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Synthesis and anti-inflammatory effects of novel pimarane diterpenoid analogs

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Abstract—Syntheses and excellent anti-inflammatory effects of a series of novel acanthoic acid analogs are reported. In particular, the mechanistic basis for their anti-inflammatory effects is also described.

1. Introduction

Inflammation is the response of an organism's immune system to the damage caused to its cells and vascularized tissues by microbial pathogens such as viruses and bacteria, as well as by injurious chemicals or physical insults. The regulation of the inflammatory response is important because insufficient responses result in immunodeficiency, which can lead to infection and cancer. However, excessive response cause morbidity and mortality in diseases such as rheumatoid arthritis, Crohn's disease, atherosclerosis, diabetes, Alzheimer's disease, multiple sclerosis, and cerebral and myocardial ischemia.[1] Inflammation is induced by a complex set of interactions among soluble factors and cells. Thus a number of points of control in inflammation are currently investigated. Such a new class of anti-inflammatory drugs includes leukotriene antagonists, cyclooxygenase-2 (COX-2) inhibitors, inducible nitric oxide synthase (iNOS) inhibitors, and tumor necrosis factor (TNF) blockers although multiple gene products have more recently been identified at the sites of inflammation and identifying intracellular signaling targets, including transcription factors.[2,3]

Recently, we have reported COX-2 inhibitory activities of acanthoic acid[4] analogs, as novel COX-2 inhibitors as well as their structure–activity relationship,[5] which revealed that C4 modification provides the enhanced in vitro activities. As an extension of studies on pimarane diterpenoids, we have synthesized the novel acanthoic acid analogs based on the previous structure–activity relationship and evaluated their anti-inflammatory effects for their therapeutic utilities. Consequently, we have discovered a number of excellent anti-inflammatory analogs as well as their interesting mechanistic aspects for the anti-inflammatory effects. We herein report syntheses and in vivo activities of the acanthoic acid analogs as novel anti-inflammatory agents. In addition, cellular mechanism associated with anti-inflammatory activities is also described.

2. Chemistry

The syntheses of the acanthoic acid analogs, which were selected based on their in vitro activities and tested for

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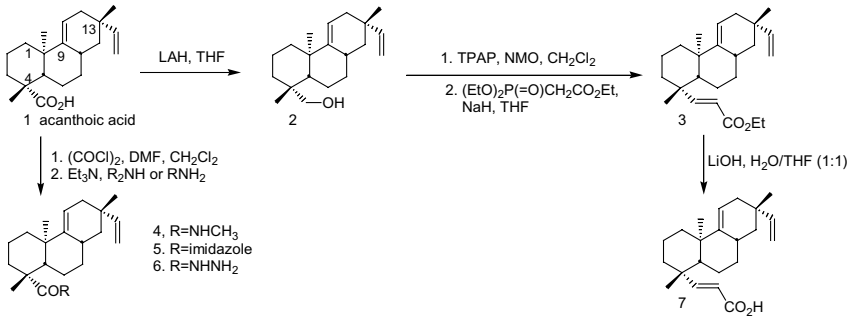
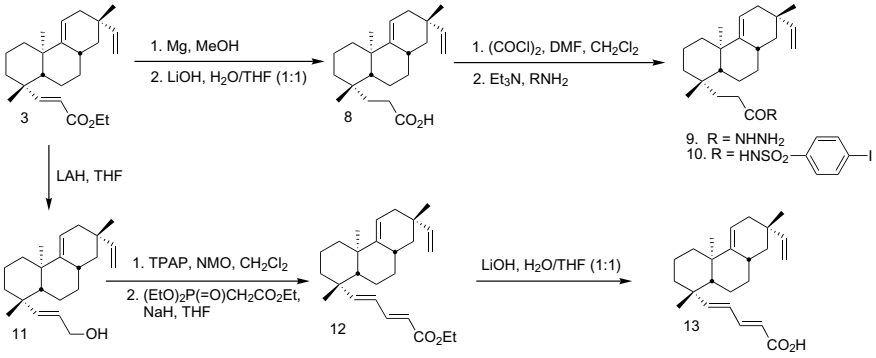
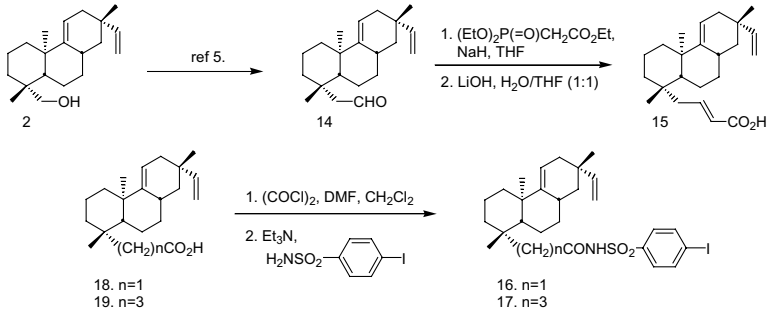


