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Therapeutic potential of the 2-alkyl and 2-alkylidene-19-nor-(20*S*)-modified analogs of 1α,25-dihydroxyvitamin D_3 ☆

Hector F. DeLuca*

Department of Biochemistry, University of Wisconsin-Madison, 433 Babcock Drive, Madison, WI 53706-1544, USA

Abstract

Five analogs of 19-nor-1α,25-dihydroxyvitamin D_3 are described that show highly selective and potent activities. The 2-methylene-19-nor-(20*S*)-1α25-dihydroxyvitamin D_3 (2MD) and its 2α-methyl sister are selectively active on the osteoblast. 2MD is bone anabolic and causes bone formation in vivo and in vitro and is being developed as a therapy for bone loss diseases such as osteoporosis. 2-Methylene-19-nor (20*S*)-bishomo-1α-hydroxypregnacalciferol (2BMP) has no activity on calcium in vivo while totally suppressing circulating parathyroid hormone. Its homologs, i.e. 2-methylene-19-nor-1α-hydroxy-homopregnacalciferol (2MP) and 2-methylene-19-nor-1α-hydroxypregnacalciferol (2MPC) act similarly but are either less selective (2MP) or not as potent (2MPC). These abbreviated side chain analogs will be developed for diseases where a rise in serum calcium is not desired, as for example, cancer, renal osteodystrophy, psoriasis and autoimmune diseases.

Keywords: Vitamin D and bone; Vitamin D analog; Bone synthesis; Non-calcemic Vitamin D analog; Parathyroid suppression

1. Introduction

It is now well accepted that the active form of Vitamin D is 1α,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) [1,2]. It is also well accepted that this active form of Vitamin D functions not only in the calcium homeostatic mechanisms and in support of bone mineralization but also elsewhere such as in the parathyroid glands, the keratinocytes, cells of the immune system, and possibly elsewhere, i.e. in the islet cells of the pancreas [1,2]. The primary and basic function of 1,25-$(OH)_2D_3$ is to elevate plasma calcium and plasma phosphorus to support bone mineralization and the prevention of neuromuscular convulsions or tetany [1,2]. The functions of 1,25-$(OH)_2D_3$ in other tissues are less prominent; however, in terms of therapy, the possible use of Vitamin D compounds is based on these less prominent functions. As for example, suppression of secondary hyperparathyroidism of renal osteodystrophy is the primary goal of Vitamin D therapy and not its role in elevating plasma calcium concentrations [3,4].

The chemical synthesis of analogs is generally carried out for a variety of reasons; one of which is to increase potency, another is to increase selectivity, and another is to diminish toxicity. The long history of synthesis of analogs of 1,25-$(OH)_2D_3$ has yielded a number of important compounds that have been clearly developed for therapeutic use. Among them are 1,25-$(OH)_2D_3$ itself, 25-hydroxyvitamin D_3 (25-OH-D_3), 1α-hydroxyvitamin D_3 (1α-OH-D3), 1α-hydroxyvitamin D_2 (1α-OH-D_2), 22-oxa-1,25-$(OH)_2D_3$, 19-nor-1,25-$(OH)_2D_2$, and calcipotriol [1,2,5–10]. The selectivity or reduced toxicity achieved in these cases is largely the result of rapid metabolic inactivation rather than selective activity of the analog at the molecular level [11–13]. There remains, therefore, a clear need for analogs of 1,25-$(OH)_2D_3$ that are selective in their activities, that are safe inasmuch as they do not produce hypercalcemia, and that retain high potency.

During the course of our investigation of A-ring derivatives of the Vitamin D system, we have focused on the 19-nor derivatives because they appear to reduce the hypercalcemic activity of 1,25-$(OH)_2D_3$ [14]. When we began to modify the A-ring beyond removing the 10,19-methylene group, we came across an interesting series of analogs modified on the carbon-2 by the addition of an alkylidene or an α-alkyl [15,16]. Thus, 2α-alkyl and 2-methylene compounds were first synthesized and yielded a bone selective series of analogs (Fig. 1) [15]. These compounds were found to bind equally well to the Vitamin D receptor (VDR) as 1,25-$(OH)_2D_3$ and were one order of magnitude more

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* Tel.: +1-608-262-1620; fax: +1-608-262-7122.
E-mail address: deluca@biochem.wisc.edu (H.F. DeLuca).

