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An Efficient Synthesis of Methyl 2-Cyano-3,12-dioxoursol-1,9-dien-28-oate (CDDU-Methyl Ester); Analogues, Biological Activities, and Comparison with Oleanolic Acid Derivatives

Liangfeng Fu, Qi-Xian Lin, Karen T. Liby, Michael B. Sporn, and Gordon W. Gribble

An efficient synthesis of methyl 2-cyano-3,12-dioxoursol-1,9-dien-28-oate (CDDU-methyl ester) from commercially available ursolic acid, which features an oxidative ozonolysis-mediated C-ring enone formation, and provides the first access to ursolic acid-derived cyano enone analogues with C-ring activation. These new ursolic acid analogues show potent biological activities, with potency of approximately five-fold less than the corresponding oleanolic acid derivatives.

Instruction

The biological importance of naturally occurring pentacyclic triterpenoids has long been recognized. The annual review of triterpenoids in *Natural Product Reports* is ample testimony to the ubiquitous worldwide distribution of these compounds, which include squalenes, lanostanes, fusetanes, dammanes, euphances, and others. Within each class is a stunning array of structural diversity and a range of biological activity.\(^1\) For example, many oleanane and ursane triterpenoids, which are derived biosynthetically by cyclization of squalene,\(^2\) have interesting biological, pharmacological, and medicinal activities not unlike those attributed to retinoids and steroids. These properties include anti-inflammatory, suppression of tumor promotion, suppression of immunoglobulin synthesis, protection of the liver against toxic injury, induction of collagen synthesis, and induction of differentiation in leukemia and teratocarcinoma cells.\(^3\)

Oleanolic acid (1), ursolic acid (2), and betulinic acid (3) (Figure 1), probably the most commonly studied triterpenoids, exhibit modest biological activity, although 2 has been marketed in China as an oral drug for the treatment of liver disorders in humans.\(^4\)

![Figure 1](image1.png)

**Figure 1** Oleanolic acid, ursolic acid, and betulinic acid

Extensive studies report the use of oleanolic acid as a skeleton motif. Indeed, the highly potent oleanolic acid derivative CDDO-methyl ester (4) (bardoxolone methyl) (Figure 2), which was developed in our laboratory years ago,\(^5\) has successfully completed a Phase I clinical trial for the treatment of cancer\(^6\) and a Phase II clinical trial for the treatment of chronic kidney disease in type 2 diabetes patients.\(^7\)

During this research we synthesized a series of CDDO-methyl ester analogues (Figure 2), such as CDDO-ethyl amide (5), CDDO-trifluoroethyl amide (6), and CDDO-imidazolide (7).\(^8\) Details of their biological properties are described in recent review articles.\(^9\)-\(^11\)

![Figure 2](image2.png)

**Figure 2** CDDO methyl ester and analogues

Due to the limited structure–activity studies involving ursolic acid, we have undertaken and now describe an efficient synthesis of the ursolic acid analogue of CDDO-methyl ester, CDDU-methyl ester (methyl 2-cyano-3,12-dioxoursol-1,9-dien-28-oate), along with related derivatives, and describe their biological activities.
To investigate the anti-inflammatory and cancer chemopreventive properties of these derivatives, we have adopted a preliminary screening assay system that measures inhibition of nitric oxide (NO) production as induced by interferon-γ (IFN-γ) in mouse macrophages. Thus, CDDO (8) is 200,000–400,000 times more active than oleanolic acid in this inducible nitric oxide synthase (iNOS) assay. Also, CDDO at nanomolar concentrations suppresses the de novo synthesis of the inflammatory enzymes iNOS and cyclooxygenase-2 (COX-2) in activated macrophages.12

Results and Discussion

Chemical Synthesis. Our rationale for this project is that because ursoic acid is often more potent than oleanolic acid,13 we deemed it important to synthesize and study the ursoic acid derivatives CDDU-methyl ester (9), CDDU-ethyl amide (10), CDDU-trifluoroethyl amide (11), and CDDU-imidazolide (12) (Figure 3).

![CDDU-Methyl Ester (9) and CDDU-Imidazolide (12)](image)

Our first goal was to prepare the C-ring enone of ursoic acid. A number of reagents and reaction conditions are known to oxidize the C11-C12 alkene of oleanolic acid, including m-CPBA,14 MMPP,15 ozone-mediated16 hydroxy lactonization, DMDO-mediated chlorolactonization,17 bismuth (III) triflate-mediated18 lactonization, and bromolactonization.19 Oxidation conditions, such as H_{2}O_{2}/AcOH20 or m-CPBA-mediated epoxidation and isomerization,21 ozonolysis22 have also been developed for oleanolic acid esters (Scheme 1).

Scheme 1. Reported C11-C12 oxidation of oleanolic acid.

