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New 1α,25-dihydroxy-19-norvitamin D_3 analogs with a frozen A-ring conformation☆

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ABSTRACT

We have recently described the synthesis of 1α,25-dihydroxy-19-norvitamin D_3 analogs **2** and **3**, possessing an additional ring connecting their 3β-oxygen and C-2. Such structural constrains prevent the A-ring conformational flexibility and the analogs exist exclusively in the α-chair form with their 1α-hydroxy groups fixed in the axial position. The analogs bind very poorly to vitamin D receptor and are devoid of transcriptional activity. Rather unexpectedly, when tested *in vivo* in rats, they exhibited calcemic response significantly delayed compared to 1α,25-dihydroxyvitamin D_3 (**1**). Such a response might be due to the metabolic conversion (ether cleavage?) of these compounds in the living organisms. It was therefore of interest to obtain and evaluate biologically the analogous compounds having an additional ring of purely hydrocarbon nature. Such analog **4** of 1α,25-dihydroxy-19-norvitamin D_3, characterized by the presence of an equatorial 1α-hydroxy group (β-chair form), has been synthesized by us and tested biologically. The geometrical isomer **5** having a fixed 3β-hydroxy group was also obtained. These compounds were formed in the Julia coupling of the sulfone derived from the Grundmann ketone, and the A-ring fragment prepared in the multi-step synthesis from the (−)-quinic acid. Contrary to its counterpart **5**, the analog **4** retained some affinity to vitamin D receptor.

1. Introduction

The importance of multiple physiological processes controlled by 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$, **1**; Fig. 1), the hormonally active metabolite of vitamin D_3, has stimulated a very intensive research directed towards its biology [1,2] and chemistry [3]. Since its discovery, an intriguing problem has attracted the attention of researchers [4–6]. Which A-ring conformer of the vitamin D compound is required for binding to the vitamin D receptor (VDR)? In 1974, Norman hypothesized that the β-chair form (Fig. 2a) and, consequently, an equatorial orientation of the 1α-hydroxy group is needed for the formation of a complex between 1α,25-$(OH)_2D_3$ and VDR [7]. Almost four decades later, the X-ray crystallographic studies of a complex of the natural hormone **1** and hVDR mutant supported this assumption [8]. To exclude the possibility of chair inversion occurring in the physiological milieu, we have synthesized the 19-norvitamin D analogs **2** and **3** in which the ring A could exist only in the α-chair form [9,10]. Although these compounds had very poor binding affinities to VDR, they both showed surprisingly high calcemic activity *in vivo*, suggesting a possible cleavage of their dihydropyran (dihydrofuran) ring. Therefore, it seemed to be of interest to synthesize the related compound **4** possessing its ring A in the β-chair conformation, prevented from an interconversion to the alternative form. It could be expected that the hydrocarbon nature of the attached cyclopentene ring will considerably increase the stability of such a vitamin D analog in the living organisms.

2. Materials and methods

2.1. Preparation of the 1α,25-dihydroxy- and 3β,25-dihydroxy-19-norvitamin D_3 analogs **4** and **5**

The vitamin D analogs **4** and **5** were synthesized at the Department of Biochemistry, University of Wisconsin-Madison and at the Department of Chemistry, University of Warsaw, according to the synthetic route presented in Scheme 1. The spectroscopic and analytical data of all obtained compounds confirmed the

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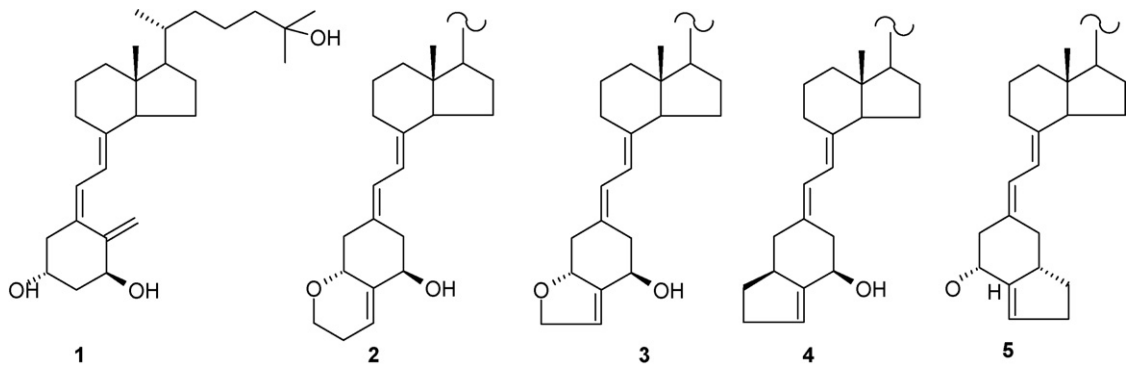


