Arch. Pharm. Chem. Life Sci. 2017, *350*, e1700178

ARCH PHARM
Archiv der Pharmazie

Full Paper

Practical Synthesis of α-Amyrin, β-Amyrin, and Lupeol: The Potential Natural Inhibitors of Human Oxidosqualene Cyclase

Dongyin Chen [1,2], *Fengguo Xu*[1], *Pu Zhang*[1], *Jie Deng*[1], *Hongbin Sun*[1], *Xiaoan Wen*[1], *and Jun Liu*[1]

[1] Jiangsu Key Laboratory of Drug Discovery for Metabolic Disease and State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing, China
[2] Department of Medicinal Chemistry, School of Pharmacy, Nanjing Medical University, Nanjing, China

A practical synthesis of α-amyrin (**1**), β-amyrin (**2**), and lupeol (**3**) was accomplished in total yields of 32, 42, and 40% starting from easily available ursolic acid (**4**), oleanolic acid (**5**), and betulin (**6**), respectively. Remarkably, these three natural pentacyclic triterpenes exhibited potential inhibitory activity against human oxidosqualene cyclase.

Keywords: α-Amyrin / β-Amyrin / Lupeol / Oxidosqualene cyclase inhibitor / Synthesis

Received: June 3, 2017; Revised: August 14, 2017; Accepted: September 5, 2017

DOI 10.1002/ardp.201700178

Additional supporting information may be found in the online version of this article at the publisher's web-site

Introduction

Pentacyclic triterpenes (PTs), one of the most important naturally occurring biologically active products, are widely distributed throughout the plant kingdom [1]. α-Amyrin (**1**), β-amyrin (**2**), and lupeol (**3**) are three representative members of the family of PTs, and have recently attracted much attention due to their reported hepatoprotective, antihyperglycemic, and hypolipidemic activity [2–5] (Fig. 1). In the past few decades, various strategies, including multi-step total synthesis [6–9], enzyme-catalyzed biosynthesis [10–13], and acid induced rearrangement of triterpene skeletons [14, 15] have been developed to prepare these three natural PTs and their derivatives. However, an efficient method for accessing these biological compounds has been rarely reported thus far.

Oxidosqualene cyclases (OSCs) are the few enzymes that are of fundamental significance for nearly all organisms ranging from microorganisms and higher plants to vertebrates [16, 17]. The central role of OSCs in construction of polycyclic triterpenes or sterols requires a sophisticated system to guide product specificity, which attracted considerable attention for more than half a century since Ruzicka proclaimed the biogenetic isoprene rule [18, 19]. In higher plants, OSCs catalyze the cyclization of (3*S*)-2,3-oxidosqualene to form PTs or other polycyclic skeletons in a highly specific manner [20]. Different from plant OSCs, human OSC converts (3*S*)-2,3-oxidosqualene into lanosterol, and thus plays a decisive role in cholesterol biosynthesis and homeostasis whose disorders are correlated with a variety of metabolic diseases [21–23]. Notably, inhibition of human OSC has been regarded as a potential therapeutic approach to hypercholesterolemia [24–28] (Fig. 1 and Supporting Information Fig. S1).

Given the similar structures of natural PTs with that of lanosterol, as well as the high sequence homology of human OSC with plant OSCs [29], we reasoned that the plant OSCs products, such as α-amyrin (**1**), β-amyrin (**2**), and lupeol (**3**), might be product analog inhibitors of human OSC (Fig. 1). That is to say, these three natural PTs might exert their hypolipidemic activity, at least in part, through inhibiting human OSC. Herein, we first report an efficient access to α-amyrin (**1**), β-amyrin (**2**), and lupeol (**3**) in total yields of 32, 42, and 40% starting from easily available ursolic acid (**4**),

Correspondence: Dr. Jun Liu, Department of Medicinal Chemistry, Jiangsu Key Laboratory of Drug Discovery for Metabolic Disease and State Key Laboratory of Natural Medicines, China Pharmaceutical University, 24 Tongjia Xiang, Nanjing 210009, China.
E-mail: junliu@cpu.edu.cn
Fax: +86 25 83271198

Additional correspondence: Dr. Xiaoan Wen,
E-mail: wxagj@126.com

Dongyin Chen and Fengguo Xu contributed equally to this article.

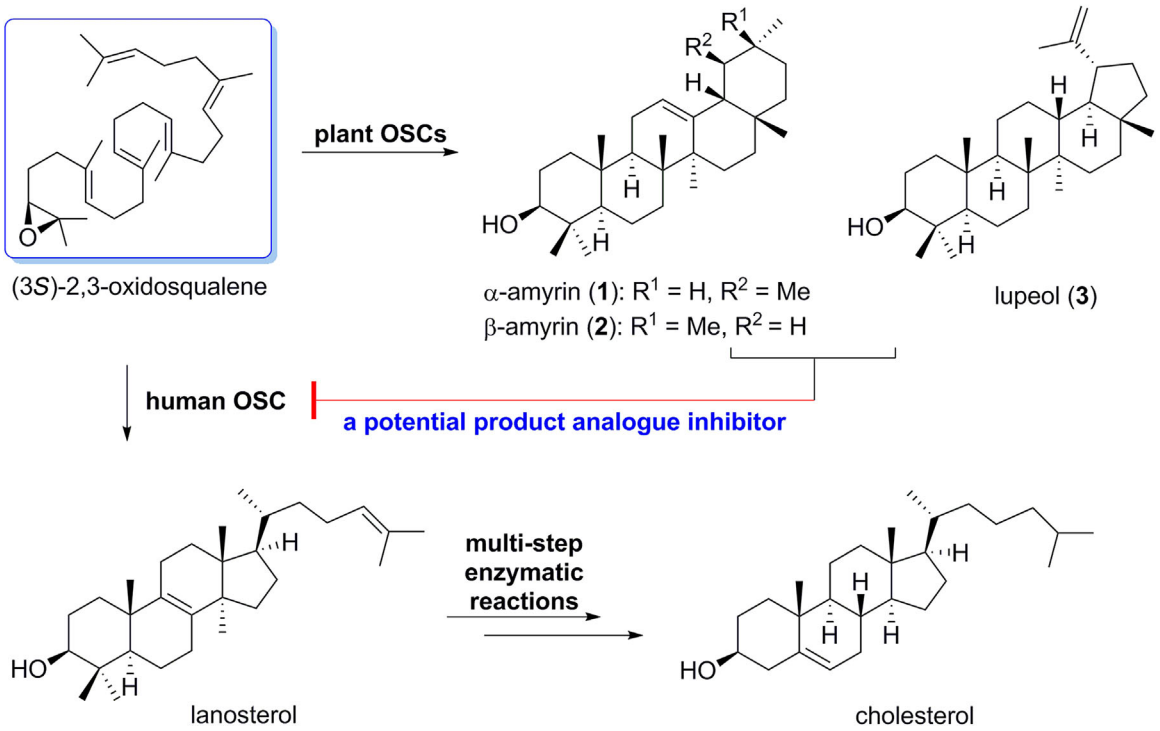


Figure 1. OSCs catalyse the conversion of (3*S*)-2,3-oxidosqualene to α-amyrin (**1**), β-amyrin (**2**), lupeol (**3**), and lanosterol.

oleanolic acid (**5**), and betulin (**6**), respectively (Schemes 1 and 2). Furthermore, a preliminary cellular assay confirmed that these three natural PTs had definite human OSC inhibitory activity (% inhibition of lanosterol biosynthesis up to 55–66% at 10 μM).

