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Synthesis and structure–activity relationships of TEI-9647 derivatives as Vitamin D_3 antagonists☆

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Abstract

The Vitamin D_3 lactone analogues, (23*S*)- and (23*R*)-25-dehydro-1α-hydroxyvitamin D_3-26,23-lactone (TEI-9647 and TEI-9648) are antagonists of the 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$) nuclear receptor (VDR)-mediated differentiation of human leukemia (HL-60) cells. In order to clarify the structure–Vitamin D antagonistic activity relationship, we paid attention to the unique lactone moiety of TEI-9647 and TEI-9648: α-exo-methylene-γ-lactone structure. We synthesized the exo-methylene-modified analogues (methylene saturated, endo-methylene, methylene-deleted, methyl-substituted, dimethyl-substituted, methylene-replaced with dimethyl and cyclopropane) and oxygen-modified analogues (oxygen atom replaced with nitrogen and carbon atom) by convergent method using palladium-catalyzed coupling reaction or direct modification of VD_3 skeleton. The antagonistic activity in HL-60 cell differentiation evaluating system of these analogues revealed that any exo-methylene-modified analogues and nitrogen analogue did not have the antagonistic activity, on the other hand carbon analogue did show. The results suggest that "α-exo-methylene carbonyl" structure of VD_3 side-chain is crucial for antagonistic activity. The structure is integral building block of many natural products which have interesting biological and it is thought that Michael-type addition of α-exo-methylene carbonyl structure with protein nucleophiles such as cysteine would play an important role for the activities. According to this theory, Michael-type reaction of TEI-9647 and TEI-9648 with cysteine residue in protein related to VDR/VDRE-mediated genomic actions such as VDR would be essential step of the antagonistic action.

Keywords: Vitamin D analogues; Antagonists; Molecular mechanism; Paget's disease of bone

1. Introduction

Vitamin D_3 lactone analogues, TEI-9647 (**1a**) and TEI-9648 (**1b**), are the first VD_3 antagonists which inhibit VDR/VDRE-mediated genomic actions of 1α,25$(OH)_2D_3$. That is, these analogues inhibit cells differentiation [1] and 24-OHase gene expression [2,3] induced by 1α,25$(OH)_2D_3$. In order to clarify the mechanism of the activity from a ligand structure standpoint, we focused on the unique lactone structure of **1a**/**1b** because original natural VD_3 lactone, a major metabolite of 1α,25$(OH)_2D_3$, has no antagonistic activity in spite of having a very similar lactone structure. Here, we report the synthesis and biological evaluation of lactone-modified **1a**/**1b** analogues (exo-methylene-modified: **2**–**8**; oxygen-modified: **9**, **10**; Fig. 1) and presumption of the mechanism of the antagonistic activity.

2. Chemistry

1a/**1b** and **2** were synthesized in our laboratory as described previously [4]. The analogues **3**–**9** were synthesized employing the convergent protocol using palladium-catalyzed coupling reaction [5]. For synthesis of **3**, double-bond isomerization of lactone derivatives **14a**/**14b** prepared from Vitamin D_2 [4] by Rh(III) gave CD-ring precursors **15a**/**15b**. The Pd-catalyzed coupling reaction of the **15a**/**15b** with A-ring enyne precursor **16a** followed by deprotection of silyl groups afforded the target **3a**/**3b** (Scheme 1).

For synthesis of **4**, non-substituted lactone derivative **17** prepared by lactonization reaction of the aldehyde **12** with