![Schema 1](image)

Reagents: (a) m-CPBA, CH_{2}Cl_{2}; (b) MMPP, acetone; (c) O_{2}, CH_{2}Cl_{2}; (d) DMDO, CHCl_{3}; (e) Br(OtBu), CHCl_{3}; (f) Br_{2}, AcOH; (g) H_{2}O_{2}, AcOH.

However, the corresponding oxidations of ursoic acid are either rare or unknown. For example, Bag and coworkers reported a selective Br_{2}/AcOH-induced bromolactonization of oleanolic acid in the presence of ursoic acid.19 Recently, Csuk and Siewert reported a m-CPBA-mediated oxidative separation of ursoic acid from oleanolic acid.23 Under these conditions, oleanolic acid was completely converted to the oxidation product, whereas ursoic acid was recovered nearly quantitatively. The different C-ring reactivity between oleanolic acid and ursoic acid is presumed to be due to the steric hindrance imparted by the C19 methyl group in ursoic acid. However, Salvador, Jing and co-workers describe the introduction of a C-12 fluorine in an ursoic acid derivative23 (Scheme 2).

Scheme 2. Reported fluorolactonization on ursoic acid.

![Scheme 2](image)

Reagent: (a) Selectfluor, dioxane, nitromethane, 80 °C.

Not surprisingly, we encountered difficulty in the oxidation of the ursoic acid C-ring. Thus, an initial reaction of ursonic ester 21 with m-CPBA as the oxidant resulted in almost quantitative recovery of 2124 (Scheme 3), and classic conditions employing hydrogen peroxide in acetic acid gave complex mixtures. Moreover, neither tert-butyl hydroperoxide nor MMPP provided satisfactory results. Reductive conditions on 21 using excess borane also failed, yielding partial reduction of both the C-3 acetate and C-17 methyl ester, but leaving the C11-C12 alkene intact.

To our delight, treatment of 21 with ozone at −40 °C afforded a mixture of 22 and 23 (4:1) (Table 1, entry 3) as an inseparable mixture, which was evidenced by our proton and carbon NMR spectra. A temperature study revealed that −78 °C is optimal (Table 1, entry 4), and these conditions provided 22 and 23 in a ratio of 8:1. Without further purification, direct treatment with pyridinium perbromide in acetonitrile afforded ring-C enones 24 and 23 in 81% and 10% yield, respectively (Scheme 3).

Mild base-catalyzed hydrolysis of 24 afforded an alcohol 25 (not shown), which was oxidized to the corresponding ketone 26 with refluxing iodoxybenzoic acid. A subsequent two-step selenation/oxidation/elimination protocol gave bis-enone 27 in good yield. Regioselective iodination using iodine in carbon tetrachloride and pyridine in the presence of catalytic amount of dimethylaminopyridine gave iodoenone 28. Final cyanation using copper(I) cyanide in dimethyl formamide unexpectedly provided a mixture of the desired cyanoenone 9 and bis-cyanoenone 29, the latter of which was not observed in our recently developed CDDO-Me synthesis.25 The formation of bis-cyanoenone 29 may indicate that CDDU-methyl ester (9) is a more reactive Michael acceptor and will prove more potent than CDDO-methyl ester (4).

In similar fashion, enone 23 was converted in five steps to cyanoenone 33 and bis-cyanoenone 34 (Scheme 5).
Scheme 3. Ozonolysis-mediated C-ring enone formation.

Scheme 4. Synthesis of CDDU-methyl ester (9).

Scheme 5. Synthesis of cyano enones 33 and 34.

**Table 1.** Optimization for ozonolysis-mediated C-ring enone formation of 22 plus 23.

<table>
<thead>
<tr>
<th>Entry</th>
<th>T (°C)</th>
<th>Ratio (22:23)</th>
<th>Yield (%)</th>
<th>Yield of 22 (%)</th>
<th>Yield of 23 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>2:1</td>
<td>84</td>
<td>56</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>2:1</td>
<td>84</td>
<td>56</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>-40</td>
<td>4:1</td>
<td>87</td>
<td>70</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>-78</td>
<td>8:1</td>
<td>91</td>
<td>81</td>
<td>10</td>
</tr>
</tbody>
</table>

**Biological Evaluation.** The inflammatory activity of our ursolic acid cyanoenones as measured by the inhibition of nitric oxide (NO) production in RAW 264.7 macrophages is summarized in Table 1. The orientation of the ring-C enone is important, as the activity significantly decreases when this moiety is moved from the 9(11)-en-12-one position in CDDU-methyl ester (9) to the 12(13)-en-11-one position in 33. Similarly, 29 is somewhat more potent than 34. Notably, the addition of a cyano group at C1 (29 and 34 compared to 9 and 33) significantly decreases biological activity, presumably because the C1-cyano group retards Michael addition with a reactive cysteine or other nucleophiles on a target protein.

