Ring-A-seco analogs of α,25-dihydroxy-19-norvitamin D_3

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Abstract

The steroid hormone $1\alpha,25$-dihydroxyvitamin D_3 [$1\alpha,25$-$(OH)_2D_3$] is the most active metabolite of vitamin D_3 which exerts its control over a multitude of biological processes related to calcium and phosphorus homeostasis, cell proliferation and differentiation, and immune regulation. Unfortunately, the therapeutic application of $1\alpha,25$-$(OH)_2D_3$ is limited by induction of hypercalcemia. The need for vitamin D compounds with selective biological profiles has stimulated the synthesis of more than three thousand analogs of $1\alpha,25$-$(OH)_2D_3$. Most of these compounds have structural modifications in the side chain and A-ring; there is also an increasing number of modifications in the CD-rings and limited number in the triene system (seco-B ring). Herein, we report the synthesis and biological evaluation of seco-A-19-nor analogs of $1\alpha,25$-dihydroxyvitamin D_3, developed to study the role of ring A in the biological activity of $1\alpha,25$-$(OH)_2D_3$. Interestingly, compounds **2** and **4** show substantial ability to bind the vitamin D receptor and the former is also characterized by selective intestinal calcium transport activity.

Keywords

1. Introduction

The importance of multiple physiological processes [1] controlled by $1\alpha,25$-dihydroxyvitamin D_3 [$1\alpha,25$-$(OH)_2D_3$, calcitriol, **1**; Fig. 1], the hormonally active metabolite of vitamin D_3, has stimulated a very intensive research directed toward developing vitamin D therapeutics that preserve clinically useful activities of $1\alpha,25$-$(OH)_2D_3$ such as blocking cell proliferation or modulating the immune system but without hypercalcemia and hyperphosphatemia. During last few decades, more than three thousand vitamin D analogs have been synthesized, most of which have focused on side chain modifications, leading to many interesting structures with more rigid side chains [2] as well as widely enlarged [3] or drastically reduced ones [4]. In recent years CD-ring system has also been extensively explored affording numerous analogs with modified hydrindane moiety, along with far-reaching modifications such as removal of the C-ring, D-ring or complete elimination of the CD-ring fragment [5]. The A-ring has been the second, most frequently modified part of the vitamin D scaffold. Thus, alterations at positions C-1, C-2, C-3, C-4 and C-10 [6] have been accomplished, with C-2 [7,8] and C-10 [9] providing the most successful and biologically interesting calcitriol analogs. Although the A-ring

modifications have been extensively explored, the closed ring structure has commonly been preserved. The only synthesis of a seco-A-ring calcitriol, namely, 2-nor-1,3-seco-1,25-dihydroxyvitamin D_3, was reported by Okamura's group [10] but without biological evaluation of the analog. Herein, we report the synthesis and biological evaluation of seco-A-ring analogs of 1α,25-dihydroxy-19-norvitamin D_3 (**2–5**, Fig. 1), developed to evaluate the importance of ring A for biological activity of vitamin D compounds. Since it is well-known that the presence of a 1α-hydroxyl group in the vitamin D molecule is crucial for receptor binding [11] and biological potency, the newly designed structures **2–5** possess a hydroxyl group capable of restoring spatial arrangement and acting like the 1α-OH.

2. Materials and methods

2.1. Preparation of the A-seco-19-norvitamin D_3 analogs 2–5

The vitamin D analogs 2–5 were synthesized at the Department of Biochemistry, University of Wisconsin-Madison according to the synthetic routes presented in Schemes 1–5. The spectroscopic and analytical data of all obtained compounds confirmed the assigned structures. The details of the synthesis will be reported elsewhere.

2.2. In vitro studies

2.2.1. Measurement of binding to the rat recombinant vitamin D receptor—The competition binding assays were performed using 1α,25-$(OH)_2$[26,27-^{3}H]D_3 as described previously [12]. 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 [13].

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 [14]. 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.

2.3. In vivo studies

Bone calcium mobilization and intestinal calcium transport studies were performed in male, weanling Sprague-Dawley rats, as described previously [7].

3. Results and discussion

3.1. Chemical synthesis of 2–5

Structures **2–5** were accomplished by modified Julia coupling reaction of the known thiazoline sulfone **10** [7] with acyclic aldehydes/ketones **6–9** (Scheme 1). This particular method of coupling was chosen for its high *E/Z* stereoselectivity that was reported for coupling of allylic sulfones with aldehydes or α,β-unsaturated aldehydes with alkylsulfones [15]. The desired 5*Z*-geometrical isomers were obtained with 2.5–12 times higher yield than their 5*E*-counterparts. Removal of the protecting groups in the obtained products by treatment with camphorsulfonic acid or tetrabutylammonium fluoride gave the expected A-seco-19-norvitamin D analogs **2–5** which were purified and separated from their minor 5*E*-isomers by reversed-phase HPLC.

The acyclic fragments **6–9** were synthesized from commercially available substrates according to the routes presented in Schemes 2–5.

3.2. Docking of analog 2 to the ligand binding pocket of the rVDR (Fig. 2)

Taking into account the selective intestinal activity of the synthesized analog **2** (see below) we decided to model its complex with rat vitamin D receptor (rVDR). Surprisingly, this 4-nor-A-seco analog, possessing only two hydroxyethyl fragments attached to C-5, and lacking the stereogenic centers in the ring A, bound vitamin D receptor only 10 times weaker than the natural hormone. We docked compound **2** into the modeled full-length [118–423] ligand binding pocket (LBP) of the rVDR and it was found this ligand anchored to the LBP similarly to $1\alpha,25\text{-(OH)}_2D_3$ (**1**) in its crystalline complex with the hVDR [14].

