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Farnesoid X-activated receptor antagonists from a marine sponge *Spongia* sp.

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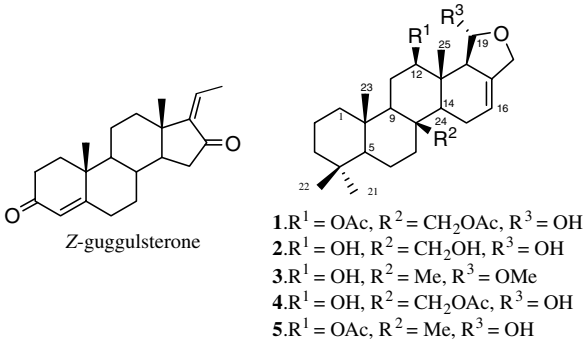
Abstract—Three novel (**1**–**3**) and two known (**4**–**5**) scalarane sesterterpenes were isolated from a marine sponge of the genus *Spongia*. The isolated compounds showed potent inhibition of transactivation for the nuclear hormone receptor, FXR (farnesoid X-activated receptor), which is a promising drug target to treat hypercholesterolemia in humans.

Hypercholesterolemia is one of the important risk factors for coronary heart disease and atherosclerosis.[1] The most well-known drugs for reducing blood cholesterol level are statins. Statins have been proven to reduce the blood cholesterol level by inhibiting the cholesterol biosynthesis enzyme HMG-CoA reductase. However, statins are reported to cause muscle damage, pharyngitis, headaches, and other pains.[2] In addition, statins do not remove accumulated cholesterol in the human body. Research is urgently needed to discover new molecular targets, as well as new therapeutic agents, to complement existing therapies. An excellent example of a new molecular target for hypercholesterolemia is the nuclear hormone receptor, FXR. Cholesterol is removed from the liver in the form of bile acids. The main bile acid biosynthetic pathway is initiated by cholesterol 7α-hydroxylase (CYP7A1), which is the rate-limiting enzyme of the bile acid biosynthetic pathway in the liver.[3] The CYP7A1 gene is suppressed by the bile-acid nuclear receptor FXR. A recent study found that *Z*-guggulsterone isolated from the guggul tree *Commiphora mukul* has directly decreased hepatic cholesterol levels through antagonizing FXR (Fig. 1).

Z-guggulsterone is the main ingredient of guggulipid, used in treating hyperlipidemia in humans.[4] However,



Z-guggulsterone

1.R^1 = OAc, R^2 = CH_2OAc, R^3 = OH
2.R^1 = OH, R^2 = CH_2OH, R^3 = OH
3.R^1 = OH, R^2 = Me, R^3 = OMe
4.R^1 = OH, R^2 = CH_2OAc, R^3 = OH
5.R^1 = OAc, R^2 = Me, R^3 = OH

Figure 1. *Z*-guggulsterone and structure of compounds **1**–**5**.

Z-guggulsterone also activates the other nuclear receptor like pregnane X receptor. The pregnane X receptor activated by guggulsterone induces the expression of CYP3A genes, which encode the cytochrome p450s in the human liver. The CYP3A are the drug-metabolizing isoenzymes and play key roles in the biotransformation of xenobiotics and drugs.[5] This suggests that patients being treated with guggulsterone should not continue taking medications metabolized by the CYP3A isoenzymes. Novel antagonists for FXR are thus needed to treat hypercholesterolemia in new ways other than the inhibition of HMG-CoA reductase in de novo cholesterol biosynthesis.

In continuing the investigation of FXR antagonists from marine organisms, five sesterterpenes **1**–**5** of scalarane class, including three novel compounds, were

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isolated from a Korean sponge of the genus *Spongia* (Fig. 1).

Many scalarane sesterterpenes have been isolated from marine sponges and many of these compounds are particularly interesting from the point of view of their pharmacological properties. In fact, these scalarane sesterterpenes have been reported to exhibit biological activities such as anti-inflammatory,[6] platelet aggregation inhibitory,[7] antimycobacterial,[8] cytotoxic,[9] mammalian phospholipase A_2 inhibitory,[10] HIV inhibitory,[11] and antineoplastic activities.[12] However, there is no report of biological activity of scalaranes in regard to metabolic disorders. In this communication, we have detailed the isolation and structural elucidation of these novel scalaranes and their FXR inhibitory activities.

