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# Vitamin D and Its Synthetic Analogs

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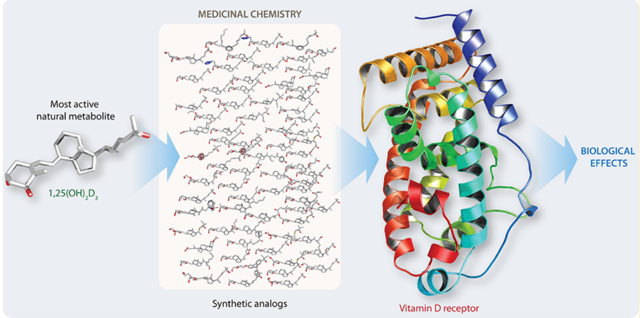
*Supporting Information*



**ABSTRACT:** For many individuals, in particular during winter, supplementation with the secosteroid vitamin $D_3$ is essential for the prevention of bone disorders, muscle weakness, autoimmune diseases, and possibly also different types of cancer. Vitamin $D_3$ acts via its metabolite 1$\alpha$,25-dihydroxyvitamin $D_3$ [**$1,25(OH)_2D_3$**] as potent agonist of the transcription factor vitamin D receptor (VDR). Thus, vitamin D directly affects chromatin structure and gene regulation at thousands of genomic loci, i.e., the epigenome and transcriptome of its target tissues. Modifications of **$1,25(OH)_2D_3$** at its side-chain, A-ring, triene system, or C-ring, alone and in combination, as well as nonsteroidal mimics provided numerous potent VDR agonists and some antagonists. The nearly 150 crystal structures of VDR's ligand-binding domain with various vitamin D compounds allow a detailed molecular understanding of their action. This review discusses the most important vitamin D analogs presented during the past 10 years and molecular insight derived from new structural information on the VDR protein.

## INTRODUCTION

An UV-B (290−315 nm)-dependent, nonenzymatic reaction in human skin converts the cholesterol precursor 7-dehydrocholesterol into previtamin $D_3$ that further isomerizes into vitamin $D_3$ (calciferol, **1**)[1] (Figure 1). Similarly, UV-B-radiated plants and mushrooms are able to produce the isomer vitamin $D_2$ (ergocalciferol, **2**) based on their membrane sterol ergosterol.[2] Both secosteroids are themselves biologically inert and have to be activated by hydroxylation first at C-25, leading to the prehormones 25-hydroxyvitamin $D_3$ [**$25(OH)D_3$**, (calcidiol, **3**)] and **$25(OH)D_2$**, and then at C-1, creating **$1,25(OH)_2D_3$** (calcitriol, **4**)[3] and **$1,25(OH)_2D_2$**, respectively.

**$25(OH)D_3$** is the metabolically most stable and abundant vitamin D metabolite, and its serum levels serve as a biomarker of the vitamin D status of individuals.[4] The biologically active form of vitamin $D_3$, **$1,25(OH)_2D_3$**, acts via activation of the transcription factor VDR as a nuclear hormone that directly affects gene regulation.[5] The physiological role of vitamin D is the regulation of calcium homeostasis for maintaining bone mineralization[6] as well as the modulation of innate and adaptive immunity[7] for improving the response to infections by microbes, such as *Mycobacterium tuberculosis*,[8] and preventing autoimmune diseases, such as multiple sclerosis.[9]

Lifestyle decisions, such as staying predominantly indoors and covered by textile outdoors, combined with changes in seasons and climate cause, for many individuals, insufficient exposure to UV-B and thus low endogenous production of vitamin $D_3$. Human diet is often rather low in vitamin D because only fatty fish and UV-B irradiated mushrooms have reasonable quantities of the vitamin $D_3$ or vitamin $D_2$, respectively. The fortification of milk, margarine, and juices with vitamin $D_3$ or vitamin $D_2$ is applied in some countries. Moreover, in winter months daily supplementation with at least 25 $\mu$g (1000 IU) of vitamin $D_3$ is recommended in order to prevent vitamin D deficiency.[10] The latter not only would result in rickets in children and in a higher risk of bone fractures due to osteoporosis or osteomalacia in adults,[11] but also will compromise the function of the immune system and the claimed preventive actions of vitamin D against cardiovascular diseases, diabetes, neuropsychiatric disorders, and cancer.[12] Supplementation with vitamin $D_3$ clearly increased in the general population, e.g., the sales of vitamin D supplementation products increased within 1 decade nearly 15-fold.[13]

There is no doubt that a sufficient vitamin D status is important for bone health,[14] but overdosing with vitamin $D_3$, **$1,25(OH)_2D_3$**, or its synthetic analogs may result in tissue calcification.[15] Symptoms of hypercalcemia are (i) digestive distress, such as vomiting, nausea, and stomach pain, (ii) fatigue, dizziness, and confusion, (iii) excessive thirst, and (iv) frequent urination. However, hypercalcemia occurs rarely and no other severe side effects or toxicity of vitamin D overdosing is known. Nevertheless, higher doses of vitamin $D_3$ are not

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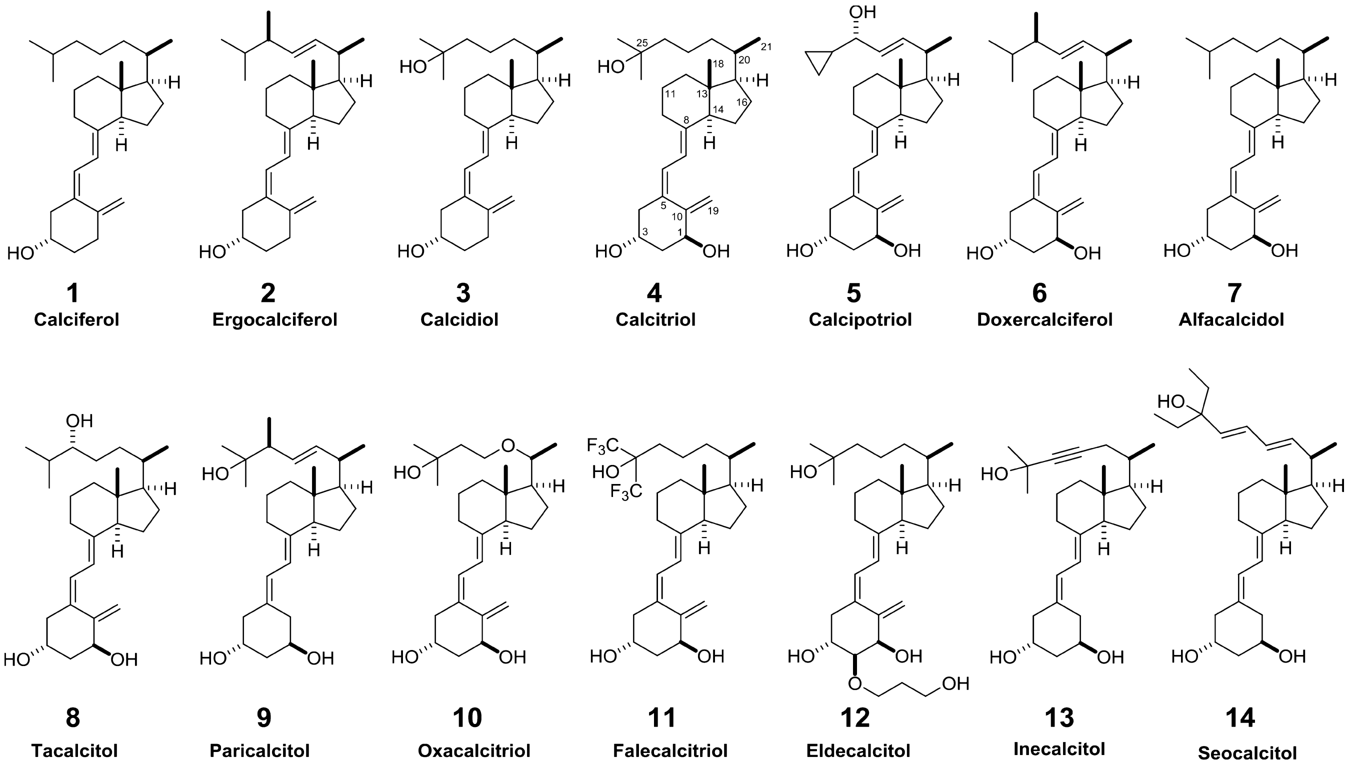


**Figure 1.** Vitamin D metabolites and analogs available on the market.

### Table 1. Vitamin D Compounds on the Market[a]

- Vitamin $D_3$ (**1**, calciferol) is used worldwide in the prevention of vitamin D deficiency and associated diseases, such as rickets [Vigantol (Bayer), Delsterol (DuPont), Duphafral-$D_3$ (multiple pharma companies), Lutavit $D_3$ (BASF), Vi-D3, Videkhol, Vigosan (multiple pharma companies)].
- Vitamin $D_2$ (**2**, ergocalciferol) is also used in the prevention of vitamin D deficiency and associated diseases, such as rickets (marketed with different names by multiple pharma companies).
- Calcidiol (**3**, **25(OH)$D_3$**) is used in the treatment of chronic hypocalcemia, renal osteodystrophy [Calderol (Upjohn), Hidroferol (Faes Farma)], rickets [Dedrogyl (Roussel), Hidroferol (Faes Farma)].
- Calcitriol [**4**, **1,25(OH)$_2$$D_3$**] is prescribed for renal osteodystrophy [Rocatrol (Roche), Calcijex (Abbott)], osteoporosis [Rocatrol (Roche)] and psoriasis [Silkis (Galderma)].
- Calcipotriol [**5**, 22-ene-26,27-dehydro-1,25(OH)$_2$$D_3$] is used for psoriasis [Davionex (Leo Pharmaceuticals), Dovonex (Warner Chilcott)].
- Doxercalciferol [**6**, 1$\alpha$(OH)$D_2$, Hectorol (Bone Care International)] is prescribed for secondary hyperparathyrodism.
- Alfacalcidol (**7**, 1$\alpha$(OH)$D_3$) is used for renal osteodystrophy [Alfarol (Chugai Pharmaceutical), One-Alpha (Leo Pharmaceuticals)], secondary hyperparathyrodism [Alfarol (Chugai Pharmaceutical)], osteoporosis [Alfarol (Chugai Pharmaceutical), Alpha $D_3$ (Teva Pharmaceuticals)] and rickets [Alfarol (Chugai Pharmaceutical)].
- Tacalcitol (**8**, 1$\alpha$,24(OH)$_2$$D_3$) is prescribed for psoriasis [Bonalfa (Teijin), Curatoderm (Merck KGaA)].
- Paricalcitol [**9**, 19-nor-1,25(OH)$_2$$D_2$, Zemplar, (Abbott Laboratories)] is used for secondary hyperparathyrodism.
- Oxacalcitriol (**10**, 22-oxa-1,25(OH)$_2$$D_3$) is used for secondary hyperparathyrodism and psoriasis [Oxarol (Chugai Pharmaceuticals)] in Japan.
- Falecalcitriol [**11**, 1,25(OH)$_2$-26,27-$F_6$-$D_3$] is prescribed for secondary hyper-parathyrodism in Japan [Hornel (Taisho Pharmaceuticals and Sumitomo Pharmaceuticals), Fulstan (Kissei Pharmaceuticals)].
- Eldecalcitol [**12**, 2$\alpha$-(3-hydroxypropoxy)-1,25(OH)$_2$$D_3$] is prescribed for osteoporosis only in Japan [Edirol (Chugai Pharmaceutical)].

[a] Only a few vitamin D compounds have reached the market.[88,89] Their applications, commercial name, and company are listed. The structures of the compounds are shown in Figure 1.

recommended as nutritional supplement for reaching non-skeletal effects of the vitamin. Similarly, the main goal of the development of vitamin D analogs is to identify compounds with a low calcemic effect versus a potent antiproliferative, prodifferentiating, and/or immune-modulatory function.

In total, more than 3000 synthetic vitamin D analogs were developed by various pharmaceutical companies and academic research groups in order to advance the biological properties of the natural compound for a applications in the therapy of (i) hyperproliferative diseases, such as different types of cancer, (ii) psoriasis, an autoimmune disease of the skin,[16] or (iii) bone disorders, such as osteoporosis.[17] However, so far only a few vitamin D compounds made it to the market (Table 1). In addition to vitamin $D_3$ being extensively used as a nutritional supplement, the commercially most successful vitamin D analog is calcipotriol (**5**), which is topical agent in clinical use for the treatment of psoriasis. Together with the compounds doxercalciferol (**6**), alfacalcidol (**7**), tacalcitol (**8**), paricalcitol (**9**), oxacalcitriol (**10**), falecalcitriol (**11**), and eldecalcitol (**12**) it had been discussed in previous reviews[18,19] (Figure 1). In contrast, despite promising in vitro results, analogs such as inecalcitol (**13**) or seocalcitol (**14**) were unsuccessful in phase II clinical trials of acute myeloid leukemia (www.hybrigenics.com/news/articles/list/type/2) or pancreatic cancer,[20] respectively. Interestingly, some immune-system-related vitamin D target genes, such as cathelicidin antimicrobial peptide

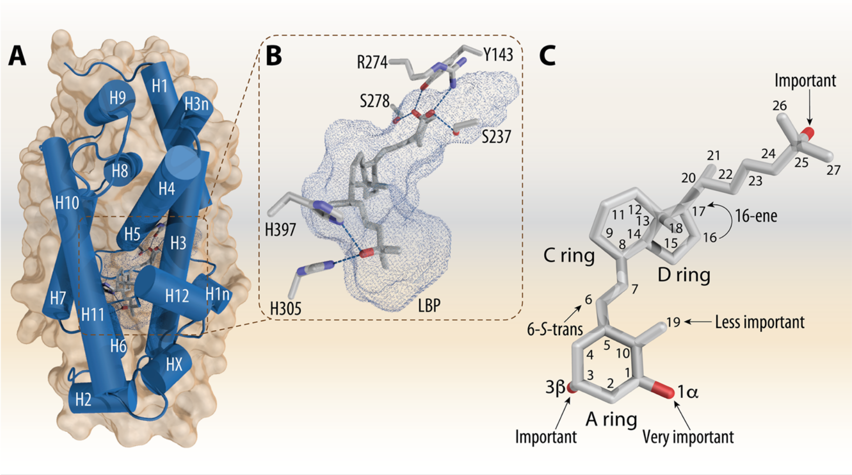


**Figure 2.** $\mathbf{1{,}25(OH)_2D_3}$ complexed to the VDR-LBD. The VDR-LBD has a conserved 3D architecture, which is made of a three-layer $\alpha$-helical sandwich. In the lower part of the LBD the LBP is located. All the helices are labeled from N-terminus toward C-terminus and numbered in white color (A). Details on the LBP with bound $\mathbf{1{,}25(OH)_2D_3}$ and critical amino acids that provide anchoring contacts for the three OH groups (B). Details on the conformation of the bound $\mathbf{1{,}25(OH)_2D_3}$ molecule with the annotated OH groups and highlights to its contribution of its activity. The numbering of the carbons atoms is indicated (C). The figure is based on the PDB code 1DB1.

