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A novel series of IKKβ inhibitors part II: Description of a potent and pharmacologically active series of analogs

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ABSTRACT

A novel series of (*E*)-1-((2-(1-methyl-1*H*-imidazol-5-yl) quinolin-4-yl) methylene) thiosemicarbazides was discovered as potent inhibitors of IKKβ. In this Letter we document our efforts at further optimization of this series, culminating in **2** with submicromolar potency in a HWB assay and efficacy in a CIA mouse model.

Previously, we described some preliminary SAR centered on **1** a compound found through high through put screening (HTS).[1] Lead compound **1** is ATP competitive, reversible, potent and selective over IKKα, (IC_{50} IKKβ = 0.25 μM, IC_{50} IKKα = 9.19 μM). Although **1** represented a promising lead with respect to its inhibition of IKKβ it demonstrated poor aqueous solubility (3.1 μg/mL, PBS buffer) and cell potency. Described herein is our efforts focused on further increasing the cellular potency and improving the physicochemical properties culminating in the pre-development candidate **2** (Fig. 1).

The highly homologous IKKβ and IKKα are members of a small class of protein kinases known as IκB Kinases (IKKs).[2] They have a distinct role in the regulation of both the adaptive and innate immunity and are critical for most aspects of host defense.[3] IKKβ and IKKα activity are required for the activation of the transcription factor Nuclear Factor kappa B NF-κB. NF-κB controls much of the innate immune system and is instrumental in the induction of genes leading to a pro-inflammatory response.

Assays used to develop the SAR in this program are a pair of chemiluminescent ELISA assays against IKKβ and IKKα, cellular

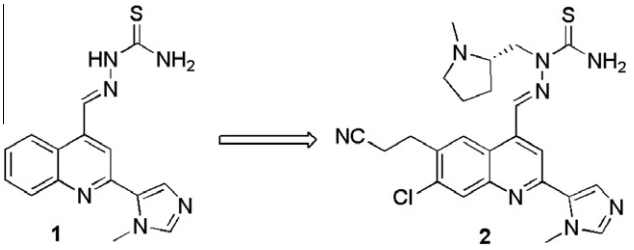

Figure 1. Lead compound **1**, and advanced analog **2**.

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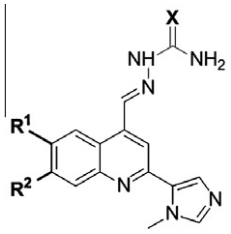
Table 1
SAR of the quinoline core

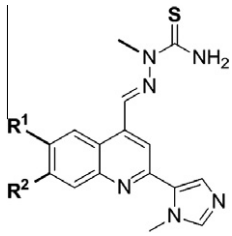


Compds	X	R^1	R^2	IKKβ IC_{50} (μM)
1	S	H	H	0.25
3	S	Cl	H	0.35
4	S	H	Cl	0.08
5	S	Cl	Cl	0.018
6	S	H	Me	0.12
7	S	Me	H	0.23
8	S	Me	Me	0.04
9	S	Me	OMe	0.37
10	S	Br	OCF_3	0.38
11	S	Me	CF_3	0.08
12	S	OMe	CF_3	0.16
13	S	OMe	Br	0.05
14	O	OMe	H	2.95
15	O	OMe	Br	0.24
16	O	Me	CF_3	0.36

assays to examine NF-κB activation via gel-shift induced TNF and a human whole blood (HWB) induced by lipopolysaccharide (LPS).

We previously reported the effect of single substitutions at the 6 or 7 position of the quinoline core. In general, small lipophilic or EWD groups enhanced potency when located at the 7-position but substitution of the 6-position with EWD or EDG failed to improve potency.[1] We now report the impact of combined substitutions at positions 6 and 7. For example, although 6-chloro analog (**3**) was less potent than the parent (**1**), the corresponding 7-chloro analog

