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Chemical modifications of natural triterpenes—glycyrrhetinic and boswellic acids: evaluation of their biological activity

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ABSTRACT

Synthetic analogues of naturally occurring triterpenoids; glycyrrhetinic acid, arjunolic acid, and boswellic acids, by modification of A-ring with a cyano- and enone-functionality, have been reported. A novel method of synthesis of α-cyanoenones from isoxazoles is reported. Bioassays using primary mouse macrophages and tumor cell lines indicate potent anti-inflammatory and cytotoxic activities associated with cyano-enones of boswellic acid and glycyrrhetinic acid.

1. Introduction

Since the first report[1] of the synthesis of 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO) **1** and its methyl ester **2**, there have been a number of studies displaying the multifarious biological activity of various analogues of this compound **1**. Various derivatives of this compound possess significant biological activities. The CDDO-Im **3** triggers[2] apoptosis in multiple myelome (MM) cells. Compounds **1** and **3** are also highly active[3] in suppressing cellular proliferation of human leukemia and breast cancer cell lines. CDDO **1** and its methyl ester **2** exhibit[4–6] high inhibitory activity against production of nitric oxide induced by interferon-γ (IFN-γ) in mouse macrophages (IC_{50}=0.1 nM). CDDO **1** was also found[7,8] to induce monocytic differentiation of human myeloid leukemia cells and adipogenic differentiation of mouse 3T3-L1 fibroblasts. The compound CDDO **1** was synthesized from an abundantly occurring triterpene, oleanolic acid. SAR studies[6] conducted on CDDO have mainly addressed to the structural variations at C-2 of the A-ring, the functionality in C-ring and the ester moiety. We now report the synthesis of the compounds, **5** from glycyrrhetinic acid **8**, **6** from arjunolic acid **16**, and **7** from boswellic acids **26** (Scheme 1), which are readily available from Indian medicinal plants. These cyano-enones **5**, **6** and **7** have been screened for nitric oxide inhibitory and cytotoxic activities in in vitro bioassays.

2. Results and discussion

With a view to study the effect of changes in the position of the carboxylic moiety and increase in the polarity of the A-ring, three naturally abundant triterpenoids from Indian medicinal plants were chosen as substrates for the synthesis of the CDDO analogues. Since glycyrrhetinic acid **8**, isolated from liquorice root,[9] already has the α,β-unsaturated system in the C-ring, it was only necessary to introduce a cyano-enone functionality in the A-ring of the triterpenoid. The formal synthesis of the cyano-ketone was carried out using Johnson's[10] protocol via the isoxazole intermediate as depicted in Scheme 2. Glycyrrhetinic acid **8**, derived from the acidic hydrolysis of glycyrrhizin, was esterified using diazomethane in quantitative yields. The ester **9** thus obtained was oxidized at 0 °C using the Jones reagent to give the 3-keto derivative **11** in 84% yield. The compound was conversely prepared by reversing the oxidation and esterification procedures to initially give the keto acid **10** that was esterified to give the keto ester **11**. The formylation of the keto ester using ethyl formate in the presence of an alkoxide base gave 2-formyl-3-keto compound **12**, the precursor for the isoxazole in 80% yield. The isoxazole **13** was prepared[11] in an acetate buffer as reported by Doorenbos in 82% yield to give the single desired isomer. This was quantitatively isomerized under basic conditions to the cyano-ketone **14** that was dehydrogenated using DDQ in benzene to give the cyano-enone ester **5** in 65% yield. An alternative protocol for the synthesis of the cyano-enone **5** was attempted via the allylic bromination–dehydrobromination. The isoxazole **13** was brominated using NBS under radical conditions and the crude product **15** gave the cyano-enone **5** when treated with a base in comparable yields.

Spectroscopic data established the assigned structure, which was further supported by X-ray crystal structure analysis (Fig. 1). This also revealed that the stereochemistry[12] of the starting material **8** had been retained in the product without any epimerization of the CD rings taking place under the basic conditions of the reactions.

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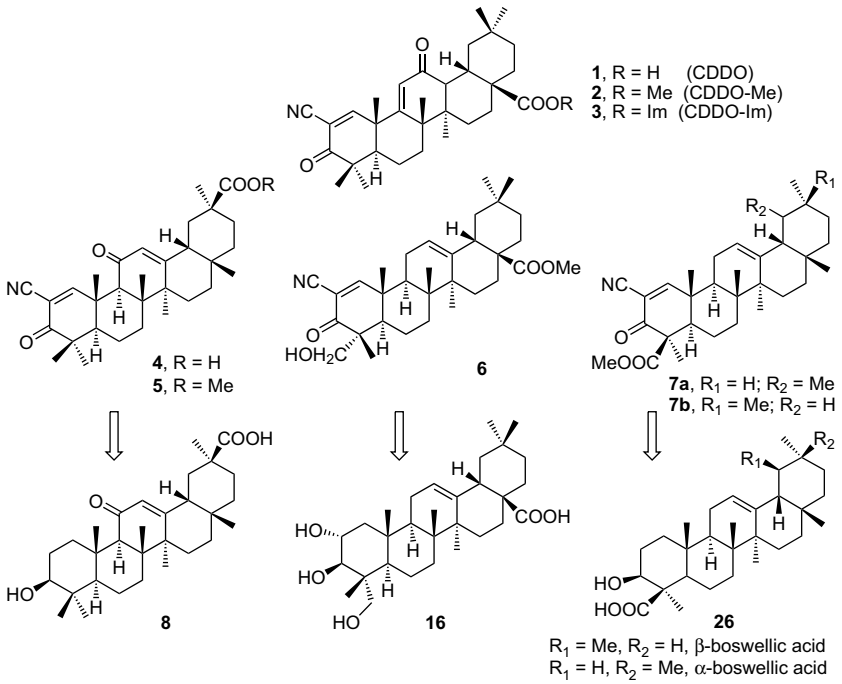
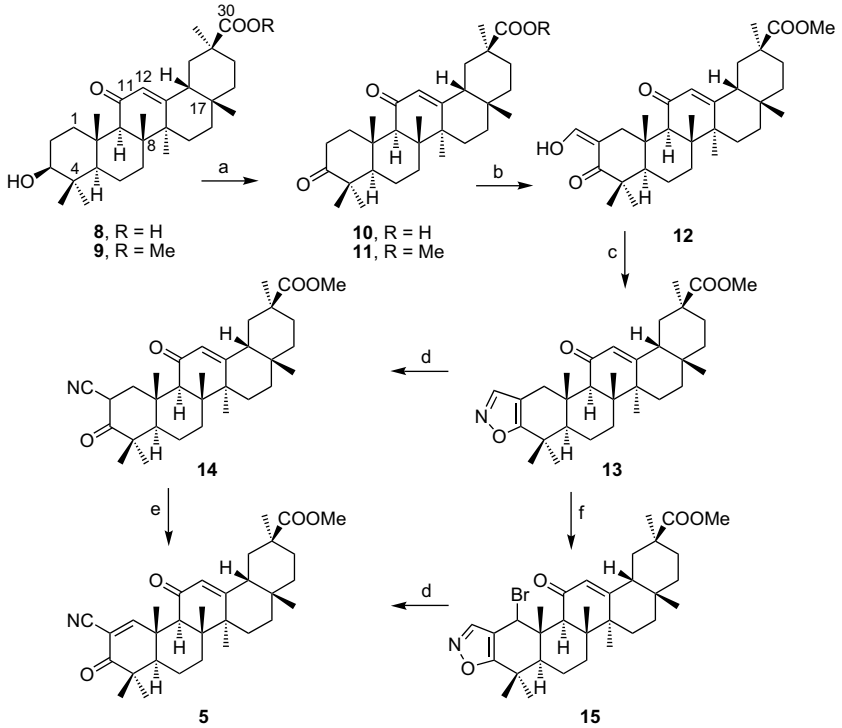


Scheme 1. Cyano-enone derivatives of triterpenes.

