CHAPTER 3

EXPERIMENTALS
3.1. General

Melting points were determined on a Buchi melting point apparatus and are uncorrected. Reactions were monitored on Merck aluminium thin layer chromatography plates and visualized by exposure to iodine vapors. Column chromatography was carried on silica gel (100-200 mesh, MERCK chemicals). IR spectra were recorded on a Perkin-Elmer 882 spectrometer using potassium bromide pellets. \(^1\)H NMR and \(^{13}\)C NMR spectra were recorded with a Bruker Avance II 400 MHz spectrometers using CDCl\(_3\)/DMSO-\(d_6\) as solvent, and tetramethyrsilane was used as internal standard. Mass spectra were obtained with Micromass 70-VSE mass spectrometer at 70 eV using electron ionization (EI). Elemental analysis of compound was within 0.04% of the theoretical values. All solvents were freshly distilled and dried prior to use according to standard procedures.

3.2. Extraction and isolation of lantadene A & B

The leaves of Lantana camara were collected in September 2010 from Palampur (HP), India. The leaves were dried in the shade and powdered. Lantana leaf powder was extracted with methanol and the extract obtained was treated with charcoal to remove the green pigments which gave golden yellow colored extract. The solvent was removed under reduced pressure and the residue was suspended in methanol-water (1:7) mixture and extracted with chloroform. The organic layer was dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure. The solid residue obtained was crystallized from methanol to obtain partially purified lantadenes as a white crystalline product [Sharma et al., 1987].

Isolation of Lantadene A (22\(\beta\)-[(2-methyl-1-oxo-2-butenyl)oxy]-3-oxoolean-12-en-28-oic acid 80)

1g of partially purified lantadenes were chromatographed over silica gel (100-200 mesh) and eluted with hexane: ethyl acetate (4:1) to obtain 22\(\beta\)-[(2-methyl-1-oxo-2-butenyl)oxy]-3-oxoolean-12-en-28-oic acid 80 as white solid (520 mg, 52%), \(R_f\) 0.63 (hexane: ethyl acetate :: 4:1), mp. 283-285 \(^0\)C.

Analysis

IR (KBr) \(\nu_{\text{max}}\): 2952.45 (C-H aliphatic), 1715.85 (C=O, ester), 1702.14 (C=O, 3-keto) cm\(^{-1}\).  

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$^1$H NMR (CDCl$_3$: 400 MHz): $\delta$ 0.82 (s, 3H, CH$_3$), 0.85 (s, 3H, CH$_3$), 1.00 (s, 3H, CH$_3$), 1.05 (s, 6H, 2 x CH$_3$), 1.09 (s, 3H, CH$_3$), 1.17 (s, 3H, CH$_3$), 3.05 (d, $J=10.40$ Hz, 1H, C-18-H), 5.09 (s, 1H, C-22α-H), 5.38 (s, 1H, C-12-H), 6.00 (q, 1H, $J=7.28$ Hz, C-3′-H).

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 37.72 (C-1), 34.14 (C-2), 217.72 (C-3), 38.45 (C-4), 55.29 (C-5), 21.48 (C-6), 30.19 (C-7), 39.21 (C-8), 50.59 (C-9), 36.78 (C-10), 24.19 (C-11), 122.49 (C 12), 143.10 (C 13), 45.94 (C 14), 26.44 (C 15), 23.51 (C 16), 46.88 (C 17), 41.99 (C-18), 47.45 (C-19), 30.05 (C-20), 33.69 (C-21), 75.84 (C-22), 27.56 (C-23), 16.84 (C-24), 15.67 (C-25), 19.48 (C-26), 26.14 (C-27), 179.28 (C-28), 32.19 (C-29), 25.79 (C-30), 166.26 (C-1′), 127.58 (C-2′), 139.06 (C-3′), 15.10 (C-4′), 20.58 (C-5′).

ESI-MS (m/z): 551.4 (M-1).

Elemental anal.: C$_{35}$H$_{52}$O$_5$ (552.5): C 76.05%; H 9.48%; found: C 76.03%; H 9.50%.

Isolation of Lantadene B (22β-[(3-methyl-1-oxo-2-butenyl)oxy]-3-oxoolcan-12-en-28-oic acid 81)

1g of partially purified lantadene Bs were chromatographed over silica gel (100-200 mesh) and eluted with hexane: ethyl acetate (4:1) to obtain 22β-[(3-methyl-1-oxo-2-butenyl)oxy]-3-oxoolcan-12-en-28-oic acid 81 as white solid (390 mg, 39%), $R_f$ 0.61 (hexane: ethyl acetate :: 4:1), mp. 293-295°C.

Analysis

IR (KBr) v max: 2950.12 (C-H aliphatic), 1713.45 (C=O, ester), 1703.09 (C=O, 3-keto) cm$^{-1}$.

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 0.83(s, 3H, CH$_3$), 0.88 (s, 3H, CH$_3$), 1.00 (s, 3H, CH$_3$), 1.05 (s, 6H, 2 x CH$_3$), 1.09 (s, 3H, CH$_3$), 1.17 (s, 3H, CH$_3$), 3.02 (d, $J=9.96$ Hz, 1H, C-18-I), 5.04 (s, 1H, C-22α-II), 5.37 (s, 1H, C-12-II), 5.55 (s, 1H, C-2′-II).

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 38.54 (C-1), 33.75 (C-2), 217.81 (C-3), 39.16 (C-4), 55.30 (C-5), 21.50 (C-6), 32.26 (C-7), 39.24 (C-8), 50.57 (C-9), 37.63 (C-10), 25.77 (C-11), 122.37 (C-12), 143.09 (C-13), 45.97 (C-14), 27.46 (C-15), 24.13 (C-16), 46.87 (C-17), 42.07 (C-18), 47.45 (C-19), 30.07 (C-20), 36.77 (C-21), 75.20 (C-22), 27.59 (C-23), 16.85 (C-24), 15.16 (C-25), 19.52 (C-26), 26.44 (C-27), 178.84 (C-28), 34.16 (C-29), 26.28 (C-30), 165.32 (C-1′), 115.96 (C-2′), 157.15 (C-3′), 20.25 (C-4′), 23.56 (C-5′).

ESI-MS (m/z): 551.5 (M-1).

Elemental anal.: C$_{35}$H$_{52}$O$_5$ (552.5): C 76.05%; H 9.48%; found: C 76.07%; H 9.49%.
3.3. Synthesis of bioactive intermediates and C-2 arylidene/aryl congeners of lantadenes

Synthesis of 22\(\beta\)-[(2-methyl-1-oxo-2-butenyl)oxy]-3\(\beta\)-hydroxyolean-12-en-28-oic acid (84)

To a solution of LA 80 (100 mg, 0.18 mM) in methanol and tetrahydrofuran mixture (20 ml, 1:1), 6.80 mg (0.18 mM) sodium borohydride was added and stirred at room temperature. After reaction completion, the solvent was removed \textit{in vacuo} and the residue was diluted with water (15ml). The mixture was acidified with dilute HCl and extracted with ethyl acetate (3 x 15ml). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed over silica gel (100-200 mesh) and eluted with hexane : ethyl acetate (4:1) to obtain 22\(\beta\)-[(2-methyl-1-oxo-2-butenyl)oxy]-3\(\beta\)-hydroxyolean-12-en-28-oic acid 84 as white solid (84 mg, 83.7%), R\(_f\) 0.58 (hexane : ethyl acetate :: 4:1), mp. 278-279 \textdegree C.

Analysis

IR (KBr) \(\nu\) \textit{max}: 3482.87 (O-H), 2948.99, 2827.53 (C-H aliphatic), 1717.87 (C=O, ester) cm\(^{-1}\).

\(^1\)H NMR (DMSO-\(d_6\), 400 MHz): \(\delta\) 0.73 (s, 3H, CH\(_3\)), 0.80 (s, 3H, CH\(_3\)), 0.89 (s, 6H, 2 x CH\(_3\)), 0.94 (s, 3H, CH\(_3\)), 0.99 (s, 3H, CH\(_3\)), 1.16 (s, 3H, CH\(_3\)), 3.00 (dd, \(J = 11.32, 8.04\) Hz, 1H, C-18-H), 3.09 (t, \(J = 7.24\) Hz, 1H, C-3\(\alpha\)-H), 4.99 (s, 1H, C-22\(\alpha\)-H), 5.31 (s, 1H, C-12-H), 6.00 (q, \(J = 6.08\) Hz, 1H, C-3\(\beta\)-H).

\(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \(\delta\) 38.77 (C-1), 36.51 (C-2), 79.28 (C-3), 45.71 (C-4), 54.77 (C-5), 20.11 (C-6), 32.30 (C-7), 40.25 (C-8), 47.06 (C-9), 38.09 (C-10), 23.74 (C-11), 121.53 (C-12), 158.43 (C-13), 41.46 (C-14), 27.90 (C-15), 25.40 (C-16), 49.59 (C-17), 38.99 (C-18), 43.96 (C-19), 29.59 (C-20), 38.29 (C-21), 75.60 (C-22), 26.96 (C-23), 15.57 (C-24), 15.18 (C-25), 16.49 (C-26), 26.68 (C-27), 177.14 (C-28), 33.43 (C-29), 25.77 (C-30), 165.70 (C-1\(^\prime\)), 127.50 (C-2\(^\prime\)), 137.42 (C-3\(^\prime\)), 15.00 (C-4\(^\prime\)), 22.89 (C-5\(^\prime\)).

ESI-MS (m/z): 553.4 (M-1).

Elemental anal.: C\(_{35}\)H\(_{54}\)O\(_5\) (554.4): cal. C 75.77%; H 9.81%; found C 75.75%; H 9.80%.

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Synthesis of 22β-[(3-methyl-1-oxo-2-butenyl)oxy]-3β-hydroxyolean-12-en-28-oic acid (85)

To a solution of LB 81 (100 mg, 0.18 mM) in methanol and tetrahydrofuran mixture (20 ml, 1:1), 6.80 mg (0.18 mM) sodium borohydride was added and stirred at room temperature. After reaction completion, the solvent was removed in vacuo and the residue was diluted with water (15ml). The mixture was acidified with dilute HCl and extracted with ethyl acetate (3 x 15ml). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed over silica gel (100-200 mesh) and eluted with hexane : ethyl acetate (4:1) to obtain 22β-[(3-methyl-1-oxo-2-butenyl)oxy]-3β-hydroxyolean-12-en-28-oic acid 85 as white solid (80 mg, 79.8%), Rf 0.56 (hexane : ethyl acetate :: 4:1), mp. 276-278 °C.

Analysis

IR (KBr) ν max: 3480.79 (O-H), 2949.59, 2875.08 (C-H aliphatic), 1717.98 (C=O, ester) cm⁻¹.

¹H NMR (CDCl₃, 400 MHz): δ 0.71 (s, 6H, 2 x CH₃), 0.81 (s, 3H, CH₃), 0.85 (s, 3H, CH₃), 0.93 (s, 6H, 2 x CH₃), 1.09 (s, 3H, CH₃), 2.94 (dd, J = 6.32, 1.60 Hz, 1H, C-18-H), 3.15 (dd, J = 4.92, 2.96 Hz, 1H, C-3α-II), 4.96 (s, 1H, C-22α-II), 5.29 (s, 1H, C-12-II), 5.48 (s, 1H, C-2′-H).

¹³C NMR (CDCl₃, 100 MHz): δ 38.75 (C-1), 33.77 (C-2), 79.05(C-3), 38.75 (C-4), 55.18 (C-5), 19.20 (C-6), 30.57 (C-7), 39.24 (C-8), 50.52 (C-9), 38.44 (C-10), 25.89 (C-11), 122.85 (C-12), 143.05 (C-13), 46.03 (C-14), 27.47 (C-15), 25.89 (C-16), 47.63 (C-17), 41.92 (C-18), 50.52 (C-19), 30.07 (C-20), 37.04 (C-21), 75.00 (C-22), 28.10 (C-23), 16.97 (C-24), 15.59 (C-25), 18.26 (C-26), 27.16 (C-27), 177.10 (C-28), 31.01 (C-29), 26.30 (C-30), 165.17 (C-1′), 115.99 (C-2′), 152.24 (C-3′), 20.24 (C-4′), 24.12 (C-5′).

