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SYNTHESIS AND ANTIRHEUMATIC ACTIVITY OF NOVEL TETRAHYDRO-6-QUINOLINEACETIC ACID DERIVATIVES

Yasushi Kohno,* Katsuya Awano, Mitsutomo Miyashita, Takayoshi Ishizaki, Kazuhiko Kuriyama, Yasuhiko Sakoe, Shinji Kudoh, Koji Saito, and Eisuke Kojima

Central Research Laboratories, Kyorin Pharmaceutical Co., Ltd., Mitarai Nogi Tochigi 329-01, Japan

Abstract: A study of the structural optimization of tetrahydroquinoline-6-acetic acid and the development of a novel DMARD with prompt action are detailed. (*S*)-(+)-8-Chloro-1,2,3,4-tetrahydro-2-trifluoromethyl-6-quinolineacetic acid (IRA-378) exhibits the effect against both acute and chronic inflammations. It also has suppressive effects of bone destruction in adjuvant arthritis and Ca release from bones *in vitro*.
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Initial drug treatment of the chronic autoimmune diseases Rheumatoid arthritis (RA) usually involves the use of nonsteroidal antiinflammatory drugs (NSAIDs) to reduce joint pain and swelling. Unfortunately, NSAIDs fundamentally do not alter the course of the disease or prevent joint destruction.[1] Disease-modifying antirheumatic drugs (DMARDs), on the other hand, have the potential to regulate immunological responses and to slow down the progress of RA,[1] but the drawback of relatively slow action. For this reason the disease is often treated by a combination therapy utilizing both NSAIDs and DMARDs.

In the preceding paper, we reported that antirheumatic activity of bicyclic anthranilic acid analogs. Interestingly, 8-carboxy-1,2,3,4-tetrahydro-6-quinolineacetic acid (**1**) has potent antirheumatic activity and suppressive effect on bone destruction.[2] However, **1** exhibits neither an effect on acetic acid-induced vascular permeability, one of the acute inflammation models, nor an effect on primary inflammation of adjuvant arthritis. For the purpose of examining the difference in rate of action between anthranilic and non-anthranilic acid structures, acid **1** was converted into tetrahydro-6-quinolineacetic acid **2** (Scheme 1). **2** then demonstrated suppressive activity against acetic acid-induced vascular permeability without cyclooxygenase (COX) inhibitory activity.

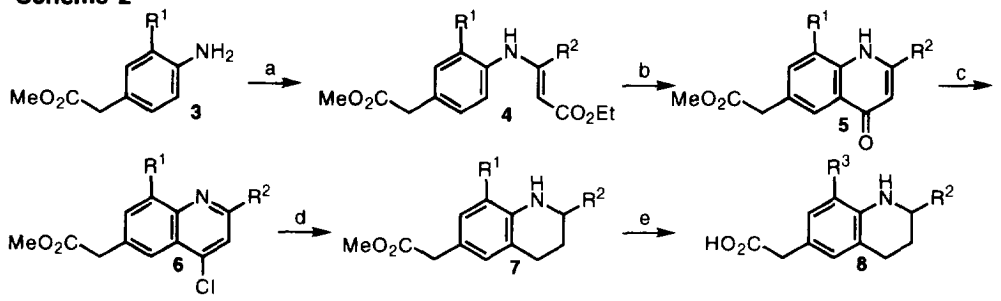
Scheme 1

The above results indicated that the tetrahydro-6-quinolineacetic acid **2** might have potential for deriving a novel DMARD with prompt action. Accordingly, we here describe the synthesis and biological activities of a series of tetrahydro-6-quinolineacetic acids.

The above results suggest that the substituent groups at the 8-position of the quinoline ring are crucial for a good activity. Hence, the introduction of substituents was aimed at the 8-position in the tetrahydroquinoline skeleton, and the effect of substituents at the 2-position was also investigated. The

general synthetic procedures are shown in Scheme 2.

