Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 18, pp. 2163-2168, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00376-2

1α,25-Dihydroxyvitamin D_3 Hybrid Analogs with Structural Changes at Both the A-Ring and the C,D-Ring Side-chain. II

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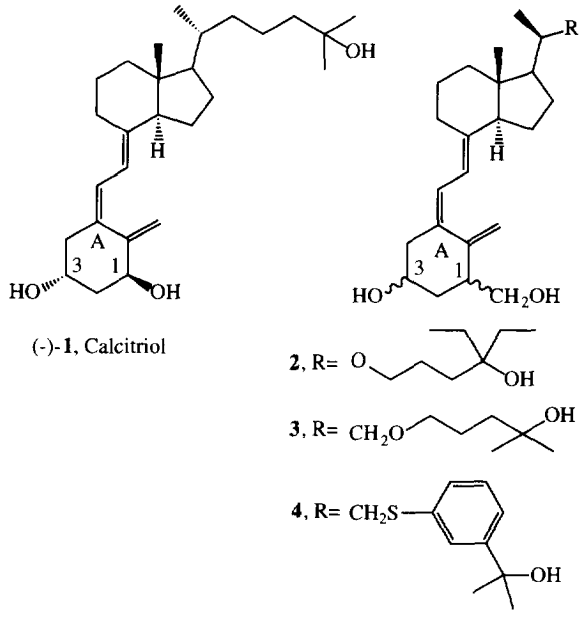
Summary: Calcitriol analogs **3** and **4**, prepared by convergent coupling of 1-hydroxymethyl A-ring phosphine oxide (±)-**5** and structurally modified C,D-ring ketones (–)-**6**, are shown to be hybrid analogs with blended and potent, but variable, biological activities.

We recently proposed and evaluated a new concept for design of physiologically active analogs of hormonally potent 1α,25-dihydroxyvitamin D_3 (calcitriol, **1**).[1] Combining a calcemia-inactivating structural change in the A-ring (*i.e.* a 1-CH_2OH substituent)[2] with a differentiation-activating structural change in the C,D-ring side-chain (*i.e.* KH-1060 side-chain)[3] produced hybrid analog (+)-**2b** with **blended and powerful biological activities**.[1] Now we report the results of further biological testing of this hybrid analog (+)-**2b** and also synthesis and initial biological testing of hybrid analogs **3** and **4** that incorporate the same 1-CH_2OH structural modification but that feature different potentiating C,D-ring side-chain structural modifications. These side-chain modifications were chosen as key structural features characteristic of the most potent vitamin D_3 analogs (*i.e.*, MC-1357 and GS-1500) described recently by the Leo Pharmaceutical Company.[4,5] Convergent syntheses of hybrid analogs **3** and **4** from previously prepared A-ring allylic phosphine oxide (±)-**5**[2] and enantiomerically pure C,D-ring ketones (–)-**6**[6] are shown in eq. 1.[7,8]

Horner-Wadsworth-Emmons coupling and silyl ether deprotection as in eq. 1 produced, in each case, two diastereomeric products that were separated easily by preparative HPLC. Characterization of each diastereomer was achieved tentatively as in previous cases[1,2] using especially 400 MHz ^1H NMR spectroscopy. For each pair of analogs **3** and **4**, the 1α-diastereomer showed the C-18 methyl group at 0.02-0.03 ppm lower field than the 1β-diastereomer. A similar trend in chemical shift was observed also for the C-19 methylene group; coupling constants also allowed distinction between the 1α- and the 1β-diastereomers (see Table I).



