Journal of Medicinal Chemistry

Volume 45, Number 22 October 24, 2002

Letters

Design and Synthesis of Tricyclic Compounds with Enone Functionalities in Rings A and C: A Novel Class of Highly Active Inhibitors of Nitric Oxide Production in Mouse Macrophages

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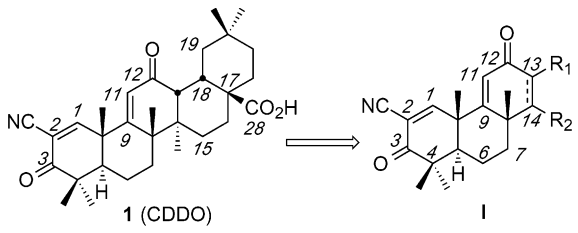
Received July 24, 2002

Abstract: Novel tricyclic compounds with enone functionalities in rings A and C, which were designed on the basis of the structure of a synthetic triterpenoid, 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid, have been synthesized. Among them, **10** shows high inhibitory activity (IC_{50} = 1 nM level) against production of nitric oxide induced by interferon-γ in mouse macrophages and is orally active at 15 mg/kg (once) in a preliminary in vivo study using mouse peritoneal inflammation induced by thioglycollate and interferon-γ.

Introduction. In a series of previous papers,[1] we reported that 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO) (**1**) shows high inhibitory activity (IC_{50} = 0.1 nM level) against production of nitric oxide (NO) induced by interferon-γ (IFN-γ) in mouse macrophages.[2] We have also reported that CDDO is a potent, multifunctional agent in various in vitro assays.[3] For example, CDDO induces monocytic differentiation and apoptosis of human myeloid leukemia cells[4] and adipogenic differentiation of mouse 3T3-L1 fibroblasts.[5] CDDO inhibits proliferation of many human tumor cell lines. CDDO blocks de novo synthesis of inducible nitric oxide synthase (iNOS) and inducible cyclooxygenase (COX-2) in mouse macrophages. These potencies are found at concentrations ranging from 10^{-6} to 10^{-9} M in cell culture. Furthermore, CDDO shows inhibitory activity against mouse peritoneal inflammation induced by thioglycollate and IFN-γ (ip administration).[6]

However, our recent studies of CDDO show that the in vivo potency (po administration) of CDDO is not optimal because of its low bioavailability. Therefore, we have modified CDDO to improve this problem and obtained some interesting analogues.[7] However, structural modifications of CDDO are severely limited because CDDO has only one functionality at C-17 that can be modified. In addition, CDDO and its analogues would have a serious cost problem for large-scale synthesis because the synthesis of CDDO requires 11 steps from oleanolic acid, an expensive triterpenoid isolated from natural sources.

Because our earlier structure−activity relationships (SARs) show that a 2-cyano-1-en-3-one functionality in ring A and a 9(11)-en-12-one functionality in ring C are essential for the extremely high potency of CDDO, we reasoned that the entire oleanane skeleton might not be necessary for potency. Therefore, we have focused our attention on tricyclic compounds having general formula **I** [tricyclic-bis-enone derivatives (TBEs)], which have the same A, B, and C rings as CDDO. A literature survey revealed that they are previously unknown compounds.



1 (CDDO) **I**

Our rationale for exploring these TBEs is as follows. (1) Because TBEs could be synthesized from commercially available small molecules, TBEs with various functionalities at different positions can be obtained.

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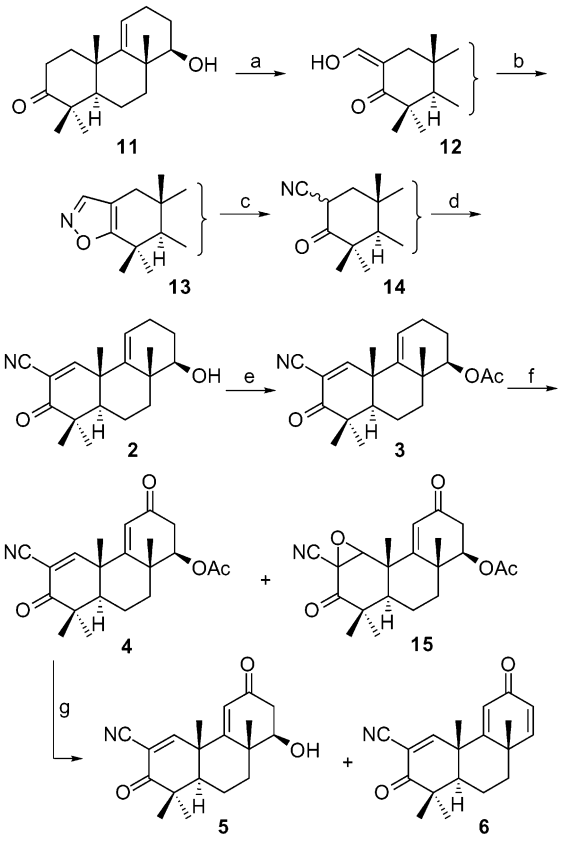
10.1021/jm025565f CCC: $22.00
Published on Web 09/24/2002