ESI-MS (m/z): 553.3 (M-1).

Elemental anal.: C₃₉H₄₈O₄ (554.3): cal. C 75.77%; H 9.81%; found C 75.72%; H 9.82%.

Synthesis of 22β-hydroxy-3-oxooolean-12-en-28-oic acid (86)

A solution of LA 80 or LB 81 (100 mg, 0.18 mM) in ethanolic potassium hydroxide (10% w/v, 25 ml) was refluxed. After reaction completion, the solvent was removed in vacuo and the residue was diluted with water (15ml). The mixture was acidified with dilute HCl and extracted with ethyl acetate (3 x 15 ml). The combined
organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed over silica gel (100-200 mesh) and eluted with hexane : ethyl acetate (2:1) to obtain 22β-hydroxy-3-oxoolean-12-en-28-oic acid 86 as white solid (54.2 mg, 63.9%); Rf 0.35 (hexane : ethyl acetate :: 1:1), mp. 234-236 °C.

Analysis
IR (KBr) ν max: 3434 (O-H), 2946 (C-H aliphatic), 1703 (C=O, keto) cm⁻¹.

1H NMR (CDCl₃, 400 MHz): δ 0.78 (s, 3H, CH₃), 0.83 (s, 3H, CH₃), 0.97 (s, 3H, CH₃), 0.99 (s, 3H, CH₃), 1.02 (s, 3H, CH₃), 1.05 (s, 3H, CH₃), 1.09 (s, 3H, CH₃), 2.28-2.33 (m, 1H, C-2α-H), 2.44-2.51 (m, 1H, C-2b-H), 2.94 (dd, J = 13.80, 4.08 Hz, 1H, C-18-H), 3.85 (t, J = 3.24 Hz, 1H, C-22α-H), 5.29 (t, J = 3.44 Hz, 1H, C-12-H).

13C NMR (CDCl₃, 100 MHz): δ 39.28 (C-1), 34.16 (C-2), 217.84 (C-3), 47.44 (C-4), 55.30 (C-5), 19.54 (C-6), 32.22 (C-7), 39.15 (C-8), 46.90 (C-9), 36.79 (C-10), 24.32 (C-11), 122.41 (C-12), 143.28 (C-13), 42.13 (C-14), 27.80 (C-15), 23.56 (C-16), 52.32 (C-17), 41.23 (C-18), 46.02 (C-19), 30.15 (C-20), 38.00 (C-21), 74.34 (C-22), 26.48 (C-23), 21.48 (C-24), 15.13 (C-25), 16.93 (C-26), 25.75 (C-27), 180.70 (C-28), 33.88 (C-29), 27.16 (C-30).

ESI-MS (m/z): 469.3 (M+).

Elemental anal.: C₃₀H₄₆O₄ (470.68): cal. C 76.55%; H 9.85%; found: C 76.55%; H 9.84%.

Synthesis of 3β,22β-dihydroxy-3-oxoolean-12-en-28-oic acid (87)

To a solution of compound 86 (100 mg, 0.21 mM) in methanol and tetrahydrofuran mixture (20 ml, 1:1), ~7.00 mg (0.21 mM) sodium borohydride was added and stirred at room temperature. After reaction completion, the solvent was removed in vacuo and the residue was diluted with water (15ml). The mixture was acidified with dilute HCl and extracted with ethyl acetate (3 x 15ml). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed over silica gel (100-200 mesh) and eluted with hexane : ethyl acetate (2:1) to obtain 3β,22β-dihydroxy-3-oxoolean-12-en-28-oic acid 87 as white solid (71.2 mg, 70.7%), Rf 0.32 (hexane : ethyl acetate :: 1:1), mp. 210-212 °C.

Analysis
IR (KBr) ν max: 3446 (O-H), 2948 (C-H aliphatic), 1707 (C=O, acid) cm⁻¹.
$^1$H NMR (DMSO-$d_6$, 400 MHz): $\delta$ 0.73 (s, 3H, CH$_3$), 0.85 (s, 3H, CH$_3$), 0.89 (s, 3H, CH$_3$), 0.93 (s, 3H, CH$_3$), 1.07 (s, 3H, CH$_3$), 1.10 (s, 3H, CH$_3$), 1.24 (s, 3H, CH$_3$), 2.94 (d, $J$ = 11.76 Hz, 1H, C-18-H), 3.07 (t, $J$ = 6.48 Hz, 1H, C-3$\alpha$-H), 3.58 (s, 2H, C-3$\beta$-OH and C-22$\alpha$-H), 4.00 (s, 1H, C-22$\beta$-OH), 5.23 (s, 1H, C-12-H).

$^{13}$C NMR (DMSO-$d_6$, 100 MHz): $\delta$ 38.09 (C-1), 27.04 (C-2), 77.20 (C-3), 38.29 (C-4), 54.79 (C 5), 17.89 (C 6), 32.41 (C 7), 40.23 (C 8), 51.02 (C 9), 37.91 (C 10), 23.89 (C-11), 120.99 (C-12), 143.78 (C-13), 41.62 (C-14), 27.31 (C-15), 22.89 (C-16), 46.03 (C-17), 41.09 (C-18), 47.09 (C-19), 29.78 (C-20), 36.52 (C-21), 72.72 (C-22), 27.93 (C-23), 15.60 (C-24), 15.01 (C-25), 16.67 (C-26), 26.79 (C-27), 176.30 (C-28), 33.70 (C-29), 25.37 (C-30).

ESI-MS ($m/z$): 471.3 (M-1).

Elemental anal.: C$_{30}$H$_{46}$O$_4$ (472.40): cal. C 76.23%; H 10.24%; found: C 76.21%; H 10.25%.

Synthesis of 2-benzylidene-22$\beta$-hydroxy-3-oxooolean-12-en-28-oic acid (88)

To a solution of compound 86 (100 mg, 0.91 mM) in ethanolic potassium hydroxide solution (5% w/v, 25 ml), benzaldehyde (1.1 eq.) was added and stirred at room temperature. After reaction completion solvent was removed in vacuo and the residue was diluted with water (15 ml). The mixture was acidified with dilute HCl and extracted with ethyl acetate (3 x 15 ml). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed over silica gel (100-200 mesh) and eluted with hexane:ethyl acetate (2:1) to obtain the 2-benzylidene-22$\beta$-hydroxy-3-oxooolean-12-en-28-oic acid 88 as white solid (83.4 mg, 71.2%), $R_f$ 0.39 (hexane:ethyl acetate :: 1:1), mp.152-154 $^\circ$C.

Analysis

IR (KBr) $\nu$ max: 3525 (O-H), 2952 (C-H aliphatic), 1671 (C=O, aryldiene) cm$^{-1}$.

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 0.85 (s, 3H, CH$_3$), 0.87 (s, 3H, CH$_3$), 0.90 (s, 3H, CH$_3$), 1.12 (s, 3H, CH$_3$), 1.14 (s, 3H, CH$_3$), 1.18 (s, 3H, CH$_3$), 1.21 (s, 3H, CH$_3$), 3.02 (t, $J$ = 7.66 Hz, 1H, C-18-H), 3.92 (s, 1H, C-22$\alpha$-H), 5.39 (s, 1H, C-12-H), 7.31-7.44 (m, 5H, Ar-H), 7.53 (s, 1H, vinylic H).

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 39.23 (C-1), 137.59 (C-2), 207.92 (C-3), 41.29 (C-4), 53.00 (C-5), 22.70 (C-6), 31.90 (C-7), 42.35 (C-8), 52.42 (C-9), 38.18 (C-10), 24.38 (C-
11), 122.44 (C-12), 143.44 (C-13), 45.20 (C-14), 27.82 (C-15), 23.78 (C-16), 45.44 (C-17),
44.21 (C-18), 46.14 (C-19), 30.22 (C-20), 36.32 (C-21), 74.36 (C-22), 29.83 (C-23), 16.59
(C-24), 15.40 (C-25), 20.36 (C-26), 27.20 (C-27), 180.74 (C-28), 33.93 (C-29), 25.71 (C-
30), 135.95 (ArCH), 133.67 (C-1”), 128.49 (C-2”), 130.47 (C-3”), 128.59 (C-4”), 130.47
(C-5”), 128.49 (C-6”).

ESI-MS (m/z): 557.3 (M - 1).

Elemental anal.: C_{37}H_{50}O_4 (558.2): cal. C 79.53%; H 9.02%; found: C 79.51%; H 9.04%.

Synthesis of 2-(4-methoxybenzylidene)-22β-hydroxy-3-oxoolean-12-en-28-oic acid (89)

To a solution of compound 86 (100 mg, 0.21 mM) in ethanolic potassium hydroxide solution (5% w/v, 25 ml), 4-methoxybenzaldehyde (1.1 eq.) was added and stirred at room temperature. After reaction completion solvent was removed in vacuo and the residue was diluted with water (15 ml). The mixture was acidified with dilute HCl and extracted with ethyl acetate (3 x 15 ml). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed over silica gel (100-200 mesh) and eluted with hexane : ethyl acetate (2:1) to obtain the 2-
(4-methoxybenzylidene)-22β-hydroxy-3-oxoolean-12-en-28-oic acid 89 as yellow solid
(81.12 mg, 65.7%), R_f 0.38 (hexane : ethyl acetate :: 1:1), mp. 188-191 °C.

Analysis

IR (KBr) ν max: 3501 (O-H), 2951 (C-H aliphatic), 1675 (C=O, aryldene) cm⁻¹.

^1H NMR (CDCl₃, 400 MHz): δ 0.78 (s, 6H, 2 x CH₃), 0.83 (s, 3H, CH₃), 1.05 (s, 6H, 2 x
CH₃), 1.10 (s, 3H, CH₃), 1.14 (s, 3H, CH₃), 2.93 (d, J = 15.24 Hz, 1H, C-18-H), 3.76 (s,
3H, OCH₃), 3.85 (s, 1H, C-22α-H), 5.33 (s, 1H, C-12-H), 6.85 (d, J = 7.72 Hz, 2H, Ar-H),
7.34 (d, J = 7.72 Hz, 2H, Ar-H), 7.44 (s, 1H, vinylic II).

^13C NMR (CDCl₃, 100 MHz): δ 39.20 (C-1), 137.48 (C-2), 207.83 (C-3), 41.20 (C-4),
52.82 (C-5), 22.63 (C-6), 31.87 (C-7), 42.32 (C-8), 52.40 (C-9), 38.18 (C-10), 24.34 (C-
11), 122.36 (C-12), 143.48 (C-13), 45.01 (C-14), 27.80 (C-15), 23.79 (C-16), 45.47 (C-17),
44.39 (C-18), 46.14 (C-19), 30.19 (C-20), 36.20 (C-21), 74.31 (C-22), 29.93 (C-23), 16.56
(C-24), 15.41 (C-25), 20.35 (C-26), 27.19 (C-27), 180.85 (C-28), 33.91 (C-29), 25.68 (C-
30). 55.35 (OCH₃). 132.33 (ArCH), 128.61 (C-1”), 132.33 (C-2”), 113.96 (C-3”), 109.88
(C-4”), 113.96 (C-5”), 131.44 (C-6”).

ESI-MS (m/z): 587.3 (M-1).
Elemental anal.: C_{37}H_{50}O_5 (588.7): cal. C 77.31%; H 8.77%; found: C 77.30%; H 8.79%.

