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Design, Semisynthesis, and Evaluation of *O*-Acyl Derivatives of (−)-Epigallocatechin-3-gallate as Antitumor Agents

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The partially purified catechin fraction isolated from green tea extract was treated with a variety of acylating agents (acyl anhydrides/chloride) to obtain (−)-epigallocatechin-3-gallate (EGCG) *O*-acyl derivatives in 20−25.4% yields. The (−)-EGCG *O*-acyl derivatives were characterized by physical data and spectral studies. These compounds were evaluated for their antitumor activity by use of a two-stage carcinogenesis model in 7,12-dimethylbenz[*a*]anthracene (DMBA)/12-*O*-tetradecanoylphorbol 13-acetate (TPA)--induced cancer in Swiss albino mice. The study showed that there was a significant decrease in the antitumor activity with the increase in size and branching of the chain length of acyl groups. The results indicated that these *O*-acyl derivatives of (−)-EGCG have the potential to be developed as cancer chemopreventive agents.

KEYWORDS: Green tea; catechins; (−)-EGCG *O*-acyl derivatives; antitumor activity

INTRODUCTION

Green tea derived from the leaves of *Camellia sinensis* has been under extensive investigations for its health benefits, and there is evidence that various catechins (polyphenolic compounds) are responsible for its biological activities (*1*, *2*). The catechins have been reported to reduce the risk of various ailments including cancer (breast, bladder, prostate, lung, skin, and liver) (*3*−*10*) and cardiovascular diseases (*11*−*13*). The green tea catechins (**Figure 1**) consist of (−)-epicatechin (EC, **1**), (−)-epigallocatechin (EGC, **2**), (−)-epicatechin-3-gallate (ECG, **3**), and (−)-epigallocatechin-3-gallate (EGCG, **4**) (*14*, *15*). Out of these compounds, (−)-EGCG (**4**) is the most abundant and has shown potent pharmacological activities including effective protection against certain forms of cancers (*16*−*19*). However, poor bioavailability has restricted its therapeutic use. After intravenous administration of catechins in rats, it was seen that the half-lives of EGCG, ECG, and EC were 191, 362, and 45 min, respectively (*20*). When pure EGCG was given, a shorter half-life was observed, suggesting the effect of other components in the extract on the plasma concentration and elimination of EGCG (*21*, *22*). It has been observed that (−)-EGCG is poorly absorbed in rats and humans due to its unstable nature in neutral and alkaline media (*23*, *24*). Under the basic environment the phenolic groups of (−)-EGCG lead to the formation of phenoxide anion, which is more reactive toward the electrophilic agents (free radicals) in the body (*25*). The formed semiquinone radical results in dimerization (*26*). However, (−)-EGCG is more stable at low pH (*25*). As pH of the intestine and body fluid is neutral or slightly alkaline, the bioavailability of (−)-EGCG is greatly reduced in vivo (*24*, *27*).

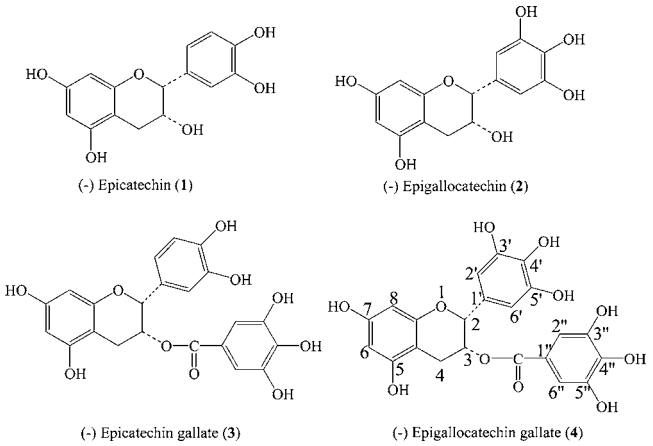

Figure 1. Structure formulas of naturally occurring green tea catechins.

Previous reports have shown that fully O-protected (−)-EGCG derivatives had potential to be developed as anticancer and cancer preventive agents with improved bioavailability (*25*, *28*−*30*). These were indeed more stable and capable of converting back to the parent (−)-EGCG in vivo.

The aim of the present study was to semisynthesize *O*-acyl derivatives of green tea catechins as anticancer agents. In this paper, we report the semisynthesis of (−)-EGCG *O*-acyl derivatives by treating partially purified catechin fraction of green tea with different acylating agents. Evaluation of these derivatives for antitumor activity on DMBA/TPA-induced skin carcinogenesis in Swiss albino mice is reported.

