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A Novel Dicyanotriterpenoid, 2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-onitrile, Active at Picomolar Concentrations for Inhibition of Nitric Oxide Production

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Abstract—New oleanane triterpenoids with various substituents at the C-17 position of 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO) and methyl 2-carboxy-3,12-dioxooleana-1,9(11)-dien-28-oate were synthesized. Among them, 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-onitrile shows extremely high inhibitory activity ($IC_{50} = 1$ pM level) against production of nitric oxide induced by interferon-γ in mouse macrophages. This potency is about 100 times and 30 times more potent than CDDO and dexamethasone, respectively.

In previous papers, we reported that 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO) (**1**), its methyl ester **2** and methyl 2-carboxy-3,12-dioxo-oleana-1,9(11)-dien-28-oate (**3**) show high inhibitory activity against production of nitric oxide (NO) induced by interferon-γ (IFN-γ) in mouse macrophages ($IC_{50} = 0.1$ nM level).[1–4] We also reported that CDDO is a potent, multifunctional agent in various in vitro assays.[5] For example, CDDO induces monocytic differentiation of human myeloid leukemia cells and adipogenic differentiation of mouse 3T3-L1 fibroblasts. CDDO also inhibits proliferation of many human tumor cell lines, and blocks de novo synthesis of inducible nitric oxide synthase (iNOS) and inducible cyclooxygenase (COX-2) in mouse macrophages. The above potencies have been found at concentrations ranging from 10^{-6} to 10^{-9} M in cell culture. Mechanism studies revealed that CDDO is a ligand for peroxisome proliferator-activated receptor γ (PPARγ)[6] and induces apoptosis in human myeloid leukemia cells.[7]

Modifications of rings A and C of oleanolic acid (**30**), a commercially available naturally occurring triterpene, led to the synthesis of CDDO. However, we had not modified the carboxyl group at C-17 of CDDO, which is very important from the perspective of structure–activity relationships (SARs). Because the synthesis of CDDO involves 11 steps from oleanolic acid, this has limited the preparation of sufficient quantities of CDDO to allow such modifications. However, we have recently produced a sufficient amount to be able to synthesize various CDDO derivatives with modified carboxyl groups (i.e., nitrile, esters, glycosides, and amides) at C-17 (see Table 1). As a result, we found that 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-onitrile (**4**) shows extremely high inhibitory activity ($IC_{50} = 1$ pM level) against production of NO in mouse macrophages. This potency is about 100 times and 30 times more potent than that of CDDO and dexamethasone, respectively. In this communication, we report the synthesis, inhibitory activity and SARs of these new analogues.

Dinitrile **4** was synthesized from CDDO by the method as shown in Scheme 1. Addition of oxalyl chloride to CDDO gave acyl chloride **31** in quantitative yield. Amide **15** was prepared in 91% yield from **31** with ammonia gas in benzene. Dehydration of **15** with thionyl chloride gave **4** in 89% yield.[8] Because the C-17 carboxyl group of CDDO is hindered, esterifications of CDDO with alcohols under acidic conditions were not successful. We found that a nucleophilic substitution

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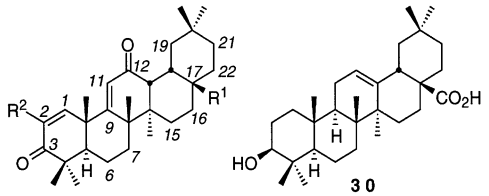
Table 1. Synthesis and biological potency of new oleanane triterpenoids



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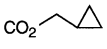
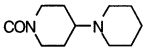
Compd	R^1	R^2	Method	Yield (%) from **1**	IC_{50} (nM)[a]
CDDO (**1**)	CO_2H	CN	Refs 1 and 4		0.44
2	CO_2Me	CN	Refs 1 and 4		0.11
3	CO_2Me	CO_2H	Refs 2 and 4		9.55
4	CN	CN	Scheme 1	81	0.0035
5	CN	CO_2H	Scheme 3		1.68
6	CO_2Et	CN	A	100	0.80
7	CO_2Et	CO_2H	Scheme 3		7.93
8	$CO_2CH_2CH{=}CH_2$	CN	B	83	1.33
9	$CO_2(CH_2)_3CH_3$	CN	A	74	6.65
10		CN	A	81	4.45
11	CO_2CH_2Ph	CN	A	97	4.35
12	$CO_2(CH_2)_7CH_3$	CN	A	89	60.4
13	CO-D-Glu(OAc)$_4$	CN	Scheme 2	75	0.070
14	CO-D-Glu	CN	Scheme 2	62	10.1
15	$CONH_2$	CN	Scheme 1	91	0.098
16	$CONHNH_2$	CN	C	55	0.26
17	CONHMe	CN	D	93	0.58
18	$CONH(CH_2)_2CH_3$	CN	D	93	1.50
19	$CONH(CH_2)_5CH_3$	CN	D	92	14.9
20	CONHPh	CN	D	100	28.6
21	$CONHCH_2Ph$	CN	D	96	9.2
22	$CONMe_2$	CN	D	89	1.55
23	$CON(n\text{-}Pr)_2$	CN	D	85	32.9
24		CN	E	86	0.80
25		CN	E	66	0.95
26		CN	E	82	1.00
27		CN	E	59	2.40
28		CN	C	83	0.014
29		CN	C	92	12.0
30	Oleanolic acid				>40,000
	Dexamethasone				0.10

