Novel Tricyclic Compounds Having Acetylene Groups at C-8a and Cyano Enones in Rings A and C: Highly Potent Anti-inflammatory and Cytoprotective Agents

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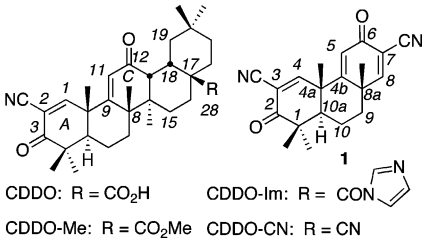
Abstract: Novel C-8a functionalized tricyclic compounds having cyano enones in rings A and C have been synthesized and biologically evaluated. Among them, compounds with acetylene groups at C-8a show extremely high potency in in vitro and in vivo bioassays for anti-inflammatory and cytoprotective activities. Both in vitro and in vivo potencies are markedly higher than those of 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO), which is being evaluated as an anticancer drug in phase I clinical trials.

We have been engaged in the improvement of anti-inflammatory and antiproliferative activity of oleanolic acid, a naturally occurring triterpenoid. This led to the discovery of CDDO[a] and related compounds (e.g., CDDO-Me, CDDO-Im, CDDO-CN, and others).[1−5] CDDO and its methyl ester (CDDO-Me) are



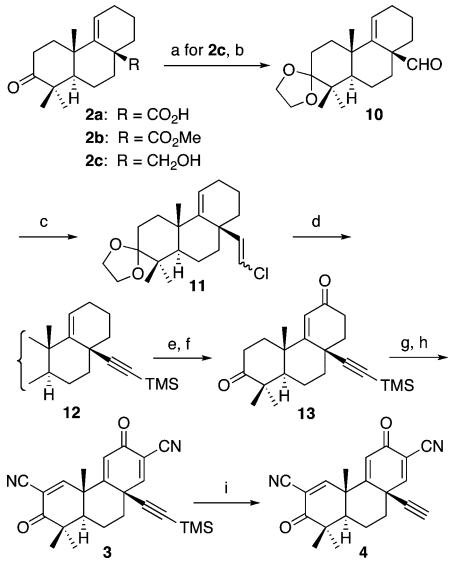
presently being evaluated in phase I clinical trials for the treatment of leukemia and solid tumors.

During the course of these investigations, we have found that three-ringed compounds with enone functionalities in rings A and C similar to those of CDDO are also a novel class of potent anti-inflammatory, cytoprotective, growth suppressive, and proapoptotic compounds. Particularly, **1** is nearly equivalent in potency to CDDO for inhibition of nitric oxide (NO) production in primary mouse macrophages stimulated with interferon-γ and is orally active in a preliminary in vivo inflammation model.[6,7] These results encouraged us to synthesize additional new analogues. Thus, we initially designed C-8a functionalized analogues because insertion of functionalities at this position would improve the potency and pharmacokinetics because of the favorable balance between hydrophilicity and hydrophobicity. Also, insertion of polar and hydrophilic functionalities (e.g., carboxyl group, amine group, etc.) would give water-soluble compounds.

We have synthesized various C-8a functionalized analogues using new tricycles **2a**−**c** (Scheme 1) as starting materials, whose efficient synthesis we have previously established for our projected synthesis,[8] and evaluated their potency for inhibition of NO production in RAW 264.7 cells stimulated with interferon-γ (RAW cell assay). These analogues include typical electron-withdrawing, electron-releasing, hydrophilic, hydrophobic, and bulky groups.[9] We have found that **3**−**9** are more potent than **1** and CDDO in the RAW cell assay (Table 1). Particularly, **3**−**5** and **7** with acetylene groups are the most potent. Their potency is higher than that of **1** and CDDO and equal to that of CDDO-Im and CDDO-CN, which have the highest potency among semisynthetic triterpenoids previously evaluated in the same assay.[3] In addition, **4** is orally and highly active for induction of the anti-inflammatory and cytoprotective enzyme heme oxygenase-1 (HO-1)[10] in the liver and stomach (Figures 1 and 2). The in vivo potency is also much higher than that of **1** and CDDO.

