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Tetrahydropyridine derivatives with inhibitory activity on the production of proinflammatory cytokines: Part 3

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ABSTRACT

In order to develop a new class of anti-rheumatic drug which inhibits production of proinflammatory cytokines such as TNFα, IL-1β, IL-6, and IL-8, a series of 3-pyridylpyrrole derivatives possessing a bicyclic tetrahydropyridine moiety at the 4-position of the pyrrole ring were synthesized and their pharmacological activities were evaluated. The derivatives were found to have potent inhibitory activities on the production of the cytokines both in vitro and in vivo. Among them, compound **4a**, (*S*)-2-(4-fluorophenyl)-4-(1,2,3,5,6,8a-hexahydroindolizin-7-yl)-3-(pyridin-4-yl)-1*H*-pyrrole (R-132811), achieved the most promising results in various in vitro and in vivo tests including several rheumatoid arthritis models ((i) inhibition of p38α, p38β, p38γ, and p38δ MAP kinases: IC_{50} = 0.034, 0.572, >10, and >10 μM, respectively; (ii) inhibition of TNFα, IL-1β, IL-6, and IL-8 production in human whole blood: IC_{50} = 0.026, 0.020, 0.88, and 0.016 μM, respectively; (iii) inhibition of LPS induced TNFα, IL-1β and IL-6 production in mice: ID_{50} = 0.93, 8.63, and 0.11 mg/kg, po, respectively; (iv) inhibition of anti-collagen antibody-induced arthritis in mice: ID_{50} = 2.22 mg/kg, po; (v) inhibition of collagen-induced arthritis in mice: ID_{50} = 2.38 mg/kg, po; (vi) prophylactic effect on adjuvant-induced arthritis in rats: ID_{50} = 3.1 mg/kg, po; (vii) therapeutic effect on adjuvant-induced arthritis in rats: ID_{50} = 4.9 mg/kg, po; (viii) analgesic effect on adjuvant-induced arthritic pain in rats: ID_{50} = 2.9 mg/kg, po). As a result, compound **4a** was chosen as a candidate for further pre-clinical studies.

p38 mitogen-activated protein (MAP) kinase is an intracellular serine/threonine (Ser/Thr) kinase that positively regulates the production and action of several proinflammatory mediators, specifically the release of TNFα and IL-1β[1] in response to stress.[2] TNFα and IL-1β are associated with the onset of inflammatory diseases and several autoimmune diseases[3] including rheumatoid arthritis (RA),[4] toxic shock syndrome, osteoarthritis, and inflammatory bowel disease.[5,6] The recent success of anti-cytokine biological agents has demonstrated clinical benefits in the treatment of inflammatory diseases.[7] However, due to the well known disadvantages common to these protein-based therapies, such as high cost and subcutaneous or intravenous administration, orally active small molecules that can effectively act as anti-cytokine agents would clearly be of additional benefit to patients.[6] Small molecule inhibitors of p38 MAP kinase have been shown to be efficacious in clinical studies as alternatives for these biological agents.[8]

We have previously reported that a series of 3-pyridylpyrrole derivatives possessing *N*-alkyl- or *N*,α-dialkyltetrahydropyridine moiety at the 4-position of the pyrrole ring potently inhibits the production of the proinflammatory cytokine TNFα (Table 1),[9] and its in vitro and in vivo activities are suggested to be significantly affected by steric hindrance around the *N*- and/or α-position of the tetrahydropyridine (THPy) moiety and lipophilicity of the molecules, respectively. Based on these results, we attempted to gain superior compounds which exhibit more potent pharmacological activities both in vitro and in vivo compared with the reported derivatives. Compound **1m** was selected as a lead compound for further optimization because it showed the most potent in vitro activity.

Herein, we report the results of the optimization research that have led us to the identification of the promising compound R-132811 (**4a**), which was chosen as a candidate for further pre-clinical studies to develop a new class of anti-rheumatic drug.

First of all, we designed a series of compound **1m** derivatives, in which an *N*-alkyl group is linked with an α-alkyl group to form a bicyclic THPy ring to reduce the steric hindrance and lipophilicity (Fig. 1).