Fig. 1. Chemical structure of 1α,25-dihydroxyvitamin D_3 (calcitriol, **1**) and its analogs.

assigned structures. The details of synthesis will be reported elsewhere.

2.2. In vitro studies

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

The procedure for obtaining the purified full-length recombinant rat receptor used in the binding studies will be reported in detail elsewhere. The competition binding assays were performed using 1α,25-$(OH)_2$[26,27-^{3}H]D_3 as described previously [11]. The experiment was in duplicate.

2.2.2. Measurement of cellular differentiation

Human promyelocytic leukemia HL-60 cells (obtained from ATTC) were plated at 1.2×10^5 cells/mL and incubated. Eighteen hours after plating the compounds tested were added, and after four days the cells were harvested and the nitro blue tetrazolium (NBT) reduction assay was performed. This method is described in detail elsewhere [12].

2.2.3. Transcriptional assay

The transcriptional activity was measured in ROS 17/2.8 (bone) cells that were stably transfected with the 24-hydroxylase (24OHase) gene promoter upstream of the luciferase reporter gene [13]. Cells were given a range of doses. Sixteen hours after dosing, the cells were harvested and luciferase activities were measured using a luminometer. Each experiment was performed twice, each time in duplicate.

3. Results and discussion

3.1. Chemical synthesis of *4* and *5*

The known cyclic diacetal **6** (Scheme 1), derived from the commercially available (−)-quinic acid [14], was subjected to reaction with iodine and triphenylphosphine, resulting in the formation of a single product **7** with an inverted configuration at the stereogenic center. The addition of the radical, generated from the iodide **7** and tributylstannane, to acrylonitrile provided compound **8** with an equatorial 2′-cyano-ethyl substituent. The nitrile **8** was further

