

Lantadenes and Their Esters as Potential Antitumor Agents

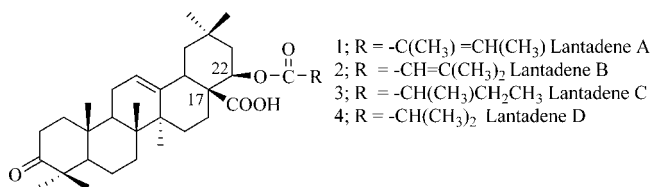
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Lantadenes are pentacyclic triterpenoids of the weed *Lantana camara*. Five new lantadenes (**14–18**) and their methyl esters (**20–24**) were synthesized, characterized, and screened for cytotoxicity against four human cancer cell lines. The parent compound (**1**) and the four most active compounds (**15**, **16**, **21**, and **22**) were further studied for their *in vivo* tumor inhibitory potential on squamous cell carcinogenesis in Swiss albino mice induced by 7,12-dimethylbenz[*a*]anthracene (DMBA) and promoted by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). These results were supported by histopathological studies and discussed in terms of structure–activity relationships. The results inferred the importance of the groups attached to C-22 and C-17 in relation to the antitumor activity of these compounds.

Lantana camara L. (Verbenaceae) is one of the most noxious weeds that grows wild in tropical and subtropical parts of the world.¹ It provides a huge amount of biomass that is of interest to exploit for natural products in drug research.² Some pentacyclic triterpenoids are known to have antibacterial, anti-inflammatory, antitumor, or anti-AIDS activity.^{3,4} Lantadenes (**1–4**) isolated from *L. camara* belong to the oleanane series, which have attracted considerable interest mainly because of their toxicity and antitumor activity. Compounds **1** and **2** were found to inhibit Epstein–Barr virus activation in Raji cells induced by 12-*O*-tetradecanoylphorbol-13 acetate (TPA) and also exhibited tumor inhibitory activity in a two-stage carcinogenesis model in mice.^{5,6} We have carried out studies on lantana and found that leaf extract showed a chemopreventive effect on DMBA-induced squamous cell carcinogenesis in Swiss albino mice.⁷ It was also found that compound **1** induces apoptosis in human leukemia HL-60 cells.⁸ These compounds differ in the structure of the group attached to C-22, and there are indications that the structural variations play an important role in pharmacological activities of these compounds. We have reported the importance of structural variations involving C-22 and C-17 in the antitumor activity of lantadene A congeners.⁹ In this paper, we report the synthesis of five new lantadenes (**14–18**) and their methyl esters (**20–24**), along with their cytotoxicity against four human cancer cell lines. The parent compound **1** and four most active compounds (**15**, **16**, **21**, and **22**) were studied for their *in vivo* tumor inhibitory potential on two-stage squamous cell carcinogenesis in Swiss albino mice, induced by 7,12-dimethylbenz[*a*]anthracene (DMBA) and promoted by 12-*O*-tetradecanoylphorbol-13-acetate (TPA).



Results and Discussion

Five new lantadenes (**14–18**) and their methyl esters (**20–24**) were synthesized as shown in Scheme 1. The crude lantadene fraction was isolated from the dried leaves of lantana and hydrolyzed to **5** in 45% yield. The carboxylic group of **5** was

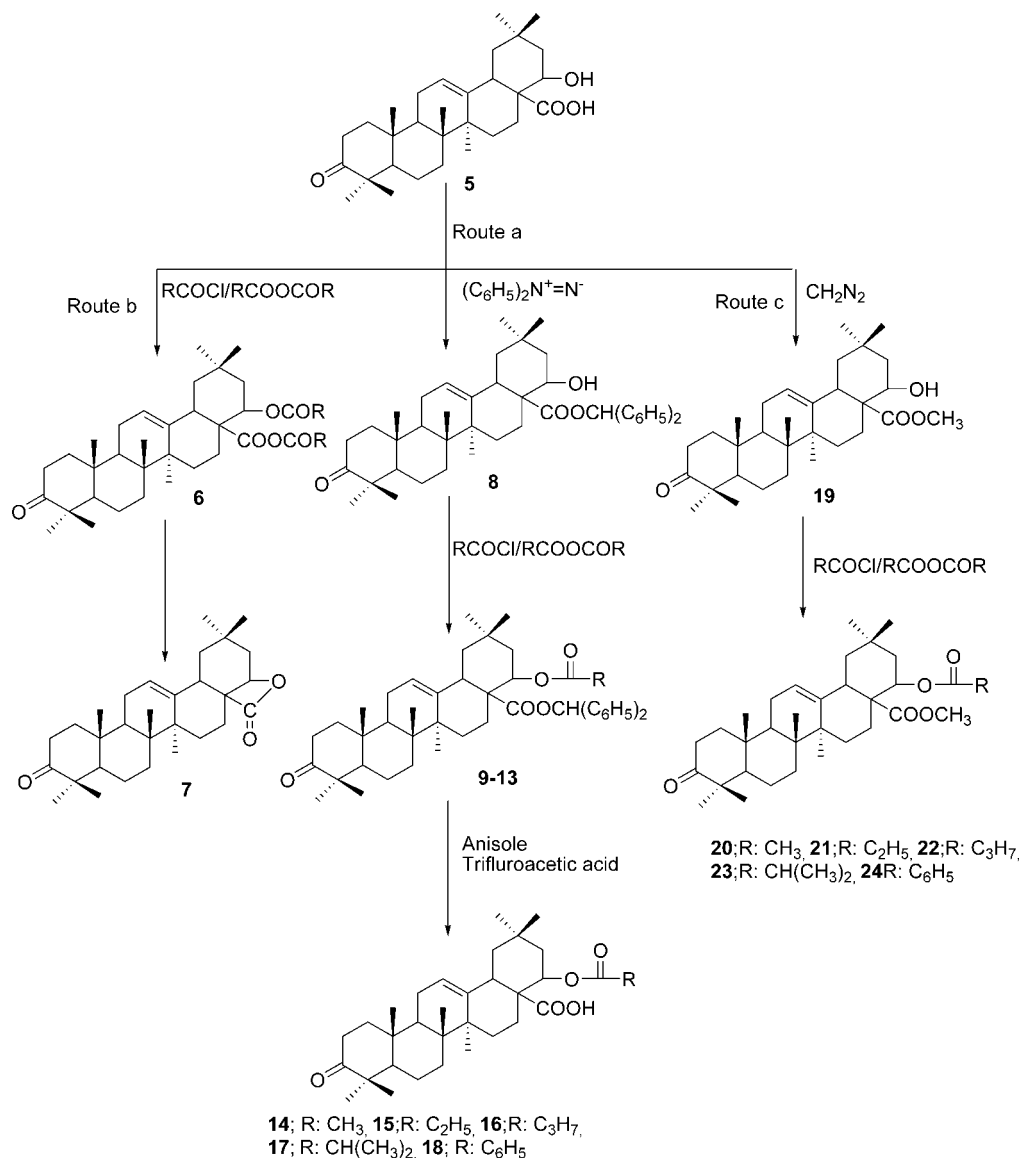
protected, followed by acylation, and finally the protecting group was removed to yield different lantadene analogues. The protecting group was selected on the basis of its ease, both in synthesis and cleavage under mild conditions without disturbing the ester at C-22. The protecting group was also selected for its selectivity toward the carboxy group at C-17. The carboxy group of **5** was protected by diphenyldiazomethane, which was prepared by treating benzophenone hydrazone with yellow mercury oxide.^{10,11} Compound **5** was treated with diphenyldiazomethane to obtain **8** in 30% yield, which was treated with various acyl chlorides, or anhydrides, to obtain diphenylmethyl esters (**9–13**). The protecting group was removed by treating with anisole and trifluoroacetic acid (1:4) to obtain the corresponding lantadenes (**14–18**). Compound **5** was also treated with acetic anhydride and benzoyl chloride to yield **14** and **18**, respectively. This reaction failed with other acyl chlorides and anhydrides, which may be due to the formation of a mixed anhydride (**6**) and finally β -lactone (**7**).¹⁷