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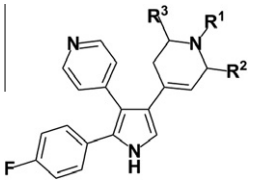
Table 1
In vitro, in vivo activities and *C* log *P* values of N-substituted or N,α-disubstituted tetrahydropyridine derivatives



Compd	R^1	R^2	R^3	IC_{50}[a] (μM)	ID_{50}[b] (mg/kg)	*C* log *P*[c]
1a	Me	H	H	3.96 (2.63–5.97)	2.89	3.48
1b	Et	H	H	1.21 (0.72–2.05)	6.67	4.01
1c	*i*-Pr	H	H	0.98 (0.75–1.28)	6.18	4.32
1d	*n*-Pr	H	H	0.52 (0.29–0.95)	6.75	4.54
1e	*n*-Octyl	H	H	1.38 (0.77–2.48	45%[d]	7.19
1f	*t*-Bu	H	H	>10	—[e]	4.72
1g	Bn	H	H	>30	-[e]	5.27
1h	Me	*di*-Me	*di*-Me	8.44 (5.26–13.54)	7.27	5.56
1i	Me	allyl	H	48.7%[f]	—[e]	4.58
1j	Me	Bn	H	30.8%[f]	—[e]	5.57
1k	Me	Me	H	0.63 (0.42–0.93)	1.42	3.83
1l	Et	Me	H	1.61 (0.94–2.75)	5.28	4.53
1m	*n*-Pr	Me	H	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 SD 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 20 mg/kg.
[e] Not tested.
[f] Inhibition at 10 μM.

The derivatives with the bicyclic THPy ring (**3a**–**d**) were synthesized in accordance with the established synthetic route, which has been reported previously (Scheme 1).[10] Introduction of the bicyclic THPy group to the 4-position of the pyrrole ring was carried out by bromine–lithium exchange of compound **2**, followed by 1,2-addition with bicyclic aminoketone derivatives to form a tertiary alcohol. Subsequent dehydroxylation of the tertiary alcohol was carried out by exposure to trifluoroacetic acid (TFA) concurrently with deprotection of a triisopropylsilyl (TIPS) group with tetrabutylammonium fluoride (TBAF) to give the bicyclic THPy derivative **3a**, **b**, **c** or **d**. Regioisomers **3a** and **3b** were separated[11] by silica gel column chromatography and their structures were determined by mass fragmentation studies (electron ionization method: EI-MS) (Scheme 2). Regioisomers **3c** and **3d** also were separated and determined in a similar manner.

The bicyclic THPy derivatives **3a**–**d** have two stereoisomers due to an asymmetric center at the α-position of the bicyclic THPy moiety. We separated the two stereoisomers of compound **3c** by a preparative HPLC method using a chiral column to gain the optically pure enantiomers **4a** (1st peak) and **4b** (2nd peak) (Scheme 3). In order to determine the absolute configuration of those enantiomers, one of them was synthesized from enantiomerically enriched (8a*S*)-hexahydroindolizin-7(1*H*)-one **5**.[12] Chiral HPLC analysis indicated that the synthesized compound corresponds with enantiomer **4a**. Thus, it was confirmed that enantiomer **4a** has an (*S*)-configuration (Scheme 4), and therefore enantiomer **4b** has an (*R*)-configuration.

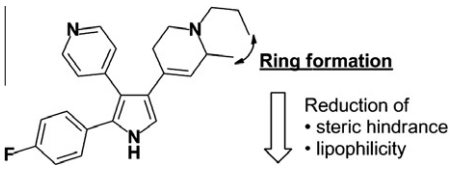
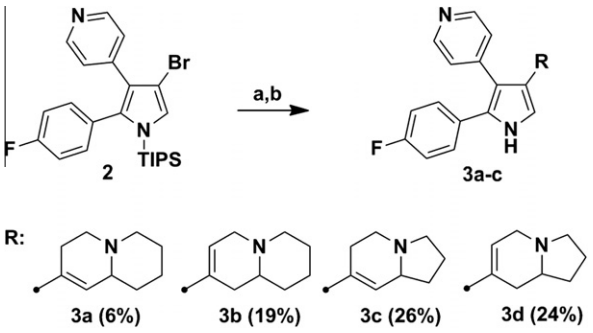


