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# SYNTHESIS AND PRELIMINARY BIOLOGICAL EVALUATION OF 2-[3-(TETRAZOLYL)PROPYL]-1α,25-DIHYDROXY-19-NORVITAMIN $D_3$

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**Abstract** – Four new 19-norvitamin $D_3$ analogs, 2α-[3-(tetrazol-1-yl)propyl]-, 2β-[3-(tetrazol-1-yl)propyl]-, 2α-[3-(tetrazol-2-yl)propyl]-, and 2β-[3-(tetrazol-2-yl)propyl]-1α,25-dihydroxy-19-norvitamin $D_3$ were synthesized. Among them, 2α-[3-(tetrazol-1-yl)propyl]-1α,25-dihydroxy-19-norvitamin $D_3$ showed weak binding affinity for vitamin D receptor (VDR) (2.6% of 1α,25-dihydroxyvitamin $D_3$: *ca*. 15% of 1α,25-dihydroxy-19-norvitamin $D_3$) and weak VDR transactivation activity in human osteosarcoma cells, which was determined by luciferase assays ($EC_{50}$ 7.3 nM, when 1α,25-dihydroxyvitamin $D_3$ 0.23 nM). Although the other three compounds could not act as VDR binders by evaluation of the competition assays, 2α-[3-(tetrazol-2-yl)propyl] analog showed weak transactivation activity ($EC_{50}$ 12.5 nM).

## INTRODUCTION

The hormonally active form of vitamin $D_3$, 1α,25-dihydroxyvitamin $D_3$ [1α,25$(OH)_2D_3$], modulates calcium homeostasis and bone mineralization as well as cellular growth, differentiation, apoptosis, anti-angiogenesis, anti-inflammation, and immune responses in many cells in a cell- and tissue-specific manner.[1-4] Actually, 1α,25$(OH)_2D_3$ and several synthetic analogs of 1α,25$(OH)_2D_3$ have been used clinically in the treatment of bone diseases, secondary hyperparathyroidism, psoriasis, and osteoporosis.[1,5] 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.[6]

The ligand binding domain (LBD) of the human VDR (hVDR) contains water molecules from the A-ring anchoring moiety to the surface of the protein, and X-ray co-crystallographic analyses of the VDR-[2α-(3-hydroxypropyl)-1α,25(OH)$_2$D$_3$ (O1C3)] and VDR-[2α-(3-hydroxypropoxy)-1α,25(OH)$_2$D$_3$ (O2C3)] complexes demonstrated that the terminal hydroxy group of both synthetic ligands (O1C3 and O2C3) forms a hydrogen bond with Arg274 and replaces one of the water molecules in the LBD of the hVDR to stabilize the complex;[7] therefore, O1C3 and O2C3 showed 3- and 1.8-times greater binding affinity for the VDR than the natural hormone, 1α,25(OH)$_2$D$_3$, respectively.[8,9] Previously, we reported synthesis and biological studies on six kinds of 2α-(2-heteroarylethyl)-1α,25(OH)$_2$D$_3$, in which 2α-[2-(tetrazol-2-yl)ethyl]-1α,25(OH)$_2$D$_3$ (**1**) showed higher osteocalcin promoter transactivation activity in human osteosarcoma (HOS) cells and a greater therapeutic effect *in vivo* in ovariectomised (OVX) rats for enhancing bone mineral density without hypercalcemic side effects than those of the natural hormone.[10] We also found that 1α,25-dihydroxy-19-norvitamin D$_3$ (**2**) derivative with the 2α-(3-hydroxypropyl) group, MART-10, was an excellent VDR binder.[11] MART-10 was non-calcemic under an effective dose of 0.3 μg/kg body weight and was more potent than 1α,25(OH)$_2$D$_3$ in repressing pancreatic cancer growth *in vivo*.[2] Here we studied the synthesis of four new 19-norvitamin D$_3$ analogs, 2α-[3-(tetrazol-1-yl)propyl]-, 2β-[3-(tetrazol-1-yl)propyl]-, 2α-[3-(tetrazol-2-yl)propyl]-, and 2β-[3-(tetrazol-2-yl)propyl]-1α,25-dihydroxy-19-norvitamin D$_3$ (**3**-**6**, Figure 1) and the effects of the heteroaromatic ring at the C2 position of MART-10 instead of the terminal OH group on binding to the hVDR and transactivation activity in HOS cells.

## RESULTS AND DISCUSSION

Synthesis of the target compounds **3**-**6** was accomplished via the convergent coupling method between A-rings (**7(N2),7(N1)**) and CD-ring (**14**) precursors using Julia olefination.[11,12] The A-ring fragments **7(N2)** and **7(N1)** were synthesized from the known compound **8**, which was available from (–)-quinic acid.[11] The primary hydroxy group was protected by the pivaloyl group, and subsequent hydroboration-oxidation reaction gave alcohol **10**. Under Mitsunobu conditions, *N*-alkylation of 1*H*-tetrazole with **10** proceeded smoothly to give regio-isomers **11(N2)** (major) and **11(N1)** (minor) in good yields. The regio-isomers **11(N2)** and **11(N1)** were separated from each other with HPLC, and the *N*2-alkyl and *N*1-alkyl structures were able to be determined using $^1$H NMR[14] and $^{13}$C NMR[15] as described in the previous paper.[10] Each pivaloyl ester was reduced by DIBAL-H, and the resulting vicinal diol was treated with $NaIO_4$ to yield ketone **7(N2)** or **7(N1)** (Scheme 1).

Horner-Emmons reaction of the known ketone **12**[13] with triethyl phosphonoacetate/NaH in THF afforded a two-carbon elongated ester, which was reduced by DIBAL-H to give allyl alcohol **13**. Subsequent

sulfonation with 2-mercaptobenzothiazole under Mitsunobu conditions followed by Mo-catalyzed oxidation furnished CD-ring sulfone **14** in moderate yield (Scheme 2).

