Vitamin D Receptor Modulators for Inflammation and Cancer

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Abstract: 1α, 25-dihydroxyvitamin D3 {1,25-$(OH)_2D_3$}, the biologically active form of vitamin D, is an important hormone that is critically required for the maintenance of mineral homeostasis and structural integrity of bones. 1,25-$(OH)_2D_3$ accomplishes this by facilitating calcium absorption from the gut and by a direct action on osteoblasts, the bone forming cells. Apart form its classical actions on the gut and bone, 1,25-$(OH)_2D_3$ and its synthetic analogs also possess potent anti-proliferative, differentiative and immunomodulatory activities. 1,25-$(OH)_2D_3$ exerts these effects through vitamin D receptor (VDR), a ligand-dependent transcription factor that belongs to the superfamily of steroid/thyroid hormone/retinoid nuclear receptors. The presence of VDR in various tissues other than gut and bone, along with their ability to exert differentiation, growth inhibitory and anti-inflammatory action, has set the stage for therapeutic exploitation of VDR ligands for the treatment of various inflammatory indications and cancer. However, the use of VDR ligands in clinic is limited by their major dose-related side effect, namely hypercalcemia/hypercalciuria. Efforts are being undertaken to develop vitamin D receptor modulators (VDRMs) that are tissue-selective and/or gene-selective in their action and these ligands may exhibit increased therapeutic indices. This review explores the recent advances in VDR biology, non-secosteroidal VDR ligands and the current and potential clinical applications of VDR ligands in inflammation and cancer.

INTRODUCTION

Calcitriol or 1α, 25-dihydroxyvitamin D3 {1,25-$(OH)_2D_3$}, the hormonally active form of vitamin D, has traditionally been associated with calcium and phosphorus homeostasis and maintenance of skeletal architecture in young and adults. Vitamin D achieves this prominent physiological role by promoting absorption of dietary calcium and phosphorus from small intestine and by influencing the number and/or activity of osteoclasts and osteoblasts. However, in the last quarter of a century, our notion of 1,25-$(OH)_2D_3$ has changed from an essential micronutrient required for bone architecture to that of a bona-fide hormone involved in cell growth, differentiation and immunomodulation. 1,25-$(OH)_2D_3$ exerts its calcemic and non-calcemic actions by binding to vitamin D receptor (VDR), an intracellular nuclear receptor that functions as a ligand-dependent transcription factor and belongs to the superfamily of steroid/thyroid hormone/retinoid receptors. The expression of VDR in small intestine, bone, kidney and parathyroid gland provides a molecular basis for the calcemic actions of vitamin D. Using a variety of techniques, involving immunohistochemistry, Northern blotting, Western blotting and polymerase chain reaction, VDR has been shown to be expressed in virtually all the tissues and cells (prostate, stomach, placenta, keratinocytes, fibroblasts, pituitary, gonads, myocytes, colon, ovary, hippocampus, glial cells, thymus, breast, pancreas, melanocytes, lymphocytes, hair follicles, heart, lung, adipocytes, dendritic cells, leukemic cells, etc.). Interestingly, in certain disease states, for example, activated T- and B-lymphocytes, rheumatoid arthritis synoviocytes and macrophages, Kaposi's sarcoma, and cancers of colon, breast and prostate, VDR protein levels are induced compared to their normal counterparts. This disease-specific induction of VDR protein indicates that these conditions could also be attractive targets of 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 drug discovery efforts towards the synthesis of VDR agonists with increased therapeutic indices and their testing in certain human diseases as well as in animals models of human diseases. These efforts have led to the development of VDR ligands for the treatment of psoriasis, secondary hyperparathyroidism (renal osteodystrophy) and osteoporosis. In addition, VDR ligands have shown some efficacy in limited open clinical trials for prostate cancer, myelodysplasia (a pre-leukemic condition), psoriatic arthritis, seborrheic dermatitis and vitiligo. VDR ligands have also shown activity in the treatment of inflammatory and autoimmune diseases (rheumatoid arthritis, type I diabetes, multiple sclerosis, inflammatory bowel disease, systemic lupus erythematosus) as well as allograft rejection in animal models.

However, still after two-decades of drug discovery efforts, the major impediment for the wider use of a VDR ligands in a clinical setting is its major side-effect, namely hypercalcemia/hypercalciuria. In other words, 1,25-$(OH)_2D_3$ and other natural and synthetic VDR ligands induce severe hypercalciuria and hypercalcemia in the therapeutically relevant dose range by promoting calcium absorption from intestine and calcium release from bone. In the case of calcipotriol (Dovonex), a VDR ligand used topically for the treatment of psoriasis, the therapeutic index was improved by decreasing systemic metabolic stability of the compound that resulted in a soft drug with a short half-life. Apart from a soft-drug approach, therapeutic index could also be

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improved by identification of tissue selective vitamin D receptor modulators (VDRMs) that function as agonists in the target organs, say bone or skin and as antagonists in intestine, an organ that is the primary contributor of the hypercalcemia/hypercalciuria side effect. More than 99% of the VDR chemistry to date has been performed on the 1,25-$(OH)_2D_3$ secosteroidal backbone. Drug discovery efforts with classical steroid hormone receptors, namely estrogen receptor (ER), progesterone receptor (PR) and androgen receptor (AR) have shown that with steroidal ligands, it has been difficult to separate the desired pharmacological activity from the undesired side effect. Non-steroidal ligands on the other hand have turned out to be tissue-selective, i.e., agonists in some tissues and antagonists in others. The classical example is tissue-selective estrogen receptor modulator (SERM), raloxifene, a non-steroidal ER ligand that functions as an agonist in bone and vascular tissue and as an antagonist in breast and uterus. Of late, VDR chemistry has taken a cue from steroid receptors and efforts have been made to synthesize non-steroidal and even non-secosteroidal VDR ligands. These ligands have shown greater separation of non-calcemic activities (*e.g.*, inhibition of cell proliferation, induction of cell differentiation, inhibition of tumor cell growth *in vivo*, etc.) from hypercalcemia *in vivo*, when compared to 1,25-$(OH)_2D_3$. The resolution of VDR crystal structure bound to VDR ligands may also help us in the identification and development of vitamin D analogs with decreased calcemic liability and enhanced tissue specificity.

VDR and Modulation of Gene Expression

VDR ligands regulate gene expression by direct binding to the ligand binding domain (LBD) of VDR in the context of VDR-retinoid X receptor (RXR) heterodimer. RXR, a nuclear receptor for 9cis-retinoic acid, is an obligate partner of VDR in mediating 1,25-$(OH)_2D_3$ action [1, 2] and contrary to the previous observation, it appears to be actively involved in mediating vitamin D-dependent regulation of gene expression [3-5]. Recent studies have shown that RXR is not a silent partner in the context of RXR-VDR heterodimer, as previously proposed, but plays an important role in mediating VDR-dependent transactivation by recruiting coactivators in response to VDR ligands. This is achieved by the "phantom ligand effect phenomenon", whereby ligand binding to VDR in the absence of an RXR ligand results in an allosteric modification of RXR, which in turn renders RXR conducive to coactivator recruitment [3]. The VDR-RXR heterodimer binds to specific DNA sequences in the target genes known as the vitamin D response elements (VDREs). Natural VDREs are direct repeat (DR) of 5'-AG(G/T)TCA-3' motifs or their variants separated by three nucleotides and commonly referred to as DR-3 motifs [1, 2]. However, DR-6 motifs and everted repeat of the same motif separated by 6 nucleotides (ER-6) have also been shown to act as VDREs [6, 7]. The VDR protein is modular in nature and can be functionally divided into three main regions with well-characterized autonomous functions. The N-terminal region of nuclear receptors contains a ligand-independent transactivation function, AF-1. However, the presence of an AF-1 in VDR is still debatable. The central region contains the DNA binding domain (DBD) consisting of two potential zinc-binding fingers that target the receptor to VDREs. The C-terminal region of the receptor contains a multifunctional domain that harbors the 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 [8]. VDR is a ligand-dependent transcription factor that can modulate the expression of vitamin D-responsive genes by two different ways. It can either positively regulate the expression of certain genes by binding to the VDREs present in their upstream regions or negatively regulate the expression of other genes by binding to negative VDREs or by antagonizing the action of certain transcription factors, such as NF-AT and NF-kB [9-12]. Genes whose expression is induced by VDR ligands and which are known to contain an authentic VDRE in their promoter are presented in Table **I**.

In order to understand vitamin D action, we need to know VDR target genes and also understand the functional and physiological implications of changes in their protein levels. These genes include osteocalcin, osteopontin, RANKL, carbonic anhydrase II and calbindin-9k, which are involved in calcium homeostasis and bone remodeling. Other genes, which contain a VDRE in their promoter region and show vitamin D-dependent upregulation in their expression, are cell adhesion molecule β3 integrin, tumor suppressor p21 and hCYP3A4, along with their mouse and rat counterparts (Table **I**). VDR ligands also induce VDRE-dependent expression of 24-hydroxylase (CYP 24) that mediates the catabolic degradation of 1,25-$(OH)_2D_3$.

Table I. Vitamin D Responsive Genes and their VDREs

Gene	VDRE		
DR-3	AGGTCA	NNN	AGGTCA
rOST	GGGTGA	ATG	AGGACA
hOST	GGGTGA	ACG	GGGGCA
mSPP-1	GGTTCA	CGA	GGTTCA
rCalbindin-9k	GGGTGT	CGG	AAGCCC
p21	AGGGAG	ATT	GGTTCA
rBSP	AGGGTT	TAT	AGGTCA
r24-(OH)ase	CGCCCT	CAC	TCACCT
aβ3integrin	GAGGCA	GAA	GGGAGA
cCAII	AGGGCA	TGG	AGTTCG
mRANKL	AGGTCA	GCC	TGGTTCA
hCYP3A4	GGGTCA	GCA	AGTTCA
mCYP3A11	AGTTCG	TAT	AGTTCA
rCYP3A1	AGTTCA	TGA	AGTTCA
Involucrin	GGCAGA	TCT	GGCAGA
PLC-γ1	AGGTCA	GACCAC	TGGACA

DR, Direct Repeat; OST, Osteocalcin; SPP, Secreted Phosphoprotein (Osteopontin);
BSP, Bone Sialoprotein; 24-(OH)ase, 24-hydroxylase; CAII, Carbonic Anhydrase II; RANKL, Receptor Activator of NF-κB Ligand; CYP, Cytochrome P450; PLC, phospholipase C; r, rat; h, human; m, mouse; a, avian; c, chicken. [6, 7, 13-16].

A number of genes, whose expression is induced by VDR ligands but have not yet been shown to contain VDREs, have been identified and are presented in Table **II**.

Table II. Positively-Regulated Vitamin D Responsive Genes

Gene	Cells	Pathway	Reference
TGase I	Keratinocytes	Differentiation	[7]
PLC-β1	Keratinocytes	Differentiation	[17]
PLC-δ1	Keratinocytes	Differentiation	[17]
VDUP1	Keratinocytes	Differentiation	[18]
Involucrin	Keratinocytes	Differentiation	[19]
IL-10	Psoriatic lesions	Anti-inflammation	[20]
IL-10 Receptor	Keratinocytes	Anti-inflammation	[21]
Hox A10	Myelomonocytic	Differentiation	[22]
Hox 8	Osteoblasts	Differentiation	[23]
p27	Promyelocytes	Anti-proliferation	[24]
	Prostate cancer cells	Anti-proliferation	[25]
	Breast cancer cells	Anti-proliferation	[26]
CD14	Prostate cells	Anti-proliferation	[25]
	Leukaemic cells	Cell adhesion	[27]
CD11b	Leukaemic cells	Cell adhesion	[27]
CD18	Leukaemic cells	Cell adhesion	[27]
NGF	Fibroblasts	CNS function	[28]
	Astrocytes, Glioma cells		[29]
E-cadherin	Prostate cells	Adhesion	[25]
	Colon cancer cells		[30]
c-fos	Osteoblasts	Differentiation	[31]
VDR	Osteoblasts	Differentiation	[31]
c-Jun	Colon cancer cells	Differentiation	[32]
Alkaline Phosphatase	Osteoblasts	Differentiation	[32]
	Colon cancer cells	Differentiation	[33]
Osteoprotegrin	Osteoblasts	Bone Remodeling	[34]
DUSP 10	Prostate cancer cells	Anti-proliferation	[35]
IGFBP2	Colon cancer cells	Anti-proliferation	[30]
IGFBP3	Prostate cancer cells	Anti-proliferation	[35-37]
	Breast cancer cells	Anti-proliferation	[38]
	Colon cancer cells	Anti-proliferation	[30]
IGFBP5	Prostate cancer cells	Anti-proliferation	[35, 36]
	Breast cancer cells	Anti-proliferation	[39]
AR	Prostate cancer cells	Proliferation	[40]
TGF-β1	Breast cancer cells	Differentiation	[41]
ZO-1	Colon cancer cells	Adhesion	[30]
ZO-2	Colon cancer cells	Adhesion	[30]
ECaC1(CAT2)	Colon cancer cells	Calcemic action	[42]
ECaC2(CAT1)	Colon cancer cells	Calcemic action	[42]
c-Jun	Colon cancer cells	Differentiation	[30]

(Table 11) contd.....

Gene	Cells	Pathway	Reference
Jun B	Colon cancer cells	Differentiation	[30]
Jun D	Colon cancer cells	Differentiation	[30]
Plectin	Colon cancer cells	Adhesion	[30]
Filamin	Colon cancer cells	Adhesion	[30]
K13	Colon cancer cells	Differentiation	[30]
Kallikrein 10	Colon cancer cells	Anti-proliferation	[30]
Protease M	SCC	Differentiation	[43]
Cystin M	SCC	Differentiation	[43]
Amphiregulin	SCC	?	[44]
GADD45α	SCC	DNA repair	[45]
Stromelysin 1	SCC	?	[43]
Collagenase 1	SCC	?	[43]

TGase I, Transglutaminase I; PLC, Phospholipase C; VDUP, Vitamin D upregulated protein; IL-10, Interleukin 10; NGF, Nerve Growth Factor; DUSP, Dual specificity phosphatase 10; IGFBP, Insulin like growth factor binding protein; AR, Androgen receptor; TGF, Transforming growth factor; ZO, Zonula occluden; GADD, Growth and DNA-damage inducible; ECaC, Epithelial calcium channel; CAT, Calcium transporter; K 13, Keratin 13; SCC, squamous cell carcinoma.

