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1 α ,25-Dihydroxyvitamin D₃-3 β -bromoacetate, a potential cancer therapeutic agent: synthesis and molecular mechanism of action

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Abstract

Synthesis of 1 α ,25-dihydroxyvitamin D₃-3 β -bromoacetate (1,25(OH)₂D₃-3-BE), a potential anti-cancer agent is presented. We also report that mechanism of action of 1,25(OH)₂D₃-3-BE may involve reduction of its catabolism, as evidenced by the reduced and delayed expression of 1 α ,25-dihydroxyvitamin D₃-24-hydroxylase (CYP24) gene in cellular assays.

Keywords

synthesis of 1,25(OH)₂D₃-3-BE; anti-cancer agent; mechanism of action of 1,25(OH)₂D₃-3-BE; 1 α ,25-dihydroxyvitamin D₃-24-hydroxylase (CYP24)

The awareness of the potential beneficial effect of vitamin D in a number of diseases, and general good health has increasingly become a public health issue [1]. In addition, the therapeutic potential of 1 α ,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), the dihydroxylated metabolite of vitamin D₃, on established tumors was realized when the potent antiproliferative property of 1,25(OH)₂D₃ was discovered in the early 1980's in leukemic cells [2]. Since then the antiproliferative and pro-differentiative effects of 1,25(OH)₂D₃ has been studied in various cancer cell lines, and as a result, 1,25(OH)₂D₃ has been investigated as a potential anti-cancer agent [3]. However, clinical trials have demonstrated strong calcemic and calciuric effects at pharmacological doses (of 1,25(OH)₂D₃) [4]. This limitation provided the search for structural analogs of 1,25(OH)₂D₃ with potent antiproliferative yet low calcemic properties [4]. This effort, based solely on a Specific Estrogen Receptor Modulator (SERM)-like concept adopted the idea that these analogs would change the conformation of nuclear vitamin D receptor (VDR), the chief modulator of the biological actions of 1,25(OH)₂D₃ sufficiently from the parent hormone to separate cell-regulatory properties from calcemic property. Numerous such 'non-calcemic' analogs (of 1,25(OH)₂D₃) have been synthesized based on this concept with the hope of achieving a favorable antiproliferative/toxicity index, yet clinical results are largely disappointing [5,6].

On the other hand, recent clinical trials of 1,25(OH)₂D₃ either alone or in combination with common cancer therapeutic drugs have produced strongly encouraging results, and brought

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back the realization that $1,25(\text{OH})_2\text{D}_3$ is probably the most promising 'drug' if its therapeutic dose can be escalated without causing toxicity [7-10]. High doses of $1,25(\text{OH})_2\text{D}_3$ are required to counter its non-tumor specific spread, and high catabolic rate. Realizing this unmet potential of $1,25(\text{OH})_2\text{D}_3$ -therapy, we adopted a hypothesis-based alternate approach to increase the half-life of $1,25(\text{OH})_2\text{D}_3$, to thus decrease effective dose with reduced toxicity.

$1\alpha,25$ -Dihydroxyvitamin D_3 -3 β -bromoacetate ($1,25(\text{OH})_2\text{D}_3$ -3-BE) was initially developed in our laboratory as an affinity labeling reagent to map the ligand-binding domain (LBD) of VDR [11]. Subsequently, we realized that since $1,25(\text{OH})_2\text{D}_3$ -3-BE covalently attaches to VDR-LBD [12-15], it might be physically protected from interacting with catabolic enzymes, thereby increasing its half-life. Furthermore, once attached to the VDR-LBD, $1,25(\text{OH})_2\text{D}_3$ -3-BE becomes a simple 3-acetate derivative of $1,25(\text{OH})_2\text{D}_3$, activating gene transcription similar to $1,25(\text{OH})_2\text{D}_3$. In other words, $1,25(\text{OH})_2\text{D}_3$ -3-BE becomes de facto $1,25(\text{OH})_2\text{D}_3$ with an increased half-life by this process. We observed that $1,25(\text{OH})_2\text{D}_3$ -3-BE has a significantly greater antiproliferative effect than $1,25(\text{OH})_2\text{D}_3$ in normal human keratinocytes, supporting our hypothesis [16]. Subsequently several publications from our group and others have demonstrated significantly stronger antiproliferative effect of $1,25(\text{OH})_2\text{D}_3$ -3-BE and its mono-hydroxylated analog, 25-hydroxyvitamin D_3 -3 β -bromoacetate than $1,25(\text{OH})_2\text{D}_3$ in various cancer cells [17-22]. We also observed strong anti-tumor effect of $1,25(\text{OH})_2\text{D}_3$ -3-BE in mouse model of renal cancer [22], and androgen-insensitive prostate cancer (manuscript in preparation).

