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

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ABSTRACT

We have previously reported the identification of a rhodanine compound (**1**) with well-balanced inhibitory activity against IKKβ and collagen-induced TNFα 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 IKKβ 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 IKKβ 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-κB (NF-κB) is related to inflammatory and immune response, and the regulation of NF-κB-mediated transcription is effective in the treatment of autoimmune and inflammatory diseases such as rheumatoid arthritis (RA).[1] NF-κB signaling pathway is triggered when the activation of IKK complex is induced by viral infection, lipopolysaccharide (LPS) and pro-inflammatory cytokines such as TNFα, IL-1.[2] In unstimulated cell, NF-κB is isolated in the cytoplasm as an inactive complex with inhibitory protein, IκB.[3] IκB is phosphorylated by the IKK complex which is composed of three subunits: the catalytic subunits IKKα and IKKβ, and the regulatory subunit IKKγ (NEMO),[4] leading to poly-ubiquitination and degradation through the 26S proteosome.[5] Among IKK complex, IKKβ is responsible for phosphorylation of the IκB in the signal induced pathway leading to NF-κB activation.[6] Also, it is known that IKKβ rather than IKKα is attractive target for treatment of the inflammatory and autoimmune diseases because of a dominant role in NF-κB signaling pathway.[7]

In the previous communication,[8] we described the identification of rhodanine compound **1** as a novel IKKβ inhibitor by hit-to-lead strategy. Compound **1** showed good selective inhibition against IKKβ over other kinases, and well-balanced inhibitory activity against IKK-β and collagen-induced TNFα 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 IKKβ. In this Letter, we describe the synthesis and biological properties of a series of rhodanine analogs. We also report a few evaluation studies to determine the possibility of drug candidate for a representative compound **12** (Fig. 1).

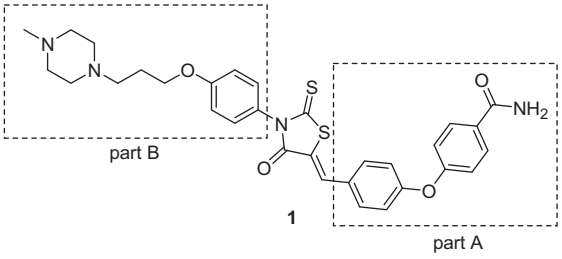

Figure 1. The structure of compound **1**.

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

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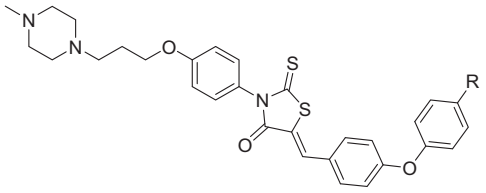
Table 1
IKKβ inhibitory activities of compounds **4–19**



Compd	R	Enzyme assay[9]		Cell-based assay[8]	
		% inhibition of IKKβ (10 μM)	IKKβ IC_{50} (μM)	% inhibition of NF-κB (10 μM)	TNFα IC_{50} (μM)
4	COOH	50.2	9.88	–	–
5	$CON(CH_3)_2$	85.7	2.10	32.3	20
6	SO_2NH_2	83.7	0.60	–	–
7	CN	82.3	0.64	55.0	4
8	OCH_3	75.3	0.84	51.6	4
9	CH_3	67.0	1.13	50.1	5
10	CF_3	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_2CH_3	80.8	0.90	60.9	4
16	NO_2	89.4	0.79	61.1	2
17	NH_2	83.8	0.85	68.8	6
18	$NHCOCH_3$	86.6	0.84	48.0	20
19	$NHSO_2CH_3$	91.6	0.82	54.0	8
1	$CONH_2$	90.1	0.35	65.4	2

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

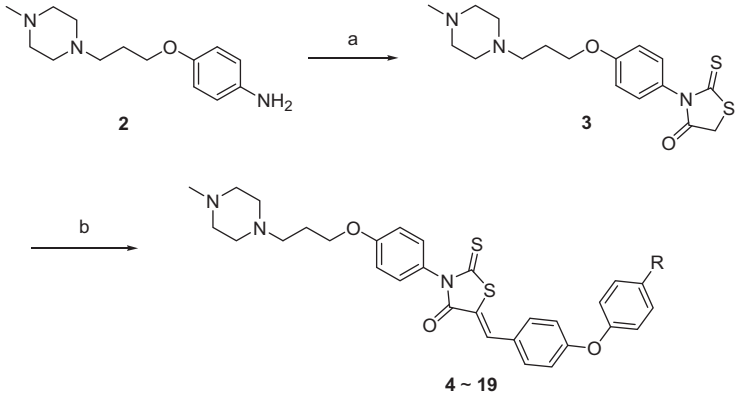
carboxamide to acid. In the previous paper,[8] 4-methylpiperazinopropyloxyphenyl moiety in part B was the optimized structure in the SAR studies. On the basis of these results, we synthesized a series of modified compounds in part A using similar method described in the earlier communication with the fixed 4-methylpiperazinylpropyloxyphenyl moiety in part B (Scheme 1), and their biological activities against IKKβ and TNFα activated cells were outlined in Table1.

As shown in Table 1, compound **4** which is a hydrolyzed form of compound **1** greatly reduced the potency against IKKβ 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 IKKβ 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,

Table 2
The stability test data of selected compound

Compd	Plasma stability[a] (%)	Microsomal stability[b] (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

[a] The remaining % of compound after 120 min in rat plasma stability test.
[b] The half life of compound in human microsomal stability test.



Scheme 1. Reagents and conditions: (a) bis(carboxymethyl)trithiocarbonate, EtOH/H_2O (4:3), reflux, 56%; (b) aldehydes, NaOAc, AcOH, reflux, 59–86%.