Synthesis of 2-(4-methylbenzylidene)-22β-hydroxy-3-oxoolean-12-en-28-oic acid (90)

To a solution of compound 86 (100 mg, 0.21 mM) in ethanolic potassium hydroxide solution (5% w/v, 25 ml), 4-methylbenzaldehyde (1.1 eq.) was added and stirred at room temperature. After reaction completion solvent was removed in vacuo and the residue was diluted with water (15 ml). The mixture was acidified with dilute HCl and extracted with ethyl acetate (3 x 15 ml). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed over silica gel (100-200 mesh) and eluted with hexane : ethyl acetate (2:1) to obtain the 2-(4-methylbenzylidene)-22β-hydroxy-3-oxoolean-12-en-28-oic acid 90 as yellow solid (97.7 mg, 81.4%, ), R_f 0.37 (hexane : ethyl acetate :: 1:1), mp. 165-167°C.

Analysis
IR (KBr) ν max: 3473 (O-H), 2949 (C-H aliphatic), 1675 (C=O, arylidene) cm⁻¹.

¹H NMR (DMSO-d₆+CDCl₃, 400 MHz): δ 0.85 (s, 3H, CH₃), 0.88 (s, 6H, 2 x CH₃), 1.12 (s, 6H, 2 x CH₃), 1.14 (s, 3H, CH₃), 1.71 (s, 3H, CH₃), 7.99 (d, J = 16.04 Hz, 1H, C-18-H), 3.81 (s, 1H, C-22α-H), 4.06 (br s, 1H, C-22β-OH), 5.34 (s, 1H, C-12-H), 7.22 (d, J = 7.96 Hz, 2H, Ar-H), 7.33 (d, J = 7.96 Hz, 2H, Ar-H), 7.42 (s, 1H, vinylic H).

¹³C NMR (DMSO-d₆+CDCl₃, 100 MHz): δ 38.65 (C-1), 138.21 (C-2), 206.78 (C-3), 41.03 (C-4), 52.19 (C-5), 20.94 (C-6), 31.45 (C-7), 41.89 (C-8), 51.18 (C-9), 38.13 (C-10), 23.87 (C-11), 120.81 (C-12), 143.76 (C-13), 44.44 (C-14), 27.28 (C-15), 22.23 (C-16), 44.76 (C-17), 43.61 (C-18), 45.92 (C-19), 29.76 (C-20), 35.66 (C-21), 72.76 (C-22), 29.28 (C-23), 16.19 (C-24), 14.88 (C-25), 19.84 (C-26), 26.98 (C-27), 176.47 (C-28), 33.61 (C-29), 23.11 (C-30), 25.10 (ArCH₃), 132.43 (ArCH), 132.38 (C-1′′′), 128.80 (C-2′′′), 129.96 (C-3′′′), 136.68 (C-4′′′), 129.96 (C-5′′′), 128.80 (C-6′′′).

ESI-MS (m/z): 571.3 (M-1).

Elemental anal.: C_{38}H_{52}O_4 (572.3): cal. C 79.68%; H 9.15%; found: C 79.66%; H 9.17%.

Synthesis of 2-(4-chlorobenzylidene)-22β-hydroxy-3-oxoolean-12-en-28-oic acid (91)

To a solution of compound 86 (100 mg, 0.21 mM) in ethanolic potassium hydroxide solution (5% w/v, 25 ml), 4-chlorobenzaldehyde (1.1 eq.) was added and stirred at room temperature. After reaction completion solvent was removed in vacuo and the residue was diluted with water (15 ml). The mixture was acidified with dilute HCl and
extracted with ethyl acetate (3 x 15 ml). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed over silica gel (100-200 mesh) and eluted with hexane : ethyl acetate (2:1) to obtain the 2-(4-chlorobenzylidene)-22β-hydroxy-3-oxoolean-12-en-28-oic acid 91 as yellow solid (83.5 mg, 67.8%), Rf 0.37 (hexane : ethyl acetate :: 1:1), mp. 132-134 °C.

**Analysis**

**IR (KBr) ν max:** 3224.81 (O-H), 1627.96 (C=O aryldene), 781.39 (C-Cl) cm⁻¹.

**¹H NMR (CDCl₃, 400 MHz):** δ 0.79 (s, 6H, 2 x CH₃), 0.84 (s, 3H, CH₃), 1.06 (s, 3H, CH₃), 1.10 (s, 3H, CH₃), 1.14 (s, 3H, CH₃), 1.18 (s, 3H, CH₃), 2.97 (dd, J = 13.88, 3.88 Hz, 1H, C-18-H), 3.87 (t, J = 3.44 Hz, 1H, C-22α-H), 5.34 (t, J = 3.18 Hz, 1H, C-12-H), 7.19 (s, 1H, vinylic H), 7.36 (dd, J = 6.84, 1.8 Hz, 2H, Ar-H), 7.94 (dd, J= 6.76, 1.76 Hz, 2H, Ar-H).

**¹³C NMR (CDCl₃, 100 MHz):** δ 39.30 (C-1), 143.38 (C-2), 207.74 (C-3), 41.30 (C-4), 52.95 (C-5), 22.64 (C-6), 31.85 (C-7), 42.32 (C-8), 52.59 (C-9), 38.20 (C-10), 25.64 (C-11), 122.83 (C-12), 140.24 (C-13), 45.19 (C-14), 27.77 (C-15), 23.74 (C-16), 45.39 (C-17), 44.13 (C-18), 46.07 (C-19), 30.17 (C-20), 36.29 (C-21), 74.35 (C-22), 29.72 (C-23), 16.43 (C-24), 15.37 (C-25), 20.30 (C-26), 27.16 (C-27), 180.88 (C-28), 33.87 (C-29), 24.35 (C-30), 136.17 (ArCH), 134.18 (C-1'''), 128.70 (C-2''''), 131.57 (C-3'''), 134.32 (C-4'''), 128.86 (C-5'''), 127.84 (C-6''').

**ESI-MS (m/z):** 591.3 (M-2).

**Elemental anal.:** C₃₇H₄₆ClO₄ (593.3); cal. C 74.91%; H 8.33%; found: C 74.90%; H 8.31%.

**Synthesis of 2-(4-hydroxy-3-methoxybenzylidene)-22β-hydroxy-3-oxoolean-12-en-28-oic acid (92)**

To a solution of compound 86 (100 mg, 0.21 mM) in ethanolic potassium hydroxide solution (5% w/v, 25 ml), 4-hydroxy-3-methoxybenzaldehyde (1.1 eq.) was added and stirred at room temperature. After reaction completion solvent was removed in vacuo and the residue was diluted with water (15 ml). The mixture was acidified with dilute HCl and extracted with ethyl acetate (3 x 15 ml). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed over silica gel (100-200 mesh) and eluted with hexane : ethyl acetate...
(2:1) to obtain the 2-(4-hydroxy-3-methoxybenzylidene)-22β-hydroxy-3-oxoolean-12-en-28-oic acid 92 as yellow solid (94.6 mg, 74.6%), R_f 0.24 (hexane : ethyl acetate :: 1:1), mp. 147-149 °C.

**Analysis**

IR (KBr) ν max: 3437 (O-H), 2969 (C-H aliphatic), 1691 (C=O, arylidene), 1275 (C-O-C) cm⁻¹.

**¹H NMR (CDCl₃, 400 MHz):** δ 0.85 (s, 3H, CH₃), 0.89 (s, 3H, CH₃), 0.91 (s, 3H, CH₃), 1.08 (s, 3H, CH₃), 1.13 (s, 3H, CH₃), 1.17 (s, 3H, CH₃), 1.21 (s, 3H, CH₃), 3.02 (t, J = 8.88 Hz, 1H, C-18-H), 3.92 (s, 3H, OCH₃), 3.94 (s, 1H, C-22α-H), 5.39 (s, 1H, C-12-H), 6.94 (dd, J = 5.44, 4.08 Hz, 2H, Ar-H), 7.02 (d, J = 1.04 Hz, 1H, Ar-H), 7.48 (1H, s, vinylic H).

**¹³C NMR (CDCl₃, 100 MHz):** δ 38.12 (C-1), 137.59 (C-2), 207.70 (C-3), 39.20 (C-4), 52.34 (C-5), 22.64 (C-6), 30.18 (C-7), 41.28 (C-8), 46.10 (C-9), 36.26 (C-10), 24.35 (C-11), 122.34 (C-12), 143.43 (C-13), 44.27 (C-14), 27.78 (C-15), 23.75 (C-16), 45.06 (C-17), 42.28 (C-18), 45.55 (C-19), 29.83 (C-20), 33.89 (C-21), 74.33 (C-22), 29.69 (C-23), 16.52 (C-24), 15.41 (C-25), 20.34 (C-26), 27.16 (C-27), 180.36 (C-28), 31.83 (C-29), 25.68 (C-30), 55.98 (OCP₃), 131.91 (ArCII), 128.85 (C-1³'), 110.86 (C-2³'), 149.51 (C-3³'), 148.64 (C-4³'), 114.09 (C-5³'), 123.47 (C-6³').

**ESI-MS (m/z):** 603.4 (M-1).

**Elemental anal.:** C_{39}H_{52}O_{6} (604.5): cal. C 75.46%; H 8.67%; found: C 75.44%; H 8.69%.

**Synthesis of 2-(3-phenylprop-2-en-1-ylidene)-22β-hydroxy-3-oxoolean-12-en-28-oic acid (93)**

To a solution of compound 86 (100 mg, 0.21 mM) in ethanolic potassium hydroxide solution (5% w/v, 25 ml), cinnamaldehyde (1.1 eq.) was added and stirred at room temperature. After reaction completion solvent was removed *in vacuo* and the residue was diluted with water (15 ml). The mixture was acidified with dilute HCl and extracted with ethyl acetate (3 x 15 ml). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed over silica gel (100-200 mesh) and eluted with hexane : ethyl acetate (2:1) to obtain the 2-(3-phenylprop-2-en-1-ylidene)-22β-hydroxy-3-oxoolean-12-en-28-oic acid 93 as yellow solid (105.9 mg, 86.7%), R_f 0.39 (hexane : ethyl acetate :: 1:1), mp. 90-92 °C.
Analysis

IR (KBr) ν max: 3662 (O-H), 2998 (C-H aliphatic), 1686 (C=O, arylidene), 1583 (C=C) cm⁻¹.

¹H NMR (CDCl₃, 400 MHz): δ 0.94 (s, 3H, CH₃), 0.97 (s, 3H, CH₃), 1.14 (s, 3H, CH₃), 1.15 (s, 6H, 2 x CH₃), 1.20 (s, 3H, CH₃), 1.25 (s, 3H, CH₃), 3.05 (dd, J = 13.72, 9.92 Hz, 1H, C 18 H), 3.93 (t, J = 2.96 Hz, 1H, C 22α H), 5.44 (t, J = 3.16 Hz, 1H, C 12 H), 6.93 (q, J = 8.72 Hz, 2H, ArCHCH, ArCHCH), 7.25 (d, J = 11.20 Hz, 1H, ArCHCHCH), 7.26 (dd, J = 9.24, 4.84 Hz, 1H, Ar-H), 7.36 (dd, J = 8.00, 1.00 Hz, 2H, Ar-H), 7.48 (d, J = 7.16 Hz, 2H, Ar-H).

