Abstract. The hormonally-active metabolite of vitamin D, 1α,25-dihydroxyvitamin D3 (I), has a wide variety of biological activities, which makes it a promising therapeutic agent for the treatment of cancer, psoriasis and osteoporosis. Insights into the structure-activity relationships of the A-ring of I are needed to assist the development of more potent and selective analogues, as well as to define the molecular mode of action. All possible A-ring stereoisomers of 2-methyl-1,25-dihydroxyvitamin D3 and 2,2-dimethyl-1,25-dihydroxyvitamin D3, which differ in stereochemistry at the C1-, C2- and C3-positions, were designed and efficiently synthesized by employing the convergent method. Biological evaluation of the analogues, in terms of the vitamin D receptor-binding affinity and HL-60 cell differentiation-inducing activity, as well as the transcriptional potency in ROS 17/2.8 cells, revealed the importance of substituents at the C2-position in certain orientations.

Cholecalciferol, known as vitamin D3, is metabolized via 25-hydroxyvitamin D3 to produce the hormonally-active form, 1α,25-dihydroxyvitamin D3 (I; Figure 1), the formation of which is strictly regulated (1). In addition to its classic role in calcium and phosphorus homeostasis, 1α,25-dihydroxyvitamin D3 dominates the cell cycle in many malignant cells, regulating proliferation, differentiation and apoptosis. Most of the biological activities of I are considered to be mediated by a ligand-inducible transcriptional factor, the vitamin D receptor (VDR), which belongs to the nuclear receptor superfamily. The specific interaction of the ligands with the ligand-binding domain of VDR has been a major focus of attention, since it triggers the whole sequence of biological responses: conformational change of the VDR, particularly of the AF-2 domain, heterodimerization with retinoid X receptors (RXRs), recruitment of co-activators and binding to the DNAs. Insights into the structure-function relationships of a variety of ligands are essential to understand how the subtype-free, singular VDR can deliver the diverse biological activities of I, as well as allowing the development of potent therapeutic agents with selective activity profiles for the treatment of cancers or osteoporosis.

Structural modification of I in the A-ring, which possesses two critical hydroxyl groups at the C1- and C3-positions, has become of interest in recent years, because the other three stereoisomers have proven to exhibit unique activity profiles, being different from the natural hormones (2, 3). Our study of all eight possible A-ring stereoisomers of 2-methyl-1,25-dihydroxyvitamin D3 and their 20-epimers showed that introduction of a simple methyl group into the parent I yielded analogues with distinct activity profiles (4-6). These methyl-introduced analogues, which differ in stereochemistry at the C1-, C2- and C3-positions, exhibited cell differentiation- or apoptosis-inducing activity towards HL-60 cells, depending on their A-ring structures (7). Some of the synthesized 2α-substituted analogues of I showed remarkably high affinity for VDR (8-10). 2β-Methyl introduction into the A-ring, on the other hand, in combination with the 1β-hydroxy or 3α-hydroxy groups, resulted in antagonists of the nongenomic, but not genomic, actions in NB-4 cells (11).

The X-ray crystal structure of VDR complexed with I (12) indicated the presence of an extra space in the vicinity of the A-ring, suggesting that the substituents of synthetic A-ring analogues could occupy this additional space. Our study of the 2-methyl analogues of I revealed that 2α-methyl-1α,25-dihydroxyvitamin D3 (2a) was a four-fold
better binder to VDR, whereas its 2-epimer, 2β-methyl-1α,25-dihydroxyvitamin D₃ (2b), showed one-eighth of the affinity of 1. In view of these results, all four possible A-ring stereoisomers of 2,2-dimethyl-1α,25-dihydroxyvitamin D₃ (3a-d), as A-ring analogues having methyl substituents projecting in both directions in this cavity, were synthesized to investigate how the second methyl group affected the activity profiles of the parent compounds.

Results and Discussion

Synthesis was carried out by using a convergent method pioneered by Trost et al. (13). The synthetic route to the A-ring precursors (11a,b) of 2,2-dimethyl-1,25-dihydroxyvitamin D₃ (3a-d) is shown in Figure 2 (14). Both of the 1,3-anti- and 1,3-syn-isomers (11a,b) were coupled with the CD-ring portion (13) in the presence of the palladium catalyst, followed by deprotection with tetrabutylammonium fluoride (TBAF), to give the vitamins 3a,b and 3c,d, respectively (Figure 3). The absolute stereochemistry of the A-ring at the C1- and C3-positions was determined by 1H NMR analyses of their bis-MTPA esters (15). Thus, the synthesis of the 2,2-dimethyl analogues of 1 was accomplished.

Table I shows the results of the VDR-binding affinity and the HL-60 cell differentiation-inducing potency of the synthesized analogues in comparison with 1 (14, 16). The 2,2-dimethyl analogue (3a) showed 3% of the VDR affinity of 1, suggesting that introduction of the second methyl group into 2a results in an approximately 100-fold reduction. The other three analogues (3b-d) exhibited weaker affinity compared with their mono-methyl-introduced analogues (4-6). The cell differentiation-inducing potency of the 2,2-dimethyl analogue 3a was approximately one-third of that of 1 (16), in accordance with the reduced VDR affinity. The transcription function of the analogues of 1 in the presence of co-activators of the 160-kDa protein family was examined and it was found that the 2,2-dimethyl analogue 3a retained the unique activity afforded by the 2α-methyl substitution, despite the reduced VDR affinity (14).

Treatment of rat osteosarcoma (ROS) 17/2.8 cells, an osteoblast-like cell line, with 1 resulted in a ligand-dependent increase in transcription of the bone-specific osteocalcin gene, which is closely related to bone formation. Figure 4 shows the potency of the vitamin D compounds (1, 3a) to induce transcription in ROS 17/2.8 cells, which were transfected with plasmids having the human osteocalcin gene promoter linked to the bacterial β-galactosidase (17). The 2,2-dimethyl compound (3a) exhibited comparable or even higher potency than the natural hormone 1. The relatively high potency of compound 3a in the established clone would suggest a unique activity profile to the bone-related cells, being different from that of 1.

In summary, various methyl-introduced A-ring analogues were synthesized and it was found that modification at the C2-position in the A-ring of 1 plays a key role in the design of selective agents. It is interesting to note that 2-methylene-19-nor-(20S)-1,25-dihydroxyvitamin D₃ (2MD) (18), which was reported to facilitate bone formation both in vitro and in vivo, has a methylene structure at the C2-position in the A-ring. The structures of the A-ring analogues of 1 could

![Figure 1. Structures of 1α,25-dihydroxyvitamin D₃ (1) and its A-ring analogues; 2α-methyl-1α,25-dihydroxyvitamin D₃ (2a), 2β-methyl-1α,25-dihydroxyvitamin D₃ (2b) and 2,2-dimethyl-1α,25-dihydroxyvitamin D₃ (3a).](image-url)
induce distinctive conformational changes in the VDR to modulate the stability of the transcriptional machinery in certain cells. The differential stability of the protein complexes caused by ligands would be an interesting approach for separating the activities of 1. The results will be reported elsewhere in detail.

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References


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