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Pyrazinoindolone inhibitors of MAPKAP-K2

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Abstract—Optimization of pyrazinoindolone inhibitors of MAPKAP-K2 (MK2) provides a reasonable balance of cellular potency and physicochemical properties. Mechanistic studies support the inhibition of MK2 which is responsible for the sub-micromolar cellular efficacy.

The mitogen-activated protein kinases (MAPKs) are important components of signal transduction pathways that mediate cell proliferation, differentiation, and death.[1] The p38 MAPK family has been shown to regulate the production of a number of pro-inflammatory cytokines such as TNFα and IL-1β, both of which are implicated in chronic inflammatory diseases.[2] MAPKAP-K2 (MK2), a direct substrate of p38, has been shown to play an important role in TNFα production in mice genetically deficient in MK2.[3–5] Lipopolysaccharide (LPS) stimulated MK2 knockout mice produce a significantly reduced amount of TNFα compared to wild type animals.[3] In addition, MK2 knock-out mice are resistant to developing disease in arthritis models.[4] Furthermore, MK2 has been shown to have fewer substrates compared to p38 which may provide for a potentially safer target.[5–7]

Recently we disclosed a class of carboline based MK2 inhibitors exemplified by compounds **1** and **3** (scaffold A, Table 1).[8] However, only analog **1** had potency in an assay of LPS-induced THF production in THP-1 cells. This report details efforts to improve the cellular potency of our MK2 inhibitors while balancing suitable physicochemical properties.

We found that transposing the indole nitrogen from the carboline scaffold to the corresponding pyrazinoindolone scaffold (scaffold **B**, compounds **2** vs **1** and **4** vs **3**, Table 1) provided us with a small improvement in molecular potency. We found this potency increase to be general and the same SAR trends were observed for compounds containing either the carboline or pyrazinoindolone scaffold.[8] Given that previous SAR on the thiazole carboxamide (R in **A**) provided us with our largest improvement in molecular potency, and that the pyrazinoindole scaffold was more potent than the carboline scaffold, we focused on improving cell potency by modifying the R-group on the pyrazinoindolone, scaffold **B**.

Compounds in Tables 1–3 were prepared according to Scheme 1. 4-Methyl-3-nitrobenzoic acid (**5**) was first

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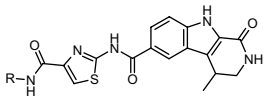
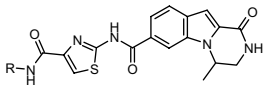
Table 1. Isomeric indole



A



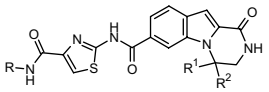
B

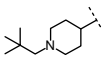
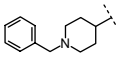
Compound	Scaffold	R	MK2 IC_{50}[a] (nM)	THP-1 IC_{50}[a] (nM)
1	**A**		44	1600
2	**B**		20	1400
3	**A**		10	>5000
4	**B**		5	>5000

[a] Values are means of ⩾2 experiments, standard deviation typically ±50% of reported value.

protected as the corresponding ethyl ester and then reacted with diethyloxalate under basic conditions to provide the corresponding aryl pyruvate ester **6**. Indole formation occurred upon heating in zinc and acetic acid to afford **7**.[9] Alkylation of the indole nitrogen with either bromoacetonitrile or 2-bromopropionitrile provided **8** and **9**, respectively. Compound **8** was alkylated with either methyl iodide or 1,3-dibromopropane to **10** or **11**.[10] Subjecting **9**–**11** to reductive conditions with either $CoCl_2/NaBH_4$ or H_2 over platinum (IV) oxide produced lactams **12**–**14**.[11] Amide bond formation with

Table 3. Indolonone SAR

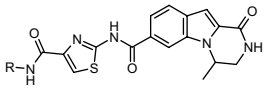


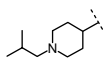
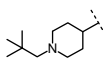
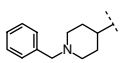
Compound	R	R^1/R^2	MK2 IC_{50}[a] (nM)	THP-1 IC_{50}[a] (nM)
21		CH_3/H	24	1600
28		CH_3/CH_3	19	1000
4		CH_3/H	5	>5000
29		CH_3/CH_3	5	2400
30		$(CH_2)_3$	2	1700
26		CH_3/H	3	735
31		CH_3/CH_3	2	430
32		$(CH_2)_3$	2	300
27		CH_3/H	9	1,600
33		CH_3/CH_3	5	900
34		$(CH_2)_3$	3	520

[a] Values are means of ⩾2 experiments, standard deviation typically ±50% of reported value.