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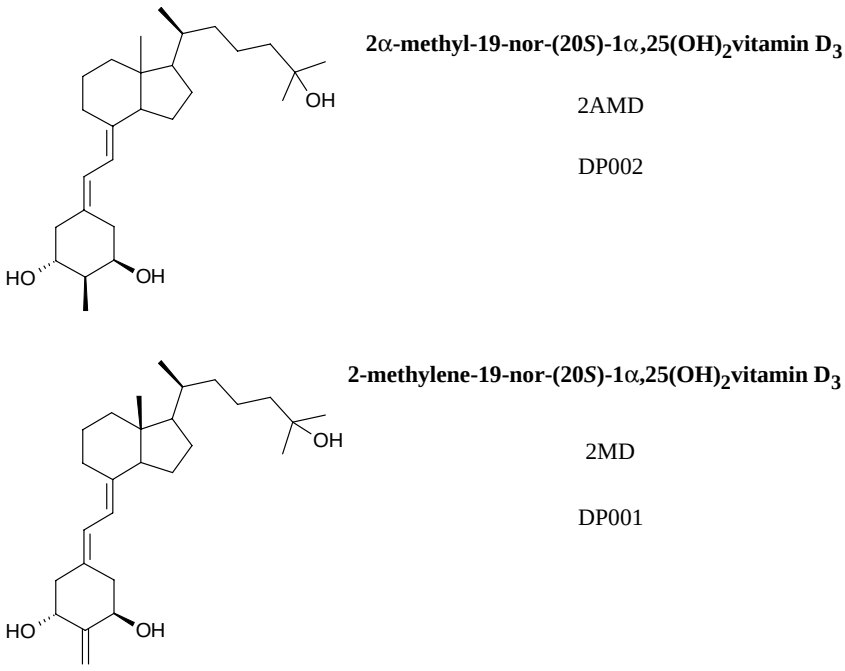


Fig. 1. Structures of the 2-carbon-modified analogs selective for bone.

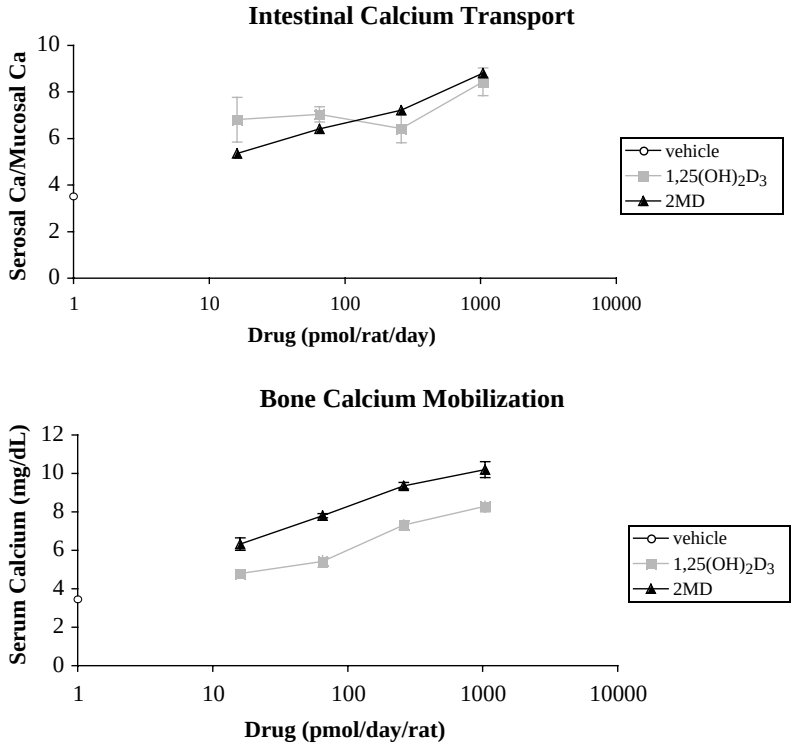


Fig. 2. Intestinal calcium transport activity and bone calcium mobilization activity of rats on a low calcium, Vitamin D-deficient diet provided daily doses of either 1,25-$(OH)_2D_3$ or 2MD. Vitamin D-deficient rats on a low calcium diet were given the various indicated doses intraperitoneally each day for a period of 4 days, and 24 h after the last dose, the animals were killed for a determination of intestinal calcium transport in vitro and for a serum calcium measurement. Bone calcium mobilization was ascertained by the elevation of serum calcium. In this model, the rise in serum calcium is at the expense of bone and hence is an in vivo measurement of bone calcium mobilization.