Results and discussion

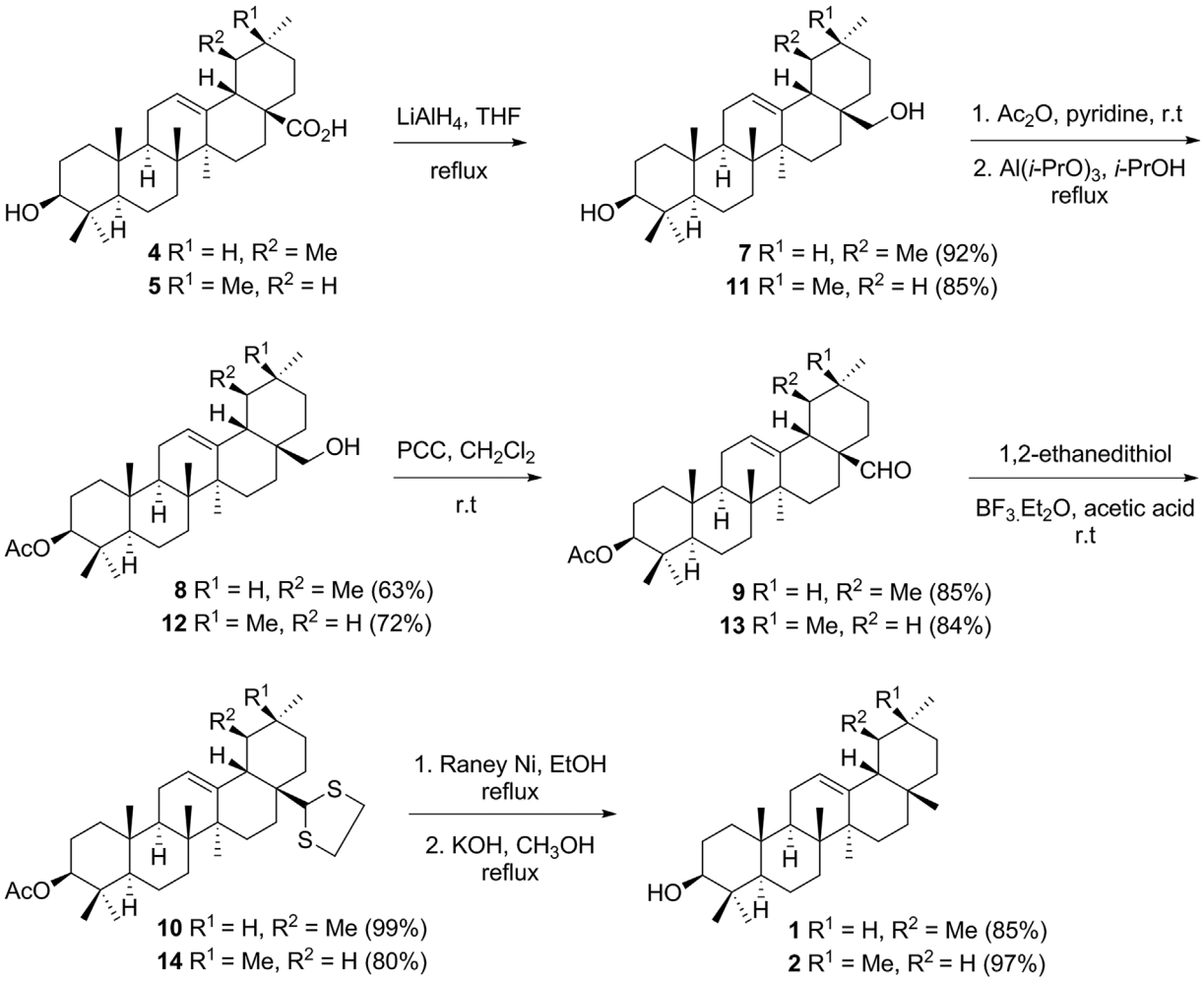
Chemistry

As shown in Scheme 1, reduction of **4** and **5** with $LiAlH_4$ in THF afforded uvaol (**7**) in 92% yield and erythrodiol (**11**) in 85% yield, respectively. Then, acetylation of **7** and **11** with acetic anhydride in anhydrous pyridine, followed by deprotection with Al(*i*-PrO)$_3$ afforded alcohol **8** (63%) and **12** (72%) over two steps. Oxidation of **8** and **12** with pyridinium chlorochromate (PCC) in CH_2Cl_2 gave aldehyde **9** (85%) and **13** (84%), which were further reacted with 1,2-ethanedithiol catalyzed by $BF_3.Et_2O$ in acetic acid to give **10** (99%) and **14** (80%). Finally, desulfurization of **10** and **14** with Raney Ni in EtOH, and followed by hydrolysis with aqueous potassium hydroxide in CH_3OH, afforded desirable product **1** (85%) and **2** (97%) over two steps, respectively.

Following the similar methodology for preparation of **1** and **2**, the synthesis of **3** was achieved as depicted in Scheme 2. Acetylation of **6** with acetic anhydride in anhydrous pyridine, followed by deprotection with Al(*i*-PrO)$_3$ afforded alcohol **15** in 57% yield for two steps. Oxidation of **15** with PCC gave aldehyde **16** (90%), which was further reacted with $Me_2AlSCH_2CH_2SAlMe_2$ in 1,2-dichloroethane at room temperature to furnish dithiolane **17** (73%). Desulfurization of **17** with Raney Ni in EtOH, followed by hydrolysis with aqueous potassium hydroxide in CH_3OH provided **3** in 87% yield over two steps.

In vitro OSC inhibitory assay

To test the above hypothesis, we established a cellular assay system to examine the inhibitory activity of the three natural PTs against human OSC. Following the literature method [30] with modification, the inhibitory activity of α-amyrin (**1**), β-amyrin (**2**), and lupeol (**3**) on human OSC-catalyzed biosynthesis of lanosterol from (3*S*)-2,3-oxidosqualene was determined. The assay was performed based on incubation of the test compounds with HL-60 cells, followed by lysis of the cells, liquid-liquid microextraction, and LC-MS/MS analysis of the resulting cellular extracts. Through comparing the substrate-product patterns of the test compounds with those of the blank control and the positive control (with known human OSC inhibitor Ro 48-8071 [31]), the relative qualitative results were obtained (Table 1). The above three compounds **1**–**3** exhibited significant human OSC inhibitory activity, and the biosynthesis of lanosterol from (3*S*)-2,3-oxidosqualene in HL-60



Scheme 1. Synthetic route to α-amyrin (**1**) and β-amyrin (**2**).

cells were inhibited up to 66, 56, and 55% at a concentration of 10 μM, respectively.