Analysis of the modeled complex revealed that flexibility of the side chain and both hydroxyalkyl substituents at C-5 allowed the ligand hydroxyl groups to form hydrogen bonds with the same set of neighboring amino acids that was found in the crystalline **1**-hVDR complex [16]. Thus, A-ring hydroxyls of **2** contacted R270, Y143 and S274, with the last contact being the strongest. The side chain 25-hydroxyl group was positioned between histidines 301 and 393, creating strong hydrogen bonds with both residues. Moreover, indole ring of tryptophan 282 was positioned parallel to the plane of the ligand 5,7-diene moiety at a distance (ca. 4.6Å) allowing π–π interactions.

3.3. Biological evaluation of the synthesized analogs 2–5

In vitro and in vivo biological activities of the vitamin D analogs **2–5** were tested. Compound **2** and **4** retained ca. 10% and 30%, respectively, of the $1\alpha,25\text{-(OH)}_2D_3$ affinity for VDR. Analogs **3** and **5** were poor binders to VDR since their activity was two and four orders of magnitude lower than that of the natural hormone (Table 1). A similar trend was observed in ability to cause differentiation of promyleocytic leukemia cells into monocytes. Analog **4** showed 8% activity compared to $1\alpha,25\text{-(OH)}_2D_3$, whereas the other compounds were approximately two (**2, 3**) and four (**5**) orders of magnitude less active than the hormone. Moreover, vitamins **2–4** showed two–three orders of magnitude lower transcriptional activity, compared to $1\alpha,25\text{-(OH)}_2D_3$. When tested in vivo, none of the new compounds **2–5** possessed any ability to mobilize calcium from bone, even given at a high doses (Figs. 3, 5 and 7). Analogs **3** and **5** did not support intestinal calcium transport (Figs. 6 and 8), whereas 2-methylene compound **4** showed some low intestinal activity (Fig. 6). Interestingly, compound **2** possessed selective activity in inducing intestinal calcium transport, significantly increasing when it was administered at higher doses (Fig. 4).

Opening of the ring A in vitamin D compounds causes large decrease in both (in vitro and in vivo) biological activities of the analogs. An entropy penalty for immobilization of flexible, acyclic fragments in the ligand binding pocket can be, at least partially, responsible for this effect. Additionally, loss of hydrophobic interactions with VDR, like in the case of **2** and **3** might also play a role.

