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(2*S*,2′*R*)-Analogue of LG190178 is a major active isomer

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Abstract—Vitamin D receptor (VDR) ligands are therapeutic agents for the treatment of psoriasis, osteoporosis, and secondary hyperparathyroidism. 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. LG190178 is a novel non-secosteroidal ligand for VDR. We synthesized and evaluated stereoisomers of LG190178 and found that only an (2*S*,2′*R*)-analogue of LG190178 (YR301) had strong activity.

The active form of vitamin D_3, 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$), is not only a regulator of calcium homeostasis and bone development and remodeling but also a potent differentiator of leukemic cells. 1α,25-$(OH)_2D_3$ and its synthetic analogues exert these effects by binding to the vitamin D receptor (VDR) which belongs to the steroid/thyroid hormone nuclear receptor superfamily. The X-ray crystal structure of the ligand binding domain (LBD) of VDR with 1α,25-dihydroxyvitamin D_3 was determined by Moras et al. in 2000 and the binding mode between ligands and LBD-VDR was revealed.[1] This enables the design of VDR ligands by structure-based drug design. We have also designed secosteroidal analogues[2] (Fig. 1).

Recently, non-secosteroidal ligands for VDR have been an attractive target for the development of new therapeutics.[3] LG190178 is the first novel non-secosteroidal ligand, with potential as therapeutic for cancer, leukemia, and psoriasis with less calcium mobilization side effects than are associated with secosteroidal 1α,25-$(OH)_2D_3$ analogues,[4] however, LG190178 includes four stereoisomers.[5] We calculated to make ducking model of four isomers and VDR, respectively,[6] and the results are summarized in Figure 2 and Table 1. The (2*S*,2′*R*)-isomer model was the most stable. There were hydrogen bonds between 2-OH and Arg-274, Ser-237 and between

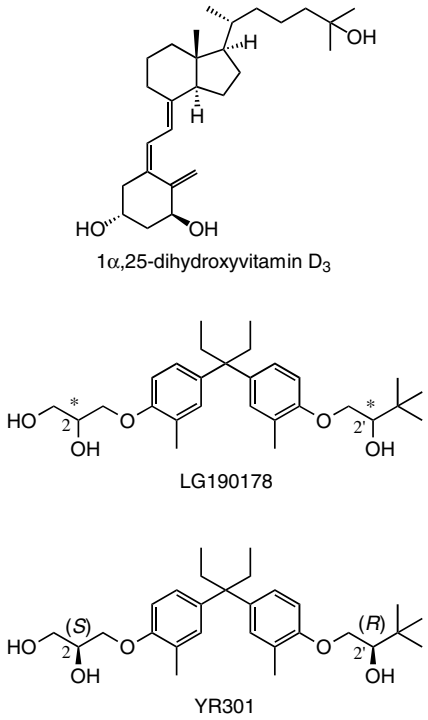

Figure 1. Structure of 1α,25-dihydroxyvitamin D_3, LG190178, and YR301.

2′-OH and His-305, His-397. (2*S*)-Isomers ((2*S*,2′*S*) and (2*S*,2′*R*)) are expected to be more important than (2*R*)-isomers.

