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NOVEL SYNTHETIC OLEANANE TRITERPENOIDS: A SERIES OF HIGHLY ACTIVE INHIBITORS OF NITRIC OXIDE PRODUCTION IN MOUSE MACROPHAGES

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Abstract: Novel oleanane triterpenoids with modified rings A and C were designed and synthesized. Among them, methyl 2-carboxy-3,12-dioxooleana-1,9-dien-28-oate showed similar high inhibitory activity (IC_{50} = 0.8 nM) to 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO), which we have synthesized previously, against production of nitric oxide induced by interferon-γ in mouse macrophages.

Introduction

In a previous communication[1] we reported that 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO) (**1**) has high inhibitory activity against production of nitric oxide (NO) induced by interferon-γ (IFN-γ) in mouse macrophages (IC_{50} = 0.1 nM level). We also showed that CDDO is a potent, multifunctional agent.[2] For example, CDDO induces monocytic differentiation of human myeloid leukemia cells and adipogenic differentiation of mouse 3T3-L1 fibroblasts. CDDO inhibits proliferation of many human tumor cell lines. CDDO blocks *de novo* synthesis of inducible nitric oxide synthase (*i*-NOS) and inducible cyclooxygenase (COX-2) in mouse macrophages. CDDO will protect rat brain hippocampal neurons from cell death induced by β-amyloid. The above activities have been found at concentrations ranging from 10^{-6} to 10^{-9} M in cell culture.

In the communication,[1] we also reported that the combination of a 1-en-3-one functionality with a nitrile group at C-2 in ring A and a 9-en-12-one functionality in ring C enhances activity very strongly in comparison with the enhancement by each functionality alone. We therefore designed and synthesized a series of novel oleanane triterpenoids to survey what combination of ring A with ring C provides highly active compounds. We have found that methyl 2-carboxy-3,12-dioxooleana-1,9-dien-28-oate (**2**) has similar high inhibitory activity to CDDO and methyl 2-cyano-3,12-dioxooleana-1,9-dien-28-oate (CDDO methyl ester) (**3**).[1,3] The new compound **2** is expected to be an alternative agent to CDDO. In this communication, the synthesis, inhibitory activity, and structure–activity relationships (SAR) are reported for these analogs.

Chemistry

Modification of Ring A (Schemes 1 and 2)

Initially, we designed and synthesized new olean-12-ene derivatives with a 1-en-3-one functionality having a substituent at C-2 in ring A, **6–9** and **12–18**, to discover which substituents enhance activity in comparison with the lead compound **4**, which was reported previously.[4] Chloride **6** was synthesized in 81% yield from

