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DESIGN AND SYNTHESIS OF 2-CYANO-3,12-DIOXOOLEAN-1,9-DIEN-28-OIC ACID, A NOVEL AND HIGHLY ACTIVE INHIBITOR OF NITRIC OXIDE PRODUCTION IN MOUSE MACROPHAGES

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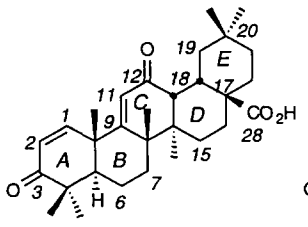
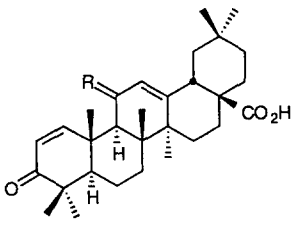
Abstract: New derivatives with electron-withdrawing substituents at the C-2 position of 3-oxoolean-1-en-28-oic acid were synthesized. Among them, 2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid (CDDO) was 400 times more potent than previous compounds we have made as an inhibitor of production of nitric oxide induced by interferon-γ in mouse macrophages (IC_{50}, 0.4 nM). The potency of CDDO was similar to that of dexamethasone, although CDDO does not act through the glucocorticoid receptor. © 1998 Elsevier Science Ltd. All rights reserved.

Introduction

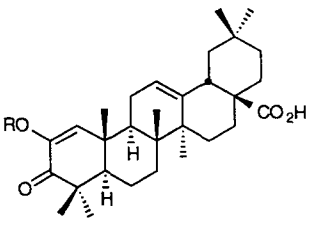
In a previous communication we reported that oleanolic acid derivatives with a 1-en-3-one functionality in ring A (e.g., **1–3**) have significant inhibitory activity against production of nitric oxide (NO) induced by interferon-γ (IFN-γ) in mouse macrophages (IC_{50}, 0.1–1 μM). We also showed that derivatives with electron-releasing substituents at the C-2 position, **4** and **5**, lose the activity.[1] Mechanism studies showed that enones **1** and **2** suppress transcription or translation of the inducible nitric oxide synthase (iNOS) and inducible cyclooxygenase (COX-2) genes, and that these compounds do not act through a glucocorticoid receptor.[2] We therefore focused on the design and synthesis of derivatives with electron-withdrawing substituents at the C-2 position to obtain more active compounds. We have now found that 2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid (CDDO) (**6**) has strong activity (IC_{50}, 0.4 nM), with a potency similar to that of dexamethasone. In this communication, the design, synthesis, and inhibitory activity are reported for these compounds.



1



2 R = O
3 R = H_2



4 R = Me
5 R = H

Design and Synthesis of New Derivatives

Initially, compounds **7** and **8** were synthesized according to the route illustrated in Scheme 1. Compound **10** was prepared by formylation of oleanonic acid (**9**)[3] with ethyl formate in the presence of sodium methoxide in

