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Development of novel lithocholic acid derivatives as vitamin D receptor agonists

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ABSTRACT

Lithocholic acid (**2**) was identified as the second endogenous ligand of vitamin D receptor (VDR), though its binding affinity to VDR and its vitamin D activity are very weak compared to those of the active metabolite of vitamin D_3, 1α,25-dihydroxyvitamin D_3 (**1**). 3-Acylated lithocholic acids were reported to be slightly more potent than lithocholic acid (**2**) as VDR agonists. Here, aiming to develop more potent lithocholic acid derivatives, we synthesized several derivatives bearing a 3-sulfonate/carbonate or 3-amino/amide substituent, and examined their differentiation-inducing activity toward human promyelocytic leukemia HL-60 cells. Introduction of a nitrogen atom at the 3-position of lithocholic acid (**2**) decreased the activity, but compound **6** bearing a 3-methylsulfonate group showed more potent activity than lithocholic acid (**2**) or its acylated derivatives. The binding of **6** to VDR was confirmed by competitive binding assay and X-ray crystallographic analysis of the complex of VDR ligand-binding domain (LBD) with **6**.

1. Introduction

Vitamin D_3 plays important roles in many physiological processes, including calcium and phosphate homeostasis, bone metabolism, and immune regulation.[1] Its active metabolite, 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$, **1**] (Fig. 1), is an endogenous agonist of vitamin D nuclear receptor (VDR),[1] and induces increased expression of target genes.

In 2001, Makishima et al. identified lithocholic acid (**2**), a bile acid formed from chenodeoxycholate, as the second endogenous agonist of VDR.[2] Although the role of lithocholic acid in VDR functions, as well as potential clinical application, has been investigated,[3] its potency as a VDR ligand is very low compared to that of 1α,25$(OH)_2D_3$ (**1**). Some 3-acylated lithocholic acid derivatives, such as 3-acetate (**3a**) and 3-propionate (**3b**), were more potent than lithocholic acid (**2**) itself, but their vitamin D activities were still low.[4,5]

We previously studied the binding of lithocholic acid (**2**) and lithocholic acid 3-acetate (**3a**) by means of docking study[4] and X-ray crystallographic analysis[6] of the complex of VDR ligand binding domain (LBD) with these ligands. The crystal structures indicated that the carboxy group of **2** and **3a** forms hydrogen bonds directly with Tyr143 and Ser274, and indirectly with Ser233 and Arg270 via a water molecule. These four amino acid residues interact with the two hydroxyl groups of 1α,25$(OH)_2D_3$ (**1**) in the complex with 1α,25$(OH)_2D_3$ (**1**). The 25-hydroxyl group of 1α,25$(OH)_2D_3$ (**1**) forms hydrogen bonds with His301 and His393, which are involved in hydrogen bonding directly with the acetyl group of lithocholic acid 3-acetate (**3a**), and indirectly with the 3-hydroxyl group of lithocholic acid (**2**) via a water molecule. Thus, introduction of an acetyl group at the 3-position of lithocholic acid (**2**) alters the interactions with the amino acid residues of VDR LBD, which presumably causes the increase of the vitamin D activity of lithocholic acid 3-acetate (**3a**). Therefore, in this study, we designed and synthesized several lithocholic acid derivatives with various polar substituents at the 3-position, and examined their vitamin D activities (Fig. 2).

2. Results and discussion

2.1. Synthesis

Various polar groups were introduced at the 3-hydroxyl group of lithocholic acid (**2**), as shown in Scheme 1. Lithocholic acid (**2**) was

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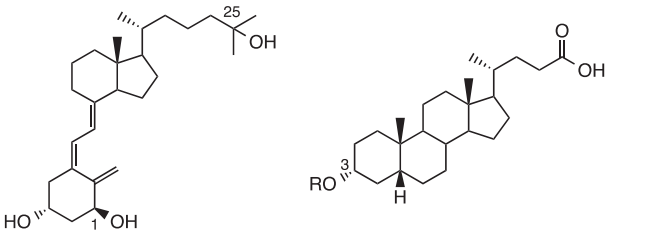
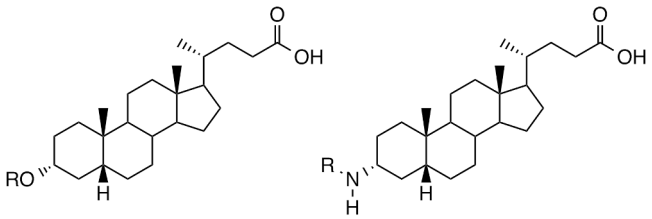


1α,25(OH)$_2$D$_3$ (**1**)

Lithocholic acid (**2**) R = H
Lithocholic acid 3-acetate (**3a**) R = CH_3CO
Lithocholic acid 3-propionate (**3b**) R = C_2H_5CO

Fig. 1. Structures of activated vitamin D_3 (1α,25(OH)$_2$D$_3$, **1**), lithocholic acid (**2**), and lithocholic acid 3-acylates (**3**).



4a R = CF_3CO-
4b R = C_6H_5CO-
5 R = CH_3OCO-
6 R = CH_3SO_2-

7 R = H
8 R = CH_3CO-
9 R = CH_3SO_2-

Fig. 2. Structures of lithocholic acid derivatives.

converted to benzyl ester **10**, which was reacted with acyl chloride, methyl chlorocarbonate, or methanesulfonyl chloride, followed by removal of the benzyl group to afford compounds **4–6**, respectively.

Compounds **7–9** bearing a 3-nitrogen functionality were synthesized as shown in Scheme 2. Mitsunobu reaction of methyl lithocholate (**14**) with diethyl azodicarboxylate (DEAD), triphenylphosphine, and formic acid afforded 3β-formate **15**. Treatment of **15** with sodium methoxide in methanol gave methyl 3-*epi*-lithocholate (**16**). Further Mitsunobu reaction of **16** with phthalimide as a nucleophile, followed by treatment with hydrazine, afforded 3α-amino derivative **18** as the pure isomer, deduced by ^{1}H NMR. Acylation or sulfonylation of **18** gave amide **19** and sulfonamide **20**, respectively. Finally, compounds **7–9** were obtained by basic hydrolysis of **18**, **19**, and **20**, respectively.

2.2. Biological evaluation

The vitamin D activity of the synthesized lithocholic acid derivatives was evaluated in terms of cell differentiation-inducing activity toward human acute promyelocytic leukemia cell line HL-60.[7] HL-60 cell differentiation was determined by measuring the ratio of nitroblue tetrazolium (NBT)-positive cells in the concentration range of 10^{-7} M–10^{-5} M test compound. Under these assay conditions, lithocholic acid (**2**) did not induce differentiation of HL-60 cells at concentrations below 10^{-5} M (data not shown), while the acetate **3a** showed differentiation-inducing activity only at 10^{-5} M (Fig. 3). Acylates **4a/b** and carbonate **5** were inactive, but the methanesulfonate **6** was more active than **3a**, showing an IC_{50} of 1.2×10^{-6} M. Among the compounds with a nitrogen-containing functionality, only compound **9** showed differentiation-inducing activity, but its potency was lower than that of the corresponding oxygen-containing derivatives (**3a** vs **8** or **6** vs **9**). Thus, 3-methanesulfonate **6** was the most active analog of lithocholic acid (**2**) among the synthesized compounds.