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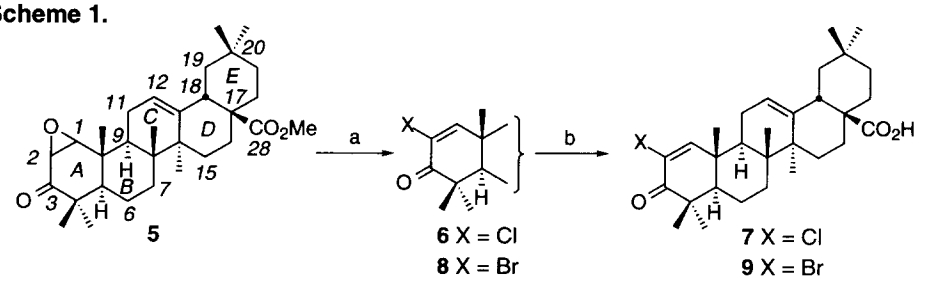
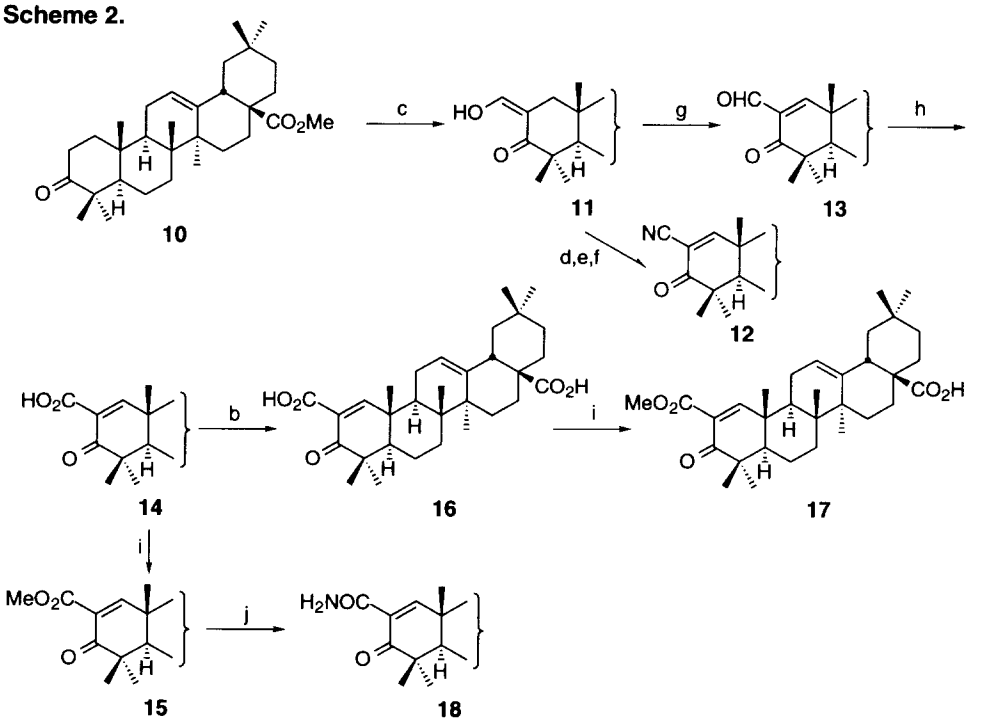
Scheme 1.

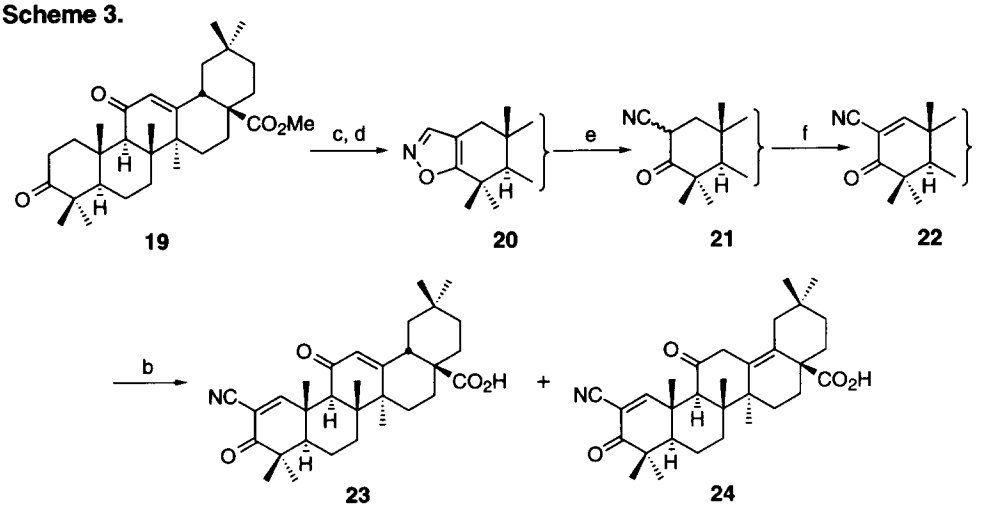


Scheme 2.

