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# Synthesis and Biological Evaluation of Phosphonate Analogues of 1α, 25-Dihydroxyvitamin $D_3$

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A new series of phosphonate side chain analogues of 1α,25-dihydroxyvitamin $D_3$ (**1**) have been synthesized. Antiproliferative activities of theses analogues (**8a,b** and **9a,b**) using human keratinocyte cell shows that analogues which have natural A-ring show higher activity than unnatural A-ring series and almost equally active to 1α,25-Dihydroxyvitamin $D_3$ (**1**) at 1 μM level.

**Key words**: vitamin $D_3$, calcitriol, analogue, phosphonate, antiproliferative activity

## INTRODUCTION

1α, 25-Dihydroxyvitamin $D_3$ (calcitriol, **1** in Fig. 1), dihydroxylated metabolite at liver and kidney from naturally occurring vitamin $D_3$, has been considered as a very active hormone for calcium homeostasis (Holick 1999, Bouillon, *et al.*, 1995). However antiproliferative and prodifferentiating activity of **1** has been recently more highlighted as a potential anticancer substance (Bouillon *et al.*, 1995). For this purpose, structural modification in C,D-ring and side chain is essential to separate desirable properties from undesirable calcemic activity (Feldman 1997, Uskokovic *et al.*, 1997, Posner *et al.*, 1999). More recently, 1α, 25-dihydroxy-24-oxo-vitamin $D_3$ (**2**), main metabolite of side chain degradation pathway, shows equal antiproliferative potency to 1α, 25-dihydroxyvitamin $D_3$ (**1**) but 10 fold less calcemic effect (Bouillon *et al.*, 1995). This suggests that structural modification of C-24 carbonyl group in **2** to other chemical functionalities (*i.e.* phosphonyl or sulfonyl) could provide biologically stable and equally or highly active analogue species. In this paper, we will discuss about synthesis and biological evaluation of phosphonate analogues which have phosponyl group (P=O) instead of carbonyl group (C=O) in lead compound **2** (Fig. 2). Scheme 1 shows general synthesis for phosphonate analogues (**8a, b** and **9a, b**).

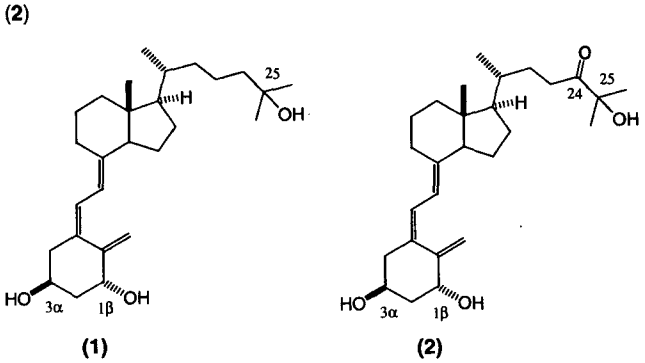


**Fig. 1.** 1α,25-dihydroxyvitamin $D_3$ (**1**) and 1α,25-dihydroxy-24-oxo-vitamin $D_3$ (**2**)

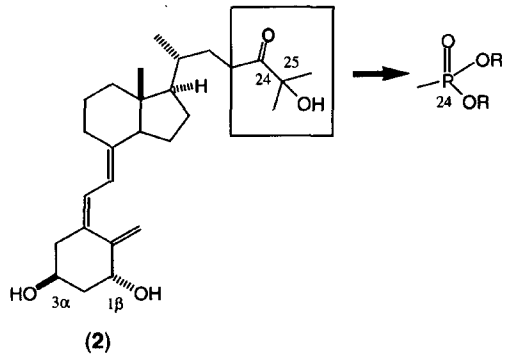


**Fig. 2.** Design of phosphonate analogue of 1α, 25-dihydroxy-24-oxo-vitamin $D_3$

## MATERIALS AND METHODS

Tetrahydrofuran (THF) was distilled from benzophenone ketyl prior to use. Methylene chloride ($CH_2Cl_2$) and triethylamine (TEA) were distilled from calcium hydride prior to use. Commercially available anhydrous solvents were used in other instances. Chemicals were purchased