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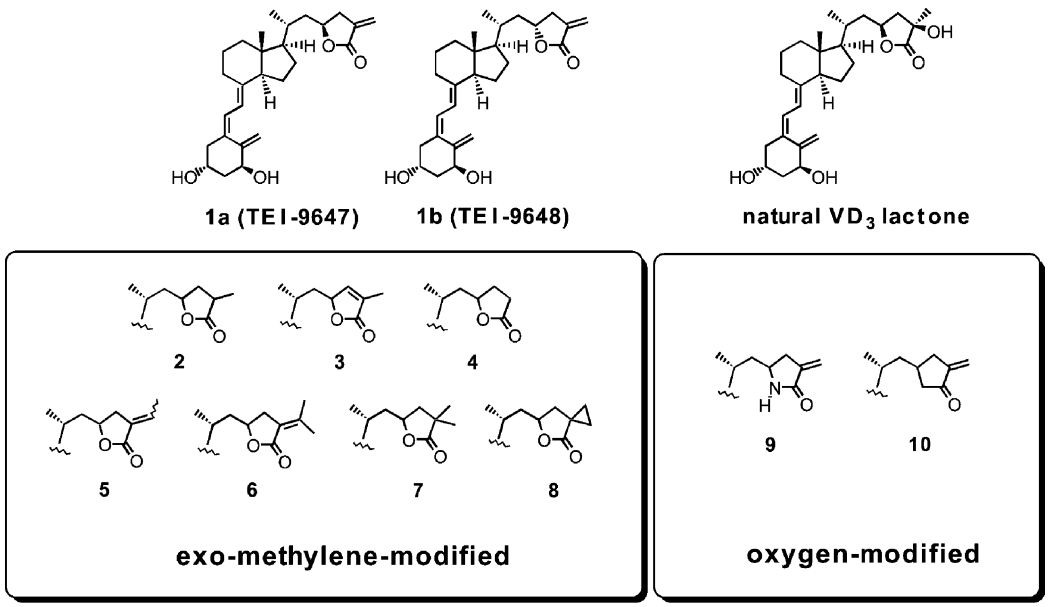
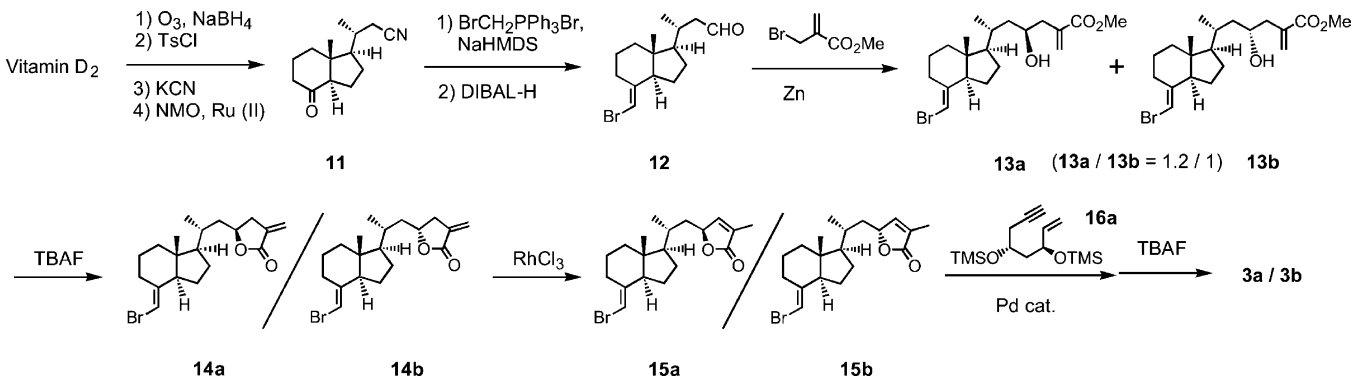
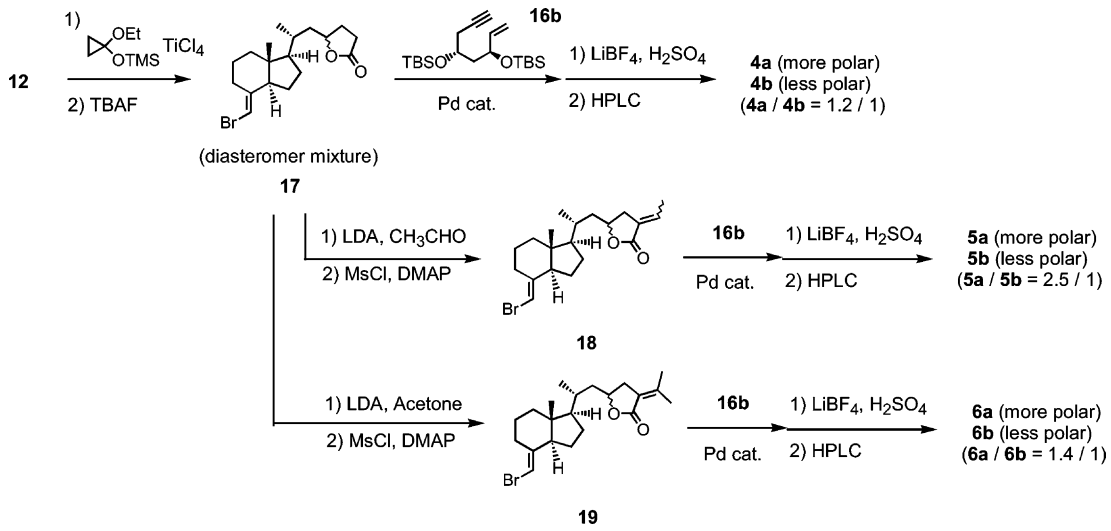


