Facile Synthesis of Stereoisomers of the Non-Secosteroidal Ligand LG190178 and their Evaluation Using the Mutant Vitamin D Receptor

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Abstract: We developed a facile synthesis process for producing optically active non-secosteroidal ligands (YR301-304), which are stereoisomers of LG190178, and evaluated their performance in transcriptional assays using mutant vitamin D receptor (VDR). It was found that all of them had stronger activities than the natural ligand 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$]. In particular, YR301 showed potent activity for both wild-type and mutant Arg274Leu VDR.

Keywords: Asymmetric reduction, vitamin D receptor, mutant vitamin D receptor, non-secosteroidal ligand, transcriptional assay, molecular modeling study.

INTRODUCTION

Vitamin D receptor (VDR) ligands are therapeutic agents used for the treatment of psoriasis, osteoporosis, and secondary hyperparathyroidism [1]. VDR ligands also show immense potential as therapeutic agents for autoimmune diseases and cancers of the skin, prostate, colon, and breast as well as leukemia [1, 2]. In 2000, Moras *et al*. revealed the binding mode between 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$] and the ligand binding domain (LBD) of VDR by X-ray analysis [3]. This enabled the novel design of VDR ligands using structure-based drug design [4]. Non-secosteroidal ligands for VDR have thus become an attractive target for the development of new therapeutics [5]. LG190178 is the first novel non-secosteroidal ligand to be developed and shows potential as a therapy for cancer, leukemia, and psoriasis with fewer calcium mobilization side effects than are associated with secosteroidal 1α,25$(OH)_2D_3$ analogues [6]; however, although LG190178 has four stereoisomers, there is no apparent active isomer of LG190178. Recently, Hashimoto's group [7] and we [8] independently synthesized four isomers of LG190178 and evaluated their interaction with VDR and revealed that the (2*S*,2'*R*)-isomer (YR301) had potent activity compared to 1α,25$(OH)_2D_3$. Furthermore, we solved the crystal structure of the rat VDR LBD bound with YR301, the overall structure of which was very similar to that of the VDR LBD-1α,25$(OH)_2D_3$ complex [9]. The mutation of VDR Arg274Leu causes a rare genetic disease called hereditary vitamin D-resistant rickets (HVDRR) [10]. The substitution of Arg274 for Leu reduced its affinity for 1α,25$(OH)_2D_3$ because of the loss of the hydrogen bond between the 1α-OH of 1α,25$(OH)_2D_3$ and Arg274 (ca. 1000-fold reduction against the wild-type receptor) [11]. Several groups have recently reported vitamin D analogues for mutant VDR [12]. As for non-secosteroidal ligands for mutant VDR, Koh *et al*. reported racemic LG190155 analogues that showed potent activity for mutant VDR (Arg274Leu) [13]; however, they showed weak activity for wild-type VDR compared with 1α,25$(OH)_2D_3$ and did not include an active isomer. In this paper, we wish to report the facile synthesis of the optically active non-secosteroidal ligands YR301-304 and that YR301 and YR303 show potent activity for mutant VDR (Arg274Leu). Furthermore, we demonstrate that YR301 exhibits strong activity for both wild-type and mutant VDR. Having activity for both wild-type and mutant receptors is an advantage for the development of drugs against rare diseases.

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RESULTS AND DISCUSSION

Synthesis of YR301-304

In our previous report, the synthesis of stereoisomers of LG190178 required several cumbersome steps including separation with a chiral column [8]. Therefore, we proposed a facile synthetic route for producing the (2*S*,2'*R*)-isomer (YR301) of LG190178 starting from bisphenol derivative **1** (Scheme **1**) [8]. The asymmetric center at the 2'-position was constructed by (*R*)-CBS-oxazaborolidine-catalyzed (CBS, Corey-Baski-Shibata) asymmetric borane reduction [14]. The results are shown in Table **1**. The use of BH_3-THF complex gave (*R*)-**2** with a moderate yield and enantioselectivity (entry 1). Borane *N*-ethyl-*N*-isopropylaniline (BACH-EI™) complex worked well to give (*R*)-**2** with a high yield and excellent enantioselectivity (entry 2). Using a low-temperature reaction reduced both the yield and enantioselectivity (entries 3 and 4). The use of 1.5 Eq. BACH-EI™ increased the chemical yield of (*R*)-**2** with a slight decrease in enantioselectivity (entry 5). After

