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# *Full Papers*

## Scalarane Sesterterpenes from a Marine Sponge of the Genus *Spongia* and Their FXR Antagonistic Activity

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Three new scalarane-based sesterterpenes, **1**–**3**, were isolated from a marine sponge of the genus *Spongia*, and their chemical structures were elucidated by analysis of HRMS and 2-D NMR spectra. The isolated compounds **1** and **3** showed inhibition against the farnesoid X-activated receptor (FXR) with $IC_{50}$ values of 2.4 and 24 $\mu$M, respectively. In particular, compound **3** directly inhibited the interaction between FXR and a coactivator peptide (SRC-1) as determined by surface plasmon resonance (SPR) spectroscopy.

FXR is a ligand-dependent transcription factor that plays crucial roles in lipoprotein metabolism, liver generation, protection from hepatotoxic agents, and repression of bacterial overgrowth in the intestine.[1] Genes targeted by FXR, including the bile salt export pump, MDR3, and multidrug-resistant related protein 2, are correlated with genetic disorders such as cholestatic liver disorders, progressive familial intrahepatic cholestasis type 2 (PFIC2), PFIC3, and Dubin-Johnson syndrome.[2] Furthermore, FXR is a key regulator of cholesterol/bile acid homeostasis. Normally cholesterol is removed in the form of bile acids in the liver. When the bile acid levels are high in the liver, they induce the activation of FXR, which subsequently represses the transcription of the CYP7Al gene. The CYP7Al gene encodes cholesterol 7α-hydroxylase, which is the key and rate-limiting enzyme in the biosynthesis of bile acids from cholesterol in the human liver.[3] Therefore, FXR antagonists have the potential to be drugs for hypercholesterolemia in humans.[4] Novel FXR ligands that tightly regulate the spatial and temporal activity of FXR are needed to treat related cholestatic liver disorders including hypercholesterolemia.

### Results and Discussion

Marine crude extracts were screened for novel FXR antagonists using a cell-based cotransfection assay. During this investigation, the crude extract of *Spongia* sp. showed potent inhibitory activity against FXR transactivation. Bioactivity-guided fractionation followed by reversed-phase HPLC gave three new scalarane sesterterpenes, 12-*O*-deacetyl-12-*epi*-19-deoxy-21-hydroxyscalarin (**1**), 12-*O*-deacetyl-12-*epi*-19-deoxy-22-hydroxyscalarin (**2**), and 12-*O*-deacetyl-12-*epi*-19-*O*-methylscalarin (**3**), along with known compounds 12-*O*-deacetyl-12-*epi*-scalarin (**4**),[5] 12-*epi*-scalarin (**5**),[6] and 12-*O*-deacetyl-12-*epi*-19-deoxyscalarin (**6**).[7] The isolated compounds showed inhibition of FXR transactivation.

A species belonging to the marine sponge genus *Spongia* was collected using scuba near Tong-Yong City in the South Sea of Korea. The wet animal was extracted with 50% MeOH in $CH_2Cl_2$. The extract was partitioned between hexanes and MeOH, and the MeOH-soluble layer was further partitioned between ethyl acetate and water. The EtOAc-soluble part was subjected to silica flash chromatography followed by reversed-phase HPLC to give compounds **1** (2.0 mg), **2** (2.0 mg), **3** (14.7 mg), **4** (46.7 mg), **5** (45.2 mg), and **6** (16.8 mg).

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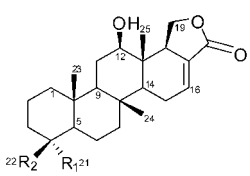
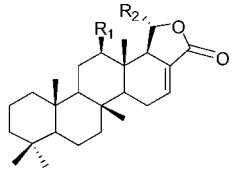


