Vitamin D Receptor as a Drug Discovery Target

Karen V. Pinette[1], Ying K. Yee[2], Bernard Y. Amegadzie[1] and Sunil Nagpal[3]*

[1]*Bioresearch Technologies and Proteins,* [2]*Discovery Chemistry and* [3]*Gene Regulation, Bone and Inflammation Research, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN-46285, USA*

Abstract: 1α, 25-dihydroxyvitamin D3 [1,25 $(OH)_2D_3$], the active metabolite of vitamin D3, is known for the maintenance of normal skeleton architecture and mineral homeostasis. Apart form these traditional calcemic actions, 1,25 $(OH)_2D_3$ and its synthetic analogs are increasingly recognized for their potent anti-proliferative, prodifferentiative and immunomodulatory activities. The calcemic and non-calcemic actions of 1,25 $(OH)_2D_3$ and its synthetic analogs are mediated through vitamin D receptor (VDR), which belongs to the superfamily of steroid/thyroid hormone nuclear receptors. Physiological and pharmacological actions of 1,25 $(OH)_2D_3$ in various systems, along with the detection of VDR in target cells, have indicated potential applications of VDR ligands in inflammation, dermatological indications, osteoporosis, cancers and autoimmune diseases. VDR ligands have shown therapeutic potential in limited clinical trials as well as in animal models of these diseases. As a result, a VDR ligand, calcipotriol is in clinic for psoriasis and another, OCT, [2-oxa-1,25 $(OH)_2D_3$] is being developed as a topical agent for the same indication. Further, 1α,-hydroxyvitamin D3 (alphacalcidol), a prodrug of 1,25 $(OH)_2D_3$ is in clinic and a synthetic VDR ligand, ED-71, is under consideration for approval in Japan for the treatment of osteoporosis. Interestingly, VDR ligands have shown not only preventive but also potent therapeutic anabolic activities in animal models of osteoporosis. However, the wide spread use of VDR ligands in above-mentioned indications is hampered by their major side effect, namely hypercalcemia. In view of this associated toxicity, synthetic VDR ligands with reduced calcemic potential have been synthesized with the ultimate aim of improving their therapeutic efficacy. This review presents recent advances in VDR biology, novel VDR ligands and therapeutic applications of VDR ligands.

INTRODUCTION

The biological actions of the hormonally active form of vitamin D3, 1,25 $(OH)_2D_3$ or calcitriol, and its synthetic analogs are mediated by the nuclear vitamin D receptor (VDR), which is a ligand dependent transcription factor belonging to the superfamily of steroid/thyroid hormone receptors [1]. Vitamin D3 has traditionally been associated with calcemic activities, namely, calcium and phosphorus homeostasis and bone maintenance. However, the observation that VDR is also present in cells other than those of the intestine, bone, kidney and parathyroid gland, led to the recognition of non-calcemic actions of VDR ligands. As a result, VDR is also known to be involved in cell proliferation, differentiation and immunomodulation. For example, activated T- and B-lymphocytes, rheumatoid arthritis synoviocytes and macrophages, Kaposi's sarcoma and colon cancer cells, exhibit upregulation of VDR expression when compared to their normal counterparts. This disease-specific upregulation of VDR expression indicates that conditions involving these components could also be responsive to VDR ligands. The expression of VDR in a variety of cell lines and primary cells, coupled with the increased evidence regarding the involvement of VDR in the processes of cell differentiation, inhibition of proliferation and immunoregulation, has prompted the testing of VDR ligands in several human diseases as well as in various animals models of human diseases. These efforts have led to the development of VDR ligands for the treatment of psoriasis, secondary hyperparathyroidism and osteoporosis. In addition, VDR ligands have shown some efficacy in limited open clinical trials for prostate cancer, myelodysplasia (a precancerous lesion) and psoriatic arthritis. VDR ligands have also shown activity in the treatment of inflammatory and autoimmune diseases in various animal models.

TISSUE DISTRIBUTION OF VDR

The distribution and possible functions of VDR in various tissues have been determined by receptor activity, immunochemical detection in the nuclei of target cells, autoradiographic localization of the ligand following administration to vitamin-D-deficient animals, and responsiveness of specific cell types to vitamin D or its analogs. Furthermore, recent cDNA library data have confirmed the tissue distribution of the receptor. This distribution of the receptor in many cell types (see Table I) coincides with Vitamin D activities in both calcemic and noncalcemic tissues, and also underscores the homeostatic function of the receptor in cellular physiology.