The above mentioned results suggest strong therapeutic potential of $1,25(\text{OH})_2\text{D}_3$ -3-BE, and warrant extensive *in vitro* and *in vivo* studies, requiring substantial quantity of $1,25(\text{OH})_2\text{D}_3$ -3-BE. Previously we reported synthesis of $1,25(\text{OH})_2\text{D}_3$ -3-BE with a starting material that is no longer available [23] mandating an alternative synthetic procedure for this compound, which is delineated in this communication. Furthermore, our hypothesis about increased stability of $1,25(\text{OH})_2\text{D}_3$ -3-BE by making it less available to catabolic enzymes remains unproven to date. In this communication we report results of cellular studies to support this hypothesis on the catabolism of this analog.

Synthesis of $1,25(\text{OH})_2\text{D}_3$ -3-BE was initiated by converting 25-hydroxyvitamin D_3 (**A**, Duphar Chemicals, Netherlands) to its trans-variety (**B**) by the sulfur-dioxide method [24] and converting the crude into the tert.butyldimethylsilylether (TBDMS) derivative (**C**) (Scheme 1) in good yield.

Success of our synthetic scheme depended critically on the introduction of the 1-hydroxyl group into (**C**) by allylic oxidation in a stereospecific manner. In an earlier publication [25] we reported that N-methylmorpholine-N-oxide/ SeO_2 allylic oxidation of 25-hydroxy-5E-[6,19,19'- H_3]vitamin D_3 -3 β -tert-butyldimethylsilyl ether to $1\alpha,25$ -dihydroxy-5E-[6,19,19'- H_3]vitamin D_3 -3 β -tert-butyldimethylsilyl ether produced an approximately 6:1 ratio of 1α -OH:1 β -OH stereoisomers. However, in the present case HPLC analysis of the crude reaction mixture showed the product to be homogeneous, indicating the presence of a single stereoisomer (**D**) in the reaction mixture, which, after purification, was identified to have the 1α -OH (desired) stereochemistry by comparison with the NMR spectrum of a known sample [25]. We speculate that the lack of three deuterium atoms in the triene system adjoining the A ring in **C** may have changed the conformation of the A-ring substantially to have the oncoming oxygen atom introduced exclusively from one side. We also found that stereochemistry at the 1-position depended on the structure of the 1-silyl ether. For example, when 1-OH was derivatized with a triethylsilyl group, the 1α -OH:1 β -OH ratio was approximately 4:1 with an overall yield of 45%.

In the next step 1 α ,25-dihydroxy-5E-vitamin D₃-3 β -tert-butyldimethylsilyl ether (**D**) was converted to its 5Z counterpart (**E**) by photolysis. Attempts to protect the 1-hydroxyl group in **E** as tetrahydropyranyl (THP) ether resulted in the derivatization of the 25-hydroxy group as well, and produced 1 α ,25-di-tetrahydropyranyloxy, 3 β -tert. butyldimethylsilyl-5Z-vitamin D₃ (**F**), which was de-silylated to produce 1 α ,25-di-tetrahydropyranyloxy-5Z-vitamin D₃ (**G**). Finally, **G** was DCC-coupled to bromoacetic acid, and the resulting product was de-protected to produce 1 α ,25-dihydroxy-5Z-vitamin D₃-3 β -bromoacetate (**I**, 1,25(OH)₂D₃-3-BE) in nearly quantitative yield.

