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Synthesis and in vitro biological activity of 4α-(2-propenyl)-5α-cholest-24-en-3α-ol: A 24,25-dehydro analog of the hypocholesterolemic agent 4α-(2-propenyl)-5α-cholestan-3α-ol

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Abstract

4α-(2-Propenyl)-5α-cholestan-3α-ol (LY295427) was previously identified from a Chinese hamster ovary (CHO) cell-based low density lipoprotein receptor/luciferase (LDLR/Luc) assay to be a potent transcriptional activator of the LDL receptor promoter in the presence of 25-hydroxycholesterol. To investigate the effect of the 24,25-unsaturation in the D-ring side chain (desmosterol D-ring side chain) on antagonizing the repressing effect of 25-hydroxycholesterol, 4α-(2-propenyl)-5α-cholest-24-en-3α-ol (17), a 24,25-dehydro analog of LY295427, was thus synthesized from lithocholic acid via the formation of 3α-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-4α-(2-propenyl)-5α-cholan-24-al (15). Test results showed that 17 had an EC_{30} value of 2.6 μM, comparable to 2.9 μM of LY295427, in the CHO cell-based LDLR/Luc assay in the presence of 25-hydroxycholesterol. Apparently, the built-in 24,25-unsaturation in the D-ring side chain of 17 had added little effect to antagonizing the repressing effect of 25-hydroxycholesterol. In the [1-^{14}C-acetate]cholesterol biosynthesis inhibition assay, 17 at 10 μg/ml (23 μM) has been shown to inhibit the cholesterol biosynthesis in CHO cells by 38% relative to the vehicle control; whereas LY295427 showed no inhibition in the same assay in our previous studies. In contrast to LY295427, the built-in 24,25-unsaturation in the D-ring side chain of 17 has conferred an inhibitory effect on cholesterol biosynthesis in CHO cells. In summary, the observed LDL receptor promoter activity of 17 is related to its ability to prevent 25-hydroxycholesterol from exerting the repressing effect via an undetermined mechanism and, in part, to inhibit the cholesterol biosynthesis.

Keywords: Oxidation; Reduction; Reductive alkylation; Sterol; LDL receptor promoter

Elevated levels of plasma cholesterol are associated with an increased risk for the development of coronary heart disease [1–3]. Clinical results have demonstrated that the lowering of plasma cholesterol levels in patients with coronary heart disease is beneficial for preventing and retarding the development of atherosclerosis and coronary heart disease [4–6].

Oxysterols metabolically derived from cholesterol were originally proposed to play a key role, via binding to the putative cytosolic oxysterol-binding protein, in down-regulating the expression of low density lipoprotein receptor (LDLR) and the synthesis of endogenous cholesterol [7–9]. However, the most recent studies by Brown and Goldstein reveal that a novel family of membrane-bound transcription factors, called sterol regulatory element-binding proteins (SREBPs), regulate genes involved in cholesterol biosynthesis as well as the LDL receptor gene [10–13]. Under sterol-deficient conditions, cells may activate the binding of the SREBP cleavage-activating protein (SCAP) [14,15] to the COOH-terminal domain of SREBP. The binding complex allows the proteolytic cleavage to occur at site-1, leading to the subsequent cleavage at site-2 and the release of the NH_2-terminal domain of SREBP for gene regulation in the nucleus [16,17]. Nevertheless, the mechanism of inhibiting the SCAP-initiated site-1 cleavage by sterols is not clear, and the investigation is still undergoing.

Recently, we reported that a new series of 3α-sterols, exemplified by 4α-(2-propenyl)-5α-cholestan-3α-ol (LY295427,

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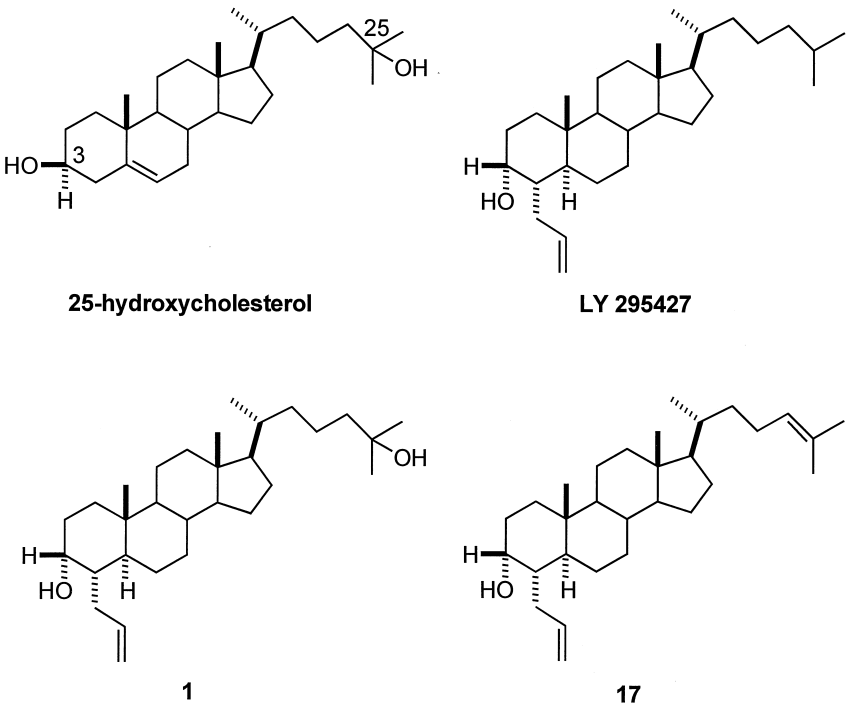


Fig. 1.

Fig. 1), could increase the LDL receptor promoter activity in the presence of 25-hydroxycholesterol in a Chinese hamster ovary (CHO) cell-based LDLR/Luc assay [18]. More importantly, these compounds have also shown efficacy in lowering the total serum cholesterol levels in hypercholesterolemic hamsters [18]. Eight compounds, including LY295427, selected from this new series showed no inhibition in the [1-^{14}C-acetate]cholesterol biosynthesis inhibition assay [18]. Presumably, the observed derepressing activity of this new series of 3α-sterols is effected by preventing 25-hydroxycholesterol from exerting its repressing effect; the mechanism of inhibition has not been determined yet.