It has been reported that esterification of C-17 carboxy-triterpenoids with MeOH showed enhancement of biological activity.¹² Thus, it was thought worthwhile to prepare methyl esters of synthesized lantadenes (**20–24**). For this purpose, instead of using the synthesized lantadenes (**14–18**) for esterification, an alternative route was used involving the preparation of **19** by treating **5** with diazomethane, followed by acylation with acyl chlorides or anhydrides in the presence of pyridine to obtain the corresponding compounds (**20–24**). This route involved fewer steps (Scheme 1, route c) and gave better yields. All structures were confirmed by using spectroscopic techniques and elemental analysis. ¹H NMR spectra of the diphenylmethyl esters showed a methyl singlet at unusually high field (δ 0.4). This chemical shift was the result of anisotropic shielding by the phenyl rings of the diphenylmethyl ester group. Hence, the high signal was assigned to the C-26 methyl group. The one proton singlet (COOCH) of diphenylmethyl ester appeared in the range δ 6.1–6.8. The free acids showed singlets for seven tertiary methyl groups in the range δ 0.83–1.18. The 12-H signal appeared in the range δ 5.30–5.41 as a triplet. Similarly, 22-H and 18-H signals appeared in the ranges δ 5.07–5.16 and 3.02–3.08 as a triplet and double doublet, respectively. Analyses of ¹³C NMR showed signals characteristic for C-28 carbonyl carbons and C-22 attached to OH groups in the ranges δ 180.1–181.4 and 75.4–77.7, respectively. The spectra also showed a downfield shift of the C-3 keto groups, which resonated in the range δ 214.3–219.2. The mass spectra of these compounds showed base peaks at *m/z* 452, which resulted from loss of the corresponding acid radical from the molecular ion. Loss of ketene from the molecular ion resulted in a peak at *m/z* 470. The RDA fragmentation

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Scheme 1. Sequence of Steps Involved in the Synthesis of Lantadenes (**14–18**) and Their Methyl Esters (**20–24**)**Table 1.** *In Vitro* Cytotoxicity of Lantadene Ester Analogues against Human Cancer Cell Lines (IC₅₀ in $\mu\text{g/mL}$)

compound	HL 60	HeLa	colon 502713	lung A-549
1	19.8 \pm 0.21a	23.3 \pm 0.08 ^b	21.3 \pm 0.05 ^b	21.8 \pm 0.21a
5	<100	>100	>100	>100
14	38.5 \pm 0.05 ^a	37.3 \pm 0.15 ^a	34.4 \pm 0.020b	36.8 \pm 0.08
15	26.4 \pm 0.17 ^b	28.6 \pm 0.05 ^a	25.4 \pm 0.05 ^a	29.6 \pm 0.14 ^b
16	21.6 \pm 0.120a	22.8 \pm 0.08 ^b	25.2 \pm 0.15 ^c	27.1 \pm 0.05 ^c
17	41.2 \pm 0.020a	43.6 \pm 0.21c	37.6 \pm 0.21c	44.2 \pm 0.21a
18	50.8 \pm 0.08 ^a	46.3 \pm 0.08 ^a	51.2 \pm 0.020b	>100
19	70.6 \pm 0.05 ^a	>100	72.4 \pm 0.05	84.2 \pm 0.05
20	37.3 \pm 0.15 ^b	36.2 \pm 0.05 ^c	34.6 \pm 0.08 ^a	36.8 \pm 0.05 ^c
21	24.2 \pm 0.21b	26.4 \pm 0.08 ^c	22.4 \pm 0.08 ^a	28.4 \pm 0.21c
22	19.3 \pm 0.08 ^a	20.1 \pm 0.21a	21.1 \pm 0.05 ^c	22.4 \pm 0.05 ^c
23	39.5 \pm 0.05 ^b	41.1 \pm 0.21b	38.1 \pm 0.05 ^a	42.2 \pm 0.08 ^a
24	48.0 \pm 0.05 ^a	50.7 \pm 0.05 ^b	>100	>100
paclitaxel ^d	<2	>3	<2	<2

^a $P < 0.05$. ^b $P < 0.01$. ^c $P > 0.001$. ^d Positive control.

gave a major peak at m/z 206. The methyl ester derivatives showed a base peak at m/z 466.

The derivatives were studied for cytotoxicity against four human cancer cell lines (HL-60, HeLa, colon 502713, and lung A-549), and the results are summarized in Table 1. Compound **20** showed cytotoxicity in the range 34.4–38.4 $\mu\text{g/mL}$. Cytotoxicity increased as the length of the side chain increased from acetoxy to propoxy.

There was a significant decrease in cytotoxicity with branching of the side chain. Introduction of a phenyl ring in the side chain further decreased the cytotoxicity. The C-17 methyl esters all were more cytotoxic than the corresponding free acids, with **22** showing the best activity (IC₅₀ 19.3–22.4 $\mu\text{g/mL}$) among the synthesized compounds. The cytotoxicity of **22** was comparable to that of **21** (IC₅₀ 22.4–28.4 $\mu\text{g/mL}$). Similarly, **23** and **24** were more cytotoxic than their corresponding free acids.

Carcinogenesis is a multistep process involving the sequential phases of initiation, promotion, and progression. Chemoprevention is one desirable strategy to reverse, arrest, or inhibit carcinogenesis.¹³ The mouse skin carcinogenesis model is often used to study the genetic and biological changes associated with chemical initiation of lesions and their subsequent transition to squamous cell carcinoma. DMBA is a polycyclic aromatic hydrocarbon capable of inducing skin lesions when applied repeatedly on mouse skin.¹⁴ The primary bioactivation of DMBA occurs via formation of DMBA-3,4-dihydrodiol. DMBA-diol-epoxide has been suggested to be the ultimate carcinogen responsible for inducing chronic inflammation, ROS production, and oxidative damage of DNA resulting in the transformation of normal cells to tumor cells.¹⁵ When mouse skin is repeatedly exposed to DMBA, the Langerhans cells will be depleted and local immunosuppression takes place.¹⁶ This leads to the induction of tumors on the skin of the mice. TPA

is a phorbol ester, which was isolated from croton oil. This is an active constituent of croton oil, responsible for the promotion of tumors.