Scheme 1. Synthesis of (±)-**2**−**6**[a]



[a] Reagents: (a) HCO_2Et, NaOMe, PhH; (b) $NH_2OH{\cdot}HCl$, aqueous EtOH; (c) NaOMe, Et_2O, MeOH; (d) DDQ, PhH; (e) Ac_2O, pyr; (f) CrO_3 (cat.), *t*-BuOOH, CH_2Cl_2; (g) KOH, aqueous MeOH.

Such "diversity-oriented synthesis" of TBEs could lead to novel drugs for the prevention and/or treatment of cancer, Alzheimer's disease, Parkinson's disease, multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, and other diseases whose pathogenesis may involve excessive production of NO and/or prostaglandins. (2) This structural flexibility might provide us with new SARs and different compounds that are appropriate and specialized for each therapeutic area. (3) Some of the activities of CDDO might be absent because of the delineation of the pharmacophore of CDDO. (4) The cost of large-scale synthesis would be overcome because TBEs could be synthesized more easily than CDDO from cheap commercially available compounds.

In this communication, we report our initial results on the synthesis and biological activity of some new TBEs.[8]

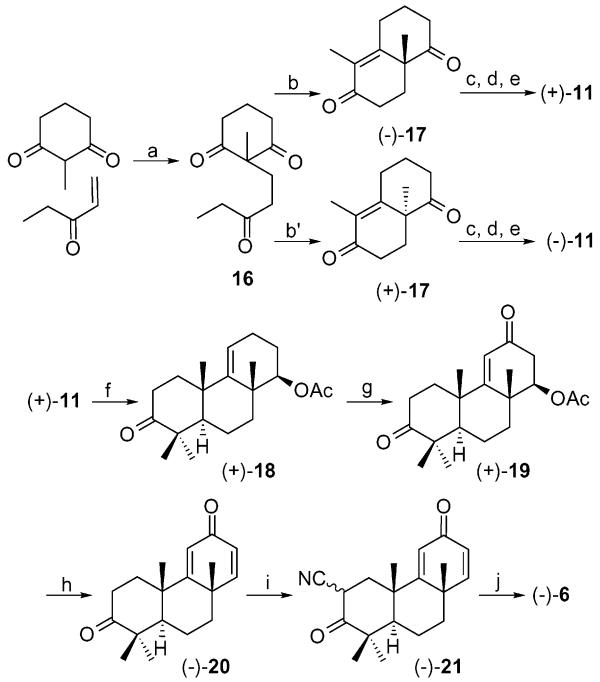
Chemistry. Our initial targets **2**−**6** were synthesized in racemic form from known compound **11**[9] (Scheme 1). Hydroxymethylene **12** was prepared in 92% yield from **11** with ethyl formate in the presence of sodium methoxide in benzene.[10] Isoxazole **13** was prepared in 73% yield by condensation of **12** and hydroxylamine hydrochloride in aqueous ethanol.[11] Cleavage of the isoxazole moiety of **13** with sodium methoxide gave nitrile **14** quantitatively.[11] Enone **2** was prepared quantitatively by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) oxidation of **14**. Acetylation of **2** gave **3** in 97% yield. Allylic oxidation of **3** with a catalytic amount of chromium trioxide and *tert*-butyl hydroperoxide in methylene chloride[12] afforded **4** and 1,2-epoxide **15** in 47% and 29% yields, respectively. Alkaline hydrolysis of **4** gave **5** and **6** in 50% and 46% yields, respectively. TBEs **4**−**6** show significant inhibitory activity (IC_{50} = 0.01 μM level, similar in potency to that of hydrocortisone) on NO production induced by IFN-γ in mouse macrophages (see Table 1).