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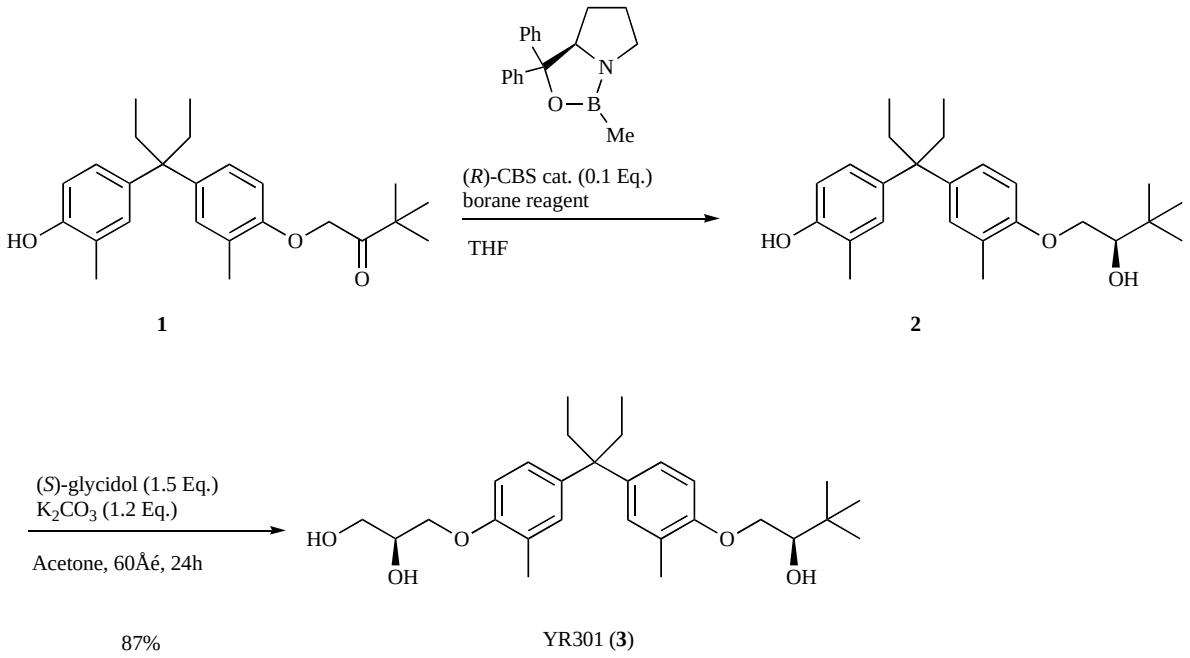


Scheme 1. Synthesis of the (2*S*,2'*R*)-isomer (YR301) of LG190178.

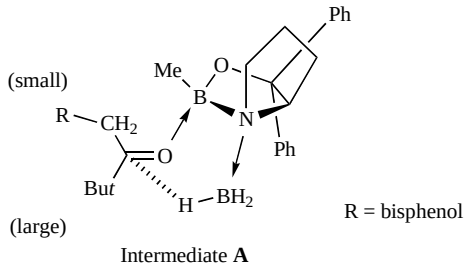
Table 1. Asymmetric Reduction of Compound 1

Entry	Borane reagent (Eq.)	Temperature (°C)	Yield (%)	Ee[a] (%)
1	BH_3-THF (1.2)	20	47	65
2	BACH-EI™ (1.2)[b]	20	75	95
3	BACH-EI™ (1.2)	0	71	73
4	BACH-EI™ (1.2)	-20	60	22
5	BACH-EI™ (1.5)	20	97	87

[a]Determined by HPLC.
[b]Borane *N*-ethyl-*N*-isopropylaniline complex.