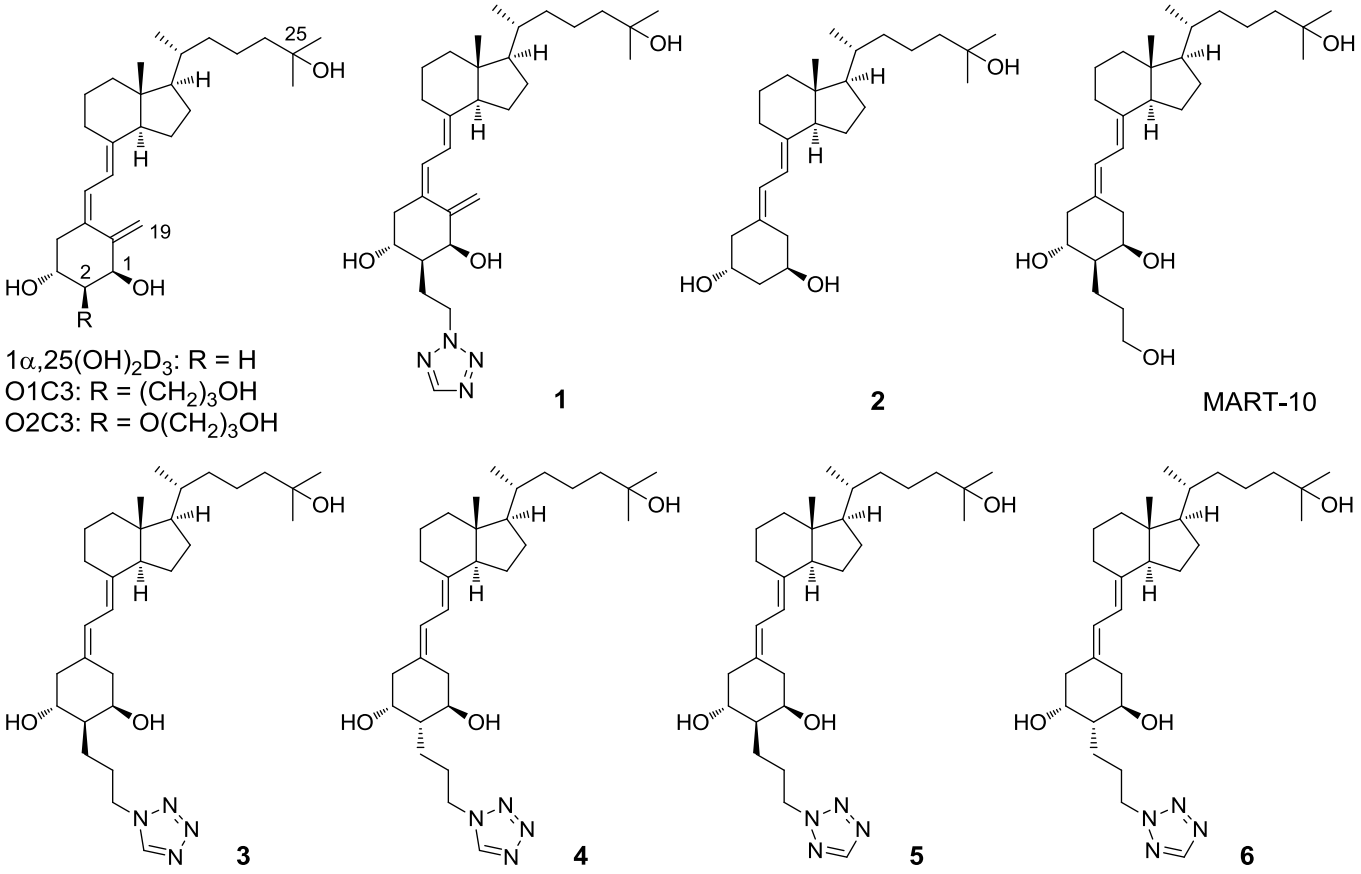
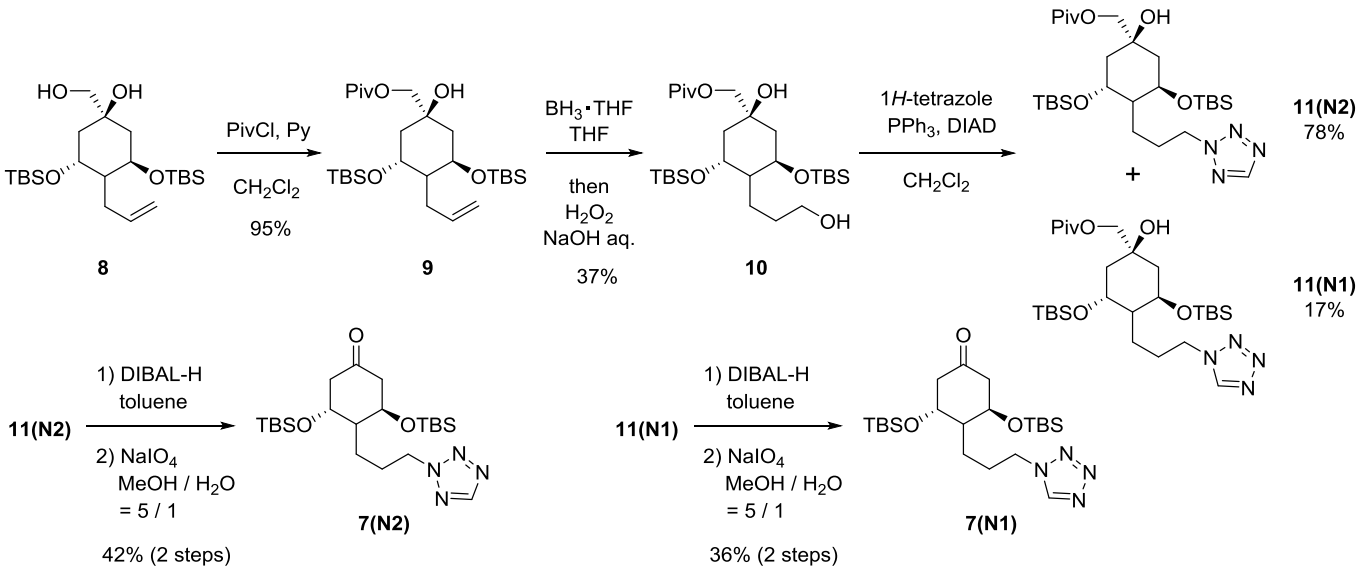
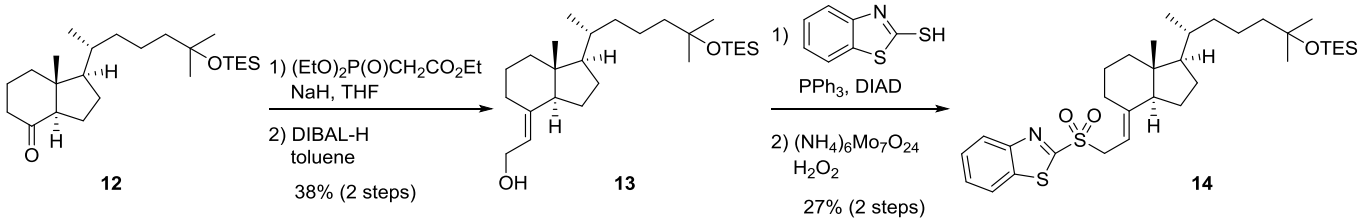



