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2-Methylene analogs of 1α-hydroxy-19-norvitamin D_3: synthesis, biological activities and docking to the ligand binding domain of the rat vitamin D receptor☆

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Abstract

In continuing efforts towards the synthesis of biologically active vitamin D compounds of potential therapeutic value, new 2-methylene-1α-hydroxy-19-norvitamin D_3 analogs **3** and **4** with modified alkyl side chains have been synthesized. The key synthetic step involved Lythgoe-type Wittig–Horner coupling of Windaus–Grundmann type ketones **9**, possessing different 17β-alkyl substituents, with the phosphine oxide **10** prepared from (-)-quinic acid. The prepared vitamins **3** and **4** were ca. eight times less potent than 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$) (**1**) in binding to the rat intestinal vitamin D receptor (VDR). In comparison with the hormone **1** they exhibited slightly lower cellular HL-60 differentiation activity. When tested in vivo; the analog **3** was characterized by very high bone calcium mobilizing potency and intestinal calcium transport activity. Unexpectedly, the 25-methyl compound **4** showed marked calcemic activity in both assays. Computational docking of the vitamin **3** into the binding pocket of the rat vitamin D receptor is also reported.

Keywords: Vitamin D analogs; 19-Norvitamin D; Calcemic activity; Vitamin D receptor; HL-60 differentiation

1. Introduction

The discovery of the most active metabolite of vitamin D_3, 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$, calcitriol, **1**; Fig. 1), has greatly stimulated research into its physiology and chemistry [1,2]. It has been established that **1** supports mineralization [3], affects the immune system, and exerts potent effects upon cell proliferation and cellular differentiation [4–6]. We synthesized interesting analogs of the natural hormone [7], in which the exocyclic methylene group is transposed, in comparison with **1**, from C-10 to C-2. Compound **2** (**2MD**), with an unnatural configuration at C-20, exhibits the same affinity to the VDR as calcitriol. It also shows a preferential activity on bone relative to intestine. Recent studies confirmed its high ability to induce bone formation both in vitro and in vivo [8]. In view of the very high calcemic potency of **2MD**, we synthesized analog **3** considering it to be a possible biological precursor of **2MD**. It might be expected that in a living organism, this compound should undergo 25-hydroxylation in the liver and slowly form the more active metabolite **2**. In our continuing investigation of the structure–activity relationship in this series of 19-norvitamin D compounds, we also prepared the 25-methyl derivative **4**, that cannot be "activated" by the 25-hydroxylation process.

2. Materials and methods

2.1. Preparation of 2-methylene analogs of 1α-hydroxy-19-norvitamin D_3 3 and 4

Vitamin D analogs **3** and **4** were synthesized at the Department of Biochemistry, University of Wisconsin-Madison according to the synthetic route presented in Scheme 1. Full synthetic details and analytical data of the prepared compounds will be reported elsewhere.

2.2. Docking procedure

Docking simulations (1,00,000 iterations each) were performed by FlexiDock software from TRIPOS. For each of the six theoretical vitamin D conformers (two 6-*s*-*trans* and four 6-*s*-*cis*), at least four docking experiments were done,

