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New derivative of 1α,25-dihydroxy-19-norvitamin D_3 with 3′-alkoxypropylidene moiety at C-2: synthesis, biological activity and conformational analysis☆

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Abstract

In pursuit of novel biologically active Vitamin D compounds of potential therapeutic value, 1α,25-dihydroxy-2-[3′-(methoxymethoxy) propylidene]-19-norvitamin D_3 (**7**) was efficiently prepared in a convergent synthesis, starting with (−)-quinic acid and the protected 25-hydroxy Grundmann ketone **16**. The key synthetic step involved Lythgoe type Wittig–Horner coupling of **16**, with the phosphine oxide **15**. Molecular modeling was employed to establish the A-ring conformation of the synthesized Vitamin **7**. Also, preliminary modeling of its complex with the rVDR was performed and interactions between ligand and the binding domain analyzed. Analog **7** was found to be only six times less potent than 1α,25-$(OH)_2D_3$ (**1**) in binding to the rat recombinant Vitamin D receptor (VDR). In comparison with hormone **1**, it also showed slightly lower cellular HL-60-differentiation activity. Preliminary in vivo tests indicated unusually high calcemic activity of **7**.

Keywords: Vitamin D compounds; 19-Norvitamin D; Conformational analysis; Vitamin D receptor; Ligand docking to rVDR

1. Introduction

It has been established that the hormonally active form of Vitamin D_3, 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$, calcitriol, **1**; Fig. 1) controls the expression of numerous genes whose products are involved in mineral metabolism and cellular differentiation [1]. These gene expressions are mediated by the Vitamin D receptor (VDR), which belongs to the nuclear receptor superfamily [2]. Taking into account the crucial role of the Vitamin D–VDR complex, it seemed desirable to design a Vitamin D analog that could not only bind effectively to VDR, but also introduce some additional interactions modifying the protein structure.

Five years ago, we reported the synthesis of the biologically active analog of the natural hormone **1**, 1α,25-dihydroxy-2-methylene-19-norvitamin D_3 (**2**) [3]. Recently, we also prepared derivatives substituted at C-2 with an ethylidene group [4]; compound **3** proved to be much more biologically potent than its geometrical *Z*-isomer **4**. Takayama's group reported synthesis of the hormone **1** analog substituted with a 2α-(3′-hydroxypropyl) group [5,6]. Such structural modification caused a three-fold increase in VDR binding and enormously (ca. 500 times) enhanced the calcium mobilizing potency of the analog **5**.

Taking into account these findings, it seemed reasonable to prepare 1α,25-dihydroxy-2-(3′-hydroxypropylidene)-19-norvitamin D_3 (**6**) or its analog with an extended substituent at C-2, capable of creating additional hydrogen bonds with VDR. Considering the possible synthetic paths, we decided to synthesize first a possible precursor of **6**, 2-(3′-methoxymethoxypropylidene)-19-nor-1α,25-$(OH)_2D_3$ (**7**). In view of preliminary results (described below) of molecular modeling of this free ligand and its complex with rVDR, the analog **7** by itself seemed to be an interesting synthetic target.

2. Materials and methods

2.1. Preparation of 1α,25-dihydroxy-2-(3′-methoxymethoxypropylidene)-19-norvitamin D_3 (7)

Vitamin D analog **7** was synthesized at the Department of Biochemistry, University of Wisconsin-Madison according to the synthetic route presented in Scheme 1. All compounds exhibited spectroscopic and analytical data in accordance