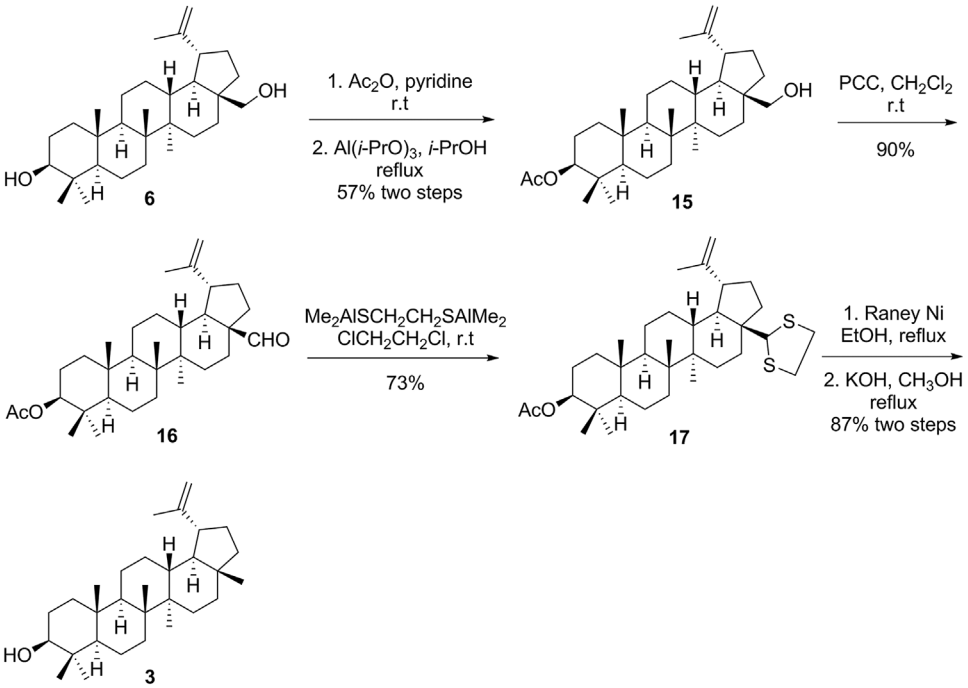
Molecular docking study

In order to expound how these three natural PTs conjugate with human OSC, as well as to afford some guidance for a reasonable design of novel OSC inhibitors in the future, the molecular docking study was conducted by use of SYBYL 1.3 as docking software. As depicted in Fig. 2, these three natural PTs almost occupied the binding position of the endogenous ligand lanosterol in the active site of human OSC (PDB ID: 1W6J), and mainly surrounded by the catalytic pocket included in the residues of ILE-338, GLY-380, THR-381, PHE-444, ASP-455, THR-502, TYR-503, PHE-696, and TYR-704. Besides, one hydrogen bond between 3β-hydroxy group of these three PTs and ASP-455 were observed, and the binding free energy of α-amyrin (**1**), β-amyrin (**2**), and lupeol (**3**) was calculated to be −11.17 kcal/mol, −9.72 kcal/mol, and −10.61 kcal/mol, respectively.

Conclusion

In summary, an efficient synthesis of α-amyrin (**1**), β-amyrin (**2**), and lupeol (**3**) was realized in a total yield of 32, 42, and 40% starting from easily available ursolic acid (**4**), oleanolic acid (**5**), and betulin (**6**), respectively. Moreover, the preliminary cellular assay results confirmed that these three naturally occurring PTs were potential human OSC inhibitors, which inhibited the biosynthesis of lanosterol up to 55–66% at 10 μM. In light of this finding, the reported hypolipidemic effect of the above three natural PTs might be at least partially due to the inhibition of human OSC, thus leading to a reduced biosynthesis of cholesterol. Further, structure modifications of these three compounds might afford a novel

ARCH PHARM
Archiv der Pharmazie



Scheme 2. Synthetic route to lupeol (**3**).

series of human OSC inhibitors that hold promise for treating hyperlipidemia.

Experimental

Chemistry

General

All commercially available solvents and reagents were used without further purification. Column chromatography was carried out on silica gel (200–300 mesh, Qindao Ocean Chemical Company, China). Melting points (mp) were measured on a RY-1 melting point apparatus. IR spectra were recorded on Shimadzu FTIR-8400S spectrometer. Nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were obtained with Bruker AV-300 or AV-500 spectrometers and are reported as chemical shifts in parts per million (ppm, δ) downfield from tetramethylsilane as an internal standard. Mass spectral data were obtained on Agilent 1100 LC/DAD/MSD or Q-Tof Micro MS/MS spectrometer.

The NMR spectra and the InChI codes of the investigated compounds together with some biological activity data are provided as Supporting Information.

*Synthesis of uvaol (**7**)*

To a solution of ursolic acid (**4**) (20.0 g, 0.044 mol) in THF (600 mL) was added $LiAlH_4$ (7.0 g, 0.183 mol). The reaction mixture was allowed to reflux for 1.5 h. At this point, the $LiAlH_4$ was quenched with MeOH and water was added, and then the mixture was extracted with EtOAc. The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO_2, petroleum ether/ethyl acetate 10:1) to give **7** (17.8 g, 92% yield) as a white amorphous solid.

$[\alpha]_D = +65.5$ ($c = 0.0965$, $CHCl_3$). FT-IR ν (cm^{-1}): 3822, 3414, 2927, 2869, 2204, 1657, 1457, 1386, 1375, 1098, 1043, 1024, 991. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.14 (t, $J = 3.7$ Hz, 1H), 3.54–3.50 (m, 1H), 3.23–3.16 (m, 2H), 1.98–1.87 (m, 3H), 1.83–1.72 (m, 1H), 1.68–0.72 (m, 19H), 1.10 (s, 3H), 1.00 (s, 3H), 0.99 (s, 3H), 0.95 (s, 3H), 0.93 (s, 3H), 0.86 (s, 3H), 0.79 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 139.0, 125.3, 79.3, 70.2, 55.4, 54.3, 47.9, 42.3, 40.3, 39.7, 39.6, 39.0, 38.3, 37.1, 35.4, 33.1, 30.9, 28.4, 27.5, 26.3, 23.6, 23.5, 21.5, 18.6, 17.6, 17.0, 15.9, 15.8. MS (ESI) *m/z*: 465.37 $[M+Na]^+$. ESI-HRMS calcd. for $C_{30}H_{50}O_2Na$ ($[M+Na]^+$): 465.3709; found: 465.3732.