Compounds **15**, **16**, **21**, and **22** were selected for two-stage carcinogenesis studies. Squamous cell carcinogenesis was induced in Swiss albino mice (LACCA/female) by DMBA and promoted by TPA. The onset of papillomas was observed in the fourth week in the DMBA/TPA-treated mice and found to be 36.3%. There was a gradual rise in the incidence of tumors that reached 100% during the eighth week. The incidence of DMBA/TPA-induced papillomas was delayed for 3 weeks by **15** and **16** and 4 weeks by **21** and **22**. The animals treated with **16** showed a significant decrease in the incidence of cancer (17.2% vs 100%) at the end of 20 weeks as compared with the DMBA/TPA-treated group ($P > 0.001$). The overall papilloma incidence in those treated with **15**, **21**, and **22** was 19.6% ($P > 0.001$, $P > 0.01$), 17.9% ($P > 0.001$, $P > 0.05$), and 12.4% ($P > 0.001$, $P > 0.05$), respectively, in comparison with the DMBA/TPA-treated group. The parent compound (**1**) group showed 18.1% incidence of tumors and a delay of three weeks.

The survival rate decreased significantly in DMBA/TPA-treated mice as compared with the vehicle-treated group (30% vs 80%). Survival rates with compound-treated animals were significantly higher in comparison to the DMBA/TPA-treated group ($P > 0.01$, $P > 0.05$). The **15**-, **17**-, **21**-, and **22**-treated groups showed similar survival rates (80%). The average body weight of DMBA/TPA-treated mice decreased significantly. However, there was slight increase in the average body weight of **15**-, **17**-, **21**-, and **22**-treated mice at the termination of the study ($P > 0.01$, $P > 0.05$). Histopathological examination of the depilated back of mice revealed normal skin and the presence of subcutaneous tissue in acetone-treated mice. Twice weekly application of DMBA for 8 weeks on depilated mice induced well-differentiated squamous cell carcinoma with formation of keratin pearls. There was marked infiltration of cancer cells in the underlying dermis. The skin section of mice treated with synthesized compounds showed hyperplastic papillomatous lesions with no evidence of infiltration or cytological atypia.

The pretreatment of animals with derivatives could be associated with their ability to interfere with the initiation of carcinogenesis, which is a relatively rapid process, and the continuous treatment after TPA with the promotion, which is a slow process. These agents also increased the survival time of the animals. Slight weight gain in **15**-, **16**-, **21**-, and **22**-treated mice could be a result of recovery from the effect of DMBA/TPA or, alternatively, with better papilloma control.

The cytotoxicity profile of these derivatives showed that, with removal of the ester at C-22, there was significant decrease in the activity. Compound **5** was inactive. Methylation of the C-17 carboxyl group resulted in some activity. Compound **19** showed better cytotoxicity than **5**, and similarly methyl esters **20**–**24** were more cytotoxic than **14**–**18**. This effect may be attributed to increased lipophilicity and better bioavailability. An increase in length of the ester side chain at C-22 gave an increase in the activity, and branching decreased the activity. An aromatic ester at C-22 also resulted in decreased activity. These results indicate the importance of the C-22 and C-17 positions in the antitumor activity of these compounds.

Experimental Section

General Experimental Procedures. Melting points were determined on a Buchi melting point apparatus and are uncorrected. For thin-layer chromatography (TLC), glass plates coated with silica gel G (E. Merck) were used. The TLC plates were activated at 110 °C for 30 min and visualized by exposure to iodine vapors. Glass columns of appropriate sizes were used. Silica gel (60–120 mesh) was used as adsorbent. IR spectra were recorded on a Perkin-Elmer 882 spectrometer using potassium bromide pellets. ^1H NMR and ^{13}C NMR spectra were recorded with a Bruker AC 300F 300 MHz spectrometer using CDCl_3 as solvent, and

tetramethylsilane 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 compounds was within $\pm 0.04\%$ of the theoretical values. All solvents were freshly distilled and dried prior to use according to standard procedures. All chemicals were purchased from SD Fine Chemicals, Qualigen, and Loba Chemicals. DMBA and TPA were purchased from Sigma Chemicals Ltd.

Plant Material. Leaves of *Lantana camara* were collected in September from Palampur (HP), India. The leaves were shade-dried and powdered. A voucher specimen (LC; 097 UIPS) was deposited in the Herbarium at the University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India.

Animals. Female Swiss albino mice (6 weeks old) weighing 18–22 g were obtained from the Central Animal House of Panjab University. Animals were kept in the Departmental Animal House with a controlled temperature of 23 ± 5 °C, $60 \pm 5\%$ humidity, and a 12 h light/dark cycle. They were fed a basal diet and water. The mice were acclimated for 1 week before experimentation.

Extraction and Isolation of Compounds 1–4. Compounds **1**–**4** were isolated from lantana leaf powder, and compound **5** was prepared by basic hydrolysis as reported previously.¹⁷

Diphenylmethyl 22 β -hydroxy-3-oxoolean-12-en-28-onate (8). In a 250 mL two-necked flask, 0.1 g (0.21 mmol) of 22 β -hydroxy-3-oxoolean-12-en-28-oic acid (**5**) was dissolved in 20 mL of CHCl_3 . The solution was stirred at 40 °C, and diphenyldiazomethane in CHCl_3 was added dropwise until the purple color of the reagent was no longer evident. The solvent was removed under reduced pressure, and the residue was chromatographed on a silica gel G column (20 g, 100–200 mesh) using CHCl_3 and a CHCl_3 –MeOH (9.5–0.5) mixture as eluting solvents. The desired product-enriched fractions were pooled, and the solvent was removed *in vacuo* to yield **8** as white crystals (0.04 g, 29.5%); mp 230 °C; IR (KBr) ν_{max} 3520 (OH), 3313 (aromatic) 1740 ($\text{C}=\text{O}$, ester), 1690 ($\text{C}=\text{O}$, keto) cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 0.4 (3H, s, C-26- CH_3), 0.90 (3H, s, CH_3), 0.97 (3H, s, CH_3), 1.02 (3H, s, CH_3), 1.06 (3H, s, CH_3), 1.12 (6H, s, $2 \times \text{CH}_3$), 3.05 (1H, dd, $J = 14.0$; 3.7 Hz, C-18-H), 4.0 (1H, t, $J = 3$ Hz, C-22-H), 5.4 (1H, t, $J = 3.4$ Hz, C-12-H), 6.8 (1H, s, COOCH), 7.4–8.1 (10H, m, Ar-H).

General Procedure for Synthesis of Diphenylmethyl 22 β -Acyloxyoleanonates (9–13). Compound **8** was stirred with different acylating agents in the presence of pyridine at room temperature, and the reactions were monitored on TLC (hexane–ethyl acetate; 18.5–1.5). The reaction mixtures were diluted with water (10 mL), stirred for 1 h, adjusted to pH 2 by adding HCl, and extracted with CHCl_3 (2×5 mL). The organic layer was dried over anhydrous Na_2SO_4 , and the solvent was removed under reduced pressure to yield compounds **9**–**13** as oily residues.

