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Synthesis and Bioactivities of Novel 4,5,6,7-Tetrahydrothieno [2,3-*c*]pyridines as Inhibitors of Tumor Necrosis Factor-α (TNF-α) Production

Masakazu Fujita,* Taketsugu Seki, Haruaki Inada and Naoko Ikeda

Omiya Research Laboratory, Nikken Chemicals Co., Ltd., 1-346, Kitabukuro-cho, Saitama-shi, Saitama 330-0835, Japan

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Abstract—Novel 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine derivatives were synthesized and evaluated for their abilities to inhibit lipopolysaccharide (LPS)-stimulated production of TNF-α in rat whole blood. Several of these compounds exhibited potent inhibitory activity.

Introduction

In the case of normal conditions, the production of cytokines helps clear viral or bacterial infections and damaged cells from injured tissues. However, the overproduction of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) is known to cause immune and inflammatory diseases.[1] Especially, the overproduction of TNF-α has been strongly implicated in the pathogenesis of such diseases as septic shock,[2] rheumatoid arthritis (RA),[3] inflammatory bowel disease (IBD),[4] multiple sclerosis,[5] type II diabetes[6] and AIDS.[7] In addition, TNF-α is a potent inducer of IL-1, IL-6, IL-8, GM-CSF and so on.[8] Therefore, agents which can inhibit the production of TNF-α have attracted much attention as potential therapies for the direct or indirect treatment of these diseases. In clinical trials, the monoclonal TNF-α antibodies, Remicade (infliximab),[9] and the soluble TNF p75 receptor fusion protein (TNFRp75: Fc), Enbrel (etanercept)[3,10] have been shown to be effective in the treatment of RA and Crohn's disease. However, these drugs as proteins are expensive and inconvenient to patients. Given these backgrounds, currently, small molecule anti-TNF-α agents are being exploited through multiple approaches.[11]

During screening of our compounds on inhibition of TNF-α production, 6-acetyl-2-amino-3-(2-chlorobenzoyl)-4,5,6,7-tetrahydothieno[2,3-*c*]pyridine (**2a**) and its derivatives (**3c** and **3e**–**g**) that were the intermediates of agents combining platelet activating factor (PAF) receptor antagonist with thromboxane synthase inhibitor (TxSI)[12] were found to have such activity. Based on this information, chemical modifications of compound **2a** were started. In this paper, we describe synthesis and the structure–activity relationships (SARs) on 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine derivatives.

Chemistry and Biology

A series of the compounds listed in Tables 1–5 were synthesized by the methods illustrated in Schemes 1 and 4.

6-Substituted 2-amino-3-acyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridines (**2**) were prepared by the Gewald synthesis.[13] Namely, reaction of *N*-substituted 4-piperidone with sulfur and acylacetonitrile in the presence of Et_3N/DMF or Et_2NH/EtOH at 60 °C-reflux yielded compounds **2**.

N-Substituted 4-piperidones were commercially available except for 1-(*c*-propanecarbonyl)-4-piperidone. 1-(*c*-Propanecarbonyl)-4-piperidone was easily prepared by acylation of 4-hydroxypiperidine followed by Swern oxidation.[14] On the other hand, *N*-substituted 4-piperidones were also prepared from the corresponding amines by Dieckmann reaction followed by decarboxylation, if the *N*-substituents were stable against acid (Scheme 2).

*Corresponding author. Tel.: +81-48-641-2334; fax: +81-48-641-5749; e-mail: mfujita-nikken@nifty.com

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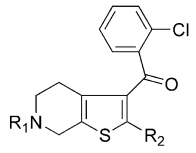
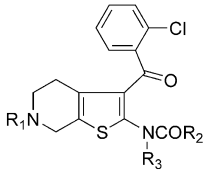
Table 1. In vitro inhibition of TNF-α production for compounds **1**, **2a**–**b**, **3c** and **3e**–**g** and the positive control



Compd	R_1	R_2	IC_{50} (μM)[a]
1	Ac	H	38
2a	Ac	NH_2	72
2b	H	NH_2	>100
3c	Ac	$ClCH_2CONH$	>100
3e	Ac	Br(Me)CHCONH	6.0
3f	Ac	Cl(Me)CHCONH	12
3g	Ac	NH_2(Me)CHCONH	8.0
FR133605			6.0±4.2
Dexamethasone			0.02
Pentoxifylline			40±2

[a]IC_{50} of LPS-stimulated TNF-α production in rat whole blood.