*3-Acetyluvaol (**8**)*

To a solution of **7** (2.0 g, 4.5 mmol) dissolved in pyridine (8 mL) was added Ac_2O (1.3 mL, 13.5 mmol). The reaction mixture was stirred overnight at room temperature, and then concentrated *in vacuo* to dryness. Then water (100 mL) was added to the reaction mixture and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4, filtered, and concentrated

Table 1. Inhibitory activity of α-amyrin (1), β-amyrin (2), and lupeol (3) against human OSC-mediated cyclization of (3*S*)-2,3-oxidosqualene to form lanosterol.[a)]

Compound	A_O/A_L[b)]	Ratio of lanosterol (%)[c)]	Inhibition of lanosterol biosynthesis (%)[d)]
1	4.50	18	66
2	3.22	24	56
3	3.12	24	55
Ro 48-8071[e)]	662.97	0.2	99.7
Control	0.85	54	0

[a)] HL 60 cells were incubated in lipid free medium, treated for 24 h with the test compounds at 10 μM, followed by lysis of the cells, liquid-liquid microextraction, and LC-MS/MS analysis of the resulting cellular extracts.
[b)] A_O, area of (3*S*)-2,3-oxidosqualene; A_L, area of lanosterol (Supporting Information Figs. S2 and S3).
[c)] Ratio of lanosterol was calculated according to the formula: % Ratio = $\{A_L/(A_O + A_L)\} \times 100$.
[d)] The percentage inhibition of lanosterol biosynthesis was calculated according to the formula: % Inhibition = $\{(R_{L.C} - R_{L.S.})/R_{L.C.}\} \times 100$; $R_{L.C.}$, ratio of lanosterol in control; $R_{L.S.}$, ratio of lanosterol in sample.
[e)] Ro 48-8071 is a known human OSC inhibitor as the positive control.

in vacuo. The residue was purified by flash chromatography (SiO_2, petroleum ether/ethyl acetate 120:1) to give 3,28-diacetyluvaol (1.6 g, 72% yield) as a white amorphous solid.

To a solution of 3,28-diacetyluvaol (0.5 g, 0.95 mmol) in *i*-PrOH (25 mL) was added Al(*i*-PrO)$_3$ (0.23 g, 1.14 mmol). The reaction mixture was allowed to reflux until the TLC indicated the consumption of the starting material. At this point, the solution was concentrated to remove the most solvent and water (100 mL) was added to the reaction mixture. Then the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO_2, petroleum ether/ethyl acetate 30:1) to give **8** (400 mg, 87% yield) as a white amorphous solid.

$[\alpha]_D$ +70.5 ($c = 0.145$, $CHCl_3$). FT-IR ν (cm^{-1}): 3706, 3681, 2972, 2923, 2866, 2844, 1735, 1275, 1261, 1054, 1033, 1015, 750. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.20 (t, $J = 3.6$ Hz, 1H), 4.60–4.54 (m, 1H), 3.59 (d, $J = 11.0$ Hz, 1H), 3.26 (d, $J = 11.0$ Hz, 1H), 2.00–1.96 (m, 3H), 1.90–1.83 (m, 1H), 1.75–0.87 (m, 19H), 2.11 (s, 3H), 1.17 (s, 3H), 1.06 (s, 3H), 1.04 (s, 3H), 1.00 (s, 3H), 0.94 (s, 3H), 0.93 (s, 3H), 0.89 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.9, 138.8, 124.9, 80.9, 70.2, 55.3, 54.0, 47.6, 42.0, 40.0, 39.4, 39.3, 38.5, 38.0, 37.7, 36.8, 35.2, 32.8, 30.6, 28.0, 26.0, 23.8, 23.6, 23.4, 23.3, 23.2, 21.3, 18.2, 17.4, 16.8, 16.7,15.7. MS (ESI) *m/z*: 507.38 $[M+Na]^+$.

*3-Acetylursolic aldehyde (**9**)*

To a solution of **8** (2.0 g, 4.1 mmol) in dichloromethane (80 mL) was added pyridinium chlorochromate (1.3 g, 6.2 mmol). The reaction mixture was allowed to stir at room temperature until the TLC indicated the consumption of the starting material. At this point, the mixture was evaporated to dryness. The residue was purified by flash chromatography (SiO_2, petroleum ether/ethyl acetate 120:1) to give **9** (1.7 g, 85% yield) as a white amorphous solid.

$[\alpha]_D$ +59.07 ($c = 0.15$, $CHCl_3$). FT-IR ν (cm^{-1}): 3437, 2970, 2934, 2870, 2851, 1732, 1715, 1456, 1377, 1247, 1025, 1003, 756. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.32 (s, 1H), 5.32 (s, 1H), 4.52–4.47 (m, 1H), 2.04 (s, 3H), 2.00–1.89 (m, 4H), 1.84–1.75 (m, 1H), 1.65–0.80 (m, 18H), 1.08 (s, 3H), 0.97 (s, 3H), 0.95 (s, 3H), 0.89 (s, 3H), 0.87 (s, 3H), 0.85 (s, 3H), 0.77 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 207.4, 171.0, 137.8, 126.1, 80.9, 55.3, 52.6, 50.1, 47.5, 42.2, 39.8, 39.0, 38.8, 38.4, 37.7, 36.8, 33.0, 31.9, 30.2, 28.1, 26.9, 23.6, 23.3, 23.2, 23.1, 21.3, 21.1, 18.2, 17.2, 16.8, 16.7, 15.6. MS (ESI) *m/z*: 505.37 $[M+Na]^+$. ESI-HRMS calcd. for $C_{32}H_{50}O_3Na$ ($[M+Na]^+$): 505.3658; found: 505.3680.

*2-(3β-Acetoxy-28-norurs-12-en-17-yl)-1,3-dithiolane (**10**)*

To a solution of **9** (4.0 g, 8.3 mmol) in AcOH (40 mL) was added 1,2-ethanedithiol (3.5 mL, 41.7 mmol) and $BF_3.Et_2O$ (46.5%, 10 mL). The reaction mixture was allowed to stir at room temperature until the TLC indicated the consumption of the starting material. At this point, the mixture was poured into water and then extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO_2, petroleum ether/ethyl acetate 130:1) to give **10** (4.6 g, 99% yield) as a white amorphous solid.

$[\alpha]_D = +33.88$ ($c = 0.66$, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.21 (s, 1H), 4.94 (m, 1H), 4.53–4.47 (m, 1H), 3.19–3.07 (m, 4H), 2.15–2.05 (m, 1H), 1.96–1.79 (m, 4H), 1.65–0.80 (m, 18H), 2.04 (s, 3H), 1.09 (s, 3H), 1.01 (s, 3H), 0.97 (s, 3H), 0.92 (s, 3H), 0.87 (s, 3H), 0.86 (s, 3H), 0.82 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.9, 138.1, 126.0, 80.8, 62.0, 57.9, 55.2, 47.5, 41.9, 41.6, 40.0, 39.1, 38.9, 38.6, 38.4, 37.7, 36.7, 32.4, 30.6, 29.6, 29.2, 28.0, 26.7, 26.1, 23.6, 23.5, 21.2, 21.1, 18.1, 17.3, 16.7, 16.4, 15.7. MS (ESI) *m/z*: 581.35 $[M+Na]^+$. ESI-HRMS calcd. for $C_{34}H_{54}O_2S_2Na$ ($[M+Na]^+$): 581.3463; found: 581.3482.

