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

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Abstract—We synthesized bicyclic thiophenes and 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine derivatives, and evaluated for their ability to inhibit LPS-stimulated production of TNF-α. Several compounds revealed excellent in vivo activity. Furthermore, an effective compound was found in adjuvant-induced arthritic model (AIA) of rat.

Introduction

Tumor necrosis factor-α (TNF-α) plays important roles in the pathology of inflammatory diseases such as rheumatoid arthritis (RA).[1] In clinical trials, the monoclonal TNF-α antibodies, Remicade (infliximab),[2] and the soluble TNF p75 receptor fusion protein (TNFRp75:Fc), Enbrel (etanercept)[1,3] have been shown to be effective in the treatment of RA and Crohn's disease. Therefore, inhibition of TNF-α has lately attracted considerable attention as target for antirheumatic agents, and it has been reported that many compounds have an inhibitory activity toward the production of TNF-α such as FR133605,[4] piperidylpyrimidine derivatives[5] and so on.[6]

In a previous paper, we have described 2-amino-3-ethoxycarbonyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine derivatives **3d** and **3u** indicate more excellent activity than 3-(2-chlorobenzoyl)-4,5,6,7-tetrahydrothieno[2,3-*c*]-pyridine analogues by oral dosing.[7] Further, based on these results, to the discovery of more potent compound, we synthesized bicyclic thiophene and 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine derivatives, and evaluated for their ability to inhibit LPS-stimulated production of TNF-α. Thus, we found an effective compound in adjuvant-induced arthritic model (AIA) of rat.

Chemistry and Biology

A series of the compounds listed in Tables 1–3 were synthesized by the methods illustrated in Scheme 1.

The syntheses of compounds **3** and **4** were started from ketones **1** and utilized synthetic sequence previously reported by us.[7]

Carboxylic acid **2b** was prepared by selective saponification of compound **2a** in 34% yield, or by *N*-acetylation of compound **2c** in 45–56% yield.

As previously described, these compounds were evaluated for their ability 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. FR133605 that was the most potent in vivo in our research into many published TNF-α inhibitors, Dexamethasone and Pentoxifylline were used as the positive control. Furthermore, representative compounds which possess excellent in vitro activity were tested in vivo after oral administration. TNF-α inhibitory activity was assessed by in vivo inhibition of serum TNF-α production in the rat.[7]

Results and Discussion

Tables 1–3 summarize the in vitro and in vivo activities.

First, as shown in Table 1, we examined to change tetrahydropyridine ring to cyclohexene, dihydrothiopyran

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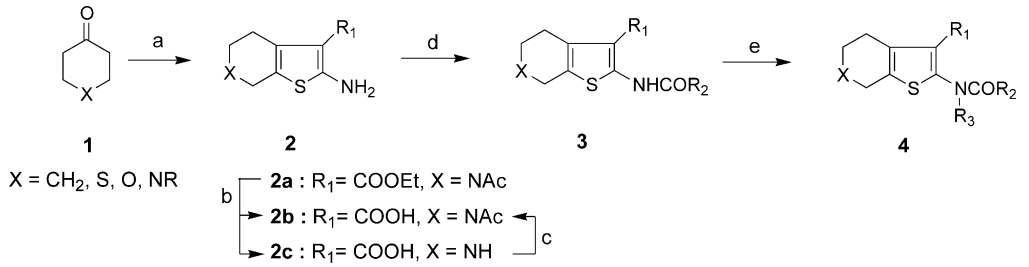


Scheme 1. Reagents and conditions: (a) $NCCH_2R_1$, S, Et_3N/DMF or Et_3N/EtOH or morpholine/MeOH, 60 °C–reflux, 37–93%; (b) NaOH, MeOH–H_2O, 0 °C–rt, **2b**: 34%, **2c**: 59%; (c) Ac_2O or AcCl, pyridine, rt, 45–56%; (d) R_2COX, pyridine, 27%–quant. or amines, triphosgene, Et_3N, CH_2Cl_2, 0 °C–rt, 32–92%; (e) R_3X, NaH, DMF, 11–87%.

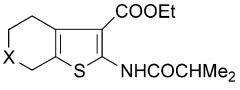
and dihydropyran ring, and *N*-substitution at the 6-position. In the case of the in vitro activity, dihydropyran (**3c**), *N*-benzoyl (**3e**) and *N*-ethyl analogues (**3g**) were more excellent than the lead compound **3d**. However, compounds **3c** and **3e** indicated weaker in the in vivo activity. From these results, it is likely that *N*-acetyl and *N*-ethyl analogues may be totally desirable, nevertheless that the modification of this moiety may not play an important role for the activity.