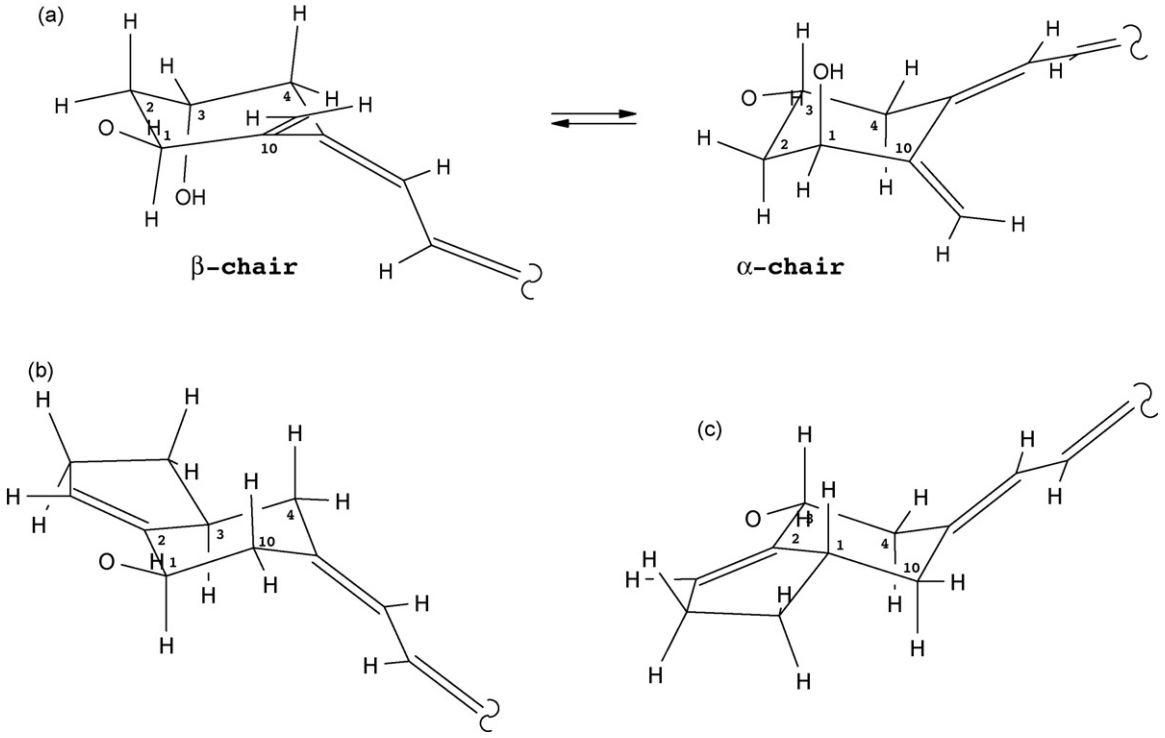
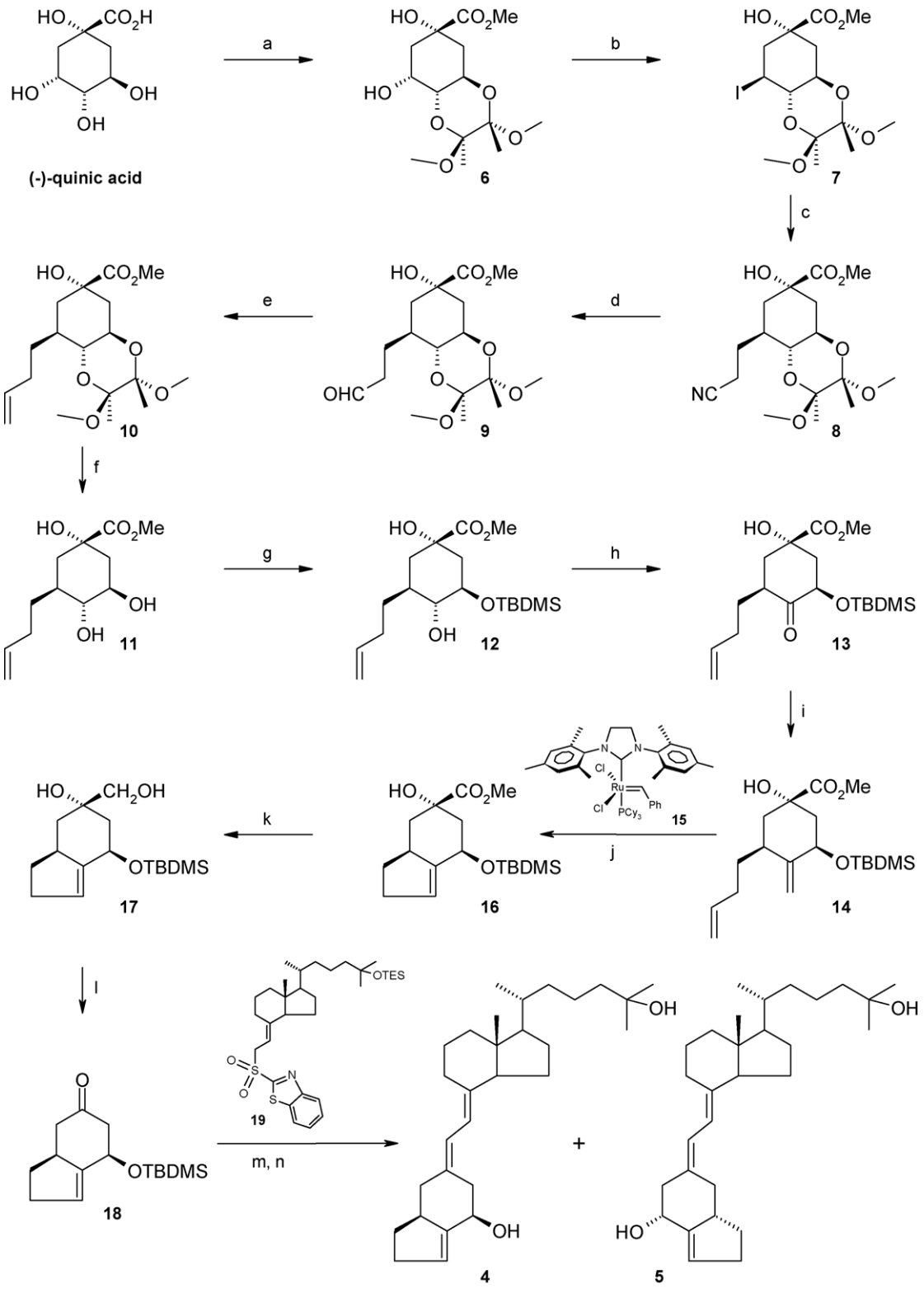


