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Design, synthesis and X-ray crystallographic study of new nonsecosteroidal vitamin D receptor ligands

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ABSTRACT

We designed and synthesized nonsecosteroidal vitamin D receptor (VDR) ligands that formed H-bonds with six amino acid residues (Tyr143, Ser233, Arg270, Ser274, His301 and His393) of the VDR ligand-binding domain. The ligand YR335 exhibited potent transcriptional activity, which was comparable to those of 1α,25-dihydroxyvitamin D_3 and YR301. The crystal structure of the complex formed between YR335 and the VDR ligand-binding domain was solved, which revealed that YR335 formed H-bonds with the six amino acid residues mentioned above.

1α,25-Dihydroxyvitamin D_3 (1α,25$(OH)_2D_3$), the physiologically active form of vitamin D, regulates various biological events including calcium and phosphorus homeostasis and cell differentiation (Fig. 1).[1,2] The biological responses of 1α,25$(OH)_2D_3$ are mediated by the vitamin D receptor (VDR), which is a member of the nuclear receptor superfamily and acts as a ligand-dependent gene transcription factor in combination with co-activators.[3,4] 1α,25$(OH)_2D_3$ has significant therapeutic potential for the treatment of osteoporosis, various types of rickets, secondary hyperparathyroidism, psoriasis, autoimmune diseases, and cancer; however, therapy using 1α,25$(OH)_2D_3$ is limited because it often causes hypercalcemia. Therefore, it is necessary to develop new VDR ligands. In 2000, Moras et al. revealed the binding mode between 1α,25$(OH)_2D_3$ and the VDR ligand-binding domain (VDR LBD) using X-ray analysis.[5] Their results showed that 1α,25$(OH)_2D_3$ formed H-bonds with six amino acid residues, Tyr143, Ser237, Arg274, Ser278, His305 and His397 (Fig. 2A).

The discovery of LG190178, the first nonsecosteroidal VDR agonistic ligand was significant,[6] and many subsequent studies of the nonsecosteroidal ligands of the VDR have been performed.[7–10] We have also reported that the (2*S*,2′*R*)-analog (YR301) of LG190178 is a major active isomer (Fig. 1).[11] Moreover, we succeeded in solving the crystal structure of the complex formed between YR301 and the rat VDR LBD at a resolution of 2.0 Å and revealed that YR301 formed H-bonds with Ser233, Arg270, His301 and His393, but not with Tyr143 or Ser274.[12] YR301 formed an H-bond network containing just four residues (Fig. 2B). We therefore attempted to design nonsecosteroidal ligands with the ability to bind with all six residues of the VDR.

We designed and synthesized nonsecosteroidal ligands (Fig. 3). Docking studies predicted that their hydroxyl groups (red spheres) formed H-bonds with Tyr143 and Ser274. A docking simulation was performed by carrying out a conformational search for ligands of the VDR LBD using *MacroModel*. The lowest energy conformer was used in the docking model.[13] Synthetic schemes for each ligand are shown in Scheme 1. Compound **1** was synthesized from a ketone via (*R*)-CBS-oxazaborolidine-catalyzed (CBS; Corey–Baski–Shibata) asymmetric borane reduction, as reported previously.[14] Compound **1** was then treated with mesylate **3**, **5** or **9**, or epoxide **7** to give the ligands through deprotection. YR333 and YR334 are diastereomers and have not been differentiated. YR335, an (*R*,*R*)-isomer, was synthesized from (2*S*)-butane-1,2,4-triol.[15] YR336, the diastereomer of YR335, was determined to be an (*S*,*R*)-isomer.

Next, the ligands were examined using a transactivation assay involving the human osteocalcin promoter.[16] The results are summarized in Table 1. YR335 exhibited potent transcriptional activity, which was comparable to those of 1α,25-dihydroxyvitamin D_3 and YR301. Most of the ligands except YR311 showed moderate activity. YR336, the diastereomer of YR335, displayed activity that was two orders of magnitude lower than that of YR335.