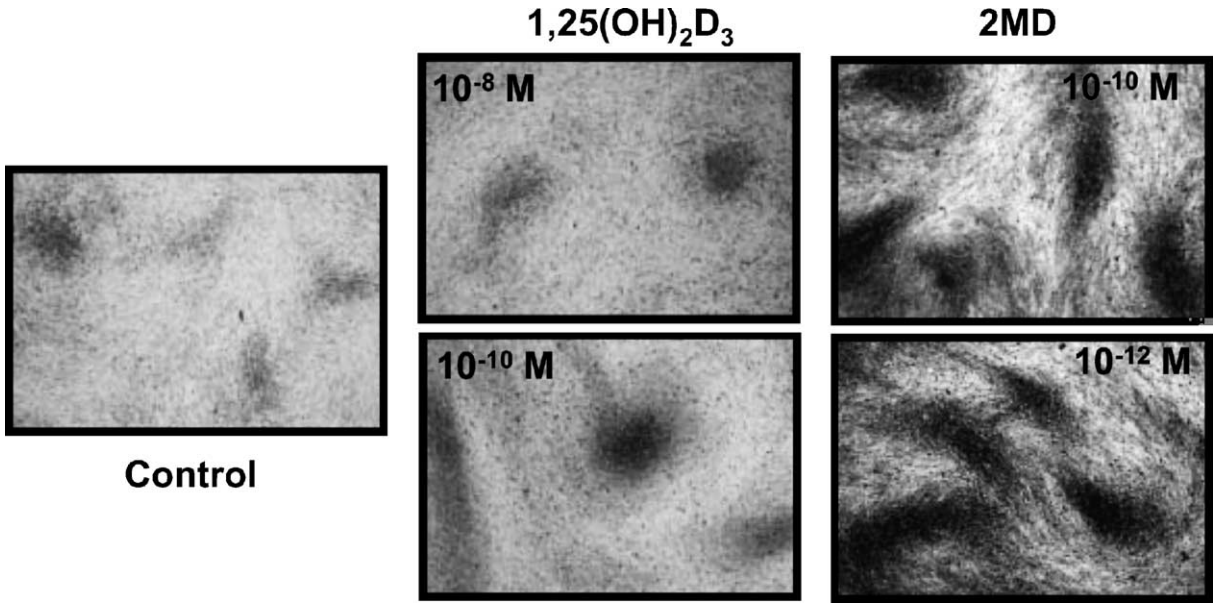


Fig. 3. 2MD induces bone nodule formation in primary cultures of human osteoblasts. Human osteoblasts isolated from surgically discarded bone from cranial sutures were incubated with the indicated compounds at the various concentrations for a period of 7 days at which time they were incubated another 7 days in the presence of β-glycerol phosphate and ascorbic acid. Even at 10^{-12} M, 2MD caused the formation of bone nodules whereas 1,25-$(OHJ)_2D_3$ at a concentration of 10^{-8} M failed to produce a significant bone nodule response.

