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Original article

Synthesis and structure–activity relationship of 3-*O*-acylated (−)-epigallocatechins as 5α-reductase inhibitors

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ABSTRACT

A series of 3-*O*-acylated (−)-epigallocatechins were synthesized and their inhibition of steroid 5α-reductase was studied. They were prepared from the reaction of EGCG with *tert*-butyldimethylsilyl chloride followed by reductive cleavage of the ester bond. The resultant (−)-epigallocatechins penta-*O*-*tert*-butyldimethylsilyl ether was esterified with different fatty acids then desilylated to provide the corresponding products. The activity of 3-*O*-acylated (−)-epigallocatechins increased with the increasing carbon numbers of the fatty acid moiety, reaching maximum for 16 carbon atoms (compound **4h**) with an IC_{50} of 0.53 μM, which was ~12-fold more potent than EGCG (IC_{50} = 6.29 μM). Introduction of monounsaturated fatty acid provided the most potent compound **6** (IC_{50} = 0.48 μM), which showed moderate anti-tumor activity *in vivo*.

1. Introduction

(−)-Epigallocatechin-3-gallate (EGCG, Fig. 1) is the most abundant catechin in green tea and has been intensively investigated for its biological activities [1]. In vitro and *in vivo* experiments have demonstrated the anti-oxidation, anti-inflammation, anti-bacterial, anti-viral, and anti-cancer properties of EGCG, which contribute to the major health benefits of tea [2]. Recent studies focused on its chemoprevention and anti-tumor properties. In animal models, EGCG is found to inhibit all stages of carcinogenesis in breast, lung, skin, prostate, and colon cancers [3,4]. A clinical trial also shows a promising result for the chemoprevention of prostate cancer by tea catechins in volunteers with high-grade prostate intraepithelial neoplasia [5].

Steroid 5α-reductase catalyzes the intracrine conversion of circulating testosterone to 5α-dihydrotestosterone (DHT), the most potent natural androgen [6,7] which is associated with the normal growth, development, and function of the prostate [8–10]. 5α-Reductase is involved in various human disorders [11–15] and plays an important role in prostate cancer [16], and its inhibition is thus helpful to the chemoprevention of prostate cancer [17]. EGCG also possesses 5α-reductase inhibitory activity with an IC_{50} value in the micromolar range [18] in addition to several cellular mechanisms with regard to chemoprevention [19]. Furthermore, intraperitoneal injection of EGCG, is found to inhibit the growth or induces the regression of human prostate cancer xenografts in nude mice [20].

For the discovery of more potent EGCG-based derivatives for the inhibition of 5α-reductase, several 3-*O*-acylated (−)-epigallocatechins are synthesized and reported in our previous study [21]. Some of them are more active than EGCG. However, the cases are limited and their structure–activity relationship is not extensively explored. In this paper, we performed a more detail study for this class of inhibitors (see the general structure in Fig. 1). A new efficient synthetic method was developed for their preparation and was suitable to prepare sufficient compounds for *in vivo* study. Among the derivatives, (−)-epigallocatechin-3-*O*-palmitoleate (**6**) was most potent for the inhibition 5α-reductase with an IC_{50} value of 0.48 μM. Using a PC-3 human prostate cancer xenograft model in SCID mice, compound **6** exhibited moderate anti-cancer activity and was more potent than EGCG.

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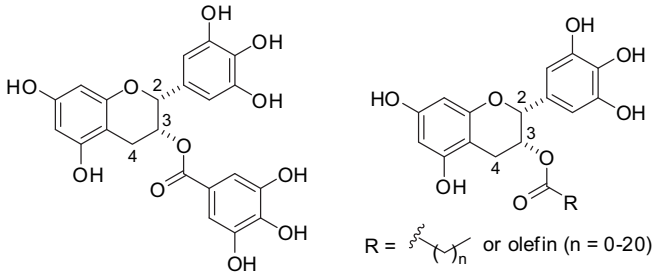


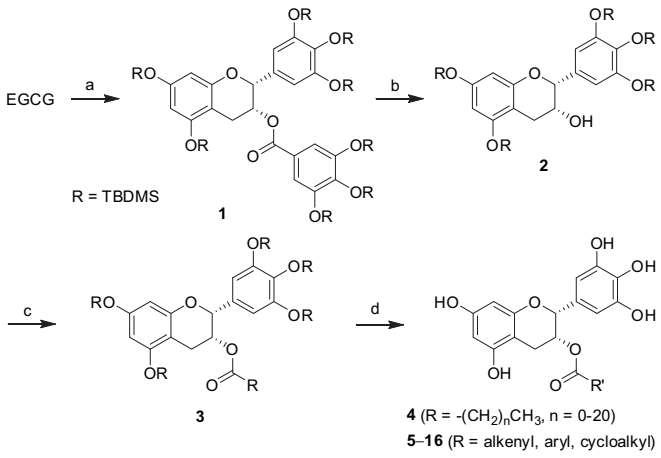
Fig. 1. Structure of EGCG and 3-*O*-Acylated (−)-Epigallocatechins

2. Chemistry

The preparation of 3-*O*-acylated (−)-epigallocatechins **4**–**16** started from the reaction of EGCG with TBDMSCl in DMF in the presence of imidazole (Scheme 1). The reaction provided EGCG octa-*O*-TBDMS (**1**) in 91% yield [22]. Reductive cleavage of the C3-O ester bond by use of $LiAlH_4$ in THF afforded EGC penta-*O*-TBDMS (**2**) in 80% yield. The free hydroxyl group in compound **2** was esterified with different C2–C22 saturated fatty acids using DCC and DMAP to give the silylated 3-*O*-acylated (−)-epigallocatechins **3**. Use of aqueous HF in THF for de-silyating compound **3** gave the corresponding 3-*O*-acylated (−)-epigallocatechins **4** presented in Table 1. Employment of unsaturated carboxylic acids, cycloalkyl carboxylic acid, and aromatic carboxylic acid to esterify compound **3** also provided the corresponding 3-*O*-acylated (−)-epigallocatechins **5**–**16** as shown in Table 2.

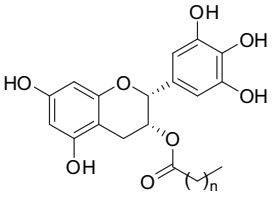
3. In vitro studies

The inhibitory activity of 3-*O*-acylated (−)-epigallocatechins **4**–**16** toward 5α-reductase was determined according to a published method using rat liver microsomal 5α-reductase [18,21,23]. The percent inhibition value at a concentration of 5.0 μM, or for more potent compounds, the IC_{50} values are presented in Table 1 and Table 2. For compound **4** where its C3-O position was acylated with straight-chain saturated fatty acids, the inhibitory activities increased with the increasing numbers of carbon atoms in the fatty acids. The inhibitory activities reached maximum at $n = 14$



Scheme 1. Synthesis of 3-*O*-acylated (−)-epigallocatechins as 5α-reductase inhibitors. Reagents and conditions: (a) TBDMSCl, imidazole, DMF, 91% yield; (b) $LiAlH_4$, THF, 80% yield; (c) R′COOH, DCC, DMAP, CH_2Cl_2, (d) HF, THF.

Table 1
Inhibition of 5α-reductase by 3-*O*-acylated (−)-epigallocatechins with straight-chain saturated fatty acids at C3-O position.



