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Review

A-ring analogs of 1,25-dihydroxyvitamin D_3

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ABSTRACT

The growing interest in1α,25$(OH)_2D_3$, the hormonally active form of vitamin D_3, has prompted numerous efforts to synthesize vitamin D analogs as potential therapeutic agents, and some of these are already on the market and in clinical development. Although most vitamin D preparations developed thus far have focused on side-chain modifications, providing many useful analogues with high potency and selectivity, in recent years, modifications of the A-ring has attracted much attention because it can afford useful analogues exhibiting unique activity profiles as well. In this review we will focus on the current understanding of the relationship between selected modifications in the A-ring of the 1α,25$(OH)_2D_3$ molecule, such as epimerization and/or substitution at C-1 and C-3, substitution at C-2, and removal of the 10,19-exocyclic methylene group, and their effect on biological potency and selectivity. Finally, suggestions for the structure-based design of therapeutically valuable A-ring vitamin D analogs will conclude the review.

Introduction

Following the discovery of the vitamin D_3 endocrine system delineated between 1970 and 1972, it became clear that the functional form of vitamin D is 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$, calcitriol, **1**, Scheme 1), which exerts its control over a multitude of biological processes related to calcium and phosphate homeostasis, cell proliferation and differentiation, and immune regulation [1–3]. Numerous studies have indicated that these biological functions of the natural hormone 1α,25-$(OH)_2D_3$ are mediated through the vitamin D receptor (VDR) [4,5], a nuclear protein which belongs to the nuclear receptor superfamily [6]. Ligand-bound VDR requires the retinoid-X receptor as a partner for binding to vitamin D-responsive elements found predominantly, although not exclusively, in the promoter region of target genes. Through a complex set of transcription factors, co-activators and co-repressors, transacetylase systems are activated to loosen chromatin, followed by the binding of transcription factors that can induce or suppress transcription of target genes [7]. As expected, the nuclear VDR is very abundant in organs that participate in calcium metabolism and bone remodeling such as the intestine, which regulates calcium absorption [8–12]; the kidney, which regulates calcium reabsorption, calcium excretion and the synthesis of 1α,25-$(OH)_2D_3$ [13]; bone, which responds to systemic calcium and phosphorous deficiency by resorption [1]; and the parathyroid gland which acts in concert with vitamin D to maintain calcium and phosphorus homeostasis [14–16]. Completely unexpected was the finding of VDR in a variety of tissues not previously considered to be targets of vitamin D action, including pancreatic islet cells, macrophages, promyelocytes, skin keratinocytes, certain specific neural cells, mammary gland, and specific cells in the reproductive organs [1,17–19]. The presence of VDR in these tissues was determined with specific monoclonal antibodies directed to VDR [20], and the presence of nuclear localization of highly radioactive 1α,25-$(OH)_2D_3$.

This discovery triggered an explosion of research directed towards finding functions of vitamin D outside calcium and phosphate homeostasis. The natural VDR ligand, 1α,25-$(OH)_2D_3$ (or its prodrug 1α-$(OH)D_3$), is an established clinical treatment for renal osteodystrophy and various types of rickets [21–23] but the investigation of pharmacological doses for treatment of a wide variety of other diseases, including breast and prostate cancers, autoimmune diseases, psoriasis, and osteoporosis [24–28] has been limited by the parallel induction of hypercalcemic effects [29,30]. These studies revealed the need to develop pharmacologically active synthetic analogs of vitamin D without the toxic side effects of hypercalcemia. The initial efforts to synthesize analogs that are more suitable than 1α,25-$(OH)_2D_3$ for clinical use (i.e., compounds that have low calcemic activity but are still effective pharmacologically) did not follow a rational design because it was not known whether its properties could be separated or which structural features of 1α,25-$(OH)_2D_3$ contributed to its calcemic activity and which to other cellular responses. However, studies of the vitamin D endocrine system had shown that the critical functional groups in the ligand were the 1α-OH and the 25-OH groups, and that catabolism of the hormone begins at the side chain [1,31]. Therefore for practical reasons most of the modifications in the first generation of vitamin D analogs were in the side chain, preserving the

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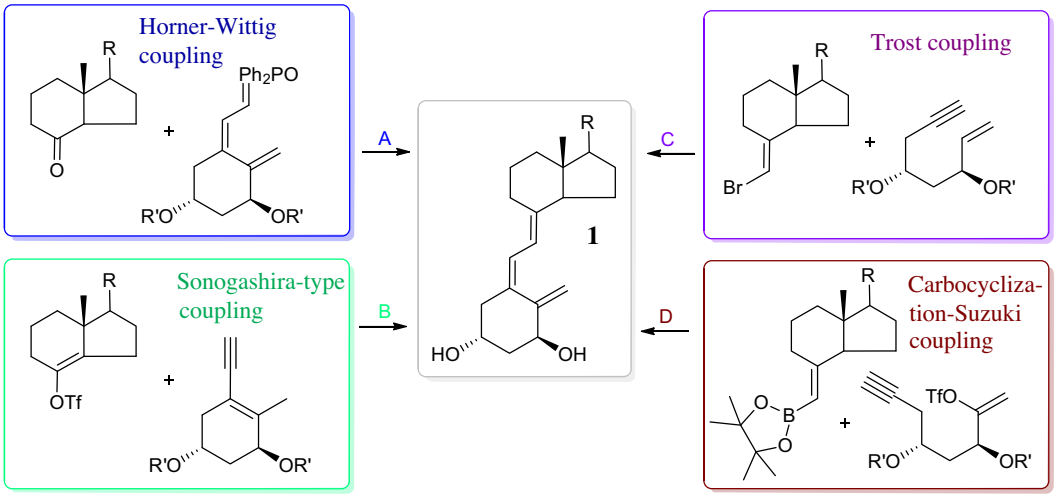

Scheme 1. Synthetic approaches to the synthesis of A-ring analogs.

