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Synthesis of C_4-Modified Acanthoic Acid Analogs and Their Biological Evaluation as Nitric Oxide Inhibitors

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Synthesis and biological evaluation of a series of C_4-modified acanthoic acid analogs are reported. Among them, the analog **7** and **10** exhibit potent cellular inhibitory activity in NO inhibition assay.

Key words: Acanthoic acid analog, NO inhibition

INTRODUCTION

Nitric Oxide (NO) which is produced by L-arginine oxidation, catalyzed by nitric oxide synthase (NOS), is an important bioregulator and ubiquitous biomessenger existing in a variety of organisms (Palmer *et al.*, 1987; Pfeiffer *et al.*, 1999). NOS bring about a five-electron oxidation of L-arginine to produce nitric oxide and L-citrulline as shown in Fig. 1. To date three NOS isoforms have been discovered and two of them, endothelial NOS and neuronal NOS, are known to be constitutive and involved in neurotransmission and vasodilatation, respectively (Rees *et al.*, 2002; Bhme *et al.*, 1993). The third isoform, which is induced by inflammatory cytokines, is concerned with host defense (White and Marletta, 1992).

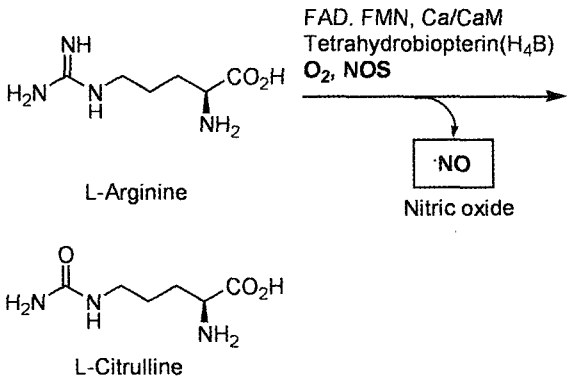

Fig. 1. Biosynthetic pathway of nitric oxide

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An excessive production of nitric oxide by *i*NOS leads to inflammatory diseases such as arthritis and inflammatory bowel disease (Kilbourn *et al.*, 1990; Jang *et al.*, 1997; Miller *et al.*, 1993). Thus, a variety of structural analogs of natural products have been synthesized and evaluated for the treatment of inflammatory diseases in animal models and in clinical trials. Acanthoic acid, isolated from the root bark of *Acanthopanax koreanum* Nakai, has recently been reported to suppress Tumor Necrosis Factor-α (TNF-α) and Interleukin-1β (IL-1β) selectively (Kang *et al.*, 1996). More recently, we have also reported anti-inflammatory effects of acanthoic acid analogs, which exhibit excellent COX-2 and NO inhibitory activities (Suh *et al.*, 2001, 2004). In particular, our previous work revealed that the length of the linker between C_4 and the terminal carboxyl group plays an important role for the anti-inflammatory effects of the acanthoic acid analogs.

As an extension of studies on acanthoic aicd analogs, we have focused on the linker optimization of the C_4-chain by investigating the effect of the linker length and the terminal functional groups on the NO inhibitory effects. We have diversified the C_4-chain on the structural basis of the analogs **4** and **7**, which exhibited NO inhibitory activities of 45.1 and 77.9 inhibition percent at 40 μM concentration, respectively (Fig. 2). We herein describe synthesis of the C_4-chain modified acanthoic acid analogs as well as evaluation of their inhibitory activities in NO generation in Raw 264.7 cells.