Scheme 1. Syntheses of the analogs **4**–**7**.



Scheme 2. Syntheses of the analogs **8**–**13**.



Scheme 3. Syntheses of the analogs **14**–**19**.

anti-inflammatory effect on adjuvant-induced arthritis in rat, are outlined in Schemes 1–3. The analogs **2**–**7** were conveniently prepared from the natural acanthoic acid (**1**) as shown in Scheme 1. LAH reduction of acanthoic acid provided the alcohol **2**, which were transformed into the α,β-unsaturated acid **7** by TPAP oxidation, olefination of the resulting aldehyde, and then ester hydrolysis. Treatment of acanthoic acid with oxalyl chloride, followed by amidation with the corresponding amines provided the amides **4** and **5** and the hydrazide **6**, respectively. Magnesium reduction of the intermediate **3** and then ester hydrolysis afforded the two carbons extended acid **8**, which was converted to the hydrazide **9** and the pipsyl amide **10** by analogy with the analogs **6** and **4**, respectively (Scheme 2). The analog **13** was also prepared from the allylic alcohol **11** by analogy with the acid **7**. The analog **15** was prepared from the known aldehyde **14**[5] by olefination and hydrolysis of the resulting ester as shown in Scheme 3. The pipsyl amides **16** and **17** were prepared by amidation of the corresponding acids with pipsyl amine. The analogs **18**–**21** were prepared by the known procedure reported by us.[5]

3. Biological assays

The purified COX-2 enzyme assay was performed according to Bohlin protocol with a slight modification.[6]

Purified COX-2 (prostaglandin endoperoxide H synthase-2) from sheep placental cotyledons was purchased from Cayman Chemical Co., Ann Arbor, MI, USA.

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 analogs tested. As a parameter of NO synthesis, nitrite concentration was measured in the supernatant of Raw 264.7 cells by the Griess reaction.[8]

Experimental arthritis was induced by an injection with 50 μL of Freund's adjuvant (100 μg of mycobacterium) to a tibiocalcanean joint of rat. The tested compounds and ketoprofen as the reference drug were individually administered with the dose of 5, 15, or 25 mg/kg by an intraperitoneal injection 10 min before adjuvant injection and the ceiling effect was observed mostly at the dose of 5–25 mg/kg (i.e. 30–40% inhibition at the dose range). The edema of the treated tibiocalcanean joint was evaluated at 1, 4, and 24 h after adjuvant injection. Anti-inflammatory effects of the tested analogs were expressed as percent inhibition of edema based on that of the control group.

4. Results and discussion

The anti-inflammatory effects of the sixteen analogs are summarized in Figure 1. Most of the analogs exhibited significant inhibition of edema formation and some inhibitory effects were comparable to that of ketoprofen. In particular, the analogs[7] **8**, **10**, **15**, and **16** appeared to be more potent than ketoprofen. The structure–activity correlation on the basis of the inhibitory activities of the tested analogs could not be clearly established. However, the pipsyl amides such as **10**, **16**, and **17** generally showed good inhibitory activity. The carbon extended carboxylic acids (**8**, **19**, and **21**) except the analog **18** showed potency equivalent to that of ketoprofen or exhibited higher efficacy.