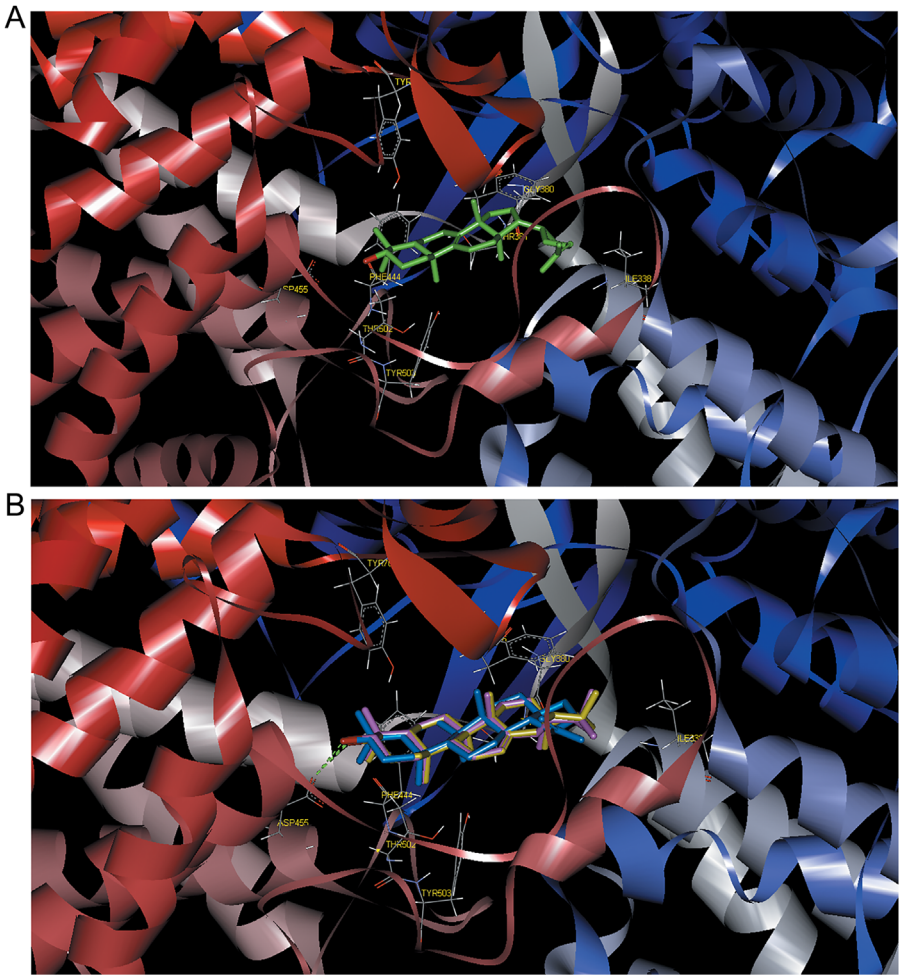


Figure 2. (A) Predicted binding mode of lanosterol docked with human OSC. (B) Binding mode of α-amyrin (**1**, yellow), β-amyrin (**2**, pink), and lupeol (**3**, blue) docked with human OSC in the similar active pocket. Hydrogen bonds are shown as green dashes. The images were generated using Accelrys Discovery Studio 2.5.

*α-Amyrin (**1**)*

To a solution of **10** (300 mg, 0.54 mmol) in EtOH (60 mL) was added Raney Ni (6.0 g). The reaction mixture was allowed to reflux until the TLC indicated the consumption of the starting material. At this point, the mixture was filtered, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, petroleum ether/ethyl acetate 250:1) to give 3-acetyl α-amyrin (197 mg, 86% yield) as a white amorphous solid.

To a solution of 3-acetyl α-amyrin (160 mg, 0.34 mmol) in MeOH (15 mL) was added KOH (0.3 g). The reaction mixture was allowed to reflux until the TLC indicated the consumption of the starting material. At this point, the mixture was added H_2O (30 mL) and then extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO_2, petroleum ether/ethyl acetate 50:1) to give **1** (150 mg, 99% yield) as a white amorphous solid.

$[\alpha]_D = +70.07$ ($c = 0.28$, $CHCl_3$). FT-IR ν (cm^{-1}): 3286, 2978, 2947, 2921, 2869, 2856, 1457, 1388, 1098, 1037, 996. ^1H NMR ($CDCl_3$, 300 MHz) δ 5.13 (t, $J = 3.6$ Hz, 1H), 3.23 (dd, $J = 5.3$, 10.2 Hz, 1H), 2.06–1.78 (m, 4H), 1.68–0.72 (m, 19H), 1.07 (s, 3H), 1.01 (s, 3H), 1.00 (s, 3H), 0.96 (s, 3H), 0.91 (s, 3H), 0.80 (s, 3H), 0.79 (s, 3H), 0.77 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 139.6, 124.4, 79.1, 59.1, 55.2, 47.7, 42.1, 41.5, 40.0, 39.7, 39.6, 38.8, 36.9, 33.8, 33.0, 31.3, 28.7, 28.1, 27.3, 26.6, 23.4, 23.3, 21.4, 18.4, 17.5, 16.9, 15.7, 15.6. ESI-HRMS calcd. for $C_{30}H_{50}ONa$ ($[M+Na]^+$): 449.3759; found: 449.3770.

*Erythrodiol (**11**)*

According to the procedure for preparation of **7**, **11** was prepared from oleanolic acid (**5**) as a white amorphous solid

(85% yield). $[\alpha]_D = +74.37$ ($c = 0.135$, $CHCl_3$). FT-IR ν (cm^{-1}): 3378, 2947, 2869, 2363, 2335, 1637, 1464, 1385, 1375, 1362, 1343, 1095, 1076, 1045, 1004, 658. ^1H NMR ($CDCl_3$, 300 MHz) δ 5.19 (t, $J = 3.6$ Hz, 1H), 3.53 (d, $J = 11.0$ Hz, 1H), 3.18–3.08 (m, 2H), 2.03–0.75 (m, 23H), 1.19 (s, 3H), 1.00 (s, 3H), 0.98 (s, 3H), 0.97 (s, 3H), 0.89 (s, 3H), 0.79 (s, 6H). MS (ESI) *m/z*: 465.37 $[M+Na]^+$.

*3-Acetylerythrodiol (**12**)*

According to the procedure for preparation of **8**, **12** was prepared from **11** as a white solid (72% yield for two steps). $[\alpha]_D = +68.39$ ($c = 0.753$, $CHCl_3$). FT-IR ν (cm^{-1}): 3490, 2945, 2873, 1709, 1464, 1370, 1268, 1050, 1029, 1008, 735. ^1H NMR ($CDCl_3$, 300 MHz) δ 5.19 (t, $J = 3.5$ Hz, 1H), 4.52–4.47 (m, 1H), 3.54 (d, $J = 11.0$ Hz, 1H), 3.20 (d, $J = 11.0$ Hz, 1H), 2.04 (s, 3H), 2.01–0.82 (m, 23H), 1.16 (s, 3H), 0.95 (s, 3H), 0.94 (s, 3H), 0.89 (s, 3H), 0.87 (s, 6H), 0.86 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.0, 144.2, 122.3, 80.9, 69.7, 55.2, 47.5, 46.4, 42.3, 41.7, 39.8, 38.3, 37.7, 36.9, 36.8, 34.1, 33.2, 32.5, 31.0, 30.9, 28.0, 25.8, 25.5, 23.5, 22.0, 21.3, 18.2, 16.7, 16.6, 15.6. MS (ESI) *m/z*: 507.37 $[M+Na]^+$.