Signal transduction via 1,25(OH)₂D₃ is a stepwise process that is initiated by the highly specific binding between 1,25(OH)₂D₃ and VDR. Ligand binding allosterically promotes formation of a heteromeric complex with RXR, which allows the complex to bind to vitamin D response element(s) in chromatin and recruitment of co-activators, leading to transcription of vitamin D target genes. According to this dogma, VDR-binding by 1,25(OH)₂D₃ as well as its analogs/derivatives such as 1,25(OH)₂D₃-3-BE is crucial for all these processes.

1,25(OH)₂D₃-3-BE is a VDR affinity alkylating agent. In an earlier study we described that 1,25(OH)₂D₃-3-BE is capable of specifically labeling native VDR in ROS 17/2.8 bone cells and calf thymus nuclear extract [12], and rapidly titrating recombinant VDR with 1:1 stoichiometry [16]. We also reported that 1,25(OH)₂D₃-3-BE covalently labels a single cysteine residue (Cys₂₈₈) in the VDR-LBD (as shown in the cartoon in Figure 1) [14]. Furthermore, we demonstrated that VDR is essential for the growth inhibitory activity of 25-hydroxyvitamin D₃-3-BE, a prototype of 1,25(OH)₂D₃-3-BE without the 1-hydroxyl group in ALVA-31 prostate cancer cells [19].

The other mechanistic aspect of our hypothesis delineates that 1,25(OH)₂D₃-3-BE, after interacting with VDR, is protected from catabolism, thus increasing its half-life, and enhancing its anti-growth property as evidenced in several studies [17-22]. 1 α ,25-Dihydroxyvitamin D₃-24-hydroxylase (CYP24) is a cytochrome P450 enzyme that introduces a hydroxyl group at the 24-position in the side chain of 1,25(OH)₂D₃ that initiates the multi-step catabolic degradation [26]. Interaction between VDR and 1,25(OH)₂D₃ is an equilibrium process so that the steady state would always contain 'free' 1,25(OH)₂D₃ (not bound to VDR) which will be rapidly catabolized by CYP24 (Figure 1).

In contrast, covalent attachment of 1,25(OH)₂D₃-3-BE inside the ligand-binding pocket of VDR [14] would eliminate/reduce 'free' 1,25(OH)₂D₃ in the steady state, thereby potentially requiring reduced and delayed expression of CYP24 (Figure 1).

We chose A498 kidney cancer cells to test our hypothesis. In the dose-response experiment A498 cells were treated with 10⁻¹⁰⁻⁶M of 1,25(OH)₂D₃ or 1,25(OH)₂D₃-3-BE or ethanol vehicle (control). As shown in Figure 2, treatment of A498 cells with 10⁻¹⁰⁻⁶M of 1,25(OH)₂D₃ strongly induced CYP24 mRNA expression. 1,25(OH)₂D₃-3-BE treatment also led to induction of CYP24 mRNA, but at a significantly higher concentration (approximately 10-fold) to achieve induction comparable to 1,25(OH)₂D₃, suggesting 1,25(OH)₂D₃-3-BE is less efficacious in inducing CYP24 than 1,25(OH)₂D₃, as predicted by our hypothesis.

In the kinetic experiment cells were treated with 10⁻⁸M of either 1,25(OH)₂D₃ or 1,25(OH)₂D₃-3-BE and CYP24 message was detected at various time-points. As shown in Figure 3, induction of CYP24 mRNA was detectable by 4 hr in 1,25(OH)₂D₃-treated cells and continued to increase over 16 hr. In contrast, 1,25(OH)₂D₃-3-BE showed a much delayed kinetics of induction of CYP24 mRNA, with CYP24 mRNA first being detectable at 16 hr.

