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Synthesis and evaluation of geometric analogs of 1α,25-dihydroxyvitamin D_2 as potential therapeutics

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ABSTRACT

An improved convergent strategy was developed for the synthesis of the previously obtained side-chain extended and rigidified analogs of 1α,25-dihydroxyvitamin D_2, PRI-1906 and PRI-1907. New (24*Z*) geometric isomers of the analogs, PRI-1916 and PRI-1917, were also obtained and identified. These side-chain isomers were separable by flash chromatography, as C-25 alcohols, from the synthetic precursors of PRI-1906 and PRI-1907, respectively. The structures of new analogs were determined by advanced techniques of ^{1}H and ^{13}C NMR, including COSY, HSQC and HMBC sequences. Binding affinities of the geometric analogs PRI-1906 and PRI-1916 and their respective C-26, C-27 homologs PRI-1907 and PRI-1917 for the full-length human vitamin D receptor were determined by a fluorescence polarization competition assay. The binding affinity of (24*Z*) methyl analog PRI-1906 was much higher than that of (24*E*) analog PRI-1906, while the affinity of (24*Z*) ethyl analog PRI-1917 was lower than that of the respective PRI-1907. Investigation of the metabolism of these compounds by human CYP24A1 revealed they are much more resistant to CYP24A1 than 1α,25-dihydroxyvitamin D_2, indicating they could have longer-term biological effects on target tissues.

1. Introduction

Over several decades numerous laboratories have endeavored to unravel the complexity of the molecular mechanisms relating to the functions of the vitamin D system [1]. In part, this has been driven by interests in identifying synthetic analogs with a desired selective activity profile for use as therapeutics [2] against not only metabolic, bone and skin diseases but also to treat cancer, cardiovascular disorders, infectious and immune diseases. In regard to these efforts we have pursued the design, synthesis and biological evaluation [3–5] of analogs of 1α,25-dihydroxyvitamin D_2 (1,25D2, Fig. 1).

Initially, we observed that out of our series of four side-chain extended and branched analogs of 1,25D2, compounds PRI-1906 and PRI-1907, with two methyl and two ethyl at C-25, respectively, were able to moderately inhibit proliferation and significantly activate expression of CYP24A1 mRNA in prostate cancer cells PC-3 [6].

We also reported, for the first time, that inhibition of cellular caspase activity can induce differentiation of AML blasts and enhance vitamin D-induced cell differentiation of these cells. Differentiation induced by 1α,25-dihydroxyvitamin D_3 (1,25D3, Fig. 1) or its analog PRI-1906 was enhanced by pan-caspase inhibitor QVD (*N*-(2-quinolyl)-L-valyl-L-aspartyl-(2,6-difluorophenoxy) methylketone) to a varying degree, depending on the subtype of the leukemia [7].

Our analogs PRI-1906 and PRI-1907 have increased cell-differentiation activities against *ex-vivo* blast cells from patients with acute myeloid leukemia as compared to 1,25D3 [8]. Our study also revealed a high variability as to the susceptibility of individual patients' blasts to our vitamin D_2 analogs.

Very recently, we have introduced 19-*nor* modification into the structure of our analogs PRI-1906 and PRI-1907. We synthesized analogs PRI-5201 and PRI-5202 [9] which have increased differentiating activity as compared to 1,25D3 and PRI-1907 [10]. In current attempts to optimize the side-chain geometry of

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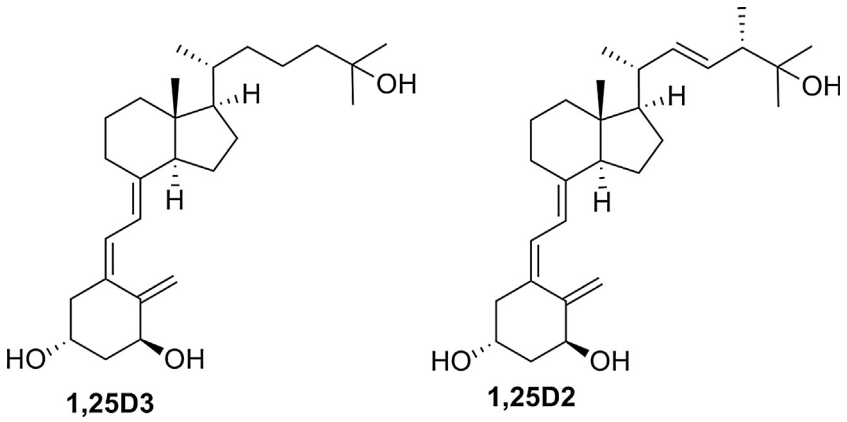