**Scheme 6.** Synthesis of CDDU-methyl ester analogues.

Reagent: (a) LiI, pyridine, reflux; (b) (COCl)₂, DMF, CH₂Cl₂; EtNH₂-HCl, Et₃N, CH₂Cl₂; (c) (COCl)₂, DMF, CH₂Cl₂; CF₃CH₂NH₂-HCl, Et₃N, CH₂Cl₂; (d) (COCl)₂, DMF, CH₂Cl₂; imidazole, PhH; (e) (COCl)₂, DMF, CH₂Cl₂; NH₃, MeOH; (f) TFAA, Et₃N, CH₂Cl₂; (g) DDQ, PhH, reflux.
Compounds 9, 10, 11, 12, 35, 36, 37, and 38 are all active at nanomolar concentrations in this assay (Table 2). Imidazolide 12, nitrile 37, and lactone 38 are the most potent. CDDU-Me (9) is slightly less active than the former three compounds, but more active than CDDU (35) itself.

As shown in Table 1, the oleanolic acid analogues are uniformly more potent than the corresponding ursolic acid derivatives, but with a similar trend. Thus, imidazolides (12 and CDDO-Im), nitriles (37 and CDDO-CN), and a lactone (38) are the most potent. As the only difference between the two scaffolds is the C19 axial methyl substituent for CDDU-methyl ester (9) versus C20 equatorial methyl for CDDO-methyl ester (Scheme 7), this simple transposition affects the biological activity by 5–10 fold. This result may be useful for future studies of structure–activity relationships related to the methyl substituents on oleanolic acid and ursolic acid. Especially important would seem to be the modification of the gem dimethyl group at C4, since the origin of the biological activity is primarily due to the reversible Michael addition mechanism at C-1 on ring-A.

Table 2. Biological potency of triterpenoids with a comparison between ursolic and oleanolic analogues.

<table>
<thead>
<tr>
<th>UA cmpds</th>
<th>IC50 (nM)</th>
<th>OA cmpds</th>
<th>IC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>17 ± 1</td>
<td>CDDO-Me</td>
<td>4 ± 1.6</td>
</tr>
<tr>
<td>29</td>
<td>380 ± 38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>61 ± 7</td>
<td>CDDO-EA</td>
<td>5 ± 3</td>
</tr>
<tr>
<td>34</td>
<td>304 ± 38</td>
<td>CDDO-TFEA</td>
<td>8 ± 4</td>
</tr>
<tr>
<td>10</td>
<td>54 ± 2</td>
<td>CDDO-Im</td>
<td>1 ± 0.4</td>
</tr>
<tr>
<td>11</td>
<td>26 ± 4</td>
<td>CDDO-CN</td>
<td>1 ± 0.05</td>
</tr>
<tr>
<td>12</td>
<td>5 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>251 ± 41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>48 ± 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>7 ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>8 ± 2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IC50 values were determined by serial dilutions of the compounds (range 1 nM–1 µM). All experiments were repeated at least 3 times.

Scheme 7. 3D models for CDDU-methyl ester and CDDO-methyl ester.

Conclusions

An efficient synthesis of methyl 2-cyano-3,12-dioxursol-1,9-dien-28-oate (CDDU-methyl ester) from commercially available ursolic acid is disclosed, which provides access to ursolic acid-derived cyanoenone analogues with C-ring enone activation. The conversion of the C ring C11-C12 alkene to a 9(11)-en-12-one features an ozonolysis-induced oxidation followed by enone formation mediated by pyridinium tribromide. Biological studies of these ursolic acid analogues display a similar structure–activity relationship to the corresponding oleanolic acid analogues, but are less potent by approximately half a log. Interestingly, introduction of a cyano substituent at C1 greatly decreases the biological activity, probably due to steric blocking of C1 to a Michael addition.

Experimental Section

Chemistry. All reactions were performed in a single-neck, round-bottomed flask fitted with rubber septa under a positive pressure of nitrogen, unless otherwise noted. Organic solutions were concentrated by rotary evaporation below 30 °C. Flash-column chromatography was performed using silica gel (0.04–0.063 mm, 230–400 mesh ASTM) purchased from DAWN RUSSUP Macherey-Nagel Inc. (Bethlehem, PA). Analytical
thin-layer chromatography (TLC) was performed using glass backed TLC extra hard layer pre-coated with silica gel (0.25 mm, 60Å pore size) impregnated with a fluorcent indicator. TLC plates were visualized by exposure to ultraviolet light (UV) or/and submersion in PAA (p-anisaldehyde) or CAM (ceric ammonium molybdate) stains followed by brief heating on a hot plate (120 °C, 10-15 s). Commercial solvents and reagents were used as received. Proton nuclear magnetic spectra (1H NMR) were recorded at 500 MHz at 24 °C, unless otherwise noted. Chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to residual protium in the NMR solvent (CDCl3, δ 7.26). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances, br = broad, app = apparent), integration, coupling constant in Hertz, and assignment. Proton-decoupled carbon nuclear magnetic resonance spectra (13C NMR) were recorded at 500 MHz at 24 °C unless otherwise noted. Chemical shifts are expressed in parts per million (ppm, δ 77.0). IR spectra were recorded on a Jasco FT-IR 4100 Series spectrophotometer, tmax (cm−1) are partially reported. High resolution mass spectra were acquired from the Mass Spectrometry Laboratory of the University of Illinois (Urbana-Champaign, IL).