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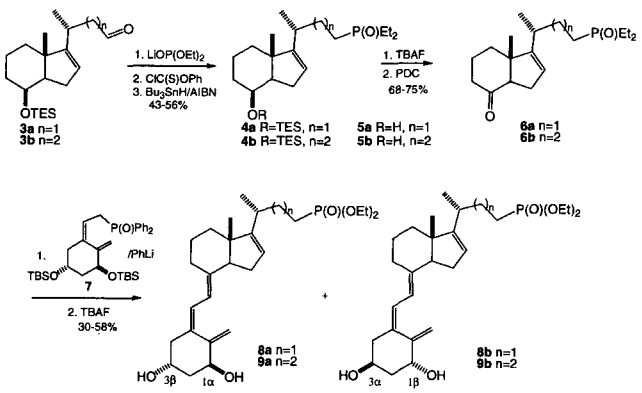



**Scheme 1.** Synthesis of Phosphonate Analogues

from Aldrich Chemical Company. Reactions were run under an atmosphere of argon. $^1H$ and $^{13}C$ NMR spectra were obtained on Varian XL-400 and XL-500 spectrometers. $^{31}P$ NMR spectra were recorded on Bruker AMX-300 spectrometer. Proton chemical shift are reported in ppm (δ) using residual $CHCl_3$ (δ 7.26) and phosphoric acid (δ 0.0) respectively. High resolution mass spectral data were obtained by using a VG-70S mass spectrometer run at 70 eV. Chemical ionization mass spectra (CIMS) were obtained using isobutane as a carrier gas. Rotations were obtained at ambient temperature on a Perkin-Elmer Model 141 polarimeter. Flash column chromatography was performed using EM Sciences Silica Gel 60 (25-40 mm). Preparative TLC was done with EM Silica Gel 60 $PF_{254}$.

**16-Ene-phosphonates (+)-4a,b.** To a solution of diethyl phosphite (0.5 ml, 3.88 mmol) in 5 ml of THF was added 2.8 ml of *n*-butyl lithium solution (1.35 M, 3.78 mmol) dropwise at -78°C. After 1 h at -78°C, 0.9 ml of above lithium diethyl phosphite solution (0.45 mmol) was added to a solution of the aldehydes (**3a**: 102 mg, 0.30 mmol, **3b**, 50 mg, 0.14 mmol) in 10 ml of THF at -78°C. After 2 h at -78°C, the reaction mixture was quenched with saturated ammonium chloride solution (20 ml) and extracted with ethyl acetate (50 ml × 3). The combined organic extract was washed with brine (30 ml), dried over $MgSO_4$ and concentrated. The crude product was purified by flash column chromatography (ethyl acetate/hexanes, 2/1) to give diasteromeric alcohols as colorless oil (for **4**a: 104 mg, 73%; for **4b**: 56 mg, 76%). For **4a**: IR (film) 3300, 2930, 1231 $cm^{-1}$; $^1H$ NMR (400 MHz/$CDCl_3$) δ 5.36 and 5.24 (s, 1H), 4.20-4.08 (m, 5H), 3.91 (m, 1H), 2.46 and 2.26 (m, 4H), 1.91-1.25 (m, 17H), 1.03 (t, *J*=6.6 Hz, 3H), 1.00 (s, 3H), 0.95 and 0.94 (t, *J*=7.8 Hz, 9H), 0.56 and 0.55 (q, *J*=8.0 Hz, 6H); EIMS *m/z* (relative intensity) 474 ($M^+$, 6), 456 ($M^+$-$H_2O$, 8), 445 ($M^+$-Et, 5); HRMS ($C_{24}H_{47}O_5PSi$) calcd 474.2930, found 474.2936. For **4b**: IR (film) 3301, 2955, 1234 $cm^{-1}$; $^1H$ NMR (400 MHz/$CDCl_3$) δ 5.37 (s, 1H), 4.14 (m, 5H), 3.80 (m, 1H), 2.31 (t, *J*=13.0 Hz, 1H), 2.01 (m, 4H), 1.86 (m, 2H), 1.80-1.44 (m, 1H), 1.31 (t, *J*=7.2 Hz, 6H), 0.98 (m, 6H), 0.94 and 0.93 (t, *J*=7.6 Hz, 9H), 0.54 (q, *J*=7.6 Hz, 6H); EIMS *m/z* (relative intensity) 488 ($M^+$, 2), 459 ($M^+$-$C_2H_5$, 4); HRMS ($C_{25}H_{49}O_5PSi$) calcd 488.3087, found 488.3088.