Fig. 1. Structures of 1α,25-dihydroxyvitamin D_3 and 1α,25-dihydroxyvitamin D_2.

these analogs, for the specific hydrogen bonding interactions of 25-hydroxyl with the amino acids residues of LBD of VDR [11], we have now obtained (22*E*,24*Z*)-analogs of 1,25D2, PRI-1916 and PRI-1917 (Fig. 2) and tested them for affinity for VDR. Metabolic stability of our analogs as to degradation by the enzyme CYP24A1 [12,13] was also determined in order to select the most resistant analog [14] for further biological evaluation.

2. Results and discussion

2.1. Synthesis of analogs PRI-1906, PRI-1907, PRI-1916 and PRI-1917

Analogs PRI-1906 and PRI-1907 were first synthesized by Julia olefination of C-22 vitamin D phenylsulphone [15].

However, the reductive dehydroxy-desulfonylation of the resulting phenylsulfonyl hydroxy ester with sodium amalgam was a very inefficient process. We have now synthesized these analogs by a modified Julia olefination [16–18] of C-22 benzothiazoyl sulfone (**1**, Scheme 1) and the side-chain aldehyde **2** [4]. In this process, the intermediate alkoxide is more reactive, than for the respective phenylsulfone, and undergoes a Smiles rearrangement to the respective sulfinate salt. This process is accompanied by the spontaneous elimination of sulfur dioxide to give the conjugated (22*E*)-ester **3** directly. Based on the mechanism of a modified Julia olefination we expected to get, as a result of olefination and Grignard reaction, (22*Z*)-isomers **12** and **13** (Fig. 3) as by-products. However, very favorably, ester **3** was obtained as a single product, not contaminated with its (22*Z*)-isomer. This was proved by a single signal of 18-CH_3 in ^{1}H NMR. Grignard reaction of ester **3** with

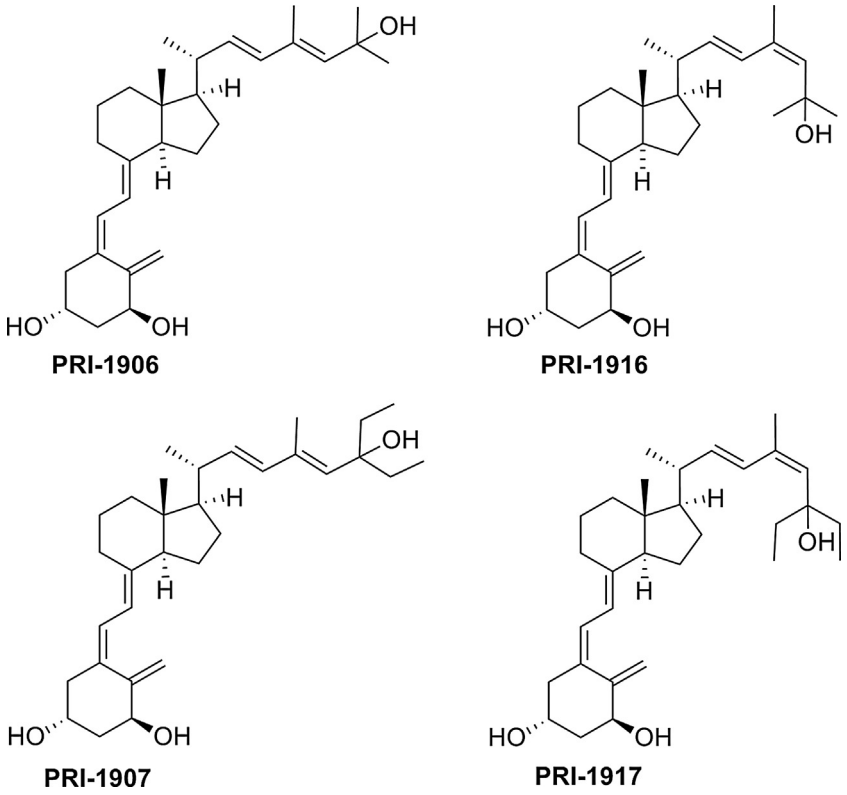

Fig. 2. Structures of side-chain homologated and unsaturated analogs of 1α,25-dihydroxyvitamin D_2.