Keywords: Vitamin D receptor; Non-secosteroidal ligand; (2*S*,2′*R*)-LG190178; YR301.
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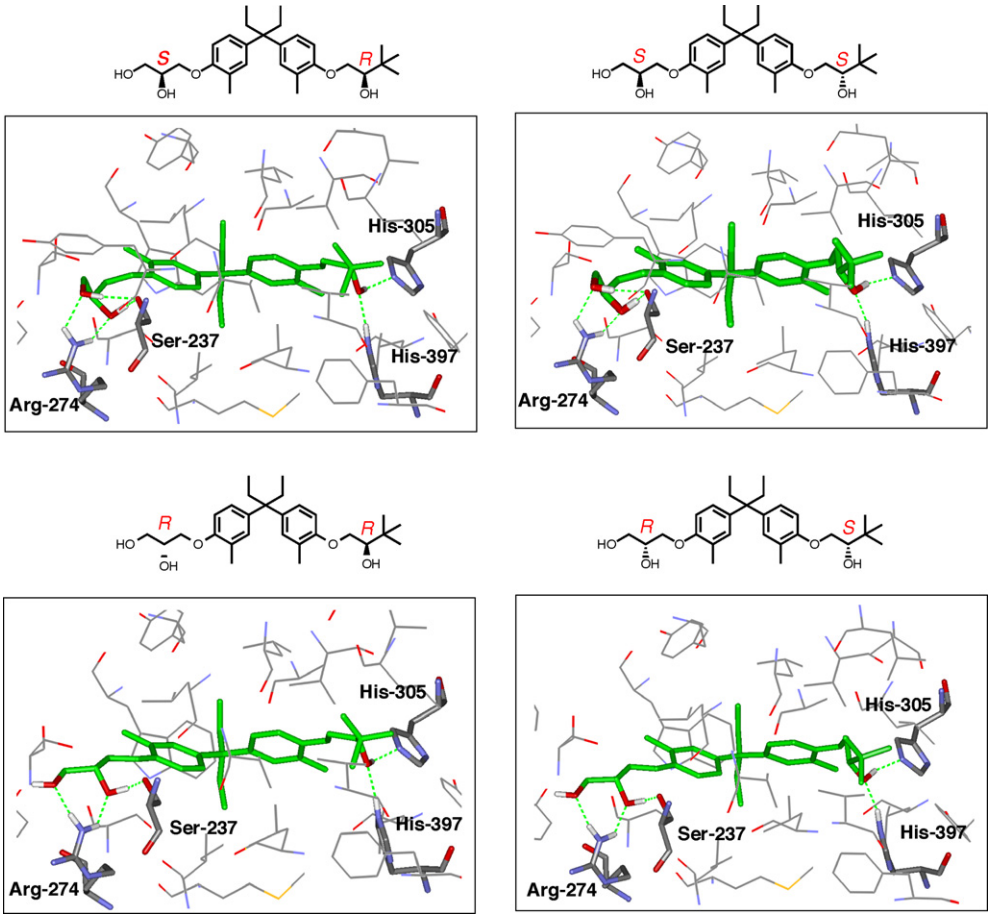


Figure 2. Modeled structure of stereoisomers of LG190178 bound to VDR.

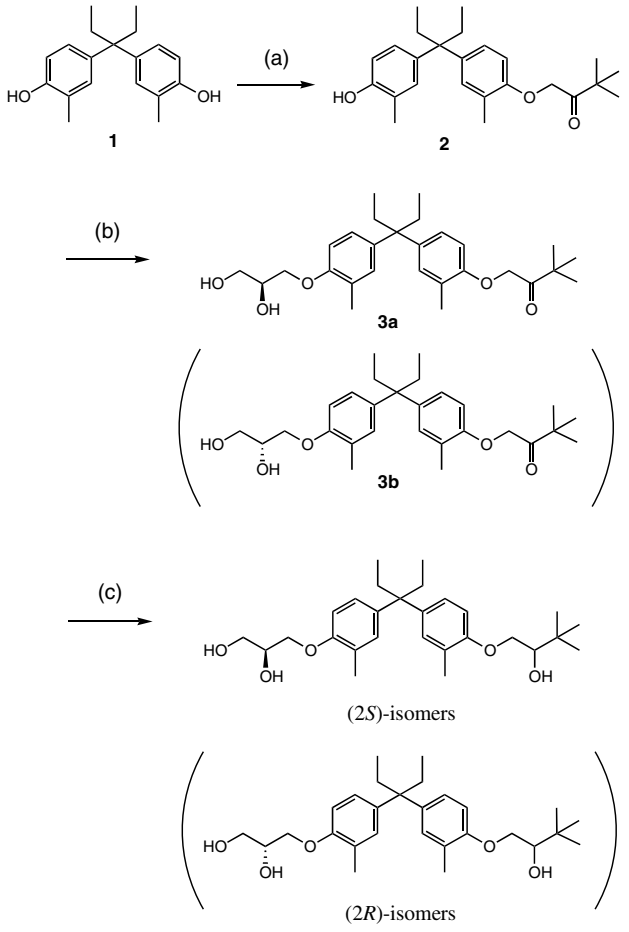
Table 1. Difference of potential energy of docking modeling of four isomers and VDR

