of indole 32 with ketone 34 (see Supporting Information for synthetic details to ketone 34). The synthesis was completed in a manner analogous to compounds 24 and 26 via condensation, oxidation of the sulfide to the sulfone followed by conversion of the ester to the amide, and finally Suzuki–Miyaura coupling.

Inhibition of IKK β has attracted attention given its role in activation of NF- κ B. To date, several optimization campaigns

have been described across a diverse range of chemical series. One key challenge in this area of drug discovery has been the identification of potent inhibitors.

Herein, we describe some of the details of the optimization of the C3 position of a series of indole-7-carboxamides. We found that while the piperidine sulfonamide could be used to modulate the solubility, *in vivo* exposure correlated with PAMPA permeability. In addition, we have been able to improve the ligand efficiency of the series through modulation of the C3 substituent. This has given rise to compounds 24 and (*R*)-28, both of which demonstrate good pharmacokinetics and *in vivo* activity.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmmedchemlett.8b00291.

Data tables, figures, experimental procedures, and characterization data (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

IKK β , inhibitor of I κ B kinase β ; NF- κ B, nuclear factor kappa B; I κ B α , inhibitory kappa B alpha; LE, ligand efficiency; COPD, chronic obstructive pulmonary disease; EDC, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide; PAMPA, parallel artificial membrane permeability assay; TNF α , tumor necrosis factor- α ; LPS, lipopolysaccharide; ATP, adenosine triphosphate; mCPBA, *meta*-chloroperoxybenzoic acid; DPPF, 1,1'-ferrocenediyl-bis(diphenylphosphine); TBTU, 2-(1*H*-benzo-1,2,4-triazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate; TFAA, trifluoroacetic anhydride; TMSOTf, trimethylsilyl triflate; DMSO, dimethyl sulfoxide; PK, pharmacokinetic; AUC, area under curve; Clp, clearance plasma; Vdss, volume of distribution steady state; $T_{1/2}$, half-life; %F, oral bioavailability

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