**1**. $R_1$ = $CH_2OH$, $R_2$ = Me
**2**. $R_1$ = Me, $R_2$ = $CH_2OH$
**6**. $R_1$ = Me, $R_2$ = Me



**3**. $R_1$ = OH, $R_2$ = OMe
**4**. $R_1$ = OH, $R_2$ = OH
**5**. $R_1$ = OAc, $R_2$ = OH

The molecular formula of **1** was deduced as $C_{25}H_{38}O_4$, on the basis of a molecular ion peak at *m/z* 403.2852 $[M + H]^+$ in the HRFABMS and $^{13}C$ NMR data. The IR spectrum showed the presence of a hydroxyl group at 3392 $cm^{-1}$ and an α,β-unsaturated carbonyl group at 1760 and 1683 $cm^{-1}$. The $^{13}C$ NMR spectrum of **1** in combination with 90° and 135° DEPT spectra revealed four methyls, nine methylenes, six methines, and six quaternary carbons. $^1H$ and $^{13}C$ NMR indicated one α,β-unsaturated-γ-lactone [δ 6.81 (H-16), 137.4 (C-16), 128.7 (C-17), and 172.0 (C-20)]. The $^1H$ NMR spectrum also revealed four downfield methylene protons at δ 4.50, 4.20, 3.33, and 2.99. The position of the oxygenated methylene protons at δ 4.50 and 4.20 (H-19's) was determined by coupling with H-18 (δ 2.92) by COSY, as well as from long-range correlations to the α,β-unsaturated-γ-lactone carbons [δ 128.7 (C-17) and 172.0 (C-20)] and to the β-methine carbon at δ 51.8 (C-18) by HMBC. Similarly, long-range HMBC correlations of downfield methylene protons at δ 3.33 and 2.99 (H-21's) with δ 36.5 (C-3), 38.4 (C-4), and 50.6 (C-5) suggested that they should be located at C-21.

Interpretation of 2D NMR data (COSY, HSQC, and HMBC) revealed that compound **1** is based on a scalarane skeleton with a hydroxyl group at C-21. In particular, cross-peaks in the HMBC spectra between four upfield singlet methyls and neighboring carbons assisted in the identification of six-membered hydrocarbon rings in the molecule (Table 1).

The relative stereochemistry of compound **1** was determined by interpretation of $^1H$ NMR coupling constants and ROESY correlations. Cross-peaks between H-22 (δ 0.75) and H-23 (δ 0.93) and between H-23 and H-24 (δ 0.97) indicated axial orientations for C-22 (δ 17.8), C-23 (δ 17.7), and C-24 (δ 17.2) on the molecular plane. Cross-peaks between H-24 and H-25 (δ 0.74) and between H-25 and H-19 (δ 4.20 and 4.50) also indicated that C-23, C-24, C-25 (δ 8.5), and C-19 (δ 71.0) were all on the same face of the molecule. In particular, the magnitude of the *J*-coupling for H-12 (δ 3.50, br d, $J$ = 11.0 Hz) to H-11 indicated that H-12 is axial. ROESY correlations were also observed between the oxygenated protons H-21's (δ 2.99 and 3.33) and H-22, between H-22 and H-23, and between H-23 and H-24. The structure of **1** was thus concluded to be 12-*O*-deacetyl-12-*epi*-19-deoxy-21-hydroxyscalarin (Table 1).

The molecular formula of **2** was deduced as $C_{25}H_{38}O_4$ on the basis of a molecular ion peak at *m/z* 403.2853 $[M + H]^+$ in the HRFABMS and $^{13}C$ NMR data. Analysis of 2D NMR data (COSY, HSQC, and HMBC) revealed that the planar structure of this compound is identical to that of **1**. However, ROESY correlations between the oxygenated protons H-22 (δ 3.51 and 3.69) and H-21 (δ 0.92), between H-22 and H-23 (δ 0.89), and between H-23 and H-24 (δ 0.96) of **2** revealed it to be the diastereomer of **1**, 12-*O*-deacetyl-12-*epi*-19-deoxy-22-hydroxyscalarin (Table 2).

The molecular formula of **3** was deduced as $C_{26}H_{40}O_4$ on the basis of $^{13}C$ NMR data and a molecular ion peak at *m/z* 417.3009 $[M + H]^+$ in the HRFABMS. The $^{13}C$ NMR spectrum of **3** in combination with 90° and 135° DEPT spectra revealed six methyls, seven methylenes, seven methines (one olefinic and two bearing oxygen), and six quaternary carbons. $^1H$ and $^{13}C$ NMR also indicated one α,β-unsaturated-γ-lactone [δ 6.87 (H-16), 136.5 (C-16), 127.2 (C-17), and 167.2 (C-20)] and an acetal group [δ 5.34 (H-19) and 105.6 (C-19)]. The position of the acetal at the γ-position of the lactone was determined by the cross-peaks between the acetal proton at δ 5.34 (H-19) and two carbons at δ 167.2 (C-20) and 58.2 (19-OMe) by HMBC. A trisubstituted double bond was located at the α-position of the lactone as determined by the long-range HMBC correlations of the olefinic proton at δ 6.87 (H-16) with both the lactone carbonyl at δ 167.2 and the β-methine carbon at δ 57.2 (C-18). Analysis of 2D NMR data revealed that compound **3** is based on a scalarane skeleton, having a methoxy group at C-19.