REGULATION OF GENE EXPRESSION BY VDR LIGANDS

At the molecular level, 1,25 $(OH)_2D_3$ and its synthetic analogs modulate gene expression through a heterodimer

*Address correspondence to this author at the [3]Gene Regulation, Bone and Inflammation Research, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN-46285, USA; Tel: (317) 433-4961; Fax: (317) 276-1414; E-mail: nagpal_sunil@lilly.com

1389-5575/03 $41.00+.00

Table I. Tissue Distribution of VDR

Noncalcemic tissues/ cells		Calcemic tissues/ cells	Overexpression
Prostate Stomach	Thymus	Small intestine	E. coli
Placenta Epidermis	Breast	Bone	Baculovirus-infected cells
Pituitary Dermis	Pancreas	Kidney	Adenovirus-infected cells
Gonads	Melanocytes	Parathyroid	Yeast (S. cerevisiae)
Myocytes	Lymphocytes		Transient mammalian cells:
Colon	Hair follicles		• CV-1
Ovary	Cardiac muscle		• COS-7
Hippocampus	Lung		
Glial cells	Adipocyte		
	Mammary		

between VDR and retinoid X receptor (RXR). RXR, a nuclear receptor for 9cis-retinoic acid, is an obligate partner of VDR in mediating 1,25 $(OH)_2D_3$ action [1]. The VDR-RXR heterodimer binds to vitamin D3 response elements (VDREs) present in the promoter regions of responsive genes. VDREs are direct repeats (DR) of 5'-AGGTCA-3' motifs separated by three nucleotides and commonly referred to as DR-3 motifs [1]. The VDR protein is modular in nature and can be functionally divided into three regions with well characterized functions. The NH2-terminal region contains a ligand independent transactivation function, AF-1. The central region contains the DNA binding domain (DBD) consisting of two C2-C2 type zinc fingers, which target the receptor to VDREs. The C-terminal region of the receptor contains a multifunctional domain that harbors the ligand binding domain (LBD), the RXR heterodimerization motif and a ligand dependent transactivation function, AF-2. A VDR ligand binds to the LBD of VDR and the ensuing conformational change results in the enhancement of VDR-RXR heterodimer formation [2]. VDR is a ligand dependent transcription factor that can modulate the expression of vitamin D responsive genes by two different ways (Fig. **1**). It can either positively regulate the expression of certain genes by binding to the VDREs present in their promoter regions or negatively regulate the expression of other genes by binding to negative VDREs [3] or by antagonizing the action of certain transcription factors, such as NF-AT and NF-kB [4-6]. Only a handful of genes whose expression is induced by VDR ligands, and which are known to contain an authentic VDRE in their promoter are presented in Fig.**1**.

These genes include osteocalcin, osteopontin and calbindin-9k, which are involved in calcium homeostasis and bone remodeling [for references, see 7]. Other genes, which contain a VDRE in their promoter region and show vitamin D dependent upregulation in their expression, are

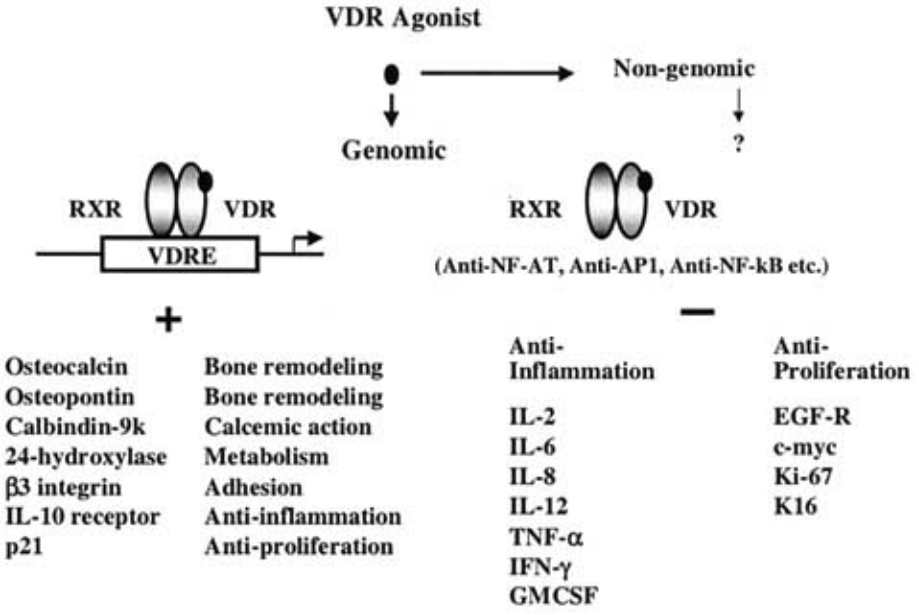


Fig. (1). Regulation of gene expresion by VDR.

the cell adhesion molecule β3 integrin and tumor suppressor p21 [7]. A number of genes, whose expression is induced by VDR ligands but have not yet been shown to contain VDREs, have also been identified. These genes include cell differentiation markers (transglutaminase I or TGase I and involucrin), which are involved in keratinocyte differentiation [8]. Differentiation regulatory genes that show VDR ligand dependent induction in their expression are Hox A10 (myelomonocytic cells) [9], c-Jun (colon cancer cells) [10], Hox 8 [11], c-fos [12] and VDR (osteoblasts) [12]. Cell adhesion molecules that fall in this category are CD14 [13], CD11b [13], CD18 [13] and E-cadherin [14]. Anti-proliferation genes in this category include the tumor suppressor, p27 [15], CD14 [13] and E-cadherin [14]. A VDR-responsive anti-inflammatory marker, which shows ligand dependent upregulation in keratinocytes, is receptor for anti-inflammatory cytokine IL-10 [16]. Osteoprotegrin, a protein involved in bone remodeling, shows vitamin D3 dependent regulation in osteoblasts [17]. Studies on VDR-knock out mice have identified calcium transport protein 1 (CAT1) and epithelial calcium channel (ECaC) as vitamin D-responsive intestinal candidate genes involved in calcium absorption and transport [18].

