European Journal of Medicinal Chemistry 85 (2014) 569–575

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and biological evaluation of 1α,25-dihydroxyvitamin D_3 analogues with aromatic side chains attached at C-17

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ARTICLE INFO

Article history:
Received 26 February 2014
Received in revised form
8 August 2014
Accepted 8 August 2014
Available online 9 August 2014

Keywords:
Vitamin D
Vitamin D analogues
Vitamin D receptor
Antiproliferative
HL-60 cell differentiation

ABSTRACT

Two new analogues of the steroid hormone 1α,25-dihydroxyvitamin D_3 with aromatic side chains attached at C-17 were designed to investigate their effects on VDR, HL-60 cell differentiation and tumor cell proliferation. These analogues were prepared by the classical photochemical ring opening approach. After the protection of both the 1α- and 3β-hydroxyl in 1α-hydroxydehydroepiandrosterone with TBS groups, followed by bromination with NBS and debromination in the presence of γ-collidine, the diene intermediate was obtained. Hydrazone formation followed by iodine oxidation gave a vinyl iodide. The aromatic side chain at C-17 was introduced via the Negishi coupling of the resulting intermediate with an in situ generated zinc reagent with the substituted aryl bromide (CD-side chain) in the presence of catalytic amount of $Pd(PPh_3)_4$. After the removal of the TBDMS and MOM protective groups, followed by UV irradiation and the subsequent thermal reaction, the 1α,25-$(OH)_2$-D_3 analogues with a substituted phenyl ring attached at C-17 to replace the C-20 and C-21 were prepared. In the VDR competitive binding assay, compounds **2** and **3** almost lost their binding ability, and were only 0.01% and 0.015% as potent as the 1α,25-dihydroxyvitamin D_3. However, compounds **2** and **3** were as potent as 1α,25-$(OH)_2$-D_3 in inducing HL-60 cell differentiation at concentrations of 30, 100, 300, 1000 nM, respectively. Moreover, compounds **2** and **3** exhibited similar or better antiproliferative potency against MCF-7 human breast cancer cells, the IC_{50} values for analogues **2**, **3** and the natural hormone were 7.08, 7.56, and 12.5 μM, respectively.

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1. Introduction

The hormonal metabolite of vitamin D, 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2$-D_3 or 1,25D] or calcitriol (**1**) (Fig. 1), initiates numerous biological functions required for human health. Besides its typical role in calcium and phosphorus homeostasis, calcitriol also regulates cell growth, differentiation, apoptosis, and adaptive/innate immune responses through the rapid activation of signal transduction pathways as well as the classic transcriptional activation pathways that require the nuclear vitamin D receptor (VDR) [1–3]. 1α,25-$(OH)_2$-D_3 and its analogues have been used in the clinic for the treatment of psoriasis, renal osteodystrophy, osteoporosis, secondary hyperparathyroidism, and vitamin D-resistant rickets. A number of 1α,25-$(OH)_2$-D_3 analogues also show extremely promising prospects for the treatment of cancer, AIDS, rheumatoid arthritis, inflammatory bowel diseases, type 1 diabetes, and Alzheimer's disease [4–6]. However, the use of calcitriol as a drug to treat hyperproliferative disorders is limited by its undesired hypercalcemic side effects [7]. Therefore, extensive efforts have been made in the design of novel 1α,25-$(OH)_2$-D_3 analogues that could dissociate between antiproliferative and/or prodifferentiating action and calcemic effects. Modifications on the A and/or CD rings or the aliphatic chain of the natural ligand have led to some potent vitamin D analogues that mediate transcriptional activity with a magnitude at least 10-fold higher than the natural ligand with identical or lower calcemic properties [8–14]. For example, the inversion of the configuration at C-20 usually resulted in increased activity both in vivo and in vitro [15]. The replacement of C-21 methyl group with a cyclopropyl ring at C-20 of 1α,25-$(OH)_2$-D_3 reduced the binding affinity for VDR and DBP, slightly improved

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http://dx.doi.org/10.1016/j.ejmech.2014.08.031
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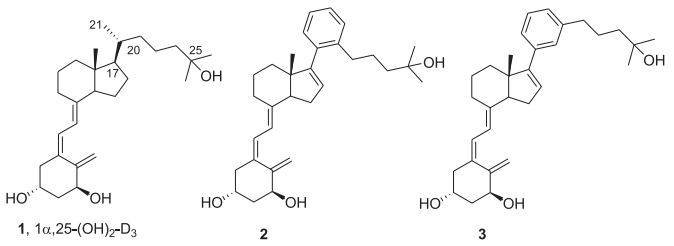

Fig. 1. 1α,25-$(OH)_2$-D_3 (**1**) and its analogues **2** and **3**.

the transactivational activity, and lowered the calcemic effects by 10-fold [16,17]. 1α,25$(OH)_2$-16-ene-20-cyclopropylvitamin D_3 proved to be several fold more potent than the natural hormone 1α,25-$(OH)_2$-D_3 as an anti-inflammatory agent [18]. Okamura's group reported a series of 1α,25-$(OH)_2$-D_3 analogues incorporating a phenyl ring at the aliphatic side chains, and found some analogues were just as potent as 1α,25-$(OH)_2$-D_3 in inducing HL-60 leukemic cell differentiation or inhibiting its proliferation, but were no toxic to the proliferation of normal human myeloid stem cells [19]. Many other side chain modifications and/or 16-ene introduction also resulted in a number of 1α,25-$(OH)_2$-D_3 analogues that exhibited more potent pro-differentiation, antiproliferative effects and transactivation potency, and some of them have the potential as anticancer agents [20–26]. Here we first report the synthesis and biological evaluations of two new vitamin D analogues **2** and **3** (Fig. 1) with a substituted phenyl ring attached at C-17 to replace the C-20 and C-21. Compared with the natural 1α,25-$(OH)_2$-D_3, this modification leads to the loss of two chiral centers at C-17 and C-20 in molecules **2** and **3**.