These genes include cell differentiation markers (TGase I, involucrin, PLC-β1, PLC-γ1, PLC-δ1 and VDUP1), which are involved in keratinocyte differentiation. Differentiation regulatory genes that show VDR-mediated induction in their expression are Hox A10 (myelomonocytic cells), c-Jun, Jun B, Jun D (colon cancer cells), and Hox 8, c-fos and VDR (osteoblasts). Cell adhesion molecules that fall in this category are CD14, CD11b, CD18 and E-cadherin. Anti-proliferation genes in this category include tumor suppressor p27, CD14 and E-cadherin. VDR-dependent anti-inflammatory markers, which show ligand-dependent upregulation, are anti-inflammatory cytokine IL-10 and its receptor. Osteoprotegrin, a protein involved in bone remodeling and NGF, which is involved in central nervous system function, also show vitamin D-dependent regulation in osteoblasts and astrocytes respectively. 1,25-$(OH)_2D_3$ also upregulated the expression of insulin like growth factor binding proteins (IGFBP2, 3 and 5) in various cancer cells (Table **II**). IGFBPs neutralize the growth promoting activities of insulin like growth factors. A number of genes that are involved in cell adhesion (ZO-1, ZO-2, filamin and plectin) and AP1-mediated differentiation pathways (c-jun, Jun B and Jun D), are also upregulated by the VDR ligand in colon cancer cells (Table **II**).

Genes that are downregulated in response to 1,25-$(OH)_2D_3$ and its synthetic analogs are presented in Table **III**.

The known hyperproliferative and inflammatory functions of these gene products indicates 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, IL-6, IL-8, IL-12, TNF-α, IFN-γ and GM-CSF. Proliferation associated genes that are transrepressed by VDR ligands include, EGF-R, c-myc, Ki-67 and K16. PTH and PTHrP, which are involved in mineral homeostasis, are also downregulated by VDR ligands. 1,25-$(OH)_2D_3$ also negatively regulates cell cycle control genes (CDK2 and cyclin D1) in cancer cells (Table **III**).

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 GM-CSF 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 GM-CSF expression [10, 11, 63]. Both VDR monomers and VDR-RXR heterodimers are involved in inhibition of IL-2 and GM-CSF promoters.

VDR Coactivators/Corepressors

Ligand binding induces conformational changes in the VDR resulting in promotion of heterodimerization with RXR and recruitment of certain proteins, called coactivators/co-factors to the RXR-VDR heterodimer complex. Ligand occupancy of VDR results in the formation of a charge clamp in the VDR and presumably RXR LBD, and as a result cofactors containing LXXLL motifs are recruited to this newly formed surface. Cofactors provide various enzymatic activities (histone acetylase, kinase and methylase) or they help in the recruitment of these activities to VDRE containing target genes promoters. These enzymatic activities promote post-translational modifications of chromatin proteins that is obligatory for efficient vitamin D-dependent pol II-mediated transcription. Cofactors constitute two classes of proteins, namely coactivators that mediate ligand-dependent transcription and corepressors that silence or suppress the expression of responsive genes. Most of the nuclear receptors associate with corepressors (N-CoR and SMRT) in an unliganded or antagonist-bound state or when bound with an antagonist or an inverse agonist. VDR was found to associate with N-CoR, SMRT and Hr corepressors in the absence of the ligand [64, 65]. However, the interaction of VDR with N-CoR and SMRT is weaker than the interaction of these corepressors with thyroid

Table III. Negatively Regulated Vitamin D-Responsive Genes

Gene	Cells	Pathway	Reference
c-myc	Keratinocytes	Proliferation	[46]
	Colon cancer cells	Proliferation	[47]
Ki-67	Psoriatic plaques	Proliferation	[48]
K16	Psoriatic plaques	Proliferation	[48]
EGF-R	Keratinocytes	Proliferation	[46]
t-PA	Keratinocytes	Protease	[49]
IL-2	T-lymphocytes	Inflammation	[50]
	PBMC	Inflammation	[51]
IL-6	PBMC	Inflammation	[52]
	Kaposi's sarcoma cells	Proliferation	[53]
IL-12	Myelomonocytes	Inflammation	[54]
IFN-γ	PBMC	Inflammation	[50]
Lymphotoxin	PBMC	Inflammation	[50]
TNF-α	PBMC	Inflammation	[50]
IL-8	Keratinocytes	Proliferation	[55]
	Kaposi's sarcoma cells	Proliferation	[53]
GM-CSF	PBMC	Inflammation	[56]
PTHrP	Osteoblasts	Mineral Homeostasis	[9]
	Keratinocytes	?	[57]
PTH	Parathyroid cells	Mineral Homeostasis	[58]
ANP	Cardiac myocytes	Hypertrophy	[59]
CDK2	Prostate cancer cells	Proliferation	[60]
	Breast cancer cells	Proliferation	[61]
PPARδ	Colon cancer cells	Proliferation	[47]
Tcf-1	Colon cancer cells	Proliferation	[47]
Cyclin D1	Colon cancer cells	Proliferation	[62]
	Breast cancer cells	Proliferation	[61]
SCCA	SCC	?	[43]

K16, Keratin 16; EGF-R, Epidermal Growth Factor Receptor; t-PA, tissue plasminogen activator; IL, Interleukin; IFN, Interferon; TNF, Tumor Necrosis Factor; GM-CSF, Granulocyte Macrophage-Colony Stimulating Factor; PTHrP, Parathyroid hormone related protein; PTH, Parathyroid hormone; ANP, Atrial Natriuretic Peptide; CDK, cyclin-dependent kinase; PPAR, peroxisome proliferator activated receptor; Tcf, T-cell factor; SCCA, squamous cell carcinoma antigen.

hormone receptor (TR) [66]. Corepressors recruit histone deacetylase (HDAC) activities to the responsive promoters that result in deacetylation of the lysine residues present in the histone tails of nucleosomes, leading to the compaction of the chromatin material and transcriptional silencing. Therefore, transcription is balanced by the "yin" and "yang" of coactivator/corepressor recruitment by the nuclear receptors. However, VDR presents a conundrum, since unlike TR and retinoic acid receptors (RARs), it has not been shown to inhibit the basal level expression of VDRE containing genes in the absence of the ligand. Therefore, the question, whether these nuclear receptor corepressors are also physiological corepressors for VDR, remains unanswered. However, we cannot rule out the possibility that corepressors are recruited by the VDR in the context of nVDREs. The identification of coactivators and their inhibitory congeners, namely corepressors have provided fresh insight into mechanism of hormone-mediated transcription. Hence, studies involving the identification of coactivators or cofactors of nuclear receptor action have been in vogue in order to obtain a more global understanding of hormone action at the level of transcription. The VDR-interacting co-factors that have been identified to interact with VDR in a ligand-dependent manner are listed in Table **IV**.

Table IV. VDR Interacting Co-factors/Coactivators

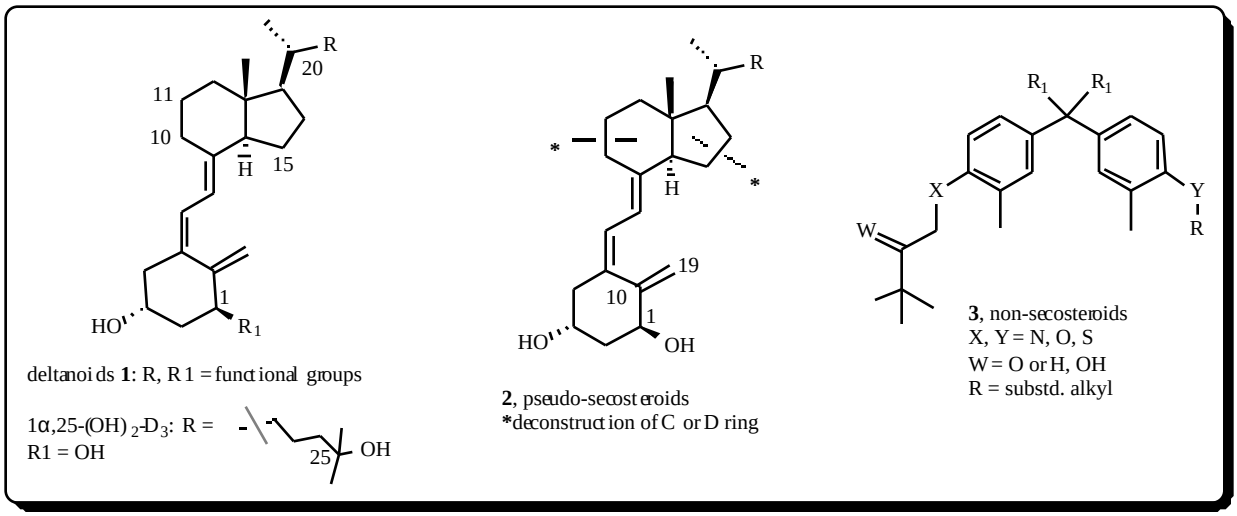
Cofactor		Function/Activity
SRC/p160 family		Chromatin modification
	SRC-1 (SRC-1/NCoA-1)	
	SRC-2 (GRIP1/TIF2/NCoA-2)	
	SRC-3 (pCIP/RAC3/ACTR/AIB1/TRAM-1/NcoA-3)	
DRIP205/TRAP220		Mediator complex recruitment
CBP/p300		Chromatin modification
TIF1		Kinase
NCoA-62/SKIP		Splicing?/ Chromatin modification
$TAF_{II}135$		TAF
$TAF_{II}55$		TAF
$TAF_{II}28$		TAF

SRC, Steroid receptor coactivator; NcoA, Nuclear receptor coactivator; GRIP, glucocorticoid receptor interacting protein; DRIP, VDR interacting protein; TRAP, TR associated protein; CBP, CREB binding protein; TIF, Transcription intermediary factor; HAT, Histone acetyltransferase; RIP, Receptor interacting protein; SKIP, ski-interacting protein; TAF, TFIID associated factor.

SRC/p160 family of coactivators includes three related (approximately 40% sequence similarity) but distinct members, namely SRC-1, SRC-2 and SRC-3. Each family member has a number of splice variants, and truncated versions of the protein, as expected, display dominant negative activity. SRCs interact with VDR in a ligand-dependent manner and the integrity of the receptor AF-2 is a must for its interaction with p160 coactivators. SRCs were initially thought to be nuclear receptor-specific coactivators but many reports are now revealing their involvement in transactivation by a number of other transcription factors. For example, SRC-1 also functions as a coactivator for NF-kB, serum response factor and p53 [67, 68]. Similarly, SRC-3 also augments the transcriptional activity of signal transducers and activators of transcription-1 (STAT-1), cAMP response element binding protein (CREB) [69] and helix-loop-helix transcription factor MEF-2 [70]. These p160 proteins interact with nuclear receptors *via* a nuclear receptor interaction domain consisting of 3 copies of the LXXLL motif, where L is leucine and X is any amino acid but for proline, and preferably one of the X amino acids should be a hydrophobic amino acid. SRC-1 contains an additional LXXLL in its C-terminal region. CBP and p300 are large proteins that interact with a wide variety of transcription factors, namely nuclear receptors, Pit-1, myoD and STATs [64]. Apart form interacting with nuclear receptors, CBP/p300 also associates with SRCs. Since CBP/p300 can also interact with TBP and TFIIB, it may bridge the nuclear receptors to the basal transcriptional machinery *via* p160 proteins [64]. 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. Increased acetylation of lysine residues in histone tails has been shown to correlate with transcriptional activity and decreased acetylation has been linked to repression of gene expression. There appears to be a quite strong association between histone acetylation, chromatin remodeling and gene expression [71, 72]. Acetylation of histones may also disrupt higher order chromatin structure since histone tails are involved in maintaining nucleosome-nucleosome interactions.

NcoA-62/SKIP is a non-LXXLL motif protein that is structurally different from SRC family members and interacts with RXR-VDR heterodimers in a ligand-dependent manner. Apart from VDR, it also induces ligand dependent transcription by RAR, ER and glucocorticoid receptor (GR). It acts synergistically with SRC coactivators by forming a ternary complex [73, 74]. There is a distinct possibility of identifying VDR-specific or tissue restricted coactivators. Such precedence exists for AR, where ARA70 specifically induces androgen-mediated gene expression and another AR coactivator, FHL2, is expressed specifically in prostate but not in other tissues [75, 76]. VDR has also been shown to directly interact with certain components of transcription machinery including TF-IIB, TF-IIA and TATA binding protein (TBP)- associated factors (TAFs), e.g., $TAFII_{135}$, $TAFII_{55}$ and $TAFII_{28}$ [77-79].

A multiprotein complex involving ~20 proteins, called DRIP (VDR interacting proteins)/TRAP (TR-associated proteins) complex that interacts with nuclear receptors and other transcription factors, has been described [80]. The DRIP complex is recruited to ligand occupied VDR by its interaction with DRIP205/TRAP220, a component of the complex that provides two LXXLL motifs for direct protein: protein interaction with RXR-VDR heterodimers. Since the DRIP complex is devoid of SRC/p160 proteins and is not associated with any detectable HAT activity, the current working model for VDR transcription assumes that ligand occupied distinct VDR-DRIP and VDR-SRC complexes are present in the nucleus [81]. First step involves the targeted recruitment of VDR-SRC or a VDR-HAT activity complex to the responsive promoter to facilitate in the destabilization of the nucleosomal core. The unwound DNA then dissociates from the VDR-SRC complex and becomes the target of the VDR-DRIP complex that contains factors required for transcription. The DRIP complex has also been shown to recruit RNA polymerase II holoenzyme to VDR in a ligand-dependent manner [82]. This two-step coactivator



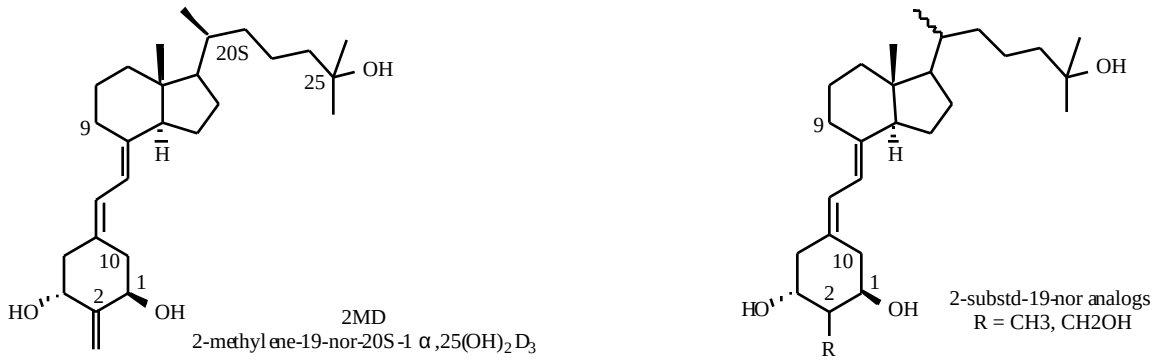
exchange model is currently in vogue but it may get more complex as more VDR interacting proteins are discovered.