Next, the VDR transactivation ability of selected compounds was examined by means of luciferase reporter gene assay (Fig. 4). Compounds **3** were used as the control, since the VDR transactivation ability of compound **2** was very low.[5] Compound **4a** showed activity only at concentrations above 1 μM, while compound **6** showed moderate potency to activate VDR, and the potency of **6** was similar to that of the 3-acetate **3a**. Competitive binding experiments showed that compound **6** binds to VDR, and its binding affinity was higher than that of the control compound, 3-propionate **3b** that was most among three known compounds **2**, **3a** and **3b**[5] (Fig. 5). These results indicate that compound **6** is a VDR agonist, and induces differentiation of HL-60 cells.

2.3. X-ray crystallographic analysis

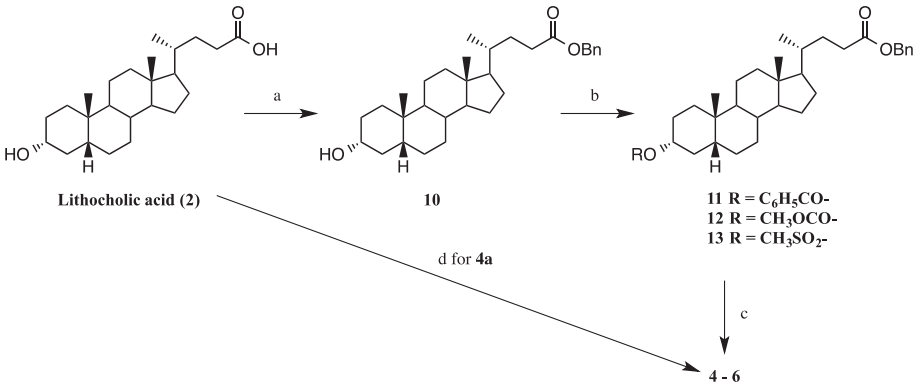
In order to clarify the VDR-binding mode of lithocholic acid derivatives, we carried out X-ray crystallographic analysis of the complex of rat VDR LBD (residues 116–423, Δ165–211) with compound **6** according to the method reported in our previous study. A synthetic peptide containing the target sequence of the coactivator MED1 (mediator of RNA polymerase II transcription subunit 1, also known as ARC205 or DRIP205) was included in the crystallization solution of VDR LBD and compound **6**.

The overall structure of VDR LBD in the ternary complex, determined at 1.8 Å resolution (Table 1), was nearly identical to that in the complex with lithocholic acid (**2**) or 3-acetate (**3a**) (Fig. 6a). Compound **6** is located in the ligand-binding pocket, which is common to proteins in the nuclear receptor superfamily, in a similar manner to 1α,25(OH)$_2$D$_3$ (**1**) and lithocholic acid (**2**) (Fig. 6b). The carboxy group of compound **6** formed hydrogen bonds with Tyr143 in helix 1 and Ser274 in helices 4/5, both of which form hydrogen bonds with the 3-hydroxyl group on the cyclohexane ring of 1α,25(OH)$_2$D$_3$ (**1**) in the crystal. The carboxy group of **6** also formed hydrogen bonds via a water molecule with Arg270 in helices 4/5 and Ser233 in helix 3. In the case of the complex of VDR LBD with 1α,25(OH)$_2$D$_3$ (**1**), these amino acid residues interacted directly with the 1-hydroxyl group of **1**. The interactions of the carboxyl group of compound **6** with VDR amino acid residues are similar to those of lithocholic acid (**2**), **3a** and **3b**. In contrast to the direct interaction of two hydroxyl groups on the cyclohexane ring of 1α,25(OH)$_2$D$_3$ (**1**) with four amino acid residues, two of them formed hydrogen bonds with the lithocholic acid derivatives via a water molecule, which may be one of the reason for the weaker vitamin D activity of the derivatives, compared to **1**.

The 3-methanesulfonyl group of **6** also interacted with amino acid residues of VDR. Thus, one of the oxygen atoms of the 3-methanesulfonyl group formed hydrogen bonds with His301 in helix 6 and His393 in helix 11, though there was no significant interaction of the other oxygen atom with VDR. These histidine residues form hydrogen bonds with the 25-hydroxyl group of 1α,25(OH)$_2$D$_3$ (**1**) or the carbonyl group of the 3-substituent of **3** in the crystal structures. In the complex of VDR with 1α,25(OH)$_2$D$_3$ (**1**), the two histidines are located at similar distances from the 25-hydroxyl group (less than 3.0 Å between the ε-nitrogen atom of histidine and the oxygen atom of the hydroxyl group). In the case of 3-propionate **3b**, the distances from the carbonyl oxygen atom are 3.80 Å and 3.31 Å for His301 and His393, respectively, which are similar to those (3.99 Å for His301 and 3.30 Å and His393) in the complex of VDR with **6**.

3. Conclusion

We designed and synthesized lithocholic acid derivatives bearing various 3-substituents as candidate VDR agonists. Compounds bearing nitrogen at the 3-position exhibited weaker activity than the corresponding oxygen analogs. Among the synthesized compounds, compound **6** bearing 3-methanesulfonate showed the most potent activity in HL-60 cell differentiation assay. Compound **6** showed VDR transactivation and binding ability. Further, we determined the crystal structure of the complex of rVDR LBD with **6**, which indicated that direct hydrogen bond formation of the functional group at the 3-position of lithocholic acid derivatives contributes to the higher potency, compared with lithocholic acid (**2**). The compounds developed in this study and



Scheme 1. Synthesis of compounds **4–6**. Reagents and conditions: (a) benzyl bromide, K_2CO_3, DMF; (b) R-Cl, pyridine, 0 °C; (c) H_2, Pd-C, ethanol; (d) TFA.

the SAR information should be helpful in the development of new-generation nonsecosteroidal VDR ligands with high potency.