4

Compound	n =	Name of $CH_3(CH_2)_nCOOH$	IC_{50} (μM) or %inhibition at 5.0 μM
4a	0	Acetic acid	(3.0%)
4b	2	Butyric acid	(35%)
4c	4	Caproic acid	(46%)
4d	6	Caprylic acid	(49.3%)
4e	8	Capric acid	2.94
4f	10	Lauric acid	0.77
4g	12	Myristic acid	0.60
4h	14	Palmitic acid	0.53
4i	16	Stearic acid	0.73
4j	18	Arachidic acid	(47.9%)
4k	20	Behenic acid	(28.3%)
EGCG	–	Gallatic acid	6.29

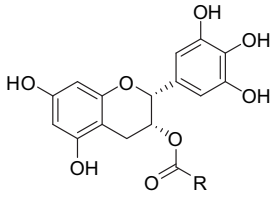
(compound **4h**, with palmitic acid, C16:0) with an IC_{50} of 0.53 μM, which was around 12-fold more potent than EGCG ($IC_{50} = 6.29$ μM). The inhibitory activity started to decrease when $n \geq 16$. For $n \geq 18$, the activity decreased dramatically. Both compounds **4j** ($n = 18$) and **4k** ($n = 20$) did not exhibit significant 5α-reductase inhibitory activities ($IC_{50} > 5.0$ μM).

The 5α-redutase inhibitory activities for (−)-epigallocatechins acylated with unsaturated fatty acids (compounds **5**–**13**), with cyclic carboxylic acids (compound **14**), and with aromatic carboxylic acids (compounds **15** and **16**) were shown in Table 2. Among compounds **5**–**7** which were derived from monounsaturated fatty acids, EGC-palmitoleate (**6**, C16:1 for the fatty acid) was the most potent compound with an IC_{50} of 0.48 μM. The activity of **6** was similar to its saturated analogue EGC-palmitate (**4h**, $IC_{50} = 0.53$ μM). The potency of compound **5** ($IC_{50} = 2.47$ μM, with C10:1 fatty acid) was slightly improved in comparison with its saturated analogue **4e** ($IC_{50} = 2.94$ μM, with C10:0 fatty acid). EGC-(*E*)-geranate (**8**, $IC_{50} = 2.24$ μM) containing two double bonds was also more active than its saturated analogue **4e** ($IC_{50} = 2.94$ μM). EGC–γ-linoleic acid (**9**, with C18:3 fatty acid) had almost identical inhibitory activity as its saturated analogs EGC-stearate (**4i**, with C18:0 fatty acid) and EGC-(*Z*)-oleate (**7**, with C18:1 fatty acid). For EGC–13-*cis*-retinoic acid (**10**) with five double bonds at the C3-O position, the potency was similar to compound **9** and its analogs. The potency for compounds **11**–**13** acylated by fatty acids with five to six carbon atoms was not significant ($IC_{50} > 5.0$ μM), which were consistent with results of compounds **4b** and **4c** derived from saturated fatty acids with less than six carbons.

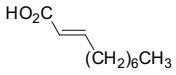
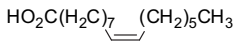
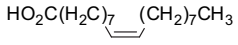
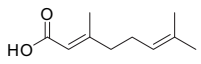
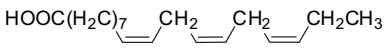
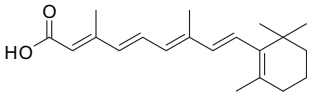
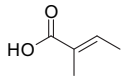
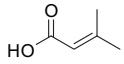
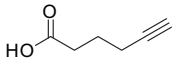
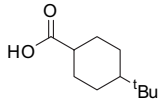
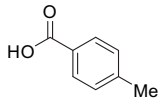
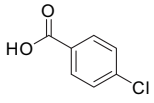
Replacement of the gallate group in EGCG with lipophilic cycloalkyl or aromatic carboxylates, including 4-*tert*-butylcyclohexylcarboxylate, *p*-methybenzoate or *p*-chlorobenzoate, enhanced the 5α-reductase inhibitory activities (see compound **14**–**16** in Table 2). Compared with EGCG ($IC_{50} = 6.29$ μM), the IC_{50} values for compounds **14**–**16** decreased to 1.19, 1.32 and 1.60 μM, respectively. In these cases, introduction of the lipophilic carboxylates generated more potent inhibitors.

For further exploration of the structure–activity relationship of the class of compounds toward the inhibition of 5α-reductase, we

Table 2
Inhibition of 5α-reductase by 3-*O*-acylated (−)-epigallocatechins with unsaturated, cyclic, or aromatic carboxylic acids at C3-O position.



5-16

Compound	Structure of RCOOH	IC_{50} (μM) or % inhibition at 5 μM
5		2.47
6		0.48
7		0.74
8		2.24
9		0.78
10		0.76
11		(29.1%)
12		(36.0%)
13		(48.8%)
14		1.19
15		1.32
16		1.60

synthesized peracylated EGCG **17** and permethylated EGCG **18** as shown in Fig. 2. Compounds **17** and **18** were almost inactive with 8.4% and 0.5% inhibition at 5.0 μM, respectively. Replacement of the ester moiety in 3-*O*-acylated (−)-epigallocatechins with a carbamate moiety decreased inhibitory activity (i.e. **15**→**19** and **16**→**20**). Introduction of the lipophilic naphthyl carbamate to the C3-O position (compound **21**) did not improve 5α-reductase inhibitory activity significantly.

4. Ligand-based pharmacophore identification

Catalyst/HipHop approach was used to evaluate the common features required for the inhibition of 5α-reductase activity by 3-*O*-acylated (−)-epigallocatechins [24]. EGCG, **4a**–**4k**, and **5**–**21** were subjected for pharmacophore model generation based on common chemical features. The resulting most reasonable pharmacophore model contained ten common features as shown in Fig. 3A: four hydrogen-bond donors, one hydrophobic group, and five excluded volumes. The most potent compound **6** was mapped onto the model as shown in Fig. 3B.

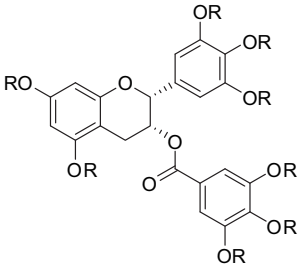
5. In vivo study

For the study of anti-cancer activity of 3-*O*-acylated (−)-epigallocatechins *in vivo*, compound **6** (20 mg/kg), EGCG (20 mg/kg and 80 mg/kg), and vehicle control (15% PEG 400 and 5% EtOH in H_2O) were injected intraperitoneally daily into PC-3 bearing SCID mice for 14 days. The tumor size was measured every two days for 28 days (see Fig. 4). Tumor size at day 28 was reduced by 38% and 32% with the administration of compound **6** at 20 mg/kg and EGCG at 80 mg/kg, respectively. No significant anti-cancer effects of dosing EGCG at 20 mg/kg were observed. There was no body weight loss or drug-related death in all treatment groups. Though PC-3 cancer cell line is androgen-insensitive [25], compound **6** showed a moderate anti-cancer activity *in vivo*.

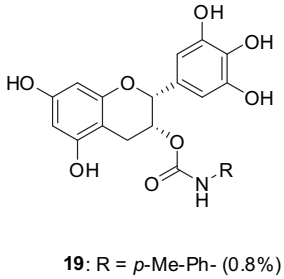
6. Discussion

6.1. Chemistry

Several methods have been reported for the synthesis of 3-*O*-acylated (−)-epigallocatechins by use of EGC as the starting material [26] or through total synthesis [27]. In this study, we chose EGCG as the starting material since it is the most abundant catechin from tea leaves and commercially available. Furthermore, it is suitable for larger scale preparation of the compounds for evaluation of the anti-cancer activity *in vivo*.



17: R = Ac (0.5%)
18: R = Me (8.4%)



19: R = *p*-Me-Ph- (0.8%)
20: R = *p*-Cl-Ph- (19.1%)
21: R = 2-naphthyl- (28.7%)

Fig. 2. Inhibitory activity of peracylated and permethylated EGCG, and EGC-carbamates toward 5α-reductase. The numbers in the parentheses are the percent inhibition at 5.0 μM.