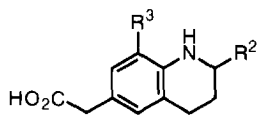
Scheme 2



a: Method A $R^2COCH_2CO_2Et$, *p*-TsOH, PhH, reflux, MethodB $CF_3C \equiv CCO_2Et$, EtOH, rt, b: Ph_2O, reflux, c: $POCl_3$, reflux, (20 - 70% from **3**) d: H_2, 10% Pd/C, Et_3N, EtOH, (80 -100%) e: aq. NaOH, EtOH, (80 - 100%) or 1) NCS, DMF, 80°C, (55 - 87%) 2) aq. NaOH, EtOH, (80 - 100%)

Treatment of methyl 4-aminophenylacetates **3** with β-ketocarboxylate derivatives in the presence of *p*-TsOH (Method A), or with ethyl 4,4,4-trifluorobutynoate (Method B) gave enamines **4**. The resultant enamines **4** were cyclized by heating to afford 4-quinolones **5**. The transformation of the quinolones **5** into tetrahydro-6-quinolineacetates **7** was performed by aromatization and reduction. Hydrolysis of the acetates **7** with aq. NaOH in EtOH or chlorination of **7** (R^1=H) with *N*-chlorosuccinimide (NCS) and successive hydrolysis produced 1,2,3,4-tetrahydro-6-quinolineacetic acids **8**.

Table 1



Acid	R^2	R^3	Inhibitory effect on vascular permeability*	Acid	R^2	R^3	Inhibitory effect on vascular permeability*
2	H	H	1.06	**8 f**	Me	Cl	1.94
8 a	H	Cl	1.44	**8 g**	Me, Me	Cl	1.22
8 b	Ph	Cl	1.63	**8 h**	Et	Cl	1.54
8 c	*o*-F-Ph	Cl	2.09	**8 i**	Pr	Cl	1.59
8 d	*p*-Meo-Ph	Cl	0.62	**8 j**	*i*-Pr	Cl	1.10
8 e	CO_2H	Cl	0.00	**8 k**	CF_3	Cl	1.48

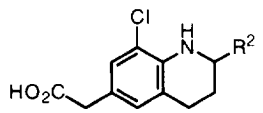
*) Each value was obtained as $\frac{\text{inhibitory rate of sample 100 mg/kg(p.o.)}}{\text{inhibitory rate of diclofenac-Na 25 mg/kg(p.o.)}}$

For the first examination, the most effective substituent group R^3 in acids **8** against acetic acid-induced vascular permeability in mice was searched.[3] The suppressive effect of acids **8** having various substituents of R^3, such as Cl, Br, NO_2, MeO, CN, Ac, Bz, and Ph, was tested under the condition that R^2 was fixed by the hydrogen atom. Among them, only chloride-substituted derivative **8a** was more effective on the vascular permeability than that of **2**. R^3 was therefore held by chloride, and the influence of substituents R^2 on the

activity was investigated (Table 1). The introductions of phenyl and alkyl groups gave good results with *o*-fluorophenyl and methyl substituted acids (**8c** and **f**) showing especially potent suppressive activity. The introduction of mono-substituent was found to be preferable to that of di-substituents for increasing the activity (**8f** and **g**). It was confirmed that the effect of most of the Table 1 compounds on vascular permeability was not owing to inhibition of COX. The acids **8** hardly suppressed malondialdehyde production in rabbit platelets at a concentration of 9×10^{-5} M *in vitro*.[4]

We selected some of the compounds demonstrating high potency in Table 1 and tested their therapeutic effects on adjuvant arthritis in rats.[5] The results are summarized in Table 2. Despite its potent effectiveness against vascular permeability, the inhibitory effect of **8c** on adjuvant arthritis was weak at a dose of 25 mg/kg. In addition, the effect of **8a** extremely decreased at doses of less than 50 mg/kg. In contrast, the alkyl-substituted compounds **8f**, **i**, and **k** significantly suppressed the swelling of adjuvant arthritic rat paws at doses less than 25 mg/kg, and the inhibitory actions of these compounds were apparent even in the early stages of drug treatment.