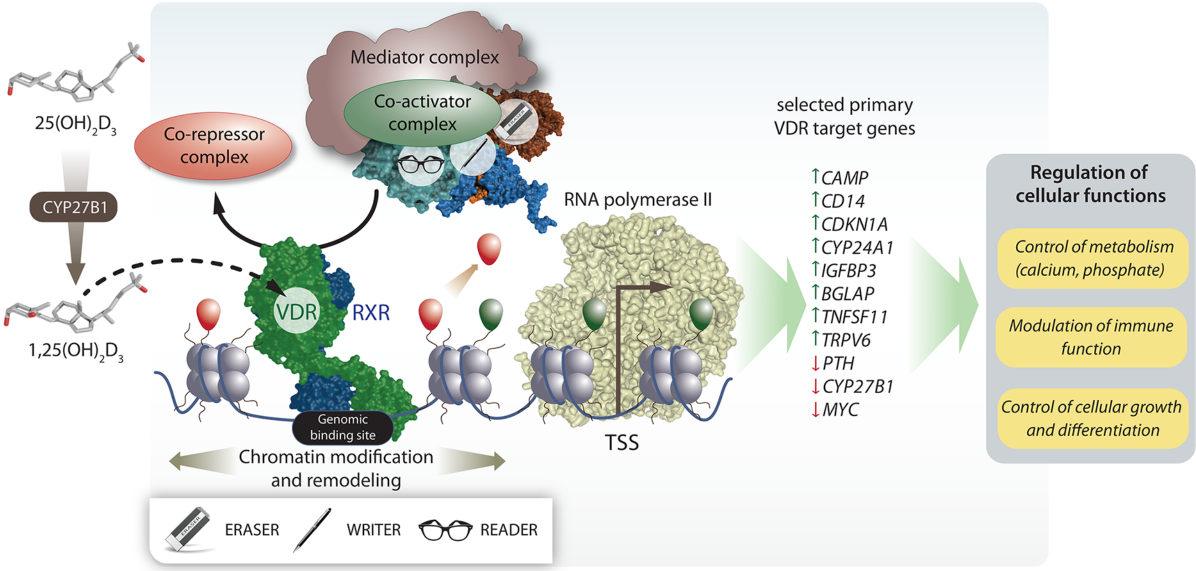
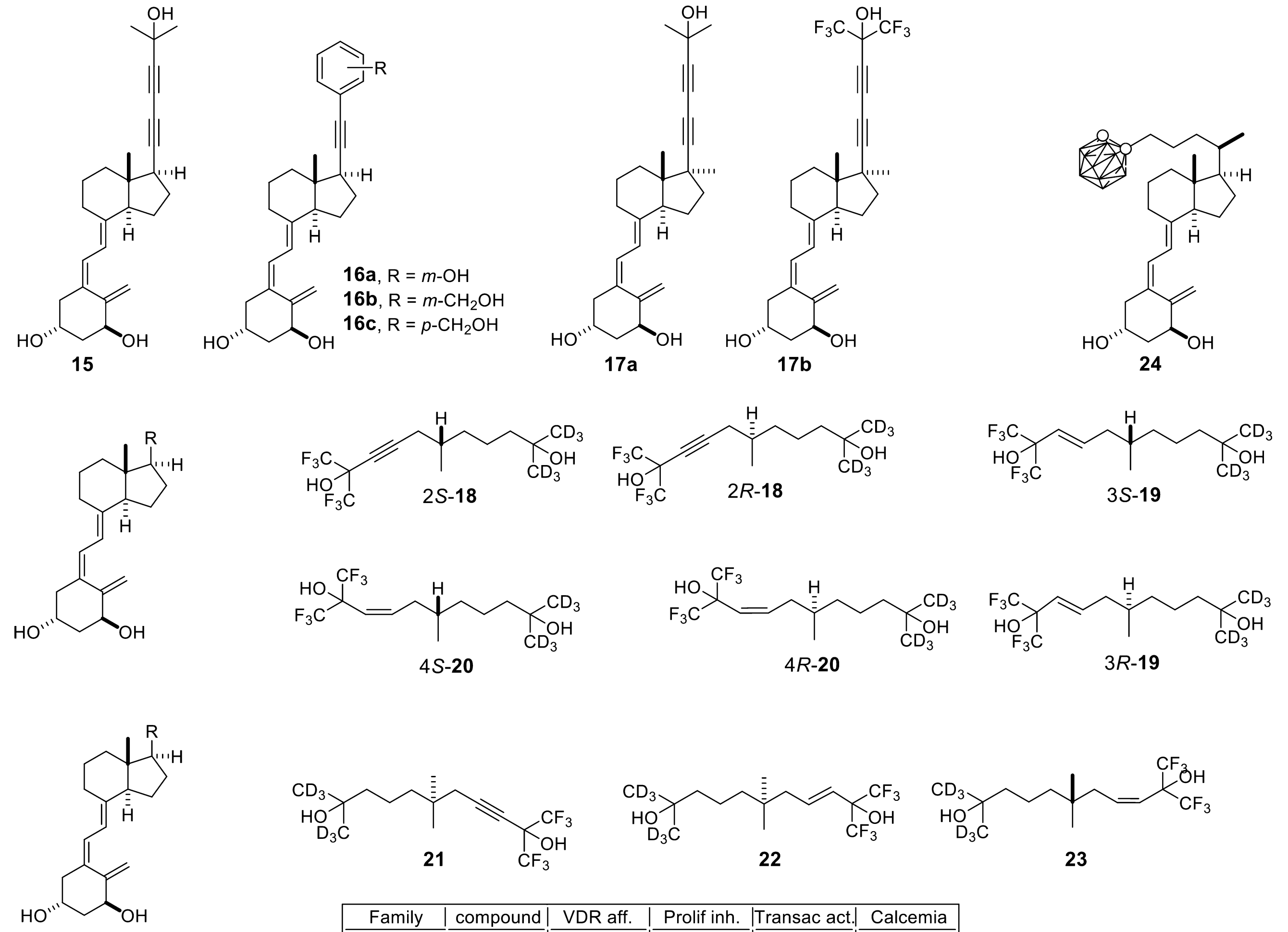


**Figure 3.** Vitamin D signaling. $\mathbf{25(OH)D_3}$ is converted by the enzyme CYP27B1 to its biologically most active form $\mathbf{1{,}25(OH)_2D_3}$, which binds to the transcription factor VDR. Upon binding of $\mathbf{1{,}25(OH)_2D_3}$ or synthetic agonists, a conformational change in the LBD is induced leading to cofactor exchanges shifting the balance toward recruitment of coactivator proteins. Co-repressor proteins dissociate from the VDR-RXR heterodimer. In parallel, the mediator complex and chromatin modifying enzymes (readers, writers, and erasers) are recruited in order to handle histone proteins of local nucleosomes around genomic VDR binding sites. In addition, chromatin remodeling complexes are recruited and rearrange nucleosomes at vitamin D-sensitive chromatin regions. Altogether, these chances lead to looping of the distal regulatory elements toward the basal transcriptional machinery with RNA polymerase II and other nuclear adaptor proteins initiating the start of $\mathbf{1{,}25(OH)_2D_3}$-dependent transcription from hundreds to thousands of TSS regions throughout the whole human genome. The ultimate outcome is the increase or decrease of the of primary vitamin D target gene expression followed by changes of indicated cellular functions.

(*CAMP*) and *CD14* (encoding for a Toll-like receptor 4 co-receptor), are very responsive,[21,22] while there are no vitamin D target genes with comparable inducibility involved in the management of cellular growth and differentiation. The failure of anticancer trials and the success in the therapy of an immune disease as well as prominent gene regulatory effects in immune cells suggest that VDR ligands, in addition to bone-related functions, may rather have a therapeutic potential in immune diseases than in cancer.

The majority of synthetic VDR ligands are direct derivatives of $\mathbf{1{,}25(OH)_2D_3}$, but within the past years an increasing number of vitamin D mimics were published. $\mathbf{1{,}25(OH)_2D_3}$ had been modified at its side-chain, A-ring (often together with side-chain changes), triene system, and C-ring. These modifications follow the strategy to increase the VDR binding affinity while in parallel modulating the metabolic stability of the molecules.[18] A reasonable number of new vitamin D analogs have been published within the past years and will be



| Family | compound | VDR aff. | Prolif inh. | Transac act. | Calcemia |
|---|---|---|---|---|---|
| 20(22)-yn- | **15** | + | | | |
| | **16a** | = | | | |
| | **16b** | = | | | |
| | **16c** | - | | | |
| | **17a** | ++ | | | |
| | **17b** | ++ | | | |
| Gemini | **2*S*-18** | +++ | | | |
| | **2*R*-18** | +++ | | | |
| | **2*S*-19** | ++ | | | |
| | **2*R*-19** | +++ | | | |
| | **2*S*-20** | + | | | |
| | **2*R*-20** | ++ | | | |
| | **21** | | +++ | | |
| | **22** | | +++ | | |
| | **23** | | +++ | | |
| Carboranyl | **24** | ++ | ++ | ++ | - |

Ref: 1,25(OH)$_2$D$_3$: = = = =

**Figure 4.** Side-chain-modified vitamin D analogs. The table summarizes the biological properties of the compounds: reference, **1,25(OH)$_2$D$_3$**; (=) similar value; (+) >10× higher; (++) >100× higher; (+++) >1000× higher; (−) >10× lower; VDR aff, VDR affinity; Prolif inh, proliferation inhibition; Transac act, VDR transactivation activity; calcemia, [Ca$^{2+}$] level changes in serum.

discussed in this review. Moreover, the number of solved VDR crystal structures with synthetic ligands has significantly grown.

## ■ CENTRAL ROLE OF VDR IN VITAMIN D SIGNALING

VDR is the only protein expressed by the human genome that is able to bind **1,25(OH)$_2$D$_3$** and its analogs at subnanomolar concentrations.[23] Thus, all physiological functions of vitamin D compounds are mediated by VDR and its target genes.[24] The *VDR* gene is expressed most prominently in intestine, kidneys, and bone, but in most of the other 400 human tissues and cell types some *VDR* expression is found.[25] This means that not only tissues that relate to calcium homeostasis and bone formation but also immune cells respond to vitamin D.[26]

VDR is an endocrine receptor and member of the superfamily of nuclear receptors; i.e., the mechanisms of its action are comparable to the receptors for glucocorticoids and estrogen.[27] VDR's ligand-binding domain (LBD) is structurally conserved and comprises 11−15 α-helices, modestly varies between solved crystal complexes, and depends on the folding of the intrinsically disordered region between helices H1 and H3 and the presence of a helix HX between helices H11 and H12[28,29] (Figure 2A). The lower part of the LBD contains a ligand-binding pocket (LBP), which is a cavity with a volume of ~700 Å$^3$ (with possible expansion beyond 1000 Å$^3$) being formed by some 40 mostly nonpolar amino acids.[30] Three pairs of polar amino acids within the LBP fix via hydrogen bonds each one of the three OH groups (at C-1$\alpha$, C-3$\beta$, and C-25) of **1,25(OH)$_2$D$_3$**. The 1$\alpha$-OH group interacts with Y143 (helix H1) and S278 (helix H5), the 3$\beta$-OH group contacts S237 (helix H3) and R274 (helix H5), and the 25-OH group interferes with H305 (loop between helices H6 and H7) and H397 (helix H11)[28] (Figure 2B).

VDR ligands induce a conformational shift to the LBD, which replaces co-repressor proteins by coactivator proteins; i.e., ligand binding induces a different protein−protein interaction profile of the receptor.[31] VDR agonists cause an efficient dissociation of co-repressors from the LBD and allow the specific binding of coactivators and the mediator complex (Figure 3). Coactivators also attract chromatin modifying enzymes that write, erase, or read post-translational marks of histones, such as acetyl and methyl groups, to histone proteins of nucleosomes in the vicinity of genomic VDR binding sites.[32] Moreover, also members of chromatin remodeling complexes interact in a ligand-dependent fashion with VDR and cause a rearrangement of nucleosomes at vitamin D-sensitive chromatin regions.[33] These epigenetic changes allow looping of VDR-bound enhancers toward accessible transcription start sites (TSSs) at hundreds to thousands of loci throughout the human genome.[34] These enhancer-TSS assemblies are triggered by ligand-activated VDR and finally result in an increase or decrease in the expression of hundreds of primary vitamin D target genes (Figure 3).

The structure of the human VDR-LBD complexed with **1,25(OH)$_2$D$_3$** was solved in the year 2000.[28] Since then altogether 143 human, rat, and zebrafish VDR-LBDs have been crystallized with a large number of synthetic analogs[35] (Table S1). In general, the analogs behave like **1,25(OH)$_2$D$_3$** by stabilizing the LBD in more or less the same conformation, since the three OH groups of each vitamin D compound take up a nearly identical position. This suggests that there is only one agonistic conformation of the LBD for which the interaction between the ligand's 25-OH group and the LBP amino acids H305 and H397 are most important (Figure 2C).

On the basis of the vitamin D analog's chemical modification, all solved VDR-ligand-complexes can be divided to the six groups: (i) A-ring modifications, (ii) side-chain modifications, (iii) triene system modifications, (iv) combined A-ring and side-chain modification, (v) modifications in the CD-ring, and (vi) nonsteroidal analogs. All modifications aim to either (i) maintain the three anchoring OH groups at the same position as in **1,25(OH)$_2$D$_3$** and/or (ii) fill the LBP most efficiently in order to form additional hydrogen network and/or hydrophobic contacts. More variant modifications of **1,25(OH)$_2$D$_3$** aim to alter the ligand conformation or to bounce the shape of the LBP by adding an additional side-chain at positions C-20 or C-22. Moreover, de novo designed nonsteroidal compounds carry modifications, such as the exchange the classical secosteroid ring structure by rings with aromatic character. The aim with these molecules is to maintain the hydrophobic interactions with amino acid residues lining the inner surface of the LBP as well as to increase the stacking interaction with aromatic amino acid residues.

In this review we discuss different classes of vitamin D analogs and, where applicable, provide molecular understanding from VDR crystal structures.