VDR Chemistry

Chemistry

VDR ligands may be classified into three broad classes of molecular structures **1**, **2**, and **3**. Deltanoids **1**, which possess the secosteroidal scaffold, have been extensively reviewed [83]. Pseudo-secosteroids **2** are relatively new VDR ligands, which retain the A-ring of vitamin D with the modified C or D rings [84]. The last class of VDR agonists is non-secosteroids **3** whose structural features have the least resemblance to secosteroids **1** [85]. Recently, we have described an overview of pseudo-secosteroids **2** and non-secosteroid **3** SAR [1].

Table V. Biological Activity of Secosteroids



Compound	VDR Binding[a] (Rel. Potency)[b]	HL-60 Differentiation.[a] (Rel. Potency)[b]	Qualitative *Ex-vivo* Ca Transport S/M[a,c]
1α-25(OH)$_2$D$_3$	100	1	+
2-methylene-19-nor-1α-25(OH)$_2$D$_3$	67	1	-
2-methylene-19-nor-20S-1α-25(OH)$_2$D$_3$ (2MD)	77	25	-
2α-methyl-19-nor-1α-25(OH)$_2$D$_3$	22	50	+
2β-methyl-19-nor-1α-25(OH)$_2$D$_3$	2.6	0.5	0
2α-hydroxymethyl-19-nor-1α-25(OH)$_2$D$_3$	8	0.2	
2β-hydroxymethyl-19-nor-1α-25(OH)$_2$D$_3$	0.9	0.04	
2β-hydroxymethyl-19-nor-20S-1α-25(OH)$_2$D$_3$	0.12	1	0

[a] Reference 87.

[b] Relative potency to 1α-25(OH)$_2$D$_3$; 100 or 1 as base potency; higher number is more potent.

[c] Qualitative results from rat *ex vivo* everted intestinal calcium transport serosal/mucosal at a daily dose of 260 pmol/day/7 days; 0) equivalent to vehicle; +) greater than vehicle; -) less than vehicle.

All three classes of VDR ligands potently activate VDR genes. However their affinity to the VDR is very different. In general, compounds **1** and **2** have high affinity to the VDR with the latter series being slightly weaker. In contrast, non-secosteroids **3** have little or no affinity to VDR under normal equilibrating conditions. Despite their poor affinity to VDR, a number of non-secosteroids **3** are active in a VDR agonist-induced cotransfection-cotransactivation assay [85]. Interestingly, it has been shown that some VDR ligand's affinity is not necessarily directly proportional to its gene activation potency [86]. The different structural features within these three classes of VDR agonists strongly suggest that they may well have drastically different metabolic pathways. These divergences of metabolic pathways may result in vast differences in residency time in different cells/tissues, which could culminate into great differences in their duration time of gene activation. This brief review will highlight recent SAR studies that contrast the attributes of these three series of VDR agonists.

Secosteroids

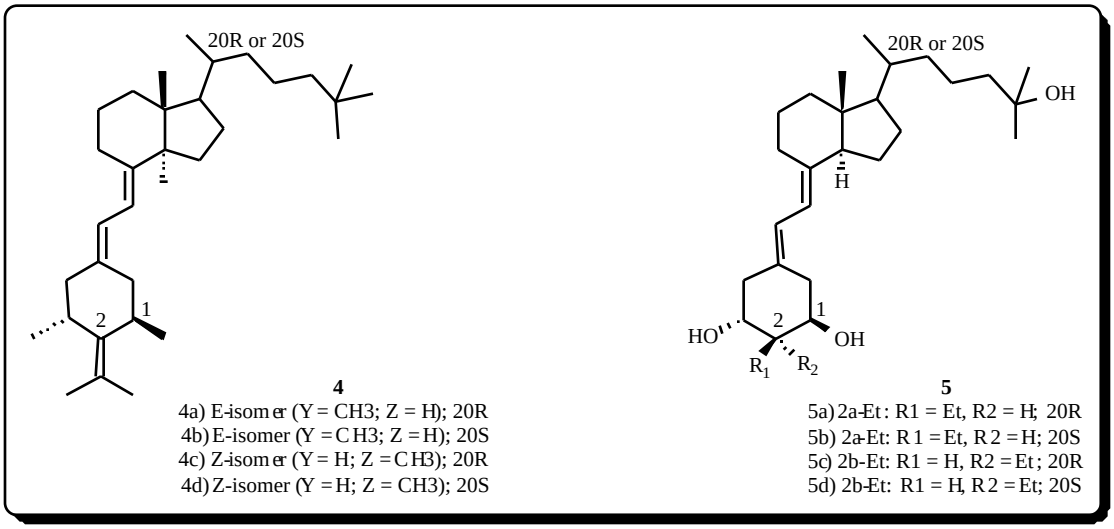
2MD represents a new class of secosteroids wherein a 2-methylene is introduced, the 19-methylene is excised (19-nor) and the C20 has the 20S unnatural stereochemistry incorporated [87]. Small structural changes within the 19-nor methylene series have a great impact on tissue selectivity and potency. 2MD has similar VDR affinity as 1,25-$(OH)_2D_3$, however its HL-60 differentiation potency is 25 times greater than the natural ligand (Table **V**).

In contrast, the 20R isomer of 2MD has both VDR affinity and HL-60 differentiation activity equivalent to 1,25-$(OH)_2D_3$. The related 2α-methyl-19-nor-1α-25$(OH)_2D_3$ exhibits a 50 times enhancement of HL-60 differentiation with a concurrent decrease of ~80% VDR affinity when compared to 1,25-$(OH)_2D_3$. Relative to 1,25-$(OH)_2D_3$, the corresponding 2β-methyl-19-nor-1α-25$(OH)_2D_3$ is nearly devoid of VDR affinity but retains half of the HL-60 activity. In general, nearly all of the 20S vitamin D analogs described (not all 20S analogs are listed in Table **V**) are more potent than 1,25-$(OH)_2D_3$ in HL-60 differentiation.

SAR was further explored in the 2-ethyl- and 2-ethylidene-19-nor series **4** and **5** [88]. At best, only some of these analogs provided a modest two to three fold increase in VDR binding (**4a**, **4b** and **5b**) over 1,25-$(OH)_2D_3$. Out of all the isomers from **4** and **5** that have a reasonable level of VDR binding (ED_{50}~$5X10^{-10}$M), only **4a** displayed no intestinal calcium transport activity.

Selectivity assessment of 2MD's ability to stimulate bone calcium mobilization versus intestinal calcium transport was determined. Using vitamin D-deficient and calcium-deficient rats and increasing doses of 1,25-$(OH)_2D_3$ or 2MD, the bone calcium mobilization and calcium intestinal transport activities were assessed. Under this diet regimen, the source of serum calcium is most probably derived from osteoclast-mediated bone resorption. Thus, the serum calcium level in this model is a surrogate of bone calcium mobilization. 2MD has 30 times greater bone calcium mobilization than 1,25-$(OH)_2D_3$. Comparison of 2MD's bone calcium mobilization and intestinal calcium transport stimulation only showed a modest 2-3-fold separation. 2MD is highly effective in stimulating *in vitro* human osteoblast bone formation at 10^{-12} M, while 1,25-$(OH)_2D_3$ is ineffective at 10^{-8} M. The enhanced transcriptional activation of 2MD over 1,25-$(OH)_2D_3$ in osteoblasts was attributed to 2MD's greater potency in promoting interaction with RXR and coactivators SRC-1 and DRIP205 [89]. The *in vitro* bone effect of 2MD is also manifested *in vivo* in a calcium diet deficient ovariectomized rat bone formation model (OVX). 2MD at a dose of 18.7 pmol/kg/day for 23 weeks caused a marked rise of 9% in total bone mineral density versus OVX controls. 2MD is a clinical candidate for osteoporosis. The pending clinical results will determine if 2MD has the requisite anabolic bone effect and calcium selectivity for a safe osteoporosis drug.

The metabolic fate of 1,25-$(OH)_2D_3$ has been investigated extensively especially with 24-hydroxylase, which ultimately leads to the deactivation of the hormone. In contrast to 1,25-$(OH)_2D_3$, the metabolic profile of 20-epi-1α,25$(OH)_2D_3$ may extend its half-life in selected tissues. Recent studies have demonstrated that 20-epi-1α-25$(OH)_2D_3$ exhibits increased transcriptional activities in Ros 17/2.8 cells *via* favorable metabolic pathways [90]. Ros 17/2.8 cells do not express 24-hydroxylase but possess 3-epimerization



4

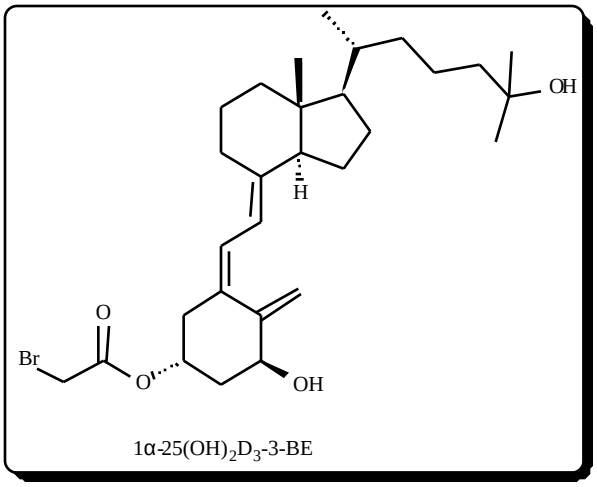
4a) E-isomer (Y = CH3; Z = H); 20R
4b) E-isomer (Y = CH3; Z = H); 20S
4c) Z-isomer (Y = H; Z = CH3); 20R
4d) Z-isomer (Y = H; Z = CH3); 20S

5

5a) 2a-Et: R1 = Et, R2 = H; 20R
5b) 2a-Et: R1 = Et, R2 = H; 20S
5c) 2b-Et: R1 = H, R2 = Et; 20R
5d) 2b-Et: R1 = H, R2 = Et; 20S

pathways. With the lack of 24-hydroxylase, Ros 17/2.8 cells are ineffective in deactivating 20-epi-1α-25$(OH)_2D_3$. The 3-epimerization pathways may serve to extend the activity of 20-epi-1α-25$(OH)_2D_3$ since its metabolite 3-epi-20-epi-1α-25$(OH)_2D_3$ is also 100 times more potent than 1,25-$(OH)_2D_3$ in Ros cell transcriptional activation. Also a number of 20-epi vitamin D analogs, when complexed to VDR, protect the receptor from degradation more effectively than 1,25-$(OH)_2D_3$ [91]. It would be of great interest to determine if any analogs within the 2MD (19-nor-20-epi) series have this beneficial metabolic profile that could extend their duration of action in Ros cell for greater bone formation effect.

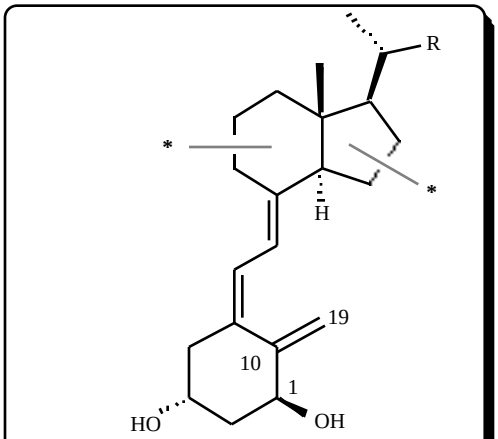
A cross-linking VDR ligand, 1α-25$(OH)_2D_3$-3-BE, is found to induce apoptosis in LNCaP and PC-3 cells [92]. Robust antiproliferative effect on LNCaP and PC-3 cells is observed with 10^{-6} M of 1α-25$(OH)_2D_3$-3-BE as measured by the decrease of 3H-thymidine incorporation. Results from controlled experiments with 1,25-$(OH)_2D_3$ and bromoacetic acid further support that the mechanism of action of 1α-25$(OH)_2D_3$-3-BE is *via* selective alkylation of the VDR. Further studies would be required to validate this VDR-LBD alkylation approach as a safe treatment for prostate cancer.



1α-25$(OH)_2D_3$-3-BE

Pseudo-Secosteroids

Since our last review no new data on pseudo-secosteroids **2** have been published [1]. Nevertheless, the novel structures in this class of compounds warrant further SAR investigation since it could produced VDR ligands with a profile quite distinct from the secosteroids and non-secosteroids. Furthermore, the lack of either the C or D ring in pseudo-secosteroids **2** could potentially abort 24-hydroxylase metabolic pathways, which in turn may increase their duration of action *in vivo.* Additional work in this series could potentially provide therapeutically useful agents.

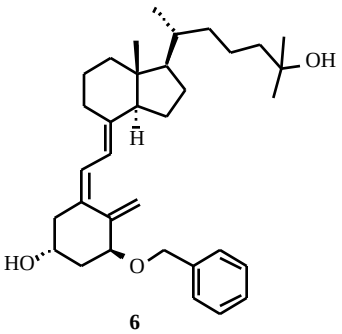


2, pseudo-secosteroids
***deconstruction of C or D ring

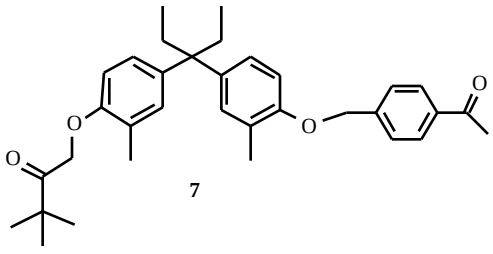
Non-Secosteroids

Koh and coworkers have designed novel VDR ligands as potential treatment for rickets-associated with VDR-LBD mutant Arg_{274}-Leu ($R_{274}L$) [93, 94]. It is known that the VDR $R_{274}L$ mutation leads to a net loss of hydrogen bonding between the Arg guanidine and the 1α-hydroxyl group of 1,25-$(OH)_2D_3$. When compared to the wild-type VDR, there is at least 1000 times decrease in gene transactivation response induced by 1,25-$(OH)_2D_3$ on VDR $R_{274}L$ mutant. Using computational models, they determined that $R_{274}L$ mutation creates a "hydrophobic hole" in place of the hydrophilic pocket within the wild type VDR-LBD. With this insight, they have replaced the 1α-hydroxy group in 1,25-$(OH)_2D_3$ with lipophilic moieties to increase interaction with the VDR $R_{274}L$ [94]. When compared to 1,25-$(OH)_2D_3$, the 1α-benzyloxy analog **6** (EC_{50} = 116 nM) is at least 20 times more potent in

Table VI. HEK293 Cell CTF Assay



6



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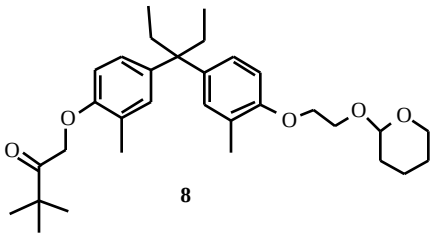


8

	1,25D3/WT	1,25D3/R274L	22/R274L	23/R274L	24/R274L
EC50 (nM)	2	>2000	116	3.3	8.4

transactivation of HEK293 cell transfected with VDR $R_{274}L$ (Table **VI**).