Table 2



Acid	R^2	Inhibitory effect on adjuvant arthritis* Dose (mg/kg)	Inhibition (%)	Acid	R^2	Inhibitory effect on adjuvant arthritis* Dose (mg/kg)	Inhibition (%)	Inhibitory effect on IgM antibody production** Conc.	Inhibition (%)
8a	H	10.0	27	**8f**	Me	12.5	42		
		50.0	20			25.0	56	2×10^{-6} M	60
		100.0	55	**8i**	Pr	12.5	32		
8c	*o*-F-Ph	25.0	19			25.0	47		N.T.
		50.0	52	**8k**	CF_3	12.5	36		
		100.0	64			25.0	45	2×10^{-6} M	71

*) Each value was obtained by comparison between the non-injected paw volumes of the arthritic and control groups.

**) Each value was obtained by the number of IgM-PFC in the tested group and that in the control group.

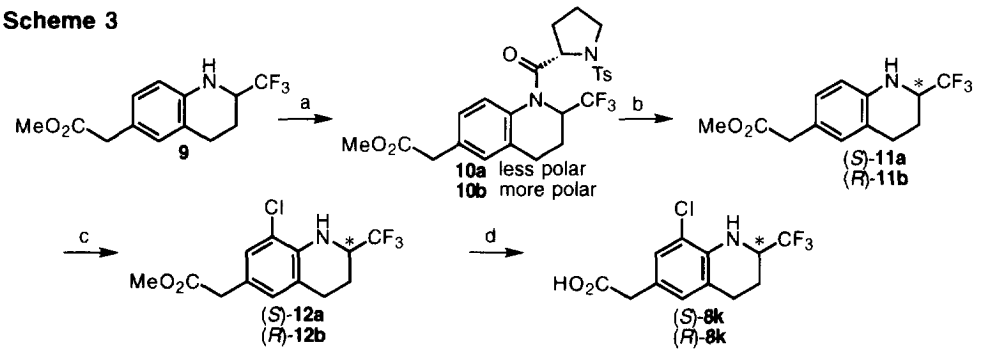
Because it was obvious that 2-alkyl-8-chloro-1,2,3,4-tetrahydro-6-quinolineacetic acids (**8f**, **i** and **k**) had potent effects against both acute and chronic inflammation, these acids' suppression of antibody production was further investigated. The methyl- and trifluoromethyl-substituted derivatives (**8f** and **k**) significantly inhibited the appearance of anti-sheep erythrocyte IgM antibody-producing cells *in vitro* (Table 2).[6] In *in vivo* as well as *in vitro* experiment, 2-trifluoromethyl-6-quinolineacetic acid **8k** showed the moderate suppressive effect on antibody production.[7]

As mentioned above, 8-chloro-1,2,3,4-tetrahydro-2-trifluoromethyl-6-quinolineacetic acid (**8k**) was found to have potential as a novel DMARD with prompt action. Our succeeding study was focused on the synthesis of optically active **8k** for comparison of the biological activities between optical isomers.

Methyl 1,2,3,4-tetrahydro-2-trifluoromethyl-6-quinolineacetate (**9**) was reacted with excess *N*-Ts-(L)-prolyl chloride in 1,2-dichloroethane at 80°C to afford a separable diastereomer mixture **10** (**a**:**b**=1:1) in 92% yield. Stereochemistry of these isomers was determined by means of X-ray analysis. The absolute configuration of the less polar isomer is *S* configuration and the more polar isomer is *R*. Hydrolysis of the each diastereomer with aq. NaOH gave the corresponding acid in good yield. The resulting acids were

reacted with $SOCl_2$ in MeOH to yield esters **11**. The transformation of the esters **11** to optically active **8k** was performed by the following synthetic procedures. After the introduction of chloride group to **11** with NCS and successive hydrolysis with aq. NaOH, produced the optically active acids (Scheme 3).[8]