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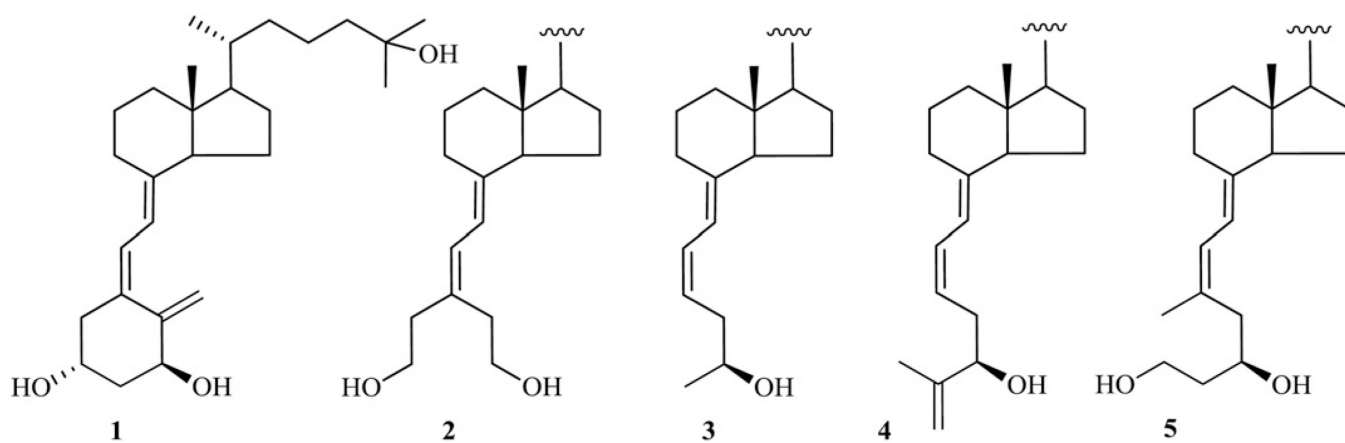
Fig. 1.
Chemical structure of 1α,25-dihydroxyvitamin D$_3$ (calcitriol, **1**) and its analogs **2–5**.
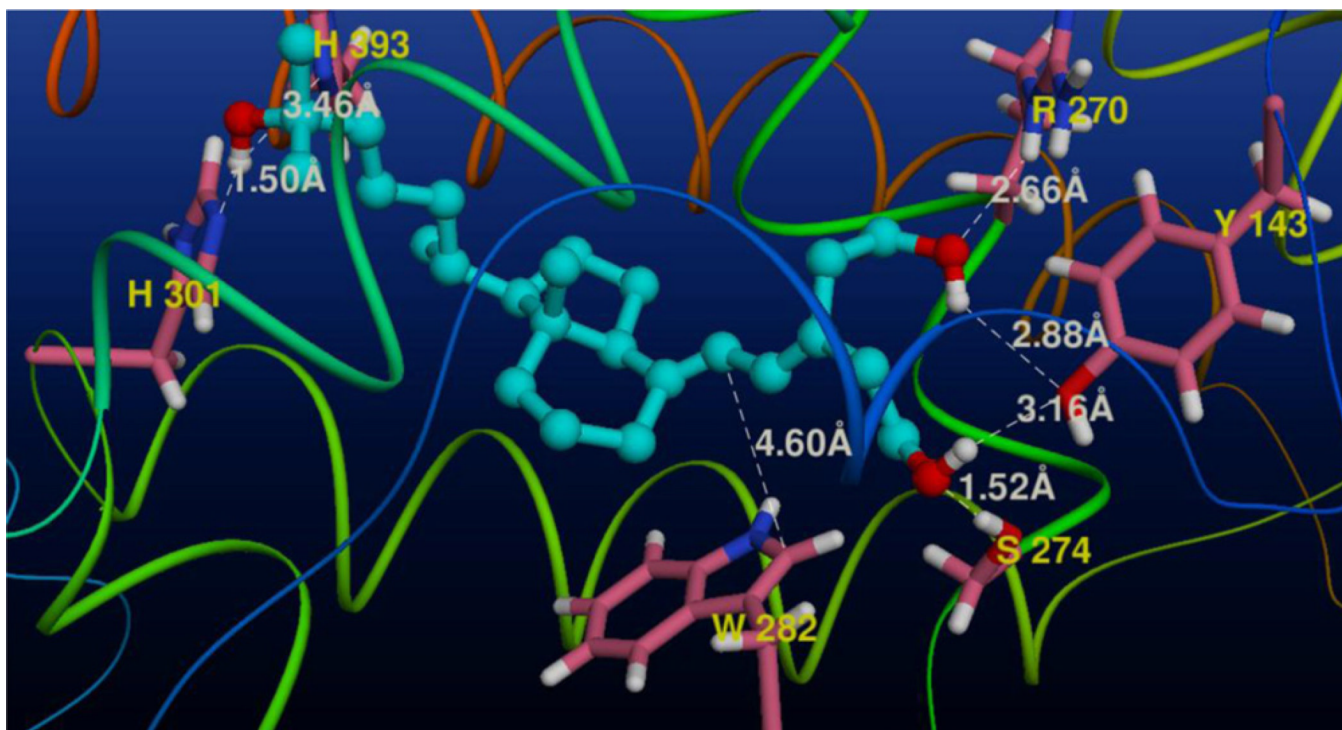
Fig. 2.
View of the three-dimensional structure of ligand binding cavity of the rat VDR with the docked analog **2**. The five amino acids (Tyr 143, Arg 