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An analog of 1α,25-dihydroxy-19-norvitamin D_3 with the 1α-hydroxy group fixed in the axial position lacks biological activity *in vitro*

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Abstract

The relationship between the A-ring chair conformation of vitamin D compounds and their ability to bind the vitamin D receptor (VDR) has long attracted the attention of many researchers. It was established that in the crystalline complexes of hVDRmt with the natural hormone, 1α,25-dihydroxyvitamin D_3 (**1**), and its side-chain analogs the vitamins exist in β-chair form with an equatorial orientation of 1α-OH. However, with all these ligands the interconversion between both A-ring forms would be possible in solution. In an attempt to verify the conformation of vitamin D compounds required for binding the VDR we prepared analog **4**, characterized by the presence of an axial 1α-hydroxy group. Since the additional ring connecting 3β-oxygen and C-2 prevents A-ring conformational flexibility, the synthesized vitamin **4** can exist exclusively in the α-chair form. The geometrical isomer **5** with a free 3β-OH group was also obtained. The analog **5** binds very poorly to VDR, whereas the vitamin **4** is practically devoid of binding ability. Both compounds also show very low HL-60-differentiating activity. When tested *in vivo* in mice the analogs **4** and **5** exhibit significant calcemic responses with analog **4** showing more activity than analog **5**.

Keywords: Vitamin D analogs; 19-Norvitamin D_3; Vitamin D receptor; VDR binding; Cellular HL-60 differentiation

1. Introduction

The discovery that 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$, **1**; Fig. 1) is the hormonal form of vitamin D marked the beginning of intense structure–activity studies aimed at discovering new analogs characterized by modified biological properties [1–3]. Parallel to these studies the conformation of the vitamin D molecule has also undergone close scrutiny. A series of interesting studies directed to the side chain conformation of different vitamin D analogs examined which spatial regions have the highest possibility of being occupied by the 25-hydroxy group [4,5]. Besides the side chain conformation, the A-ring conformational equilibrium of vitamin D compounds has attracted considerable research interest for more than 30 years [4]. Advances in NMR spectroscopy and development of force field calculation methods made it possible to establish, or even predict, the proportion of equilibrating α- and β-chair A-ring forms (Fig. 2a). Particularly interesting was another closely related problem discussed in the literature, namely the correlation of A-ring conformation with biological activities of vitamin D compounds. As early as in 1974 it was proposed [6] that equatorial orientation of the 1α-hydroxy group (the β-chair form shown in Fig. 2a) is necessary for calcium regulation ability. Six years ago Moras reported the crystal structures of hVDR ligand binding domain (LBD) bound to the hormone **1** [7] as well as to the ligands with an unnatural configuration at C-20 [8], and it became clear that the vitamin D receptor binds (at least in the crystalline state) vitamin D analogs with their A-rings in the β-chair conformation. It seemed, therefore, interesting to synthesize a vitamin D analog that could only

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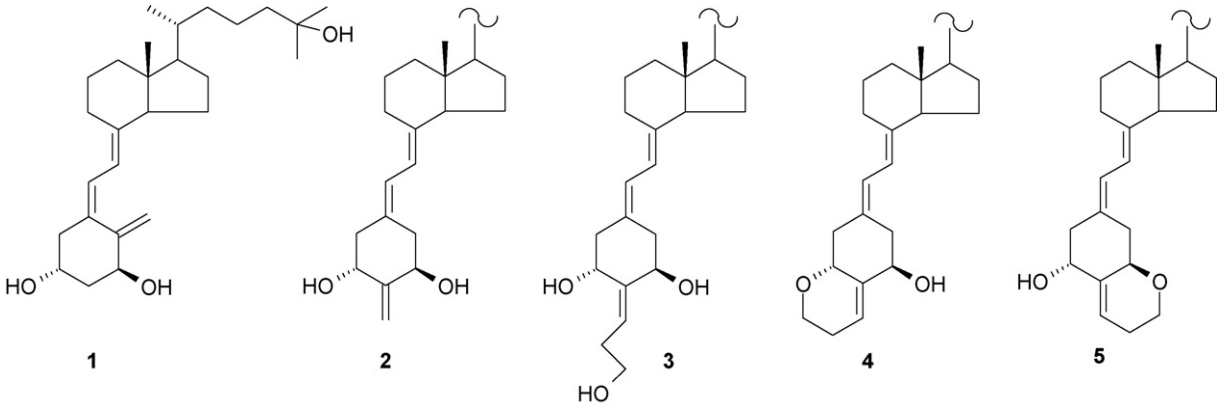

Fig. 1. Chemical structure of 1α,25-dihydroxyvitamin D_3 (calcitriol, **1**) and its analogs.

assume the opposite α-chair conformation of its ring-A, and as a consequence, possess the 1α-hydroxy group in the axial orientation.