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MATERIALS AND METHODS

Chemicals. 7,12-Dimethylbenz[*a*]anthracene (DMBA) and 12-*O*-tetradecanoylphorbol 13-acetate (TPA) were purchased from Sigma-Aldrich (St. Louis, MO). The acylating agents (acetic anhydride, propionic anhydride, butyric anhydride, isobutyric anhydride, and valeryl chloride) and various solvents (AR or HPLC grade) were purchased from E. Merck/S. D. Fine Chemicals Ltd. India. Precoated silica gel G plates of appropriate sizes (0.2 mm thickness, plastic base, E. Merck, India) were used for thin layer chromatographic (TLC) analyses.

Plant Material. Green tea was collected from Himachal Pradesh Krishi Vishvavidyalya (HPKV) Palampur, Himachal Pradesh, India. The voucher specimen (no. 1453) was deposited in the herbarium of University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India.

Extraction and Isolation of Partially Purified Catechin Fraction. Dry green tea leaves (20 g) were grounded to coarse powder and soaked in water (500 mL) contained in a conical flask at room temperature for 1 h. The contents were heated at 80 °C for 1 h. The mixture was cooled to room temperature and filtered. The filtrate was treated with aqueous solution of aluminum chloride (15 mL, 20% w/v) followed by saturated sodium bicarbonate solution (15 mL) to adjust the pH (5.5−6.5). The reaction mixture was allowed to stand for 1 h (25 °C). The precipitated material was collected by filtration and then suspended in water (20 mL) and sulfuric acid (5 mL, 40% w/v). The reaction mixture was extracted with ethyl acetate (3 × 100 mL) and the organic layer was dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure. The residue was washed with chloroform (3 × 50 mL) to yield partially purified catechin fraction as amorphous brown solid (4.2 g). The above process was repeated several times to obtain more of partially purified catechin fractions.

General Procedure for Semisynthesis and Purification of *O*-Acyl Derivatives of EGCG. The partially purified catechin fraction (4.2 g) was treated with an acylating agent in the presence of pyridine. The mixture was stirred at 45−50 °C for 8 h, poured into ice-cold water (100 mL), and allowed to stand for 1 h. The solid product formed was collected by filtration and dissolved in dichloromethane (100 mL). The organic layer was washed with water (2 × 50 mL) and brine (2 × 10 mL) and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure to give a brown semisolid product (4 g). TLC analysis of acylated material indicated the presence of a number of spots including one of the EGCG *O*-acyl derivatives. The acylated mixture was subjected to tandem preparative scale elution chromatography (*31*) (SGC I and SGC II). A number of fractions were collected. Fractions with similar R_f values were combined and evaporated under reduced pressure to give corresponding catechins. It was further purified by PTLC. The R_f values were recorded. The purity (>98%) of each compound was determined by HPLC in methanol/water (70:30, flow rate 1 mL/min) at wavelength (λ_{max}) 270 nm. The yields were calculated on the basis of partially purified catechin fraction. It has been assumed that partially purified catechin (4.2 g) contains (60%) (−)-EGCG (*32*). This has been converted to the corresponding acylated derivatives **5**−**9**.

Identification of *O*-Acyl Derivatives of (−)-EGCG. ^{1}H NMR and ^{13}C NMR spectra were recorded on a 400 MHz Bruker AC 30 NMR spectrometer (Bruker, Switzerland), with $SiMe_4$ as an internal standard. IR spectra were measured on a Perkin-Elmer RX-1 spectrometer (Perkin-Elmer, Switzerland). Melting points were determined on a Boetius stage apparatus and are uncorrected (*33*). Optical rotations were measured on Rudolph automatic Polarimeter Autopol III (Rudolph). UV spectra were recorded on a Perkin-Elmer UV−vis spectrophotometer Lambda-15 (Bodenseewerk, Perkin-Elmer & Co. GmbH, Germany). Mass spectra were performed on LC Waters Allianz 2695, MS detector ESI, Software MassLynx 4.0 (Waters). Solvents used in the above LC/MS were (A) ammonium acetate buffer (pH 6.7) and (B) acetonitrile (1:9). The spectra were recorded in positive ion mode between *m*/*z* 120 and 1500. Column chromatography was performed with silica gel 60−120 mesh, 30 g/g of each acylated mixture loaded; column size 750 × 30 mm; fraction size 50 mL; solvent chloroform/ethyl acetate for compound **5** and hexane/ethyl acetate for **6**−**9** in gradient mode. A Waters HPLC system equipped with automated gradient controller, 510 pump, U6K injector, 481 UV detector, and 746 data module (Waters) and a Lichrosphere C-18 column (250 mm length × 4.6 mm diameter, E. Merck, India) was used for qualitative analyses.