Diphenylmethyl 22 β -acetoxy-3-oxoolean-12-en-28-oate (9): oil (0.04 g, 31.1%); IR (KBr) ν_{max} 3037 (aromatic), 1742 ($\text{C}=\text{O}$, ester), 1682 ($\text{C}=\text{O}$, keto) cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 0.4 (3H, s, C-26- CH_3), 0.91 (3H, s, CH_3), 0.98 (3H, s, CH_3), 1.03 (3H, s, CH_3), 1.05 (3H, s, CH_3), 1.10 (6H, s, $2 \times \text{CH}_3$), 2.09 (3H, s, OCOCH_3) 3.05 (1H, dd, $J = 14.0$; 3.7 Hz, C-18-H), 5.0 (1H, t, $J = 3$ Hz, C-22-H), 5.4 (1H, t, $J = 3.4$ Hz, C-12-H), 6.6 (1H, s, COOCH), 7.4–8.0 (10H, m, Ar-H).

Diphenylmethyl 22 β -propoxy-3-oxoolean-12-en-28-oate (10): oil (0.017 g, 45.7%); IR (KBr) ν_{max} 3042 (aromatic), 1738 ($\text{C}=\text{O}$, ester), 1685 ($\text{C}=\text{O}$, keto) cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 0.4 (3H, s, C-26- CH_3), 0.91 (3H, s, CH_3), 0.97 (3H, s, CH_3), 1.04 (3H, s, CH_3), 1.08 (3H, s, CH_3), 1.14 (6H, s, $2 \times \text{CH}_3$), 1.73 (3H, t, $J = 7.1$ Hz, $-\text{OCOCH}_2\text{CH}_3$), 3.04 (1H, dd, $J = 14.2$; 3.7 Hz, C-18-H), 3.61 (2H, q, $J = 7.1$ Hz, $-\text{OCOCH}_2\text{CH}_3$), 5.9 (1H, t, $J = 3$ Hz, C-22-H), 5.6 (1H, t, $J = 3.4$ Hz, C-12-H), 6.5 (1H, s, COOCH), 7.5–8.2 (10H, m, Ar-H).

Diphenylmethyl 22 β -butyryloxy-3-oxoolean-12-en-28-oate (11): oil (0.04 g, 36%); IR (KBr) ν_{max} 3061 (aromatic), 1735 ($\text{C}=\text{O}$, ester), 1689 ($\text{C}=\text{O}$, keto) cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 0.4 (3H, s, C-26- CH_3), 0.84 (3H, s, CH_3), 0.90 (3H, s, CH_3), 0.96 (3H, t, $J = 7.1$ Hz, $-\text{OCOCH}_2\text{CH}_2\text{CH}_3$), 1.04 (3H, s, CH_3), 1.09 (3H, s, CH_3), 1.17 (6H, s, $2 \times \text{CH}_3$), 1.72 (2H, sextet, $J = 7.1$ Hz, $-\text{OCOCH}_2\text{CH}_2\text{CH}_3$), 2.25 (2H, t, $J = 7.1$ Hz, $-\text{OCOCH}_2\text{CH}_2\text{CH}_3$), 3.04 (1H, dd, $J = 14.1$; 3.7 Hz, C-18-H), 5.1 (1H, t, $J = 3$ Hz, C-22-H), 5.3 (1H, t, $J = 3.4$ Hz, C-12-H), 6.1 (1H, s, $-\text{COOCH}$), 7.5–8.2 (10H, m, Ar-H).

Diphenylmethyl 22 β -isobutyryloxy-3-oxoolean-12-en-28-oate (12): oil (0.05 g, 45%); IR (KBr) ν_{max} 3062 (aromatic), 1745 ($\text{C}=\text{O}$, ester),

1690 (C=O, keto) cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 0.4 (3H, s, C-26- CH_3), 0.84 (3H, s, CH_3), 0.90 (3H, s, CH_3), 1.04 (3H, s, CH_3), 1.09 (3H, s, CH_3), 1.17 (6H, s, $2 \times \text{CH}_3$), 1.20 (6H, d, $J = 7.4$ Hz, $2 \times -\text{OCOCH}(\text{CH}_3)_2$), 2.58 (1H, septet, $J = 7.4$ Hz, $-\text{OCOCH}(\text{CH}_3)_2$), 3.04 (1H, dd, $J = 14.1$; 3.7 Hz, C-18-H), 5.1 (1H, t, $J = 3$ Hz, C-22-H), 5.3 (1H, t, $J = 3.4$ Hz, C-12-H), 6.3 (1H, s, $-\text{COOCH}$), 7.4–8.0 (10H, m, Ar-H).

Diphenylmethyl 22 β -benzyloxy-3-oxoolean-12-en-28-oate (13): oil (0.05 g, 34.6%); IR (KBr) ν_{max} 3043 (aromatic), 1738 (C=O, ester), 1693 (C=O, keto) cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 0.4 (3H, s, C-26- CH_3), 0.85 (3H, s, CH_3), 0.90 (3H, s, CH_3), 1.04 (3H, s, CH_3), 1.08 (3H, s, CH_3), 1.18 (6H, s, $2 \times \text{CH}_3$), 3.04 (1H, dd, $J = 14.1$; 3.6 Hz, C-18-H), 5.2 (1H, t, $J = 3$ Hz, C-22-H), 5.3 (1H, t, $J = 3.4$ Hz, C-12-H), 6.1 (1H, s, $-\text{COOCH}$), 7.6–8.1 (15H, m, Ar-H).

General Procedure for Synthesis of 22 β -Acyloxy-3-oxoolean-12-en-28-oic Acids (14–18). Diphenylmethyl 22 β -acyloxy-3-oxoolean-12-en-28-oate (0.25 g, 0.37 mmol), anisole, and trifluoroacetic acid were stirred vigorously for 5 min at room temperature. The solvent was removed under reduced pressure, and 10 mL of light petroleum was added to the residue. The solid product was collected by filtration, washed with light petroleum, and recrystallized from MeOH and H_2O to give compounds 14–18.

22 β -Acetoxy-3-oxoolean-12-en-28-oic acid (14): white crystals (0.12 g, 63.5%); mp 296 °C; IR (KBr) ν_{max} 3303 (COOH), 1743 (C=O, ester), 1721 (C=O, COOH), 1688 (C=O, keto) cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 0.83 (3H, s, C-26- CH_3), 0.90 (3H, s, CH_3), 1.04 (6H, s, $2 \times \text{CH}_3$), 1.06 (3H, s, CH_3), 1.09 (3H, s, CH_3), 1.18 (3H, s, CH_3), 1.94 (3H, s, $-\text{OCOCH}_3$), 3.03 (1H, dd, $J = 14.2$; 3.08 Hz, C-18-H), 5.07 (1H, t, $J = 3$ Hz, C-22-H), 5.37 (1H, t, $J = 3.4$ Hz, C-12-H); ^{13}C NMR (75 MHz) δ 39.3 (C-1), 34.2 (C-2), 214.3 (C-3), 47.5 (C-4), 55.4 (C-5), 19.5 (C-6), 32.3 (C-7), 39.2 (C-8), 46.9 (C-9), 36.8 (C-10), 24.4 (C-11), 121.2 (C-12), 143.3 (C-13), 42.0 (C-14), 27.7 (C-15), 23.6 (C-16), 52.2 (C-17), 39.1 (C-18), 45.8 (C-19), 30.1 (C-20), 38.1 (C-21), 75.4 (C-22), 26.5 (C-23), 21.5 (C-24), 15.1 (C-25), 16.7 (C-26), 25.8 (C-27), 180.2 (C-28), 33.8 (C-29), 26.8 (C-30), 166.2 (C-1'), 15.3 (C-2'); EMIS m/z 512 [M^+], 494 (16), 469 (32), 468 (40), 452 (100), 306 (36), 291 (25), 206 (20), 203 (18); *anal.* $\text{C}_{32}\text{H}_{48}\text{O}_5$ C, 74.96%; H, 9.44%; found C, 74.96%; H, 9.47%.

