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Novel vitamin D receptor ligands having a carboxyl group as an anchor to arginine 274 in the ligand-binding domain☆

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ABSTRACT

Vitamin D_3 is metabolized into the hormonally active form, 1α,25-dihydroxyvitamin D_3 (**1**), via 25-hydroxyvitamin D_3 (**2**) which is the most abundant circulating metabolite. Introduction of the 1α-hydroxyl group into 25-hydroxyvitamin D_3 (**2**) to produce 1α,25-dihydroxyvitamin D_3 (**1**) increases the VDR binding affinity by approximately 1000-fold. The X-ray crystal structure of human VDR in complex with 1α,25-dihydroxyvitamin D_3 (**1**) shows that, together with Ser-237, the 1α-hydroxyl group of 1α,25-dihydroxyvitamin D_3 (**1**) makes hydrogen bonds with Arg-274, single mutation of which results in impaired ligand recognition. In 2002, lithocholic acid, which possesses a carboxyl group at position C24, was demonstrated to be a weak VDR ligand. We speculated that the carboxylic acid of lithocholic acid could be recognized by Arg-274 in the ligand-binding domain of VDR. In view of the significance of Arg-274 to direct the 1α-hydroxyl group, as well as the results with lithocholic acid and its derivatives, we designed the C2 modified analogues of 25-hydroxylvitamin D_3 (**2**) having a carboxyl group, instead of the 1-hydroxyl group, for better electrostatic interaction to the guanidinium side-chain of arginine.

1. Introduction

Vitamin D_3 is metabolized into the hormonally active form, 1α,25-dihydroxyvitamin D_3 (**1**), via 25-hydroxyvitamin D_3 (**2**) which is the most abundant circulating metabolite (Fig. 1) [1–3]. Introduction of the 1α-hydroxyl group into 25-hydroxyvitamin D_3 (**2**) to produce 1α,25-dihydroxyvitamin D_3 (**1**) increases the vitamin D receptor (VDR) binding affinity by approximately 1000-fold. Several enzymes, including CYP27A1 and CYP27B1, strictly regulate formation and degradation of the most active metabolite **1**, depending upon serum calcium concentration [4].

Most actions of **1** as well as its fellow metabolites are mediated via a ligand-inducible transcription factor, VDR, belonging to the nuclear receptor super-family. Binding mode of ligands to VDR, as well as the stability of the complex, is now a focus of interest for chemists, because formation of the ligand–receptor complex triggers the whole sequential reactions, in vitamin D-responsive gene activation and/or suppression, resulting in specific biological responses.

The X-ray crystal structures of human VDR in complex with **1** and its analogues show that each of the three hydroxyl groups at positions C1, C3 and C25 makes hydrogen bonds with two amino acid residues in the ligand-binding domain (LBD) of VDR (Fig. 2) [5,6]. In particular, together with Ser-237, the 1α-hydroxyl group in **1** makes hydrogen bonds with Arg-274, single mutation of which results in impaired ligand recognition [7]. In return, the importance of the 1α-hydroxyl group over the other hydroxyl groups at position C3 and C25 in the *seco*-steroid structure has been demonstrated by the data of A-ring diastereomers of **1** [4,8,9]. In comparison with the case of the 3-hydroxyl group, epimerization or loss of the hydroxyl group at position C1 severely affects the VDR affinity.

In 2002, lithocholic acid (**3**: LCA) and 3-ketolithocholic acid (**4**), which possess a carboxyl group at position C24, were demonstrated to be weak VDR ligands [10]. Although acylation at the 3-hydroxyl group in **3** increased the potency, the transformation of the 24-carboxylic acid in LCA **3** into its methyl, ethyl or benzyl ester decreased the potency of the VDR activation, which suggested the importance of the free carboxylic acid to be recognized by VDR [11]. We envisioned that the carboxylic acid of **3**, instead of the hydroxyl group, could make interaction with the critical Arg-274 in the LBD of VDR. In view of the significance of Arg-274 to direct the 1α-hydroxyl group in vitamin D compounds, as well as the results with lithocholic acid derivatives, we designed the C2 modified analogues of 25-hydroxyvitamin D_3 (**2**) having a carboxyl group, instead of the 1-hydroxyl group, for better electrostatic interaction to the guanidinium side-chain of arginine (Fig. 3). Novel VDR ligands, 2α-carboxy-25-hydroxyvitamin D_3 (**5**) as well as its methyl ester (**6**), as a reference compound of **5**, have been synthesized (Fig. 4).

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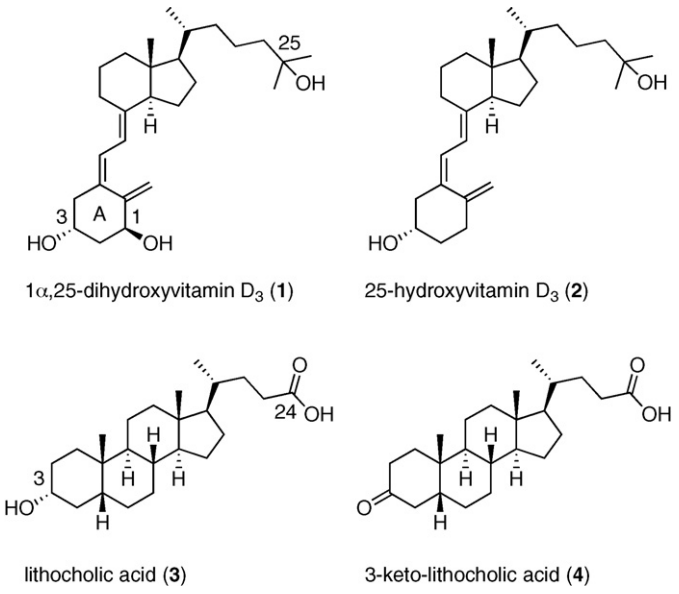


Fig. 1. Chemical structures of 1α,25-dihydroxyvitamin D_3 (**1**), 25-hydroxyvitamin D_3 (**2**), lithocholic acid (**3**) and 3-keto-lithocholic acid (**4**).