¹³C NMR (CDCl₃, 100 MHz): δ 39.19 (C-1), 136.68 (C-2), 207.33 (C-3), 41.22 (C-4), 53.06 (C-5), 22.63 (C-6), 31.94 (C-7), 42.33 (C-8), 52.42 (C-9), 38.24 (C-10), 24.36 (C-11), 122.38 (C-12), 143.42 (C-13), 45.08 (C-14), 27.79 (C-15), 23.76 (C-16), 45.35 (C-17), 42.42 (C-18), 46.11 (C-19), 30.19 (C-20), 36.07 (C-21), 74.33 (C-22), 29.64 (C-23), 16.61 (C-24), 15.50 (C-25), 20.29 (C-26), 27.20 (C-27), 180.74 (C-28), 33.90 (C-29), 25.64 (C-30), 140.66 (ArCHCHCH), 132.76 (ArCH), 123.37 (ArCHCH), 137.25 (C-1′′), 127.15 (C-2′′), 128.84 (C-3′′), 128.79 (C-4′′), 128.82 (C-5′′), 127.13 (C-6′′).

ESI-MS (m/z): 583.3 (M-1).

Elemental anal.: C₃₀H₅₂O₄ (584.3): cal. C 80.09%; H 8.96%; found: C 80.06%; H 8.97%.

Synthesis of 2-(4-dimethylaminobenzylidene)-22β-hydroxy-3-oxoolean-12-en-28-oic acid (94)

To a solution of compound 86 (100 mg, 0.21 mM) in ethanolic potassium hydroxide solution (5% w/v, 25 ml), 4-dimethylaminobenzaldehyde (1.1 eq.) was added and stirred at room temperature. After reaction completion solvent was removed in vacuo and the residue was diluted with water (15 ml). The mixture was acidified with dilute HCl and extracted with ethyl acetate (3 x 15 ml). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed over silica gel (100-200 mesh) and eluted with hexane : ethyl acetate (2:1) to obtain the 2-(4-dimethylaminobenzylidene)-22β-hydroxy-3-oxoolean-12-en-28-oic acid 94 as yellow solid (89.10 mg, 70.6%). Rr 0.31 (hexane : ethyl acetate :: 1:1). mp. 175-177 °C.

Analysis

IR (KBr) ν max: 3456 (O-H), 2998 (C-H aliphatic), 1668 (C=O, arylidene) cm⁻¹.
$^1$H NMR (CDCl$_3$, 400 MHz): δ 0.79 (s, 3H, CH$_3$), 0.80 (s, 3H, CH$_3$), 0.83 (s, 3H, CH$_3$), 1.05 (s, 6H, 2 x CH$_3$), 1.10 (s, 3H, CH$_3$), 1.14 (s, 3H, CH$_3$), 2.94 (s, 6H, 2 x NH$_3$), 2.98 (s, 1H, C-18-H), 3.86 (s, 1H, C-22α-H), 5.34 (s, 1H, C-12-H), 6.64 (d, $J = $ 8.76 Hz, 2H, Ar-H), 7.33 (d, $J = $ 8.92 Hz, 2H, Ar-H), 7.46 (s, 1H, vinylic H).

$^{13}$C NMR (CDCl$_3$, 100 MHz): δ 38.21 (C-1), 138.65 (C-2), 207.59 (C-3), 40.23 (C-4), 52.75 (C-5), 22.66 (C-6), 31.92 (C-7), 41.25 (C-8), 52.44 (C-9), 37.09 (C-10), 25.73 (C-11), 122.51 (C-12), 143.49 (C-13), 44.85 (C-14), 27.83 (C-15), 23.84 (C-16), 45.59 (C-17), 42.35 (C-18), 46.19 (C-19), 30.22 (C-20), 36.14 (C-21), 74.34 (C-22), 30.11 (C-23), 15.46 (C-24), 16.59 (C-25), 20.42 (C-26), 27.23 (C-27), 180.98 (C-28), 33.95 (C-29), 24.41 (C-30), 132.62 (ArCH), 123.97 (C-1’’), 132.62 (C-2’’), 111.78 (C-3’’), 150.40 (C-4’’), 111.78 (C-5’’), 128.94 (C-6’’), 39.24 (NCH$_3$), 39.31 (NCH$_3$).

ESI-MS (m/z): 600.4 (M-1).

Elemental anal.: C$_{39}$H$_{55}$NO$_4$ (601.4): cal. C 77.83%; H 9.21%; found: C 77.81%; H 9.19%.

**Synthesis of 2-((pyridine-4-ylmethylidene)-22β-hydroxy-3-oxoolean-12-en-28-oic acid (95)**

To a solution of compound 86 (100 mg, 0.21 mM) in ethanolic potassium hydroxide solution (5% w/v, 25 ml), pyridine-4-aldehyde (1.1 eq.) was added and stirred at room temperature. After reaction completion solvent was removed in vacuo and the residue was diluted with water (15 ml). The mixture was acidified with dilute HCl and extracted with ethyl acetate (3 x 15 ml). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed over silica gel (100-200 mesh) and eluted with hexane : ethyl acetate (2:1) to obtain the 2-((pyridine-4-ylmethylidene)-22β-hydroxy-3-oxoolean-12-en-28-oic acid 95 as yellow solid (75.81 mg, 64.7%), R$_f$ 0.18 (hexane : ethyl acetate :: 1:1), mp. 123-125 0°C.

**Analysis**

IR (KBr) ν max: 3479 (O-H), 2948 (C-H aliphatic), 1667 (C=O, aryldiene) cm$^{-1}$.

$^1$H NMR (DMSO-$d_6$+CDCl$_3$, 400 MHz): δ 0.79 (s, 3H, CH$_3$), 0.84 (s, 3H, CH$_3$), 0.93 (s, 3H, CH$_3$), 0.99 (s, 3H, CH$_3$), 1.04 (s, 3H, CH$_3$), 1.08 (s, 3H, CH$_3$), 1.10 (s, 3H, CH$_3$), 2.93 (dd, $J = $ 13.92, 3.92 Hz, 1H, C-18-H), 3.73 (s, 1H, C-22α-H), 4.08 (br s, 1H, C-22β-OH),
5.26 (t, J= 3.12 Hz, 1H, C-12-H), 6.85 (s, 1H, vinylic H), 7.02 (d, J = 5.84 Hz, 2H, Ar-H),
8.36 (d, J= 5.68 Hz, 2H, Ar-H).

$^{13}$C NMR (DMSO-$d_6$+CDCl$_3$, 100 MHz): $\delta$ 38.49 (C-1), 156.17 (C-2), 203.39 (C-3),
41.05 (C-4), 52.47 (C-5), 21.23 (C-6), 31.97 (C-7), 41.37 (C-8), 51.15 (C-9), 38.16 (C-10),
27.00 (C-11), 120.37 (C-12), 144.17 (C-13), 43.85 (C-14), 27.88 (C-15), 23.81 (C-16),
43.85 (C 17), 42.00 (C 18), 45.77 (C 19), 29.77 (C 20), 35.48 (C 21), 72.69 (C 22), 29.77
(C-23), 18.55 (C-24), 17.04 (C-25), 18.70 (C-26), 27.27 (C-27), 180.25 (C-28), 33.63 (C-
29), 25.26 (C-30), 132.34 (ArCH), 148.90 (C-1”’), 123.48 (C-2”’), 149.04 (C-3”’), 149.04
(C-5”’), 123.48 (C-6”’).

ESI-MS (m/z): 558.3 (M-1).

Elemental anal.: C$_{36}$H$_{50}$NO$_4$ (559.3): cal. C 77.10%; H 8.99%; found: C 77.12%; H
8.98%.

Synthesis of 2-(3-nitrobenzylidene)-22$\beta$-hydroxy-3-oxoolean-12-en-28-oic acid (96)

To a solution of compound 86 (100 mg, 0.21 mM) in ethanolic potassium hydroxide solution (5% w/v, 75 ml), 3-nitrobenzaldehyde (1.1 eq.) was added and stirred at room temperature. After reaction completion solvent was removed in vacuo and the residue was diluted with water (15 ml). The mixture was acidified with dilute HCl and extracted with ethyl acetate (3 x 15 ml). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed over silica gel (100-200 mesh) and eluted with hexane : ethyl acetate (2:1) to obtain the 2-
(3-nitrobenzylidene)-22$\beta$-hydroxy-3-oxoolean-12-en-28-oic acid 96 as brown solid
(78.37 mg, 62.1%), R$_f$ 0.12 (hexane : ethyl acetate :: 1:1), mp. 142-144 $^0$C.

Analysis

IR (KBr) $\nu$ max: 3376 (O-H), 2948 (C-H aliphatic), 1667 (C=O, arylicene), 1459 (N-O,
asym), 1261 (N-O, sym) cm$^{-1}$.

$^1$H NMR (DMSO-$d_6$+CDCl$_3$, 400 MHz): $\delta$ 0.81 (s, 6H, 2 x CH$_3$), 0.99 (s, 3H, CH$_3$), 1.02
(s, 6H, 2 x CH$_3$), 1.17 (s, 3H, CH$_3$), 1.24 (s, 3H, CH$_3$), 2.92 (d, J = 12.68 Hz, 1H, C-18-H),
3.70 (s, 1H, C-22$\alpha$-H), 4.38 (br s, 1H, C-22$\beta$-OH), 5.24 (s, 1H, C-12-H), 6.43-8.10 (m, 5H,
Ar-H and vinylic H).

$^{13}$C NMR (DMSO-$d_6$+CDCl$_3$, 100 MHz): $\delta$ 35.73 (C-1), 132.56 (C-2), 206.16 (C-3),
38.36 (C-4), 51.00 (C-5), 22.33 (C-6), 29.87 (C-7), 38.67 (C-8), 44.73 (C-9), 35.49 (C-10),
23.89 (C-11), 120.89 (C-12), 148.38 (C-13), 41.93 (C-14), 27.34 (C-15), 22.40 (C-16), 41.99 (C-17), 41.14 (C-18), 44.36 (C-19), 29.30 (C-20), 33.81 (C-21), 72.54 (C-22), 29.05 (C-23), 15.11 (C-24), 14.36 (C-25), 16.37 (C-26), 27.14 (C-27), 176.05 (C-28), 31.53 (C-29), 25.18 (C-30), 128.74 (ArCH), 128.74 (C-1’’), 118.24 (C-2’’), 135.74 (C-3’’), 115.54 (C-4’’), 120.89 (C-5’’), 128.60 (C-6’’).

ESI-MS (m/z): 600.4 (M 3).

Elemental anal.: C_{37}H_{49}NO_{6} (600.4): cal. C 73.60%; H 8.18%; found: C 73.59%; H 8.19%.

Synthesis of 2-(4-fluorobenzylidene)-22β-hydroxy-3-oxoolean-12-en-28-oic acid (97)

To a solution of compound 86 (100 mg, 0.21 mM) in ethanolic potassium hydroxide solution (5% w/v, 25 ml), 4-fluorobenzaldehyde (1.1 eq.) was added and stirred at room temperature. After reaction completion solvent was removed in vacuo and the residue was diluted with water (15 ml). The mixture was acidified with dilute HCl and extracted with ethyl acetate (3 x 15 ml). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed over silica gel (100-200 mesh) and eluted with hexane : ethyl acetate (2:1) to obtain the 2-(4-fluorobenzylidene)-22β-hydroxy-3-oxoolean-12-en-28-oic acid 97 as yellow solid (89.9 mg, 74.4%), Rf 0.37 (hexane : ethyl acetate :: 1:1), mp.159-161 ⁰C.

Analysis

IR (KBr) ν max: 3529 (O-H), 2949 (C-H aliphatic), 1672 (C=O, arylidene), 1020 (C-F) cm⁻¹.

¹H NMR (DMSO-d₆+CDCl₃, 400 MHz): δ 0.85 (s, 3H, CH₃), 0.87 (s, 6H, 2 x CH₃), 1.11 (s, 6H, 2 x CI₂), 1.13 (s, 3H, CI₃), 1.24 (s, 3H, CI₃), 2.96 (t, J = 13.64 Hz, 1H, C-18-II), 3.78 (s, 1H, C-22α-H), 4.23 (br s, 1H, C-22β-0H), 5.32 (s, 1H, C-12-H), 7.14 (t, J = 8.60 Hz, 2H, Ar-H), 7.40 (s, 1H, vinylc H), 7.46 (t, J = 8.04 Hz, 2H, Ar-H).