## ■ SIDE-CHAIN MODIFICATIONS

The first locked side-chain vitamin D analogs nor-21-20(22),23(24)-diyn-1,25(OH)$_2$D$_3$ (**15**), nor-21,23,24,25,26,27-20(22)-yn-22-(3-hydroxyphenyl)-1,25-(OH)$_2$D$_3$ (**16a**), nor-21,23,24,25,26,27-20(22)-yn-22-[3-(hydroxymethyl)phenyl]-1,25(OH)$_2$D$_3$ (**16b**), and nor-21,23,24,25,26,27-20(22)-yn-22-[4-(hydroxymethyl)phenyl]-1,25(OH)$_2$D$_3$ (**16c**) have been synthesized by convergent route through a Wittig−Horner approach starting from Inhoffen−Lythgoe diol[36] (Figure 4). These analogs lead to significant activation of VDR-dependent transcription in comparison to **1,25(OH)$_2$D$_3$**. An unique structural modification on the C-22-diyne analog, a C-17-methyl substitution, was provided through a vinyl(pinacolo)boronate approach and resulted in the C-17-methyl-substituted vitamin D analogs nor-21-20(22),23(24)-diyn-17-methyl-1,25(OH)$_2$D$_3$ (**17a**) and nor-21-20(22),23(24)-diyn-17-methyl-26,26,26,27,27,27-hexafluoro-1,25(OH)$_2$D$_3$ (**17b**).[37] The C-22-aromatic-substituted analogs are less potent in activating VDR than the C-22-diyne isomers. The C-17-methyl analogs bind more efficiently to VDR than **1,25(OH)$_2$D$_3$**.

The two side-chain analog Gemini comprises an unaltered side-chain of **1,25(OH)$_2$D$_3$** and a second chain at C-20.[38,39] Although the volume of Gemini is increased by some 25%, it still fits into VDR's LBP.[30] One side-chain of Gemini takes the same place as that of **1,25(OH)$_2$D$_3$**, whereas an extra subcavity opens within the LBP for the second side-chain.[40] The increase in transcriptional activity of Gemini[41] motivated the preparation of Gemini-type analogs with side-chains containing double or triple bonds and isohexafluoro-2-propanol or isohexadeutero-2-propanol side-chain ends. Compounds **18**−**20** have been synthesized with both configurations at C-20 by a convergent approach through Wittig−Horner coupling starting from Inhoffen−Lythgoe diol.[42] (*R*)-Analogs showed higher antiproliferative potency in MCF10CA1 human breast cancer cells than their (*S*)-counterparts, and both were 100−1000 times more potent than **1,25(OH)$_2$D$_3$**. Furthermore, both configurations of the Gemini derivatives are also more potent than **1,25(OH)$_2$D$_3$** in inducing the differentiation of NB4 human leukemia cells. Thus, Gemini compounds have enhanced potency in inhibiting proliferation and inducing differentiation with reduced induction of hypercalcemia when compared to **1,25(OH)$_2$D$_3$**. Moreover, C-20 methyl-substituted Gemini analogs (**21**−**23**) are also potent in the inhibition of HL-60 human leukemia cell proliferation and the induction of *CAMP* gene expression.[43]

1$\alpha$-Hydroxy-25,26,27-trinor-24-*o*-carboranyl-vitamin D$_3$ (**1,25cD$_3$**, **24**, Figure 4) is a rather new vitamin D analog, in which an *o*-carborane moiety replaces the 25-OH group.[44] Despite the lack of this critical group, **1,25cD$_3$** is as effective as **1,25(OH)$_2$D$_3$** in inhibiting the growth of MCF-7 human breast cancer cells and in inducing the differentiation of HaCaT human keratinocytes. VDR binds **1,25cD$_3$** 2 times tighter than **1,25(OH)$_2$D$_3$** and is equally potent as the natural hormone in inducing reporter gene activity while not showing adverse calcemic effects. Moreover, like most other vitamin D analogs, the conformation of the complex of **1,25cD$_3$** with

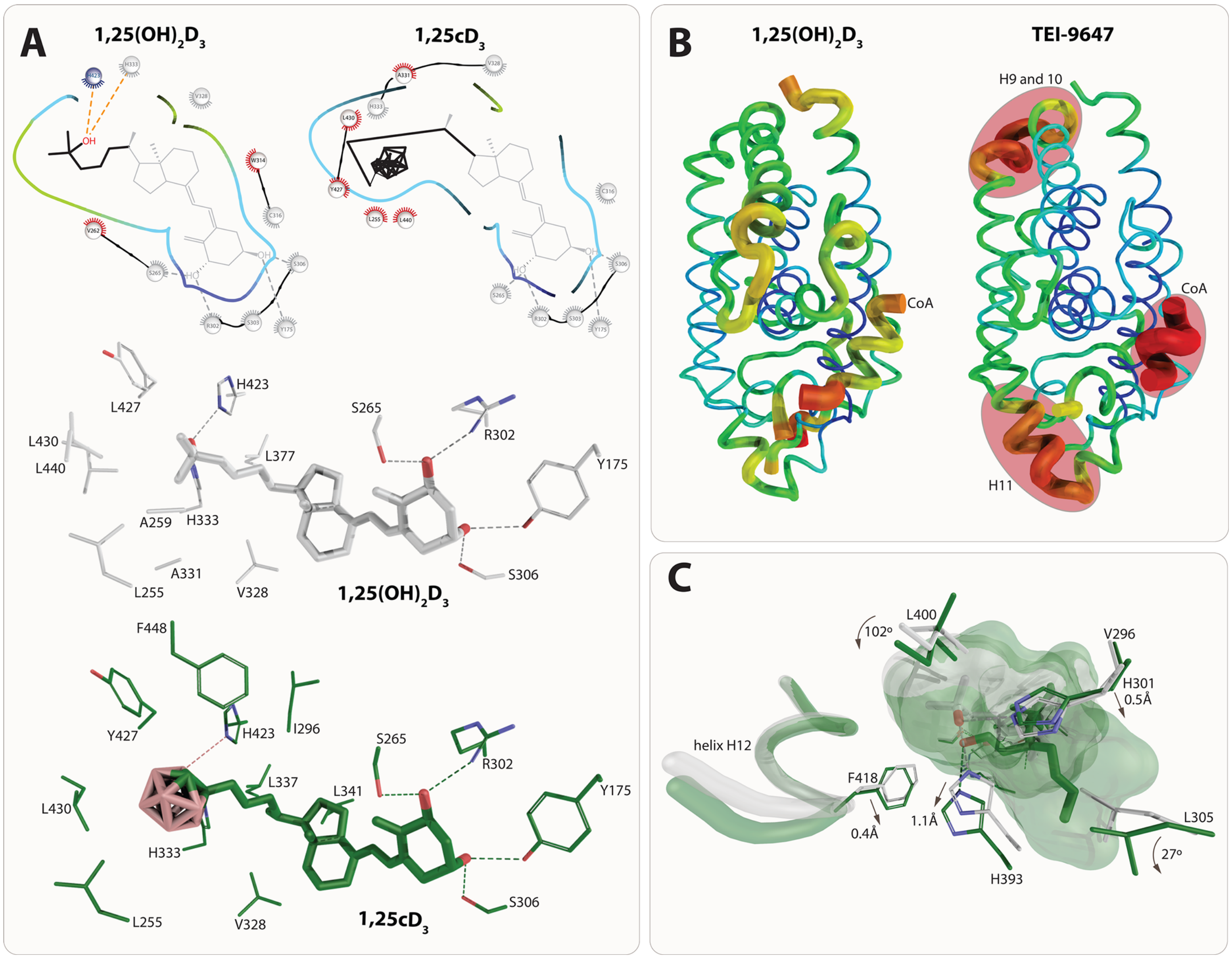


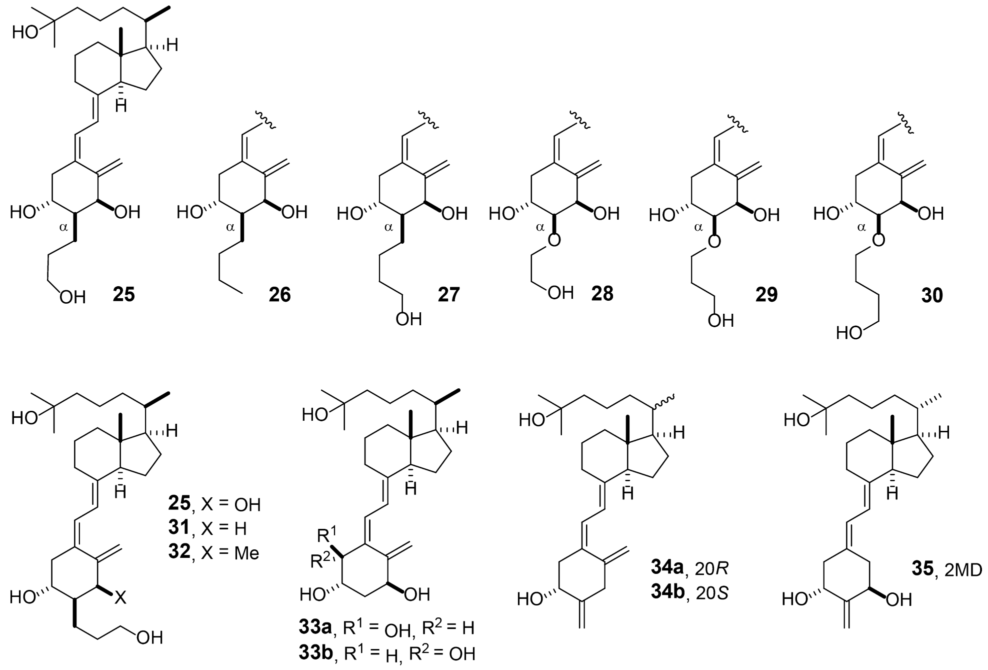
**Figure 5.** Structure−function relationship of VDR ligands (I). The carborane group of **$1,25cD_3$** creates additional hydrophobic interactions that compensate for the loss of the 25-OH group. All residues that have conserved interactions are shown in gray (top). Detailed interaction with residues based on PDB code structures 2HC4 (**$1,25(OH)_2D_3$**) and 5E7V (**$1,25cD_3$**). The displayed interactions are identified under cutoff 3.5 Å (A). Destabilization of the VDR-LBD upon binding of 23,36-lactone analogs. Representation of crystal structure *b*-factors using structures PDB codes 1RK3 (**$1,25(OH)_2D_3$**, left) and 3A2H (TEI-9647, right). Regions with the highest *b*-factors are highlighted in red and they are helices H9−10 that may affect heterodimerization with RXR, helix H11 affecting the position of helix H12, and the coactivator peptide showing very high *b*-factors overall. The most stabile part of the VDR is shown in blue through green, yellow and red monitoring the highest *b*-factor values (B). Structural implication of 22S-alkyl-2-methylene-19-nor-**$1,25(OH)_2D_3$** binding. The position of the helix H12 takes the same conformation in both 22S-alkyl-2-methylene-19-nor-**$1,25(OH)_2D_3$** and **$1,25(OH)_2D_3$**. Many residues around the two aliphatic chains move or rotate, such as H301 (loop helices H6/7), H393 (helix H11), or F418 (helix H12). Structural elements from 22S-alkyl-2-methylene-19-nor-$1,25(OH)_2D_3$ (PDB code 2ZXM) and **$1,25(OH)_2D_3$** (PDB code 1RK3) complexes are highlighted in green and white color, respectively (C).

VDR's LBD is highly similar to that of **$1,25(OH)_2D_3$**; i.e. the protein shows the same topology. Nevertheless, the loop between helices H6 and H7, which is a critical region for the activation of the receptor, and the last part of helix H11 show shifts by 0.6 Å. The carborane side-chain is 2.4 Å longer than that of **$1,25(OH)_2D_3$**, but it is hydrophobic and therefore favors the interaction with hydrophobic amino acid in this part of the VDR-LBP. This compensates for the loss of the 25-OH group (Figure 5A). Thus, the collection of small changes stabilizes helices H3, H11, and H12 and overall causes higher stability of VDR's LBD.

## ■ A-RING MODIFICATION

The biological profiles of the C-2-substituted vitamin D analogs 2-(3′-hydroxypropyl)-$1,25(OH)_2D_3$ (**25**), 2-butyl-$1,25(OH)_2D_3$ (**26**), 2-(4′-hydroxybutyl)-$1,25(OH)_2D_3$ (**27**), 2-(2′-hydroxyethoxy)-$1,25(OH)_2D_3$ (**28**), 2-(3′-hydroxypropoxy)-$1,25(OH)_2D_3$ (**29**), and 2-(4′-hydroxybutoxy)-$1,25(OH)_2D_3$ (**30**) (Figure 6) indicated that C-2$\beta$-substituted analogs have higher affinity for the serum vitamin D binding protein (DBP) and lower affinity for VDR but are superior to C-2$\alpha$ analogs in reporter gene assays.[45]

The analog 2-(3′-hydroxypropyl)-1$\alpha$-methyl-$25(OH)_2D_3$ (**32**) was synthesized through a Pd-catalyzed ring-closure of enyne, and coupling with vinyl bromide and showed a 2-fold higher potency than **$1,25(OH)_2D_3$** in reporter gene assays.[46] Interestingly, when VDR's amino acid R274, which contacts the 1$\alpha$-OH group of the ligand (Figure 2), is mutated to a hydrophobic residue (R274L), the compound is even 7 times more potent than the natural hormone, suggesting that the 1$\alpha$-



| Family | compound | VDR aff. | 24OH trans | HL-60 diff |
|---|---|---|---|---|
| 2α-subst | **25** | ++ | - | = |
| | **26** | -- | -- | -- |
| | **27** | = | -- | = |
| | **28** | = | -- | = |
| | **29** | ++ | -- | = |
| | **30** | -- | - | -- |
| | **31** | | ++ | |
| | **32** | | -- | |
| 4-OH | **33a** | - | | |
| | **33b** | = | | |
| 2-methylen | **34a** | -- | -- | - |
| | **34b** | = | - | + |
| | **35** | = | ++ | ++ |
| Ref: $1,25(OH)_2D_3$: | | = | = | = |

**Figure 6.** Vitamin D analogs with A-ring modifications. The table summarizes the biological properties of the compounds: reference, **$1,25(OH)_2D_3$**; (=) similar value; (+) >10× higher; (−) >10× lower; (--), >100× lower; VDR aff, VDR affinity; 24OH trans, *CYP24A1* transactivation activity; HL60 diff, HL-60 cell differentiation induction.

methyl group is stabilized primarily by hydrophobic interactions.

The compounds 1*α*,4*α*,$25(OH)_3D_3$ (**33a**) and 1*α*,4*β*,25-$(OH)_3D_3$ (**33b**) were created through a Pd-catalyzed ring-closure and coupling and showed lower affinity for VDR than **$1,25(OH)_2D_3$**.[47] However, the 4*β*-analog (**33b**) displays higher VDR affinity and potency in reporter gene assays than the 4*α*-compound (**33a**).