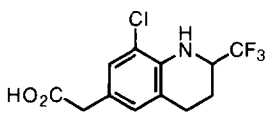
Scheme 3



a: (L)-Ts-Pro-Cl, $ClCH_2CH_2Cl$, 80°C, 92% (**a**:**b**=1:1) b: 1) aq. NaOH, EtOH, reflux, (97 - 100%) 2) $SOCl_2$, MeOH, rt, (88 - 90%) c: NCS, DMF, 80°C, (85 - 90%) d: aq. NaOH, EtOH, rt, (quant.)

The optical isomers showed striking different potencies against the acute and chronic inflammatory models (Table 3). (*S*)-(+)-8-Chloro-1,2,3,4-tetrahydro-2-trifluoromethyl-6-quinolineacetic acid (*S*-**8k**, IRA-378) significantly inhibited the vascular permeability in mice at a dose of 50 mg/kg and moderately reduced the swelling of the non-injected paws of arthritic rats even at a dose of 6.3 mg/kg. On the other hand, (*R*)-**8k** did not exhibit the effect against vascular permeability at a dose of 50 mg/kg. Furthermore, *R*-isomer required a four fold dosage increase over (*S*)-**8k** to exhibit the same effect on adjuvant arthritis (25.0 mg/kg vs 6.3 mg/kg).

Table 3



Optical form	Inhibitory effect on vascular permeability*		Inhibitory effect on adjuvant arthritis**	
	Dose (mg/kg)	Inhibition (%)	Dose (mg/kg)	Inhibition (%)
racemic-**8k**	50	0.99	25.0	46
	100	1.48		
(*S*)-**8k**	50	1.28	6.3	25
(IRA-378)	100	1.62	12.5	34
			25.0	60
(*R*)-**8k**	50	0.43	12.5	3
	100	1.50	25.0	20

*, **) See the footnotes of Tables 1 and 2, respectively.

Thus, the optically active (*S*)-**8k** (IRA-378) was more effective than (*R*)-**8k** not only against the acute inflammatory model but also against the chronic one. The effect of IRA-378 on adjuvant arthritis was then investigated in further detail, and its suppressive patterns were compared to those of NSAIDs. Figure 1 compares the effectiveness of IRA-378 (25 mg/kg) and diclofenac-Na (0.5 mg/kg) in inhibiting the swellings

of non-injected paws.[9] IRA-378 displayed suppressive patterns similar to those of diclofenac-Na. This result suggests that IRA-378 is a novel DMARD with prompt action.

In the articular of adjuvant arthritic rats, bone destruction advances along with the disease.[10] Table 4 shows the results of radiologic analysis of non-injected paws on Day 27.[11] In the adjuvant control, bone damage was developed and its mean score was 7.8. IRA-378 significantly suppressed the bone damage ($p<0.05$) at a dose of 25 mg/kg. In addition, it was confirmed that IRA-378 suppressed the Ca release from bones stimulated with IL-1β or PTH *in vitro* in a concentration dependent manner (Table 5).[12]

Fig. 1

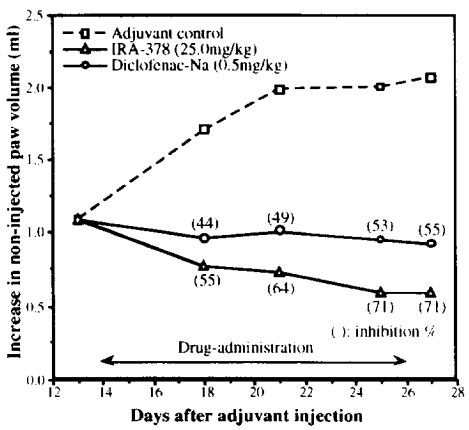


Table 4

Group	Dose (mg/kg)	n[a]	Bone damage score on Day 27
Adjuvant control	–	8	7.8±1.0
IRA-378	25.0	10	2.5±0.6*
Diclofenac-Na	0.5	9	4.3±0.8

Value are means±SE. a) Number of rats.
*) significantly different from the adjuvant control, $p<0.05$ (Scheffé 's test).