**Table 1.** NMR Data of **1** (methanol-$d_4$)[a]

| no. | $\delta_C$, mult.[b] | $\delta_H$ (*J* in Hz) | COSY | HMBC |
|---|---|---|---|---|
| 1 | 40.8, $CH_2$ | 0.85, m, 1.73, m | 2 | 3, 5, 9, 10 |
| 2 | 18.9, $CH_2$ | 1.44, m, 1.50, m | 3 | 1, 4 |
| 3 | 36.5, $CH_2$ | 1.20, m, 1.50, m | 2 | 1, 4, 5, 21, 22 |
| 4 | 38.4, qC | | | |
| 5 | 50.6, CH | 1.20, m | 6 | 23 |
| 6 | 19.1, $CH_2$ | 1.44, m, 1.47, m | 5, 7 | 5, 8 |
| 7 | 42.3, $CH_2$ | 1.02, m, 1.47, m | 6 | 5, 6 |
| 8 | 38.5, qC | | | |
| 9 | 60.2, CH | 1.00, m | 11 | 8, 10, 11 |
| 10 | 38.8, qC | | | |
| 11 | 19.1, $CH_2$ | 1.44, m, 1.50, m | 9, 12 | 8, 9, 10, 12 |
| 12 | 81.8, CH | 3.40, dd (11.0, 4.2) | 11 | 18, 25 |
| 13 | 41.3, qC | | | |
| 14 | 54.7, CH | 1.33, d (12.1) | 15 | 7, 8, 13, 15, 18, 24, 25 |
| 15 | 25.0, $CH_2$ | 2.25, m, 2.35, m | 14, 16 | 14, 20 |
| 16 | 137.4, CH | 6.81, dd (6.8, 3.3) | 15 | 14, 15, 18, 20 |
| 17 | 128.7, qC | | | |
| 18 | 51.8, CH | 2.92, m | 19 | 12, 13, 16, 17, 25 |
| 19 | 71.0, $CH_2$ | 4.20, dd (9.5, 9.5), 4.50, dd (9.5, 9.5) | 18 | 18, 20, |
| 20 | 172.0, qC | | | |
| 21 | 72.0, $CH_2$ | 2.99, d (11.0), 3.33, d (11.0) | | 3, 4, 5, 22 |
| 22 | 17.8, $CH_3$ | 0.75, s | | 3, 4, 21 |
| 23 | 17.7, $CH_3$ | 0.93, s | | 1, 9, 10 |
| 24 | 17.2, $CH_3$ | 0.97, s | | 7, 9, 14 |
| 25 | 8.5, $CH_3$ | 0.74, s | | 12, 13, 14 |

[a] 600 MHz for $^1H$ NMR and 150 MHz for $^{13}C$ NMR. [b] Multiplicity was determined by analysis of DEPT spectra.

The relative stereochemistry of **3** was established by analysis of $^1H$ NMR coupling constants and NOESY data (Figure 1). The magnitude of the coupling between H-12 (δ 3.50, br d, $J$ = 10.2 Hz) and H-11 and a correlation between H-12 and H-18 (δ 2.52) in the NOESY spectra both suggested an axial orientation for H-12. The α-configuration of the methoxy group at C-19 was determined by a NOESY correlation between H-19 (δ 5.34) and H-25 (δ 0.82). The structure of **3** was concluded to be 12-*O*-deacetyl-12-*epi*-19-*O*-methylscalarin.

To investigate the FXR antagonistic effect of compounds **1**–**6**, a cotransfection assay was performed using a full-length human FXR and an ecdysone receptor response element (EcRE)-driven fly luciferase reporter.[8] Transfected cells (CV-1, a monkey kidney cell line) were treated with or without the tested compounds **1**–**6** in the presence of 50 μM chenodeoxycholic acid (CDCA, a natural ligand for FXR). As a result, compounds **1**–**6** showed inhibition of FXR transactivation. In particular, 12-*O*-deacetyl-12-*epi*-19-deoxy-21-hydroxyscalarin (**1**) showed the most potent inhibitory activity against FXR with an $IC_{50}$ of 2.4 μM without any significant cytotoxicity. It was clear that the stereochemistry of C-4 was critical for the biological activity, as compounds **2**, **4**, **5**, and **6** showed almost no activity against FXR below the $IC_{50}$ value of cytotoxicity (Figure 2 and Table 3).