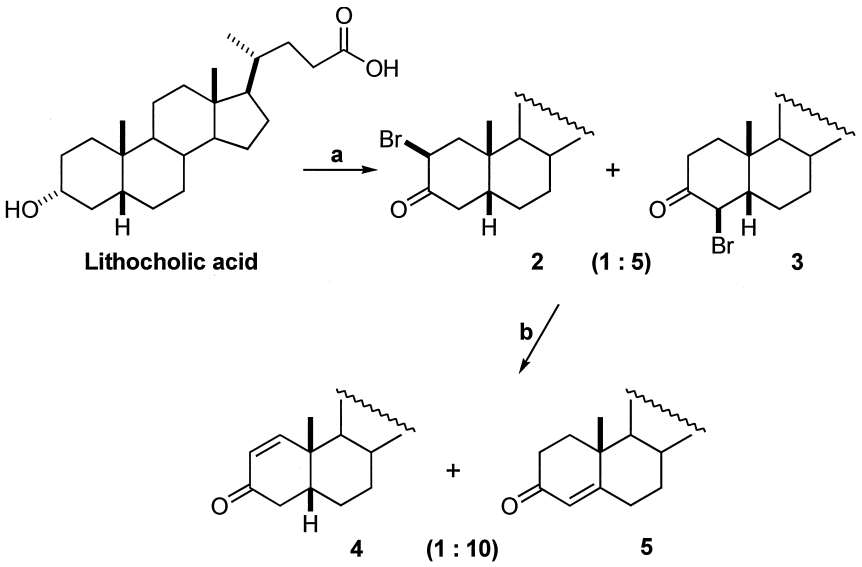
In our previous studies [18], we had extensively explored the structure-activity relationships (SAR) in the A-ring of LY295427. However, few SAR studies had been done in the D-ring side chain. Nonetheless, we observed that compound 1 (Fig. 1), a 25-hydroxyl analog of LY295427, exerted a weaker effect on derepressing the transcriptional activity of the LDL receptor promoter in the presence of 25-hydroxycholesterol in the LDLR/Luc assay with an EC_{30} value of 9.4 μM versus 2.9 μM of LY295427. It appears that the 25-hydroxyl functionality in 1 may somewhat confer the repressing effect as does the 25-hydroxyl functionality in 25-hydroxycholesterol. Consequently, 1 turns out to be less effective in antagonizing the repressing effect caused by 25-hydroxycholesterol.

As part of our continued SAR studies in the D-ring side chain of LY295427, we also investigated the effect of the 24,25-unsaturation (desmosterol D-ring side chain) on the transcriptional activity of the LDL receptor promoter in the presence of 25-hydroxycholesterol as well as the effect on the cholesterol biosynthesis. 4α-(2-Propenyl)-5α-cholest-24-en-3α-ol (17), a 24,25-dehydro analog of LY295427, is thus synthesized from lithocholic acid via the formation of 3α-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-4α-(2-propenyl)-5α-cholan-24-al (15) (Schemes 1–4).

1. Experimental

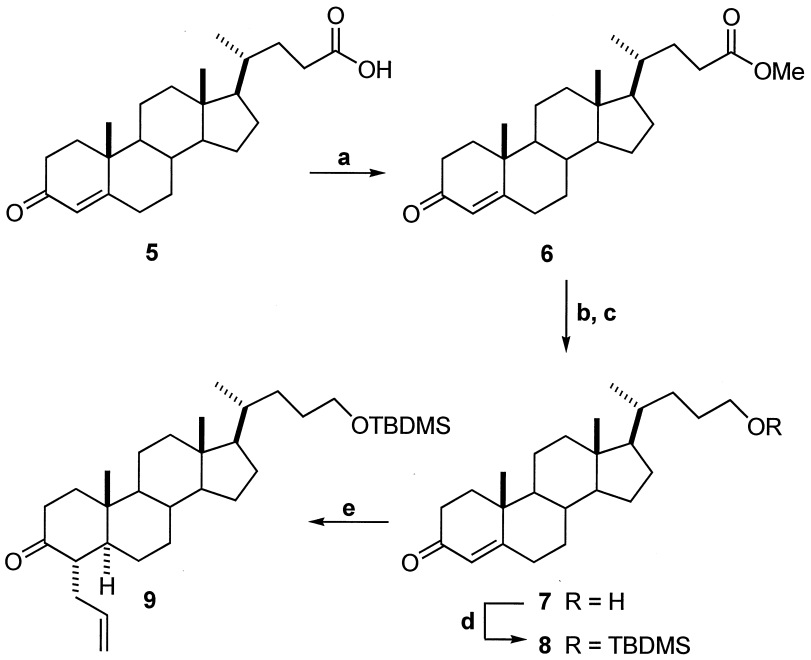
1.1. Materials

Reagents were used as supplied from Aldrich, unless otherwise noted. Reactions were run under an anhydrous nitrogen atmosphere, unless otherwise noted. Powdered magnesium sulfate was used for drying the organic extract. Silica gel (E. Merck, 230–400 mesh ASTM) was used for flash column chromatography. Reactions were monitored, and the homogeneity of the products was checked, by thin-layer chromatography on silica gel 60 F_{254} plates (E. Merck). ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on a General Electric QE-300 instrument. Chemical shifts are expressed as parts per million downfield from internal tetramethylsilane. Infrared spectra were determined on a Nicolet MX-1 FT-IR. Field desorption mass spectra (FDMS) were recorded on a VG Analytical ZAB-3F spectrometer. Elemental analyses were determined on a (Perkin-Elmer Model 240C) elemental analyzer. Melting points were determined on a Thomas-Hoover melting point apparatus, but are uncorrected.



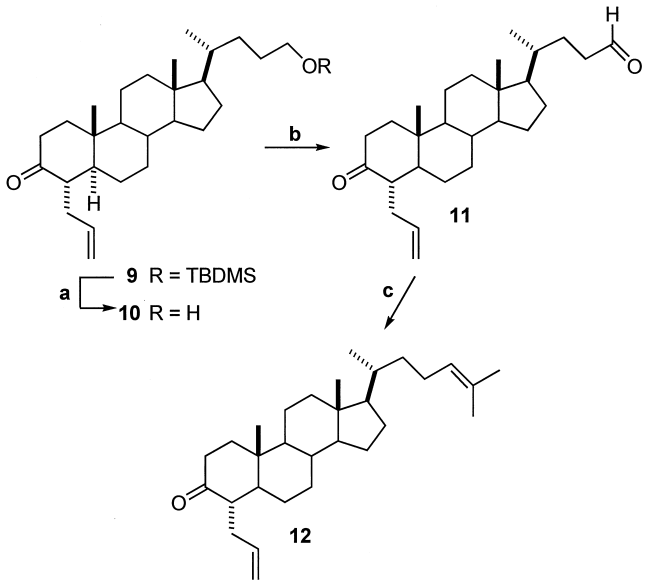
Reagents: (a) NBS, aqueous dioxane, 50 °C; (b) LiCl, DMF, 100 °C.

Scheme 1.



Reagents: (a) MeOH, $HCl_{(g)}$; (b) DIBALH, CH_2Cl_2, -10 °C; (c) MnO_2, $ClCH_2CH_2Cl$; (d) TBDMSCl, imidazole, DMF; (e) Li, NH_3, *t*-BuOH, THF, -78 °C; allyl iodide, -78 °C.

Scheme 2.