Genes that are downregulated in response to 1,25 $(OH)_2D_3$ and its synthetic analogs are also listed in Fig. **1**. The known hyperproliferative and inflammatory functions of these gene products indicate that many of the therapeutic effects of 1,25 $(OH)_2D_3$ and its analogs could result from their negative gene regulatory or transrepression activities. VDR ligands have been documented to inhibit the expression of cytokines, namely, IL-2 (T-cell lines and PBMCs) [19,20], IL-6 (psoriatic plaques and PBMCs) [20,21], IL-8 (fibroblasts) [22], IL-12 (myelomonocytes) [23], TNF-α (PBMCs) [20], IFN-γ (PBMCs) [20] and GMCSF (PBMCs) [24]. Proliferation associated genes that are transrepressed by VDR ligands include, EGF-R (keratinocytes) [25], c-myc (keratinocytes) [25], Ki-67 and K16 (psoriatic plaques) [26]. PTH (parathyroid cells) [27] and PTHrP (osteoblasts and keratinocytes) [3,28], which are involved in mineral homeostasis, are also downregulated by VDR ligands.

Negative regulation of PTH and PTHrP gene expression appears to occur through an entirely different class of DNA motif, called negative VDRE (nVDRE). However, the mechanism of VDR-dependent inhibition of IL-2 and GMCSF expression appears to be more complex than the involvement of positive or negative VDREs. In the case of these cytokines, VDR first competes with NF-AT1 for binding to the composite NF-AT1-AP1 enhancer motif, and then it interacts with c-Jun. This apparent co-occupancy of the composite site by VDR-c-Jun leads to inhibition of activated IL-2 and GMCSF expression [4,5,29]. Both VDR monomers and VDR-RXR heterodimers are involved in inhibition of IL-2 and GMCSF promoters.

TRANSCRIPTIONAL ACTIVATION BY VDR

Transcription is a complex multi-step process that is initiated at the promoter regions of responsive genes. The process requires the binding of ligand occupied VDR-RXR heterodimers to VDREs present in the upstream regions of responsive genes. The targeted recruitment of various chromatin modifying enzymatic activities and co-factors then act as a bridge between the enhancer and the Pol II transcription machinery. Using yeast two-hybrid and biochemical strategies, a number of proteins or co-factors that interact with VDR and other nuclear receptors, in a ligand dependent manner, have been identified. The co-factors, which interact with VDR, are listed in Table II.

Table II. VDR Interacting Co-factors

Cofactor	Function/Activity
SRC/p160 family	HAT
SRC-1 (SRC-1/NCoA-1)	
SRC-2 (GRIP1/TIF2/NCoA-2)	
SRC-3 (pCIP/RAC3/ACTR/AIB1/TRAM-1/NcoA-3)	
CBP/p300	HAT
RIP 140	Not known
TIF1	Kinase
$TAF_{II}135$	TAF
$TAF_{II}55$	TAF
$TAF_{II}28$	TAF
NCoA-62	Not known

HAT, Histone Acetyltransferase; TAF, TFIID Associated Factor.

For example, the SRC/p160 family of cofactors includes three members, namely SRC-1, SRC-2 and SRC-3. SRC family members and CBP/p300 proteins are histone acetyltransferases (HATs) that destabilize the nucleosomal core by catalyzing the acetylation of lysine residues present in the N-terminal tails of histones [for references, see 30]. VDR has also been shown to directly interact with certain components of the transcription machinery including TATA binding protein (TBP)- associated factors (TAFs), e.g., TF-IIB, TF-IIA, $TAFII_{135}$, $TAFII_{55}$ and $TAFII_{28}$ [31-33]. The SRC family members, CBP/p300, NcoA-62 and TAFs act as transcriptional coactivators and strongly potentiate ligand dependent activation of transcription by VDR and other members of the nuclear receptor superfamily. The activities associated with other VDR interacting proteins mentioned in Table I are not well characterized.

A complex of 15-20 proteins called DRIP (VDR interacting proteins) complex that interacts with VDR, other nuclear receptors and transcription factors, has been described [34]. The DRIP complex has been shown to be sufficient for *in vitro* ligand dependent transcription by the VDR-RXR heterodimer [34]. The complete DRIP complex could be recruited to ligand occupied VDR via its interaction with a single protein, DRIP 205, a component of the complex. The current working model for VDR transcription states that ligand occupied VDR-DRIP and VDR-SRC complexes are present in the nucleus. The first step involves the targeted recruitment of VDR-SRC or a VDR-HAT activity complex to a responsive promoter to facilitate in the destabilization of

the nucleosomal core. The unwound DNA then becomes a target for the VDR-DRIP complex, which contains factors required for transcription. This two-step model is currently in vogue but it may get more complex as additional VDR interacting proteins are discovered.