Table 1. Inhibitory Activity of New Compounds **2**−**10**

compd	activity[a] IC_{50} (nM)	compd	activity[a] IC_{50} (nM)
(±)-**2**	310	(±)-**7**	91
(±)-**3**	480	(±)-**8**	1600
(±)-**4**	53	(±)-**9**	61
(±)-**5**	75	(±)-**10**	2.1
(±)-**6**	61	**1** (CDDO)	0.5
(−)-**6**	64	oleanolic acid	>40000
(+)-**6**	58	hydrocortisone	10

[a] IC_{50} values of compounds **1**−**10** and hydrocortisone were determined in the range of 0.1 pM to 10 μM (10-fold dilutions). Values are an average of two separate experiments. None of the compounds were toxic to primary mouse macrophages at 10 μM.

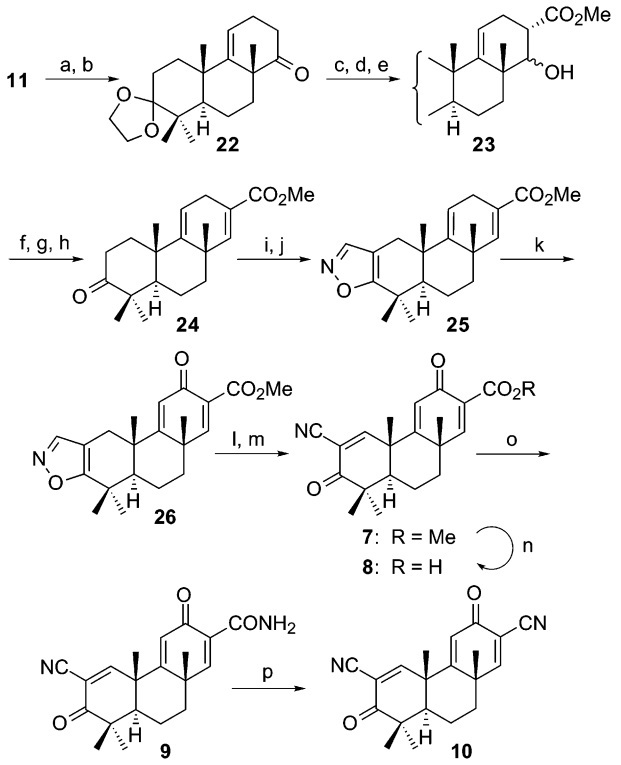
Scheme 2. Synthesis of TBEs (−)- and (+)-**6**[a]



[a] Reagents: (a) Et_3N, THF; (b) (*R*)-(+)-phenylalanine, d-CSA, DMF; (b′) (*S*)-(−)-phenylalanine, d-CSA, DMF; (c) $NaBH_4$, EtOH; (d) ethyl vinyl ketone, Na, MeOH; (e) Li, liquid NH_3, THF, CH_3I; (f) Ac_2O, pyr; (g) CrO_3 (cat.), *t*-BuOOH, CH_2Cl_2; (h) DBU, CH_2Cl_2; (i) LDA, *p*-TsCN, THF; (j) DDQ, PhH.

It is often the case that one enantiomer of a drug has greater potency and/or less toxicity than its antipode. Therefore, we synthesized both optically pure (−)-**6**, with the same configuration as naturally occurring triterpenoids, and its antipode, (+)-**6** (Scheme 2).[13] Optically pure (+)- and (−)-**17** were prepared via achiral intermediate **16** from ethyl vinyl ketone and 2-methyl-1,3-cyclohexanedione by a known method.[14] Enantiomer (+)-**11** and its antipode (−)-**11** were synthesized from (−)- and (+)-**17**, respectively, according to the same sequence as for racemic **11**.[9] The enantiomeric excess of each enantiomer (+)- or (−)-**11** was determined to be 90% by ^1H and ^{19}F NMR of the (−)-*R*-MTPA ester[15] derived from each enantiomer. TBE (−)-**6** was synthesized from (+)-**11** according to the alternative route.

