

Synthesis of 2 α -Heteroarylalkyl Active Vitamin D₃ with Therapeutic Effect on Enhancing Bone Mineral Density in Vivo

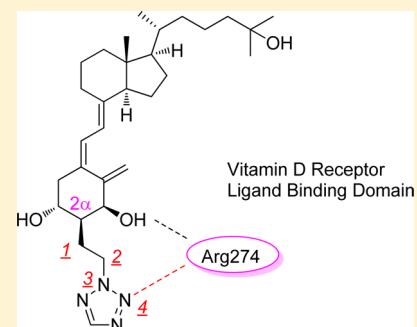
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S Supporting Information

ABSTRACT: 2 α -Heteroarylethyl-1 α ,25-dihydroxyvitamin D₃ 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 2 α -position, were synthesized. Among them, 2 α -[2-(tetrazol-2-yl)ethyl]-1 α ,25-dihydroxyvitamin D₃ 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 D₃. 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 D₃, 1 α ,25-dihydroxyvitamin D₃ [1 α ,25(OH)₂D₃], 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 1 α ,25(OH)₂D₃ plays pivotal roles in maintaining calcium and phosphorus homeostasis and bone remodeling. 1 α ,25(OH)₂D₃ 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 1 α ,25(OH)₂D₃ binds to the VDR is hydrogen bond formation between the 1 α -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-[1 α ,25(OH)₂D₃] complex by forming hydrogen bond networks.⁵ X-ray crystallographic analyses of the VDR-[2 α -(3-hydroxypropyl)-1 α ,25(OH)₂D₃ (O1C3)] and VDR-[2 α -(3-hydroxypropoxy)-1 α ,25(OH)₂D₃ (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 2 α -[2-(tetrazol-2-yl)ethyl]-1 α ,25(OH)₂D₃ (**1a**) and the related compounds **1b–f** since the number of atoms from the 2 α -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).

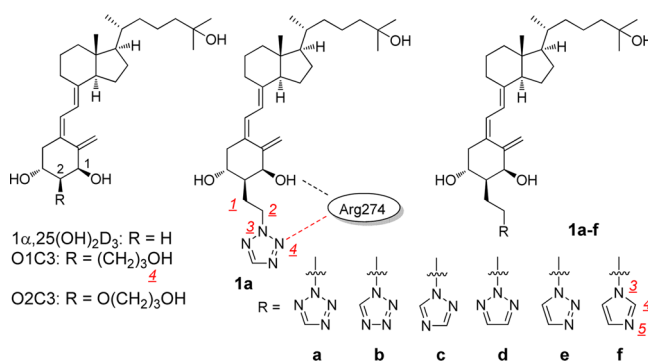


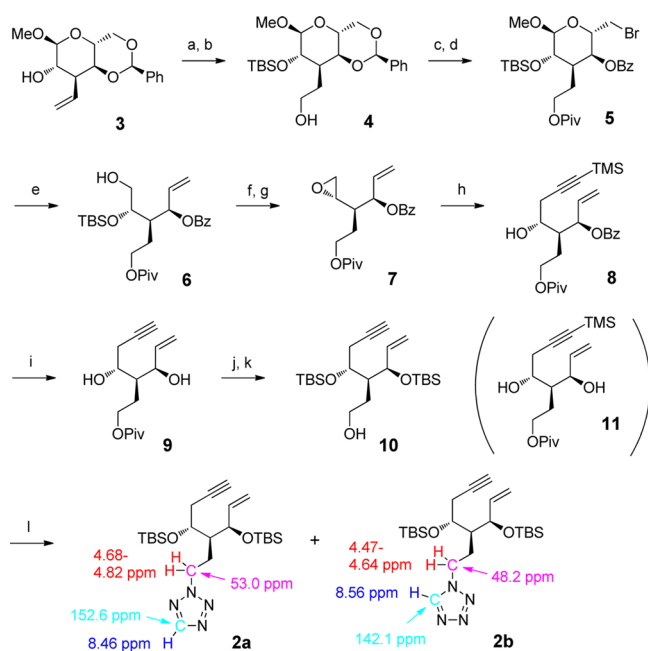
Figure 1. Structures of active vitamin D₃ and 2 α -substituted synthetic analogues, O1C3, O2C3, and **1a–f**.