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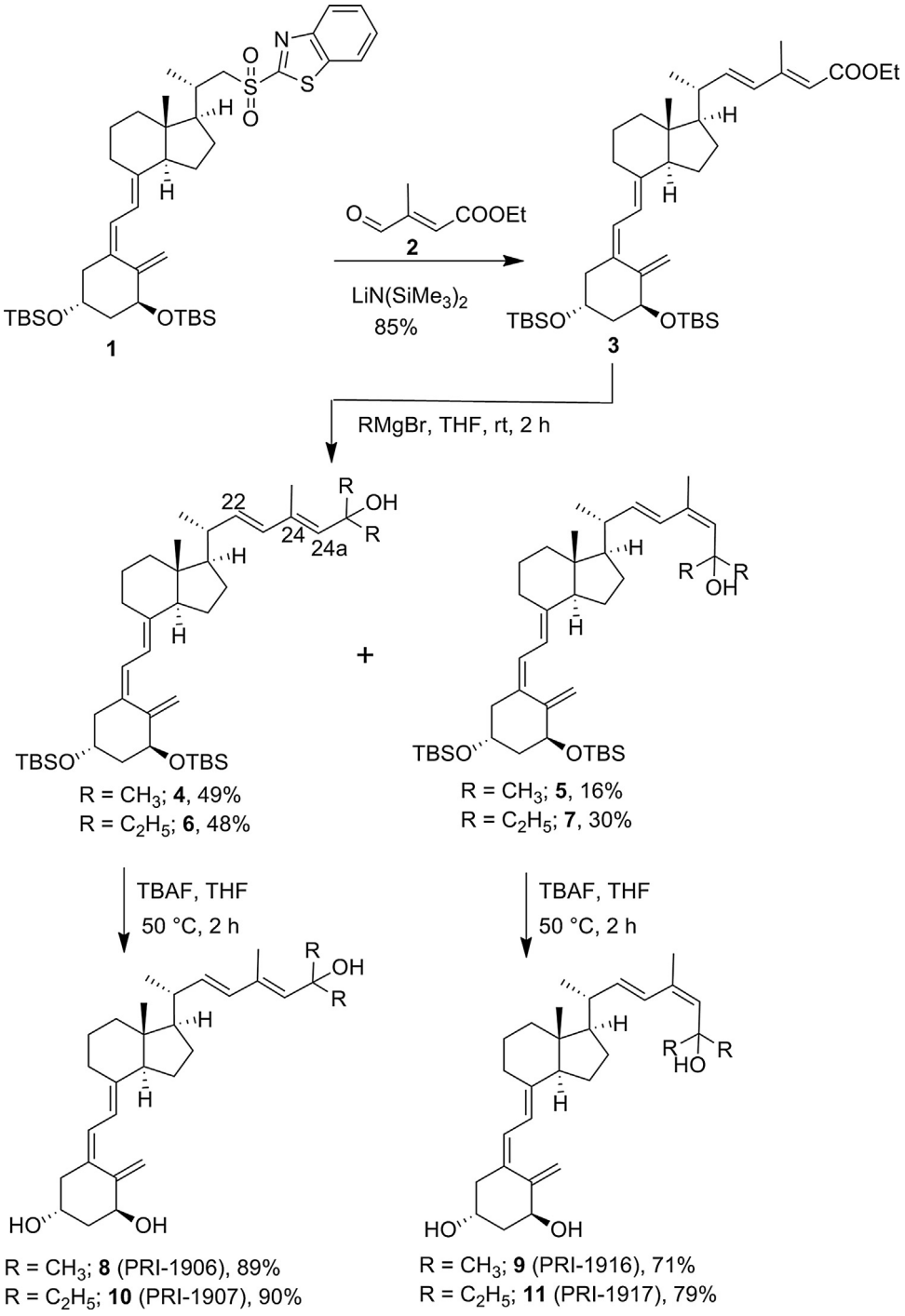
MeMgBr and EtMgBr, gave the respective alcohols **4** and **6**. Quite unexpectedly, in the course of the Grignard reaction, the (24*Z*)-isomers **5** and **7** were also formed, as isomerization by-products.