Direct binding of scalarins **1**–**6** to the ligand binding domain (LBD) of FXR was monitored by using surface plasmon resonance (SPR) spectroscopy using a BIAcore system.[9] FXR LBD (4 μM), preincubated for 1 h with *E*-guggulsterone or scalarins in the presence of 50 μM CDCA, was injected over the sensor chip surface on which a coactivator peptide (SRC-1) was immobilized. Ligand-

**Table 2.** $^1$H and $^{13}$C NMR Data of **2** (methanol-$d_4$)[a] and **3** ($CDCl_3$)[a]

| | **2** | | **3** | |
|---|---|---|---|---|
| | $\delta_C$, mult.[b] | $\delta_H$ ($J$ in Hz) | $\delta_C$, mult. | $\delta_H$ ($J$ in Hz) |
| 1 | 41.4, $CH_2$ | 0.90, m, 1.76, m | 40.1, $CH_2$ | 0.78, 1.71, m |
| 2 | 19.4, $CH_2$ | 1.45, m, 1.65, m | 18.7, $CH_2$ | 1.37, 1.56, m |
| 3 | 43.1, $CH_2$ | 0.97, m, 1.76, m | 42.2, $CH_2$ | 1.13, dt (12.4, 4.5), 1.36, m |
| 4 | 39.9, qC | | 33.4, qC | |
| 5 | 58.6, CH | 0.99* | 56.9, CH | 0.81* |
| 6 | 19.4, $CH_2$ | 1.45, m, 1.65, m | 18.2, $CH_2$ | 1.53, 1.69, m |
| 7 | 36.9, $CH_2$ | 0.96, m, 1.85, m | 41.6, $CH_2$ | 0.90, 1.70, m |
| 8 | 38.6, qC | | 37.6, qC | |
| 9 | 60.4, CH | 0.97* | 59.0, CH | 0.90* |
| 10 | 38.6, qC | | 40.1, qC | |
| 11 | 28.3, $CH_2$ | 1.44, m, 1.68, m | 26.1, $CH_2$ | 1.53, m, 1.72, m |
| 12 | 81.8, CH | 3.39, dd (11.0, 4.2) | 80.4, CH | 3.50, dd (10.2, 1.5) |
| 13 | 41.3, qC | | 40.1, qC | |
| 14 | 54.6, CH | 1.32, d (12.1) | 52.9, CH | 1.21, dd (11.2, 5.4) |
| 15 | 25.0, $CH_2$ | 2.24, m, 2.36, m | 23.8, $CH_2$ | 2.17, m, 2.31, m |
| 16 | 137.4, CH | 6.80, dd (6.8, 3.3) | 136.5, CH | 6.87, dd (6.9, 3.4) |
| 17 | 128.7, qC | | 127.2, qC | |
| 18 | 51.7, CH | 2.92, m | 57.2, CH | 2.55, m |
| 19 | 71.0, $CH_2$ | 4.19, dd (9.5, 9.5)<br>4.49, dd (9.5, 9.5) | 105.6, CH | 5.34, d (6.3) |
| 20 | 172.8, qC | | 167.2, qC | |
| 21 | 27.2, $CH_3$ | 0.92, s | 33.4, $CH_3$ | 0.84, s |
| 22 | 65.1, $CH_2$ | 3.51, d (11.0), 3.69, d (11.0) | 21.5, $CH_3$ | 0.81, s |
| 23 | 17.8, $CH_3$ | 0.89, s | 16.9, $CH_3$ | 0.90, s |
| 24 | 17.1, $CH_3$ | 0.96, s | 16.7, $CH_3$ | 0.86, s |
| 25 | 8.4, $CH_3$ | 0.75, s | 9.3, $CH_3$ | 0.82, s |
| OH | | | | 2.52, br s |
| 19-OMe | | | 58.2, $CH_3$ | 3.62, s |

[a] Assignments were aided by a combination of COSY, HSQC, and HMBC experiments. [b] Multiplicity was determined by analysis of DEPT spectra. *The coupling constant was not determined because of overlapped signals.

