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Biological activity and conformational analysis of C20 and C14 epimers of CD-ring modified *trans*-decalin 1α,25-dihydroxyvitamin D analogs☆

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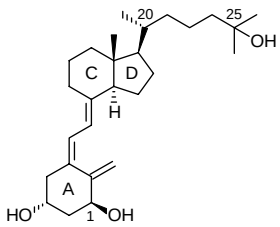
Abstract

In the context of our ongoing study of vitamin D structure–function relationships and in an attempt to obtain a better dissociation of their prodifferentiating (HL-60) and/or antiproliferative (MCF-7) activities and their calcemic activity, further 20-*epi* and 14-*epi* modifications were made to three *trans*-decalin CD-ring analogs of 1,25-dihydroxyvitamin D_3, the hormonally active metabolite of vitamin D_3, possessing a natural 20*R* side chain and featuring additional structural modifications in the *seco*-B-ring and in the A-ring. Following a previously observed trend and in agreement with the conformational analysis results, all three 20-*epi* derivatives show substantially lower biological activities, opposite to what is usually observed for analogs having the natural CD-ring. The 14-*epi* modification (*cis*-decalins) has little effect on the biological activity of the ynediene type and the saturated derivative, but results in an approximate 10-fold reduction in activity of the previtamin derivative. No better dissociation of the prodifferentiating and/or antiproliferative activities and the calcemic activity was achieved.

Keywords: Structure–function relationships; Vitamin D; Synthesis; Conformational analysis

1. Introduction

The last two decades has witnessed an active search for analogs of 1α,25-dihydroxyvitamin D_3 (**1**, calcitriol, further abbreviated as 1,25-D_3), the hormonally active form of vitamin D [1]. Next to its classical calciotropic activity, 1,25-D_3 (**1**) has been shown to possess immunosuppressive activity, to inhibit cellular proliferation and to induce cellular differentiation. Its therapeutic utility in the treatment of certain cancers and skin diseases is however limited since effective doses provoke calcemic side effects such as hypercalcemia, hypercaliuria and bone decalcification. This has stimulated the development of analogs of the natural hormone in which the prodifferentiating and/or antiproliferative activities and the calcemic activity are dissociated [2].



1 1,25-D_3

In this context our laboratories in Gent and Leuven have focussed in recent years on the development of analogs that vary in the structure of the central CD-ring system (see references 17–20 cited in [3]). Recently we have reported the surprising biological activities of a series of *trans*-fused decalin CD-ring 1,25-D_3 analogs possessing the natural side chain (20*R* configuration), but which are further mod-

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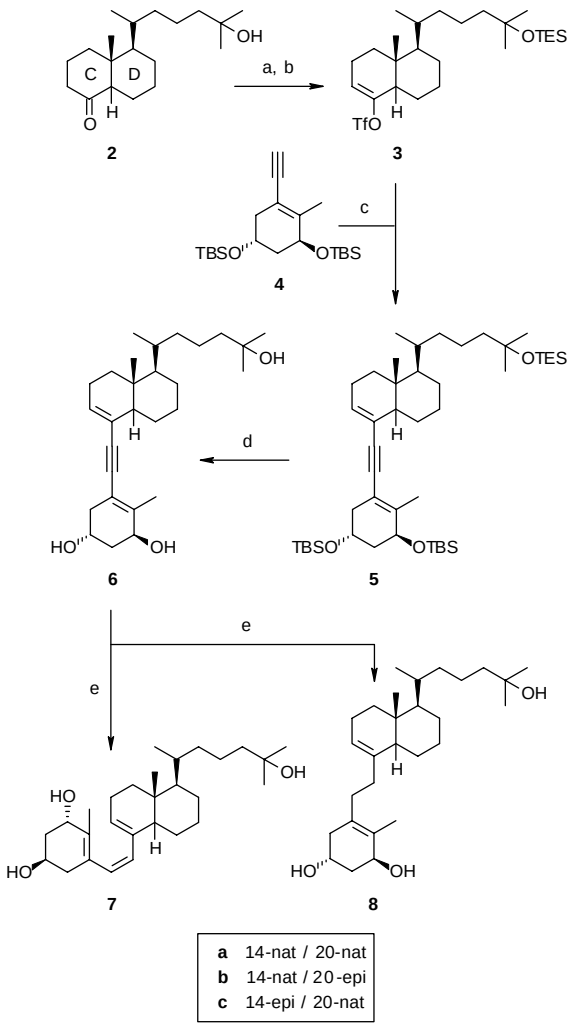