This successful approach has been extended to the diaryl alkanes which provided **7** and **8** [94]. The HEK293 cell transactivation EC_{50} for **7** and **8** are 3.3 nM and 8.4 nM, respectively. These results show that these ligands' transactivation potency on VDR $R_{274}L$ mutant is nearly equivalent to 1,25-$(OH)_2D_3$ effect on the wild type VDR. These molecules have promise as possible agents for the treatment of rickets caused by the VDR $R_{274}L$ mutant.

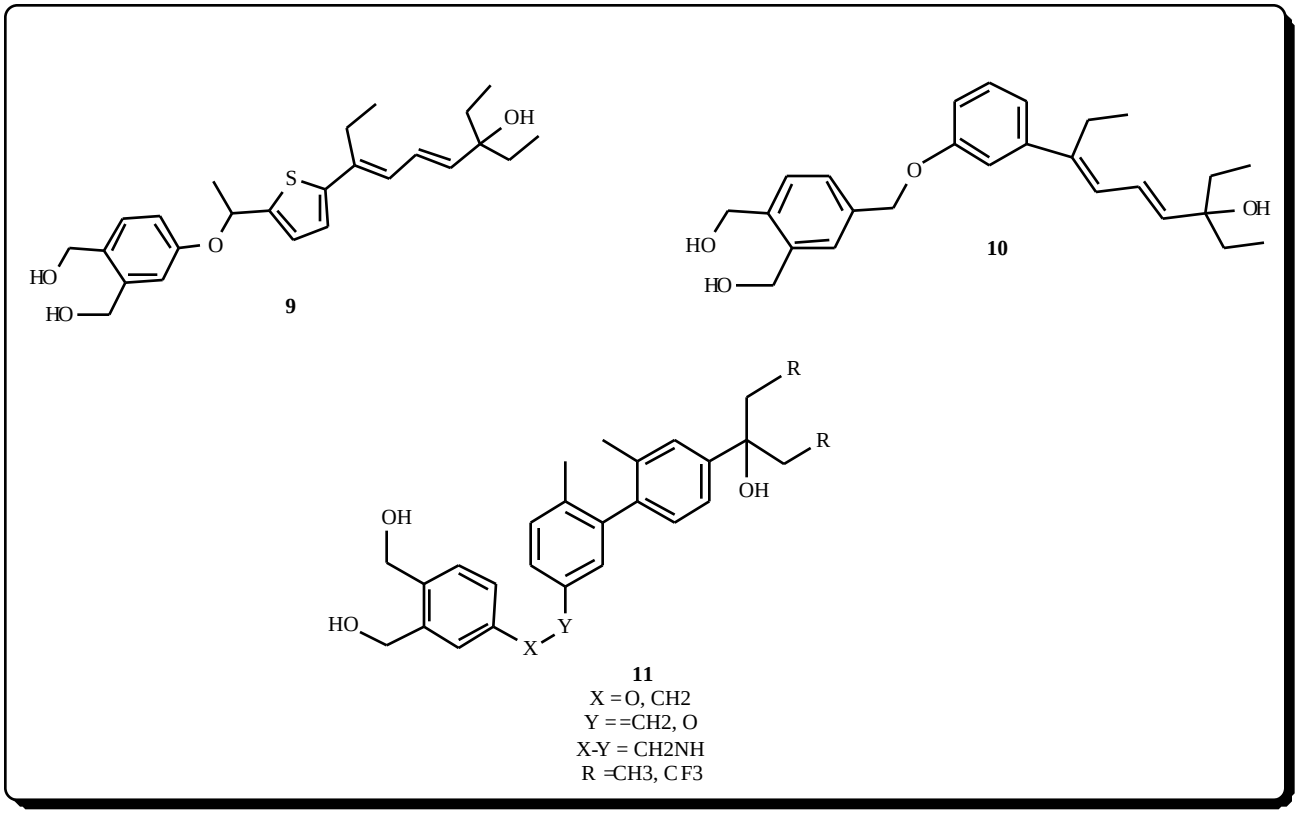
The Galderma group has disclosed work in the non-secosteroid area in a number of patent publications [95-97]. Some of the representative compounds described are **9**, **10**, and **11** wherein the scaffold is based on two aryl groups connected by two-atom linkers. These VDR agonists also possess a hydrophilic (dihydroxy group) and hydrophobic side chains that resemble the 1,3-dihydroxy and C25 groups of 1,25-$(OH)_2$D3, respectively. Compounds **11** exhibits nanomolar VDR transactivation potency in HL-60 transfected with hVDR-p24(OH)ase-chloramphenicol-acetyltransferase (promoter-reporter) assay. Future disclosures will provide a clearer assessment of the calcium selectivity of these VDR agonists.

VDRMs as Potential Therapeutics for Inflammatory Diseases

VDR and Psoriasis

Psoriasis is an inflammatory skin disorder that affects ~2% of the population. The presence of the VDR protein in activated T cells [98, 99], and the anti-proliferative and prodifferentiation activities of 1,25-$(OH)_2D_3$ and its synthetic analogs in keratinocytes [7] provided a reasonable basis for the clinical use of VDR ligands for the treatment of psoriasis. The proof of principle for the use of 1,25-$(OH)_2D_3$ analogs came from a fortuitous observation when a patient treated orally with alfacalcidol (1α-hydroxyvitamin D3) for osteoporosis showed remission of psoriatic lesions [100]. Subsequently, promising results were obtained in clinical studies using oral alfacalcidol (1 μg/day), topical 1α,24-dihydroxyvitamin D3 (tacalcitol) and topical 1,25-$(OH)_2D_3$ in patients with plaque-type psoriasis [101-103]. Orally administered 1,25-$(OH)_2D_3$, was also efficacious but demonstrated potent hypercalcemic activity which could result in nephrocalcinosis and a reduction in bone density [104]. These calcemic effects could be reduced by oral administration of 1,25-$(OH)_2D_3$ at night and by limiting dietary intake of calcium. However, topical 1,25-$(OH)_2D_3$ was found to be efficacious, and at 15μg/g concentration, once daily application resulted in approximately 90% improvement in psoriatic lesions with no skin irritation [105].

The use of 1,25-$(OH)_2D_3$ in psoriasis is limited by its hypercalcemic/hypercalciuric activity. Medicinal chemists have tried to develop 1,25-$(OH)_2D_3$ analogs with decreased hypercalcemic activity by minor modifications of the secosteroidal backbone. Calcipotriol (calcipotriene, Dovonex), a synthetic vitamin D analog containing a double bond and a cylcopropane ring in the side chain, was found to be 100-200 times less potent than 1,25-$(OH)_2D_3$ in hypercalcemic activity as a result of its low systemic half life [106]. In clinical studies, significant improvement was observed in ~70% of the patients after two weeks of topical therapy with twice daily application of the drug. Complete clearance of lesions has been observed in ~25% of the



patients. The full response of the drug was observed after 6-8 weeks of treatment. Calcipotriol ointment was also compared with betamethasone 17-valerate and found to be slightly superior to the steroid [106, 107]. The most common side effect of topical calcipotriol in clinic was cutaneous irritant reaction that was observed in approximately 20% of the patients [107].

Postmarketing surveillance study of tacalcitol ointment (4μg/g) has been performed on more than 5000 psoriasis patients. Once a day treatment with topical tacalcitol resulted in good efficacy in 70 % of the patients and it was tolerated well by 90 % of the patients [108]. In an effort to discover topical anti-psoriatic agents superior to calcipotriol and tacalcitol, 1α,25-dihydroxy-22-oxacalcitriol (maxacalcitol) has been studied in a phase II, double blind, randomized, left *vs* right, concentration dose response study with once a day application in patients with mild to moderate chronic plaque psoriasis. At a concentration of 25 μg/g, maxacalcitol resulted in marked improvement or complete clearance in 55% of the patients compared to calcipotriol (50 μg/g), which produced similar result in 46% of the patients [109].

The anti-psoriatic activity of 1,25-$(OH)_2D_3$ and its analogs results from their potent differentiation, anti-proliferation and immunomodulatory activities. 1,25-$(OH)_2D_3$ treatment inhibited the proliferation of cultured keratinocytes and resulted in their differentiation [7, 106]. 1,25-$(OH)_2D_3$-mediated keratinocyte differentiation was exemplified by increased levels of transglutaminase I (TGase I) and involucrin as well as enhanced cornified envelop formation in suprabasal cells [7]. The expression of PLC-β1, γ1 and δ1 that induce AP1 activity and the expression of proteins that form the cornified envelop, was also induced by 1,25-$(OH)_2D_3$ [7, 17]. Some markers of cell proliferation which were down-regulated by VDR ligands in keratinocytes included EGF-R, c-myc, Ki-67 and K16. The immunomodulatory activity of VDR also appears to account for some of the therapeutic effects of 1,25-$(OH)_2D_3$ and its analogs in psoriasis. VDR ligands decreased the expression and/or protein levels of IL-2, IL-6, IL-8, IFN-γ and GM-CSF (Table **III**), all of which play an important role in processes of cutaneous inflammation and proliferation of T-lymphocytes and keratinocytes. The expression of IL-2 and GM-CSF was negatively regulated through VDR by ligand-dependent inhibition of NF-AT-AP1 composite element activity [11, 63]. VDR ligands also increased the expression of the anti-inflammatory cytokine IL-10 in psoriatic lesions [20].

VDR and Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a common human autoimmune disease affecting approximately 1% of the people worldwide. Although there has been progress in defining its etiology and pathogenesis, these are still incompletely understood. In addition to inflammation in the synovium, which is the joint lining, the aggressive front of tissue called pannus invades and destroys local articular structures. In RA, CD4+ T cells, B cell and macrophages infiltrate the synovium and sometimes organize into discrete lymphoid aggregates with germinal centers. Hyperplasia of the intimal lining results from a marked increase in macrophage-like and fibroblast-like synoviocytes. Locally expressed degradative enzymes, including matrix metalloproteinases (MMPs), serine proteases and aggrecanases digest the extracellular matrix and destroy the articular structures [110]. Epidemiological studies have reported low serum 25-hydroxyvitamin D and 1,25-$(OH)_2D_3$ levels in RA patients. The lowest values of serum 1,25-$(OH)_2D_3$ levels were found in patients with high disease activity [111]. Interestingly, low serum 1,25-$(OH)_2D_3$ levels were also observed in rats with adjuvant-induced arthritis [112].

VDR has been detected in immune cells like activated CD8 (highest concentration), CD4 lymphocytes and macrophages [99]. 1,25-$(OH)_2D_3$ and its analogs potently inhibited antigen- and mitogen-induced T-cell proliferation and cytokine production [113-117]. Several key cytokines in T cells are direct targets of 1,25-$(OH)_2D_3$ and its analogs, in particular Th1 cytokines, such as IL-2 and IFN-γ. 1,25-$(OH)_2D_3$ inhibits IL-2 secretion *via* impairment of transcription factor NF-AT complex formation, because the ligand-bound VDR complex itself binds to the distal NF-AT binding site of the human IL-2 promoter [10, 11]. IFN-γ has been found to be directly inhibited by 1,25-$(OH)_2D_3$ through interaction of the ligand-bound VDR complex with a VDRE in the promoter region of this cytokine [118]. 1,25-$(OH)_2D_3$ inhibited the expression of both subunits (p35 and p40) of IL-12, which directs Th1 development and plays an important role in many autoimmune diseases. The repressive effect on p40 is mediated by RXR/VDR-dependent antagonism of the enhancer activity of NF-kB, which is a major enhancer factor of the p40 gene [54]. The antagonism between VDR and NF-kB is mutual since over expression of p65 but not p50 subunit of NF-kB inhibited VDRE-mediated transcription in transfected cells [119]. Similar NF-kB dependent mechanism has also been implicated in the inhibition of IL-8 promoter expression by 1,25-$(OH)_2D_3$ [12]. VDR ligands also inhibited the induced production of pro-inflammatory cytokines, IL-1α, IL-6, and TNF-α [50]. These activities form the basis of VDR ligand-mediated suppression of T-lymphocyte driven immune reactivity and inflammation.

Among the different animal models of RA, two have been used to test the effects of VDR ligands on the course of the disease, namely Lyme arthritis and collagen-induced arthritis (mouse and rat). Mice infection with Borrelia burgdorferi, the causative agent of human Lyme arthritis, produces acute arthritis lesions with footpad and ankle swelling. Infected mice supplemented with diet containing 1,25-$(OH)_2D_3$ minimized or prevented these symptoms [120]. The same prophylactic treatment could also prevent collagen-induced arthritis in mice [121]. In another study, the immunomodulatory activity of a 20-epi-1 α, 25-dihydroxyvitamin D3 (MC1288), was evaluated in a rat collagen-induced arthritis. The vitamin D analogue MC 1288 has the ability to prevent (prophylactic treatment), and furthermore to suppress, already established CIA (therapeutic treatment) by its immunomodulatory properties without inducing hypercalcaemia [122]. Finally, in an open-label trial of oral 1,25-$(OH)_2D_3$ in patients with psoriatic arthritis, a significant improvement in the arthritis symptoms was observed [123].

VDR expression has been observed in chondrocytes, and synoviocytes from rheumatoid lesions but not in normal

cartilage [124]. *In vitro* studies suggest that 1,25-$(OH)_2D_3$ contributes to the regulation of MMPs and PGE2 production by human articular chondrocytes in osteoarthritic cartilage [125]. These *in vitro* and pre-clinical animals model studies strengthen our view that appropriate VDR ligands (non-hypercalcemic compounds) are promising therapeutic candidates for the treatment and/or inhibition of progression of RA.

VDR and Multiple Sclerosis

Multiple sclerosis (MS), an inflammatory disease that affects nearly one million people worldwide, arises when the immune system mistakenly attacks self-molecules within the white matter of the brain and spinal cord [126]. Epidemiological studies have shown that the geographical areas with least sunlight exposure (e.g. Scandinavian countries) have the highest rate of the disease, whereas it is absent from the equatorial regions [127]. Since UV light plays an important role in the biosynthesis of 1,25-$(OH)_2D_3$, various studies have tried to explore the plausible connection between Vitamin D and MS. MS patients were found to be vitamin D deficient and experimental diets rich in vitamin D improved their symptoms [128, 129].