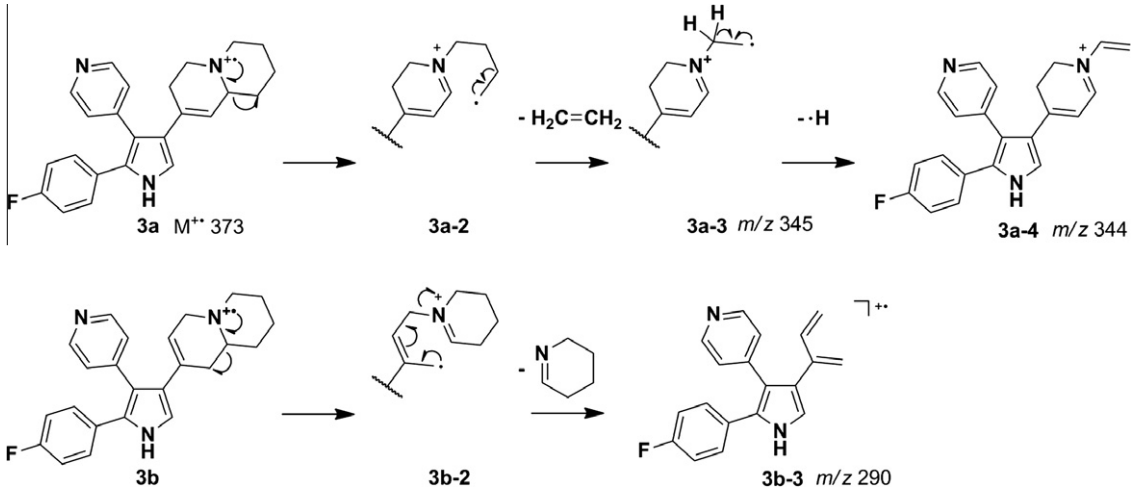
Figure 1.



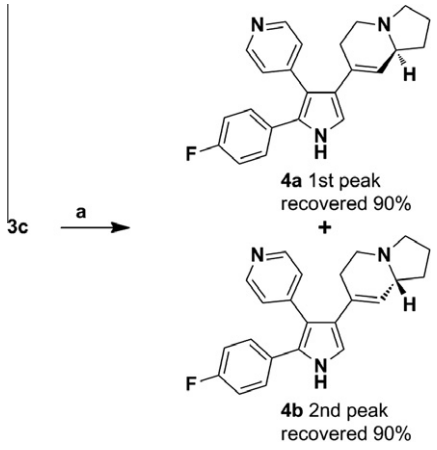
Scheme 1. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C, then bicyclic aminoketone derivatives, and then rt; (b) TFA, CH_2Cl_2, rt, then TBAF, THF, rt.

The inhibitory activities of the bicyclic THPy derivatives on LPS-induced TNFα production were evaluated in vitro and in vivo.[9,10] Their IC_{50}s, ID_{50}s and *C* log *P*s[13] are summarized together with those of compound **1m** in Table 2.

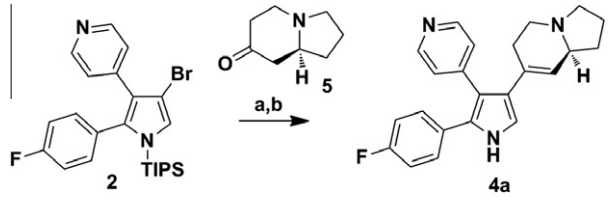
Compound **3a**, which has a six-membered ring within the bicyclic THPy moiety and a lower *C* log *P* value (4.23), showed more potent in vitro and in vivo activities than those of the corresponding monocyclic THPy analog **1m** (IC_{50}: 0.31 vs 0.44 μM; ID_{50}: 2.27 vs 2.79 mg/kg). Compound **3c** with a five-membered ring and further lowered *C* log *P* value (3.67) showed much more potent in vitro and in vivo activities than those of the corresponding monocyclic THPy analog, compound **1l** (IC_{50}: 0.042 vs 1.61 μM; ID_{50}: 1.09 vs 5.28 mg/kg). Compound **3c** exceeded even compound **3a** in both in vitro and in vivo activities (IC_{50}: 0.042 vs 0.31 μM; ID_{50}: 1.09 vs 2.27 mg/kg). In addition, it is noteworthy that the in vitro activity of compound **3c** was sevenfold as potent as that of compound **3a**. This remarkable increase in the in vitro activity of compound **3c** is supposed to be mainly attributed to further reduction of the steric hindrance due to the five-membered ring formation in its bicyclic THPy moiety. The bicyclic THPy ring of compound **3c** is thought to have a more rigid conformation than that of compound



Scheme 2. Proposed pathway for generation of *m*/*z* 344 from compound **3a** and generation of *m*/*z* 290 from compound **3b**.