Scheme 3. Synthesis of TBEs (±)-**7**−**10**[a]

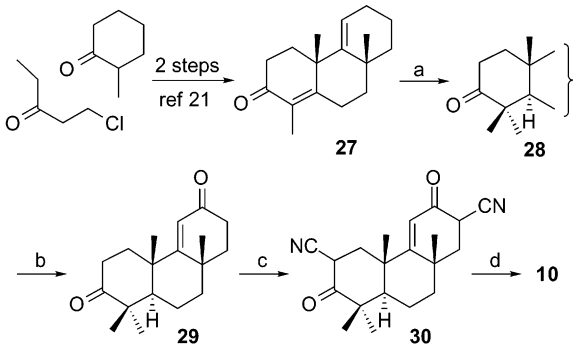


[a] Reagents: (a) $HOCH_2CH_2OH$, PPTS, PhH; (b) CrO_3, pyr, CH_2Cl_2; (c) MMC, DMF; (d) CH_2N_2, Et_2O, THF; (e) $NaBH_4$, EtOH, CH_2Cl_2; (f) PPTS, aqueous acetone; (g) MsCl, pyr, CH_2Cl_2; (h) DBU, THF; (i) HCO_2Me, NaOMe, PhH; (j) $NH_2OH{\cdot}HCl$, aqueous EtOH; (k) CrO_3 (21 equiv), pyr, CH_2Cl_2; (l) NaOMe, Et_2O, MeOH; (m) DDQ, PhH; (n) KOH, aqueous MeOH; (o) NH_3, MeOH; (p) $SOCl_2$, toluene.

Acetylation of (+)-**11** gave (+)-**18** in quantitative yield. Allylic oxidation of (+)-**18** gave (+)-**19** in 79% yield. Deacetylation of (+)-**19** with DBU gave (−)-**20** in 97% yield.[16] Cyanation of the enolate of (−)-**20** with *p*-TsCN in THF gave (−)-**21** in 93% yield.[17] DDQ oxidation of (−)-**21** gave (−)-**6** ($[\alpha]_D$ −115° ($CHCl_3$)) in 73% yield. The overall yield of (−)-**6** from (+)-**11** was 52%. This latter route is much improved over our original one (Scheme 1) in which the overall yield of **6** from **11** was only 14%. Similarly, (+)-**6** ($[\alpha]_D$ +115° ($CHCl_3$)) was synthesized from (−)-**11** by this sequence.

Because **6** shows good potency in our assay, this compound may be a good scaffold from which to discover new, more potent TBEs. Thus, we targeted **7**−**10**, analogues of **6** with electron-withdrawing groups at C-13, to discern the influence of substituents at C-13 on biological activity because we found previously that substitution at the α-position of an α,*β*-unsaturated ketone strongly affects the potency of synthetic triterpenoids.[1b] Racemic **7**−**10** were synthesized according to the synthetic route shown in Scheme 3. Ketal **22** was synthesized in 93% yield by ketalization of **11**, followed by oxidation. Ester **23** (epimeric mixture) was prepared in 71% yield from **22** by carboxylation with Stiles' reagent,[18] methylation with diazomethane, and reduction with $NaBH_4$. Deketalization of **23**, and subsequent mesylation of the hydroxyl group at C-14, followed by dehydration with DBU gave **24** in 74% yield.[19] Isoxazole **25** was obtained in 97% yield by formylation at C-2 of **24** with methyl formate, followed by condensation with hydroxylamine. Allylic oxidation of **25** gave dienone **26** in 58% yield. TBE **7** was obtained in 81% yield by cleavage of the isoxazole moiety of **26** with base, followed by DDQ oxidation. Base hydrolysis of **7** gave **8** in 77% yield. Amidation of **7** with saturated ammonia in methanol gave **9** in 64% yield. Dehydration of **9** with thionyl chloride in toluene gave **10** in 40% yield. Of these TBEs, **10** shows high potency (IC_{50} = 1 nM level) and is approaching the potency of CDDO in our bioassay (see Table 1).

Scheme 4. Efficient Synthesis of TBE (±)-**10**[a]



[a] Reagents: (a) Li (4.5 equiv), H_2O (1 equiv), liquid NH_3, THF, CH_3I; (b) CrO_3 (cat.), *t*-BuOOH, CH_2Cl_2; (c) *p*-TsCN (4 equiv), LDA (2.4 equiv), THF; (d) DDQ (2.5 equiv), PhH.