Scheme 1. (a) TESCl, DMAP or imidazole, CH_2Cl_2, rt, 3–12 h; (b) *n*-BuLi, *i*-Pr_2NH, THF, 0 °C, 20 min; ketone/silyl ether from (a), THF, −78 °C, 45 min; then → rt over 2 h; $(CF_3SO_2)_2NC_6H_5$, THF, −78 °C, 10 min; then → rt over 4 h; (c) **4**, CuI, $Pd(PPh_3)_2(OAc)_2$, Et_2NH, MeCN, rt, 1 h; (d) TBAF, THF, rt, 12 h; (e) H_2, Lindlar catalyst, quinoline, EtOAc, rt.

ified in the *seco*-B-ring region, including the ynediene derivative **6a**, the previtamin **7a**, and the saturated derivative **8a** (Scheme 1) [3]. For better understanding of the structure–function relationships involved, we wish to describe here the effect on the biological activity of the latter analogs of either the 20-*epi* modification (**6b**–**8b**; 20*S* configuration) or the 14-*epi* modification (**6c**–**8c**; *cis*-fused decalin analogs).

2. Materials and methods

2.1. Biological evaluation

1,25-D_3 (**1**) was a gift from Duphar (Weesp, The Netherlands; J.P. van de Velde).

2.1.1. Binding studies

The affinity of the decalin analogs of 1,25-D_3 to the vitamin D receptor was evaluated by their ability to compete with [^{3}H]1,25-D_3 for binding to high speed supernatant from intestinal mucosa homogenates obtained from normal pigs as described previously [4]. The relative affinity of the analogs was calculated from their concentration needed to displace 50% of [^{3}H]1,25-D_3 from its receptor compared with the activity of 1,25-D_3. Binding to hDBP was performed at 4 °C essentially as described previously [5].

2.1.2. MCF-7 proliferation assay

The human breast carcinoma (MCF-7) cell line was obtained from the American Tissue Culture Company (Rockville, MD). The antiproliferative activity on MCF-7 cells was assessed by evaluating [^{3}H]thymidine incorporation. Cells were seeded in 96-well plates (7500 cells per well) and 1 μCi [methyl-^{3}H]thymidine (ICN Biomedicals, Costa Mesa, CA) was added 72 h after the initiation of treatment. Cells were semi-automatically harvested after an additional 6 h of incubation on filter plates only retaining incorporated thymidine (GF/C Filter and Filtermate Universal Harvester, Packard Instrument, Meriden, CT). Counting was performed using a microplate scintillation counter (Topcount, Packard).

2.1.3. Differentiation of HL-60 cells

The human promyelocytic leukemia cell line (HL-60) was obtained from the American Tissue Culture Company (Rockville, MD). HL-60 cells were seeded at 4×10^4 cells cm^{-3} in 25 cm^2 Falcon tissue chambers using RPMI 1640 medium supplemented with 20% fetal calf serum (Sera-Lab, W. Sussex, UK) and gentamycin (50 μg cm^{-3}; Gibco, Roskilde, Denmark). 1,25-D_3, analogs or vehicle were added the day after plating. After 4 days of culture, differentiation was measured by using the NBT reduction assay as described previously [6].

2.1.4. In vivo studies

NMRI mice were obtained from the Proefdierencentrum of Leuven (Belgium) and fed on a vitamin D-replete diet (0.2% calcium, 1% phosphate, 2000 U vitamin D per kilogram; Hope Farms, Woerden, The Netherlands). The calcemic effect of the decalin analogs was tested in NMRI mice by daily subcutaneous injection of 1,25-D_3 its analogs or the solvent during seven consecutive days, using serum calcium concentration as parameter.

2.2. Synthesis

The synthesis of the key intermediates **2b** and **2c**, and general procedures for their further conversion into derivatives **6b**–**8b** and **6c**–**8c**, respectively, according to the synthetic route outlined in Scheme 1, have been described in detail previously [3].