Reagents: (a) $(n\text{-Bu})_4NF$, THF; (b) PCC, 4Å MS, CH_2Cl_2; (c) $(Ph)_3P{=}CMe_2$, THF, -15 °C.

Scheme 3.

1.2. Syntheses

1.2.1. Synthesis of a mixture of 2β-bromo-3-oxo-5β-cholan-24-oic acid (2) and 4β-bromo-3-oxo-5β-cholan-24-oic acid (3)

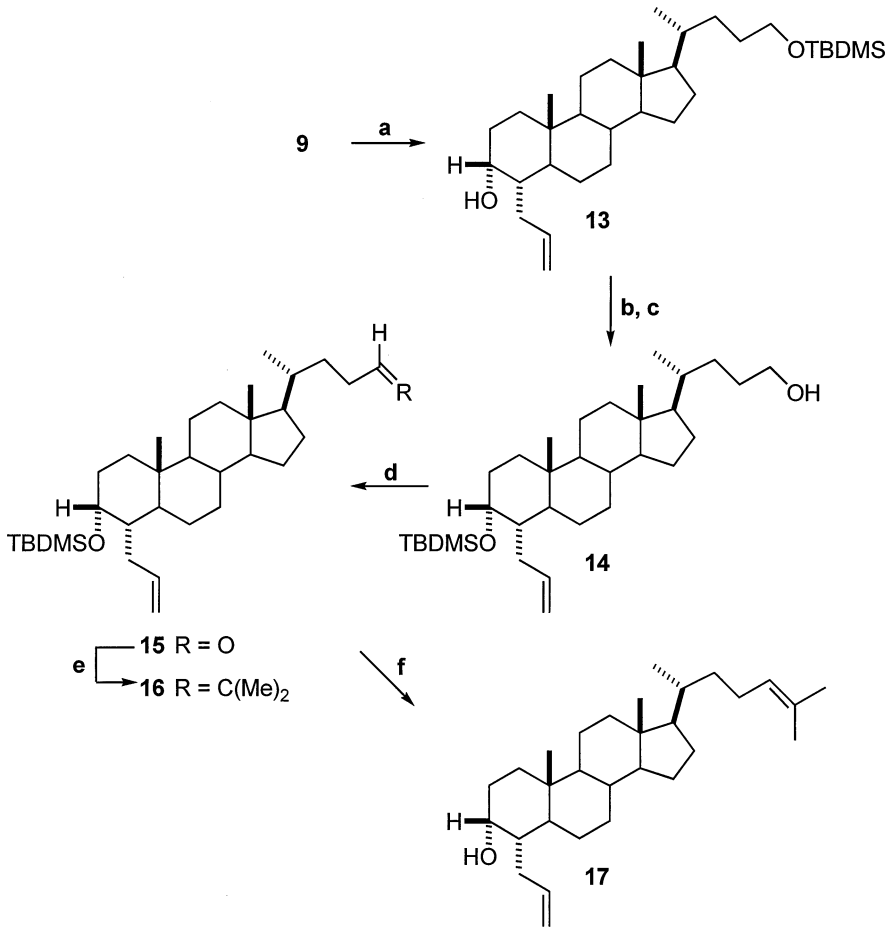
Synthesis of 3 by using *N*-bromosuccinimide was reported in the literature [19]. A mixture of lithocholic acid (50.0 g, 133 mmol) and *N*-bromosuccinimide (54.4 g, 305 mmol) in water (96 ml) and dioxane (864 ml) was stirred at 50°C for 3 h. The mixture was concentrated in vacuo at 35°C to give a solid residue. It was dissolved in CH_2Cl_2 (500 ml), washed with water (500 ml × 2), dried, filtered, and concentrated to give an inseparable mixture of 2 and 3 (60.0 g, 100%) in a 1:5 ratio as a white solid. It was recrystallized from EtOAc/CH_3CN. mp 173.0–176.0°C; IR ($CHCl_3$) 3350–2550 (br), 1720, 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.69 (s, 3H, C-18 CH_3 of 3), 0.92 (d, J = 6.3 Hz, 3H, C-21 CH_3 of 3), 1.08 (s, 3H, C-19 CH_3 of 3), 2.62 (dd, J = 14.0, 5.6 Hz, 1H, C-4 equatorial proton of 2), 2.83 (t, J = 14.0 Hz, 1H, C-4 axial proton of 2), 4.69 (dd, J = 14.0, 5.4 Hz, 1H, C-2 proton of 2), 4.96 (d, J = 12.1 Hz, 1H, C-4 proton of 3); FDMS *m/e* 453 ($M^+ + 1$, ^{79}Br), 455 ($M^+ + 1$, ^{81}Br); Analysis calculated for $C_{24}H_{37}BrO_3$: C, 63.57; H, 8.22. Found: C, 63.73; H, 8.32.

1.2.2. Syntheses of 3-oxo-5β-chol-1-en-24-oic acid (4) and 3-oxochol-4-en-24-oic acid (5) using lithium chloride as a dehydrobrominating agent.

Lithium chloride (26.0 g, 615 mmol) was added to a stirred solution of 2 and 3 (1:5, 62.0 g, 137 mmol) in anhydrous dimethylformide (DMF) (650 ml); the resultant mixture was heated in an oil bath at 100°C for 4 h. After concentration in vacuo at 40°C, the resultant sludge was diluted with ethyl acetate (650 ml), washed with water (200 ml × 2), dried, filtered, and concentrated to give a solid mixture of 4 and 5 in a 1:10 ratio. Following recrystallization from CH_2Cl_2/CH_3CN, 5 (30.0 g, 59%) was obtained as a white solid. Mixture of 4 and 5: ^1H NMR ($CDCl_3$) δ 5.72 (s, 1H, —CH═ of 5), 5.89 (d, J = 10.2 Hz, 1H, —CH═ of 4), 6.83 (d, J = 10.2 Hz, 1H, —CH═ of 4). Five: mp 183.5–185.0°C; IR ($CHCl_3$) 3300–2600 (br), 1709, 1661 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.71 (s, 3H, C-18 CH_3), 0.93 (d, J = 6.4 Hz, 3H, C-21 CH_3), 1.17 (s, 3H, C-19 CH_3), 0.92–2.08 (m, 19H), 2.23–2.50 (m, 6H, two C-2, two C-6, and two C-23 protons), 5.72 (s, 1H, —CH═); FDMS *m/e* 372 (M^+); Analysis Calculated for $C_{24}H_{36}O_3$: C, 77.38; H, 9.74. Found: C, 77.18; H, 9.64.