VDR lignads have been tested in mouse and rat experimental allergic encephalomyelitis (EAE), a model for MS. Garcion *et al.* reported that curative treatment of chronic relapsing-EAE (CR-EAE) of Lewis rats with 1,25-$(OH)_2D_3$ led to rapid clinical improvement accompanied by an inhibition of CD4, MHC class I and II, nitric oxide synthase expression in the posterior areas of the central nervous system, without any effect on transforming growth factor-β1 (TGF-β1) expression [130]. Administration of a less calcemic analog of vitamin D (Ro 63-2023) prevented CR-EAE induced by MOG peptide 35-55 in Biozzi AB/H mice, and this was associated with a profound reduction of MOG_{35-55}-specific proliferation and Th1 cell development. Neuropathological analysis showed a significant reduction of inflammatory infiltrates, demyelinated areas, and axonal loss in brains and spinal cords of treated mice. Inhibition of IL-12-dependent Th1 cell development is associated with effective treatment of CR-EAE, further suggesting the feasibility of this approach in the treatment of MS [131]. These results demonstrate a correlation between the capacity of Ro 63-2023 to inhibit IL-12-dependent Th1 development and EAE treatment. Conversely, a systemic increase in the transcripts for TGF-β1 and IL-4 was suggested to be responsible for the efficacy of 1,25-$(OH)_2D_3$ in EAE. The reasons for this discrepancy might pertain to different EAE models analyzed. TGF-β1 and IL-4 have been reported to be beneficial in EAE, but this activity has been ascribed to indirect inhibition of encephalitogenic Th1 cells. IL-10 also appears to be critical in the control of pathogenic Th1 responses in EAE, and 1,25-$(OH)_2D_3$ has been shown *in vitro* to strongly enhance IL-10 production by human DCs and to favor the induction of IL-10 producing regulatory T cells [132, 133].

Vitamin D analogs can cross the intact blood-brain barrier and could therefore directly inhibit CNS APCs like microglia, that regulate intracerebral T cells responses [134] or target infiltrating T cells as well as recruited APCs. Alternatively, the immunomodulatory effect of VDR ligands could be mainly exerted in the peripheral lymphoid organs leading to inhibition of encephalitogenic T cell development. Therefore, VDR ligands appear to have immune potential as therapeutic agents for MS.

VDR and Type I Diabetes

In humans, insulin-dependent (type I) diabetes mellitus (IDDM) is classified as an autoimmune disorder with both genetic and environmental determinants [135]. A state of hyperglycemia results from the T-cell mediated destruction of insulin-secreting β-cells in the pancreatic Islets of Langerhans. The disease pathogenesis is not fully understood, although the elucidation of some mechanisms is due to studies with animal models such as non-obese diabetic (NOD) mouse. Zella *et al.*, have reported that vitamin D status is a determining factor of disease susceptibility and oral administration of 1,25-$(OH)_2D_3$ prevents diabetes onset in NOD mice through 200 days of age. Similarly, nonhypercalcemic vitamin D analog (Ro 26-2198) treatment effectively blocked ongoing type I diabetes in adult NOD mice. Ro 26-2198 inhibited IL-12 production and pancreatic infiltration of Th1 cells while increasing the frequency of CD4+CD25+ regulatory cells in pancreatic lymph nodes, arresting the immunological progression and preventing the clinical onset of type I diabetes in the NOD mice [136]. Protection from type I diabetes was found to be associated with a selective decrease in Th1 cells in the pancreatic lymph nodes and pancreas, without a marked deviation of Th2 phenotype. The frequency of CD4+CD25+ regulatory cells in pancreatic lymph nodes of Ro26-2198-treated NOD mice was two fold higher than untreated mice. These cells were anergic, as demonstrated by their impaired capacity to proliferate and secrete IFN-γ in response to T-cell receptor ligation, and they inhibited the T cell response to the pancreatic autoantigen IA-2.

The observation that ongoing type I diabetes in the adult NOD mouse could be arrested by VDR ligands suggest that a similar treatment may also inhibit disease progression in prediabetic or newly diagnosed type I diabetes patients. Hence, VDR ligands because of their potent immunomodulatory properties may find therapeutic usefulness in the prevention and treatment of type I diabetes.

VDR and Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a T cell-dependent antibody-mediated autoimmune disease and the mouse strain MRL/lpr spontaneously develops an SLE-like syndrome sharing many immunological features with human SLE. Administration of the vitamin D analog, 22-oxa-1,25$(OH)_2D_3$ significantly prolonged the average life span of MRL/lpr mice and induced a significant reduction in proteinuria, renal arthritis, granuloma formation and knee joint arthritis [137]. Lemire *et al.*, showed that dermatological lesions, like alopecia, necrosis of the ear, and scab formation, were completely inhibited by 1,25$(OH)_2D_3$ [138]. VDR ligands also significantly reduced cell proliferation and IgG production (both polyclonal and anti-dsDNA), and enhanced B cell apoptosis in lymphocytes from SLE patients [139]. These results suggest a beneficial role of VDR ligands in the treatment of human SLE.

VDRMs as Potential Therapeutics for Cancers

VDR and Prostate Cancer

Prostate cancer is the second leading malignancy and cause of cancer deaths among men in the US [140]. Epidemiological studies have shown an inverse correlation between mortality rates due to prostate cancer and UV light exposure. These results are noteworthy since UV light is required for the biosynthesis of vitamin D in skin [141]. Further, low serum level of vitamin D was one of the major risk factors for developing prostate cancer [142]. The expression of VDR was observed in normal prostate, benign prostate hyperplasia (BPH), malignant prostate, and prostate cancer cell lines. These observations strengthened the hypothesis prostate cancer and BPH could be therapeutically amenable to VDR ligands [143-145]. 1,25-$(OH)_2D_3$ inhibited the proliferation of prostate cancer cell lines as well as that of primary epithelial cells from normal prostate, BPH and prostate cancer [35, 146, 147]. VDR ligands also inhibited tumor cell growth and metastasis *in vivo* [148-151]. 1,25-$(OH)_2D_3$ appears to inhibit the proliferation of prostate cancer cells by arresting their growth at G0/G1 stage of cell cycle, apoptosis, tumor cell differentiation and interaction with androgen signaling pathways. Although the exact molecular mechanism of 1,25-$(OH)_2D_3$ action is just beginning to emerge, it is becoming clear that VDR ligands influence various pathways involved in signal transduction. Gene expression studies in LNCaP cells have identified various interesting and relevant vitamin D targets and shown that the growth inhibition engendered by VDR ligands is achieved by reduction of cyclin-dependent kinase2 (CDK2) activity as well as the induction of p21, p27, IGFBP-3, IGFBP-5 and E-cadherin expression [25, 35-37]. p21 and p27 are tumor suppressors that act as inhibitors of cyclin-dependent kinase and arrest the cell growth at G0/G1 stage. IGFBP-3 and 5 are IGF-binding proteins that inhibit the IGF-dependent proliferation of tumor cells. E-adherin is a cell adhesion molecule and is expressed in differentiated non-proliferating cells. A gene expression profiling performed on LNCaP, normal prostate or prostatic adenocarcinoma cells using cDNA microarray was used to identify 1,25-$(OH)_2D_3$ target genes. 24-hydroxylase, a VDRE containing gene, was found to be maximally upregulated by 1,25-$(OH)_2D_3$ in both normal as well as primary cancer cells. However, in LNCaP cell, 24-hydroxylase expression was not induced by 1,25-$(OH)_2D_3$. In LNCaP cell, the most vitamin D-responsive gene was found to be IGFBP-3, whereas primary cells did not show any upregulation of IGFBP-3 expression. In normal as well as primary cancer cells, DUSP-10 showed maximum 1,25-$(OH)_2D_3$-dependent expression. DUSP-10 inactivates mitogen activated protein kinase (MAPK), indicating that inhibition of the growth promoting actions of MAPK may in part explain the growth inhibitory actions of 1,25-$(OH)_2D_3$ in prostate cancer cells. The anti-oxidant effects of 1,25-$(OH)_2D_3$ in prostatic primary cultures were highlighted by the induced expression of thioredoxin reductase 1 (TRR1) and superoxide dismutase 2 (SOD2) [35].

Growth inhibition by 1,25-$(OH)_2D_3$ was found to be inversely proportional to its 24-hydroxylase induction activity. DU145, a prostate cancer cell line that showed higher 1,25-$(OH)_2D_3$ induced expression of 24-hydroxylase, was less responsive to 1,25-$(OH)_2D_3$-mediated growth inhibition than LNCaP cells that showed very low basal and induced expression of 24-hydroxylase. Accordingly, inhibitors of P450 hydroxylases augmented the growth inhibitory effects of 1,25-$(OH)_2D_3$ and its synthetic analog EB 1089 in prostate cancer cells [152, 153]. Therefore the use of 24-hydroxylase inhibitors may enhance the growth inhibitory activity of 1,25-$(OH)_2D_3$ and its synthetic analogs in prostate cancer. Interestingly, 1,25-$(OH)_2D_3$ has been shown to inhibit the proliferation of both androgen-dependent as well as androgen-independent prostate cancer cells. The growth inhibitory effects of 1,25-$(OH)_2D_3$ are androgen-dependent, since, casodex, an AR antagonist, blocked the anti-proliferative activity of the VDR ligand in LNCaP cells. Accordingly, expression of androgen receptor (AR) was induced by 1,25-$(OH)_2D_3$ in prostate cancer cells [40]. Interestingly, 1,25-$(OH)_2D_3$ also inhibited the growth of AR negative prostate cancer cells as well as cells derived from a patient with advanced androgen-independent prostate cancer [40, 147, 154].

In a pilot study of patients with recurrent prostate cancer, oral calcitriol (starting with 0.5 μg/day and escalating the maximum dose to 2.5 μg/day) treatment for 6 to 15 months resulted in a significant decrease in the rate of prostate specific antigen (PSA) rise during therapy (in comparison to PSA increase before therapy) in 6 out of 7 patients. In the 7^{th} patient, the decrease in PSA rise did not reach statistical significance. As expected, the unwanted side effect of 1,25-$(OH)_2D_3$ treatment was the development of dose-dependent hypercalciuria [155]. Cancers are generally treated by a therapeutic regimen involving a combination of drugs. Since chemotherapeutic/cytotoxic agents and VDR ligands work by different mechanisms, it was hypothesized that differentiating agents like 1,25-$(OH)_2D_3$, retinoids and steroids may sensitize tumor cells to cytotoxic effects of chemotherapeutic regimens. Indeed, a number of *in vitro* and *in vivo* studies showed 1,25-$(OH)_2D_3$-mediated enhancement of the anti-tumor activities of cisplatin, paclitaxel and adriamycin [156-158]. These studies provided the basis for the use of combination therapy involving calcitriol and taxol in prostate cancer. In a clinical study, androgen-independent prostate cancer patients were treated with oral 1,25-$(OH)_2D_3$ (0.5 μg/kg) on day 1, followed by intravenous docetaxel (36 mg/m^2) on day 2 and the regimen was repeated weekly for 6 weeks on an 8-week cycle. Patients were kept on a low calcium diet (400-500 mg calcium/day) and increased oral hydration to reduce the risk of developing hypercalcemia. Thirty of 37 patients achieved significant PSA response (50% decrease in PSA) and 22 patients showed >75 % reduction in PSA levels. This study demonstrated that the combination was better than the docetaxel treatment alone when PSA response rate or overall survival was compared with contemporary phase II clinical trials involving treatment of patients with docetaxel as a single agent [159]. Still, the main hurdle to the therapeutic use of VDR ligands is their hypercalcemia/hypercalciuria side effects. Therefore, prostate cancer treatment could benefit from further development of truly non-calcemic/less calcemic VDR ligands.

VDR and Breast Cancer

Breast cancer is a major cause of death among women in the United States. Annually 200,000 women are diagnosed

with breast cancer and of these approximately 40,000 die from the disease [160]. Like prostate cancer, epidemiological studies have shown an inverse relationship between exposure to solar radiation (UV exposure) and higher breast cancer incidence and mortality [161, 162]. Interestingly, chromosomal region 20q13.2 that contains 24-hydroxylase (CYP24) is amplified in breast cancer. Since 24-hydroxylase is involved in the catabolism of 1,25-$(OH)_2D_3$, the amplification of this gene may result in decreased serum 1,25-$(OH)_2D_3$ levels and may lead to cell proliferation in the absence of vitamin D-mediated growth control [163]. Serum 1,25-$(OH)_2D_3$ levels were also found to be reduced in advanced bone metastatic breast cancer patients than in early stage patients [164]. VDR protein was found in breast cancer cell lines, carcinogen-induced rat primary mammary tumors, normal breast tissues as well as in primary breast tumors. Further, RXR and VDR protein levels were increased in cancerous than normal breast tissue [165, 166]. VDR ligands inhibited the growth of breast cancer cells *in vitro* and *in vivo* [1, 166]. Interestingly, 1,25-$(OH)_2D_3$ inhibited the proliferation of both ER-positive and ER-negative breast cancer cells [1, 13, 167]. Although the exact mechanism underlying the growth inhibitory actions of vitamin D in breast cancer cells is just beginning to emerge, the data support a multi-pronged effect involving growth arrest at G0/G1 stage, cell apoptosis, disruption of estrogen and other growth factor-mediated cell survival signals. At molecular level, 1,25-$(OH)_2D_3$ induced the expression of tumor suppressor cyclin dependent kinase inhibitors, p21 and p27 [26, 167, 168]. 1,25-$(OH)_2D_3$-dependent induction of p21 is mediated *via* a VDRE, whereas that of p27 is not mediated *via* a VDRE but involves transcription factors Sp1 and NF-Y [169, 170]. 1,25-$(OH)_2D_3$ also decreased the protein levels of cyclins and cyclin dependent kinases (CDK2, CDK4, cyclin D1 and cyclin A) in MCF-7 cells [168, 171]. 1,25-$(OH)_2D_3$ prevented the activation of cyclin D1-CDK4 and also resulted in the loss of cyclin D3, which leads to repression of E2F transcription factors and decreased cyclin A expression [26]. The antiproliferative effects of vitamin D on breast cancer cells could also be mediated by the induction of transforming growth factor-β1 (TGF-β1) and suppression of oncogene c-myc [172, 173]. Interestingly, 1,25-$(OH)_2D_3$ indirectly inhibits c-myc by inducing the expression of homeobox transcription factor HOXB4, that binds to a specific DNA sequence in the c-myc promoter and blocks its transcription [174]. 1,25-$(OH)_2D_3$ also inhibited insulin and IGF-I-induced growth of breast cancer cells by inducing the expression of IGFBP-3 and IGFBP-5 [38, 39]. VDR ligands also inhibited angiogenesis and decreased the metastatic potential of breast cancer cell *in vitro* and *in vivo* [175-177]. VDR ligands increased the cytotoxicity of doxorubicin, paclitaxel, adriamycin and irradiation in breast cancer cell cultures, thus providing a rationale for their combination with chemotherapeutic and radiation regimens [157, 160, 178-180]. In an *in vivo* xenograft study, additive antiproliferative effects were observed following combination treatment with a synthetic vitamin D analog (CB 1093) with either paclitaxel or cisplatin [156]. VDR ligands also have immense potential to act as chemopreventive agents since they blocked the progression of mammary carcinogenesis *in vivo* [181].