In this communication, we report our initial results on the synthesis and biological potency of some new C-8a functionalized tricyclic compounds.

Scheme 1. Synthesis of Tricyclic **3** and **4** in Racemic Form[a]



[a] Reagents and yields: (a) EG, PPTS, PhH, 100%; (b) DMSO, $(COCl)_2$, Et_3N, 100%; (c) $Ph_3PCH_2Cl_2$, *n*-BuLi, THF, HMPA, 80%; (d) MeLi, THF; TMSCl, 93%; (e) aqueous HCl, MeOH, 97%; (f) CrO_3, *t*-BuOOH, CH_2Cl_2, 65%; (g) *p*-TsCN, LDA, THF; (h) DDQ, PhH; 61% (i) TBAF, THF, 71%.

Compounds **3** and **4** in racemic form were synthesized by the sequence shown in Scheme 1. Compound **10** was obtained in quantitative yield by ketalization of **2c** with ethylene glycol (EG) in the presence of pyridinium *p*-toluenesulfonate (PPTS) in benzene, followed by Swern oxidation.[11] Compound **11** was prepared in 80% yield as a mixture of *E*/*Z* chlorovinyl isomers (*E*/*Z* = 4:1) by a Wittig reaction of **10** with (chloromethyl)-triphenylphosphonium chloride.[12] Dehydrochlorination of **11** with MeLi, followed by trapping the acetylide with chlorotrimethylsilane (TMSCl), gave **12** in 93% yield.[13] Deketalization

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[a] Abbreviations: CDDO, 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid; CDDO-Me, methyl 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oate; CDDO-Im, 1-[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole; CDDO-CN, 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-onitrile; DXM, dexamethasone.

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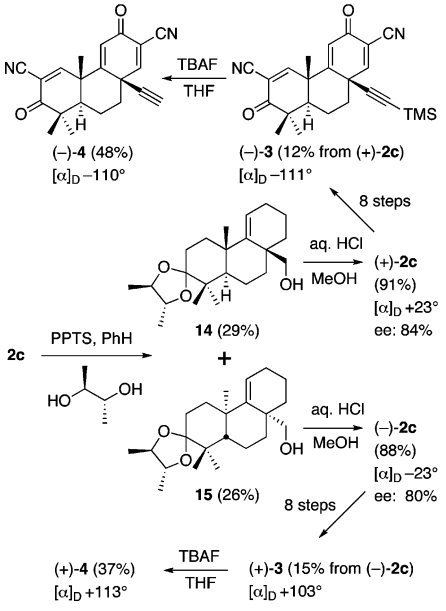
of **12** under acidic conditions, followed by a chromium-mediated allylic oxidation,[14] afforded **13** in 63% yield. Compound **3** was synthesized in 61% yield by double cyanation of **13** with LDA and *p*-toluenesulfonyl cyanide (*p*-TsCN),[15] followed by DDQ oxidation (29% overall yield from **2c**). The TMS group was removed by tetra-*n*-butylammonium fluoride (TBAF)[16] to give **4** in 71% yield (20% overall yield from **2c**).

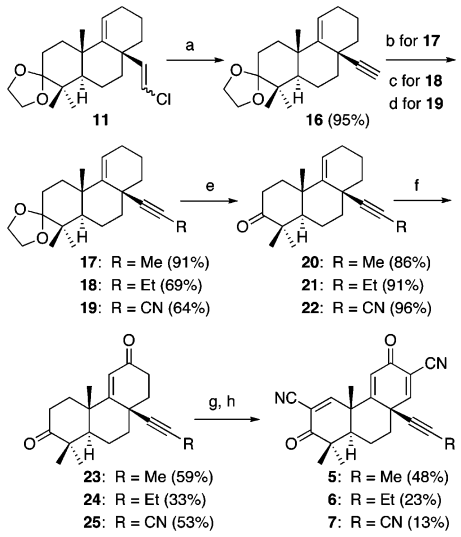
Scheme 2. Synthesis of Optically Active (−)-**3**, (+)-**3**, (−)-**4**, and (+)-**4**