1.2.3. Methyl 3-oxochol-4-en-24-oate (6)

Synthesis of 6 was reported in the literature [19]. A suspension of 5 (14.4 g, 38.7 mmol) in anhydrous CH_3OH (200 ml) was bubbled with HCl gas under ice-cooling; the resultant mixture was stirred at ambient temperature for 8 h. After concentration, the solid residue was dissolved in EtOAc (150 ml); the solution was washed sequentially with saturated aqueous $NaHCO_3$ (50 ml) and water (50 ml), dried, filtered, and concentrated to give a yellowish solid. The solid was dissolved in a small amount of CH_2Cl_2 and passed through a short pad of silica (eluded with 40% ethyl acetate in hexane) to provide 6 (14.6 g, 98%) as a white solid. mp 122.0–124.0°C; IR (KBr) 1728, 1674 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.73 (s, 3H, C-18 CH_3), 0.94 (d, J = 6.3 Hz, 3H, C-21 CH_3), 1.19 (s, 3H, C-19 CH_3), 0.90–2.10 (m,



Reagents: (a) K-selectride, THF, -10 °C; (b) TBDMSOTf, pyridine, CH_2Cl_2, 0 °C; (c) $(n\text{-}Bu)_4NF$, THF; (d) PCC, NaOAc, 4Å MS, CH_2Cl_2; (e) $(Ph)_3P{=}CMe_2$, THF, -15 °C; (f) $(n\text{-}Bu)_4NF$, THF, reflux.

Scheme 4.

19H), 2.18–2.52 (m, 6H, two C-2, two C-6, and two C-23 protons), 3.68 (s, 3H, OCH_3), 5.74 (s, 1H, C-4 proton); FDMS *m*/*e* 386 (M^+); Analysis Calculated for $C_{25}H_{38}O_3$: C, 77.68; H, 9.91. Found: C, 77.65; H, 9.77.

1.2.4. 3-Oxochol-4-en-24-ol (7)

Diisobutylaluminum hydride (41.4 ml, 1.0 M in toluene) was added to a stirred solution of 6 (5.00 g, 13.0 mmol) in anhydrous CH_2Cl_2 (50 ml) at −10°C. Upon the completion of the addition, the cold bath was removed, then the mixture was stirred for 1 h. The mixture was cooled to −10°C, when it was treated with 1 N HCl (30 ml). The two-layered mixture was stirred vigorously at ambient temperature for 40 min before it was extracted with EtOAc/THF (1:1; 100 ml × 2). The combined organic layers were dried, filtered, and concentrated to give a solid. After recrystallization from CH_2Cl_2/CH_3CN, a mixture of diols (4.61 g, 99%) was obtained as a white solid.

The above diols (2.80 g, 7.77 mmol) were added to a suspension of activated MnO_2 (3.37 g, 38.9 mmol) in anhydrous 1,2-dichloroethane (100 ml). The resultant suspension was sonicated for 5 h. The suspension was filtered through a short pad of celite and washed with CH_2Cl_2 (10 ml). The filtrate was concentrated to give a solid. After recrystallization from CH_2Cl_2/C_6H_{14}, **7** (2.74 g, 98%) was obtained as a white solid. mp 131.0–132.5°C; IR (KBr) 3379 (br), 1656 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.70 (s, 3H, C-18 CH_3), 0.92 (d, J = 6.4 Hz, 3H, C-21 CH_3), 1.17 (s, 3H, C-19 CH_3), 0.85–2.05 (m, 22H), 2.20–2.50 (m, 4H, two C-2 and two C-6 protons), 3.61 (br t, J = 6.0 Hz, 2H, —CH_2O—), 5.71 (s, 1H, C-4 proton); FDMS *m*/*e* 358 (M^+); Analysis Calculated for $C_{24}H_{38}O_2$: C, 80.39; H, 10.68. Found: C, 80.57; H, 10.94.

1.2.6. 24-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]chol-4-en-3-one (8)

A stirred solution of 7 (1.80 g, 5.02 mmol) and imidazole (479 mg, 7.03 mmol) in anhydrous DMF (20 ml) was

treated with *tert*-butyldimethylsilyl chloride (1.06 g, 7.03 mmol) at 0°C; the resultant mixture was stirred at ambient temperature for 1.5 h. The mixture was concentrated in vacuo, and the residue was chromatographed on silica (gradient 0–15% ethyl acetate/toluene) to give 8 (2.26 g, 95%) as a white solid. mp 75.0–77.5°C; IR (KBr) 1679 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.04 [s, 6H, -Si(CH_3)$_2^-$], 0.70 (s, 3H, C-18 CH_3), 0.88 (s, 9H, *tert*-butyl), 0.92 (d, J = 6.4 Hz, 3H, C-21 CH_3), 1.17 (s, 3H, C-19 CH_3), 0.75–2.07 (m, 21H), 2.18–2.48 (m, 4H, two C-2 and two C-6 protons), 3.56 (t, J = 6.4 Hz, 2H, —CH_2O—), 5.71 (s, 1H, C-4 proton); FDMS m/e 472 (M^+); Analysis Calculated for $C_{30}H_{52}O_2$: C, 76.21; H, 11.09. Found: C, 76.45; H, 11.16.

1.2.7. 24-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-4α-(2-propenyl)-5α-cholan-3-one (9)

Lithium chips (23.0 mg, 3.31 mmol) and a glass-coated stir bar were placed in a flame-dried, three-necked, round-bottomed flask fitted with a dry ice condenser under argon. Liquid ammonia (15 ml) was collected in the flask at −78°C to form a deep blue solution, then followed by the addition of anhydrous tetrahydrofuran (THF) (15 ml). A solution of 8 (614 mg, 1.30 mmol) and *t*-BuOH (0.122 ml, 1.30 mmol) in anhydrous THF (15 ml) was added dropwise to the deep blue solution. Upon completion of the addition, the resultant blue solution was stirred for 15 min before it was treated with 1,3-pentadiene (0.25 ml) to quench the excess lithium. After 15 min, allyl iodide (0.238 ml, 2.60 mmol) was added to the white suspension, and the resultant mixture was stirred at −78°C for 2.5 h. Saturated aqueous NH_4Cl (15 ml) was cautiously added to the white suspension. The cold bath was removed, and the mixture, with the evaporation of ammonia, was allowed to warm to ambient temperature. After extraction with EtOAc (30 ml × 2), the combined organic layers were washed with saturated aqueous NaCl (15 ml), dried, filtered, and concentrated. After flash chromatography on silica (gradient 0–5% ethyl acetate/toluene), 9 (402 mg, 60%) was obtained as an oil. IR (film) 1711 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.04 [s, 6H, -Si(CH_3)$_2^-$], 0.66 (s, 3H, C-18 CH_3), 0.88 (s, 9H, *tert*-butyl), 0.90 (d, J = 6.4 Hz, 3H, C-21 CH_3), 1.04 (s, 3H, C-19 CH_3), 0.68–2.06 (m, 24H), 2.15–2.52 (m, 5H, two C-2, one C-4, and two allylic protons), 3.56 (t, J = 6.4 Hz, 2H, —CH_2O—), 4.93–5.04 (m, 2H, ═CH_2), 5.68–5.84 (m, 1H, —CH═); FDMS m/e 514 (M^+); Analysis Calculated for $C_{33}H_{58}O_2Si$: C, 76.97; H, 11.35. Found: C, 77.09; H, 11.30.