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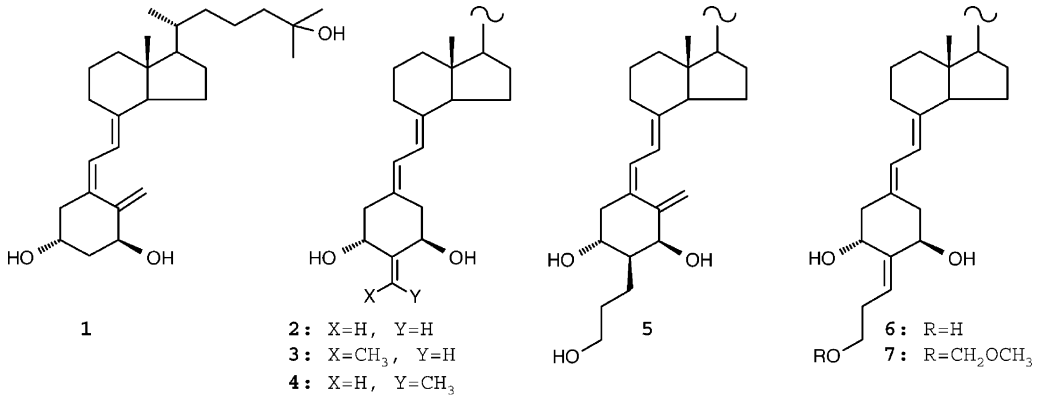
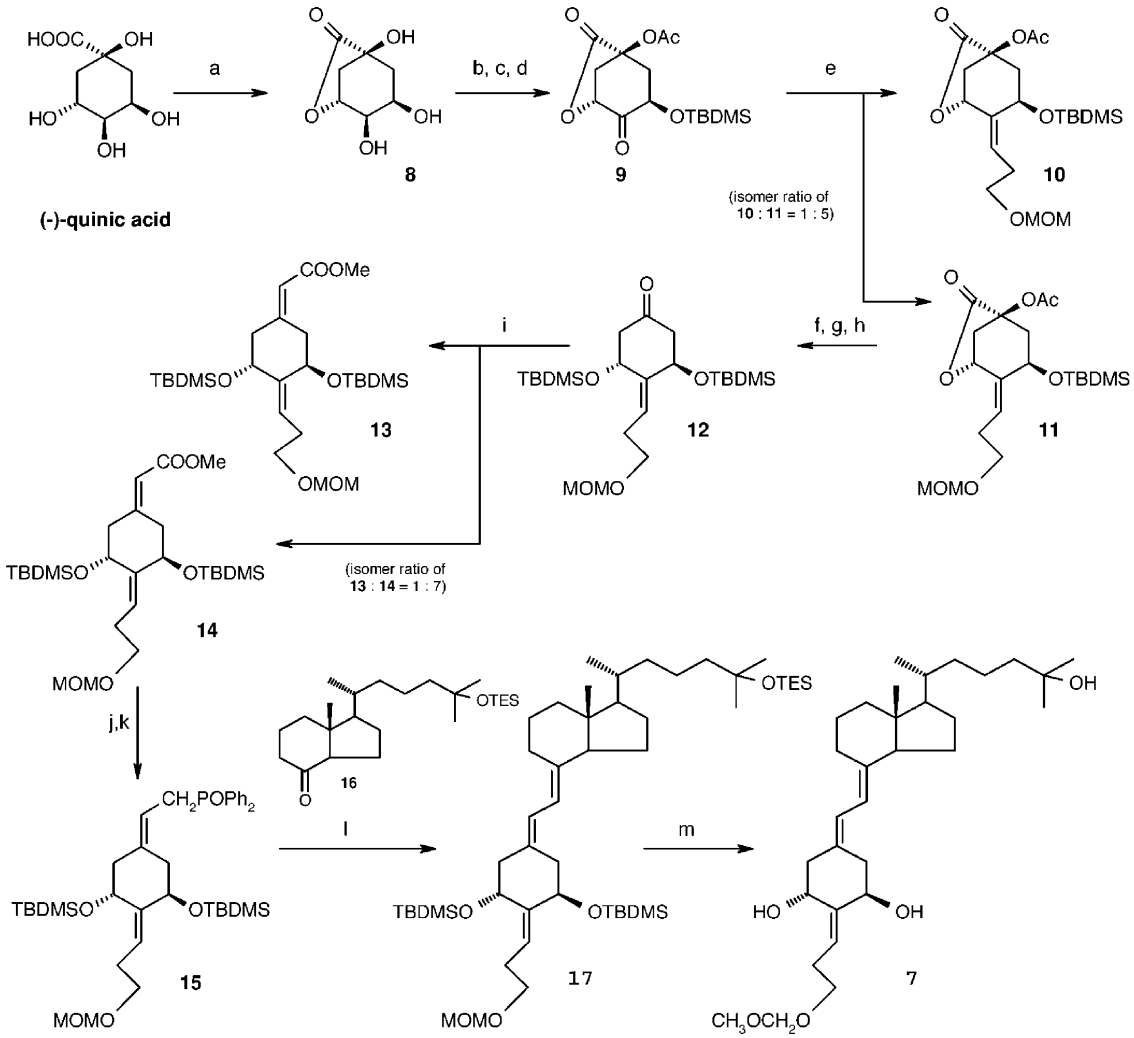