VDR and Colon Cancer

Colon cancer is the second leading cause of cancer deaths in the USA. Epidemiological studies have shown an inverse correlation between calcium, vitamin D, milk intake, sunlight exposure, serum levels of 25-hydroxyvitamin D3 and colon cancer incidence/mortality [182, 183]. Further, increased VDR protein levels were reported in colonic tumors than normal colon [183]. Colonic cells also possess the ability to synthesize 1,25-$(OH)_2D_3$ from its precursor 25-hydroxyvitamin D3 by the action of 1α-hydroxylase [184]. Therefore, VDR appears to be a clinically relevant potential therapeutic target for the prevention and treatment of colorectal cancer. As observed for prostate and breast cancer cells, 1,25-$(OH)_2D_3$ inhibits the growth of colon cancer cells by modulating multiple pathways involving G1 cell-cycle block, apoptosis and cell differentiation [47, 185, 186]. 1,25-$(OH)_2D_3$ induced differentiation of tumor cells and it also potentiated butyrate/tributyrin-induced differentiation of HT-29 colon cancer cells [187, 188]. Treatment of colon carcinoma cells (RG/C2, AA/C1, PC/JW, HT-29 and SW620) with 1,25-$(OH)_2D_3$ and a synthetic vitamin D analog (EB 1089) resulted in growth inhibition, induction of differentiation (increased alkaline phosphatase) and apoptosis [33]. 1,25-$(OH)_2D_3$ and a synthetic analog (ZK 156718) also induced the expression of p21 and p27 in Caco-2 cells [189]. Therefore VDR ligands induce tumor suppressors p21 and p27 in breast, prostate and colon cancer cells. Treatment of SW480 colon cancer cells with 1,25-$(OH)_2D_3$ resulted in cell differentiation that was accompanied with an increase in E-cadherin and other adhesion proteins (occludin, zonula occludens [ZO-1 and ZO-2] and vinculin). 1,25-$(OH)_2D_3$-induced differentiation was also accompanied by the translocation of β-catenin, plakoglobin and ZO-1 from the nucleus to the plasma membrane for a better cell-to-cell contact. 1,25-$(OH)_2D_3$ repressed the expression of c-myc, PPARδ, Tcf-1 and CD44, all of which are involved in cell proliferation [47]. Since ligand occupied VDR competed with T-cell transcription factor (TCF)-4 for interaction with β-catenin and c-myc, PPARδ, tcf-1 and CD44 are target genes for β-catenin-TCF-4 transcriptional activity. Therefore, inhibition of β-catenin-TCF-4 signaling may be one of the molecular pathways involved in 1,25-$(OH)_2D_3$-mediated growth inhibition in colon cancer cells. In Caco-2 cells, a decrease in cyclin D1 was found to be the major mechanism of the antiproliferative effects of two synthetic VDR ligands (Ro 23-7553 and JK-1624-3) [62].

Recently a novel complex involving VDR-Ser/Thr protein phosphatase PP1c/PP2A and p70S6 kinase was identified [186]. In this complex, 1,25-$(OH)_2D_3$ induced the VDR-associated phosphatase activity. The modulation of PP1c/PP2A activity by VDR resulted in a ligand-dependent, rapid and specific dephosphorylation and inactivation of their substrate p70S6 kinase. Since p70S6 kinase is essential for G1 to S phase transition, these results also provide one of the molecular pathways of 1,25-$(OH)_2D_3$-mediated G1 block in Caco-2 cells [186]. VDR ligands not only reduced the proliferation of colon cancer cell *in vitro*, but also inhibited the proliferation of normal and premalignant human rectal epitheliomas *in vitro* and reduced tumorigenesis in xenograft, Apc^{min} mutant mouse and

chemically-induced tumors *in vivo* [33, 47, 62, 190-194]. These observation suggest immense potential of a less calcemic VDR ligand for the prevention and treatment of colon cancer.

VDR and Myelodysplasia /Leukemia

Monocytes, macrophages, activated lymphocytes and myeloid leukemic cells express VDR message and protein. VDR ligands differentiated human myeloid cells into monocyte/macrophage-like cells [195]. 1,25-$(OH)_2D_3$ and its synthetic analogs prolonged survival time in murine models of leukemia *in vivo* [196-198]. In clinical trials involving myelodysplastic syndrome (a precancerous condition) patients, treatment with vitamin D analogs produced sustained hematological responses and maturation of cells in more than one lineage [199, 200]. Therefore, the development of less calcemic analogs of VDR ligands may result in the realization of the differentiation therapy dream for leukemia and myelodysplasia. Less calcemic analogs of vitamin D have been synthesized and some of these analogs are potent inducers of differentiation in HL-60 cells. These analogs belong to the 23-oxa-, 19-nor-23-oxa-, iso-19-nor-23-oxa, 20-methyl-23-oxa-, 20-ene-23-oxa-, 20-ene-19-nor-23-oxa-, 20,21-cyclo-23-oxa-, and 25-oxa-calcitriol series of compounds [201]. Non-secosteroidal and non-steroidal (C- and D-ring analogs) vitamin D analogs with less calcium mobilization activities were also potent stimulators of HL-60 differentiation *in vitro* [84, 85, 202]. However, their activities have not been tested *in vivo* and thus, we do not know whether these analogs show the required separation between induction of myeloid differentiation and hypercalcemia.

VDR and Kaposi's Sarcoma (KS) /Primary Effusion Lymphoma (PEL)

KS, a highly vascular tumor, occurs predominantly in men with HIV infection. The herpesvirus that causes KS also results in PEL and both these tumor types appear to be therapeutic targets of VDR ligands. KS cell lines as well as primary KS and PEL tumor tissues showed high level of expression of VDR mRNA and protein and the proliferation of KS and PEL cells was inhibited by 1,25-$(OH)_2D_3$ *in vitro* (KS and PEL) and *in vivo* (KS) [53, 203]. Further, topical treatment of KS lesions with calcipotriol (Dovonex, 0.005 %, twice daily application) showed anti-tumor activity in patients [53]. These observations also indicate that even B-cell lymphoma and cutaneous T-cell lymphoma could show responsiveness to VDR ligands either as stand-alone agents or in a combination regimen.

CONCLUSIONS

VDR plays an important physiological role in mineral homeostasis and bone maintenance. We have also come to realize that VDR is a pharmacologically important target for various autoimmune diseases and cancers. These desired properties stem from the anti-proliferative, differentiative and immunomodulatory activities of the ligand-activated receptor. The therapeutic efficacy of VDR ligands in osteoporosis, osteomalacia, psoriasis and renal osteodystrophy has been well established. A plethora of preclinical and clinical studies have identified arthritis, multiple sclerosis, type I diabetes, myelodysplasia, leukemia and cancers of prostate, colon, breast and skin as additional diseases where appropriate VDR ligands may exert their therapeutic effects. However, still the major roadblock for the development of a successful orally available VDR ligand for any of these indications is hypercalcemia/hypercalciuria. In the past decade, a number of studies have provided the molecular basis of both therapeutic as well as side effects. Various recent cell and molecular advances in the field of VDR biology has resulted in the development of novel assays that are currently being applied for the identification of tissue and/or gene selective VDRMs. Medicinal chemists have taken a cue from steroid medicinal chemistry approaches and have come out with non-steroidal and non-secosteroidal VDR ligands that appear to show better separation between therapeutic efficacy and hypercalcemia. Finally, combination of VDRMs with other differentiation inducing and chemotherapeutic agents may offer additive or synergistic activities with favorable therapeutic indices.

REFERENCES

[1] Pinette, K. V.; Yee, Y. K.; Amegadzie, B. Y.; Nagpal, S. *Mini Rev. Med. Chem.*, **2003**, *3*, 19.
[2] Sutton, A. L.; MacDonald, P. N. *Mol. Endocrinol.*, **2003**, *17*, 777.
[3] Bettoun, D. J.; Burris, T. P.; Houck, K. A.; Buck, D. W. 2nd.; Stayrook, K. R.; Khalifa, B.; Lu, J.; Chin, W. W.; Nagpal, S. *Mol. Endocrinol.*, **2003**, *17*, 2320.
[4] Pathrose, P.; Barmina, O.; Chang, C. Y.; McDonnell, D. P.; Shevde, N. K.; Pike, J. W. *J. Bone Miner. Res.*, **2002**, *17*, 2196.
[5] Thompson, P. D.; Remus, L. S.; Hsieh, J.-C.; Jurutka, P. W.; Whitfield, G. K.; Galligan, M.; Encinas Dominguez, C.; Haussler, C. A.; Haussler, M. R. *J. Mol. Endocrinol.*, **2001**, *27*, 211.
[6] Thompson, P. D.; Jurutka, P. W.; Whitfield, G. K.; Myskowski, S. M.; Eichhorst, K. R.; Dominguez, C. E.; Haussler, C. A.; Haussler, M. R. *Biochem. Biophys. Res. Commun.*, **2002**, *299*, 730.
[7] Bikle, D. D.; Tu, C.-L.; Xie, Z.; Oda, Y. *J. Cell. Biochem.*, **2003**, *88*, 290.
[8] Cheskis, B.; Freedman, L. P. *Mol. Cell. Biol.*, **1994**, *14*, 3329.
[9] Falzon, M. *Mol. Endocrinol.*, **1996**, *10*, 672.
[10] Alroy, I.; Towers, T. L.; Freedman, L. P. *Mol. Cell. Biol.*, **1995**, *15*, 5789.
[11] Takeuchi, A.; Reddy, G. S.; Kobayashi, T.; Okano, T.; Park, J.; Sharma, S. *J. Immunol.*, **1998**, *160*, 209.
[12] Harant, H.; Andrew, P. J.; Reddy, G. S.; Foglar, E.; Lindley, I. J. *Eur. J Biochem.*, **1997**, *250*, 63.
[13] Nagpal, S.; Lu, J.; Boehm, M. F. *Curr. Med. Chem.*, **2001**, *8*, 1661.
[14] Quelo, I.; Machuca, I.; Jurdic, P. *J. Biol. Chem.*, **1998**, *273*, 10638.
[15] Kitazawa, R.; Kitazawa, S. *Biochem. Biophy. Res. Commun.*, **2002**, *290*, 650.
[16] Makishima, M.; Lu, T. T.; Xie, W.; Whitfield, G. K.; Domoto, H.; Evans, R. M.; Haussler, M. R.; Mangelsdorf, D. J. *Science*, **2002**, *296*, 1313.
[17] Xie, Z.; Bikle, D. D. *J. Invest. Dermatol.*, **2001**, *117*, 1250.
[18] Champliaud, M. F.; Viel, A.; Baden, H. P. *J. Invest. Dermatol.*, **2003**, *121*, 781.
[19] Pillai, S.; Bikle, D. D. *J. Cell. Physiol.*, **1991**, *146*, 94.
[20] Kang, S.; Yi, S.; Griffiths, C. E.; Fancher, L.; Hamilton, T. A.; Choi, J. H. *Br. J. Dermatol.*, **1998**, *138*, 77.
[21] Michel, G.; Gailis, A.; Jarzebska-Deussen, B.; Muschen, A.; Mirmohammadsadegh, A.; Ruzicka, T. *Inflamm. Res.*, **1997**, *46*, 32.
[22] Rots, N. Y.; Liu, M.; Anderson, E. C.; Freedman, L. P. *Mol. Cell Biol.*, **1998**, *18*, 1911.
[23] Hodgkinson, J. E.; Davidson, C. L.; Beresford, J.; Sharpe, P. T. *Biochim. Biophys. Acta*, **1993**, *1174*, 11.
[24] Muto, A.; Kizaki, M.; Yamato, K.; Kawai, Y.; Kamata-Matsushita, M.; Ueno, H.; Ohguchi, M.; Nishihara, T.; Koeffler, H. P.; Ikeda, Y. *Blood*, **1999**, *93*, 2225.
[25] Campbell, M. J.; Elstner, E.; Holden, S.; Uskokovic, M.; Koeffler, H. P. *J. Mol. Endocrinol.*, **1997**, *19*, 15.
[26] Jensen; Skjode, S.; Madsen; Winkel, M.; Lukas, J.; Binderup, L.; Bartek, J. *Mol. Endocrinol.*, **2001**, *15*, 1370.