(−)-5,7-*O*-Diacetyl-3′,4′,5′-*O*-triacetylepigallocatechin-3-*O*-(3″,4″,5″-*O*-triacetyl)gallate (5). The mp, ^{1}H NMR, and ^{13}C NMR data were consistent with those previously reported (*25*, *28*, *29*): amorphous solid, yield 20%; $[\alpha]_D^{25} = -36.00$ (*c* 10 g L^{-1}, $CHCl_3$); $R_f = 0.42$ (chloroform/ethyl acetate 5.5:4.5); IR ν_{max} (KBr) 1777 (s), 1728 (m) cm^{-1}; UV−vis (MeOH) λ_{max} (log ϵ) = 264 (4.1), 222 (4.4), 207 (4.5) nm; LC-MS *m*/*z* 812.11 $[M + NH_4]^+$.

(−)-5,7-*O*-Di-*n*-propionyl-3′,4′,5′-*O*-tri-*n*-propionylepigallocatechin-3-*O*-(3″,4″,5″-*O*-tri-*n*-propionyl)gallate (6). The mp, ^{1}H NMR, and ^{13}C NMR data were consistent with those previously reported (*29*): amorphous solid, yield 20%; $[\alpha]_D^{25} = -42.59$ (*c* 10.8 g L^{-1}, $CHCl_3$); $R_f = 0.45$ (*n*-hexane/ethyl acetate 6:4); IR ν_{max} (KBr) 1770 (s), 1728 (m) cm^{-1}; UV−vis (MeOH) λ_{max} (log ϵ) = 266 (3.6), 218 (4.3), 199 (4.1) nm; LC-MS *m*/*z* 924.71 $[M + NH_4]^+$.

(−)-5,7-*O*-Di-*n*-butyryl-3′,4′,5′-*O*-tri-*n*-butyrylepigallocatechin-3-*O*-(3″,4″,5″-*O*-tri-*n*-butyryl)gallate (7). The mp, ^{1}H NMR, and ^{13}C NMR data were consistent with those previously reported (*29*): semisolid, yield 25.4%; $[\alpha]_D^{25} = -28.30$ (*c* 10.6 g L^{-1}, $CHCl_3$); R_f = 0.47 (*n*-hexane/ethyl acetate 7:3); IR ν_{max} (KBr) 1773 (s), 1725 (m) cm^{-1}; UV−vis (MeOH) λ_{max}(log ϵ) = 271 (3.4), 225 (4.2), 207 (4.3) nm; LC-MS *m*/*z* 1036.85 $[M + NH_4]^+$.

(−)-5,7-*O*-Diisobutyryl-3′,4′,5′-*O*-triisobutyrylepigallocatechin-3-*O*-(3″,4″,5″-*O*-triisobutyryl)gallate (8). Semisolid, yield 14.2%; $[\alpha]_D^{25} = -35.07$ (*c* 10.55 g L^{-1}, $CHCl_3$); $R_f = 0.45$ (*n*-hexane/ethyl acetate 7:3); IR (KBr) 3513 (br, moist), 2978 (s), 2938 (m), 2879 (w), 1768 (s), 1724 (m), 1622 (w), 1596 (w), 1494 (w), 1468 (w), 1430 (w), 1388 (w), 1348 (w), 1319 (w), 1183 (m), 1125 (s), 1089 (s), 966 (w), 910 (w), 874 (w), 837 (w), 763 (m), 666 (w) cm^{-1}; UV−vis (MeOH) λ_{max} (log ϵ) = 270 (3.4), 225 (4.3), 206 (4.5) nm; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25−1.34 (m, 48H, $2CH_3 \times 8$), 2.68−2.83 (m, 8H, CH × 8), 5.16 (br s, 1H, H-2), 5.70 (m, 1H, H-3), 2.96 (dd, 1H, H-4b, *J* = 1.76, 17.6 Hz), 3.06 (dd, 1H, H-4a, *J* = 4.76, 18.0 Hz), 6.58 (d, 1H, H-8, *J* = 2.2 Hz), 6.73 (d, 1H, H-6, *J* = 2.2 Hz), 7.20 (s, 2H, H-2′,6′), 7.57 (s, 2H, H-2″,6″); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.7−18.9 ($2CH_3 \times 8$), 33.7−34.0 (CH × 8), 26.0 (C-4), 67.8 (C-3), 76.5 (C-2), 107.7 (C-8), 108.8 (C-6), 109.3 (C-4a), 118.3 (C-2″,6″), 122.0 (C-2′,6′), 127.2 (C-1″), 134.4 (C-4′), 134.8 (C-1′), 139.1 (C-4″), 143.5 (C-3″,5″), 143.6 C-3′,5′), 149.7 (C-7), 149.9 (C-5), 154.8 (C-8a), 163.5 (−C═O), 172.0 (−C═O), 172.5 (−C═O), 173.5 (−C═O × 2), 173.9 (−C═O × 2), 174.4 (−C═O), 174.8 (−C═O); LC-MS *m*/*z* 1036.85 $[M + NH_4]^+$.