Optically active (−)-**3**, (+)-**3**, (−)-**4**, and (+)-**4** were synthesized by the sequence shown in Scheme 2. The resolution of **2c** in racemic form was carried out in the manner described by Grieco.[17] Treatment of **2c** with (−)-(*R*,*R*)-2,3-butanediol afforded the pair of diastereomers **14** and **15**. Separation of the two diastereomers was achieved by iterative flash column chromatography to give **14** (including 8% of **15**) and **15** (including 10% of **14**) in 29% and 26% yields, respectively. The diastereomeric purity was determined by ^{1}H NMR (300 MHz, $CDCl_3$) using the integration values of the methyl signals (δ 0.92 and 0.88 of **14**; δ 0.96 and 0.86 of **15**) for the two diastereomers. Diastereomer **14** was then treated with acidic methanol to give (+)-**2c** in 91% yield. The deketalization conditions required much longer time (45 min) than deketalization of **12** (5 min) because of the bulky ketal of **14**. Similarly, **15** afforded (−)-**2c** in 88% yield under the same conditions. On the basis of the diastereomeric purity, we concluded that (+)-**2c** includes 8% of (−)-**2c** [enantiomeric excess (ee), 84%] and (−)-**2c** includes 10% of (+)-**2c** (ee, 80%). The circular dichroism (CD) values for the two enantiomers (+)-**2c** and (−)-**2c** are $\Delta\epsilon_{288} = +0.22$ (*c* 0.0025, EtOH) and $\Delta\epsilon_{288} = -0.22$ (*c* 0.0025, EtOH), respectively. On the basis of these CD values and application of the octant rule,[18] we have determined that (+)-**2c** has the same configuration as that of CDDO and that (−)-**2c** has the opposite configuration.

We did not synthesize optically active **3** and **4** directly from **14** (or **15**) because the TMS group might be cleaved under the conditions for removal of the bulky ketal. Thus, we synthesized optically active **3** and **4** from (+)-**2c** (or (−)-**2c**) by the same sequence as for **3** and **4** in racemic form. Optically active (−)-**3** was obtained in 12% yield from (+)-**2c**. Removal of the TMS group was achieved by treatment of (−)-**3** with TBAF to give (−)-**4** in 48% yield. Similarly, (+)-**3** was synthesized in 15% yield from (−)-**2c**, and then removal of the TMS group of (+)-**3** afforded (+)-**4** in 37% yield.

Scheme 3. Synthesis of Tricyclic **5**−**7** in Racemic Form[a]



[a] Reagents: (a) MeLi, THF, aqueous NH_4Cl; (b) MeLi, THF, MeI; (c) MeLi, THF, EtI; (d) *n*-BuLi, THF, PhOCN; (e) aqueous HCl, MeOH; (f) CrO_3, *t*-BuOOH, CH_2Cl_2; (g) *p*-TsCN, LDA, THF; (h) DDQ, PhH.

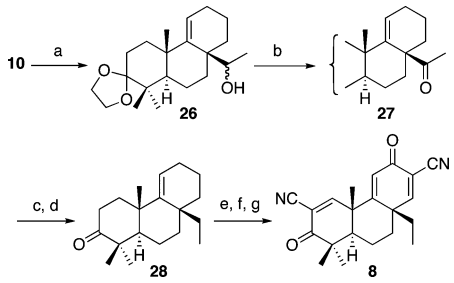
Compounds **5**−**7** in racemic form were synthesized by the sequence shown in Scheme 3. Dehydrochlorination of **11** with MeLi followed by quenching of the acetylide with aqueous NH_4Cl solution afforded **16** in 95% yield.[12] Insertion of the methyl group into the acetylene moiety was achieved by treating **16** with MeLi and trapping the resulting anion with methyl iodide to give **17** in 91% yield. The ketal **17** was subjected to acidic conditions to give **20** in 86% yield. Allylic oxidation of **20** afforded **23** in 59% yield. Double cyanation of **23**, followed by DDQ oxidation, gave **5** in 48% yield (21% overall yield from **11**). Ethylacetylene **18** was obtained in 69% yield by quenching lithium acetylide of **16** with iodoethane. Compound **6** was synthesized in 7% yield from **18** by the same methods as for **5**. Treatment of the acetylide of **16** with phenyl cyanate (PhOCN) afforded **19** in 64% yield.[19] Compound **7** was obtained in 7% yield from **19** by the same procedures as for **5**.