¹³C NMR (DMSO-d₆+CDCl₃, 100 MHz): δ 38.65 (C-1), 135.34 (C-2), 206.48 (C-3), 41.08 (C-4), 52.15 (C-5), 22.25 (C-6), 31.46 (C-7), 41.91 (C-8), 51.11 (C-9), 38.16 (C-10), 25.09 (C-11), 120.76 (C-12), 143.77 (C-13), 44.45 (C-14), 27.28 (C-15), 23.10 (C-16), 44.70 (C-17), 43.43 (C-18), 45.95 (C-19), 29.79 (C-20), 35.68 (C-21), 72.65 (C-22), 29.26 (C-23), 16.22 (C-24), 14.91 (C-25), 19.84 (C-26), 27.03 (C-27), 176.33 (C-28), 33.68 (C-30).
29), 23.87 (C-30), 133.05 (ArCH), 132.01 (C-1”), 131.93 (C-2”), 115.26 (C-3”), 160.65 (C-4”), 115.05 (C-5”), 131.52 (C-6”);

ESI-MS (m/z): 575.3 (M-1).

Elemental anal.: C_{37}H_{49}FO_4 (576.4): cal. C 77.05%; H 8.56%; found: C 77.03%; H 8.58%.

**Synthesis of 2-(3-phenylprop-2-en-1-ylidene)-22β-[2-methyl-1-oxo-2-butenyl]oxy]-3-oxoolean-12-en-28-oic acid (98)**

To a solution of compound 80 (100 mg, 0.18 mM) in ethanolic potassium hydroxide solution (5% w/v, 25 ml), cinnamic aldehyde (1.1 eq.) was added and stirred at room temperature. After reaction completion solvent was removed in vacuo and the residue was diluted with water (15 ml). The mixture was acidified with dilute HCl and extracted with ethyl acetate (3 x 15 ml). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed over silica gel (100-200 mesh) and eluted with hexane : ethyl acetate (4:1) to obtain the yellow solid as 2-(3-phenylprop-2-en-1-ylidene)-22β-[2-methyl-1-oxo-2-butenyl]oxy]-3-oxoolean-12-en-28-oic acid 98 (85.2 mg, 71.1%), R_f (hexane : ethyl acetate :: 4:1), mp 145-147 °C.

**Analysis**

IR (KBr) v max: 2951 (C-H aliphatic), 1715 (C=O, ester), 1672 (C=O, aryldene) cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ 0.86 (s, 3H, CH₃), 0.91 (s, 6H, 2 x CH₃), 1.02 (s, 3H, CH₃), 1.11 (s, 3H, CH₃), 1.15 (s, 3H, CH₃), 1.18 (s, 3H, CH₃), 2.97 (dd, J = 9.24, 8.40 Hz, 1H, C-18-H), 5.10 (t, J = 2.68 Hz, 1H, C-22α-H), 5.34 (s, 1H, C-12-H), 6.00 (dd, J = 5.44 Hz, 1H, C-14-H), 6.04 (dd, J = 10.04, 3.44 Hz, 2H, ArCH₂, ArCH₂), 7.26 (d, J = 9.80 Hz, 1H, ArCHCHCH), 6.94 (dd, J = 9.80 Hz, 1H, ArCHCHCH), 7.30 (dd, J = 5.32 Hz, 2.20 Hz, 1H, ArH), 7.36 (dd, J = 7.88, 1.6 Hz, 2H, ArH), 7.48 (d, J = 8.52 Hz, 2H, ArH).

¹³C NMR (100 MHz, CDCl₃): δ 39.13 (C-1), 137.30 (C-2), 207.18 (C-3), 37.72 (C-4), 53.06 (C-5), 22.62 (C-6), 30.07 (C-7), 38.64 (C-8), 50.70 (C-9), 36.07 (C-10), 25.70 (C-11), 122.49 (C-12), 143.22 (C-13), 42.36 (C-14), 29.34 (C-15), 24.22 (C-16), 45.06 (C-17), 42.18 (C-18), 45.32 (C-19), 29.72 (C-20), 33.72 (C-21), 76.73 (C-22), 29.64 (C-23), 15.68 (C-24), 15.45 (C-25), 16.49 (C-26), 27.55 (C-27), 179.93 (C-28), 31.94 (C-29), 26.19 (C-30), 166.28 (C-1’), 128.79 (C-2’), 140.70 (C-3’), 20.60 (C-4’), 14.15 (C-5’), 123.36
(ArCHCH), 132.65 (ArCH), 139.02 (ArCHCHCH), 136.67 (C-1’’), 127.15 (C-2’’), 128.79 (C-3’’), 128.48 (C-4’’), 128.84 (C-5’’), 127.61 (C-6’’).

**ESI-MS (m/z):** 665.4 (M-1).

**Elemental anal.:** C_{44}H_{58}O_5 (666.43); cal. C 79.24%; H 8.77%; found: C 79.25%; H 8.78%.

**Synthesis of 2-(3-phenylprop-2-en-1-ylidene)-22β-[(3-methyl-1-oxo-2-butenyl)oxy]-3-oxoolean-12-en-28-oic acid (99)**

To a solution of compound 81 (100 mg, 0.18 mM) in ethanolic potassium hydroxide solution (5% w/v, 25 ml), cinnamic aldehyde (1.1 eq.) was added and stirred at room temperature. After reaction completion solvent was removed in vacuo and the residue was diluted with water (15 ml). The mixture was acidified with dilute HCl and extracted with ethyl acetate (3 x 15 ml). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed over silica gel (100-200 mesh) and eluted with hexane: ethyl acetate (4:1) to obtain the yellow solid as 2-(3-phenylprop-2-en-1-ylidene)-22β-[(3-methyl-1-oxo-2-butenyl)oxy]-3-oxoolean-12-en-28-oic acid 99 as yellow solid (79.2 mg, 66.7%), R_f (hexane : ethyl acetate :: 4:1), mp. 159-161 °C.

**Analysis**

**IR (KBr) ν max:** 2947 (C-H aliphatic), 1682 (C=O, arylidene) cm^{-1}.

**^1H NMR (CDCl₃, 400 MHz):** δ 0.82 (s, 3H, CH₃), 0.94 (s, 6H, 2 x CH₃), 1.04 (s, 3H, CH₃), 1.13 (s, 3H, CH₃), 1.18 (s, 3H, CH₃), 1.25 (s, 3H, CH₃), 2.97 (d, J = 20.84 Hz, 1H, C-18-H), 5.05 (s, 1H, C-22α-H), 5.45 (s, 1H, C-12-H), 5.57 (s, 1H, C-2’’), 6.98 (q, J = 16.49, 15.44 Hz, 2H, ArC=CH₂), 7.27 (d, J = 11.52 Hz, 1H, ArC=CH₂), 7.32 (d, J = 4.92 Hz, 1H, ArH), 7.35 (dd, J = 8.24, 7.64 Hz, 2H, ArH), 7.48 (d, J = 7.28 Hz, 2H, ArH).

**^13C NMR (CDCl₃, 100 MHz):** δ 39.14 (C-1), 138.69 (C-2), 207.13 (C-3), 39.14 (C-4), 53.07 (C-5), 20.24 (C-6), 31.88 (C-7), 42.19 (C-8), 50.69 (C-9), 38.56 (C-10), 24.03 (C-11), 122.48 (C-12), 143.24 (C-13), 45.06 (C-14), 27.55 (C-15), 22.61 (C-16), 45.34 (C-17), 42.21 (C-18), 46.06 (C-19), 30.06 (C-20), 37.71 (C-21), 75.94 (C-22), 29.63 (C-23), 63 (C-24), 15.45 (C-25), 16.53 (C-26), 26.18 (C-27), 179.95 (C-28), 36.08 (C-29), 25.69 (C-30), 166.32 (C-1’’), 115.97 (C-2’’), 157.32 (C-3’’), 20.54 (C-4’’), 14.47 (C-5’’), 123.37 (ArCH),
136.69 (ArCHC), 140.69 (ArCHCCH), 137.29 (C-1”), 127.14 (C-2”), 132.66 (C-3”), 128.78 (C-4”), 128.83 (C-5”), 127.14 (C-6”).

**ESI-MS (m/z):** 665.4 (M-1).

**Elemental anal.:** C_{44}H_{58}O_{5} (666.43): cal. C 79.24%, H 8.77%; found: C 79.27%, H 8.77%.

**Synthesis of 2-(2-methyl-3-phenylprop-2-en-1-ylidene)-22β-[2-methyl-1-oxo-2-butenyloxy]-3-oxoolean-12-en-28-oic acid (100)**

To a solution of compound 80 (100 mg, 0.18 mM) in ethanolic potassium hydroxide solution (5% w/v, 25 ml), α-methyl-trans-cinnamic aldehyde (1.1 eq.) was added and stirred at room temperature. After reaction completion solvent was removed in vacuo and the residue was diluted with water (15 ml). The mixture was acidified with dilute HCl and extracted with ethyl acetate (3 x 15 ml). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed over silica gel (100-200 mesh) and eluted with hexane : ethyl acetate (4:1) to obtain the 2-(2-methyl-3-phenylprop-2-en-1-ylidene)-22β-[2-methyl-1-oxo-2-butenyloxy]-3-oxoolean-12-en-28-oic acid 100 as light yellow solid (83.1 mg, 67.1%), R_1 0.66 (hexane : ethyl acetate :: 4:1), mp.152-154 °C.

**Analysis**

**IR (KBr) ν max:** 2950 (C-H aliphatic), 1716 (C=O, ester), 1670 (C=O, arylidene) cm^{-1}.

**¹H NMR (CDCl₃, 400 MHz):** δ 0.84 (s, 3H, CH₃), 0.89 (s, 3H, CH₃), 1.00 (s, 3H, CH₃), 1.05 (s, 6H, 2 x CH₃), 1.09 (s, 3H, CH₃), 1.17 (s, 3H, CH₃), 3.07 (t, J = 8.28 Hz, 1H, C-18-H), 5.03 (t, J = 7.68 Hz, 1H, C-22α-H), 5.31 (s, 1H, C-12-H), 5.92 (dd, J = 4.16, 1.12 Hz, 1H, C-2′-H), 7.11-7.17 (m, 3H, ArH), 7.20-7.34 (m, 4H, ArH).

**¹³C NMR (CDCl₃, 100 MHz):** δ 38.47 (C-1), 138.75 (C-2), 206.96 (C-3), 39.23 (C-4), 55.31 (C-5), 19.49 (C-6), 30.04 (C-7), 39.23 (C-8), 50.61 (C-9), 37.72 (C-10), 24.20 (C-11), 122.50 (C-12), 143.10 (C-13), 45.97 (C-14), 26.46 (C-15), 23.52 (C-16), 46.89 (C-17), 42.00 (C-18), 47.43 (C-19), 27.57 (C-20), 36.78 (C-21), 75.89 (C-22), 26.92 (C-23), 15.63 (C-24), 15.10 (C-25), 19.49 (C-26), 26.14 (C-27), 179.62 (C-28), 33.68 (C-29), 25.78 (C-30), 166.29 (C-1′), 123.39 (C-2′), 155.48.10 (C-3′), 21.48 (C-4′), 20.53 (C-5′), 16.86 (ArCHCCH₃), 127.69 (ArCHC), 130.41 (ArCH), 143.10 (ArCHCCH), 138.75 (C-1′′), 127.69 (C-2′′), 129.30 (C-3′′), 128.43 (C-4′′), 129.30 (C-5′′), 128.24 (C-6′′).
ESI-MS (m/z): 679.6 (M-1).

Elemental anal.: C_{45}H_{60}O_{5} (680.44): cal. C 79.37%, H 8.80%; found: C 79.35%, H 8.81%.