(−)-5,7-*O*-Di-*n*-valeryl-3′,4′,5′-*O*-tri-*n*-valerylepigallocatechin-3-*O*-(3″,4″,5″-*O*-tri-*n*-valeryl)gallate (9). Semisolid, yield 24.1%; $[\alpha]_D^{25} = -42.37$ (*c* 11.8 g L^{-1}, $CHCl_3$); $R_f = 0.68$ (*n*-hexane/ethyl acetate 7:3); IR (KBr) 3402 (br, moist), 2960 (s), 2934 (s), 2872 (m), 1772 (s), 1727 (m), 1621 (w), 1595 (w), 1493 (w), 1463 (w), 1429 (w), 1361 (w), 1318 (w), 1227 (m), 1134 (s), 1092 (s), 1045 (m), 912 (w), 763 (w), 558 (w) cm^{-1};. UV−vis (MeOH) λ_{max} (log ϵ) = 268 (3.5), 224 (4.3), 206 (4.5) nm; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90−0.98 (24H, $CH_3 \times 8$), 1.36−1.46 (16H, $CH_2 \times 8$), 1.64−1.74 (16H, $CH_2 \times 8$), 2.46−2.56 (16H, $CH_2 \times 8$), 5.17 (br s, 1H, H-2), 5.67 (m, 1H, H-3), 2.96 (dd, 1H, H-4b, *J* = 2.8, 17.4 Hz), 3.05 (dd, 1H, H-4a, *J* = 4.7, 18.6 Hz), 6.59 (d, 1H, H-8, *J* = 2.2 Hz), 6.72 (d, 1H, H-6, *J* = 2.2 Hz), 7.21 (s, 2H, H-2′,6′), 7.59 (s, 2H, H-2″,6″); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.6 ($CH_3 \times 8$), 22.2 ($CH_2 \times 8$), 26.8−26.9 ($CH_2 \times 8$), 33.4−34.0 ($CH_2 \times 8$), 26.0 (C-4), 67.9 (C-3), 76.5 (C-2), 107.9 (C-8), 109.0 (C-6), 109.4 (C-4a), 118.6 (C-2″, 6″), 122.2 (C-2′, 6′), 127.3 (C-1″), 134.4 (C-4′), 134.9 (C-1′), 139.0 (C-4″), 143.4 (C-3″,5″), 143.5 (C-3′,5′), 149.7 (C-7), 149.8 (C-5), 154.8 (C-8a), 163.6 (−C═O), 168.9 (−C═O), 169.4 (−C═O), 170.2 (−C═O × 2), 170.4 (−C═O × 2), 171.3 (−C═O), 171.7 (−C═O); LC-MS *m*/*z* 1148.58 $[M + NH_4]^+$.

Animals. Female Swiss albino mice (6 weeks old, weighing 13−28 g) were obtained from the Central Animal House of Panjab University, Chandigarh, India, and kept in the Departmental Animal Rooms at controlled temperature (23 ± 5 °C) and humidity (60% ± 5%) with a 12 h light/dark cycle. They were fed on basal diet and

water. The mice were acclimatized for 1 week before experimentation. The 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.