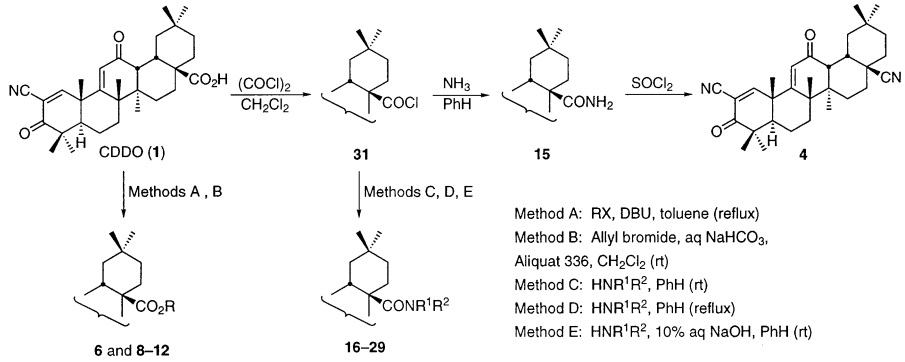
[a]IC_{50} values of compounds **1**–**29** and dexamethasone were determined in the range of 0.01 pM–1 μM (10-fold dilutions). Values are an average of several separate experiments. None of the compounds was toxic to primary mouse macrophages at 1 μM.

method using an alkyl halide and DBU in toluene (reflux)[9] gives esters **6** and **9**–**12** from CDDO in good yield (see Table 1). Allyl ester **8** was successfully prepared in 83% yield from allyl bromide and CDDO using a phase-transfer catalyst.[10] Amides **16**–**29** were synthesized in good yield by condensation reactions (Methods C and D, see Scheme 1) between acyl chloride **31** and the corresponding amines. Tetra-*O*-acetyl-β-D-glucopyranoside **13** was prepared in 75% yield from tetra-*O*-acetyl-α-D-glucopyranosyl bromide[11] and CDDO using a phase-transfer catalyst.[12] Because in the ^1H NMR spectrum (300 MHz, $CDCl_3$) of **13** the anomeric proton was observed at δ 5.70 ppm (1H, d, $J = 7.8$ Hz), the proton was assigned the β-configuration. Acetyl groups of **13** were removed with saturated ammonia methanol solution to afford β-D-glucopyranoside **14** in 83% yield (Scheme 2). In addition to these CDDO derivatives, we have synthesized derivatives of compound **3**, nitrile **5** and ethyl ester **7** (Scheme 3). Their syntheses require many more steps than the syntheses of CDDO derivatives because the carboxyl group at C-2 must be introduced after the carboxyl group at C-17 is modified. Acid **33** was prepared in 83% yield by cleavage of the known methyl ester **32**[1,4] with LiI in DMF.[13] The same sequence as for **4** gave nitrile **34** in 25% yield (chlorination, 100%; amidation, 100%; and dehydration, 25%). The desired nitrile **5** was synthesized in 4 steps from **34** (yield, 24%) according to the known synthetic sequence for **3**[2,4] (insertion of carboxyl group at C-2 of **34** with Stiles' reagent,[14] followed by methylation with diazomethane, 48%; insertion of double bond at C-1 with phenylselenenyl chloride–pyridine and subsequent H_2O_2 oxidation,[15] followed by selective hydrolysis of the C-2 methyl ester with KOH in aqueous methanol, 51%). Ethyl ester **35** was prepared in 99% yield by ethyl iodide and DBU in toluene. The desired ethyl ester **7** was synthesized in 57% yield from **35** by the same sequence as for **5**.

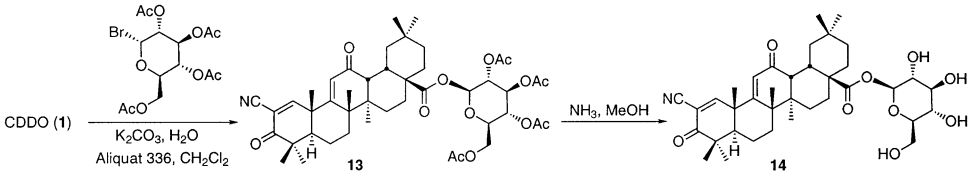
The inhibitory activities [IC_{50} (nM) value] of new synthetic triterpenoids **4**–**29**,[16] oleanolic acid, and dexamethasone on NO production induced by IFN-γ in mouse macrophages[17] are shown in Table 1. Dinitrile **4** shows extremely high potency ($IC_{50} = 1$ pM level); it is about 100 times and 30 times more potent than CDDO and dexamethasone, respectively.