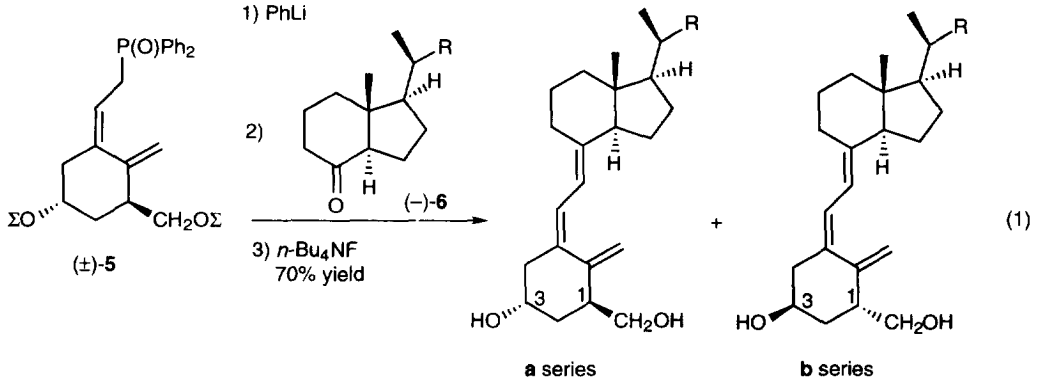


Table I. 400 MHz ^{1}H NMR data (ppm) for Analogs 3 and 4

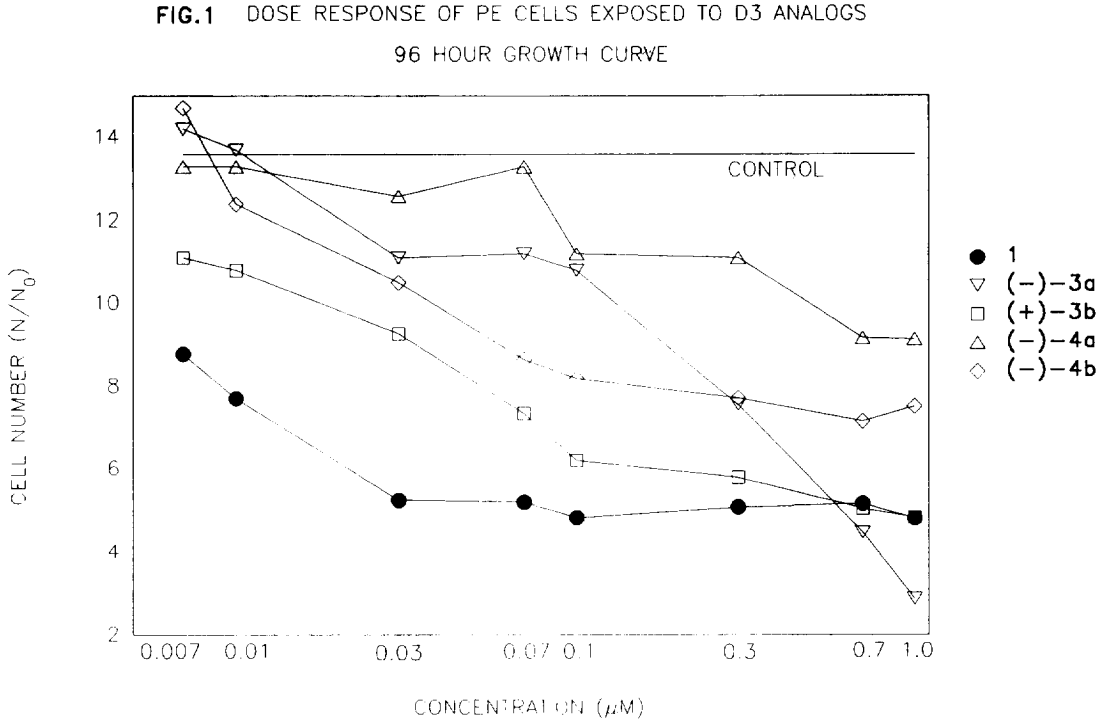
Compound	C-18	C-19a	C-19b
3a	0.55	5.18 (dd, 1.6 & 0.8 Hz)	5.02
3b	0.53	5.14 (d, 1.2 Hz)	4.98
4a	0.52	5.17 (dd, 1.6 & 0.4 Hz)	5.00
4b	0.49	5.15 (d, 1.2 Hz)	4.98