Taken together, results of **Figures 2 and 3** suggest that 1,25(OH)₂D₃-3-BE is less effective in inducing CYP24 gene expression than an equivalent amount of 1,25(OH)₂D₃. CYP24 is a catabolic enzyme that initiates the degradation of 1,25(OH)₂D₃. Therefore, reduced and delayed expression of CYP24 gene implies decreased catabolism of 1,25(OH)₂D₃-3-BE, as predicted by our hypothesis.

We reported earlier that 1,25(OH)₂D₃-3-BE changes the conformation of VDR differently from 1,25(OH)₂D₃ as reflected in the enhanced stabilization of VDR-hOCVDRE (human osteocalcin vitamin D response element) complex in COS-1 cells and promotion of a longer stimulation of CYP24 mRNA expression in keratinocytes compared with 1,25(OH)₂D₃ [16]. Furthermore, recently we reported that compounds similar to 1,25(OH)₂D₃-3-BE with alkylating bromoacetate group at 1 and 11 positions of 1,25(OH)₂D₃ specifically labeled VDR-LBD, yet their antiproliferative activity in keratinocytes was similar to that of 1,25(OH)₂D₃ [27]. Therefore, considering all the information we ascribe enhanced antiproliferative activity of 1,25(OH)₂D₃-3-BE to a combination of its increased half-life (by covalent labeling of VDR-LBD) and a unique change in VDR-conformation upon binding (Figure 1).

Several non-genomic pathways for the action of 1,25(OH)₂D₃ and its analogs have also been reported [28]. We have observed that PI3K/Akt non-genomic pathway is involved in the activity of 1,25(OH)₂D₃-3-BE in kidney cancer cells [22]. Therefore, mechanism of action of 1,25(OH)₂D₃-3-BE may involve a combination of genomic and non-genomic pathways.

In conclusion, we report an efficient synthesis of 1,25(OH)₂D₃-3-BE, a potential therapeutic agent for cancer. In addition, we provide data from cellular studies to support our hypothesis about its intrinsic mechanism of action.

Synthesis

(i) A mixture of (**C**) (760 mg) and SeO₂ (192 mg) in 15 ml of anhydrous CH₂Cl₂ was refluxed under argon for 30 min followed by cooling to room temperature and addition of a solution of N-methylmorpholine-N-oxide (850 mg) in 15 ml of anhydrous CH₂Cl₂. The mixture was refluxed for an additional 60 min. After usual workup and purification trans-1 α ,25-dihydroxyvitamin D₃-3 β -TBDMS ether (**D**) was obtained in 95% yield.

(ii) A toluene (10 ml) solution of **D** (80 mg), anthracene (10 mg), Et₃N (40 μ l) in a quartz test tube was irradiated from a Hanovia medium pressure mercury arc lamp for 75 min. After preparative TLC purification 1 α ,25-dihydroxyvitamin D₃-3-TBDMS ether (**E**) was obtained in 67% yield.

Cellular studies

(i) Induction of 1 α ,25-dihydroxyvitamin D₃-24hydroxylase (CYP24) gene expression by 1,25(OH)₂D₃ and 1,25(OH)₂D₃-3-BE (dose response): A498 kidney cancer cells (ATCC, Manassas, VA) were treated with 10⁻¹⁰⁻⁶M of 1,25(OH)₂D₃, 1,25(OH)₂D₃-3-BE or ethanol (vehicle control) for 24 hr. Total RNA was prepared and subjected to reverse transcription using Moloney murine leukemia virus (MMLV) reverse transcriptase and random hexamers under standard conditions. Following cDNA synthesis, PCR was performed using gene specific primers to the vitamin D target gene, CYP24 and β -actin (control). The products were analyzed on 1% agarose gels.