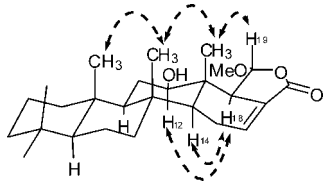



**Figure 1.** Key NOESY correlations of **3**.

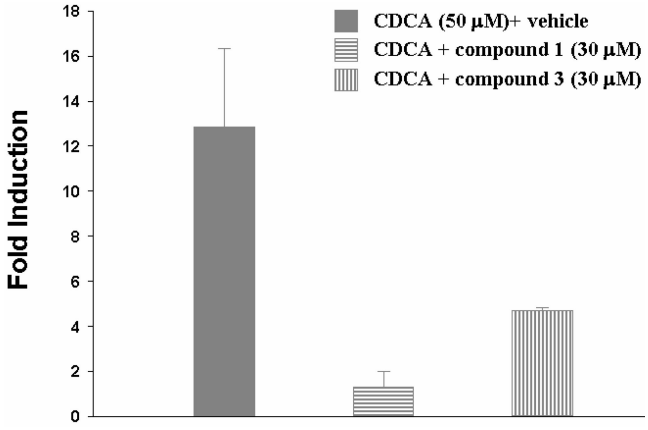



**Figure 2.** Repression of FXR transactivation by scalarins. The cotransfected cells with FXR, luciferase reporter, and $\beta$-galactosidase expression plasmids were treated with tested compounds (30 $\mu$M) or solvent (DMSO) in the presence of 50 $\mu$M CDCA. The luciferase activity was normalized to the $\beta$-galactosidase activity and expressed as fold induction. For simplicity compounds **2**, **4**, **5**, and **6** were excluded from the graph since they did not show any repression at 30 $\mu$M.

induced association of the FXR LBD with the SRC-1 peptide was monitored by a change in resonance units (RUs). Scalarins **1**–**6** decreased the affinity of FXR LBD for SRC-1 peptide, which was

**Table 3.** Inhibition of FXR Transactivation and Cytotoxicity for **1**–**6**

| compd | inhibition of FXR transactivation $IC_{50}$, $\mu$M[a] | cytotoxicity $IC_{50}$, $\mu$M[a,b] (CV-1 cell line) |
|---|---|---|
| **1** | 2.4 | 49.4 |
| **2** | >100 | >100 |
| **3** | 24.0 | 48.0 |
| **4** | 75.0 | 77.2 |
| **5** | 60.4 | 75.1 |
| **6** | 31.6 | 41.4 |
| *E*-guggulsterone | 4.1 | not determined |

[a] Each experiment was repeated more than three times. [b] Cytotoxicity was measured using the MTT method.

facilitated by CDCA (Figure 2).12-*O*-Deacetyl-12-*epi*-19-deoxy-21-hydroxylscalarin (**1**), which showed the most potent antagonistic activity against FXR in the cell-based cotransfection assay, was only a weak inhibitor or no inhibitor at all of the specific interaction between FXR and the SRC-1 peptide. This result suggests that compound **1** should inhibit FXR transactivation by an indirect mechanism or by interaction with one of the other cofactors such as SRC-2 or -3 in cells, which was not tested in this study. Compounds **3**, **4**, **5**, and **6** showed very strong direct interactions with FXR, although they were not especially potent in the cell-based assay. This may be caused by the fact that FXR controls target gene expression in a ligand- and promoter-specific fashion.[10] They may interact with FXR very well on a natural promoter such as a bile salt export pump or cholesterol 7α-hydroxylase, a well-known target for FXR, while they activate the luciferase reporter gene poorly on a nonmammalian promoter, ecdysone receptor response element. The mechanism of action for scalarins is currently underway in our laboratory.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured on a Rudolph Research Autopol III, #A7214. Infrared spectra were recorded on a Thermo Electron Corp. Nicolet 570. $^1$H