Model	AE (kcal/mol)
I (2*S*,2′*R*)	0
II (2*S*,2′*S*)	+1.87
III (2*R*,2*R*)	+3.56
IV (2*R*,2′*S*)	+4.94

We synthesized (2*S*)-LG190178 and (2*R*)-LG190178. The synthetic routes of (2*S*)-isomers and (2*R*)-isomers are shown in Scheme 1. The 2-position asymmetric center was constructed using optically active glycidols. (2*S*)-Analogues and (2*R*)-analogues were analyzed by HPLC with a chiral column (CHIRALPAK IA, solvent: hexane/EtOH 9:1). HPLC charts are shown in Figure 3. The four isomers are YR301, YR302, YR303, and YR304, respectively. To determine the stereochemistry of the 2′-position, we synthesized the (2*S*,2′*R*)-isomer from (*R*)-diol **6**. (*R*)-1-(Benzyloxy)-3,3-dimethylbutan-2-ol **5** was separated using HPLC with a chiral column (CHIRALPAK IA, DAICEL; solvent: hexane/EtOH 9:1) and hydrogenated in the presence of $Pd(OH)_2/C$ to afford compound **6** ($[\alpha]_D^{25}$ −31.6 (*c* = 1.00, methanol)) in 70% yield. Absolute configuration of **6** was determined by comparing the optical rotation of **6** with that of the literature.[7] Scheme 3 shows the synthesis of the (2*S*,2′*R*)-isomers from (*R*)-diol **6**. Then YR301 was determined to be the (2*S*,2′*R*)-isomer. Using the same procedures YR302, 303, and 304 were determined to be (2*S*,2′*S*)-isomer, (2*R*,2′*R*)-isomer, and (2*R*,2′*S*)-isomer, respectively (Scheme 2).

Isomers were examined for transcriptional assays and VDR affinity. The results are summarized in Table 2. (2*S*,2′*R*)-Isomer (YR301) exhibited potent transcriptional activity, comparable to natural ligand 1α,25-$(OH)_2D_3$. Moreover, in the cases of human colon carcinoma cell, YR301 exhibited stronger activities than 1α,25-$(OH)_2D_3$. (2*S*,2′*S*)- and (2*R*,2′*R*)-isomers, diastereomers of (2*S*,2′*R*), decreased the activity by two orders of magnitude, compared with (2*S*,2′*R*)-isomer (YR301). (2*R*,2′*S*)-Isomer, the enantiomer of (2*S*,2′*R*) exhibited the weakest activity. It is very interesting that only YR301 showed strong activity. Figure 4 shows an overlay of the X-ray structure of 1α,25-$(OH)_2D_3$ and modeled structure of (2*S*,2′*R*)-isomer (YR301) in LBD of VDR. YR301 is well overlapped with 1α,25-$(OH)_2D_3$, especially with the hydrophobic region, but YR301 is not expected to be H-bonded to Tyr-143 and Ser-278, which are H-bonded to 1α,25-$(OH)_2D_3$. It is important to remember that considering H-bonds to Tyr-143 and Ser-278 is important in the design of new ligands.

In summary, we revealed that YR301, an (2*S*,2′*R*)-analogue of LG190178, was a major active isomer. YR301



Scheme 1. Reagents and conditions: (a) $ClCH_2CO^tBu$, NaH, DMF, 80 °C, 12 h, 40%; (b) (*S*)-glycidol (or (*R*)-glycidol), NaH, DMF, 80 °C, 3 h, 59%; (c) $NaBH_4$, MeOH, rt, 2 h, 89%.