To solution of the above alcohols (for **4a**: 32 mg, 0.068 mmol; for **4b**: 55 mg, 0.11 mmol) in 7 ml of dry $CH_2Cl_2$ was added *N,N*-dimethylaminopyridine (for **4a**: 72 mg, 0.59 mmol; for **4a**: 120 mg, 0.98 mmol) and phenyl chlorothioformate (for **4a**: 0.40 ml, 0.29 mmmol; for **4b**: 0.66 ml, 0.48 mmol) at 0°C. The mixture was stirred for 1 h at 0°C and then at rt for 17 h. The solution was concentrated in reduced pressure. The residue was purified by preparative TLC (ethyl acetate/hexanes, 1/2) to give formates as pale yellow oil (for **4a**: 38 mg, 91%; for **4b**: 59 mg, 86%). For **4a**: IR (film) 2932, 1284, 1201 $cm^{-1}$; $^1H$ NMR (400 MHz/$CDCl_3$) δ 7.41 (m, 2H), 7.29 (m, 1H), 7.10 (m, 2H), 5.40 and 5.38 (m, 1H), 5.39 and 5.35 (s, 1H), 4.22 (m, 4H), 4.12 (br t, 1H), 2.41-2.07 (m, 4H), 1.91-1.66 (m, 6H), 1.46 (m, 2H), 1.38 (m, 6H), 1.10 (t, *J*=6.8 Hz, 3H), 1.06 (d, *J*=3.6 Hz, 3H), 0.95 and 0.93 (t, *J*=7.9 Hz, 9H), 0.56 (m, 6H); CIMS *m/z* (relative intensity) 611 ($MH^+$, 18), 518 ($M^+$-Et, 5), 456 ($M^+$-HOCSOPh, 100); HRMS (for $MH^+$, $C_{31}H_{52}O_6PSSi$) calcd 611.2992, found 611.2983. For **4b**: IR (film) 2930, 1261, 1203 $cm^{-1}$; $^1H$ NMR (400 MHz/$CDCl_3$) δ 7.41 (t, *J*=7.8 Hz, 2H), 7.30 (t, *J*=7.4 Hz, 1H), 7.10 (m, 2H), 5.83 (m, 1H), 5.31 and 5.29 (s, 1H), 4.19 (m, 4H), 4.12 (s, 1H), 2.26 (dd, *J*=13.6, 12.6 Hz, 1H), 2.10-1.85 (m, 6H), 1.70 (m, 3H), 1.35 (dq, *J*=7.2, 3.6 Hz, 4H), 1.01 (t, *J*=5.2 Hz, 6H), 0.95 (t, *J*=8.0 Hz, 9H), 0.56 (q, *J*=8.0 Hz, 6H); CIMS *m/z* (relative intensity) 625 ($MH^+$, 5); HRMS ($MH^+$, $C_{32}H_{54}O_6PSSi$) calcd 625.3148, found 625.3139.