treatment of (*R*)-**2** with (*S*)-glycidol, the desired (2*S*,2'*R*)-isomer YR301 (**3**) was obtained in a sufficient yield. In a similar manner, the (2*S*,2'*S*)-isomer [by use of (*S*)-CBS and (*S*)-glycidol, YR302 (**4**)], (2*R*,2'*R*)-isomer [by use of (*R*)-CBS and (*R*)-glycidol, YR303 (**5**)], and (2*R*,2'*S*)-isomer [by use of (*S*)-CBS and (*R*)-glycidol, YR304 (**6**)] were synthesized, respectively.

Fig. (**1**) shows the stereochemical course of the asymmetric reduction of compound **1**. The transition-state complex **A**, which is generated by compound **1**, (*R*)-CBS catalyst, and borane reagent, might be attacked by hydride on the *si* face to afford desired product (*R*)-**2**.



Intermediate **A**

Fig. (1). Plausible stereochemical course.

Transcriptional Assays for Mutant and Wild-Type VDR

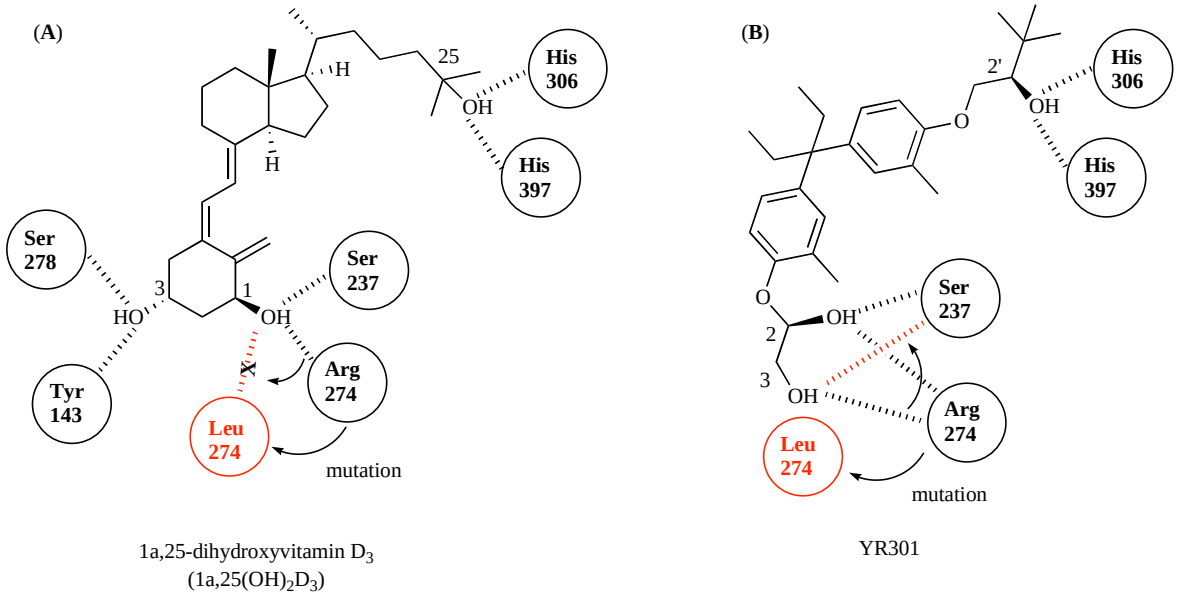
Four isomers, YR301 (**3**), YR302 (**4**), YR303 (**5**), and YR304 (**6**), were examined in transcriptional assays with mutant VDR (Arg274Leu). Their activity for mutant VDR was not as strong as that for wild-type VDR [8]. The results are summarized in Table **2**. For mutant VDR (Arg274Leu), all non-secosteroidal ligands (YR301-304) exhibited stronger activities than $1\alpha,25(OH)_2D_3$; in particular, YR301 and YR303 were more than 20 times more potent than $1\alpha,25(OH)_2D_3$.