*3-Acetyloleanolic aldehyde (**13**)*

According to the procedure for preparation of **9**, **13** was prepared from **12** as a white amorphous solid (84% yield). $[\alpha]_D = +63.5$ ($c = 0.532$, $CHCl_3$). FT-IR ν (cm^{-1}): 3441, 2948, 2709, 2361, 2339, 1729, 1465, 1439, 1375, 1362, 1249, 1148, 1026, 991, 928, 653. ^1H NMR ($CDCl_3$, 500 MHz) δ 9.40 (s, 1H), 5.34 (t, $J = 2.0$ Hz, 1H), 4.51–4.47 (m, 1H), 2.64–2.61 (m, 1H), 2.04 (s, 3H), 2.01–1.95 (m, 1H), 1.90–1.87 (m, 2H), 1.71–0.82 (m, 19H), 1.14 (s, 3H), 0.93 (s, 3H), 0.92 (s, 3H), 0.91 (s, 3H), 0.87 (s, 3H), 0.85 (s, 3H), 0.74 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 207.3, 170.9, 143.0, 123.1, 80.8, 55.3, 49.0, 47.5, 45.6, 41.7, 40.4, 39.6, 38.2, 37.7, 36.9, 33.1, 33.0, 32.7, 30.6, 28.0, 27.7, 26.7, 25.5, 23.5, 23.4, 22.1, 21.2, 18.2, 17.0, 16.6, 15.4. ESI-HRMS calcd. for $C_{32}H_{50}O_3Na$ ($[M+Na]^+$): 505.36522; found: 505.36546.

*2-(3β-Acetoxy-28-norolean-12-en-17-yl)-1,3-dithiolane (**14**)*

According to the procedure for preparation of **10**, **14** was prepared from **13** as a white amorphous solid (80% yield). $[\alpha]_D = +45$ ($c = 0.12$, $CHCl_3$). FT-IR ν (cm^{-1}): 2947, 2922, 2853, 2360, 2334, 1734, 1657, 1555, 1275, 1260, 1049, 764, 751. ^1H NMR ($CDCl_3$, 300 MHz) δ 5.27 (t, $J = 3.5$ Hz, 1H), 4.96 (s, 1H), 4.52–4.47 (m, 1H), 3.22–3.08 (m, 4H), 2.05 (s, 3H), 2.00–0.83 (m, 23H), 1.16 (s, 3H), 0.98 (s, 3H), 0.96 (s, 3H), 0.93 (s, 3H), 0.89 (s, 3H), 0.88 (s, 3H), 0.87 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.0, 143.5, 123.2, 80.9, 62.0, 55.3, 47.5, 46.9, 46.7, 41.6, 40.6, 39.8, 39.0, 38.8, 38.3, 37.7, 36.8, 34.2, 33.0, 32.3, 31.1, 28.0, 26.2, 25.6, 25.5, 25.4, 23.7, 23.6, 23.5, 21.3, 18.2, 16.7, 16.5, 15.6. MS (ESI) *m/z*: 581.35 $[M+Na]^+$. ESI-HRMS calcd. for $C_{34}H_{54}O_2S_2Na$ ($[M+Na]^+$): 581.3463; found: 581.3482.

*β-Amyrin (**2**)*

According to the procedure for preparation of **1**, **2** was prepared from **14** over two steps as a white amorphous solid (97% yield). $[\alpha]_D = +83.1$ ($c = 0.22$, $CHCl_3$). FT-IR ν (cm^{-1}): 3295, 2947, 2869, 2853, 1639, 1464, 1385, 1360, 1190, 1035, 996, 951, 814, 659. ^1H NMR ($CDCl_3$, 500 MHz) δ 5.19 (t, $J = 2.1$ Hz, 1H), 3.22 (dd, $J = 2.9$, 6.8 Hz, 1H), 2.03–0.73 (m, 23H), 1.14 (s, 3H), 1.00 (s, 3H), 0.97 (s, 3H), 0.94 (s, 3H), 0.87 (s, 6H), 0.83 (s, 3H), 0.79 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 145.2, 121.8, 79.0, 55.2, 47.7, 47.3, 46.9, 41.8, 39.8, 38.8, 38.6, 37.2, 37.0, 34.8, 33.3, 32.7, 32.5, 31.1, 28.4, 28.1, 27.3, 27.0, 26.2, 26.0, 23.7, 23.5, 18.4, 16.8, 15.6, 15.5. ESI-HRMS calcd. for $C_{30}H_{50}ONa$ ($[M+Na]^+$): 449.3758; found: 449.3770.

*3-Acetylbetulin (**15**)*

According to the procedure for preparation of **8**, **15** was prepared from betulin (**6**) as a white solid (57% yield for two steps). FT-IR ν (cm^{-1}): 3416, 2944, 2872, 1732, 1642, 1453, 1384, 1374, 1247, 1027, 979, 887, 750. ^1H NMR ($CDCl_3$, 300 MHz) δ 4.68 (s, 1H), 4.59 (s, 1H), 4.50–4.45 (m, 1H), 3.80 (d, $J = 11.0$ Hz, 1H), 3.34 (d, $J = 10.8$ Hz, 1H), 2.43–2.34 (m, 1H), 2.04 (s, 3H), 2.00–0.78 (m, 24H), 1.69 (s, 3H), 1.03 (s, 3H), 0.98 (s, 3H), 0.85 (s, 6H), 0.84 (s, 3H). MS (ESI) *m/z*: 507.36 $[M+Na]^+$.

*3-Acetylbetulinic aldehyde (**16**)*

According to the procedure for preparation of **9**, **16** was prepared from **15** as a white amorphous solid (90% yield). FT-IR ν (cm^{-1}): 3438, 2941, 2865, 1727, 1641, 1447, 1377, 1245, 1029, 979, 884. ^1H NMR ($CDCl_3$, 500 MHz) δ 9.68 (s, 1H), 4.76 (s, 1H), 4.63 (s, 1H), 4.47 (dd, $J = 5.8$, 10.6 Hz, 1H), 2.89–2.83 (m, 1H), 2.09–0.77 (m, 24H), 2.04 (s, 3H), 1.70 (s, 3H), 0.97 (s, 3H), 0.92 (s, 3H), 0.85 (s, 3H), 0.84 (s, 3H), 0.83 (s, 3H). MS (ESI) *m/z*: 505.36 $[M+Na]^+$.