[27] James, S. Y.; Williams, M. A.; Kelsey, S. M.; Newland, A. C.; Colston, K. W. *Leukemia* , **1997**, *11*, 1017.
[28] Musiol, I. M.; Feldman, D. *Endocrinology*, **1997**, *138*, 12.
[29] Neveu, I.; Naveilhan, P.; Jehan, F.; Baudet, C.; Wion, D.; De Luca, H. F.; Brachet, P. *Brain Res. Mol. Brain Res.*, **1994**, *24*, 70.
[30] Palmer, H. G.; Sanchez-Carbayo, M.; Ordonez-Moran, P.; Larriba, M. J.; Cordon-Cardo, C.; Munoz, A. *Cancer Res.*, **2003**, *63*, 7799.
[31] Mahonen, A.; Pirskanen, A.; Maenpaa, P. H. *Biochim. Biophys. Acta.*, **1991**, *1088*, 111.
[32] Chen, A.; Davis, B. H.; Bissonnette, M.; Scaglione-Sewell, B.; Brasitus, T. A. *J. Biol. Chem.*, **1999**, *274*, 35505.
[33] Diaz, G. D.; Paraskeva, C.; Thomas, M. G.; Binderup, L.; Hague, A. *Cancer Res.*, **2000**, *60*, 2304.
[34] Hofbauer, L. C.; Dunstan, C. R.; Spelsberg, T. C.; Riggs, B. L.; Khosla, S. *Biochem. Biophys. Res. Commun.*, **1998**, *250*, 776.
[35] Krishnan, A. V.; Peehl, D. M.; Feldman, D. *J. Cell. Biochem.*, **2003**, *88*, 363.
[36] Drivdahl, R. H.; Loop, S. M.; Andress, D. L.; Ostenson, R. C. *Prostate*, **1995**, *26*, 72.
[37] Huynh, H.; Pollak, M.; Zhang, J. C. *Int. J. Oncol.*, **1998**, *13*, 137.
[38] Colston, K. W.; Perks, C. M.; Xie, S. P.; Holly, J. M. *J. Mol. Endocrinol.*, **1998**, *20*, 157.
[39] Rozen, F.; Yang, X. F.; Huynh, H.; Pollak, M. *J. Natl. Cancer Inst.*, **1997**, *89*, 652.
[40] Zhao, X. Y.; Ly, L. H.; Peehl, D. M.; Feldman, D. *Endocrinology*, **1997**, *138*, 3290.
[41] Koli, K.; Keski-Oja, J. *Cancer Res.*, **1995**, *55*, 1540.
[42] Van Cromphaut, S. J.; Dewerchin, M.; Hoenderop, J. G.; Stockmans, I.; Van Herck, E.; Kato, S.; Bindels, R. J.; Collen, D.; Carmeliet, P.; Bouillon, R.; Carmeliet, G. *Proc. Natl. Acad. Sci. USA*, **2001**, *98*, 13324.
[43] Lin, R.; Nagai, Y.; Sladek, R.; Bastien, Y.; Ho, J.; Petrecca, K.; Sotiropoulou, G.; Diamandis, E. P.; Hudson, T. J.; White, J. H. *Mol. Endocrinol.*, **2002**, *16*, 1243.
[44] Akutsu, N.; Bastien, Y.; Lin, R.; Mader, S.; White, J. H. *Biochem. Biophys. Res. Commun.*, **2001**, *281*, 1051.
[45] Akutsu, N.; Lin, R.; Bastien, Y.; Bestawros, A.; Enepekides, D. J.; Black, M. J.; White, J. H. *Mol. Endocrinol.*, **2001**, *15*, 1127.
[46] Matsumoto, K.; Hashimoto, K.; Higashiyama, M.; Nishida, Y.; Yoshikawa, K. *Br. J. Dermatol.*, **1990**, *123*, 93.
[47] Palmer, H. G.; Gonzalez-Sancho, J.; Espada, J.; Berciano, M. T.; Puig, I.; Baulida, J.; Quintanilla, M.; Cano, A.; de Herreros, A. G.; Lafarga, M.; Munoz, A. *J. Cell Biol.*, **2001**, *154*, 369.
[48] van de Kerkhof, P. C. M. *J. Invest. Deramtol. Symp. Proc.*, **1996**, *1*, 78.
[49] Koli, K.; Keski-Oja, J. *J. Investig. Dermatol. Symp. Proc.*, **1996**, *1*, 33.
[50] Muller, K.; Bendtzen, K. *J. Invest. Dermatol. Symp. Proc.*, **1996**, *1*, 68.
[51] Monolagas, S. C.; Provvedini, D. M.; Tsoukas, C. D. *Mol. Cell. Endocrinol.*, **1985**, *43*, 113.
[52] Oxholm, A.; Oxholm, P.; Staberg, B.; Bendtzen, K. *Acta Derm. Venereol.*, **1989**, *69*, 385.
[53] Masood, R.; Nagpal, S.; Zheng, T.; Cai, J.; Tulpule, A.; Smith, D. L.; Gill, P. S. *Blood*, **2000**, *96*, 3188.
[54] D'Ambrosio, D.; Cippitelli, M.; Cocciolo, M. G.; Mazzeo, D.; Lucia, P. D.; Lang, R.; Sinigaglia, F.; Panina-Bordignon, P. *J. Clin. Invest*., **1998,** *101***,** 252.
[55] Gurlek, A.; Pittelkow, M. R.; Kumar, R. *Endocr. Rev.*, **2002**, *23*, 763.
[56] Tobler, A.; Gasson, J.; Reichel, H.; Norman, A. W.; Koeffler, H. P. *J. Clin. Invest.*, **1987**, *79*, 1700.
[57] Kremer, R.; Karaplis, A. C.; Henderson, J.; Gulliver, W.; Banville, D.; Hendy, G. N.; Goltzman, D. *J. Clin. Invest.*, **1991**, *87*, 884.
[58] Russell, J.; Lettieri, D.; Sherwood, L. M. *Endocrinology*, **1986**, *119*, 2864.
[59] Li, Q.; Gardner, D. G. *J. Biol. Chem.*, **1994**, *269*, 4934.
[60] Zhuang, S. H.; Burnstein, K. L. *Endocrinology*, **1998**, *139*, 1197.
[61] Narvaez, C. J.; Zinser, G.; Welsh, J. *Steroids*, **2001**, *66*, 301.
[62] Hofer, H.; Ho, G.; Peterlik, M.; Uskokovic, M.; Lee, J. K.; White, M. C.; Posner, G. H.; Cross, H. S. *J. Pharmacol. Exp. Ther.*, **1999**, *291*, 450.
[63] Towers, T. L.; Staeva, T. P.; Freedman, L. P. *Mol. Cell Biol.*, **1999**, *19*, 4191.
[64] Hermanson, O.; Glass, C. K.; Rosenfeld, M. G. *Trends Endocrinol. Metabol.*, **2002**, *13*, 55.
[65] Hsieh, J. C.; Sisk, J. M.; Jurutka, P. W.; Haussler, C. A.; Slater, S. A.; Haussler, M. R.; Thompson, C. C. *J. Biol. Chem.*, **2003**, *278*, 38665.
[66] Tagami, T.; Lutz, W. H.; Kumar, R.; Jameson, J. L. *Biochem. Biophys. Res. Commun.*, **1998**, *253*, 358.
[67] Kim, H. J.; Kim, J. H.; Lee, J. W. *J. Biol. Chem.*, **1998**, *273*, 28564.
[68] Lee, S. K.; Kim, H. J.; Kim, J. W.; Lee, J. W. *Mol. Endocrinol.*, **1999**, *13*, 1924.
[69] Torchia, J.; Rose, D. W.; Inostroza, J.; Kamei, Y.; Westin, S.; Glass, C. K.; Rosenfeld, M. G. *Nature*, **1997**, *387*, 677.
[70] Chen, S. L.; Dowhan, D. H.; Hosking, B. M.; Muscat, G. E. *Genes Dev.*, **2000**, *14*, 1209.
[71] Struhl, K. *Genes Dev.*, **1998**, *12*, 194.
[72] Tyler, J. K.; Kadonaga, J. T. *Cell*, **1999**, *99*, 443.
[73] Zhang, C.; Baudino, T. A.; Dowd, D. R.; Tokumaru, H.; Wang, W.; MacDonald, P. N. *J. Biol. Chem.*, **2001**, *276*, 40614.
[74] Barry, J. B.; Leong, G. M.; Church, W. B.; Issa, L. L.; Eisman, J. A.; Gardiner, E. M. *J. Biol. Chem.*, **2003**, *278*, 8224.
[75] Yeh, S.; Chang, C. *Proc. Natl. Acad. Sci. USA*, **1996**, *93*, 5517.
[76] Muller, J. M.; Isele, U.; Metzger, E.; Rempel, A.; Moser, M.; Pscherer, A.; Breyer, T.; Holubarsch, C.; Buettner, R.; Schule, R. *EMBO J.*, **2000**, *19*, 359.
[77] Mengus, G.; May, M.; Carre, L.; Chambon, P.; Davidson, I. *Genes Dev.*, **1997**, *11*, 1381.
[78] Mengus, G.; Gangloff, Y. G.; Carre, L.; Lavigne, A. C.; Davidson, I. *J. Biol. Chem.*, **2000**, *275*, 10064.
[79] Lavigne, A. C.; Mengus, G.; Gangloff, Y. G.; Wurtz, J. M.; Davidson, I. *Mol. Cell Biol.*, **1999**, *19*, 5486.
[80] Rachez, C.; Suldan, Z.; Ward, J.; Chang, C. P.; Burakov, D.; Erdjument-Bromage, H.; Tempst, P.; Freedman, L. P. *Genes Dev.*, **1998**, *12*, 1787.
[81] Rachez, C.; Gamble, M.; Chang, C.-P. B.; Atkins, G. B.; Lazar, M. A.; Freedman, L. P. *Mol. Cell. Biol.*, **2000**, *20*, 2718.
[82] Chiba, N.; Suldan, Z.; Freedman, L. P.; Parvin, J. D. *J. Biol. Chem.*, **2000**, *275*, 10719.
[83] Bouillon, R.; Okamura, W. H.; Norman, A. W. *Endocr. Rev.*, **1995**, *16*, 200.
[84] Verstuyf, A.; Verlinden, L.; van Etten, E.; Shi, L.; Wu, Y.; D'Halleweyn, C.; Van Haver, D.; Zhu, G. D.; Chen, Y. J.; Zhou, X.; Haussler, M. R.; De Clercq, P.; Vandewalle, M.; Van Baelen, H.; Mathieu, C.; Bouillon, R. *J. Bone Miner. Res.*, **2000**, *15*, 237.
[85] Boehm, M. F.; Fitzgerald, P.; Zou, A.; Elgort, M. G.; Bischoff, E. D.; Mere, L.; Mais, D. L.; Bissonnette, R. P.; Heyman, R. A.; Nadzan, A. M.; Reichman, M.; Allegretto, E. A. *Chem. Biol.*, **1999**, *6*, 265.
[86] Peleg, S.; Nguyen, C.; Woodard, B. T.; Lee, J. K.; Posner, G. H. *Mol. Endocrinol.*, **1998**, *12*, 525.
[87] Sicinski, R. R.; Prahl, J. M.; Smith, C. M.; DeLuca, H. F. *J. Med. Chem.*, **1998**, *41*, 4662.
[88] Sicinski, R. R.; Rotkiewicz, P.; Kolinski, A.; Sicinska, W.; Prahl, J. M.; Smith, C. M.; DeLuca, H. F. *J. Med. Chem.*, **2002**, *45*, 3366.
[89] Yamamoto, H.; Shevde, N. K.; Warrier, A.; Plum, L. A.; DeLuca, H. F.; Pike, J. W. *J. Biol. Chem.*, **2003**, *278*, 31756.
[90] Siu-Caldera, M. L.; Rao, D. S.; Astecker, N.; Weiskopf, A.; Vouros, P.; Konno, K.; Fujishima, T.; Takayama, H.; Peleg, S.; Reddy, G. S. *J. Cell. Biochem.*, **2001**, *82*, 599.
[91] Maenpaa, P. H.; Vaisanen, S.; Jaaskelainen, T.; Ryhanen, S.; Rouvinen, J.; Duchier, C.; Mahonen, A. *Steroids*, **2001**, *66*, 223.
[92] Swamy, N.; Persons, K. S.; Chen, T. C.; Ray, R. *J. Cell. Biochem.*, **2003**, *89*, 909.
[93] Swann, S. L.; Bergh, J. J.; Farach-Carson, M. C.; Koh, J. T. *Org. Lett.*, **2002**, *4*, 3863.
[94] Swann, S. L.; Bergh, J.; Farach-Carson, M. C.; Ocasio, C. A.; Koh, J. T. *J. Am. Chem. Soc.*, **2002**, *124*, 13795.
[95] Bernardon, J.-M. WO-00026167, published May 11, 2000., **2000**.
[96] Bernardon, J.-M. WO-00138320, published May 31, 2001., **2001**.
[97] Bernardon, J.-M.; Biadatti, T. *US2003/0195259, published Oct. 16, 2003.*, **2003**.
[98] Provvedini, D. M.; Tsoukas, C. D.; Deftos, L. J.; Manolagas, S. C. *Science*, **1983**, *221*, 1181.
[99] Veldman, C. M.; Cantorna, M. T.; DeLuca, H. F. *Arch. Biochem. Biophys.*, **2000**, *374*, 334.
[100] Morimoto, S.; Kumahara, Y. *Med. J. Osaka Univ.*, **1985**, *35*, 51.
[101] Morimoto, S.; Yoshikawa, K.; Kozuka, T.; Kitano, Y.; Imanaka, S.; Fukuo, K.; Koh, E.; Kumahara, Y. *Br. J. Dermatol.*, **1986**, *115*, 421.

[102] Takamoto, S.; Onishi, T.; Morimoto, S.; Imanaka, S.; Yukawa, S.; Kozuka, T.; Kitano, Y.; Seino, Y.; Kumahara, Y. *Calcif. Tissue Int.*, **1986**, *39*, 360.

[103] Kato, T.; Rokugo, M.; Terui, T.; Tagami, H. *Br. J. Dermatol.*, **1986**, *115*, 431.

[104] Perez, A.; Raab, R.; Chen, T. C.; Turner, A.; Holick, M. F. *Br. J. Dermatol.*, **1996**, *134*, 1070.

[105] Perez, A.; Chen, T. C.; Turner, A.; Raab, R.; Bhawan, J.; Poche, P.; Holick, M. F. *Br. J. Dermatol.*, **1996**, *134*, 238.

[106] Kragballe, K. *Pharmacol. Toxicol.*, **1995**, *77*, 241.

[107] Ramsay, C. A.; Berth-Jones, J.; Brundin, G.; Cunliffe, W. J.; Dubertret, L.; van de Kerkhof, P. C.; Menne, T.; Wegmann, E. *Dermatology*, **1994**, *189*, 260.

[108] Gollnick, H.; T., M. *Curr. Med. Res. Opin.*, **1998**, *14*, 213.

[109] Barker, J. N.; Ashton, R. E.; Marks, R.; Harris, R. I.; Berth-Jones, J. *Br. J. Dermatol.*, **1999**, *141*, 274.

[110] Firestein, G. S. *Nature*, **2003**, *423*, 356.

[111] Kroger, H.; Penttila, I. M.; Alhava, E. M. *Scand. J. Rheumatol.*, **1993**, *22*, 172.

[112] Langman, C. B.; Ford, K. K.; Pachman, L. M.; Glorieux, F. *J. Bone Miner. Res.*, **1990**, *5*, 905.

[113] Bhalla, A. K.; Amento, E. P.; Serog, B.; Glimcher, L. H. *J. Immunol.*, **1984**, *133*, 1748.

[114] Rigby, W. F.; Stacy, T.; Fanger, M. W. *J. Clin. Invest.*, **1984**, *74*, 1451.

[115] Tsoukas, C. D.; Provvedini, D. M.; Manolagas, S. C. *Science*, **1984**, *224*, 1438.

[116] Matsui, T.; Takahashi, R.; Nakao, Y.; Koizumi, T.; Katakami, Y.; Mihara, K.; Sugiyama, T.; Fujita, T. *Cancer Res.*, **1986**, *46*, 5827.

[117] Reichel, H.; Koeffler, H. P.; Tobler, A.; Norman, A. W. *Proc. Natl. Acad. Sci. USA*, **1987**, *84*, 3385.

[118] Cippitelli, M.; Santoni, A. *Eur. J. Immunol.*, **1998**, *28*, 3017.

[119] Farmer, P. K.; He, X.; Schmitz, M. L.; Rubin, J.; Nanes, M. S. *Am. J. Physiol. Endocrinol. Metab.*, **2000**, *279*, E213.

[120] Cantorna, M. T.; Hayes, C. E.; DeLuca, H. F. *J. Nutr.*, **1998**, *128*, 68.

[121] Tsuji, M.; Fujii, K.; Nakano, T.; Nishii, Y. *FEBS Lett.*, **1994**, *337*, 248.

[122] Larsson, P.; Mattsson, L.; Klareskog, L.; Johnsson, C. *Clin. Exp. Immunol.*, **1998**, *114*, 277.

[123] Huckins, D.; Felson, D. T.; Holick, M. *Arthritis Rheum.*, **1990**, *33*, 1723.

[124] Tetlow, L. C.; Woolley, D. E. *Arthritis Res.*, **1999**, *1*, 63.