Antitumor Activity of *O*-Acyl Derivatives of (−)-EGCG. Skin tumors were induced in Swiss albino mice (LACCA/female) according to the method of Azuine and Bhide (*34*). Depilatory was used to remove hair from the back of mice. The animals were divided into seven groups and given treatments 2 days after removal of hair. The animals in group I ($n = 10$) as a control were treated with acetone (100 μL). The acetone was topically applied two times per week on the depilated back of each mice for 20 weeks. The animals of group II ($n = 15$) were topically treated with DMBA (100 nmol/100 μL) on the depilated back of each mice for 2 weeks followed by application by TPA (1.7 nmol/100 μL of acetone) two times per week for next 18 weeks. The dose of all the derivatives (**5**−**9**) was calculated on the basis of ED_{50}, which falls between 50 and 53 mg/kg body weight of mice. The animals of groups III−VII ($n = 10$) were orally treated with compounds **5**−**9**, respectively, suspended in water and carboxymethyl cellulose (5%, 50 mg/kg body weight). After 1 week the animals were topically treated with DMBA (100 nmol/100 μL), followed by two times per week application of TPA (1.7 nmol/100 μL of acetone). This treatment was continued for next 20 weeks. Body weight, incidence of skin papillomas, and number of animals that survived after the 20-week treatment period were recorded. The body weight and number of deaths and papillomas were recorded weekly. The number of papillomas that persisted for 2 weeks or more has been taken into consideration for final evaluation.

Histology of Papillomas. The skin papillomas and normal skin tissue were fixed in Zenker, routinely processed, and embedded in paraffin. Sections (7 μm thick) were stained with hematozylin and eosin and examined under a light microscope to carry out histopathology.

Statistical Analysis. The data were analyzed by analysis of variance (ANOVA), Student's t test, and χ^2 test. The value of $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Semisynthesis of *O*-Acyl Derivatives of (−)-EGCG. We have semisynthesized (−)-EGCG *O*-acyl derivatives by treatment of partially purified catechin fraction of green tea leaves with various acylating agents. Although such derivatives of (−)-EGCG have been reported from commercially available (−)-EGCG as the starting material (*25*, *28*−*30*), it is the first report of using this compound isolated from natural source, that is, green tea as the starting material. The dry green tea leaves were extracted with hot water. The aqueous extract was treated with an aqueous aluminum chloride solution to precipitate out tea catechins as complex with aluminum chloride. The complex was decomposed with sulfuric acid and the reaction mixture was extracted with ethyl acetate. The removal of solvent under reduced pressure followed by washing with chloroform gave partially purified catechin fraction. This novel procedure is cost-effective and provided sufficient amount of (−)-EGCG *O*-acyl derivatives to carry out the biological studies.

For the semisynthesis of *O*-acyl derivatives, the partially purified catechin fraction was treated with an acylating agent in the presence of pyridine (**Figure 2**). In each reaction, TLC analysis of the acylated material showed 4−5 spots. The acylated material was subjected to tandem preparative-scale elution chromatography (*31*) (SGC I and SGC II) and preparative TLC to obtain *O*-acylated derivatives of different catechins present in green tea. In this paper, only (−)-EGCG *O*-acyl derivatives (**5**−**9**) obtained in the reaction of partially purified catechin fraction with different acylating agents have been reported. Purity of (−)-EGCG *O*-acyl derivatives was checked by HPLC (>98%). Structures of these compounds were confirmed by physical data and spectral studies. Of these compounds, *O*-acetyl (**5**) and *O*-propionyl (**6**) derivatives are amorphous powders