1.2.8. 3-Oxo-4α-(2-propenyl)-5α-cholan-24-ol (10)

A stirred solution of 9 (907 mg, 1.77 mmol) in THF (9 ml) was treated with tetrabutylammonium fluoride (1 M in THF, 3.54 ml), and the resultant mixture was stirred at ambient temperature for 2.5 h. After dilution with ethyl acetate (40 ml), the solution was washed with water (20 ml × 2), dried, filtered, and concentrated. The residue was chromatographed on silica (gradient 10–30% ethyl acetate/toluene) to give 10 (650 mg, 92%) as a white solid. mp 136.0–138.0°C; IR (KBr) 3549 (br), 1706 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.67 (s, 3H, C-18 CH_3), 0.91 (d, J = 6.4 Hz, 3H, C-21 CH_3), 1.04 (s, 3H, C-19 CH_3), 0.68–2.05 (m, 25H), 2.16–2.50 (m, 5H, two C-2, one C-4, and two allylic protons), 3.60 (br t, J = 6.0 Hz, 2H, —CH_2O—), 4.93–5.03 (m, 2H, ═CH_2), 5.69–5.83 (m, 1H, —CH═); FDMS m/e 400 (M^+); Analysis Calculated for $C_{27}H_{44}O_2$: C, 80.94; H, 11.07. Found: C, 81.19; H, 11.19.

1.2.9. 3-Oxo-4α-(2-propenyl)-5α-cholan-24-al (11)

Pyridinium chlorochromate (733 mg, 3.40 mmol) was added to a stirred suspension of 10 (545 mg, 1.36 mmol) and 4Å molecular sieve powder (222 mg) in anhydrous CH_2Cl_2 (22 ml) at ambient temperature, and the resultant suspension was stirred for 1.5 h. After filtration through a short pad of silica (30% ethyl acetate/hexane), the filtrate was concentrated, and the residue was chromatographed on silica (gradient 10–20% ethyl acetate/hexane) to give 11 (450 mg, 83%) as a white solid. mp 133.0–135.0°C; IR (KBr) 1722, 1705 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.69 (s, 3H, C-18 CH_3), 0.93 (d, J = 6.5 Hz, 3H, C-21 CH_3), 1.07 (s, 3H, C-19 CH_3), 0.70–2.08 (m, 22H), 2.18–2.55 (m, 7H, two C-2, one C-4, two C-23, and two allylic protons), 4.95–5.06 (m, 2H, ═CH_2), 5.70–5.87 (m, 1H, —CH═), 9.78 (s, 1H, CHO); FDMS m/e 398 (M^+); Analysis Calculated for $C_{27}H_{42}O_2$: C, 81.35; H, 10.62. Found: C, 81.76; H, 10.65.

1.2.10. 4α-(2-Propenyl)-5α-cholest-24-en-3-one (12)

Potassium bistrimethylsilylamide (0.5 M in toluene, 1.00 ml) was added to a stirred suspension of isopropyltriphenylphosphonium iodide (233 mg, 0.540 mmol) in anhydrous THF (3 ml) at −15°C, and the resultant red suspension was stirred for 15 min. A solution of 11 (181 mg, 0.455 mmol) in anhydrous THF (2 ml) was added dropwise to the red suspension at −15°C, then the mixture was stirred at 0°C for 1 h. Acetone (0.1 ml) was added to the mixture to quench the excess phosphonium ylide, also followed by the addition of hexane (10 ml). The mixture was filtered through a short pad of silica (10% ethyl acetate/hexane). The filtrate was concentrated, and the residue was chromatographed on silica (gradient 2–10% ethyl acetate/hexane) to give 12 (90.0 mg, 47%) as a white solid. mp 134.0–135.0°C; IR (KBr) 1705 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.67 (s, 3H, C-18 CH_3), 0.92 (d, J = 6.3 Hz, 3H, C-21 CH_3), 1.05 (s, 3H, C-19 CH_3), 1.60 and 1.68 (both s, 6H, C-26 and -27 CH_3), 0.75–2.10 (m, 24H), 2.20–2.53 (m, 5H, two C-2, one C-4, and two allylic protons), 4.95–5.12 (m, 3H, ═CH_2 and C-24 protons), 5.72–5.85 (m, 1H, —CH═); FDMS m/e 424 (M^+); Analysis Calculated for $C_{30}H_{48}O$: C, 84.84; H, 11.39. Found: C, 84.61; H, 11.32.

1.2.11. 24-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-4α-(2-propenyl)-5α-cholan-3α-ol (13)

K-Selectride (1M in THF, 1.17 ml) was added to a stirred solution of 9 (400 mg, 0.779 mmol) in anhydrous THF (10 ml) at −10°C. Upon completion of the addition, the result-

ant yellowish solution was allowed to warm to ambient temperature, when it was stirred for 1.5 h. The reaction mixture was then cooled to −10°C and sequentially treated with MeOH (1 ml), 5 N NaOH (0.93 ml), and 30% H_2O_2 (0.477 ml). After stirring for 30 min, the cold bath was removed, and the mixture was stirred vigorously for 2 h. Half-saturated aqueous NaCl (10 ml) and EtOAc (60 ml) were added to the mixture. The organic layer was separated, washed with half-saturated aqueous NaCl (10 ml), dried, filtered, and concentrated. After flash chromatography on silica (gradient 5–10% ethyl acetate/hexane), 13 (369 mg, 92%) was obtained as a white solid. mp 101.0–102.0°C; IR (KBr) 3486 (br) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.04 [s, 6H, -Si$(CH_3)_2^-$], 0.64 (s, 3H, C-18 CH_3), 0.81 (s, 3H, C-19 CH_3), 0.89 (s, 9H, *tert*-butyl), 0.90 (d, J = 6.5 Hz, 3H, C-21 CH_3), 0.55–2.05 (m, 29H), 2.22–2.35 (m, 1H, allylic proton), 3.56 (t, J = 6.5 Hz, 2H, —CH_2O—), 3.90 (br s, 1H, C-3 proton), 4.98–5.15 (m, 2H, ═CH_2), 5.78–5.95 (m, 1H, —CH═); FDMS m/e 516 (M^+); Analysis Calculated for $C_{33}H_{60}O_2Si$: C, 76.68; H, 11.70. Found: C, 76.49; H, 11.51.

1.2.12. 3α-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-4α-(2-propenyl)-5α-cholan-24-ol (14)

tert-Butyldimethylsilyl trifluoromethanesulfonate (0.130 ml, 0.560 mmol) was added to a stirred solution of 13 (192 mg, 0.373 mmol) and anhydrous pyridine (0.060 ml, 0.75 mmol) in anhydrous CH_2Cl_2 (4 ml) at 0°C. The resultant mixture was then allowed to stir at ambient temperature for 2 h. The crude mixture was filtered through a short pad of silica (5% ethyl acetate/hexane), then the filtrate was concentrated to give an oily residue.