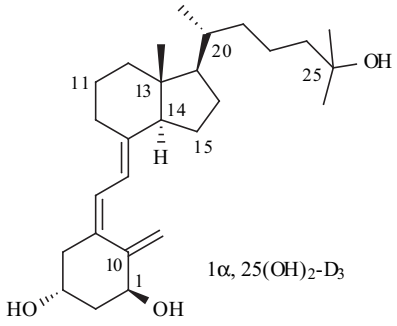
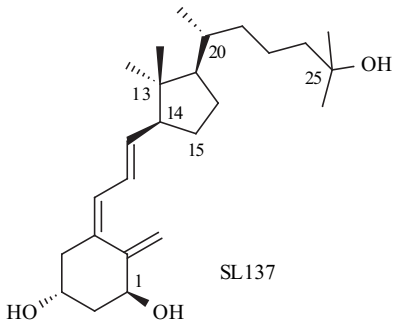
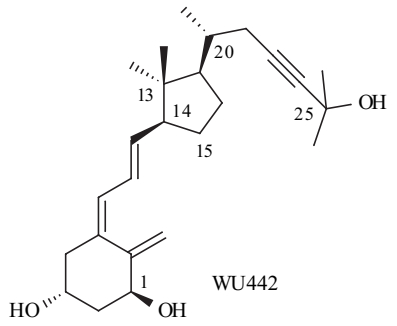
CHEMISTRY

SAR (structure-activity relationship) on non-calcemic VDR agonists based on secosteroid analogs has been extensively reviewed [35]. The current review summarizes recent results from several series of non-secosteroidal VDR agonists.

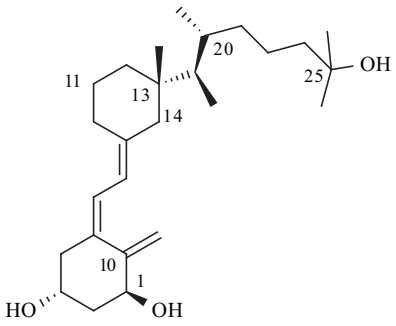
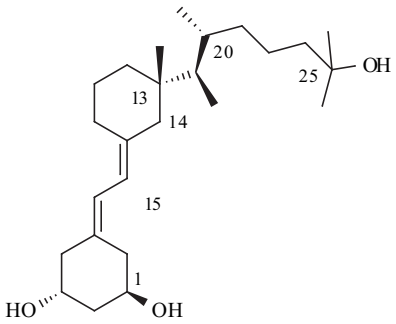
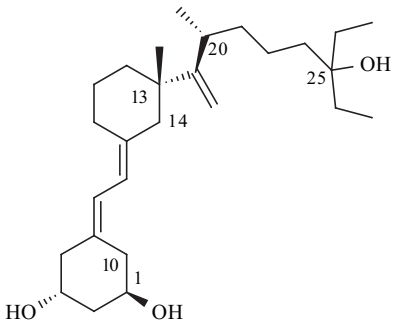
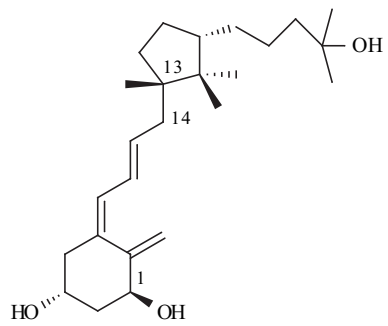
RING MODIFICATIONS OF 1,25 $(OH)_2D_3$

Three series of non-secosteroidal VDR agonists derived from the modification of the C or D ring of calcitriol have been identified (Fig. **2**) [36,37]. In general, these analogs have weaker affinity to VDR but still exhibit greater MCF-7 antiproliferative activity than calcitriol. These non-secosteroidal VDR agonists have non-calcemic activity dissociated from their calcemic function. They possess excellent selectivity of differentiation and antiproliferation activity over the hypercalcemia effect. Nearly all analogs have low or no DBP (plasma vitamin D binding protein) binding.

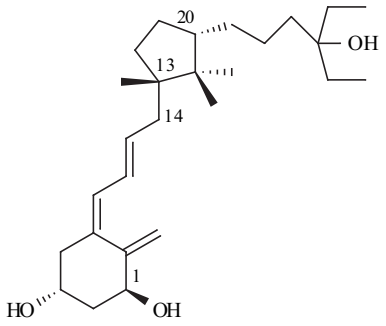
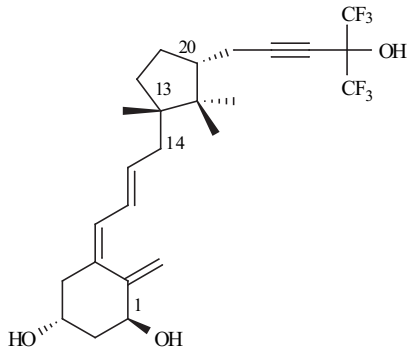
WU442, lacking a C-ring, exhibited twice the antiproliferative potency of calcitriol but had an 800 fold reduction in calcemic activity (%MCF-7/% Ca) [36,37]. CY616, a D-ring modified analog without a C10 methylidene, had a twelve fold enhancement of antiproliferative activity over calcitriol and more than 1000 fold decrease in calcemic potency. A third series of non-secosteroidal VDR agonists was constructed by removal of C and D rings and forming a new "E" ring by linking C13-C20. Examples of this series are represented by KS176, KS291, and CD483 [37]. KS291 and CD483 have low affinity for VDR but still exhibited high antiproliferative activity. Both compounds showed 1000 fold lower calcemic activity than calcitriol.