Fig. 1. Chemical structure of 1α,25-dihydroxyvitamin D_3 (calcitriol, **1**) and its analogs.



Scheme 1. (a) *p*-TsOH, DMF, benzene, 90%; (b) TBDMSCl, DMF, 76%; (c) Dess–Martin periodinane, CH_2Cl_2, 95%; (d) Ac_2O, pyr, 81%; (e) Ph_3P^+ $(CH_2)_3OMOM$ Br^-, *n*-BuLi, THF, 77%; (f) $LiAlH_4$, THF, 59%; (g) $NaIO_4$, MeOH, 88%; (h) *t*-$BuMe_2SiOTf$, 2,6-lutidine, CH_2Cl_2, 66%; (i) Me_3SiCH_2COOMe, LDA, THF, 88%; (j) DIBALH, THF, 97%; (k) *n*-BuLi, TsCl, THF; *n*-BuLi, Ph_2PH, THF; H_2O_2, CH_2Cl_2, 68% (three steps); (l) **16**, PhLi, THF, 48%; (m) *n*-Bu_4NF, Et_3N, THF, 71%.

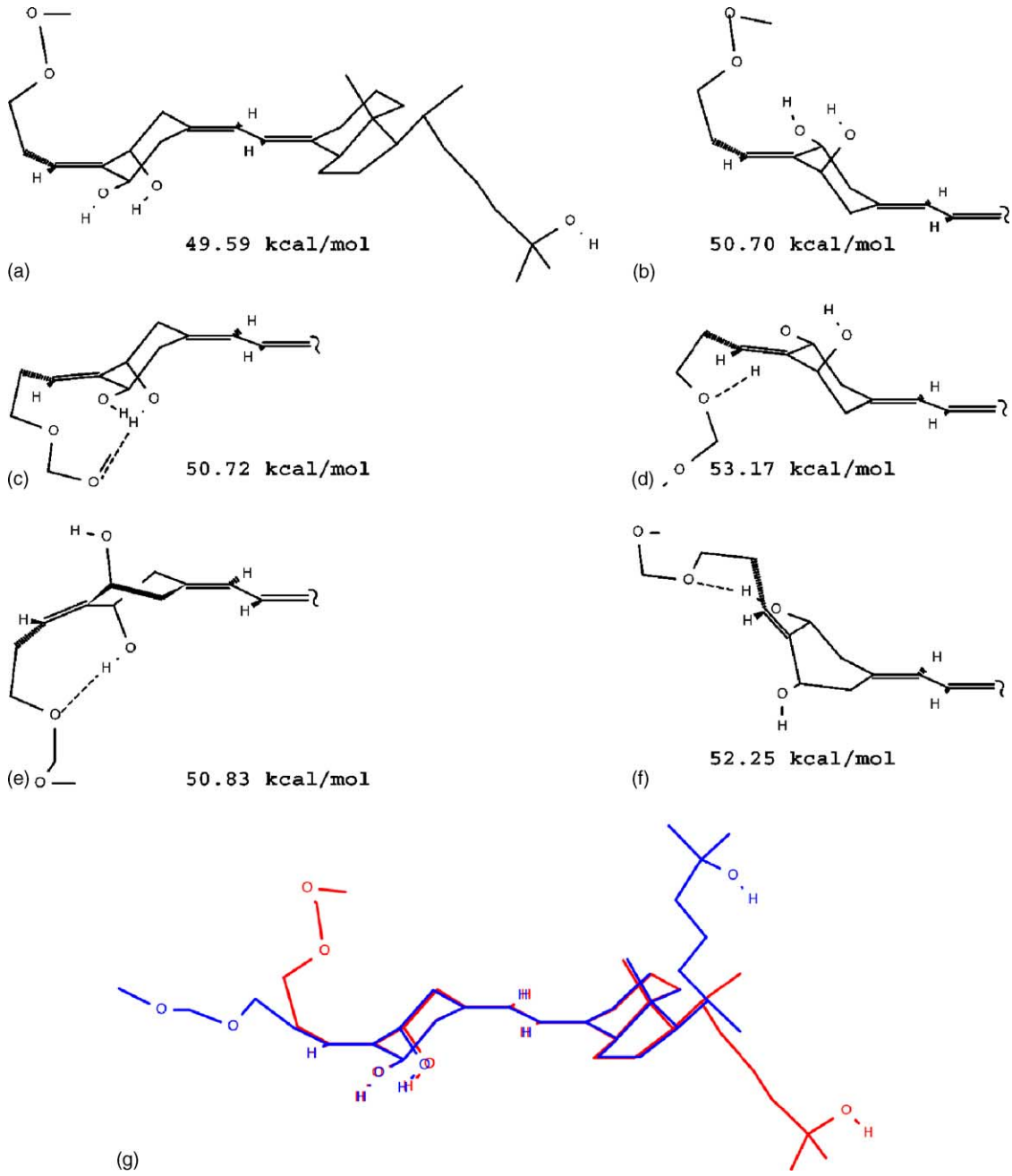


Fig. 2. Preferred, energy minimized conformations (and their steric energies) of the synthesized analog **7** with the ring A in the β-chair (a) and α-chair form (b). The lowest energy conformers of **7**, possessing intramolecular hydrogen bonds, and the ring A in the: β-chair (c), α-chair (d), and flexible forms (e, f). Overlaid structures (g) of the ligand **7** docked to LBD (blue) and its global minimum conformer (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

with their structure. Full details of their synthesis will be reported elsewhere.

2.2. Molecular modeling

2.2.1. Conformational search