For some in vivo assays, we needed to synthesize more than 1 g of **10**. Since we synthesized **10** in 19 steps via **11** from ethyl vinyl ketone and 2-methyl-1,3-cyclohexanedione (Scheme 3), this route is too long for a practical synthesis of **10**. Therefore, we have succeeded in preparing **10** in six steps from commercially available simple compounds (Scheme 4). This much shorter route indicates that we could supply a large number of useful TBE compounds at low cost. Noteworthy in this new route is the reductive methylation step using 1 equiv of water[20] to give the new compound **28** in 63% yield from **27**,[21] which is easily synthesized in two steps, and in good yield from 2-methylcyclohexanone and 1-chloro-3-pentanone. Interestingly, the reductive methylation of **27** without a proton donor or with proton donors other than water gave no **28**. Allylic oxidation of **28** gave **29** in 64% yield. Double cyanation of the enolate of **29** with *p*-TsCN in THF successfully gave dinitrile **30** (isomeric mixture). Finally, **10** was prepared in 67% yield (from **29**) by DDQ oxidation of **30**.

Biological Results and Discussion. The inhibitory activities [IC_{50} (nM)] of racemic **2**−**10**, (−)- and (+)-**6**, triterpenoids [**1** (CDDO) and oleanolic acid], and hydrocortisone (a positive control) on NO production induced by IFN-γ in mouse macrophages are shown in Table 1. Important results are the following. (1) TBE **10** approaches the potency of CDDO in this assay; it is only about 4 times less than CDDO and about 5 and 30 times greater than hydrocortisone and **6**, respectively. A nitrile group enhances potency among the typical electron-withdrawing groups surveyed at C-13. (2) Since **4** and **5** are more potent than **2** and **3**, the importance of the bis-enone structure for high potency even in relatively small molecules is confirmed. (3) Both enantiomers (−)-**6** and (+)-**6** show the same potency. For example, it is known that for CC-1065 and some related compounds both natural (+) and unnatural (−) enantiomers are active.[22]

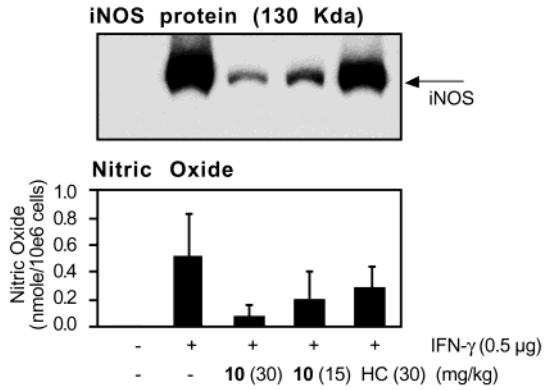


Figure 1. TBE **10** (30 and 15 mg/kg) and hydrocortisone (HC) (30 mg/kg) suppress both NO production and iNOS protein synthesis in vivo. Female CD-1 mice were injected ip with thioglycollate 3 days before IFN-γ stimulation. On day 3, mice (6 per group) were injected ip with IFN-γ (0.5 μg/mouse). TBE **10** and hydrocortisone were gavaged once, 1 h before IFN-γ injection. Nitric oxide was measured by the Griess reaction. Cell lysates were obtained for Western blot analysis of iNOS protein.

The inhibitory activity of **10** on NO production induced by IFN-γ in mouse macrophages was not blocked by the glucocorticoid antagonist mifepristone (RU486)[23] (at 1 μM), which reverses the action of hydrocortisone. This strongly implies that the actions of TBEs on the iNOS system are not mediated by their interaction with the glucocorticoid receptor.

In a preliminary in vivo study using mouse peritoneal inflammation induced by thioglycollate and IFN-γ, **10** is orally active at 30 and 15 mg/kg. Toxicity was not observed at either dosage. The potency at 15 mg/kg is still much better than hydrocortisone (30 mg/kg), which is clinically used as an orally active antiinflammatory drug (see Figure 1).

In conclusion, these biological results suggest that TBEs may be a new class of potential drug candidates for inflammatory diseases. Further syntheses and biological evaluation (in vitro and in vivo) of new TBEs, including water-soluble derivatives, are in progress.

Acknowledgment. We thank Dr. Carl Nathan for expert advice on the preparation of macrophages and the nitric oxide assay. We also thank Dr. Steven Mullen (University of Illinois) for the mass spectra. This investigation was supported by funds from NIH Grant 1 R01-CA78814, the Norris Cotton Cancer Center, and the National Foundation for Cancer Research. M.B.S. is Oscar M. Cohn Professor. F.G.F., Jr. is an Oscar M. Cohn Scholar.