Compound	VDR[a]	COS-7[a]	MCF-7[a]	Ca serum[a]	% MCF-7/% Ca
 1α, 25$(OH)_2$-D_3	100	100	100	100	1
C-Ring Removal  SL137	70	100	85	3	28
 WU442	40	ND	200	0.25	800

(Fig. 2). contd.....

Compound	VDR[a]	COS-7[a]	MCF-7[a]	Ca serum[a]	% MCF-7/% Ca
D-Ring Removal  ZG1368	60	200[b]	6000	50	120
 ZG1423	45	180[b]	2000	13	154
 CY616	40	180[b]	1275	1	1275
"E" Ring  KS176	10	ND	30	<0.1	>300

(Fig. 2). contd.....

Compound	VDR[a]	COS-7[a]	MCF-7[a]	Ca serum[a]	% MCF-7/% Ca
 KS291	36	70	100	0.1	1000
 CD483	10	63	200	0.2	1000

[a] Results are expressed as % activity at 50% dose response relative to 1,25 $(OH)_2D_3$.
[b] Estimated from dose response curves
ND = not determined

Fig. (2). Nonsecosteroidal vitamin D analogs.

DIARYL ALKANES

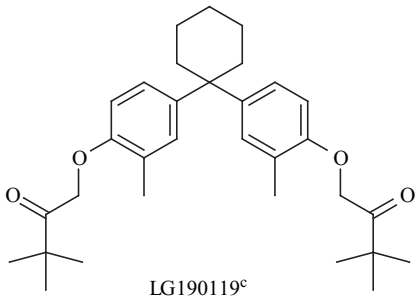
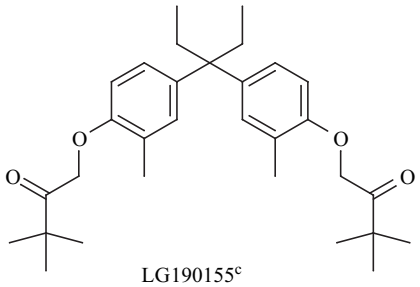
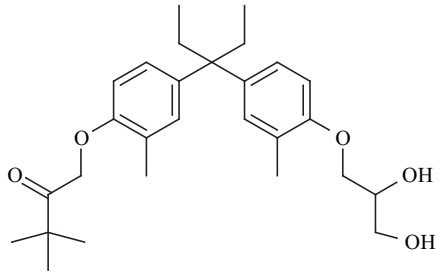
A novel series of VDR agonists based on diaryl alkanes was identified from screening (Fig. **3**) [38]. The lack of the vitamin D backbone makes these diaryl alkanes structurally distinct from 1,25$(OH)_2D_3$ and C/D ring modified non-secosteroidal analogs. Despite this lack of resemblance to calcitriol, the diaryl alkanes do retain the characteristic VDR agonist hydrophilic and lipophilic sidechains. LG190178, a diastereomeric mixture of four isomers, displaces ^{3}H-1,25 $(OH)_2D_3$ with a modest Ki of 150 nM, while LG190119 and LG190155 only bind to VDR under non-equilibrating conditions. These results were presumed to be due to faster K_{on} and slower K_{off} binding kinetics of calcitriol to VDR. Despite their low or poor affinity to VDR, these analogs mimic the activity profile of 1,25 $(OH)_2D_3$. LG190155 and LG190178 are active in a VDR agonist induced cotransfection assay (CTF), inhibit proliferation of the human breast cancer cell line (SK-BR-3), prostate cell line (LNCaP) and keratinocytes, and induce monocytic differentiation of HL60 leukemic cells. LG190119 and L190155 also induced the VDR dependent 24-hydroxylase target gene in rodents but with a lower increase in serum calcium as compared to 1,25 $(OH)_2D_3$. This lower calcemic effect was credited to their lack of binding to DBP.

The discovery of these novel non-secosteroid VDR mimics provides more opportunities for developing cell and tissue selective VDR modulators. Investigation of sidechain modification and stereochemical SAR within these series could lead to more potent VDR agonists that are devoid of calcemic effects. The dissimilarity of these scaffolds to calcitriol, especially in the diaryl alkane series, may minimize metabolism and improve their pharmacodynamic profile. As a result, these VDR agonists are promising leads toward safer and more orally effective drugs for psoriasis, osteoporosis and cancer.

THERAPEUTIC APPLICATIONS OF VITAMIN D_3

Osteoporosis

VDR is expressed at high levels in osteoblasts, and the primary biological effects of 1,25 $(OH)_2D_3$ are also mediated

Compound	VDR[a] Ki (nM)	CTF EC50 (nM)	LNCaP EC50 (nM)	Kidney 24(OH)ase (fold induction)	Serum Ca (ug/dl)	24(OH)ase /Ca
Calcitriol[b]	**2-5**	**9**	**2**	**5.7**	**>15**	**0.38**
 LG190119[c]	>10,000	2000	2000	13.5	9.1	1.48
 LG190155[c]	>10,000	800	300	6.0	9.6	0.63
 LG190178[c]	150	40	20	ND	ND	ND

[a] CTF assay used pRShVDR and VDRE(1)-ΔMTV-LUC in Hep G2 cells.

[b] Calcitriol was administered at 15 μg/kg. LG190119 was administered at 10 mg/kg. LG190155 was administered at 0.1 mg/kg.