The analogs 2-methylene-25-$(OH)D_3$ (**34a**) and 2-methylen-20-epi-25-$(OH)D_3$ (**34b**) belong to the family of the potent lead compound 2-methylene-$1,25(OH)_2D_3$ (**35**, **2MD**) and were synthesized through a Pd-catalyzed coupling between an enol triflate and an enyne.[48] These molecules are defined by the relocation of the exocyclic methylene group from C-10 to C-2 and the inversion of the C-20 configuration. Compound **34a** shows lower affinity for VDR than **$1,25(OH)_2D_3$**, lower potency in inducing HL-60 cell differentiation and in reporter gene assays, while compound **34b** displays the same affinity for VDR as **$1,25(OH)_2D_3$**, higher potency in HL-60 cell differentiation induction, and lower activity in reporter gene assays.

## TRIENE SYSTEM MODIFICATIONS

The compounds PRI-1731 (**36**), PRI-1732 (**37**), PRI-1733 (**38**), and PRI-1734 (**39**) represent a series of vitamin D analogs with a branched side-chain (*E*)-stereochemistry at the C-5/C-6 double bond, both configurations at C-24 and a C-22/C-23 double bond or an OH group at C-22[49,50] (Figure 7). They have moderate prodifferentiating activities on HL-60 cells and their maximal inhibition of proliferation ranged from

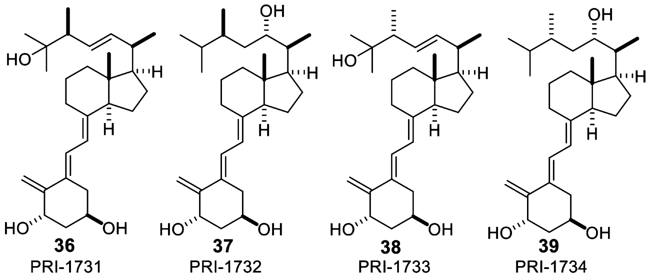
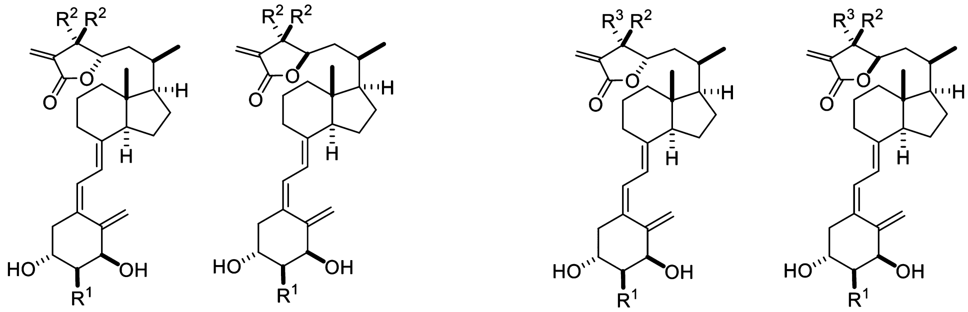


**Figure 7.** Triene system modified vitamin D analogs.



**40**, $R^1 = R^2 = H$ (**TEI-9647**)
**42**, $R^1 = Me, R^2 = H$
**44**, $R^1 = (CH_2)_3OH, R^2 = H$
**46**, $R^1 = O(CH_2)_3OH, R^2 = H$
**48**, $R^1 = H, R^2 = Me$
**50**, $R^1 = H, R^2 = \text{-}CH_2CH_2\text{-}$
**52**, $R^1 = Me, R^2 = Me$

**41**, $R^1 = R^2 = H$ (**TEI-9648**)
**43**, $R^1 = Me, R^2 = H$
**45**, $R^1 = (CH_2)_3OH, R^2 = H$
**47**, $R^1 = O(CH_2)_3OH, R^2 = H$
**49**, $R^1 = H, R^2 = Me$
**51**, $R^1 = H, R^2 = \text{-}CH_2CH_2\text{-}$
**53**, $R^1 = Me, R^2 = Me$

**54a**, $R^1 = R^2 = H, R^3 = Me$
**54b**, $R^1 = Me, R^2 = H, R^3 = Me$
**54c**, $R^1 = (CH_2)_3OH, R^2 = H, R^3 = Me$
**54d**, $R^1 = O(CH_2)_3OH, R^2 = H, R^3 = Me$
**55a**, $R^1 = R^2 = H, R^3 = Me$
**55b**, $R^1 = H, R^2 = Me, R^3 = Me$
**55c**, $R^1 = H, R^2 = (CH_2)_3OH, R^3 = Me$
**55d**, $R^1 = \mathbf{H}, R^2 = O(CH_2)_3OH, R^3 = Me$
**56a**, $R^1 = R^2 = H, R^3 = Et$
**56b**, $R^1 = Me, R^2 = H, R^3 = Et$
**56c**, $R^1 = (CH_2)_3OH, R^2 = H, R^3 = Et$
**56d**, $R^1 = O(CH_2)_3OH, R^2 = H, R^3 = Et$
**57a**, $R^1 = R^2 = H, R^3 = Et$
**57b**, $R^1 = H, R^2 = Me, R^3 = Et$
**57c**, $R^1 = H, R^2 = (CH_2)_3OH, R^3 = Et$
**57d**, $R^1 = H, R^2 = O(CH_2)_3OH, R^3 = Et$
**58a**, $R^1 = R^2 = H, R^3 = Pr$
**58b**, $R^1 = Me, R^2 = H, R^3 = Pr$
**58c**, $R^1 = (CH_2)_3OH, R^2 = H, R^3 = Pr$
**58d**, $R^1 = O(CH_2)_3OH, R^2 = H, R^3 = Pr$
**59a**, $R^1 = R^2 = H, R^3 = Pr$
**59b**, $R^1 = H, R^2 = Me, R^3 = Pr$
**59c**, $R^1 = H, R^2 = (CH_2)_3OH, R^3 = Pr$
**59d**, $R^1 = H, R^2 = O(CH_2)_3OH, R^3 = Pr$
**60a**, $R^1 = R^2 = H, R^3 = Bu$
**60b**, $R^1 = Me, R^2 = H, R^3 = Bu$
**60c**, $R^1 = (CH_2)_3OH, R^2 = H, R^3 = Bu$
**60d**, $R^1 = O(CH_2)_3OH, R^2 = H, R^3 = Bu$
**61a**, $R^1 = R^2 = H, R^3 = Bu$
**61b**, $R^1 = H, R^2 = Me, R^3 = Bu$
**61c**, $R^1 = H, R^2 = (CH_2)_3OH, R^3 = Bu$
**61d**, $R^1 = H, R^2 = O(CH_2)_3OH, R^3 = Bu$
**62a**, $R^1 = R^2 = H, R^3 = i\text{-Bu}$
**62b**, $R^1 = Me, R^2 = H, R^3 = i\text{-Bu}$
**62c**, $R^1 = (CH_2)_3OH, R^2 = H, R^3 = i\text{-Bu}$
**62d**, $R^1 = O(CH_2)_3OH, R^2 = H, R^3 = i\text{-Bu}$
**63a**, $R^1 = R^2 = H, R^3 = i\text{-Bu}$
**63b**, $R^1 = H, R^2 = Me, R^3 = i\text{-Bu}$
**63c**, $R^1 = H, R^2 = (CH_2)_3OH, R^3 = i\text{-Bu}$
**63d**, $R^1 = H, R^2 = O(CH_2)_3OH, R^3 = i\text{-Bu}$

**64a**, $R^1 = R^2 = H, R^3 = Me$
**64b**, $R^1 = Me, R^2 = H, R^3 = Me$
**64c**, $R^1 = (CH_2)_3OH, R^2 = H, R^3 = Me$
**64d**, $R^1 = O(CH_2)_3OH, R^2 = H, R^3 = Me$
**65a**, $R^1 = R^2 = H, R^3 = Me$
**65b**, $R^1 = H, R^2 = Me, R^3 = Me$
**65c**, $R^1 = H, R^2 = (CH_2)_3OH, R^3 = Me$
**65d**, $R^1 = \mathbf{H}, R^2 = O(CH_2)_3OH, R^3 = Me$
**66a**, $R^1 = R^2 = H, R^3 = Et$
**66b**, $R^1 = Me, R^2 = H, R^3 = Et$
**66c**, $R^1 = (CH_2)_3OH, R^2 = H, R^3 = Et$
**66d**, $R^1 = O(CH_2)_3OH, R^2 = H, R^3 = Et$
**67a**, $R^1 = R^2 = H, R^3 = Et$
**67b**, $R^1 = H, R^2 = Me, R^3 = Et$
**67c**, $R^1 = H, R^2 = (CH_2)_3OH, R^3 = Et$
**67d**, $R^1 = H, R^2 = O(CH_2)_3OH, R^3 = Et$
**68a**, $R^1 = R^2 = H, R^3 = Pr$
**68b**, $R^1 = Me, R^2 = H, R^3 = Pr$
**68c**, $R^1 = (CH_2)_3OH, R^2 = H, R^3 = Pr$
**68d**, $R^1 = O(CH_2)_3OH, R^2 = H, R^3 = Pr$
**69a**, $R^1 = R^2 = H, R^3 = Pr$
**69b**, $R^1 = H, R^2 = Me, R^3 = Pr$
**69c**, $R^1 = H, R^2 = (CH_2)_3OH, R^3 = Pr$
**69d**, $R^1 = H, R^2 = O(CH_2)_3OH, R^3 = Pr$
**70a**, $R^1 = R^2 = H, R^3 = Bu$
**70b**, $R^1 = Me, R^2 = H, R^3 = Bu$
**70c**, $R^1 = (CH_2)_3OH, R^2 = H, R^3 = Bu$
**70d**, $R^1 = O(CH_2)_3OH, R^2 = H, R^3 = Bu$
**71a**, $R^1 = R^2 = H, R^3 = Bu$
**71b**, $R^1 = H, R^2 = Me, R^3 = Bu$
**71c**, $R^1 = H, R^2 = (CH_2)_3OH, R^3 = Bu$
**71d**, $R^1 = H, R^2 = O(CH_2)_3OH, R^3 = Bu$
**72a**, $R^1 = R^2 = H, R^3 = i\text{-Bu}$
**72b**, $R^1 = Me, R^2 = H, R^3 = i\text{-Bu}$
**72c**, $R^1 = (CH_2)_3OH, R^2 = H, R^3 = i\text{-Bu}$
**72d**, $R^1 = O(CH_2)_3OH, R^2 = H, R^3 = i\text{-Bu}$
**73a**, $R^1 = R^2 = H, R^3 = i\text{-Bu}$
**73b**, $R^1 = H, R^2 = Me, R^3 = i\text{-Bu}$
**73c**, $R^1 = H, R^2 = (CH_2)_3OH, R^3 = i\text{-Bu}$
**73d**, $R^1 = H, R^2 = O(CH_2)_3OH, R^3 = i\text{-Bu}$

| Family | compound | VDR aff. | Antag act. |
|---|---|---|---|
| lactone | **40** | -- | ref |
| | **42** | -- | ++ |
| | **44** | -- | +++ |
| | **46** | -- | ++ |
| | **48** | - | + |
| | **50** | ++ | ++ |
| | **52** | - | ++ |
| | **41** | -- | -- |
| | **43** | - | - |
| | **45** | - | - |
| | **47** | - | - |
| | **49** | - | - |
| | **51** | -- | -- |
| | **53** | + | + |

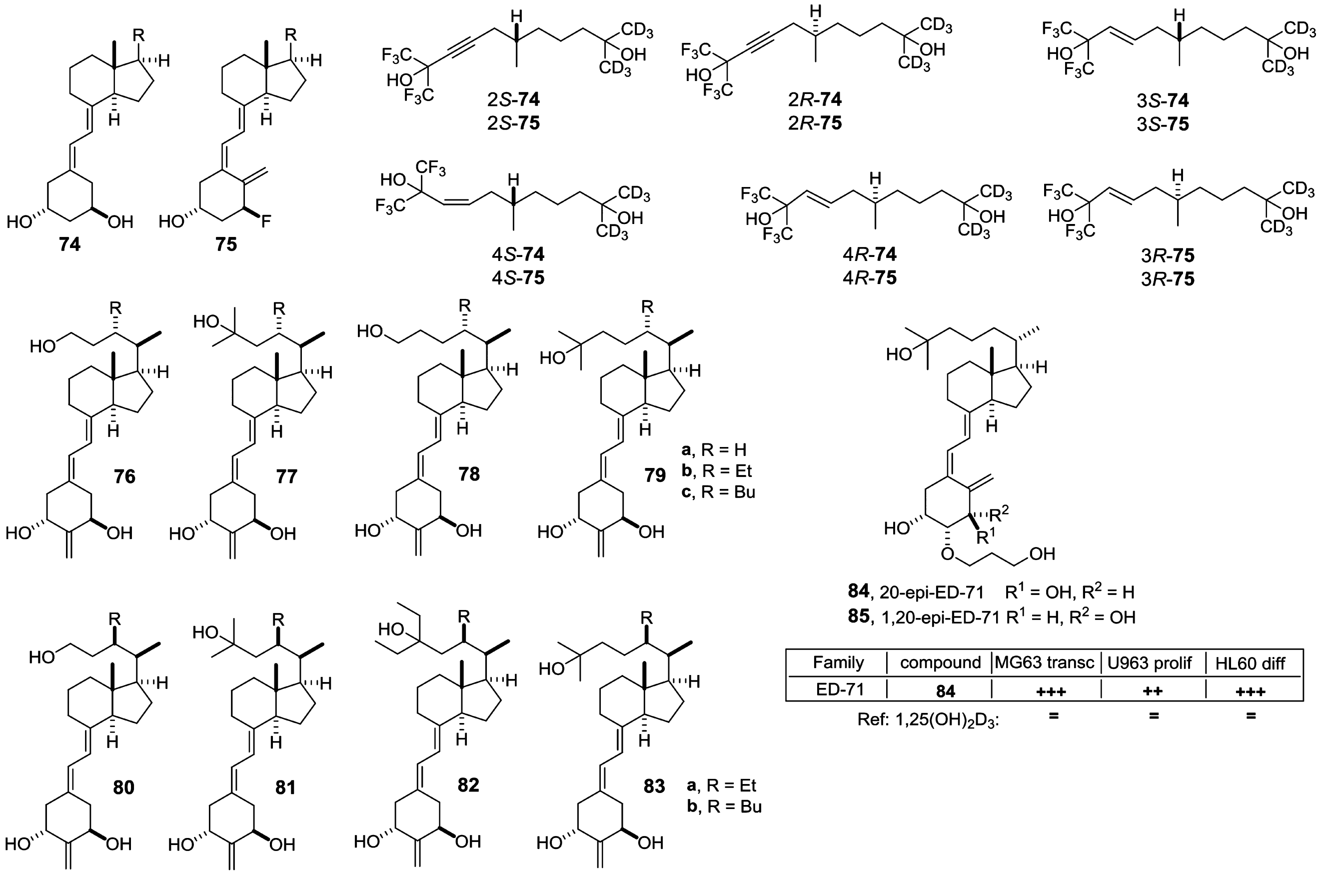
Ref: $1,25(OH)_2D_3$: =

**Figure 8.** Vitamin D analogs with side-chain and A-ring modifications (I). The table summarizes the biological properties of the compounds: reference, **$1,25(OH)_2D_3$**; (=) similar value; (+) >10× higher; (++) >100× higher; (−) >10× lower; (--) >100× lower; VDR aff, VDR affinity; Antag act, antagonistic activity.