In binding to the calf thymus vitamin D receptor using the previously described protocol,[9] each of the four new vitamin D analog diastereomers (–)-**3a**, (+)-**3b**, (–)-**4a**, and (–)-**4b** binds with less than 1/1000 the affinity of calcitriol. In rat osteoscarcoma cells, using the previously described patch-clamp technique,[10] hybrid analogs **3** and **4** stimulate calcium channels via an instantaneous, non-genomic process (Table II); calcium current is stimulated strongly by analogs **3**, more so than by our first hybrid analog (+)-**2b**, even at the physiologically very low 0.01 nM concentration level.

Table II. Calcium Current Measurements

Compound	Calcium currents (mV) at Various Concentrations of Analogs (mean ± SE, n=3-5)			
	5.0 nM	**0.5 nM**	**0.05 nM**	**0.01 nM**
1	9.79 ± 0.19	5.73 ± 1.33	1.78 ± 0.82	
3a	8.99 ± 0.67	8.80 ± 0.72	7.33 ± 0.22	5.42 ± 1.53
3b	8.93 ± 0.61	10.00 ± 0.08	7.30 ± 1.47	4.94 ± 1.47
4a	11.18 ± 1.77	6.60 ± 1.94		
4b	8.58 ± 1.02	6.24 ± 0.17		

In inhibiting proliferation of murine keratinocytes using the previously described protocol,[2] hybrid analogs (+)-**3b** and (–)-**4b**, characterized by a 1β-oriented hydroxymethyl group, are more potent than the corresponding 1α-hydroxymethyl diastereomers (–)-**3a**, and (–)-**4a**; at 1 μM concentration, diastereomers (+)-**3b** and (–)-**4b** are comparable to calcitriol (**1**) in potency, and they may be only slightly less potent than calcitriol (**1**) even at the physiologically relevant 0.1 μM concentration level (Fig. 1). Our first hybrid analog (+)-**2b** with the KH-1060 side chain appears in keratinocytes to be the most antiproliferative compound in this series of analogs.[1]



Further biological testing of our original hybrid analog (+)-**2b** has shown the following results: (1) in the human breast cancer cell line SKBr3, (+)-**2b** is slightly to moderately **more** active than calcitriol (**1**) at suppressing DNA synthesis; (2) (+)-**2b** is comparable in potency to calcitriol (**1**) at inducing differentiation of U937 cells *in vitro*; (3) (+)-**2b** weakly inhibits growth of human T-cells, thereby making it the first vitamin D_3 analog lacking the 1α-hydroxyl group that shows any immunological activity;[11] and (4) (+)-**2b** is 200-300 less calcemic than calcitriol (**1**), as determined by measuring urinary calcium excretion in female rats 3-7 days after oral administration of this analog. In depth molecular biology study of all of these hybrid analogs **2-4** in terms especially of their ability to activate genes in leukemic cells and to induce phosphorylation in chondrocytes is in progress and will be reported fully in due course.

Thus, our original expectation of blended and powerful biological activities associated with doubly-modified analogs like **2**[1] has now been confirmed also with hybrid analogs **3** and **4**. Especially important is the desirably low *in vivo* calcemic effect of analog (+)-**2b** and the low *in vitro* VDR binding activities of analogs (+)-**3b** and (–)-**4b** combined with their desirably high antiproliferative activity in normal skin cells and in human cancer cells.

Acknowledgment

We thank the NIH for generous financial support, Dr. Michael Sporn for the SKBr3 data, Professor William Rigby for the T-cell data, and Dr. Lise Binderup and Ms. Christina Hansen for the U937 cell assay and for the *in vivo* calcemia study.