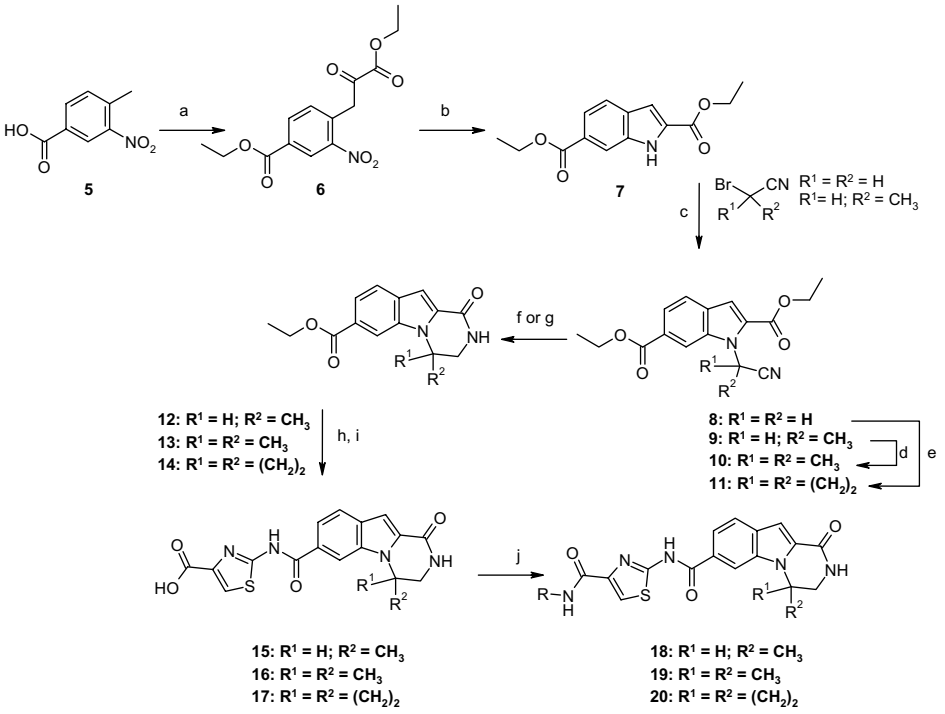
EDC and 2-aminothiazole-4-carboxylate followed by ester hydrolysis gave **15**–**17**. Finally, amide analogs **18**–**20** were prepared via EDC mediated coupling of the corresponding thiazole acids with a variety of amines.

Table 2. Thiazole carboxamide SAR



Compound	R	MK2 IC_{50}[a] (nM)	THP-1 IC_{50}[a] (nM)	PAMPA (pE $\times 10^{-6}$)
4		5	>5000	0
2		20	1400	9
21		12	5800	Not tested
22		45[b]	1800[b]	Not tested
23		46	2700	8.6
24		7	8900	0.1
25		4	1670	4.9
26		3	735	20.8
27		9	1600	5.0

[a] Values are means of ⩾2 experiments, standard deviation typically ±50% of reported value.
[b] Values $n = 1$.



Scheme 1. Reagents and conditions: (a) 1—HCl (g), EtOH, 90 °C, 98%; 2— NaOEt, EtOH, diethyloxalate, rt, 77%; (b) Zn, HOAc–H_2O, 75 °C, 81%; (c) K_2CO_3, DMF, 80 °C, 88–91%; (d) LiHMDS, CH_3I, THF, 0 °C–rt, 84–95%; (e) 1,3-dibromopropane, 60% NaH, DMSO-ether, 65 °C, 61–95%; (f) PtO_2, 50 psi H_2, EtOH, 54–95%; (g) $CoCl_2$, $NaBH_4$, MeOH–THF, 70 °C, 59%; (h) Methyl-2-aminothiazole-4-carboxylate EDC, HObT, DMAP (cat.), DMF, rt–60 °C, 53–94%; (i) NaOH, H_2O–MeOH, 60 °C, 93–98%; (j) RNH_2, EDC, HObT, DMAP (cat.), DMF, rt–60 °C, 25–90%.

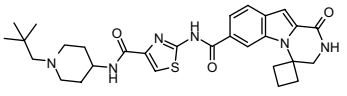
Table 2 highlights a number of amide R-groups on the thiazole with scaffold **B**. While analogs of our **4** that lacked the piperidene nitrogen (**2** and **21**–**23**) consistently had measurable cell potency, these compounds had poor physicochemical properties (solubility at pH 7.4 $\leqslant$1.5 μg/mL vs >96 μg/mL for compound **4**). Given that the piperidene had reasonable physicochemical properties, but not cellular efficacy, we wanted to determine if we could build from the piperidine nitrogen in order to provide a balance of cellular potency and suitable physicochemical properties. We found that the addition of lipophilic alkyl or aryl groups on the piperidene nitrogen (**24**–**27**) allowed us to maintain reasonable physicochemical properties (pH 7.4 solubility generally >30 μg/mL) while providing THP-1 activity. While a number of factors likely govern cell efficacy, we found that compounds which demonstrated better permeability in the parallel artificial membrane permeability assay (PAMPA) tended to provide better cellular efficacy versus **4**.[12,13]

From exploring SAR at other regions of **4**, we found that substitution of the 4-position of the indole with either a *gem*-dimethyl or spirocyclobutyl group provided small increases in both molecular and cellular potency without affecting our improved PAMPA permeability (Table 3). Overall, compound **32** provided us our best combination of molecular, cellular, and physicochemical properties and we therefore profiled this compound further to assess the potential for this class of MK2 inhibitors.