Fig. 2. Conformational equilibrium in ring A in 1α-hydroxyvitamin D analogs (a) and the A-ring conformation of the synthesized analogs **4** (b) and **5** (c).



Scheme 1. (a) $(MeCO)_2$, $CH(OMe)_3$, CSA, MeOH, 85%; (b) I_2, Ph_3P, Im, PhMe, 54%; (c) CH_2=CHCN, AIBN, PhMe, Bu_3SnH, 67%; (d) DIBALH, PhMe, 33%; (e) $(PPh_3)_3RhCl$, Ph_3P, Me_3SiCHN_2, *i*-PrOH, THF, 21%; (f) TFA, H_2O, 58%; (g) *t*-$BuMe_2SiCl$, Im, DMF, 50%; (h) Dess-Martin periodinane, CH_2Cl_2, 88%; (i) $Ph_3P^+CH_3$ Br^-, *n*-BuLi, THF, 60%; (j) **15**, PhMe, 82%; (k) $LiAlH_4$, THF, 81%; (l) $NaIO_4$, MeOH, 95%; (m) **19**, LiHMDS, THF; (n) *n*-Bu_4NF, Et_3N, THF, 26% (over two steps).

reduced to the aldehyde **9** which was subsequently methylenated using the rhodium catalyzed process developed by Lebel and Paquet [15]. The formed terminal alkene **10** was then converted in several steps into the diolefin **14** which underwent smoothly the ring-closing metathesis carried out in the presence of the 2nd generation Grubbs catalyst **15** [16]. The obtained bicyclic hydroxy ester **16** was transformed into the desired A-ring fragment, protected hydroxycyclohexanone **18**. This ketone was then subjected to the modified Julia olefination with the known thiazoline sulfone **19** [17] and lithium bis(trimethylsilyl)amide. Removal of the silyl protect-

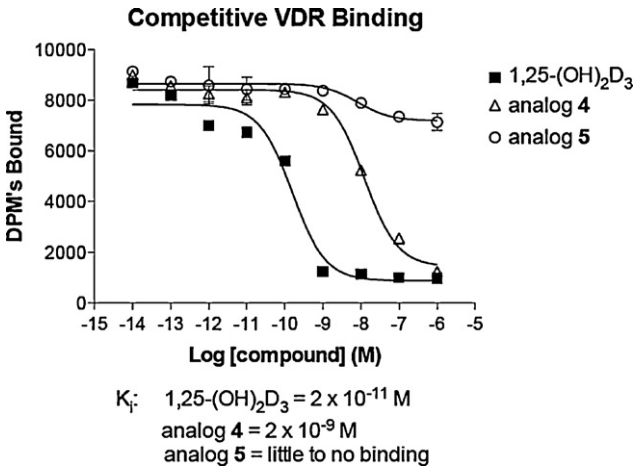


Fig. 3. Competitive binding of 1α,25-$(OH)_2D_3$ (**1**) and the synthesized analogs **4** and **5** to the rat recombinant vitamin D receptor. This experiment was carried out twice, each time in duplicate.