A species of marine sponge of the genus *Spongia* was collected using SCUBA near Tong-Yong City in the South Sea, Korea. The wet animal (23 kg) was extracted three times with 50% MeOH in DCM. These extracts were combined and partitioned three times between hexane and MeOH. Then the MeOH soluble layer was further partitioned between ethyl acetate (10 g) and water three times. The EtOAc-soluble part was found to decrease FXR transactivation by nearly 90% at 100 μg/ml. The EtOAc-soluble part was subjected to silica flash chromatography using stepped gradient mixtures of EtOAc and hexane as eluents to provide 21 fractions. Among them, active fractions (90% inhibition at 25 μg/ml) against FXR were further separated by using reversed-phase HPLC (Optimapak, 250 × 10 mm, 5 μm, 100 Å, UV = 210 nm), eluting with 85% acetonitrile in H_2O to afford compounds **1** (41.8 mg), **2** (1.5 mg), **3** (3.5 mg), **4** (88.1 mg), and **5** (31.4 mg), as colorless oils.

The molecular formula of compound **1**, $[\alpha]_D^{25}$ −5.0° (*c* 0.002, $CHCl_3$), was deduced as $C_{29}H_{44}O_6$ based on the fragmentation at *m/z* 470.3034 $[M-H_2O]^+$ in HREIMS and ^{13}C NMR data. The IR spectrum showed the presence of a hydroxyl group at 3392 cm^{-1} and an ester at 1737 cm^{-1} and 1238 cm^{-1}. The ^{13}C NMR spectrum of **1** showed the presence of 29 carbon signals. At 90° and 135° DEPT spectra, six methyls, nine methylenes, seven methines (one olefin and two bearing oxygen), and seven quaternary carbons were revealed. Thus, compound **1** has an exchangeable proton[13] and, as indicated by the molecular formula, eight degrees of unsaturation. The ^1H NMR spectrum revealed the presence of one olefinic proton at δ 5.47 (H-16) together with an acetal hydrogen at δ 5.39 (H-19), two downfield methylene systems at δ 4.31 and δ 4.51 (H-24s), and δ 4.13 and δ 4.41 (H-20s). The ^1H NMR spectrum of **1** also featured a downfield methylene proton at δ 4.69 (H-12), four methyl groups with singlets at δ 0.85 (H-21), δ 0.79 (H-22), δ 0.88 (H-23), and δ 0.95 (H-25). Analysis of 2D NMR data (COSY, HSQC, and HMBC) revealed that compound **1** is based on the scalarane skeleton, having acetate groups at C-12 and C-24. The position of the acetyl group was assigned at C-12 (δ 82.3) by an HMBC cross-peak between H-12 (δ 4.69) and δ 170.8 (12-OAc). The presence of the remaining acetyl group at C-24 (δ 64.9) was also evident from an HMBC cross-peak between the methylene protons at δ 4.51 and δ 4.31 (H-24s) and carbons at δ 36.6 (C-7), δ 54.7 (C-14), and the carbonyl carbon at δ 171.1 (24-OAc). Complete assignment of the NMR data is given in Table 1.

The relative stereochemistry of **1** was established by analysis of coupling constants and NOSEY spectra (Fig. 2). The cross-peaks between H-23 (δ 0.88) and H-24 (δ 4.51), H-24 (δ 4.31) and H-25 (δ 0.95), and H-25 and H-19 (δ 5.39) indicated that C-23 (δ 16.3), C-24 (δ 64.9), C-25 (δ 9.3), and C-19 (δ 99.1) all have the same orientation on the molecular plane. In particular, a coupling constant (4.6 Hz) between H-19 (δ 5.39) and H-18 (δ 2.28), as well as a cross-peak between H-19 (δ 5.39) and H-25 (δ 0.95) in the NOESY spectra, supported unambiguously the *trans* relationship between H-18 (δ 2.28) and H-19 (δ 5.39). Alternatively, H-12 (δ 4.69) is coupled to H-11 (δ 1.88) with $J = 11.3$ Hz, and also correlates with H-18 (δ 2.28) strongly in the NOESY spectra, which suggest the *cis* relationship between H-12 (δ 4.69) and H-18 (δ 2.28). The gross structure of 12,24-diacetoxy-deoxoscalarin **1** was thus determined.