ND = not determined

Fig. (3). Diaryl alkanes.

by this cell type [39]. Following stimulation with 1,25 $(OH)_2D_3$, osteoblasts increase the expression of several marker genes including osteocalcin, osteopontin, and alkaline phosphatase, while inhibiting collagen synthesis, thus supporting its role of hormone action in bone formation. However, the ability to use 1,25 $(OH)_2D_3$ as a treatment for osteoporosis is hindered by its serious side effect of hypercalcemia. Hypercalcemia results from an increase in calcium absorption through the intestine leading to increased plasma and urine levels of calcium that can ultimately lead to the mineralization of soft tissues. Nevertheless, prevention of osteoporosis has been observed following the administration of 1,25 $(OH)_2D_3$ to post-menopausal women. These individuals exhibited both an increase in bone mineral density, and a decrease in the incidence of vertebral fractures [40-42].

Several approaches have been explored to utilize less calcemic analogs of vitamin D_3 for the treatment of osteoporosis. Alfacalcidol, 1α-hydroxyvitamin D_3, a precursor of 1,25 $(OH)_2D_3$, has been found to reduce the incidence of vertebral fractures and increase bone mass in several clinical trials [43,44]. The mechanism of action of alfacalcidol is not completely understood, however, it has been shown to exhibit anabolic activity in bone, independent of parathyroid hormone suppression [45]. Success in treating osteoporosis with alfacalcidol is believed to be the result of the ability to administer higher doses of this compound as compared to 1,25 $(OH)_2D_3$, prior to the detection of hypercalcemia [43-46]. Alfacalcidol is currently an approved treatment for osteoporosis in Japan.

Several attempts have been made to synthesize analogs of 1,25 $(OH)_2D_3$ that exhibit a lower occurrence of

hypercalcemia *in vivo*. ED-71, 1α, 25-dihydroxy-2β-(3-hydroxypropoxy) vitamin D_3, is one example of such a compound [47]. In studies using normal, ovariectomized, and prednisolone-treated rats, ED-71 increased calcium absorption in the gut, decreased bone resorption and increased bone mineralization [48,49]. ED-71 has also been found to be as effective as PTH in ovariectomized rats [50]. At a dose of 0.08 μg/kg/day for 5 weeks, ED-71 decreased bone resorption and increased bone mass without inducing hypercalcemia [50]. However, optimal analogs have yet to be identified, and most 1,25 $(OH)_2D_3$ analogs have a small therapeutic window due to the development of hypercalcemia as a result of extended dosing.

SECONDARY HYPERPARATHYROIDISM

Secondary hyperparathyroidism is characterized by hyperplasia of the cells of the parathyroid gland, in combination with elevated levels of parathyroid hormone (PTH). The cause of this condition has been linked to a defect in the renal synthesis of 1,25 $(OH)_2D_3$, leading to a reduction in intestinal absorption of calcium. A low level of circulating 1,25 $(OH)_2D_3$ then causes a secondary increase in parathyroid hormone secretion. This feedback mechanism is required for the maintenance of appropriate levels of serum calcium [51,52]. Patients with renal failure who are undergoing chronic dialysis treatment most often exhibit symptoms of hyperparathyroidism. Retention of phosphate in the kidneys of these patients leads to the inhibition of normal calcium homeostasis. Suppression of PTH secretion is the ultimate goal of secondary hyperparathyroidism treatment. 1,25 $(OH)_2D_3$ has been used to successfully treat secondary hyperparathyroidism due to its ability to suppress transcription of the pre-PTH gene [53,54]. Unfortunately, the use of this treatment is limited by the development of hypercalcemia and hyperphosphatemia, due to the action of 1,25 $(OH)_2D_3$ on cells of the intestine and skeleton. An analog of 1,25 $(OH)_2D_3$, 19-nor-1α, 25-dihydroxyvitamin D_2 (Paricalictol, Zemplar), that shows a 10-100 fold reduction in calcemic activity, as compared to 1,25 $(OH)_2D_3$, is now used for the treatment of secondary hyperparathyroidism in patients undergoing chronic dialysis treatment [52,55].

RHEUMATOID ARTHRITIS

Epidemiological studies have reported low serum levels of vitamin D, and its metabolites, in rheumatoid arthritis patients [56], and several animal studies have highlighted the therapeutic potential of VDR ligands. 1,25 $(OH)_2D_3$ was found to inhibit the progression of Lyme disease- and collagen-induced arthritis in both murine and rat models [57,58]. A 1,25 $(OH)_2D_3$ synthetic analog, 22-oxa-1α, 25-dihydroxyvitamin D_3 (MC1288), was also found to arrest the development of collagen-induced arthritis in rats, and treatment administered at the onset of the disease greatly reduced the severity of joint inflammation [59]. In addition to current animal models, an open-label trial involving patients with psoriatic arthritis showed an improvement in disease symptoms following the oral administration of 1,25 $(OH)_2D_3$ [60]. These data clearly support the potential therapeutic utility of VDR ligands in the treatment of rheumatoid arthritis.