4. Experimental

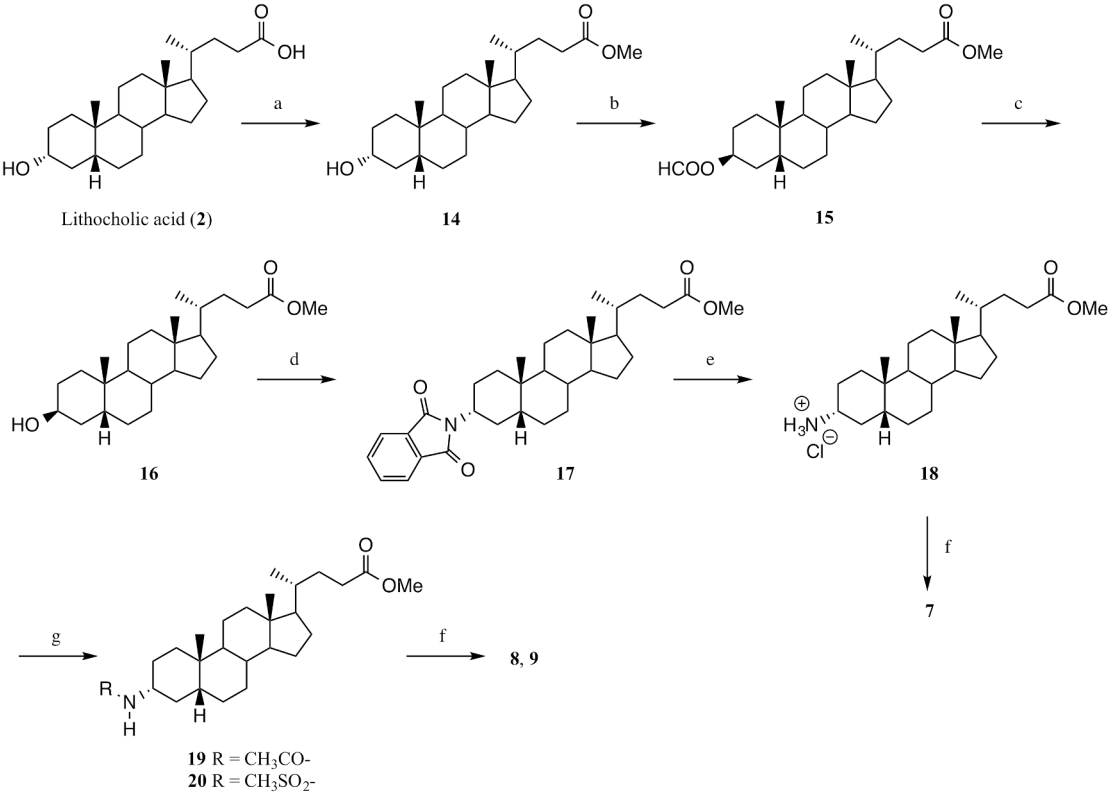
4.1. General

All reagents were purchased from Sigma-Aldrich Chemical Co., Tokyo Kasei Kogyo Co., Wako Pure Chemical Industries, and Kanto Chemical Co., Inc. Silica gel for column chromatography was purchased from Kanto Chemical Co., Inc. ^{1}H NMR spectra were recorded on at 600 MHz on a Bruker AVANCE 600 spectrometer or at 500 MHz on a Bruker AVANCE 500 spectrometer or at 400 MHz on a Bruker AVANCE 400 spectrometer. ^{13}C NMR spectra were recorded on at 125 MHz on a Bruker AVANCE 500 spectrometer. Chemical shifts are reported in ppm as δ values from tetramethylsilane. ^{19}F NMR spectra were recorded on at 376.5 MHz on a Bruker AVANCE 400 spectrometer. Trifluorotoluene (δ-63 ppm) was used as external standard in ^{19}F NMR spectra. Data are reported as follows: chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q quartet; br, broad; and m, multiplet), coupling constants (Hz), and integration. Mass spectra were collected on a Bruker Daltonics micrO TOF focus-II in the positive ion mode.

4.2. Synthesis

4.2.1. Compound *4a*

A solution of lithocholic acid (**2**, 110 mg, 0.30 mmol) in trifluoroacetic acid (2 ml) was stirred at room temperature for 4 h, then aqueous sodium bicarbonate was added, and the reaction mixture was extracted with ethyl acetate. The organic layer was washed with aqueous sodium bicarbonate, and water, dried over magnesium sulfate, and evaporated. The residue was chromatographed on silica gel (ethyl acetate/hexane 1:9) to give **4a** (110 mg, 80%). Colorless Powder (ethanol/H_2O); mp 177–178 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.93 (m,



Scheme 2. Synthesis of compounds **7–9**. Reagents and conditions: (a) AcCl, MeOH; (b) DEAD, PPh_3, HCOOH, THF; (c) NaOMe, MeOH; (d) DEAD, PPh_3, phthalimide, toluene; (e) 1) hydrazine monohydrate, MeOH, 2) HCl aq; (f) NaOH aq, MeOH; (g) R-Cl, Et_3N, CH_2Cl_2.

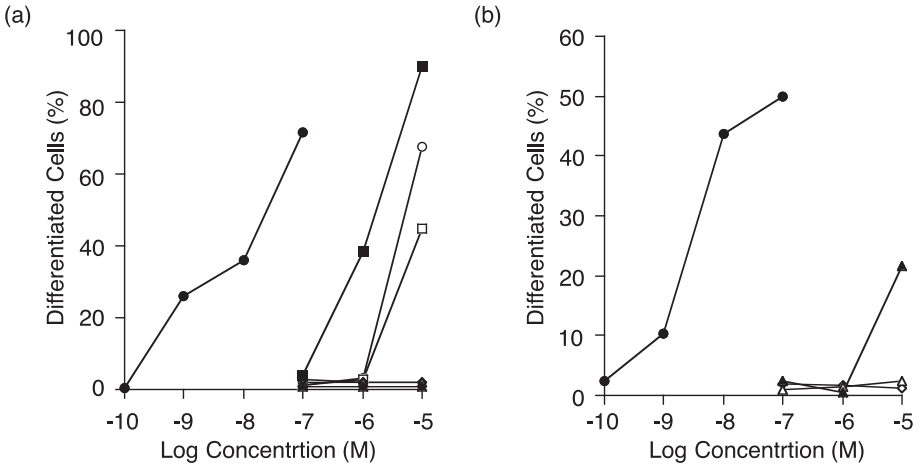


Fig. 3. HL-60 cell differentiation-inducing activity of lithocholic acid derivatives. (a) ●: 1α,25(OH)$_2$D$_3$ (**1**), ○: **3a**, □: **3b**, △: **4a**, ▲: **4b**, ◊: **5**, ■: **6**; (b) ●: 1α,25(OH)$_2$D$_3$ (**1**), ◊: **7**, △: **8**, ▲: **9**.

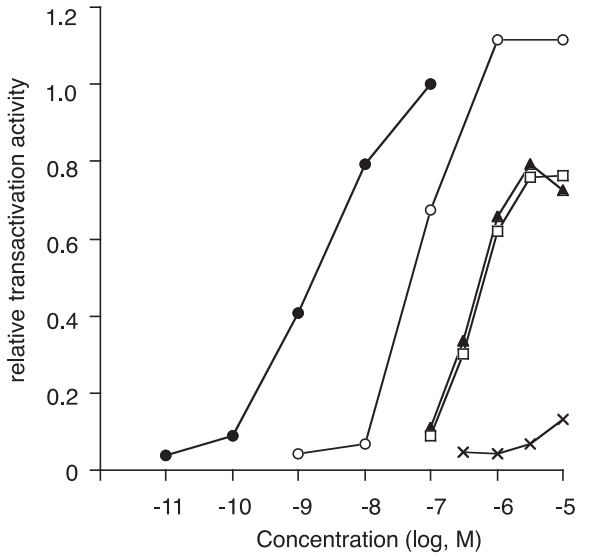


Fig. 4. VDR transactivation ability of lithocholic acid derivatives. ●: 1α,25(OH)$_2$D$_3$ (**1**), □: **3a**, ▲: **3b**, ×: **4a**, ○: **6**.

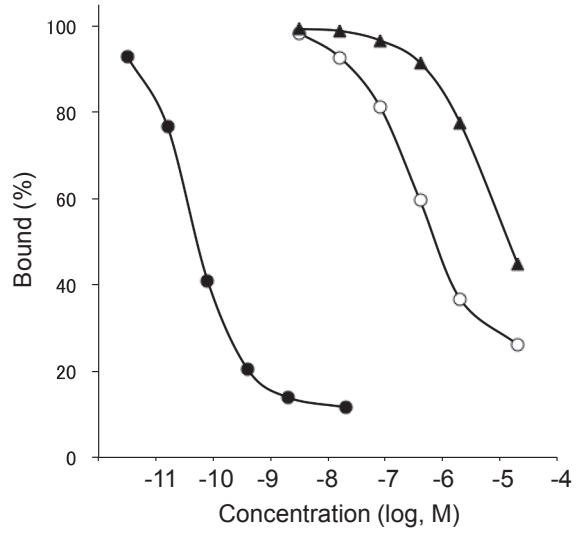


Fig. 5. VDR-binding ability of lithocholic acid derivatives. ●: 1α,25(OH)$_2$D$_3$ (**1**), ▲: **3b**, ○: **6**.