Biological Evaluation. NO Assay. RAW 264.7 cells (5 x 10^5 cells per well) were plated in 96-well plates. The next day, cells were incubated with synthetic triterpenoids and 10 ng/mL IFNγ (R & D systems) for 24 h. NO was measured as nitrite by the Griess reaction.

Methyl 3-Acetoxy-12-oxoursoyl-9(11)-en-28-oate (24). To a stirred solution of ester 21 (500 mg, 0.98 mmol, 1.0 equiv) in methylene chloride (10 mL) was subjected to ozonolysis at -78 °C. Upon completion of the reaction, it was allowed to slowly warm to room temperature and kept at room temperature for 3 h. The solvent was then removed to give crude inseparable reaction mixtures with the desired ketone 22 and 23 (~81%). The crude mixture was dissolved in acetonitrile (10 mL) and pyridinium perbromide (416 mg, 1.30 mmol, 1.3 equiv) was added. The resulting mixture was then heated to 50 °C for 18 h. After the completion of the reaction, it was allowed to cool to room temperature and quenched with 20% aqueous sodium thiosulfate (20 mL). It was then extracted with methylene chloride (20 mL, 1.0 M solution, 20.0 mmol, 1.0 equiv) in one portion. The resulting suspension was cooled in ice bath and was then filtered through Celite. The resulting filtrate was concentrated and flash column chromatography over silica gel using hexanes:EtOAc (2:1) gave keto 26 (4.8 g, 99%) as a yellowish solid. 1H NMR (500 MHz, CDCl3) δ 5.87 (s, 1H), 4.62 (dd, 1H, J = 11.2 Hz, J = 2.7 Hz), 2.57 (m, 1H), 2.45 (m, 2H), 2.18 (m, 1H), 1.35-1.95 (m, 14H), 1.00-1.30 (m, 2H), 1.24 (s, 3H), 1.12 (s, 3H), 1.08 (s, 3H), 1.07 (s, 3H), 1.03 (s, 3H), 0.83 (d, 3H, J = 6.1 Hz), 0.67 (d, 3H, J = 6.3 Hz); 13C NMR (500 MHz, CDCl3) δ215.4, 199.9, 177.9, 177.6, 124.2, 51.8, 50.9, 50.5, 49.9, 47.3, 45.3, 42.2, 40.7, 39.4, 39.3, 38.7, 36.8, 36.7, 36.7, 34.1, 32.0, 31.1, 28.3, 26.5, 24.3, 24.2, 23.8, 21.4, 20.8, 19.7, 19.5, 19.0; IR (solution, CHCl3, cm−1): 3019, 2430, 2340, 1698, 1684, 1652, 1585, 1540, 1507, 1217, 772, 669, 624, 432.

Methyl 3-Hydroxy-12-oxoursoyl-9(11)-en-28-oate (25). To a stirred solution of enone 24 (7.0 g, 13.3 mmol, 1.0 equiv) in methanol (100 mL) was added potassium carbonate (7.0 g, 50.0 mmol, 3.0 equiv). The reaction mixture was allowed stir at room temperature for 24 h. After completion of the reaction, it was quenched with water (200 mL) and extracted with methylene chloride (4 x 100 mL). The combined organic extracts were washed with brine (80 mL) and dried over Na2SO4. Removal of solvent and flash column chromatography over silica gel using hexanes:EtOAc (2:1) gave alcohol 25 (5.6 g, 87%) as a white solid. 1H NMR (500 MHz, CDCl3) δ 5.80 (s, 1H), 3.56 (s, 3H), 3.13 (dd, 1H, J = 11.6 Hz, J = 4.5 Hz), 2.83 (dd, 1H, J = 11.4 Hz, J = 3.0 Hz), 2.32 (d, 1H, J = 3.8 Hz), 2.25 (brs, 1H), 1.78-1.91 (m, 2H), 1.37-1.72 (m, 12H), 1.24-1.35 (m, 2H), 1.03-1.20 (m, 2H), 1.09 (s, 3H), 1.04 (s, 3H), 1.01 (s, 3H), 0.95 (s, 3H), 0.79 (d, 3H, J = 6.1 Hz), 0.73 (s, 3H), 0.65 (d, 3H, J = 6.6 Hz); 13C NMR (500 MHz, CDCl3) δ 201.0, 179.9, 178.4, 123.0, 77.9, 52.0, 51.1, 50.1, 45.4, 42.3, 40.8, 40.2, 39.5, 39.4, 38.8, 36.9, 36.4, 33.0, 31.3, 28.4, 27.6, 24.5, 24.4, 23.9, 20.9, 19.9, 19.7, 18.0, 15.9; IR (solution, CHCl3, cm−1): 3019, 2410, 2360, 2340, 1716, 1652, 1558, 1539, 1520, 1507, 1217, 772, 669, 624, 432.