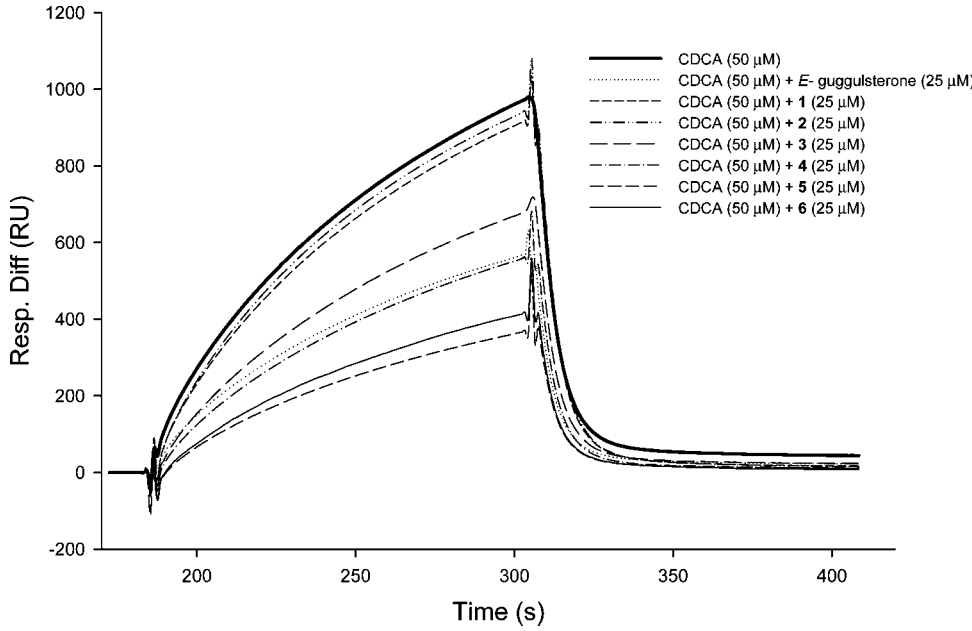



**Figure 3.** *E*-Guggulsterone or scalarins (**1**–**6**) in the presence of 50 $\mu$M CDCA was injected over the sensor chip surface on which a coactivator peptide (SRC-1) was immobilized and SPR data were graphed. Some scalarins (**3**–**6**) disrupted a specific CDCA-mediated interaction between FXR and the coactivator peptide SRC-1, showing their direct binding to FXR *in vitro*.

and $^{13}$C NMR spectra were recorded in $CDCl_3$ or methanol-$d_4$ solution containing $Me_4Si$ as internal standard on Bruker Avance DPX-500 and DPX-600 spectrometers. FABMS was measured on a JEOL JMS-AX505WA mass spectrometer. Solvents used in partitioning were first grade products of Dae Jang & Metals Co., Korea. HPLC grade solvents from Burdick & Jackson were used in adsorption chromatography, TLC, and HPLC. Younglin SDV 30 plus HPLCs were used for the isolation of compounds with Younglin M 720 UV detectors.

**Animal Material.** A marine sponge specimen was collected at a depth of 10 m near Tong-Yong City in the South Sea of Korea. The sponge was immediately frozen by packing with dry ice and then stored at −18 °C until processed. The sponge was identified as a species of the genus *Spongia*. The sponge was brown with a compact, round shape. The skeleton was comprised of a tightly meshed system, and its consistency was compressible. Primary fibers were recognized only on the surface with broken spicules, and they were all the same thickness as secondary fibers, around 25–40 $\mu$m. A voucher specimen was deposited at the Center for Marine Natural Products and Drug Discovery, Seoul National University, Korea.

**Extraction and Isolation.** The frozen sponge (23.0 kg, wet wt) was extracted three times with 50% MeOH in $CH_2Cl_2$. The dried extract was dissolved in MeOH and partitioned three times with fresh portions of hexanes. Then the MeOH-soluble layer was dried, resuspended in $H_2O$, and partitioned with fresh portions of ethyl acetate three times. The EtOAc-soluble part (10 g) was found to decrease FXR transactivation by nearly 90% at 100 $\mu$g/mL. The EtOAc-soluble part was subjected to Si flash chromatography using a step gradient with EtOAc and hexanes as eluents to provide 21 fractions. Among them, active fractions (90% inhibition at 25 $\mu$g/mL) against FXR were further separated by using repeated RP HPLC (Optimapak, 250 × 10 mm, 5 $\mu$m, 100 Å, UV = 210 nm), eluting with 85% $CH_3CN$ in $H_2O$ to afford compounds **1** (2.0 mg), **2** (2.0 mg), **3** (14.7 mg), **4** (46.7 mg), **5** (45.2 mg), and **6** (16.8 mg), as colorless oils.

**12-*O*-Deacetyl-12-*epi*-19-deoxy-21-hydroxyscalarin (1):** $[\alpha]_D^{25}$ +12.0 (*c* 0.001, $CHCl_3$); IR (KBr) $\nu_{max}$ 3392, 1760, 1683, 1238 cm$^{-1}$; $^1$H, $^{13}$C, and 2D NMR data, see Table 1; LRFABMS obsd $[M + H]^+$ *m/z* 403; HRFABMS 403.2852 (calcd for $C_{25}H_{38}O_4$, 403.2848).

**12-*O*-Deacetyl-12-*epi*-19-deoxy-22-hydroxyscalarin (2):** $[\alpha]_D^{25}$ −16.0 (*c* 0.0005, $CHCl_3$); IR (KBr) $\nu_{max}$ 3392, 1760, 1683, 1238 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Table 2; LRFABMS obsd $[M + H]^+$ *m/z* 403; HRFABMS 403.2853 (calcd for $C_{25}H_{38}O_4$, 403.2848).