*2.2.1. (6S)-2-Methyl-6-[(1R,4aR,8aR)-5-(trifluoromethanesulfonyloxy)-8a-methyl-1,2,3,4,4a,7,8,8a-octahydro-1-naphthyl]-2-(triethylsilyloxy)heptane (**3b**) and (6R)-2-methyl-6-[(1R,4aS,8aR)-5-(trifluoromethanesulfonyloxy)-8a-methyl-1,2,3,4,4a,7,8,8a-octahydro-1-naphthyl]-2-(triethylsilyloxy)heptane (**3c**)*

According to the described procedure, enol triflate **3b** (**3c**) was prepared from hydroxyketone **2b** (**2c**) in two steps. Yield: **3b**, 68%; **3c**, 73%.

Data of **3b**: R_f (cyclohexane–EtOAc, 5:1) 0.77; $[\alpha]_D^{20}$ +4.6 (*c* 0.86, $CHCl_3$); IR (neat) 3418, 2953, 2876, 1681, 1448, 1417, 1382, 1209, 1144, 1043, 1016, 725 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.67 (1H, d, J = 3.3 Hz), 2.21 (3H, m), 1.88 (2H, m), 1.75 (2H, m), 1.19 (3H, s), 1.18 (3H, s), 0.94 (9H, t, J = 7.9 Hz), 0.85 (3H, s), 0.79 (3H, d, J = 6.9 Hz), 0.56 (6H, q, J = 7.9 Hz) ppm; ^{13}C NMR ($CDCl_3$) δ 150.9, 116.7, 73.4, 65.9, 50.2, 48.9, 44.9, 39.9, 39.8, 33.6, 30.7, 30.0, 29.9, 26.2, 22.6, 22.4, 22.1, 20.0, 16.9, 12.7, 7.2, 7.2, 7.2, 6.6, 6.6, 6.6 ppm; MS *m/z* 540 (M^+), 525, 512, 511, 468, 458, 429, 393, 377, 363, 324, 307, 293, 259, 235, 203, 173, 149, 135, 103, 75, 69, 55; ESI-MS *m/z* 563 $[M+Na]^+$. Anal. Calcd. for $C_{26}H_{47}F_3O_4SSi$: C, 57.74; H, 8.76. Found: C, 57.57; H, 8.88.

Data of **3c**: R_f (cyclohexane–EtOAc, 5:1) 0.70; $[\alpha]_D^{20}$ +24 (*c* 0.86, $CHCl_3$); IR (neat) 3419, 2954, 2875, 1469, 1417, 1381, 1364, 1244, 1209, 1145, 1047, 870 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.79 (1H, d, J = 2.7 Hz), 2.35 (1H, m), 2.11 (2H, m), 1.87 (2H, m), 1.61 (2H, m), 1.18 (6H, s), 1.02 (3H, s), 0.94 (9H, t, J = 7.9 Hz), 0.93 (3H, d, J = 6.9 Hz), 0.56 (6H, q, J = 7.8 Hz) ppm; MS *m/z* 540 (M^+), 536, 513, 511, 450, 429, 401, 390, 377, 363, 333, 293, 259, 235, 203, 173, 149, 135, 103, 75, 69, 55; ESI-MS *m/z* 558 $[M + NH_4]^+$.

*2.2.2. (1R,3S)-5-[(1R,4aR,8aR)-1-((1S)-5-Hydroxy-1,5-dimethylhexyl)-8a-methyl-1,2,3,4,4a,7,8,8a-octahydro-5-naphthyl]ethynyl-4-methylcyclohex-4-ene-1,3-diol (**6b**) and (1R,3S)-5-[(1R,4aS,8aR)-1-((1R)-5-hydroxy-1,5-dimethylhexyl)-8a-methyl-1,2,3,4,4a,7,8,8a-octahydro-5-naphthyl]-ethynyl-4-methylcyclohex-4-ene-1,3-diol (**6c**)*

According to the general procedure, enol triflate **3b** (**3c**) was coupled with A-ring synthon **4** to give the protected 1,25-D_3 derivative **5b** (**5c**), which was consequently deprotected using tetrabutylammonium fluoride (TBAF) to give the ynediene type analog **6b** (**6c**). Yield: **6b**, 77%; **6c**, 80%.