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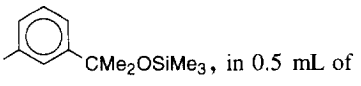
7. A solution of 53 mg (0.11 mmol, 1.5 eq) of phosphine oxide (±)-**5** in 1mL of anhydrous THF was cooled to -78 ℃ and treated dropwise under argon with 110 μL (0.11 mmol, 1.5 eq) of 1 M solution of phenyllithium in THF. The resulting orange solution was stirred for 30 min at -78 °C. To this solution, was added a solution of 25.3 mg (0.066 mmol, 1eq) of C,D-ring ketone (–)-**6**, R = $CH_2O(CH_2)_3CMe_2OSiMe_3$, in 0.5 mL of anhydrous THF dropwise. After being stirred for 1 h at the same temperature, the reaction mixture was allowed to warm up to rt for 10 h, quenched with 2 mL of a 1:1 mixture of 2 N sodium potassium tartrate and 2 N K_2CO_3, extracted with EtOAc (30 mL x 2) and washed with brine (15 mL x 2). The combined organic portion was dried with anhydrous $MgSO_4$, concentrated *in vacuo* and then purified by chromatography (3% EtOAc/hexane) to afford 44.4 mg (0.058 mmol, 88%) of the coupled product as a colorless oil. The silyl ethers were dissolved in 2 mL of anhydrous THF. To the solution, were added 0.35 mL (0.35 mmol, 6 eq) of 1 M tetrabutylammonium fluoride solution in THF, and 50 μL (0.35 mmol, 6 eq) of triethylamine. After 12 h at rt, the mixture was extracted with EtOAc (30 mL x 2) and washed with brine (15 mL x 2). The combined organic portion was dried with anhydrous $MgSO_4$, concentrated *in vacuo*

and then purified by chromatography (EtOAc / MeOH / NEt_3) to afford 25.5 mg (0.055 mmol, 95%) of mixture of two diastereomers as a viscous colorless oil. The diastereomers were separated by reverse phase HPLC (C-18 semipreparative column, 50% MeCN / H_2O, 3 ml / min) to afford 8.5 mg (26.8 %) of (–)-**3a** (RT=38.9 min) as a white solid, and 11.5 mg (42.8%) of (+)-**3b** (RT=46.0 min) as a colorless oil. Rf=0.39 (3 % MeOH/EtOAc). (–)-**3a** (1β, 3α); $[\alpha]^{28}_D$ -131° (c=2 mg/mL, $CHCl_3$); mp 129 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.32 (d, *J* =11.2 Hz, 1H), 5.95 (d, *J* =11.2 Hz, 1H), 5.18 (dd, *J* =1.6, 0.8 Hz, 1H), 5.02 (d, *J* =2 Hz, 1H), 3.99-3.93 (m, 1H), 3.54-3.52 (m, 1H), 3.50 (dd, *J* =5.2, 4 Hz, 1H), 3.47-3.36 (m, 2H), 3.20 (dd, *J* =9.2, 7.6 Hz, 1H), 2.83 (dd, *J* =12.4, 4 Hz, 1H), 2.67-2.59 (m, 2H), 2.56 (br s, OH), 2.26 (dd, *J* =12.0, 9.6 Hz, 1H), 2.00-1.21 (m, 21H), 1.22 (s, 6H), 0.95 (d, *J* =6.4, 3H) 0.55 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.11, 142.79, 134.01, 123.71, 117.14, 114.56, 75.34, 71.58, 70.14, 67.15, 64.32, 56.14, 53.56, 46.36, 45.69, 45.07, 41.19, 39.73, 37.43, 36.14, 29.48, 29.26, 28.98, 26.77, 24.81, 23.54, 22.12, 17.29, 12.37; IR ($CHCl_3$, cm^{-1}) 3607, 3398, 3008, 2934, 2874, 1644, 1453, 1377, 1247, 1100, 1036; UV (MeOH) λ_{max} 266 nm (ε=66,000); MS *m/z* (70 eV, EI) 460 (10.3%, M^+), 148(100%); HRMS *m/z* (M^+) Calcd. for $C_{29}H_{48}O_4$ 460.3553, found 460.3556. (+)-**3b** (1β, 3α); $[\alpha]^{28}_D$ + 45 ° (c=1 mg/ mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.30 (d, *J* =11.2 Hz, 1H), 5.94 (d, *J* =11.2 Hz, 1H), 5.14 (d, *J* =1.2 Hz, 1H), 4.98 (d, *J* =2 Hz, 1H), 4.03-3.97 (m, 1H), 3.64-3.54 (m, 1H), 3.50 (dd, *J* =5.2, 4 Hz, 1H), 3.46-3.36 (m, 2H), 3.50 (dd, *J* =9.2, 7.6 Hz, 1H), 2.82 (dd, *J* =12.4, 4.0 Hz, 1H), 2.65-2.57 (m, 2H), 2.56(br s, OH), 2.27 (dd, *J* =12.8, 8.8 Hz, 1H), 2.00-1.25 (m, 21H), 1.22 (s, 6H), 0.94 (d, *J* =6.8, 3H) 0.53 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.33, 142.84, 134.28, 123.57, 117.07, 113.84, 75.33, 71.55, 70.15, 67.09, 64.29, 58.09, 53.47, 46.23, 45.63, 44.46, 41.16, 39.73, 37.36, 36.07, 29.45, 29.24, 28.97, 26.81, 24.79, 23.43, 22.05, 17.28, 12.32; IR ($CHCl_3$, cm^{-1}) 3613, 3402, 3014, 2943, 2873, 1602, 1467, 1350, 1238, 1114; UV (MeOH) λ_{max} 262 nm (ε=16,000); MS *m/z* (70 eV, EI) 460 (11.43%, M^+), 148(100%); HRMS *m/z* (M^+) Calcd. for $C_{29}H_{48}O_4$ 460.3553, found 460.3552.