To a solution of the above formates (for **4a**: 110 mg, 0.18 mmol; for **4b**: 75 mg, 0.12 mmol) in 7 ml of dry toluene were added tributyltin hydride (for **4a**: 0.095 ml, 0.35 mmol; for **4b**: 0.13 ml, 0.48 mmol) and 2,2-azobisisobutyronitrile (0.03 M in toluene, for **4a**: 1.4 ml, 0.045 mmol; for **4b**: 0.7 ml, 0.021 mmol). The solution was refluxed for 2 h and then allowed to cool to rt. The reaction mixture was concentrated *in vacuo*. The residue was purified by flash chromatography (ethyl acetate/hexanes, 1/1) to give phosphonate (+)-**4a,b** as a colorless oil (**4a:** 70 mg, 85%; for **4b**: 37 mg, 66%). (+)-**4a**: $[\alpha]_D^{20}$ = (+)-18.7° (c=1.5, $CHCl_3$); IR (film) 2930, 1247, $cm^{-1}$; $^1H$ NMR (400 MHz/$CDCl_3$) δ 5.23 (d, *J*=7.6 Hz, 1H), 4.04 (m, 5H), 2.22 (t, *J*=13.2 Hz, 1H), 2.02 (m, 1H), 1.87-1.38 (m, 10H), 1.29 (t, *J*=7.0 Hz, 6H), 0.95 (d, *J*=8.0 Hz, 3H), 0.92 (t, *J*=8.0 Hz, 9H), 0.56 (q, *J*=8.0 Hz, 6H); $^{13}C$ NMR (100 MHz/$CDCl_3$) δ 158.6, 120.5, 68.9, 61.4, 55.0, 46.5, 35.7, 34.9, 32.6 (d, *J*=19 Hz), 30.8, 28.5, 23.9 (d, *J*=139 Hz), 22.4, 18.6, 18.0, 16.4, 6.9, 4.9; $^{31}P$ NMR (300 MHz/$CDCl_3$) δ 33.8; EIMS *m/z* (relative intensity) 458 ($M^+$, 31), 429 ($M^+$-Et, 100); HRMS ($C_{24}H_{47}O_4PSi$) calcd 458.2981, found 468.2987. (+)-**4b**: $[\alpha]_D^{20}$ =(+)-45° (c=1.4, $CHCl_3$); IR (film) 2951, 1243, $cm^{-1}$; $^1H$ NMR (400 MHz/$CDCl_3$) δ

5.24 (s, 1H), 4.07 (m, 5H), 2.23 (t, *J*=13.2 Hz, 1H), 2.02 (m, 1H), 1.86 (m, 2H), 1.70-1.39 (m, 12H), 1.29 (t, *J*= 7.0 Hz, 6H), 0.98 (s, 3H), 0.95 (d, *J*= 6.8 Hz, 3H), 0.93 (t, *J*=8.0 Hz, 9H), 0.54 (q, *J*=8.0 Hz, 6H); $^{13}$C NMR (100 MHz/$CDCl_3$) δ 159.8, 119.8, 68.9, 61.3, 55.0, 46.5, 37.4 (d, *J*=16 Hz), 35.7, 34.9, 31.2, 30.7, 25.8 (d, *J*=139 Hz), 22.2, 20.5, 18.7, 18.0, 16.4, 6.9, 4.9; $^{31}$P NMR (300 MHz/$CDCl_3$) δ 32.9; EIMS *m/z* (relative intensity) 472 ($M^+$, 2), 443 ($M^+$-$C_2H_5$, 100); HRMS ($C_{25}H_{49}O_4PSi$) calcd 472.3138, found 472.3145.

**16-Ene-8-Hydroxy-Phosphonate (+)-5a,b.** A solution of triethylsilyl-ether ((+)-**4**: 56 mg, 0.12 mmol; (+)-**4b**: 51 mg, 0.11 mmol) in 10 ml of THF and Tetrabutylammonium fluoride (1 M in THF, 0.13 ml, 0.13 mmol) was stirred for 5 h at rt. The mixture was concentrated in reduced pressure and the residue was purified by flashy chromatography (ethyl acetate) to give alcohol (+)-**5a,b** as a coloress oil (**5a**: 34 mg, 83 %; **5b**: 27 mg, 70%). (+)-**5a**: $[\alpha]_D^{20}$ =(+)-8.1° (c=0.8, $CHCl_3$); IR (film) 3414, 2927, 1243 $cm^{-1}$; $^1$H NMR (400 MHz/$CDCl_3$) δ 5.29 (br t, 1H), 4.14 (s, 1H), 4.04 (m, 5H), 2.26 (t, *J*= 12.0 Hz, 1H), 2.05 (m, 1H), 1.94 (dq, *J*=14.8, 3.2 Hz, 1H), 1.78-1.28 (m, 12H), 1.29 (t, *J*=7.0 Hz, 6H), 1.00 (s, 3H), 0.98 (d, *J*= 6.8 Hz, 3H); $^{13}$C NMR (100 MHz/$CDCl_3$) δ 158.6, 120.6, 68.9, 61.3, 54.4, 46.2, 35.4, 33.9, 32.6 (d, *J*=18 Hz), 30.2, 29.6, 28.5, 23.9 (d, *J*= 140 Hz), 22.0, 18.2, 17.7, 16.4; EIMS *m/z* (relative intensity) 344 ($M^+$, 6), 326 ($M^+$-$H_2O$, 4); HRMS ($C_{18}H_{33}O_4P$) calcd 344.2116, found 344.2108. (+)-**5b**: $[\alpha]_D^{20}$ =(+)-4.8° (c=2.7, $CHCl_3$); IR (film) 3419, 2928, 1228 $cm^{-1}$; $^1$H NMR (400 MHz/$CDCl_3$) δ 5.29 (s, 1H), 4.15 (s, 1H), 4.06 (m, 4H), 2.45 (t, *J*=13.2 Hz, 1H), 2.04-1.92 (m, 2H), 1.89-1.80 (m, 3H), 1.74-1.38 (m, 13H), 1.29 (t, *J*=7.0 Hz, 6H) 1.01 (s, 3H), 0.97 (d, *J*=6.8 Hz, 3H); $^{13}$C NMR (100 MHz/$CDCl_3$) δ 159.8, 120.1, 69.1, 61.3, 54.5, 46.4, 37.5 (d, *J*=16 Hz), 35.6, 34.0, 31.4, 30.2, 25.9 (d, *J*=139 Hz), 20.0, 20.6, 18.4, 17.8; EIMS *m/z* (relative intensity) 358 ($M^+$, 8), 340 ($M^+$-$H_2O$, 13); HRMS ($C_{19}H_{35}O_4P$) calcd 358.2273, found 358.2276.