Table 2
SAR of the quinoline core



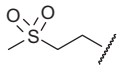
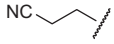
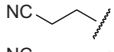
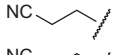
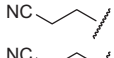
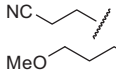
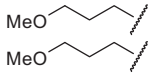
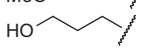
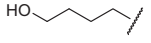
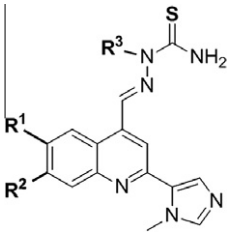
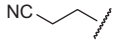
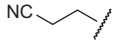
No	R^1	R^2	IKKβ IC_{50} (μM)	IKKα IC_{50} (μM)	NFκB IC_{50} (μM)	HWB IC_{50} (μM)
17		Cl	0.003	0.041	0.4	1.85
18		H	0.006	1.4	0.32	3.4
19		Me	0.002	0.14	0.14	0.42
20		Cl	0.002	0.071	0.2	1.5
21		CF_3	—	—	0.8	6
22		F	—	—	0.3	0.7
23		Me	0.031	6.7	0.6	7.8
24		H	0.094	>30	5.5	>30
25		Me	0.012	1.4	0.5	1.3
26		Me	0.013	1.8	0.3	3.7

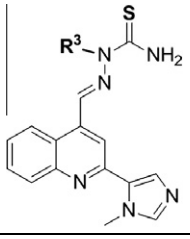
Table 3
Preliminary PK

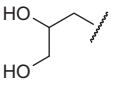
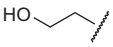
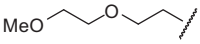
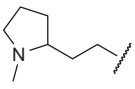


No	R^3	R^1	R^2	HWB IC_{50} (μM)	Sol. (μM)	Rat Cl (L/h/kg)	Rat %F
27	*n*-Bu	H	H	>30	>100	0.36	20
18	Me		H	3.4	20	2.9	17
20	Me		Cl	1.5	20	3.3	15

(**4**) had a threefold boost relative to **1** and the 6,7-dichloro analog (**5**) was 12-fold more potent than the parent. Similarly, while the mono-methyl analogs (**6**) and (**7**) had similar potencies, the 6,7-dimethyl analog (**8**) was sixfold more potent than **1**. Increasing the steric demand at the 7-position with a methoxy group (**9**) was not well tolerated. A similar effect was seen with a bulky EWD group, $-OCF_3$ (**10**). Using a less bulky EWD group, $-CF_3$ for compound **11** showed only a slight loss in potency relative to **8.** In this context, at the 6-position a methoxy group (**12**) was tolerated with only a minimal loss in activity relative to **11**. Exchanging the trifluoromethyl group with the more electronegative bromine (**13**) provided a threefold boost in potency relative to **12**. As noted earlier,[1] semicarbazones are less potent than the corresponding semithiocarbazones, but potency SAR follows a similar pattern as noted for the semicarbazones **14–16** relative to compounds **13** and **11**. Despite the enhanced biochemical potency of 6,7-disubstituted analogs such as **8**, **11** and **13**, cellular activities of all compounds in Table 1 were >1 μM.

Table 4
Improvements in solubility



No	R^3	IKKβ IC_{50} (μM)	IKKα IC_{50} (μM)	NFκB IC_{50} (μM)	HWB IC_{50} (μM)	Sol. PBS buffer (μM)
28		2.7	30	—	—	>100
29		0.24	30	4.8	10	>100
30		0.28	6.2	5	16	>100
31		0.24	30	3.1	9	>100
32		0.026	4.3	0.9	2	>100

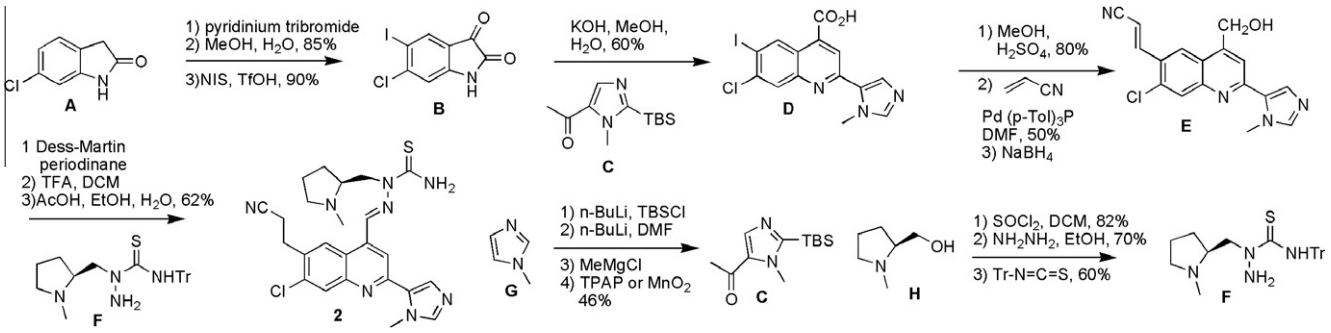
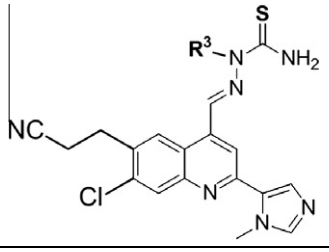
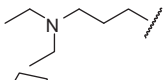
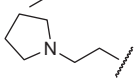
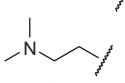
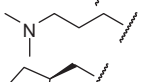
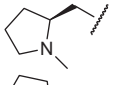
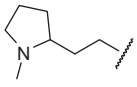