Arjunolic acid **16**, isolated[13] from *Terminalia arjuna*, while retaining an olean skeleton with a C-28 carboxylic acid has an additional hydroxyl group (C-23) in the A-ring providing additional polar functionality. It was initially attempted to selectively activate the C-2 hydroxy and displace with a nitrile, however various attempts gave intractable mixtures.

As an alternate protocol, the hydroxyl group at C-2 of methyl arjunolate **17**, was removed by following Barton's xanthate protocol. Initially, the two hydroxyl groups at C-3 and C-23 of **17** were protected as an acetonide **18**, which readily formed a xanthate ester **19** with carbon disulfide. Reduction of the xanthate ester **19** with tributyltin hydride, followed by hydrolysis afforded the diol **21** in



Scheme 2. Reagents: (a) (i) CH_2N_2/MeOH; (ii) Jones oxidation (b) NaOMe/HCOOEt; (c) NH_2OH/AcOH; AcONa/MeOH/C_6H_6; (d) NaOMe/MeOH/Et_2O; (e) DDQ/C_6H_6; (f) NBS/AIBN/$CHCl_3$.

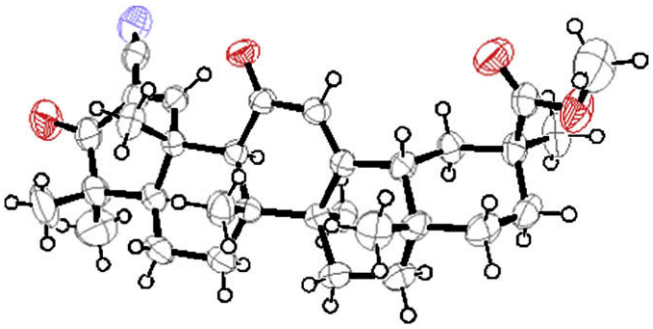


Figure 1. Crystal structure of methyl 2-cyano-3,11-dioxo-30-norolean-1,12-dien-30-oate **5**.

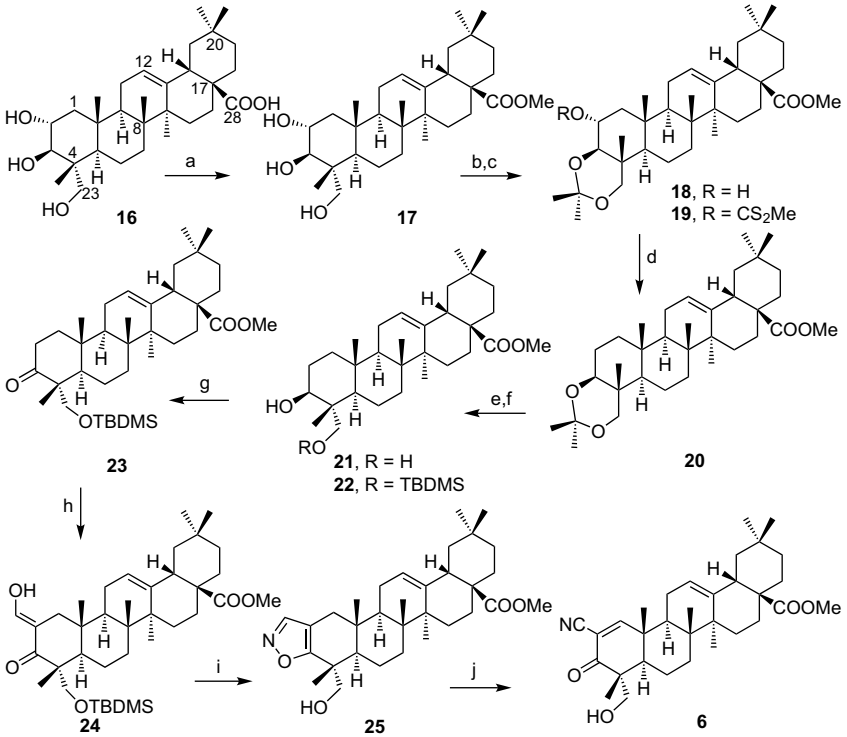
good yield. This compound was identical with methyl hederagenin, obtained by the esterification of hederagenin, a natural triterpene isolated[14] from *Sapindus emarginatus*. Selective protection of the primary alcohol as its TBDMS ether **22**, followed by the oxidation of the secondary alcohol at C-3 using PDC afforded the ketone **23**. Following Johnson's protocol,[10] the isoxazole **25** was prepared, which subsequently afforded the desired cyano-enone **6** in good yield as depicted in Scheme 3. The silyl protecting group was cleaved under the acidic conditions during the preparation of the isoxazole.

Boswellic acids, isolated[15] from the gum of *Boswellia serrata*, were obtained as a mixture of α and β isomers in the ratio of (9:1), belonging to the olean and ursane skeletons and differing only in the position of the methyl groups in the ring-E of the triterpene moiety. Both α and β isomers, however, retain a carboxylic moiety as a pendant at C-4 in the A-ring of the triterpenoid. The synthesis of the cyano-enone derivative of boswellic acid was depicted in Scheme 4. The free acids **26** were esterified to furnish **27** in order to ensure safe oxidation of the C-3 hydroxyl group to the keto group **28**. Formylation of **28** afforded compound **29**. Since this compound decomposed on standing, the crude compound was directly employed in the subsequent steps without purification. Thus, reaction of **29** with hydroxylamine hydrochloride in refluxing methanol gave the isoxazole **30**. Bromination–dehydrobromination protocol was employed to afford the desired mixture of cyano-enones **7** of boswellic acids in a (9:1) ratio as indicated from its NMR spectrum. Further work on the purification of **7** is in progress.

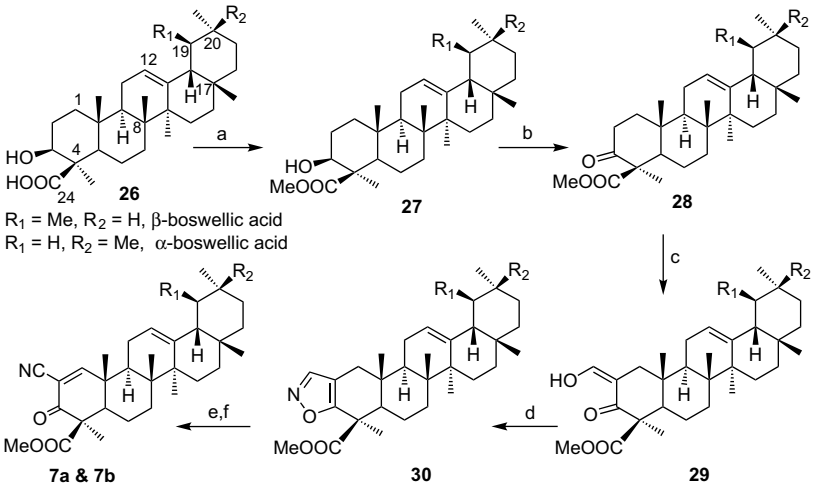
3. Bioassays

In a previous study Suh et al.[7] reported that 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO) shows potent anti-inflammatory, anti-proliferative, apoptotic, and differentiating activities. Subsequently its methyl and imidazolide analogues have been found to be more potent in these activities than CDDO.[3,8,16,17] Here we have screened **5**, **6**, and **7** for their ability to inhibit nitric oxide production (Table 1) induced by IFN-γ in primary mouse macrophages and for cytotoxicity using MTT assay (Table 2).