**16-Ene-8-Ketone 6a,b.** To a solution of alcohol (**5a**: 27 mg, 0.76 mmol; **5b**: 34 mg, 0.10 mmol) in 10 ml of $CH_2Cl_2$ was added 57 mg of oven dried celite and pyridium dichlomate (for **6a**: 57 mg, 0.15 mmol; for **6b**: 75 mg, 0.20 mmol) at rt. After 17 h the reaction mixture filtered through flashy silica pad, and then eluted with ethyl acetate. The filtrate was concentrated and purified by flash chromatography (ethyl acetate) to give ketone (+)-**6a,b** as a light yellow oil (**6a**: 24 mg, 90%; **6b**: 32 mg, 97%). (+)-**6a** : $[\alpha]_D^{20}$ = (+)-13.4° (c=2.4, $CHCl_3$); IR (film) 2991, 1710, 1236 $cm^{-1}$; $^1$H NMR (400 MHz/$CDCl_3$) δ 5.28 (s, 1H), 4.07 (m, 4H), 2.82 (dd, *J*=10.8, 6.6 Hz, 1H), 2.44 (dd, *J*=15.6, 10.8 Hz, 1H), 2.26 (m, 2H), 2.11-1.42 (m, 12H), 1.28 (t, *J*=7.0 Hz, 6H), 1.02 (d, *J*=6.8 Hz, 3H), 0.77 (s, 3H); $^{13}$C NMR (100 MHz/$CDCl_3$) δ 210.5, 157.7, 120.5, 63.2, 61.3, 53.7, 40.4, 37.3 (d, *J*= 16 Hz), 34.5, 32.5, 27.1, 25.9 (d, *J*=140 Hz), 23.9, 21.5, 20.5, 17.3, 16.4; EIMS *m/z* (relative intensity) 356 ($M^+$, 61); HRMS ($C_{19}H_{33}O_4P$) calcd 356.2117, found 356.2124. (-)-**6b**: $[\alpha]_D^{20}$ =(-)-19.3° (c=3.8, $CHCl_3$); IR (film) 2960, 1716, 1244 $cm^{-1}$; $^1$H NMR (400 MHz/$CDCl_3$) δ 5.30 (s, 1H), 4.07 (m, 4H), 2.84 (dd, *J*=10.4, 6.8 Hz, 1H), 2.48-1.58 (m, 13H), 1.31 (t, *J*=6.8 Hz, 6H), 1.07 (d, *J*=6.8 Hz, 3H), 0.80 (s, 3H); $^{13}$C NMR (100 MHz/$CDCl_3$) δ 210.2, 156.7, 121.2, 63.1, 61.3, 53.6, 40.4, 34.3, 33.5 (d, *J*= 17 Hz), 29.6, 28.6, 27.1, 23.7 (d, *J*=140 Hz), 23.8, 21.3, 17.2, 16.4; EIMS *m/z* (relative intensity) 342 ($M^+$, 42); HRMS ($C_{18}H_{31}O_4P$) calcd 342.1960, found 342.1961.