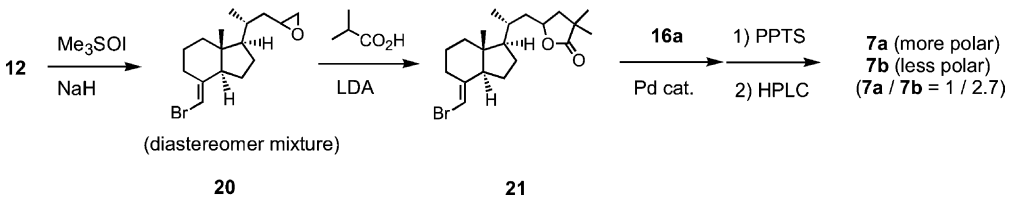
Fig. 1. Structures of TEI-9647 (**1a**), TEI-9648 (**1b**), natural VD_3 lactone and lactone-modified analogues **2**–**10**.



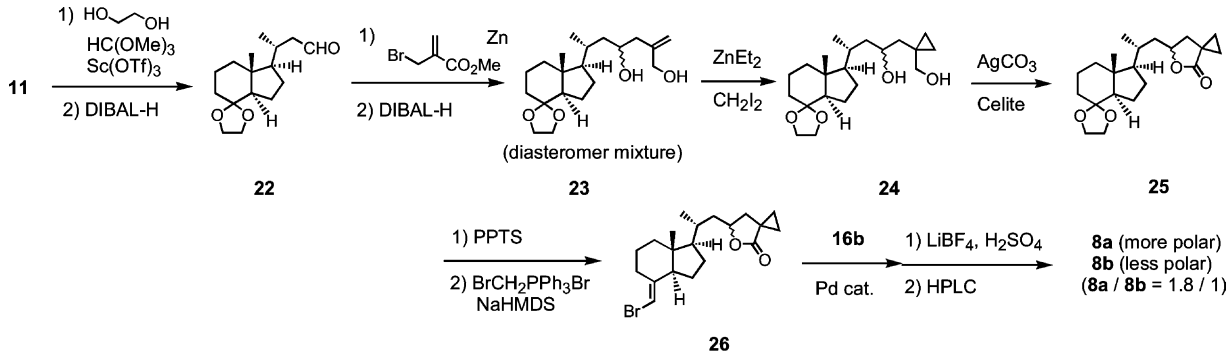
Scheme 1.



Scheme 2.



Scheme 3.



Scheme 4.

cyclopropane derivative and $TiCl_4$ was used for CD-ring part (Scheme 2). **4a** and **4b** were diastereomers caused by asymmetric center at 23-position and these were able to separate by reverse-phase HPLC but have not been determined their absolute configurations. For syntheses of **5** and **6**, corresponding CD-ring parts **18** or **19** were prepared by aldol reaction of **17** with acetaldehyde or acetone followed by dehydration (Scheme 2).

For synthesis of **7**, epoxydation of aldehyde **12** with trimethyloxosulfonium ylide followed by lactonization with isobutyric acid gave desired CD-ring precursor **21** (Scheme 3).

For synthesis of **8**, diol **23** was prepared from ketone **11** by acetal protection, cyano group reduction, Reformatosky type allylation with bromomethylacrylate methyl ester and reduction of ester group. Cyclopropanation of **23** then oxidation of primary hydroxyl group to generate lactone-ring followed by deprotection of acetal group and bromomethylenation afforded target CD-ring part **26** (Scheme 4).

For synthesis of **9**, target CD-ring **29a**/**29b** was synthesized by Reformatosky type allylation of imine which was prepared from **12** and *p*-methoxyaniline with bromomethylacrylate ester, lactonization, then deprotection of *p*-methoxyphenyl group with CAN (Scheme 5).

The analogue **10** was synthesized by direct modification of Vitamin D skeleton. That is, bromination of alcohol **30** prepared from Vitamin D_2 [6] gave **31**. Lithiation of **31** then conjugation addition to cyclopentenone afforded **32**. α-Exo-methylenation with *N*-methylaniline-TFA salts and paraformaldehyde followed by deprotection of silyl groups gave **10a**/**10b** with regioisomers **33** which were separated by HPLC.