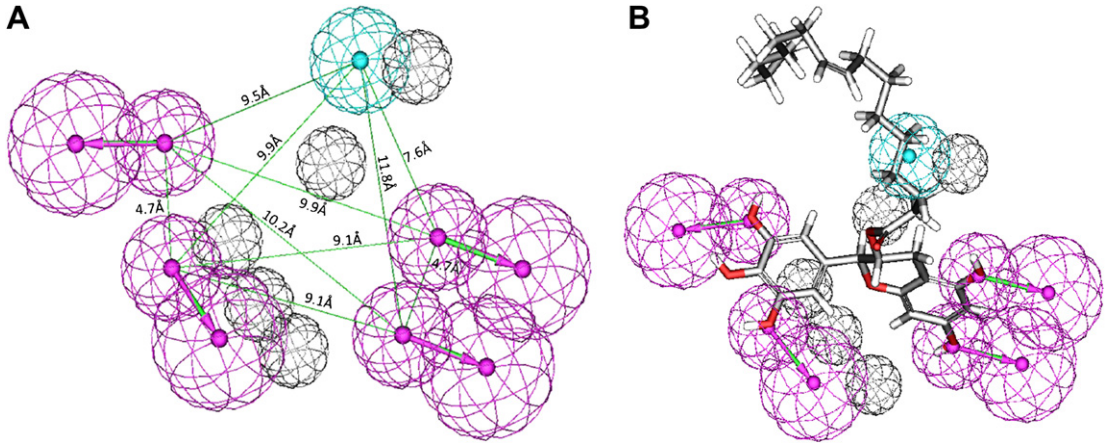


Fig. 3. (A) Pharmacophore generated using Catalyst methodology that describes the inhibition of 5α-reductase by 3-*O*-acylated (−)-epigallocatechins. Phamacophore features are color-coded (hydrogen-bond donor, light red; hydrophobic, light blue; excluded volume, black). (B) Compound 6 mapped onto the pharmacophore. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

The traditional method to derive (−)-epigallocatechins esterified at the C3-O position involves the use of benzyl chloride [28] to mask the hydroxyl groups in EGCG followed by saponifying the gallate group by use of methanolic NaOH. However, the yield was lower than that from the use of TBDMSCl and epimerization takes place at C-2 position due to the use of the strong base [29]. In addition, the methodology is not suitable for unsaturated fatty acids since hydrogenation is required to remove the benzyl groups and the condition also reduces the double or triple bonds. For the deprotection of TBDMS groups in compound **3**, we first tried the use of TBAF as the desilylating agent. However, only trace amount of the corresponding products were obtained. Since it is reported that EGCG and (−)-epigallocatechin is extremely unstable in alkaline solutions (pH > 8) while stable in acidic solutions (pH < 4) [30], we used HF instead of TBAF to maintain the acidic condition to obtain the products in satisfactory yields.

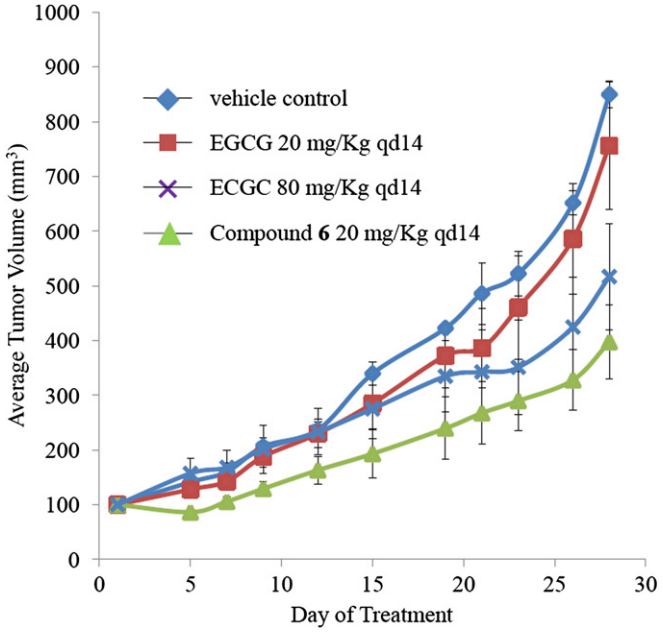


Fig. 4. *In vivo* anti-cancer activity of compound **6** and EGCG for PC-3 human prostate cancer xenograft.

6.2. Biological studies

Since blocking all the hydroxyl groups in EGCG dramatically reduced the activity (see the %inhibition of compounds **17** and **18** in Fig. 2), the hydroxyl groups in EGCG was important to interact with 5α-reductase and might act as hydrogen bonding donors. Nevertheless, the contribution of each hydroxyl group could not be assessed. A selective protection method should be used to rule out their different roles. It could be postulated that the gallate group was not important since its replacement with fatty acids retained or improved the 5α-reductase inhibitory activity (see Tables 1 and 2). More evidences come from the compounds derived from the replacement of gallate group in EGCG with close-in-size tert-butylcyclohexyl, methylphenyl, or chlorophenyl group (see compounds **14**–**15** in Table 2). Their 5α-reductase inhibitory was more potent. Though the structure of 5α-reductase is unknown, it would be reasonable to assume a hydrophobic pocket to interact with the long-chain fatty acid in 3-*O*-acylated (−)-epigallocatechins. Previous kinetic study has shown that EGCG does not compete with testosterone or NADPH for binding to microsomal 5α-reductase; the inhibition could not be overcome by increasing the concentration of NADPH or testosterone [18]. The 3-*O*-acylated (−)-epigallocatechins would not be substrate analogs and bound to the different pocket from testosterone and NADPH.

The range of inhibitory activity (~1–2 log units) for 3-*O*-acylated (−)-epigallocatechins toward 5α-reductase was not sufficient to generate a meaningful activity-based (predictive) pharmacophore model using Catalyst/Hypogen technology. Catalyst/HipHop approach was thus used as an alternative and the model generated was presented in Fig. 3. For providing more information on the pocket bound with the compounds (e.g., through receptor surface analysis) [31,32], the synthesis of more diverse compounds is required. The roles of hydroxyl groups at the different position of 3-*O*-acylated (−)-epigallocatechin should be addressed and the receptor surface around them needs to be probed by different functionalities.

As shown in Fig. 4, dosing compound **6** at 20 mg/kg [33] for 14 days showed better anti-cancer activity than dosing EGCG at 80 mg/kg and 20 mg/kg in PC-3 xenograft model. The results seem to correlate to the 5α-reductase inhibitory activity of the respective compounds (IC_{50} for **6**, 0.48 μM; IC_{50} for EGCG, 6.29 μM). However, other mechanism for the anti-cancer activity of **6** could not be ruled out since EGCG also alters several signal transduction pathway associated the proliferation and survival of cells [19]. The better cell-penetrating ability of compound **6** arisen from the lipophilicity would also attribute to its better *in vivo* anti-cancer activity.

7. Conclusions

In summary, we have synthesized a series of 3-*O*-acylated (−)-epigallocatechins with inhibitory activity toward steroid 5α-reductase. 5α-Reductase inhibition increased with increasing number of carbon atoms of a saturated fatty acid coupled with EGC, reaching a maximum when the fatty acid was sixteen carbons in length (palmitic acid, compound **4h**, $IC_{50} = 0.53$ μM). Replacement of the saturated fatty acids with unsaturated fatty acid slightly increased activity, providing the most potent compound **6** with an IC_{50} of 0.48 μM, which showed moderate anti-tumor activity on PC-3 human prostate cancer xenograft *in vivo*.