Fig. (**2**) shows our proposed schematic representation of the hydrogen bonds between the ligand [$1\alpha,25(OH)_2D_3$ or YR301 (**3**)] and mutant VDR (Arg274Leu). The substitution of Arg274 for Leu causes the loss of a crucial hydrogen bond to the 1α-OH of $1\alpha,25(OH)_2D_3$ and leads to a decrease in the *in vitro* ligand-dependent transactivation function (Fig. **2A**) [11]. On the other hand, it seems that the non-secosteroidal ligand **3** shows potent activity for mutant VDR (Arg274Leu) because its 3-OH assists in hydrogen binding to Ser237 (red dashed line in Fig. **2B**) and enhances its affinity compared with $1\alpha,25(OH)_2D_3$. YR303 (**5**) might also show potent activity for the same reason. This conformational change was supported by a molecular modeling study (Fig. **3**).

Table 2. Transcriptional Assays Using Mutant VDR (Arg274Leu) and Wild-type VDR [8]

Compound	Transcription EC_{50} (nM)	
	Mutant VDR MCF7[a]	Wild-type VDR HOS/SF[b]
1α,25$(OH)_2D_3$	440	0.0106
YR301(2*S*,2'*R*)	22	0.0396
YR302(2*S*,2'*S*)	100	1.66
YR303(2*R*,2'*R*)	17	4.68
YR304(2*R*,2'*S*)	69	15.6

[a]Human breast cancer cell.
[b]Human osteosarcoma cell, serum free.



1a,25-dihydroxyvitamin D_3 (1a,25$(OH)_2D_3$)

YR301

Fig. (2). Schematic representation of the hydrogen bonds (black and red dashed lines) between the ligand [1α,25$(OH)_2D_3$ or YR301 (**3**)] and mutant VDR based on the reported cocrystal structure.

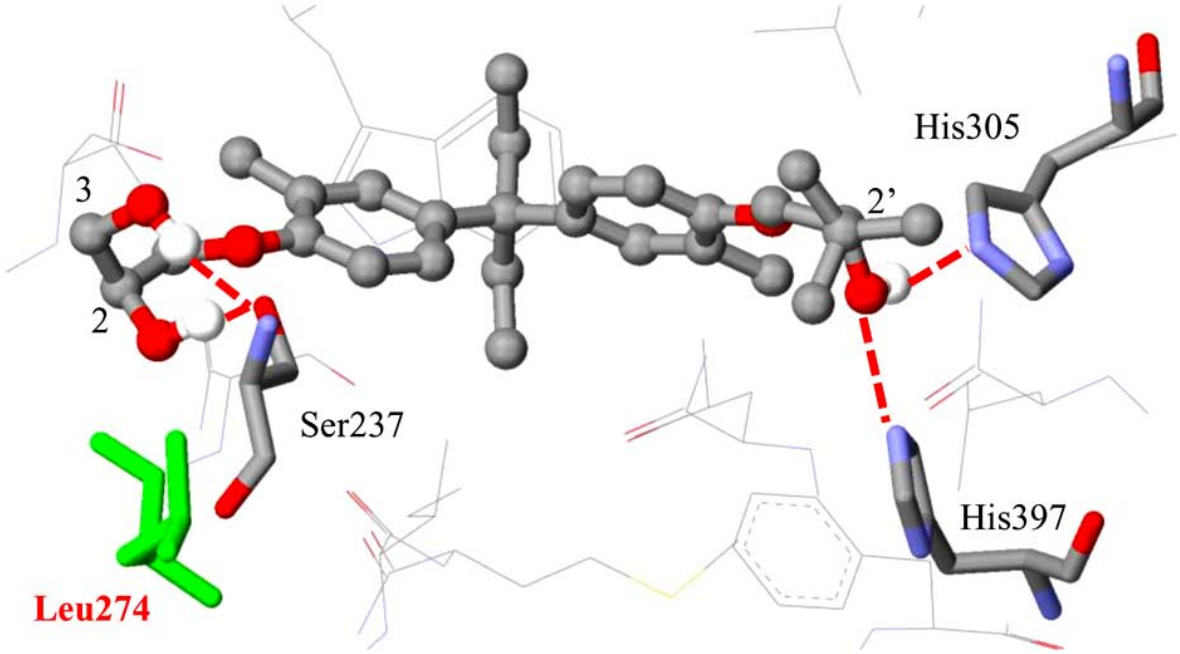


Fig. (3). Modeled structure of YR301 bound to the mutant VDR (Arg274Leu). Hydrogen bonds are shown as red dashed lines.