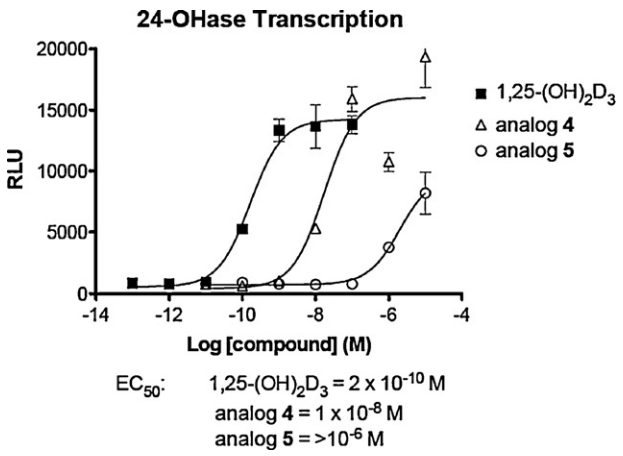


Fig. 5. Transcriptional activity of 1α,25-$(OH)_2D_3$ (**1**) and the synthesized analogs **4** and **5**. Transcriptional assay was carried out in rat osteosarcoma cells stably transfected with a 24-hydroxylase gene reporter plasmid. Each experiment was performed twice, each time in duplicate.

ing groups in the obtained products gave the expected mixture of two 19-norvitamin D analogs **4** and **5** which were purified and separated by reversed-phase HPLC. ^{1}H NMR spectra of both vitamins confirmed that their ring A is fixed in the single chair conformation (Fig. 2b and c).

3.2. Biological evaluation of the synthesized analogs *4* and *5*

Due to the presence of an exocyclic double bond being a part of an additional five-membered ring [18], the cyclohexane ring A in the synthesized analogs **4** and **5** exists as a "frozen" chair conformer with the secondary hydroxy group (1α or 3β, respectively) assuming only an equatorial orientation. First, an ability of both vitamins to bind the rat recombinant VDR has been assessed. Analog **4**, characterized by a bridge between C-2 and C-3, is less able to bind to VDR by two orders of magnitude compared to the native hormone (Fig. 3). Also, it has been found that the vitamin **4** is able to stimulate differentiation of promyleocytic leukemia cells into monocytes, being 15 times less potent than 1α,25-$(OH)_2D_3$ in the HL-60 assay (Fig. 4). The isomeric compound **5**, with a hydrocarbon bridge connecting C-1 and C-2, is practically devoid of any binding affinity to the receptor and it also lacks activity in promoting cancer cell differentiation. These data are consistent with the transcription results. In the reporter cell assay, the activity of the vitamin **4** is reduced 50 times compared to the native hormone, whereas its counterpart **5** is less active by four orders of magnitude (Fig. 5).

The results of binding studies show that the vitamin **4** with an equatorial 1α-hydroxyl has an affinity for VDR of one order of magnitude higher than the previously synthesized analog **3** despite the fact that the latter one can form additional hydrogen bonds involving the oxygen atom from its furan ring as an acceptor. This confirms that the ligand-binding pocket of the VDR easily accommodates vitamin D analogs possessing their A ring in the β-chair form. Because vitamin **4** competes for the VDR binding at 10 nM, *in vivo* activity is expected and these studies are in progress.

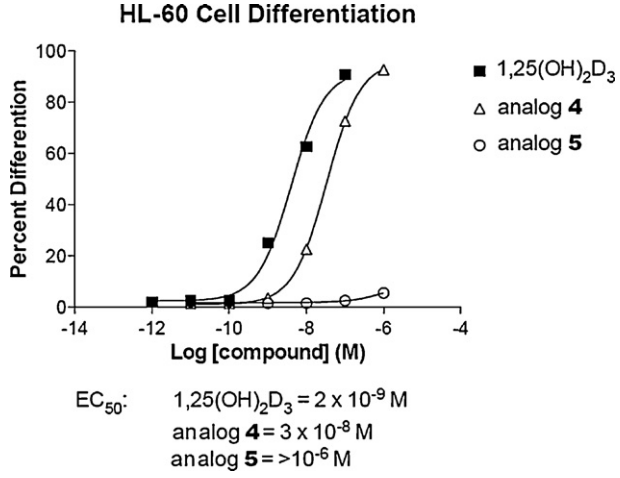


Fig. 4. Differentiation activity of 1α,25-$(OH)_2D_3$ (**1**) and the synthesized analogs **4** and **5**. Differentiation state was determined by measuring the percentage of cells reducing nitro blue tetrazolium (NBT).

Acknowledgements

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