10% to 15% of that for **$1,25(OH)_2D_3$** and 20−30% of that for $1,25(OH)_2D_2$.

## SIDE-CHAIN AND A-RING MODIFICATIONS

25-Dehydro-1*α*-hydroxy-vitamin $D_3$-26,23 lactones with double modifications of C-24 and C-2*α* were synthesized via a convergent approach by Pd-catalyzed ring closure of a enyne and subsequent coupling with a functionalized vinyl bromide.[51,52] Numerous analogs have been synthesized with variations in their C-23 configuration, C-24 mono- or disubstitution [H, Me, Et, *c*-Pr, *n*-Pr, *n*-Bu, *i*-Bu (**40**, **41**, **48**, **50**, **52**, **54**, **56**, **58**, **60**, **62**, **64**, **66**, **68**, **70**, **72**)] and C-2*α* substitution [H, Me, $CH_2CH_2CH_2OH$, $OCH_2CH_2CH_2OH$, (**42**−**47**, **49**, **51**, **53**, **55**, **57**, **59**, **61**, **63**, **65**, **67**, **69**, **71**, **73**)] (Figure 8). The principal characteristic of these analogs is their antagonist activity, probably due to locking the VDR-LBD in a conformation where it does not effectively interact with coactivator proteins. This can be seen also from the values of the *β*-factors found in the crystal structure (PDB code 3A2H) of the lactone analog TEI-9647, where the coactivator peptide shows very high values compared to **$1,25(OH)_2D_3$**. In addition, helices H9 and 10 as well as H11 and to some



| Family | compound | MG63 transc | U963 prolif | HL60 diff |
|---|---|---|---|---|
| ED-71 | **84** | +++ | ++ | +++ |
| Ref: 1,25(OH)$_2$D$_3$: | | = | = | = |

| Family | compound | MCF10 prol | NB4 diff |
|---|---|---|---|
| Gemini | **2*S*-74** | ++ | +++ |
| | **2*R*-74** | ++ | +++ |
| | **3*S*-74** | +++ | ++ |
| | **3*R*-74** | ++ | +++ |
| | **4*S*-74** | = | + |
| | **4*R*-74** | ++ | ++ |
| | **2*S*-75** | + | ++ |
| | **2*R*-75** | + | ++ |
| | **3*S*-75** | ++ | +++ |
| | **3*R*-75** | - | + |
| | **4*S*-75** | ++ | + |
| | **4*R*-75** | - | = |
| Ref: 1,25(OH)$_2$D$_3$: | | = | = |

| Family | compound | VDR aff |
|---|---|---|
| 22-subst | **76a** | -- |
| | **76b** | -- |
| | **76c** | - |
| | **77a** | = |
| | **77b** | -- |
| | **77c** | - |
| | **78a** | -- |
| | **78b** | - |
| | **78c** | - |
| | **79a** | + |
| | **79b** | + |
| | **79c** | + |
| Ref: 1,25(OH)$_2$D$_3$: | | = |

| Family | compound | VDR aff |
|---|---|---|
| 22-subst | **80a** | -- |
| | **80b** | -- |
| | **80c** | - |
| | **81a** | = |
| | **81b** | -- |
| | **81c** | -- |
| | **82a** | ++ |
| | **82b** | - |
| | **82c** | - |
| | **83a** | = |
| | **83b** | + |
| | **83c** | ++ |
| Ref: 1,25(OH)$_2$D$_3$: | | = |

**Figure 9.** Vitamin D analogs with side-chain and A-ring modifications (II). The table summarizes the biological properties of the compounds: reference, **1,25(OH)$_2$D$_3$**; (=) similar value; (+) >10× higher; (++) >100× higher; (+++) >1000× higher; (−) >10× lower; (--) >100× lower; VDR aff, VDR affinity; MCF10 prol, MCF10 cell proliferation inhibition; NB4 diff, NB4 cell differentiation induction; MG63 trans, MG63 cell transactivation activity; U963 prol, U963 cell proliferation inhibition; HL60 diff, HL-60 cell differentiation induction.

extent H12 show higher fluctuation leading to overall destabilization of the LBD (Figure 5B). Structure−activity relationship studies demonstrated that the exomethylene group of the lactone is indispensable, the C-23*S* configuration provides higher activity, and an appropriate combination of C-24 and C-2*α* substitution obtains the highest antagonist potency.

19-nor (**74**) and 1*α*-F (**75**) Gemini analogs containing triple bonds and isohexafluoro-2-propanol or isohexadeutero-2-propanol side-chain end groups[42] (Figure 9) were synthesized. Again (*R*)-analogs are more potent in inhibiting MCF10CA1 cell proliferation than their (*S*)-counterparts. Both isomers are active already at 100−1000 times lower concentrations than **1,25(OH)$_2$D$_3$**. In both configurations the Gemini analogs are equally potent to **1,25(OH)$_2$D$_3$** in inducing NB4 cell differentiation but are less calcemic than the natural hormone.

A series of 2-methylene-19-nor vitamin D analogs with or without C-22*S* alkyl substitution, such as 2-methylene-19,25,26,27-tetranor-vitamin D$_3$ (**76**), 2-methylene-19,25-dinor-vitamin D$_3$ (**77**), 2-methylene-19,26,27-trinor-vitamin D$_3$ (**78**), and 2-methylene-19-nor-vitamin D$_3$ (**79**) and their C-22*S* alkyl derivatives (**a**, R = H; **b**, R = Et; **c**, R = Bu) have been prepared and biologically tested.[53] The side-chain modifications in **76**, **77**, and **78** reduce the VDR binding affinity 10-fold compared to **1,25(OH)$_2$D$_3$**. Interestingly, an increasing size of the C-22 substituent in 2-methylene-19-nor-vitamin D$_3$ (**79**) results in a decreased VDR binding affinity compared to **1,25(OH)$_2$D$_3$**. Compounds with a normal side-chain (**79a**,

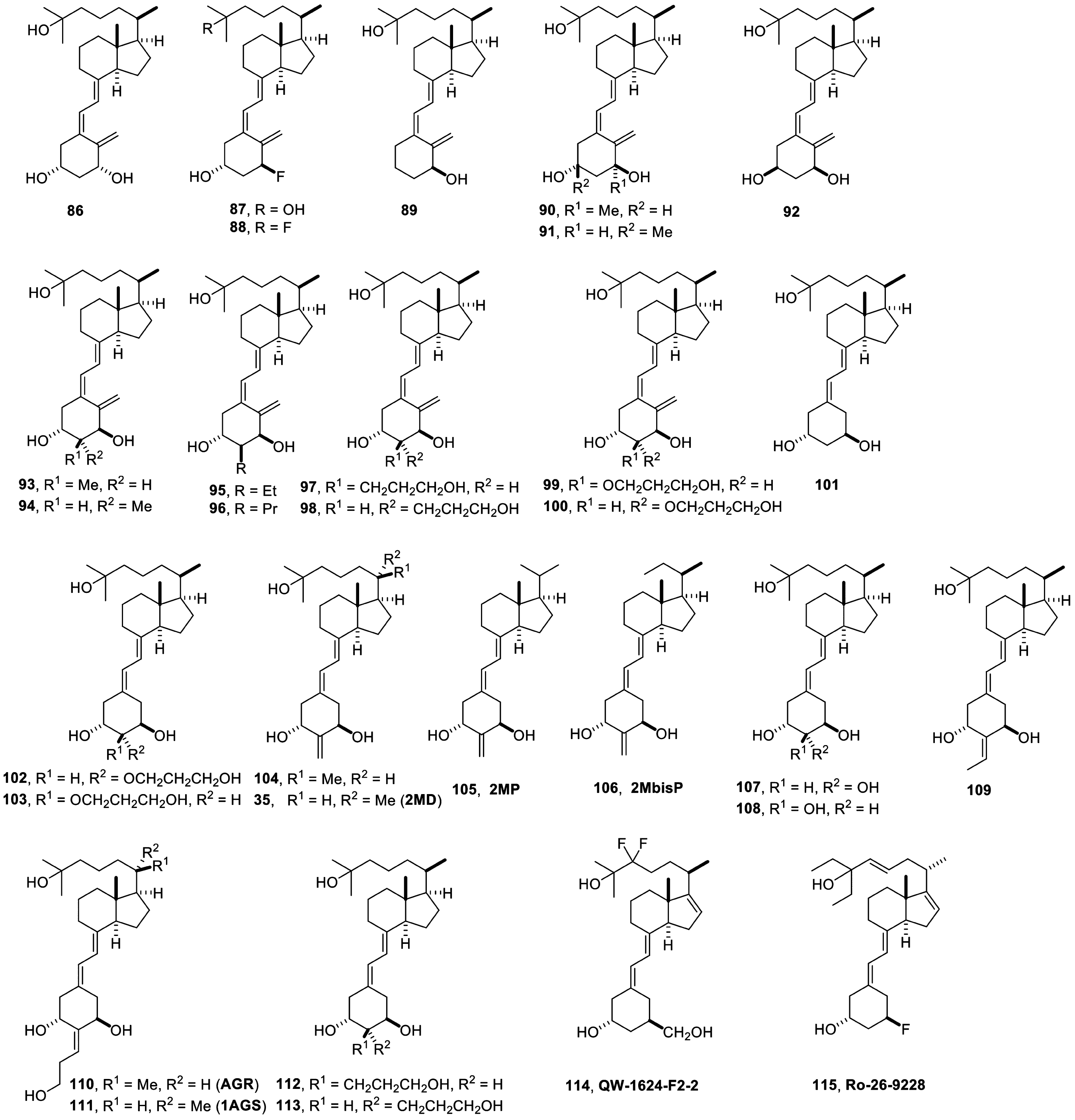
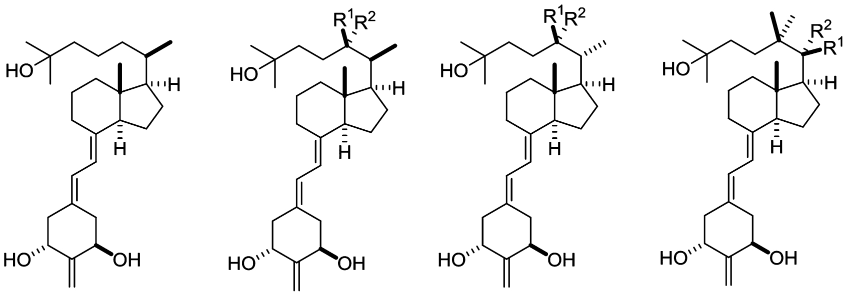


**Figure 10.** Vitamin D analogs with side-chain and A-ring modifications (III).

**79b**, and **79c**) show strong activation in reporter gene assays and compounds without C-22 substitution (**76a**, **77a**, **78a**, and **79a**) even full agonist activity. In contrast, C-22*S* butyl-substituted molecules (**76c**, **76c**, **78c**, and **79c**) present little transactivation potency, while C-22*S* ethyl-substituted compounds (**76b**, **77b**, **78b**, and **79b**) display intermediate activity. Moreover, the analogs **76a**, **77a**, **78a**, and **79a** induce the recruitment of the VDR partner receptor retinoid X receptor (RXR, Figure 3) and of a coactivator peptide in a concentration dependent manner, while C-22*S*-substituted compounds cause only moderate effects.

VDR-LBD crystal structures complexed with further 22*S*-alkyl-2-methylene-19-nor-1,25(OH)$_2$D$_3$ derivatives (**80**–**83**)[54] confirmed that the compounds trigger the creation of an extra cavity of the LBP by rotating L305 about 27° outward, in order to shelter the butyl group (Figure 5C). Ligands act as VDR antagonists when they do not interact with the C-terminal helix H12 of the receptor. Interestingly even though the position of helix H12 is maintained in the agonistic position, most likely forced by the presence of the coactivator peptide, there is a shift or rotation of multiple residues away from the 22-butyl analog. These changes weaken the interaction with H301 (loop H6/7), H393 (H11), or F418 (H12) and destabilize this

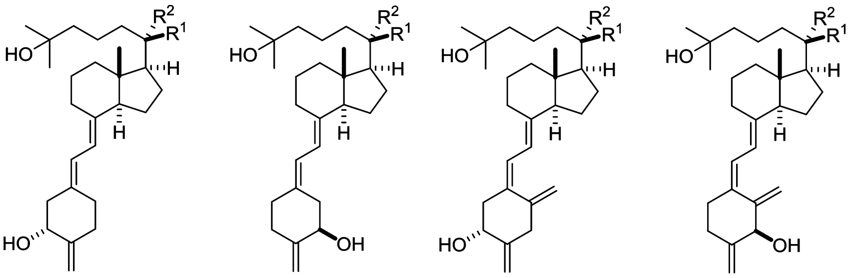


**104**, $R^1$ = Me, $R^2$ = H
**35**, $R^1$ = H, $R^2$ = Me

**116a**, $R^1$ = Me, $R^2$ = H
**116b**, $R^1$ = H, $R^2$ = Me

**117a**, $R^1$ = Me, $R^2$ = H
**117b**, $R^1$ = H, $R^2$ = Me

**118a**, $R^1$ = Me, $R^2$ = H
**118b**, $R^1$ = H, $R^2$ = Me



**119a**, $R^1$ = Me, $R^2$ = H
**119b**, $R^1$ = H, $R^2$ = Me

**120a**, $R^1$ = Me, $R^2$ = H
**120b**, $R^1$ = H, $R^2$ = Me

**121a**, $R^1$ = Me, $R^2$ = H
**121b**, $R^1$ = H, $R^2$ = Me

**122a**, $R^1$ = Me, $R^2$ = H
**122b**, $R^1$ = H, $R^2$ = Me

| Family | compound | VDR aff | 24OH trans | HL60 diff |
|---|---|---|---|---|
| 2-methylen | **116a** | - | -- | - |
| | **116b** | = | ++ | = |
| | **117a** | ++ | +++ | +++ |
| | **117b** | -- | -- | -- |
| | **118a** | ++ | ++ | - |
| | **119a** | -- | -- | -- |
| | **119b** | -- | -- | -- |
| | **120a** | -- | = | + |
| | **120b** | - | + | + |
| | **121a** | -- | -- | -- |
| | **121b** | - | -- | -- |
| | **122a** | -- | = | + |
| | **122b** | - | = | + |
| Ref: 1,25(OH)$_2$D$_3$: | | = | = | = |

**Figure 11.** Vitamin D analogs with side-chain and A-ring modifications (IV). The table summarizes the biological properties of the compounds: reference, **1,25(OH)$_2$D$_3$**; (=) similar value; (+) >10× higher; (++) >100× higher; (+++) >1000× higher; (−) >10× lower; (--) >100× lower; VDR aff, VDR affinity; 24OH trans, *CYP24A1* transactivation activity; HL60 diff, HL-60 cell differentiation induction.

region of the LBD (Figure 5C). Interestingly, in the presence of a coactivator peptide some of the antagonistic 22-butyl analogs take the agonistic conformation. Whether this is a technical artifact of the crystallization or has a physiological meaning, such as sensing of cofactor balance in the cellular context, needs to be clarified.