Table 5

Model	Inhibitory effect on bone resorption*	
	Concentration (μg/ml)	Inhibition (%)
IL-1β-stimulated	0.4	24
	2.0	78**a)
	10.0	97**a)
PTH-stimulated	0.4	41
	2.0	61*b)
	10.0	94**b)

*, **) significantly different from the positive control, $p<0.05$ and $p<0.01$, respectively.
a) Dunnett's test. b) Scheffé 's test.

Nitric oxide (NO) is a unique biological messenger molecule, and recently its close relationship to inflammation has been discussed.[13] The macrophage inducible nitric oxide synthase (iNOS) is regulated at the transcriptional level and expressed to a significant level only after cell activation. Excessive NO production not only mediates the bactericidal and tumouricidal actions of macrophages, but may also contribute to cell and tissue damage in immunological diseases. IRA-378 suppressed the expression of iNOS mRNA and inhibited induction of NO generation from murine macrophages stimulated with LPS and IFN-γ. Hence, it seems that IRA-378 may exhibit its antiinflammatory and immunomodulating effects by inhibiting NO production.

In conclusion, we were able to develop the novel antirheumatic agent, (*S*)-(+)-8-chloro-1,2,3,4-tetrahydro-2-trifluoromethyl-6-quinolineacetic acid (IRA-378) by structural optimization of 1,2,3,4-tetrahydro-6-quinolineacetic acid (**2**). IRA-378 has various unique biological activities and its pharmacological properties are distinguished from those of ordinary DMARDs and NSAIDs. Studies to clarify the actions of IRA-378 on the other models are currently on going.

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the University of Tokyo for their X-ray analysis. We would also like to thank Dr. S. Suzue, Kyorin Pharmaceutical CO., LTD., for his many useful suggestions.