Compound **8** in racemic form was synthesized by the sequence shown in Scheme 4. The aldehyde **10** was treated with MeMgBr in THF to give a diastereomeric mixture (2:1) of alcohol **26** in 87% yield. Ratcliffe oxidation[20] of **26** gave **27** in 73% yield. A forced Wolff−Kishner reduction of **27** with anhydrous hydrazine and KOH in diethylene glycol (at 165 °C for 2 h and then at 217 °C for 24 h),[21] followed by removal of the ketal, produced **28** in 51% yield. Compound **8** was synthesized in 48% yield from **28** by the same sequence as for **5** (16% overall yield from **10**).

Compound **9** in racemic form was synthesized by the sequence shown in Scheme 5. A Wittig reaction on **10** with methyltriphenylphosphonium iodide in the presence of potassium *tert*-butoxide in THF gave **29** in 74% yield. Removal of the ketal of **29** afforded **30** in quantitative yield. Compound **9** was obtained in 48% yield via **31** from **30** by the same methods as for **5** (36% overall yield from **10**).

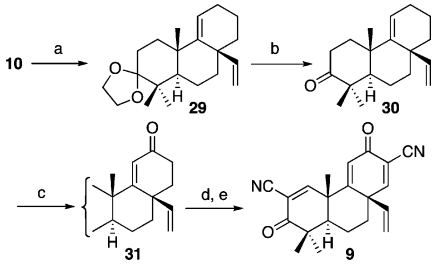
We have evaluated the inhibitory activity of new C-8a functionalized tricyclic compounds **3**−**9** including optically active analogues in the RAW cell assay. The IC_{50} values are shown in Table 1. All new compounds **3**−**9** are more potent than the lead **1**, CDDO, and DXM. Noteworthy is that **3**−**5** and **7** having acetylene groups are significantly more potent

Scheme 4. Synthesis of Tricyclic **8** in Racemic Form[a]



[a] Reagents and yields: (a) MeMgBr, THF, 87%; (b) CrO_3, pyr, CH_2Cl_2, 73%; (c) NH_2NH_2, KOH, diethylene glycol; (d) aqueous HCl, MeOH, 51% from **27**; (e) CrO_3, *t*-BuOOH, CH_2Cl_2; (f) LDA, *p*-TsCN, THF; (g) DDQ, PhH, 48% from **28**.

Scheme 5. Synthesis of Tricyclic **9** in Racemic Form[a]



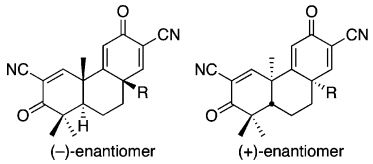
[a] Reagents and yields: (a) Ph_3PMeI, *t*-BuOK, THF, 74%; (b) aqueous HCl, MeOH, 100%; (c) CrO_3, *t*-BuOOH, CH_2Cl_2, 83%; (d) LDA, *p*-TsCN, THF; (e) DDQ, PhH, 58%.

than **1** and CDDO. The potency is equal to that of CDDO-CN and CDDO-Im, which have the highest potency among semisynthetic triterpenoids previously evaluated in the same assay.[3]

It is often the case that one enantiomer of a drug has greater potency and/or less toxicity than its antipode. Indeed, we have found that (−)- and (+)-**1** have different biological properties.[7] In this assay, we have not found a significant difference between (−)- and (+)-enantiomers of **3** and **4**.

Presently, we are engaged in synthesizing various new tricyclic compounds having acetylene groups in order to develop clear structure−activity relationships for these compounds.