The molecular formula of **2**, $[\alpha]_D^{25}$ +9.0° (*c* 0.002, $CHCl_3$), was deduced as $C_{25}H_{40}O_4$ based on the fragmentation at *m/z* 427.2809 $[M+Na]^+$ in HRFABMS in combination with ^{13}C NMR data. The IR spectrum showed the presence of a hydroxyl group at 3392 cm^{-1}. At 90° and 135° DEPT spectra, four methyls, nine methylenes, seven methines (one olefin and two bearing oxygen), and five quaternary carbons were revealed. Thus, compound **2** has three exchangeable protons and, as indicated by the molecular formula, six degrees of unsaturation. The spectral data for this compound were very similar to those of **1**, with the loss of signals for the two acetoxyl groups in compound **1**. Analysis of 2D NMR revealed that compound **2** possesses the carbon skeleton identical to that of **1**. The structural difference is that the two acetoxyl groups at δ 82.7 (C-12) and δ 62.4 (C-24) are replaced by two hydroxyl groups, as evidenced by the significant upfield shifts of H-12 (δ 3.53), two methylene proton at δ 3.89 and δ 3.97 (H-24) in ^1H NMR spectrum, which was demonstrated by its acetylation.[14] Therefore, the structure of **2** was determined as 12-*O*-deacetoxyl-24-hydroxyl-deoxoscalarin (Table 2).

Compound **3**, $[\alpha]_D^{25}$ +2.0° (*c* 0.002, $CHCl_3$), possessed the molecular formula $C_{26}H_{42}O_3$ on the basis of HRFABMS [obsd *m/z* 401.3048 $[M+Na]^+$]. The spectral data for this compound were very similar to those of **2**, with an additional methoxy peak and the loss of signals for downfield methylene protons in ^1H and ^{13}C NMR data (Table 2). Analysis of 2D NMR also confirmed that compound **3** possesses the same carbon skeleton as that of **2**. The structural difference is that the hydroxyl group at C-24 is replaced by a proton, as evidenced by the appearance of a singlet methyl signal (δ 0.85) as well as the loss of two downfield proton

Table 1. NMR data of compound **1** ($CDCl_3$)[a]

Compound	δ_C, m[b]	δ_H, m, *J* (Hz)	COSY	HMBC
1	40.3 t	0.85		C-2, 5, 9, 10
		1.66 dt 12.5 1.5		
2	18.4 t	1.38 m, 1.57 m	3	
3	41.8 t	1.15 m		C-21, 22
		1.33 m	2	
4	33.1 s			
5	56.5 d	0.85 s		
6	18.6 t	1.57 m		
		2.14 dt 13.2 1.5		
7	36.6 t	0.89 m		
		2.14 m		
8	39.8 s			
9	58.1 d	1.12 br d 12.1	11	C-5, 7, 8, 11, 12, 14, 23, 24
10	37.2 s			
11	23.2 t	1.43 m	9, 12	C-9, 12, 13
		1.88 dd 12.7 3.4	12	C-9, 12, 10, 13
12	82.3 d	4.69 dd 11.2 4.2	11	C-11, 18, 25
13	38.0 s			
14	54.7 d	1.39	15	C-7, 9, 12, 15, 16, 18, 24, 25
15	23.3 t	2.20 m	14, 16	
		2.40 m	14	
16	116.6 d	5.47 br s	15	C-20
17	135.9 s			
18	61.5 d	2.28 br s	19	
19	99.1 d	5.39 t 2.4	18	C-18
20	68.2 t	4.13 d 11.3		C-16, 17, 18
		4.41 d 11.3		
21	33.2 q	0.85 s		C-3, 4, 5, 22
22	21.8 q	0.79 s		C-3, 4, 5, 21
23	16.3 q	0.88 s		
24	64.9 t	4.31 d 12.5		C-7, 14, Carbonyl (24)
		4.51 d 12.5		
25	9.3 q	0.95 s		
12-OAc	170.8 s			
	21.4 q	2.05 s		
24-OAc	171.1 s			
	21.1 q	2.08 s		
OH		3.61 br s		