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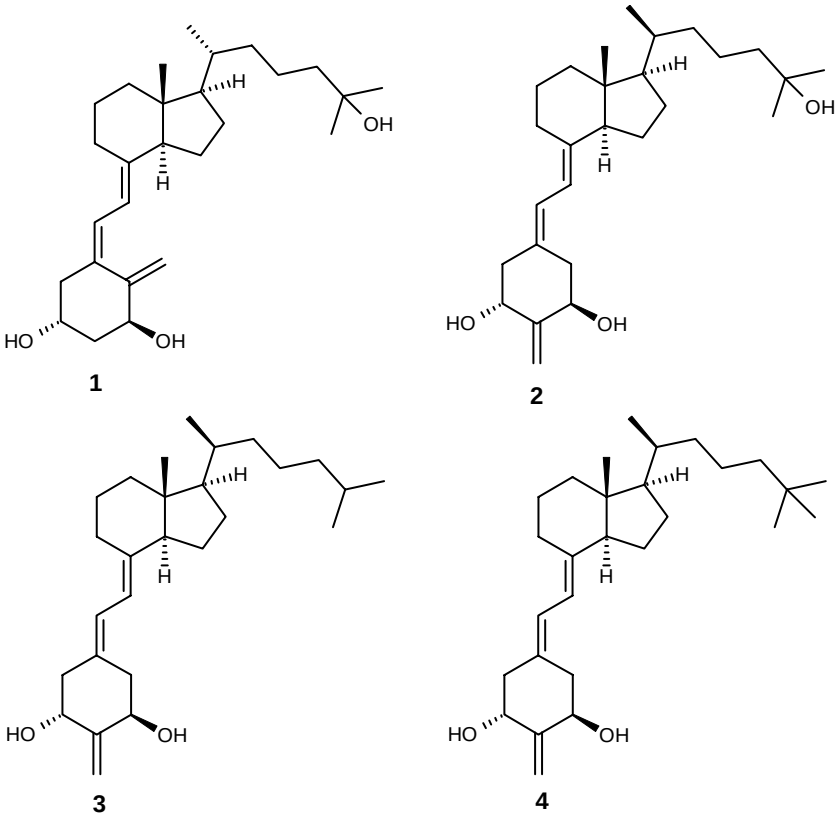


Fig. 1. Chemical structure of 1α,25-dihydroxyvitamin D_3 (calcitriol, **1**) and its 19-nor analogs.

starting with different pre-positioning of the ligand [9]. For final consideration, the lowest energy complex, possessing parallel orientation of the tryptophan 282 ring to the steroid 5,7-diene moiety was selected. The 3.5 Å contacts between **3** and rVDR were detected by the program Biodesigner.

2.3. In vitro studies

2.3.1. Measurement of binding to the rat recombinant vitamin D receptor

Purified rat recombinant vitamin D receptor was prepared and will be reported in detail elsewhere. Competition binding assays were performed using 1α,25-$(OH)_2$[26,27-^{3}H]D_3 as previously described [10]. The experiments were carried out in triplicate on two different occasions.

2.3.2. Measurement of cellular differentiation

Human leukemia HL-60 cells were plated at 2×10^5 cells per plate and incubated for 4 days. At the end of the 4th day, superoxide production was measured by nitro blue tetrazolium (NBT) reduction [6].

2.4. In vivo studies

2.4.1. Intestinal calcium transport

Female CD-1 mice (5–6 weeks old) were obtained from Harlan (Madison, WI). They were fed a purified diet containing 0.47% calcium. After 1 week of acclimation, the animals were administered alendronate intraperitoneally to prevent bone calcium mobilization. Twenty-four hours later the animals were given a single oral dose of the test compounds dissolved in Neobee M-5 oil. Blood was collected 24 h after the oral dose and serum calcium levels determined using atomic absorption spectrometry.