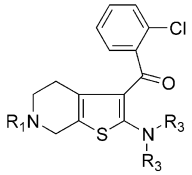
Table 2. In vitro inhibition of TNF-α production for the amides and the ureas



Compd	R_1	R_2	R_3	IC_{50} (μM)[a]
3a	Ac	Me	H	9.5
3b	Ac	Et	H	15
3c	Ac	$ClCH_2$	H	>100
3d	Ac	$Me(CH_2)_5$	H	>100
3e	Ac	Br(Me)CH	H	6.0
3f	Ac	Cl(Me)CH	H	12
3g	Ac	NH_2(Me)CH	H	8.1
3h	Ac	Me_2CH	H	2.6
3i	Ac	Me_2CH	Me	33
3j	Ac	Me_3C	H	3.4
3k	Ac	*c*-Propane	H	3.0
3l	Ac	*c*-Propane	Me	74
3m	Ac	*c*-Butane	H	2.9
3n	Ac	*c*-Hexane	H	39
3o	Ac	Ph	H	46
3p	Ac	4-FPh	H	100
3q	Ac	3-Pyridyl	H	12
3r	Ac	4-Pyridyl	H	21
3s	Ac	$Ph(CH_2)_2$	H	>100
3t	Ac	PhCH=CH	H	>100
4a	Ac	EtNH	H	10
4b	Ac	Et_2N	H	0.51
4c	Ac	Et_2N	Me	10
5a	*c*-PropaneCO	Me_2CH	H	2.6
5b	*c*-PropaneCO	*c*-Propane	H	0.9
5c	*c*-PropaneCO	Ph	H	>100
5d	*c*-PropaneCO	3-Pyridyl	H	3.9
6a	Bn	Me_2CH	H	5.0
6b	Bn	*c*-Propane	H	6.5
6c	Bn	Ph	H	>100
6d	Bn	3-Pyridyl	H	7.1
7a	Me	Me_2CH	H	2.0
7b	Me	*c*-Propane	H	1.3
10a	H	Me_2CH	H	4.0
10b	Et	Me_2CH	H	0.92
10c	Et	Me_2CH	Et	10

[a]IC_{50} of LPS-stimulated TNF-α production in rat whole blood.

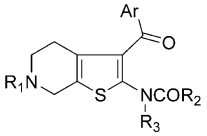
Table 3. In vitro inhibition of TNF-α production for the *N*-dialkyl substitution



Compd	R_1	R_3	IC_{50} (μM)[a]
8a	Ac	Me_2CHCH_2	30
8b	Ac	*c*-HexylCH_2	100
9a	*c*-PropaneCO	Me_2CHCH_2	>100
9b	*c*-PropaneCO	*c*-HexylCH_2	>100

[a]IC_{50} of LPS-stimulated TNF-α production in rat whole blood.

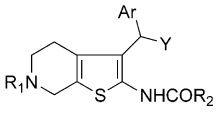
Table 4. In vitro inhibition of TNF-α production for the amides and the ureas without 3-(2-chlorobenzoyl) group