Table 1. Inhibition of NO Production in RAW 264.7 Cells Stimulated with Interferon-γ by New Tricyclic **3**−**9**[a]



compd	R	IC_{50} (nM)	compd	R	IC_{50} (nM)
3[b]	−C≡C−TMS	3	**7**[b]	−C≡C−CN	4
(+)-**3**	−C≡C−TMS	2	**8**[b]	−Et	10
(−)-**3**	−C≡C−TMS	3	**9**[b]	−CH=CH_2	10
4[b]	−C≡CH	1	**1**[b]	−Me	30
(+)-**4**	−C≡CH	1	CDDO		20
(−)-**4**	−C≡CH	3	CDDO-Im		1
5[b]	−C≡C−Me	5	CDDO-CN		1
6[b]	−C≡C−Et	20	DXM		20

[a] RAW 264.7 cells were treated with various concentrations of compounds and interferon-γ (10 ng/mL) for 24 h. Supernatants were analyzed for NO by the Griess reaction. IC_{50} values are an average of two separate experiments. [b] A 1:1 mixture of (−)- and (+)-enantiomers (racemic form).

Subsequent to the in vitro assay, we have evaluated the potency of **4**, which is one of the most potent compounds in the RAW cell assay, for induction of the anti-inflammatory and cytoprotective enzyme heme oxygenase-1 in the liver and stomach (in vivo, oral administration). There is major interest in stimulating HO-1 as a protective enzyme in many chronic disease conditions in which inflammation and oxidative stress play an important role.[10] As shown in Figures 1 and 2, oral dosing of 1 μmol of **4** causes significant induction of HO-1 in the liver and stomach while **1** and CDDO are markedly less potent at this low dose. Compound **4** is as potent as CDDO-Im in the liver but is clearly more potent than CDDO-Im in the stomach, which is the most potent semisynthetic triterpenoid we have developed for induction of HO-1.[22]

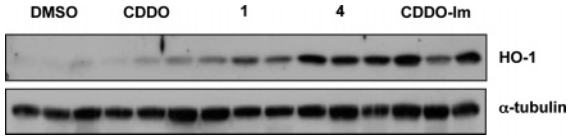


Figure 1. Tricyclic **4** is a potent inducer of HO-1 in liver when given by gavage. Male CD-1 mice (three mice per group) were separately gavaged with 1 μmol of the following: tricyclic compounds **1** and **4**, CDDO, and CDDO-Im. After 6 h, livers were collected and analyzed by Western blot for HO-1.

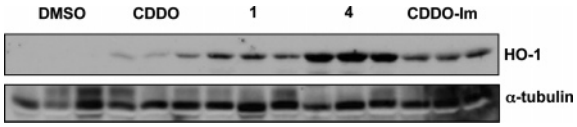


Figure 2. Tricyclic **4** is a potent inducer of HO-1 in stomach when given by gavage. Male CD-1 mice (three mice per group) were separately gavaged with 1 μmol of the following: tricyclic compounds **1** and **4**, CDDO, and CDDO-Im. After 6 h, stomachs were collected and analyzed by Western blot for HO-1.

In conclusion, although a series of tricyclic compounds was designed on the basis of CDDO, the structure of **4** is clearly different from that of CDDO because **4** has an acetylene group at C-8a. Tricyclic compounds, diterpenoids, and triterpenoids with an acetylene group at C-8a (C-8 in terpenoid nomenclature) have not been reported prior to our synthesis of **4**. Currently, **4** is one of the most bioactive compounds in in vitro and in vivo bioassays in our pool of drug candidates including semisynthetic triterpenoids and tricyclic compounds. Therefore, **4** may represent a new class of potential drug candidates having an entirely new structure for inflammatory diseases and cancer chemoprevention. Further syntheses and biological evaluation of new tricyclic compounds having acetylene groups at C-8a, including water-soluble analogues, are in progress.

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Supporting Information Available: Synthetic procedures and characterization data for new compounds **3**−**9** and ^{1}H NMR spectra for (−)-**3**, (+)-**3**, **4**, (−)-**4**, (+)-**4**, **5**, and **7**−**9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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