The above disilyl product was diluted in THF (4 ml) and treated with tetrabutylammonium fluoride (1 M in THF, 0.933 ml); the resultant mixture was stirred at ambient temperature for 2 h. After dilution with ethyl acetate (50 ml), the solution was washed with water (20 ml × 2), dried, filtered, and concentrated. The residue was chromatographed on silica (gradient 0–25% ethyl acetate/hexane) to give 14 (190 mg, 99%) as a white solid. mp 107.5–109.0°C; IR (KBr) 3355 (br) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.02 and 0.04 [both s, 6H, -Si$(CH_3)_2^-$], 0.65 (s, 3H, C-18 CH_3), 0.79 (s, 3H, C-19 CH_3), 0.90 (s, 9H, *tert*-butyl), 0.91 (d, J = 6.5 Hz, 3H, C-21 CH_3), 0.60–2.00 (m, 29H), 2.06–2.19 (m, 1H, allylic proton), 3.61 (t, J = 6.5 Hz, 2H, —CH_2O—), 3.87 (br s, 1H, C-3 proton), 4.92–5.05 (m, 2H, ═CH_2), 5.72–5.90 (m, 1H, —CH═); FDMS m/e 459 (M^+-C_4H_9); Analysis Calculated for $C_{33}H_{60}O_2Si \cdot 0.5C_6H_{14}$: C, 77.21; H, 12.06. Found: C, 77.59; H, 11.68.

1.2.13. 3α-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-4α-(2-propenyl)-5α-cholan-24-al (15)

Pyridinium chlorochromate (150 mg, 0.696 mmol) was added to a stirred suspension of 14 (180 mg, 0.348 mmol), sodium acetate (57.0 mg, 0.696 mmol), and 4Å molecular sieve powder (57 mg) in anhydrous CH_2Cl_2 (5 ml) at ambient temperature. The resultant suspension was stirred for 1 h. After filtration through a short pad of silica (10% ethyl acetate/hexane), the filtrate was concentrated, and the residue was chromatographed on silica (gradient 0–7% ethyl acetate/hexane) to give 15 (160 mg, 89%) as a white solid. mp 119.0–120.0°C; IR (KBr) 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.02 and 0.04 [both s, 6H, -Si$(CH_3)_2^-$], 0.65 (s, 3H, C-18 CH_3), 0.79 (s, 3H, C-19 CH_3), 0.90 (s, 9H, *tert*-butyl), 0.91 (d, J = 6.4 Hz, 3H, C-21 CH_3), 0.60–1.95 (m, 26H), 2.08–2.15 (m, 1H, allylic proton), 2.25–2.52 (m, 2H, C-23 protons), 3.87 (br s, 1H, C-3 proton), 4.93–5.03 (m, 2H, ═CH_2), 5.72–5.85 (m, 1H, —CH═), 9.76 (s, 1H, CHO); FDMS m/e 458 (M^+-C_4H_8); Analysis Calculated for $C_{33}H_{58}O_2Si \cdot 0.2C_6H_{14}$: C, 77.19; H, 11.52. Found: C, 77.19; H, 11.84.

1.2.14. 3α-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-4α-(2-propenyl)-5α-cholest-24-ene (16)

Potassium bistrimethylsilylamide (0.5 M in toluene, 0.815 ml) was added to a stirred suspension of isopropyltriphenylphosphonium iodide (189 mg, 0.437 mmol) in anhydrous THF (4 ml) at −15°C, and the resultant red suspension was stirred for 15 min. A solution of 15 (150 mg, 0.291 mmol) in anhydrous THF (2 ml) was added dropwise to the red suspension at −15°C, and the mixture was stirred at 0°C for 1 h. Acetone (0.1 ml) was added to the mixture to quench the excess phosphonium ylide, then followed by the addition of hexane (10 ml). The mixture was filtered through a short pad of silica (5% ethyl acetate/hexane), then the filtrate was concentrated, and the residue was chromatographed on silica (hexane) to give 16 (150 mg, 95%) as a white solid. mp 88.5–89.0°C; IR (KBr) 2932 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.02 and 0.04 [both s, 6H, -Si$(CH_3)_2^-$], 0.64 (s, 3H, C-18 CH_3), 0.79 (s, 3H, C-19 CH_3), 0.90 (s, 9H, *tert*-butyl), 0.91 (d, J = 6.5 Hz, 3H, C-21 CH_3), 1.60 and 1.68 (both s, 6H, C-26 and -27 CH_3), 0.60–2.18 (m, 29H), 3.87 (br s, 1H, C-3 proton), 4.90–5.03 (m, 2H, ═CH_2), 5.09 (t, J = 6.4 Hz, 1H, C-24 proton), 5.74–5.88 (m, 1H, —CH═); FDMS m/e 541 (M^+ + 1); Analysis Calculated for $C_{36}H_{64}OSi$: C, 79.93; H, 11.92. Found: C, 79.87; H, 12.10.

1.2.15. 4α-(2-Propenyl)-5α-cholest-24-en-3α-ol (17)

Tetrabutylammonium fluoride (1 M in THF, 2.22 ml) was added to a stirred solution of 16 (120 mg, 0.222 mmol) in THF (2 ml), and the resultant mixture was heated to reflux for 1 d. At ambient temperature, the mixture was diluted with EtOAc (50 ml), washed with water (20 ml × 3), dried, filtered, and concentrated. The residue was chromatographed on silica (gradient 0–10% ethyl acetate/hexane) to give 17 (92.7 mg, 98%) as a white solid. mp 83.0–84.0°C; IR (KBr) 3453 (br) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.64 (s, 3H, C-18 CH_3), 0.81 (s, 3H, C-19 CH_3), 0.91 (d, J = 6.4 Hz, 3H, C-21 CH_3), 1.60 and 1.68 (both s, 6H, C-26 and -27 CH_3), 0.65–2.06 (m, 29H), 2.22–2.32 (m, 1H, allylic proton), 3.89 (br s, 1H, C-3 proton), 4.98–5.15 (m,

3H, ═CH_2 and C-24 protons), 5.78–5.94 (m, 1H, —CH═); FDMS *m/e* 426 (M^+); Analysis Calculated for $C_{30}H_{50}O$: C, 84.44; H, 11.81. Found: C, 84.24; H, 11.54.