270, Ser 274, His 301 and His 393) forming the shortest hydrogen bonds (the Å distances are marked in white) with the ligand are depicted; also the Trp 282 residue is shown.
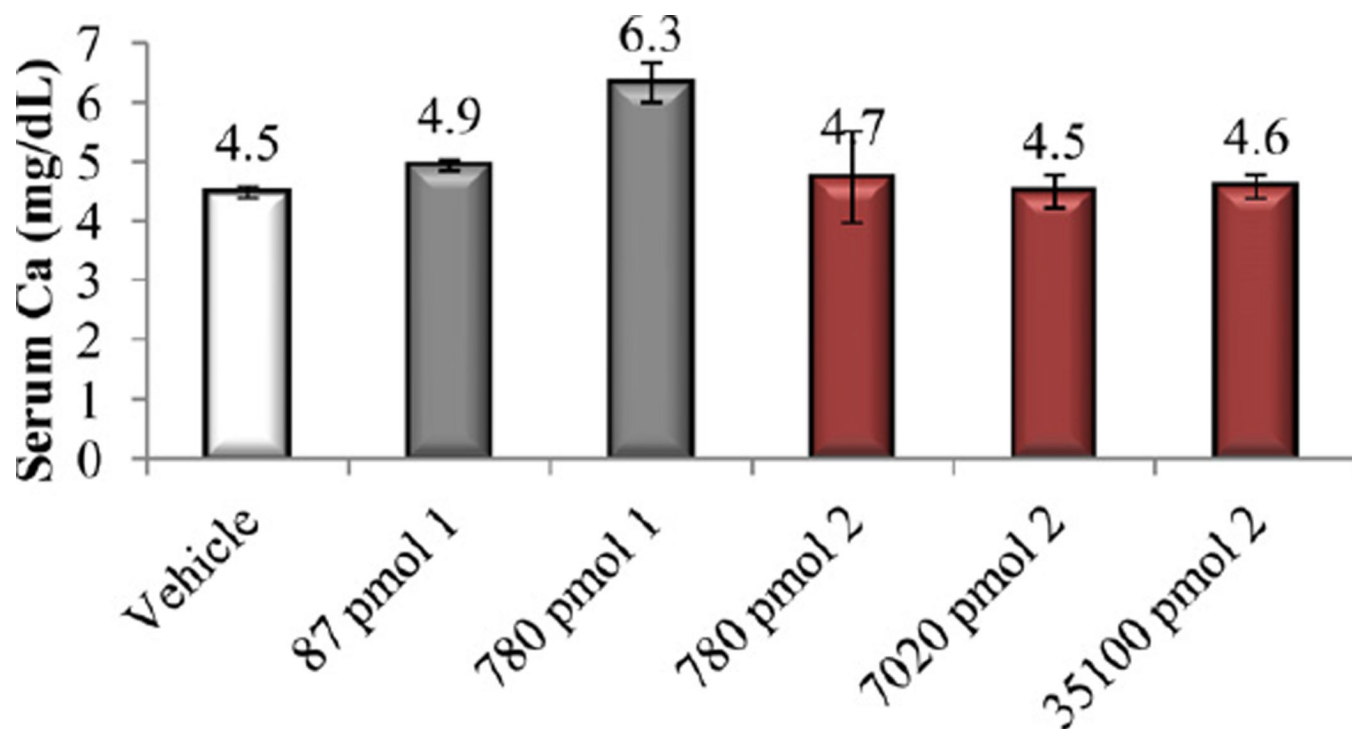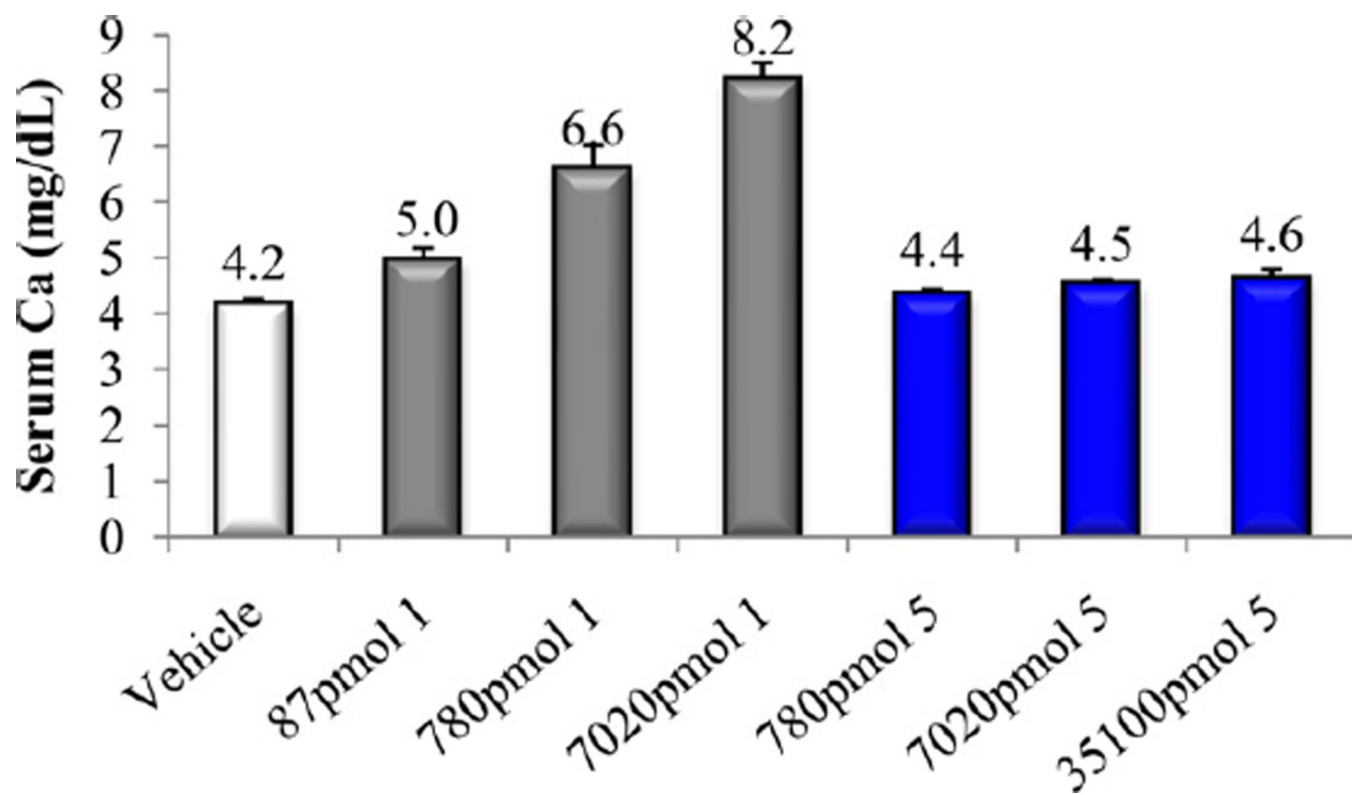
Fig. 3.
Bone calcium mobilization activity of calcitriol (**1**) and compound **2**.
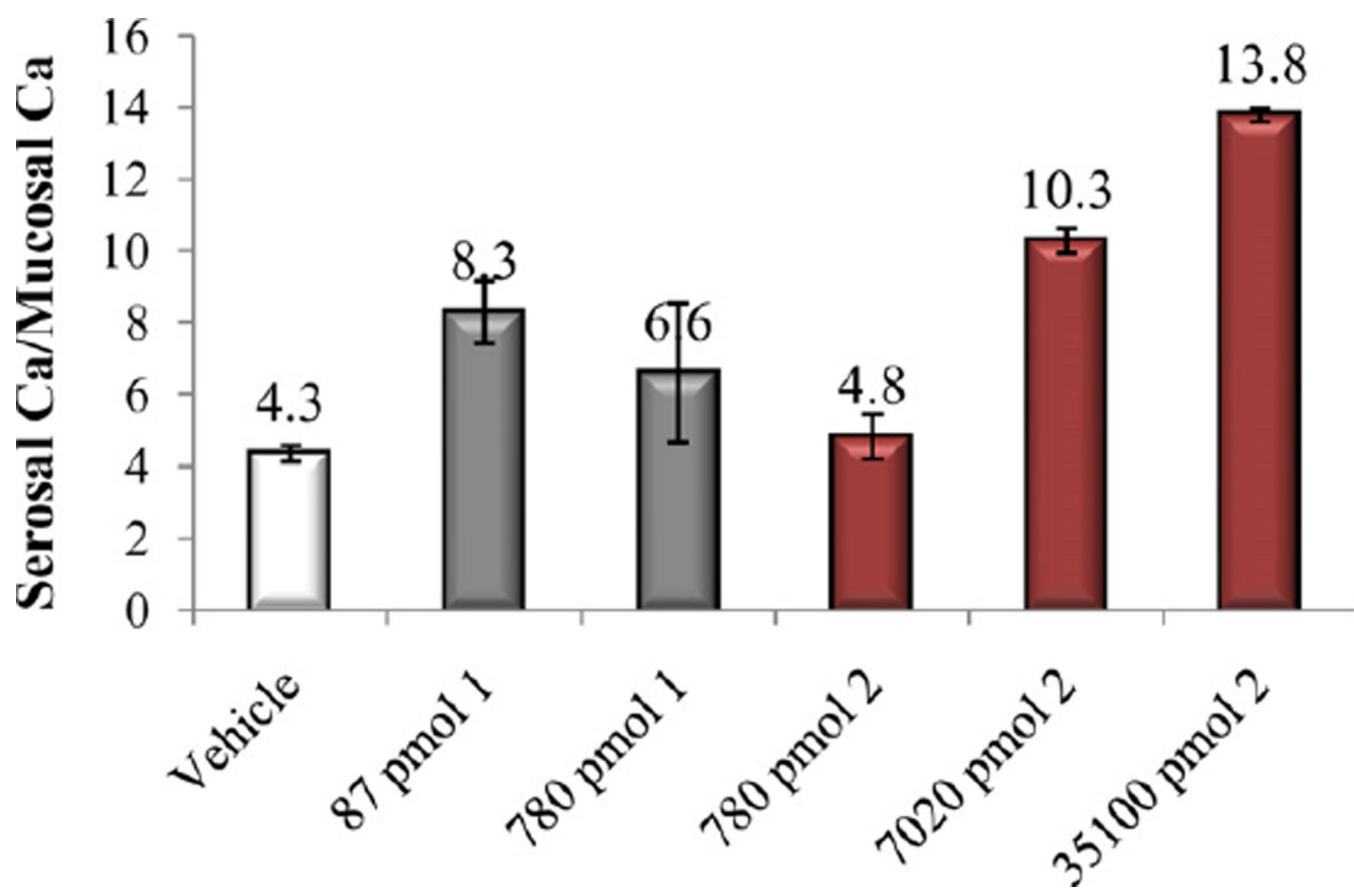
Fig. 4.