AUTOIMMUNE DISEASES

Multiple Sclerosis

Multiple sclerosis (MS) is an autoimmune inflammatory demyelinating disease of the central nervous system (CNS), and can be fatal [61]. The animal model that is most successfully utilized to study multiple sclerosis is a model of murine experimental inflammatory encephalomyelitis (EAE). In this model, Th-1 cells, specific for myelin basic protein, become activated and cause cytokine mediated demyelination of CNS cells [61]. It had been shown that 1,25 $(OH)_2D_3$ and its analogs could suppress the cytokine production of activated T-cells, therefore the EAE model system was used to evaluate the possible use of VDR ligands for the treatment of MS. Several studies found that EAE disease symptoms could be improved by treatment with 1,25 $(OH)_2D_3$ [61,62], and a less calcemic analog, Ro 63-2023 [61,63,64]. The mechanism of the immunoregulatory actions of 1,25 $(OH)_2D_3$ in this model has been linked to the *in vivo* induction of IL-4 production. VDR ligands were found to be less effective in reducing the progression of EAE in IL-4 knockout animals [65].

Type I Diabetes Mellitus

Type I diabetes mellitus is a result of the autoimmune destruction of pancreatic β cells. When NOD mice were treated with 1,25 $(OH)_2D_3$ there was an observed shift of autoantigen-specific T-cells from a Th-1 to Th-2 phenotype [66]. The ability of VDR ligands to shift T-cell phenotype appears to be the underlying mechanism of the therapeutic action of these compounds, and is likely to be the result of increased levels of IL-4 production. In addition to the natural ligand, a non-calcemic VDR ligand, 20-epi-22-oxa-24, 26,27-trishomo-1α, 25-dihydroxyvitamin D_3 (KH 1060), has also been shown to prevent the onset of type I diabetes in NOD mice [67]. Due to the multiple immunoregulatory properties of VDR ligands, further development of these compounds focused on reducing hypercalcemic side effects may result in compounds that are efficacious in the treatment of type I diabetes.

Systemic Lupus Erythematosus

Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease. Patients with SLE produce autoantibodies to many tissue antigens including DNA, histones, red blood cells, platelets, and leukocytes; and as a result these individuals present with varying symptoms. The therapeutic potential of VDR ligands for the treatment of SLE was explored using an analog of 1,25 $(OH)_2D_3$, 22-oxa-1α, 25-dihydroxyvitamin D_3. Symptoms of SLE were alleviated in MRL/lpr mice following treatment with 22-oxa-1α, 25-dihydroxyvitamin D_3. In addition, at therapeutically effective doses of 22-oxa-1α, 25-

dihydroxyvitamin D_3, symptoms of hypercalcemia were not observed in the autoimmune animals [68].

CANCERS

Prostate

VDR expression has been reported in normal, hyperplastic, and malignant prostate, and prostate cancer cell lines. The receptor expression levels suggested the prostate could be a potential target organ for VDR ligands [69,70]. Data to support the role of vitamin D in prostate cancer was established upon the observation that one of the major risk factors for developing prostate cancer was a low serum level of vitamin D [71]. Several *in vitro* studies have shown the ability of 1,25 $(OH)_2D_3$ to control proliferation of prostate cancer cell lines [72,73], and inhibit tumor cell growth and metastasis *in vivo* [72]. The mechanism by which 1,25 $(OH)_2D_3$ and its analogs elicit their effects on prostate cancer cells is not well understood. However, evidence now exists to indicate that the reduction of cyclin-dependent kinase-2 activity, and the induction of p21, p27 and E-cadherin may contribute to the inhibitory activity of these compounds [73]. Inhibition of LNCaP prostate cancer cell proliferation has also been obtained by non-secosteroidal and non-calcemic analogs of 1,25 $(OH)_2D_3$ [38]. Recently, a non-secosteroidal VDR modulator, LG 190119 [Fig. **3**], has been shown to inhibit LNCaP xenograft tumor growth in athymic mice without causing hypercalcemia or other apparent side effects [74].

It has also been reported that when 1,25 $(OH)_2D_3$ was used in combination therapy with other chemotherapeutic agents, including cisplatin, paclitaxel, and docitaxel, enhanced anti-tumor effects were observed in a model of human xenograft prostatic adenocarcinoma [75]. It is clear from these and other studies that prostate cancer treatment could benefit from further development of non-calcemic vitamin D analogs.

Breast

There is some evidence of the potential protective effects of sunlight and dietary vitamin D consumption on breast cancer risk in women [71]. The VDR is expressed on most breast cancer cell types [76] and 1,25 $(OH)_2D_3$ has been shown to inhibit the proliferation of breast cancer cells *in vitro*, and tumor progression *in vivo* [76-79]. The use of vitamin D analogs in breast cancer treatment offers an alternative to hormonal treatment, and provides a choice in the treatment of estrogen receptor-negative tumors. One such analog, 22-oxa-1α, 25-dihydroxyvitamin D_3, has been shown to inhibit the growth of both ER positive (MCF-7, T-47D, and ZR-75-1) and ER negative (MDA-MB-231 and BT-20) cell lines without inducing hypercalcemia *in vivo* [80]. Similar to the rationale described for prostate cancer treatment, treatment of breast cancer could be expanded to include combination therapies of VDR ligands with more common treatment regimes including tamoxifen and taxol. An *in vivo* xenograft study has demonstrated the utility of such a treatment. In this model, antiproliferative effects were observed following combination treatment with the vitamin D_3 analog CB 1093 with either paclitaxel of cisplatin [81].