8. Experimental

8.1. Chemistry

(−)-Epigallocatechin-3-gallate (EGCG, >98% purity) were purchased from Ryss Co. Chemical reagents and starting materials including imidazole, TBDMSCl, fatty acids, carboxylic acids, and isocyanates were purchase from Acros Organics or Aldrich. Purification by gravity column chromatography was carried out by use of Merck Silica Gel 60 (particle size 0.063–0.200 mm, 70–230 mesh ASTM). Infrared (IR) spectra were measured on a PerkinElmer Spectrum GX FT-IR spectrometer. The wavenumbers reported are referenced to the polystyrene absorption at 1601 cm^{-1}. Absorption intensities are recorded by the following abbreviations: s, strong; m, medium; w, weak. Proton NMR spectra were obtained on a Bruker AVANCE DRX 500 NMR spectrometer (500 MHz) by use of $CDCl_3$, CD_3OD, or DMSO-d_6 as solvent. Carbon-13 NMR spectra were obtained a Bruker spectrometer (125 MHz) by use of $CDCl_3$ or DMSO-d_6 as solvent. Carbon-13 chemical shifts are referenced to the center peak of $CDCl_3$ (δ 77.0 ppm) or DMSO-d_6 (δ 39.5 ppm). Multiplicities are recorded by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; *J*, coupling constant (Hz). ESI-MS spectra were obtained from an Applied Biosystems API 300 mass spectrometer.

8.1.1. Synthesis of (−)-epigallocatechin-3-gallate octa(tert-butyldimethylsilyl) ether (**1**) [22]

To a solution of (−)-epigallocatechin-3-gallate (10.0 g, 21.8 mmol, 1.0 equiv) in DMF (50 mL) was added imidazole (14.9 g, 219 mmol, 10 equiv) followed by TBDMSCl (32.9 g, 218 mmol, 10 equiv) at 0 °C. The solution was warmed to room temperature and stirred for 12 h. The reaction mixture was quenched with water (50 mL) and extracted with hexanes (100 mL × 3). The combined organic layers were washed with water (50 mL × 3), saturated aqueous NaCl (50 mL), dried over $MgSO_4$(s), filtered, and concentrated under reduced pressure. The residue was purified by gravity column chromatography using silica gel (2.0% EtOAc in hexanes) to provide (−)-epigallocatechin-3-gallate octa(*tert*-butyldimethylsilyl) ether (**1**, 27.3 g, 19.9 mmol) as white solids in 91% yield: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.05 (s, 2H, ArH), 6.58 (s, 2H, ArH), 6.16 (d, $J = 2.2$ Hz, 1H, ArH), 5.93 (d, $J = 2.2$ Hz, 1H, ArH), 5.58 (s, 1H, C2-H), 5.01 (s, 1H, C3-H), 2.93 (brs, 2H, C4-H), 0.86–0.98 (m, 72H, 8 × $C(CH_3)_3$), 0.08–0.20 (m, 48H, 8 × $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ−4.22, −3.98, −3.77, −3.65, −3.54, −2.77, 18.30, 18.38, 18.58 (2 × C), 18.85, 18.91, 25.84, 25.88 (2 × C), 26.26, 26.33, 26.40, 27.01, 68.08, 77.42, 101.84, 103.89, 103.98, 112.57, 115.62, 121.97, 130.09, 138.01, 143.08, 148.34, 148.57, 154.86, 155.12, 155.78, 165.19; ESI-MS *m*/*z* 1372.0 $(M + H)^+$.

8.1.2. Synthesis of (−)-epigallocatechin penta(tert-butyldimethylsilyl) ether (**2**) [22]

To a THF solution (100 mL) containing (−)-epigallocatechin-3-gallate octa(*tert*-butyldimethylsilyl) ether (**1**, 26.0 g, 18.9 mmol, 1.0 equiv) was slowly added $LiAlH_4$ (1.44 g, 37.9 mmol, 2.0 equiv) in an ice bath. The solution was warmed to room temperature and stirred for 4.0 h. The reaction was quenched with saturated Na_2SO_4(aq) in an ice bath and filtered. The cake was washed with Et_2O (100 mL) and the combined filtrates were condensed under reduced pressure. The residue was purified by gravity column chromatography using silica gel (3.0% EtOAc in hexanes) to provide (−)-epigallocatechin penta(*tert*-butyldimethylsilyl) ether (**2**, 13.3 g, 15.2 mmol) as viscous liquid in 80% yield: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.61 (s, 1H, ArH), 6.11 (d, $J = 2.1$ Hz, 1H, ArH), 5.96 (d, $J = 2.1$ Hz, 1H, ArH), 4.84 (s, 1H, C2-H), 4.17 (brs, 1H, C3-H), 2.83–2.86 (m, 2H, C4-H), 0.93–1.00 (m, 45H, 5 × $C(CH_3)_3$), 0.12–0.23 (m, 30H, 5 × $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ −4.23, −4.06, −3.77, −3.45, 18.39 (2 × C), 18.57, 18.98, 25.89 (2 × C), 26.36 (2 × C), 28.39, 66.77, 78.04, 101.50, 103.96, 104.07, 112.26, 129.95, 138.06, 148.94, 155.10 (2 × C), 155.47; ESI-MS *m*/*z* 879 $(M + H)^+$.

8.1.3. General procedure for the synthesis of 3-O-acylated (−)-epigallocatechin penta(tert-butyldimethylsilyl) ether (**3**)

To a solution of compound **2** (1.0 equiv), *N*,*N*′-dicyclohexylcarbodiimide (DCC, 2.2 equiv), and 4-dimethylaminopyridine (DMAP, 0.40 equiv) in CH_2Cl_2 was added with various fatty acids or carboxylic acids (2.0 equiv). The solution was stirred at room temperature for 12 h and the insoluble urea was filtered. The filtrate was concentrated under reduced pressure to provide crude compound **3**, which was directly used for next step.

8.1.4. General procedure for the synthesis of 3-O-acylated (−)-epigallocatechin (**4**)

To a plastic beaker containing compound **3** in THF was added aqueous HF (100 equiv). The reaction was stirred at room temperature and the reaction was monitored by TLC. After the reaction was completed, the solution was quenched with water and extracted with EtOAc. The combined organic layer was concentrated under reduced pressure and the residue was purified by gravity column chromatography using silica gel (EtOAc:hexanes :formic acid = 1:1:0.01) to provide compound **4**.

8.1.5. (2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl acetate (**4a**)

Total yield from **2**, 61%; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.46 (s, 2H, ArH), 5.94 (s, 1H, ArH), 5.90 (s, 1H, ArH), 5.34 (brs, 1H, C2-H), 4.86 (s, 1H, C3-H), 2.89 (dd, $J = 17.2$, 4.5 Hz, 1H, C4-H), 2.78 (dd, $J = 17.2$, 2.2 Hz, 1H, C4-H), 1.92 (s, 3H, $CH_3C(=O)$); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 21.55, 22.18, 67.68, 76.27, 94.20, 95.45, 97.26, 105.42, 128.35, 132.31, 145.50, 155.46, 156.50, 156.55, 170.38; ESI-MS *m*/*z* 349.0 $(M + H)^+$.

8.1.6. (2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl butyrate (**4b**)

Total yield from **2**, 51%; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.49 (s, 2H, ArH), 5.94 (s, 1H, ArH), 5.91 (s, 1H, ArH), 5.34 (brs, 1H, C2-H), 4.62 (s, 1H, C3-H), 2.92 (dd, $J = 17.0$, 4.4 Hz, 1H, C4-H), 2.76 (dd, $J = 17.0$, 2.0 Hz, 1H, C4-H), 2.18 (t, $J = 7.1$ Hz, 2H, $-CH_2C(=O)$), 1.28–1.33 (m, 2H, CH_2), 0.76 (t, $J = 7.6$ Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 13.56, 18.43, 22.18, 36.28, 67.71, 76.28, 94.21, 95.49, 97.28, 105.43, 128.39, 132.34, 145.56, 155.49, 156.52, 156.59, 172.26; ESI-MS *m*/*z* 377.0 $(M + H)^+$.

