#### Accepted Manuscript

Identification and characterization of potent, selective and metabolically stable IKK $\beta$  inhibitor

Doyeon Kim, Yun Gyeong Kim, Jae Hong Seo, Kye Jung Shin

PII: DOI: Reference:	S0960-894X(16)30066-X http://dx.doi.org/10.1016/j.bmcl.2016.01.065 BMCL 23530
To appear in:	Bioorganic & Medicinal Chemistry Letters
Received Date: Revised Date: Accepted Date:	<ul><li>22 December 2015</li><li>19 January 2016</li><li>22 January 2016</li></ul>



Please cite this article as: Kim, D., Kim, Y.G., Seo, J.H., Shin, K.J., Identification and characterization of potent, selective and metabolically stable IKKβ inhibitor, *Bioorganic & Medicinal Chemistry Letters* (2016), doi: http://dx.doi.org/10.1016/j.bmcl.2016.01.065

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

# **Bioorganic & Medicinal Chemistry Letters**

# Identification and characterization of potent, selective and metabolically stable IKKβ inhibitor

Doyeon Kim, Yun Gyeong Kim, Jae Hong Seo and Kye Jung Shin\*

Integrated Research Institute of Pharmaceutical Sciences, College of Pharmacy, The Catholic University of Korea, 43 Jibong-ro, Wonmi-gu, Bucheon, Gyeonggi-do, 14662, Republic of Korea

#### Correspondence to:

C

Name	:	Prof. Kye Jung Shin
Tel/Fax No.	:	+82-2-2164-4060/ +82-2-2164-4059
E-mail Address	:	kyejung@catholic.ac.kr

#### Abstract

We have previously reported the identification of a rhodanine compound (1) with wellbalanced inhibitory activity against IKK $\beta$  and collagen-induced TNF $\alpha$  activated cells. However, we need more optimized compounds because of its instability over plasma and microsome. As part of a program directed toward the optimization of IKK $\beta$  inhibitor, we modified a substituent of parent compound to a series of functional groups. Among substituted compounds, fluorine substituent (12) on the para position of phenyl ring restored the stability toward plasma and microsome while retaining inhibitory potency and selectivity against IKK $\beta$  over other kinases. Also, we have demonstrated that compound 12 is an ATP non-competitive inhibitor and safe enough to apply to animal experiment from an acute toxicity test.

The transcription factor called the nuclear factor- $\kappa$ B (NF- $\kappa$ B) is related to inflammatory and immune response, and the regulation of NF- $\kappa$ B-mediated transcription is effective in the treatment of autoimmune and inflammatory diseases such as rheumatoid arthritis (RA).<sup>1</sup> NF- $\kappa$ B signaling pathway is triggered when the activation of IKK complex is induced by viral infection, lipopolysaccharide (LPS) and pro-inflammatory cytokines such as TNF $\alpha$ , IL-1.<sup>2</sup> In unstimulated cell, NF- $\kappa$ B is isolated in the cytoplasm as an inactive complex with inhibitory protein, I $\kappa$ B.<sup>3</sup> I $\kappa$ B is phosphorylated by the IKK complex which is composed of three subunits: the catalytic subunits IKK $\alpha$  and IKK $\beta$ , and the regulatory subunit IKK $\gamma$  (NEMO)<sup>4</sup>, leading to poly-ubiquitination and degradation through the 26S proteosome.<sup>5</sup> Among IKK complex, IKK $\beta$  is responsible for phosphorylation of the I $\kappa$ B in the signal induced pathway leading to NF- $\kappa$ B activation.<sup>6</sup> Also, it is known that IKK $\beta$  rather than IKK $\alpha$  is attractive target for treatment of the inflammatory and autoimmune diseases because of a dominant role in NF- $\kappa$ B signaling pathway.<sup>7</sup>

In the previous communication,<sup>8</sup> we described the identification of rhodanine compound **1** as a novel IKK $\beta$  inhibitor by hit-to-lead strategy. Compound **1** showed good selective inhibition against IKK $\beta$  over other kinases, and well-balanced inhibitory activity against IKK- $\beta$  and collagen-induced TNF $\alpha$  activated cells. Prior to *in vivo* animal study with compound **1** in type-II collagen-induced rheumatoid arthritis animal model, we performed the rat plasma and human hepatic microsomal stability test. However, the stability of compound **1** in plasma and microsome was less than satisfactory to the application for *in vivo* test; the remaining % of compound **1** after 120 min in rat plasma stability test was 58%, and the half life of compound **1** in human microsomal stability test was 72 min. Thus, we carried out the structural modification of compound **1** to obtain more stable compounds in plasma and microsome without the loss of selectivity and inhibitory activity against IKK $\beta$ . In this paper, we describe

the synthesis and biological properties of a series of rhodanine analogues. We also report a few evaluation studies to determine the possibility of drug candidate for a representative compound **12**.