Scheme 3. Reagents and conditions: (a) HPLC separation. Column, CHIRALPAK AD (Φ 4.6 × 250 mm, DAICEL CHEMICAL INDUSTRIES, LTD, Japan), eluent with *n*-hexane/EtOH 80:20. The flow rate was 1.0 mL/min and the temperature was adjusted to 40 °C. UV detection was performed at 254 nm.

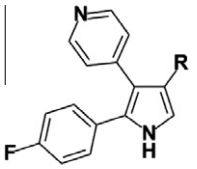


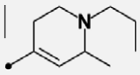
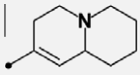
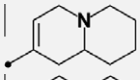
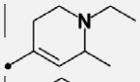
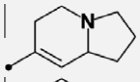
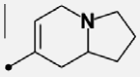
Scheme 4. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C, then (8a*S*)-hexahydroindolizin-7(1*H*)-one, and then rt; (b) TFA, CH_2Cl_2, rt, then TBAF, THF, rt.

3a because it contains a five-membered ring. That rigid conformation of the bicyclic THPy moiety might also contribute to the excellent in vitro activity of compound **3c**. The increase in the in vivo activities of compound **3a** and **3c** is probably due to both the increased in vitro activity and the improved pharmacokinetic character brought about by the lower lipophilicity.

Compounds **3b** and **3d** are regioisomers of compounds **3a** and **3c**, respectively, and they are distinguished from their counterparts based on the position of C–C double bond within their bicyclic THPy rings. Both compounds **3b** and **3d** showed lower in vitro and in vivo activities compared with their counterpart, as expected based on the previous letter.[9]

Table 2
In vitro, in vivo activities and *C* log *P* values of bicyclic tetrahydropyridine derivatives



Compd	R	IC_{50}[a] (μM)	ID_{50}[b] (mg/kg)	*C* log *P*[c]
1m		0.44 (0.32–0.61)	2.79	5.06
3a		0.31 (0.26–0.38)	2.27	4.23
3b		0.81 (0.59–1.10)	3.55	4.23
1l		1.61 (0.94–2.75)	5.28	4.53
3c		0.042 (0.028–0.063)	1.09	3.67
3d		0.60 (0.40–0.91)	2.85	3.67

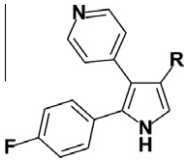
[a] Inhibition of LPS-induced TNFα production in human whole blood. Results are given as mean and SD of three to four determinations.
[b] Inhibition of LPS-induced TNFα production in mice $N = 5$.
[c] *C* log *P* values calculated using Pallas® (INFOCOM CORPORATION).

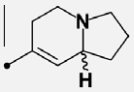
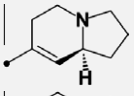
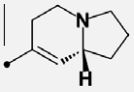
Biological activities of compounds **3c** (racemate), **4a** ((*S*)-enantiomer) and **4b** ((*R*)-enantiomer) were examined (Table 3). (*S*)-Enantiomer **4a** was found to be much more active than (*R*)-enantiomer **4b**, that is (*S*)-enantiomer **4a** was 40- and 7-fold as potent as (*R*)-enantiomer **4b** in vitro and in vivo, respectively.

In order to assess the potential of compound **4a** for a novel type of anti-rheumatic drug, we performed a variety of pharmacological tests related to proinflammatory cytokine production and rheumatoid arthritis (Table 4).

There have been four isoforms of p38 MAP kinase, namely, α-, β-, γ- and δ-isoforms,[14] that have been identified so far. Compound **4a** inhibited α-isoform potently (IC_{50} = 0.034 μM) and β-isoform weakly (IC_{50} = 0.572 μM). On the other hand, it inhibited neither

Table 3
In vitro and in vivo activities of hexahydroindolizine derivatives **3c**, **4a**, and **4b**



Compd	R	IC_{50}[a] (μM)	ID_{50}[b] (mg/kg)
3c		0.042 (0.028–0.063)	1.09
4a		0.026 (0.018–0.041)	0.93
4b		49%[c]	6.59

[a] Inhibition of LPS-induced TNFα production in human whole blood. Results are given as mean and SD of three to four determinations.
[b] Inhibition of LPS-induced TNFα production in mice $N = 5$.
[c] Inhibition at 1 μM.