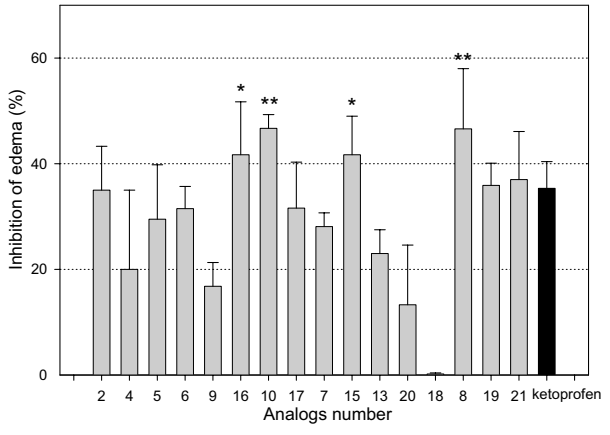


Figure 1. Inhibition of edema formation by acanthoic acid analogs. Data represent the mean ± SE with at least four separate experiments (significant compared to ketoprofen, $^{**}p < 0.01$, $^{*}p < 0.05$).

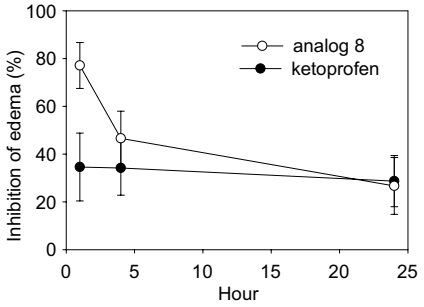


Figure 2. The time-related inhibitory effects of the analog **8** and ketoprofen on edema formation in rat. Data represent the mean ± SE with at least four separate experiments.

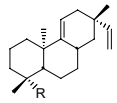
In subsequent experiments, we further monitored the time-related inhibitory effect of the representative analog **8** to determine that their inhibitory effects were persistent. The inhibitory effect of the analog **8** was observed up to 24 h after a single dose of treatment. The pharmacological effect gradually declined as a function of time as shown in Figure 2. This implies that the analog **8** seems to be metabolized more rapidly than ketoprofen.

The mechanistic aspects of the acanthoic acid analogs presented in Figure 1 were analyzed on the basis of their in vitro activities. Our investigation was particularly focused on the COX-2 and NO inhibition because our in vitro assays revealed the characteristic features on these two proinflammatory factors for most of the anti-inflammatory analogs. The in vitro activities of COX-2 inhibition and suppression of NO production are summarized in Table 1.

For the analogs **2**, **4**, **5**, **10**, and **16**, NO inhibition activity seems to play more important role for their anti-inflammatory effects while COX-2 inhibition is responsible for the analogs **6**, **18**, and **19**. The analogs **13**, **15**, **20**, and **21** exhibited good inhibitory activities in both in vitro assays. The analogs **10**, **16**, and **21** exhibited highly potent NO inhibitory activities with IC_{50} of 1.2, 8.8, and 2.3 μM, respectively. The significant in vivo anti-inflammatory activity of analog **8** in spite of its poor in vitro activities suggests the possibility that the pharmacological effect of the analog may result from other mechanistic basis.

As mentioned earlier, inflammation is not a single process, but a complex, multifaceted mosaic of individual events, which may also differ in their responses to NO.[4] However, the excellent inhibition of edema formation by many acanthoic acid analogs are likely due to the suppression of NO production rather than COX-2 inhibition although it is, at present, not clear whether the excellent suppression of NO production arises from the direct NO inhibition or the iNOS inhibition. However, our observation of COX-2 inhibition and suppression of NO production is considered to be quite important for

Table 1. In vitro COX-2 inhibition and suppression of NO production of acanthoic acid analogs



Entry	Analogs	R	In vitro assay	
			COX-2 inhibition[a]	NO inhibition[b]
1	**2**	CH_2OH	NA	84.8
2	**4**	$CONHCH_3$	NA	85.3
3	**5**	COim[c]	NA	99
4	**6**	$CONHNH_2$	41.9	3.4
5	**16**	$CH_2CONHSO_2Ph(4\text{-}I)$	72.2	94.6
6	**10**	$(CH_2)_2CONHSO_2Ph(4\text{-}I)$	60.7	99.5
7	**7**	$CH{=}CHCO_2H$	69.7	45.1
8	**15**	$CH_2CH{=}CHCO_2H$	32.0	77.9
9	**13**	$CH{=}CHCH{=}CHCO_2H$	26.8	96.5
10	**20**	$(CH_2)_3CH{=}CHCO_2H$	37.8	98.2
11	**18**	CH_2CO_2H	82.3	NA
12	**8**	$(CH_2)_2CO_2H$	105	23.0
13	**19**	$(CH_2)_3CO_2H$	49.4	65.2
14	**21**	$(CH_2)_4CO_2H$	38.7	99.0

