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Synthesis of 2α-Heteroarylalkyl Active Vitamin D_3 with Therapeutic Effect on Enhancing Bone Mineral Density in Vivo

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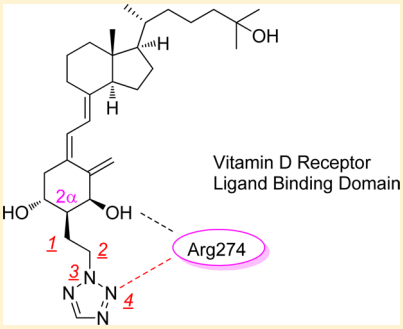
Supporting Information

ABSTRACT: 2α-Heteroarylethyl-1α,25-dihydroxyvitamin D_3 analogues, which were designed to form a hydrogen bond between Arg274 of human vitamin D receptor (hVDR) and a nitrogen atom of the heteroaromatic ring at the 2α-position, were synthesized. Among them, 2α-[2-(tetrazol-2-yl)ethyl]-1α,25-dihydroxyvitamin D_3 showed higher osteocalcin promoter transactivation activity in human osteosarcoma (HOS) cells and a greater therapeutic effect in ovariectomized (OVX) rats, osteoporosis model animals, on enhancing bone mineral density than those of active vitamin D_3. X-ray cocrystallographic analysis of the hVDR-ligand complex confirms that the new hydrogen bond formation stabilized the complex.



KEYWORDS: *Vitamin D, vitamin D receptor, X-ray cocrystallographic analysis, osteocalcin, bone mineral density, in vivo antiosteoporotic effect*

The pleiotropically hormonal active metabolite form of secosteroid vitamin D_3, 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$], exerts its biological activity via binding and modulation of the vitamin D receptor (VDR), a member of the nuclear receptor superfamily of transcriptional regulators.[1] 1α,25$(OH)_2D_3$ plays pivotal roles in maintaining calcium and phosphorus homeostasis and bone remodeling. 1α,25$(OH)_2D_3$ also acts as a regulator in a wide range of fundamental physiological processes including cell growth and differentiation, immune function, embryonic development, and inflammatory reactions.[2,3] One of the most important factors when 1α,25$(OH)_2D_3$ binds to the VDR is hydrogen bond formation between the 1α-OH group and the Arg274 residue of the hVDR.[4]

The ligand binding domain (LBD) of the hVDR contains water molecules from the A-ring anchoring moiety to the surface of the protein, called a water channel, to stabilize the VDR-[1α,25$(OH)_2D_3$] complex by forming hydrogen bond networks.[5] X-ray crystallographic analyses of the VDR-[2α-(3-hydroxypropyl)-1α,25$(OH)_2D_3$ (O1C3)] and VDR-[2α-(3-hydroxypropoxy)-1α,25$(OH)_2D_3$ (O2C3)] complexes have clearly demonstrated that the terminal hydroxy group of both synthetic ligands forms a hydrogen bond with Arg274 and replaces one of the water molecules in the LBD of the hVDR to stabilize the complex;[5] therefore, O1C3 and O2C3 showed 3- and 1.8-times greater binding affinity for the VDR than the natural hormone, respectively.[6−8] Here we studied the effects of a heteroaromatic ring on binding to the hVDR instead of the OH group and on biological activities in vitro and in vivo.

We designed 2α-[2-(tetrazol-2-yl)ethyl]-1α,25$(OH)_2D_3$ (**1a**) and the related compounds **1b−f** since the number of atoms from the 2α-position to the terminal oxygen of O1C3 is four, and compound **1a**, for example, also consists of four atoms to the nitrogen that could coordinate to Arg274, but only **1f** has five to the nitrogen atom (Figure 1).

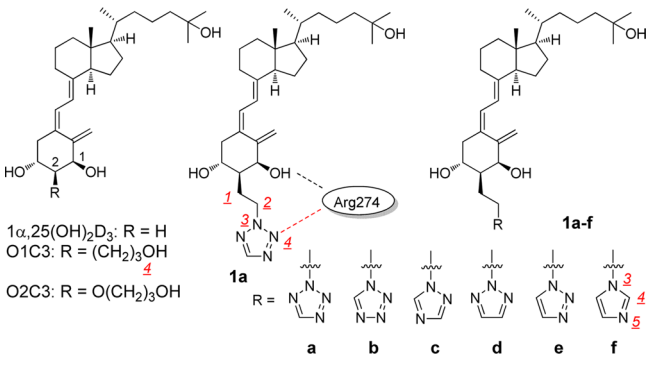


Figure 1. Structures of active vitamin D_3 and 2α-substituted synthetic analogues, O1C3, O2C3, and **1a−f**.