Figure 2. Synthesis of the key 6-cyanoethyl-7-chloroquinoline (**2**).

In our investigations, we found that replacing the semithiocarbazone N–H with a methyl group had a positive effect on the PK properties and that flexible bulkier groups at the quinoline 6-position enhanced the cellular activity.[1] With this in mind we set out to explore this SAR more fully. Several analogs containing longer, flexible moieties at the quinoline 6-position coupled with small EWG at the quinoline 7-position had sub-micromolar potency in the NF-κB assay. These included a methylethylsulfone (**17**) a methoxypropyl (**23**) a propanol (**25**) and an *n*-butanol (**26**). Interestingly, analogs **18**, **19, 20, 21** and **22** with an ethyl cyano moiety at the 6-quinoline position were all <1.0 μM in the NF-kB assay and **19** and **22** had HWB potency <1.0 μM. Generally, a 7-methyl group was most favored relative to a single hydrogen, with the overall order for potency in cells being Me > F > Cl > CF_3 > H. For example, while **18** and **19** were only threefold apart in the IKKβ biochemical assay and NF-κB cellular assay, **19** was eightfold more potent than **18** in the HWB assay. The methylpropyl ether analogs (**23**) and (**24**) were found to be threefold apart in the biochemical assay but **23** was still ninefold more potent in the NF-κB assay and >4-fold in the HWB assay (Table 2).

Table 5
Improvements in cellular potency



No	R^3	NFκB IC_{50} (μM)	HWB IC_{50} (μM)	Sol. PBS (μM)	Rat Cl (L/h/kg)
33		0.05	0.4 ± 0.1	>100	3.6–2.9
34		0.08	0.2 ± 0.1	10	2.4
35		0.04 ± 0.02	0.2 ± 0.1	15	4.4
36		0.1	0.2	>100	3.1
2		0.02 ± 0.01	0.095 ± 0.07	>100	1.9
37		0.05 ± 0.02	0.2 ± 0.1	75	3.3

Our attempts at improving the physicochemical properties were encouraged by the PK profile of the *n*-butyl-semithiocarbazone (**27**). Although this compound suffered from poor cellular potency it had increased solubility, lowered rat clearance and reasonable oral bioavailability. Unfortunately, the potent analogs **18** and **20** had lower solubility and the rat clearance was quite high, though the effect on oral bioavailability was muted (Table 3). To explore and extend these observations, a series of substituted semithiocarbazones were prepared. While simple alkyl side chains such as **28** did improve solubility, the effect on potency was minimal. Improvements in potency came from alcohols **29**, **30**, and ethers such as **31**. However, amine containing analogs like **32** proved to have the greatest increase in cellular activity while retaining solubility (Table 4).

We then undertook an SAR exploration to determine this optimal amine side chain. We chose the 6-cyanoethyl-7-chloroquinoline scaffold because of it relatively superior physicochemical properties and ease of synthesis (Fig. 2). The cellular activity was only slightly less than the 7-methyl or 7-fluoro substitutions (**20** vs **19** and **22**). The incorporation of an amino-alkyl semithiocarbazone gave many compounds with potency in the HWB assay below 0.5 μM, presumably by decreasing the lipophilicity thereby decreasing the plasma protein binding (Table 5).

As a comparison of **35** versus **36** and **33** versus **34** indicates, there was an increase in solubility upon moving to a slightly longer side chain**.** Only a few analogs had rat clearance values near or below 50% liver blood flow (Table 5).