NA: no activity.
The analogs **18**–**21** were reported in Ref. 5.
[a] Unit: IC_{50} (μM).
[b] NO inhibition was measured at 40 μM concentration. unit: inhibition (%).
[c] im: imidazole.

the anti-inflammatory effects of the acanthoic acid analogs since both enzyme activities are the major contributing factors for the inflammatory process.

In summary, we have developed novel anti-inflammatory pimarane diterpenoids and found their mechanistic basis, providing valuable information for development of new anti-inflammatory agents. Furthermore, the analogs may serve as novel and promising anti-inflammatory agents. The detailed mechanisms for anti-inflammatory effects of the acanthoic acid analogs such as regulation of proinflammatory cytokine and transcription factors as well as iNOS inhibition are currently being explored and the successful results will be reported in due courses.

Acknowledgements

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7. Spectral data for the analog **10**: IR (neat) 3248, 2925, 1706, 1442, 1174 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.21 (s, 1H), 7.85 (d, 2H, $J = 8.5$ Hz), 7.71 (d, 2H, $J = 8.5$ Hz), 5.74 (dd, 1H, $J = 17.4$, 10.4 Hz), 5.27 (m, 1H), 4.85 (dd, 1H, $J = 17.4$, 1.2 Hz), 2.20–0.64 (m, 20H), 0.98 (s, 3H), 0.88 (s, 3H), 0.70 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.2, 151.5, 150.3, 138.3, 138.1, 129.7, 116.1, 109.5, 102.9, 47.4, 41.6, 41.1, 37.9, 37.6, 37.2, 35.5, 34.8, 32.0, 29.2, 28.5, 27.0, 26.8, 26.1, 22.5, 19.1, 18.1; HR-MS (EI): calcd for $C_{28}H_{38}INO_3S$ [M]: 595.1657; found: 595.1604.
Spectral data for the analog **15**: IR (neat) 2928, 1692, 1638, 1292 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.02 (dt, 1H, $J = 15.6$, 7.8 Hz), 5.75 (m, 2H), 5.30 (m, 1H), 4.86 (d, 1H, $J = 17.5$ Hz), 4.80 (d, 1H, $J = 10.7$ Hz), 2.48–0.69 (m, 18H), 1.04 (s, 3H), 0.90 (s, 3H), 0.84 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.4, 157.7, 150.2, 149.9, 117.2, 116.3, 109.1, 55.1, 47.5, 41.7, 41.1, 39.7, 38.3, 37.9, 37.6, 34.9, 30.7, 29.2, 26.9, 24.5, 22.4, 19.1, 18.3; HR-MS (EI): calcd for $C_{23}H_{34}O_2$ [M]: 342.2559; found: 342.2564.
Spectral data for the analog **16**: IR (neat) 3434, 2922, 1715, 1385, 1079 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.84 (d, 2H, $J = 8.8$ Hz), 7.69 (d, 2H, $J = 8.5$ Hz), 5.73 (dd, 1H, $J = 17.5$, 10.7 Hz), 5.29 (m, 1H), 4.85 (d, 1H, $J = 17.5$ Hz), 4.79 (d, 1H, $J = 10.5$ Hz), 2.36–0.82 (m, 18H), 0.91 (s, 3H), 0.88 (s, 3H), 0.85 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.1, 151.3, 150.3, 141.6, 138.4, 127.9, 116.0, 109.1, 100.1, 47.8, 41.7, 41.2, 38.5, 37.9, 37.6, 37.2, 34.8, 29.7, 29.2, 28.1, 27.0, 25.4, 22.4, 19.2, 18.7; HR-MS (EI): calcd for $C_{27}H_{36}INO_3S$ [M]: 581.1461; found: 581.1465.
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