EXPERIMENTAL SECTION

General

All chemicals were of commercial grade without further purification. ^{1}H and ^{13}C NMR spectra were recorded on a *Varian AS 400* spectrometer, and measurements were carried out in $CDCl_3$ with tetramethylsilane used as an internal standard. FT-IR spectra were recorded on a *JASCO FT/IR-4100* spectrometer at 1 cm^{-1} resolution, with a mean of 16 scans used for the solution ($CDCl_3$) method and a 0.1 mm path length adopted for NaCl cells. Optical rotations $[\alpha]_D$ were measured with a *Jasco DIP-316* polarimeter using a 1.0 dm cell. ESI-MS spectra were measured on a Waters Micromass ZQ LC Mass spectrometer.

Procedure for the Asymmetric Reduction

In an inert gas atmosphere, to a mixture of 1.0 M (*R*)-CBS catalyst in toluene (0.05 mL, 0.05 mmol) and 2.0 M BACH-EI™ in THF (0.3 mL, 0.6 mmol) in THF (3 mL) was slowly added **1** (191 mg, 0.5 mmol) in THF (3 mL) over a period of 1 h at room temperature. The reaction mixture was then stirred for 10 min and quenched with MeOH (0.2 mL). The solvent was removed under reduced pressure and then purified by silica gel column chromatography (*n*-hexane/AcOEt = 5/1) to afford (*R*)-**2** (144 mg, 75%, 95% ee) as a colorless oil. IR (in $CDCl_3$) 3404 (br), 2875, 2350, 1501, 1238, 1122 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.60 (t, *J* = 5.7 Hz, 6H), 1.01 (s, 9H), 2.01 (q, *J* = 5.7 Hz, 4H), 2.17 (s, 3H), 2.20 (s, 3H), 2.48 (br s, 1H), 3.71 (d, *J* = 4.5 Hz, 1H), 3.86 (t, *J* = 6.6 Hz, 1H), 4.09 (dd, *J* = 1.8, 5.1 Hz, 1H), 4.46 (s, 1H), 6.65 (d, *J* = 6.3 Hz, 1H), 6.70 (d, *J* = 6.3 Hz, 1H), 6.90 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 8.7, 16.3, 16.9, 26.3, 29.5, 33.8, 48.6, 69.4, 110.3, 114.2, 122.7, 125.7, 126.4, 126.9, 130.8, 130.9, 141.4, 151.5, 154.5; $[\alpha]^{21}_D$ = —51.5 (*c* 0.15, $CHCl_3$, 95% ee); ESI(+)-MS: 385 (M^++H).

The absolute stereoconfiguration was determined by chiral HPLC: Daicel Chiralpak IA column (4.6 mmϕ, 250 mm), *n*-hexane : *i*-PrOH = 10 : 1, wavelength: 254 nm, flow rate: 1.0 ml/min, retention time: 8.5 min ((*R*)-**2**), 13.2 min ((*S*)-**2**).