**16-Ene-Phosphonate Calcitriol Analogs (+)-8a, (-)-8b and (+)-9a, (-)-9b.** To a solution of phosphine oxide (+/-)-**7** (for **8**: 88 mg, 0.15 mmol; for **9**: 79 mg, 0.13 mmol) in 1 ml of anhydrous THF was treated dropwise with phenyl lithium (for **8**: 1.5 M in cyclohexane-ether, 0.1 ml, 0.15 mmol; for **9**: 1.58 M in cyclohexane-ether, 0.82 mL, 0.13 mmol) at -78°C. The resulting reddish orange solution was stirred at -78°C for 30 min and then a solution of ketone (**6a**: 33 mg, 0.10 mmol; **6b**: 23 mg, 0.065 mmol) in 1 ml of anhydrous THF was added dropwise. The reaction mixture was stirred until reddish color turned to pale yellow, and then quenched with 3 ml of a 1/1 mixture of 2 N sodium potassium tartrate solution and 2 N $K_2CO_3$ solution. The aqueous layer was extracted with ethyl acetate (50 ml × 3). The combined organic extract was with brine (50 ml), dried over $MgSO_4$, and concentrated. The residue was purified by preparative TLC (ethyl acetate) to give coupled prducts as colorless oil (for **8**: 35 mg, 50%; for **9**: 35 mg, 83%) and unreacted ketone **6a** (15 mg, 45%).

To a solution of silyl ether (for **8**: 35 mg, 0.50 mmol; for **9**: 35 mg) in 10 mL of anhydrous THF was addedtetrabutylammonium fluoride (for **8**: 1 M in THF, 0.16 mL, 0.16 mmol; for **9**: 0.26 mL, 0.26 mmol) and TEA (for **8**: 0.025 ml, 0.18 mmol; for **9**: 0.04 mL, 0.29 mmol). The solution was stirred at rt for 15 h in dark. The reaction mixture was diluted with 20 ml of brine and the aqueous layer was extracted with ethyl acetate (30 ml × 3). The combined organic extract was washed with brine (20 ml), dried over $MgSO_4$, and concentrated. The residue was purified by preparative TLC (ethyl acetate/TEA, 97/3) to give diastereomeric diols as colorless oil (**8a,b**: 14 mg, 59%). The diastereomers **8a,b** were separated by reverse phase HPLC (C-18 semi preparative column, 55% MeCN/45% $H_2O$, 3 ml/min) to give (+)-**8a** as a colorless oil (6 mg, 13%, ret. time, 45.0 min) and (-)-**8b** as a colorless oil (8 mg, 17%, ret. time, 41.5 min). (+)-**8a**: $[\alpha]_D^{20}$ =(+)-9.0° (c=0.4, EtOH); $\lambda_{max}$=263 nm, ε=11,000; IR (film) 3608, 2930, 1239 $cm^{-1}$; $^1$H NMR (500 MHz/$CDCl_3$) δ 6.37 (d, *J*= 11.5 Hz, 1H), 6.11 (d, *J*= 11.0 Hz, 1H), 5.34 (s, 1H), 5.32 (m, 1H), 5. 01 (s, 1H), 4.45 (m, 1H), 4.24 (m, 1H), 4.07 (m,