Compd	R_1	R_2	R_3	Ar	IC_{50} (μM)[a]
3u	Ac	Me_2CH	H	3-ClPh	7.6
3v	Ac	*c*-Propane	H	3-ClPh	20
3w	Ac	3-Pyridyl	H	3-ClPh	36
3x	Ac	Me_2CH	H	4-ClPh	>100
3y	Ac	Me_2CH	H	2-MePh	3.9
3z	Ac	*c*-Propane	H	2-MePh	2.9
3aa	Ac	Me_2CH	H	Ph	3.0
3bb	Ac	*c*-Propane	H	Ph	3.0
3cc	Ac	Ph	H	Ph	48
3dd	Ac	3-Pyridyl	H	Ph	14
3ee	Ac	Me	H	4-MeOPh	26
3ff	Ac	Me	H	3,4-$(MeO)_2$Ph	46
3gg	Ac	Me	H	2,6-$(MeO)_2$Ph	88
3hh	Et	Me_2CH	H	3,4-(OCH_2O) Ph	78
3ii	Ac	Me_2CH	H	Me	35
3jj	Ac	*c*-Propane	H	Me	28
3kk	Ac	Me_2CH	H	OEt	22
3ll	Ac	*c*-Propane	H	OEt	10
3mm	Ac	3-Pyridyl	H	OEt	22
4d	Ac	Et_2N	H	2,6-$(MeO)_2$Ph	3.8

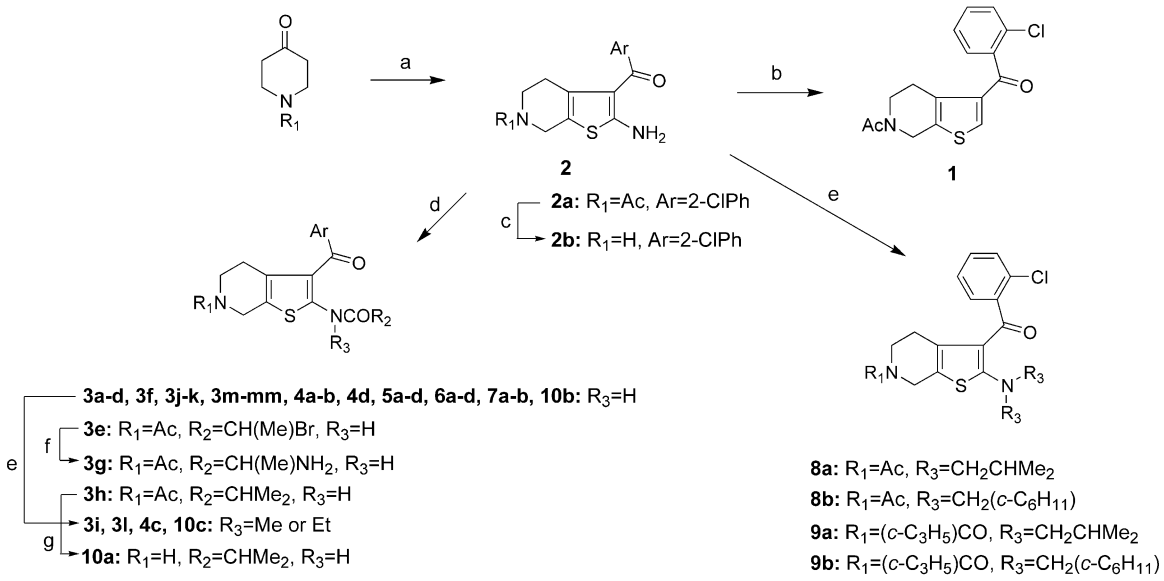
[a]IC_{50} of LPS-stimulated TNF-α production in rat whole blood.

Table 5. In vitro inhibition of TNF-α production for modification of the oxygen atom

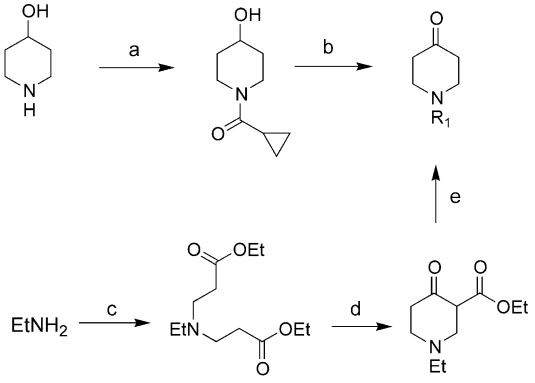


Compd	R_1	R_2	Ar	Y	IC_{50} (μM)[a]
3j	Ac	Me_3C	2-ClPh	=O	3.4
11a	Ac	Me_3C	2-ClPh	OH	19
12a	Ac	Me_3C	2-ClPh	H	29
3ii	Ac	Me_2CH	Me	=O	35
11b	Ac	Me_2CH	Me	OH	38
12b	Ac	Me_2CH	Me	H	23
10b	Et	Me_2CH	2-ClPh	=O	0.92
11c	Et	Me_2CH	2-ClPh	OH	100

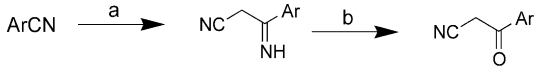
[a]IC_{50} of LPS-stimulated TNF-α production in rat whole blood.