Table 1
Data collection and refinement statistics for crystal structure of VDR LBD/coactivator peptide/compound **6** complex. Values in parentheses are for the highest-resolution shell.

Data Collection		
Unit cell dimensions		
a (Å)	153.9	
b (Å)	43.7	
c (Å)	41.8	
β (deg)	96.1	
Space group	*C*2	
Resolution (Å)	50–1.8	(1.83–1.80)
Completeness (%)	98.6	(91.9)
Redundancy	3.5	(2.9)
No of unique reflections	25,497	(1198)
Average I/σ(I)	16.3	(1.3)
$CC_{1/2}$		(0.760)
Refinement		
R-cryst (%)	21.3	
R-free (%)	25.7	
RMS bond length (Å)	0.010	
RMS bond angles (deg)	1.41	
Atoms		
Protein	1984	
Ligand and water	57	

1H), 2.40 (ddd, 1H, J = 5.2, 10.2, 15.6 Hz), 2.26 (ddd, 1H, J = 6.4, 9.4, 15.8 Hz), 0.95 (s, 3H), 0.92 (d, 3H, J = 6.4 Hz), 0.65 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.9, 156.99 (q, J_{CF} = 41.7 Hz), 114.6 (q, J_{CF} = 286.2 Hz), 79.4, 56.4, 55.9, 42.7, 41.9, 40.4, 40.0, 35.7, 35.3, 34.7, 34.5, 31.6, 31.1, 30.7, 28.1, 26.9, 26.2, 26.1, 24.1, 23.2, 20.8, 18.2, 12.0. ^{19}F NMR (376.5 MHz, $CDCl_3$) δ-75.6. HRMS-ESI (m/z): $[M-H]^-$ calcd for $C_{26}H_{38}F_3O_4$ 471.2728; found, 471.2726.

*4.2.2. Compound **10***

Potassium carbonate (810 mg, 5.86 mmol) was added to a solution of lithocholic acid (2.00 g, 5.31 mmol) in DMF (14 ml). After 30 min, benzyl bromide (950 µl, 7.99 mmol) was added, and resulting solution was stirred at room temperature for 3 h. Ethyl acetate and water were added to the reaction mixture. The organic layer was washed with water, dried over magnesium sulfate, and evaporated. The residue was chromatographed on silica gel (ethyl acetate/hexane 1:9 to 1:1) to give compound **10** (2.22 g, 89%). Colorless powder (ethyl acetate/hexane; mp 123–124 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (m, 5H), 5.11 (ABq. 2H, J = 12.3 Hz), 3.62 (m, 1H), 2.40 (ddd, J = 15.4, 9.9, 5.1 Hz), 2.27 (ddd 1H, J = 15.4, 8.9, 6.5 Hz), 0.91 (s, 3H), 0.49 (d, 3H, J = 6.5 Hz), 0.62 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.1, 136.1, 128.5, 128.2, 128.1, 71.8, 66.1, 56.5, 55.9, 42.7, 42.1, 40.4, 40.1, 36.4, 35.8, 35.3,

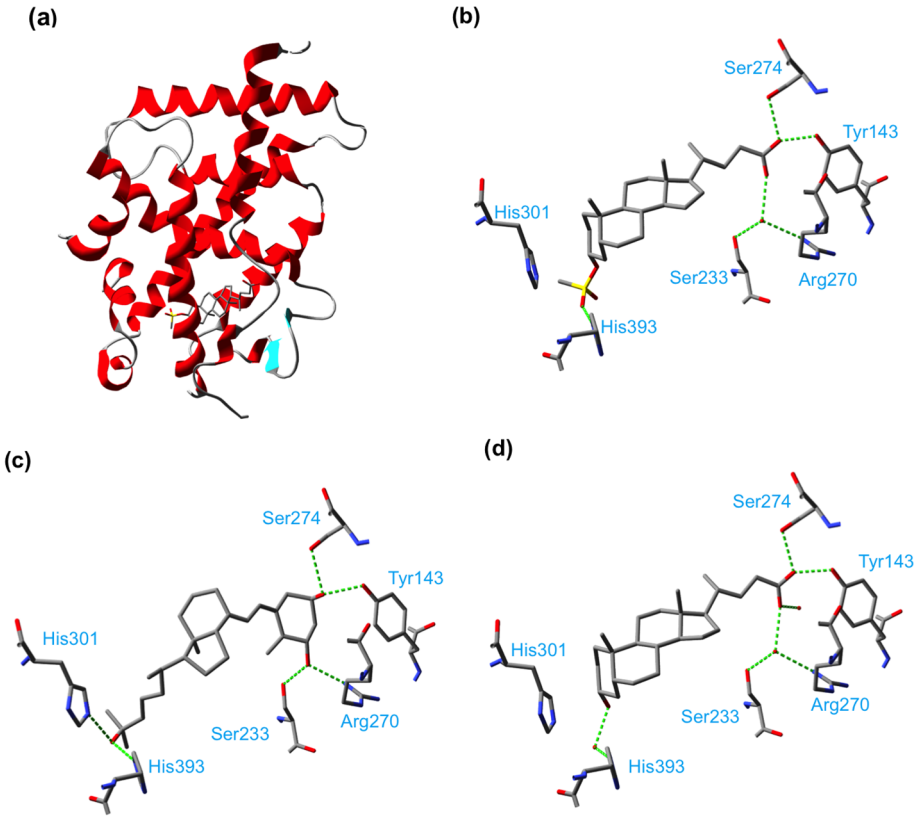


Fig. 6. Crystal structure of the complex of VDR with **6**. (a) Overall structure, and (b) VDR binding feature of **6**, compared with those of (c) 1α,25(OH)$_2$D$_3$ (**1**) (PDB: 1RK3)[9] and (d) lithocholic acid (**2**) (PDB: 3W5P)[6].

34.5, 31.3, 31.0, 30.5, 28.2, 27.2, 26.4, 24.2, 23.4, 20.8, 18.2, 12.0. HRMS-ESI (*m/z*): [M + Na]$^+$ calcd for $C_{31}H_{46}NaO_3$ 489.3339; found, 489.3338.