A-ring and the 25-OH group and either increasing or decreasing availability to catabolic enzymes of 1α,25-$(OH)_2D_3$ and vitamin D binding protein (DBP) but only slightly altering the VDR binding activity. These compounds included analogs with modifications at the C-23, C-24 and C-16–17 positions, fluorines at positions 26 and 27, and various combinations of these modifications [32]. Later on, the approach to the design of vitamin D analogs changed; more recent studies in which structural groups thought to be essential for 1α,25-$(OH)_2D_3$ function, such as the 1α-OH or 25-OH, were replaced, showed that the resulting deltanoids have significant biological activity both *in vivo* and *in vitro* despite their lower affinity for the VDR. These deltanoids included analogs with A-ring modifications such as 1α-fluoride (**2**, Fig. 3) [33] or 1β-hydroxymethyl-3-epi-25-$(OH)D_3$ (**3**) [34,35] and modifications at C-25, which prevent 25-hydroxylation [36–38]. Although most vitamin D analogs developed thus far have focused on side-chain modifications, an intense interest has been directed more recently at modifications of the A-ring moiety as well. In this review, we focus on the current understanding of the relationship between selected modifications in the A-ring of the 1α,25-$(OH)_2D_3$ molecule and their effect on biological potency and selectivity.

Synthetic approaches to A-ring analogs of active vitamin D

A-ring modification of 1α,25$(OH)_2D_3$ is the second most extensive area of analog studies next to side chain modifications, and many different approaches to modifying the A-ring have been developed over the years. Current synthetic approaches are based on convergent methodologies in which a preformed A-ring fragment is attached to a CD fragment [39]. Among these, application of the Horner–Wittig reaction in vitamin D synthesis, pioneered by Lythgoe and developed by the Roche group [40] and others, probably provides one of the methods of choice because the vitamin D conjugated triene unit is set up with predictable configurations (C_5–C_6-*Z* and C_7–C_8-*E*) in a single step from A-ring and CD-ring units (Route A, Scheme 1), thus making this method highly convergent and efficient. Furthermore, this methodology that involves the coupling of A-ring phosphine oxides with C,D-ring ketones, is also highly accessible and pragmatic, given the availability of a number of elegant synthetic approaches to the enantiopure A-ring allylic phosphine oxides. Another major pathway to constructing the conjugated triene unit of vitamin D is the enyne approach pioneered by Lythgoe in 1971 [41] and further developed by Mourino [42]. The Mourino's Sonogashira-type method involves the coupling of A-ring enynes and CD-ring Grundmann enol triflates (Route B). In 1992, Trost reported a conceptually novel approach to vitamin D_3 synthesis. This new convergent strategy takes advantage of a Pd-catalyzed alkylative enyne cyclization that allows the one-pot coupling of an acyclic A-ring precursor to CD-ring fragment and the consecutive cyclization of A-ring (Route C) [43]. The shortness and high overall yield (~10%) of this sequence, as well as, the excellent control of regioselectivity, make this method very competitive with other approaches and therefore largely used by chemists in recent years. Besides these relatively mature approaches to the triene unit mentioned above, Mourino has recently proposed a new method that might potentially find application in the synthesis of new analogs [44]. It relies on a Pd-catalyzed carbocyclization–Suzuki coupling approach that employs CD-ring alkenyl-boronic ester and acyclic A ring precursor, enol-triflate (Route D). It is interesting to notice that most of the routes to the A-ring enynes start from naturally occurring substances. Among these, (*S*)-carvone, quinic acid, and chiral monosaccharides, such as D-glucose, D-xylose, and D-arabinose, or sugar alcohols, such as D-mannitol, are examples of commonly used chiral pool material.

Conformation–activity relationships in the A-ring

The conformational flexibility of the A ring and its effect on biological activity have been the focus of several structure–function studies over the past few decades [45–50]. In 1974 Okamura et al. first reported that the A-ring of 1,25-$(OH)_2D_3$ equilibrates between two chair conformers, the α- and β-form (Fig. 1), in a 1:1 ratio as determined by ^{1}H NMR analysis [48], and proposed that the β-form, in which the 1α-hydroxyl occupies the equatorial position, may be responsible for the biological activity [51].

To experimentally alter the conformational equilibrium of the A-ring, 2-methyl analogues of the natural hormone (**4** and **5**, Fig. 2), and 19-nor-1α,25-$(OH)_2D_3$ (**6** and **7**) were synthesized by Japanese scientists [52] and by our group [53]. The results of biological and conformational analysis studies on these compounds seemed to contradict Okamura's suggestion [54]. In fact, it was found that 2α-alkylated vitamins characterized by strong bias (above 90% for 19-norvitamin) toward conformers with the axial hydroxyl at C-1, were much more biologically potent then the respective 2β-isomers existing in solution primarily in the opposite β-chair form [53,55]. Afterward, however, the Moras group reported the crystal structure of the hVDR ligand binding domain (LBD) complexed with hormone **1** [56] and several other ligands characterized by an unnatural configuration at C-20 [57]. The results of these structural studies clearly indicated that the receptor binds (at least in the crystalline state) vitamin D compounds having their A-rings in the β-chair