**Figure 1.** Structures of $1\alpha,25(OH)_2D_3$, its analogs of O1C3, O2C3, and **1** as well as the 19-nor-$1\alpha,25(OH)_2D_3$ analog of MART-10 and the derivatives with heterocyclic ring of **3-6**

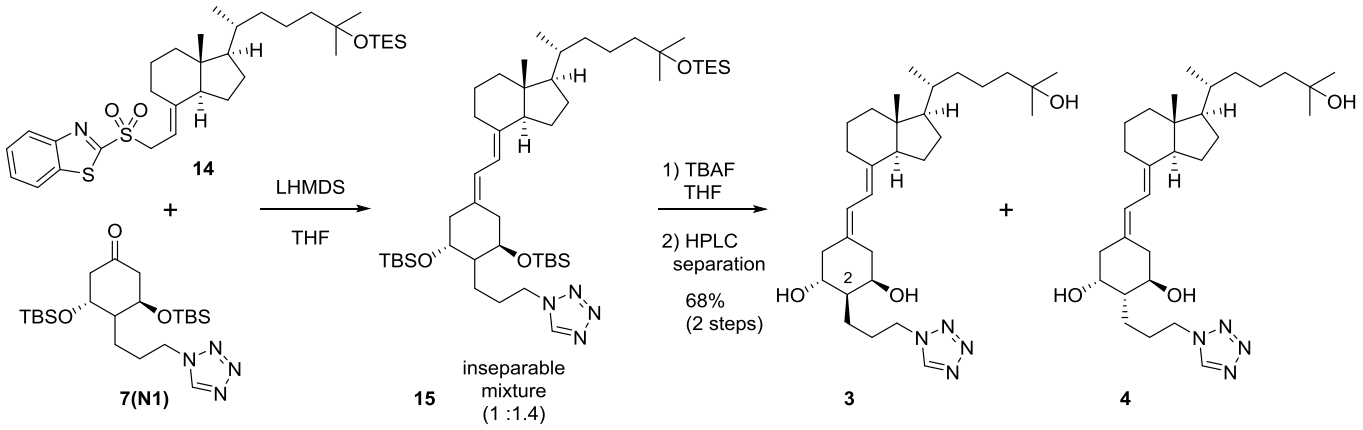



**Scheme 1.** Synthesis of A-ring ketones **7(N2)** and **7(N1)**




**Scheme 2.** Synthesis of the CD-ring moiety

The coupling reaction between A-ring ketone **7(N1)** and CD-ring sulfone **14** using Julia olefination gave an inseparable C2-diastereo mixture of **15α**(N1) and **15β**(N1) with the *O*-protecting groups in 1:1.4 ratio in 89% yield, and subsequent TBAF treatment afforded deprotected 2α-[3-(tetrazol-1-yl)propyl]- (**3**) and 2β-[3-(tetrazol-1-yl)propyl]-1α,25-dihydroxy-19-norvitamin $D_3$ (**4**), which were separated from each other with HPLC (Scheme 3). Stereochemistry of the C2 position was determined by NOE experiments




**Scheme 3.** Julia coupling reaction, deprotection, and separation steps for **3** and **4**

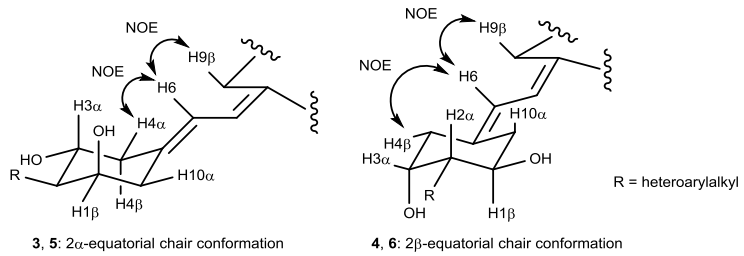


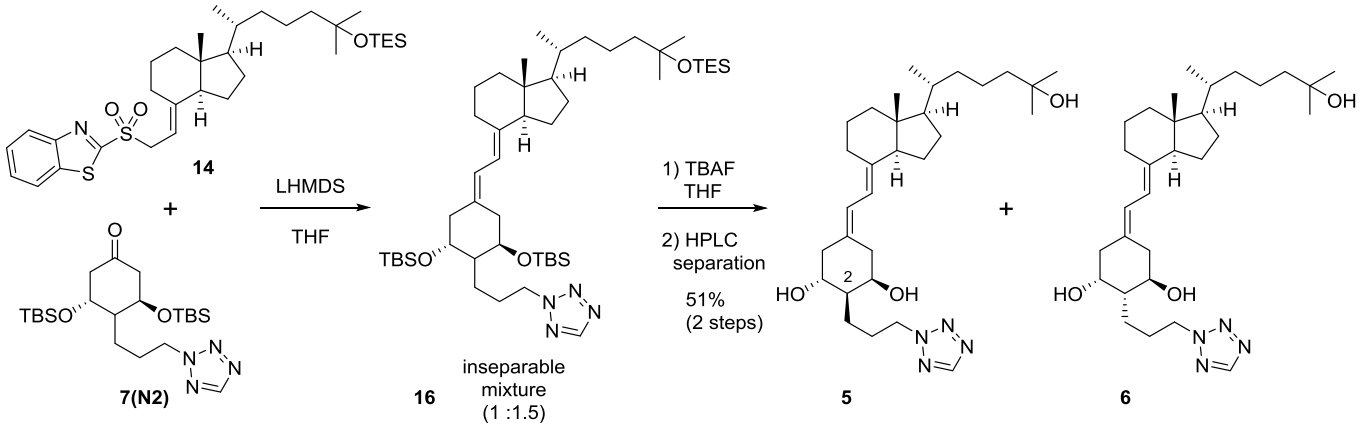

**Figure 2.** Preferred conformation of the 2-substituted 19-norvitamin $D_3$ analogs **3**-**6**. Left (**3** and **5**, 2α-equatorial chair conformation): NOE between H6 and H4α was observed, 3.6% for R = 3-(tetrazol-1-yl)propyl (**3**: $J_{3\alpha,4\beta}$ = 9.6, $J_{1\beta,10\alpha}$ = 3.7 Hz) and 3.5% for R = 3-(tetrazol-2-yl)propyl (**5**: $J_{3\alpha,4\beta}$ = 9.6, $J_{1\beta,10\alpha}$ = 4.1 Hz). Right (**4** and **6**, 2β-equatorial chair conformation): NOE between H6 and H4β was observed, 4.9% for R = 3-(tetrazol-1-yl)propyl (**4**: $J_{3\alpha,4\beta}$ = 3.2, $J_{1\beta,10\alpha}$ = 10.1 Hz), 4.8% for R = 3-(tetrazol-2-yl)propyl (**6**: $J_{3\alpha,4\beta}$ = 3.2, $J_{1\beta,10\alpha}$ = 10.1 Hz)