The calculation of optimized geometries and steric energies were carried out using the algorithm from the MM^{+} HyperChem (release 5.0) software package (Autodesk Inc.). The energy-minimized structure of the hormone **1** was used for modeling of **7** [7]. The procedure used for generation of the respective conformers of alkoxypropylidene substituent was analogous to that described previously by us for the Vitamin D side chain conformers and involved Conformational Search module of ChemPlus (release 1.5) program (Hypercube Inc.) [7].

2.2.2. Docking procedure

Docking simulations (100,000 iterations each) of Vitamin **7** to the full-length LBD of the rVDR were performed

using FlexiDock software from TRIPOS. The global minimum conformer of **7** (Fig. 2, $E_s = 49.59$ kcal/mol) was chosen and several docking experiments were done, starting with different pre-positioning of the ligand [8]. For final consideration, the lowest energy complex, possessing a parallel orientation of tryptophan rings to the steroid 5,7-diene moiety, was selected. The 3.5 Å contacts between **7** and the 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 [9]. The experiments were carried out in triplicate on two different occasions.

2.3.2. Measurement of cellular differentiation

Human leukemia HL-60 cells (obtained from ATTC) were plated at 2×10^5 cells per plate and incubated. Then the compounds tested were added, and after 4 days superoxide production was measured by nitro blue tetrazolium (NBT) reduction. This method is described in detail elsewhere [10].