Synthesis of 2-(2-methyl-3-phenylprop-2-en-1-ylidene)-22β-[(3-methyl-1-oxo-2-butenyl)oxy]-3-oxoolean-12-en-28-oic acid (101)

To a solution of compound 81 (100 mg, 0.18 mM) in ethanolic potassium hydroxide solution (5% w/v, 25 ml), α-methyl-trans-cinnamic aldehyde (1.1 eq.) was added and stirred at room temperature. After reaction completion solvent was removed in vacuo and the residue was diluted with water (15 ml). The mixture was acidified with dilute HCl and extracted with ethyl acetate (3 x 15 ml). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed over silica gel (100-200 mesh) and eluted with hexane : ethyl acetate (4:1) to obtain the 2-(2-methyl-3-phenylprop-2-en-1-ylidene)-22β-[(3-methyl-1-oxo-2-butenyl)oxy]-3-oxoolean-12-en-28-oic acid 101 as light yellow solid (86.4 mg, 70.8%), Rf 0.64 (hexane : ethyl acetate :: 4:1), mp. 167-169 °C.

Analysis

IR (KBr) ν max: 2951 (C-H aliphatic), 1714 (C=O, ester), 1652 (C=O, aryldiene) cm⁻¹.

¹H NMR (CDCl₃, 400 MHz): δ 0.77 (s, 3H, CH₃), 0.83 (s, 3H, CH₃), 0.96 (s, 6H, 2 x CH₃), 1.00 (s, 3H, CH₃), 1.06 (s, 3H, CH₃), 1.18 (s, 3H, CH₃), 3.04 (dd, J = 5.72, 2.39 Hz, 1H, C-18-H), 4.97 (t, J = 2.88 Hz, 1H, C-22α-H), 5.30 (t, J = 3.48 Hz, 1H, C-12-H), 5.49 (s, 1H, C-2'-'H), 7.15-7.47 (m, 7H, ArH, ArCH, ArCHCH).

¹³C NMR (CDCl₃, 100 MHz): δ 38.47 (C-1), 138.82 (C-2), 206.77 (C-3), 39.23 (C-4), 55.31 (C-5), 19.49 (C-6), 30.04 (C-7), 39.23 (C-8), 50.61 (C-9), 37.72 (C-10), 24.20 (C-11), 122.50 (C-12), 143.10 (C-13), 45.97 (C-14), 26.46 (C-15), 23.52 (C-16), 46.89 (C-17), 42.00 (C-18), 47.43 (C-19), 27.57 (C-20), 36.78 (C-21), 75.89 (C-22), 26.92 (C-23), 15.63 (C-24), 15.10 (C-25), 16.91 (C-26), 26.14 (C-27), 179.58 (C-28), 33.68 (C-29), 25.78 (C-30), 166.30 (C-1’), 115.95 (C-2’), 157.20 (C-3’), 21.48 (C-4’), 20.53 (C-5’), 16.86 (ArCH₂CH₃), 127.61 (ArCH), 134.47 (ArCHCH₃), 143.10 (ArCCCH), 138.82 (C-1’), 127.69 (C-2’), 129.44 (C-3’), 128.24 (C-4’), 130.47 (C-5’), 128.24 (C-6’).

ESI-MS (m/z): 679.6 (M-1).
**Elemental anal.:** C_{45}H_{66}O_{5} (680.44); cal. C 79.37%; H 8.80%; found: C 79.38%, H 8.80%.

**Synthesis of 2-(2-methyl-3-phenylprop-2-en-1-ylidene)-22β-hydroxy-3-oxoolean-12-en-28-oic acid (102)**

To a solution of compound 86 (100 mg, 0.21 mM) in ethanolic potassium hydroxide solution (5% w/v, 25 ml), α-methyl-trans-cinnamic aldehyde (1.1 eq.) was added and stirred at room temperature. After reaction completion solvent was removed in vacuo and the residue was diluted with water (15 ml). The mixture was acidified with dilute HCl and extracted with ethyl acetate (3 x 15 ml). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed over silica gel (100-200 mesh) and eluted with hexane: ethyl acetate (2:1) to obtain the 2-(2-methyl-3-phenylprop-2-en-1-ylidene)-22β-hydroxy-3-oxoolean-12-en-28-oic acid 102 as yellow solid (85.1 mg, 68.3%), R_f 0.41 (hexane : ethyl acetate :: 1:1), mp.135-137 °C.

**Analysis**

**IR (KBr) ν max:** 3416 (O-H), 2944 (C-H stretching), 1679 (C=O, arylidene) cm\(^{-1}\).

**\(^1\)H NMR (CDCl\(_3\), 400 MHz):** δ 0.78 (s, 3H, CH\(_3\)), 0.80 (s, 3H, CH\(_3\)), 0.83 (s, 3H, CH\(_3\)), 1.06 (s, 6H, 2 x CH\(_3\)), 1.08 (s, 3H, CH\(_3\)), 1.14 (s, 3H, CH\(_3\)), 2.93 (dd, J = 15.64, 4.56 Hz, 1H, C-18-H), 3.86 (s, 1H, C-22α-H), 5.34 (s, 1H, C-12-H), 7.11-7.46 (m, 7H, ArH, ArCH, ArCH\(_2\)CH).

**\(^{13}\)C NMR (CDCl\(_3\), 100 MHz):** δ 38.15 (C-1), 135.90 (C-2), 207.92 (C-3), 39.19 (C-4), 52.96 (C-5), 22.66 (C-6), 31.86 (C-7), 41.25 (C-8), 52.38 (C-9), 36.79 (C-10), 24.34 (C-11), 122.37 (C-12), 143.40 (C-13), 44.17 (C-14), 27.77 (C-15), 23.74 (C-16), 45.16 (C-17), 42.31 (C-18), 45.39 (C-19), 30.18 (C-20), 36.27 (C-21), 74.30 (C-22), 29.77 (C-23), 15.36 (C-24), 14.22 (C-25), 20.32 (C-26), 27.16 (C-27), 180.79 (C-28), 33.89 (C-29), 25.66 (C-30), 16.50 (ArCH\(_2\)CH\(_2\)CH), 128.25 (ArCH), 130.42 (ArCH\(_2\)CH\(_2\)CH), 137.55 (ArCH\(_2\)CH\(_2\)CH), 133.63 (C-1”), 128.32 (C-2”), 130.21 (C-3”), 129.09 (C-4”), 129.32 (C-5”), 128.44 (C-6”).

**ESI-MS (m/z):** 597.4 (M-1).

**Elemental anal.:** C_{40}H_{54}O_{4} (598.40); cal. C 80.22%; H 9.09%; found: C 80.20%; H 9.08%.
Synthesis of 2-(3,4-dimethoxybenzylidene)-22β-hydroxy-3-oxoolean-12-en-28-oic acid (103)

To a solution of compound 86 (100 mg, 0.21 mM) in ethanolic potassium hydroxide solution (5% w/v, 25 ml), 3,4-dimethoxybenzaldehyde (1.1 eq.) was added and stirred at room temperature. After reaction completion solvent was removed in vacuo and the residue was diluted with water (15 ml). The mixture was acidified with dilute HCl and extracted with ethyl acetate (3 x 15 ml). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was chromatographed over silica gel (100-200 mesh) and eluted with hexane : ethyl acetate (2:1) to obtain the 2-(3,4-dimethoxybenzylidene)-22β-hydroxy-3-oxoolean-12-en-28-oic acid 103 as yellow solid (93.1 mg, 72.1%), Rf 0.27 (hexane : ethyl acetate :: 1:1), mp. 195-197 °C.

Analysis

IR (KBr) ν max: 3433 (O-H), 2946 (C-H aliphatic), 1686 (C=O, arylidene), 1272 (C-O-C, ether) cm⁻¹.

¹H NMR (CDCl₃, 400 MHz): δ 0.86 (s, 3H, CH₃), 0.89 (s, 3H, CH₃), 0.91 (s, 3H, CH₃), 1.11 (s, 6H, 2 x CH₃), 1.17 (s, 3H, CH₃), 1.21 (s, 3H, CH₃), 3.04 (d, J = 15.04 Hz, 1H, C-18-1H), 3.85 (dd, J = 5.52, 2.84 Hz, 1H, C-22e-II), 3.90 (s, 3H, OCH₃), 3.91 (s, 3H, OCH₃), 5.40 (s, 1H, C-12-H), 6.90 (d, J = 8.44 Hz, 1H, Ar-H), 6.96 (d, J = 1.56 Hz, 1H, ArH), 7.07 (dd, J = 3.48, 1.44 Hz, 1H, ArH), 7.49 (s, 1H, vinylic H).

¹³C NMR (CDCl₃, 100 MHz): δ 38.28 (C-1), 137.59 (C-2), 207.73 (C-3), 39.18 (C-4), 60.44 (C-5), 20.34 (C-6), 29.82 (C-7), 41.18 (C-8), 52.83 (C-9), 36.23 (C-10), 22.62 (C-11), 122.15 (C-12), 143.59 (C-13), 44.29 (C-14), 25.64 (C-15), 21.06 (C-16), 45.03 (C-17), 42.28 (C-18), 45.54 (C-19), 27.82 (C-20), 33.25 (C-21), 74.32 (C-22), 27.22 (C-23), 15.40 (C-24), 14.20 (C-25), 16.52 (C-26), 24.36 (C-27), 180.70 (C-28), 30.16 (C-29), 23.75 (C-30), 55.91 (OCH₃), 55.98 (OCH₃), 128.85 (ArC=H), 131.88 (C-1’’), 110.18 (C-2’’), 149.53 (C-3’’), 148.63 (C-4’’), 114.13 (C-5’’), 123.49 (C-6’’).

ESI-MS (m/z): 617.3 (M-1).

Elemental anal. C₃₉H₅₄O₆ (618.84). cal. C 75.69%, H 8.80%, found. C 75.67%, H 8.81%.
Synthesis of 2-(2-methyl-3-phenylpropyl)-22β-hydroxy-3-oxoolean-12-en-28-oic acid (104)

To a solution of 2-(2-Methyl-3-phenylprop-2-en-1-ylidene)-22β-hydroxy-3-oxoolean-12-en-28-oic acid 102 (100 mg, 0.16 mM) in methanol (20 ml), palladium on carbon (0.15 mM) and ammonium formate (8.00 mM) was added and stirred at room temperature. After reaction completion the reaction mixture was filtered, dried over anhydrous sodium sulphate and evaporated to dryness. The residue was chromatographed over silica gel (100-200 mesh) and eluted with hexane : ethyl acetate (2:1) to obtain 2-(2-methyl-3-phenylpropyl)-22β-hydroxy-3-oxoolean-12-en-28-oic acid 104 as colourless solid (48.6 mg, 48.4%), $R_f$ 0.44 (hexane : ethyl acetate :: 1:1), mp. 61-63 °C.

Analysis

IR (KBr) ν max: 3466 (O-H), 2947 (C-H aliphatic), 1706 (C=O, keto) cm⁻¹.

$^1$H NMR (CDCl₃, 400 MHz): δ 0.78 (s, 3H, CH₃), 0.83 (s, 3H, CH₃), 0.85 (s, 1H, CH₃), 1.05 (s, 3H, CH₃), 1.07 (s, 3H, CH₃), 1.10 (s, 3H, CH₃), 1.14 (s, 3H, CH₃), 2.98 (dd, $J = 20.08, 16.02$ Hz, 1H, C-18-H), 3.84 (t, $J = 3.00$ Hz, 1H, C-22α-H), 5.31 (t, $J = 3.44$ Hz, 1H, C-12-H), 7.11-7.46 (m, 5H, ArH).