Data of **6b** (IM 915): R_f (CH_2Cl_2–MeOH, 9:1) 0.32; $[\alpha]_D^{20}$ −39 (*c* 1.52, $CHCl_3$); UV (EtOH) λ_{max} 283, 269, 259 nm; IR (neat) 3412, 2933, 2855, 2281, 1448, 1363, 1211, 1152, 1104, 1048, 957, 797 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.09 (1H, d, J = 3.0 Hz), 4.26 (1H, s), 4.11 (1H, m), 2.56 (1H, m), 2.16 (2H, m), 1.99 (3H, s), 1.88 (2H, m), 1.78 (2H, m), 1.21 (6H, s), 0.79 (3H, d, J = 6.9 Hz), 0.78 (3H, s) ppm; ^{13}C NMR ($CDCl_3$) δ 139.1, 134.2, 123.7, 116.0, 93.5, 88.0, 70.9, 69.2, 63.2, 50.7, 47.6, 43.9, 39.9, 39.0, 36.2, 33.5, 29.9, 29.1, 29.1, 26.7, 25.2, 23.6, 22.2, 21.8, 20.2, 18.7, 16.6, 12.2 ppm; MS *m/z* 428 (M^+), 419, 410, 392, 377, 353, 326, 297, 281, 239, 221, 199, 183, 171, 142, 105, 103, 91, 75, 43; ESI-MS *m/z* 469 $[M + K]^+$, 451 $[M + Na]^+$, 446 $[M + NH_4]^+$, 411 $[M + H - H_2O]^+$.

Data of **6c** (IM 724): R_f (CH_2Cl_2–MeOH, 9:1) 0.29; UV (EtOH) λ_{max} 258, 268, 282 nm; IR (neat) 3414, 2930, 2854, 2281, 1447, 1378, 1215, 1150, 1046, 757 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.17 (1H, d, J = 3.2 Hz), 4.20 (1H, s), 4.06 (1H, m), 2.50 (2H, m), 1.95 (3H, s), 1.82 (1H, m), 1.73 (2H, m), 1.64−1.50 (3H, m), 1.39 (5H, m), 1.18 (6H, s), 0.97 (3H, s), 0.88 (3H, d, J = 6.9 Hz) ppm; ^{13}C NMR ($CDCl_3$) δ 139.1, 136.2, 124.1, 116.0, 93.5, 87.4, 70.9, 69.1, 63.1, 46.0, 44.1, 41.0, 39.9, 38.9, 35.2, 33.7, 33.2, 30.7, 29.0, 29.0, 25.2, 23.7, 23.1, 22.7, 21.5, 21.2, 18.6 ppm; MS *m/z* 428 (M^+), 419, 410, 392, 328, 317, 299, 271, 199, 185, 159, 142, 81, 75, 43; ESI-MS *m/z* 469 $[M+K]^+$, 451 $[M+Na]^+$, 446 $[M + NH_4]^+$, 411 $[M + H - H_2O]^+$.

*2.2.3. (1R,3S)-5-[(Z)-2-[(1R,4aR,8aR)-1-((1S)-5-Hydroxy-1,5-dimethylhexyl)-8a-methyl-1,2,3,4,4a,7,8,8a-octahydro-5-naphthyl]ethenyl]-4-methylcyclohex-4-ene-1,3-diol (**7b**) and (1R,3S)-5-[2-[(1R,4aR,8aR)-1-((1S)-5-hydroxy-1,5-dimethylhexyl)-8a-methyl-1,2,3,4,4a,7,8,8a-octahydro-5-naphthyl]ethyl]-4-methylcyclohex-4-ene-1,3-diol (**8b**); (1R,3S)-5-[(Z)-2-[(1R,4aS,8aR)-1-((1R)-5-hydroxy-1,5-dimethylhexyl)-8a-methyl-1,2,3,4,4a,7,8,8a-octahydro-5-naphthyl]ethenyl]-4-methylcyclohex-4-ene-1,3-diol (**7c**) and (1R,3S)-5-[2-[(1R,4aS,8aR)-1-((1R)-5-Hydroxy-1,5-dimethylhexyl)-8a-methyl-1,2,3,4,4a,7,8,8a-octahydro-5-naphthyl]ethyl]-4-methylcyclohex-4-ene-1,3-diol (**8c**)*

According to the general procedure, ynediene analog **6b** (**6c**) was hydrogenated using Lindlar catalyst in the presence of quinoline to afford a mixture of the previtamin **7b** (**7c**) and the saturated derivative **8b** (**8c**). Yield: **7b** + **8b**, 86%; **7c** + **8c**, 88%.