Geometric isomers **4** and **5** as well as **6** and **7** were separable by flash chromatography, which was not feasible in the case of triols **8** and **9** as well as **10** and **11**. Desilylation of alcohols **4**–**7** gave the respective final analogs **8**–**11**. The structures of new (24*Z*)-analogs **9** and **11** were assigned by ^{1}H and ^{13}C NMR.

2.2. Structural assignment of analogs PRI-1916 and PRI-1917

The ^{1}H and ^{13}C NMR spectra of PRI-1917 and PRI-1907, supported by MS data, indicated [19] that both compounds are geometric isomers. The most significant differences were observed in the range 7.00–5.00 ppm in the ^{1}H NMR spectra and 140–120 ppm in the ^{13}C NMR spectra. Based on the advanced 2D NMR experiments (including COSY, HSQC and HMBC sequences) all signals in the ^{1}H and ^{13}C NMR spectra were assigned to the respective protons and carbon atoms. In the ^{1}H NMR spectrum of PRI-1907 protons at 5.48 (H-22) and 5.97 ppm (H-23) were of



Scheme 1. Convergent synthesis of side-chain (22*E*,24*E*)-analogs of 1α,25-dihydroxyvitamin D_2 (**8**, PRI-1906 and **10**, PRI-1907) and (22*E*,24*Z*)-analogs of 1α,25-dihydroxyvitamin D_2 (**9**, PRI-1916 and **11**, PRI-1917).

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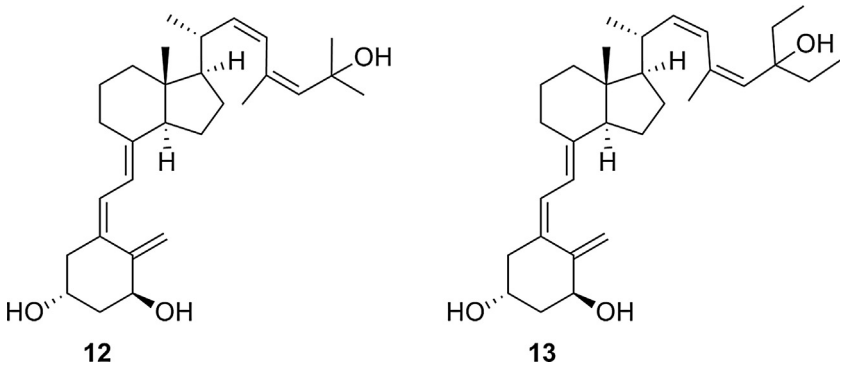

Fig. 3. Structures of hypothetic (22*Z*)-analogs of 1α,25-dihydroxyvitamin D_2.

(22*E*)-geometry, as the coupling constant ^{3}J(H22-H23) was equal to 15 Hz. The same was the value of the ^{3}J(H22-H23) for PRI-1917, indicating unequivocally that both, PRI-1907 and PRI-1917 are (22*E*)-isomers. The same was also a case for analogs PRI-1906 and PRI-1916. 1D Selective ^{1}H NOESY sequence allowed us to determine the mutual relation between all protons in the side-chain of analogs PRI-1907 (Fig. 4) and PRI-1917 (Fig. 5), as well as PRI-1906 and PRI-1916.

Irradiation of H-23 proton at 5.97 ppm in the ^{1}H NMR spectrum of PRI-1907 [19] gave the NOE enhancement at H-24a proton at 5.28 ppm, whereas irradiation of H-22 proton at 5.48 ppm resulted in a strong NOE effect at C-28 methyl (1.92 ppm). The same NOE effect was also observed for H-22 proton at 5.52 ppm and C-28 methyl (1.82 ppm) for PRI-1917. On the contrary, irradiation of the isolated signal at 7.00 ppm in PRI-1917 did not result in any NOE effects in this range of ^{1}H NMR shifts. These NOE enhancements clearly indicated (22*E*) geometry [20] in both PRI-1907 and PRI-1917, whereas the C-24 geometry was (24*E*) and (24*Z*) for PRI-1907 and PRI-1917, respectively. The same geometries were found for PRI-1906 and PRI-1916, respectively.