4.2.3. Compound *11*

4-Dimethylaminopyridine (13 mg, 0.11 mmol) and benzoyl chloride (20 µl, 0.22 mmol) were added to a solution of **10** (50 mg, 0.11 mmol) in pyridine (0.5 ml) at 0 °C. The mixture was stirred at room temperature for 24 h, then aqueous hydrochloric acid was added, and the whole was extracted with ethyl acetate. The organic layer was washed with aqueous sodium bicarbonate, and water, dried over magnesium sulfate, and evaporated. The residue was chromatographed on silica gel (ethyl acetate/hexane 1:19 to 1:10) to give **11** (36 mg, 64%), together with unreacted **10** (18 mg, 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (m, 2H), 7.54 (tt, J = 1.4, 7.4 Hz, 1H), 7.43 (t, J = 7.6 Hz, 2H), 7.35 (m, 6H), 5.13 (d, J = 12.4 Hz, 2H), 5.09 (d, 2H, J = 12.3 Hz), 4.97 (m, 1H), 2.40 (ddd, 1H, J = 5.1, 10.0, 15.3 Hz), 2.27 (ddd, 1H, J = 6.6, 9.1, 15.6 Hz), 0.96 (s, 3H), 0.91 (d, 3H, J = 6.1 Hz), 0.63 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.1, 166.1, 136.1, 132.6, 130.9, 129.5, 128.5, 128.2, 128.1, 75.0, 66.1, 56.5, 56.0, 42.7, 41.9, 40.5, 40.1, 35.8, 35.3, 35.1, 34.6, 32.4, 31.3, 31.0, 28.2, 27.0, 26.7, 26.3, 24.2, 23.4, 20.9, 18.3, 12.0. HRMS-ESI (*m/z*): [M + Na]$^+$ calcd for $C_{38}H_{50}NaO_4$ 593.3601; found, 593.3599.

4.2.4. Compound *12*

Methyl chloroformate (17 µl, 0.22 mmol) was added to a solution of **10** (51 mg, 0.11 mmol) in pyridine (0.5 ml) at 0 °C, and the mixture was stirred at room temperature for 7 h. Then, methyl chloroformate (240 µl, 3.1 mmol) was added, and the mixture was stirred overnight. After addition of methanol, the solvent was removed in vacuo. The residue was chromatographed on silica gel (ethyl acetate/hexane 1:99 to 1:19) to give **12** (39 mg, 68%), together with unreacted **10** (4 mg, 7%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (m, 5H), 5.18 (ABq, 2H, J = 12.4 Hz), 4.58 (m, 1H), 3.76 (s, 3H), 2.40 (ddd, 1H, J = 5.0, 9.9, 15.0 Hz), 2.27 (ddd, 1H, J = 6.8, 9.2, 15.6 Hz), 0.93 (s, 3H), 0.90 (d, 1H, J = 6.3 Hz), 0.61 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.0, 155.2, 136.1, 128.5, 128.2, 128.1, 78.3, 66.0, 56.4, 55.9, 54.4, 42.7, 41.8, 40.3, 40.1, 35.7, 35.3, 34.9, 34.5, 32.1, 31.2, 30.9, 28.1, 27.0, 26.5, 26.2, 24.1, 23.2, 20.8, 18.2, 12.0. HRMS-ESI (*m/z*): [M + Na]$^+$ calcd for $C_{33}H_{48}NaO_5$ 547.3394; found, 547.3390.

4.2.5. Compound *13*

Methanesulfonyl chloride (35 µl, 0.46 mmol) was added to a solution of **10** (43 mg, 0.091 mmol) in pyridine (0.5 ml) at 0 °C. After 2 h, the mixture was poured into water, and extracted with ethyl acetate. The organic layer was washed with water, dried over magnesium sulfate, and evaporated. The residue was chromatographed on silica gel (ethyl acetate/hexane 1:9) to give **13** (50 mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (m, 5H), 5.11 (ABq, 2H, J = 12.3 Hz), 4.65 (m, 1H), 3.00 (s, 3H), 2.40 (ddd, 1H, J = 5.0, 9.9, 15.0 Hz), 2.27 (ddd, 1H, J = 6.8, 9.2, 15.6 Hz), 0.93 (s, 3H), 0.90 (d, 1H, J = 6.3 Hz), 0.62 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.0, 136.1, 128.5, 128.2, 128.1, 82.9, 66.1, 56.4, 55.9, 42.7, 42.1, 40.4, 40.0, 38.8, 35.7, 35.3, 35.0, 34.4, 33.3, 31.2, 30.9, 28.1, 27.8, 26.8, 26.2, 24.1, 23.1, 20.8, 18.2, 12.0. HRMS-ESI (*m/z*): [M + Na]$^+$ calcd for $C_{32}H_{48}NaO_5S$ 567.3115; found, 567.3109.

4.2.6. Compound *4b*

A suspension of **11** (85 mg, 0.15 mmol) and 10% palladium on carbon (16 mg) in methanol (1 ml) and formic acid (0.02 ml) was stirred in a hydrogen atmosphere for 15 h. The reaction mixture was filtered on Celite, and the filtrate was evaporated. The residue was chromatographed on silica gel (ethyl acetate/hexane 1:4) to give **4b** (63 mg, 88%). Colorless Powder (methanol); mp 100–102 °C; ^{1}H NMR

(400 MHz, $CDCl_3$) δ 8.05 (m, 2H), 7.54 (t, J = 7.6 Hz, 1H), 7.43 (t, J = 7.6 Hz, 2H), 4.98 (m, 1H), 2.40 (ddd, 1H, J = 5.2, 10.3, 15.6 Hz), 2.26 (ddd, 1H, J = 6.3, 9.6, 15.8 Hz), 0.96 (s, 3H), 0.93 (d, J = 6.4 Hz, 3H), 0.66 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.2, 166.1, 132.7, 130.9, 129.5, 128.2, 77.3, 77.0, 76.7, 75.0, 56.5, 56.0, 42.7, 41.9, 40.5, 40.1, 35.8, 35.3, 35.1, 34.6, 32.3, 30.9, 30.7, 28.2, 27.0, 26.7, 26.3, 24.2, 23.3, 20.9, 18.2, 12.0. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{31}H_{44}NaO_4$ 503.3132; found, 503.3133.

4.2.7. Compound **5**

A suspension of **12** (85 mg, 0.15 mmol) and 10% palladium on carbon (16 mg) in ethanol (2 ml) was stirred in a hydrogen atmosphere for 1 h. The reaction mixture was filtered on Celite, and the filtrate was evaporated. The residue was chromatographed on silica gel (ethyl acetate/hexane 1:9 to 1:4) to give **5** (63 mg, 88%). Colorless Powder (ethanol); mp 200–202 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.58 (m, 1H), 3.76 (s, 3H,), 2.26 (ddd, 1H, J = 6.5, 9.6, 15.8 Hz), 2.40 (ddd, 1H, J = 5.1, 10.1, 15.4 Hz), 0.93 (s, 3H), 0.92 (d, 1H, J = 7.1 Hz), 0.64 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.4, 167.7, 155.3, 132.4, 130.8, 128.8, 78.4, 77.3, 77.0, 76.7, 68.1, 56.4, 55.9, 54.4, 42.7, 41.8, 40.3, 40.1, 38.7, 35.7, 35.3, 34.9, 34.5, 32.1, 31.0, 30.7, 30.3, 28.9, 28.1, 27.0, 26.5, 26.2, 24.1, 23.7, 23.2, 22.9, 20.8, 18.2, 14.0, 12.0, 10.9. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{26}H_{42}NaO_5$ 457.2924; found, 457.2920.