Scheme 1. Reagents and conditions: (a) $NCCH_2COAr$, S, Et_3N/DMF or Et_2NH/EtOH, 60 °C–reflux, 70–93%; (b) (i) $NaNO_2$, 75% H_2SO_4, 0 °C; (ii) 50% H_3PO_2, 0 °C, 51%; (c) NaOH, MeOH–H_2O, reflux, quant; (d) for all compounds except for **4a–b** and **4d**: R_2COX, pyridine, 51%–quant. For **4a–b** and **4d**: $EtNH_2$ or Et_2NH, triphosgene, Et_3N, CH_2Cl_2, 0 °C–rt, 21%–quant; (e) R_3X, NaH, DMF, 11–87%; (f) (i) NaI, THF, reflux; (ii) NH_3, THF, −20 °C, 81%; (g) NaOH, MeOH–H_2O, 0 °C–rt, 77%.



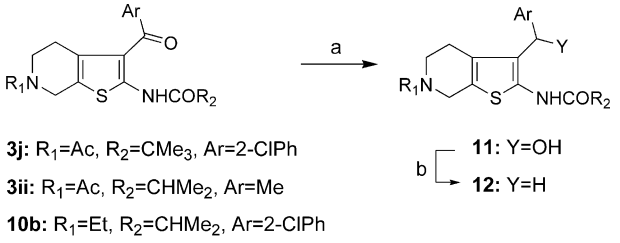
Scheme 2. Reagents and conditions: (a) *c*-propaneCOCl, Et_3N, CH_2Cl_2, −60 °C, 95%; (b) Swern oxidation, 72%; (c) $Br(CH_2)_2COOEt$, Et_3N, MeCN, 80 °C, 84%; (d) NaH, PhMe–EtOH, reflux, quant; (e) concd HCl, reflux, 93%.



Scheme 3. Reagents and conditions: (a) MeCN, LDA, −78 to −70 °C, 20–92%; (b) concd HCl, rt–reflux, 90%–quant.

Acylacetonitrile were commercially available or easily prepared by the condensation of acetonitrile anion with the corresponding nitriles shown in Scheme 3.[15]

6-Acetyl-3-(2-chlorobenzoyl)-4,5,6,7-tetrahydrothieno [2,3-*c*] pyridine **1** was synthesized by treatment of compound **2a** with sodium nitrite followed by denitrogenation with phosphinic acid. *N*-Acylation at the 2-position of compound **2a** with the requisite acid halide were easily accomplished in pyridine (**3a–f**, **3h**, **3j–k**, **3m–mm**, **5a–d**, **6a–d**, **7a–b** and **10b**). Iodoacetamide was prepared by the reaction of compound **3e** with NaI in refluxing THF and was ammonolyzed by passing NH_3 gas in



Scheme 4. Reagents and conditions: (a) $NaBH_4$, MeOH, 89%–quant; (b) Et_3SiH, CF_3COOH, 85–94%.

THF solution to give compound **3g**. Compounds **4a–b** and **4d** were obtained by treatment with triphosgene and amine in the presence of Et_3N. Alkylation of amines gave dialkylamines without monoalkylamines using alkyl halide and NaH in 11–30% yield (**8a–b** and **9a–b)**. Amides and urea were alkylated by similar methodology at rt–60 °C in 52–87 and 80% yields, respectively (**3i**, **3l**, **10c** and **4c)**. Saponification of compound **2a** and selective saponification of compound **3h** afforded compound **2b** in quantitative yield and compound **10a** in 77% yield, respectively.