Figure 1. The structure of compound 1

We focused on our initial efforts to replace the carboxamido substituent of phenylether (part A) in compound **1** with a variety of functional groups since the instability of compound **1** in plasma and microsome was considered to be caused by the hydrolysis of carboxamide to acid. In the previous paper,<sup>8</sup> 4-methylpiperazinopropyloxyphenyl moiety in part B was the optimized structure in the SAR studies. On the basis of these results, we synthesized a series of modified compounds in part A using similar method described in the earlier communication with the fixed 4-methylpiperazinylpropyloxyphenyl moiety in part B (Scheme 1), and their biological activities against IKK $\beta$  and TNF $\alpha$  activated cells were outlined in Table1.



**Scheme 1**. Reagents and conditions: (a) bis(carboxymethyl)trithiocarbonate, EtOH/H<sub>2</sub>O (4:3), reflux, 56%; (b) aldehydes, NaOAc, AcOH, reflux, 59~86%.



Table 1. IKK $\beta$  inhibitory activities of compounds 4–19

	_	enzyme a	ussay <sup>9</sup>	cell-based a	cell-based assay <sup>8</sup>	
compd	R	% inhibition of	IKKβ IC <sub>50</sub>	% inhibition of	TNF $\alpha$ IC <sub>50</sub>	
		ΙΚΚβ (10 μΜ)	(µM)	NF-κB (10 μM)	(µM)	
4	СООН	50.2	9.88	-	-	
5	CON(CH <sub>3</sub> ) <sub>2</sub>	85.7	2.10	32.3	20	
6	SO <sub>2</sub> NH <sub>2</sub>	83.7	0.60	-	-	
7	CN	82.3	0.64	55.0	4	
8	OCH <sub>3</sub>	75.3	0.84	51.6	4	
9	CH <sub>3</sub>	67.0	1.13	50.1	5	
10	CF <sub>3</sub>	17.0	-	-	-	
11	t-Bu	27.2	-	-	-	
12	F	93.3	0.79	84.8	0.5	
13	Cl	88.7	0.82	66.6	3	
14	Br	78.9	0.96	76.6	6	
15	SO <sub>2</sub> CH <sub>3</sub>	80.8	0.90	60.9	4	

16	NO <sub>2</sub>	89.4	0.79	61.1	2
17	$NH_2$	83.8	0.85	68.8	6
18	NHCOCH <sub>3</sub>	86.6	0.84	48.0	20
19	NHSO <sub>2</sub> CH <sub>3</sub>	91.6	0.82	54.0	8
1	CONH <sub>2</sub>	90.1	0.35	65.4	2
The eccert n	notheds for any me and	call lines are describ	ad in raf 8 and 0		

The assay methods for enzyme and cell-lines are described in ref. 8 and 9.

As shown in Table 1, compound 4 which is a hydrolyzed form of compound 1 greatly reduced the potency against IKK\beta compared to compound 1 and showed no activity against cell-lines. The replacement of carboxamide with dimethylcarboxamide (5) which is more stable and bulkier than simple amide 1 was detrimental to enzymatic and cellular activities. Bioisosteric transformation to sulfonamide (6) was found to inhibit IKKB enzyme at low concentration comparable with compound 1, however, its cellular activity was not observed in cell-based assay. A series of compounds (7-17) were prepared for investigating the change of activity according to the electronic effect. Substitution with electron withdrawing groups such as nitrile (7), halogens (12-14), methanesulfonyl (15) and nitro (16) except for trifluoromethyl group (10) maintained the activities against enzyme and cell-lines, especially, fluoro substituent compound (12) showed better cellular activity than compound 1. Electron donating substituents (8, 9, 17) exhibited good activities with  $IC_{50}$  values ranging from 1 to 0.8 µM except for bulky t-butyl substituent. These results indicated that the electronic effects on *para* position did not impact significantly the potency of parent compound 1, but rather the bulkiness was more likely to affect on activities. Chemical modification of compound 1 to reversed amides (18, 19) was tolerated in enzyme activities, but led to somewhat decrease in cellular potency. In the study on substituent effect at part A, fluorine substituted compound 12 was the most potent representative of this small series against enzyme and cell.