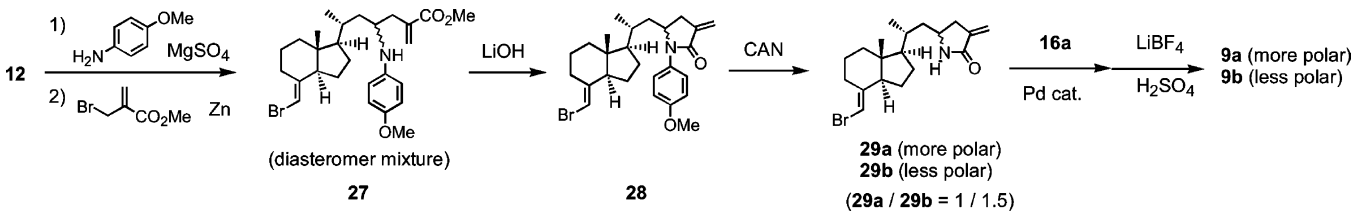
3. Biological evaluations

Binding affinity to VDR and HL-60 cell differentiation assay were performed as described previously [1].

4. Results

Biological activities of the analogues **2**–**10** on binding affinity to chick intestinal VDR and antagonistic activity in HL-60 cell differentiation evaluating system were summarized in Table 1.

The VDR binding affinity of the analogues became weaker than original antagonist **1a**/**1b** except dimethyl



Scheme 5.

Table 1
VDR binding affinity and antagonistic activity of TEI-9647 side-chain analogues

Compound	VDR binding affinity[a]	Antagonistic activity[b]
1α,25(OH)$_2$D$_3$	100	NA
1a/1b (TEI-9647/TEI-9648)	12.3/7.2	100/41
2a	0.5	NA
3a/3b	0.9/1.1	NA/NA
4a/4b	0.6/0.5	NA/NA
5a/5b	4.1/2.4	NA/NA
6a/6b	66.7/9.9	NA/NA
7a/7b	22.7/0.7	NA/NA
8a/8b	8.2/1.0	NA/NA
9a/9b	4.4/1.4	NA/NA
10a/10b	1.4/3.5	34/26

[a] Relative activity which normalized by the potency of 1α,25(OH)$_2$D$_3$ (=100) using chick intestinal VDR.
[b] Relative activity which normalized by the potency of **1a** (=100) in HL-60 cell differentiation system induced by 1α,25(OH)$_2$D$_3$, NA = not antagonist.

analogues **6a**/**6b** and **7a**. Any exomethylene-modified analogues **2**–**8** and the nitrogen analogue **9** did not have the antagonistic activity, on the other hand the carbon analogue **10** kept the antagonistic activity.

5. Discussion

The results of the antagonistic activities of the side-chain analogues except the nitrogen analogue **9** reveal that "α-exo-methylene carbonyl" structure of VD$_3$ side-chain is crucial for antagonistic activity. The structure is integral building block of many natural products which have interesting biological activities such as cytotoxic, antitumoral and bactericidal [7]. It is thought that Michael-type addition of α-exo-methylene carbonyl structure with protein nucleophiles such as cysteine would play an important role in the activities [8]. According to this theory, Michael-type reaction of **1a**/**1b** with cysteine residue in protein related to VDR/VDRE-mediated genomic actions such as VDR would be essential step of the antagonistic action (Fig. 2).

The reason why the nitrogen analogue **9** has no antagonism is thought that side-chain of **9** would not occur the Michael-type reaction because the ability of the Michael acceptor of **9** is very weak due to the existence of electron-donating nitrogen atom at α-position of the carbonyl group. It is reported that antagonistic mechanism of ZK159222, another VD$_3$ antagonist from Schering, may attribute to be pushing helix 12 of the VDR by bulky side-chain extensions [9]. Our hypothesis of TEI-9647 antagonistic

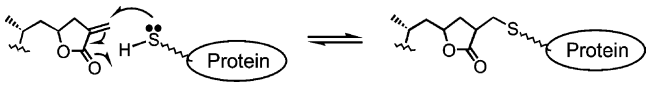


Fig. 2. Michael-type reaction of **1a**/**1b** with cysteine residue of protein.

mechanism is obviously different from that of ZK159222 and compatible with the fact that each antagonist stabillize different antagonistic conformation [10]. The VD$_3$ antagonist is expected to be potent therapeutic agent for some diseases caused by hypersensitivity to 1α,25(OH)$_2$D$_3$ such as Paget's disease of bone [11,12]. We expect that this study would contribute to discovery and development of such treating agent. Further studies will be reported in due course.

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