Compound 14 was also prepared by stirring a solution of 0.12 g (0.25 mmol) of 22 β -hydroxy-3-oxoolean-12-en-28-oic acid in 2 mL of pyridine and 1 mL (1.079 g, 1.1 mmol) of acetic anhydride overnight at room temperature using magnetic stirring. The progress of the reaction was monitored by TLC. The reaction mixture was diluted with 10 mL of H_2O , acidified with HCl, and extracted with ether (3×10 mL). The combined organic layer was washed with 5 mL of H_2O and dried over anhydrous Na_2SO_4 , and the solvent was removed under reduced pressure. The residue obtained was recrystallized from a MeOH and H_2O mixture to afford white crystals of 14 (0.05 g, 38.2%), mp 296 °C. The spectroscopic and elemental data were identical with 14 reported above.

22 β -Propoxy-3-oxoolean-12-en-28-oic acid (15): white crystals (0.15 g, 77.3%); mp 274–276 °C; IR (KBr) ν_{max} 3437 (COOH), 1747 (C=O, ester), 1726 (C=O, COOH), 1691 (C=O, keto) cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 0.86 (3H, s, C-26- CH_3), 0.91 (3H, s, CH_3), 1.02 (6H, s, $2 \times \text{CH}_3$), 1.04 (3H, s, CH_3), 1.06 (3H, s, CH_3), 1.19 (3H, s, CH_3), 1.70 (3H, t, $J = 7.2$ Hz, $-\text{OCOCH}_2\text{CH}_3$), 3.03 (1H, dd, $J = 14.0$; 3.08 Hz, C-18-H), 3.64 (2H, q, $J = 7.2$ Hz, $-\text{OCOCH}_2\text{CH}_3$), 5.11 (1H, t, $J = 3$ Hz, C-22-H), 5.41 (1H, t, $J = 3.4$ Hz, C-12-H); ^{13}C NMR (75 MHz) δ 36.3 (C-1), 34.4 (C-2), 216.6 (C-3), 46.3 (C-4), 55.4 (C-5), 19.3 (C-6), 32.5 (C-7), 39.1 (C-8), 46.5 (C-9), 36.7 (C-10), 24.1 (C-11), 121.6 (C-12), 143.2 (C-13), 42.3 (C-14), 27.8 (C-15), 23.5 (C-16), 52.3 (C-17), 39.4 (C-18), 45.7 (C-19), 30.3 (C-20), 38.3 (C-21), 76.5 (C-22), 26.2 (C-23), 21.4 (C-24), 15.3 (C-25), 16.8 (C-26), 25.9 (C-27), 180.3 (C-28), 33.8 (C-29), 26.9 (C-30), 172.2 (C-1'), 26.7 (C-2'), 9.1 (C-3'); EMIS m/z 526 [M^+], 511 (27), 498 (10), 497 (13), 484 (21), 482 (33), 469 (37), 452 (100), 296 (12), 320 (34), 305 (10), 248 (16), 246 (19), 206 (21), 203 (17); *anal.* $\text{C}_{33}\text{H}_{50}\text{O}_5$ C, 75.25%; H, 9.57%; found C, 75.21%; H, 9.59%.

22 β -Butyryloxy-3-oxoolean-12-en-28-oic acid (16): white crystals (0.15 g, 73.4%); mp 241–243 °C; IR (KBr) ν_{max} 3411 (COOH), 1752 (C=O, ester), 1731 (C=O, COOH), 1686 (C=O, keto) cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 0.86 (3H, s, C-26- CH_3), 0.91 (3H, s, CH_3), 0.95 (3H, t, $J = 7.2$ Hz, $-\text{OCOCH}_2\text{CH}_2\text{CH}_3$), 1.02 (6H, s, $2 \times \text{CH}_3$), 1.04 (3H, s, CH_3), 1.09 (3H, s, CH_3), 1.17 (3H, s, CH_3), 1.71 (2H, sextet,

$J = 7.2$ Hz, $-\text{OCOCH}_2\text{CH}_2\text{CH}_3$), 2.23 (2H, t, $J = 7.2$ Hz, $-\text{OCOCH}_2\text{CH}_2\text{CH}_3$), 3.03 (1H, dd, $J = 14.2$; 3.08 Hz, C-18-H), 5.10 (1H, t, $J = 3$ Hz, C-22-H), 5.30 (1H, t, $J = 3.4$ Hz, C-12-H); ^{13}C NMR (75 MHz) δ 37.2 (C-1), 33.4 (C-2), 217.9 (C-3), 46.3 (C-4), 55.3 (C-5), 19.1 (C-6), 32.6 (C-7), 39.6 (C-8), 46.2 (C-9), 36.4 (C-10), 24.4 (C-11), 121.2 (C-12), 143.3 (C-13), 42.2 (C-14), 27.7 (C-15), 23.6 (C-16), 52.1 (C-17), 39.2 (C-18), 45.5 (C-19), 30.3 (C-20), 38.1 (C-21), 76.9 (C-22), 26.5 (C-23), 21.3 (C-24), 15.1 (C-25), 16.6 (C-26), 25.5 (C-27), 180.1 (C-28), 33.6 (C-29), 26.8 (C-30), 172.0 (C-1'), 36.1 (C-2'), 18.1 (C-3'), 13.4 (C-4'); EMIS m/z 540 [M^+], 525 (10), 522 (17), 497 (14), 496 (22), 452 (100), 469 (22), 435 (8), 409, (10) 407 (3), 334 (19), 319 (42), 206 (34), 203 (22); *anal.* $\text{C}_{34}\text{H}_{52}\text{O}_5$ C, 75.51%; H, 9.69%; found C, 75.56%; H, 9.70%.