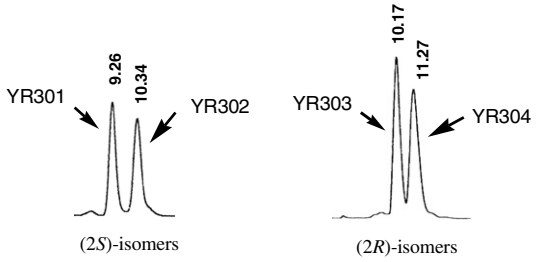
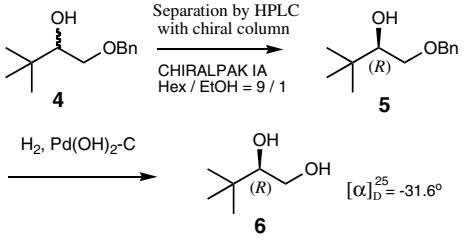
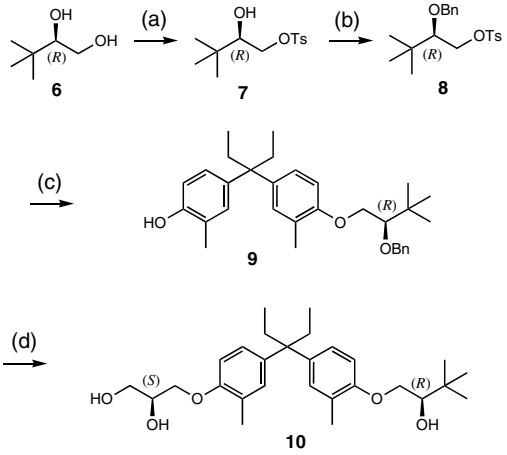


Figure 3. HPLC chart.



Scheme 2. Separation and determination of chiral.

exhibited potent biological activity, comparable to natural ligand 1α,25-$(OH)_2D_3$. Non-secosteroidal ligands for VDR are novel candidates for therapeutic agents.



Scheme 3. Reagents and conditions: (a) TsCl, C_5H_5N, CH_2Cl_2, rt, 19 h, 52%; (b) benzy trichloroacetimidate, TMSOTf, DMF, rt, 5 h, 72%; (c) **1**, NaH, DMF, rt, 1 h, 32%; (d) (*S*)-glycidol, NaH, DMF, 80 °C, 3 h, 43%; H_2, $Pd(OH)_2$/C, rt, 24 h, 93 h.

Table 2. Biological activities for transcriptional assays and VDR affinity

Compound	Transcription EC_{50} (nM)			VDR affinity
	HOS/SF[a]	HOS/5% FCS[b]	Caco-2/5% FCS[c]	
1α,25$(OH)_2D_3$	0.0106	0.555	3.52	100
YR301 (2*S*,2′*R*)	0.0396	0.79	1.80	28.3
YR302 (2*S*,2′*S*)	1.66	9.9	21.1	0.831
YR303 (2*R*,2′*R*)	4.68	16.8	22.1	0.482
YR304 (2*R*,2′*S*)	15.6	83.2	131.4	0.112

[a] Human osteosarcoma cell, serum free.
[b] Human osteosarcoma cell, 5% fetal calf serum.
[c] Human colon carcinoma cell, 5% fetal calf serum.

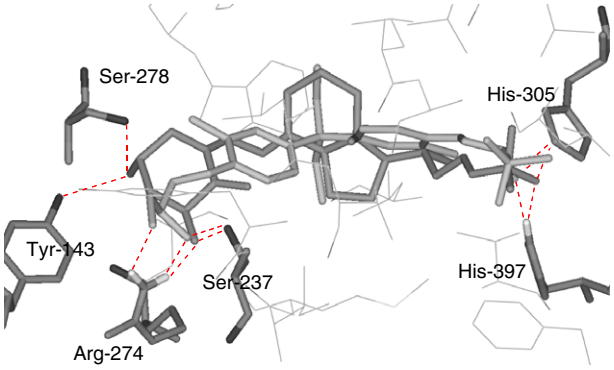


Figure 4. Overlay of the X-ray structure of 1α,25-$(OH)_2D_3$ and modeled structure of YR301 in VDR. 1α,25-$(OH)_2D_3$ and YR301 are shown in orange and green, respectively. Hydrogen bonds are drawn as dotted lines.

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