8. A solution of 30 mg (0.06 mmol, 1.5 eq) of phosphine oxide (±)-**5** in 0.7 mL of anhydrous THF was cooled to -78 °C and treated dropwise under argon with 63 μL (0.06 mmol, 1.5 eq) of 1 M solution of phenyllithium in THF. The resulting orange solution was stirred for 30 min at -78 °C. To this solution, was added a solution of 19.0 mg (0.044 mmol, 1 eq) of C,D-ring (–)-**6**, R =  , in 0.5 mL of anhydrous THF dropwise. After being stirred for 1 h at the same temperature, the reaction mixture was allowed to warm up to rt for 10 h, quenched with 2 mL of a 1:1 mixture of 2 N sodium potassium tartrate and 2 N K_2CO_3, extracted with EtOAc (30 mL x 2) and washed with brine (15 mL x 2). The combined organic portion was dried with anhydrous $MgSO_4$, concentrated *in vacuo* and then purified by chromatography (3% EtOAc/hexane) to afford 27.8 mg (0.058 mmol, 78%) of the coupled product as a colorless oil. The silyl ethers (60.0 mg, 0.074 mmol) were dissolved in 3 mL of anhydrous THF. To the solution, were added 0.44 mL (0.44 mmol, 6 eq) 1 M tetrabutylammonium fluoride solution in THF, and 65 μL (0.35 mmol, 5 eq) of triethylamine. After 12 hr, the mixture was extracted with EtOAc (30 mL x 2) and washed with brine (15 mL x 2). The combined organic portion was dried with anhydrous $MgSO_4$, concentrated *in vacuo* and then purified by chromatography (EtOAc/MeOH/NEt_3) to afford 37.0 mg (0.073 mmol, 98%) of mixture of two diastereomers as a viscous colorless oil. The diastereomers were separated by reverse phase HPLC (C-18 semipreparative column, 60% MeCN/H_2O, 3 ml/min) to afford 9.5 mg (31.7%) of (–)-**4a** (RT=28.5 min) as a white solid, and 14.9 mg (42.8%) of (–)-**4b** (RT=35.5 min) as a colorless oil. Rf=0.40 (3%