To determine whether the substituted compounds instead of carboxamido functional group

could enhance the stability over plasma and microsome, the selected compounds (7, 8, 12-16) which revealed good activities were performed to stability tests (Table 2).

Table 2. The stability test data of selected compound					
Compd	Plasma stability <sup>a</sup> (%)	Microsomal stability <sup>b</sup> (min)			
7	49	72.9			
8	73	223.5			
12	87	239.0			
13	78	126.0			
14	72	157.5			
15	72	70.5			
16	45	57.8			
1	58	72.0			

RIP

<sup>a</sup>The remaining % of compound after 120 min in rat plasma stability test <sup>b</sup>The half life of compound in human microsomal stability test

As illustrated in Table 2, the stability of selected compounds was evaluated with rat plasma and human microsome. Nitrile (7), methanesulfonyl (15) and nitro (16) substituents were very unstable to microsome and(or) plasma presumably due to the high polarity and reactivity toward liver microsome, meanwhile, the remarkable metabolic stability enhancement compared to parent molecule 1 was observed in methoxy (8) and halogen (12-14) substituents, especially, fluorine substituent (12) had stable enough to plasma and 3-fold longer half-life to microsome compared with compound 1. From the results of inhibitory activity and metabolic stability, fluorine compound 12 had equipotent IKK $\beta$  inhibitory activity and excellent metabolic stability in comparison with compound 1. Taken results together, compound 12<sup>10</sup> was selected to be a program lead for further biological evaluation.

In the drug discovery process of kinase inhibitor, kinase selectivity profiling is an essential element to allow more accurate evaluation of on- and off-target effects of the inhibitor that can be expected in relation to efficacy and toxicity possibly working on other kinases.<sup>11</sup> Next

efforts were made to perform the kinase panel test against more than 40 other kinases with the lead compound **12** in 10  $\mu$ M concentration, and the results of % inhibition data are summarized in Table 3.

Kinase	% inhibition <sup>a</sup>	Kinase	% inhibition <sup>a</sup>
Abl1	20.0	ΙΚΚε	0
Aurora A	0	JNK1	2.0
Aurora B	0	JNK2	0
b-Raf	0	JNK3	0
c-Src	14.0	LCK	0
CAMK1a	58.6	LYN/LYN A	27.8
CDK1/cyclin B	3.1	MAPKAPK2	42.0
CDK2/cyclin A	6.4	MAPKAPK5	5.2
CDK4/cyclin D1	17.3	MNK1	0
CDK5/p25	5.5	MNK2	33.1
CDK6/cyclin D1	12.9	MSK1	27.1
CDK7/cyclin H	2.5	MSK2	0
CDK9/cyclin T1	0	P8a/MAPK14	14.8
CHK1	0	P70S6K	24.9
CHK2	47.0	PDK1	0
СК1б	0	PIM1	0
<b>CK2</b> α	0	PIM2	0
EGFR	0	РКА	0
EPHA2	29.0	ΡΚϹα	18.4
ERK2/MAPK2	6.8	ROCK1	61.1
FGFR1	20.5	TBK1	0
GSK3β	0	TIE2/TEK	57.5
ΙΚΚα	0	ТРКА	24.7
		ΙΚΚβ	93.3

Table 3. Kinase selectivity of compound 12

<sup>a</sup>Duplicate mode at 10  $\mu$ M of compound **12** 

As described in Table 3, compound 12 showed excellent selectivity over most of kinases regardless of serine/threonine or tyrosine kinase. It is noteworthy that there was a big difference between IKK $\alpha$  and IKK $\beta$  in inhibitory activity of compound 12. Generally, the majority of kinase inhibitors located in the ATP-binding site, and bind to the hinge region through hydrogen bonding which involves a bidentate interaction with a donor-acceptor pair pattern. However, compound 12 structurally does not contain the functional group to

participate in a bidentate hydrogen bonding. We conceived that the excellent selectivity of compound **12** came from binding to allosteric region of IKK $\beta$ . To determine what type of inhibition was occurring to IKK $\beta$ , enzyme kinetics study was carried out. We examined the Lineweaver-Burk plot to determine whether compound **12** of IKK $\beta$ -catalyzed reaction was competitive or non-competitive (allosteric). The reciprocals of the reaction rate and ATP concentration were plotted with compound **12** being present, and its plot was displayed in Figure 2.