8.1.7. (2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl hexanoate (**4c**)

Total yield from **2**, 56%; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.47 (s, 2H, ArH), 5.94 (s, 1H, ArH), 5.91 (s, 1H, ArH), 5.33–5.34 (m, 1H, C2-H), 4.63 (s, 1H, C3-H), 2.88 (dd, $J = 17.2$, 4.6 Hz, 1H, C4-H), 2.79 (dd, $J = 17.2$, 2.4 Hz, 1H, C4-H), 2.21 (t, $J = 7.2$ Hz, 2H, $-CH_2C(=O)$),

1.08–1.28 (m, 6H, 3 × CH_2), 0.82 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 14.07, 22.18, 23.98, 30.95, 31.44, 34.68, 67.73, 76.29, 94.24, 95.47, 97.26, 105.42, 128.38, 132.33, 145.55, 155.48, 156.51, 156.58, 172.25; ESI-MS m/z 405.0 $(M + H)^+$.

*8.1.8. (2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl octanoate (**4d**)*

Total yield from **2**, 57%; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.47 (s, 2H, ArH), 5.92 (s, 1H, ArH), 5.88 (s, 1H, ArH), 5.30 (s, 1H, C2-H), 4.80 (s, 1H, C3-H), 2.78–2.81 (m, 2H, C4-H), 2.20 (t, J = 7.2 Hz, 2H, $-CH_2C(=O)$), 1.17–1.29 (m, 10H, 5 × CH_2), 0.88 (t, J = 7.3 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 14.01, 22.16, 23.57, 24.45, 28.99, 29.23, 31.36, 34.51, 67.72, 76.28, 94.23, 95.46, 97.24, 105.41, 128.36, 132.39, 145.57, 155.49, 156.52, 156.58, 172.23; ESI-MS m/z 433.0 $(M + H)^+$.

*8.1.9. (2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl decanoate (**4e**)*

Total yield from **2**, 55%; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.45 (s, 2H, ArH), 5.96 (s, 1H, ArH), 5.91 (s, 1H, ArH), 5.34 (s, 1H, C2-H), 4.83 (s, 1H, C3-H), 2.76–2.85 (m, 2H, C4-H), 2.13 (t, J = 7.2 Hz, 2H, $-CH_2C(=O)$), 1.13–1.31 (m, 14H, 7 × CH_2), 0.91 (t, J = 7.4 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 14.03, 22.15, 23.54, 24.43, 28.37, 28.76, 28.91, 29.06, 31.34, 34.50, 67.68, 76.29, 94.21, 95.45, 97.23, 105.45, 128.39, 132.41, 145.63, 155.47, 156.52, 156.64, 172.32; ESI-MS m/z 461.0 $(M + H)^+$.

*8.1.10. (2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl dodecanoate (**4f**)*

Total yield from **2**, 61%; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.24 (s, 1H, OH), 8.99 (s, 1H, OH), 8.75 (s, 2H, OH), 7.98 (s, 1H, OH), 6.34 (s, 2H, ArH), 5.92 (s, 1H, ArH), 5.75 (s, 1H, ArH), 5.20 (s, 1H, C2-H), 4.85 (s, 1H, C3-H), 2.75–2.85 (m, 2H, C4-H), 2.12 (t, J = 7.2 Hz, 2H, $-CH_2C(=O)$), 1.09–1.35 (m, 18H, 9 × CH_2), 0.85 (t, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 14.01, 22.17, 23.58, 24.44, 28.35, 28.73, 28.80, 28.91, 29.05, 29.08, 31.37, 34.51, 67.71, 76.30, 94.23, 95.46, 97.26, 105.45, 128.39, 132.41, 145.63, 155.48, 156.50, 156.59, 172.27; ESI-MS m/z 489.0 $(M + H)^+$.

*8.1.11. (2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl tetradecanoate (**4g**)*

Total yield from **2**, 58%; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.47 (s, 2H, ArH), 5.94 (s, 1H, ArH), 5.90 (s, 1H, ArH), 5.33 (m, 1H, C2-H), 4.86 (s, 1H, C3-H), 2.79–2.89 (m, 2H, C4-H), 2.18–2.21 (m, 2H, $-CH_2C(=O)$), 1.20–1.45 (m, 22H, 11 × CH_2), 0.90 (t, J = 6.8 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 14.00, 22.15, 23.53, 24.41, 28.36, 28.72, 28.81, 28.86, 28.93, 28.97, 29.07, 29.11, 31.32, 34.50, 67.69, 76.33, 94.24, 95.47, 97.21, 105.46, 128.38, 132.42, 145.61, 155.49, 156.47, 156.56, 172.28; ESI-MS m/z 517.0 $(M + H)^+$.

*8.1.12. (2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl palmitate (**4h**)*

Total yield from **2**, 60%; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.46 (s, 2H, ArH), 5.94 (s, 1H, ArH), 5.91 (s, 1H, ArH), 5.33 (s, 1H, C2-H), 4.88 (s, 1H, C3-H), 2.90 (dd, J = 17.4, 4.5 Hz, 1H, C4-H), 2.77 (dd, J = 17.4, 1.5 Hz, 1H, C4-H), 2.19 (t, J = 7.3 Hz, 2H, $-CH_2C(=O)$), 1.11–1.33 (m, 26H, 13 × CH_2), 0.90 (t, J = 7.1 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 14.03, 22.11, 23.56, 24.40, 28.38, 28.73, 28.81, 28.87, 28.92–29.12 (6 × CH_2), 31.33, 34.55, 67.66, 76.38, 94.23, 95.48, 97.20, 105.47, 128.35, 132.43, 145.60, 155.52, 156.48, 156.57, 172.29; ESI-MS m/z 545.0 $(M + H)^+$.

*8.1.13. (2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl stearate (**4i**)*

Total yield from **2**, 65%; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.47 (s, 2H, ArH), 5.94 (d, J = 2.2 Hz, 1H, ArH), 5.91 (d, J = 2.2 Hz, 1H, ArH), 5.33 (brs, 1H, C2-H), 4.88 (s, 1H, C3-H), 2.92 (dd, J = 17.5, 4.5 Hz, 1H, C4-H), 2.78 (dd, J = 17.5, 4.5 Hz, 1H, C4-H), 2.20 (t, J = 7.2 Hz, 2H, $-CH_2C(=O)$), 1.11–1.45 (m, 30H, 15 × CH_2), 0.89 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 14.01, 22.15, 24.43, 25.49, 28.35, 28.70, 28.76, 28.82, 28.95–29.11 (8 × CH_2), 31.36, 33.67, 67.74, 76.33, 94.22, 95.45, 97.29, 105.47, 128.40, 132.46, 145.61, 155.44, 156.52, 156.62, 172.21; ESI-MS m/z 573.0 $(M + H)^+$.

*8.1.14. (2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl icosanoate (**4j**)*

Total yield from **2**, 51%; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.46 (s, 2H, ArH), 5.94 (s, 1H, ArH), 5.91 (s, 1H, ArH), 5.32 (s, 1H, C2-H), 4.87 (s, 1H, C3-H), 2.73–2.90 (m, 2H, C4-H), 2.14 (t, J = 7.2 Hz, 2H, $-CH_2C(=O)$), 1.19–1.38 (m, 34H, 17 × CH_2), 0.89 (t, J = 6.8 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 13.92, 22.17, 24.42, 25.47, 28.36, 28.72, 28.95–29.11 (12 × CH_2), 31.37, 33.63, 67.73, 76.32, 94.21, 95.43, 97.28, 105.43, 128.39, 132.42, 145.60, 155.46, 156.51, 156.61, 172.24; ESI-MS m/z 601.0 $(M + H)^+$.