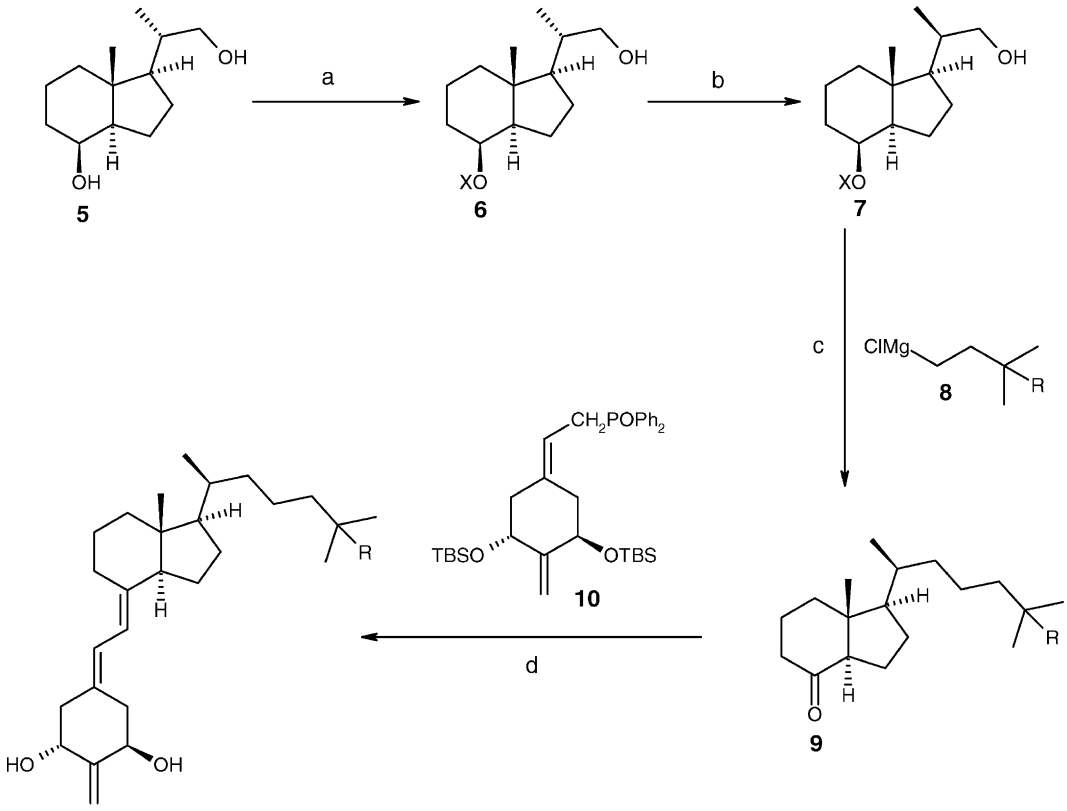
2.4.2. Bone calcium mobilization

Female CD-1 mice (5–6 weeks old) were obtained from Harlan (Madison, WI). They were fed a purified diet containing 0.02% calcium. After 1 week of acclimation, the animals were administered a single oral dose of the test compounds dissolved in Neobee M-5 oil. Blood was collected 48 h after the oral dose and serum calcium levels determined using atomic absorption spectrometry.

3. Results and discussion

3.1. Chemical synthesis of the analogs *3* and *4*

The strategy of our synthesis of 2-substituted 19-norvitamins was based on the Lythgoe-type Wittig–Horner coupling. The A-ring fragment, phosphine oxide **10** (Scheme 1), was prepared from commercially available (-)-quinic acid [7]. The corresponding CD-fragment **9**, required for the



For X = Bz and R = H:
a) 1. BzCl, py, 2. KOH, EtOH (**6**, 93%); b) 1. PDC, 2. n-Bu_4NOH, 3. $NaBH_4$ (**7**, 27%); c) 1. TsCl, Et_3N, 2. **8**, Li_2CuCl_4, 3. PDC (**9**, 60%); d) 1. **10**, PhLi, 2. TBAF (**3**, 45%).

For X = TBS and R = Me:
a) 1. TBSOTf, 2. TBAF (**6**, 93%); b) 1. SO_3·py, 2. n-Bu_4NOH, 3. $NaBH_4$ (**7**, 36%); c) 1. TsCl, Et_3N, 2. **8**, Li_2CuCl_4, 3. HF·py, 4. PDC (**9**, 59%); d) 1. **10**, PhLi, 2. TBAF (**3**, 36%).

Scheme 1.

synthesis of vitamins **3** and **6**, was obtained from the known Inhoffen–Lythgoe diol **5**. Epimerization at C-20 was accomplished at the stage of intermediate 22-aldehydes [11]. The coupling of 8-ketones **9** with the anion generated from **10**, followed by hydroxyls deprotection, gave the corresponding 2-methylene-19-norvitamins **3** and **4**.

*3.2. Docking of analog **3** to the ligand binding pocket of the VDR*

The synthesized vitamin D compound **3** is a 25-deoxy analog of the biologically active **2MD**. Calculated energy of the most stable complex of VDR and **3** was −51.6 kcal/mol. This analog settles in the binding pocket (Fig. 2) similar to the parent hormone **1**, with 6-*s-trans* conformation of the 5,7-diene moiety and equatorial orientation of the 1α-OH group. Also the vitamin orientation in the binding pocket, with an A ring directed towards Y143 and a side chain between His 301 and His 393, resembles the position of 1α,25-$(OH)_2D_3$ in the hVDR deletion mutant [12]. The same set of amino acids, found in the hormone-hVDR crystalline complex, creates hydrogen bonds with 1α- and 3β-hydroxyls. Hydrophilic amino acids R270 and S233 contact 1α-OH, while S274 and Y143 contact 3β-OH group. The lengths of these hydrogen bonds are: 2.94, 3.72, 3.03, and 2.46 Å, respectively.