Selective reduction of the ketones **3j**, **3ii** and **10b** with $NaBH_4$ gave compounds **11** in high yield. With LAH, reduction of the amido group at the 6-position or decomposition were easily accomplished. Further, selective reduction of the alcohols **11** by treatment with Et_3SiH in CF_3COOH easily afforded compounds **12** in excellent yield (Scheme 4).

These compounds were evaluated for their abilities to inhibit LPS-stimulated production of TNF-α in rat whole blood. IC_{50} of TNF-α production was determined by comparison of yield with a control to which no test compound was added.[16] FR133605[17] of TNF inhibitor,

Dexamethasone and Pentoxifylline were used as the positive control.

Results and Discussion

We initially investigated in vitro SAR concerning the amino group at the 2-position and the acetyl group at the 6-position (Table 1). Compounds **3e–g** showed excellent activity. These initial results appeared to indicate that nonbasic amino group at the 2-position and the 6-substituents were important for potent activity. Therefore, based on this information, chemical modifications of their substituents were started.

Table 2 summarizes the in vitro activity for the amides and the ureas. Keeping an acetyl group at the 6-substituent, acetyl and propionyl derivatives were tolerated, whereas heptanoyl derivative was inactive (**3a–b** and **3d**). From this result, it was clear that long-chain alkanoyl group was not desirable. Introduction of the branching alkanoyl group such as isobutyryl, pivaloyl, 2-chloropropionyl and so on exhibited potency comparable to the straight-chain alkanoyl derivatives (e.g., **3h**, **3j** vs **3b** and **3f** vs **3c**). Moreover, substituents at the 2-position of propionyl group led to increase in activity in the order of $Me > Br > NH_2 > Cl$ (**3e–h**). Substitution of a cycloalkanoyl group makes little difference to excellent activity between *c*-propanecarbonyl analogue (**3k**) and *c*-butanecarbonyl analogue (**3m**). However, *c*-hexanecarbonyl analogue (**3n**) displayed much weaker activity. Nicotinoyl derivatives (**3q–r**) were tolerated despite benzoyl derivatives (**3o–p**) and phenylalkanoyl derivatives (**3s–t**) had diminished potency. The activity of the ureas compared with the amides showed increased potency (**3b** vs **4a** and **3h** vs **4b**). These appeared to demonstrate the importance of the pyridine nitrogen and the urea nitrogen for activity. *N*-Methylation of the amides and the urea indicated 13–25 times weaker activity (**3h**, **3k**, **4b** vs **3i**, **3l**, **4c**, respectively). Replacement of acetyl group with *c*-propanecarbonyl or benzyl group at the 6-position showed similar results in activity as with 6-acetyl. Namely, isobutyryl analogues (**5a** and **6a**) and *c*-propanecarbonyl analogues (**5b** and **6b**) revealed more excellent activity. Likewise, in the case of 6-methyl, they exhibited excellent activity (**7a–b**). Furthermore, based on these results, we investigated the in vitro activity for isobutyryl analogues. In particular, non-6-substituted isobutyryl analogue (**10a**) was approximately equipotent with 6-acetyl, 6-(*c*-propanecarbonyl) and 6-benzyl isobutyryl analogues (**3h**, **5a** and **6a**) The activity of compounds **10b** and **10c** demonstrated that 3-(2-chlorobenzoyl)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine derivatives bearing alkyl group such as methyl or ethyl at the 6-position and acylamino group such as isobutyramido at the 2-position were desirable.

On the other hand, dialkyl analogues except for compound **8a** showed considerably less activity (Table 3).