*2-(3β-Acetoxy-28-norlup-20(29)-en-17-yl)-1,3-dithiolane (**17**)*

A solution of Me_3Al (8.1 mL, 2.0 M in toluene) in CH_2Cl_2 (16 mL) was cooled to −78°C, and treated slowly with 1,2-ethanedithiol (0.68 mL). After 5 min of stirring at −78°C, the resultant white slurry was warmed to 0°C and stirred for an additional 30 min. Upon completion, the reaction contents were concentrated to afford $Me_2AlSCH_2CH_2SAlMe_2$ (1.66 g, 8.1 mmol) as a white free flowing powder. A solution of **6** (1.3 g, 2.7 mmol) in 1,2-dichloroethane (30 mL) was added slowly to a solution of $Me_2AlSCH_2CH_2SAlMe_2$ (1.66 g, 8.1 mmol) in 1,2-dichloroethane (30 mL) at room temperature. After stirring for overnight, the reaction content was quenched by the slow and sequential addition of Et_3N (4 mL) and $NaHCO_3$ saturated aqueous (30 mL), and then extracted with EtOAc (3 × 10 mL). The combined organic layer were washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by flash chromatography (SiO_2, petroleum ether/ethyl acetate 350:1) to give **17** (1.1 g, 73% yield) as a white amorphous solid.

$[\alpha]_D = -9.3$ ($c = 0.3$, $CHCl_3$). FT-IR ν (cm^{-1}): 3436, 2939, 2873, 1733, 1639, 1452, 1384, 1251, 1030, 978, 890. ^1H NMR ($CDCl_3$, 300 MHz) δ 4.99 (s, 1H), 4.75 (s, 1H), 4.61 (s, 1H), 4.50–4.45 (m, 1H), 3.34–3.25 (m, 1H), 3.18–3.06 (m, 3H), 2.92–2.82 (m, 1H), 2.25–0.78 (m, 24H), 2.04 (s, 3H), 1.73 (s, 3H), 1.04 (s, 3H), 1.00 (s,

3H), 0.86 (s, 3H), 0.85 (s, 3H), 0.84 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.5, 149.6, 109.7, 80.4, 58.6, 54.9, 50.5, 49.9, 49.7, 47.9, 42.4, 40.5, 38.9, 38.3, 37.9, 37.3, 36.8, 36.6, 35.1, 33.6, 33.5, 31.8, 27.8, 27.4, 24.4, 23.2, 20.8, 20.4, 18.4, 17.7, 16.0, 15.7, 15.6, 14.7. MS (ESI) *m/z*: 581.35 $[M+Na]^+$. ESI-HRMS calcd. for $C_{34}H_{54}O_2S_2Na$ ($[M+Na]^+$): 581.3463; found: 581.3482.

*Lupeol (**3**)*

According to the procedure for preparation of **1**, **3** was prepared from **17** over two steps as a white amorphous solid (87% yield). mp: 212–213°C. $[\alpha]_D = +21.2$ ($c = 1.0$, $CHCl_3$). FT-IR ν (cm^{-1}): 3416, 2944, 2871, 2360, 2342, 1639, 1454, 1381, 1190, 1106, 1043, 1014, 983, 881. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.68 (s, 1H), 4.57 (s, 1H), 3.18 (dd, $J = 5.3$, 10.8 Hz, 1H), 2.42–2.33 (m, 1H), 1.99–1.85 (m, 1H), 1.68–0.67 (m, 23H), 1.68 (s, 3H), 1.03 (s, 3H), 0.97 (s, 3H), 0.94 (s, 3H), 0.83 (s, 3H), 0.79 (s, 3H), 0.76 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.0, 109.3, 79.0, 55.3, 50.4, 48.3, 48.0, 43.0, 42.8, 40.8, 40.0, 38.8, 38.7, 38.0, 37.2, 35.6, 34.3, 29.8, 28.0, 27.4, 27.3, 25.1, 20.9, 19.3, 18.3, 18.0, 16.1, 16.0, 15.4, 14.5. ESI-HRMS calcd. for $C_{30}H_{50}ONa$ ($[M+Na]^+$): 449.3759; found: 449.3770.

Biological evaluation

According to the literature method [30] with modification, the inhibitory activity of the test compounds on human OSC-mediated lanosterol biosynthesis from (3*S*)-2,3-oxidosqualene was determined based on incubation with HL-60 cells, followed by lysis of the cells, liquid–liquid microextraction, and LC-MS/MS analysis of the resulting cellular extracts. Through comparing the substrate-product patterns of the test compounds with those of the blank control and the positive control (with known human OSC inhibitor Ro 48-8071 [31]), the relative qualitative results were obtained.

Cell culture and sample preparation

HL-60 cell line was purchased from American Type Culture Collection, USA. RPMI 1640 medium, fetal bovine serum and lipid free medium were purchased from Gibco-BRL, Invitrogen, USA. Ro 48-8071 was a selective OSC inhibitor that was prepared according to the literature method [31]. HL-60 cells were cultured in RPMI 1640 medium containing 10% (v/v) fetal bovine serum (FBS) without antibiotics at 37°C in a humidified atmosphere of 5% CO_2/95% air. (3*S*)-2,3-Oxidosqualene and lanosterol were purchased from Sigma–Aldrich (St. Louis, MO, USA). The cellular incubation experiment was performed as below: HL 60 cells (5×10^6) were suspended in 6-well plates in the presence or absence of the test compounds in 2 mL lipid free medium without antibiotics. The compounds were dissolved in dimethyl sulfoxide (DMSO) at a stock concentration (10^{-2} M), then diluted with lipid free medium to reach the final test concentration of 10 μM (DMSO final concentration at 0.1%). After a 24 h incubation period, the content of each well was transferred into a plastic tube and the wells were washed with 750 μL of cold phosphate-buffered saline (PBS). The cells were centrifuged at 1000 rpm for 5 min, and the pellets were washed twice with cold PBS. The supernatant was removed, and then the sediments were collected in the plastic tubes for further LC-MS/MS analysis.

LC-MS/MS analysis

The sediments from the above cellular incubation experiment were extracted by *n*-hexane after being freeze-dried. Chromatographic separation was achieved by HPLC on an Agilent ZORBAX SB-C18 (2.1 mm × 50 mm, 1.8 μm). An isocratic elution was used at a flow rate of 0.3 mL/min and the mobile phase was comprised of acetonitrile and water (95:5, v/v). The column oven was maintained at 50°C. Analytes were determined on a Shimadzu LC-MS8040 triple-quadrupole mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) source (Shimadzu, Kyoto, Japan) in positive ionization mode. Semi-quantitative analysis was performed using multiple reaction monitoring (MRM) with precursor to product transition: *m/z* 427.45 → 427.25 for (3*S*)-2,3-oxidosqualene and *m/z* 409.40 → 109.00 for lanosterol, respectively. Parameters for MRM were all automatic optimized with a 100 ms dwell time. The optimal MS detection parameters were set as follows: interface voltage −4.5 kV, desolvation line (DL) temperature 150°C, and the heat block temperature 200°C. Nitrogen was used as nebulizing gas and drying gas with a flow rate of 4.0 and 5.0 L/min, respectively. The collision induced dissociation (CID) gas was argon. Data acquisition and quantitative analysis were carried out on the LabSolution software (Shimadzu). The method was validated for accuracy, precision, and stability using QC samples. The selected LC-MS/MS analysis chromatograms are showed in Supporting Information Figs. S2 and S3.