While **32** shows negligible inhibition of the following kinases at 10 μM: Erk2, ANKRD3, CDK2, smLck, MKK1, PTK2, prkCDK, NEK7, MAPK3, CK1, PRK2, MST4, ITK, MAPKAP-K3, p38-α, -β, -γ, -δ, AURORAb, PLK1, Lyn, NEK6, MSKk1, MAP3K11, CHK1, NEK2A, MNK1, CHK2, PDK1, PKCa, PRKγ1, CK2, PKA, PIM2, MBK5, SGK, DAPK1, PLKK, CSK, EF2K, VEGFR, PKBdph, Src, PKBβ, Jnk1α1, Jnk3α1; the kinases listed in Table 4 were all inhibited to a significant extent.

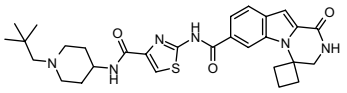
Given the kinase selectivity profile of **32**, we wanted to confirm that the potency in the THP-1 cell assay was primarily due to MK2 inhibition. We therefore tested both

Table 4. Kinase selectivity of **32**



Kinase	IC_{50} (nM) (%inh. 10 μM)	Kinase IC_{50} (nM) (%inh. 10 μM)	
MAPKAP-K1	25	NIK	480
Erk2	>3000	PDK1	(92)
PBK	(75)	CHK2	(84)
DYRK1A	(94)	PRAK	(98)
ERK8	(82)	SRPK1	(60)
MST2	(86)	ROCKII	(63)
CLK1	93	GSK3β	920
AMPK	(98)	MNK2α	(80)

Table 5. Cellular efficacy studies of **32**[a]



MK2 IC_{50} (nM)	2
LPS stimulation of THP-1 cells IC_{50} (nM)	
TNFα	300
Anisomycin-stimulated HeLa cells IC_{50} (nM)	
P-p38α	>30,000
P-HSP27 cellular efficacy IC_{50} (nM)	
THP-1	710

[a] Values are means of ⩾2 experiments, standard deviation typically ±50% of reported value.

Table 6. BalbC mouse pharmacokinetics

Compound	**1**	**2**	**32**
IV dose 0.5 mg/kg (70:30 PEG, 400:H_2O)			
Cl (%Q)	18	18	30
MRT (h)	0.8 ± 0.07	2.0 ± 1.0	6.1 ± 0.7
$AUC_{0-\infty}$ (ng h/mL)	525 ± 140	535 ± 165	165 ± 34
PO dose 1.0 mg/kg (PEG 400)			
C_{max} (μM)	0.06 ± 0.01	0.08 ± 0.05	0.06 ± 0.01
$AUC_{0-\infty}$ (ng h/mL)	88 ± 1.8	115 ± 17	446 ± 56
%*F*	8 ± 0.2	11 ± 2	>100 ± 17

the upstream and downstream effects of our inhibitors in the p38 MAPK pathway. This was assessed in two different ways (Table 5). First, inhibitor treated HeLa cells that were stimulated with anisomycin to maximally activate the p38/MK2 pathway did not show any inhibition of p38 phosphorylation by flow cytometry ruling out compound effects on signaling events upstream of MK2. Second, **32** inhibited phosphorylation of HSP27, a direct downstream substrate of MK2, to a similar level of potency to that observed in the THP-1 cell assay.[13,14] Taken together, this supports that the cellular efficacy we are observing is driven by MK2 inhibition.

The mouse pharmacokinetic properties for this class of compounds are described in Table 6 and indicate that overall, **32** is better than either compounds **1** and **2**. This may in part be again due to the poor aqueous solubility of **1** and **2** (pH 7.4 <0.1 μg/mL) while **32** is considerably more soluble (pH 7.4 >96 μg/mL).

In order to assess the activity of our compounds in vivo, a close analog of **32** (compound **26**) was tested in a mouse model of LPS-stimulated TNFα production.[15] However, even at an oral dose of 60 mg/kg, we did not observe any inhibition of TNFα. This is not surprising given the low plasma levels which are barely above the THP-1 cell potency (90 min post-dose = 690 nM; THP-1 IC_{50} = 735 nM).

Overall, we have been able to improve the profile of our initially disclosed carboline based MK2 inhibitors. We have demonstrated a better balance of physicochemical properties and cellular efficacy. Furthermore, we have demonstrated this potency is due to inhibition of MK2 via examining both upstream and downstream effects of our inhibitors in the MK2 pathway.

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