Bioorganic & Medicinal Chemistry Letters 20 (2010) 2435–2437

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Tetrahydropyridine derivatives with inhibitory activity on the production of proinflammatory cytokines: Part 2

Akira Nakao [a,*], Nobuyuki Ohkawa [b], Takayoshi Nagasaki [c], Takashi Kagari [d], Hiromi Doi [d], Takaichi Shimozato [d], Shigeru Ushiyama [e], Kazumasa Aoki [a]

[a] *Medicinal Chemistry Research Laboratories II, Daiichi Sankyo Co., Ltd, 1-16-13 Kitakasai, Edogawa-ku, Tokyo134-8630, Japan*
[b] *Medicinal Chemistry Research Laboratories I, Daiichi Sankyo Co., Ltd, 1-2-58 Hiromachi, Shinagawa-ku, Tokyo140-8710, Japan*
[c] *Research Department II, Daiichi Sankyo RD Associe Co., Ltd, 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan*
[d] *Biological Research Laboratories III, Daiichi Sankyo Co., Ltd, 1-16-13 Kitakasai, Edogawa-ku, Tokyo 134-8630, Japan*
[e] *R&D Planning Department, Daiichi Sankyo Co., Ltd, 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan*

A R T I C L E I N F O

Article history:
Received 12 January 2010
Revised 27 February 2010
Accepted 4 March 2010
Available online 10 March 2010

Keywords:
Analgesic and anti-inflammatory agent
p38 MAP kinase
Cytokine
TNFα
IL-1β

A B S T R A C T

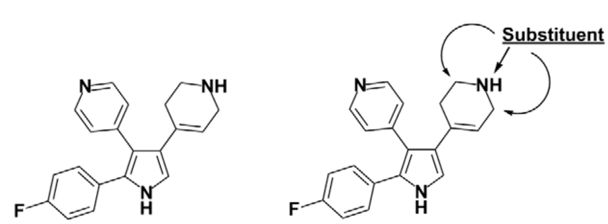
We previously reported a novel pyrrole derivative **1** which possesses a tetrahydropyridine group at the β-position with a proinflammatory cytokine TNFα production inhibitor. Herein, we report the synthesis and biological activity of N- and α-position substituted tetrahydropyridine derivatives. In this series, we found that compound **3o** showed good inhibitory activity in vitro (inhibition of lipopolysaccharide (LPS)-induced TNFα production in human whole blood, IC_{50} = 0.44 μM) and compound **3i** demonstrated potent inhibitory activity in vivo (inhibition of LPS-induced TNFα production in mice, ID_{50} = 1.42 mg/kg).

Excessive production of proinflammatory cytokines, including TNFα and IL-1β,[1] is implicated in many inflammatory diseases[2] and its inhibition is a proven therapeutic strategy in suppressing inflammation. Anti-cytokine biological agents are proven to be clinically effective in the treatment of rheumatoid arthritis (RA), ankylosing spondylitis, Crohn's disease and psoriasis by the blockade of TNFα function.[3] Therefore, the discovery and development of orally active proinflammatory cytokine inhibitors for the treatment of numerous inflammatory diseases have been pursued by many pharmaceutical research groups.[4]

We have previously reported[5] the pyridylpyrrole derivative possessing tetrahydropyridine at the β-position of the pyrrole ring **1** (Fig. 1) as a potent inhibitor of proinflammatory cytokine TNFα production. In an attempt to find more potent compounds than **1**, we designed and synthesized the alkyl substituent to be introduced to a N- and α-position on the tetrahydoropyridinyl group. Herein, we report our results which have led to the identification of potent pyrrole-based inhibitors of the proinflammatory cytokine TNFα, **3i** and **3o**.

Tetrahydropyridine derivatives **3a–o** were synthesized in accordance with our established synthetic route which has been reported previously (Scheme 1).[5] The introduction of a N- and α-substituted tetrahydropyridinyl group to the β-position of the pyrrole ring was carried out by a bromine–lithium exchange of **2** followed by 1,2-addition with piperidin-4-one derivatives. Subsequent dehydroxylation of the tertiary alcohol was carried out in excellent yield by exposure to trifluoroacetic acid (TFA) concurrently with deprotection of the triisopropylsilyl (TIPS) group with tetrabutylammonium fluoride (TBAF) to give the tetrahydropyridine derivatives **3a–o**. All of the α-substituted tetrahydropyridinyl analogues **3i–o** were racemic compounds.

