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2-(3′-Hydroxypropylidene)-1α-hydroxy-19-norvitamin D compounds with truncated side chains

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Abstract

Our recent studies with 2-(3′-hydroxypropylidene) analogs of 1α,25-dihydroxy-19-norvitamin D_3 showed that this 2-substituent creates compounds with very potent biological activity. In the continuing search for vitamin D compounds with selective activity profiles, we prepared a series of 1α-hydroxy-19-norvitamin D analogs characterized by the presence of a 3′-hydroxypropylidene substituent at C-2 and a truncated side chain. These vitamin D compounds were efficiently prepared using convergent syntheses. The C,D-fragments, namely the Grundmann ketones **19**, **20**, **27**, **36** and **37** were synthesized from the known 8β-benzoyloxy-22-aldehydes **12** and **29**. These hydrindanones were subjected to Lythgoe type Wittig–Horner coupling with phosphine oxide **21**, prepared by us previously, and after hydroxyl deprotection the set of 19-norvitamins **7**–**11** was successfully obtained. According to our expectations, all analogs (with an exception of the 20*R*-compound **7**) have pronounced *in vitro* activity. When compared to the natural hormone 1α,25-$(OH)_2D_3$ (**1**), they show the same or only slightly reduced affinity for the vitamin D receptor while being similarly effective as **1** in differentiation of HL-60 cells into monocytes.

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

1. Introduction

The discovery of the active, hormonal form of vitamin D, 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$, calcitriol, **1**; Fig. 1) and realization of its broad range of biological functions [1,2] stimulated synthetic efforts directed at the generation of analogs with selective activity profiles [3]. Vitamin D compounds with suppressed calcemic potency which retain some other important functions of the natural hormone **1** are of particular interest [4]. During our structure–activity studies we synthesized analogs of **1** in which the A-ring exocyclic methylene group was transposed from C-10 to C-2 [5]. The analog **2** (2MD), also possessing an "unnatural" configuration at C-20, showed significantly enhanced calcemic activity [6]. We continued the studies on 2-alkylidene-19-norvitamins [7] and we recently established that some 2-methylene-1α-hydroxy-19-norvitamin D compounds, possessing an abbreviated side chain (**3** and **4**), are practically devoid of calcemic activity while still retaining other genomic functions, e.g. effectively suppressing parathyroid hormone levels [8]. Very recently, we synthesized [9] derivatives of 1α,25-dihydroxy-19-norvitamin D_3 with the 3′-hydroxypropylidene moiety at C-2 (**5** and **6**). Interestingly, their *in vivo* calcemic activity significantly exceeded that of 2MD (**2**), especially in stimulating intestinal calcium transport. Molecular modeling studies of these analogs indicated that the presence of an oxygen function, located at the terminus of the propylidene fragment, could introduce additional interactions with the vitamin D receptor. As a continuation of our search for biologically active 2-alkylidene-19-norvitamin D compounds we