Methyl 3,12-Dioxy-12-oxoursoyl-9(11)-en-28-oate (26). To a stirred solution of alcohol 25 (4.9 g, 10.1 mmol, 1.0 equiv) in ethyl acetate (80 mL) was added iodoxybenzoic acid (3.7 mg, 13.1 mmol, 1.3 equiv) in one portion. The resulting suspension was heated to reflux for 24 h. After completion of the reaction, it was cooled in ice bath and was then filtered through Celite. The resulting filtrate was concentrated and flash column chromatography over silica gel using hexanes:EtOAc (2:1) gave keto 26 (4.8 g, 99%) as a yellowish solid. 1H NMR (500 MHz, CDCl3) δ 5.87 (s, 1H), 3.60 (s, 3H), 2.86 (dd, 1H, J = 11.2 Hz, J = 2.7 Hz), 2.57 (m, 1H), 2.45 (m, 2H), 2.18 (m, 1H), 1.35-1.95 (m, 14H), 1.00-1.30 (m, 2H), 1.24 (s, 3H), 1.12 (s, 3H), 1.08 (s, 3H), 1.07 (s, 3H), 1.03 (s, 3H), 0.83 (d, 3H, J = 6.1 Hz), 0.67 (d, 3H, J = 6.3 Hz); 13C NMR (500 MHz, CDCl3) δ215.4, 199.9, 177.9, 177.6, 124.2, 51.8, 50.9, 50.5, 49.9, 47.3, 45.3, 42.2, 40.7, 39.4, 39.3, 38.7, 36.8, 36.7, 36.7, 34.1, 32.0, 31.1, 28.3, 26.5, 24.3, 24.2, 23.8, 21.4, 20.8, 19.7, 19.5, 19.0; IR (solution, CHCl3, cm−1): 3019, 2430, 2340, 1698, 1684, 1652, 1585, 1540, 1507, 1217, 772, 668; HRMS-ESI (calcd for C17H14O3 [M+H]+) 483.3474, found 483.3471.
Methyl 2-iodo-3,12-dioxoursol-1(2),9(11)-dien-28-oate (28). To a stirred solution of bisoneone (1.07 g, 2.3 mmol, 1.0 equiv) in a 1:1 mixture of pyridine and carbon tetrachloride (10 mL) was added dimethylaminopyridine (56 mg, 0.46 mmol, 0.2 equiv) and iodine (1.75 g, 6.9 mmol, 3.0 equiv), and the resulting mixture was heated to 90 °C for 24 h without light. After the completion of the reaction, the solution was removed under vacuum, and the residue was diluted with ethyl acetate (100 mL). The resulting solution was successively washed with 20% aqueous sodium thiosulfate (3 x 10 mL), 1 N aqueous HCl (4 equiv) in a 1:1 mixture of pyridine and carbon tetrachloride (10 mL), 20% aqueous sodium thiosulfate (3 x 10 mL), saturated NaHCO₃ (10 mL), brine (10 mL), and dried over Na₂SO₄. Removal of solvent and flash column chromatography over silica gel using hexanes:EtOAc (4:1 & 2:1) gave iodo-enone 28 (1.18 g, 87%) as a yellowish solid. ¹H NMR (500 MHz, CDCl₃) δ 8.11 (s, 1H), 6.11 (s, 1H), 3.66 (s, 3H), 2.95 (dd, 1H, J₁ = 11.3 Hz, J₂ = 3.0 Hz), 2.48 (d, 1H, J = 3.7 Hz), 1.90-1.99, (m, 1H), 1.70-1.87 (m, 7H), 1.59-1.65 (m, 1H), 1.44-1.57 (m, 3H), 1.41 (s, 3H), 1.15-1.26 (m, 3H), 1.18 (s, 3H), 1.22 (s, 3H), 1.17 (s, 3H), 0.89 (d, 3H, J = 6.1 Hz), 0.75 (d, 3H, J = 6.6 Hz); ¹³C NMR (500 MHz, CDCl₃) δ 199.8, 197.0, 178.3, 171.5, 163.8, 154.2, 124.6, 122.0, 112.1, 51.2, 51.0, 45.7, 45.4, 45.2, 42.1, 39.6, 38.9, 36.9, 31.9, 31.8, 28.6, 28.5, 20.0, 24.0, 22.4, 20.9, 20.0, 19.7, 18.7; IR (solution, CHCl₃ cm⁻¹): 3019, 2360, 2340, 1717, 1652, 1540, 1520, 1507, 1217, 770, 669; HRMS-E SI (calcd for C₃₁H₄₅O₃Na [M+Na]⁺ 667.3057), found 667.3050.