Regioisomers **3i** and **3j** were separated[6] by silica gel column chromatography and their structures were determined by NMR studies. The NOE experiments, with a strong NOE from H^A to H^B

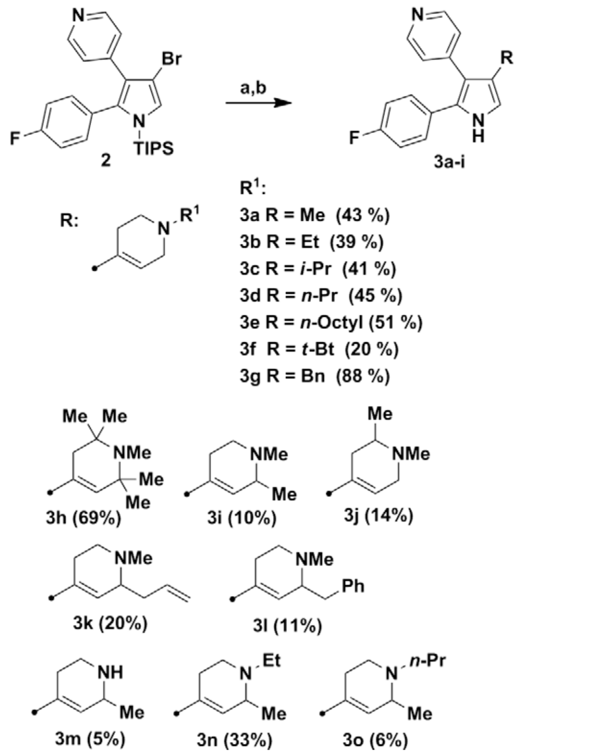


1

Figure 1.

* Corresponding author.
E-mail address: nakao.akira.g5@daiichisankyo.co.jp (A. Nakao).

0960-894X/$ - see front matter Crown Copyright © 2010 Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.03.022



Scheme 1. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C then piperidin-4-one derivatives, then rt; (b) TFA, CH_2Cl_2, rt, then TBAF, THF, rt.

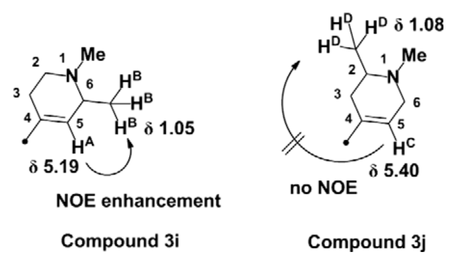


Figure 2.

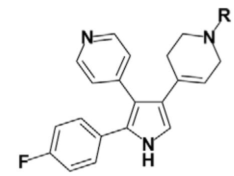
had no enhancement between H^C and H^D (Fig. 2). Compounds **3k–o** were synthesized and determined in a similar manner.

First, the designed and synthesized N-substituted tetrahydropyridine derivatives were evaluated in terms of their inhibitory activities in LPS-induced TNFα production in human whole blood.[5] N-unsubstituted analogue **1** was used as a reference for the comparison of the in vitro and in vivo potencies of the new analogues (Table 1).

Compound **3a**, having a methyl group on nitrogen, showed lower inhibitory activity than that of **1**, while ethyl (**3b**) and isopropyl (**3c**) analogues were more effective. In particular, *n*-propyl derivative **3d** showed the most potent inhibitory activity (IC_{50} = 0.98 μM). Octyl analogue **3e**, possessing an alkyl chain elongated substituent, exhibited good inhibitory activity, but *tert*-butyl (**3f**) and benzyl (**3g**) derivatives which have a rather sterically hindered group, their potency decreased by less than IC_{50} = 10 μM.

Subsequently, we evaluated the inhibitory activity of these derivatives in the LPS-induced TNFα production in mice.[5] Compound **3a** showed the most effective activity (ID_{50} = 2.89 mg/kg), which was more than twofold as potent as that of **1**. However, analogues **3b**, **3c**, and **3d** demonstrated good in vitro efficacy and showed lower activity than that of **1**. Furthermore, **3e** dropped unexpectedly in in vivo potency.