The side chain, with the 25-OH group removed, is stabilized in the VDR pocket by efficient hydrophobic interactions. Short (<3.5 Å) specific contacts were found between methyl groups of amino acids L223, L226, A227, V230, A299, L410, and 26- and 27-methyls from the ligand. Also hydrophobic interactions between the 21-methyl group and I264, L305, and L309 help anchor the side chain in a manner similar to that found in the hormone complex.

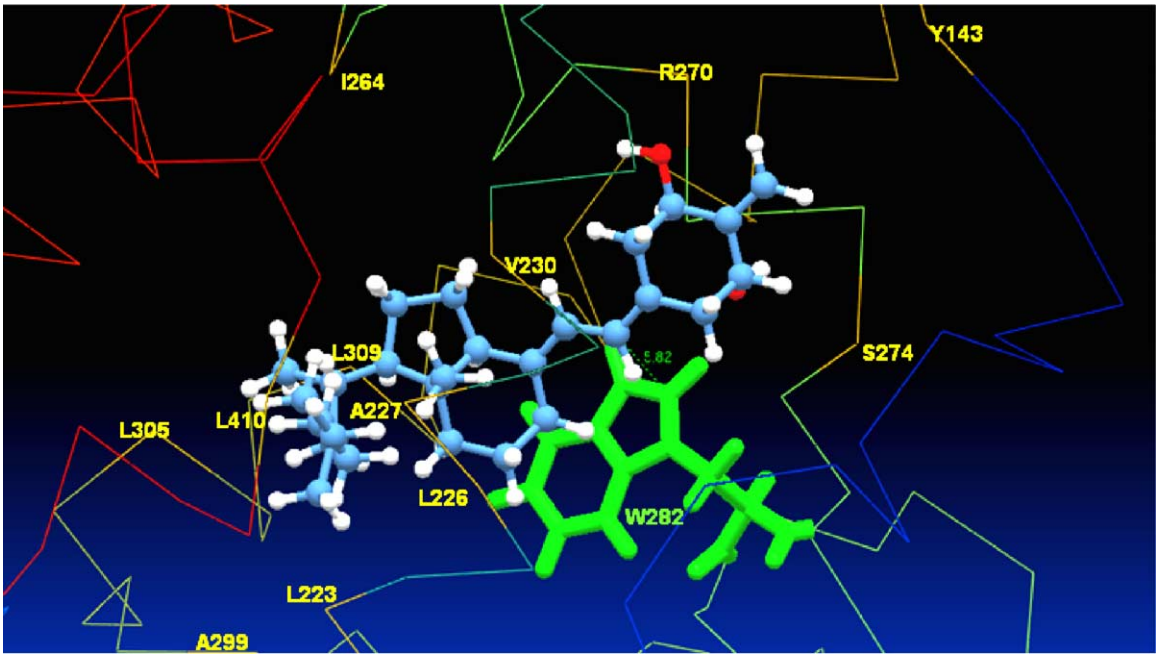


Fig. 2. View of the three-dimensional structure of ligand binding cavity of the rat VDR with the docked vitamin D analog **3**. The common contacts (distances shorter than 3.5 Å) found between any atom of the ligand and receptor are marked in yellow. The molecule of tryptophan 282 is marked in green.