γ-isoform nor δ-isoform even at 10 μM.[15] Compound **4a** potently inhibited not only the production of TNFα (IC_{50} = 0.026 μM), but also that of other proinflammatory cytokines such as IL-1β, IL-6, and IL-8[16] in vitro (IC_{50} = 0.020, 0.88, and 0.016 μM, respectively). Also in the in vivo activities, compound **4a** potently inhibited the production of TNFα[10,17] and IL-6[19] (ID_{50} = 0.93 and 0.11 mg/kg, respectively). Compound **4a** was effective on both anti-collagen antibody-induced arthritis[20] and collagen-induced arthritis in mice[16,21] (ID_{50} = 2.22 and 2.38 mg/kg, respectively). Furthermore, compound **4a** demonstrated not only prophylactic effect, but also therapeutic effect on adjuvant arthritis in rats[22,23] (ID_{50} = 3.1 mg/kg and 4.9 mg/kg, respectively). Interestingly, compound **4a** also showed analgesic effect on adjuvant-induced arthritic pain in rats[24–26] (ID_{50} = 2.9 mg/kg). Thus, compound **4a** was found to selectively inhibit p38α MAP kinase, block the production of the proinflammatory cytokines such as TNFα, IL-1β, IL-6 and IL-8, and exhibit the inhibitory, prophylactic, therapeutic or analgesic effect on rheumatoid arthritis in animals.

In order to develop a new class of anti-rheumatic drug, which inhibits the production of the proinflammatory cytokines such as TNFα, IL-1β, IL-6, and IL-8, we synthesized a series of 3-pyridylpyrrole derivatives possessing a bicyclic THPy moiety at the 4-position of the pyrrole ring and evaluated its pharmacological activities. Compound **3c** with a specific bicyclic THPy ring, namely, the hexahydroindolizine ring, showed excellent in vitro and in vivo activities. (*S*)-Enantiomer **4a** separated from racemic compound **3c** showed much more potent inhibitory activities than those of (*R*)-enantiomer **4b**. We evaluated compound **4a** in various pharmacological assay systems related to proinflammatory cytokine production and rheumatoid arthritis, and thus gained promising results. Therefore, compound **4a** (R-132811) was chosen as the candidate for further pre-clinical studies such as pharmacological, pharmacokinetic, toxicological, and physicochemical studies.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.06.122.

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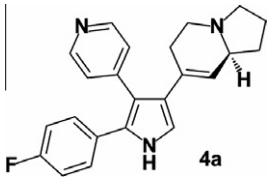
Table 4
Representative results of pharmacological tests on compound **4a**



Biological evaluations		
Inhibition of p38 MAP kinase[a] IC_{50} (μM)	p38α[15]	0.034 (0.028–0.042)
	p38β[15]	0.572 (0.281–1.165)
	p38γ[15]	>10[c]
	p38δ[15]	>10[c]
Inhibition of LPS-induced cytokine production in human whole blood[a] IC_{50} (μM)	TNFα[11,16]	0.026 (0.018–0.041)
	IL-1β[16]	0.020 (0.016–0.026)
	IL-6[16]	0.88 (0.62–1.3)
	IL-8[16]	0.016 (0.011–0.022)
Inhibition of LPS-induced cytokine production in mice[b] ID_{50} (mg/kg)	TNFα[11,17]	0.93
	IL-1β[18]	8.63
	IL-6[19]	0.11
Inhibition of anti-collagen antibody-induced arthritis in mice[b,20] ID_{50} (mg/kg)		2.22
Inhibition of collagen-induced arthritis in mice[b,5,16,21] ID_{50} (mg/kg)		2.38
Prophylactic effect on adjuvant arthritis in rats[b,22] ID_{50} (mg/kg)		3.1
Therapeutic effect on adjuvant arthritis in rats[b,23] ID_{50} (mg/kg)		4.9
Analgesic effect on adjuvant-induced arthritic pain in rats[b,24] ID_{50} (mg/kg)		2.9

[a] Results are given as mean and SD of three to four determinations.
[b] $N = 5$.
[c] No inhibition at 10 μM.