**12-*O*-Deacetyl-12-*epi*-19-*O*-methylscalarin (3):** $[\alpha]_D^{25}$ +9.0 (*c* 0.002, $CHCl_3$); IR (KBr) $\nu_{max}$ 3392, 1760, 1683, 1238 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Table 2; LRFABMS obsd $[M + H]^+$ *m/z* 417; HRFABMS 417.3009 (calcd for $C_{26}H_{41}O_4$, 417.3005).

**Acetylation of 12-*O*-Deacetyl-12-*epi*-19-deoxy-21-hydroxyscalarin (1).** Acetic anhydride (200 $\mu$L) and dimethylaminopyridine (DMAP) were added to a solution of 12-*O*-deacetyl-12-*epi*-19-deoxy-21-hydroxylscalarin (**1**, 300 $\mu$g) in pyridine (300 $\mu$L). The reaction mixture was then stirred for 6 h at room temperature. The reaction mixture was concentrated under reduced pressure, and the residue was purified by Si gel thick-layer chromatography (hexanes/ethyl acetate, 65:35) to give the diacetylated compound as a colorless oil: $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 6.87 (d, 1H, $J$ = 3.3 Hz), 4.67 (dd, 1H, $J$ = 11.4, 4.1 Hz), 4.23 (dd, 1H, $J$ = 9.2, 9.2 Hz), 4.10 (dd, 1H, $J$ = 9.2, 9.2 Hz), 3.86 (d, 1H, $J$ = 10.9 Hz), 3.61 (d, 1H, $J$ = 10.9 Hz), 2.86 (m, 1H), 2.35 (m, 1H), 2.15 (m, 1H), 2.06 (s, 6H), 1.84 (dd, 1H, $J$ = 10.9, 2.9 Hz), 1.72 (dt, 1H, $J$ = 12.6, 1.2 Hz), 1.64 (dt, 1H, $J$ = 12.6, 1.2 Hz), 1.61 (m, 1H), 1.45–1.40 (m, 3H), 1.37–1.34 (m, 2H), 1.13 (dd, 2H, $J$ = 5.7, 0.4 Hz), 1.05 (d, 1H, $J$ = 11.9 Hz), 0.97 (s, 3H), 0.92 (m, 2H), 0.88 (s, 3H), 0.86 (s, 3H), 0.82 (s, 3H); FABMS $[M + H]^+$ *m/z* 487; HRFABMS *m/z* 487.3069 (calcd for $C_{29}H_{43}O_6$, 487.3060).

**Acetylation of 12-*O*-Deacetyl-12-*epi*-19-deoxy-22-hydroxyscalarin (2).** 12-*O*-Deacetyl-12-*epi*-19-deoxy-22-hydroxyscalarin (**2**) was acetylated following the same procedure as for the acetate of 12-*O*-deacetyl-12-*epi*-19-deoxy-21-hydroxylscalarin (**1**). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 6.87 (dd, 1H, $J$ = 6.7, 3.3 Hz), 4.67 (dd, 1H, $J$ = 11.4, 4.1 Hz), 4.23 (dd, 1H, $J$ = 9.2, 9.2 Hz), 4.18 (d, 1H, $J$ = 10.9 Hz), 4.10 (dd, 1H, $J$ = 9.2, 9.2 Hz), 3.89 (d, 1H, $J$ = 10.9 Hz), 2.86 (m, 1H), 2.35 (m, 1H), 2.15 (m, 1H), 2.07 (s, 6H), 1.84 (dd, 1H, $J$ = 10.9, 2.9 Hz), 1.72 (dt, 1H, $J$ = 12.6, 1.2 Hz), 1.64 (dt, 1H, $J$ = 12.6, 1.2 Hz), 1.61 (m, 1H), 1.45–1.40 (m, 3H), 1.37–1.34 (m, 2H), 1.13 (dd, 2H, $J$ = 5.7, 0.4), 1.05 (d, 1H, $J$ = 11.9 Hz), 0.97 (s, 3H), 0.95 (s, 3H), 0.88 (m, 2H), 0.86 (s, 3H), 0.86 (s, 3H); FABMS $[M + H]^+$ *m/z* 487; HRFABMS *m/z* 487.3063 (calcd for $C_{29}H_{43}O_6$, 487.3060).