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

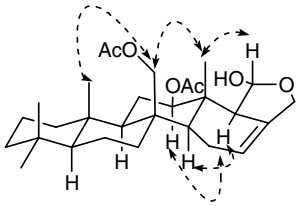

Figure 2. Key NOESY correlations of compound **1**.

signals in ^1H NMR spectrum. An additional methoxy group (δ 3.50) also shows a strong long range correlation with C-19 (δ 106.1) in HMBC spectra. Therefore, the structure of **3** was assigned as 12-*O*-deacetoxyl-19-*O*-methydeoxoscalarin.[15]

Together with **1**–**3**, the two previously reported scalarane-based sesterterpenes (**4**–**5**) were also isolated.[16,17]

To investigate the FXR antagonistic effect of compounds **1**–**5**, a co-transfection assay was performed using a full length FXR receptor and a reporter.[18] The plasmids containing human FXR and luciferase reporter were transfected into CV-1 cells, monkey kidney cells. The transfected cells were treated with or without the tested compounds **1**–**5** in the presence of 50 μM chenodeoxycholic acid (CDCA, a natural ligand for FXR). As a result, the compounds **1**–**5** showed inhibitory activities against FXR transactivation. In particular, 12,24-diacetoxy-deoxoscalarin **1** showed the most potent inhibitory activity against FXR transactivation with an IC_{50} value of 8.1 μM without any significant cytotoxicity. Interestingly, 12,24-diacetoxy-deoxoscalarin **1** with two acetyl groups at C-12 and C-24 showed a marked inhibitory transactivation against FXR compared with scalarane sesterterpenes **2**–**4**, as shown in Table 3. These results suggest that the acetyl groups at C-12 and C-24 are critical for the FXR antagonistic activity. Further study into the structure and activity relationship based on a molecular modeling experiment is in progress in our laboratory.

Table 2. ^{1}H, ^{13}C data of **2** (methanol-d_4) and **3** ($CDCl_3$)

Compound	**2**[a]		**3**[a]	
	δ_C, m[b]	δ_H, m, *J* (Hz)	δ_C, m[b]	δ_H, m, *J* (Hz)
1	41.6 t	1.18	42.0 t	1.13
		1.73		1.36
2	20.1 t	1.43 m, 1.54 m	18.5 t	1.40 m, 1.61 m
3	41.9 t	1.12 m	41.6 t	0.92 m
		1.36 m		1.72 m
4	34.3 s		33.2 s	
5	58.2 d	0.89	56.4 d	0.78 s
6	19.8 t	1.45 m, 1.54 m	18.1 t	1.36 m, 1.51 m
7	37.7 t	0.75 m	39.8 t	0.87 m
		2.35 dd 12.0 2.9		1.74 m
8	43.2 s		37.4 s	
9	60.2 d	1.01 d 11.5	58.8 d	0.91
10	38.6 s		37.3 s	
11	26.8 t	1.44 m	25.5 t	1.41 m
		1.64 m		1.70 m
12	82.7 d	3.53 dd 11.4 4.5	81.1 d	3.47 dd 11.2 3.6
13	44.0 s		39.8 s	
14	56.3 d	1.25 d 12.1	53.2 d	1.11 d 12.1
15	25.1 t	2.18 m	21.9 t	1.77 m
		2.65 m		2.04 dd 12.1 1.1
16	118.9 d	5.48 s	117.1 d	5.51 t
17	136.3 s		134.9 s	
18	62.6 d	2.20 br s	59.0 d	2.29 br s
19	100.8 d	5.28 d 6.0	106.1 d	4.91 dt 3.2 1.4
20	69.1 t	4.41 dd 11.7 1.7	68.6 t	4.45 dd 11.8 1.4
		4.13 dd 11.7 1.7		4.20 dd 11.8 1.4
21	33.9 q	0.86 s	33.2 q	0.83 s
22	21.9 q	0.83 s	21.2 q	0.82 s
23	17.2 q	0.92 s	16.5 q	0.92 s
24	62.4 t	3.89 d 12.1	17.0 q	0.85 s
		3.97 d 12.1		
25	9.7 q	0.91 s	8.7 q	0.80 s
19-OMe			56.2 q	3.50 s
OH				3.27 br s

[a] Assignments were aided by a combination of COSY, HSQC, and HMBC experiments.
[b] Multiplicity was determined by analysis of DEPT spectra.