22 β -Isobutyryloxy-3-oxoolean-12-en-28-oic acid (17): white crystals (0.11 g, 53.1%); mp 199 °C; IR (KBr) ν_{max} 3485 (COOH), 1738 (C=O, ester), 1734 (C=O, COOH), 1688 (C=O, keto) cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 0.84 (3H, s, C-26- CH_3), 0.90 (3H, s, CH_3), 1.01 (6H, s, $2 \times \text{CH}_3$), 1.04 (3H, s, CH_3), 1.09 (3H, s, CH_3), 1.17 (3H, s, CH_3), 1.20 (6H, d, $J = 7.5$ Hz, $2 \times -\text{OCOCH}(\text{CH}_3)_2$), 2.58 (1H, septet, $J = 7.5$ Hz, $-\text{OCOCH}(\text{CH}_3)_2$), 3.02 (1H, dd, $J = 14.3$; 3.8 Hz, C-18-H), 5.13 (1H, t, $J = 3$ Hz, C-22-H), 5.32 (1H, t, $J = 3.3$ Hz, C-12-H); ^{13}C NMR (75 MHz) δ 37.9 (C-1), 33.3 (C-2), 217.2 (C-3), 46.4 (C-4), 55.1 (C-5), 19.4 (C-6), 32.2 (C-7), 39.6 (C-8), 46.4 (C-9), 36.5 (C-10), 24.2 (C-11), 121.2 (C-12), 143.3 (C-13), 42.2 (C-14), 27.7 (C-15), 23.6 (C-16), 52.2 (C-17), 39.1 (C-18), 45.3 (C-19), 30.1 (C-20), 38.2 (C-21), 77.1 (C-22), 26.5 (C-23), 21.3 (C-24), 15.1 (C-25), 16.7 (C-26), 25.5 (C-27), 180.2 (C-28), 33.8 (C-29), 26.6 (C-30), 174.5 (C-1'), 38.5 (C-2'), 17.4 (C-3' and C-4'); EMIS m/z 540 [M^+], 525 (12), 522 (21), 452 (100), 469 (36), 334 (34), 319 (31), 248 (12), 246 (15), 231 (16), 206 (27), 203 (20); *anal.* $\text{C}_{34}\text{H}_{52}\text{O}_5$ C, 75.51%; H, 9.69%; found C, 75.56%; H, 9.70%.

22 β -Benzoyloxy-3-oxoolean-12-en-28-oic acid (18): brown crystals (0.13 g, 59.0%); mp 237 °C; IR (KBr) ν_{max} 3478 (COOH), 1735 (C=O, ester), 1738 (C=O, COOH), 1693 (C=O, keto) cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 0.85 (3H, s, C-26- CH_3), 0.90 (3H, s, CH_3), 1.02 (6H, s, $2 \times \text{CH}_3$), 1.04 (3H, s, CH_3), 1.08 (3H, s, CH_3), 1.18 (3H, s, CH_3), 3.06 (1H, dd, $J = 14.3$; 3.8 Hz, C-18-H), 5.16 (1H, t, $J = 3$ Hz, C-22-H), 5.36 (1H, t, $J = 3.4$ Hz, C-12-H), 7.4–8.0 (15H, m, Ar-H); ^{13}C NMR (75 MHz) δ 39.5 (C-1), 32.3 (C-2), 219.2 (C-3), 47.4 (C-4), 55.6 (C-5), 19.7 (C-6), 32.7 (C-7), 39.9 (C-8), 46.7 (C-9), 35.5 (C-10), 24.2 (C-11), 121.9 (C-12), 143.8 (C-13), 42.7 (C-14), 27.9 (C-15), 23.9 (C-16), 52.7 (C-17), 39.8 (C-18), 45.7 (C-19), 31.4 (C-20), 37.2 (C-21), 77.7 (C-22), 26.9 (C-23), 21.8 (C-24), 15.7 (C-25), 16.9 (C-26), 25.9 (C-27), 181.4 (C-28), 33.9 (C-29), 26.9 (C-30), 167.0 (C-1'), 130.5 (C-2'), 129.7 (C-3' and C-7'), 128.8 (C-4' and C-6'), 132.8 (C-5'); EMIS m/z 574 [M^+], 548 (12), 546 (32), 532 (27), 530 (22), 523 (20), 470 (8), 469 (27), 452 (100), 278 (29), 353 (24), 248 (13), 246 (19), 231 (15), 206 (16), 203 (21); *anal.* $\text{C}_{37}\text{H}_{50}\text{O}_5$ C, 77.31%; H, 9.77%; found C, 77.31%; H, 8.78%.

Compound 18 was prepared by stirring a solution of 22 β -hydroxy-3-oxoolean-12-en-28-oic acid (0.12 g, 0.25 mmol) in 2.4 mL of pyridine and 1.480 g (1.3 mL, 1.5 mmol) of benzoyl chloride for 4.5 h at room temperature. Progress of the reaction was monitored by TLC. The reaction mixture was diluted with 10 mL of H_2O , acidified with HCl, and extracted with ether (3×10 mL). The combined organic layer was washed with H_2O (5 mL) and dried over anhydrous Na_2SO_4 , and the solvent was removed by distillation. The residue was recrystallized from a MeOH and H_2O mixture to afford 18 (0.08 g, 54.5%), mp 237 °C.

Methyl 22 β -hydroxy-3-oxoolean-12-en-28-onate (19). Excess ethereal diazomethane was added to 0.15 g (0.32 mmol) of 5, and the reaction mixture was kept overnight. Excess diazomethane was neutralized by adding acetic acid. The solvent was removed under reduced pressure, and the residue was crystallized from MeOH to obtain 19 as white crystals (0.12 g, 77.6%); mp 179–181 °C; IR (KBr) ν_{max} 3595 (OH), 2911 (aliphatic), 1711 (C=O, ester), 1684 (C=O, keto) cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 0.85 (3H, s, CH_3), 0.89 (3H, s, CH_3), 1.04 (6H, s, $2 \times \text{CH}_3$), 1.09 (3H, s, CH_3), 1.11 (3H, s, CH_3), 1.15 (3H, s, CH_3), 3.01 (1H, dd, $J = 14.2$; 3.7 Hz, C-18-H), 3.59 (3H, s, $-\text{COOCH}_3$), 3.71 (1H, t, $J = 3$ Hz, C-22-H), 4.81 (1H, broad, C-22-OH, exchanged with D_2O), 5.28 (1H, t, $J = 3.5$ Hz, C-12-H); ^{13}C NMR (75 MHz) δ 39.4 (C-1), 34.2 (C-2), 217.4 (C-3), 47.5 (C-4), 55.4 (C-5), 19.5 (C-6), 32.1 (C-7), 39.2 (C-8), 46.9 (C-9), 36.6 (C-10), 24.3 (C-11), 121.2 (C-12), 143.3 (C-13), 42.0 (C-14), 27.7 (C-15), 23.6 (C-16), 52.1 (C-17), 39.1 (C-18), 45.8 (C-19), 30.1 (C-20), 38.1 (C-21),

76.6 (C-22), 26.5 (C-23), 21.5 (C-24), 15.1 (C-25), 16.7 (C-26), 25.8 (C-27), 180.5 (C-28), 33.8 (C-29), 26.8 (C-30), 51.1 (COOCH₃); EMIS *m/z* 484 [M⁺], 466 (100), 409 (21), 407 (15), 248 (31), 246 (17), 203, (26) 201 (11); *anal.* C₃₁H₄₈O₄ C, 76.82%; H, 9.98%; found C, 76.82%; H, 9.99%.