and $^1$H NMR chemical shifts as compared with the known C2-substituted 19-norvitamin $D_3$ analogs.[11] (Figure 2, Table 1). The other A-ring ketone **7(N2)** was also converted to the target compounds 2α-[3-(tetrazol-2-yl)propyl]- (**5**) and 2β-[3-(tetrazol-2-yl)propyl]-1α,25-dihydroxy-19-norvitamin $D_3$ (**6**) in the same manner (Scheme 4).

**Table 1.** $^1$H NMR Chemical shifts of representative protons of 2-[3-(tetrazole-1-yl)propyl] and 2-[3-(tetrazole-2-yl)propyl] analogs **3**-**6**

| | 2α-Me[a] | 2α-Et[b] | 2α-allyl[c] | 3 | 5 | 2β-Me[a] | 2β-Et[b] | 2β-allyl[c] | 4 | 6 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1β-H | 3.96 | 4.14 | 4.08 | 4.06 | 4.07 | 3.51 | 3.54 | 3.64 | 3.52 | 3.53 |
| 3α-H | 3.61 | 3.64 | 3.70 | 3.62 | 3.62 | 3.90 | 4.09 | 4.02 | 4.01 | 4.02 |
| 4α-H | 2.60 | 2.60 | 2.61 | 2.59 | 2.58 | 2.43 | 2.34-2.42 | 2.40 | 2.42 | 2.41 |
| 4β-H | 2.13 | 2.14 | 2.13-2.17 | 2.12-2.19 | 2.12-2.19 | 2.34 | 2.34-2.42 | 2.34 | 2.34 | 2.34 |
| 10α-H | 2.80 | 2.87 | 2.86 | 2.88 | 2.86 | | | 1.88-1.92 | 1.85-1.93 | 1.85-1.93 |
| 10β-H | 2.22 | 2.15 | 2.13-2.17 | 2.12-2.19 | 2.12-2.19 | 3.08 | 3.10 | 3.11 | 3.09 | 3.09 |
| 7-H | 5.82 | 5.82 | 5.81 | 5.79 | 5.80 | 5.87 | 5.87 | 5.87 | 5.85 | 5.85 |
| 6-H | 6.37 | 6.38 | 6.35 | 6.38 | 6.37 | 6.26 | 6.26 | 6.26 | 6.25 | 6.25 |
| 9β-H | 2.80 | 2.80 | 2.80 | 2.79 | 2.79 | 2.80 | 2.80 | 2.80 | 2.79 | 2.79 |
| 18-H | 0.54 | 0.53 | 0.53 | 0.52 | 0.52 | 0.55 | 0.55 | 0.55 | 0.54 | 0.54 |
| 21-H | 0.94 | 0.94 | 0.94 | 0.94 | 0.94 | 0.94 | 0.94 | 0.94 | 0.94 | 0.94 |
| 26,27-H | 1.13 | 1.22 | 1.22 | 1.22 | 1.22 | 1.22 | 1.22 | 1.22 | 1.22 | 1.22 |

[a]ref. 16. [b]ref. 17. [c]ref. 11.




**Scheme 4.** Julia coupling reaction, deprotection, and separation steps for **5** and **6**

Next, preliminary biological activity was evaluated for the new four 19-norvitamin $D_3$ analogs **3**-**6**, and the results are shown in Table 2. According to the hVDR competition assay, only 2α-[3-(tetrazol-1-yl)propyl]-1α,25-dihydroxy-19-norvitamin $D_3$ (**3**) showed weak binding affinity for hVDR, i.e., 2.6% of the natural hormone, 1α,25$(OH)_2D_3$. Although MART-10 showed almost the same level of VDR binding affinity as 1α,25$(OH)_2D_3$, 1α,25-dihydroxy-19-norvitamin $D_3$ (**2**) showed 17%

VDR binding affinity compared with 1α,25(OH)$_2$D$_3$;[11] therefore, **3** exhibited *ca*. 15% of the VDR binding affinity of **2**. In contrast, transactivation assay demonstrated that **3** and **5** showed weak VDR transactivation activity in HOS cells, EC$_{50}$ 7.3 nM and 12.5 nM with 1α,25-dihydroxyvitamin D$_3$ 0.23 nM, respectively. As compared with the previous results of **1**,[10] two methylene lengths between tetrazole and the C2 position of the A-ring was better for biological responses than the three methylene lengths of **3** and **5**, even though **3** and **5** were 19-nor analogs.

**Table 2.** Relative hVDR binding affinity and transactivation activity through hVDR in HOS cells

| Compound | Relative hVDR binding affinity (%) | Transactivation activity through hVDR in HOS cells (EC$_{50}$, nM) |
|---|---|---|
| 1α,25(OH)$_2$D$_3$ | 100 | 0.23 |
| **1** | 64 | 0.010[a)] |
| **3** | 2.6 | 7.3 |
| **4** | < 1 | > 80.7 |
| **5** | < 1 | 12.5 |
| **6** | < 1 | > 61.9 |

[a)] Osteocalcin promoter transactivation activity in HOS cells, when EC$_{50}$ of 1α,25(OH)$_2$D$_3$ was 0.026 nM: see, ref. 10.