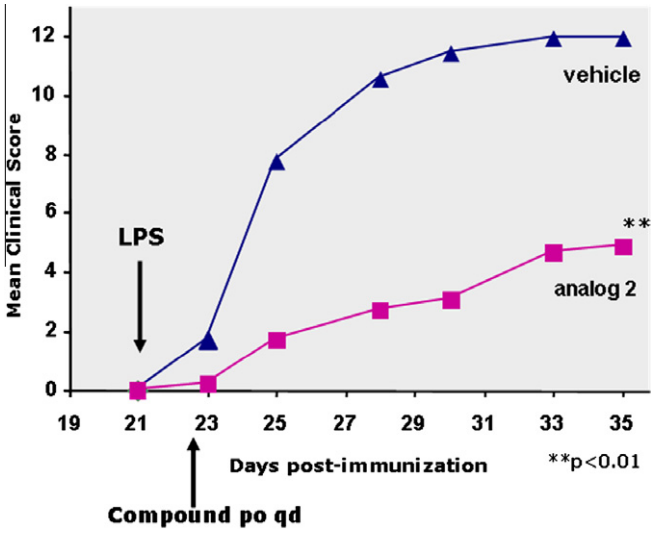


Figure 3. CIA mouse model with **2**: collagen/CFA: administered (id) on day 0. Analog **2** administered (100 mg/kg) orally on day 22–35. Clinical scoring: Visual clinical score (4 paws) day 23–35: edema, erythema, severe joint damage. Scoring for each paw 0–4 (normal–very severe).

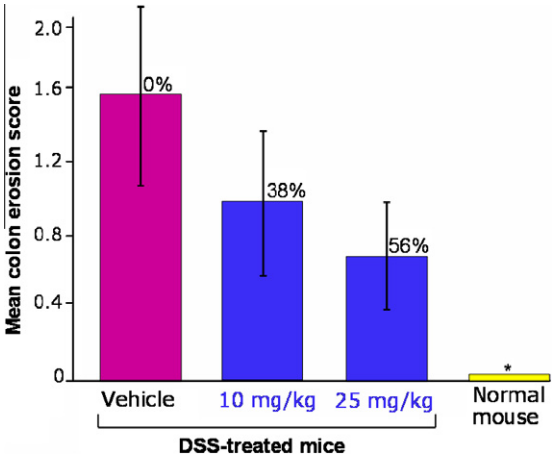


Figure 4. IBD mouse model with **2**: $N = 5$ mice. DSS administered in H_2O for 7 days. **2** administered orally 25 mg/kg and 10 mg/kg BID. Vehicle: 1% Tween, 80 + 1% methycellulose. Colon erosion: reflects loss of surface epithelium, generally associated with mucosal hemorrhage. Clinical scoring: 0 = normal, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, 5 = severe. $^{*}p \leqslant 0.05$ 2 tailed T test to 'dss + vehicle'.

Based on the HWB potency, the relatively low rat clearance, and the solubility, compound **2** was chosen for testing in vivo. In our primary in vivo model of NF-κB activation, (TNFα induction by LPS stimulation in mice) analog **2** had an ED_{50} <0.1 mg/kg.[4] Compound **2** was further evaluated in a mouse collagen induced arthritis (CIA) model.[5] Analog **2** elicited a sharp MCS reduction relative to the vehicle control with only a reversal to the severe state (score of 4) at day 33, still considerably below the vehicle treated group (Fig. 3). As part of our program targeting diseases of inflammation we were very interested in inflammatory bowel diseases (IBD) such as ulcerative Colitis and Crohn's disease. One of the most reliable models to determine drug effectiveness in these diseases is the mouse dextran sulfate sodium (DSS) model of IBD.[6] At the 10 mg/kg dose **2** lowered the colon erosion score by 38% and at 25 mg/kg it lowered the score by 56% relative to the DSS-vehicle control (Fig. 4). Additional histopathology determinations were made by scoring the colon inflammation and colon glandular epithelial loss. With **2** the colon inflammation inhibition relative to the DSS-vehicle control was 19% and 31% for doses of 10 and 25 mg/kg, respectively. The colon glandular epithelial loss was 28% and 36% for 10 and 25 mg/kg, respectively. The total of all three histopathology scored parameters provided an inhibition relative to the DSS-vehicle control of 27% and 40% for the 10 and 25 mg/kg doses, respectively. The PK exposure post 16–18 h was 1.4 ± 0.94 ng/mL and 8.5 ± 15 ng/mL for the 10 mg/kg and 25 mg/kg doses, respectively.