In 1998 we synthesized an analog of **1** possessing the A-ring exocyclic methylene group transposed from C-10 to C-2 [9] and we established that such modification of the structure (compound **2**) practically did not change the affinity for the VDR. Very recently, we synthesized a 3′-hydroxypropylidene derivative of 1α,25-dihydroxy-19-norvitamin D_3 (**3**) that also bound to VDR almost as well as **1** [10]. These results prompted us to attempt a synthesis of a tetracyclic vitamin D analog of a structure **4**; it could be expected that a three-carbon bridge between C-2 and 3β-oxygen should not interfere with the LBD. On the other hand, structural constrains of this molecule should prevent the ring-A from flipping over to the alternative form, effectively "freezing" its α-chair conformation (Fig. 2b).

2. Materials and methods

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

Vitamin D analogs **4** and **5** were synthesized at the Department of Biochemistry, University of Wisconsin-Madison and at the Department of Chemistry, Warsaw University according to the synthetic route presented in Schemes 1 and 2.

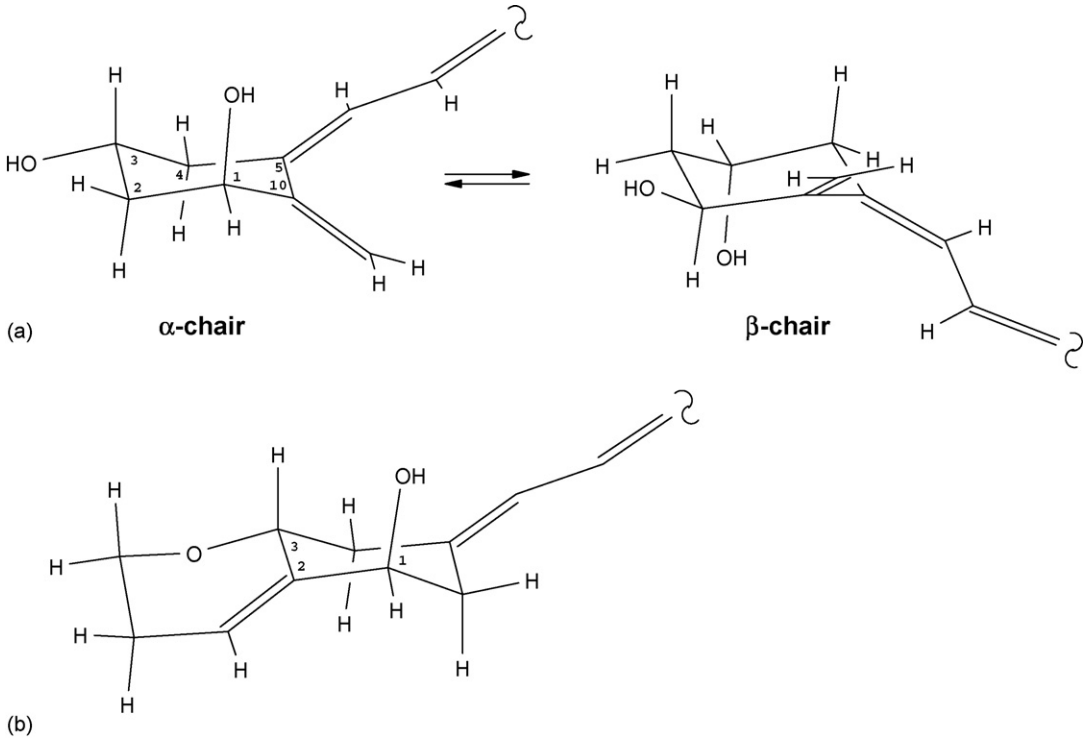

Fig. 2. Conformational equilibrium in ring-A in 1α-hydroxyvitamin D analogs (a) and the A-ring conformation of the synthesized analog **4** (b).