active in causing HL-60 differentiation [15,17]. These properties are not unique or surprising; however, when we examined in vivo activities of these compounds, we learned that as expected, 2-methylene-19-nor-(20*S*)-1,25-$(OH)_2D_3$ (2MD) is approximately equal to 1,25-$(OH)_2D_3$ in supporting intestinal calcium transport in Vitamin D-deficient rats (shown in Fig. 2 for 2MD). Most surprising, however, was when we examined the biopotency of these compounds in the mobilization of calcium from bone, the 2-carbon-modified analogs were between 30 and 100 times more effective than the native hormone [17]. This then suggested that these analogs act selectively on the osteoblast because it is the osteoblast that mediates the mobilization of calcium from bone by providing the RANKL for both osteoclastogenesis and osteoclast-activation [18,19]. When measured in vitro in osteoclastogenesis, 2MD turned out to be two orders of magnitude more effective than the native hormone when tested in bone marrow cultures [17]. Although this proved to be surprising, further examination of the effect of these compounds on human osteoblasts resulted in the demonstration that 2MD (and not calcitriol, even at concentrations of 10^{-7} M), at 10^{-12} M is able to induce human osteoblasts to form bone nodules as shown in Fig. 3 [17]. This activity required the presence of the receptor inasmuch as osteoblasts derived from receptor knockout mice were unable to respond to 2MD in this system. These results again supported the idea that 2MD is much more effective on bone than 1,25-$(OH)_2D_3$ while being approximately equally active in the intestine.

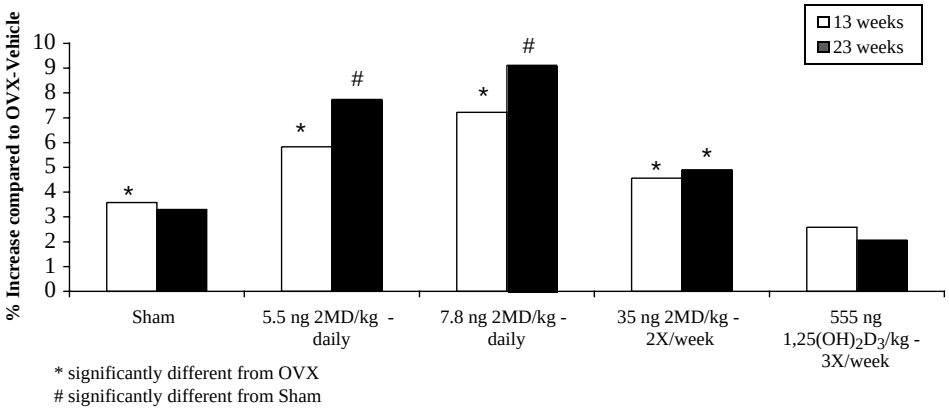


Fig. 4. 2MD given orally causes a marked increase in total body bone mineral density in aged, ovariectomized, female rats. Retired female breeders were ovariectomized and following a 5-week period, they were given the indicated doses orally of either 2MD or 1,25-$(OH)_2D_3$ for 23 weeks. At a dose of 7 ng/kg of body weight, 2MD produced a 9% elevation in total body bone mineral density, whereas a much higher dose of 1,25-$(OH)_2D_3$ given three times a week was relatively ineffective.

We have now conducted a total of four ovariectomy studies in which we can show that 2MD has a very marked ability to increase the formation of new bone. Fig. 4 provides total body bone mineral density studies in retired, female breeder rats obtained from Sprague–Dawley that have also been ovariectomized and allowed to lose mineral for approximately 6 weeks ([17]; unpublished results). They were then treated for a total of 35 weeks and shown here is a 28-week scan. The results show that total body bone density is markedly increased by a dose of 5 or 7 ng/kg body weight per day not only above ovariectomized control but also above the sham-operated control. This was found without any marked increases in serum calcium concentration. Serum calcium was increased between 0.5 and 1 mg% by the 7 ng/kg per day dose. The examination of the skeleton by a variety of means now has shown that trabecular and cortical bone volume were increased by 25% during this treatment ([20]; unpublished results). Bone histomorphometry reveals that this is the result of increased bone synthesis rather than inhibition of bone resorption. We have repeated these experiments in younger animals with much lower doses, and have found that as little as 0.5 ng/kg per day will cause an increase in bone mass as will 1 and 2.5 ng/kg per day. These low doses produce little or no change in serum calcium. Fig. 5 shows tibia sections taken from the animals shown in Fig. 4 with the Goldner's Masson-Trichrome Stain