In summary, we synthesized new 19-norvitamin D$_3$ analogs **3-6** with a tetrazole ring at the C2 position possessing the propyl linker, and the 2α-derivatives **3** and **5** showed higher biological activity than the 2β-counterparts **4** and **6**, respectively. The terminal OH group of MART-10 (Figure 1), which showed the highly potent anti-cancer activities *in vitro* and *in vivo*,[2] was important for strong binding for VDR, since corresponding compounds **3** and **5** with the tetrazole ring did not show efficient VDR binding affinity. The methylene lengths between the A-ring and the tetrazole ring was also important for great binding affinity for VDR, since compound **1** showed potent VDR binding affinity and anti-osteoporosis activity *in vivo*.[10] These findings will be important for the design of vitamin D analogs for anti-cancer, anti-osteoporosis, and regulating immune system chemotherapy.[1]

## EXPERIMENTAL

$^1$H and $^{13}$C NMR spectra were recorded on JEOL JNM-ECS 400 NMR (400 MHz) spectrometer. $^1$H NMR spectra were referenced with (CH$_3$)$_4$Si (δ 0.00 ppm) as an internal standard. $^{13}$C NMR spectra were

referenced with deuterated solvent (δ 77.0 ppm for $CDCl_3$). IR spectra were recorded on a JASCO FT-IR-4200 Fourier Transform Infrared Spectrophotometer. High resolution mass spectra were obtained on a Shimadzu LCMS-IT-TOF mass spectrometer in positive electrospray ionization (ESI) mode. Optical rotations were measured on a JASCO P-2200 digital polarimeter. Column chromatography was performed on silica gel 60N (Kanto Chemical Co., Inc., 40-63 μm or 100-210 μm). High performance liquid chromatography (HPLC) was carried out on a Shimadzu HPLC system consisting of the following equipments: pump, LC-6AD; detector, SPD-10A; column, YMC-Pack ODS-A. All experiments were performed under anhydrous conditions in an atmosphere of argon, unless stated otherwise.

**(3*R*,5*R*)-3,5-Bis(*tert*-butyldimethylsilyloxy)-1-hydroxy-4-(3-hydroxypropyl)cyclohexylmethyl pivalate (10)**

To a solution of compound **8**[11] (101.0 mg, 0.234 mmol) in $CH_2Cl_2$ (3.0 mL) were added pyridine (0.06 mL, 0.703 mmol) and PivCl (0.06 mL, 0.469 mmol) at 0 °C, and the mixture was stirred at rt for 7 h. To the mixture was added sat. $NH_4Cl$ aq. at 0 °C, and the aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over $Na_2SO_4$, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 6/1) to give the inseparable diastereomeric mixture of compound **9** (114.5 mg, 0.222 mmol, 95%) as a colorless oil.

To a solution of the compound **9** (114.5 mg, 0.222 mmol) in THF (3.0 mL) was added $BH_3$·THF (0.95 M solution in THF, 0.59 mL, 0.556 mmol) at 0 °C, and the mixture was stirred at rt for 2 h. To the reaction mixture were added 3 M NaOH (0.5 mL) and 30% $H_2O_2$ (0.5 mL), and the mixture was stirred at rt for 1 h. To the mixture was added sat. $Na_2S_2O_3$ aq. at 0 °C, and the aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over $Na_2SO_4$, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 3/1) to afford the inseparable diastereomeric mixture of alcohol **10** (43.3 mg, 0.0813 mmol, 37%) as a colorless oil.

**(3*R*,5*R*)-4-[3-(2*H*-Tetrazol-2-yl)propyl]-3,5-bis(*tert*-butyldimethylsilyloxy)-1-hydroxycyclohexyl-methyl pivalate (11(N2))** and **(3*R*,5*R*)-4-[3-(1*H*-tetrazol-1-yl)propyl]-3,5-bis(*tert*-butyldimethyl-silyloxy)-1-hydroxycyclohexylmethyl pivalate (11(N1))**

To a solution of alcohol **10** (41.8 mg, 0.0784 mmol) in THF (2.0 mL) were added $PPh_3$ (123.4 mg, 0.470 mmol), 1*H*-tetrazole (24.7 mg, 0.353 mmol) and DIAD (1.9 M solution in toluene, 0.25 mL, 0.470 mmol) at 0 °C, and the mixture was stirred at the same temperature for 1 h. The mixture was concentrated, and the residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 4/1-1/1) to give **11(N2)** (35.9 mg, 0.0613 mmol, 78%) and **11(N1)** (7.9 mg, 0.0135 mmol, 17%), as an inseparable diastereomeric mixture and as a colorless oil.

**(3*R*,5*R*)-4-[3-(2*H*-Tetrazol-2-yl)propyl]-3,5-bis(*tert*-butyldimethylsilyloxy)cyclohexan-1-one (7(N2))**

To a solution of compound **11(N2)** (35.9 mg, 0.0613 mmol) in toluene (2.0 mL) was added DIBAL-H (1.01 M solution in toluene, 0.15 mL, 0.153 mmol) at -78 °C, and the mixture was stirred at the same temperature for 2 h. To the reaction mixture was added sat. Rochell Salt aq. (2.0 mL) at -78 °C, and the mixture was stirred for 16 h at rt. The aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over $Na_2SO_4$, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 2/1) to give (3*R*,5*R*)-4-[3-(2*H*-tetrazol-2-yl)propyl]-3,5-bis(*tert*-butyldimethylsilyloxy)-1-hydroxymethylcyclohexan-1-ol (22.6 mg, 0.0451 mmol, 74%) as a colorless oil.

To a solution of the above diol (22.6 mg, 0.0451 mmol) in MeOH (2.0 mL) and $H_2O$ (0.4 mL), $NaIO_4$ (28.9 mg, 0.135 mmol) was added at 0 °C, and the mixture was stirred at rt for 3 h. To the mixture was added brine at 0 °C, and the mixture was concentrated. To the residue were added brine and EtOAc, and the aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over $Na_2SO_4$, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 4/1) to give compound **7(N2)** (12.1 mg, 0.0257 mmol, 57%) as a colorless oil.

$[\alpha]_D^{20}$ -42.2 (c 1.08, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$) δ 0.014 (s, 3H), 0.024 (s, 3H), 0.042 (s, 3H), 0.056 (s, 3H), 0.84 (s, 9H), 0.86 (s, 9H), 1.45-1.75 (m, 3H), 2.01-2.24 (m, 2H), 2.33 (dd, *J* = 8.7, 14.2 Hz, 1H), 2.43 (dd, *J* = 4.2, 14.6 Hz, 1H), 2.47 (dd, *J* = 4.2, 14.6 Hz, 1H), 2.61 (dd, *J* = 4.6, 14.2 Hz, 1H), 3.99 (ddd, *J* = 4.6, 8.3, 8.7, Hz, 1H), 4.29 (ddd, *J* = 2.3, 6.4, 6.4 Hz, 1H), 4.67 (ddd, *J* = 6.9, 6.9, 13.8 Hz, 1H), 4.72 (ddd, *J* = 6.9, 6.9, 13.8 Hz, 1H), 8.50 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ -5.1, -4.9, -4.4, -4.3, 18.0, 18.0, 24.0, 25.7(3C), 25.8(3C), 27.7, 48.6, 48.7, 49.6, 53.3, 68.2, 70.1, 152.9, 207.3; IR (neat) 1720, 1466, 1362, 1254 cm$^{-1}$. ESI-HRMS calcd for $C_{22}H_{44}N_4O_3Si_2$ ($[M+Na]^+$) 491.2850, found 491.2850.