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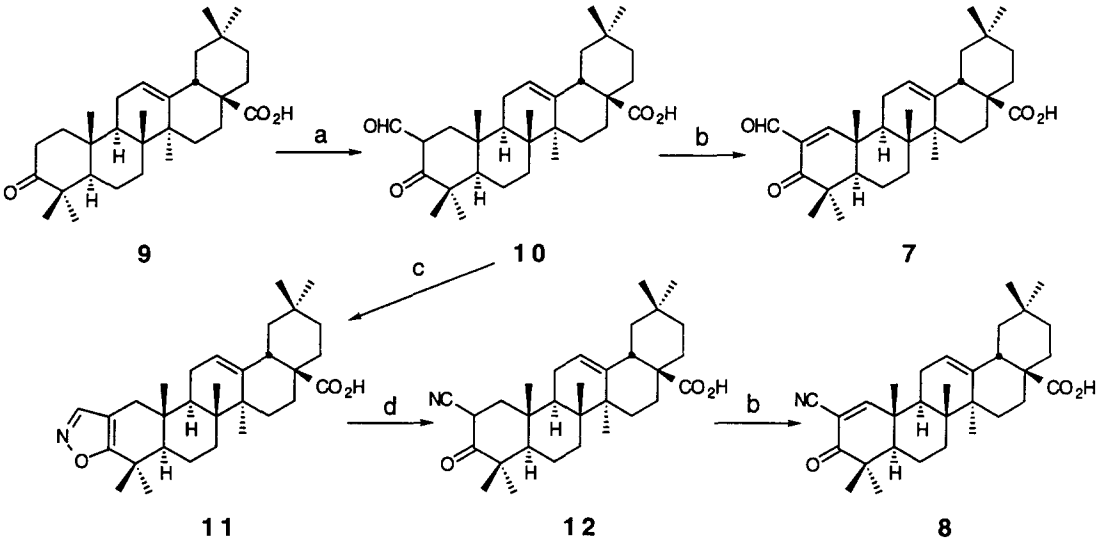
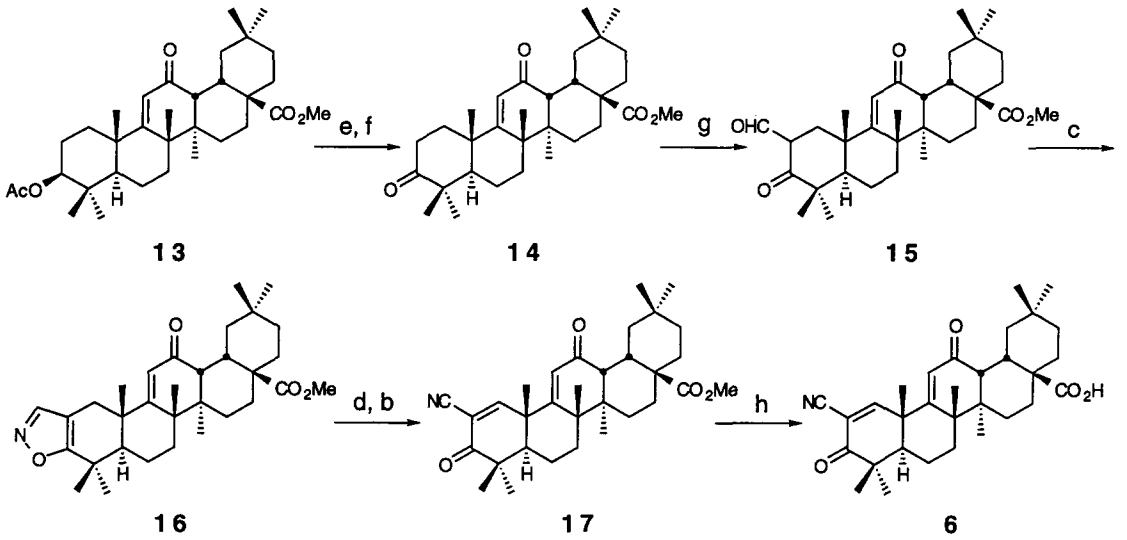
Scheme 1.



Scheme 2.



a: HCO_2Et / MeONa / THF, b: PhSeCl / AcOEt; 30%H_2O_2 / THF, c: NH_2OH·HCl / EtOH / H_2O,
d: MeONa / MeOH / Et_2O, e: KOH / MeOH, f: Jones, g: HCO_2Et / MeONa / PhH, h: LiI / DMF

THF[4] [yield, 45% (66% based on recovered **9**)]. Aldehyde **7** was obtained in 29% yield by introduction of a double bond at C-1 of **10** with phenylselenenyl chloride in ethyl acetate and sequential addition of 30% hydrogen peroxide[5] ($PhSeCl-H_2O_2$). Nitrile **12** was synthesized via isoxazole **11** from **10** according to Johnson's method.[6] Isoxazole **11** was synthesized in 99% yield from **10** by addition of hydroxylamine in aqueous ethanol.