2. Chemistry

The classical strategy, including photo-reaction of provitamin D_3 into previtamin D_3 and the subsequent thermal conversion of previtamin D_3 into vitamin D_3, was applied for the synthesis of analogues **2** and **3**. To achieve this purpose, we first built the aromatic side chains through a new synthetic route. As shown in Scheme 1, the commercial available alcohol, **4a** or **4b**, was first converted to an iodide, which was reacted with the sodium salt of diethyl malonate to give a diester. Upon hydrolysis, compounds **5a** and **5b** were obtained in 63% and 65% yields, respectively. Decarboxylation and subsequent esterification produced the ethyl esters **6a** and **6b** in excellent yields. Coupling reaction of **6a** or **6b** with methylmagnesium bromide gave the tertiary alcohol, which was treated with chloromethyl methyl ether (MOMCl) to furnish the target aromatic side chains **7a** or **7b** in good yield.

1α-Hydroxydehydroepiandrosterone **8** (Scheme 2) was prepared from commercial available dehydroepiandrosterone by a modified procedure developed by our group, applying recoverable Pd/C catalyst mediated dehydrogenation of sterols as a key step [27]. After the TBDMS ether formation to protect the diols, diene **9** was obtained via sequential treatment with NBS and γ-collidine [28]. Diene **9** was then reacted with hydrazine hydrate in the presence of hydrazine sulfate to give hydrazone **10** in 87% yield. Oxidation of hydrazone **10** with iodine in the presence of a hindered guanidine base yielded the vinyl iodide **11**. The iodide **11** was instable when exposed to air, so it was used for the next reaction without further purification. Attaching the aromatic side chains to the C-17 position was successfully achieved through the Negishi coupling approach [29]. Bromide **7a** or **7b** was treated with *n*-butyllithium at −78 °C and then with zinc chloride in THF solution, the resulting zinc derivative was allowed to react with a preformed mixture of iodide **11** and $Pd(PPh_3)_4$ to give compounds **12a** and **12b**, which were treated sequentially with TBAF (tetrabutylammonium fluoride) and TsOH (*p*-toluenesulfonic acid) to remove the TBDMS and MOM protective groups to give the provitamins **13a** and **13b** in 31% and 30% yields (from **10**), respectively. Finally, these provitamins were converted to the desired vitamin D analogues **2** and **3** by the biomimetic sequence of photoirradiation with a high-pressure mercury lamp, followed by thermal isomerization and HPLC purification, in 16% and 14% yields, respectively.

3. Pharmacology

3.1. Competitive VDR binding assay

To examine the binding affinity of compounds **2** and **3** to the VDR, competitive VDR binding assay using bovine thymus was carried out by the standard method [30]. The relative potency of the analogues was calculated from the concentration required to displace 50% of the [^{3}H]-1α,25-dihydroxyvitamin D_3 from the receptor compared with the activity of 1α,25-dihydroxyvitamin D_3, which was assigned as 100 by definition.

3.2. Cell proliferation assay

The ability of compounds **2** and **3** to inhibit the proliferation of human MCF-7 breast cancer cells was examined by the conventional MTT assay. MCF-7 cells were incubated with compounds **2** and **3** at the indicated concentrations for 72 h, and their inhibiting rates were determined. 1α,25-Dihydroxyvitamin D_3 was used as a reference drug.

3.3. HL-60 cell differentiation assay

To evaluate the effects of compounds **2** and **3** on cell differentiation, HL-60 cells were incubated and treated with compound **2**, **3** or calcitriol (**1**) for 48 h at the indicated concentrations. 0.1% DMSO was used as vehicle. After the treatment, cells were transferred to slides with cytospin and applied for Wright's–Giemsa staining [31]. Forty microscopic view fields were analyzed and photographed.

4. Results and discussion

1α,25-Dihydroxyvitamin D_3 functions as the ligand for VDR, with the hormone–receptor complex inducing calcemic and phosphatemic effects that result in normal bone mineralization and remodeling. In the VDR binding assay, compounds **2** and **3** exhibited very low VDR affinity (see Supplementary data). They were comparable in potency to that of 25-OH-D_3, but were only 0.01% and 0.015% as potent as the parent 1α,25-$(OH)_2$-D_3 (Table 1). It is apparent that introducing a phenyl ring at the C-17 has much influence on the binding ability to the VDR.

X-ray crystal structure of Moras VDR(LBD)-1,25D complex was used for molecular docking studies by Sybyl–x1.3 software (PDB code: 1DB1) [32]. Docking analysis of compounds **2** and **3** demonstrated that the A, seco-B, C, and D rings present conformations that are similar to those observed in the presence of the natural ligand (Fig. 2). The A-ring hydroxyl groups make the same hydrogen bonds as the hVDR LBD bound to 1α,25-$(OH)_2$-D_3 complex, 1-OH with Ser237 and Arg274, 3-OH with Tyr143 and Ser278. However, the side chain tertiary hydroxyl group in compounds **2** and **3** form hydrogen bonding with His397, while the natural 25-OH binds to His305. In addition, the phenyl ring at the C-17 makes the side chain longer than that of natural hormone. All these

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