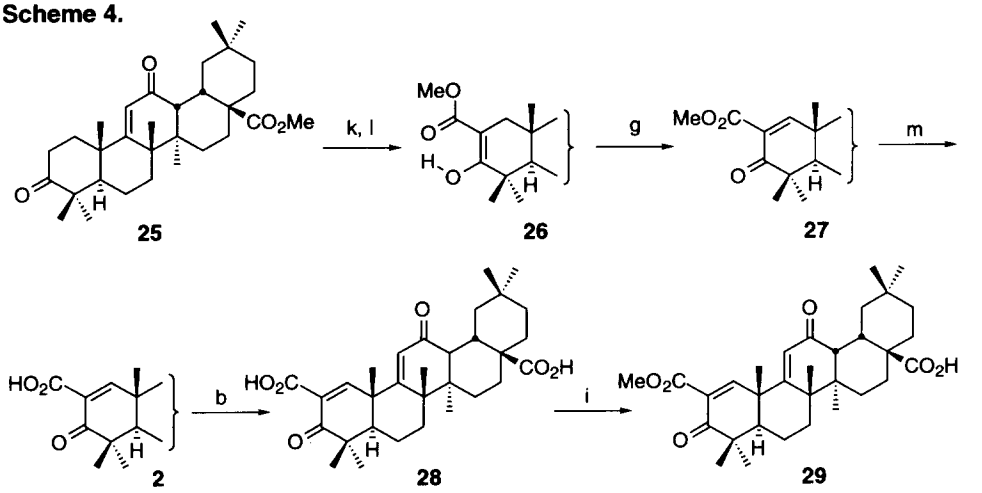


epoxide **5**[4] with hydrogen chloride in acetic acid and $CHCl_3$.[5] Halogenolysis of **6** with LiI in DMF[6] gave chloride **7** in 77% yield. Similarly, bromides **8** and **9** were prepared from **5** and **8** (yield, 96% and 76%), respectively. Compound **11**[7] was prepared in 95% yield by formylation of C-3 ketone **10**[4] with ethyl formate in the presence of sodium methoxide in benzene.[8] Nitrile **12** was synthesized in three steps (yield, 30%) from **11** according to the same synthetic route as for **30**, which was prepared previously.[1] Enal **13** was prepared from **11** by phenylselenenyl chloride-pyridine in CH_2Cl_2 and sequential addition of 30% H_2O_2[9] (yield, 71%; 79% based on recovered **11**). Jones oxidation of **13** gave acid **14** in 30% yield. Methylation of **14** with MeOH under acidic conditions gave ester **15** in 80% yield. Halogenolysis of **14** gave dicarboxylic acid **16** in 58% yield. Methylation of **16** with MeOH under acidic conditions gave ester **17** selectively in 70% yield because the carboxylic acid at C-17 of **16** is very sterically hindered. Amide **18** was prepared selectively in 72% yield from **15** with saturated ammonia-MeOH. Compounds **12** and **14–17** were found to be more active than the lead compound **4** (see Table 1).

Scheme 3.



Scheme 4.



a: HX/AcOH/$CHCl_3$, b: LiI/DMF, c: HCO_2Et/NaOMe/PhH, d: NH_2OH·HCl/aq EtOH, e: NaOMe/Et_2O/MeOH, f: PhSeCl/AcOEt; 30%H_2O_2/THF, g: PhSeCl/pyr./CH_2Cl_2; 30%H_2O_2/CH_2Cl_2, h: Jones, i: H_2SO_4/MeOH, j: NH_3/MeOH, k: Stiles' reagent/DMF, l: CH_2N_2/Et_2O/THF, m: KOH/aq MeOH

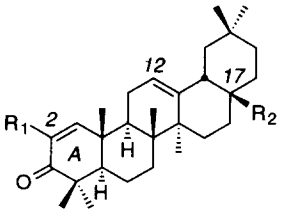
Modification of Ring C

We already reported the synthesis and inhibitory activity of 3-oxoolean-1-ene derivatives with various structures of ring C, and among them enones **31–33** are more active than the lead compound **4** (see Table 2).[4]