Figure 2. The double reciprocal plot for ATP concetration in IKK $\beta$  inhibition by compound 12

In the Lineweaver-Burk plot of compound 12, the lines had the same intercept point on the xaxis, but had different slopes and different intercepts on the y-axis, which is a typical plot of non-competitive inhibitor. With this result, we could infer that compound 12 is a noncompetitive IKK $\beta$  inhibitor and its excellent selectivity over other kinases is brought by

binding to allosteric region not ATP-binding site.

Next, to observe acute toxicity and animal mortality, and provide a reference for clinical trials, compound **12** were orally administered to ICR mice. Twenty ICR mice were given 2000 mg/kg of compound **12** by single oral dose and closely observed 3 hours after administration for 14-day period, and were weighed daily before and after administration. After the observation period, all surviving animals underwent gross anatomy. Throughout the experiment period, no animal was died, all animals did not show any abnormal symptoms, and the average weight of male and female animals were normal (Table 4). Major organs had no visible changes via gross anatomy in all of sacrificed animals at the15th day. From this acute toxicity experiment, compound **12** was found to be safe in the single 2000 mg/kg dose by oral administration for ICR mice.

Group	Number	Before administration	Day1	Day2	Day3	Day7	Day10	Day14
Female	10	18.9±0.9	19.7±0.9	21.7±1.4	23.3±1.2	28.9±1.3	31.3±1.1	35.1±1.4
Male	10	20.3±0.4	21.5±1.1	22.1±1.2	23.2±1.0	26.2±1.6	27.4±1.7	29.2±1.5

Table 4. Average body weight after single oral dose of compound 12 in ICR mice ( g ,  $\bar{x}\pm SD$  )

To examine *in vivo* kinetics of compound **12**, the pharmacokinetic properties, after dissolving in 20% HP- $\beta$ -cyclodextron, were evaluated intravenously and orally in rat (Table 5). Compound **12** had moderate clearance, large volume distribution, and long half-life after intravenous and oral administration, while had a relatively low oral AUC value resulting from low C<sub>max</sub> level compared to intravenous administration. The bioavailability of compound **12** was 23% which was a possible level to administer orally.

Parameter	Intravenous <sup>a</sup>	Oral <sup>b</sup>
$AUC_{0-\infty}$ (ng h/mL)	175.6±41.3	79.4±9.8
T <sub>1/2</sub> (h)	5.50±2.20	5.81±2.16
$C_{\rm max}$ (ng/mL)	93.99±9.96	8.6±2.5
CL (L/h/kg)	5.88±1.22	5.86±0.74
V (L/kg)	44.33±10.35	50.58±25.54
F (%)	23	3.0

Table 5. Pharmacokinetic profiles of compound 12

<sup>a</sup>IV dose: 1 mg/kg

<sup>b</sup>PO dose: 2 mg/kg

Formulation: 20% HP- $\beta$ -cyclodextron (yellow transparent solution) Species: male SD rat (n = 3 per 0.25, 0.5, 1, 2, 4, 8, 12, 24 h time point)

In summary, we established the structure activity relationship for a series of rhodanine compounds for IKK $\beta$  inhibitors. We showed that the metabolic instability of compound **1** could be overcome by the introduction of appropriate substituents on the phenyl ring instead of carboxamido substituent which was a metabolically labile substituent. Among substituted compounds, fluorine substituent (**12**) on the *para* position of phenyl ring restored the stability toward plasma and microsome while retaining inhibitory potency and selectivity against IKK $\beta$  over other kinases. Also, we have demonstrated that compound **12** is an ATP noncompetitive inhibitor and safe enough to apply to animal experiment from an acute toxicity test. In conclusion, compound **12** has a high potential of drug candidate in the development of therapeutics for the treatment of NF- $\kappa$ B associated immune disease such as rheumatoid arthritis, Crohn's disease, chronic obstructive pulmonary disease (COPD), and cancer.

#### Acknowledgements

This work was financially supported by Research Fund 2012 of The Catholic University of Korea and the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea. (HI12C1087).