As shown in Table 1, **7** shows highest activity (EC_{50}=74.14 nM) for the inhibition of nitric oxide followed by **5** (EC_{50}=172. 8 nM). In contrast, **6** showed lower potency (EC_{50}=1.6 μM). All others (intermediates) showed activities at >5 μM. The ability of the modified triterpenes to inhibit growth has been demonstrated previously. Hence, we tested the growth inhibitory/cytotoxic activities of modified triterpenoids derived from boswellic acid (**7**), glycyrrhetinic acid (**5**), and arjunolic acid (**6**) using MTT assay. As shown in Table 2, compound **7** shows a potent activity with an EC_{50} of 0.108 μM on HL60 leukemia cells. The cytotoxic activity of **5** and **6** on HL60 was much less at EC_{50} of 0.925 μM and 0.548 μM, respectively. In other cell lines the EC_{50} ranged from 0.3 to 0.6 μM for **7**, 4.2 to 7.8 μM for **5**, and 1.7 to 8.0 μM for **6** (Table 2). These results suggest that **7** has a very potent anti-inflammatory and growth inhibitory activities among the three modified triterpenoids. Although, cyano-enone modification results in more potent activities of the



Scheme 3. Reagents: (a) CH_2N_2/Et_2O; (b) acetone, HCl; (c) CS_2/NaH, imidazole; (d) TBTH, AIBN, PhH; (e) Amberlite resin; (f) *t*-BDMSi-Cl, imidazole, CH_2Cl_2; (g) PCC/CH_2Cl_2; (h) HCOOEt, NaOtAm; (i) $NH_2OH{\cdot}HCl$/MeOH; (j) (i) NaOMe; (ii) DDQ/C_6H_6.



Scheme 4. Reagents: (a) CH_2N_2/MeOH; (b) PDC/CH_2Cl_2; (c) HCOOEt/NaOMe; (d) NH_2OH/HOAc; (e) NBS/AIBN/$CHCl_3$; (f) NaOMe/CH_3OH.

triterpenoids, not all the compounds are comparable. This is obvious when we compare CDDO[7] and **5**–**7** of this study. Recently, modified betulinic acid was reported[18] to have a very potent activity as compared to CDDO. Taken together, modified triterpenoids are a good source of lead molecules for drug development targeting inflammation and cancer.

4. Experimental

4.1. General methods

Melting points were recorded on a Buchi B-540 apparatus and are uncorrected. Infrared spectra were recorded on JASCO FTIR 410. Proton (^{1}H NMR) and carbon magnetic resonance (^{13}C NMR) spectra were generally recorded on a JEOL JNM-LA 300 spectrometer. Mass spectra measurements were carried out on a JEOL JMS DX 303 spectrometer. Elemental analyses were carried out on a Carlo Erba Element Analyzer 1106 at the department of organic chemistry, Indian Institute of Science. Analytical thin-layer chromatography (TLC) was performed on (10×5 cm) glass plates coated with Acme silica gel G or GF_{254} (containing 13% calcium sulfate as binder). Visualization of the spots on TLC plates was achieved either by exposure to iodine vapor or UV light or by spraying sulfuric acid and heating the plates at 120 °C. Column chromatography was performed by using Acme silica gel (100–200 mesh) or neutral alumina. All solvents were freshly distilled over CaH_2 or Na/benzophenone as appropriate. X-ray data were collected at 293 K on a SMART CCD–BRUKER diffractometer with graphite monochromated Mo Kα radiation (λ=0.7107 Å). Structure was solved by direct methods (SIR92). Refinement was by full-matrix least-squares procedures on F^2 using SHELXL-97. The non-hydrogen atoms were refined anisotropically whereas hydrogen atoms were refined isotropically.

Table 1
Inhibition of IFN-γ induced nitric oxide production by modified triterpenoids

Triterpenoids	EC_{50}
Boswellic acids (**26a** and **26b**)	No effect till 20 μM
Methyl 3-oxoboswellate (**28**)	No effect till 20 μM
Isoxazole from methyl boswellate (**30**)	No effect till 20 μM
Cyano-enone from methyl boswellate (**7**)	0.074 μM
Glycyrrhetinic acid (**8**)	>20 μM
Isoxazole from methyl glycyrrhetinate (**13**)	>20 μM
Cyano-ketone from methyl glycyrrhetinate (**14**)	14.5 μM
Cyano-enone from methyl glycyrrhetinate (**5**)	0.173 μM
Arjunolic acid (**16**)	>20 μM
Cyano-ketone of methyl arjunolate	7.5 μM
Isoxazole of methyl arjunolate (**25**)	8.7 μM
Cyano-enone from methyl arjunolate (**6**)	1.6 μM

4.1.1. *Glycyrrhetinic acid, (3β-hydroxy-11-oxo-30-norolean-12-en-30-oic acid)* ***8***[9]

Mp 295 °C; ν_{max} (neat)/cm^{-1} 3440, 2945, 1705, 1664, 1457, 1386, and 867; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.72 (1H, s), 3.21 (1H, dd, *J* 9.0 and 6.8 Hz), 2.80 (1H, d, *J* 13.5 Hz), 2.32 (1H, s), 2.21–0.66 (42H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 199.6, 181.0, 168.5, 128.4, 100.4, 85.7, 78.6, 61.7, 55.0, 48.0, 45.3, 43.6, 43.0, 40.9, 39.0, 37.6, 37.0, 32.7, 31.8, 30.8, 28.5, 28.3, 28.0, 27.3, 26.5, 23.4, 18.6, 17.4, 16.2, and 15.5; *m*/*z* (DI) 470 (M^+), 303, 262, 175, and 135 (100).

4.1.2. *3,11-Dioxo-30-norolean-12-en-30-oic acid* ***10***

Jones reagent (prepared from 18.24 g of CrO_3) was added drop wise to a mechanically stirred suspension of glycyrrhetinic acid **7** (9.21 g, 19.6 mmol) in acetone (400 mL) at 0 °C over a period of 30 min till the brown color persisted. The mixture was stirred for further 30 min. Propan-2-ol (5 mL) was added, reaction mixture filtered, and the residue was thoroughly washed with dichloromethane. The solution was concentrated to 100 mL and was diluted with dichloromethane (400 mL). The organic layer was washed with water (2×200 mL), brine and dried over sodium sulfate. The organic layer was concentrated under vacuum and the residual solid was recrystallized from methanol/dichloromethane to give the pure ketone **10** (8.43 g, 92%) as a colorless crystalline solid. Mp 308–310 °C (from dichloromethane/methanol); (Found: C, 76.7; H, 9.5. $C_{30}H_{44}O_4$ requires: C, 76.9; H, 9.4%.) ν_{max} (KBr)/cm^{-1} 3311, 2965,

Table 2
Cytotoxic activities of **5**, **6**, and **7** (EC_{50} values) on different tumor cell lines as determined by MTT assay

No.	Cell line	Description	EC_{50} (nM) of the compounds		
			7	**5**	**6**
1	A549	Lung carcinoma	599	7598	7936
2	A431	Skin carcinoma	505	4271	1697
3	HL60	Leukemia	108	925	548
4	MCF-7	Breast carcinoma	522	6213	2249
5	T47D	Breast carcinoma	327	5541	3907
6	HT1080	Acetabulum fibro sarcoma	393	7747	3029

1726, 1682, 1644, 1455, and 1386; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.75 (1H, s), 2.97 (1H, m), 2.64 (1H, m), 2.41 (1H, s), 2.41–0.87 (40H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 217.3, 199.7, 181.2, 169.8, 128.4, 76.6, 61.0, 55.4, 48.2, 47.8, 45.3, 43.8, 43.3, 40.9, 39.7, 37.7, 36.7, 34.2, 32.1, 31.9, 30.9, 28.6, 28.4, 26.5, 26.3, 23.3, 21.4, 18.8, 18.5, and 15.6.