Intestinal calcium transport activity of calcitriol (**1**) and compound **2**.
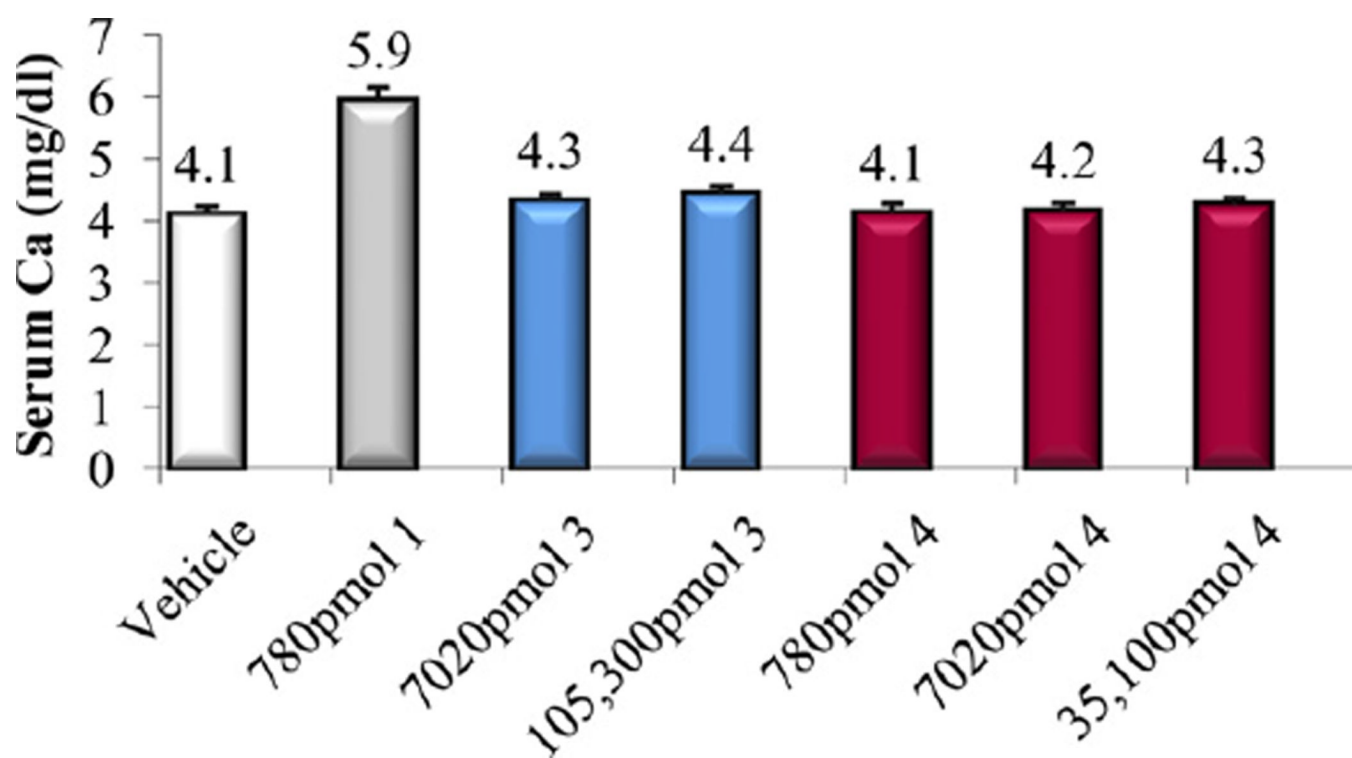
Fig. 5.
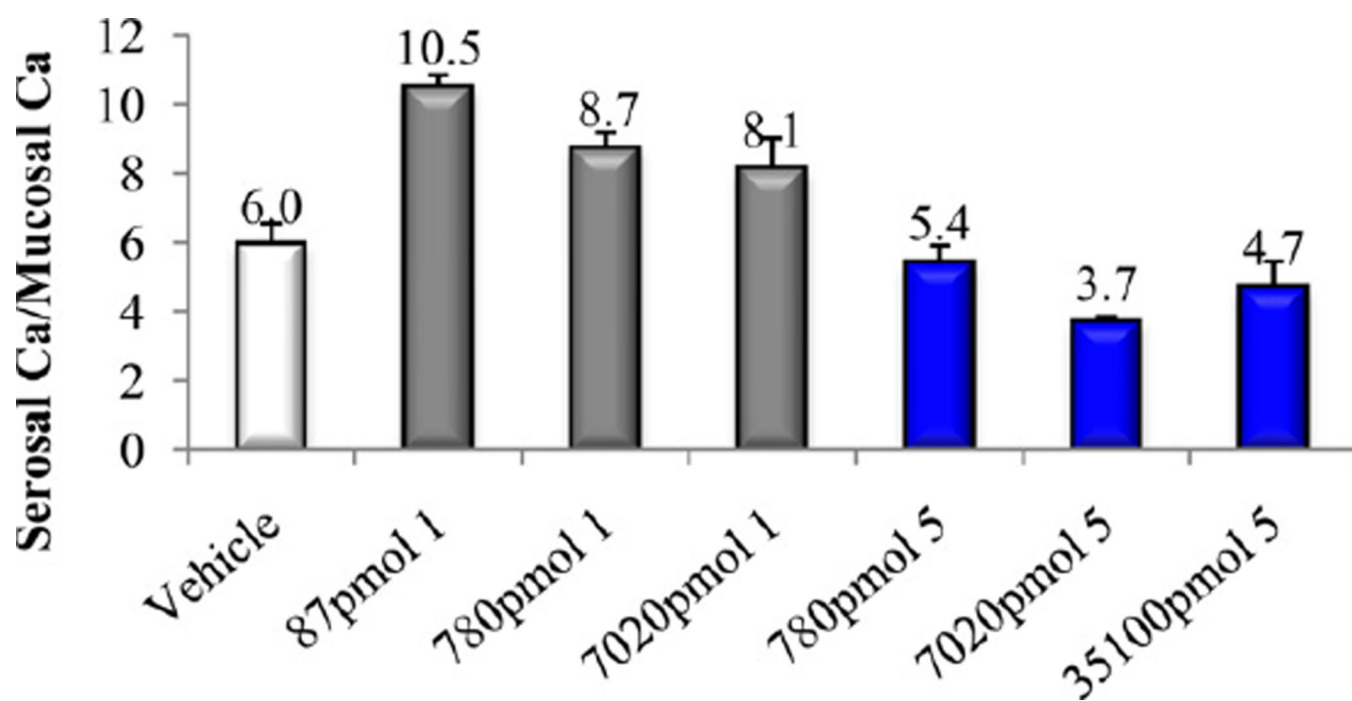Bone calcium mobilization activity of calcitriol (**1**) and compounds **3** and **4**.
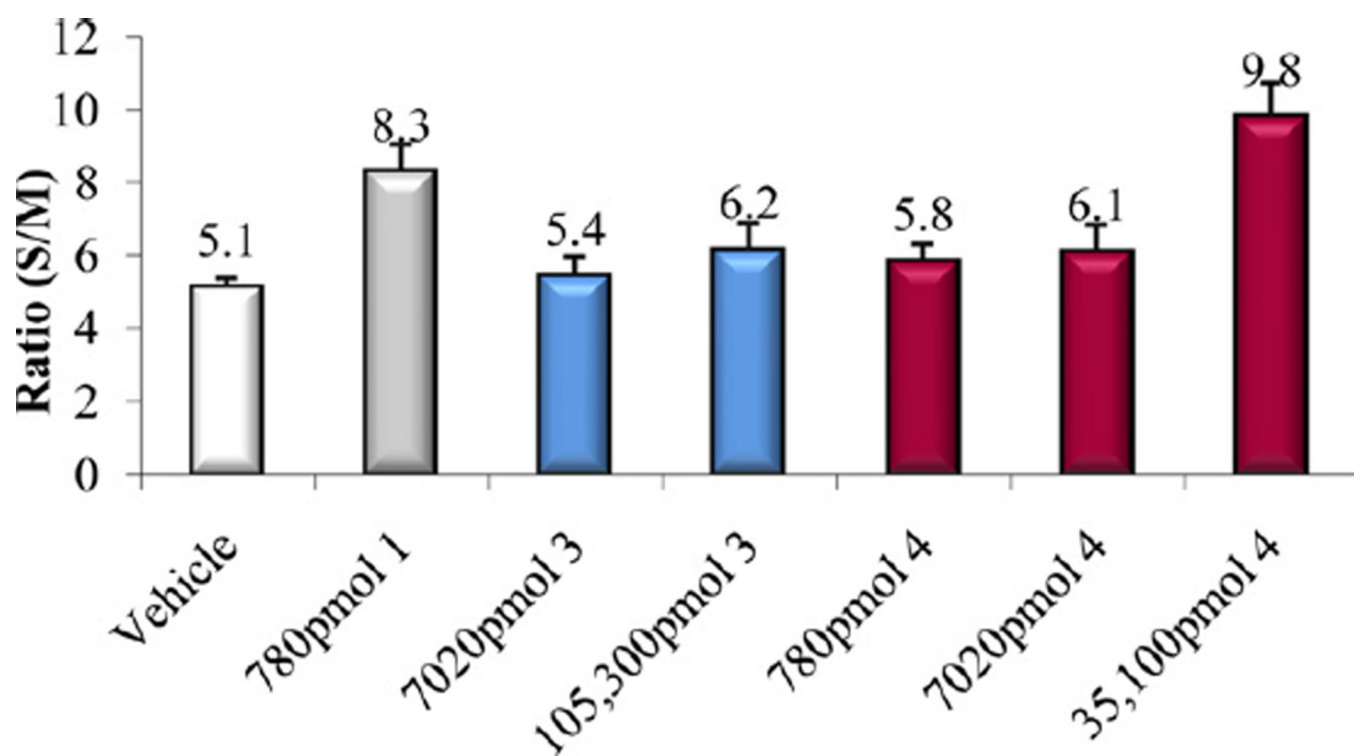
Fig. 6.
Intestinal calcium transport activity of calcitriol (**1**) and compounds **3** and **4**.