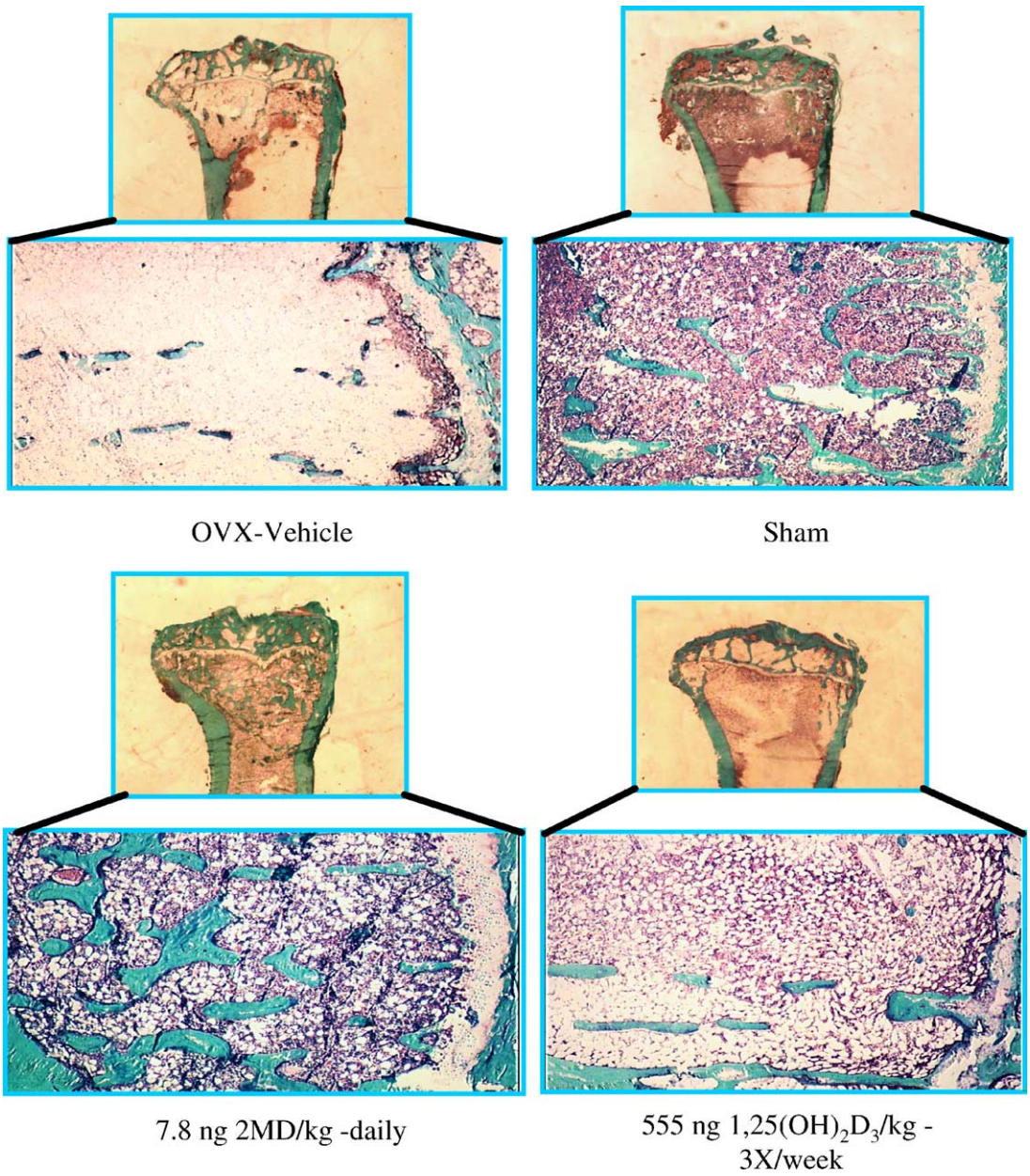
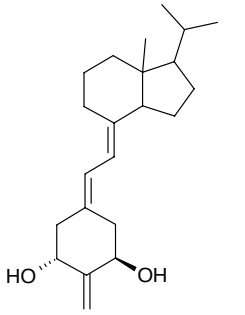


Fig. 5. Histologic examination of distal ends of tibia taken from animals in Fig. 3. The bones were sectioned and stained with Goldner's Masson-Trichrome Stain to reveal mineralized bone (green stain).

illustrating the marked activity of 2MD in increasing total bone mass. Thus, 2MD and its 2α-methyl analog appear to be bone anabolic and represent possible new compounds to be used for the treatment of diseases where there is a lack of total bone mass such as osteoporosis (post-menopausal, age-related and steroid-induced).