4.1.3. Methyl 3β-hydroxy-11-oxo-30-norolean-12-en-30-oate **9**

A solution of ethereal diazomethane (in excess) was added to a suspension of glycyrrhetinic acid **7** (9.4 g, 20 mmol) in methanol (150 mL) at 0 °C. The reaction mixture was allowed to stand overnight. The excess of diazomethane was quenched with acetic acid (six drops) and the mixture was concentrated under vacuum. The solid was recrystallized from methanol/dichloromethane mixture to give colorless crystals **9** (9.6 g, 99%). Mp 254–256 °C (from dichloromethane/methanol); (Found: C, 76.65; H, 10.1. $C_{31}H_{48}O_4$ requires: C, 76.85; H, 9.9%.) ν_{max} (neat)/cm^{-1} 3357, 2946, 1722, 1656, and 1467; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.63 (1H, s), 3.69 (3H, s), 3.20 (1H, m), 2.80 (1H, d, *J* 13.5 Hz), 2.30 (1H, s), and 2.15–0.65 (41H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 199.2, 176.2, 168.2, 128.5, 78.5, 61.7, 54.9, 51.5, 48.2, 45.2, 43.9, 43.0, 41.1, 39.1 (2C), 37.7, 37.0, 32.8, 31.7, 31.0, 28.5, 28.2, 28.1, 27.3, 26.4, 23.4, 18.6, 17.4, 16.3, and 15.6; *m*/*z* (DI) 484 (M^+), 317, 276, 175, and 135 (100%).

4.1.4. Methyl 3,11-dioxo-30-norolean-12-en-30-oate **11**

Following the procedure for the synthesis of **10** from **8** described earlier, the oxidation of the methyl ester **9** (9.21 g, 19 mmol) gave **11** as amorphous solid (8.80 g, 95%) [This compound was found to be identical to that obtained by diazomethane esterification of the acid **10**.] Mp 248–250 °C (from dichloromethane/methanol); (Found: C, 77.6; H, 9.8. $C_{31}H_{46}O_4$ requires: C, 77.2; H, 9.5%.) ν_{max} (neat)/cm^{-1} 2942, 1725, 1704, 1655, 1625, 1463, and 1386; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.68 (1H, s), 3.69 (3H, s), 2.97 (1H, m), 2.62 (1H, m), 2.40 (1H, s), and 2.37–0.83 (39H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 215.6, 198.4, 176.1, 168.7, 128.3, 60.9, 55.3, 51.5, 48.2, 47.5, 45.1, 43.8, 43.2, 41.1, 39.6, 37.7, 36.6, 33.9, 32.1, 31.7, 31.0, 28.5, 28.2, 26.5, 26.4, 26.3, 23.3, 21.3, 18.7, 18.5, and 15.5; *m*/*z* (DI) 482 (M^+), 317, 276, and 135 (100%).

4.1.5. Methyl 2-hydroxymethylene-3,11-dioxo-30-norolean-12-en-30-oate **12**

To an ice-cold suspension of sodium methoxide (440 mg, 8 mmol) in dry benzene (20 mL) was added a solution of ketone **11** (1.9 g, 4 mmol) and ethyl formate (634 mg, 8 m mol) over a period of 10 min under an atmosphere of nitrogen. The resulting mixture was allowed to stand overnight at room temperature. Ice water (20 mL) was added and the two layers separated. The organic layer was extracted with 20% sodium hydroxide solution (3×20 mL). The combined aqueous layer was acidified with 2 N hydrochloric acid and extracted with ethyl acetate (3×20 mL). The ethyl acetate layer was washed with water, brine, dried over sodium sulfate, and concentrated under vacuum. Recrystallization of the solid from dichloromethane/methanol afforded the pure colorless formyl derivative **12** (1.60 g, 78%). Mp 232–234 °C (from dichloromethane/methanol); (Found: C, 75.1; H, 9.15. $C_{32}H_{46}O_4$ requires: C, 75.3; H, 9.0%.) ν_{max} (KBr)/cm^{-1} 3438, 2934, 1732, 1653, 1618, 1457, and 1386; ^{1}H NMR (300 MHz, $CDCl_3$): δ 14.84 (1H, d, *J* 3.0 Hz), 8.62 (1H, d, *J* 3.0 Hz), 5.71 (1H, s), 3.71 (3H, s), 3.46 (1H, d, *J* 14.1 Hz), 2.40 (1H, s), and 2.15–0.84 (38H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 198.5, 189.2, 188.2, 176.1, 169.1, 128.4, 105.5, 59.5, 52.2, 51.5, 48.1, 44.8, 43.8, 43.1, 41.2, 39.9, 39.6, 37.7, 36.1, 31.7, 31.6, 31.0, 28.5, 28.4, 28.1, 26.4, 26.3, 23.2, 20.8, 18.7, 18.2, and 14.6; *m*/*z* (DI) 510 (M^+), 317, 276, 135, and 83 (100%).

4.1.6. Isoxazole derivative of the methyl ester of glycyrrhetinic acid **13**

The formyl derivative **12** (0.80 g, 1.6 mmol) in benzene (6 mL) was mixed with a solution of hydroxylamine hydrochloride (134 mg, 1.92 mmol), sodium acetate (312 mg, 1.92 mmol) in acetic acid (1 mL) and water (1 mL). The mixture was homogenized by the addition of methanol (2 mL) and refluxed for a period of 5 h. The solvents were removed under reduced pressure and the residue was diluted with water (15 mL) and extracted with ethyl acetate (3×15 mL). The combined organic layer was washed with brine, dried over sodium sulfate, and filtered. The solvent was removed under vacuum and the resulting solid was recrystallized from dichloromethane/methanol to give a colorless solid **13** (0.64 g, 80%). Mp 279–280 °C (from dichloromethane/methanol); (Found: C, 75.7; H, 9.05; N, 2.4. $C_{32}H_{45}NO_4$ requires: C, 75.7; H, 8.9; N, 2.7%.) ν_{max} (neat)/cm^{-1} 2972, 1730, 1658, 1620, 1460, 1385, and 870; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.97 (1H, s), 5.72 (1H, s), 3.71 (3H, s), 3.65 (1H, d, *J* 15.1 Hz), 2.50 (1H, s), and 2.16–0.84 (38H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 198.4, 176.2, 171.7, 169.0, 149.8, 128.5, 108.8, 59.9, 53.2, 51.5, 48.1, 45.1, 43.8, 43.2, 41.2, 38.3, 37.7, 35.9, 34.6, 31.8 (2C), 31.0, 28.8, 28.5, 28.2, 26.5, 26.4, 23.2, 21.5, 18.2, 18.0, and 15.6; *m*/*z* (DI) 507 (M^+), 135 and 83 (100%).