**(3*R*,5*R*)-4-[3-(1*H*-Tetrazol-1-yl)propyl]-3,5-bis(*tert*-butyldimethylsilyloxy)cyclohexan-1-one (7(N1))**

The title compound **7(N1)** (2.3 mg) was obtained using a similar procedure to that described above for **7(N2)**, starting from compound **11(N1)** (7.9 mg, 0.0135 mmol), as a colorless oil (36% for two steps).

$[\alpha]_D^{20}$ -41.8 (c 1.05, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$) δ 0.013 (s, 3H), 0.031 (s, 3H), 0.052 (s, 3H), 0.062 (s, 3H), 0.84 (s, 9H), 0.87 (s, 9H), 1.48-1.75 (m, 3H), 1.96-2.19 (m, 2H), 2.33 (dd, *J* = 8.6, 14.6 Hz, 1H), 2.43 (dd, *J* = 4.2, 14.6 Hz, 1H), 2.47 (dd, *J* = 4.2, 14.6 Hz, 1H), 2.62 (dd, *J* = 4.6, 14.2 Hz, 1H), 3.99 (ddd, *J* = 4.6, 8.7, 8.7, Hz, 1H), 4.28 (ddd, *J* = 2.3, 6.4, 6.4 Hz, 1H), 4.44 (ddd, *J* = 6.9, 6.9, 13.8 Hz, 1H), 4.50 (ddd, *J* = 6.9, 6.9, 13.8 Hz, 1H), 8.50 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ -5.0, -4.8, -4.3, -4.2, 18.0, 18.0, 24.1, 25.7(3C), 25.8(3C), 28.3, 48.6, 48.6, 48.7, 49.6, 68.3, 70.2, 141.4, 207.1; IR (neat) 1720, 1466, 1362, 1254 cm$^{-1}$. ESI-HRMS calcd for $C_{22}H_{44}N_4O_3Si_2$ ($[M+Na]^+$) 491.2850, found 491.2846.

**2-[(1*R*,2*E*,6*R*,7*R*)-7-[(*R*)-6-Triethylsilyloxy-6-methylheptan-2-yl]-6-methylbicyclo[4.3.0]nonan-2-ylidene]ethanol (13)**

To a suspension of NaH (109.0 mg, 4.56 mmol) in THF (5.0 mL) was added $(EtO)_2P(O)CH_2CO_2Et$ (1.1 mL, 5.32 mmol) at 0 °C, and the mixture was stirred at rt for 2 h. To the above mixture was added compound **12**[13] (300.0 mg, 0.76 mmol) in THF (2.0 mL) at 0 °C, and the mixture was stirred at the same temperature for 14 h. To the reaction mixture was added sat. $NH_4Cl$ aq. at 0 °C, and the aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over $Na_2SO_4$, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 40/1) to give ester (152.7 mg, 0.33 mmol, 43%) as a colorless oil. This was used without further purification for the next step.

To a solution of the above ester (152.7 mg, 0.33 mmol) in toluene (3.0 mL) was added DIBAL-H (1.01 M solution in toluene, 0.81 mL, 0.82 mmol) at -78 °C, and the mixture was stirred at the same temperature for 1 h. To the reaction mixture were added $Et_2O$ (4.0 mL) and sat. Rochell Salt aq. (10.0 mL) at -78 °C, and the mixture was stirred for 19 h at rt. The aqueous layer was extracted with $Et_2O$. The organic layer was washed with brine, dried over $Na_2SO_4$, and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 6/1) to give **13** (123.8 mg, 0.294 mmol, 89%) as a colorless oil.

$[\alpha]_D^{20}$ +35.5 (c 1.01, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$) δ 0.55 (s, 3H), 0.56 (q, *J* = 7.8 Hz, 6H), 0.93 (d, *J* = 6.4 Hz, 3H), 0.95 (t, *J* = 7.8 Hz, 9H), 0.97-1.08 (m, 1H), 1.19 (s, 6H), 1.21-1.71 (m, 14H), 1.87-2.02 (m, 3H), 2.63 (dd, *J* = 3.6, 12.4 Hz, 1H), 4.18 (dd, *J* = 6.9, 12.4 Hz, 1H), 4.23 (dd, *J* = 6.9, 12.4 Hz, 1H), 5.22 (dd, *J* = 6.9, 6.9 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 6.9(3C), 7.2(3C), 11.9, 18.9, 20.9, 22.3, 23.6, 27.7, 28.8, 29.9, 30.1, 36.2, 36.5, 40.5, 45.4, 45.6, 55.7, 56.7, 58.7, 73.5, 119.3, 143.8; IR (neat) 3317, 1670, 1462, 1416, 1377 $cm^{-1}$. ESI-HRMS calcd for $C_{26}H_{50}O_2Si$ ($[M+Na]^+$) 445.3478, found 445.3460.

**(1*R*,2*E*,6*R*,7*R*)-2-[2-(Benzothiazole-2-sulfonyl)ethylidene]-7-[(*R*)-6-triethylsilyloxy-6-methylheptan-2-yl]-6-methylbicyclo[4.3.0]nonane (14)**

To a solution of compound **13** (122.0 mg, 0.290 mmol) in $CH_2Cl_2$ (3.0 mL) were added 2-mercaptobenzothiazole (72.0 mg, 0.430 mmol), $PPh_3$ (113.0 mg, 0.430 mmol) and DIAD (0.06mL, 0.29 mmol) at 0 °C and the mixture was stirred at the same temperature for 1 h. The mixture was concentrated. To the residue in EtOH (1.6 mL) were added 30% $H_2O_2$ (0.16 mL) and $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (72.0 mg, 0.058 mmol) at 0 °C, and the mixture was stirred at rt for 2 h. To the mixture was added sat. $Na_2SO_3$ aq. at 0 °C, and the aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over $Na_2SO_4$, and concentrated. The residue was purified by flash column

chromatography on silica gel (hexane/EtOAc = 10/1) to give **14** (47.8 mg, 0.079 mmol, 27%) as a colorless oil.