2.3. Human VDR binding assay

Binding affinities of the new analogs PRI-1916 and PRI-1917 for human full-length VDR were evaluated using a 1,25D3 assay kit and compared to that of 1,25D3, PRI-1906 and PRI-1907 [10]. In this fluorescence polarization competition assay, VDR was added to a fluorescent VDR ligand to form a receptor/tracer complex giving a high polarization value. This complex was then added to individual test compounds to displace the tracer from the complex. The activity of each compound is shown (Table 1) as a percentage whereby the activity of 1,25D3 was normalized to 100%. This data was derived from dose–response curves (data not shown)

2.4. Metabolic resistance of analogs to CYP24A1

The metabolism of each analog by human CYP24A1 (hCYP24A1) was analyzed using the membrane fraction prepared from the recombinant *Escherichia coli* cells expressing hCYP24A1 [21]. The conversion ratios of each analog to its metabolites for PRI-1906, PRI-1916, PRI-1907 and PRI-1917 were 2.3, 11, 0.8 and 10%, respectively (Table 2, Fig. 6 and SI). Meanwhile, native 1,25D3 and 1,25D2 were sequentially metabolized by CYP24A1 [21,22], and their conversion ratios were 49% and 39%, respectively. These results indicated that all of the analogs are more resistant to CYP24A1-dependent metabolism than native 1,25D3 and 1,25D2. In particular, PRI-1906 and PRI-1907 showed a remarkable resistance against CYP24A1. Since CYP24A1 is transcriptionally induced by VDR agonists in the target tissues to inactivate them, our analogs, especially PRI-1906 and PRI-1907, should have longer-term biological effects in the target tissues [23].

The complete evaluation of biological activities of our analogs is underway in this laboratory, according to the previously outlined scheme [3].

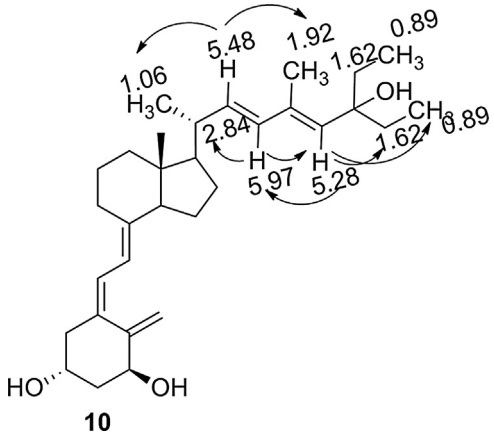

Fig. 4. Structure of **10** (PRI-1907) showing NOE assignments for characteristic protons in the side-chain.

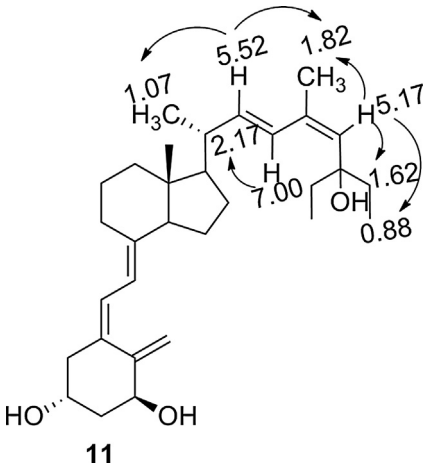

Fig. 5. Structure of **11** (PRI-1917) showing NOE assignments for characteristic protons in the side-chain.

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Table 1
Binding affinity of analogs for the full-length human vitamin D receptor in a fluorescence polarization competition assay.

Compound	1,25D3	PRI-1906	PRI-1916	PRI-1907	PRI-1917
IC_{50}	$2.232e^{-09}$	$5.561e^{-008}$	$6.048e^{-09}$	$6.172e^{-09}$	$6.848e^{-008}$
Relative binding affinity[a]	100	4	37	38	3

[a] Potency of 1,25D3 normalized to 100.

Table 2
Metabolic conversion of active forms of vitamins D and of analogs by human CYP24A1.

Compound	1,25D3	1,25D2	PRI-1906	PRI-1916	PRI-1907	PRI-1917
Metabolic conversion[a] (%)	49	39	2.3	11	0.8	10

[a] Data represent the mean of at least 2 independent experiments.