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22. The experiment was carried out according to the method described by Winder et al. (Winder, C. V.; Lembke, L. A.; Stephens, M. D. *Arthritis Rheum.* **1969**, *12*, 472–482). Heat-killed, dried *Mycobacterium butyricum* was ground with an agate mortar, and then suspended in dry-sterilized liquid paraffin to make a 4.0 mg/mL suspension. Then, this suspension was sonicated. Adjuvant arthritis was induced in rats by injecting the adjuvant (*M. butyricum*, 200 μg/0.05 mL/paw) intradermally in the heel of the right hind paw on day 0. The test compounds were suspended in 0.5% CMC aqueous solution at the appropriate concentrations, and orally administrated at 5 mL/kg once a day from day 0 through day 20. The vehicle was administrated to the control group. The volume of both feet was measured on days 3, 5, 7, 10, 13, 15, 18, and 21 by a plethysmometer (Ugo Basile) by soaking the hind paw from the toe to the hairline in the bath of the plethysmometer. The swelled foot volume and the % inhibition of treated animals over the control on day 21 were calculated with the equations below. We used the volume of adjuvant-injected foot (right hind foot) for the calculation because the swelling of the adjuvant-injected foot was more prominent and stable than that of the uninjected foot. The swelled foot volume (mL) = right hind foot volume of animals − mean right hind foot volume of normal animals. % Inhibition = {1 − (swelled foot volume of animal on days 21)/(mean swelled foot volume of the control group on day 21)} × 100
23. Adjuvant arthritis was induced as described above. On day 18, adjuvant arthritis was established in most adjuvant-injected animals and the swelled foot volume almost reached a plateau. We selected animals with prominent swelling of the adjuvant-injected foot (foot volume ⩾ 2.75 mL). These animals were divided into groups so that the mean value of the adjuvant-injected foot volume in each group was about the same. The test compounds were suspended in 0.5% CMC aqueous solution at the appropriate concentrations, and orally administered at 5 mL/kg twice a day from day 18 to day 24 (7 days). The volume of both feet was measured on days 18, 20, 23, and 25 (0, 2, 5, and 7 days after the first administration) by a plethysmometer (Ugo Basile) by soaking the hind paw from the toe to the hairline in the bath of the plethysmometer. The swelled foot volume and the ratio of the swelled foot volume were calculated with the equations below, and the % inhibition against control group was obtained. We used the volume of adjuvant-injected foot (right hind foot) for the calculation because the swelling of the adjuvant-injected foot was more prominent and stable than that of the uninjected foot. The swelled foot volume (mL) = right hind foot volume of animals − mean right hind foot volume of normal animals. The ratio of swelled foot volume = {(right hind foot volume of animals on day 25 − mean right hind foot volume of normal animal on day 25)/(right hind foot volume of animals on day 18 − mean right hind foot volume of normal animal on day 18)} × 100
24. The experiment was performed according to the method by Kuzuna et al. (Kuzuna, S.; Kawai, K. *Chem. Pharm. Bull.* **1975**, *23*, 1184) with slight modifications. Heat-killed, dried *M. butyricum* was ground with an agate mortar, and was suspended in dry, sterilized liquid paraffin to make a 4.0 mg/mL suspension. Then, the suspension was sonicated. Adjuvant arthritis was induced in rats by injecting the adjuvant (*M. butyricum*, 200 μg/0.05 mL/paw) intradermally in the heel of the right hind paw on day 0. The rats with well-established arthritis were fasted overnight on day 17. On the day of the experiment (day 18), 'pain-positive' rats were selected as follows: the tarso-tibial joint of the uninjected foot of rats was gently flexed five times at intervals of 4–5 s, and rats squeaking at every flection were defined as 'pain-positive'. The pain-positive rats were then divided into groups at 0.5, 1, 2, 4, 6, 8, 24, 48, and 72 h after administration in the same way. The pain score of each rat was recorded by counting the number of times the animal squeaked. Statistical analysis: IC_{50} and ID_{50} values were calculated from liner regression curves obtained from the % inhibition and the logarithmic value of the concentrations or dosages of the test compounds by the last-squares method. The 95% confidence intervals (C.I.) were calculated by Fieller's theorem. ID_{30} value (dose which decreased the ratio of swelled foot volume of the control group by 30%) was calculated by the following equation: the % inhibition = $60/[1 + \exp\{-b_0 - b_1 \log(\text{dose})\}]$ (b_0 and b_1; parameters to be estimated). IC_{50} and ID_{50} values, and their 95% C.I. values were calculated using the SAS System for Windows (SAS Institute Inc.).
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