The crystal structure of the LBD of the complex formed between the rat VDR and YR335 was solved to a resolution of 1.8 Å and is

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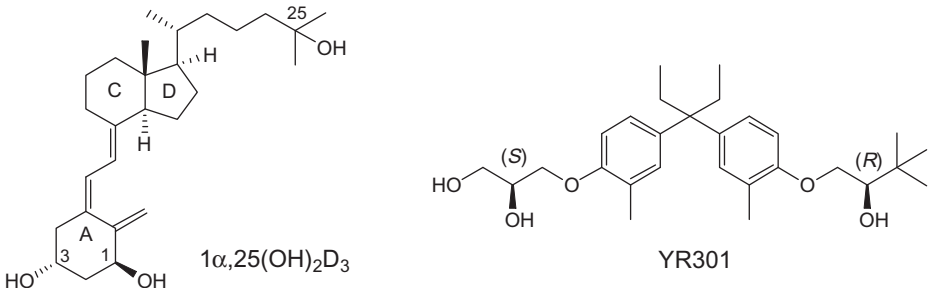

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Figure 1. Chemical structures of $1\alpha,25(OH)_2D_3$ and YR301.

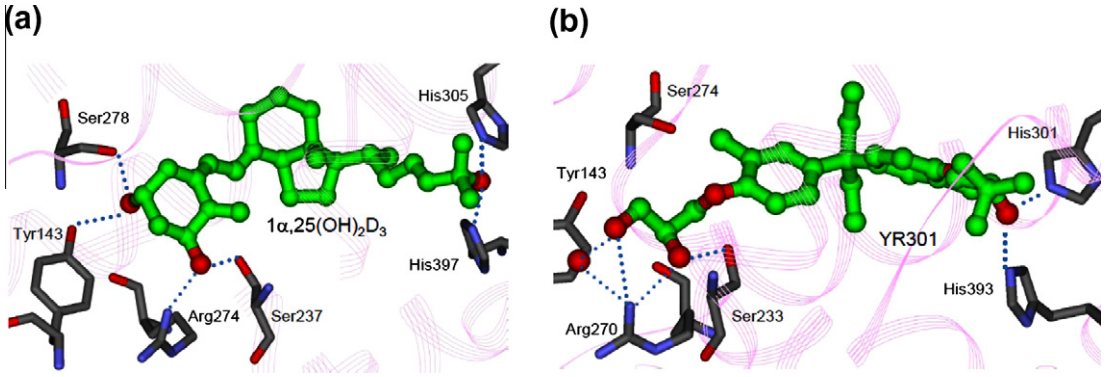


Figure 2. H-bond networks detected in the X-ray structures of the complexes (a) between the VDR LBD and $1\alpha,25(OH)_2D_3$, and (b) between the VDR LBD and YR301.