*8.1.15. (2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl docosanoate (**4k**)*

Total yield from **2**, 56%; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.24 (s, 1H, OH), 8.99 (s, 1H, OH), 8.75 (s, 2H, OH), 7.98 (s, 1H, OH), 6.34 (s, 2H, ArH), 5.92 (s, 1H, ArH), 5.75 (s, 1H, ArH), 5.20 (s, 1H, C2-H), 4.85 (s, 1H, C3-H), 2.76–2.83 (m, 2H, C4-H), 2.16 (t, J = 7.0 Hz, 2H, $-CH_2C(=O)$), 1.15–1.38 (m, 38H, 19 × CH_2), 0.85 (t, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 13.96, 22.16, 24.44, 25.48, 28.39, 28.79, 28.96–29.12 (14 × CH_2), 31.37, 33.62, 67.70, 76.30, 94.22, 95.45, 97.25, 105.46, 128.39, 132.41, 145.62, 155.47, 156.50, 156.59, 172.23; ESI-MS m/z 627.0 $(M - H)^-$.

*8.1.16. (E)-((2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl) chroman-3-yl) dec-2-enoate (**5**)*

Total yield from **2**, 55%; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.85–6.88 (m, 1H, C=CH), 6.46 (s, 2H, ArH), 5.95 (s, 1H, ArH), 5.91 (s, 1H, ArH), 5.74 (d, J = 15.6 Hz, 1H, C=CH), 5.37 (m, 1H, C2-H), 4.90 (s, 1H, C3-H), 2.75–2.90 (m, 2H, C4-H), 2.14–2.15 (m, 2H, $C{=}CCH_2$), 1.28–1.41 (m, 10H, 5 × CH_2), 0.88 (t, J = 6.8 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 14.12, 23.12, 22.54, 28.58, 29.74, 30.01, 31.24, 33.56, 68.18, 76.71, 94.60, 95.84, 97.88, 105.76, 121.38, 128.81, 132.76, 146.00, 150.19, 155.86, 156.74, 157.21, 168.54; ESI-MS m/z 459.0 $(M + H)^+$.

*8.1.17. (Z)-((2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl) chroman-3-yl) hexadec-9-enoate (**6**)*

Total yield from **2**, 52%; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.48 (s, 2H, ArH), 5.96 (d, J = 2.3 Hz, 1H, ArH), 5.93 (d, J = 2.3 Hz, 1H, ArH), 5.32–5.39 (m, 3H, −CH = CH− + C2-H), 4.89 (s, 1H, C3-H), 2.91 (dd, J = 17.4, 4.6 Hz, 1H, C4-H), 2.79 (dd, J = 17.4, 2.1 Hz, 1H, C4-H), 2.17–2.20 (m, 2H, $-CH_2C(=O)$), 1.98–2.04 (m, 4H, $-CH_2CH = CHCH_2-$), 1.13–1.33 (m, 18H, 9 × CH_2), 0.89 (t, J = 6.8 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 14.39, 22.55, 24.85, 25.91, 27.07 (2 × CH_2), 28.75 (2 × CH_2), 28.92, 29.00, 29.57 (2 × CH_2), 31.61, 34.05, 68.15, 76.73, 94.67, 95.90, 97.70, 105.90, 128.83, 130.02, 130.19, 132.85, 146.06, 155.90, 156.92, 157.02, 172.67; IR (diffuse reflectance) 3364 (br), 2928 (s), 2977 (2854), 2092 (s), 1705 (s), 1607 (s), 1518 (m), 1464 (s), 1307 (s), 1251 (s), 1184 (s), 1141 (s), 1094 (s), 1032 (s), 1016 (s), 889 (w), 822 (s), 784 (m), 728 (m), 700 (s), 628 (w), 538 (w), 460 (w) cm^{-1}; ESI-MS m/z 543.1 $(M + H)^+$.

*8.1.18. (Z)-((2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl) chroman-3-yl) octadec-9-enoate (**7**)*

Total yield from **2**, 68%; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.48 (s, 2H, ArH), 5.96 (s, 1H, ArH), 5.93 (s, 1H, ArH), 5.32–5.35 (m, 3H, CH = CH + C2-H), 4.89 (s, 1H, C3-H), 2.82–2.93 (m, 2H, C4-H), 2.16–2.18

(m, 2H, $-CH_2C(=O)$), 2.00–2.02 (m, 4H, $-CH_2CH = CHCH_2-$), 1.20–1.33 (m, 22H, 11 × CH_2), 0.89 (t, J = 6.8 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 14.48, 22.66, 23.76, 26.10, 28.14, 29.43, 30.21, 30.26, 30.33, 30.37, 30.48, 30.64, 30.81, 30.86, 33.08, 34.95, 68.23, 76.75, 94.69, 95.74, 97.95, 106.01, 128.67, 130.79, 130.88, 132.74, 146.02, 155.93, 156.91, 157.12, 172.56; ESI-MS m/z 571.0 $(M + H)^+$.

*8.1.19. (E)-((2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl) chroman-3-yl)-3,7-dimethylocta-2,6-dienoate (**8**)*

Total yield from **2**, 50%; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.66 (s, 2H, ArH), 6.01 (s, 1H, ArH), 5.95 (m, 2H, ArH + C=CH), 5.29–5.33 (m, 2H, C=CH + C2-H), 4.87 (s, 1H, 1H, C3-H), 2.79–2.82 (m, 2H, C4-H), 2.00–2.25 (m, 4H, 2 × CH_2), 1.55–1.58 (m, 9H, 3 × CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 18.54, 18.62, 22.42, 24.60, 26.15, 39.12, 68.21, 76.64, 94.55, 95.81, 97.60, 105.91, 114.23, 123.55, 128.82, 132.06, 132.76, 146.04, 155.91, 156.90, 157.12, 161.23, 166.02; ESI-MS m/z 457.0 $(M + H)^+$.

*8.1.20. (9Z,12Z,15Z)-((2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxy-phenyl)chroman-3-yl)octadeca-9,12,15-trienoate (**9**)*

Total yield from **2**, 22%; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.47 (s, 2H, ArH), 5.95 (s, 1H, ArH), 5.91 (s, 1H, ArH), 5.28–5.35 (m, 7H, 3 × −CH = CH− + C2-H), 4.89 (s, 1H, C3-H), 2.77–2.81 (m, 6H, 3 × CH_2), 2.12–2.22 (m, 2H, CH_2), 2.01–2.12 (m, 2H, CH_2), 1.95–2.00 (m, 2H, CH_2), 1.29–1.37 (m, 10H, 5 × CH_2), 0.89 (t, J = 6.9 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 14.46, 22.39, 23.63, 25.71, 26.56, 27.90, 28.19, 30.20, 30.46, 32.66, 34.83, 68.12, 76.68, 94.55, 95.84, 97.72, 105.91, 128.81, 128.95, 129.08, 129.24, 129.26, 130.60, 131.14, 132.74, 146.16, 155.83, 156.91, 157.10, 172.60; ESI-MS m/z 567.5 $(M + H)^+$.