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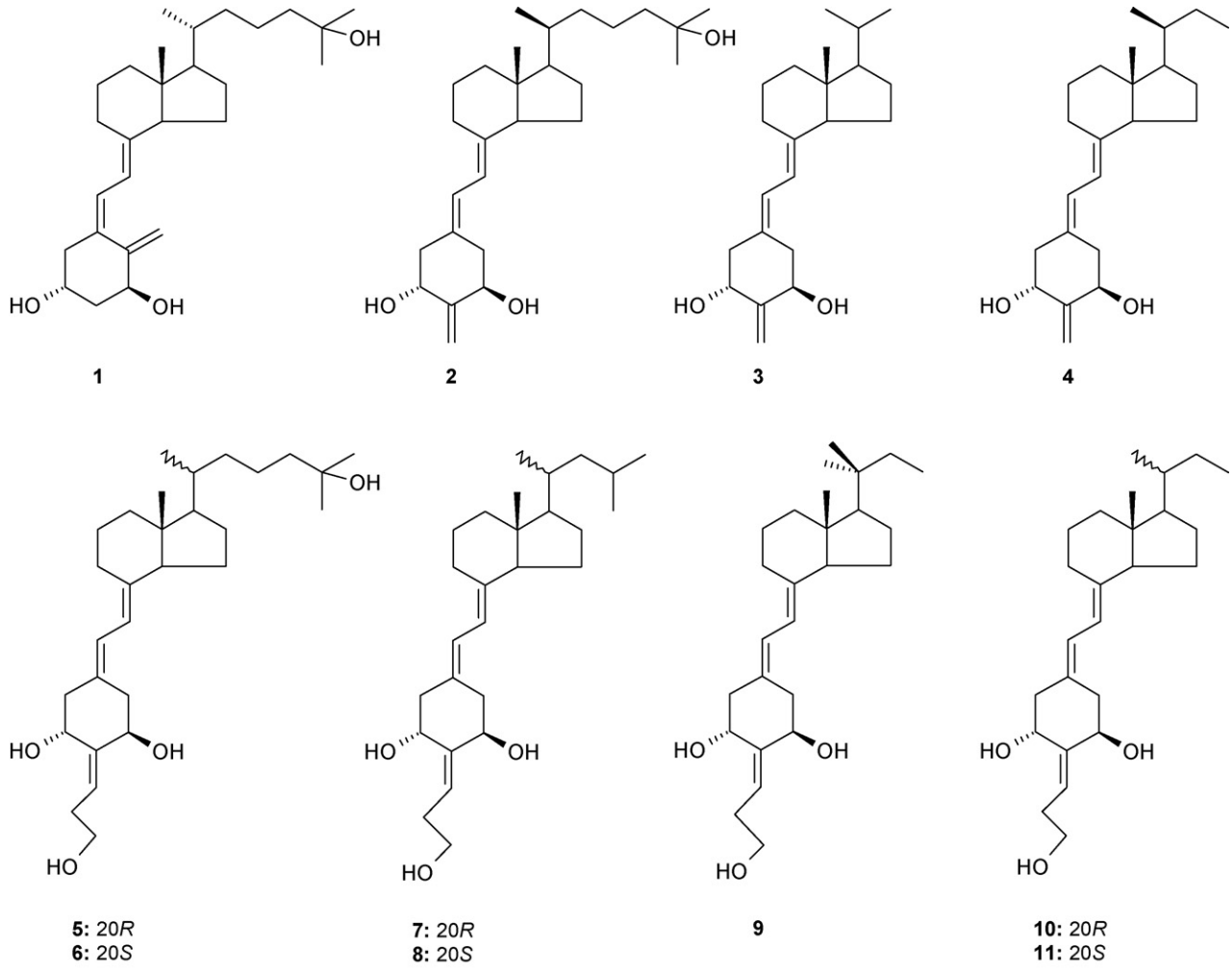

Fig. 1. Chemical structure of 1α,25-dihydroxyvitamin D_3 (calcitriol, **1**) and its analogs.

have prepared and biologically tested "hybrid analogs" **7**–**11** characterized by the presence of a 3′-hydroxypropylidene moiety at C-2 and a truncated alkyl side chain containing no hydroxyl.

2. Materials and methods

*2.1. Preparation of (20R)- and (20S)-1α-hydroxy-2-[3′-hydroxypropylidene]-19,23,24-trinorvitamin D_3 (**7** and **8**), 1α-hydroxy-2-[3′-hydroxypropylidene]-20-methyl-19,24,25,26,27-pentanorvitamin D_3 (**9**), (20R)- and (20S)-1α-hydroxy-2-[3′-hydroxypropylidene]-19,24,25,26,27-pentanorvitamin D_3 (**10** and **11**)*

2-(3′-Hydroxypropylidene)-1α-hydroxy-19-norvitamin D compounds **7**–**11** 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 1–3. All prepared compounds exhibited spectroscopic and analytical data consistent with their structure. Full details of their 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 rat recombinant vitamin D receptor used in the binding studies 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 duplicate on two-three different occasions.

2.2.2. 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 four days superoxide production was measured by nitro blue tetrazolium (NBT) reduction. This method is described in detail elsewhere [11].