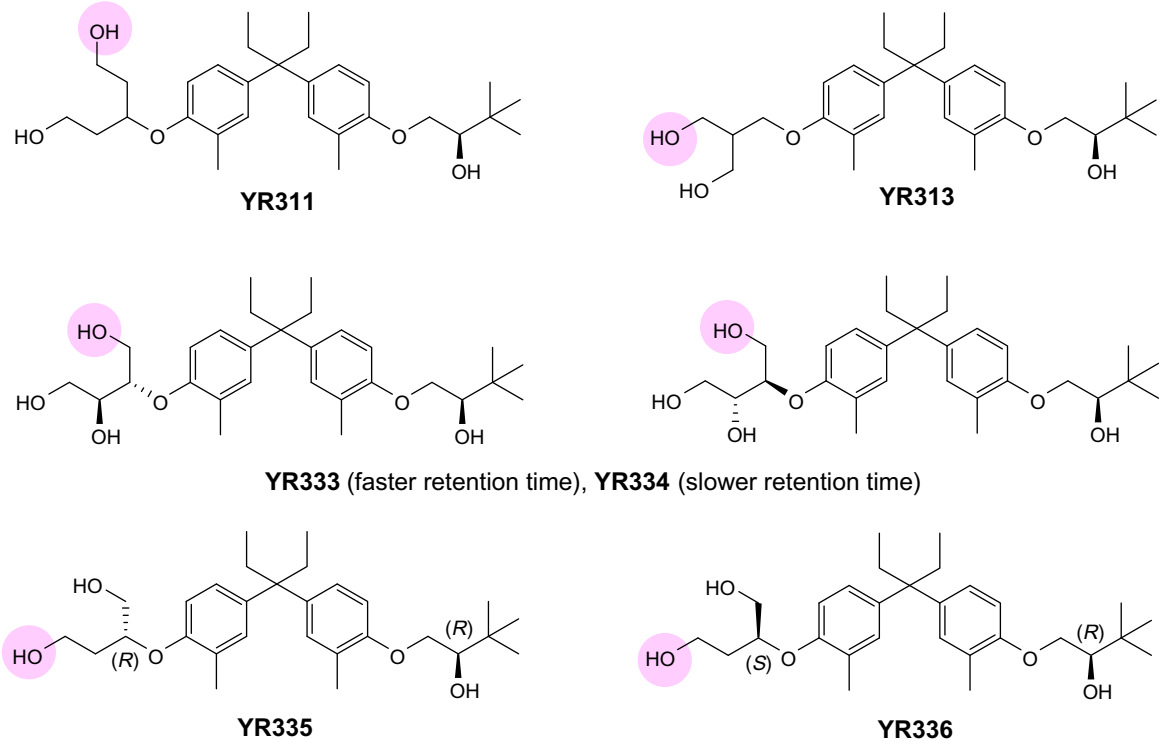
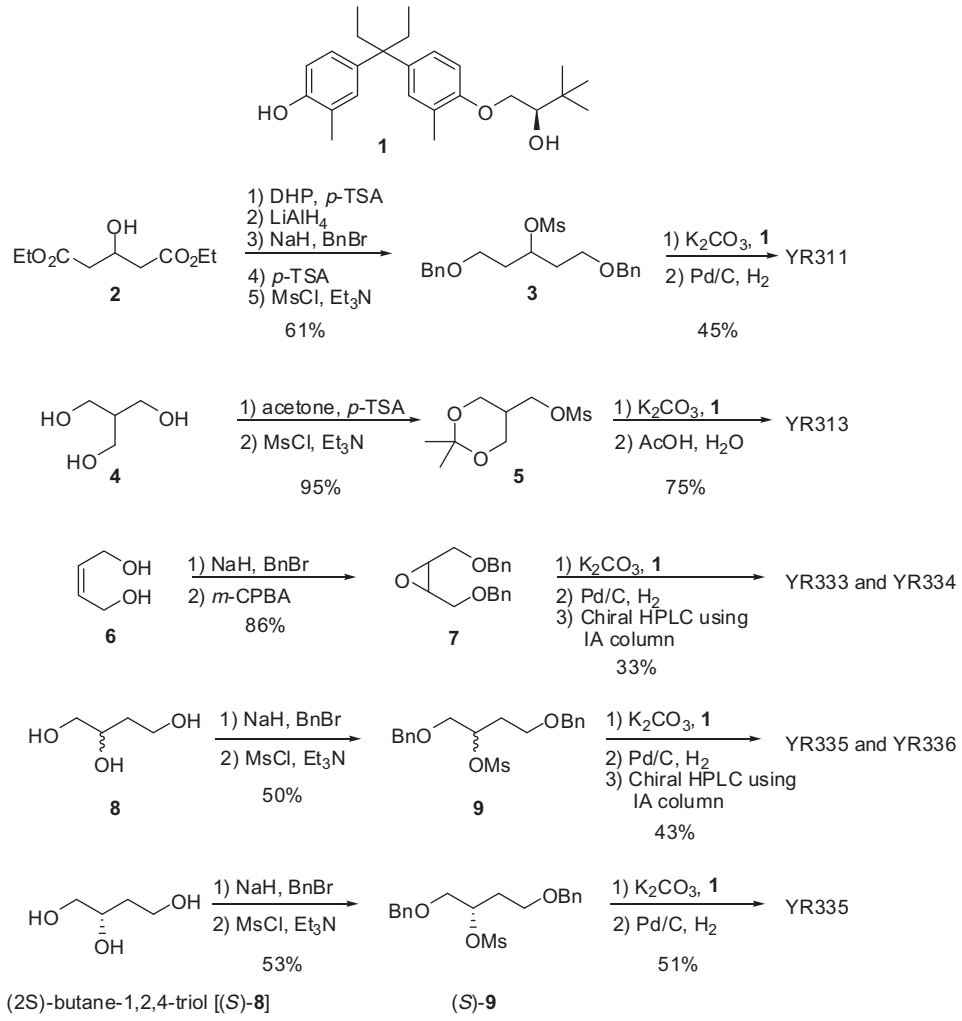