1.3. In vitro LDL receptor/luciferase assay

Compound 17 was evaluated for its derepressing capability against 25-hydroxycholesterol in the LDLR/Luc assay, in which stably transfected, stationary CHO cells were exposed to 25-hydroxycholesterol (0.5 μg/ml) and 17 at different concentrations (0, 0.6, 1.3, 2.5, 5.0, and 10.0 μg/ml) in serum-free medium for 24 h. Two ethanol stock solutions of 25-hydroxycholesterol (0.5 mg/ml) and 17 (4.0 mg/ml) were prepared and diluted in the assay medium to achieve the previously indicated concentrations. The one containing 25-hydroxycholesterol, but without 17, is a repressed control. Cells were then washed and lysed, and homogenates were evaluated for luciferase-catalyzed light production. The procedure is described in detail in our previous publication [18]. EC_{30} refers to the effective concentration of 17 required to increase the specific light induction by 30% over the repressed control.

1.3. [1-^{14}C-Acetate]cholesterol biosynthesis inhibition assay

Replicate, 100 mm dishes of confluent CHO cells were incubated with 8 ml assay media (without the 25-hydroxycholesterol supplement) containing the ethanol vehicle (0.5% v/v), 17 (10 μg/ml or 23 μM), or mevinolin (10 μM). After a 20-h incubation, [1-^{14}C]-acetic acid, sodium salt (8 μl) was added directly to each dish (final concentration of 1 μCi/ml), and the dishes were incubated for an additional 4 h. Cell monolayers were then harvested by scraping, and the cell pellets were suspended in water and sonicated for 10 s. After an aliquot was removed for protein determination [20], lipids were extracted with chloroform/methanol (2:1 v/v) [21]. After normalizing for protein content, the lipid fraction was resuspended using chloroform/methanol (4;1, v/v) in a small volume (100 μl or less), and lipids were separated by high performance thin-layer chromatography on Kieselgel 60 silica plates with a mobile phase consisting of hexane/heptane/diethyl ether/acetic acid (63:18.5:18.5:1, v/v). Lipids were visualized by dipping the plate for 3 s in a solution of hexane/ethanol/sulfuric acid (64:35:1, v/v), then placed for 15 min in a 110°C oven [22]. The cholesterol band was scraped and counted for radioactivity on a Beckman LS6500 Scintillation Counter.

1.4. Statistics

Data were analyzed by ANOVA, and differences between means of the repressed control and 17-treated CHO cells were determined by the statistical method of Dunnett [23]. The EC_{30} value was determined by the nonlinear regression analysis method using Prism (Graphpad, San Diego, CA, USA).

2. Results and Discussion

2.1. Synthesis

Synthesis of the target 3α-sterol 17 was envisioned to proceed through Stork's reductive alkylation of enone 8 [24–26], then followed by the stereoselective K-selectride reduction of the C-3 keto group [18,27]. Enone 8 could be derived from compound 6, which in turn could be synthesized from lithocholic acid according to known procedures [19].

Following these procedures [19], lithocholic acid was treated with 2.3 equivalents of *N*-bromosuccinimide in aqueous dioxane at 50°C for 3 h to give an inseparable mixture of 2β-bromoketone 2 and 4β-bromoketone 3 in a 1:5 ratio in quantitative yield (Scheme 1). In the ^{1}H NMR spectrum, the signal, because of the axial proton at C-2 in 2, appears at δ 4.69 as a doublet of doublets (J = 14.0, 5.4 Hz), whereas the axial proton at C-4 in 3 appears at δ 4.96 as a doublet (J = 12.1 Hz). In the literature, compound 3 was reported to be the only isolated product [19].

Subsequent dehydrobromination of the mixture of 2 and 3 (1:5) using Li_2CO_3 in DMF at 100°C for 4 h delivered an inseparable mixture of enones 4 and 5 in a 1:4.5 ratio. The ratio was improved to 1:10 by replacing Li_2CO_3 with LiCl as the dehydrobrominating agent [28,29], and pure 5 was isolated in a 59% yield after recrystallization from CH_2Cl_2/CH_3CN (Scheme 1). In the ^{1}H NMR spectrum of the mixture of 4 and 5, the signals, because of the olefinic protons in 4, appear at δ 5.89 and 6.83 as an AB pattern (J = 10.2 Hz), whereas the olefinic proton in 5 appears at δ 5.72 as a singlet, which is in agreement with the literature [19].

As depicted in Scheme 2, compound 5 was readily converted to its methyl ester 6. Subsequent reduction of 6 with diisobutylaluminum hydride in CH_2Cl_2 at −10°C gave a mixture of diols, which were then oxidized with activated MnO_2 in 1,2-dichloroethane to provide enone 7 in an overall 97% yield. The reaction rate of this heterogeneous oxidation was greatly accelerated by sonication. Utilizing the C-24 primary alcohol as an internal proton source, an attempt to perform Stork's reductive alkylation of 7 with allyl iodide gave a complex mixture, which only yielded 14% of the desired allylated product. Thus, the C-24 hydroxyl in 7 was protected as a *tert*-butyldimethylsilyl ether in a 95% yield. The resultant protected enone 8 was reductively alkylated following Stork's protocols to deliver the ketone 9 in a respectable 60% yield.

As shown in Scheme 3, there appeared to be only four steps starting from compound 9 to the synthesis of the target 3α-ol 17. Compound 9 was thus treated with tetrabutylammonium fluoride to give the primary alcohol 10 in a 92% yield; then, in turn, it was oxidized with pyridinium chlo-

rochromate in CH_2Cl_2 in the presence of 4Å molecular sieves to provide the aldehyde 11 in an 83% yield preparing for the installation of the isopropylidene functionality [30]. The initial attempt to selectively couple the aldehyde group in 11 with the Wittig reagent, generated from isopropyltriphenylphosphonium iodide and potassium *tert*-butoxide in THF, only afforded the desired compound 12 in a <10% yield. We were able to improve the coupling yield to 47% by employing potassium bistrimethylsilylamide as the base.

At this juncture, we decided to go back to compound 9 and adopt a different synthetic approach toward the synthesis of the target 3α-ol 17. The new synthetic route would allow for the synthesis of 3α-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-4α-(2-propenyl)-5α-cholan-24-al (15), which could serve as a versatile intermediate that was suitable for future SAR studies in the D-ring side chain (Scheme 4). It is known that K-selectride, because of its bulkiness, almost exclusively delivers its hydride via the equatorial course to the carbonyl group in cyclohexanone to provide the cyclohexanol with the hydroxyl group in the axial position [18, 27]. As anticipated, the desired 3α-ol 13 in a 92% yield was obtained as the sole product after the reduction of 9 with K-selectride. In its ^{1}H NMR spectrum, the C-3 equatorial proton appears at δ 3.90 as a singlet. The hindered 3α-hydroxyl in 13 was then protected as a *tert*-butyldimethylsilyl ether, whereas the less hindered *tert*-butyldimethylsilyl ether of the C-24 hydroxyl was selectively deprotected with tetrabutylammonium fluoride in THF to give 14 in a 99% yield. Subsequent pyridinium chlorochromate oxidation of 14 in CH_2Cl_2 in the presence of sodium acetate and 4Å molecular sieves provided the target intermediate 15 in an 89% yield [30,31]. Wittig coupling of 15 with (isopropylidene)triphenylphosphorane in THF at −15°C went smoothly and delivered the compound 16 in a 95% yield, compared to a 47% yield for the formation of 12. The hindered *tert*-butyldimethylsilyl ether of the 3α-hydroxyl was deprotected with tetrabutylammonium fluoride in THF at reflux to complete the synthesis of our target 3α-ol 17 in a 98% yield.