Data of **7b** (IM 742): R_f (CH_2Cl_2–MeOH, 9:1) 0.32; $[\alpha]_D^{20}$ −23 (*c* 1.65, $CHCl_3$); UV (EtOH) λ_{max} 283, 269, 259 nm; IR (neat) 3414, 2930, 2854, 1448, 1363, 1215, 1154, 1103, 1047, 957, 759 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.96 (1H, d, J = 12.0 Hz), 5.83 (1H, d, J = 12.0 Hz), 5.45 (1H, s), 4.18 (1H, s), 4.02 (1H, m), 2.43 (1H, d, J = 16.1 Hz), 2.06 (4H, m), 1.93 (1H, d, J = 5.6 Hz), 1.77 (3H, s), 1.42 (6H, m), 1.20 (6H, s), 0.81 (3H, s), 0.77 (3H, d, J = 6.9 Hz) ppm; ^{13}C NMR ($CDCl_3$) δ 137.2, 131.9, 130.3, 129.9, 127.6, 125.0, 70.9, 70.5, 64.2, 50.7, 48.7, 44.7, 40.7, 39.2, 38.9, 36.3, 34.0, 29.9, 29.1, 29.1, 27.0, 24.5, 22.7, 22.1, 21.7, 16.8, 16.6, 12.6 ppm; MS *m/z* 430 (M^+), 412, 395, 379, 376, 351, 331, 305, 291, 278, 259, 237, 227, 209, 199, 174, 161, 141, 134, 131, 121, 105, 91, 81, 59, 43; ESI-MS *m/z* 469 $[M+K]^+$, 453 $[M+Na]^+$, 413 $[M+H-H_2O]^+$, 395 $[M + H - 2H_2O]^+$, 377$[M + H - 3H_2O]^+$.

Data of **8b** (IM 6383): R_f (CH_2Cl_2–MeOH, 9:1) 0.32; $[\alpha]_D^{20}$ −57 (*c* 3.15, $CHCl_3$); IR (neat) 3406, 2932, 2853, 1447, 1378, 1212, 1154, 1116, 1045, 936, 738 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.31 (1H, s), 4.08 (1H, s), 4.02 (1H, m), 2.30 (1H, m), 1.99 (5H, m), 1.85 (3H, m), 1.74

(3H, s), 1.42 (4H, m), 1.18 (6H, s), 1.00 (1H, dd, J = 3.2, 12.9 Hz), 0.76 (3H, d, J = 6.9 Hz), 0.75 (3H, s) ppm; ^{13}C NMR ($CDCl_3$) δ 138.2, 132.5, 127.0, 120.3, 70.9, 70.8, 63.8, 50.8, 48.6, 44.2, 40.7, 39.2 36.8, 34.0, 33.9, 33.0, 32.6, 29.9, 29.0, 29.0, 27.0, 23.5, 23.1, 22.7, 21.7, 16.6, 15.9, 12.6 ppm; MS m/z 432 (M^+), 414, 396, 378, 363, 356, 343, 341, 325, 301, 283, 277, 273, 259, 227, 211, 199, 175, 161, 149, 140, 135, 105, 95, 55, 43; ESI-MS m/z 471 $[M+K]^+$, 455 $[M + Na]^+$, 450 $[M + NH_4]^+$, 433 $[M + H]^+$, 415 $[M + H - H_2O]^+$, 397 $[M + H - 2H_2O]^+$, 379 $[M + H - 3H_2O]^+$.

Data of **7c** (IM 7382): R_f (CH_2Cl_2–MeOH, 9:1) 0.32; $[\alpha]_D^{20}$ −48 (c 1.80, $CHCl_3$); UV (EtOH) λ_{max} 283, 269, 259 nm; IR (neat) 3412, 2928, 2853, 1449, 1378, 1215, 1156, 1044, 759 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.95 (1H, AB, J = 12.3 Hz), 5.90 (1H, AB, J = 12.3 Hz) 5.63 (1H, brs), 4.18 (1H, s), 4.01 (1H, m), 2.54 (1H, dd, J = 4.0, 16.3 Hz), 2.03 (2H, m), 1.96 (3H, m), 1.75 (3H, s), 1.58 (2H, m), 1.19 (6H, s), 0.95 (3H, s), 0.90 (3H, d, J = 6.9 Hz) ppm; ^{13}C NMR ($CDCl_3$) δ 137.4, 131.6, 130.5, 130.3, 128.1, 127.7, 70.9, 70.9, 63.8, 46.6, 44.1, 42.2, 40.6, 37.8, 35.5, 33.7, 33.3, 30.9, 29.1, 29.0, 26.7, 24.3, 23.3, 23.0, 22.9, 21.2, 21.2, 16.9 ppm; MS m/z 430 (M^+), 412, 394, 376, 369, 354, 338, 324, 301, 295, 283, 271, 255, 229, 185, 159, 121, 119, 81, 59, 43; ESI-MS m/z 469 $[M + K]^+$, 453 $[M + Na]^+$, 413 $[M + H - H_2O]^+$.