Table 1
In vitro, in vivo activities and Clog *P* values of N-substituted tetrahydropyridine derivatives



Compd	R	IC_{50}[a] (μM)	ID_{50}[b] (mg/kg)	Clog *P*[c]
1	H	1.86 (1.45–2.31)	5.98	3.04
3a	Methyl	3.96 (2.63–5.97)	2.89	3.48
3b	Ethyl	1.21 (0.72–2.05)	6.67	4.01
3c	*i*-Propyl	0.98 (0.75–1.28)	6.18	4.32
3d	*n*-Propyl	0.52 (0.29–0.95)	6.75	4.54
3e	*n*-Octyl	1.38 (0.77–2.48)	45%[d]	7.19
3f	*t*-Butyl	>10	—[e]	4.72
3g	Benzyl	>30	—[e]	5.27

[a] Inhibition of LPS-induced TNFα production in human whole blood. Results are given as mean and S.D. of three to four determinations.
[b] Inhibition of LPS-induced TNFα production in mice. *N* = 5.
[c] Clog *P* values calculated using PALLAS (INFOCOM CORPORATION).
[d] % Inhibition at 20 mg/kg.
[e] Not tested.

In order to search for the relationship between the in vivo efficacy and molecular property, we calculated Clog *P* values[7] as a polar parameter of the molecule, and these values were shown in the left column in Table 1. The Clog *P* value of **3a**, which exhibited the most effective in vivo potency, was 3.48. In comparison, Clog *P* values of **3b**, **3c**, **3d**, and **3e** showed unexpectedly lower efficacy in vivo than that of **3a** and were 4.01, 4.32, 4.54, and 7.19, respectively. Despite the slightly lower in vitro potency of **3a** compared to those of **3b–e**, it is speculated that the good in vivo efficacy of **3a** (ID_{50} ca. 3 mg/kg) demonstrated that compounds having a Clog *P* value between ca. 3.5 and 4.0 is preferable to this in vivo system.

Next, we designed and synthesized α-substituted *N*-methyltetrahydropyridine derivatives, which were evaluated in terms of their inhibitory activities in vitro (Table 2). Tetramethyl analogue **3h** showed lower activity than that of **3a**, while methyl geometric isomers **3i** and **3j** showed potent inhibitory activity, IC_{50} = 0.63 and 1.28 μM, respectively. Therefore, regarding the α-substitution of the 1,2,3,6-tetrahydropyridin-4-yl group, a substitution of the 6-position was preferable to that of the 2-position in demonstrating good inhibitory activity. Although, allyl (**3k**) and benzyl (**3l**) derivatives, having a rather sterically hindered group at the 6-position, showed less potency. These results suggest that the introduction of a methyl group to the 6-position of the 1,2,3,6-tetrahydropyridin-4-yl group exhibited the most enhanced inhibitory activity in vitro.

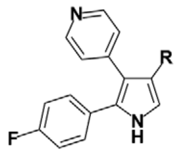
We then evaluated the in vivo efficacy of these derivatives. While **3h** decreased in in vivo efficacy as with in vitro, **3i** showed excellent inhibitory activity (ID_{50} = 1.42 mg/kg), which was twofold potent compared to that of regioisomer **3j** (ID_{50} = 2.73 mg/kg), Clog *P* values of **3h** and **3i** were 5.56 and 3.83, respectively. In this series, the Clog *P* values of **3i** and **3j**, similarly analogue **3a** (ID_{50} = <3 mg/kg), indicated good in vivo efficacy and were less than four.

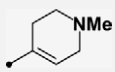
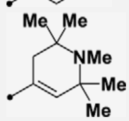
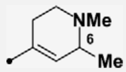
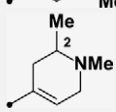
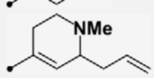
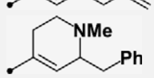
Based on these results, we further examined the effects of substituent on nitrogen atom of 6-methyl 1,2,3,6-tetrahydropyridin-4-yl. The biological activities are summarized in Table 3.

Regarding in vitro activities as compared with **3i**, unsubstituted analogue **3m** kept its potency (IC_{50} = 0.71 μM), while ethyl derivative **3n** decreased in efficacy. Compound **3o**, possessing a *n*-propyl group, showed the most potent inhibitory activity (IC_{50} = 0.44 μM).

In terms of in vivo activities, contrary to our expectation, **3o** showed lower efficacy than that of **3i** and was the same potency as that of **3m**, ID_{50} = 2.29 and 2.79 mg/kg, respectively.