We have investigated a possible molecular basis of the high potency and selectivity of 2MD for osteoblastic activity. The results suggest that 2MD causes a marked increased in VDR affinity for the Vitamin D responsive elements and increases interaction with retinoid X receptor and coactivators SRC-1 and Drip 205 [21].

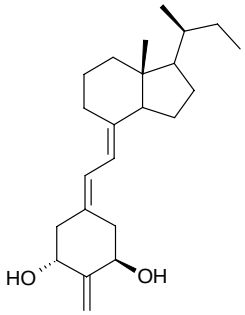
Fig. 6 presents the structures of three important new analogs of this series in which the side chain is largely abbreviated, and there is an absence of an hydroxyl in the side chain. Therefore, the 25-hydroxyl anchor point in the receptor is no longer present. Fig. 6 shows that these compounds bind as well to the receptor as 1,25-$(OH)_2D_3$ and that they are approximately equal in their ability to cause HL-60 cell differentiation. Not shown here is that these compounds are just as active as 1,25-$(OH)_2D_3$ in inducing transcription of Vitamin D-responsive genes as illustrated by the 24-hydroxylase (CYP-24) promoter driving luciferase reporter gene system. Thus, these compounds without the 25-hydroxyl and without the side chain are nevertheless transcriptionally active. Of considerable importance is that only one of these analogs show a tendency to increase intestinal calcium transport and this, at only very high doses. That compound is 2-methylene-19-nor-1α-hydroxy-homopregnacalciferol (2MP). Note especially the 2-methylene-bis-homo-(20*S*)-1α-hydroxypregnacalciferol (2BMP) has no activity whatsoever in the calcium systems (Table 1). However, these compounds are clearly active in vivo as revealed by the suppression of plasma parathyroid hormone (PTH) levels when given at doses that do not change serum calcium concentration (Table 2). In fact, the bishomo-compound and the homo compound are completely able to suppress circulating PTH levels. The pregnacalciferol is the least active of the three by suppressing PTH levels by 60%.



2-methylene-19-nor-1α-hydroxyhomopregnacalciferol

2MP

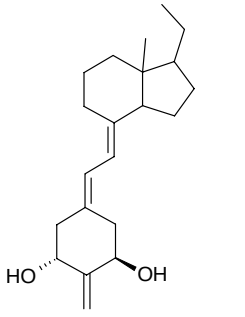
DP003



2-methylene-19-nor-(20*S*)-1α-hydroxybishomopregnacalciferol

2MbisP

DP006



2-methylene-19-nor-1α-hydroxypregnacalciferol

2Mpregna

DP004

Fig. 6. Structures of 2-carbon-modified analogs that possess little or no calcium mobilizing activity.

Table 1
Activity of analogs in intestine and bone

Compound	Dose level (μg/kg)	Intestinal calcium transport (serosal Ca/mucosal Ca)	Bone calcium mobilization (mg/dl)
Vehicle		3.6 ± 0.4	4.1 ± 0.2
1β,25(OH)$_2$D$_3$	0.8	7.2 ± 0.9*	5.0 ± 0.3*
2-Methylene-19-nor-1β-hydroxy-homopregnacalciferol (2MP)	0.2	4.8 ± 0.1	4.0 ± 0.1
	0.6	3.1 ± 0.3	4.1 ± 0.0
	1.9	4.9 ± 0.5	4.0 ± 0.1
	5.6	6.0 ± 0.9	4.3 ± 0.1
Vehicle		4.1 ± 0.1	4.6 ± 0.1
1β,25(OH)$_2$D$_3$	0.8	7.5 ± 0.8*	5.3 ± 0.2
2-Methylene-19-nor-1β-hydroxy-bis-homopregnacalciferol (2MbisP)	0.2	3.5 ± 0.1	4.4 ± 0.2
	0.6	3.5 ± 0.5	4.2 ± 0.2
	1.9	3.0 ± 0.2	4.1 ± 0.0
	5.6	3.0 ± 0.3	4.4 ± 0.2
Vehicle		7.3 ± 0.4	4.3 ± 0.1
1β,25(OH)$_2$D$_3$	1.0	10.9 ± 0.8*	5.4 ± 0.2*
2-Methylene-19-nor-1β-hydroxypregnacalciferol (2Mpregna)	0.8	7.9 ± 0.9	4.8 ± 0.1
	3.9	7.6 ± 0.5	4.5 ± 0.1