Methyl 2-Cyano-3,12-dioxoursol-1(2),9(11)-dien-28-oate (9) and Methyl 1,2-Dicyano-3,12-dioxoursol-1(2),9(11)-dien-28-oate (29). To a stirred solution of iodoenone 28 (322 mg, 0.5 mmol, 1.0 equiv) in anhydrous dimethylformamide (5 mL) was added copper (I) cyanide (54 mg, 0.66 mmol, 1.2 equiv), and the resulting mixture was allowed to heat to 120 °C for 12 h. After the completion of the reaction, it was cooled to room temperature, diluted with ethyl acetate (100 mL). The resulting solution was washed with water (3 x 10 mL), brine (3 x 10 mL), and dried over Na₂SO₄. Removal of solvent and flash column chromatography over silica gel using hexanes:EtOAc (4:1 & 2:1) gave cyanoneone 9 (142 mg, 53%) and bicycnoenone 29 (76 mg, 27%) as yellowish solids, respectively. For 9: ¹H NMR (500 MHz, CDCl₃) δ 8.04 (s, 1H), 6.11 (s, 1H), 3.67 (s, 3H), 2.96 (dd, 1H, J₁ = 11.5 Hz, J₂ = 2.7 Hz), 2.51 (d, 1H, J = 3.7 Hz), 1.91-1.99 (m, 1H), 1.72-1.85 (m, 7H), 1.60-1.68 (m, 1H), 1.47-1.58 (m, 3H), 1H, 1.16-1.30 (m, 2H), 1.26 (s, 3H), 1.21 (s, 3H), 1.18 (s, 3H), 1.12 (s, 3H), 1.09 (d, 3H, J = 6.1 Hz), 0.83-0.90 (m, 1H), 0.74 (d, 3H, J = 6.6 Hz); ¹³C NMR (500 MHz, CDCl₃) δ 199.3, 196.9, 178.3, 169.8, 166.4, 124.9, 115.1, 114.7, 52.2, 51.4, 50.2, 47.9, 46.0, 45.3, 42.9, 42.6, 41.2, 39.6, 38.9, 36.9, 31.9, 31.3, 28.5, 27.6, 25.7, 25.4, 23.9, 21.8, 20.9, 20.0, 19.6, 18.4; IR (solution, CHCl₃ cm⁻¹): 3019, 2360, 2340, 1716, 1652, 1558, 1540, 1520, 1507, 1217, 772, 669, 464; HRMS-E SI (calcd for C₃₃H₄₇NO₄ [M+H]⁺ 656.3270), found 656.3267. For 29: ¹H NMR (500 MHz, CDCl₃) δ 6.22 (s, 1H), 3.68 (s, 3H), 3.02 (dd, 1H, J₁ = 11.6 Hz, J₂ = 2.0 Hz), 2.51 (d, 1H, J = 3.4 Hz), 1.74-2.02 (m, 7H), 1.40-1.67 (m, 4H), 1.62 (s, 3H), 1.18-1.34 (m, 2H), 1.26 (s, 3H), 1.22 (s, 3H), 1.21 (s, 3H), 1.17 (s, 3H), 1H, 0.83-0.97 (m, 2H), 0.74 (d, 3H, J = 5.9 Hz), 0.74 (d, 3H, J = 6.6 Hz); ¹³C NMR (500 MHz, CDCl₃) δ 198.0, 195.2, 178.3, 166.3, 146.8, 129.6, 124.9, 113.9, 112.1, 53.2, 51.6, 50.3, 47.3, 46.4, 46.2, 46.1, 42.7, 42.1, 39.8, 38.8, 36.8, 31.4, 29.1, 28.8, 26.6, 26.2, 26.1, 24.1, 21.0, 20.9, 20.0, 19.3, 19.2; IR (solution, CHCl₃ cm⁻¹): 3019, 2929, 2360, 2340, 1732, 1716, 1683, 1668, 1558, 1540, 1520, 1507, 1472, 1456, 1217, 772, 669, 473; HRMS-E SI (calcd for C₃₃H₄₇NO₄ [M+H]⁺ 351.3474), found 351.3468.