(ii) Induction of CYP24 gene expression by 1,25(OH)₂D₃-3-BE (kinetics): In this experiment A498 cells were treated with 10⁻⁸M of 1,25(OH)₂D₃-3-BE and 1,25(OH)₂D₃

and RNA prepared over the course of 16 hr for the analysis of CYP24 mRNA levels by RT-PCR.

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Abbreviations

1,25(OH)₂D₃	1 α ,25-dihydroxyvitamin D ₃
1,25(OH)₂D₃-3-BE	1 α ,25-dihydroxyvitamin D ₃ -3 β -bromoacetate
VDR	vitamin D receptor
VDR-LBD	vitamin D receptor-ligand binding domain

References cited

- [1]. Holick MF. Vitamin D: a D-Lightful health perspective. *Nutr. Rev.* 2008; 66:S182. [PubMed: 18844847]
- [2]. Abe E, Miyaara C, Sakagami H, Takeda M, Konno K, Yamazaki T, Yoshiki S, Suda T. Differentiation of mouse myeloid leukemia cells induced by 1 α ,25-dihydroxyvitamin D₃. *Proc. Natl. Acad. Sci. USA.* 1981; 78:4990. T. [PubMed: 6946446]
- [3]. Deeb KK, Trump DL, Johnson CS. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. *Nat. Rev. Cancer.* 2007; 7:684. [PubMed: 17721433]
- [4]. Matsuda S, Jones G. Promise of vitamin D analogues in the treatment of hyperproliferative conditions. *Mol. Cancer Thera.* 2006; 5:797.
- [5]. Dalhoff K, Dancey J, Astrup L, Skovsgaard T, Hamberg KJ, Lofts FJ, Rosmorduc O, Erlinger S, BachHansen J, Steward WP, Skov T, Burcharth F, Evans TR. A phase II study of the vitamin D analogue Seocalcitol in patients with inoperable hepatocellular carcinoma. *Brit. J. cancer.* 2003; 89:252. [PubMed: 12865912]
- [6]. Evans TR, Colston KW, Lofts FJ, Cunningham D, Anthoney DA, Gogas H, de Bono JS, Hamberg KJ, Skov T, Mansi JL. A phase II trial of the vitamin D analogue Seocalcitol (EB1089) in patients with inoperable pancreatic cancer. *Br. J. Cancer.* 2002; 86:680. [PubMed: 11875725]
- [7]. Muindi JR, Johnson CS, Trump DL, Christy R, Engler KL, Fakih MG. A phase I and pharmacokinetics study of intravenous calcitriol in combination with oral dexamethasone and gefitinib in patients with advanced solid tumors, *Cancer Chemother. Pharmacol.* 2009; 65:33.
- [8]. Fakih MG, Trump DL, Muindi JR, Black JD, Bernardi RJ, Creaven PJ, Schwartz J, Brattain MG, Hutson A, French R, Johnson CS. A phase I pharmacokinetic and pharmacodynamic study of intravenous calcitriol in combination with oral gefitinib in patients with advanced solid tumors. *Clin. Cancer Res.* 2007; 13:1216. [PubMed: 17317832]
- [9]. Beer TM, Javle MM, Ryan CW, Garzotto M, Lam GN, Wong A, Henner WD, Johnson CS, Trump DL. Phase I study of weekly DN-101, a new formulation of calcitriol, in patients with cancer, *Cancer Chemother. Pharmacol.* 2007; 59:581.
- [10]. Trump DL, Potter DM, Muindi J, Brufsky A, Johnson CS. Phase II trial of high-dose, intermittent calcitriol (1,25 dihydroxyvitamin D₃) and dexamethasone in androgen-independent prostate cancer. *Cancer.* 2006; 106:2136. DL. [PubMed: 16598750]
- [11]. Ray R, Ray S, Holick MF. 1 α ,25-dihydroxyvitamin D₃-3-deoxy-3-bromoacetate, an affinity labeling analog of 1 α ,25-dihydroxyvitamin D₃. *Bioorg. Chem.* 1994; 22:276.
- [12]. Ray R, Swamy N, MacDonald PN, Ray S, Haussler MR, Holick MF. Affinity labeling of 1 α ,25-dihydroxyvitamin D₃ receptor. *J. Biol. Chem.* 1996; 271:2012. MF. [PubMed: 8567652]