4.2.8. Compound **6**

A suspension of **12** (19 mg, 0.035 mmol) and 10% palladium on carbon (17 mg) in ethanol (1 ml) was stirred in a hydrogen atmosphere for 1 h. The reaction mixture was filtered on Celite, and the filtrate was evaporated. The residue was chromatographed on silica gel (ethyl acetate/hexane 1:9 to 1:4) to give **6** (11 mg, 70%). Colorless Powder (ethanol); mp 156–157 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.65 (m, 1H), 3.00 (s, 3H), 2.40 (ddd, 1H, J = 5.0, 9.9, 15.0 Hz), 2.27 (ddd, 1H, J = 6.8, 9.2, 15.6 Hz), 0.93 (s, 3H), 0.92 (d, 1H, J = 6.1 Hz), 0.64 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.0, 83.0, 56.4, 55.9, 42.7, 42.1, 40.4, 40.0, 38.8, 35.7, 35.3, 35.0, 34.4, 33.3, 30.9, 30.7, 28.1, 27.8, 26.8, 26.2, 24.1, 23.1, 20.8, 18.2, 12.0. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{25}H_{42}NaO_5S$ 477.2645; found, 477.2651.

4.2.9. Compound **14**

Acetyl chloride (50 µl) was added to a solution of lithocholic acid (**2**, 497 mg, 1.32 mmol) in methanol (5 ml). The mixture was stirred for 4 h at room temperature, then poured into water. The resulting precipitate was taken up in ethanol. The solution was filtered, and the filtrate was evaporated. The residue was recrystallized from hexane to give **14** (438 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.66 (s, 3H), 3.61 (m, 1H), 2.35 (ddd, 1H, J = 15.4, 10.4, 5.0 Hz), 2.21 (ddd, 1H, J = 15.4, 9.9, 6.6 Hz), 0.91 (s, 3H), 0.90 (d, 3H, J = 6.5 Hz,), 0.64 (s, 3H ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.79, 71.86, 56.47, 55.92, 51.47, 47.71, 42.07, 40.41, 40.15, 36.44, 35.83, 35.36, 35.33, 34.56, 31.04, 30.98, 30.53, 28.18, 27.17, 26.40, 24.20, 23.36, 20.81, 18.25, 12.03. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{25}H_{42}NaO_3$ 413.3026; found 413.3026.

4.2.10. Compound **15**

Triphenylphosphine (79 mg, 0.30 mmol), formic acid (12 µl, 0.3 mmol), and DEAD (2.2 M solution in toluene, 140 µl, 0.30 mmol) were successively added to a solution of compound **14** (50 mg 0.13 mmol) in toluene (1 ml). The mixture was stirred at 0 °C for 1 h, and at room temperature for 17 h, then poured into aqueous sodium bicarbonate, and extracted with ethyl acetate. The organic layer was washed with water, dried over sodium sulfate, and evaporated. The residue was chromatographed on silica gel (ethyl acetate/hexane 1:9) to give **15** (37 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H), 5.22 (brs, 1H), 3.66 (s, 3H), 2.34 (ddd, 1H, J = 15.2, 10.0, 5.2 Hz), 2.21 (ddd, 1H, J = 16.8, 10.4, 6.8 Hz), 0.96 (s, 3H), 0.91 (d, 3H, J = 6.4 Hz), 0.65 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.0, 161.1, 71.2, 56.8, 56.2, 51.7, 43.0, 40.4, 40.1, 37.5, 35.8, 35.6, 35.1, 31.3, 31.2, 30.8, 30.8, 28.4, 26.6, 26.3, 25.2, 24.4, 24.0, 21.3, 18.5, 12.3. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{26}H_{42}NaO_4$ 411.2975; found 441.2964.

4.2.11. Compound **16**

Sodium methoxide (155 mg) was added to a solution of **15** (699 mg, 1.67 mmol) in methanol (150 ml). After 2 h, the reaction mixture was poured into 2 M hydrochloric acid. The resulting precipitate was washed with water, and dissolved in ethanol and ethyl acetate. The solution was filtered, and the filtrate was evaporated. The residue was chromatographed on silica gel (ethyl acetate/hexane 1:4 to 1:1) to afford **16** (606 mg, 93%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.10 (brs, 1H), 3.66 (s, 3H), 2.35 (ddd, 1H, J = 14.8, 9.9, 4.9 Hz), 2.21 (ddd, 1H, J = 15.9, 9.9, 6.6 Hz), 0.95 (s, 3H), 0.91 (d, 3H, J = 6.1 Hz), 0.64 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.0, 67.4, 56.8, 56.2, 51.7, 43.0, 40.4, 39.9, 36.8, 35.8, 35.6, 35.3, 33.7, 31.3, 31.2, 30.1, 28.4, 28.1, 26.9, 26.5, 24.4, 24.1, 21.3, 18.5. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{25}H_{42}NaO_3$ 413.3026; found 413.3026.

4.2.12. Compound **17**

Triphenylphosphine (379 mg, 1.4 mmol), phthalimide (231 mg, 1.6 mmol), and DEAD (2.2 M solution in toluene, 620 µl, 1.4 mmol) were added to a solution of **16** (281 mg, 0.72 mmol) in toluene (4 ml). After 2.5 h, the reaction mixture was poured into water, and extracted with ethyl acetate. The organic layer was washed with water, dried over sodium sulfate, and evaporated. The residue was chromatographed on silica gel (ethyl acetate/hexane 1:19 to 1:9) to afford **17** (218 mg, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (dd, J = 5.4, 2.9 Hz), 7.69 (dd, J = 5.6, 3.2 Hz), 4.18 (m, 1H), 3.67 (s, 3H), 2.73 (q, 1H, J = 12.7 Hz), 2.35 (m, 2H), 2.22 (ddd, 1H, J = 16.1,9.8,7.0 Hz), 0.97 (s, 3H), 0.92 (d, 3H, J = 6.3 Hz), 0.66 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.1, 168.8, 168.8, 134.0, 134.0, 132.3, 132.3, 123.2, 123.2, 56.6, 56.0, 51.7, 51.5, 43.3, 43.0, 40.7, 40.3, 36.8, 36.0, 35.6, 34.8, 31.2, 31.2, 30.0, 28.4, 27.3, 26.5, 24.5, 24.4, 23.7, 21.1, 18.5, 12.3. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{33}H_{45}NNaO_4$ 542.3241; found 542.3220.

4.2.13. Compound **18**

Hydrazine monohydrate (250 µl, 5.0 mmol) was added to a solution of **17** (520 mg 1.00 mmol) in methanol (25 ml). The mixture was heated at reflux for 5.5 h, poured into brine, and extracted with dichloromethane. The organic layer was washed with aqueous sodium hydrogen carbonate, dried over sodium sulfate, and evaporated. The residue was dissolved in diethyl ether, and 1 M HCl was added. The precipitate was collected to afford **18** (372 mg, 87%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.30 (s, 3H), 3.66 (s, 3H), 3.18 (brs, 1H), 2.36 (ddd, 1H, J = 15.1, 10.3, 4.8 Hz), 2.21 (ddd, 1H, J = 15.1, 10.3, 6.8 Hz), 0.91 (s, 3H), 0.90 (d, 3H, J = 6.2 Hz), 0.63 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.77, 55.07, 55.77, 51.95, 51.47, 42.66, 42.22, 40.30, 39.80, 35.80, 35.35, 35.10, 34.56, 31.63, 31.09, 30.97, 28.13, 26.79, 26.25, 24.20, 23.39, 20.85, 18.27, 12.01. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{25}H_{44}NO_2$ 390.3367; found 390.3356.