Next, we modified the substituents at the 3-position (Table 2). In the in vitro activity, the esters (**3j–k**) and the nitrile (**3n**) compared with compound **3d** showed more potent and equipotent activity, respectively, despite the carboxylic acid (**3l**) and the carboxamide (**3m**) were inactive. In the in vivo activity, compounds **3j–k** exhibited considerably weaker activity, considering the in vitro activity. Therefore, these suggest that the bulky substituents of the ester moiety are less suitable for the in vivo activity.

Finally, as shown in Table 3, we investigated *N*-substitution at the 2-position. The methyl, ethyl and *t*-butyl groups showed more potent activity, whereas the other alkyl groups were almost equipotent in both the in vitro and in vivo activities (**3d** vs **3o–t**). In particular, compound **3r** revealed excellent activity. Replacement of the ethyl group with an ethoxy or an ethylamino group had diminished potency (**3w** and **3aa**). Moreover, diethylamino analogue **3cc** compared with compound **3aa** showed more potent activity and especially excellent in vivo activity, while cyclohexylethylamino analogue **3dd** showed similar activity. Capping the ethyl with a 4-morpholinyl group makes little difference to activity (**3w** vs **3x**). Furthermore, replacement of the ethylamino (**3aa**) with heterocyclic amino groups showed equally or slightly more potent activity (**3y–z**), whilst changing from the diethylamino (**3cc**) to cyclic secondary amino groups had diminished potency except for **3jj** (see **3ff–ll**). These results suggest that neutral or basic natures in this region are not an important factor for the activity. The introductions of both polar and bulky groups were found to lead to increase in the in vitro activity, but to decrease in the in vivo activity (**3bb** and **3ee**). As previously described, *N*-methylation of the amide **3r** and the urea **3cc** indicated weaker activity despite slightly in the case of **3r** (see **4a–b**). Further, changing the acetyl at the 6-position to ethyl group, **3r** had diminished potency, but **3cc** had increased in vitro activity despite equally in the in vivo activity (**3mm–nn**).[7]

As the target of development of arthritis, compound **3cc** which possesses excellent in vivo activity was evaluated

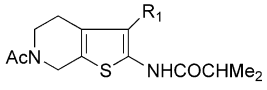
Table 1. In vitro and vivo inhibitory activities of TNF-α production for compounds **3a–i**



Compd	X	In vitro IC_{50} (μM)[a]	In vivo inhibition (%) at 50 mg/kg, po[b]
3a	CH_2	28	1.8
3b	S	34	21.2
3c	O	10	17.6
3d	AcN	22	49.1
3e	BzN	10	31.8
3f	MeN	32	22.7
3g	EtN	15	69.2
3h	$Me(CH_2)_2N$	40	NT[c]
3i	BnN	42	NT
FR133605		6.0±4.2	90.3±5.9
Dexamethasone		0.02	NT[d]
Pentoxifylline		40±2	NT[e]

[a]IC_{50} of LPS-stimulated TNF-α production in rat whole blood.
[b]Inhibition of LPS-stimulated serum TNF-α production in the rat.
[c]NT, not tested.
[d]ED_{50} = 0.12±0.06 mg/kg, po.
[e]74.4% at 100 mg/kg, po.

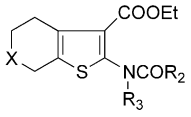
Table 2. In vitro and vivo inhibitory activities of TNF-α production for compounds **3d** and **3j–n**



Compd	R_1	In vitro IC_{50} (μM)[a]	In vivo inhibition (%) at 50 mg/kg, po[b]
3d	COOEt	22	49.1
3j	$COOCHMe_2$	7.1	42.1
3k	$COOCH_2CH(Et)(CH_2)_3Me$	10	10.6
3l	COOH	>100	NT[c]
3m	$CONH_2$	>100	NT
3n	CN	22	23.2

[a]IC_{50} of LPS-stimulated TNF-α production in rat whole blood.
[b]Inhibition of LPS-stimulated serum TNF-α production in the rat.
[c]NT, not tested.