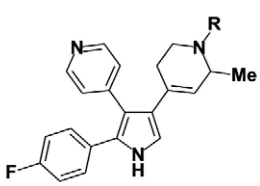
Table 2
In vitro, in vivo activities and *C*log *P* values of α-substituted *N*-methyltetrahydropyridine derivatives



Compd	R	IC_{50}[a] (μM)	ID_{50}[b] (mg/kg)	Clog *P*[c]
3a		3.96 (2.63–5.97)	2.89	3.48
3h		8.44 (5.26–13.54)	7.27	5.56
3i		0.63 (0.42–0.93)	1.42	3.83
3j		1.28 (0.96–1.70)	2.73	3.83
3k		48.7%[d]	–[e]	4.58
3l		30.8%[d]	–[e]	5.57

[a] Inhibition of LPS-induced TNFα production in human whole blood. Results are given as mean and S.D. of three to four determinations.
[b] Inhibition of LPS-induced TNFα production in mice. *N* = 5.
[c] *C*log *P* values calculated using PALLAS (INFOCOM CORPORATION).
[d] Inhibition at 10 μM.
[e] Not tested.

Table 3
In vitro, in vivo activities and *C*log *P* values of N- substituted α-methyltetrahydropyridine derivatives



Compd	R	IC_{50}[a] (μM)	ID_{50}[b] (mg/kg)	Clog *P*[c]
3m	H	0.71 (0.15–1.72)	2.29	3.56
3i	Methyl	0.63 (0.42–0.93)	1.42	3.83
3n	Ethyl	1.61 (0.94–2.75)	5.28	4.53
3o	*n*-Propyl	0.44 (0.32–0.61)	2.79	5.06

[a] Inhibition of LPS-induced TNFα production in human whole blood. Results are given as mean and S.D. of three to four determinations.
[b] Inhibition of LPS-induced TNFα production in mice. *N* = 5.
[c] *C*log *P* values calculated using PALLAS (INFOCOM CORPORATION).
[d] Inhibition at 10 μM.

The *N*-*n*-propyl analogues, both α-unsubstituted and α-methyl substituted tetrahydropyridine **3d** and **3o**, demonstrated unexpectedly low potency in vivo, despite their potent in vitro activities. This result could be explained by their *C*log *P* values such as 4.5–5.0, which are large for the in vivo system. On the other hand, *N*-methyl, α-methyltetrahydropyridine derivative **3i**, showed the most potent in vivo efficacy (ID_{50} = 1.42 mg/kg) and had a 3.83 *C*log *P* value. From these results, the in vivo efficacy of this series would rest on the balance of the in vitro potency and lipophilicity, therefore, in order to obtain more effective compounds in vivo, it would be necessary for the *C*log *P* value to be less than four with higher inhibitory activity in vitro.

In order to develop a new anti-inflammatory agent, we synthesized and evaluated derivatives possessing a N-and α-substituted tetrahydropyridinyl group at the β-position of the pyrrole ring as scaffold **1**. Analogue **3o**, which has *N*-*n*-propyl, an α-methyl tetrahydropyridinyl group, showed potent inhibitory activity in LPS-induced TNFα production in human whole blood. Furthermore, *N*-methyl, α-methyl analogue **3i**, exhibited the most potent inhibitory activity in the LPS-induced TNFα production in mice. Based on these results, we plan to further investigate pyridylpyrroles possessing N- and α-substituted chiral tetrahydoropyridinyl groups.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.03.022.