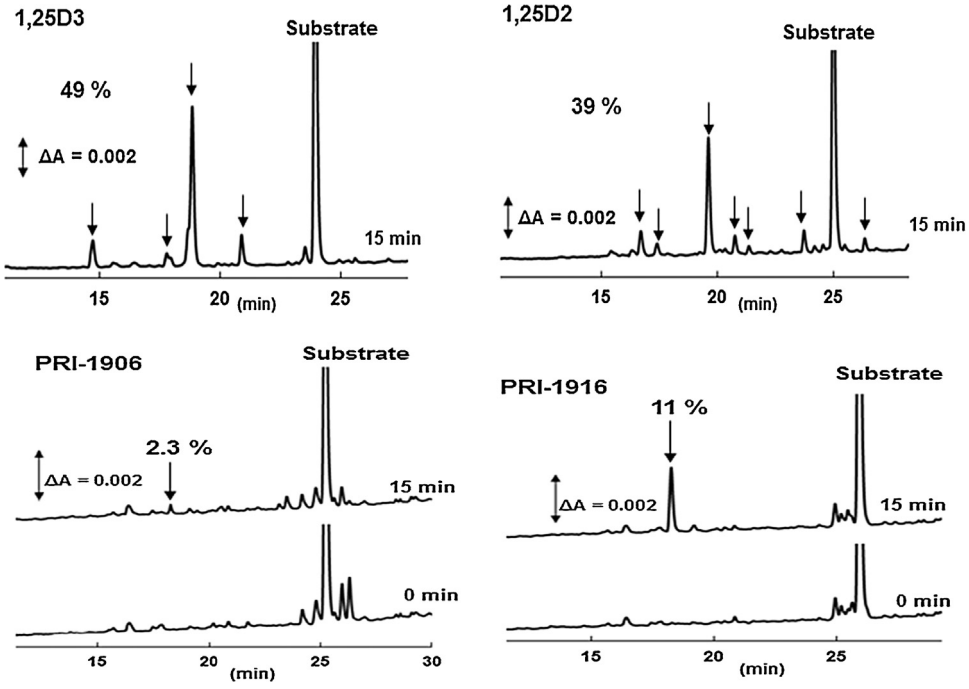

Fig. 6. HPLC profiles of 1,25D3, 1,25D2, analogs PRI-1906 and PRI-1916 and their metabolites generated by human CYP24A1. The peaks marked with arrows indicate putative metabolites. Profiles for PRI-1907 and PRI-1917 are included in the supporting information (SI).

3. Conclusions

We synthesized side-chain extended and homologated (24*Z*)-analogs PRI-1916 and PRI-1917 of 1,25D2 and have developed improved method for the synthesis of our leading 1,25D2 analogs PRI-1906 and PRI-1907, by a modified Julia olefination and from the previously developed C-22 benzothiazoyl sulfone as the advanced intermediate. New analogs were separable from their geometric isomers by flash chromatography at the level of C-25 alcohols. The structures of side-chains of analogs PRI-1916 and PRI-1917 was assigned by advanced techniques of ^{1}H and ^{13}C NMR. Binding affinities of the new analogs for the human full-length VDR were determined using fluorescence polarization competition assay. The VDR binding affinity of new analogs were lower than that of 1,25D3. Futhermore, introducing (24*Z*) geometry increased the affinity for methyl analog PRI-1916, as compared to (24*E*) analog PRI-1906, while it decreased the affinity for ethyl analog PRI-1917, as compared to (24*E*) analog PRI-1907, meaning that the terminal alkyls at C-25 strongly influence the binding affinity.

Isomerization of side-chain (24*E*)-analogs of 1,25D2 (PRI-1906 and PRI-1907) at C24 resulted in the lowered resistance to metabolism by CYP24A1, although (24*Z*)-analogs (PRI-1916 and PRI-1917) were still more resistant than native 1,25D2 and 1,25D3. These results reveal that side-chain extended and rigidified analogs of 1,25D2, especially (24*E*)-analogs, may be useful to elicit longer-term biological effects against target tissues.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jsbmb.2015.08.025.