2-Cyano-3,12-dioxoursol-1(2),9(11)-dien-28-carboxylic acid ethyl amide (10). To a stirred solution of acid 35 (400 mg, 0.81 mmol, 1.0 equiv) in methylene chloride (15 mL) was added oxalyl chloride (0.35 mL, 4.05 mmol, 5.0 equiv) and anhydrous dimethylformamide (0.11 µL, 2.0 µmol, catalytic) slowly at 0 °C, and it was allowed to warm to room temperature for 2 h. The solvent was removed, and toluene (10 mL) was added and removed by vacuum, which was repeated for three times to provide the corresponding acyl chloride. To a stirred solution of the resulting acyl chloride obtained above in methylene chloride (10 mL) was added triethylamine (0.58 mL, 4.05 mmol, 5.0 equiv) followed by slow addition of ethylamine hydrochloride (340 mg, 4.05 mmol, 5.0 equiv) at 0 °C. After the addition, the resulting mixture was allowed to warm to room temperature and kept stirring until the disappearance of acyl chloride. After the completion of the reaction, the solvent was removed and it was diluted with methylene chloride (80 mL),
washed with 1 N HCl (10 mL), saturated aqueous NaHCO₃ (10 mL), brine (10 mL), and dried over Na₂SO₄. Removal of solvent and flash column chromatography over silica gel using hexanes:EtOAc (2:1 & 1:1) gave ethyl amide 10 (418 mg, 99%) as a yellowish solid. ¹H NMR (500 MHz, CDCl₃) δ 8.05 (1H, 2.56 (d, 1H, J = 11.7 Hz), 2.12 (m, 1H), 1.71-1.92 (m, 4H), 1.60-1.70 (m, 3H), 1.57 (3H, J, 1.50 (s, 3H), 1.39-1.50 (m, 2H), 1.22-1.44 (m, 1H), 1.28 (s, 3H), 1.21 (s, 3H), 1.18 (s, 3H), 1.00-1.10 (m, 1H), 0.95 (d, 3H, J, = 6.3 Hz), 0.82-0.90 (m, 1H), 0.82 (d, 3H, J, = 6.4 Hz); ¹³C NMR (500 MHz, CDCl₃) δ 196.6, 192.2, 178.3, 174.3, 165.7, 123.4, 115.4, 114.5, 87.8, 55.1, 48.1, 46.4, 45.3, 45.1, 43.2, 42.9, 39.8, 37.5, 32.8, 31.3, 31.2, 30.8, 28.0, 27.6, 26.3, 21.9, 21.8, 20.3, 19.5, 18.6, 17.8; IR (solution, CHCl₃, cm⁻¹): 3019, 2969, 2936, 2360, 2340, 1772, 1715, 1679, 1675, 1558, 1540, 1520, 1507, 1456, 1215, 909, 749, 669; HRMS-ESI (calcld. for C₁₃H₁₂NO₄ [M+H]⁺) 490.2957, found 490.2961.

2-Cyano-3,12-dioxoaryl(1,2),9(11)-dien-28-carboxylic acid trifluorethyl amide (36). To a stirred solution of acid 35 (860 mg, 1.75 mmol, 1.0 equiv) in methylene chloride (15 mL) was added oxalyl chloride (0.50 mL, 5.72 mmol, 3.3 equiv) and anhydrous dimethylformamide (0.15 µL, 2.7 µmol, catalytic) slowly at 0 °C, and it was allowed to warm to room temperature for 2 h. The solvent was removed, and toluene (10 mL) was added and removed by vacuum, which was repeated for three times to provide the corresponding acyl chloride. To a stirred solution of the resulting acyl chloride obtained above in methylene chloride (20 mL) was added ammonia (8.75 mL, 1.0 M in MeOH, 8.75 mmol, 5.0 equiv), and the resulting mixture was stirred at room temperature until the disappearance of acyl chloride. After the completion of the reaction, the solvent was removed and flash column chromatography over silica gel using hexanes:EtOAc (1:1 & 1:2) gave ethyl amide 36 (791 mg, 92%) as a yellowish solid. ¹H NMR (500 MHz, CDCl₃) δ 8.06 (1H, 6.28 (s, 1H), 2.51 (d, 1H, J = 11.7 Hz), 2.10 (m, 1H), 1.69-1.90 (m, 6H), 1.51-1.67 (m, 4H), 1.54 (3H, 1.34-1.50 (m, 2H), 1.46 (s, 3H), 1.10-1.30 (m, 2H), 1.23 (s, 3H), 1.17 (s, 3H), 1.15 (s, 3H), 0.82-0.92 (m, 1H), 0.91 (d, 3H, J, = 6.3 Hz), 0.78 (d, 3H, J, = 6.1 Hz); ¹³C NMR (500 MHz, CDCl₃) δ 200.3, 197.0, 180.4, 171.1, 167.7, 129.4, 115.0, 114.9, 51.2, 49.8, 47.8, 46.2, 45.3, 43.9, 43.1, 42.1, 39.2, 39.0, 37.9, 31.9, 31.4, 31.9, 31.4, 28.2, 27.5, 25.9, 23.7, 21.8, 20.9, 20.1, 19.5, 18.4; IR (solution, CHCl₃, cm⁻¹): 3019, 2969, 2936, 2360, 2340, 1772, 1715, 1679, 1675, 1558, 1540, 1520, 1507, 1456, 1215, 909, 749, 669; HRMS-ESI (calcld. for C₁₃H₁₂NO₄ [M+H]⁺) 491.3114, found 491.3105.