Fig. 7.
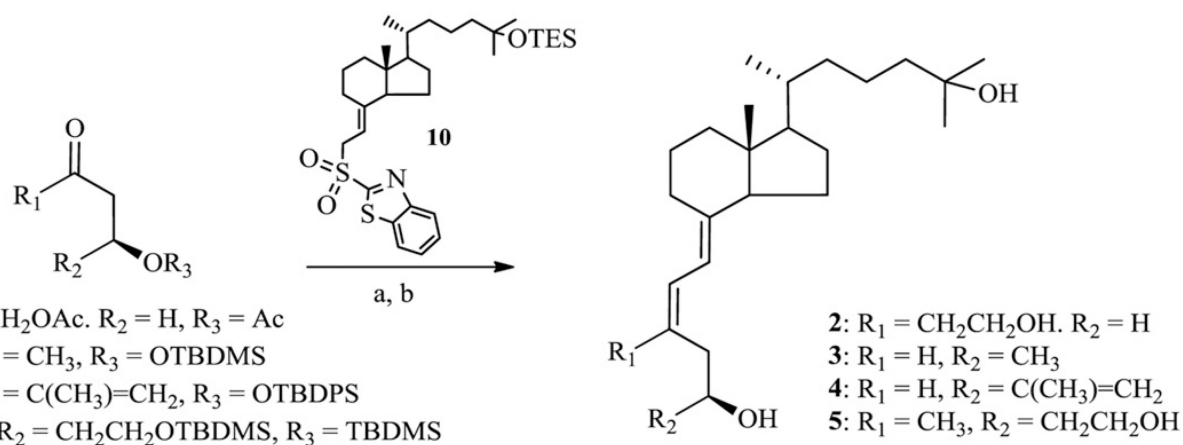Bone calcium mobilization activity of calcitriol (**1**) and compound **5**.
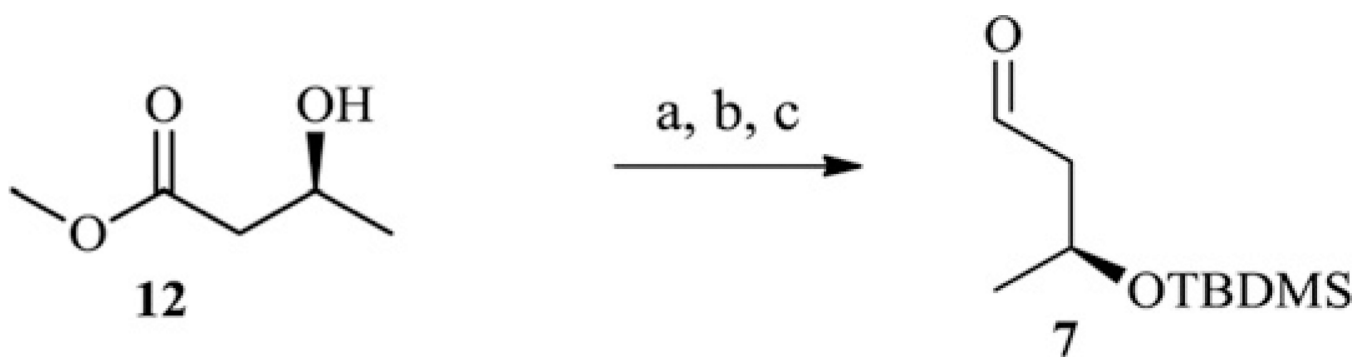
Fig. 8.
Intestinal calcium transport activity of calcitriol (**1**) and compound **5**.