4.1.7. Methyl 2-cyano-3,11-dioxo-30-norolean-1,12-dien-30-oate **5**

The isoxazole **13** (270 mg, 0.5 mmol) in diethyl ether (5 mL) was added to a stirred solution of sodium (60 mg, 2.6 mmol) in dry methanol (3 mL) under an atmosphere of argon at 0 °C. It was allowed to stir for 1 h. Cold water (5 mL) was added to the reaction mixture and the organic layer was washed thoroughly with 5% potassium hydroxide solution (3×10 mL). The combined alkaline extracts were acidified and extracted with ethyl acetate (3×15 mL). The organic layer was washed with brine, dried over sodium sulfate, and concentrated under reduced pressure. The crude residue **14** (248 mg, 91%) was directly used in the next step without purification. ν_{max} (KBr)/cm^{-1} 2963, 2204, 1726, 1657, 1461, 1388, and 801; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.70, (1H, s), 3.70 (3H, s), 2.40 (1H, s), and 2.34–0.83 (40H, m); *m*/*z* (DI) 507 (M^+), 310, 276, and 135 (100). A mixture of the crude cyano-ketone **14** (63 mg, 0.12 mmol) and DDQ (33 mg, 0.14 mmol) in benzene (3 mL) was refluxed for 8 h. After cooling the reaction mixture to ambient temperature, acetone was added till it became homogenous and the clear solution was filtered through a pad of alumina. The filtrate was concentrated and the solid residue was recrystallized from dichloromethane/methanol to give the pure cyano-enone **5** (40 mg, 65%). Mp 237–239 °C (from dichloromethane/methanol); (Found: C, 76.1; H, 8.5; N, 2.8. $C_{32}H_{43}NO_4$ requires: C, 76.0; H, 8.6; N, 2.8%.) ν_{max} (neat)/cm^{-1} 2951, 2232, 1727, 1685, 1655, 1610, 1457, 1387, 802, and 736; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.49 (1H, s), 5.78 (1H, s), 3.71 (3H, s), 2.70 (1H, s), and 2.20–0.84 (37H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 197.4, 196.8, 176.2, 171.9, 170.9, 127.8, 114.5, 113.3, 54.2, 51.7, 51.6, 48.3, 45.5, 44.9, 43.8, 43.4, 41.1, 39.5, 37.6, 31.7, 31.6, 31.0, 28.5, 28.1, 27.4, 26.4, 26.2, 23.3, 21.4, 19.4, 18.8 and 18.0; *m*/*z* (DI) 83 (100%).

Crystal data: Compound **5**: $C_{32}H_{43}O_4N$, MW=505, colorless crystal, crystal system: monoclinic, space group: *P*2(1), cell parameters: *a*=7.282 (4) Å, *b*=12.262 (8) Å, *c*=16.173 (10) Å, β=94.57° (1), *V*=1440.00 (6) Å^3, *Z*=2, D_c=1.162 g cm^{-3}, *F*(000)=544.0, μ=0.08 mm^{-1}. Total number of l.s. parameters=342, R_1=0.0494 for 4676 $F_o > 4\sigma(F_o)$ and 0.0655 for all 5812 data. wR_2=0.1230, GOF=1.019, restrained GOF=1.024 for all data. Crystallographic data (without structure factor) have deposited with the Cambridge Crystallographic Data Center and the depository number is CCDC 213948.

4.1.8. Synthesis of **5** via allylic bromination–dehydrobromination

A mixture of the isoxazole **13** (270 mg, 0.5 mmol), NBS (98 mg, 0.55 mmol), AIBN (catalytic amount) in chloroform (5 mL) was allowed to reflux under the influence of tungsten lamp for 6 h. The solution was cooled, and the precipitated succinimide was filtered. Removal of the solvent afforded the crude bromo compound **15**, which was added to an ice-cold solution of sodium (46 mg,

2 mmol) in methanol (2 mL). The reaction mixture was stirred at room temperature for 1 h. The solvent was removed under vacuum and the residue was diluted with water (10 mL). The aqueous layer was repeatedly extracted with ether (3×15 mL). The combined organic layer was dried over sodium sulfate and concentrated under vacuum. The crude solid was purified by column chromatography to give the cyano-enone **5** (63%). Mp 237–239 °C.

4.1.9. Arjunolic acid **16**[13]

Arjunolic acid was isolated[13] from the acetone extract of the heartwood of *T. arjuna* Bedd. It was crystallized from acetone, mp 320–325 °C; ν_{max} (KBr)/cm^{-1} 3544, 3466, 3389, 2931, 1708, and 1639; ^{1}H NMR (300 MHz, py-d_5): δ 5.48 (1H, br t), 4.25–4.23 (3H, s), 3.74 (1H, d, *J* 10.2 Hz), 3.30 (1H, d, *J* 10.2 Hz), 2.34 (1H, d, *J* 9.6 Hz), 2.18–0.92 (41H, m); *m*/*z* (DI) 248 (M^+), 203 (100), 191, 173, and 133.

4.1.10. Methyl arjunolate **17**

Ethereal diazomethane was added to an ice cooled suspension of arjunolic acid **16** (9.8 g, 2 mmol) in methanol (25 mL). The resultant mixture was left at room temperature for 4 h. The excess diazomethane was quenched with drops of acetic acid. Removal of the solvent afforded a residue, which was crystallized from methanol to give the methyl ester **17** (9.5 g, 95%).[13] Mp 248–250 °C; ν_{max} (neat)/cm^{-1} 3395, 2946, and 1725; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.28 (1H, br s), 3.71 (1H, br s), 3.61 (3H, s), 3.57 (1H, br s), 3.48 (2H, m), 2.84 (1H, d, *J* 13.2 Hz), 1.95–0.67 (41H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 178.3, 144.0, 122.3, 80.1, 69.8, 69.7, 58.3, 51.7, 48.1, 47.7, 46.9, 46.0, 41.9, 41.5, 39.5, 38.3, 38.2, 34.0, 33.1, 32.7, 32.5, 30.7, 28.2, 27.8, 26.1, 23.6, 23.5, 23.2, 18.4, 17.1, and 12.9.

4.1.11. Preparation of the isopropylidene derivative **18**

A mixture of methyl arjunolate **17** (500 mg, 1 mmol) in dry acetone (50 mL) containing a catalytic amount of concd. hydrochloric acid and activated 4 Å molecular sieves was stirred for 6 h. The reaction mixture was filtered, concentrated, and the residue was purified by column chromatography (hexane/ethyl acetate, 7:3) to give **18** (412 mg, 76%); mp 179–181 °C (from ethyl acetate); (Found: C, 75.3; H, 10.1. $C_{34}H_{54}O_5$ requires: C, 75.2; H, 10.0%.) R_f (30% ethyl acetate/hexane 0.4); ν_{max} (neat)/cm^{-1} 3492, 2945, and 1725; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.28 (1H, br t), 3.78 (1H, dt, *J* 4.5 and 9.7 Hz), 3.62 (3H, s), 3.61 (1H, d, *J* 9.7 Hz), 3.48 (1H, d, *J* 3.3 Hz), 3.32 (1H, d, *J* 9.3 Hz), 2.87 (1H, dd, *J* 14.4 and 5.5 Hz), 2.23 (1H, s), 2.07–0.88 (44H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.6, 143.4, 122.0, 99.4, 82.0, 72.5, 64.9, 51.4, 51.3, 47.6, 46.4, 46.2, 45.7, 41.5, 41.1, 39.3, 37.9, 36.8, 33.8, 33.1, 32.2, 32.0, 30.6, 29.6, 27.5, 25.9, 23.6, 23.1, 22.9, 19.3, 17.7, 17.4, 16.7, and 13.4.