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[19] ^{1}H and ^{13}C NMR data of new analogs: **9**, PRI-1916: ^{1}H NMR (600 MHz, CD_3OD, 25.8(C, TMS) δ = 6.988 (d, J = 15.6Hz, 1H, 23-H), 6.324 (d, J = 11.4Hz, 1H, 7-H), 6.084 (d, J = 10.8Hz, 1H, 6-H), 5.523 (dd, J_1 = 15Hz, J_2 = 9Hz, 1H, 22-H), 5.377 (s, 1H, 24a-H), 5.286 (s, 1H, 19E-H), 4.897 (s, 1H, 19Z-H), 4.349 (t, J_1 = J_2 = 6Hz, 1H, 1-H), 4.126 (t, J_1 = J_2 = 5.4Hz, 1H, 3-H), 1.774 (s, 3H, 28-CH_3), 1.352 (s, 6H, 26-CH_3, 27-CH_3), 1.078 (d, J = 7.2 Hz, 3H, 21-CH_3), 0.601 (s, 3H, 18-CH_3); ^{13}C NMR (600 MHz, CD_3OD, 25.8(C, TMS): δ = 149.79 (C=CH2, C-10), 142.33 (C=C H2, C-8), 139.29 (C=CH2, C-5), 136.39 (C=CH2, C-24a), 135.78 (C=CH2, C-23), 133.72 (C=CH2, C-24), 126.84 (C=CH2, C-22), 124.83 (C=CH2, C-6), 119.05 (C=CH2, C-7), 112.07 (C=CH2, C-19), 77.10 (C—OH, C-25), 71.46 (C—OH, C-1), 67.38 (C—OH, C-3), 57.72 (C—CH2, C-14), 57.59 (C—CH2, C-17), 46.97 (C—CH2, C-4), 46.15 (C—CH2, C-13), 43.70 (C—CH2, C-2), 42.26 (C—CH3, C-20), 41.72 (C—CH2, C-12), 29.98 (C—CH2, C-9), 28.88 (C—CH2, C-16), 24.60 (C—CH2, C-15), 23.25 (C—CH2, C-11), 21.29 (C-21), 21.19 (C-28), 12.96 (C-18), 12.69 (C-26), 12.68 (C-27). HRMS: calc'd for $C_{29}H_{44}O_3$ [M + Na]$^+$, 463.3188 found: 463.3190. **11**, PRI-1917: ^{1}H NMR (600 MHz, CD_3OD, 25.8(C, TMS): δ = 6.997 (d, J = 15.6Hz, 1H, 23-H), 6.321 (d, J = 11.4Hz, 1H, 7-H), 6.082 (d, J = 11.4Hz, 1H, 6-H), 5.515 (dd, J_1 = 15.6Hz, J_2 = 8.4 Hz, 1H, 22-H), 5.284 (s, 1H, 24a-H), 5.168 (s, 1H, 19E-H), 4.896 (s, 1H, 19Z-H), 4.347 (t, J_1 = 6, J_2 = 5.4Hz, 1H, 1-H), 4.123 (t, J_1 = 6.6Hz, J_2 = 4.8Hz, 1H, 3-H), 1.811 (s, 3H, 28-CH_3), 1. 441-1.372 (m, 6H, 26-CH_3, 27-CH_3), 1.078 (d, J = 7.2Hz, 3H, 21-CH_3), 0.601 (s, 3H, 18-CH_3). ^{13}C NMR (600 MHz, CD_3OD, 25.8 °C, TMS): δ = 149.79 (C=CH_2, C-10), 142.42 (C=C H_2, C-8), 138.00 (C=CH_2, C-5), 135.73 (C=CH_2, C-24a), 135.00 (C=CH_2, C-23), 133.58 (C=CH_2, C-24), 127.84 (C=CH_2, C-22), 124.85 (C=CH_2, C-6), 119.02 (C=CH_2, C-7), 112.07 (C=CH_2, C-19), 77.32 (C—OH, C-25), 71.48 (C—OH, C-1), 67.39 (C—OH, C-3), 57.87 (C—CH2, C-14), 57.60 (C—CH2, C-17), 46.96 (C—CH2, C-4), 46.15 (C—CH2, C-13), 43.69 (C—CH2, C-2), 42.24 (C—CH3, C-20), 41.74 (C—CH2, C-12), 35.49 (C—CH3, C-26), 35.35 (C—CH3, C-27), 29.98 (C—CH2, C-9), 28.79 (C—CH2, C-16), 24.61 (C—CH2, C-15), 23.27 (C—CH2, C-11), 21.90 (C-21), 21.31 (C-28), 12.68 (C-18), 8.59 (C-26′), 8.522 (C-27′). HRMS: calc'd for $C_{31}H_{48}O_3$ [M + Na]$^+$, 491.3501 found: 491.3502..
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