3. Results and discussion

3.1. Chemical synthesis of 7

The synthesis of 19-norvitamin **7**, based on the Wittig–Horner coupling approach, involved first the preparation of the corresponding A-ring fragments with the desired orientation of alkoxypropylidene substituent. A new synthetic route has been developed (Scheme 1) starting from bicyclic lactone **8** that was obtained from commercial (1*R*,3*R*,4*S*,5*R*)-(−)-quinic acid. The prepared allylic ester **14** was transformed to the A-ring phosphine oxide **15** and the latter subjected to the Wittig–Horner coupling with the protected 25-hydroxy Grundmann's ketone **16** [11]. The obtained 19-norvitamin D compound **17**, after hydroxy group deprotection, afforded 1α,25-dihydroxy-2-[3′-(methoxymethoxy)propylidene]-19-norvitamin D_3 (**7**). Configurations of the alkoxypropylidene unit at C′-4 in the isomeric compounds **10**, **11** and **13**, **14**, were determined by analysis of their ^{1}H NMR spectra and NOE measurements.

3.2. Conformational analysis of free ligand 7

Our previous studies on Vitamin D analogs possessing hydroxymethyl substituents at C-2 or C-10 showed significantly diminished biological potency of compounds in which the A-ring hydroxyls (especially 1α-OH) were involved in hydrogen bond formation with the neighboring hydroxy groups [3]. Therefore, molecular modeling was employed to establish the preferred A-ring conformation of the analog **7**. According to our expectations, the global minimum conformer of **7** ($E_s = 49.59$ kcal/mol; Fig. 2a) possesses ring A in the β-chair conformation [4]; its counterpart with α-chair form (Fig. 2b) has steric energy higher by 1.11 kcal/mol. The lowest energy conformers of **7**, having intramolecular hydrogen bonds, are all characterized by higher steric energies, independently of their A-ring rigid (Fig. 2c and d) or flexible (Fig. 2e and f) conformations.

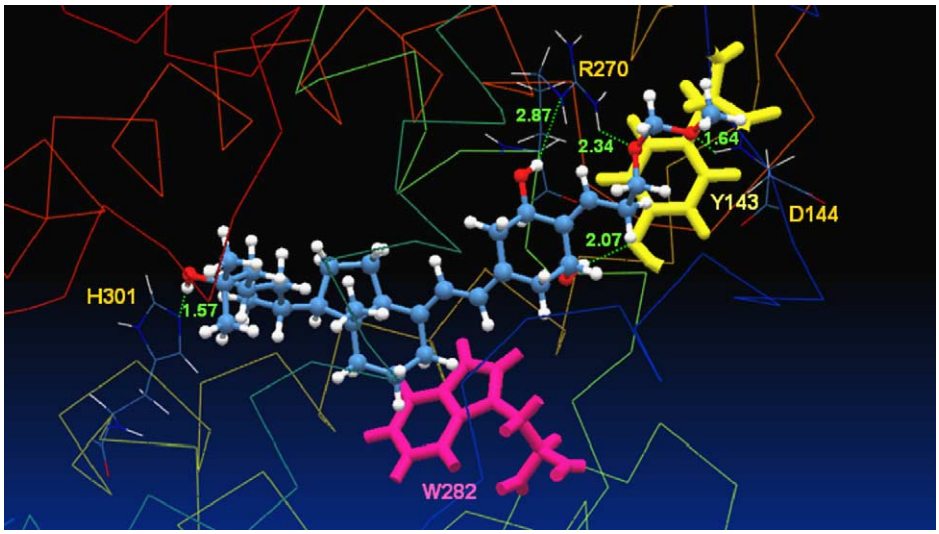


Fig. 3. View of the three-dimensional structure of ligand binding cavity of the rat VDR with the docked Vitamin D analog **7**. They are indicated four amino acids (Tyr 143, Asp 144, Arg 270, and His 301) forming the shortest hydrogen bonds (the Angstrom distances are marked in green) with the ligand. Also the Trp 282 residue is shown (pink). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