Colon

A number of epidemiological studies have shown an inverse correlation between vitamin D intake and the risk of colon or colorectal cancer [71]. The VDR is also expressed in colon cancer cell lines, normal mucosa and colonic tumors, and 1,25 $(OH)_2D_3$ has been found to exert antiproliferative actions in colorectal cancer [82,83]. However, the underlying molecular mechanism of action is not fully understood at this time. It was reported that the reduction of cyclin D1 levels was a key factor in the antiproliferative effects of two vitamin D_3 analogs, Ro 23-7553 and JK-1624-3, on Caco-2 tumor cell growth [84]. Another study reported the VDR ligand-dependent differentiation of SW480 colon carcinoma cells via the induction of E-cadherin and the inhibition of beta-catenin signaling pathway [14].

Leukemia

Uncontrolled proliferation of hematopoietic cells, that are unable to further differentiate into mature cell types, gives rise to leukemia. Activated lymphocytes, monocytes, macrophages and myeloid cells have been found to express VDR, and 1,25 $(OH)_2D_3$ is able to induce the differentiation of human myeloid cells into a monocyte/macrophage cell type [85,86]. Animal studies and clinical trials using 1,25 $(OH)_2D_3$ and its analogs have identified the major limitation of this course of leukemia treatment to be the development of hypercalcemia. Many VDR ligands have been synthesized in an effort to alleviate the side effects of 1,25 $(OH)_2D_3$, and several have been found to be potent stimulators of HL-60 differentiation *in vitro* without inducing hypercalcemia [38,87]. A therapy to induce cellular differentiation in leukemia patients may result from the continued development of therapeutically active non-calcemic VDR ligands.

PSORIASIS

Psoriasis is a skin disease that is characterized by hyperproliferation of epidermal keratinocytes and complex immune disturbances. It was observed that keratinocytes and dermal fibroblasts expressed VDR and that 1,25 $(OH)_2D_3$ could stimulate keratinocyte differentiation [88,89]. Clinical evidence to support use of 1,25 $(OH)_2D_3$ and its analogs for the treatment of psoriasis occurred when an individual receiving oral administration of 1,25 $(OH)_2D_3$ for osteoporosis, showed remission of psoriatic lesions [90]. The utility of this approach to treat psoriasis is currently undesirable due to the potent hypercalcemic activity of orally administered 1,25 $(OH)_2D_3$, and the ability of this compound to cause complete remission of psoriatic lesions in only 30% of patients [91]. The use of less calcemic synthetic analogs of 1,25 $(OH)_2D_3$ has been useful for developing a treatment for this skin disease. One prominent example is the use of calcipotriol [92]. Calcipotriol was

found to be over 100 times less calcemic than 1,25 $(OH)_2D_3$ [92,93]. A topical calcipotriol treatment has also shown success in the reduction of psoriatic plaques. The application of calcipotriol ointment twice a day yielded significant improvement of psoriatic lesions in 60-70% of patients (n=167), with complete disease remission in 26% [93]. The most common side effect observed during this trial was the development of a cutaneous irritation in approximately 20% of patients.

The anti-psoriatic activity of 1,25 $(OH)_2D_3$ is a result of the differentiation, anti-proliferative, and immunomodulatory characteristics of this class of compounds. 1,25 $(OH)_2D_3$ has been found to promote differentiation and inhibition of proliferation in cultured keratinocytes [89,92,94]. Expression of epidermal growth factor receptor, c-myc, Ki-67 and keratin 16 were also downregulated following VDR ligand treatment [25,26]. VDR ligands are also responsible for decreasing the expression of pro-inflammatory cytokines including IL-2, IFN γ, IL-6, IL-8, and GM-CSF [19-22,24], all of which play a role in cutaneous inflammation, and proliferation of T-lymphocytes and keratinocytes. Also, calcipotriene-induced clinical improvement of psoriasis has been observed to follow an increase in patient IL-10 with a concomitant decrease in IL-8 levels [95]. In an independent study, it had previously been observed that IL-10 receptor gene expression was also induced in response to treatment with 1,25 $(OH)_2D_3$ [16]. It is believed that the development of more efficacious VDR ligands, with improved side effect profiles, will further expand the treatment options for patients with severe psoriasis.

CONCLUSION

The therapeutic utility of VDR ligands in osteoporosis, psoriasis and secondary hyperparathyroidism has been well established. Based upon preclinical and clinical data, rheumatoid arthritis, psoriatic arthritis, autoimmune diseases, myelodysplastic syndrome and certain cancers have emerged as additional indications of VDR ligands. Still the major impediment in the wider use of VDR ligands has been hypercalcemia. Although less calcemic analogs have been synthesized, truly non-calcemic analogs are still elusive. Therefore, a SAR for the identification and development of non-calcemic VDR ligands still appears to be extremely important. The combination of current VDR ligands with retinoids, steroids or chemotherapeutic agents may offer additive or synergistic effects in target indications. The next major breakthrough in the VDR field will be the identification of tissue selective VDR ligands. Expanded SAR and increased understanding of VDR biology have the potential to ultimately yield tissue selective VDR ligands that may exhibit improved therapeutic indices compared to those of existing ones.

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