4.1.12. Xanthate derivative **19**

A solution of **18** (1.1 g, 2 mmol), sodium hydride (240 mg, 10 mmol), imidazole (50 mg, 0.74 mmol) in THF (10 mL) was stirred for 30 min and freshly distilled carbon disulphide (1 mL, excessive amount) was added and the mixture was heated under reflux for 2 h. The reaction mixture was cooled to ambient conditions and methyl iodide (1.5 mL, excess) added. The mixture was further heated under reflux for 1 h. Water (15 mL) was added to the cooled mixture and extracted with ether (3×20 mL). The organic layer was washed with brine and dried. Removal of the solvent under reduced pressure afforded a residue, which was purified by chromatography on silica gel (hexane/ethyl acetate, 10:1) to obtain **19** (1.16 g, 92%). Mp 214–216 °C; (Found: C, 68.55; H, 9.0. $C_{36}H_{56}O_5S_2$ requires: C, 68.3; H, 8.9%.) ν_{max} (neat)/cm^{-1} 2946, 1725, and 1197; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.78 (1H, dd, *J* 10.3 and 4.2 Hz), 5.26 (1H, br s), 3.80 (1H, d, *J* 10.3 Hz), 3.62 (3H, s), 3.44 (1H, d, *J* 10.3 Hz), 2.86 (1H, dd, *J* 4.0 and 13.0 Hz), 2.51 (3H, s), 2.36 (1H, dd, *J* 4.8 and 12.3 Hz), 2.00–0.70 (44H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 178.2, 143.7, 121.8, 99.4, 78.8, 78.6, 77.20, 72.6, 51.5, 51.1, 47.7, 46.6, 45.8, 43.4, 41.6, 41.2, 39.4, 38.8, 37.8, 33.8, 33.1, 32.3, 32.0, 30.7, 29.7, 27.6, 26.9, 26.0, 23.6, 23.3, 23.0, 19.2, 18.5, 17.4, 17.3, 16.6, and 13.5.

4.1.13. Methyl hederagenin **21** (from arjunolic acid)

A catalytic amount of AIBN was added to a mixture of **19** (1.0 g, 1.58 mmol) and TBTH (0.5 mL) in dry benzene (10 mL). The reaction mixture was heated under reflux for 2 h, the solvent was removed under reduced pressure and the residue refined by chromatography (hexane/ethyl acetate, 10:1) to obtain a white powder **20** (673 mg, 81%). This compound was sufficiently pure to be used for the subsequent operation. ν_{max} (neat)/cm^{-1} 2942 and 1724; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.28 (1H, t, *J* 3.6 Hz), 3.62 (3H, s), 3.47 (1H, m), 3.53 (1H, d, *J* 10.0 Hz), 3.44 (1H, d, *J* 10.0 Hz), 2.86 (1H, dd, *J* 14.0 and 4.8 Hz), 2.04–0.70 (46H, m). Amberlite-450 was added to **20** (600 mg, 1.14 mmol) in methanol (6 mL) and the resultant mixture allowed to stand at ambient temperature for 12 h with occasional shaking. The mixture was filtered through a pad of Celite and the filtrate concentrated under reduced pressure. The resultant residue was purified by column chromatography (hexane/ethyl acetate, 3:2) to give methyl hederagenin as a colorless solid **21** (482 mg, 87%).[14] Mp 240–242 °C; R_f (40% ethyl acetate/hexane 0.42); identical with an authentic sample prepared from hederagenin, ν_{max} (neat)/cm^{-1} 3507, 2942, and 1724; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.28 (1H, br t), 3.74 (1H, d, *J* 10.0 Hz), 3.63 (1H, br s), 3.62 (3H, s), 3.44 (1H, d, *J* 10.0 Hz), 2.86 (1H, dd, *J* 13.2 and 3.3 Hz), 2.32–0.70 (42H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 178.5, 144.2, 122.4, 118.2, 80.2, 69.6, 69.5, 51.5, 48.0, 47.6, 46.5, 45.0, 41.7, 41.5, 39.6, 38.3, 38.2, 34.0, 33.4, 32.5, 32.3, 30.3, 28.4, 27.8, 26.2, 23.5, 23.4, 23.1, 17.1, and 13.1.

4.1.14. Isolation of hederagenin (3,23-dihydroxyolean-12-en-28-oic acid)

Dry pericap of soap nuts, *Sapindus mukorossi*, (*S. emarginatus*) abundantly available in southern states of India were collected. The pulverized, dried pericaps of soap nuts (125 g) were defatted by extraction with hexane (200 mL) in a Soxhlet extractor. This was further extracted with ethyl acetate (300 mL) followed by methanol (300 mL). The methanol extract (16.2 g) was submitted for hydrolysis with 6 N hydrochloric acid (60 mL) for 2 h. The acidic mass was carefully neutralized with 5% sodium hydroxide solution and extracted with ethyl acetate (5×60 mL). The combined ethyl acetate extract was evaporated and dried under vacuum. The crude sapogenin, hederagenin (6.5 g) was crystallized from methanol to afford pure hederagenin, mp 333–335 °C; ν_{max} (neat)/cm^{-1} 3447, 2930, 1698, and 756; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.46 (1H, br s), 4.22–4.24 (3H, m), 3.72 (1H, d, *J* 10.2 Hz), 3.32 (1H, d, *J* 10.2 Hz), 2.34 (1H, d, *J* 9.6 Hz), 2.16–0.91 (41H, m); *m*/*z* (DI) 248, 203 (100%), 191, 173, and 133.

4.1.15. Methyl hederagenin **21** (from hederagenin)

Ethereal diazomethane was added to an ice-cold suspension of hederagenin (600 mg, 1.20 mmol) in methanol (4 mL). The resultant mixture was left at ambient temperature for 4 h. The excess diazomethane was quenched using drops of acetic acid. The solvent was removed by evaporation under reduced pressure and the residue purified by column chromatography on silica gel (hexane/ethyl acetate, 3:2) to give the methyl ester (570 mg, 95%). This compound was identical with compound **21**.[14] Mp 240–242 °C.

4.1.16. 23-O-tert-Butyldimethylsilyl derivative of methyl hederagenin **22**

tert-Butyldimethylsilyl chloride (159 mg, 1.06 mmol) in dichloromethane (1 mL) was added to a mixture of the diol **21** (432 mg, 0.88 mmol) and imidazole (108 mg, 1.59 mmol) in dry dichloromethane (1.5 mL) at 0 °C over a period of 30 min. The reaction mixture was stirred for 6 h and worked up to give a residue, which was purified by chromatography. Elution with hexane/ethyl acetate

(9:1) afforded a viscous oil **22** (480 mg, 91%). (Found: C, 73.94; H, 10.73. $C_{37}H_{64}O_4Si$ requires: C, 74.0; H, 10.33%.) R_f (5% ethyl acetate/hexane 0.3); ν_{max} (neat)/cm^{-1} 3420, 2949, and 1726; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.27 (1H, br t), 3.67 (1H, d, J 9.0 Hz), 3.61 (3H, s), 3.60 (1H, d, J 5.4 Hz), 3.35 (1H, d, J 9.0 Hz), 2.85 (1H, dd, J 14.0 and 4.0 Hz), 2.16 (1H, s), 1.95–0.70 (55H, m).

4.1.17. Methyl 23-O-tert-butyldimethylsilyl-3-oxoolean-12-en-28-oate **23**

A solution of alcohol **22** (376 mg, 0.67 mmol) in dry dichloromethane was added to mixture of pyridinium chlorochromate (731 mg, 3.40 mmol) and sodium acetate (730 mg, 8.9 mmol) in dry dichloromethane. The reaction mixture was stirred for 4 h before filtering through a pad of Celite. The concentrated residue was refined by column chromatography on silica gel (hexane/ethyl acetate, 95:5) to give viscous oil **23** (292 mg, 78%). (Found: C, 73.65; H, 10.75. $C_{37}H_{62}O_4Si$ requires: C, 74.25; H, 10.36%.) R_f (5% ethyl acetate/hexane 0.5); ν_{max}(neat)/cm^{-1} 2927, 2856, 1731, and 1703; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.33 (1H, br t), 3.62 (3H, s), 3.61 (1H, d, J 9.0 Hz), 3.27 (1H, d, J 9.0 Hz), 2.86 (1H, m), 2.38 (2H, m), 2.04–0.8 (53H, m).