Supporting Information Available: Spectral data and elemental analyses of new compounds and experimental details of biological evaluation, including data with RU486. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Honda, T.; Rounds, B. V.; Gribble, G. W.; Suh, N.; Wang, Y.; Sporn, M. B. Design and synthesis of 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid, a novel and highly active inhibitor of nitric oxide production in mouse macrophages. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2711−2714. (b) Honda, T.; Gribble, G. W.; Suh, N.; Finlay, H. J.; Rounds, B. V.; Bore, L.; Favaloro, F. G., Jr.; Wang, Y.; Sporn, M. B. Novel synthetic oleanane and ursane triterpenoids with various enone functionalities in ring A as inhibitors of nitric oxide production in mouse macrophages. *J. Med. Chem.* **2000**, *43*, 1866−1877. (c) Honda, T.; Rounds, B. V.; Bore, L.; Finlay, H. J.; Favaloro, F. G., Jr.; Suh, N.; Wang, Y.; Sporn, M. B.; Gribble, G. W. Synthetic oleanane and ursane triterpenoids with modified rings A and C: A series of highly active inhibitors of nitric oxide production in mouse macrophages. *J. Med. Chem.* **2000**, *43*, 4233−4246.

(2) (a) Ding, A.; Nathan, C. F.; Graycar, J.; Derynck, R.; Stuehr, D. J.; Srimal, S. Macrophage deactivating factor and transforming growth factors-β1, -β2, and -β3 inhibit induction of macrophage nitrogen oxide synthesis by IFN-γ. *J. Immunol.* **1990**, *145*, 940−944. (b) Bogdan, C.; Paik, J.; Vodovotz, Y.; Nathan, C. Contrasting mechanisms for suppression of macrophage cytokine release by transforming growth factor-β and interleukin-10. *J. Biol. Chem.* **1992**, *267*, 23301−23308.

(3) Suh, N.; Wang, Y.; Honda, T.; Gribble, G. W.; Dmitrovsky, E.; Hickey, W. F.; Maue, R. A.; Place, A. E.; Porter, D. M.; Spinella, M. J.; Williams, C. R.; Wu, G.; Dannenberg, A. J.; Flanders, K. C.; Letterio, J. J.; Mangelsdorf, D. J.; Nathan, C. F.; Nguyen, L.; Porter, W. W.; Ren, R. F.; Roberts, A. B.; Roche, N. S.; Subbaramaiah, K.; Sporn, M. B. A novel synthetic oleanane triterpenoid, 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid, with potent differentiating, anti-proliferative, and anti-inflammatory activity. *Cancer Res.* **1999**, *59*, 336−341.

(4) Ito, Y.; Pandey, P.; Place, A.; Sporn, M. B.; Gribble, G. W.; Honda, T.; Kharbanda, S.; Kufe, D. The novel triterpenoid 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid induces apoptosis of human myeloid leukemia cells by a caspase-8-dependent mechanism. *Cell Growth Differ.* **2000**, *11*, 261−267.

(5) Adipogenic differentiation of mouse 3T3-L1 fibroblasts is due to CDDO being a ligand for the peroxisome proliferator-activated receptor γ: Wang, Y.; Porter, W. W.; Suh, N.; Honda, T.; Gribble, G. W.; Leesnitzer, L. A.; Plunket, K. D.; Mangelsdorf, D. J.; Blanchard, S. G.; Willson, T. M.; Sporn, M. B. A synthetic triterpenoid, 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO), is a ligand for the peroxisome proliferator-activated receptor γ. *Mol. Endocrinol.* **2000**, *14*, 1550−1556.

(6) Suh, N.; Wang, Y.; Williams, C.; Risingsong, R.; Honda, T.; Rounds, B. V.; Bore, L.; Gribble, G. W.; Sporn, M. B.CDDO, a novel synthetic oleanane triterpenoid, suppresses nitric oxide production and synthesis of inducible nitric oxide synthase (iNOS) in female CD-1 mice. *Proceedings of the 91st Annual Meeting of the American Association for Cancer Research*, San Francisco, CA, April 1−5, 2000; American Association of Cancer Research: Philadelphia, PA; p 663.