These results provide the following SARs about substituents at C-17: (1) A nitrile group enhances potency. Dinitrile **4** is much more potent than **1** and **2**, nitrile **5** is more potent than **3**. (2) Ester moieties decrease potency. The less polar the ester, the less is its potency. Ester **12** is much less potent than **1** and **2**. (3) Tetra-*O*-acetyl-D-glucopyranoside **13** is more potent than **1** and **2**. D-Glucopyranoside **14** is much less potent than **1**, **2**, and **13**. Interestingly, in this case, the more polar the compound, the less is its potency. However, because we have only one example, we cannot conclude that this will be a general relationship. (4) Amide moieties decrease potency, although amide **15** and hydrazide **16** show similar potency to those of **1** and **2**. The less polar the amide, the less is its potency. (5) Although carbonyl imidazole **28** is about 30 times more potent than **1**, because this moiety is much more reactive than the other moieties with nucleophiles, it is difficult to compare it with the other moieties. Interestingly, the carbonyl pyrazole **29**, with less reactivity than **28**, is much less potent than **1** and **28**.

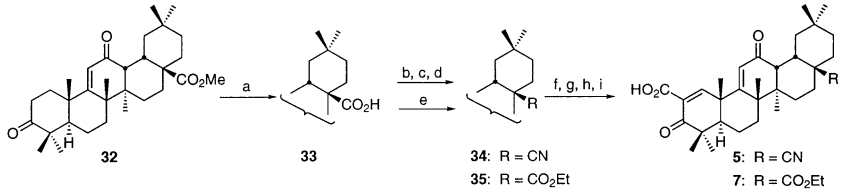
Some of these compounds including **4** had good in vivo antiinflammatory activity, when given ip or po, against peritoneal inflammation induced by thioglycollate and



Scheme 1.



Scheme 2.



Scheme 3. (a) LiI, DMF; (b) $(COCl)_2$, CH_2Cl_2; (c) NH_3, PhH; (d) $SOCl_2$; (e) EtI, DBU, toluene; (f) Stiles' reagent, DMF; (g) CH_2N_2, Et_2O, THF; (h) PhSeCl, pyr, CH_2Cl_2; 30% H_2O_2, CH_2Cl_2; (i) KOH, aq MeOH.

IFN-γ. We will report these data elsewhere. Further biological evaluation of dinitrile **4** is also in progress.

Acknowledgements

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16. All new compounds **4**–**29** exhibited satisfactory spectral data including high-resolution mass spectra and elemental analyses. Dinitrile **4**: amorphous solid; $[\alpha]_D^{25}$ +21° (*c* 0.29, $CHCl_3$). UV (EtOH) λ_{max} (log ε) 244 (4.30) nm. IR (KBr) 2947, 2871, 2253, 2233, 1690, 1666 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.04 (1H, s), 6.01 (1H, s), 3.26 (1H, d, $J=4.8$ Hz), 2.78 (1H, ddd, $J=3.3$, 4.8, 13.5 Hz), 1.55, 1.53, 1.26, 1.18, 1.01, 1.00, 0.91 (each 3H, s). ^{13}C NMR ($CDCl_3$) δ 197.9, 196.6, 169.4, 165.6, 125.0, 123.9, 114.9, 114.5, 50.1, 47.9, 46.1, 45.2, 42.8, 42.3, 38.4, 35.1, 34.2, 33.7, 33.3, 32.5, 31.9, 30.7, 28.2, 27.2, 26.9, 25.2, 23.9, 23.1, 21.8, 21.7, 18.4. EIMS (70 eV) *m*/*z* 491 $[M]^+$ (100), 472 (29), 457 (14), 269 (100). HREIMS calcd for $C_{31}H_{40}N_2O_2$: 472.3090. Found: 472.3095. Anal. calcd for $C_{31}H_{40}N_2O_2 \cdot H_2O$ C, 75.88; H, 8.63; N, 5.71. Found: C, 75.53; H, 8.58; N, 5.69.
17. Briefly, the procedure for this assay is as follows: Macrophages were harvested from female mice injected intraperitoneally four days previously with 4% thioglycollate. These cells were seeded in 96-well tissue culture plates and incubated with 4 ng/mL IFN-γ in the presence or absence of inhibitory test compounds. After 48 h NO production (measured as nitrite by the Griess reaction) was determined. Full details of the assay are given in ref 18.
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