Molecular docking mode

Molecular docking studies were performed using SYBYL 1.3 software package. All structures were minimized with the Tripos force field, and the hydrogen atoms were added. Powel optimized the energy gradient, the maximum times to 1000 times the energy convergence criterion reaching 0.005 kcal/mol, by use of Gasteiger–Hückel charges. Ligand-protein docking was performed by the Surflex Dock in SYBYL 1.3. The crystal structure of human OSC was retrieved from RCSB Protein Data Bank (PDB ID: 1W6J). Biopolymer module was then used to repair the crystal structure of the protein termini-treatment, to fix side chain amides and residues and to add charges. The potent target compounds docking with human OSC selected catalytic pocket of lanosterol as active site. The active pocket was formed through computing, and all the docking parameters were set to the defaults. Results were then aggregated and the best poses inspected. The images were generated using Accelrys Discovery Studio 2.5.

This work was supported by the National Natural Science Foundation of China (81373303, 81473080, 81573299, 81673443, and 81602957), the Natural Science Foundation

of Jiangsu Province, China (BK20161035), the "111 Project" from the Minisry of Education of China, the State Administration of Foreign Expert Affairs of China (111-2-07), and the Program for Changjiang Scholars and Innovative Research Team in University (IRT1193).

The authors have declared no conflict of interest.

References

[1] H. Sheng, H. Sun, *Nat. Prod. Rep*. **2011**, *28*, 543–593.
[2] F. A. Santos, J. T. Frota, B. R. Arruda;, T. S. de Melo, A. A. da Silva, G. A. Brito, M. H. Chaves, V. S. Rao, *Lipids Health Dis*. **2012**, *11*, 98–105.
[3] F. A. Oliveira, M. H. Chaves, F. R. Almeida, R. C. Lima, Jr., R. M. Silva, J. L. Maia, G. A. Brito, F. A. Santos, V. S. Rao, *J. Ethnopharmacol*. **2005**, *98*, 103–108.
[4] V. Sudhahar, S. A. Kumar, P. T. Sudharsan, P. Varalakshmi, *Vascul. Pharmacol*. **2007**, *46*, 412–418.
[5] V. Sudhahar, S. Ashok Kumar, P. Varalakshmi, V. Sujatha, *Mol. Cell Biochem*. **2008**, *317*, 11–20.
[6] E. J. Corey, H. J. Hess, S. Proskow, *J. Am. Chem. Soc*. **1959**, *81*, 5258–5259.
[7] E. J. Corey, J. Lee, *J. Am. Chem. Soc*. **1993**, *115*, 8873–8874.
[8] G. Stork, S. Uyeo, T. Wakamatsu, P. Grieco, J. Labovitz, *J. Am. Chem. Soc*. **1971**, *93*, 4945–4947.
[9] K. Surendra, E. J. Corey, *J. Am. Chem. Soc*. **2009**, *131*, 13928–13929.
[10] E. J. Corey, P. R. Ortiz de Montellano, *J. Am. Chem. Soc*. **1967**, *89*, 3362–3363.
[11] E. E. Van Tamelen, M. P. Seiler, W. Wierenga, *J. Am. Chem. Soc*. **1972**, *94*, 8229–8231.
[12] H. H. Rees, E. I. Mercer, T. W. Goodwin, *Biochem. J*. **1966**, *99*, 726–734.
[13] H. Shan, W. K. Wilson, D. R. Phillips, B. Bartel, S. P. Matsuda, *Org. Lett*. **2008**, *10*, 1897–1900.
[14] G. Brownlie, M. B. E. Fayez, F. S. Spring, R. Stevenson, W. S. Strachan, *J. Chem. Soc*. **1956**, 1377–1381.
[15] T. Kikuchi, M. Niwa, M. Takayama, T. Yokoi, T. Shingu, *Chem. Pharm. Bull*. **1980**, *28*, 1999–2007.
[16] D. H. Barton, T. R. Jarman, K. C. Watson, D. A. Widdowson, R. B. Boar, K. Damps, *J. Chem. Soc. Perkin Trans*. *1*, **1975**, *12*, 1134–1138.
[17] P. D. Dean, *Steroidologia* **1971**, *2*, 143–157.
[18] L. Ruzicka, *Experientia* **1953**, *9*, 357–367.
[19] A. Eschenmoser, D. Arigoni, *Helv. Chim. Acta* **2005**, *88*, 3011–3050.
[20] K. U. Wendt, G. E. Schulz, E. J. Corey, D. R. Liu, *Angew. Chem. Int. Ed*. **2000**, *39*, 2812–2833.
[21] R. Thoma, T. Schulz-Gasch, B. D'Arcy, J. Benz, J. Aebi, H. Dehmlow, M. Hennig, M. Stihle, A. Ruf, *Nature* **2004**, *432*, 118–122.
[22] D. Staedler, C. Chapuis-Bernasconi, H. Dehmlow, H. Fischer, L. Juillerat-Jeanneret, J. D. Aebi, *J. Med. Chem*. **2012**, *55*, 4990–5002.
[23] V. Leduc, S. Jasmin-Bélanger, J. Poirier, *Trends Mol. Med*. **2010**, *16*, 469–477.
[24] B. Eisele, R. Budzinski, P. Müller, R. Maier, M. Mark, *J. Lipid Res*. **1997**, *38*, 564–575.
[25] M. Mark, P. Müeller, R. Maier, B. Eisele, *J. Lipid Res*. **1996**, *37*, 148–158.
[26] O. H. Morand, J. D. Aebi, H. Dehmlow, Y.-H. Ji, N. Gains, H. Lengsfeld, J. Himber, *J. Lipid Res*. **1997**, *38*, 373–390.
[27] G. R. Brown, D. M. Hollinshead, E. S. Stokes, D. S. Clarke, M. A. Eakin, A. J. Foubister, S. C. Glossop, D. Griffiths, M. C. Johnson, F. McTaggart, D. J. Mirrlees, G. J. Smith, R. Wood, *J. Med. Chem*. **1999**, *42*, 1306–1311.
[28] G. R. Brown, D. M. Hollinshead, E. S. Stokes, D. Waterson, D. S. Clarke, A. J. Foubister, S. C. Glossop, F. McTaggart, D. J. Mirrlees, G. J. Smith, R. Wood, *J. Med. Chem*. **2000**, *43*, 4964–4972.
[29] T. Kushiro, M. Shibuya, Y. Ebizuka, *Eur. J. Biochem*. **1998**, *256*, 238–244.
[30] A. Horling, C. Müller, R. Barthel, F. Bracher, P. Imming, *J. Med. Chem*. **2012**, *55*, 7614–7622.
[31] A. Lenhart, D. J. Reinert, J. D. Aebi, H. Dehmlow, O. H. Morand, G. E. Schulz, *J. Med. Chem*. **2003**, *46*, 2083–2092.