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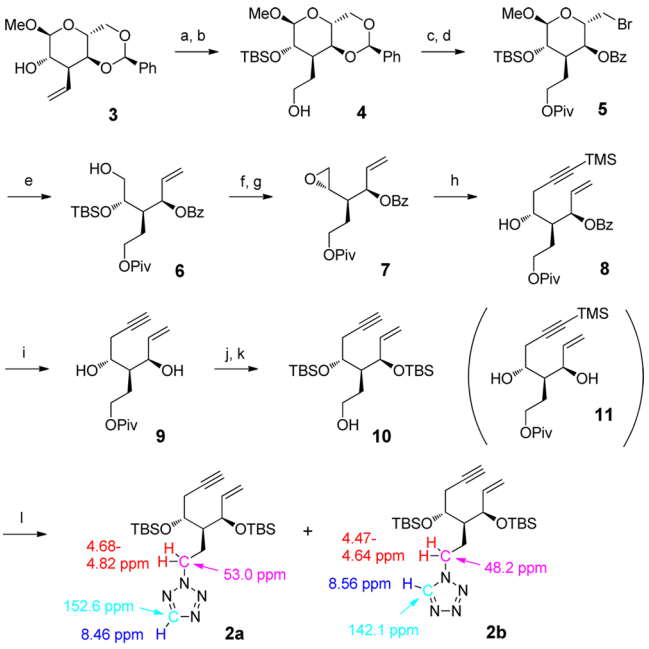
Synthesis of the A-ring precursors enyne **2a,b** is shown in Scheme 1. Previously, we reported the preparation of methyl 3-deoxy-3-*C*-ethenylaltropyranoside **3** from methyl α-D-glucoside,[9] and **3** was converted to alcohol **4** via a hydroboration−oxidation reaction after TBS protection at the 2-OH group of **3**. The OH group of **4**, protected by a pivaloyl group followed by our usual protocol, gave enyne **10** in good yield.[10] Briefly, NBS treatment of benzylidene acetal gave bromide **5**, which reacted with activated zinc powder in the presense of $NaBH_3CN$ to provide alcohol **6**. The alcohol was converted to epoxide **7** through sulfonylation of the primary alcohol followed by TBAF treatment. Ethynylation of **7** using lithium TMS-acetylide in the presence of $BF_3{\cdot}OEt_2$ in THF and subsequent solvolysis in K_2CO_3/MeOH supplied enyne **9**.[11] Bis-*O*-silylation with TBSOTf/2,6-lutidine followed by hydride reduction of the ester part afforded **10**.[10] Mitsunobu reaction between **10** and 1*H*-tetrazole gave the desired protected enynes **2a** and **2b** in 81% and 19% yields, respectively. When comparing ^{1}H NMR[12] and ^{13}C NMR[13] chemical shifts of appropriate methylene and methyne CHs of **2a** and **2b**, these two isomers were distinguishable, i.e., methylene protons of 2-*N*-alkyl substituted tetrazole **2a** appeared in a lower field (4.68−4.82 ppm) than those of the corresponding 1-*N*-alkyl substituted tetrazole **2b** (4.47−4.64 ppm). Instead, the methyne proton of **2a** appeared in a higher field (8.46 ppm) than that of **2b** (8.56 ppm) in ^{1}H NMR; the methylene and methyne carbons of **2a** (53.0 and 152.6 ppm) appeared in a lower field than those of **2b** (48.2 and 142.1 ppm) in ^{13}C NMR, respectively.[12,13]

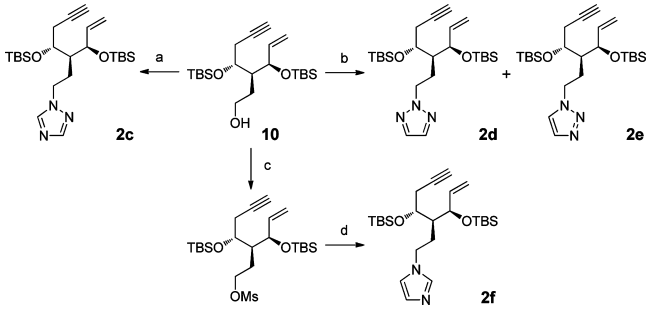
Scheme 1. Synthesis of A-Ring Precursors 2a,b[a]



[a]Reagents and conditions: (a) TBSOTf, 2,6-lutidine, CH_2Cl_2, 85%. (b) 9-BBN, THF, and then, H_2O_2, aq. NaOH, 92%. (c) PivCl, py, CH_2Cl_2, 95%. (d) NBS, $BaCO_3$, CCl_4, 87%. (e) Zn powder, $NaBH_3CN$, 1-PrOH−H_2O (5:1), 70%. (f) TsCl, py, 96%. (g) TBAF, THF, 80%. (h) BuLi, TMS-acetylene, $BF_3{\cdot}OEt_2$, THF, 78%. (i) K_2CO_3, MeOH, 71%. (j) TBSOTf, 2,6-lutidine, CH_2Cl_2, 93%. (k) DIBAL-H, CH_2Cl_2, 92%. (l) DIAD, PPh_3, 1*H*-tetrazole, THF, **2a** 81% and **2b** 19%.