20-epi-Eldecalcitol (**84**), a 20-epi derivative of the antiosteoporotic drug eldecalcitol (**12**) (Table 1 and Figure 1), was synthesized through a convergent approach by Pd-catalyzed ring-closure of an enyne and coupling with vinyl bromide.[55] Since 20-epi-1,25(OH)$_2$D$_3$, a diasteromer of **1,25(OH)$_2$D$_3$** possessing an inverted C-21 methyl-substituent at C-20, shows enhanced biological activities compared to **1,25(OH)$_2$D$_3$**, compound **84** displays a 50-fold increased inhibition of U937 human leukemia cell proliferation.[56]

Since the presence of a 1$\alpha$-OH group in **1,25(OH)$_2$D$_3$** is crucial for VDR binding, its replacement with a 1$\beta$-OH group [1$\beta$,25(OH)$_2$D$_3$, **86**] causes loss of physiological activity[57] (Figure 10). Replacing the 1$\alpha$-OH group with one fluor atom [1$\alpha$-F,25(OH)$_2$D$_3$, **87**] also markedly diminishes biological activity,[58] while a compound with each a fluorine atom at C-1 and C-25 [1,25(F)$_2$(OH)$_2$D$_3$, **88**] is devoid of all activity.[59] Interestingly, the 3-OH group is not necessary if the 1$\alpha$-OH group is already in position, but the lack of the 3-OH group [1$\alpha$,25(OH)$_2$-3-deoxy-D$_3$, **89**] reduces the biological activity.[60,61] Additional methyl groups at C-1$\beta$ [1$\beta$-methyl-1,25-(OH)$_2$D$_3$, **90**] and C-3$\alpha$ [3$\alpha$-methyl-1,25(OH)$_2$D$_3$, **91**] significantly reduce VDR binding affinity. Switching the 3-OH group from $\beta$ to $\alpha$ position [3-epi-1,25(OH)$_2$D$_3$, **92**] causes a drastic reduction of physiological activity.[62] In fact,

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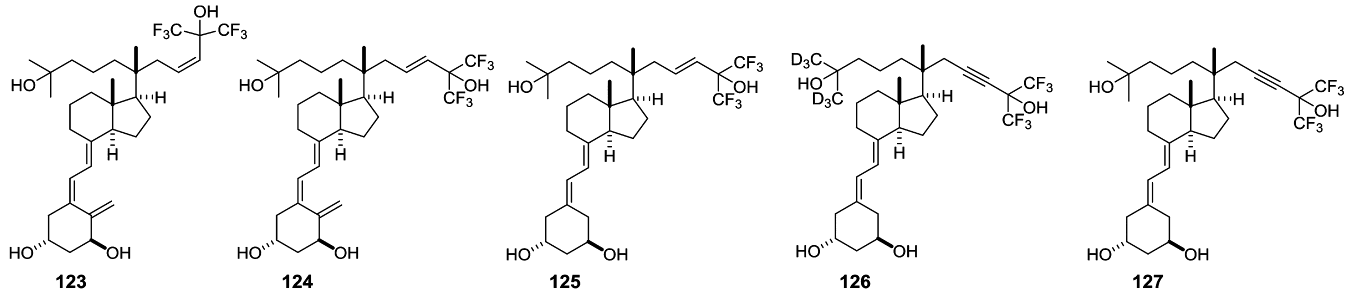
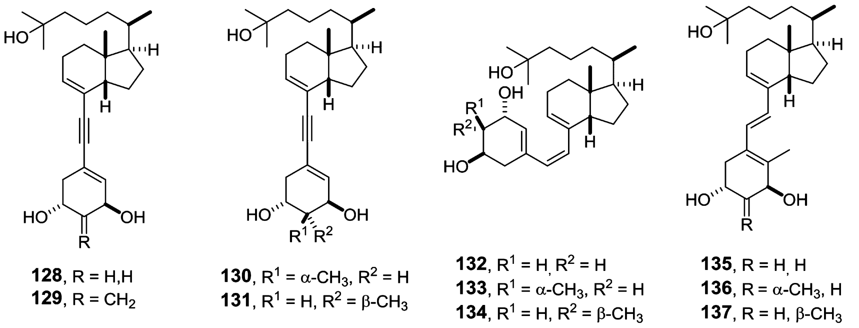


**123** **124** **125** **126** **127**



**128**, R = H,H
**129**, R = $CH_2$

**130**, $R^1$ = α-$CH_3$, $R^2$ = H
**131**, $R^1$ = H, $R^2$ = β-$CH_3$

**132**, $R^1$ = H, $R^2$ = H
**133**, $R^1$ = α-$CH_3$, $R^2$ = H
**134**, $R^1$ = H, $R^2$ = β-$CH_3$

**135**, R = H, H
**136**, R = α-$CH_3$, H
**137**, R = H, β-$CH_3$

| Family | compound | HL60 prol |
|---|---|---|
| Gemini | **123** | ++ |
| | **124** | - |
| | **125** | - |
| | **126** | ++ |
| | **127** | ++ |

Ref: $1,25(OH)_2D_3$: =

| Family | compound | VDR aff |
|---|---|---|
| 14-epi | **129** | - |
| | **130** | - |
| | **131** | -- |
| | **132** | -- |
| | **133** | -- |
| | **134** | -- |
| | **135** | --- |
| | **136** | = |
| | **137** | - |

Ref: $1,25(OH)_2D_3$: =

**Figure 12.** Vitamin D analogs with side-chain and A-ring modifications (V). The table summarizes the biological properties of the compounds: reference, **$1,25(OH)_2D_3$**; (=) similar value; (+) >10× higher; (++) >100× higher; (−) >10× lower; (--) >100× lower; VDR aff, VDR affinity; HL60 diff, HL-60 cell differentiation induction.

compound **92** is an intermediate of **$1,25(OH)_2D_3$** degradation displaying lower VDR binding affinity and lower calcemic effects compared to **$1,25(OH)_2D_3$**. Despite its decreased potency, the in vivo action of compound **92** is tissue-specific.

Taken together, most modifications of the A-ring result in decreased biological activity except for those modified at C-2. Therefore, a large number of C-2-substituted vitamin D analogs have been synthesized and were studied intensively for their biological activity. 2*α*-Substitutions [2*α*-methyl-$1,25(OH)_2D_3$ (**93**)] are more potent than 2*β*-substitutions [2*β*-methyl-$1,25(OH)_2D_3$ (**92**)].[63] Elongation of the C-2-alkyl group [2*α*-ethyl-$1,25(OH)_2D_3$ (**94**) and 2*α*-propyl-$1,25(OH)_2D_3$ (**95**)] reduces VDR binding affinity and biological potency, but *ω*-hydroxylation restores the activity.[64] 2*α*-Methyl-$1,25(OH)_2D_3$ (**93**) is twice as calcemic as **$1,25(OH)_2D_3$**. In combination with 20-epimerization [2*α*-methyl-20-epi-$1,25(OH)_2D_3$] VDR binding affinity increases even 12-fold.[63] 2*α*-(3′-Hydroxypropyl)-$1,25(OH)_2D_3$ (**97**) has a 3-fold increased VDR binding affinity than **$1,25(OH)_2D_3$**, while 2*β*-(3′-hydroxypropyl)-$1,25(OH)_2D_3$ (**98**) is 1.4 times more potent. Terminal hydroxylation of 2*α*- and 2*β*-propoxy groups at C-2, 2*α*-hydroxypropoxy-$1,25(OH)_2D_3$ (**99**) and 2*β*-hydroxypropoxy-$1,25(OH)_2D_3$ (**100**) also increases the VDR binding potential.

19-nor-Vitamin D analogs are known to be devoid of hypercalcemic and hyperphosphatemic effects.[65,66] For example, 19-nor-$1,25(OH)_2D_3$ (**101**) has a 5 times reduced the VDR binding affinity compared to **$1,25(OH)_2D_3$** paired with low or no bone calcification activity, while 19-nor-$1,25(OH)_2D_2$ (paricalcitol, **9**, Figure 1) has similar affinity as the natural hormone. Selected modifications at the C-2 position of 19-nor-vitamin D analogs are more potent inducers of gene activity. 2*β*-(3′-Hydroxypropoxy)-19-nor-$1,25(OH)_2D_3$ (**102**) and 2*α*-(3′-hydroxypropoxy)-19-nor-$1,25(OH)_2D_3$ (**103**) have reduced potency in bone and intestine.[67] 2-Methylene-19-nor-20-epi-$1,25(OH)_2D_3$ (**2MD**, **35**) shows VDR binding affinity comparable to **$1,25(OH)_2D_3$** but a 100 times enhanced ability to mobilize calcium from bone.[68] Moreover, **2MD** is 10 times more potent than **$1,25(OH)_2D_3$** in activating *CYP24A1* gene activity and inducing HL-60 cell differentiation. Analogs of **2MD** with a shortened side-chain, such as **2MP** (**105**) and **2MbisP** (**106**) are able to reduce parathyroid hormone (PTH) production.[69]

The compound 19-nor-1*α*,2*β*,$25(OH)_3D_3$ (**107**) is as potent as **$1,25(OH)_2D_3$** in intestinal calcium transport, while its epimer 19-nor-1*α*,2*α*,$25(OH)_3D_3$ (**108**) possesses less activity.[70] Importantly, both compounds are not calcemic. Compound **107** has higher VDR binding affinity than **108** and is more potent in inhibiting MCF-7 cell proliferation. When the 2-ethylidene group is in *E*-configuration (**109**), VDR binding affinity is 2.4-fold increased. Moreover, a 3′-hydroxypropylidene group at C-2 in *E*-configuration resulted in the potent compounds **AGR** (**110**) and **1AGS** (**111**), which seem to be intestine-selective.[60]

2*α*-(3′-Hydroxypropyl)-19-nor-$1,25(OH)_2D_3$ (**112**), which is modified at both C-2 and C-10, shows a similar VDR binding affinity as **$1,25(OH)_2D_3$**, displays a 36-fold higher potential in inducing HL-60 cell differentiation, and has a 500-fold higher antiproliferative potency in PZ-HPV-7 prostate cells. Its epimer 2*β*-(3′-hydroxypropyl)-19-nor-$1,25(OH)_2D_3$

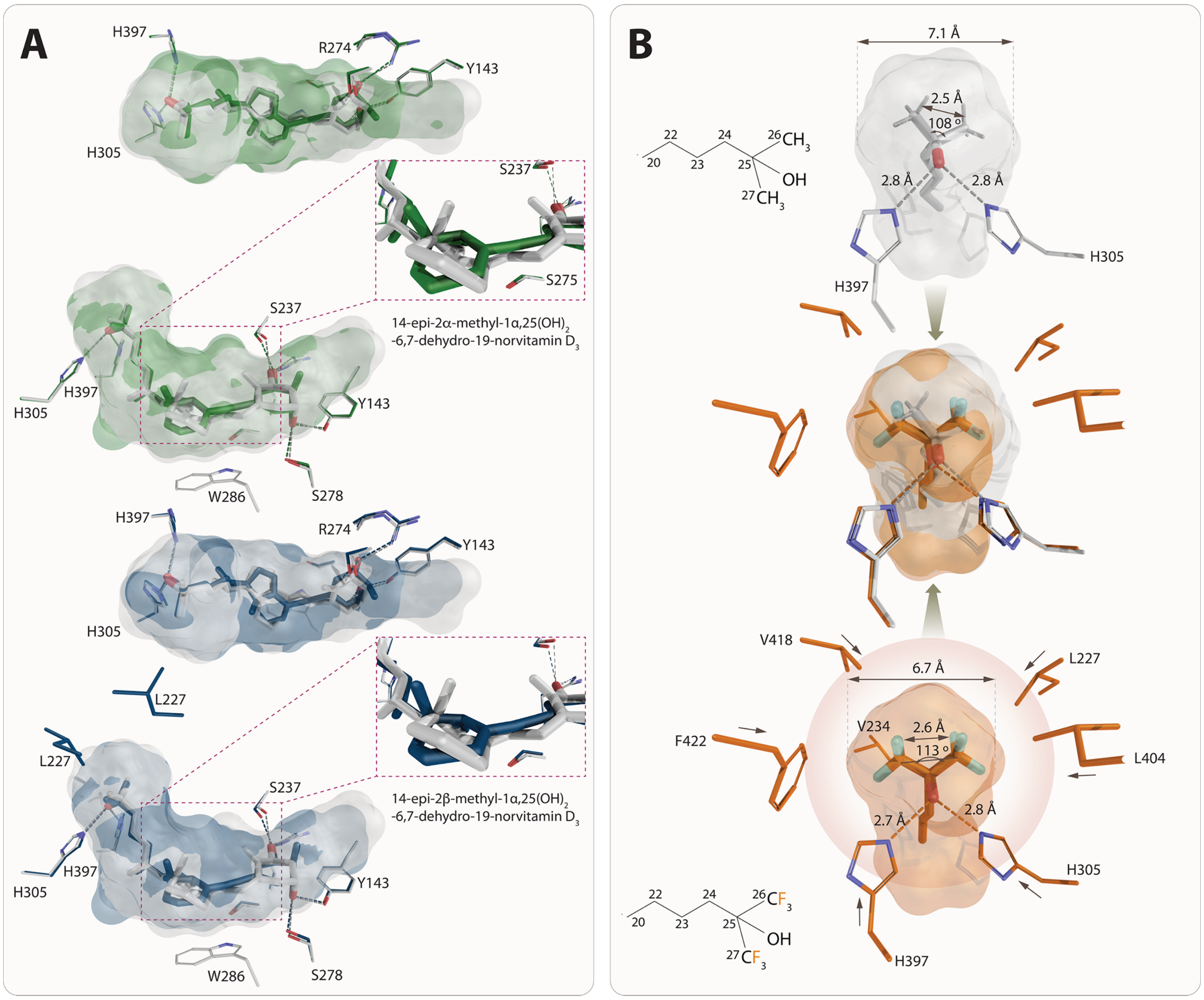
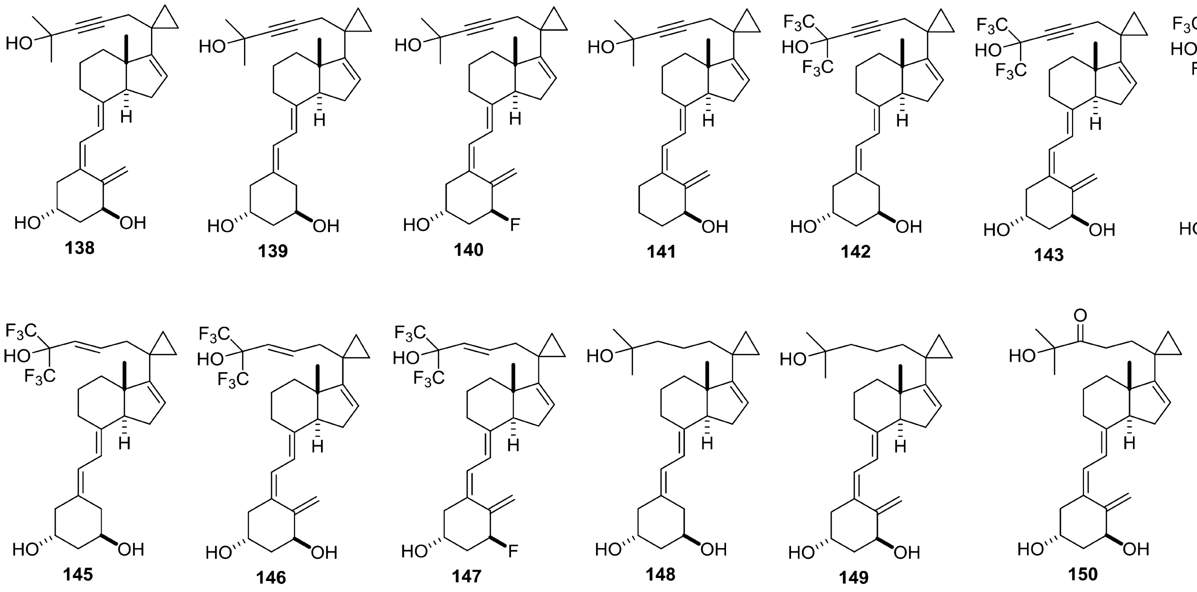