4.2.14. Compound **7**

2 M aqueous sodium hydroxide (1 ml) was added to a solution of **18** (40 mg, 0.093 mmol) in methanol (3 ml). The mixture was stirred at 0 °C for 1 h, and at room temperature for 8 h, then poured into 2 M hydrochloric acid. The resulting precipitate was dissolved in methanol. The solution was filtered, and the filtrate was evaporated to give **7** (27 mg, 77%). ^{1}H NMR (600 MHz, CD_3OD) δ 3.07 (m, 1H), 2.18 (ddd, 1H, J = 13.7, 10.3, 4.8 Hz), 2.02 (m, 2H), 0.97 (s, 3H), 0.92 (d, 3H,J = 6.2 Hz), 0.67 (s, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 183.59, 58.11, 57.77, 52.49, 44.03, 43.47, 41.96, 41.67, 37.35, 37.28, 36.25, 36.16, 35.74, 34.12, 32.76, 29.41, 28.11, 27.64, 27.03, 25.39, 23.88, 22.07, 19.11, 12.62. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{24}H_{42}NO_2$ 376.3210; found 376.3199.

4.2.15. Compound **19**

Acetic anhydride (200 μl) was added to a solution of **18** (68.5 mg, 0.16 mmol) in dichloromethane (1.5 ml) and pyridine (0.5 μl). The mixture was stirred at room temperature for 22.5 h, poured into water, and extracted with dichloromethane. The organic layer was washed with water, dried with sodium sulfate, and evaporated. The residue was recrystallized from ethyl acetate to afford **19** (36 mg, 53%). Colorless prisms (ethyl acetate); mp 208 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.29 (br d, J = 7.6 Hz, 1H), 3.66 (s, 3H), 3.78 (m, 1H), 2.35 (ddd, 1H, J = 15.8, 10.3, 5.5 Hz), 2.22 (ddd, 1H, J = 15.1, 9.6, 6.2 Hz), 1.95 (s, 1H), 0.93 (s, 3H), 0.91 (d, 3H, J = 6.2 Hz), 0.64 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.78, 169.12, 56.66, 56.06, 51.49, 49.44, 42.75, 42.30, 40.58, 40.22, 35.78, 35.38, 34.55, 33.70, 31.08, 31.01, 28.20, 27.98, 26.97, 26.41, 24.18, 23.64, 23.53, 20.81, 18.27, 12.04. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{27}H_{46}NO_3$ 432.3472; found 432.3461.

4.2.16. Compound **20**

Methanesulfonyl chloride (300 μl) was added to a solution of **18** (69 mg, 0.16 mmol) in dichloromethane (1.5 ml) and pyridine (0.5 μl). The mixture was stirred at room temperature for 17 h, poured into water, and extracted with dichloromethane. The organic layer was washed with water, dried over sodium sulfate, and evaporated. The residue was chromatographed on silica gel (ethyl acetate/hexane 1:4 to 2:3) to afford **20** (58 mg, 77%). Colorless prisms (ethyl acetate); mp 155 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.13 (m, 1H), 3.66 (s, 3H), 3.32 (m, 1H), 2.35 (ddd, 1H, J = 15.1, 9.6, 4.8 Hz), 2.22 (ddd,1H, J = 15.8, 9.6, 6.2 Hz), 1.96 (dt, 1H, J = 13.8, 2.7 Hz), 0.93 (s, 3H), 0.91 (d, 3H, J = 6.9 Hz), 0.64 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.76, 56.48, 55.96, 54.12, 51.49, 42.72, 42.57, 42.25, 40.51, 40.09, 35.86, 35.74, 35.35, 35.07, 34.40, 31.04, 30.98, 29.35, 28.16, 26.92, 26.31, 24.15, 23.45, 20.80, 18.26, 12.03. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{26}H_{45}NNaO_4S$ 490.2967; found 490.2951.

4.2.17. Compound **8**

2 M aqueous hydroxide (4 ml) was added to a solution of **19** (19 mg, 0.045 mmol) in methanol (12 ml). The mixture was stirred at room temperature for 3.5 h, then poured into 2 M hydrochloric acid, and extracted with dichloromethane. The organic layer was washed with water, dried over sodium sulfate, and evaporated. The residue was recrystallized from ethyl acetate to yield compound **8** (13 mg, 69%). Colorless prisms (ethyl acetate); mp 268 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 4.98 (br s, 1H), 3.70–3.63 (m, 1H), 3.32 (s, 3H), 2.33 (ddd, J = 15.2, 10, 5.2 Hz, 1H), 2.20 (ddd, J = 15.2, 8.4, 6.8 Hz, 1H), 2.04 (dt, J = 12, 8.4 Hz, 1H), 1.99–1.02 (m, 25H), 0.98 (s, 3H), 0.96 (d, J = 6.4 Hz, 3H), 0.71 (s, 3H) .^{13}C-NMR (126 MHz, CD_3OD) δ 179.0, 172.5, 58.2, 57.7, 51.1, 50.5, 44.1, 42.0, 41.7, 37.4, 37.2, 36.9, 35.8, 34.4, 32.7, 32.6, 29.4, 28.6, 28.4, 27.8, 25.4, 24.2, 22.9, 22.1, 18.9, 12.6. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{26}H_{44}NO_3$ 418.3316. Found 418.3308.

4.2.18. Compound **9**

2 M aqueous hydroxide (7 ml) was added to a solution of compound **20** (24 mg, 0.050 mmol) in methanol (21 ml). The mixture was stirred at room temperature for 2 h, poured into 2 M hydrochloric acid, and extracted with dichloromethane. The organic layer was washed with water, dried over sodium sulfate, and evaporated. The residue was recrystallized from ethyl acetate to afford **9** (8 mg, 37%). Colorless prisms (ethyl acetate); mp 160–161.5 °C; ^{1}H NMR (600 MHz, $CDCl_3$,) δ 4.13 (m, 1H), 3.332 (m, 1H), 2.35 (ddd,1H, J = 15.1, 9.6, 4.8 Hz), 2.22 (ddd, 1H, J = 15.8, 9.6, 6.2 Hz), 1.96 (dt, 1H, J = 13.8, 2.7 Hz), 0.93 (s, 3H), 0.91 (d, 3H, J = 6.9 Hz), 0.64 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$x) δ 178.51, 56.51, 55.99, 54.09, 42.75, 42.58, 42.25, 40.52, 40.11, 35.88, 35.75, 35.34, 35.06, 34.40, 30.75, 30.71, 29.36, 28.17, 26.93, 26.32, 24.17, 23.46, 20.81, 18.25, 12.04. HRMS-ESI (m/z): $[M+Na]^+$ calcd for $C_{25}H_{43}NNaO_4S$ 476.2810. Found 476.2823.

4.3. HL-60 cell differentiation assay[7]

HL-60 cells were cultured in RPMI-1640 medium supplemented with 5% FBS and penicillin G and streptomycin at 37 °C under 5% CO_2 in air. The cells were diluted to 8.0×10^4 cells/mL with RPMI-1640 (5% FBS), and an ethanol solution of a test compound was added to give 10^{-9}–10^{-6} M final concentration. Control cells were treated with the same volume of ethanol alone. 1α,25(OH)$_2$D$_3$ was always assayed at the same time as a positive control. The cells were incubated at 37 °C under 5% CO_2 in air for 4 days. The percentage of differentiated cells was determined by nitro-blue tetrazolium (NBT) reduction assay. Cells were incubated at 37 °C for 20 min in RPMI-1640 (5% FBS) and an equal volume of phosphate-buffered saline (PBS) containing NBT (0.2%) and 12-*O*-tetradecanoylphorbol 13-acetate (TPA; 200 ng/mL). The percentage of cells containing blue-black formazan was determined in a minimum of 200 cells.