Other azols, 1,2,4-triazole, 1,2,3-triazole, and imidazole, were also attached to enyne unit **10** through S_N2 reactions (Scheme 2). Alkylation of 1,2,3-triazole gave two products, and 2-*N*-alkyl triazole **2d** was the main product. For imidazole, it was necessary to produce imidazole anion by NaH since the *N*-alkylation reaction failed under the Mitsunobu conditions.

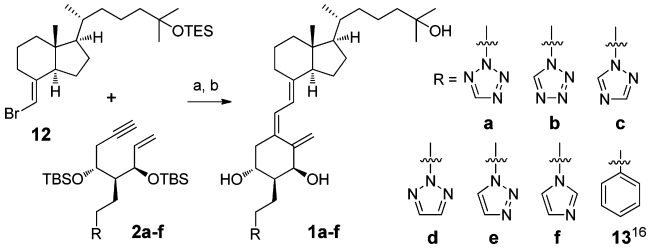
Scheme 2. Preparation of A-Ring Precursors 2c−f from Enyne Alcohol 10[a]



[a]Reagents and conditions: (a) DIAD, PPh_3, 1,2,4-triazole, THF, 94%. (b) DIAD, PPh_3, 1*H*-1,2,3-triazole, THF, **2d** 85% and **2e** 15%. (c) MsCl, Et_3N, CH_2Cl_2, 91%. (d) NaH, imidazole, THF, 71%.

As shown in Scheme 3, the CD-ring bromoolefin **12**[14] and enynes **2a−f** were coupled under Trost coupling conditions[15] followed by deprotection of *O*-silyl groups using TBAF to give the new 2α-heteroarylalkyl vitamin D_3 analogues **1a−f**, respectively.

Scheme 3. Trost Coupling between 12 and 2a−f[a]



[a]Reagents and conditions: (a) $Pd(PPh_3)_4$, Et_3N−toluene (1:1), reflux. (b) TBAF, THF, for two steps: **1a** 40%, **1b** 37%, **1c** 29%, **1d** 27%, **1e** 49%, and **1f** 48%.

The preliminary biological activities, hVDR binding affinity and osteocalcin promoter transactivation activity in human osteosarcoma (HOS) cells, of the new compounds **1a−f** were evaluated (Table 1). Among them, 2α-[2-(tetrazol-2-yl)ethyl]-

Table 1. hVDR Binding Affinity and Osteocalcin Promoter Transactivation Activity in HOS Cells

compd	relative hVDR binding affinity (%)	osteocalcin transactivation activity in HOS/SF cells (EC_{50}, nM)
1α,25(OH)$_2$D$_3$	100	0.026
1a	64	0.010
1b	<1	0.29
1c	4	0.15
1d	12	0.044
1e	<1	0.21
1f	2	0.68
13	1	–

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1α,25(OH)$_2$D$_3$ (**1a**) showed potent binding affinity for hVDR and greater transactivation activity (EC_{50} 9.8 pM) than that of the natural hormone, 1α,25(OH)$_2$D$_3$. Analogue **1d** showed moderate hVDR binding affinity and almost the same level of transactivation activity as 1α,25(OH)$_2$D$_3$. Compounds **1b**, **1e**, and **1f** showed no effective binding affinity for hVDR, just like 2α-phenethyl-1α,25(OH)$_2$D$_3$ (**13**, *R* = Ph in Scheme 3).[16]

Effects of the 2-*N*-alkylated tetrazole ring of **1a** on the in vitro biological results were strong, and next we tested the in vivo therapeutic effect of **1a** using an ovariectomized (OVX) rat as an osteoporosis model animal. Twelve-week-old Sprague–Dawley female rats were ovariectmized and fed a normal diet containing 1.0% Ca ad libitum for 4 weeks. The rats were then administrated **1a** at doses of 0.007 μg/kg/day and 0.02 μg/kg/day, 5 times a week for 4 weeks. Twenty-four hours after the final administration, blood was collected under anesthesia, the rats were euthanized, and bone mineral density (BMD) of the spine (L4–L5) bone mass was measured by dual X-ray absorption meter. The results of BMD and serum Ca density are shown in Tables 2 and 3. The newly synthesized analogue