**Figure 13.** Structure−function relationship of various VDR ligands (II). The binding of 14-epi-2$\alpha$-methyl- and 14-epi-2$\beta$-methyl-**1,25(OH)$_2$**-6,7-dehydro-19-norvitamin $D_3$. The overall spatial conservation of the OH groups is maintained, but the CD-ring structure for the epimers shows higher flexibility by changing the more planar conformation in **1,25(OH)$_2$D$_3$** to reverse V-letter shape. This is largely due to the bending of the C-ring downward. The other notable changes involve the proximal location of L227 in 2$\beta$-methyl epimer and W286 for **1,25(OH)$_2$D$_3$** under 3.5 Å cutoff. Structural elements from 14-epi-2$\alpha$-methyl- (PDB code 3AUQ), 14-epi-2$\beta$-methyl-1,25(OH)$_2$-6,7-dehydro-19-norvitamin $D_3$ (PDB code 3AUR), and **1,25(OH)$_2$D$_3$** (PDB code 1DB1) complexes are highlighted in green, blue, and white color, respectively (A). Possible mechanism governing the potency of fluorinated analogs. The effect of a fluorinated functional group is illustrated on $CF_3$ group in comparison with $CH_3$ group located at the terminal carbons C-26 and C-27 of the ligand's aliphatic chain. The high electronegativity of the fluor atom has a pulling effect for the hydrophobic residues located in the proximity of the functional group (bottom), which cannot be seen for the **1,25(OH)$_2$D$_3$** under the same 3.5 Å cutoff (top). However, the hydrogen bonds between the conserved histidines and the 25-OH group are maintained in both scenarios. The positions of the $CF_3$ groups show a moderate opening about 5° with maintained distance between carbon C-26 and C-27. These changes have effect on the LBP, which is a bit smaller (middle panel) in the presence of $CF_3$ functional groups (B). Structural elements for the $CF_3$ and $CH_3$ groups are highlighted in orange and white color, respectively.

(**113**) has lower VDR binding and prodifferentiation activity, but it is as potent as **110** in inhibiting prostate cell proliferation.[70,71]

Promising analogs with three different alterations in the vitamin D skeleton (the A-ring, the side-chain, and the CD-ring) are 1$\beta$-hydroxymethyl-16-ene-24,24-$F_2$-26,27-bishomo-25$(OH)_2D_3$ (QW-1624-F2-2, **114**)[72] and 1$\alpha$-fluor-16-ene-20-epi-23-ene-26,27-bishomo-25$(OH)_2D_3$ (Ro-26.9228, **115**).[73] In a skin cancer model compound **114** inhibits progression and molecule **115** restores bone loss, while both are not hypercalcemic.

Six new derivatives of compound **104** have been prepared by a convergent synthesis using the Wittig−Horner approach[74] in order to evaluate the influence of methyl groups at C-22 on biological activity (Figure 11). Single methylation of the (20*R*)-25-hydroxylated side-chain (**117**) did not change the VDR binding affinity in comparison to the parent compound **104**. However, the addition of a 22-methyl group to the (20*S*)-25-hydroxylated side-chain (**118**) caused a much stronger effect. The 22*R*-compound **118a** has a 2.5 times higher VDR binding affinity than **104** and is 250-fold more potent than its 22-epimer **118b**. The prodifferentiation potential of an analog



| Family | compound | MLR IFN-γ | LPS TNF-α |
|---|---|---|---|
| Gemini | 138 | = | +++ |
| | 139 | = | +++ |
| | 140 | + | -- |
| | 141 | -- | |
| | 142 | + | -- |
| | 143 | = | + |
| | 144 | -- | |
| | 145 | + | -- |
| | 146 | = | -- |
| | 147 | - | -- |
| | 148 | = | = |
| | 149 | +++ | +++ |
| | Ref: $1,25(OH)_2D_3$: | = | = |

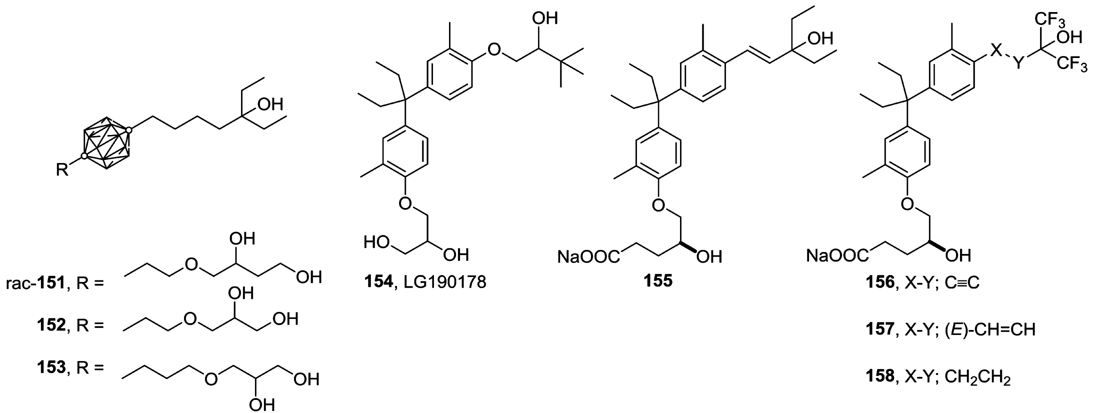
**Figure 14.** A-ring, D-ring, and side-chain modified vitamin D analogs. The table summarizes the biological properties of the compounds: reference, **$1,25(OH)_2D_3$**; (=) similar value; (+) >10× higher; (+++) >1000× higher; (−) >10× lower; (--) >100× lower; MLR IFN-γ, INFG inhibition; LPS TNFα, TNF inhibition.

with a 22*S*-methyl group in the "natural" side-chain (20*R*) (**116a**) is 10-fold higher than that of its 22-epimer (**116b**), whereas in the case of "unnatural" 20*S*-compound the 22*R*-epimer (**117a**) is 1000 times more potent than its 22-epimer (**117b**) and 4-fold more potent than the parent compound **104**. When two methyl groups were introduced at C-22, such as in the 20*R*-compound (**118a**) and the 20*S*-compound (**118b**), VDR binding affinity is increased compared to their parent molecules.

C-20-isomers of 25(OH)-2-methylene-vitamin $D_3$ and 3-desoxy-1α,25$(OH)_2$-2-methylene-vitamin $D_3$ (**117**−**120**) were synthesized through a convergent approach using a Sonorogashira coupling[75] (Figure 11). The biological activities of compounds **119**−**122** are clearly lower than those of the parent compound **104**. With the exception of the 1α-hydroxylated compounds they were also less active than **$1,25(OH)_2D_3$**. Analogs without a 1α-OH group show lower VDR binding affinity, HL-60 cell prodifferentiation activity, and *CYP24A1* activation than those hydroxylated at C-1. The addition of the 10-exo-methylene group improved the in vitro activity of the (20*S*)-1-desoxy compounds. In contrast, in the (20*S*)-series only VDR binding affinity augmented. The presence of the 2-exomethylene group resulted in enhanced intestinal calcium transport compared to **$1,25(OH)_2D_3$**, but bone calcium mobilization was 10-fold decreased in the (20*R*)-series.

A large structure−function analysis of 39 Gemini derivatives[43] showed five compounds (**123**−**127**) with enhanced antiproliferative activity (Figure 12). Compound **127** was stronger than **$1,25(OH)_2D_3$** in inhibiting cancer cell growth, while both were equipotent in their calcemic effect.

The Sonogashira approach was used to synthesize novel 14-epi derivatives of 19-nor-1α,25$(OH)_2$-previtamin $D_3$ (**132**) and 19-nor-1α,25$(OH)_2$-tachysterol $D_3$ (**135**).[76] Dienynic compounds (**129**−**131**) showed moderate VDR binding affinity, where the 2-methylene compound (**129**) has higher activity than the 2-methyl-substituted diastereomers **130** and **131**. Previtamin $D_3$ compounds (**132**−**134**) showed low VDR binding affinity. 14-epi-19-nor-tachysterol) $D_3$ compounds displayed higher VDR binding affinity, of which 2-methylene-14-epi-19-nor-tachysterol $D_3$ (**137**) is most potent. Crystal structure analysis indicated unique binding conformations. The binding of both epimers, 14-epi-2α-methyl- (PDB code 3AUQ) and 14-epi-2β-methyl-1,25$(OH)_2$-6,7-dehydro-19-nor-



rac-**151**, R =

**152**, R =

**153**, R =

**154**, LG190178

**155**

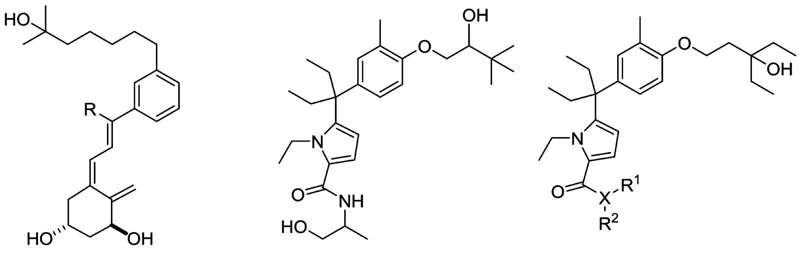
**156**, X-Y; C≡C

**157**, X-Y; (*E*)-CH=CH

**158**, X-Y; $CH_2CH_2$

| Family | compound | VDR aff | HL60 diff |
|---|---|---|---|
| carboranyl | **rac-151** | | -- |
| | **152** | -- | +++ |
| | **153** | -- | - |
| diphenyl hybrids | **154** | + | + |
| | **155** | = | = |
| | **156** | - | ++ |
| | **157** | ++ | ++ |
| | **158** | = | = |

Ref: $1,25(OH)_2D_3$: = =



**166a**, R = Et
**166b**, R = n-Pr
**166c**, R = n-Bu
**166d**, R = n-Hex
**166e**, R = n-Hep

**159**

**160a**, X = N, $R^1 = H$, $R^2 = (CH_2)_3N(CH_3)_2$
**160b**, X = N, $R^1 = H$, $R^2 = (CH_2)_3N(CH_2CH_3)_2$
**160c**, X = N, $R^1 = H$, $R^2 = (CH_2)_2N(CH_3)_2$
**160d**, X = N, $R^1 = H$, $R^2 = (CH_2)_2N(CH_2CH_3)_2$
**160e**, X = N, $R^1 = H$, $R^2 = CH_2CN$
**160f**, X = N, $R^1 = H$, $R^2 = (CH_2)_2NH_2$
**160g**, X = N, $R^1 = H$, $R^2 = (CH_2)_3N$ O
**160h**, X = N, $R^1 = (CH_2)_2CN$, $R^2 = (CH_2)_2CN$
**160i**, X = N, $R^1 = CH_3$, $R^2 = CH_2CH{=}CH_2$
**161a**, X = O, $R^1 = CH(CH_3)C{\equiv}CH$
**161b**, X = O, $R^1 = (CH_2)_2C{\equiv}CH$

**162a**, X = N, $R^1$ = Ph-*p*-$CH_2CO_2CH_3$
**162b**, X = N, $R^1 = H$, $R^2$ = Ph-*p*-$OCF_3$
**162c**, X = N, $R^1 = H$, $R^2$ = Ph-*m*-$OCF_3$
**162d**, X = N, $R^1 = H$, $R^2$ = Ph-*p*-$OCH_2CH_3$
**163a**, X = N, $R^1 = H$, $R^2 = CH[CH(CH_3)C_2H_5]CO_2CH_3$
**163b**, X = N, $R^1 = CH_3$, $R^2 = CH_2CO_2CH_2CH_3$
**163c**, X = N, $R^1 = H$, $R^2 = CH(CO_2CH_3)CH_2CO_2CH_3$
**164a**, X = N, $R^1 = H$, $R^2 = CH[CH(CH_3)C_2H_5]CO_2H$
**164b**, X = N, $R^1 = CH_3$, $R^2 = CH_2CO_2H$
**164c**, X = N, $R^1 = H$, $R^2 = CH(CO_2CH_3)CO_2H$
**164d**, X = N, $R^1 = H$, $R^2 = CH(CH_3)CO_2H$
**165a**, X = N, $R^1 = H$, $R^2 = CH[CH(CH_3)C_2H_5]CH_2OH$
**165b**, X = N, $R^1 = CH_3$, $R^2 = CH_2CH_2OH$
**165c**, X = N, $R^1 = H$, $R^2 = CH(CH_2OH)CH_2CH_2OH$
**165d**, X = N, $R^1 = H$, $R^2 = CH(CH_3)CH_2OH$

| Family | comp | VDR aff | 24OH tr | MCF7 pr | Ca |
|---|---|---|---|---|---|
| triene hybrids | **166a** | - | - | = | -- |
| | **166b** | -- | -- | = | - |
| | **166c** | -- | -- | + | - |
| | **166d** | -- | -- | + | -- |
| | **166e** | -- | -- | + | -- |