4.1.18. Isoxazole of methyl hederagenin **25**

A mixture of the ketone **23** (250 mg, 0.42 mmol), ethyl formate (0.2 mL, excess), and sodium tertiary amylate {prepared from sodium (48 mg, 2.09 mmol) and *t*-amyl alcohol (1.5 mL)} in benzene (2 mL) was stirred at room temperature for 8 h. The reaction mixture was acidified with 2 N hydrochloric acid and extracted with ether (3×15 mL), washed with brine and dried over anhydrous sodium sulfate. The organic layer was concentrated to afford a glassy solid **24**, which was directly used in the next step. ν_{max} (neat)/cm^{-1} 2948, 2856, and 1728; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.31 (1H, d, J 3.3 Hz), 5.30 (1H, br s), 3.70 (1H, d, J 9.0 Hz), 3.63 (3H, s), 3.62 (1H, br s), 3.34 (1H, d, J 9.0 Hz), 2.95–2.85 (1H, m), 2.21–0.80 (55H, m). The crude formyl derivative **24** in methanol (3 mL), hydroxylamine hydrochloride (58 mg, 0.84 mmol), and sodium acetate (68 mg, 0.84 mmol) were heated under reflux for 8 h. The reaction mixture was cooled and the solvent removed under reduced pressure. The residue in water (6 mL) was extracted with ethyl acetate (15×3 mL). The combined organic layers were washed with water, brine, and dried over anhydrous sodium sulfate. The residue was purified by column chromatography. Elution with (hexane/ethyl acetate, 4:1) afforded the pure isoxazole **25** (146 mg, 68% for two steps). Mp 218–220 °C; (Found: C, 74.95; H, 9.3; N, 2.45. $C_{32}H_{47}NO_4$ requires: C, 75.4; H, 9.3; N, 2.75%.) R_f (20% ethyl acetate/hexane 0.5); ν_{max} (neat)/cm^{-1} 2974, 1732, 1656, 1620, 1460, 1385, and 870; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.02 (1H, s), 5.34 (1H, br t), 3.80 (1H, d, J 11.0 Hz), 3.63 (3H, s), 3.55 (1H, d, J 11.0 Hz), 2.9 (1H, dd, J 4.0 and 14.4 Hz), 2.5–0.8 (39H, m); m/z (DI) 507 (M$^+$), 310, 276, and 135 (100).

4.1.19. Methyl 2-cyano-23-hydroxy-3-oxooleana-1,12-dien-28-oate **6**

The cyano-ketone (179 mg, 96%), R_f (20% ethyl acetate in hexane 0.3) was prepared from the isoxazole **25** (186 mg, 0.36 mmol) by hydrolysis using sodium methoxide in methanol. Since this compound could not be purified, it was directly used in the next step. The crude cyano-ketone (155 mg, 0.30 mmol) was oxidized using DDQ to give the cyano-enone **6** (98 mg, 65%). Mp 221–223 °C; (Found: C, 75.5; H, 8.7; N, 2.6. $C_{32}H_{45}NO_4$ requires: C, 75.7; H, 8.9; N, 2.8%.) R_f (20% ethyl acetate in hexane 0.25); ν_{max} (neat)/cm^{-1} 2951, 2232, 1727, 1685, 1655, 1610, and 802; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.74 (1H, s), 5.34 (1H, t, J 3.3 Hz), 3.76 (1H, d, J 10.8 Hz), 3.63 (3H, s), 3.62 (1H, br s), 3.43 (1H, d, J 10.8 Hz), 2.89 (1H, dd, J 5.7 and 14.7 Hz), 2.15–2.12 (2H, m), 2.00–1.92 (2H, m), 1.71–0.83 (32H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 198.0, 177.6, 169.5, 144.8, 121.0, 115.0, 114.3, 67.4, 51.6, 50.2, 46.7, 45.7, 45.3, 42.2, 41.6, 41.3, 40.5, 40.4, 34.0, 33.4, 32.3, 32.0, 30.9, 27.8, 26.0, 23.8, 23.4, 23.0, 19.1, 18.5, 17.6, and 17.2.

4.1.20. Isolation of β-boswellic acid from the gum of *B. serrata*[15]

The commercially available gum (100 g) of *B. serrata* was extracted with ethyl acetate (3×100 mL) and the ethyl acetate extract was filtered and concentrated under vacuum. The syrupy mass (45 g) was treated with 5% sodium hydroxide solution and stirred till a uniform emulsion is formed. The emulsion was extracted with hexane (3×100 mL). The aqueous extract was acidified with dilute hydrochloric acid and the precipitate was extracted with ethyl acetate (3×100 mL). The ethyl acetate extract was thoroughly washed with water, dried over anhydrous sodium sulfate, and concentrated to afford a creamy powder (22 g) consisting a mixture of boswellic acids. This mixture (5 g) was chromatographed on a silica gel column. Elution with hexane–ethyl acetate (4:1) afforded a mixture of β-, and α- boswellic acids (2.2 g) in the ratio of 9:1, R_f (20% ethyl acetate/hexane 0.3).

4.1.21. Methyl boswellates **27**

The methyl esters of α- and β-boswellic acids **27** (606 mg, 98%) were obtained from boswellic acids **26** (602 mg, 1.32 mmol) by esterification with ethereal diazomethane.[15] Mp 195 °C; (Found: C, 78.83; H, 10.50. $C_{31}H_{50}O_3$ requires: C, 79.10; H, 10.78%.) R_f (20% ethyl acetate/hexane 0.6); ν_{max} (neat)/cm^{-1} 3511, 2921, and 1722; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.13 (1H, s), 4.10 (1H, br s), 3.61 (3H, s), 2.23–0.78 (45H, m); m/z (DI) 470 (M$^+$), 394, 161.

4.1.22. Methyl 3-ketoboswellates **28**

A solution of the alcohol **27** (658 mg, 1.40 mmol) and PDC (602 mg, 1.60 mmol) was taken in dry dichloromethane (6 mL). The reaction mixture was stirred for 4 h before filtering through a pad of Celite. The concentrated residue was purified by column chromatography on silica gel (20% ethyl acetate in hexane) to give a colorless solid **28** (467 mg, 71%).[15] Mp 160 °C; (Found: C, 79.35; H, 10.25. $C_{31}H_{48}O_3$ requires: C, 79.40; H, 10.30%.) R_f (20% ethyl acetate/hexane 0.8); ν_{max} (neat)/cm^{-1} 2949, 1742, 1714, and 1662; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.54 and 5.30 (1H, br s), 3.68 (3H, s), 2.92 (1H, dt, J 6.0 and 14.7 Hz), 2.34 (1H, d, J 14.7 Hz), 2.16–0.76 (42H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 207.9, 173.9, 139.9, 124.0, 59.2, 58.2, 57.4, 52.0, 46.6, 42.3, 41.5, 41.1, 39.9, 39.7, 37.2, 36.5, 33.9, 33.0, 31.3, 28.9, 28.1, 26.6, 23.7, 23.2, 21.5, 21.0, 20.6, 17.6, 16.9, and 13.4; m/z (DI) 468 (M$^+$), 218 (100%).