Figure 3. Chemical structures of the synthesized nonsecosteroidal ligands.



Scheme 1. Synthesis of ligands.

Table 1
Transcriptional activity of ligands[16]

Ligands	Transcriptional EC_{50} (nM)
YR311	19.4
YR313	1.11
YR333	0.92
YR334	1.25
YR335	0.06
YR336	1.29
YR301	0.04
$1\alpha,25(OH)_2D_3$	0.01

shown in Figure 4. The crystal structure of the complex formed between YR335 and the VDR ligand-binding domain was solved to reveal that YR335 formed H-bonds with six amino acid residues (Tyr143, Ser233, Arg270, Ser274, His301 and His393).[17] Contrary to YR301, YR335 was confirmed to form H-bonds with Ser278 and Tyr143.

Although YR335 was able to form additional H-bonds with Ser274 and Tyr143, its transcriptional activity was almost the same as that of YR301. This was probably because YR335 and YR301 contained the same number of H-bonds and that the stabilization energy of YR335 was similar to that of YR301. YR335 formed six H-bonds with the six amino acid residues shown in Figure 4. YR301 formed six H-bonds with four amino acid residues, that is to say, the 2′-OH was H-bonded to His301 and His393, the 2-OH was H-bonded to Arg270 and Ser233, and the 1-OH was directly H-bonded to Arg270 and was bound to Arg270 via a water molecule-mediated H-bond (Fig. 2B).

Although all of the designated ligands were able to form additional H-bonds with Ser274 and Tyr143 in the docking simulation, as shown in Figure 3, except for YR335, none of them displayed potent activity. Therefore, it is highly probable that even if the designated ligands formed H-bonds with the desired amino acid residues, their steric energies were not lowered and their activities were not increased.

In conclusion, we designed and synthesized nonsecosteroidal VDR ligands that formed H-bonds with six amino acid residues (Tyr143, Ser233, Arg270, Ser274, His301 and His393) of the VDR LBD. The ligand YR335 showed potent transcriptional activity, which was comparable to that of YR301. Furthermore, to understand the strong activity of YR335, the crystal structure of the complex formed between YR335 and the rat VDR LBD was solved, which revealed that YR335 formed H-bonds with the six amino acid residues mentioned above.