Cleavage of isoxazole **11** with sodium methoxide gave nitrile **12** in 98% yield. Compound **8** was obtained in 36% yield by introduction of a double bond at C-1 of **12** with PhSeCl-H_2O_2. Compound **7** was toxic to cells in culture. Compound **8** was more potent than **3** (see Table). We therefore designed the new target **6** based on both structures of **1** and **8**, because **1** is also much more active than **3** (see Table and ref 1). The synthesis of **6** is illustrated in Scheme 2. Compound **14** was prepared in 89% yield from known compound **13**[7] by alkali hydrolysis, followed by Jones oxidation. Compound **15** was prepared in quantitative yield by formylation of **14** with ethyl formate in the presence of sodium methoxide in benzene. Isoxazole **16** was synthesized in 61% yield from **15** by the addition of hydroxylamine. Nitrile **17** was obtained by cleavage of isoxazole **16** with sodium methoxide (yield, 100%), followed by introduction of a double bond at C-1 with PhSeCl-H_2O_2 (yield, 40%). CDDO (**6**) was prepared in 71% yield by halogenolysis of **17** with lithium iodide in DMF.[8]

Biological Results and Discussion

The inhibitory activities [IC_{50} (μM) value] of compounds **1–8**,[9] oleanolic acid, and dexamethasone (a positive control) on production of NO induced by IFN-γ in mouse macrophages[10] are shown in the Table. Compound **8** was more active than **3** but less active than **1**. CDDO (**6**) was a strong inhibitor (IC_{50}, 0.4 nM), equivalent to dexamethasone. However, the inhibitory activity of **6** was not blocked by the glucocorticoid antagonist, RU-486,[11] which reverses the action of dexamethasone.

Table. IC_{50} (μM)[a] Values for Inhibition of Production of NO Induced by IFN-γ in Mouse Macrophages[10]

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
dexamethasone	0.0003	**5**	37
1	0.17	CDDO (**6**)	0.0004
2	1.4	**7**	> 1[b]
3	7.1	**8**	0.6
4	19	oleanolic acid	> 40

[a]IC_{50} (μM) values of compounds **1–5**, **7** and **8** were determined in the range of 0.01–40 μM (4-fold dilutions); dexamethasone and **6** were assayed in the range of 0.1 pM–1 μM (10-fold dilutions). Values are an average of two separate experiments.
[b]Compound **7** was toxic to cells above 1 μM and was not active below 1 μM.

These results provide the following interesting structure–activity relationships:

(1) A nitrile group at C-2 enhances activity. Compounds **6** and **8** are more potent than **1** and **3**, respectively.

(2) Hydroxyl and methoxy groups at C-2 decrease activity. Compounds **4** and **5** were much less potent than **3**.

(3) The above results suggest that electron-withdrawing groups at C-2 increase potency, and electron-releasing groups decrease potency.

(4) A 9-en-12-one functionality is also a strong enhancer of potency. Compounds **1** and **6** are more active than **3** and **8**, respectively.

(5) The combination of a 9-en-12-one functionality, together with a nitrile group at C-2, provides a particularly potent compound for suppression of production of NO.

On the basis of these structure–activity relationships, further lead optimization is in progress. Further biological evaluation of CDDO (**6**) is also in progress.[12]

Acknowledgments

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9. All new compounds **6–8** exhibited satisfactory spectral data including high-resolution mass spectra and elemental analyses. CDDO (**6**): amorphous solid; $[\alpha]^{22}_{D}$ +33 ° (*c* 0.28, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 240.4 (4.21) nm; IR (KBr) 2950, 2867, 2235, 1692, 1665 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.05 (1H, s), 5.99 (1H, s), 3.10-3.00 (2H, m), 1.49, 1.35, 1.26, 1.17, 1.02, 1.00, 0.91 (each 3H, s); ^{13}C NMR ($CDCl_3$) δ 199.0, 196.8, 183.6, 168.8, 166.0, 124.3, 114.9, 114.6, 50.0, 47.9, 47.2, 46.0, 45.3, 42.8, 42.4, 35.9, 34.7, 33.5, 33.1, 31.9, 31.7, 30.9, 28.2, 27.2, 26.9, 24.9, 23.3, 22.7, 21.8, 18.5; EIMS (70 eV) *m/z* 491 $[M]^+$ (100), 476 (62), 445 (29), 430 (27), 269 (94). HREIMS Calcd for $C_{31}H_{41}NO_4$: 491.3036; Found: 491.3020. Anal. Calcd for $C_{31}H_{41}NO_4 \cdot 1/4H_2O$ C, 75.04; H, 8.43. Found: C, 75.29; H, 8.79.
10. 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 hours NO production (measured as nitrite by the Griess reaction) was determined. Full details of the assay are given in reference 13.
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