# 2α-(3-Hydroxypropyl)- and 2α-(3-Hydroxypropoxy)-1α,25-dihydroxyvitamin $D_3$ Accessible to Vitamin D Receptor Mutant Related to Hereditary Vitamin D-Resistant Rickets

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**Hereditary vitamin D-resistant rickets (HVDRR) is a genetic disorder caused by mutations in the vitamin D receptor, which lead to resistance to 1α,25-dihydroxyvitamin $D_3$ [1α,25$(OH)_2D_3$]. We found that the A ring-modified analogues, 2α-(3-hydroxypropyl)- and 2α-(3-hydroxypropoxy)-1α,25$(OH)_2D_3$, (O1C3 and O2C3) can bind better than the natural hormone to the mutant VDR (R274A), which similar to the HVDRR mutant, R274L, had lost the hydrogen bond to the 1α-hydroxyl group of 1α,25$(OH)_2D_3$.**

**Key words** 2α-substituted 1α,25-dihydroxyvitamin $D_3$; mutant vitamin D receptor; rickets; potent ligand; docking study

1α,25-Dihydroxyvitamin $D_3$ [1α,25$(OH)_2D_3$], the active metabolite of vitamin $D_3$, mediates its actions mostly through binding to the vitamin D receptor (VDR), a nuclear receptor that acts as a ligand-dependent transcription factor.[2—4)] It is known that hereditary vitamin D-resistant rickets (HVDRR) is caused by mutations to the VDR gene. Over 20 VDR mutations that cause HVDRR have been reported. Most of these mutations occur at the DNA binding domain of the VDR, but few are localized at the ligand binding domain (LBD). Two LBD mutations which cause HVDRR substitute Arg-274 (Arg274Leu) and His-305 (His305Gln) which are essential for anchoring 1α,25$(OH)_2D_3$ in the LBD *via* hydrogen bonds with the 1α- and 25-hydroxyls of 1α,25$(OH)_2D_3$.[5,6)] The HVDRR mutation, Arg274Leu, causes a 1000-fold decrease in the affinity of 1α,25$(OH)_2D_3$ for VDR.[5)] Recently, Peleg *et al.* have demonstrated the rationale for using A ring-modified analogues to restore loss of binding and transcriptional activity of the Arg274Leu mutant.[7)] Another study, by Koh *et al.* extended these findings by examination of steroidal and non-steroidal vitamin D mimics for their ability to restore activities of this mutant VDR.[8,9)] This prompted us to report our preliminary results on two analogues, O1C3 and O2C3 that appear to bind the VDR mutant Arg274Ala in which, similarly to Arg274Leu, the amino acid substitution causes a hydrophobic hole that prohibits the 1α-OH group of 1α,25$(OH)_2D_3$ from forming hydrogen bond to the LBD of VDR.[10)]

As shown in Fig. 1, the analogues O1C3 and O2C3 possess 3-hydroxypropyl and 3-hydroxypropoxy groups, respectively, at the 2α position of 1α,25$(OH)_2D_3$. Each terminal hydroxyl group of the 2α-side chain (anchor side chain) was

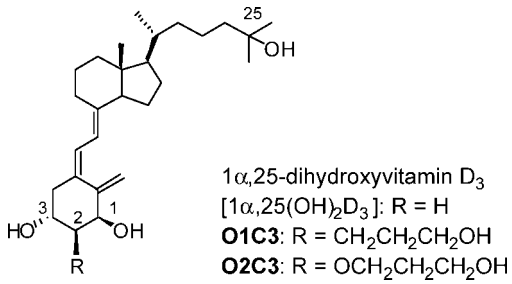


Fig. 1. Structures of 1α,25-Dihydroxyvitamin $D_3$ and Its 2α-Substituted Analogues of O1C3 and O2C3