4.4. Luciferase reporter assay

COS-7 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal calf serum (FCS). Cells were seeded on 24-well plates at a density of ca. 2×10^4 per well. After 24 h, cells were transfected with a reporter plasmid containing three copies of the mouse osteopontin VDRE (5-GGTTCAcgaGGTTCA, SPPx3-TK-LUC), a hVDR expression plasmid (pCMX-hVDR), and the internal control plasmid containing sea pansy luciferase expression construct (pRL-CMV) by the lipofection method as described previously. After 4 h incubation, the medium was replaced with fresh DMEM containing 1% FCS (HyClone, UT). The next day, the cells were treated with the indicated concentration of 1α,25(OH)$_2$D$_3$ (**1**) or lithocholic acid derivatives, or ethanol vehicle, and cultured for 24 h. Cells in each well were harvested with cell lysis buffer, and the luciferase activity was measured with a luciferase assay kit (Tokyo Ink, Inc., Japan) according to the manufacturer's instructions. Transactivation measured in terms of luciferase activity was normalized with the internal control. All experiments were done in triplicate.

4.5. Competitive binding assay

Binding affinity was investigated according to our previous report.[8] Bovine thymus VDR purchased from Yamasa Biochemical (Choshi, Chiba, Japan) was dissolved in 0.05 M phosphate buffer (pH 7.4) containing 0.3 M KCl and 5 mM dithiothreitol just before use. The receptor solution (500 μl) in an assay tube was incubated with 0.072 nM [^{3}H]1α,25-(OH)$_2$D$_3$, together with graded amounts of each vitamin D analog or vehicle for 19 h at 4 °C. Bound and free [^{3}H] 1α,25(OH)$_2$D$_3$ (**1**) were separated on dextran-coated charcoal for 20 min at 4 °C. The assay tubes were centrifuged at 1000*g* for 10 min. The radioactivity of the supernatant was counted. These experiments were done in duplicate.

4.6. X-ray crystallographic analysis

Crystals of VDR complexes were prepared according to the method of Vanhooke et al.[9] with some modifications. The rat VDR LBD (residues 116–423, Δ165–211) was cloned as an *N*-terminal His6-tagged fusion protein into the pET14b expression vector and overproduced in *Escherichia coli* C41. The cells were grown at 37 °C in LB medium (including ampicillin 100 mg/L) and subsequently induced for 6 h with 15 μM isopropyl-β-D-thiogalactopyranoside (IPTG) at 23 °C. The purification procedure included affinity chromatography on a Ni-NTA column, followed by dialysis and cation-exchange chromatography (SP-Sepharose). After tag removal by thrombin digestion, protease was removed by filtration through a HiTrap benzamidine column and the protein was further purified by gel filtration on a Super-dex200 column. The purity and homogeneity of the rVDR LBD were assessed by SDS-PAGE.

Purified rVDR LBD solution was concentrated to about 0.75 mg/mL by ultrafiltration. To an aliquot (800 µl) of the protein solution was added a ligand (ca. 10 equiv), then the solution was further concentrated to about 1/8, and a solution (25 mM Tris-HCl, pH 8.0; 50 mM NaCl; 10 mM DTT; 0.02% NaN_3) of coactivator peptide (H_2N-KNHPMLMNLLKDN-$CONH_2$) derived from DRIP205 was added. This solution of VDR/ligand/peptide was allowed to crystallize by the vapor diffusion method using a series of precipitant solutions containing 0.1 M MOPS–NaOH (pH 7.0), 0.1–0.4 M sodium formate, 12–22% (w/v) PEG4000, and 5% (v/v) ethylene glycol. Droplets for crystallization were prepared by mixing 2 µl of complex solution and 1 µl precipitant solution, and droplets were equilibrated against 500 µl precipitant solution at 20 °C.

Prior to diffraction data collection, crystals were soaked in a cryoprotectant solution containing 0.1 M MOPS-NaOH (pH 7.0), 0.1–0.4 M sodium formate, 15–20% PEG4000, and 17–20% ethylene glycol. Diffraction data sets were collected at 100 K in a stream of nitrogen gas at beamline BL-6A of KEK-PF (Tsukuba, Japan). Reflections were recorded with an oscillation range per image of 1.0°. Diffraction data were indexed, integrated, and scaled using the program HKL2000 (HKL Research Inc., USA). The structures were solved by molecular replacement with the program Phaser in the Phenix[10] using rat VDR LBD coordinates (PDB code: 2ZLC), and finalized sets of atomic coordinates were obtained after iterative rounds of model modification with the program COOT[11] and refinement with REFMAC[12]. The coordinates and structure factors have been deposited to Protein Data Bank (Entry ID: 6K5O).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmc.2019.07.003.

References

1. Feldman D, Glorieux FH, Pike JW, eds. *Vitamin D.* 2nd ed. Elsevier Academic Press; 2005.
2. Makishima M, Lu TT, Xie W, et al. Vitamin D receptor as an intestinal bile acid sensor. *Science (80-).* 2002;296:5571.
3. Nehring JA, Zierold C, DeLuca HF. Lithocholic acid can carry out in vivo functions of vitamin D. *Proc Natl Acad Sci USA.* 2007;104:10006–10009.
4. Adachi R, Honma Y, Masuno H, et al. Selective activation of vitamin D receptor by lithocholic acid acetate, a bile acid derivative. *J Lipid Res.* 2005;46:46–57.
5. Ishizawa M, Matsunawa M, Adachi R, et al. Lithocholic acid derivatives act as selective vitamin D receptor modulators without inducing hypercalcemia. *J Lipid Res.* 2008;49:763–772.
6. Masuno H, Ikura T, Morizono D, et al. Crystal structures of complexes of vitamin D receptor ligand-binding domain with lithocholic acid derivatives. *J Lipid Res.* 2013;54:2206–2213.
7. Mangelsdorf DJ, Koeffler HP, Donaldson CA, Pike JW, Haussler MR. 1,25-Dihydroxyvitamin D3-induced differentiation in a human promyelocytic leukemia cell line (HL-60): receptor-mediated maturation to macrophage-like cells. *J Cell Biol.* 1984;98:391–398.
8. Fujii S, Masuno H, Taoda Y, et al. Boron cluster-based development of potent non-secosteroidal vitamin D receptor ligands: direct observation of hydrophobic interaction between protein surface and carborane. *J Am Chem Soc.* 2011;133:20933–20941.
9. Vanhooke JL, Benning MM, Bauer CB, Pike JW, DeLuca HF. Molecular structure of the rat vitamin D receptor ligand binding domain complexed with 2-carbon-substituted vitamin D 3 hormone analogues and a LXXLL-containing coactivator peptide. *Biochemistry.* 2004;43:4101–4110.
10. Adams PD, Afonine PV, Bunkoczi G, et al. PHENIX: a comprehensive python-based system for macromolecular structure solution. *Acta Crystallogr D.* 2010;66:213–221.
11. Emsley P, Lohkamp B, Scott WG, Cowtan K. Features and development of coot. *Acta Crystallogr D.* 2010;66:486–501.
12. Murshudov GN, Skubák P, Lebede AA, et al. (2011) REFMAC5 for the refinement of macromolecular crystal structures. *Acta Crystallogr D.* 2011;67:355–367.