Next, we considered that phenyl moiety and oxygen atom were the very important factor for activity, so that the modification of the 3-position affected the activity. Therefore, first, we investigated in vitro SAR concerning substituents of the phenyl group (Table 4). The position of the chlorine atom showed the decrease in activity in the order of 4- < 3- < 2- (**3h**, **3k**, **3q** and **3u–x**). In particular, the 4-position was essentially inactive at 100 μM. The 2-Me phenyl derivatives **3y–z** and the non-substituted phenyl derivatives **3aa–dd** were approximately equipotent with **3h**, **3k**, **3o** and **3q**, respectively. The mono- and di-alkoxyphenyl derivatives **3ee–hh** indicated weak activity. Urea **4d** compared with analogue **4b** was 7-fold weaker activity. Thus, there was little difference in activity in terms of the electron withdrawing or donating nature of the phenyl moiety. However, the position of the substituents appeared to be important for the activity. On the other hand, as shown in Table 6, in the in vivo activity, the 2-Me phenyl derivative **3z** showed more potent activity than the other phenyl analogues. Replacement of the phenyl moiety with methyl and ethoxy groups displayed decreased potency. Namely, the substitutions led to increase in activity in the order of 2-ClPh > EtO > Me (**3h**, **3k** and **3q** vs **3ii–jj** vs **3kk–mm**). However, as shown in Table 6, in the in vivo activity, the ethoxy derivatives **3kk–ll** exhibited most potent activity.

Lastly, we modified a part of the oxygen atom at the 3-position (Table 5). Changing acetyl to hydroxyethyl and ethyl made little difference to activity (**3ii** vs **11b** vs **12b**). Nevertheless, in the case of benzoyl, the modification led to increase in activity in the order of benzoyl > phenylhydroxymethyl > benzyl (**3j** vs **11a** vs **12a**). Especially, compound **11c** compared with compound **10b** was 100-fold weaker activity. It seems that the oxygen atom of carbonyl group is the important factor for the activity.

Moreover, from the result of Table 6, logP values appear to almost never affect the in vivo activity.

All compounds which possess excellent in vitro activity ($IC_{50} < 10$ μM) were tested in vivo after oral

Table 6. In vivo inhibition of TNF-α production and logP for representative compounds

Compd	logP[a]	Inhibition (%) at 50 mg/kg, po[b]
3h	3.54	2.4
3k	3.33	35.0
3y	3.60	3.9
3z	3.25	58.6
3aa	3.19	35.0
3bb	2.84	34.1
3ii	2.54	22.7
3jj	2.19	31.3
3kk	3.19	49.1
3ll	2.84	64.2
FR133605	NT[c]	90.3 ± 5.9
Dexamethasone	NT	NT[d]
Pentoxifylline	NT	NT[e]

[a]Determined by HPLC analysis.
[b]Inhibition of LPS-stimulated serum TNF-α production in the rat.
[c]NT, not tested.
[d]$ED_{50} = 0.12 \pm 0.06$ mg/kg, po.
[e]74.4% at 100 mg/kg, po.

administration. TNF-α inhibitory activity was assessed by in vivo inhibition of serum TNF-α production in the rat.[18] As the results, the ED_{50} values for these compounds except for **3z** and **3ll** were >50 mg/kg.

In conclusion, starting from 3-(2-chlorobenzoyl)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine analogues, we have found that 3-ethoxycarbonyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine derivatives indicate more excellent activity by oral dosing. It is likely that esters may play an important role in the in vivo activity. Therefore, to our discovery of orally more excellent agents, the SAR of 3-alkoxycarbonyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine derivatives are in progress in our work.