[125] Tetlow, L. C.; Woolley, D. E. *Arthritis Res.*, **1999**, *1*, 63.

[126] Visconti, A.; Santucci, S.; Figa Talamanca, L.; Cannoni, S.; Ristori, G.; Salvetti, M. *Neurol. Sci.*, **2003**, *24*, (Suppl. *5*), S287.

[127] Hayes, C. E.; Cantorna, M. T.; DeLuca, H. F. *Proc. Soc. Exp. Biol. Med.*, **1997**, *216*, 21.

[128] Goldberg, P.; Fleming, M. C.; Picard, E. H. *Med. Hypotheses*, **1986**, *21*, 193.

[129] Nieves, J.; Cosman, F.; Herbert, J.; Shen, V.; Lindsay, R. *Neurology*, **1994**, *44*, 1687.

[130] Garcion, E.; Sindji, L.; Nataf, S.; Brachet, P.; Darcy, F.; Montero-Menei, C. N. *Acta Neuropathol.*, **2003**, *105*, 438.

[131] Mattner, F.; Smiroldo, S.; Galbiati, F.; Muller, M.; Di Lucia, P.; Poliani, P. L.; Martino, G.; Panina-Bordignon, P.; Adorini, L. *Eur. J. Immunol.*, **2000**, *30*, 498.

[132] Penna, G.; Adorini, L. *J. Immunol.*, **2000**, *164*, 2405.

[133] Barrat, F. J.; Cua, D. J.; Boonstra, A.; Richards, D. F.; Crain, C.; Savelkoul, H. F.; de Waal-Malefyt, R.; Coffman, R. L.; Hawrylowicz, C. M.; O'Garra, A. *J. Exp. Med.*, **2002**, *195*, 603.

[134] Aloisi, F.; Ria, F.; Adorini, L. *Immunol. Today*, **2000**, *21*, 141.

[135] Hayes, C. E.; Nashold, F. E.; Spach, K. M.; Pedersen, L. B. *Cell. Mol. Biol. (Noisy-le-grand)*, **2003**, *49*, 277.

[136] Zella, J. B.; McCary, L. C.; DeLuca, H. F. *Arch. Biochem. Biophys.*, **2003**, *417*, 77.

[137] Abe, J.; Nakamura, K.; Takita, Y.; Nakano, T.; Irie, H.; Nishii, Y. *J. Nutr. Sci. Vitaminol.*, **1990**, *36*, 21.

[138] Lemire, J. M.; Ince, A.; Takashima, M. *Autoimmunity*, **1992**, *12*, 143.

[139] Linker-Israeli, M.; Elstner, E.; Klinenberg, J. R.; Wallace, D. J.; Koeffler, H. P. *Clin. Immunol.*, **2001**, *99*, 82.

[140] Hellerstedt, B. A.; Pienta, K. J. *CA Cancer J. Clin.*, **2002**, *52*, 154.

[141] Schwartz, G. G.; Hulka, B. S. *Anticancer Res.*, **1990**, *10*, 1307.

[142] Guyton, K. Z.; Kensler, T. W.; Posner, G. H. *Annu. Rev. Pharmacol. Toxicol.*, **2001**, *41*, 421.

[143] Skowronski, R. J.; Peehl, D. M.; Feldman, D. *Endocrinology*, **1993**, *132*, 1952.

[144] Feldman, D.; Skowronski, R. J.; Peehl, D. M. *Diet and Cancer* (Book); Plenum Press, New York, **1995**, 53.

[145] Kivineva, M.; Blauer, M.; Syvala, H.; Tammela, T.; Tuohimaa, P. *J. Steroid Biochem. Mol. Biol.*, **1998**, *66*, 121.

[146] Konety, B. R.; Johnson, C. S.; Trump, D. L.; Getzenberg, R. H. *Semin. Urol. Oncol.*, **1999**, *17*, 77.

[147] Peehl, D. M.; Skowronski, R. J.; Leung, G. K.; Wong, S. T.; Stamey, T. A.; Feldman, D. *Cancer Res.*, **1994**, *54*, 805.

[148] Getzenberg, R. H.; Light, B. W.; Lapco, P. E.; Konety, B. R.; Nangia, A. J.; Acierno, J. S.; Dhir, R.; Shurin, Z.; Day, R. S.; Trump, D. L.; Johnson, C. S. *Urology*, **1997**, *50*, 999.

[149] Lokeshwar, B. L.; Schwartz, G. G.; Selzer, M. G.; Burnstein, K. L.; Zhuang, S.-H.; Block, N. L.; Binderup, L. *Cancer Epidemiol. Biomarkers Prev.*, **1999**, *8*, 241.

[150] Polek, T. C.; Murthy, S.; Blutt, S. E.; Boehm, M. F.; Zou, A.; Weigel, N. L.; Allegretto, E. A. *Prostate*, **2001**, *49*, 224.

[151] Vegesna, V.; O'Kelly, J.; Said, J.; Uskokovic, M.; Binderup, L.; Koeffler, H. P. *Anticancer Res.*, **2003**, *23*, 283.

[152] Ly, L. H.; Zhao, X. Y.; Holloway, L.; Feldman, D. *Endocrinology*, **1999**, *140*, 2071.

[153] Peehl, D. M.; Seto, E.; Hsu, J. Y.; Feldman, D. *J. Urol.*, **2002**, *168*, 1583.

[154] Zhao, X. Y.; Peehl, D. M.; Navone, N. M.; Feldman, D. *Endocrinology*, **2000**, *141*, 2548.

[155] Gross, C.; Stamey, T.; Hancock, S.; Feldman, D. *J. Urol.*, **1998**, *159*, 2035.

[156] Koshizuka, K.; Koike, M.; Kubota, T.; Said, J.; Binderup, L.; Koeffler, H. P. *Int. J. Oncol.*, **1998**, *13*, 421.

[157] Wang, Q.; Yang, W.; Uytingco, M. S.; Christakos, S.; Weider, R. *Cancer Res.*, **2000**, *60*, 2040.

[158] Hershberger, P. A.; Yu, W.-D.; Modzelewski, R. A.; Rueger, R. M.; Johnson, C. S.; Trump, D. L. *Clin. Cancer Res.*, **2001**, *7*, 1043.

[159] Beer, T. M.; Eilers, K. M.; Garazotto, M.; Egorin, M. J.; Lowe, B. A.; Henner, W. D. *J. Clin. Oncol.*, **2003**, *21*, 123.

[160] Polar, M. K.; Gennings, C.; Park, M.; Gupta, M. S.; Gewirtz, D. A. *Cancer Chemother. Pharmacol.*, **2003**, *51*, 415.

[161] Garland, F. C.; Garland, C. F.; Gorham, E. D.; Young, J. F. *Preventive Med.*, **1990**, *19*, 614.

[162] John, E. M.; Schwartz, G. G.; Dreon, D. M.; Koo, J. *Cancer Epidemiol. Biomarkers Prev.*, **1999**, *8*, 399.

[163] Albertson, D. G.; Ylstra, B.; Segraves, R.; Collins, C.; Dairkee, S. H.; Kowbel, D.; Kuo, W. L.; Gray, J. W.; Pinkel, D. *Nat. Genet.*, **2000**, *25*, 144.

[164] Mawer, E. B.; Walls, J.; Howell, A.; Davies, M.; Ratcliffe, W. A.; Bundred, N. J. *J. Clin. Enndocrinol. Metabolism*, **1997**, *82*, 118.

[165] Friedrich, M.; Axt-Fliedner, R.; Villena-Heinsen, C.; Tilgen, W.; Schmidt, W.; Reichrath, J. *Histochem. J.*, **2002**, *34*, 35.

[166] Bortman, P.; Folgueira, M.A.A.K.; Katayama, M. L. H.; Snitcovsky, I. M. L.; Brentani, M. M. *Braz. J. Med. Biol. Res.*, **2002**, *35*, 1.

[167] Flanagan, L.; Packman, K.; Juba, B.; O'Neill, S.; Tenniswood, M.; Welsh, J. *J. Steroid Biochem. Molec. Biol.*, **2003**, *84*, 181.

[168] Wu, G.; Fan, R. S.; Li, W.; Ko, T. C.; Brattain, M. G. *Oncogene*, **1997**, *15*, 1555.

[169] Liu, M.; Lee, M. H.; Cohen, M.; Bommakanti, M.; Freedman, L. P. *Genes Dev.*, **1996**, *10*, 142.

[170] Inoue, T.; Kamiyama, J.; Sakai, T. *J. Biol. Chem.*, **1999**, *274*, 32309.

[171] Verlinden, L.; Verstuyf, A.; Convents, R.; Marcelis, S.; Van Camp, M.; Bouillon, R. *Mol. Cell. Endocrinol.*, **1998**, *142*, 57.

[172] Koli, K.; Keski-Oja, J. *Cancer Res.*, **1995**, *55*, 1540.

[173] Saunders, D. E.; Christensen, C.; Wappler, N. L.; Schultz, J. F.; Lawrence, W. D.; Malviya, V. K.; Malone, J. M.; Deppe, G. *Anticancer Drugs*, **1993**, *4*, 210.

[174] Pan, Q.; Simpson, R. U. *J. Endocrinol.*, **2001**, *169*, 153.

[175] Hensen, C. M.; Frandsen, T. L.; Brunner, N.; Binderup, L. *Clin. Exp. Metastasis*, **1994**, *12*, 195.

[176] El Abdaimi, K.; Dion, N.; Papavasiliou, V.; Cardinal, P. E.; Binderup, L.; Goltzman, D.; Stemarie, L. G.; Kremer, R. *Cancer Res.*, **2000**, *60*, 4412.

[177] Mantell, D. J.; Owens, P. E.; Bundred, N. J.; Mawer, E. B.; Canfield, A. E. *Circulation Res.*, **2000**, *87*, 214.

[178] Sundaram, S.; Gewirtz, D. A. *Radiat. Res.*, **1999**, *152*, 47.

[179] Sundaram, S.; Chaudhry, M.; Reardon, D.; Gupta, M.; Gewirtz, D. A. *Breast Cancer Res. Treat.*, **2000**, *63*, 1.

[180] Chaudhry, M.; Sundaram, S.; Gennings, C.; Carter, H.; Gewirtz, D. A. *Cancer Chemother. Pharmacol.*, **2001**, *47*, 429.
[181] Mehta, R. G.; Hussain, E. A.; Mehta, R. R.; Das Gupta, T. K. *Mutation Res.*, **2002**, *523-524*, 253.
[182] Kallay, E.; Pietschmann, P.; Toyokuni, S.; Bajna, E.; Hahn, P.; Mazzucco, K.; Bieglmayer, C.; Kato, S.; Cross, H. S. *Carcinogenesis*, **2001**, *22*, 1429.
[183] Vandewalle, B.; Adenis, A.; Hornez, L.; Revillion, F.; Lefebvre, J. *Cancer Lett.*, **1994**, *86*, 67.
[184] Cross, H. S.; Kallay, E.; Farhan, H.; Weiland, T.; Manhardt, T. *Recent Results Cancer Res.*, **2003**, *164*, 413.
[185] Evans, S. R. T.; Soldatenkov, V.; Shchepotin, E. B.; Bogrash, E.; Shechepotin, I. B. *Int. J. Oncol.*, **1999**, *14*, 979.
[186] Bettoun, D. J.; Buck, D. W., 2nd; Lu, J.; Khalifa, B.; Chin, W. W.; Nagpal, S. *J. Biol. Chem.*, **2002**, *277*, 24847.
[187] Zhao, X.; Feldman, D. *Endocrinology*, **1993**, *132*, 1808.
[188] Gaschott, T.; Steinhilber, D.; Milovic, V.; Stein, J. *J. Nutr.*, **2001**, *131*, 1839.
[189] Gaschott, T.; Steinmeyer, A.; Steinhilber, D.; Stein, J. *Biochem. Biophys. Res. Commun.*, **2002**, *290*, 504.
[190] Eisman, J. A.; Barkla, D. H.; Tutton, P. J. M. *Cancer Res.*, **1987**, *47*, 21.
[191] Belleli, A.; Shany, S.; Levy, J.; Guberman, R.; Lamprecht, S. A. *Carcinogenesis*, **1992**, *13*, 2293.
[192] Wali, R. K.; Bissonnette, M.; Khare, S.; Hart, J.; Sitrin, M. D.; Brasitus, T. A. *Cancer Res.*, **1995**, *55*, 3050.
[193] Huerta, S.; Irwin, R. W.; Heber, D.; Go, V. L. W.; Koeffler, H. P.; Uskokovic, M. R.; Harris, D. M. *Cancer Res.*, **2002**, *62*, 741.
[194] Wali, R. K.; Khare, S.; Tretiakova, M.; Cohen, G.; Nguyen, L.; Hart, J.; Wang, J.; Wen, M.; Ramaswamy, A.; Joseph, L.; Sitrin, M.; Brasitus, T. A.; Bissonnette, M. *Cancer Epidemiol. Biomarkers Prev.*, **2002**, *11*, 1653.
[195] Freedman, L. P. *J. Nutr.*, **1999**, *129*, 581.
[196] Honma, Y.; Hozum, i. M.; Abe, E.; Konno, K.; Fukushima, M.; Hata, S.; Nishii, Y.; DeLuca, H. F.; Suda, T. *Proc. Natl. Acad. Sci. USA*, **1983**, *80*, 201.
[197] Potter, G. K.; Mohamed, A. N.; Dracopoli, N. C.; Groshen, S. L.; Shen, R. N.; Moore, M. A. *Exp. Hematol.*, **1985**, *13*, 722.
[198] Kasukabe, T.; Honma, Y.; Hozumi, M.; Suda, T.; Nishii, Y. *Cancer Res.*, **1987**, *47*, 567.
[199] Kelsey, S. M.; Newland, A. C.; Cunningham, J.; Makin, H. L.; Coldwell, R. D.; Mills, M. J.; Grant, I. R. *Lancet*, **1992**, *340*, 316.
[200] Mellibovsky, L.; Diez, A.; Perez-Vila, E.; Serrano, S.; Nacher, M.; Aubia, J.; Supervia, A.; Recker, R. R. *Br. J. Haematol.*, **1998**, *100*, 516.
[201] Steinmeyer, A.; Kirsch, G.; Neef, G.; Schwarz, K. *Curr. Pharm. Des.*, **2000**, *6*, 767.
[202] Verstuyf, A.; Verlinden, L.; Van Baelen, H.; Sabbe, K.; D'Hallewyn, C.; De Clercq, P.; Vandewalle, M.; Bouillon, R. *J. Bone Miner. Res.*, **1998**, *13*, 549.
[203] Jenner, R. G.; Maillard, K.; Cattini, N.; Weiss, R. A.; Boshoff, C.; Wooster, R.; Kellam, P. *Proc. Natl. Acad. Sci. USA*, **2003**, *100*, 10399.