References and Notes

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2. Kohno Y.; Awano K.; Miyashita M.; Fujimori S.; Sakoe Y.; Kudoh S.; Saito K.; Kojima E., *Bioorg. Med. Chem. Lett* .submitted (the preceding paper).
3. We carried out the acetic acid-induced vascular permeability experiment according to the method of Whittle A., *Br. J. Pharmacol.*, **22**, 246 (1964).
4. Inhibitory activity on COX was measured according to the method described by Hiroi et al.: Hiroi J.; Ohara K.; Fujitsu T.; Hirai O.; Satoh S.; Ochi T.; Senoh H.; Mori J.; Kikuchi H., *Folia Parmacol. Japan*, **86**, 441 (1985). Inhibitory rate (%) of each compound at a concentration of $9X10^{-5}$ M was as follows; **8b**, 76; **8c**, 61; **8i**, 13; **8j** , 60. The other derivatives showed no inhibitory activity at the concentration. Particularly, **8k** did not exhibit inhibition at a concentration of $3x10^{-4}$ M. In contrast, the obtained IC_{50} value of diclofenac-Na was $3.0x10^{-8}$ M.
5. Adjuvant arthritis was induced in SD rats according to the method of Kameyama et al. : Kameyama T.; Nabeshima T.; Yamada S.; Sato M., *Arzneim-Forsch/Drug Res.*, **37**, 19 (1987). After the adjuvant injection, drug treatment (p.o.) was started on Day 14 and continued until Day 20 (once daily, 7 treatments). Suppressive rate on edema was calculated on Day 21.
6. The spleen cells of BDF_1 mice were isolated by finely mincing the spleen, then centrifuging and suspending in medium (RPMI-1640 + 10% FCS). Each 50 µl of spleen cell suspension ($2x10^7$ cells/ml) and sheep erythrocyte suspension ($1x10^7$ cells/ml) was mixed in a 96-well plate. Test compounds were dissolved in DMSO and diluted in medium and added to the 96-well plate 100 µl each. After incubation at 37 °C for 5 days in an incubator with 5% CO_2, the number of IgM antibody-producing cells was counted by Jerne method: Jerne N. K.; Nordin A. A., *Science*, **140**, 405 (1963).
7. Sheep erythrocytes ($5x10^8$ cells/0.1 ml PBS) were intraperitoneally injected to a BALB/c mice. The test compounds were administered orally once daily for 4 consecutive days after the erythrocyte injection. On the next day of final administration, the spleen cells were isolated by finely mincing the spleen, then centrifuging and suspending in medium (RPMI-1640 + 10% FCS). The number of IgM antibody-producing cells was counted by Jerne method. **8k** showed inhibition of 35% ($p<0.05$) at a dose of 12.5 mg/kg.
8. (*S*)-**8k** : $[\alpha]_D^{25}$ 21.2°(c 0.7, $CHCl_3$); mp 107°C (from AcOEt-n-hexane); Anal. calcd. for $C_{12}H_{11}ClF_3NO_2$: C, 49.08; H, 3.78; N, 4.77. Found: C, 49.12; H, 3.61; N, 4.70. (*R*)-**8k** : $[\alpha]_D^{25}$ -19.3°(c 0.7, $CHCl_3$); mp 106°C (from AcOEt-n-hexane); Anal. calcd. for $C_{12}H_{11}ClF_3NO_2$: C, 49.08; H, 3.78; N, 4.77. Found: C, 49.15; H, 3.61; N, 4.76.
9. During our preliminary examining of the effect of diclofenac-Na on adjuvant arthritis, the obtained ED_{50} value of diclofenac-Na on Day 21 was 0.5 mg/kg. On the other hand, by considering the effect of IRA-378 on adjuvant arthritis in Table 3, we presumed that ED_{50} value of IRA-378 was about 25 mg/kg. In comparing suppressive patterns of them, the above doses (diclofenac-Na 0.5 mg/kg, IRA-378 25 mg/kg) which both of compounds would display same effect were adopted.
10. (a) Pozo E. D.; Graeber M.; Elford P.; Payne T., *Arth. Rheum.*, **33**, 247 (1990). (b) Binderup L., *Acta Pharmacol. Toxicol.*, **59**, 228 (1986).
11. On day 27, radiographic assessment of non-injected paws was done by a modification of the method of Tanaka et al.: Tanaka A.; Kakushi H.; Shike T.; Yoshida T.; Kojima Y.; Nishiyama S.; Yahara I., *Oyo Yakuri-Pharmacometrics*, **24**, 571 (1982). Bone damage was assessed blindly on a scale of 0-4+ (with 0 equaling normal and 4+ equaling severe changes) for metatarsus, tarsus, calcaneum and the end of the distal tibia of the non-injected paw. The maximum total severity score by this method was 16+.
12. Bone resorption was measured according to a modification of the method in Tsuda et al.: Tsuda M.; Kitazaki T.; Ito T.; Fujita T., *J. Bone Miner. Res.*, **1**, 207 (1986).
13. (a) Stefanovic-Racic M.; Meyers K.; Meschter C.; Coffey J. W.; Hoffman R. A.; Evans C. H., *Arthritis & Rheumatism*, **37**, 1062 (1994). (b) Connor J. R.; Manning P. T.; Settle S. L.; Moore W. M.; Jerome G. M.; Webber R. K.; Tjoeng F. S.; Currie M. G., *Eur. J. Pharmacl.*, **273**, 15 (1995). (c) Salvemini D.; Manning P. T.; Zweifel B. S.; Seibert K.; Connor J.; Currie M. G.; Needleman P.; Masferrer J. L., *J. Clin. Invest.*, **96**, 301 (1995).

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