Combination of Modified Ring A with Ring C (Schemes 3 and 4)

On the basis of the above results, new oleanane derivatives with modified rings A and C, **2**, **22–24**, and **27–29**, were designed and synthesized. Isoxazole **20** was prepared from C-3 ketone **19**[4] by formylation (yield, 98%), followed by condensation with hydroxylamine (yield, 74%).[10] Cleavage of the isoxazole moiety of **20** with sodium methoxide gave nitrile **21** in 92% yield.[10] Nitrile **22** was prepared from **21** by phenylselenenyl

Table 1. IC_{50} (μM)[a] Values of Olean-12-ene Derivatives with Modified Ring A



compd	R_1 at C-2	R_2 at C-17	Taft's σ* value of R_1	activity IC_{50} (μM)
34[4]	OH	CO_2H	1.34	27
18	$CONH_2$	CO_2Me	1.68	14
35[4]	OMe	CO_2H	1.81	30
15	CO_2Me	CO_2Me	2	0.9
17	CO_2Me	CO_2H		2.2
14	CO_2H	CO_2Me	2.08	0.8
16	CO_2H	CO_2H		0.07
13	CHO	CO_2Me	2.15	toxic[b]
36[1]	CHO	CO_2H		toxic[b]
8	Br	CO_2Me	2.84	> 40
9	Br	CO_2H		7.3
6	Cl	CO_2Me	2.96	> 40
7	Cl	CO_2H		> 40
12	CN	CO_2Me	3.3	0.7
30[1]	CN	CO_2H		0.6
4[4]	H	CO_2H	-	5.6
oleanolic acid			-	> 40
hydrocortisone			-	0.01

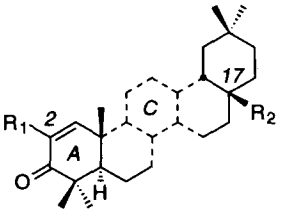
chloride in ethyl acetate and sequential addition of 30% H_2O_2[11] (yield, 33%; 57% based on recovered **21**). Halogenolysis of **22** gave acids **23** and **24** in 37% and 16% yield, respectively. Compounds **2** and **27–29** could not be synthesized according to the similar synthetic route as for **14–17** because Jones oxidation of the precursor of **2** (aldehyde at C-2) gives an unknown compound instead of **2**. They were synthesized according to the alternative route illustrated in Scheme 4. Ester **26** was prepared in 78% yield from C-3 ketone **25**[4] by Stiles' reagent (methoxymagnesium methyl carbonate) in DMF,[12] followed by methylation with diazomethane. Enone **27** was prepared from **26** according to the same method as for **13** (yield, 71%; 88% based on recovered **26**). Hydrolysis of **27** with potassium hydroxide in aqueous MeOH gave acid **2** selectively in 78% yield again because of the steric hindrance of the methoxycarbonyl group at C-17 of **27**. Halogenolysis of **2** gave dicarboxylic acid **28** and monocarboxylic acid **31** in 47% and 24% yield, respectively. Methylation of **28** with MeOH under acidic conditions gave ester **29** selectively in 82% yield.

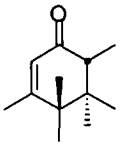
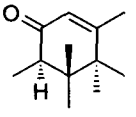
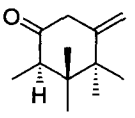
Biological Results and Discussion

Inhibitory Activity of Olean-12-ene Derivatives with Modified Ring A

The inhibitory activities [IC_{50} (μM) value] of olean-12-ene derivatives with a 1-en-3-one functionality with a substituent at C-2 in ring A,[13] oleanolic acid, and hydrocortisone (a positive control) on production of NO induced by IFN-γ in mouse macrophages[14] are shown in Table 1. These derivatives are arranged according to