$[\alpha]_D^{20}$ -7.6 (c 1.02, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$) δ 0.26 (s, 3H), 0.55 (q, *J* = 7.8 Hz, 6H), 0.85 (d, *J* = 6.0 Hz, 3H), 0.94 (t, *J* = 7.8 Hz, 9H), 1.18 (s, 6H), 0.86-1.90 (m, 18H), 2.55 (d, *J* = 12.8 Hz, 1H), 4.21 (dd, *J* = 6.9, 14.2 Hz, 1H), 4.43 (dd, *J* = 9.2, 14.2 Hz, 1H), 5.02 (dd, *J* = 6.9, 9.2 Hz, 1H), 7.58 (ddd, *J* = 1.4, 7.3, 8.3 Hz, 1H), 7.63 (ddd, *J* = 1.4, 7.3, 8.3 Hz, 1H), 8.00 (dd, *J* = 1.4, 7.3 Hz, 1H), 8.21 (dd, *J* = 1.4, 8.3 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 6.9(3C), 7.2(3C), 11.6, 18.8, 20.9, 22.2, 23.3, 27.5, 29.1, 29.9, 30.1, 36.0, 36.4, 40.1, 45.5, 45.8, 54.0, 56.1, 56.5, 73.5, 104.2, 122.3, 125.4, 127.7, 128.0, 137.1, 152.2, 152.9, 166.0; IR (neat) 1740, 1659, 1554, 1466 $cm^{-1}$. ESI-HRMS calcd for $C_{33}H_{53}NO_3S_2Si$ ($[M+Na]^+$) 626.3134, found 626.3134.

**2α-[3-(Tetrazol-1-yl)propyl]-1α,25-dihydroxy-19-norvitamin $D_3$ (3) and 2β-[3-(tetrazol-1-yl)-propyl]-1α,25-dihydroxy-19-norvitamin $D_3$ (4)**

To a solution of **14** (331.1 mg, 0.55 mmol) in THF (1.0 mL) was added LHMDS (1.0 M solution in THF, 0.53 mL, 0.53 mmol) at -78 °C, and the mixture was stirred at the same temperature for 30 min. To this solution was added **7(N1)** (129.0 mg, 0.28 mmol) in THF (1.0 mL), and the mixture was stirred at -78 °C for 1 h, and then warmed to rt in 2 h. To the mixture was added sat. $NH_4Cl$ aq. at 0 °C, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $Na_2SO_4$ and concentrated. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 2/1) to give a diastereomeric mixture of the protected coupling product (196.1 mg, oil), which was used in the next step without further purification.

To the solution of the coupling product (191.6 mg) in THF (2.5 mL) was added TBAF (1.0 M solution in THF, 1.12 mL, 1.12 mmol) at 0 °C, and the mixture was stirred at rt for 12 h. To the mixture was added sat. $NH_4Cl$ aq. at 0°C, and the aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over $Na_2SO_4$, and concentrated. The residue was purified by column chromatography on silica gel ($CH_2Cl_2$/MeOH = 20/1) to give 96.7 mg of the mixture of **3** and **4** (1:1.4) as a colorless oil (68% for two steps). The products were separated by preparative HPLC (YMC-Pack ODS-A 250×20 mm, MeCN/$H_2O$= 9/1, 10 mL/min) for biological evaluations.

**3**: $[\alpha]_D^{20}$ +32.7 (c 1.00, $CHCl_3$); UV(EtOH) $\lambda_{max}$ 243, 252, 261 nm, $\lambda_{min}$ 247, 259 nm; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.52 (s, 3H), 0.94 (d, *J* = 6.4 Hz, 3H), 1.02-1.12 (m, 1H), 1.22 (s, 6H), 1.24-2.22 (m, 25H), 2.59 (dd, *J* = 4.6, 12.4 Hz, 1H), 2.79 (dd, *J* = 4.1, 12.4 Hz, 1H), 2.88 (dd, *J* = 3.7, 14.2 Hz, 1H), 3.62 (ddd, *J* = 4.7, 9.6, 9.6 Hz, 1H), 4.06 (ddd, *J* = 3.7, 6.5, 6.5 Hz, 1H), 4.48 (t, J = 7.3 Hz, 2H), 5.79 (d, *J* = 11.0 Hz, 1H), 6.38 (d, *J* = 11.0 Hz, 1H), 8.69 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 12.1, 18.9, 20.9, 22.3, 23.5,

24.6, 27.6, 27.7, 29.0, 29.3, 29.4, 35.8, 36.2, 36.5, 40.5, 44.5, 45.8, 45.9, 48.6, 48.7, 56.4, 56.6, 68.6, 71.2, 71.3, 115.1, 124.5, 130.6, 142.6, 143.7; IR (neat) 3398, 1616, 1443, 1377, 1215 $cm^{-1}$. ESI-HRMS calcd for $C_{30}H_{50}N_4O_3$ ($[M+Na]^+$) 537.3781, found 537.3780.

**4**: $[\alpha]_D^{20}$ +14.4 (c 1.01, $CHCl_3$); UV(EtOH) $\lambda_{max}$ 244, 252, 261 nm, $\lambda_{min}$ 247, 258 nm; $^1H$ NMR (400 MHz, $CDCl_3$) δ 0.54 (s, 3H), 0.94 (d, *J* = 6.4 Hz, 3H), 1.02-1.10 (m, 1H), 1.22 (s, 6H), 1.27-2.22 (m, 24H), 2.34 (dd, *J* = 3.2, 13.7 Hz, 1H), 2.42 (brd, *J* = 13.7 Hz, 1H), 2.79 (dd, *J* = 3.7, 12.4 Hz, 1H), 3.09 (dd, *J* = 3.7, 12.9 Hz, 1H), 3.52 (ddd, *J* = 4.6, 10.1, 15.6 Hz, 1H), 4.01 (ddd, *J* = 3.2, 7.3, 10.1 Hz, 1H), 4.47 (t, J = 7.3 Hz, 2H), 5.85 (d, *J* = 11.4 Hz, 1H), 6.25 (d, *J* = 11.4 Hz, 1H), 8.67 (s, 1H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 12.1, 18.9, 20.9, 22.4, 23.6, 24.9, 27.5, 27.7, 29.1, 29.3, 29.4, 36.2, 36.5, 38.3, 40.5, 44.2, 44.5, 45.9, 48.7, 48.8, 56.4, 56.6, 68.3, 70.9, 71.2, 115.2, 123.9, 130.5, 142.6, 143.6; IR (neat) 3460, 1616, 1439, 1373, 1215 $cm^{-1}$. ESI-HRMS calcd for $C_{30}H_{50}N_4O_3$ ($[M+Na]^+$) 537.3781, found 537.3775.