$^{13}$C NMR (CDCl₃, 100 MHz): δ 38.20 (C-1), 40.20 (C-2), 206.87 (C-3), 40.60 (C-4), 51.91 (C-5), 23.22 (C-6), 32.88 (C-7), 41.26 (C-8), 51.34 (C-9), 37.16 (C-10), 24.68 (C-11), 121.31 (C-12), 142.36 (C-13), 44.37 (C-14), 27.32 (C-15), 23.65 (C-16), 44.94 (C-17), 43.15 (C-18), 46.02 (C-19), 30.90 (C-20), 35.19 (C-21), 73.27 (C-22), 29.16 (C-23), 15.45 (C-24), 14.32 (C-25), 21.62 (C-26), 26.80 (C-27), 179.59 (C-28), 35.19 (C-29), 26.14 (C-30), 19.27 (ArCH₂CHCH₃), 28.73 (ArCH₂CH), 41.07 (ArCH₂CHCH₂), 44.14 (ArCH₂), 134.85 (C-1’’), 128.27 (C-2’’), 132.58 (C-3’’), 127.20 (C-4’’), 129.37 (C-5’’), 127.39 (C-6’’).

ESI-MS (m/z): 601.3 (M-1).

Elemental anal.: C₄₀H₅₈O₄ (602.43): cal. C 79.69%; H 9.70%; found: C 79.68%; H 9.71%.

Synthesis of 2-(3,4-dimethoxybenzyl)-22β-hydroxy-3-oxoolean-12-en-28-oic acid (105)

To a solution of 2-(3,4-Dimethoxybenzylidene)-22β-hydroxy-3-oxoolean-12-en-28-oic acid 103 (100 mg, 0.16 mM) in methanol (20 ml), palladium on carbon (0.15 mM) and ammonium formate (8.00 mM) was added and stirred at room temperature. After reaction
completion the reaction mixture was filtered, dried over anhydrous sodium sulphate and evaporated to dryness. The residue was chromatographed over silica gel (100-200 mesh) and eluted with hexane : ethyl acetate (2:1) to obtain 2-(3,4-dimethoxybenzyl)-22β-hydroxy-3-oxoolean-12-en-28-oic acid 105 as white solid (59.2 mg, 58.9%), \( R_f \) 0.35 (hexane : ethyl acetate :: 1:1), mp. 103-105\(^\circ\)C.

**Analysis**

IR (KBr) \( \nu \) max: 3491 (O-H), 2947 (C-H aliphatic), 1709 (C=O, keto), 1253 (C-O-C, ether) cm\(^{-1}\).

\(^1^H\) NMR (CDCl\(_3\), 400 MHz): \( \delta \) 0.77 (s, 3H, CH\(_3\)), 0.80 (s, 3H, CH\(_3\)), 0.87 (s, 6H, 2 x CH\(_3\)), 0.99 (s, 3H, CH\(_3\)), 1.09 (s, 6H, 2 x CH\(_3\)), 2.97 (dd, \( J = 6.80, 3.88 \) Hz, 1H, C-18-H), 3.87 (s, 3H, OCH\(_3\)), 3.89 (s, 3H, OCH\(_3\)), 3.91 (s, 1H, C-22α-H), 5.32 (s, 1H, C-12-H), 6.70-6.88 (m, 3H, ArH).

\(^1^C\) NMR (CDCl\(_3\), 100 MHz): \( \delta \) 35.52 (C-1), 32.79 (C-2), 217.23 (C-3), 37.07 (C-4), 62.67 (C-5), 21.68 (C-6), 28.68 (C-7), 38.32 (C-8), 51.33 (C-9), 33.65 (C-10), 22.43 (C-11), 121.80 (C-12), 141.94 (C-13), 41.02 (C-14), 26.14 (C-15), 21.98 (C-16), 41.14 (C-17), 40.19 (C-18), 45.04 (C-19), 28.68 (C-20), 33.60 (C-21), 73.35 (C-22), 28.35 (C-23), 14.53 (C-24), 13.11 (C-25), 15.70 (C-26), 24.65 (C-27), 179.73 (C-28), 29.09 (C-29), 23.35 (C-30), 54.77 (OCH\(_3\)), 54.81 (OCH\(_3\)), 30.89 (ArCH\(_2\)), 133.00 (C-1′′), 108.62 (C-2′′), 147.54 (C-3′′), 146.81 (C-4′′), 109.56 (C-5′′), 117.73 (C-6′′).

ESI-MS (m/z): 619.4 (M-1).

**Elemental anal.**: C\(_{39}\)H\(_{56}\)O\(_8\) (620.41): C 75.45%; H 9.09%; found: C 75.43%; H 9.10%.

**Synthesis of 2-(3-phenylpropyl)-22β-hydroxy-3-oxoolean-12-en-28-oic acid (106)**

To a solution of 2-(3-Phenylprop-2-en-1-ylidene)-22β-hydroxy-3-oxoolean-12-en-28-oic acid \( \mathbf{93} \) (100 mg, 0.17 mM) in methanol (20 ml), palladium on carbon (0.15 mM) and ammonium formate (8.00 mM) was added and stirred at room temperature. After reaction completion the reaction mixture was filtered, dried over anhydrous sodium sulphate and evaporated to dryness. The residue was chromatographed over silica gel (100-200 mesh) and eluted with hexane : ethyl acetate (2:1) to obtain 2-(3-phenylpropyl)-22β-hydroxy-3-oxoolean-12-en-28-oic acid \( \mathbf{106} \) as semi solid (47.4 mg, 46.6%). \( R_f \) 0.43 (hexane : ethyl acetate :: 1:1).

**Analysis**
IR (KBr) ν max: 3437 (O-H), 2944 (C-H aliphatic), 1700 (C=O, keto) cm⁻¹.

¹H NMR (CDCl₃, 400 MHz): δ 0.78 (s, 3H, CH₃), 0.89 (s, 3H, CH₃), 1.02 (s, 6H, 2 x CH₃), 1.07 (s, 3H, CH₃), 1.17 (s, 3H, CH₃), 1.22 (s, 3H, CH₃), 2.98 (t, J = 3.40 Hz, 1H, C₁₈-H), 3.89 (s, 1H, C-22α-H), 5.34 (t, J = 6.80 Hz, 1H, C-12-H), 7.14-7.17 (m, 2H, ArH), 7.24-7.28 (m, 3H, ArH).

¹³C NMR (CDCl₃, 100 MHz): δ 40.05 (C 1), 41.05 (C 2), 216.84 (C 3), 47.57 (C 4), 51.61 (C-5), 18.50 (C-6), 32.82 (C-7), 39.98 (C-8), 45.98 (C-9), 35.63 (C-10), 24.61 (C-11), 121.15 (C-12), 142.04 (C-13), 44.96 (C-14), 28.68 (C-15), 23.29 (C-16), 51.36 (C-17), 41.19 (C-18), 45.72 (C-19), 30.72 (C-20), 38.39 (C-21), 73.26 (C-22), 26.73 (C-23), 22.58 (C-24), 15.26 (C-25), 17.04 (C-26), 26.12 (C-27), 179.80 (C-28), 35.23 (C-29), 28.21(C-30), 28.92 (ArCH₂CH₂), 29.11(ArCH₂CH₂CH₂), 37.22 (ArCH₂), 141.54 (C-1′′), 127.24 (C-2′′), 127.36 (C-3′′), 124.23 (C-4′′), 127.36 (C-5′′), 127.24 (C-6′′).

ESI-MS (m/z): 587.4 (M-1).

Elemental anal.: C₅₉H₅₆O₄ (588.42): C 79.55%; H 9.59%; found: C 79.53%; H 9.60%.

3.4. In-vitro cytotoxicity study on NCI-60 cell lines and NCI’s COMPARE analysis

The NCI in-vitro anticancer screening was two-stage process, beginning with the evaluation of selected compounds 80, 81, 84, 85, 86, 87, 92 and 93 against the NCI’s 60 human tumor cell lines composite of nine different type of cancers at a single high dose (10 μM) concentration. The output from the single dose screen was reported as a mean graph and available for analysis by the NCI’s COMPARE program. Compounds which exhibited significant growth inhibition were further evaluated against the same 60 human tumor cell lines at five concentration levels ranging from 0.01-100 μM concentration.

The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96 well microtiter plates in 100 μL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37°C, 5 % CO₂, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line were fixed in situ with trichloroacetic acid (TCA), to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs were solubilized in dimethyl sulfoxide at 400-fold
the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/ml gentamicin. Additional four, 10-fold or ½ log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 μl of these different drug dilutions were added to the appropriate microtiter wells already containing 100 μl of medium, resulting in the required final drug concentrations.

Following drug addition, the plates were incubated for an additional 48 h at 37°C, 5 % CO₂, 95 % air, and 100 % relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 μl of cold 50 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded, and the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μl) at 0.4 % (w/v) in 1 % acetic acid was added to each well, and plates were incubated for 10 minutes at room temperature. After staining, unbound dye was removed by washing five times with 1 % acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology was the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 μl of 80 % TCA (final concentration, 16 % TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as:

\[
\frac{[(T_i-T_z)/(C-T_z)]}{(T_i-T_z)/T_z} \times 100 \text{ for concentrations for which } T_i \geq T_z
\]

\[
\frac{[(T_i-T_z)/T_z]}{T_i-T_z} \times 100 \text{ for concentrations for which } T_i < T_z
\]

Three dose response parameters were calculated for each experimental agent. Growth inhibition of 50 % (GI₅₀) is calculated from \([(T_i-T_z)/(C-T_z)] \times 100 = 50\), which was the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) is calculated from Ti = Tz. The LC₅₀ (concentration of drug resulting in a 50% reduction in the measured protein at the end
of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50. Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested [Boyd and Paull, 1995].

The NCI’s COMPARE algorithm utilizes the in vitro antitumor results in determining and expressing the degree of similarity, or mean-graph profiles generated on a similar compound or different compounds [Shoemaker, 2006]. Dose response parameters were used as seed to calculate Pearson correlation coefficient (PCC) with various set compounds and prediction of probable molecular mechanistic targets.

3.5. Selective cancer cytotoxicity, mechanistic studies and docking analysis of novel congeners of lantadene

3.5.1. Cell culture and MTT assay

The human leukemic cells HL-60, colorectal carcinoma cells HCT116, breast adenocarcinoma cells MCF7, human lung cancer cells A549 and African green monkey kidney fibroblast VERO cells were grown in RPMI medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin (Gibco, Invitrogen, USA). For assay, phenol red-free RPMI medium (Sigma-Aldrich, USA) supplemented with 5% fetal bovine serum and 1% penicillin-streptomycin was used. HL-60 (15,000 cells/well), HCT116 and MCF7 (3000 cells/well), and A549 (4000 cells/well) were seeded into 96-well plates and incubated overnight for cell attachment. For treatment, compounds were added at concentrations ranging from 0.01 to 100 μM and incubated for 48 hours. At the end of incubation, 20 μl/well of 5 mg/mL thiazoyl blue tetrazolium bromide (MTT) (Amresco, USA) was added and cells were further incubated for 4 hours. Supernatant was then removed and the purple formazan which has formed was dissolved using 100 μl of DMSO (Fisher Scientific, UK). Absorbance was read at 570 nm using Spectra Max M4 microplate reader (Molecular Devices Inc., US).

3.5.2. NF-κB Luciferase assay

The A549 cells were cultured in 12-well plates and transiently co-transfected with 0.2μg of a pNF-κB-Luc vector (Stratagene, La Jolla, CA) and 0.2 μg of pSV-β-galactosidase dissolved in 3μL lipofectamine™ or lipofectamine™ 2000 (Invitrogen,
Carlsbad, CA) as the internal control. The plasmids were transfected according to the manufacturer’s instructions. After 6 h, the medium was changed to complete medium and cultured for 6 hours, and then the transfected cells were treated with different compounds in complete medium for 24 hours. The A549 cell extracts were harvested using 150μL of lysis buffer (Tropix, Inc., Bedford, MA) per well. To measure the luciferase and β-galactosidase activities, cell extracts (20μL each) were assayed separately using the Luciferase Assay Kit and Galacto-Light Plus™ system (Tropix, Inc.), respectively. Luciferase activity was measured and analyzed using an FB12 luminometer (Zylux Corporation, Oak Ridge, TN) [Liang et al., 2009].