*8.1.21. (2E,4E,6E,8E)-((2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxy-phenyl)chroman-3-yl)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenoate (**10**)*

Total yield from **2**, 15%; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.64–7.67 (m, 1H, C=CH), 6.95–7.02 (m, 1H, C=CH), 6.48 (s, 2H, ArH), 6.08–6.24 (m, 4H, C=CH), 5.97 (s, 1H, ArH), 5.94 (s, 1H, ArH), 5.32 (s, 1H, C2-H), 4.92 (s, 1H, C3-H), 2.73–2.95 (m, 2H, C4-H), 2.18 (s, 3H, CH_3), 2.01–2.03 (m, 2H, CH_2), 1.92 (s, 3H, CH_3), 1.63 (s, 3H, CH_3), 1.29–1.50 (m, 4H), 1.04 (s, 3H, CH_3), 0.96 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 12.85, 13.91, 20.31, 21.91, 22.56, 29.40, 33.98, 35.25, 40.77, 68.23, 76.97, 94.76, 95.91, 97.88, 105.92, 119.83, 128.85, 129.49, 130.68, 130.94, 132.21, 132.83, 136.52, 138.96, 139.06, 140.45, 146.26, 154.28, 156.14, 157.02, 157.22, 165.78; ESI-MS m/z 589.0 $(M + H)^+$.

*8.1.22. (E)-((2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl) chroman-3-yl)-2-methylbut-2-enoate (**11**)*

Total yield from **2**, 57%; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.73–6.74 (m, 1H, C=CH), 6.55 (s, 2H, ArH), 5.96 (s, 1H, ArH), 5.93 (s, 1H, ArH), 5.34 (s, 1H, C2-H), 4.92 (s, 1H, C3-H), 2.80–2.90 (m, 2H, C4-H), 1.70–1.73 (m, 6H, 2 × CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 12.86, 15.32, 22.43, 67.28, 76.26, 94.54, 95.83, 97.88, 105.75, 128.40, 128.68, 132.65, 138.67, 145.72, 155.96, 157.21, 157.30, 166.20; ESI-MS m/z 389.0 $(M + H)^+$.

*8.1.23. (2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl-3-methylbut-2-enoate (**12**)*

Total yield from **2**, 51%; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.55 (s, 2H, ArH), 6.46 (s, 1H, C=CH), 5.97 (s, 1H, ArH), 5.91 (s, 1H, ArH), 5.36 (s, 1H, C2-H), 4.94 (s, 1H, C3-H), 2.83–2.94 (m, 2H, C4-H), 1.58 (s, 3H, CH_3), 1.57 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 20.41, 22.38, 26.45, 68.15, 76.31, 94.59, 95.88, 97.50, 105.76, 116.12, 128.85, 132.60, 145.96, 155.88, 156.92, 157.02, 157.20, 166.20; ESI-MS m/z 389.0 $(M + H)^+$.

*8.1.24. (2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl-hex-5-ynoate (**13**)*

Total yield from **2**, 48%; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.46 (s, 2H, ArH), 5.94 (s, 1H, ArH), 5.90 (s, 1H, ArH), 5.35 (m, 1H, C2-H), 4.88 (s, 1H, C3-H), 2.79–2.90 (m, 2H, C4-H), 2.31–2.33 (m, 2H, $C\equiv CCH_2$), 2.16 (s, 1H, $C\equiv CH$), 2.01–2.06 (m, 2H, $-CH_2C(=O)$), 1.62–1.65 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 20.72, 22.55, 23.91, 33.20, 67.98, 71.32, 76.28, 84.71, 94.55, 95.94, 97.47, 105.68, 128.74, 132.75, 145.87, 155.06, 156.90, 157.26, 173.44; ESI-MS m/z 401.0 $(M + H)^+$.

*8.1.25. (2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl-4-tert-butylcyclohexanecarboxylate (**14**)*

Total yield from **2**, 52%; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.47 (s, 2H, ArH), 5.96 (s, 1H, ArH), 5.93 (s, 1H, ArH), 5.32 (m, 1H, C2-H), 4.90 (m, 1H, C3-H), 2.76–2.89 (m, 2H, C4-H), 2.02–2.08 (m, 1H, CH), 1.70–1.85 (m, 4H, 2 × CH_2), 1.36–1.40 (m, 1H, CH), 1.29–1.34 (m, 4H, 2 × CH_2), 0.82 (s, 9H, 3 × CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 22.43, 25.54, 27.62, 29.88, 32.52, 43.62, 48.23, 67.94, 76.38, 94.75, 95.84, 97.37, 105.55, 128.65, 132.87, 145.98, 155.16, 157.01, 157.28, 175.42; ESI-MS m/z 473.0 $(M + H)^+$.

*8.1.26. (2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl-4-methylbenzoate (**15**)*

Total yield from **2**, 60%; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.76 (d, J = 8.2 Hz, 2H, ArH), 7.19 (d, J = 8.2 Hz, 2H, ArH), 6.51 (s, 2H, ArH), 5.97 (s, 1H, ArH), 5.95 (s, 1H, ArH), 5.33–5.35 (m, 1H, C2-H), 4.91 (s, 1H, C3-H), 2.90 (dd, J = 17.4, 4.6 Hz, 1H, CH), 2.80 (dd, J = 17.4, 2.1 Hz, 1H, CH), 2.36 (s, 3H, $ArCH_3$); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 21.12, 22.57, 67.84, 76.43, 94.80, 95.72, 97.27, 105.48, 128.62, 128.33, 129.39, 129.62, 133.66, 142.86, 146.92, 155.06, 157.04, 157.15, 170.21; ESI-MS m/z 425.0 $(M + H)^+$.

*8.1.27. (2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl-4-chlorobenzoate (**16**)*

Total yield from **2**, 55%; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.84 (d, J = 8.6 Hz, 2H, ArH), 7.41 (d, J = 8.6 Hz, 2H, ArH), 6.50 (s, 2H, ArH), 5.97 (s, 1H, ArH), 5.96 (s, 1H, ArH), 5.33–5.35 (m, 1H, C2-H), 4.97 (s, 1H, C3-H), 2.95 (dd, J = 17.2, 4.5 Hz, 1H, C4-H), 2.85 (dd, J = 17.2, 2.2 Hz, 1H, C4-H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 22.67, 67.75, 76.45, 94.82, 95.49, 97.20, 105.34, 128.62, 128.69, 129.74, 131.23, 132.62, 138.04, 146.83, 155.07, 157.11, 157.15, 170.37; ESI-MS (for ^{35}Cl) m/z 445.0 $(M + H)^+$.

*8.1.28. 5-(((2R,3R)-5,7-Diacetoxy-2-(3,4,5-triacetoxyphenyl) chroman-3-yloxy)carbonyl)benzene-1,2,3-triyl triacetate (**17**) [34]*

Prepared from the reaction of EGCG with excess acetyl anhydride in pyridine. Yield, 65%; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.11 (s, 2H, ArH), 7.26 (s, 2H, ArH), 6.76 (s, 1H, ArH), 6.64 (s, 1H, ArH), 5.66 (brs, 1H, C2-H), 5.21 (s, 1H, C3-H), 3.00–3.15 (m, 2H, C4-H), 2.22–2.45 (m, 24H, 3 × $(C=O)CH_3$); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 20.29, 20.40, 20.59, 20.72, 20.84, 20.93, 26.05, 68.18, 76.91, 108.26, 109.18, 118.38, 118.72, 122.27, 122.53, 134.53, 135.25, 139.11, 143.47, 143.57, 149.82, 149.90, 154.91, 163.72, 166.39, 166.94, 167.63, 167.82, 168.64, 168.83; ESI-MS m/z 715.0 $(M + H)^+$.