- [13]. Swamy N, Kounine M, Ray R. Identification of the subdomain in the nuclear receptor for the hormonal form of vitamin D₃, 1,25-dihydroxyvitamin D₃, vitamin D receptor, that is covalently modified by an affinity labeling reagent. *Arch. Biochem. Biophys.* 1997; 348:91. [PubMed: 9390178]
- [14]. Swamy N, Xu W, Paz N, Hsieh J-C, Haussler MR, Maalouf GJ, Mohr SC, Ray R. Molecular modeling, affinity labeling and site-directed mutagenesis define the key points of interaction between the ligand-binding domain of the vitamin D nuclear receptor and 1,25-dihydroxyvitamin D₃. *Biochem.* 2000; 39:12162. [PubMed: 11015194]
- [15]. Mohr SC, Swamy N, Xu W, Ray R. Why do we need a three-dimensional architecture of the ligand-binding domain of the nuclear 1 α ,25-dihydroxyvitamin D₃ receptor? *Steroids.* 2001; 66:189. [PubMed: 11179726]
- [16]. Chen ML, Ray S, Swamy N, Holick MF, Ray R. Anti-proliferation of human keratinocytes with 1 α , 25-dihydroxyvitamin D₃-3-bromoacetate, an affinity labeling analog of 1 α ,25-dihydroxyvitamin D₃: mechanistic studies. *Arch. Biochem. Biophys.* 1999; 370:34. [PubMed: 10496974]
- [17]. Swamy N, Persons KS, Chen TC, Ray R. 1 α ,25-Dihydroxyvitamin D₃-3 β -(2)-bromoacetate, an affinity labeling derivative of 1,25-dihydroxyvitamin D₃ displays strong antiproliferative and cytotoxic behavior in prostate cancer cells. *J. Cell. Biochem.* 2003; 89:909. R. [PubMed: 12874825]
- [18]. Swamy N, Chen TC, Peleg S, Dhawan P, Christakos S, Stewart LV, Weigel NL, Mehta RG, Holick MF, Ray R. Inhibition of proliferation and induction of apoptosis by 25-hydroxyvitamin D₃-3-bromoacetate in prostate cancer cells. *Clin.Cancer Res.* 2004; 10:8018. [PubMed: 15585637]
- [19]. Lambert JL, Young CD, Persons KS, Ray R. Mechanistic and pharmacodynamic studies of a 25-hydroxyvitamin D₃ derivative in prostate cancer cells. *Biochem. Biophys.Res. Comm.* 2007; 361:189. [PubMed: 17658477]
- [20]. Lange TS, Singh RK, Kim KK, Zou Y, Kalkunte SS, Sholler GL, Swamy N, Brard L. Anti-proliferative and pro-apoptotic properties of 3-bromoacetoxycalcidol in high risk neuroblastoma. *Chem. Biol. Drug Des.* 2007; 70:302. [PubMed: 17937776]
- [21]. Persons KS, Eddy VJ, Chadid S, Deoliveira R, Saha AK, Ray R. Anti-growth effect of 1,25-dihydroxyvitamin D₃-3-bromoacetate alone or in combination with 5-amino-imidazole-4-carboxamide-1-beta-4-ribofuranoside in pancreatic cancer cells. *Anticancer Res.* 2010; 30:1875. [PubMed: 20651329]
- [22]. Lambert JR, Eddy VJ, Young CD, Persons KS, Sarkar S, Kelly JA, Genova E, Lucia MS, Faller DV, Ray R. A vitamin D receptor-alkylating derivative of 1 α ,25-dihydroxyvitamin D₃ inhibits growth of human kidney cancer cells and suppresses tumor growth. *Cancer Prev. Res. (Phila).* 2010; 3:1596. [PubMed: 21149334]
- [23]. Ray R, Holick SA, Holick MF. Synthesis of a photoaffinity-labelled analog of 1,25-dihydroxyvitamin D₃. *J. Chem. Soc., Chem. Comm.* 1985; 11:702.
- [24]. Yamada S, Suzuki t, Takayama H, Miyamoto K, Matsunaga I, Nawata Y. Stereoselective synthesis of (5E)- and (5Z)-vitamin D₃-alkanoic acids via vitamin D₃-sulfur sulfur dioxide adducts. *J. Org. Chem.* 1983; 48:3483.
- [25]. Ray R, Vicchio D, Yergey A, Holick MF. Synthesis of 25-hydroxy-[6,19,19'-²H₃]vitamin D and 1 α ,25-dihydroxy-[6,19,19'-²H₃]vitamin D₃. *Steroids.* 1992; 57:142. [PubMed: 1621268]
- [26]. Omdahl JL, Swamy N, Serda R, Annalora A, Berne M, Ray R. Affinity labeling of rat cytochrome P450C24 (CYP24) and identification of Ser57 as an active site residue. *J. Steroid Biochem. Mol. Biol.* 2004; 89-90:159. [PubMed: 15225765]
- [27]. Kaya T, Swamy N, Persons KS, Ray S, Ray R. Covalent labeling of nuclear vitamin D receptor with affinity labeling reagents containing a cross-linking probe at three different positions of the parent ligand: structural and biochemical implications. *Bioorg.Chem.* 2009; 37:57. [PubMed: 19223058]
- [28]. Bouillon R, Carmeliet G, Verlinden L, van Etten E, Verstuyf A, Luderer HF, Lieben L, Mathieu C, Demay M. Vitamin D and human health: lessons from vitamin D receptor null mice. *Endocr. Rev.* 2008; 6:726. [PubMed: 18694980]