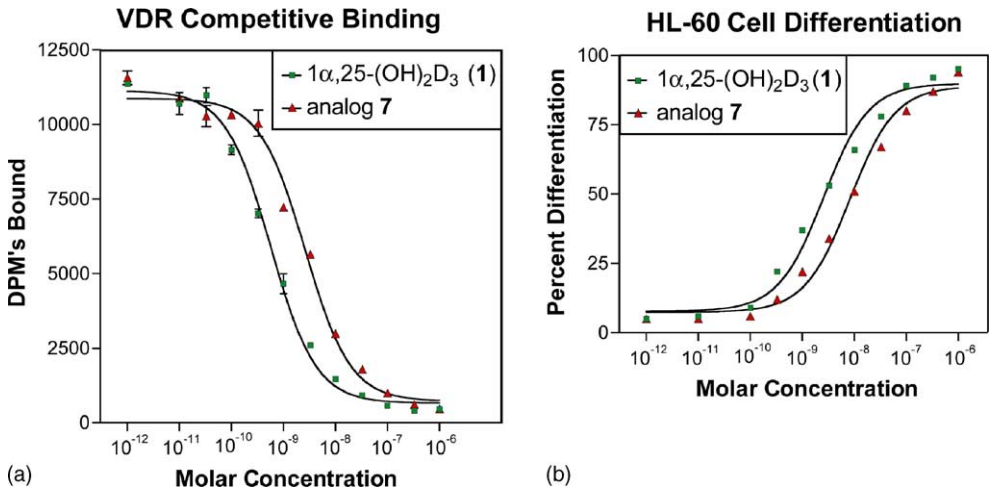


Fig. 4. (a) Competitive binding of 1α,25-$(OH)_2D_3$ (**1**) and the synthesized analog **7** to the rat recombinant Vitamin D receptor. This experiment was carried out in triplicate on two different occasions. (b) Differentiation activity of 1α,25-$(OH)_2D_3$ (**1**) and the analog **7**. Differentiation state was determined by measuring the percentage of cells reducing nitro blue tetrazolium (NBT).

3.3. Docking of analog 7 to the ligand binding pocket of the rVDR

We confirmed that, irrespectively to different arbitrarily chosen pre-positions of the ligand in the LBD of the rVDR, this compound docked in the binding pocket similar to the hormone **1** in its crystalline complex with the hVDR [12]. The analog is anchored in the LBD (Fig. 3) with the A ring and side chain directed towards Y143 and H301, respectively. Tryptophan 282 is positioned under the B-seco ring of **7** and parallel to the plane of the intercyclic 5,7-diene; a distance (5.2 Å) allows π–π interaction. A similar set of amino acids (as in the case of the hormone–hVDR complex) create hydrogen bonds with the A-ring hydroxyls of **7** (Fig. 3): hydrophilic amino acid R270 contacts 1α-OH, while S274 and Y143 contact 3β-OH group. The side chain 25-hydroxy group is contacted only by H301. Interestingly, both oxygens from 2′-alkoxypropylidene moiety create three strong hydrogen bonds: the "terminal" oxygen (from methoxy group) with the backbone NH of D144 (l.64 Å) and the "central" oxygen with R270 (2.34 and 2.45 Å). Superimposition of the ligand, found in the most stable complex of **7** and rVDR, and the global minimum conformer shows their significant differences (Fig. 2g). In the former, the conformation of the 2-alkoxypropylidene group is more extended (directed outwards the ring A). Moreover, its 25-hydroxyl occupies now the spatial region EA (not G as in the case of energetically preferred side chain conformation), as defined by Yamada et al. [13].

3.4. Biological evaluation of the analog 7

The synthesized 19-norvitamin D analog **7** was tested for its ability to bind the rat recombinant Vitamin D receptor. The competitive binding analysis showed (Fig. 4a) that **7** exhibits six times lower affinity for the receptor than 1α,25-$(OH)_2D_3$ (**1**). Studies on the ability of the tested compound to induce HL-60 cell differentiation indicated a slightly decreased (five times) potency in comparison with the hormone **1** (Fig. 4b). Preliminary in vivo studies indicate an unexpectedly high calcemic activity of the Vitamin **7**. Results of these tests will be published elsewhere.

Acknowledgements

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