Table 6
PK parameters of **2**

	Rat (mean $n = 3$)	Dog (mean $n = 3$)	Rhesus (mean $n = 3$)
Cl (L/h/kg)	1.9	1.3 ± 0.4	1.4 ± 0.2
Vdss (L/kg)	4.6	4.4 ± 3.5	5.0 ± 1.4
MRT (h)	2.2	3.3 ± 1.7	3.6 ± 0.4
C_{max} (μg/L)	2.2	3.3 ± 0.4	14.8 ± 1.4
	@3 mg/kg	@ 4 mg/kg	@ 4 mg/kg
F%	15	37 ± 34	5 ± 0.5

Although **2** showed in vivo efficacy and excellent HWB activity, it suffered from high intrinsic clearance (CL_{int}) as judged by human hepatocytes (high metabolizing donor) in the absence and presence of plasma proteins[7] and CYP P450 inhibition (2D6 = 0.54 μM, 3A4 = 1.7 μM). Additionally, **2** had medium to high clearance across three species and variable oral bioavailability (Table 6). In any case, the results from the in vivo models coupled with the potency as found in the HWB assay demonstrated a robust proof of concept for this series. Further elaboration on the development of these analogs will be described subsequently.

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Lawrence McGee for discussions on the manuscript.

References and notes

1. See Cushing, T. D.; Baichwal, V.; Berry, K.; Billedeau, R.; Bordunov, V.; Broka, C.; Cardozo, M.; Cheng, P.; Clark, D.; Dalrymple, S.; DeGraffenreid, M.; Gill, G.; Hao, X.; Hawley, R. C.; He, X.; Jaen, J. C.; Labadie, S. S.; Labelle, M.; Lehel, C.; Lu, P.-P.; McIntosh, J.; Miao, S.; Parast, C.; Shin, Y.; Sjogren, E. B.; Smith, M.-L.; Talamas, F. X.; Tonn, G.; Walker, K. M.; Walker, N. C. P.; Wesche, H.; Whitehead, C.; Wright, M.; Browner, M. F. *Bioorg. Med. Chem. Lett.* **2010**, *21*, 417. for details pertaining to the assays, synthesis and initial SAR.
2. (a) Solt, L. A.; May, M. J. *Immunol. Res.* **2008**, *42*, 3; (b) Hacker, H.; Karin, M. *Sci. STKE* **2006**, 357; (c) Woronicz, J. D.; Gao, X.; Cao, Z.; Rothe, M.; Goeddel, D. V. *Science* **1997**, *278*, 866; (d) Regnier, C. H.; Song, H. Y.; Gao, X.; Goeddel, D. V.; Cao, Z.; Rothe, M. *Cell* **1997**, *90*, 373.
3. Bonizzi, G.; Karin, M. *Trends Immunol.* **2004**, *25*, 280.
4. BALB/C mice were treated with compound **2** (PO) at various doses, followed by IP delivery of LPS in 0.5 h. Mice were bled 1.5 h later and their blood plasma analyzed for TNFα levels by ELISA.
5. Collagen/CFA was delivered subcutaneously at day zero, followed by LPS (50 mg, IP) stimulation on day 21. On day 22, compound **2** (100 mg/kg) in a HPMC vehicle was administered orally (QD) until day 35. The study was blinded with respect to treatment. Mean clinical score (MCS) was based on observed edema, erythema and severe joint damage.
6. This experiment was performed by Alison Bendele at Bolder BIOPATH Inc. Swiss-Webster female mice were administered 5% DSS in H_2O for 7 days, followed by plain H_2O thereafter. The compound **2** was dosed at 25 mg/kg and 10 mg/kg orally (BID) from day 0 until day 9. At sacrifice (day 10) the 3 mm colon tissues biopsy was incubated for 24 h and the resulting supernatant assayed for TNFα and IL-1B via ELISA. The colons were clinically scored and tissues evaluated histopathologically.
7. CL_{int}: −HSA/AAG = 3.4/6.8 ; +HSA/AAG = 2.1/2/4 μL/min * 10^6 Cells. {[HSA (human serum albumin)] = 40 mg/mL, [AAG (α1-acid glycoprotein) = 2 mg/mL]}.