Ref: $1,25(OH)_2D_3$: = = = =

| Family | compound | HL60 diff | MCF7 prol | Family | compound | HL60 diff | MCF7 prol |
|---|---|---|---|---|---|---|---|
| pyrrolyl phenyl hybrids | **160a** | + | +++ | pyrrolyl phenyl hybrids | **162d** | -- | -- |
| | **160b** | = | ++ | | **163a** | --- | = |
| | **160c** | -- | ++ | | **163b** | -- | + |
| | **160d** | +++ | -- | | **163c** | - | +++ |
| | **160e** | -- | ++ | | **164a** | -- | - |
| | **160f** | -- | +++ | | **164b** | - | +++ |
| | **160g** | -- | -- | | **164c** | - | - |
| | **160h** | --- | -- | | **164d** | - | - |
| | **160i** | --- | -- | | **165a** | -- | - |
| | **161a** | --- | -- | | **165b** | - | ++ |
| | **161b** | --- | -- | | **165c** | -- | - |
| | **162a** | --- | -- | | **165d** | - | - |
| | **162b** | --- | -- | | | | |
| | **162c** | --- | -- | | | | |

Ref: $1,25(OH)_2D_3$: = = = =

**Figure 15.** Nonsteroidal VDR ligands. The table summarizes the biological properties of the compounds: reference, **$1,25(OH)_2D_3$**; (=) similar value; (+) >10× higher; (++) >100× higher; (+++) >1000× higher; (−) >10× lower; (--), >100× lower; (---), >1000× lower; VDR aff, VDR affinity; calcemia, [$Ca^{2+}$] level changes in serum; 24OH trans, *CYP24A1* transactivation activity; HL60 diff, HL-60 cell differentiation induction; MCF7 pr, MCF7 proliferation inhibition.

vitamin $D_3$ (PDB code 3AUR) is very similar in maintaining the position of the anchoring OH groups seen from the **$1,25(OH)_2D_3$** complex (PDB code 1DB1). However, the modification between C-6 and C-7 provides rigidity for this

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region introducing an unforeseen compensation in flexibility for the CD rings. Compared to their more planar conformation in **1,25(OH)$_2$D$_3$**, here they take a reverse V-letter conformation such as by the bending of the C ring about 9° downward. For the 2*β*-methyl isomer the residue L227 is closer to the ligand under 3.5 Å cutoff but under similar cutoff W286 seems to be closer to **1,25(OH)$_2$D$_3$**, which is due to more planar conformation of the CD-rings (Figure 13A).

## SIDE-CHAIN, D-RING, AND A-RING MODIFICATIONS

The synthesis of 12 analogs (**138**−**149**) of 1*α*,25(OH)$_2$-16-ene-20-cyclopropylvitamin D$_3$ relied on Wittig−Horner coupling[77] (Figure 14). These compounds have an unsaturated D-ring between C-16 and C-17 and a cyclopropyl group located at C-20. The structural diversity on the side-chain covered triple CC bonds (**138**−**144**), double CC bonds (**145**−**147**) and single CC bonds (**148** and **149**) together with the A-ring covered 1*α*,3*β*-dihydroxy (**138**, **143**, **146**, and **149**), 19-nor (**139**, **142**, **145**, and **148**), 3-deoxy-1*α*-hydroxy (**141**), and 1*α*-fluor-3*β*-hydroxy (**140** and **144**). The anti-inflammatory properties of these compounds were studied via analyzing the inhibition of the secretion of the cytokines interferon-*γ* (IFNG) and tumor necrosis factor (TNF). Most of 16-ene-20-cyclopropyl analogs inhibited IFNG with similar potency to **1,25(OH)$_2$D$_3$**, but compound **149** was more potent. The inhibition of TNF showed wide differences, some analogs (**138**, **140**, **143**−**145**) failed to induce TNF inhibition, whereas analog **149** inhibited TNF more efficiently than **1,25(OH)$_2$D$_3$**. The metabolism of **149** was studied and the stable 24-oxo metabolite **150** accumulated during metabolism. Compound **150** mediates similar induction of primary vitamin D target genes as analog **149** but has a lower calcemic activity.

## NONSTEROIDAL VDR LIGANDS

The synthesis of nonsteroidal VDR agonists containing a hydrophobic 1,12-dicarba-*closo*-dodecaborane (*p*-carborane) unit was achieved through bimolecular nucleophilic substitution[78] (Figure 15). The carborane cage replaced the CD-rings of the natural hormone exploiting the hydrophobicity of *p*-carborane. Despite their simple and flexible structure, the carborane-based VDR ligands show moderate binding affinity for VDR compared to **1,25(OH)$_2$D$_3$**. The analogs are flexible acyclic triols; i.e., they lack an A-ring and conjugated triene structures. Their structures shared a branched side-chain on a carborane carbon, and in the other carbon three different chains are bound either to 3-oxaheptan-5,7-diol (**151**), 3-oxahexan-5,6-diol (**152**) or 4-oxaheptan-6,7-diol (**153**). The flexibility of the diol is favorable for VDR binding affinity, which, however, is more then 100 times lower than for **1,25(OH)$_2$D$_3$**. Nevertheless, these mimics are rather active in inducing HL-60 cell differentiation [rac-**151**, 5%; (*S*)-**151**, 8%; (*R*)-**151**, 2%; **152**, 0.05%; **153**, 0.001%)]. The *S*-isomers showed, compared to the *R*-enantiomer, higher prodifferentiation activity and VDR binding affinity.

**LG190178** (**154**) is the first published nonsteroidal vitamin D analog.[79] In general, VDR ligands based on bisphenyl core compounds with *γ*-hydroxycarboxylic acid moiety (**155**) show agonist activity. From compounds with a fluorine-containing bisphenyl core[80] the hexafluoro analog (**157**) is 5 times more potent in reporter gene assays than the parent compound (**155**), shows 2 times higher prodifferentiation activity, and is 7 times more effective in inducing bone *γ*-carboxyglutamate protein (*BGLAP*) expression. Like in secosteroidal vitamin D analogs, fluorination is an effective modification as shown by crystal structure analysis of the VDR-LBD complexed with **155**. Also in this case helix H12 is stabilized in the agonistic position allowing interaction with coactivator proteins. From physicochemical point of view the fluorine atom's ionic radius is 100% larger than that of the hydrogen, the van der Waals radius is only 27% larger. However, the high electronegativity of fluor has a possible "pulling" effect for residues residing in the nearest proximity of a fluorinated functional group. This can be illustrated on the comparison of the aliphatic chain of the ligands that have CH$_3$ or CF$_3$ functional groups at carbon C-26 and C-27. The latter shows additional five hydrophobic residues that cannot be seen for CH$_3$ at the cutoff 3.5 Å (Figure 13B). The only maintained interactions are with conserved histidine residues. In addition, the possible reaction to the strong van der Waals forces from hydrophobic residues is a moderate opening of the functional groups by 5° with maintained distance between carbon C-26 and C-27 due to additional twist in the absolute position of the C-25 carbon. These net effects show also small variation the LBP size, which is slightly confined in the presence of CF$_3$ functional groups resulting in a tighter packing of the cavity (Figure 13B).

Nonsteroidal vitamin D mimics with phenylpyrrolyl pentane skeletons have been designed (**159**−**165**).[81,82] Among them, **159** shows clear antiproliferative effects on MCF-7 cells. In order to improve the biological activity of compound **159**, derivatives were designed comprising side-chains terminated in a diethylcarbinol, hydrophilic groups or hydrophobic groups (**160**−**165**). The antiproliferative activities of the compounds were tested in MCF-7 cells, PC3 human prostate cancer cells, Caco2 human colon cancer cells, and HepG2 human liver cancer cells. Compound **160b** exhibits the best antiproliferative activity, being more potent than the prototype compound **159** and **1,25(OH)$_2$D$_3$**. Also the compounds **160a**, **160c**, **160d**, **160f**, **160g**, **164b**, and **165b** show in all four model systems better antiproliferative activities than **159** and **1,25(OH)$_2$D$_3$**. The R$^2$ substitutions at the pyrrole-ring side-chains are crucial for the antiproliferative activity of the compounds. Molecules with hydrophilic groups at the end of the pyrrole-ring side-chain (**160a**, **160b**, **160d**, **160g**, and **164b**) are more potent than those bearing hydrophobic groups (**161a**, **161b**, **162a**−**d**, **163a**, and **164b**). Moreover, compounds **160a**, **160c**, **160d**, **160g**, and **164b** were less cytotoxic than **159** and **1,25(OH)$_2$D$_3$**. Compounds **160a**−**d**, **164b**, and **165b** also display prodifferentiating activity. In reporter gene assays **164b** is the most potent compound, whereas the transactivation potential of **160b** and **160g** is comparable to that of **1,25(OH)$_2$D$_3$**.

A novel class of analogs,[83] where the C-ring and D-ring were replaced by an aromatic *m*-phenylene D-ring and an alkyl chain, were synthesized based on the formation of the triene system through a Pd-catalyzed ring-closure of an enol trifate and a subsequent Suzuki−Miyaura reaction with appropriate boronate in aqueous medium.[84] Compounds **166a**−**e** efficiently induce the differentiation of human keratinocytes and show antiproliferative activity in MCF-7, PC-3, SKOV-3 (human ovary cancer), and HaCaT cells comparable to **1,25(OH)$_2$D$_3$**. Compound **166a** with the shortest chain at C-8 is most active not only in antiproliferative tests but also in reporter gene assays. Importantly, none of compounds **166a**−**e** induce hypercalcemia. In a SCID mice xenograph model of aggressive MDA-MB-231 human breast cancer cells compound

**166a** shows high efficacy for tumor growth inhibition and overall survival.

## CONCLUSIONS

This review demonstrated that clever and relevant chemistry significantly increased the number and variety of synthetic vitamin D analogs. Analog design had advanced and led to functional molecules, such as the *o*-carborane compounds, that are devoid of a 25-OH group. Moreover, there are now molecules that completely lack A- and/or CD-rings, such as *p*-carborane compounds, but still interact with VDR. Some of these nonsteroidal vitamin D analogs display high activity in vitro in combination with low calcemic effects in vivo. Thus, the area of nonsteroidal analogs and mimics is expected to further rise in future.

The assessment of the biological profile of VDR ligands is still primarily reduced to in vitro assays, such as VDR binding affinity, reporter gene assays, and antiproliferative and prodifferentiation measurements in different cancer cell lines. The variety in the assays makes a direct comparison of the different types of vitamin D analogs difficult. Moreover, a reliable extrapolation of the in vivo potential of the compounds is impossible without changing to a different set of assays, such as gene expression profiles in freshly isolated human peripheral blood mononuclear cells.[85]

Nowadays research on vitamin D analogs is nearly exclusively performed in academia and many interesting approaches for optimizing the profile of VDR ligands have not been explored to their limits. Accordingly, a complete picture is still missing and there is potential for improvements. The number of nearly 150 solved crystal structures of the VDR-LBD complexed with synthetic ligands is impressive and demonstrates the active interest of academia in understanding the molecular actions of VDR agonists and antagonists.

Unfortunately, failures of clinical trails focused on cancer have majorly dampened the interest of pharma industry in further developing vitamin D compounds. Since the natural hormone **$1,25(OH)_2D_3$** primarily prevents bone- and immune-system-related diseases, the molecule and it synthetic derivatives may not be perfect drugs for the therapy of cancer. Nevertheless, calcipotriol-activated VDR in stroma of human pancreatic tumors had been shown to markedly reduce markers of inflammation and fibrosis in pancreatitis and human tumor stroma.[86] This suggests that vitamin D compounds rather affect immune cells of the microenvironment of tumors than directly inhibiting the proliferation of the cancer cells. In fact, to date, most genome-wide data on the action of VDR and its ligands are available from cells of the hematopoietic system.[87] This further emphasizes the impact of vitamin D and VDR for innate and adaptive immunity and suggests that these areas should be further explored for a commercial application.

## ASSOCIATED CONTENT

### Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.9b00208.

> Table S1 describing all 143 publically available VDR ligand crystal structures with individual hyperlinks to the PDB and PubMed databases and citations of refs 90−144 (PDF)

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The authors declare no competing financial interest.

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**Ferdinand Molnár** received his Ph.D. in Biochemistry from the University of Kuopio (Kuopio, Finland) in 2006. He did his postdoctoral training in Structural Biology at the IGBMC (Illkirch, France). In 2008 he joined the School of Pharmacy at the University of Eastern Finland (Kuopio, Finland) studying nuclear receptor−ligand, −protein, and −DNA interactions. In 2018 he moved to the Nazarbayev University (Astana, Kazakhstan) where he holds an Associate Professor position at the Department of Biology. Prof. Molnár's interests are integrative structural biology and bioinformatics, eukaryotic transcriptional regulation in health and disease, and recombinant protein production.

**Carsten Carlberg** graduated in 1989 with a Ph.D. in Biochemistry at the Free University Berlin (Germany). After positions as postdoc at Roche (Basel, Switzerland), group leader at the University of Geneva (Switzerland), and docent at the University of Düsseldorf (Germany) he is since 2000 Full Professor of Biochemistry at the University of Eastern Finland in Kuopio (Finland). His work focuses on mechanisms of gene regulation by nuclear hormones, in particular on vitamin D. At present Prof. Carlberg has projects on epigenome-wide effects of vitamin D on the human immune system.

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## ABBREVIATIONS USED

**$1,25(OH)_2D_3$**, 1$\alpha$,25-dihydroxyvitamin $D_3$; **$1,25(OH)_2D_2$**, 1$\alpha$,25-dihydroxyvitamin $D_2$; **$25(OH)D_3$**, 25-hydroxyvitamin $D_3$; BGLAP, bone $\gamma$-carboxyglutamate protein (previously called osteocalcin); CAMP, cathelicidin antimicrobial peptide; CYP24A1, cytochrome P450, family 24, subfamily A, polypeptide 1; DBP, vitamin D binding protein; IFNG, interferon-$\gamma$; LBD, ligand-binding domain; LBP, ligand-binding pocket; PDB, Protein Data Base; PTH, parathyroid hormone; RXR, retinoid X receptor; TNF, tumor necrosis factor; TSS, transcription start site; VDR, vitamin D receptor

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