Structural Refinement of seco-Steroidal Skeleton and the Biological Activity through Nuclear Receptors

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1α,25-Dihydroxyvitamin D_3 (1α,25$(OH)_2D_3$, **1**) regulates a variety of biological actions through vitamin D receptor (VDR), including calcium and phosphorus homeostasis, bone remodeling as well as cellular proliferation and differentiation. To enhance its potency and to study the structure and function relationship, we synthesized a series of 1α,25$(OH)_2D_3$ analogs with modification at the C-2α position (Figure 1). We found that substitution with 2α-methyl, 2α-(3-hydroxypropyl), and 2α-(3-hydroxypropoxy) groups increased the binding affinity for the VDR 2- to 4-fold compared to **1**. The crystal structures of the VDR with **2a**, **3c**, and **4b** provide a molecular explanation for the interaction between the 2α-substituents and the water molecules in the ligand binding domain of the VDR. The 2α-terminal hydroxyl group of **3c** and **4b** replaces one of the water molecules to form hydrogen bonds with the VDR, and additional effect of hydrophobic interaction with elongated 2α-CH_2 groups gave the stronger complex than the VDR-**1**. Based on accumulated knowledge in VDR agonists **1**-**5**, we synthesized 2α-substituted analogs of '*double side chain*' (gemini, **6**), 19-norvitamin D_3 (**7**), TEI-9647 (VDR antagonist **8**), 1-alkylated vitamin D_3 (**9**), and 14-*epi*-previtamin D_3 (**10**). Gemini analogs **6a-c** showed potent HL-60 cell differentiation activity (1.3-3.8 times compared to **1**), and MART-10 (**7c**) had potent antiproliferative activity on PZ-HPV-7 cells even at 10^{-10} M. Modification of TEI-9647 (**8**) at both C-2α with a 3-hydroxypropoxy group and C-24 with a propyl group generated an analog with an IC_{50} of 7.4 pM against 10 nM of 1α,25$(OH)_2D_3$. 1α-Methyl-2α-(3-hydroxypropyl)-25-hydroxyvitamin D_3 (**9a**) improved binding affinity for the mutant VDR (Arg274Leu). Compound **10a** (2α-methyl) showed moderate osteocalcin transcriptional activity on HOS cells. We theorize that modification at A-ring alone and in combination with functionalization of the other part of the vitamin D molecule would provide new and important information on the mechanism of vitamin D actions, that would lead to the development of new therapeutic regimes for the treatment of various diseases.

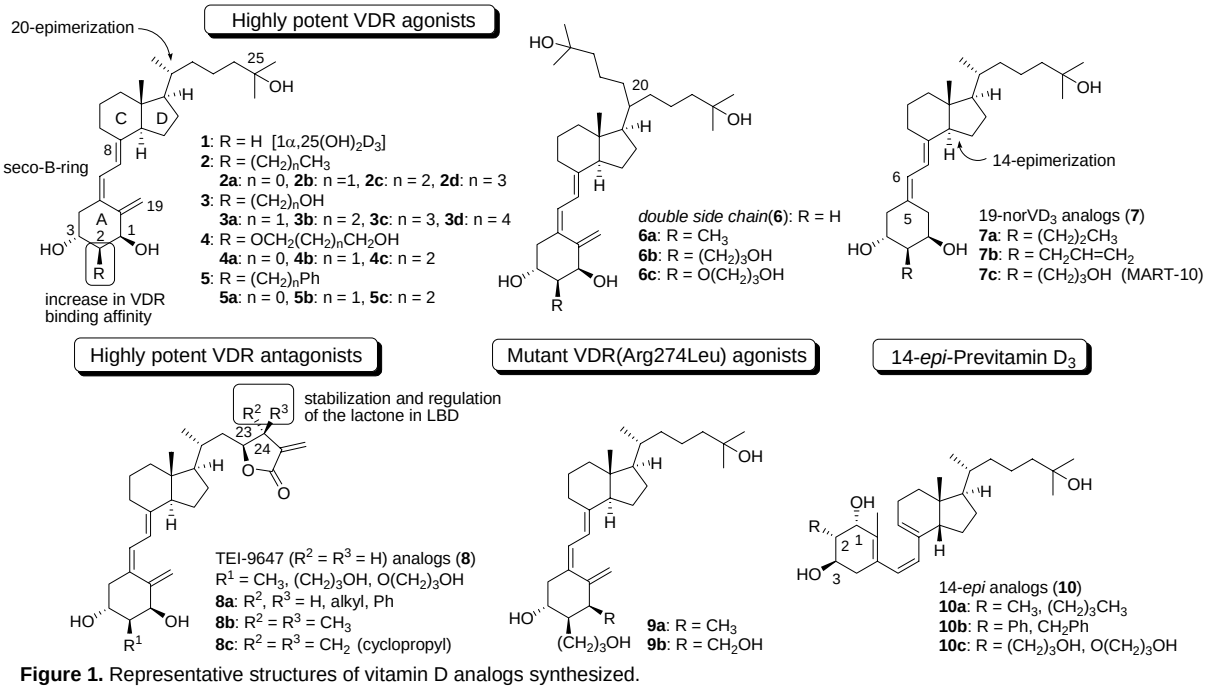

Figure 1. Representative structures of vitamin D analogs synthesized.