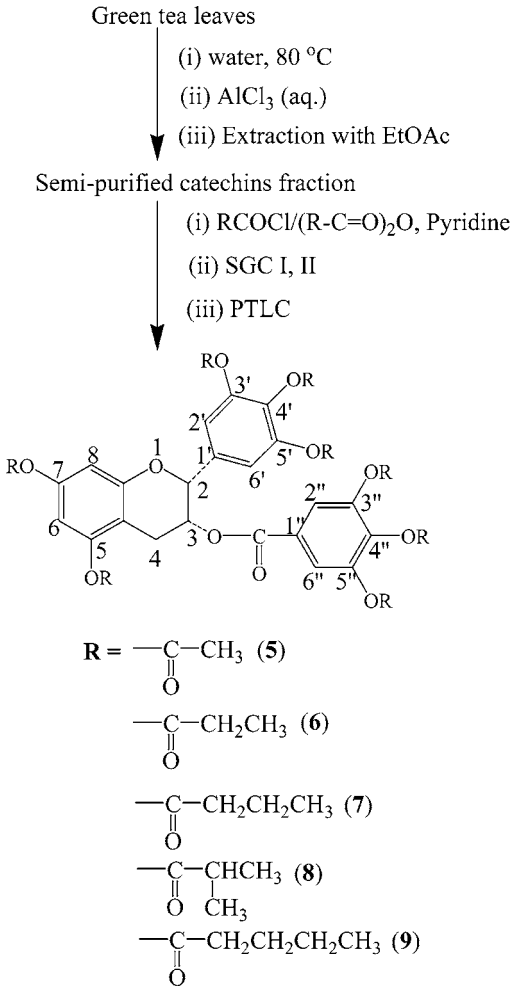

Figure 2. Semisynthesis of *O*-acyl derivatives of (−)-EGCG from green tea leaves.

whereas *O*-butyryl (**7**), *O*-isobutyryl (**8**), and *O*-valeryl (**9**) derivatives are semisolids. The relative R_f values show that the polarity of *O*-acyl derivatives decreases as the acyl chain increases (**5**−**9**). The UV spectra of these compounds have characteristics of acyloxyphenyl rings (260−270 nm) and a band in the 218−225 nm region for substituted gallate moiety. The vibrational bands around 1770 and 1725 cm^{-1} in the IR spectra show the presence of acyl and benzoyl functional groups, respectively. ^1H NMR spectra displayed signals for corresponding alkyl groups in the expected range of δ 2.83−0.96. Similarly, in ^{13}C NMR spectra, the carbonyl carbons of acyl groups appeared in the range of δ 163−175 and alkyl carbons at δ 21.0−20.0 (**5**), 29.6−8.9 (**6**), 36.2−13.6 (**7**), 34.0−18.7 (**8**), and 34.0−13.6 (**9**). The mass spectra showed the molecular ion peaks for compounds **5**−**9** at m/z 812.11, 924.71, 1036.85, 1036.85, and 1148.58 $[M + NH_4]^+$, respectively. The structure formulas have been supported on the basis of the above spectral data (UV, IR, NMR, and MS). Although the amount of each acylating agent (moles) was taken in excess of the phenolic contents of the naturally occurring catechins, the yield of the acylated derivatives depends on the nature of reagent.

Antitumor Activity of *O*-Acyl Derivatives of (−)-EGCG. Cancer is a multistep process involving the sequential phases of initiation, promotion, and progression. Chemoprevention is one of the most desirable strategies to reverse, arrest, or inhibit the carcinogenesis (*35*). Green tea exhibits a wide spectrum of biological activities including cancer chemoprevention (*1*, *2*). The mouse skin carcinogenesis model is a well-characterized

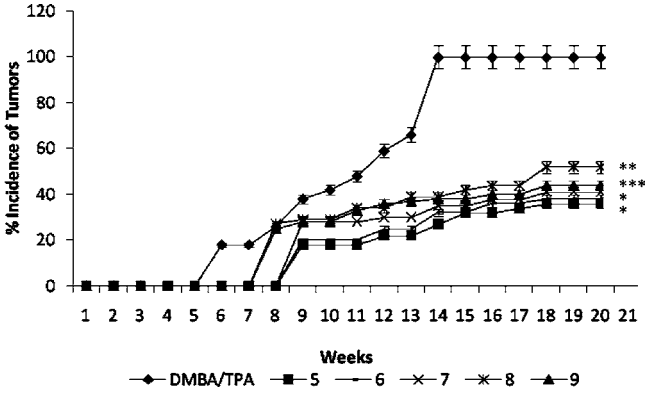

Figure 3. Effect of EGCG prodrugs on percent incidence of tumors in mice induced by DMBA/TPA. ***P < 0.001, ** P < 0.01, *P < 0.05 vs DMBA/TPA alone.

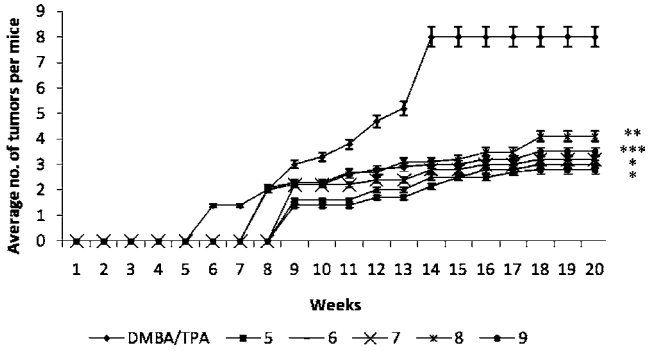

Figure 4. Effect of EGCG prodrugs on average number of tumors per mouse (tumor yield) induced by DMBA/TPA. ***P < 0.001, **P < 0.01, *P < 0.05 vs DMBA/TPA alone.