Table 2. IC_{50} (μM)[a] Values of Oleanane Derivatives with Modified Rings A and C



compd	structure of ring C	R_1 at C-2	R_2 at C-17	activity IC_{50} (μM)
3 [1]		CN	CO_2Me	0.0001
1 [1]		CN	CO_2H	0.0002
27		CO_2Me	CO_2Me	toxic[b]
29		CO_2Me	CO_2H	0.1
2		CO_2H	CO_2Me	0.0008
28		CO_2H	CO_2H	0.2
31 [4]		H	CO_2H	0.2
22		CN	CO_2Me	0.02
23		CN	CO_2H	0.04
32 [4]		H	CO_2H	1.4
24		CN	CO_2H	0.07
33 [4]		H	CO_2H	2.6
dexamethasone				0.0001

[a] IC_{50} (μM) values of compounds **1–3**, **16**, **22–24**, hydrocortisone and dexamethasone were determined in the range of 0.1 pM–1 μM (tenfold dilutions). The other compounds were assayed in the range of 0.01–40 μM (fourfold dilutions). Values are an average of two separate experiments.
[b] Compounds **13**, **27** and **36** were toxic to cells above 1 μM and were not active below 1 μM.

the strength of Taft's σ* values[15] of substituents at C-2. These results provide the following interesting SAR:

(1) The relationship between Taft's σ* value and activity is not observed.

(2) Methoxycarbonyl, carboxyl, and nitrile groups at C-2 enhance activity. Compounds **12**, **14–16**, and **30** are about 10–100 times more active than the lead compound **4**.

(3) Hydroxyl, aminocarbonyl, methoxy, chloride, and bromide groups decrease activity.

(4) Formyl group does not show activity, but only toxicity.

(5) Methoxycarbonyl and carboxyl groups at C-17 show similar activity.

Inhibitory Activity of Oleanane Derivatives with Modified Rings A and C

The inhibitory activities [IC_{50} (μM) value] of oleanane derivatives with modified rings A and C,[13] and dexamethasone (a positive control) on production of NO induced by IFN-γ in mouse macrophages are shown in Table 2. These results provide the following interesting SAR:

(1) A 9-en-12-one functionality is the strongest enhancer of activity among structures of ring C. Compound **31** is about 10 times more active than **4**.

(2) 12-En-11-one and 13-en-11-one functionalities also enhance activity. Compounds **32** and **33** are about 2–4 times more active than **4**.

(3) The combination of a 9-en-12-one functionality with nitrile and carboxyl groups at C-2 provides extremely highly active compounds. Compounds **2**, **3**, and CDDO (**1**) are about 10,000 times more active than **4**.

(4) The combination of 12-en-11-one and 13-en-11-one functionalities with a nitrile group at C-2 also provides highly active compounds. Compounds **22–24** are about 100 times more active than **4**.

(5) Although compounds **27–29** were also expected to show similar high activity to CDDO from the perspective of SAR, they did not show high activity.

Currently, further evaluation in vivo for both antiinflammation and chemoprevention of CDDO, **2**, and **3** are in progress. Studies on the mode of action of these compounds also are in progress.

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References and Notes

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3. CDDO methyl ester (**3**) was found to show the same high activity as CDDO after our previous communication[1] was published.
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7. ^{1}H and ^{13}C NMR of compound **11** in $CDCl_3$ showed that it is the single isomer as depicted in Scheme 2.
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13. All new compounds, **2**, **6–9**, **12–18**, **22–24**, and **27–29** exhibited satisfactory spectral data including high-resolution mass spectra and elemental analyses.
14. Briefly, the procedure for this assay is as follows: Macrophages were harvested from female mice injected intraperitoneally four days earlier with 4% thioglycollate. These cells were seeded in 96-well tissue culture plates and incubated with 20 ng/mL IFN-γ in the presence or absence of inhibitory test compounds. After 48 hours NO production (measured as nitrite by the Griess reaction) was determined. Full details of the assay are given in reference 16.
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