CHEMISTRY

The syntheses of acanthoic acid analogs are outlined in Schemes, 1-3. The syntheses of the analogs **4** and **7** are

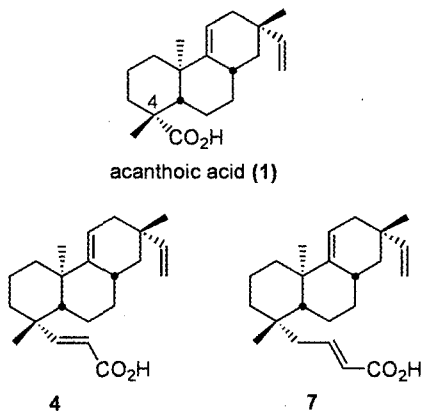
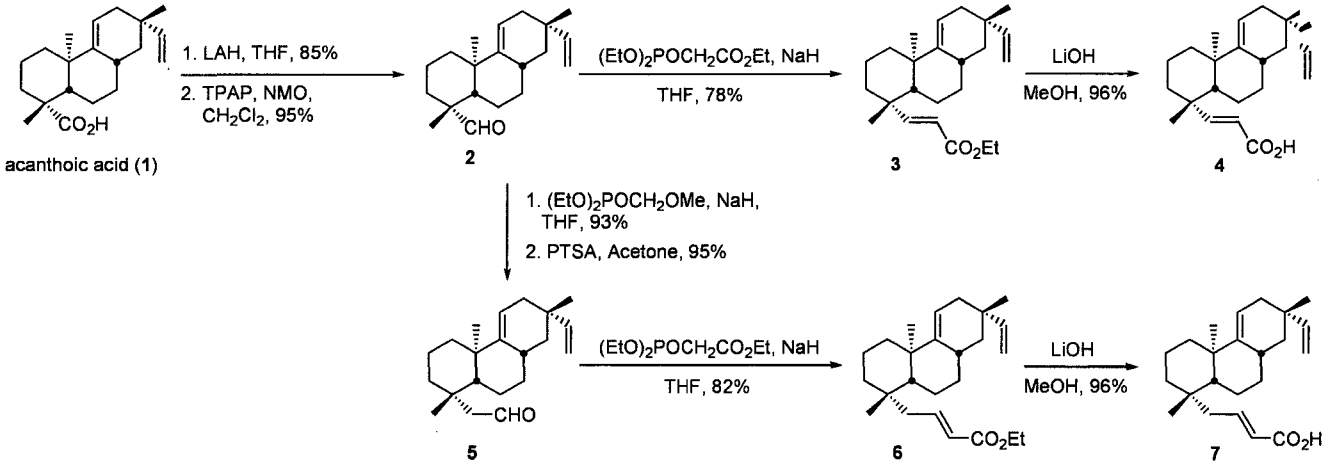


Fig. 2. Structrues of acanthoic acid and acanthoic acid analogs

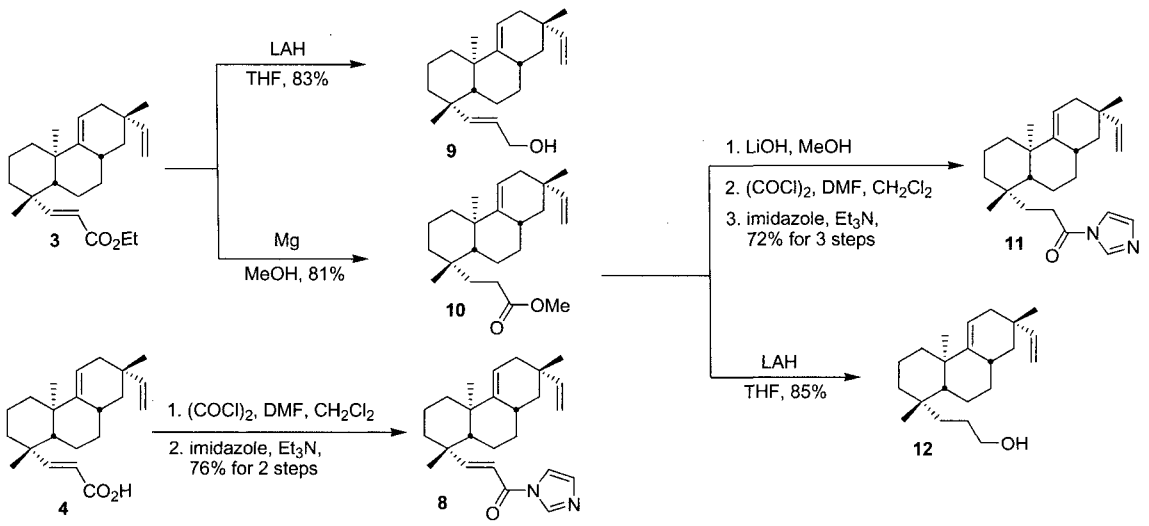
presented in Scheme 1. LAH reduction of acanthoic acid (**1**) and TPAP oxidation of the resulting alcohol provided the aldehyde **2**, which were transformed into the analog **4** by olefination and ester hydrolysis. One carbon homologation of the aldehyde **2** and hydrolysis of the resulting enol ether provided the aldehyde **5**, which were transformed into the analog **7** by the same procedures for the analog **4**.