Table 3. In vitro and vivo inhibitory activities of TNF-α production for compounds **3d**, **3o–nn** and **4a–b**



Compd	X	R_2	R_3	In vitro IC_{50} (μM)[a]	In vivo inhibition (%) at 50 mg/kg, po[b]
3d	AcN	Me_2CH	H	22	49.1
3o	AcN	Me_2CHCH_2	H	20	33.1
3p	AcN	Me_3C	H	11	63.7
3q	AcN	Me	H	13	78.4
3r	AcN	Et	H	7.9	90.1
3s	AcN	$Me(CH_2)_2$	H	28	33.3
3t	AcN	$Me(CH_2)_3$	H	18	42.9
3u	AcN	Cyclopropyl	H	10	64.2
3v	AcN	3-Pyridyl	H	22	23.5
3w	AcN	EtO	H	20	57.1
3x	AcN	4-Morpholinyl$(CH_2)_2O$	H	13	57.6
3y	AcN	3-PyridylNH	H	13	48.7
3z	AcN	4-MorpholinylNH	H	12	22.1
3aa	AcN	EtNH	H	19	17.0
3bb	AcN	Bn(MeOOC)CHNH	H	0.9	8.2
3cc	AcN	Et_2N	H	6.2	92.3
3dd	AcN	Cyclohexyl(Et)N	H	12	7.6
3ee	AcN	Cyclpropyl$CH_2(Me(CH_2)_2)N$	H	1.3	34.8
3ff	AcN	4-Methyl-1-piperazinyl	H	17	40.7
3gg	AcN	4-Formyl-1-piperazinyl	H	22	41.9
3hh	AcN	Piperazinyl	H	16	51.4
3ii	AcN	Piperidino	H	13	46.8
3jj	AcN	Morpholino	H	1.0	90.3
3kk	AcN	Thiomorpholino	H	12	20.0
3ll	AcN	3-Thiazolidinyl	H	6.0	5.4
4a	AcN	Et	Me	9.0	81.0
4b	AcN	Et_2N	Me	12	51.9
3mm	EtN	Et	H	22	9.2
3nn	EtN	Et_2N	H	2.2	88.7

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

by AIA (Table 4).[8] Indomethacin inhibited paw edema to a significant degree, but showed no effects for cartilage destruction and femur weight. Auranofin, one of disease modifying antirheumatic drugs (DMARD), did not affect paw edema and femur weight, whilst the loss of GAG content was restored. FR133605 significantly suppressed paw edema and restored femur weight, and the loss of GAG content improved. Compound **3cc** also significantly suppressed paw edema and improved femur weight and the loss of GAG content. In particular, compound **3cc** drastically affected femur weight and the loss of GAG content. This suggests that compound **3cc** possesses excellent effects for bone and cartilage.

Table 4. Effects of compound **3cc**, FR133605 and antirheumatic drugs in the adjuvant-induced arthritic model of rat

Drug	Dose (mg/kg)	Percentage recovery		
		Paw edema	GAG	Femur weight
3cc	50	42.5*	119**	110**
FR133605	50	78.4**	90	98*
Auranofin	10	−4.7	73	17
Indomethacin	2	72.1**	7.4	20

Data are the means ± SE ($n = 7–10$); $^{**}p < 0.01$, $^{*}p < 0.05$ compared to the value of control.

In conclusion, we have found that several of ethoxycarbonyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine derivatives indicate more excellent inhibitory activity for TNF-α production by oral dosing. Among these compounds, the representative compound **3cc** exhibited some excellent profiles in AIA as a candidate for antirheumatic drugs. Further, next stage including action mechanism, toxicity and pharmacokinetics studies of these compounds is currently in progress and will be reported elsewhere.

Acknowledgement

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8. Induction of adjuvant arthritis:[4] Arthritis was induced on day 1 by the injection of 100 μg of adjuvant (suspension of heat killed *Mycobacterium tuberculosis* H37RA in paraffin oil) into the tail base of male Lewis rats (200–250 g, Charles River Laboratories). Drugs suspended in 0.5% CMC were orally administered once a day starting on day 1 up to day 17. The volume of the right hind paw was measured at day 18 by aqueous plethysmography. The right femora were dissected and removed on the final day (day 18), and the dry femora weight and the glycosaminoglycan (GAG) content in the articular cartilages were measured. The condyles were removed from the right femora and digested with papain (300 μg/mL) in 50 mM phosphate buffer (pH 6.5) containing 2 mM *N*-acetyl cysteine and 2 mM EDTA at 65 °C for 2 h. The released sulfated GAG were measured by the dimethyl methylene blue dye spectrophotometric method.