References and Notes

1. Ivashkiv, L. B. *Adv. Immunol.* **1996**, *63*, 337.
2. Engelberts, I.; von Asmuth, E. J. U.; van der Linden, C. J.; Buurman, W. A. *Lymphokine Cytokine Res.* **1991**, *10*, 127.
3. Moreland, L. W.; Baumgartner, S. W.; Schiff, M. H.; Tindall, E. A.; Fleischmann, R. M.; Weaver, A. L.; Ettlinger, R. E.; Cohen, S.; Koopman, W. J.; Mohler, K.; Widmer, M. B.; Blosch, C. M. *N. Engl. J. Med.* **1997**, *337*, 141.
4. Feldman, M.; Taylor, P.; Paleolog, E.; Brennan, F. M.; Maini, R. N. *Transplant. Proc.* **1998**, *30*, 4126.
5. Clements, J. M.; Cossins, J. A.; Wells, G. M. A.; Corkill, D. J.; Helfrich, K.; Wood, L. M.; Pigott, R.; Stabler, G.; Ward, G. A.; Gearing, A. J. H.; Miller, K. M. *J. Neuroimmunol.* **1997**, *74*, 85.
6. Morimoto, Y.; Nishikawa, K.; Ohashi, M. *Life Sci.* **1997**, *61*, 795.
7. Dezube, B. J.; Pardee, A. B.; Beckett, L. A.; Ahlers, C. M.; Ecto, L.; Allen-Ryan, J.; Anisowicz, A.; Sager, R.; Crumpacker, C. S. *J. Acquir. Immune Defic. Syndr.* **1992**, *5*, 1099.
8. Feldmann, M.; Elliott, M. J.; Woody, J. N.; Maini, R. N. *Adv. Immunol.* **1997**, *64*, 283.
9. Elliott, M. J.; Maini, R. N.; Feldmann, M.; Long-Fox, A.; Charles, P.; Bijl, H.; Woody, J. N. *Lancet* **1994**, *344*, 1125.
10. Maini, R. N.; Breedveld, F. C.; Kalden, J. R.; Smolen, J. S.; Davis, D.; Macfarlane, J. D.; Antoni, C.; Leeb, B.; Elliott, M. J.; Woody, J. N.; Schaible, T. F.; Feldmann, M. *Arthritis Rheum.* **1998**, *41*, 1552.
11. Newton, R. C.; Decicco, C. P. *J. Med. Chem.* **1999**, *42*, 2295.
12. Fujita, M.; Seki, T.; Inada, H.; Shimizu, K.; Takahama, A.; Sano, T. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 771.
13. Sabnis, R. W. *Sulfur Reports* **1994**, *16*, 1.
14. Miyazawa, S.; Okano, K.; Kawahara, T.; Machida, Y.; Yamatsu, I. *Chem. Pharm. Bull.* **1992**, *40*, 762.
15. Ridge, D. N.; Hanifin, J. W.; Harten, L. A.; Johnson, B. D.; Menschik, J.; Nicolau, G.; Sloboda, A. E.; Watts, D. E. *J. Med. Chem.* **1979**, *22*, 1385.
16. TNF-α inhibition assay was performed according to previously described method with slight modification.[17,19] Whole blood from male SD rats (Charles River Laboratories) was collected into 2% heparinized vacuum tubes. The blood was suspended in RPMI-1640 supplemented with 100 μg/mL streptomycin and 100 U/mL penicillin at a concentration of 50%, and seeded into 24- well plate (950 μL). Vehicle (DMSO) or test compounds in DMSO was added to each well, and the plate was incubated at 37 °C in 5% CO_2 for 30 min. Finally, 10 μg/mL LPS (*Escherichia coli* B055:B5) in RPMI-1640 was added, and plates were incubated at 37 °C in 5% CO_2 for 4 h. Supernatants were then harvested, and assayed for TNF-α using ELISA kits. IC_{50} of TNF-α production was determined by comparison of yield with a control to which no test compound was added.
17. Yamamoto, N.; Sakai, F.; Yamazaki, H.; Kawai, Y.; Nakahara, K.; Okuhara, M. *J. Rheumatol.* **1996**, *23*, 1778.
18. TNF-α inhibition assay was performed according to previously described method with slight modification.[17,19] Male SD rats (6 weeks old, Charles River Laboratories) were dosed intravenously or orally with test compounds suspended in 0.5% CMC. 1 h later, each rat was injected iv with LPS (*E. coli* B055:B5, 0.1 mg/kg). The rats were sacrificed and bled 90 min later, which is a time point of maximal elevation of serum TNF-α activity. Serum TNF-α activity was measured using ELISA kits. ED_{50} of TNF-α production was determined by comparison of yield with a control to which no test compound was added.
19. Wadsworth, S. A.; Cavender, D. E.; Beers, S. A.; Lalan, P.; Schafer, P. H.; Malloy, E. A.; Wu, W.; Fahmy, B.; Olini, G. C.; Davis, J. E.; Pellegrino-Gensey, J. L.; Wachter, M. P.; Siekierka, J. J. *J. Pharmacol. Exp. Ther.* **1999**, *291*, 680.