2.2. In vitro biology

A LDLR/Luc assay [18] was set up by stably transfecting a gene construct in CHO cells in which a DNA segment containing the promoter and regulatory control elements of the LDL receptor gene was fused to the firefly luciferase reporter gene. Compound 17 was evaluated for its derepressing capability in the presence of 25-hydroxycholesterol in the CHO cell-based LDLR/Luc assay. In the assay stably transfected, stationary CHO cells were exposed to 25-hydroxycholesterol (0.5 μg/ml) and 17 at different concentrations (0, 0.6, 1.3, 2.5, 5.0, and 10.0 μg/ml) in serum-free medium for 24 h. The one containing 25-hydroxycholesterol, but without 17, is a repressed control. Cells were then washed and lysed, and homogenates were evaluated for luciferase-catalyzed light production.

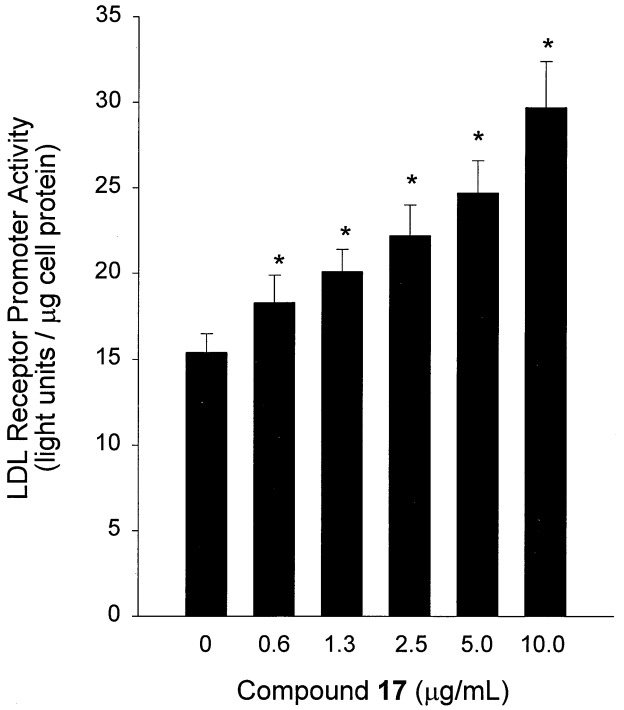


Fig. 2. Activation of LDL receptor transcriptional activity by 17 in the presence of 25-hydroxycholesterol. CHO cells, transfected with a LDL receptor promoter/luciferase gene construct, were treated for 24 h with 25-hydroxycholesterol (0.5 μg/ml) and 17 (0–10 μg/ml). The column containing no 17 represents the repressed control. The EC_{30} value was determined to be 1.1±0.1 μg/ml (2.6±0.3 μM) by the nonlinear regression analysis method. Values are means±SE for triplicates run at three different times. Asterisks denote a significant difference from the repressed control, $P < 0.05$.

As shown in Fig. 2, there is a good dose-response relationship between the LDL receptor transcriptional activity versus the concentration of 17 in the LDLR/Luc assay. EC_{30} refers to the effective concentration of 17 required to increase the specific light induction by 30% over the repressed control; therefore, the value was determined to be 2.6 μM, comparable to 2.9 μM of LY295427, by the nonlinear regression analysis method. Apparently, the built-in 24,25-unsaturation in the D-ring side chain had conferred, on 17, little effect on antagonizing the repressing effect of 25-hydroxycholesterol in transfected CHO cells.

A [1-^{14}C-acetate]cholesterol biosynthesis inhibition assay was also set up to investigate the inhibition of cholesterol biosynthesis by 17 in CHO cells. Confluent CHO cells were incubated with assay media containing either the ethanol vehicle or 17 (10 μg/ml or 23 μM) or mevinolin (10 μM) for 20 h, then they were treated with [1-^{14}C]-acetic acid and incubated for an additional 4 h. The [^{14}C]-cholesterol was isolated and counted for radioactivity according to the procedure described in the Experimental section.

As indicated in Fig. 3, 17 shows a 38% inhibition of the cholesterol biosynthesis compared to the control medium, whereas LY295427 did not show any inhibition in the same assay in our previous studies [18]. Mevinolin showed a 90%

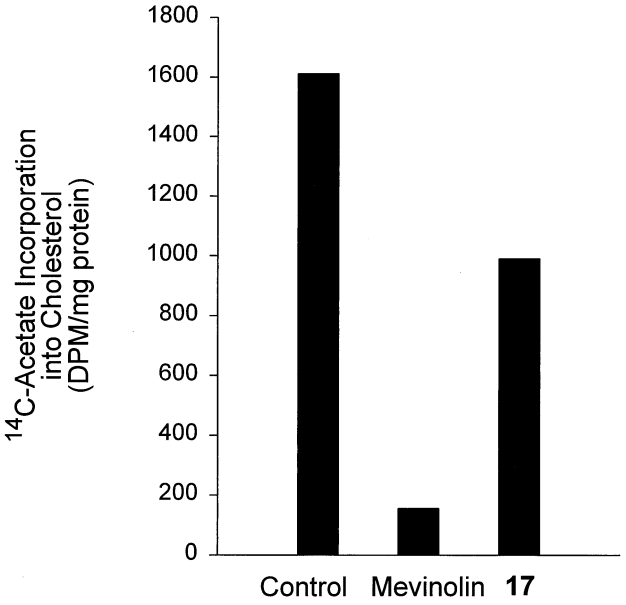


Fig. 3. Cholesterol biosynthesis inhibition was assayed in CHO cells by the 1-^{14}C-acetate incorporation method. The procedure is described in the Experimental section. Assay medium was used as a control. Mevinolin (10 μM) was used as a positive control and showed a 90% inhibition. Seventeen (10 μg/ml or 23 μM) showed a 38% inhibition. Values are means for duplicates with $<$10% variations.

inhibition in the assay. In contrast to LY295427, the built-in 24,25-unsaturation in the D-ring side chain of 17 has conferred an inhibitory effect on cholesterol biosynthesis in CHO cells; the site of inhibition in the biosynthetic pathway has not been identified.

Presumably, the observed LDL receptor promoter activity of 17 is related to its ability to prevent 25-hydroxycholesterol from exerting the repressing effect via a yet undetermined mechanism and, in part, to inhibit the cholesterol biosynthesis.

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