Preparation of YR301 (3)

In an inert gas atmosphere, a mixture of compound **2** (115 mg, 0.30 mmol), K_2CO_3 (62 mg, 0.45 mmol), and (*S*)-glycidol (33 mg, 0.45 mmol) in acetone (3 mL) was stirred for 24 h at 60 ºC. The solvent was removed under reduced pressure and then purified by silica gel column chromatography (*n*-hexane/AcOEt = 2/1) to afford YR301 (**3**) (120 mg, 87%) as a colorless oil. YR301 is known compound [7, 8]. IR (in $CDCl_3$) 3399 (br), 2964, 2934, 2875, 1500, 1234, 1120 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.60 (t, *J* = 5.7 Hz, 6H), 1.00 (s, 9H), 2.01 (q, *J* = 5.7 Hz, 4H), 2.18 (s, 3H), 2.20 (s, 3H), 2.48 (br s, 1H), 3.71 (d, *J* = 4.5 Hz, 1H), 3.78 (dd, *J* = 5.7, 10.1 Hz, 1H), 3.84 (m, 3H), 4.01-4.15 (m, 4H), 6.62 (d, *J* = 6.3 Hz, 1H), 6.68 (d, *J* = 6.3 Hz, 1H), 6.90 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 8.7, 16.9, 26.6, 29.5, 33.8, 43.5, 48.7, 53.7, 64.1, 69.3, 69.4, 69.8, 70.8, 110.3, 125.6, 125.7, 126.5, 130.9, 141.5, 154.3, 154.6; $[\alpha]^{21}_D$ = —14.2 (*c* 1.2, $CHCl_3$); ESI(+)-MS: 481 (M^++Na).

YR302 (4): $[\alpha]^{21}_D$ = +22.9 (*c* 1.4, $CHCl_3$).

YR303 (5): $[\alpha]^{21}_D$ = —23.2 (*c* 0.4, $CHCl_3$).

YR304 (6): $[\alpha]^{21}_D$ = +14.0 (*c* 1.2, $CHCl_3$).

Method for the Reporter Assay

Transcriptional assays were performed using optically pure YR301-304 (**3**)-(**6**). Reporter assays were performed using luciferase as a reporter as follows: cells of the human breast cancer cell line MCF7 were grown at 37 °C in DMEM supplemented with 10% FBS and 1% P/S in an atmosphere of 95% air and 5% CO_2. The cells were collected, suspended in DMEM supplemented with 5% FBS (stripped with dextran-coated charcoal) and 1% P/S without phenol red, and plated in a 24-well plate (2.5 x 10^4 cells/well). Then, the cells were incubated in a CO_2 incubator at 37 °C overnight. Ligand stock solutions were then prepared at various concentrations in DMSO (10^{-7} to 10^{-3} M). DMSO was used as a vehicle. The plasmids used in our assays were as follows: receptor plasmids pM(GAL4-hVDR(R274L)(DEF)) for mutant type hVDR prepared by site-directed mutagenesis using QuikChange II XL Site-Directed Mutagenesis Kits (Stratagene), reporter plasmids (17M2-G-Luc), and internal standard plasmids (pRL-CMV). The plasmids were diluted in OPTI-MEM medium at a concentration of 50 ng/well for the receptor plasmid, 0.2 μg/well for the reporter plasmid, and 2.5 ng/well for the internal plasmid. Transfection was carried out using TransFast reagent (Promega) according to the manufacturer's instructions. After 3-6 h of transfection, ligand stock solutions were added at final concentrations of 10^{-10} to 10^{-6} M, and the cells were further incubated overnight. Luciferase assays were performed using a Dual-Luciferase Reporter Assay System Kit (Promega). All experiments were carried out at least three times, and data are shown as mean ± SD.

Molecular Modeling Study

The mutant binding-site model was generated by changing the side chain of Arg274 to that of Leu from wild type VDR (PDB ID: 1DB1), and a docking model of ligands bound to mutant VDR (Arg274Leu) was constructed *via* a conformational search using *MacroModel* (ver. 8.1). AMBER* was used as a force field. Differences in potential energy were estimated by molecular mechanics calculations.

CONCLUSION

In conclusion, we developed a facile synthesis process for producing optically active non-secosteroidal ligands YR301-304 (**3**)-(**6**), which are stereoisomers of LG190178, and evaluated their performance in transcriptional assays using mutant VDR (Arg274Leu). YR301 (**3**) demonstrated potent activity for both wild-type and mutant VDR (Arg274Leu). Non-secosteroidal ligands for VDR are novel candidates for therapeutic agents, and further derivatization of them is underway.

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