**2α-[3-(Tetrazol-2-yl)propyl]-1α,25-dihydroxy-19-norvitamin $D_3$ (5) and 2β-[3-(tetrazol-2-yl)-propyl]-1α,25-dihydroxy-19-norvitamin $D_3$ (6)**

The title compounds **5** and **6** (140.9 mg, 1 : 1.5 mixture) were obtained using similar procedures to those described above for **3** and **4**, starting from ketone **7(N2)** (203.0 mg, 0.433 mmol) and arylsulfone **14** (521.3 mg, 0.866 mmol), each as a colorless oil (51% for two steps).

**5**: $[\alpha]_D^{20}$ +30.9 (c 1.03, $CHCl_3$); UV(EtOH) $\lambda_{max}$ 245, 251, 261 nm, $\lambda_{min}$ 248, 258 nm; $^1H$ NMR (400 MHz, $CDCl_3$) δ 0.52 (s, 3H), 0.94 (d, *J* = 6.4 Hz, 3H), 1.01-1.10 (m, 1H), 1.22 (s, 6H), 1.26-2.30 (m, 25H), 2.58 (dd, *J* = 4.6, 12.9 Hz, 1H), 2.79 (dd, *J* = 4.1, 12.9 Hz, 1H), 2.86 (dd, *J* = 4.1, 14.2 Hz, 1H), 3.62 (ddd, *J* = 4.6, 9.6, 9.6 Hz, 1H), 4.07 (brs, 1H), 4.69 (t, J = 7.3 Hz, 2H), 5.80 (d, *J* = 11.0 Hz, 1H), 6.37 (d, *J* = 11.0 Hz, 1H), 8.50 (s, 1H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 12.1, 18.9, 20.9, 22.3, 23.5, 24.5, 27.2, 27.7, 29.0, 29.3, 29.4, 35.7, 36.2, 36.5, 40.5, 44.5, 45.6, 45.9, 48.6, 53.4, 56.4, 56.6, 68.4, 71.2, 71.3, 115.2, 124.3, 130.9, 143.5, 152.8; IR (neat) 3402, 1616, 1446, 1373, 1215 $cm^{-1}$. ESI-HRMS calcd for $C_{30}H_{50}N_4O_3$ ($[M+Na]^+$) 537.3781, found 537.3786.

**6**: $[\alpha]_D^{20}$ +18.4 (c 1.01, $CHCl_3$); UV(EtOH) $\lambda_{max}$ 243, 252, 261 nm, $\lambda_{min}$ 247, 258 nm; $^1H$ NMR (400 MHz, $CDCl_3$) δ 0.54 (s, 3H), 0.94 (d, *J* = 6.4 Hz, 3H), 1.02-1.10 (m, 1H), 1.22 (s, 6H), 1.26-2.31 (m, 24H), 2.34 (dd, *J* = 3.2, 13.7 Hz, 1H), 2.41 (brd, *J* = 13.7 Hz, 1H), 2.79 (dd, *J* = 3.7, 12.4 Hz, 1H), 3.09 (dd, *J* = 3.7, 12.9 Hz, 1H), 3.53 (ddd, *J* = 5.1, 10.1, 15.6 Hz, 1H), 4.02 (ddd, *J* = 3.2, 6.9, 10.1 Hz, 1H), 4.69 (t, J = 7.3 Hz, 2H), 5.85 (d, *J* = 11.4 Hz, 1H), 6.25 (d, *J* = 11.4 Hz, 1H), 8.50 (s, 1H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 12.1, 18.9, 20.9, 22.4, 23.6, 24.8, 27.0, 27.7, 29.1, 29.3, 29.4, 36.2, 36.5, 38.1, 40.5, 44.2, 44.5, 45.9, 48.8, 53.4, 56.4, 56.6, 68.2, 70.9, 71.2, 115.2, 123.8, 130.7, 143.4, 152.9; IR (neat) 3413, 1616, 1446, 1373, 1215 $cm^{-1}$. ESI-HRMS calcd for $C_{30}H_{50}N_4O_3$ ($[M+Na]^+$) 537.3781, found 537.3780.

**human VDR binding assay**

Binding affinity to hVDR was evaluated using a 1α,25(OH)$_2$D$_3$ assay kit (Polarscreen Vitamin D Receptor Competitor Assay, RED, Cat. No. PV4569) purchased from Invitrogen. The solution of test compound (1 mM in EtOH) was diluted to 10 times with DMSO. The solution was diluted to 50 times with the assay buffer included in the kit. The solution was defined as the compound solution. Meanwhile, VDR/Fluoromone and VDR RED, both of which are included in the kit, were diluted with the assay buffer included in the kit so that the concentration of VDR/Fluoromone was 2.8 nM, and that of VDR RED was 2 nM in the mixture. The solution was defined as the VDR/Fluoromone and VDR RED complex. To a 384 well Black plate (Coring, #3677) was added the compound solution (10 μL), and the VDR/Fluoromone and VDR RED complex (10 μL) were added to each well. The mixture was incubated at 20-25 °C for 2 h. The polarized fluorescence in each wells was measured (384 nm, emission: 595 nm, excitation: 535 nm, time: 250 ms/well). All compounds were evaluated with N = 2 within the range from $10^{-6}$ M to $10^{-10}$ M. $IC_{50}$ values were calculated by using the average of measured values. The activities of each compound were shown as relative value in which the activity of the natural hormone 1α,25(OH)$_2$D$_3$ was normalized to 100%.

**General procedure for transactivation assay of human osteocalcin promotor**

(1) Procedure of VDR expressed HOS cells

A mixture of plasmid of pGL4.26 DR3 and pcDNA3-human VDR (Full length) (ratio = 5:1) and pGL4-CMV-Rluc (Promega) were transfected into HOS cells (purchased from ATCC) by using MaxCyte STX (MaxCyte Co. Ltd), and the transfected cells were incubated at 37 °C under 5% $CO_2$ for 20 h. After incubation, cells were cryopreserved.

(2) Transactivation assay

Frozen VDR expressed Hos cells were thawed and suspended into DMEM media containing 5% charcoal stripped Fatal Bovine Serum. Transfected cells were seeded onto 384-well plate (4000 cells / 10 μL / well) and incubated under 5% $CO_2$ at 37 °C for 4 h. Test compounds were dissolved in 100% DMSO and added to the wells (The final concentration of DMSO in the assay was 0.1%). After 20 h incubation at 37 °C under 5% $CO_2$ in a cell culture incubator, the Dual-Glo Luciferase Assay System (Pro-Mega) was used to detect activities according to the manufacture's instructions. Data Plotted and $pEC_{50}$ values were calculated using the XLfit program (ID business Solution Ltd.).

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