Scheme 1.
(i) (a) **10**, LiHMDS, THF, then the ketone **6** in THF; (b) CSA, MeOH, 35% (two steps); (ii)
(a) **10**, LiHMDS, THF, then the ketone **7–9** in THF; (b) TBAF, THF, for **7**: 30% (two steps),
$5E{:}5Z = 1{:}2.5$; for **8**: 35% (two steps), $5E{:}5Z = 1{:}6$; for **9**: 31% (two steps), $5E{:}5Z = 1{:}12$.
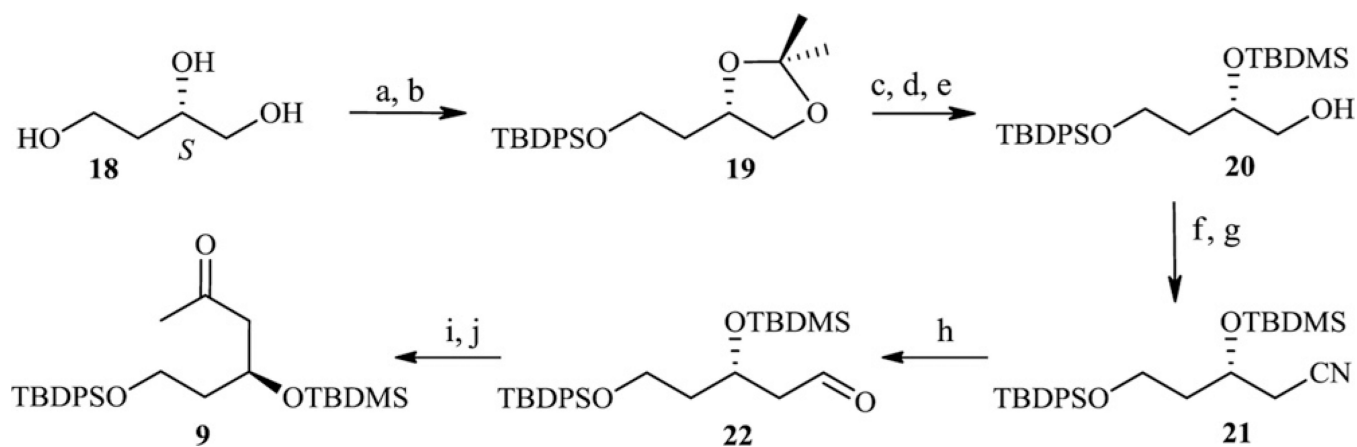
Scheme 2.
(a) TBAF, THF, 54%; (b) PCC, CH$_2$Cl$_2$, 68%.