#### **References and notes**

- (a) Breedveld, F. C.; Dayer, J. M. Ann. Rheum. Dis. 2000, 59, 841. (b) Yamamoto, Y.; Gaynor, R. B. Trends Biochem. Sci. 2004, 29, 72, (c) Karin, M.; Yamamoto, Y.; Wang, Q. M. Nat. Rev. Drug Discov. 2004, 3, 17, (d) Strnad, J.; Burke, J. R. Trends Pharmacol. Sci. 2007, 28, 142, (e) Coish, P. D. G.; Wickens, P. L.; Lowinger, T. B. Expert Opin. Ther. Patents 2006, 16, 1.
- (a) Barnes, P. J.; Karin, M. N. Engl. J. Med. 1997, 336, 1066. (b) Morwick, T.; Berry, A.; Brickwood, J.; Cardozo, M.; Catron, K.; DeTuri, M.; Emeigh, J.; Homon, C.; Hrapchak, M.; Jacober, S.; Jakes, S.; Kaplita, P.; Kelly, T. A.; Ksiazek, J.; Liuzzi, M.; Magolda, R.; Mao, C.; Marshall, D.; McNeil, D.; Prokopowicz, A. 3rd; Sarko, C.; Scouten, E.; Sledziona, C.; Sun, S.; Watrous, J.; Wu, J. P.; Cywin, C. L. J. Med. Chem. 2006, 49, 2898.
- (a) Tak, P. P.; Firestein, G. S. J. Clin. Invest. 2001, 107, 7. (b) Ghosh, S.; Karin, M. Cell 2002, 109, S81.
- 4. Gilmore, T. D. Oncogene 2006, 25, 6680.
- 5. Karin, M. Oncogene 1999, 18, 6867.
- Li, Z. W.; Chu, W. M.; Hu, Y. L.; Delhase, M.; Deerinck, T.; Ellisman, M.; Johnson, R.; Karin, M. J. Exp. Med. 1999, 189, 1839.
- 7. Schmid, J. A.; Birbach, A. Cytokine Growth Factor Rev. 2008, 19, 157.
- Song , H.; Lee, Y. S.; Roh, E. J.; Seo, J. H.; Oh, K. S.; Lee, B. H.; Han, H.; Shin, K. J. Bioorg. Med. Chem. Lett. 2012, 22, 5668.
- 9. Oh, K. S.; Lee, S.; Choi, J. K.; Lee, B. H. Comb. Chem. High Throughput Screening 2010, 13, 790.
- 10. Compound 12 (2HCl salt): <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 2.26 (bs, 2H), 2.83 (s, 3H),
  3.33–3.83 (m, 10H), 4.16 (t, *J* = 5.7 Hz, 2H), 7.11 (d, *J* = 8.0 Hz, 2H), 7.13 (d, *J* = 8.9 Hz, 2H), 7.23 (dd, *J* = 4.6, 9.1 Hz, 2H), 7.30–7.35 (m, 4H), 7.72 (d, *J* = 8.9 Hz, 2H), 7.83

(s, 1H), 12.07 (bs, 2H); <sup>13</sup>C NMR (125 MHz, DMSO-*d*<sub>6</sub>) δ 23.81, 42.55, 48.71, 50.11, 53.65, 65.66, 115.48, 117.35, 117.53, 118.44, 121.93, 122.57, 122.64, 128.19, 128.26, 130.42, 132.58, 133.49, 151.38, 151.40, 158.49, 159.23, 160.17, 160.41, 167.54, 194.55. MS: calcd for  $C_{30}H_{30}FN_3O_3 S_2 + H^+$ , 564.18; found  $[M + H]^+$ , 564.14.

11. (a) Davies, S. P.; Reddy, H.; Caivano, M.; Cohen, P. Biochem. J. 2000, 351, 95. (b) Bain, J.; Plater, L.; Elliott, M.; Shpiro, N.; Hastie, C. J.; Mclauchlan, H.; Klevernic, I.; Arthur,

...K

# Identification and characterization of potent, selective and metabolically stable IKKβ inhibitor

Doyeon Kim, Yun Gyeong Kim, Jae Hong Seo and Kye Jung Shin

We describe an optimized compound 12 to have desired activity against IKK $\beta$ , good stability to plasma and microsome, and excellent selectivity over other kinases.

12

Remaining % of rat plasma stability after 120 min: 87% Half life in human microsomal stability: 239 min IKK $\beta$  IC<sub>50</sub>: 0.79  $\mu$ M, TNF $\alpha$  IC<sub>50</sub>: 0.5  $\mu$ M Acute toxicity: >2,000 mg/kg