MeOH/ EtOAc). (–)-**4a**; $[\alpha]^{28}_D$ -127 ° (c=1.4 mg/ mL, $CHCl_3$); mp 148 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.50-7.47 (m, 1H), 7.27-7.23 (m, 3H), 6.31 (d, *J* =11.6 Hz, 1H), 5.95 (d, *J* =11.6 Hz, 1H), 5.17 (dd, *J* =2.0, 0.8 Hz, 1H), 5.01 (d, *J* =1.2 Hz, 1H), 3.99-3.92 (m, 1H), 3.57-3.54 (m, 2H), 3.26 (dd, *J* =12.4, 3.6 Hz, 1H), 2.83-2.79 (m, 1H), 2.75 (dd, *J* =12.0, 8.4 Hz, 1H), 2.66-2.58 (m, 2H), 2.25 (dd, *J* =12.0, 9.6 Hz, 1H), 2.03-1.28 (m, 20H), 1.04 (d, *J* =6.8, 3H) 0.52 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.77, 145.14, 142.50, 137.49, 134.17, 128.64, 127.24, 125.21, 123.68, 117.27, 114.52, 72.45, 67.13, 64.31, 56.03, 55.74, 46.34, 45.80, 45.01, 40.82, 40.27, 37.41, 35.41, 31.71, 28.95, 26.84, 23.57, 22.07, 18.89, 12.46; IR ($CHCl_3$, cm^{-1}) 3605, 2934, 1642, 1379, 1100, 1036, 914; UV (MeOH) λ_{max} 258 nm (ε=23,000); MS *m/z* (70 eV, EI) 510 (45.12%, M^+), 148 (100%); HRMS *m/z* (M^+) Calcd. for $C_{32}H_{46}O_3S$ 510.3168, found 510.3173. (–)-**4b**; $[\alpha]^{28}_D$ -16 ° (c=11.9 mg/mL, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.48-9-7.49 (m, 1H), 7.27-7.18 (m, 3H), 6.30 (d, *J* =11.2 Hz, 1H), 5.94 (d, *J* =11.2 Hz, 1H), 5.15 (dd, *J* =1.2, 1H), 4.98 (d, *J* =2.0 Hz, 1H), 4.03-3.97 (m, 1H), 3.61-3.58 (m, 2H), 3.25 (dd, *J* =12.4, 3.6 Hz, 1H), 2.84-2.79 (m, 1H), 2.74 (dd, *J* =12.4, 8.8 Hz, 1H), 2.66-2.57 (m, 2H), 2.27 (dd, *J* =12.6, 8.0 Hz, 1H), 2.03-1.33 (m, 20H), 1.04 (d, *J* =6.8, 3H) 0.49 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.77, 145.33, 142.58, 137.49, 134.41, 128.63, 127.23, 125.22, 123.52, 117.18, 113.86, 72.43, 67.09, 64.29, 56.00, 55.68, 46.22, 45.74, 44.47, 40.82, 40.29, 37.36, 35.37, 31.71, 28.95, 26.89, 23.46, 22.00, 18.88, 12.43; IR ($CHCl_3$, cm^{-1}) 3604, 3015, 2932, 1590, 1452, 1381, 1036; UV (MeOH) λ_{max} 258 nm (ε=53,000); MS *m/z* (70 eV, EI) 510 (68.75%, M^+), 135 (100%); HRMS *m/z* (M^+) Calcd. for $C_{32}H_{46}O_3S$ 510.3168, found 510.3163.

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(*Received in USA* 27 *June* 1995; *accepted* 18 *August* 1995)