Scheme 3.
(a) TBDMSCl, imidazole, DMF, 76%; (b) DIBALH, CH_2Cl_2, 62%; (c) PCC, CH_2Cl_2, 51%.
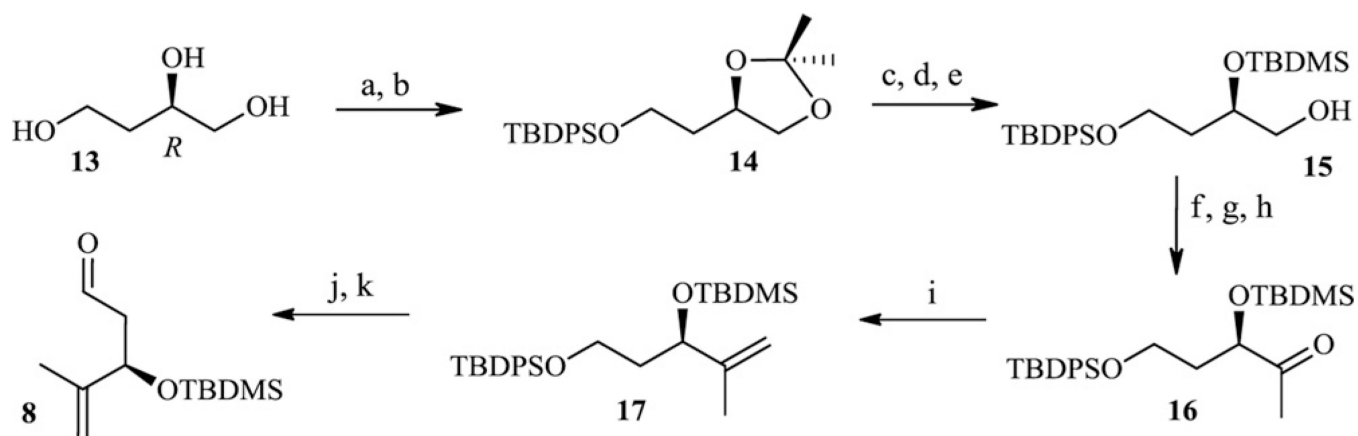
Scheme 4.
(a) CH₃C(OCH₃)CH₃, *p*-TsOH, DMF, 98%; (b) TBDPSCl, imidazole, DMF, 100%; (c) AcOH, THF, H₂O, 92%; (d) TBDMSCl, imidazole, DMF, 95%; (e) AcOH, THF, H₂O, 56%; (f) PCC, CH₂Cl₂, 92%; (g) CH₃MgBr, THF, 75%; (h) (COCl)₂, DMSO, TEA, 82%; (i) Ph₃PCH₃Br, *n*-BuLi, THF, 60%; (j) KOH, MeOH, 55%; (k) PCC, CH₂Cl₂, 88%.

Scheme 5.
(a) $CH_3C(OCH_3)CH_3$, *p*-TsOH, DMF, 98%; (b) TBDPSCl, imidazole, DMF, 100%; (c) AcOH, THF, H_2O, 92%; (d) TBDMSCl, imidazole, DMF, 95%; (e) AcOH, THF, H_2O, 56%; (f) *p*-TsCl, Pyr, 92%; (g) NaCN, DMSO, 74%; (h) DIBALH, CH_2Cl_2, 78%; (i) CH_3MgBr, THF, 82%; (j) NMO, TPAP, CH_2Cl_2, 90%.

Table 1

Relative VDR binding properties, HL-60 differentiation activities and transcriptional activities of the vitamin D compounds.[a]

Compound	VDR binding	HL-60 cell differentiation	24-OHase transcription
1	100	100	100
2	10	1	0.5
3	2	2	0.13
4	30	8	1
5	0.01	0.01	–

[a]The activity of $1\alpha,25\text{-}(OH)_2D_3$ is normalized to 100.