References and notes

1. (a) Adamus, J. L.; Badger, A. M.; Kumar, S.; Lee, J. C. *Prog. Med. Chem.* **2001**, *38*, 1; (b) Lee, J. C.; Kumar, S.; Griswold, D. E.; Underwood, D. C.; Votta, B. J.; Adams, J. L. *Immunopharmacology* **2000**, *47*, 185.
2. (a) Foster, M. L.; Halley, F.; Souness, J. E. *Drug News Perspect.* **2000**, *13*, 488; (b) Feldmann, M.; Brennan, F. M.; Maini, R. N. *Annu. Rev. Immunol.* **1996**, *14*, 397; (c) Tracey, K. J.; Cerami, A. *Annu. Rev. Cell Biol.* **1993**, *9*, 317.
3. (a) Goldsmith, D. R.; Wagstaff, A. J. *BioDrugs* **2005**, *19*, 401; (b) Feldman, S. R.; Gordon, K. B.; Bala, M.; Evans, R.; Li, S.; Dooley, L. T.; Guzzo, C.; Patel, K.; Menter, A.; Gottlieb, A. B. *Br. J. Dermatol.* **2005**, *152*, 954; (c) Moreland, L. W. *Dis. Clin. North Am.* **1998**, *24*, 579; (d) Bang, L. M.; Keating, G. M. *BioDrugs* **2004**, *18*, 121.
4. (a) Graneto, M. J.; Kurumbail, R. G.; Vazquez, M. L.; Shieh, H.-S.; Pawlitz, J. L.; Williams, J. M.; Stallings, W. C.; Geng, L.; Naraian, A. S.; Koszyk, F. J.; Stealey, M. A.; Xu, X. D.; Weier, R. M.; Hanson, G. J.; Mourey, R. J.; Compton, R. P.; Minch, S. J.; Anderson, G. D.; Monahan, J. B.; Devraj, R. *J. Med. Chem.* **2007**, *50*, 5712; (b) McClure, K. F.; Letavic, M. A.; Kalgutkar, A. S.; Gabel, C. A.; Audoly, L.; Barberia, J. T.; Braganza, J. F.; Carter, D.; Carty, T. J.; Cortina, S. R.; Dombroski, M. A.; Donahue, K. M.; Elliott, N. C.; Gibbons, C. P.; Jordan, C. K.; Kuperman, A. V.; Labasi, J. M.; LaLiberte, R. E.; McCoy, J. M.; Naiman, B. M.; Nelson, K. L.; Nguyen, H. T.; Peese, K. M.; Sweeney, F. J.; Taylor, T. J.; Trebino, C. E.; Abramov, Y. A.; Laird, E. R.; Volberg, W. A.; Zhou, J.; Bach, J.; Lombardo, F. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4339; (c) McClure, K. F.; Abramov, Y. A.; Laird, E. R.; Barberia, J. T.; Cai, W.; Carty, T. J.; Cortina, S. R.; Danley, D. E.; Dipesa, A. J.; Donahue, K. M.; Dombroski, M. A.; Elliott, N. C.; Gabel, C. A.; Han, S.; Hynes, T. R.; LeMotte, P. K.; Mansour, M. N.; Marr, E. S.; Letavic, M. A.; Pandit, J.; Ripin, D. B.; Sweeney, F. J.; Tan, D.; Tao, Y. *J. Med. Chem.* **2005**, *48*, 5728; (d) Dominguez, C.; Powers, D. A.; Tamayo, N. *Curr. Opin. Drug Discov. Devel.* **2005**, *8*, 421; (e) Dominguez, C.; Tamayo, N.; Zhang, D. *Expert Opin. Ther. Patents* **2005**, *15*, 801; (f) Goldstein, D. M.; Gabriel, T. *Curr. Top. Med. Chem.* **2005**, *5*, 1017; (g) Hynes, J.; Leftheris, K. *Curr. Top. Med. Chem.* **2005**, *5*, 967; (h) Regan, J.; Breitfelder, S.; Cirillo, P.; Gilmore, T.; Graham, A. G.; Hickey, E.; Klaus, B.; Madwed, J.; Moriak, M.; Moss, N.; Pargellis, C.; Pav, S.; Proto, A.; Swinamer, A.; Tong, L.; Torcellini, C. *J. Med. Chem.* **2002**, *45*, 2994.
5. (a) Nakao, A.; Ohkawa, N.; Nagasaki, T.; Doi, H.; Shimozato, T.; Ushiyama, S.; Aoki, K. *Bioorg. Med. Chem. Lett.* **2009**, *16*, 4607; (b) IC_{50} values were calculated from liner regression curves obtained from the% inhibition and the logarithmic value of the concentrations of the test compounds by the last-squares method. The 95% confidence intervals (C.I.) were calculated by Fieller's theorem. IC_{50} values and their 95% C.I. values were calculated using the SAS System for Windows (SAS Institute Inc.).
6. The crude ratio of regioisomers were approximately 1:1 by NMR studies. The isolated yield indicated in Scheme 1.
7. The program, PALLAS from INFOCOM CORPORATION, was used to calculate the log *P* value.