General Procedure for Synthesis of Methyl 22 β -acyloxyoleanonates (20–24). A solution of **19** (0.15 g, 0.31 mmol) was stirred with pyridine and the appropriate acylating agents. Progress of reactions was monitored by TLC. The reaction mixtures were diluted with 10 mL of H₂O, acidified with HCl, and extracted with ether (3 \times 10 mL). The organic layer was dried over anhydrous Na₂SO₄, and solvent was removed under reduced pressure. The residue obtained was recrystallized from a MeOH–H₂O mixture to afford **20–24**.

Methyl 22 β -acetoxy-3-oxoolean-12-en-28-oate (20): white crystals (0.10 g, 61.3%); mp 216–218 °C; IR (KBr) ν_{\max} 2950 (aliphatic), 1746 (C=O, ester), 1715 (C=O, ester), 1689 (C=O, keto) cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 0.85 (3H, s, CH₃), 0.89 (3H, s, CH₃), 1.04 (6H, s, 2 \times CH₃), 1.09 (3H, s, CH₃), 1.11 (3H, s, CH₃), 1.15 (3H, s, CH₃), 2.09 (3H, s, -OCOCH₃), 3.05 (1H, dd, *J* = 14.2; 3.7 Hz, C-18-H), 3.78 (3H, s, -COOCH₃), 5.10 (1H, t, *J* = 3 Hz, C-22-H), 5.39 (1H, t, *J* = 3.5 Hz, C-12-H); ¹³C NMR (75 MHz) δ 39.7 (C-1), 34.6 (C-2), 217.8 (C-3), 47.8 (C-4), 55.7 (C-5), 19.3 (C-6), 32.6 (C-7), 39.5 (C-8), 47.2 (C-9), 36.6 (C-10), 24.3 (C-11), 121.6 (C-12), 143.8 (C-13), 42.3 (C-14), 27.9 (C-15), 23.7 (C-16), 52.1 (C-17), 39.4 (C-18), 46.1 (C-19), 30.4 (C-20), 38.2 (C-21), 77.6 (C-22), 26.8 (C-23), 21.6 (C-24), 15.8 (C-25), 16.6 (C-26), 26.1 (C-27), 180.1 (C-28), 34.1 (C-29), 26.8 (C-30), 51.4 (COOCH₃), 166.1 (C-1'), 15.3 (C-2'); EMIS *m/z* 526 [M⁺], 511 (11), 483 (25), 466 (100), 435 (8), 409 (10), 320 (24), 305 (14), 231 (44), 206 (22), 203 (13); *anal.* C₃₃H₅₀O₅ C, 75.25%; H, 9.57%; found C, 75.27%; H, 9.59%.

Methyl 22 β -propoxy-3-oxoolean-12-en-28-oate (21): white crystals (0.12 g, 71.7%); mp 231 °C; IR (KBr) ν_{\max} 2959 (aliphatic), 1742 (C=O, ester), 1713 (C=O, 17 ester), 1693 (C=O, keto) cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 0.85 (3H, s, CH₃), 0.89 (3H, s, CH₃), 1.04 (6H, s, 2 \times CH₃), 1.09 (3H, s, CH₃), 1.11 (3H, s, CH₃), 1.14 (3H, t, *J* = 7.1 Hz, -OCOCH₂CH₃), 1.15 (3H, s, CH₃), 2.21 (2H, q, *J* = 7.1 Hz, -OCOCH₂CH₃), 3.05 (1H, dd, *J* = 14.1; 3.7 Hz, C-18-H), 3.77 (3H, s, -COOCH₃), 5.10 (1H, t, *J* = 3 Hz, C-22-H), 5.39 (1H, t, *J* = 3.5 Hz, C-12-H); ¹³C NMR (75 MHz) δ 39.8 (C-1), 34.7 (C-2), 217.2 (C-3), 47.6 (C-4), 55.8 (C-5), 19.7 (C-6), 32.7 (C-7), 39.6 (C-8), 47.4 (C-9), 36.7 (C-10), 24.6 (C-11), 121.7 (C-12), 143.9 (C-13), 42.6 (C-14), 28.2 (C-15), 23.8 (C-16), 52.4 (C-17), 39.5 (C-18), 46.3 (C-19), 30.5 (C-20), 38.3 (C-21), 77.9 (C-22), 26.5 (C-23), 21.5 (C-24), 15.9 (C-25), 16.5 (C-26), 26.3 (C-27), 180.8 (C-28), 34.3 (C-29), 26.9 (C-30), 51.5 (COOCH₃), 166.3 (C-1'), 26.7 (C-2'), 9.1 (C-3'); EMIS *m/z* 540 [M⁺], 525 (16), 512 (16), 511 (12), 483 (24), 466 (100), 334 (17), 319 (27), 248 (12), 246 (16), 203 (14); *anal.* C₃₄H₅₂O₅ C, 75.51%; H, 9.69%; found C, 75.53%; H, 9.71%.

Methyl 22 β -butyryloxy-3-oxoolean-12-en-28-oate (22): white crystals (0.017 g, 34.9%); mp 192–194 °C; IR (KBr) ν_{\max} 2962 (aliphatic), 1737 (C=O, ester), 1714 (C=O, ester), 1696 (C=O, keto) cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 0.84 (3H, s, CH₃), 0.89 (3H, s, CH₃), 1.04 (6H, s, 2 \times CH₃), 1.09 (3H, s, CH₃), 1.11 (3H, s, CH₃), 1.15 (3H, s, CH₃), 1.32 (3H, t, *J* = 7.2 Hz, -OCOCH₂CH₂CH₃), 1.46 (2H, sextet, *J* = 7.2 Hz, -OCOCH₂CH₂CH₃), 2.33 (2H, t, *J* = 7.2 Hz, -OCOCH₂CH₂CH₃), 3.03 (1H, dd, *J* = 14.0; 3.7 Hz, C-18-H), 3.87 (3H, s, COOCH₃), 5.04 (1H, t, *J* = 3 Hz, C-22-H), 5.38 (1H, t, *J* = 3.5 Hz, C-12-H); ¹³C NMR (75 MHz) δ 38.9 (C-1), 34.5 (C-2), 217.1 (C-3), 47.9 (C-4), 55.3 (C-5), 19.6 (C-6), 32.2 (C-7), 39.3 (C-8), 47.7 (C-9), 36.6 (C-10), 24.7 (C-11), 121.3 (C-12), 143.5 (C-13), 42.8 (C-14), 28.5 (C-15), 23.9 (C-16), 52.6 (C-17), 39.7 (C-18), 46.5 (C-19), 30.7 (C-20), 38.6 (C-21), 78.2 (C-22), 27.1 (C-23), 21.7 (C-24), 16.2 (C-25), 16.7 (C-26), 26.6 (C-27), 181.2 (C-28), 34.5 (C-29), 27.3 (C-30), 51.7 (COOCH₃), 166.1 (C-1'), 36.1 (C-2'), 18.2 (C-3'), 13.4 (C-4'); EMIS *m/z* 554 [M⁺], 526 (10), 511 (22), 510 (19), 483 (21), 466 (100), 348 (17), 333 (23), 248 (13), 231 (7), 206 (12), 203 (15); *anal.* C₃₅H₅₄O₅ C, 75.77%; H, 9.81%; found C, 75.75%; H, 9.82%.