4.1.23. Preparation of the isoxazole **30**

Employing a protocol similar to the synthesis of **25** from **23**, the crude of the 2-hydroxymethylene derivative **29** was obtained from **28** (456 mg, 0.98 mmol), which was directly used for the synthesis of isoxazole **30** (358 mg, 74%). (Found: C, 77.8; H, 9.6; N, 2.85. $C_{32}H_{47}NO_3$ requires: C, 77.9; H, 9.53; N, 2.84%.) R_f (20% ethyl acetate/hexane 0.57); ν_{max} (neat)/cm^{-1} 2918, 1732, and 1646; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.05 (1H, s), 5.24 and 5.19 (1H, br t), 3.63 (3H, s), 2.53 (1H, m), 2.15–0.77 (41H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.9, 166.9, 150.1, 139.7, 123.9, 121.27, 111.2, 77.2, 59.1, 55.3, 52.1, 47.3, 46.8, 46.3, 45.1, 42.2, 41.8, 41.4, 39.8, 39.7, 39.5, 38.2, 38.2, 37.0, 35.9, 35.6, 34.6, 33.8, 33.3, 32.5, 32.4, 32.0, 31.2, 31.1, 28.8, 28.4, 28.1, 26.9, 26.6, 26.1, 25.6, 24.4, 23.6, 23.0, 21.3, 19.8, 17.4, 16.4, 16.3, 14.5, and 14.3; m/z (DI) 493 (M$^+$), 218 (100%).

4.1.24. Cyano-enone of methyl boswellates **7**

Following the bromination–dehydrobromination protocol employed for the synthesis of **4** from **13**, the cyano-enone of α- and β-methyl boswellates **7** (90 mg, 54% yield) was obtained from **30** (174 mg, 0.34 mmol) as an amorphous solid. The isomers were observed in a (1:9) ratio, based on the integration of the ^{1}H proton signals. (Found: C, 78.4; H, 9.0; N, 2.5. $C_{32}H_{45}NO_3$ requires: C, 78.2; H, 9.2; N, 2.85%.) R_f (20% ethyl acetate/hexane 0.4); ν_{max} (neat)/cm^{-1} 2922, 2234, 1729, 1698, and 1612; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62 and 7.60 (1H, s), 5.17 and 5.12 (1H, br t), 3.59 (3H, s), 2.17–0.79 (40H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 190.3, 172.5, 168.0, 146.0,

140.7, 122.5, 120.0, 114.7, 113.6, 59.0, 54.5, 54.4, 54.3, 52.5, 47.3, 46.5, 42.5, 41.4, 41.3, 41.0, 40.8, 40.7, 39.54, 39.5, 36.9, 34.6, 33.7, 33.3, 32.6, 32.5, 32.3, 31.6, 31.1, 31.0, 28.3, 27.8, 26.9, 26.6, 26.4, 25.9, 25.7, 23.6, 23.5, 23.3, 23.1, 22.6, 21.3, 20.9, 19.3, 17.5, 17.4, 14.1, 14.0, and 13.9; *m*/*z* (DI) 491 (M^+), 476, 432, and 218 (100%), 203.

4.1.25. Bioassays

4.1.25.1. Nitric oxide assay. Thioglycolate (4%) elicited peritoneal macrophages were isolated from two months old C57black6 male mice after sacrificing by cervical dislocation. The cells were cultured for 72 h in presence of mouse interferon gamma (10 U/mL) either alone or in presence of test compounds. Induced nitric oxide rapidly oxidized into nitrite in culture medium (RPMI+10%FBS) and the nitrite concentration in supernatants was taken as a measure of nitric oxide production.[19] Griess assay was done to measure the nitrite in the medium.

4.1.25.2. MTT assay for cytotoxicity. Cancer cell lines were plated in 96-well plates (Nunc, Denmark) at a density of 5×10^3 to 8×10^3 cells/well depending on the cell line. The plates were incubated at 37 °C in a humidified 5% CO_2 atmosphere. Cells were allowed to adhere to the surface for 3–4 h and different concentrations of the compounds were added and incubated for 72 h. Later, 20 μL of MTT solution (5 mg/mL) was added to each well and the incubation continued for an additional 3 h. The dark blue formazan crystals formed within the healthy cells were solubilized with DMSO and the absorbance was measured in an ELISA plate reader at 550 nm. The EC_{50} values of the compounds were calculated by Graphpad Prism.

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References and notes

1. Honda, T.; Rounds, B. V.; Gribble, G. W.; Suh, N.; Wang, Y.; Sporn, M. B. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2711.
2. Chauhan, D.; Li, G.; Poddar, K.; Hideshima, T.; Shringarpure, R.; Catley, L.; Mitsiades, C.; Munshi, N.; Tai, Y. T.; Suh, N.; Gribble, G. W.; Honda, T.; Schlossman, R.; Richardson, P.; Sporn, M. B.; Anderson, K.; Lipper, J. *Blood* **2004**, *103*, 3158.
3. Place, A. E.; Suh, N.; Williams, C. R.; Risingsong, R.; Honda, T.; Honda, Y.; Gribble, G. W.; Leesnitzer, L. M.; Stimmel, J. B.; Wilson, T. M.; Rosen, E.; Sporn, M. B. *Clin. Cancer Res.* **2003**, *9*, 2798.
4. Honda, T.; Rounds, B. V.; Bore, L.; Favaloro, F. G., Jr.; Gribble, G. W.; Suh, N.; Wang, Y.; Sporn, M. B. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3429.
5. Honda, T.; Gribble, G. W.; Suh, N.; Finlay, H. J.; Rounds, B. V.; Bore, L.; Favaloro, F. G., Jr.; Wang, Y.; Sporn, M. B. *J. Med. Chem.* **2000**, *43*, 1866.
6. Honda, T.; Rounds, B. V.; Bore, L.; Finlay, H. J.; Favalaro, F. G., Jr.; Suh, N.; Wang, Y.; Sporn, M. B.; Gribble, G. W. *J. Med. Chem.* **2000**, *43*, 4233.
7. 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, C.; 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. *Cancer Res.* **1999**, *59*, 336.
8. Wang, Y.; Porter, W. W.; Suh, N.; Honda, T.; Gribble, G. W.; Leesnitzer, L. M.; Plunket, K. D.; Mangelsdorf, D. J.; Blanchard, S. G.; Wilson, T. M.; Sporn, M. B. *Mol. Endocrinol.* **2000**, *14*, 1550.
9. Beaton, J. M.; Spring, F. S. *J. Chem. Soc.* **1955**, 3126.
10. Johnson, W. S.; Shelberg, W. E. *J. Am. Chem. Soc.* **1945**, *67*, 1745.
11. Doorenbos, N. J.; Milewich, L. *J. Org. Chem.* **1966**, *31*, 3193.
12. Campsteyn, H.; Dupont, L.; Lamotte, J.; Dideberg, O.; Vermeire, M. *Acta Crystallogr.* **1977**, *B33*, 3443.
13. Row, L. R.; Subba Rao, G. S. R. *Tetrahedron* **1962**, *18*, 827.
14. Jacobs, W. A. *J. Biol. Chem.* **1925**, *63*, 621.
15. Taneja, S. C.; Sethi, V. K.; Dhar, K. L.; Kapil, R. S. European Patent 755940, 1997; Simpson, J. C. E.; Williams, N. E. *J. Chem. Soc.* **1938**, 686.
16. Konopleva, M.; Tsao, T.; Ruvolo, P.; Stiouf, I.; Estroy, Z.; Leysath, C. E.; Zhao, S.; Harris, D.; Chang, S.; Jackson, C. E.; Munsell, M.; Suh, N.; Gribble, G. W.; Honda, T.; May, W. S.; Sporn, M. B. *Blood* **2002**, *99*, 326.
17. Ji, Y.; Lee, H. J.; Goodman, C.; Uskokovic, M.; Liby, K.; Sporn, M. B.; Suh, N. *Mol. Caner Ther.* **2006**, *5*, 1452.
18. Honda, T.; Liby, K. L.; Su, X.; Sundararajan, C.; Honda, Y.; Suh, N.; Risingsong, R.; Williams, C. R.; Royce, D. B.; Sporn, M. B.; Gribble, G. W. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 6306.
19. Ding, A.; Nathan, C.; Graycar, J.; Derynck, R.; Stuehr, D. J.; Srimal, S. *J. Immunol.* **1990**, *145*, 940.