model for studying the genetic and biological changes associated with the chemical initiation of the lesions and their subsequent transition to squamous cell carcinoma. The various *O*-acyl derivatives of (−)-EGCG (**5**−**9**) were semisynthesized and screened for their tumor inhibitory potential against DMBA/TPA-induced skin carcinogenesis in Swiss albino mice. The administration of (−)-EGCG derivatives (**5**−**9**) at maximal tolerated dose (50 mg/kg body weight) has shown a significant delay in the onset and overall reduction of papillomas in mice, slight increase in the average body weight, and better survival rate in comparison to DMBA/TPA alone, suggesting chemopreventive activity of (−)-EGCG derivatives. The onset of papillomas was observed in the fifth week in the DMBA/TPA-treated mice and found to be 18%. There was a gradual rise in the incidence of tumors that reached 100% during the 13th week (**Figure 3**). The incidence of DMBA/TPA-induced papillomas was delayed for 3 weeks by (−)-EGCG derivatives **5**−**7**, whereas in **8** and **9** the delay was for 2 weeks. Compounds **5**−**9** showed significant decrease in the incidence of cancer (36%, 38%, 41%, 52%, and 44% vs 100% respectively) at the end of 20 weeks as compared with DMBA/TPA alone (P < 0.001, P < 0.01, P < 0.05) (**Figure 3**). Compounds **5**−**9** also showed significant decrease in the average number of tumors per mice (2.8, 3, 3.2, 4.1, and 3.5 vs 8 respectively) at the end of the 20-week study as compared with DMBA/TPA alone (P < 0.001, P < 0.01, P < 0.05) (**Figure 4**). The antitumor profile of these compounds showed that there was significant decrease in activity with increased branching of the *O*-acyl chain (P < 0.05, P < 0.01). Compound **5** showed better antitumor activity than all other derivatives (P < 0.05, P < 0.001), and compound **8** was the least active compound among all EGCG derivatives. The survival rate of the mice decreased significantly in DMBA/TPA-treated mice as compared with vehicle-treated group (37% vs 85%). The survival rate of (−)-EGCG derivative-treated animals was significantly higher in comparison to the DMBA/TPA-treated group (**Figure 5**). The acetyl derivative- (**5**) treated group showed 85% survival rate, whereas the groups treated with **6**−**9** showed 75% survival rate (P < 0.001, P < 0.05). The average body weight of **6**-, **7**-, **9**-, and DMBA/TPA-treated mice did not differ from that of the acetone-treated mice throughout the study. However, there was a slight increase in the average body weight of the compound **5**-treated group and a slight decrease in the average body weight of compound **8**-treated mice at the termination of the study (P < 0.001, P < 0.05) (**Figure 6**). The histopathological examination (*36*) of depilated backs of mice revealed normal skin and presence of subcutaneous tissue in acetone-treated mice, whereas DMBA/TPA-treated animals showed 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 (−)-EGCG *O*-acyl derivative-treated mice showed hyperplastic papillomatous lesions without evidence of infiltration or cytological atypia.

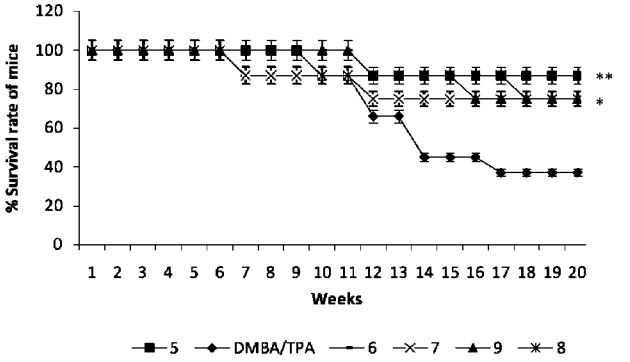

Figure 5. Effect of EGCG prodrugs and DMBA/TPA on percent survival rate of mice. **P < 0.01, *P < 0.05 vs DMBA/TPA alone.