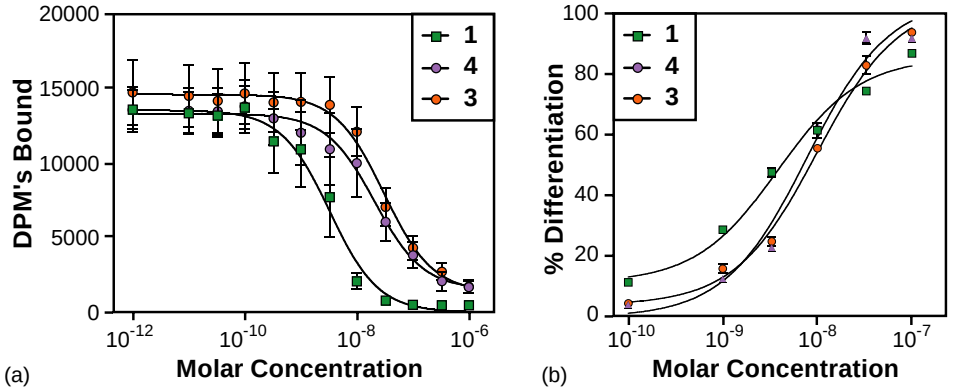


Fig. 3. (a) Competitive binding of 1α,25-$(OH)_2D_3$ (**1**) and the synthesized 2-methylene substituted 19-nor-1α-$(OH)D_3$ analogs **3** and **4** to the rat recombinant vitamin D receptor. This experiment was carried out in triplicate on two different occasions. (b) Differentiation activity of 1α,25-$(OH)_2D_3$ (**1**) and the synthesized analogs **3** and **4**. Differentiation state was determined by measuring the percentage of cells reducing nitro blue tetrazolium (NBT).

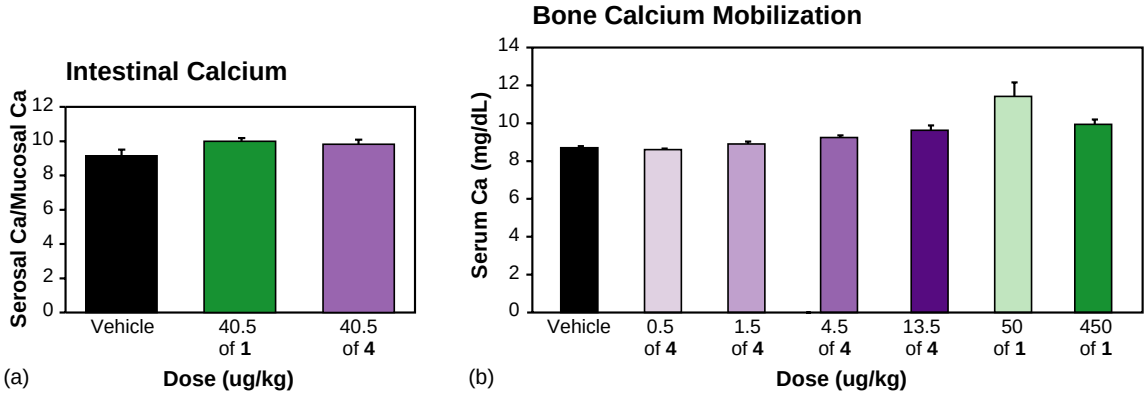


Fig. 4. (a) Support of intestinal calcium transport of the 25-methyl-2-methylene-19-nor-1α-$(OH)D_3$ **4** in mice. (b) Bone calcium mobilization activity of the analog **4** in mice on a low-calcium diet.

3.3. Biological evaluation of the synthesized analogs **3** and **4**

The presented results (Fig. 3) indicate that the binding ability of synthesized 2-methylene analogs, **3** and **4**, to the rat recombinant vitamin D receptor is ca. eight times lower than that of 1α,25-$(OH)_2D_3$ (**1**). The established HL-60 cellular differentiation activity of the tested compounds is slightly weaker in comparison with the hormone **1** (Fig. 3). 19-Norvitamin **3**, as expected, has an intestinal calcium transport activity and an extremely high ability to mobilize calcium from bone (data not shown). Surprisingly, the 25-methyl compound **4** shows similar intestinal activity as 1α,25-$(OH)_2D_3$ and retains bone calcium mobilizing activity (Fig. 4).

Highly elevated calcemic activities of **3**, approaching that of its corresponding 25-hydroxy counterpart [7], suggest in vivo 25-hydroxylation of the tested analog. However, replacing the 25-hydroxyl group with a methyl substituent does not abolish the in vivo activity as shown by the results of biological tests of 25-methyl vitamin **4**.

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