Vitamin D-deficient rats were given the indicated doses of analog each day for 4 days and on day 5, the intestinal calcium transport activity and bone calcium mobilization activity was determined as described in Fig. 2.

* Indicates statistically significantly different from vehicle control at $P < 0.05$.

In results not shown here, these compounds given orally are fully able to induce 24-hydroxylase mRNA in the keratinocyte. Thus, it is very clear that these compounds are systemically active, but they are unable to support elevation of serum calcium. We have elevated the dose in normal animals to as high as 300 μg/kg body weight without increasing serum calcium concentration when given orally. These compounds, therefore, in vivo seem to be very selective in terms of lacking the ability to stimulate the intestine and bone to mobilize calcium whereas they are clearly active in suppressing PTH levels, and are active in inducing 24-hydroxylase in the keratinocytes. These results suggest that these compounds are extremely promising as being truly selectively non-calcemic while retaining in vivo activity to suppress the parathyroid on one hand and to induce changes in the keratinocyte on the other. These results suggest that this series of compounds may be useful in the treatment of secondary hyperparathyroidism of renal osteodystrophy; they may be very useful in the treatment of cancer; or in the treatment of autoimmune disease. They may also be useful as an oral treatment for the skin disorder, psoriasis.

Table 2
Analog suppression of serum PTH

Compound	Dose level (μg/kg)	PTH suppression (%)	Serum calcium (mg/dl)
Vehicle			9.3 ± 0.0.4
1β,25(OH)$_2$D$_3$	0.2	100 ± 0*	10.6 ± 0.1*
2-Methylene-19-nor-1β-hydroxy-homopregnacalciferol (2MP)	0.8	14 ± 17	9.5 ± 0.1
	2.3	52 ± 12*	9.8 ± 0.3
	6.8	100 ± 0*	10.3 ± 0.1*
	67		11.2 + 0.2*
2-Methylene-19-nor-1β-hydroxybishomopregnacalciferol (2MbisP)	0.8	42 ± 26*	9.2 ± 0.1
	2.3	66 ± 24*	9.1 ± 0.1
	7.0	86 ± 67*	9.1 ± 0.1
	70		9.5 + 0.1
2-Methylene-19-nor-1β-hydroxypregnacalciferol (2Mpregna)	0.7	40 ± 14*	9.2 ± 0.1
	2.2	39 ± 16*	8.9 ± 0.1
	6.5	67 ± 21*	9.0 ± 0.1
	64		9.3 + 0.1

Normal rats fed a 0.47% calcium, 0.3% phosphorus diet were given the indicated doses intraperitoneally each day for 7 days and serum PTH determined by ELIZA.

* Significantly different from vehicle at $P < 0.05$ or 0.001.

2. Conclusions

The 2-alkyl and 2-alkylidene analogs of 19-nor-1,25-$(OH)_2D_3$ are extremely interesting inasmuch as they display selective activity. It seems clear that 2-methylene-19-nor-(20*S*)-1,25-$(OH)_2D_3$ and its 2α-methyl analog are selectively active in bone and cause new bone synthesis in animals depleted of bone mass. These compounds seem ideal for the treatment of bone loss disease such as osteoporosis and their possible use for fracture healing and any circumstance where synthesis of new bone is desired. On the other hand, the 2-alkyl and 2-alkylidene derivatives of 19-nor-1α-OH-D_3 that lack the side chain and the hydroxyl thereon appear to be truly non-calcemic yet are effective systemically suggest that they may be useful in the treatment of malignancy and may be useful in the treatment of psoriasis by oral administration or in the treatment of autoimmune disease.

Acknowledgements

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