**Acetylation of 12-*O*-Deacetyl-12-*epi*-19-*O*-methylscalarin (3).** 12-*O*-Deacetyl-12-*epi*-19-*O*-methylscalarin (**3**) was acetylated following the same procedure as for the acetate of 12-*O*-deacetyl-12-*epi*-19-deoxy-21-hydroxylscalarin (**1**). $^1$H NMR (600 MHz, $CDCl_3$) $\delta$ 6.87 (dd, 1H, $J$ = 6.7, 3.3 Hz), 5.31 (d, 1H, $J$ = 5.5 Hz), 4.65 (dd, 1H, $J$ = 11.3, 4.4 Hz), 4.12 (d, 1H, $J$ = 7.2 Hz), 3.49 (s, 3H), 2.58 (m, 1H), 2.35 (m, 1H), 2.15 (m, 1H), 2.01 (s, 3H), 1.89 (dd, 1H, $J$ = 10.9, 2.9 Hz), 1.70 (dt, 1H, $J$ = 12.6, 1.2 Hz), 1.64 (dt, 1H, $J$ = 12.6, 1.2 Hz), 1.61 (m, 1H), 1.43–1.38 (m, 2H), 1.37–1.30 (m, 3H), 1.15 (dd, 1H, $J$ = 5.7, 0.4), 1.00 (d, 1H, $J$ = 11.9 Hz), 0.93 (s, 3H), 0.88 (s, 3H), 0.86 (m, 2H), 0.84 (s, 3H), 0.83 (s, 3H), 0.80 (s, 3H); FABMS $[M + Na]^+$ *m/z* 481; HRFABMS *m/z* 481.2927 (calcd for $C_{28}H_{42}O_5Na$, 481.2930).

**Cotransfection Assay.** CV-1 cells were seeded in 96-well plates in Dulbecco's modified Eagle's medium (GIBCO) supplemented with 10% lipid-depleted fetal bovine serum in humidified air containing 5% $CO_2$ at 37 °C for 24 h. Transient cotransfection with pCMX-hFXR, pCMX-$\beta$-GAL, and Tk-(EcRE)$_6$-LUC was carried out using SuperFect (Qiagen), according to the manufacturer's instructions. The pCMX vector contains the cytomegalovirus promoter-enhancer followed by a bacteriophage T7 promoter for transcription *in vitro*.[11] Tk-(EcRE)$_6$-LUC is a luciferase reporter that contains the six direct repeat of ecdysone receptor response element. pCMX-$\beta$-GAL is a control plasmid for normalization to minimize pipetting error that expresses $\beta$-galactosidase. After 24 h incubation, cotransfected cells were treated with a control vehicle (DMSO) or the indicated compounds in the presence of 50 $\mu$M CDCA. Cells were harvested at 24 h, and luciferase activity was assayed as described in the literature.[12] Luciferase activity was normalized to the $\beta$-galactosidase activity expressed from the control plasmid pCMX-$\beta$-GAL to minimize pipetting error. The experiment was performed in triplicate and repeated three times.

**Cytotoxicity Assay.** CV-1 cells were seeded in 96-well plates in DMEM supplemented with 10% fetal bovine serum in humidified air containing 5% $CO_2$ at 37 °C. After 24 h incubation, indicated compounds were administrated at various concentrations. Cells were harvested at 24 h and were incubated with 1 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution for 1 h at 37 °C. DMSO was added to each well in order to dissolve the produced purple formazan crystals, and the absorbance of each well was measured at 450 nm using an ELISA reader. The experiment was carried out in triplicate and repeated three times.

**Surface Plasmon Resonance (SPR) Spectroscopy.** SPR analysis was performed using a BIAcore 3000 system and BIACORE 3000 Control software 3.1.1 (BIAcore AB, Uppsala, Sweden). Briefly, biotinylated wild-type peptide from human SRC-1 (CPSSHSSLTERHKILHRLLQEGSPS-$CONH_2$) containing the LXXLL nuclear receptor interaction motif was immobilized on the surfaces of CM5 sensor chips (BIAcore Inc.). Human FXR LBD (4 $\mu$M) preincubated with ligands for 1 h was injected over the surfaces in a running buffer composed of 50 mM HEPES (pH 7.4), 150 mM NaCl, 3 mM EDTA, 0.005% Tween 20, and 1.1% DMSO at 25 °C. After completion of the injection (120 s), the complex was washed with buffer for an additional 120 s. The flow cell surfaces were regenerated by subsequent application of a 10 s pulse of 10 mM NaOH.

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**Supporting Information Available:** The $^1$H and $^{13}$C NMR spectra of **1**–**3** and a photo of the sponge are available free of charge via the Internet at http://pubs.acs.org.

## References and Notes

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