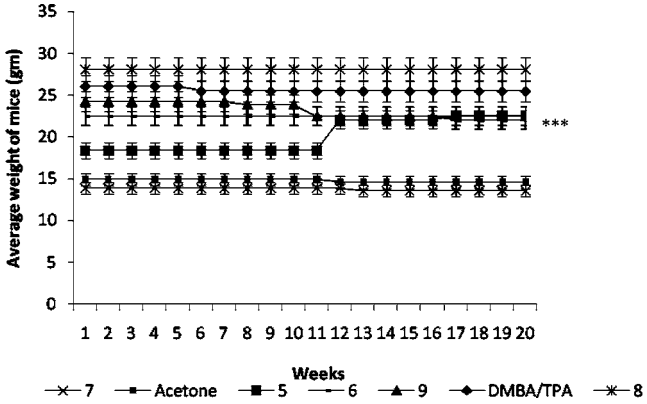

Figure 6. Effect of EGCG prodrugs and DMBA/TPA on average body weight of mice. ***P < 0.001 vs DMBA/TPA alone.

The results showed that antitumor activity is decreased with the increase in the hydrocarbon chain from acetyl to valeryl group (**5**−**9**). The finding that a compound with branched-acyl chain (**8**) led to reduction in the inhibitory activity may be due to the steric hindrance by the two methyl groups. This would avoid the enzyme-catalyzed hydrolytic cleavage of the ester bond of the *O*-isobutyryl derivative of (−)-EGCG (**8**). The mechanism by which these compounds inhibited the DMBA/TPA-induced skin carcinogenesis was not clearly understood. However, it was hypothesized that these derivatives reverse the depletion of skin Langerhans cells and local immunosupression. It is evident from the histopathological studies that the severity

of DMBA/TPA-induced malignancy was reduced markedly by these derivatives. The pretreatment of animals with EGCG derivatives shows their ability to interfere with the initiation of tumors, which is relatively a rapid process, and the continuous treatment after TPA interferes with the promotion, which is a slow process (*36*). The compounds also increased the survival rate of the animals. This effect could be associated with low papilloma burden as result of inhibitory effect of compounds on carcinogenesis. Slight weight gain with compound **5** could be the result of recovery from the effect of DMBA/TPA or, alternatively, better papilloma control.

In conclusion, there is enough evidence that EGCG is the most potent catechin present in green tea and is responsible for the antitumor properties. However, it has poor bioavailability, due to its instability and poor aqueous solubility. Although the instability of EGCG is responsible for its potent antitumor activity due to its antioxidant properties, such compounds are difficult to formulate in therapeutic dosage forms used clinically. Many investigators have prepared EGCG derivatives to overcome these problems using commercially available EGCG as the starting material. We have devised a method for the derivatization of (−)-EGCG and other tea catechins starting from partially purified catechin fraction by the use of acyl chlorides/anhydrides to impart stability followed by isolation of these derivatives. Out of these, *O*-acyl derivatives of EGCG were obtained in adequate quantity and evaluated for their antitumor activity. Further modification and optimization of the pharmacophore of (−)-EGCG may result in the development of potent antitumor agents. This strategy may be helpful to utilize green tea as a resource for drug development, which provided a sufficient amount of (−)-EGCG *O*-acyl derivatives in hand to carry out the biological activity. Physicochemical experiments are in process to evaluate the pharmacokinetic and prodrug potential of these derivatives.

ABBREVIATIONS USED

5, (−)-5,7-*O*-diacetyl-3′,4′,5′-*O*-triacetylepigallocatechin-3-*O*-(3′,4″,5″-*O*-triacetyl)gallate; **6**, (−)-5,7-*O*-dipropionyl-3′,4′,5′-*O*-tripropionylepigallocatechin-3-*O*-(3″,4″,5″-*O*-tripropionyl)-gallate; **7**, (−)-5,7-*O*-dibutyryl-3′,4′,5′-*O*-tributyrylepigallocatechin-3-*O*-(3″,4″,5″-*O*-tributyryl)gallate; **8**, (−)-5,7-*O*-diisobutyryl-3′,4′,5′-*O*-triisobutyrylepigallocatechin-3-*O*-(3″,4″,5″-*O*-triisobutyryl)gallate; **9**, (−)-5,7-*O*-divaleryl-3′,4′,5′-*O*-trivaleryl-epigallocatechin-3-*O*-(3″,4″,5″-*O*-trivaleryl)gallate; SGC, silica gel chromatography; PTLC, preparative thin layer chromatography; TMS, tetramethylsilane.

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Supporting Information Available: Details of SGC I, SGC II, and PTLC with yield calculation. This material is available free of charge via the Internet at http://pubs.acs.org.

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