3.5.3. Akt kinase inhibition assay

AKT1/PKBa KinEASE™ FP Fluorescein Green Assay kit for fluorescence polarization experiments and Akt1 enzyme were purchased from Upstate, Millipore Corporation (Charlottesville, VA). The enclosed experimental protocol of the KinEASE™ kit was followed. Total reaction volume per well was 25μL. In Corning Costar 384-well black plates, various concentrations of compound in DMSO was diluted with buffer containing 50mM HEPES (pH 7.2), 0.01% BSA, 5mM MgCl₂, 1mM DTT. STK Substrate 3 (final concentration 10 μM) and Akt1 (concentration needed to achieve 70% activity) were added to each well and incubated for 10 min at 25 °C, ATP (final concentration 100μM) was added to start the reactions. After 1 h incubation at 25 °C, the reactions were quenched with 5 μL STK stop mix including the phosphorylated STK tracer, 5μL STK antibody mix were then added and the mixture were incubated for 6 h at 25 °C before reading. Fluorescence polarization were recorded by using a TECAN infinite® M1000 multimode reader at 25 °C, excitation: 470nm, emission: 530nm, z-position: 23,580μm. The data was fitted by using nonlinear regression in GraphPad Prism 5, and the IC₅₀ values were obtained from the dose response curves. All data are obtained as average values from triplicate samples, and the experiments were repeated twice [Lindsley et al., 2008].

3.5.4. Western blot analysis

The A549 cells (2 x 10⁶ cells) were incubated at 37 °C for 12 hr in 2 mL of RPMI containing 10% FBS and the corresponding concentrations of each test compounds. After incubation, the cells were washed three times with PBS, dipped in 150 mL of ice-cold lysis buffer (20 mM HEPES, pH 7.4, 1% Triton-X 100, 10% glycerol, 1M sodium fluoride,
2.5 mM \( p \)-nitrophenylene phosphate, 10 mg/mL of phenylmethylsulfonylfluoride, 1 mM Na\(_3\)VO\(_4\), 5 mg/mL of leupeptin, and 1 mM EDTA) for 15 min, and disrupted with a Sonic Disruptor (UR-20P, TOMY). The lysis buffer containing the disrupted cells was centrifuged at 13,000 x g and 37 \(^\circ\)C for 20 min. The supernatant fraction obtained was boiled for 5 min in 3 x sample buffer (50 mM Tris, pH 7.4, 4% SDS, 10% glycerol, 4% 2-mercaptoethanol, and 0.05 mg/mL of bromophenol blue) at a ratio of 2:1 (v/v), loaded on an acrylamide gel (8 or 10%) and subjected to electrophoresis (150 min at 125 V). The antibody for NF-\( \kappa \)B and phospho-GSK3\( \beta \)(S9), was purchased from Santa Cruz Biotechnology and Cell Signalling Technologies respectively and Western blotting was carried out as described previously [Ban et al., 2002]. The levels of each protein were quantified by scanning densitometry, and the individual band density value for each point was expressed as the relative density signal.

3.5.5. Docking analysis

The 2D structures of the compounds were constructed using ACD/ChemSketch 11.0 and prepared by using UCSF Chimera-1.6.1 [Pettersen et al., 2004]. The 3D crystal structure of the nuclear factor kappa-B (NF-\( \kappa \)B) P50 homodimer (PDB ID: 1NFK), structure of the NF-kappa B p50,p65 heterodimer bound to the interferon \( \beta \)-\( \kappa \)B site (PDB ID: 1LE9) was obtained from the RCSB protein data bank (http://www.pdb.org). Automated molecular docking was performed to find out molecular interaction and optimized geometry by using docking software AutoDock 4.2. On the basis of Lamarckian genetic algorithm principle [Cosconati et al., 2010] all docking parameters were set to default values.

3.6. Anti-melanoma activity and apoptotic studies of 2-(3-phenylprop-2-en-1-ylidene)-22\( \beta \)-hydroxy-3-oxoolean-12-en-28-oic acid (93)

3.6.1. In-vitro antitumor activity

3.6.1.1. Cell culture and cytotoxicity assay

The African green monkey kidney fibroblast (VERO) cells were grown in RPMI medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin (Gibco, Invitrogen, USA). For assay, phenol red-free RPMI medium (Sigma-Aldrich, USA) supplemented with 5% fetal bovine serum and 1% penicillin-streptomycin was
used. The B16F10 (mouse melanoma cell) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Hyclone Laboratories, Logan, USA), 100 U/ml penicillin (Sigma) and 100 μg/ml streptomycin (Sigma).

3.6.1.2. Morphology assessment

The B16F10 cells (1×10⁵ cells/well) were seeded in 6-well plates and after 24 h of incubation; the cells were treated with different concentrations of compound 93 for 24 h. Then cells were then washed with PBS, fixed with 70% ethanol for 15 min and then washed with PBS. The cells were stained with DAPI (1 μg/ml) for 15 min, washed with PBS again, and then observed under a fluorescence microscope equipped with a Cool SNAP-Pro color digital camera.

3.6.1.3. DNA fragmentation assay

B16F10 cells were incubated with 0, 5 and 10μM of 93 for 24 h at 37 °C. DNA fragmentation was analyzed by electrophoresis as described earlier [Smith et al., 1989]. Briefly, after exposure to trypsin, the cells (10⁷ cells per sample) were washed with Tris-buffered saline (TBS) buffer (pH 7.6) and collected by centrifugation at 1000 g for 10 minutes. The pellet was re-suspended for 2 h at 50 °C in a lysis solution made up of 10 mM Tris-HCl (500 μL, pH 8.0), 150 mM NaCl, 10 mM ethylenediamine tetraacetic acid (EDTA, edetic acid), 0.4% sodium dodecyl sulfate (SDS) and 100 μg/ml proteinase K. The lysate was then extracted with equal volumes of phenol/ CHCl₃/ isoamyl alcohol (25:24:01). The DNA was precipitated with ethanol (EtOH), air-dried and dissolved in TE buffer (5mM Tris-HCl (pH 8.0) and 20 mM edetic acid containing RNase A [0.1 mg/ml, Sigma]). The samples were run in agarose gel containing ethidium bromide (0.5 μg/ml) and were visualized under ultraviolet (UV) light.

3.6.1.4. Caspase-3 activity

Cells (1.5×10⁶ cells/ml) were treated with compound 93 (at 5 and 10 μM) in 12-well plates for 24 h. Caspase-3 activity was measured using a Caspase-3 colorimetric assay kit (BioVision, USA). Briefly, compound 93 treated cells were washed with PBS, and were lysed with cell lysis buffer for 1 min on ice. Each cell lysate was centrifuged at 10000g for 1 min, and the supernatant was collected. After protein quantification using a DC protein assay kit (Bio-Rad Laboratories, USA), 50 μg protein was diluted to 50 μL with cell lysis
buffer and to that, 50 μL reaction buffer was added. The absorbance of each sample mixture was measured at 400 nm. Finally, 5 μL 4 mM DEVD-pNA (caspase-3 substrate) was added to the mixture and incubated at 37 °C for 1 h. The absorbance of the final reaction mixture was measured at the same wavelength.

3.6.1.5. Western blot analysis

The B16F10 cells (2 x 10^6 cells) were incubated at 37 °C for 12 hr in 2 mL of DMEM containing 10% FBS and the corresponding concentrations of test compound. After incubation, the cells were washed three times with PBS, dipped in 150 mL of ice-cold lysis buffer (20 mM HEPES, pH 7.4, 1% Triton-X 100, 10% glycerol, 1M sodium fluoride, 2.5mM p-nitrophenylec phosphate, 10 mg/mL of phenylmethysulfonyl fluoride, 1mM Na3VO4, 5mg/mL of leupeptin, and 1mM EDTA) for 15 min, and disrupted with a Sonic Disrupter (UR-20P, TOMY). The lysis buffer containing the disrupted cells was centrifuged at 13,000 x g and 37 °C for 20 min. The supernatant fraction obtained was boiled for 5 min in 3 x sample buffer (50 mM Tris, pH 7.4, 4% SDS, 10% glycerol, 4% 2-mercaptoethanol, and 0.05 mg/mL of bromophenol blue) at a ratio of 2:1 (v/v), loaded on an acrylamide gel (8 or 10%) and subjected to electrophoresis (150 min at 125 V). The antibody for NF-κB and c-jun, Bcl-2, Bax and caspase-3 was purchased from Santa Cruz Biotechnology and Cell Signalling Technologies respectively and Western blotting was carried out as described previously [Ban et al., 2002]. The levels of each protein were quantified by scanning densitometry, and the individual band density value for each point was expressed as the relative density signal.

3.6.1.6. Flow cytometric analysis

The effects of compound 93 treatment on B16F10 cell cycle progression was determined by flow cytometric analysis. The DNA content was assessed by staining ethanol fixed cells with propidium iodide (PI). Briefly, the B16F10 cells were seeded in a 6 well plate at a density of 5 x 10^5 cells per well and allowed to attach overnight. The cell growth medium was replaced with fresh medium dosed with various concentration of 93 or DMSO (control). After 24 h of incubation (37°C, 5% CO2) the cells were harvested, washed twice with ice-cold PBS, fixed with 75% ethanol at 4 °C for 30 min, and then stained using a DNA staining kit with propidium iodide (Cycle Test Plus kit, Becton-Dickinson, San Jose, CA, USA). The DNA content at sub-G1, G1, S and G2/M phases was
then determined using a FACS Calibur (Becton-Dickinson, San Jose, CA, USA) and analyzed by Cell Quest software.

3.6.2. *In-vivo* antitumor activity

3.6.2.1. *In-vivo* anti-melanoma activity

B16F10 mouse melanoma cells were collected from cell cultures by trypsinization and then injected subcutaneously (1×10^6 cells) into the right flank region of C57BL/6 mice. After 24h of tumor induction, the mice were intraperitoneally injected with either olive oil vehicle alone (tumor control), 10mg/kg of 93 in olive oil or 5mg/kg cisplatin in PBS at 3 days intervals over 28 day treatment period. The length (A) and width (B) of the tumor from each mouse was measured every 3 days and the tumor volume was calculated using the formula, V= AB^2/2. After the treatment period, the animals were sacrificed and the tumors were removed and immediately weighed. The tumor inhibition ratio was calculated by the formula: inhibition ratio (%) = [(A-B)/A] × 100, whereby A was the average tumor weight of the negative control and B was the average tumor weight of the treated group. The death of each animal was recorded starting from the first day of tumor allograft. The percentage of surviving mice was determined at the designated time.

3.6.2.2. Liver enzymes quantification

To access the safety of compound 93 in animals, we evaluated a possible hepatotoxic effect, evaluating three enzymes associated with liver injury, the glutamic-oxalacetic transaminase (GOT), the glutamic pyruvic transaminase (ALT) and gamma glutamyl transferase (gamma-GT). The experimental procedure consists in a simple addition of the 50 μL of the serum sample to 1000 μL of commercial reagent for each tested enzyme [Stark et al., 1986]. After 28 days of melanoma cells allograft and compound 93 treatments has started the blood was collect.

3.6.2.3. Effects on blood cells

The blood samples were collected after 28 days of tumor allograft from the axillary plexus of the animals in all experimental groups, which were used to perform blood and reticulocyte count, according to the techniques used in the Laboratory of Experimental Hematology of FCF/USP [Fock et al., 2008].