Data of **8c** (IM 7381): R_f (CH_2Cl_2–MeOH, 9:1) 0.31; $[\alpha]_D^{20}$ +48 (c 2.10, $CHCl_3$); IR (neat) 3414, 2928, 2854, 1637, 1618, 1448, 1380, 1214, 1153, 1044, 759 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.47 (1H, d, J = 4.5 Hz), 4.12 (1H, s), 4.06 (1H, m), 2.35 (1H, dd, J = 5.1, 16.5 Hz), 1.74 (3H, s), 1.20 (6H, s), 0.96 (3H, s), 0.90 (1H, d, J = 6.8 Hz) ppm; ^{13}C NMR ($CDCl_3$) δ 138.5, 132.6, 127.1, 122.7, 70.9, 70.8, 63.9, 45.8, 41.4, 40.7, 38.9, 35.7, 34.4, 33.3, 33.2, 33.0, 32.6, 32.4, 30.8, 29.1, 29.0, 26.7, 23.8, 23.4, 22.9, 21.5, 21.1, 15.9 ppm; MS m/z 432 (M^+), 414, 396, 378, 363, 343, 341, 325, 301, 285, 277, 273, 259, 199, 161, 140, 105, 95, 55, 43; ESI-MS m/z 471 $[M + K]^+$, 455 $[M + Na]^+$, 450 $[M + NH_4]^+$, 433 $[M + H]^+$.

2.3. Conformational analysis

Modelling was performed by molecular mechanics calculations using the MacroModel V6.0 molecular modelling programme of Still and co-workers [7] run on a Silicon Graphics Octane workstation and using the programme's standard parameters. Conformational analysis of the side chain of compounds **6**–**8** was carried out on model compounds with only a Me group on C8, in the assumption that side-chain conformations are little or not affected by the distant *seco*-B-ring and A-ring parts of the molecule. Because of the conformational flexibility of the *cis*-fused decalin CD-ring in the 14-*epi* derivatives **c**, the prior generation of the necessary 23,328 starting conformations (using MULT) followed by minimisation as done previously [3,9] was replaced by the use of the programme's Low Mode Conformational Search (LMCS) Monte–Carlo method (3×10^4 steps) to search the conformational space. In the case of the 14-*epi* derivatives **c** all possible conformations of the *cis*-decalin CD-ring were allowed in the search. The conformations found were minimised using the MM2* force field implementation of the programme and the conformations within 20 kJ mol^{-1} of the minimum energy form were retained. All conformations of each compound were then overlaid using C13 as common origin (x, y, z = 0), C14 was positioned in the yz-plane (x = 0) and the position of C18 was made to coincide with the positive y-axis (x, z = 0) to obtain the respective dot maps. The procedure for the calculation of the active volume has been reported earlier [9].

Table 1
Biological activities of 1,25-D_3 analogs **6**–**8**[a]

Code	Binding affinity		Cell differentiation and proliferation		Calcemic effect serum (mice)
	VDR (pig)	DBP (human)	HL-60	MCF-7	
1,25-D_3	100	100	100	100	100
IM 902 (**6a**)	85	80	100	100	10
IM 915 (**6b**)	1	5	3	9	<1
IM 724 (**6c**)	50	–	100	300	10
IM 9053 (**7a**)	0.5	7	700	2000	20
IM 742 (**7b**)	4	–	70	70	0.5
IM 7382 (**7c**)	9	–	75	150	4
IM 9102 (**8a**)	70	20	85	100	0.5
IM 6383 (**8b**)	0.8	5	0	0	–
IM 7381 (**8c**)	8	–	70	80	2

[a] All data resulted from evaluation in the Legendo laboratory at the KU Leuven. The activities are presented as relative values, the reference value of 1,25-D_3 being defined as 100.

3. Results

The biological evaluation of the parent compound (**1**) and the analogs **6**–**8** has included the determination of (1) the affinity for the pig intestinal mucosa vitamin D receptor (VDR) and for the human vitamin D binding protein (hDBP), (2) the prodifferentiating activity, tested on promyelocytic leukemia HL-60 cells, (3) the in vitro antiproliferative effects, determined on breast cancer MCF-7 cells and (4) the in vivo calcemic activity, tested in vitamin D-replete NMRI mice. Results are shown in Table 1.