Table 3. Inhibition of FXR transactivation and cytotoxicity for compounds **1**–**5**

Compound	Inhibition of FXR transactivation IC_{50}[a] (μM)	Cytotoxicity IC_{50}[a,b] (μM) (CV-1 cell)
1	8.1	32.7
2	64.5	>100
3	24.8	86.9
4	25.3	29.2
5	81.1	98.5
Z-Guggulsterone	10.0	Not determined

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

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13. Triacetate of compound **1** to a solution of compound **1** (0.7 mg) in pyridine (300 μL) were added acetic anhydride (200 μL) and dimethylaminopyridine. 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 thicker layer chromatography [TLC development with hexane/ethyl acetate (65:35)] to give triacetate as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.30 (d, 1H, J = 3.0 Hz), 5.56 (br s, 1H), 4.68 (dd, 1H, J = 11.1, 4.5 Hz), 4.52 (d, 1H, J = 12.5 Hz), 4.33 (d, 1H, J = 11.0 Hz), 4.29 (d, 1H, J = 12.5 Hz), 4.22 (d, 1H, J = 11.0 Hz), 2.60 (br s, 1H), 2.43 (m, 1H), 2.24 (m, 1H), 2.16 (dt, 1H, J = 13.2, 1.5 Hz), 2.08 (s, 3H), 2.05 (s, 3H), 1.98 (s, 3H), 1.85 (dd, 1H, J = 12.0, 3.5 Hz), 1.66 (dt, 1H, J = 12.5, 1.5 Hz), 1.57 (m, 2H), 1.56 (m, 1H), 1.39 (m, 2H), 1.38 (m, 2H), 1.33 (m, 1H), 1.15 (m, 1H), 1.12 (br d, 1H, J = 12.1 Hz), 0.96 (s, 3H), 0.89 (m, 1H), 0.88 (s, 3H), 0.85 (s, 1H), 0.84 (s, 3H), 0.79 (s, 3H). FABMS *m/z* 553 $[M+Na]^+$; HRFABMS *m/z* 553.3140 (calcd for $C_{31}H_{46}O_7$ Na, 553.3141).
14. Triacetate of compound **2** 12-*O*-deacetoxyl-24-hydroxyl-deoxoscalarin (**2**) was prepared following the same procedure for triacetate of compound **1**. ^{1}H NMR data of triacetate of compound **2** were identical with those of triacetate of compound **1**. FABMS *m/z* 553 $[M+Na]^+$; HRFABMS *m/z* 553.3139 (calcd for $C_{31}H_{46}O_7$ Na, 553.3140).
15. Monoacetate of compound **3** 12-*O*-deacetoxyl-19-*O*-methydeoxoscalarin (**3**) was also prepared following the same procedure for triacetate of compound **1**. ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.51 (br s, 1H), 5.01 (d, 1H, J = 3.2 Hz), 4.65 (dd, 1H, J = 11.4, 4.3 Hz), 4.22

(d, 1H, J = 12.1 Hz), 4.12 (d, 1H, J = 12.1 Hz), 3.31 (s, 3H), 2.61 (s, 1H), 2.35 (m, 1H), 2.15 (m, 1H), 2.04 (s, 3H), 1.84 (dd, 1H, J = 10.9, 2.9 Hz), 1.68 (dt, 1H, J = 12.6, 1.2 Hz), 1.62 (dt, 1H, J = 12.6, 1.2 Hz), 1.58 (m, 1H), 1.42–1.38 (m, 3H), 1.37–1.30 (m, 4H), 1.13 (dd, 1H, J = 5.7, 0.4 Hz), 0.98 (d, 1H, J = 11.9 Hz), 0.92 (s, 3H), 0.86 (m, 2H), 0.87 (s, 3H), 0.83 (s, 3H), 0.82 (s, 3H), 0.79 (s, 3H). FABMS m/z 467 $[M+Na]^+$; HRFABMS m/z 467.3144 (calcd for $C_{28}H_{44}O_4Na$, 467.3137).

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