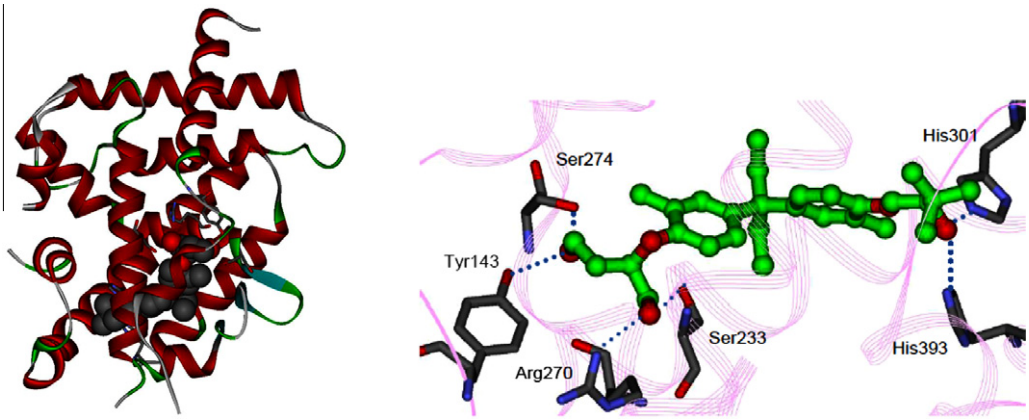


Figure 4. H-bond network formed between the VDR LBD and YR335 in the X-ray structure.

Acknowledgment

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15. Spectroscopic data for YR335: Colorless oil; $[\alpha]_D^{22} = -13.5$ (*c* 1.5, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 6.91–6.96 (m, 4H), 6.79 (d, $J = 8.4$ Hz, 1H), 6.69 (d, $J = 8.0$ Hz, 1H), 4.55 (m, 1H), 4.12 (m, 1H), 3.70–3.90 (m, 6H), 2.46 (br s, 1H), 2.18 (s, 3H), 2.16 (s, 3H), 1.97–2.06 (m, 8H), 1.00 (s, 9H), 0.59 (t, $J = 7.2$ Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.5, 153.5, 141.7, 141.3, 131.0, 130.8, 126.5, 126.4, 125.7, 112.4, 110.3, 77.0, 76.5, 69.4, 64.7, 64.4, 59.4, 48.6, 34.3, 33.8, 29.5, 26.3, 25.6, 17.0, 16.9, 8.7; ESI(+)-MS *m/z* 495 $[M+Na]^+$.
16. The human osteocalcin gene promoter fragment −838/+10 was cloned into the pGL3 reporter plasmid (Promega), and the human VDR and RXR genes were cloned into the pCDNA3 expression vector (Invitrogen). Hos cells were maintained in phenol red free DMEM (Invitrogen) containing 10% FCS (Invitrogen). Prior to the transfection, the cells were plated in a 96-well plates at a density of 400,000 cells per well in Opti-MEM (Invitrogen). The cells were then transfected with human osteocalcin reporter vector (pGL3-hOc: 100 ng/well), the human VDR and RXR expression vectors (pCDNA-hVDR and pCDNA-hRXR, respectively, 10 ng/well) and phRL-TK (Promega: 25 ng/well) using 50 μl of Lipofectamine 2000 reagent (Invitrogen). Three hours after the transfection, they were treated with ethanol vehicle and various concentrations of the VDR ligands (from 10 pM to 10 μM). Luciferase activity was quantitated the next day using a luminometer (Berthold) and the Dual-Glo luciferase assay reagent (Promega).
17. VDR-LBD protein had the same structure and was purified and complexed by the same protocol as described in reference 12. Its crystal structure has been deposited in the RCSB under PDB code: 3AUN. The initial crystals of the VDR LBD complexed with YR335 were obtained under the following crystallization conditions: 100 mM MOPS (pH 7.0), 200 mM ammonium citrate, 20% PEG 4000, and 4% 2-propanol. The crystals were cryoprotected in 30% glycerol and cooled at 79 K, and X-ray data were collected using SPring-8 beamline BL41XU. The data were reduced and scaled using HKL2000.[18] Molecular replacement was achieved with MOLREP[19] from CCP4 (Collaborative Computational Project, No. 4, 1994), and the structure was built using COOT[20] and refined using REFMAC.[21] Crystal data: spacer group: $P2_12_12$, $a = 44.24$, $b = 47.33$, $c = 136.52$ Å, data collection: SPring-8, beamline BL41XU, wavelength: 1.000 Å, resolution = 50.00–1.80 Å, total number of reflections = 185203, unique reflection = 27038, $R_{merge} = 0.046$, completeness = 98.6%, multiplicity = 3.7, average $(I/\sigma(I)) = 41.8$, $R_{factor} = 19.6\%$, $R_{free} = 23.7\%$.
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