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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-

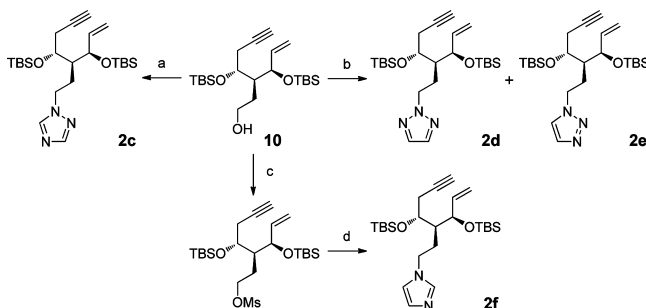
Scheme 1. Synthesis of A-Ring Precursors **2a,b^a**



deoxy-3-C-ethenylaltropyranoside **3** from methyl α -D-glucoside, 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 presence of NaBH₃CN 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 BF₃·OEt₂ in THF and subsequent solvolysis in K₂CO₃/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 ¹H NMR¹² and ¹³C NMR¹³ 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 ¹H 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 ¹³C NMR, respectively.^{12,13}

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

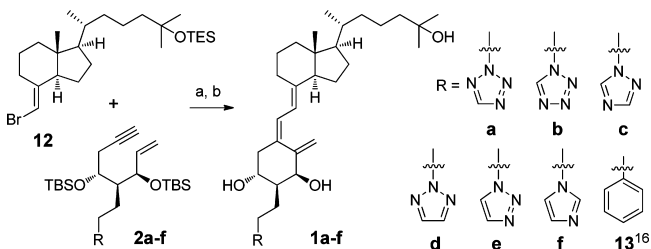
Scheme 2. Preparation of A-Ring Precursors **2c–f from Enyne Alcohol **10**^a**



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.

As shown in Scheme 3, the CD-ring bromoolefin **12**¹⁴ and enynes **2a–f** were coupled under Trost coupling conditions¹⁵

Scheme 3. Trost Coupling between **12 and **2a–f**^a**



followed by deprotection of O-silyl groups using TBAF to give the new 2*α*-heteroarylalkyl vitamin D₃ analogues **1a–f**, respectively.

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, 2*α*-[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) |
|--|------------------------------------|--|
| 1 <i>α</i> ,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 | – |

$1\alpha,25(\text{OH})_2\text{D}_3$ (**1a**) showed potent binding affinity for hVDR and greater transactivation activity (EC_{50} 9.8 pM) than that of the natural hormone, $1\alpha,25(\text{OH})_2\text{D}_3$. Analogue **1d** showed moderate hVDR binding affinity and almost the same level of transactivation activity as $1\alpha,25(\text{OH})_2\text{D}_3$. Compounds **1b**, **1e**, and **1f** showed no effective binding affinity for hVDR, just like 2α -phenethyl- $1\alpha,25(\text{OH})_2\text{D}_3$ (**13**, $R = \text{Ph}$ 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 ovariectomized 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 $\mu\text{g}/\text{kg}/\text{day}$ and 0.02 $\mu\text{g}/\text{kg}/\text{day}$, 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 ($\mu\text{g}/\text{kg}/\text{day}$) | 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 $1\alpha,25(\text{OH})_2\text{D}_3$ Using Ovariectomized (OVX) Rat Model

| | dose ($\mu\text{g}/\text{kg}/\text{day}$) | 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 |
| $1\alpha,25(\text{OH})_2\text{D}_3$ (OVX) | 0.025 | 0.218 ± 0.010 | 10.14 ± 0.37 |
| $1\alpha,25(\text{OH})_2\text{D}_3$ (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 $\mu\text{g}/\text{kg}/\text{day}$ without the significant side effect of increased serum calcium, i.e., hypercalcemia compared to $1\alpha,25(\text{OH})_2\text{D}_3$ (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– $1\alpha,25(\text{OH})_2\text{D}_3$ complex with a root-mean-square deviation (rmsd) of 0.45 Å on all C α 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 2 α -terminal OH group of O1C3 and Arg274 to stabilize the complex after missing two water molecules from the original hVDR LBD– $1\alpha,25(\text{OH})_2\text{D}_3$ 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*-

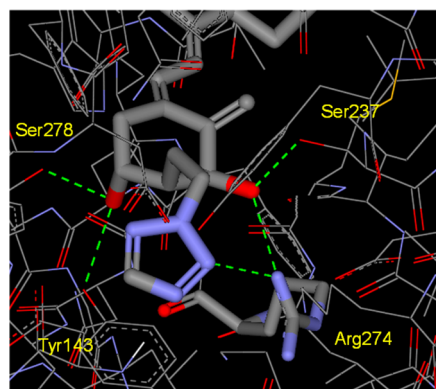


Figure 2. X-ray studies on hVDR LBD–**1a** complex. The A-ring part is magnified.

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 2 α -heteroarylalkyl- $1\alpha,25$ -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 2 α -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>.

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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, diisobutylaluminum hydride; DIAD, diisopropyl azodicarboxylate

(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.

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