2-Cyano-3,12-dioxoaryl(1,2),9(11)-dien-28-nitrile (37). To a stirred solution of amide 36 (600 mg, 1.22 mmol, 1.0 equiv) in methylene chloride (40 mL) at 0 °C was added triethylamine (0.44 mL, 3.13 mmol, 2.5 equiv) and trifluoroacetic anhydride (0.26 mL), and the resulting mixture was stirred at 0 °C until the disappearance of starting material. After the completion of the reaction, it was quenched with saturated aqueous NaHCO₃ (20 mL), and extracted with methylene chloride (3 x 20 mL). The combined organic extracts were washed with brine (10 mL) and dried over Na₂SO₄. Removal of solvent and flash column chromatography over silica gel using hexanes:EtOAc (4:1 & 2:1) gave the desired product 37 (407 mg, 89%) as a white solid. ¹H NMR (500 MHz, CDCl₃) δ 8.06 (s, 1H), 6.16 (s, 1H), 5.08 (s, 1H), J = 3.7 Hz), 2.72 (dd, 1H, J = 11.2 Hz, J = 3.2 Hz), 1.95-2.18 (m, 4H), 1.74-1.86 (m, 3H), 1.55-1.66 (m, 3H), 1.53 (3H, J, 1.34-1.50 (m, 2H), 1.45 (s, 3H), 1.27 (s, 3H), 1.16-1.26 (m, 2H), 1.20 (s, 3H), 1.12 (s, 3H), 0.92 (d, 3H, J, = 5.6 Hz), 0.82-0.92 (m, 1H), 0.76 (d, 3H, J, = 6.6 Hz); ¹³C NMR (500 MHz, CDCl₃) δ 198.1, 196.8, 170.4, 166.1, 125.0, 124.8, 115.2, 114.6, 51.5, 48.0, 46.1, 45.3, 44.3, 43.0, 41.5, 41.2, 39.0, 38.7, 36.7, 32.0, 30.6, 28.8, 27.6, 25.7, 25.8, 25.6, 21.8, 20.6,
2-Cyano-3,12-dioxosulphol-1(2),9(11)-di-en-28-carboxylic acid imidazolide (12). To a stirred solution of acid 35 (460 mg, 1.0 mmol, 1.0 equiv) in methylene chloride (20 mL) was added oxalyl chloride (0.50 mL, 5.58 mmol, 5.8 equiv) and anhydrous dimethylformamide (0.15 mL, 2.0 μmol, catalytic) slowly at 0 °C, and it was allowed to warm to room temperature for 2 h. The solvent was removed, and toluene (10 mL) was added and removed by vacuum, which was repeated for three times to provide the corresponding acyl chloride. To a stirred solution of the resulting acyl chloride obtained above in benzene (10 mL) was added amidazole (350 mg, 5.0 mmol, 5.0 equiv) and the resulting mixture was stirred at room temperature until the disappearance of acyl chloride. After the completion of the reaction, removal of solvent and flash column chromatography over silica gel using hexanes:EtOAc (4:1 & 2:1) gave ethyl amide 12 (458 mg, 90%) as a yellowish solid. 1H NMR (500 MHz, CDCl3) δ 8.34 (s, 1H), 8.05 (s, 1H), 7.66 (s, 1H), 7.06 (s, 1H), 6.10 (s, 1H), 3.27 (d, 1H, J = 9.8 Hz), 2.43 (s, 3H), 0.98-2.06 (m, 11H), 1.44 (s, 3H), 1.23 (s, 3H), 1.16 (s, 9H), 0.96 (d, 3H, J = 5.1 Hz), 0.78 (d, 3H, J = 5.9 Hz); 13C NMR (500 MHz, CDCl3) δ 197.9, 196.8, 175.5, 170.2, 166.3, 137.8, 130.6, 124.6, 117.9, 115.1, 114.6, 52.6, 51.1, 47.9, 46.0, 45.2, 43.1, 42.9, 41.3, 39.9, 38.8, 35.8, 31.7, 31.0, 28.1, 27.5, 25.7, 25.4, 21.8, 20.8, 20.0, 19.7, 18.3, IR (solution, CHCl3, cm−1): 3019, 2930, 2870, 2640, 1716, 1698, 1520, 1214, 909, 773, 669, 450; HRMS-ESI (calcd for C34H24N2O3 [M+H]+) 473.3168, found 473.3165.

Acknowledgements
We acknowledge support from Reata Pharmaceuticals.

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