*8.1.29. (2R,3R)-5,7-Dimethoxy-2-(3,4,5-trimethoxyphenyl) chroman-3-yl-3,4,5-trimethoxybenzoate (**18**) [35]*

Prepared from the reaction of EGCG with excess dimethylsulfate in the presence of K_2CO_3 using acetone as the solvent. Yield, 43%; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.11 (s, 2H, ArH), 6.78 (s, 2H, ArH), 6.22 (s, 1H, ArH), 6.16 (s, 1H, ArH), 5.63 (m, 1H, C2-H), 5.12 (s, 1H, C3-H), 3.65–3.82 (m, 24H, 3 × CH_3), 2.90–3.12 (m, 2H, C4-H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 22.38, 55.38, 56.14, 56.52, 56.88, 57.02, 61.54, 68.50, 76.81, 92.17, 92.95, 99.66, 100.88, 106.75, 125.90, 129.45,

138.19, 143.01, 153.89, 154.24, 156.38, 159.28, 160.75, 164.09, ESI-MS m/z 571.0 $(M + H)^+$.

*8.1.30. (2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl-p-tolylcarbamate (**19**)*

From the reaction of **2** with *p*-tolyl isocyanate followed by desilylation with HF. Total yield from **2**, 44%; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.37 (s, 1H), 9.27 (s, 1H), 8.99 (s, 1H), 8.73 (s, 2H), 8.03 (s, 1H), 7.27 (brs, 2H, ArH), 7.00 (d, J = 8.2 Hz, 2H, ArH), 6.41 (s, 2H, ArH), 5.93 (s, 1H, ArH), 5.77 (s, 1H, ArH), 5.28 (s, 1H, C2-H), 4.93 (s, 1H, C3-H), 2.85–2.95 (m, 2H, C4-H), 2.19 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 20.37, 25.97, 67.78, 76.42, 94.46, 95.54, 97.66, 105.63, 118.41, 128.57, 129.03, 131.16, 132.49, 136.56, 145.72, 153.07, 155.53, 156.58, 156.61; ESI-MS m/z 438.0 $(M - H)^-$.

*8.1.31. (2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl-4-chlorophenylcarbamate (**20**)*

From the reaction of **2** with 4-chlorophenyl isocyanate followed by desilylation with HF. Total yield from **2**, 67%; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.29 (brs, 2H, ArH), 7.18 (d, J = 8.8 Hz, 2H, ArH), 6.53 (s, 2H, ArH), 5.95 (s, 1H, ArH), 5.90 (s, 1H, ArH), 5.37 (s, 1H, C2-H), 4.84 (s, 1H, C3-H), 2.80–2.93 (m, 2H, C4-H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 25.91, 68.11, 76.32, 94.44, 95.54, 97.54, 105.54, 119.82, 125.98, 128.46, 128.52, 132.48, 138.12, 145.72, 152.95, 155.47, 156.59, 156.63; ESI-MS (for ^{35}Cl) m/z 458.0 $(M - H)^-$.

*8.1.32. (2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl-naphthalen-1-ylcarbamate (**21**)*

From the reaction of **2** with 1-naphthyl isocyanate followed by desilylation with HF. Total yield from **2**, 55%; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.37 (s, 1H), 9.31 (s, 1H), 9.02 (s, 1H), 8.78 (s, 2H), 8.10 (s, 1H), 7.75–7.87 (m, 1H, ArH), 7.70–7.75 (m, 2H, ArH), 7.32–7.48 (m, 4H), 6.50 (s, 2H, ArH), 5.96 (s, 1H, ArH), 5.80 (s, 1H, ArH), 5.34 (s, 1H, C2-H), 4.95 (s, 1H, C3-H), 2.65–2.98 (m, 2H, C4-H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 25.93, 67.92, 76.67, 94.46, 95.48, 97.71, 105.74, 122.36, 123.43, 123.66, 125.42, 125.94, 126.77, 127.78, 128.67, 128.70, 132.53, 133.65, 133.75, 145.75, 154.58, 155.66, 156.59, 156.62; ESI-MS m/z 474.0 $(M - H)^-$.

8.2. Biological procedures

[^{3}H]Testosterone (80.4 Ci/mmol, [^{3}H]T) was purchased from New England Biochemical (Boston, MA). Testosterone (T), dihydrotestosterone (DHT), dithiothreitol and finasteride were purchased from Sigma. Phosphomolybdic acid (PMA), nicotinamide adenine dinucleotide phosphate (NADPH), and analytical grade organic solvents were purchased from Merck. Ready Organic™ liquid scintillation cocktail was purchased from Beckman Coulter, Inc.

8.2.1. Assay of 5α-reductase

Microsomes were isolated from rat liver as described by Sun et al. [23]. The enzymatic assay was based on the measurement of DHT production from testosterone in the presence of microsomes from rat liver containing both the type 1 and type 2 5α-reductase. The assay mixture, in a final volume of 200 μL, contained 35 nM [^{3}H]testosterone, 1.0 mM NADPH, 1 mM dithiothreitol, and 40 mM potassium phosphate, pH 6.5, with or without a test compound. The reaction was started by the addition of 500 ng rat liver microsome. After incubation at 37 °C for 30 min, the reaction was stopped by the addition of 200 μL ethyl acetate containing 500 ng testosterone and 500 ng DHT (both radio-inactive). The reaction mixture was vortexed for 1.0 min and 50 μL upper organic layer was collected and separated by TLC (silica gel 60 F-254 plate, 6 × 1.5 cm). These plates were developed in a solvent system (cyclohexane:ethyl acetate = 50:50, v/v) and the testosterone and DHT on the TLC plate were exposed by spraying with PMA (12 g in 250 mL of 95% ethanol). The spots on the TLC were cut separately and placed into counting vials, followed by the addition of 0.50 mL of ethyl acetate to extract [^{3}H]T or [^{3}H]DHT. Five mL of Ready Organic™ liquid scintillation cocktail was added to vials and the radioactivity was measured using an LS6500 multi-purpose scintillation counter (Beckman Instruments, Inc.). The concentration of test compound inhibiting the conversion of [^{3}H]testosterone to [^{3}H]DHT by 50% (IC_{50}) was determined by interpolation between appropriate data points.

8.2.2. In vivo anti-cancer activity studies

PC-3 (1×10^6 cell/mouse) tumor cells were subcutaneously injected into the right flank of 5 week old male NOD/SCID mice (BioLASCO, Taiwan). Tumor volume was measured with a digital caliper once tumor was palpable (within 10 to 15 days after implantation). The tumor-bearing animals were treated when the size of tumor have reached to an average volume of ~50 to 100 mm^3. Twenty mice were divided into 4 groups and treated intraperitoneally with compound **6** (20 mg/kg), EGCG (20 mg/kg or 80 mg/kg) and vehicle control (15% PEG 400 and 5% EtOH in H_2O) with one dose per day for 14 days. The test compound **6** and EGCG were formulated in a solution of 15% PEG 400, 5% EtOH, and 80% H_2O. Body weights of the mice were measured every two days and the size of tumor was measured by a digital caliper every two days. The tumor size plotted in Fig. 4 represents the mean +/− S.E. of data from 5 mice.

8.3. Computational method

The HipHop module in the CATALYST 4.11 package (Accelrys Inc., San Diego, CA) was used to identify the common features for 5α-reductase inhibitors [24]. The HipHop algorithm was forced to determine pharmacophore models that contain 0–5 hydrogen-bond acceptor, 0–5 hydrogen-bond donor, 0–3 positive charge, 0–3 negative charge, 0–3 hydrophobic group and 0–5 excluded volume features. All parameters were set default, except for the Principal value and MaxOmitFeat value. Finally, the top 10 scoring hypotheses composed of these pharmacophore features for each set were exported. CATALYST produced 10 hypotheses, and all include ten common features: four hydrogen-bond donor, one hydrophobic group and five excluded volume. Among these 10 hypotheses, Hypol is the most reasonable (Fig. 3) as characterized.

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