4. Discussion

4.1. The 20-epi modification

Opposite to what is usually observed for 1,25-D_3 analogs with the reverse stereochemistry at C20 (e.g. 20-*epi*-**1**), 20-*epi trans*-decalin analogs possessing the natural C-ring and *seco*-B-ring (and the natural or 19-*nor* A-ring) show a much lower biological activity when compared to the

corresponding derivatives with the natural 20*R* side chain [3]. This is explained by the much lower occupation by the 25-OH group of the side chain of the "active" volume, a volume in space determined by conformational analysis of side-chain analogs based on the dot-map approach developed by Okamura et al. [8], the preferred occupation of which would correspond to high prodifferentiating (HL-60) and antiproliferative (MCF-7) activity [3,9]. This volume coincides with the region in space designated EA in the comprehensive structure–function relationship studies of Yamada [10]. Application of our conformational analysis procedure (Section 2) for the side-chain orientation to the *trans*-decalins **6a**–**8a** and **6b**–**8b**, possessing a double bond in the C-ring, showed that also in this case substantial occupation of the active volume is realised by the 20*R* analogs **a**, but not by the 20-*epi* analogs **b**.[1] Inspection of Table 1 learns that the biological activities of analogs **6**–**8** all follow this previously observed trend: the cell differentiative and antiproliferative, but also the calcemic activities of the 20-*epi* analogs **6b**–**8b** are substantially lower than of **6a**–**8a**. The 20-epimerisation modification therefore does not result in a better dissociation of the prodifferentiating and/or antiproliferative activities and the calcemic activity in these types of derivatives. The affinity of **6b**–**8b** for the VDR is also lower than of **6a**–**8a**, except in the case of **7b** for which a 10-fold increase to a moderate 4% is observed. Remarkably the latter previtamin still possesses vitamin D-like prodifferentiative and antiproliferative activity, which has to be attributed to a *trans*-decalin effect rather then to a genuine previtamin triene effect [3,11].

4.2. The 14-epi modification

Conformational analysis of the *cis*-decalins **6c**–**8c** showed that 14-epimerisation has little or no influence on the preferred orientations of the side chain and that substantial occupation of the active volume is equally realised by the 14-*epi* analogs **c** as by the *trans*-decalin analogs **a** (see footnote 1). This is reflected in the results of the biological evaluation: the cell differentiative, antiproliferative and calcemic activities of **6c** and **6a**, and of **8c** and **8a** are comparable. However, in the case of the previtamin **7c** an approximate 10-fold reduction as compared to **7a** is observed. The affinity for the VDR of **6c** is still high, in the case of the previtamin **7c** has increased 20-fold, and in the case of **8c** has decreased 10-fold as compared to their corresponding *trans*-decalin analogs **a**. As is the case for the 20-epimerisation modification (see above), also 14-epimerisation does not improve the desired dissociation of the prodifferentiating and/or antiproliferative activities and the calcemic activity.

In conclusion, further modification by 20-epimerisation of ynediene **6a**, previtamin **7a** and saturated derivative **8a**, *trans*-decalin CD-ring analogs of 1,25-D_3, results in substantially lower prodifferentiating (HL-60) and antiproliferative (MCF-7) activities of **6b**–**8b**. These are largely understood on the basis of their side chain orientation as determined by conformational analysis. This is also the case for the activities observed for **6c** and **8c**, derivatives resulting from modification by 14-epimerisation of **6a** and **8a**, respectively, which are hardly different. However, 14-epimerisation of previtamin **7a** to **7c** leads to an approximate 10-fold reduction in activity. Clearly the structural features that determine the biological activity of previtamins require additional study. Neither 20-epimerisation nor 14-epimerisation leads to a further dissociation of the prodifferentiating and/or antiproliferative activities and the calcemic activity of **6a**–**8a**.

[1] According to a Boltzmann distribution at 298 K, the occupation of the active volume by the 25-hydroxy group of the side chain was calculated to correspond to 68 mol% in the case of **6a**–**8a** and to 0.2 mol% in the case of **6b**–**8b**. For the 14-*epi* decalin analogs **6c**–**8c** the occupation was calculated to correspond to 67 mol%, and to 0.2 mol% in the case of their 20-*epi* analogs (not discussed).

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