Table 2. Therapeutic Effects of 1a Using Ovariectomized (OVX) Rat Model

	dose (μg/kg/day)	BMD (g/cm^2)	serum Ca (mg/dL)
control (sham)		0.231 ± 0.015	9.71 ± 0.27
control (OVX)		0.209 ± 0.011	9.25 ± 0.11
1a (OVX)	0.007	0.214 ± 0.012	9.41 ± 0.20
1a (OVX)	0.02	0.223 ± 0.017	9.66 ± 0.25

Table 3. Therapeutic Effects of 1α,25(OH)$_2$D$_3$ Using Ovariectomized (OVX) Rat Model

	dose (μg/kg/day)	BMD (g/cm^2)	serum Ca (mg/dL)
control (sham)		0.245 ± 0.010	9.72 ± 0.40
control (OVX)		0.209 ± 0.019	9.14 ± 0.24
1α,25(OH)$_2$D$_3$ (OVX)	0.025	0.218 ± 0.010	10.14 ± 0.37
1α,25(OH)$_2$D$_3$ (OVX)	0.1	0.228 ± 0.013	10.35 ± 0.27

1a (Table 2) showed an increase of BMD at low doses of 0.007 and 0.02 μg/kg/day without the significant side effect of increased serum calcium, i.e., hypercalcemia compared to 1α,25(OH)$_2$D$_3$ (Table 3).

Next, we studied the crystal structure of the complex between truncated hVDR LBD and **1a** to see new interactions of the tetrazole ring and amino acid residues of the LBD (Figure 2).[17] The hVDR LBD–**1a** complex was superimposed on the hVDR LBD–1α,25(OH)$_2$D$_3$ complex with an root-mean-square deviation (rmsd) of 0.45 Å on all Cα atoms. Clearly, one of the nitrogen atoms of the 2-*N*-alkylated tetrazole ring formed a direct hydrogen bond with Arg274 (2.99 Å distance), which was also shown in the hVDR LBD–O1C3 complex between the 2α-terminal OH group of O1C3 and Arg274 to stabilize the complex after missing two water molecules from the original hVDR LBD–1α,25(OH)$_2$D$_3$ complex.[5] Although we were also able to analyze the crystal structure of the hVDR LBD–**1b** complex and found that ligands **1a** and **1b** were superimposable in the hVDR (data not shown), it was difficult to explain why **1a** showed much higher binding affinity than **1b** from the crystal structures. 1-*N*-

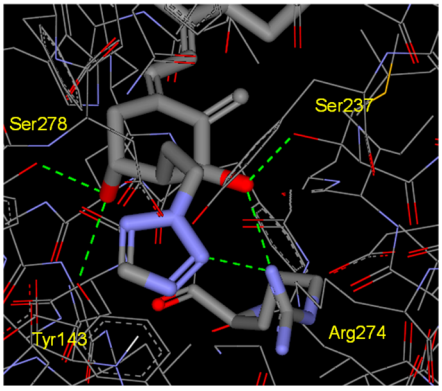

Figure 2. X-ray studies on hVDR LBD–**1a** complex. The A-ring part is magnified.

Alkylated tetrazole ring might have an equilibrium relationship between nitrogen and carbon atoms against Arg274 by free rotation of the 1-*N*-alkylated single bond in the solution. The dynamic difference in solution might reflect the lower binding affinity of the 1-*N*-alkylated tetrazole ring.

In summary, we synthesized 2α-heteroarylalkyl-1α,25-dihydroxyvitamin D$_3$ analogues **1a–f** for the first time and tested their biological activity. Among them, we found that **1a** showed higher osteocalcin promoter transactivation activity in HOS cells and a greater therapeutic effect in vivo in OVX rats on enhancing bone mineral density without hypercalcemic side effects than those of the natural hormone. X-ray cocrystallographic analysis of the hVDR LBD–**1a** complex confirmed the new hydrogen bond formation between the nitrogen atom of the tetrazole ring and Arg274. We believe that 2α-heteroarylalkyl active vitamin D analogues would be potent candidates for osteoporosis chemotherapy.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures and spectral data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

VDR, vitamin D receptor; LBD, ligand binding domain; OVX, ovariectomized; BMD, bone mineral density; TBS, *tert*-butyldimethylsilyl; 9-BBN, 9-borabicyclo[3.3.1]nonane; PivCl,

pivaloyl chloride; NBS, *N*-bromosuccinimide; TBAF, tetrabutylammonium fluoride; TMS, trimethylsilyl; DIBAL-H, diisobutylaluminium hydride; DIAD, diisopropyl azodicarboxylate

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(10) Previously, we synthesized **10** from D-xylose; see ref 6 (spectral data included).

(11) Under high dilution conditions (0.02 M), **8** was converted to **9** and **11** in 52% and 36% yields, respectively.

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(17) The Protein Data Bank accession numbers for the coordinates of the structures of the VDR complex with **1a** and **1b** reported in this letter are 4ITE and 4ITF, respectively.