(7) Honda, T.; Honda, Y.; Favaloro, F. G., Jr.; Gribble, G. W.; Suh, N.; Place, A. E.; Rendi, M. H.; Sporn, M. B. A novel dicyanotriterpenoid, 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-onitrile, active at picomolar concentrations for inhibition of nitric oxide production. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1027−1030.

(8) Throughout this article, for the ease of comparison, the same atom numbering as used with the oleanane skeleton is used for TBEs. Racemic TBEs and their intermediates are shown by the enantiomers with the same configuration as naturally occurring triterpenoids.

(9) Hirota, H.; Nakamura, T.; Tsuyuki, T.; Takahashi, T. Stereoselective total synthesis of (±)-labdane-8α,15-diol and (±)-eperuane-8β,15-diol. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 4023−4028.

(10) Clinton, R. O.; Manson, A. J.; Stonner, F. W.; Neumann, H. C.; Christiansen, R. G.; Clarke, R. L.; Ackerman, J. H.; Page, D. F.; Dean, J. W.; Dickinson W. B.; Carabateas, C. Steroidal[3,2-*c*]-pyrazoles. II. Androstanes, 19-norandrostanes and their unsaturated analogs. *J. Am. Chem. Soc.* **1961**, *83*, 1478−1491.

(11) Johnson, W. S.; Shelberg, W. E. A plan for distinguishing between some five- and six-membered ring ketones. *J. Am. Chem. Soc.* **1945**, *67*, 1745−1754.

(12) Muzart J. Synthesis of unsaturated carbonyl compounds via a chromium-mediated allylic oxidation by 70% tert-butylhydroperoxide. *Tetrahedron Lett.* **1987**, *28*, 4665−4668.

(13) Development of TBEs **4** and **5** was excluded because they are expected to be unstable because of their β-hydroxyketone structures.

(14) Hagiwara, H.; Uda, H. Optically pure (4a*S*)-(+)- or (4a*R*)-(−)-1,4a-dimethyl-4,4a,7,8-tetrahydronaphthalene-2,5(3*H*,6*H*)-dione and its use in the synthesis of an inhibitor of steroid biosynthesis. *J. Org. Chem.* **1988**, *53*, 2308−2311.

(15) Dale, J. A.; Dull, D. L.; Mosher, H. S. α-Methoxy-α-trifluoromethylphenylacetic acid, a versatile reagent for the determination of enantiomeric composition of alcohols and amines. *J. Org. Chem.* **1969**, *34*, 2543−2549.

(16) Srikrishna, A.; Jagadeeswar Reddy, T. Enantiospecific synthesis of (+)-(1*S*,2*R*,6*S*)-1,2-dimethylbicyclo[4,3,0]nonan-8-one and (−)-7-epibakkenolide-A. *Tetrahedron* **1998**, *54*, 11517−11524.

(17) Kahne, D.; Collum, D. B. Kinetic cyanations of ketone enolates. *Tetrahedron Lett.* **1981**, *22*, 5011−5014.

(18) Finkbeiner, H. L.; Stiles, M. Chelation as a driving force in organic reactions. IV. Synthesis of α-nitro acids by control of the carboxylation−decarboxylation equilibrium. *J. Am. Chem. Soc.* **1963**, *85*, 616−622.
(19) Hirota, H.; Yokoyama, A.; Miyaji, K.; Nakamura, T.; Igarashi, M.; Takahashi, T. Total synthesis of (±)-amarolide, a quassinoid bitter principle. *J. Org. Chem.* **1991**, *56*, 1119−1127.
(20) Smith, H. A.; Huff, B. J. L.; Powers, W. J.; Caine, D. The reduction−methylation of cyclohexanone derivatives. *J. Org. Chem.* **1967**, *32*, 2851−2856.
(21) Heathcock, C. H.; Mahaim, C.; Schlecht, M. F.; Utawanit, T. A synthetic approach to the quassinoids. *J. Org. Chem.* **1984**, *49*, 3264−3274.
(22) Smith, J. A.; Bifulco, G.; Case, D. A.; Boger, D. L.; Gomez-Paloma, L.; Chazin, W. J. The structural basis for in situ activation of DNA alkylation by duocarmysin SA. *J. Mol. Biol.* **2000**, *300*, 1195−1204.
(23) Gagne, D.; Pons, M.; Philibert, D. RU38486: a potent antiglucocorticoid in vitro and in vivo. *J. Steroid Biochem.* **1985**, *23*, 247−251.

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