Figure 1.

Cartoon depicting interaction between 1,25(OH)₂D₃, 1,25(OH)₂D₃-3-BE and VDR. Points to note: (i) reversible and irreversible nature of interaction of 1,25(OH)₂D₃ and 1,25(OH)₂D₃-3-BE respectively with VDR, leading to different catabolic outcomes, (ii) covalent attachment of 1,25(OH)₂D₃-3-BE via Cys288 in VDR-LBD, (iii) differential conformational changes of VDR-LBD induced by 1,25(OH)₂D₃ and 1,25(OH)₂D₃-3-BE.

Figure 2.

Comparison of the dose dependent increase of CYP24 mRNA by 1,25(OH)₂D₃ and 1,25(OH)₂D₃-3-BE. A498 cells were treated with ethanol control or 10⁻¹⁰⁻⁶ M of 1,25(OH)₂D₃ and 1,25(OH)₂D₃-3-BE for 24 hours, total RNA was prepared and CYP24 mRNA levels assessed by RT-PCR. β-Actin mRNA levels were determined for each sample as control. The results are representative of two independent experiments.

Figure 3.

Kinetics of CYP24 message induction by 1,25(OH)₂D₃ and 1,25(OH)₂D₃-3-BE. A 498 cells were treated with 10⁻⁸M of 1,25(OH)₂D₃ and 1,25(OH)₂D₃-3-BE or ethanol control for 16 hours, total RNA was prepared at specified times and CYP24 mRNA levels assessed by RT-PCR. β-Actin mRNA levels were determined for each sample as control. The results are representative of two independent experiments.

Scheme 1. Scheme for the synthesis of 1,25(OH)₂D₃-3-BE.