The analogs **4** and **7** were transformed into the corresponding alcohol or amide and then reduced to the analogs **11**, **12**, **16**, and **17** by selective olefin reduction with magnesium as shown in Scheme 2 and 3. Treatment of the analog **4** with oxalyl chloride, followed by amidation with imidazole provided the analog **8**. LAH reduction of the analog **3** provided the analog **9** while conjugated reduction with magnesium of the analog **3** provided the analog **10**, which was transformed into the analog **11** by sequential ester hydrolysis and amidation. Direct LAH reduction of the analog **10** afforded the analog **12**.

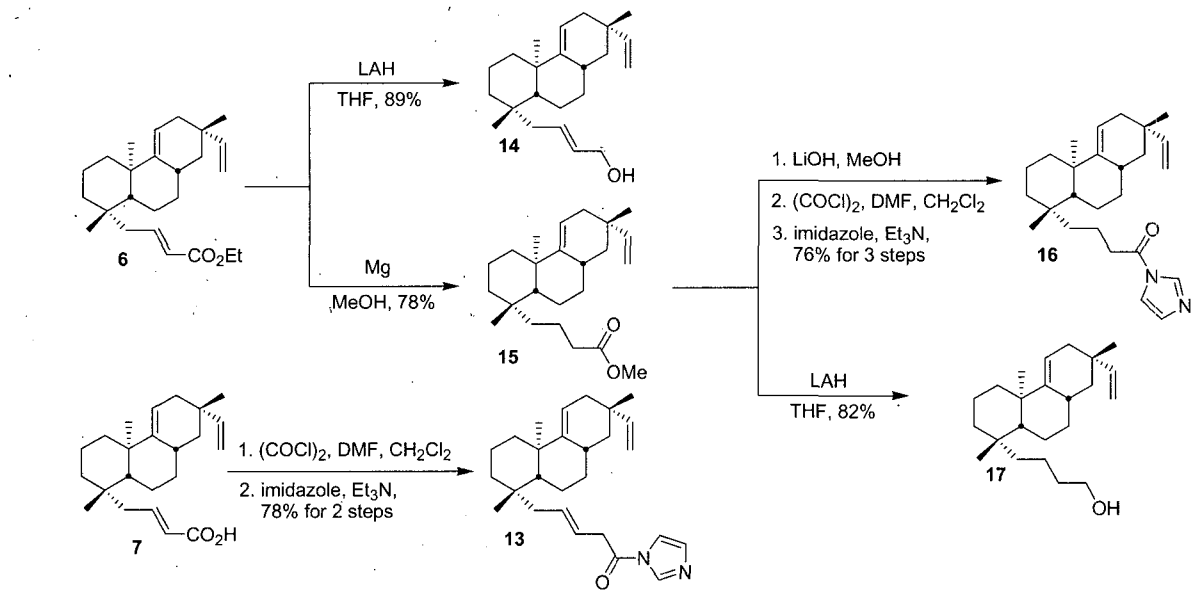
The analogs **13-17** were prepared from the analogs **6** and **7** as shown in Scheme 3. The synthetic pathways for



Scheme 1. Synthesis of the analogs **4** and **7**



Scheme 2. *Synthesis of the analogs* **8-12**



Scheme 3. Synthesis of the analogs, **13-17**

the analogs **13-17** are same as those for the analogs **8-12**.