Methyl 22 β -isobutyryloxy-3-oxoolean-12-en-28-oate (23): white crystals (0.08 g, 41.1%); mp 163 °C; IR (KBr) ν_{\max} 2958 (aliphatic), 1739 (C=O, ester), 1721 (C=O, ester), 1698 (C=O, keto) cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 0.86 (3H, s, CH₃), 0.89 (3H, s, CH₃), 1.03 (6H, s, 2 \times CH₃), 1.09 (3H, s, CH₃), 1.10 (3H, s, CH₃), 1.15 (3H, s, CH₃), 1.25 (6H, d, *J* = 7.0 Hz, 2 \times -OCOCH(CH₃)₂), 2.61 (1H, m, -OCOCH(CH₃)₂), 3.05 (1H, dd, *J* = 14.1; 3.5 Hz, C-18-H), 3.80 (3H,

s, -COOCH₃), 5.20 (1H, t, *J* = 3 Hz, C-22-H), 5.49 (1H, t, *J* = 3.5 Hz, C-12-H); ¹³C NMR (75 MHz) δ 38.9 (C-1), 34.6 (C-2), 217.6 (C-3), 47.4 (C-4), 55.5 (C-5), 19.6 (C-6), 32.3 (C-7), 39.4 (C-8), 47.8 (C-9), 36.5 (C-10), 24.8 (C-11), 121.4 (C-12), 143.6 (C-13), 42.7 (C-14), 28.7 (C-15), 24.1 (C-16), 52.7 (C-17), 39.9 (C-18), 46.7 (C-19), 30.9 (C-20), 38.7 (C-21), 78.3 (C-22), 26.7 (C-23), 21.7 (C-24), 16.6 (C-25), 16.9 (C-26), 25.8 (C-27), 180.9 (C-28), 34.5 (C-29), 27.4 (C-30), 51.8 (COOCH₃), 166.1 (C-1'), 35.8 (C-2'), 17.4 (C-3'), 17.4 (C-4'); EMIS *m/z* 554 [M⁺], 526 (12), 511 (26), 483 (13), 466 (100), 348 (12), 333 (17), 248 (7), 206 (16), 203 (19); *anal.* C₃₅H₅₄O₅ C, 75.77%; H, 9.81%; found C, 75.79%; H, 9.84%.

Methyl 22 β -benzyloxy-3-oxoolean-12-en-28-oate (24): white crystals (0.12 g, 61.7%); mp 186–188 °C; IR (KBr) ν_{\max} 3433 (aromatic), 2956 (aliphatic), 1740 (C=O, ester), 1709 (C=O, ester), 1702 (C=O, keto) cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 0.85 (3H, s, CH₃), 0.89 (3H, s, CH₃), 1.04 (6H, s, 2 \times CH₃), 1.09 (3H, s, CH₃), 1.12 (3H, s, CH₃), 1.17 (3H, s, CH₃), 3.04 (1H, dd, *J* = 14.3; 3.8 Hz, C-18-H), 4.26 (3H, s, -COOCH₃), 5.07 (1H, t, *J* = 3 Hz, C-22-H), 5.46 (1H, t, *J* = 3.5 Hz, C-12-H), 7.2–8.1 (5H, m, Ar-H); ¹³C NMR (75 MHz) δ 39.5 (C-1), 34.9 (C-2), 219.6 (C-3), 47.5 (C-4), 55.4 (C-5), 19.9 (C-6), 32.8 (C-7), 39.6 (C-8), 48.3 (C-9), 36.6 (C-10), 24.9 (C-11), 121.7 (C-12), 143.8 (C-13), 42.9 (C-14), 28.8 (C-15), 24.4 (C-16), 52.8 (C-17), 39.9 (C-18), 46.9 (C-19), 30.7 (C-20), 38.9 (C-21), 78.6 (C-22), 27.5 (C-23), 21.9 (C-24), 16.7 (C-25), 17.4 (C-26), 25.9 (C-27), 181.5 (C-28), 34.8 (C-29), 27.4 (C-30), 51.8 (COOCH₃), 167.2 (C-1'), 122.3, 122.9, 129.1, 130.2, 133.5 (C-Ph); EMIS *m/z* 588 [M⁺], 560 (14), 557 (13), 511 (21), 483 (18), 466 (100), 292 (18), 367 (23), 246 (15), 206 (37), 203 (19); *anal.* C₃₈H₅₂O₅ C, 77.51%; H, 8.90%; found C, 77.56%; H, 8.92%.

Cell Culture and MTT Assay. Four cell lines (HL-60, HeLa, colon 502713, and A-549) were obtained from NCCS, Pune, India, and maintained in RPMI medium supplemented with 10% fetal bovine serum, 100 μ g/mL streptomycin, and 100 IU/mL penicillin. Cells were incubated at 37 °C in a humidified atmosphere of 5% CO₂ for 48 h for comparison of cytotoxicity between compounds. The cell lines (2 \times 10⁻⁴/0.1 mL well) were treated with serial dilutions of compounds in 96-well culture plates (Costar) for 48 h. During the last 4 h, cells were reacted with MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) at 37 °C for a colorimetric MTT-based cytotoxicity assay. The reaction product, formazan, was extracted with DMSO, and the absorbance was read at 540 nm.¹⁸ Data represent the mean values and standard deviation of triplicate assays in at least one experiment.

In Vivo Antitumor Activity. Squamous cell carcinogenesis was induced in Swiss albino mice (LACCA/female) according to the established method.¹⁹ Animal care and handling was done according to the guidelines set by the World Health Organization (WHO), Geneva, Switzerland, and the Indian National Science Academy (INSA), New Delhi, India. Depilatory cream was used to remove hairs from the back of mice. The animals were left for 2 days and divided into 17 groups. Animals in group I (*n* = 10) were treated with 100 μ L of vehicle (acetone). The acetone was topically applied on the depilated back of each mouse twice weekly for 20 weeks. The animals of group II (*n* = 10) were topically treated with DMBA (100 nmol/100 μ L of acetone) on the depilated back of each mouse for 2 weeks and promoted with twice weekly applications of TPA (1.7 nmol/100 μ L of acetone) for the next 18 weeks. The animals of groups III–VII (*n* = 10) were orally treated with test compounds suspended in water and carboxymethyl cellulose. Treatments started 1 week before DMBA application and continued for 20 weeks thereafter. Body weight, incidence of skin papillomas, and number of animals that survived after the 20-week treatment period were recorded. Body weight, number of deaths, and papillomas were recorded at weekly intervals. Only those papillomas that persisted for 2 weeks or more were taken into consideration for final evaluation of the data.

Histology. The incidence of skin lesions, tumors per mouse, body weight of mice, and number of mice that survived the 20-week period were recorded. The body weight, number of deaths, and papillomas appearing on depilated skin were recorded at weekly intervals. Only those papillomas that persisted for 2 weeks or more were considered for final evaluation of the data. The skin nodules were excised and fixed in Zenker, routinely processed, and embedded in paraffin. Sections (7 μ m thick) were stained with hematoxylin and eosin and examined under a light microscope to carry out histopathology.

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