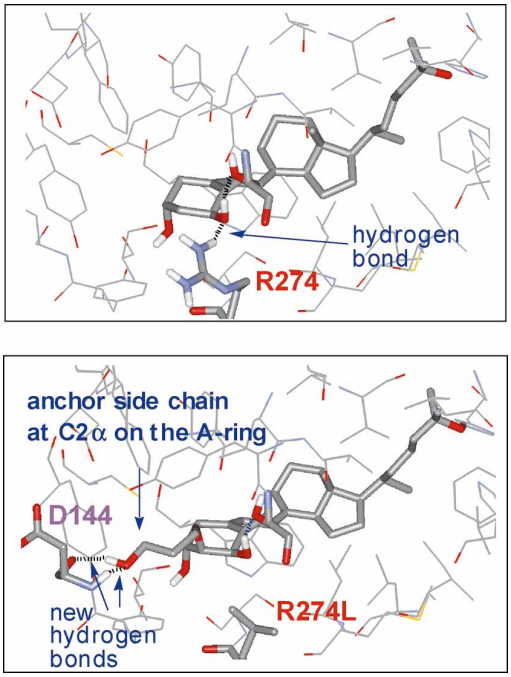


Fig. 2. Upper: Crystal Structure of VDR Bound to 1α,25$(OH)_2D_3$ by Moras *et al.*[6)] Showing the Normal Hydrogen Bond between the 1α-OH Group and Arg-274

Lower: Modeled Structure of O2C3 in LBD of VDR (R274L) Forming Additional Hydrogen Bonds between the Terminal Hydroxyl Group with Asp-144[13)]

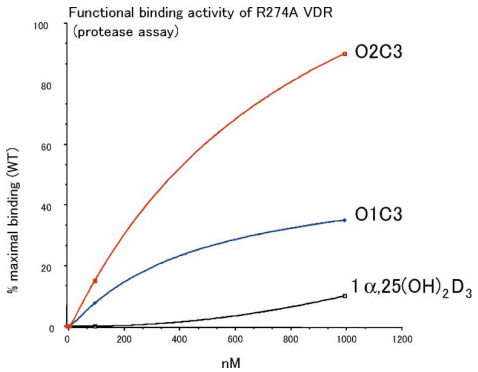


Fig. 3. $^{35}$S-WT VDR or $^{35}$S-R274A VDR Were Incubated in the Presence or Absence of 1α,25$(OH)_2D_3$ or the Analogues O1C3 and O2C3 and Then Subjected to Trypsin Digestion. Trypsin-resistant Fragments Were Separated by SDS-PAGE, and Visualized by Autoradiography of the Gels. The Intensity of the Main Ligand-stabilized Product (a 34 kDa Fragment) Was Assessed by Densitometry.[7)] The Results Are Expressed as % of Maximal Stabilization of WT-VDR by 1α,25$(OH)_2D_3$.

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designed to form an additional hydrogen bond to Asp-144. In previous studies we found that, indeed, these analogues had higher affinity for the wild-type VDR than $1\alpha,25(OH)_2D_3$.[11,12] We hypothesized that the putative hydrogen bond with Asp-144 may compensate for the loss of hydrogen bond between the $1\alpha$-OH group and Arg-274 in the Arg274Ala mutant VDR (Fig. 2).[13]

To examine this hypothesis, the substitution Arg274Ala was introduced into the wild-type VDR expression plasmid by site-directed mutagenesis, using the quickchange system of Startagene. This mutant does not have binding activity that can be assessed by saturation or competition assays with $^3$H-$1\alpha,25(OH)_2D_3$. Therefore, we used the protease (trypsin) sensitivity assay to compare binding of $1\alpha,25(OH)_2D_3$ and the analogues to this mutant. We have previously shown that stabilization of $^{35}$S-VDR conformation by the ligand *in vitro* correlates very well with ligand-dependent transcriptional potency of VDR in cultured cells.[14] $^{35}$S-VDR was incubated with either $1\alpha,25(OH)_2D_3$ or the analogues O1C3 and O2C3 and then subjected to trypsin digestion. The trypsin-resistant fragments were detected by autoradiography.[7] We found that O1C3 stabilized the mutant VDR conformation twice as well, and the analogue O2C3 stabilized the mutant VDR conformation 10-times as well as $1\alpha,25(OH)_2D_3$ (Fig. 3).

The results suggest that the relative binding affinities of O1C3 and O2C3 for the mutant VDR were 200% and 1000%, respectively, when the potency of $1\alpha,25(OH)_2D_3$ was normalized to 100%. It is possible that the length of the anchor side chain of O2C3 is more suitable than that of O1C3 for the putative hydrogen bonding to Asp-144.

In summary, we have found ligands that bind the mutant VDR, R274A, better than the natural hormone. The position and polarity of the substituted amino acid (Arg274Ala) lead to an identical disruption of ligand binding and transcriptional activities as in the HVDRR mutant Arg274Leu. We propose that this design strategy would provide a potential therapeutic approach for treatment of genetic disease.[7—9]

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**References and Notes**

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