Spectral data for representative analogs

Spectral data for the analog 9

IR (neat) 3331, 2924, 1621, 1455, 1374, 1114 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) d 5.85 (d, 1H, *J* = 16.3 Hz), 5.75 (dd, 1H, *J* = 17.5, 10.7 Hz), 5.44 (m, 1H), 5.28 (m, 1H), 4.86 (dd, 1H, *J* = 17.5, 1.2 Hz), 4.79 (dd, 1H, *J* = 10.7, 1.2 Hz), 0.95-1.98 (m, 18H), 0.90 (s, 3H), 0.87 (s, 3H), 0.85 (s, 3H); HR-MS (EI): calcd for $C_{22}H_{34}O$ [M]: 314.2610; found: 314.2615.

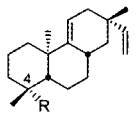
Spectral data for the analog 12

IR (neat) 3321, 2921, 1456, 1372, 1058; ^{1}H-NMR ($CDCl_3$, 300 MHz) d 5.75 (dd, 1H, *J* = 17.5, 10.7 Hz), 5.27 (m, 1H), 4.86 (dd, 1H, *J* = 17.5, 1.2 Hz), 4.79 (dd, 1H, *J* = 10.7, 1.2 Hz), 3.54 (t, 2H, *J* = 6.5 Hz), 0.90-1.99 (m, 20H), 1.02 (s, 3H), 0.90 (s, 3H), 0.80 (s, 3H); HR-MS (EI): calcd for $C_{22}H_{36}O$ [M]: 316.2766; found: 316.2758.

BIOLOGICAL ASSAYS

Inhibition of NO production in Raw 264.7 cells was assayed by measuring the accumulation of nitrite in the culture medium by the Griess reaction. Raw 264.7 cells were transferred in 96 well plates at a density of 1×10^5 cells/well. After 3 h incubation, the cells were stimulated with LPS (1 μg/mL) for 24 h in the absence or presence of the acanthoic acid analogs tested. As a parameter of NO synthesis, nitrite concentration was measured in the supernatant of Raw 264.7 cells by Griess reaction (Jin *et al.*, 2002).

Table I. The *in vitro* data of NO inhibition of acanthoic acid analogs



entry	Structure (R)	analogs	NO inhibition IC_{50}, μM
1	$CH=CHCO_2H$	**4**	>10
2	$CH=CHCOim^a$	**8**	1.02
3	$CH=CHCH_2OH$	**9**	0.05
4	CH_2CH_2COim	**11**	19.4
5	$CH_2CH_2CH_2OH$	**12**	0.21
6	$CH_2CH=CHCO_2H$	**7**	>10
7	$CH_2CH=CHCOim$	**13**	>10
8	$CH_2CH=CHCH_2OH$	**14**	>10
9	$CH_2CH_2CH_2COim$	**16**	>10
10	$CH_2CH_2CH_2CH_2OH$	**17**	>10

[a]im: imidazole.

RESULTS AND DISCUSSION

The *in vitro* data of NO inhibition of the synthesized compounds are summarized in Table I.

For the extension of the C_4-linker, the two-carbon homologation turned out to be optimal for the increased NO inhibitory activity. In case of the terminal functional groups (the analogs **4**, **8**, **9**, **11**, and **12**), the analogs bearing alcohol and amide exhibited the better NO inhibitory activities. In addition, the alcohol analogs, **9** and **12,** showed the higher potencies compared to the amide analogs, **8** and **11**. In particular, the alcohol analog **9**

exhibited the most potent activity with an IC_{50} of 50 nM. This also partly support that the presence of double bond in the C_4-chain is beneficial for the higher NO inhibitory activity.

In conclusion, we have established the partial structure-activity relationship (SAR) of the C_4-chain length of the acanthoic acid analogs although our study does not provide clear SAR yet. In addition, our study confirmed that the C_4-chain length plays an important role for the NO inhibitory activity of the acanthoic acid analogs. Moreover, we have identified an acanthoic acid analog, which possesses an excellent inhibitory activity in NO production in Raw 264.7 cells through the SAR studies. The enzyme assay in three NOS isozymes are currently being explored to validate the selectivity for *i*NOS and the successful results will be reported in due courses.

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