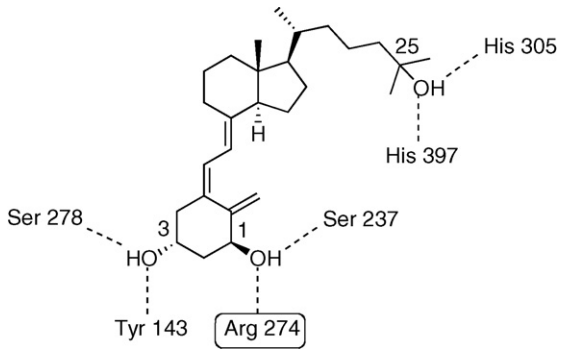


Fig. 2. Each of the three hydroxyl groups at positions C1, C3 and C25 in **1** was recognized with the two amino acid residues in the ligand-binding domain (LBD) of human VDR. The crucial 1α-hydroxyl group in **1** made hydrogen bonds with Arg-274 and Ser-237.

Novel VDR ligands with a carboxyl group

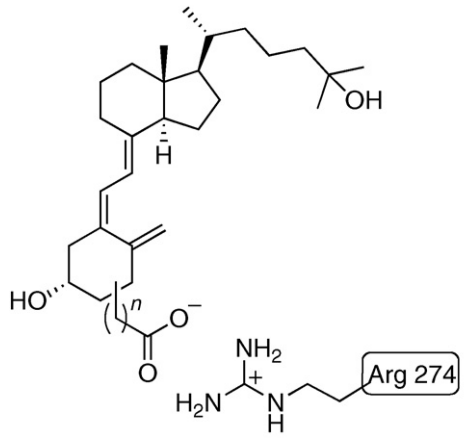


Fig. 3. Design of novel VDR ligands with a carboxyl group. The carboxyl group instead of the 1α-hydroxyl group could interact the guanidinium side-chain of Arg-274 in the LBD more favourably.

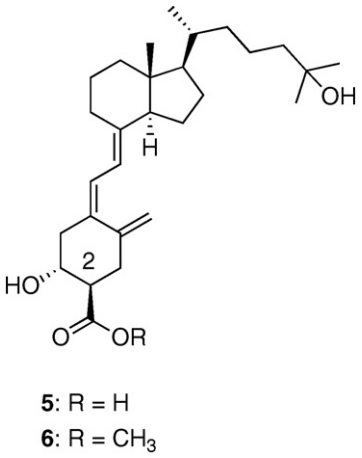
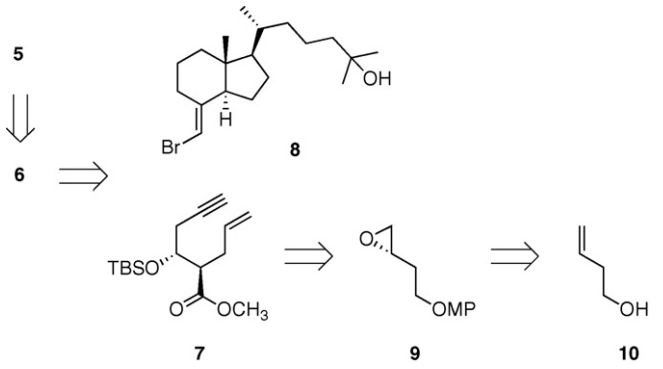


Fig. 4. Chemical structure of 2α-carboxy-25-hydroxyvitamin D_3 (**5**) and its methyl ester (**6**).



Scheme 1. Retrosynthesis of 2α-carboxy-25-hydroxyvitamin D_3 (**5**). MP: 4-methoxyphenyl.

2. Results and discussion

Synthesis of 2α-carboxy-25-hydroxyvitamin D_3 (**5**) and its methyl ester (**6**) has been carried out by a convergent method using a palladium catalyst [12]. Scheme 1 depicts the retrosynthetic analysis of the novel VDR ligands (**5**, **6**) (Scheme 1). Separate synthesis of the requisite A-ring enyne precursor **7** and the CD-ring portion **8**, followed by coupling of the two units, produced the targeted compounds. Nine-step conversion of the known epoxide **9** prepared from 3-buten-1-ol **10** yielded the requisite A-ring enyne precursors of **6**, which was then converted to **5** by hydrolysis. Detailed synthetic procedures will be published elsewhere soon.

Preliminary testing of the novel VDR ligands (**5**, **6**) showed weak affinity to bovine thymus VDR in comparison with 1α,25-dihydroxyvitamin D_3 (**1**), however their potencies were comparable to 25-hydroxyvitamin D_3 (**2**). Under the conditions that we employed, lithocholic acid **3** itself exhibited low VDR affinity; our novel compounds showed more than 100-fold better binding to VDR in comparison with **3**.

In summary, we have designed novel VDR ligands introduced a carboxyl group into the A-ring of the *seco*-steroid to interact the critical amino acid residue, Arg-274 in the LBD of VDR. Synthesis of the compounds was carried out by a convergent method using palladium catalyst. Novel VDR ligands showed comparable VDR binding affinities to VDR in comparison with 25-hydroxyvitamin D_3.

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