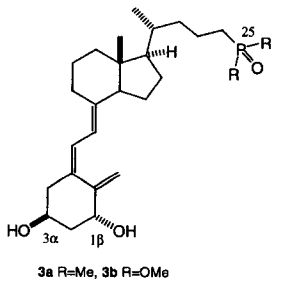
4H), 3.64 (s, 1H), 3.38 (m, 1H), 2.81 (dd, *J*=12.0, 4.5 Hz, 1H), 2.60 (dd, *J*=13.0, 3.5 Hz, 1H), 2.35 (m, 2H), 2.22 (t, *J*=11.0 Hz, 1H), 2.16 (t, *J*=7.7 Hz, 2H), 2.07-1.48 (m, 8H), 1.32 (t, *J*=7.0 Hz, 6H), 1.04 (d, *J*=7.0 Hz, 3H), 0.68 (s, 3H); $^{13}$C NMR (100 MHz/$CDCl_3$) δ 158.2, 147.7, 142.3, 133.1, 124.8, 121.2, 116.9, 111.6, 70.6, 66.9, 61.4, 58.3, 49.9, 45.2, 43.9, 36.7, 33.5 (d, *J*=18 Hz), 31.9, 28.7, 27.2, 23.7 (d, *J*=140 Hz), 23.6, 21.4, 16.8, 16.5; CIMS *m/z* (relative intensity) 479 ($MH^+$, 56), 461 ($MH^+$-$H_2O$, 45); HRMS (for $MH^+$, $C_{27}H_{44}O_5P$) calcd 479.2926, found 479.2916. (-)-**8b**: $[\alpha]_D^{20}$ = (-)-2.0° (c=0.8, EtOH); $\lambda_{max}$=264 nm, ε=11,200; IR (film) 3608, 2930, 1240 $cm^{-1}$; $^1$H NMR (400 MHz/$CDCl_3$) δ 6.39 (d, *J*=11.2 Hz, 1H), 6.10 (d, *J*=11.6 Hz, 1H), 5.31 (s, 2H), 5. 01 (d, *J*=1.2 Hz, 1H), 4.45 (m, 1H), 4.21 (m, 1H), 4.09 (m, 4H), 3.63 (s, 1H), 3.38 (m, 1H), 2.82 (dd, *J*=12.0, 4.0 Hz, 1H), 2.62 (dd, *J*=12.8, 4.0 Hz, 1H), 2.39-1.98 (m, 16H) 1.32 (t, *J*=7.2 Hz, 6H), 1.04 (d, *J*=6.8 Hz, 3H), 0.67 (s, 3H); $^{13}$C NMR (100 MHz/$CDCl_3$) δ 158.2, 147.1, 142.4, 133.9, 124.9, 121.3, 116.9, 112.9, 71.5, 66.8, 61.4, 58.3, 50.0, 45.5, 42.8, 35.2, 33.7 (d, *J*=17 Hz), 31.9, 28.6, 27.1, 23.8 (d, *J*=140 Hz), 23.7, 21.3, 16.8, 16.5; CIMS *m/z* (relative intensity) 479 ($MH^+$, 26), 461 ($MH^+$-$H_2O$, 45); HRMS (for $MH^+$, $C_{27}H_{44}O_5P$) calcd, 479.2926 found 479.2911.

The diastereomers **9a,b** were separated by reverse phase HPLC (C-18 semi preparative column, 55% MeCN/45% $H_2O$, 4 ml/min) to give (+)-**9a** (10mg, 34%, ret. time, 48.8 min) as a colorless oil and (-)-**9b** (7mg, 24%, ret. time, 41.3 min) as a colorless oil. (+)-**9a**: $[\alpha]_D^{20}$ =(+)-2.0° (c=1.0, EtOH); $\lambda_{max}$=262 nm, ε=11,180; IR (film) 3384, 2931, 1233 $cm^{-1}$; $^1$H NMR (400 MHz/$CDCl_3$) δ 6.37 (d, *J*=11.6 Hz, 1H), 6.10 (d, *J*=11.6 Hz, 1H), 5.30 (d, *J*= 16.4 Hz, 2H), 5. 00 (s, 1H), 4.43 (m, 1H), 4.30 (m, 1H), 4.08 (m, 4H), 3.64 (m, 1H), 2.81 (d, *J*= 11.6 Hz, 1H), 2.59 (d, *J*= 10.8 Hz, 1H), 2.33 (m, 2H), 2.23-1.41 (m, 13H), 1.30 (t, *J*=7.2 Hz, 6H), 1.01 (d, *J*=7.2 Hz, 3H), 0.67 (s, 3H); $^{13}$C NMR (100 MHz/$CDCl_3$) δ 159.3, 147.7, 142.3, 133.2, 124.8, 120.5, 116.9, 111.6, 70.6, 66.8, 61.4, 58.3, 50.0, 45.1, 42.8, 37.3 (d, *J*= 16 Hz), 35.3, 32.4, 29.4, 28.7, 25.2 (d, *J*= 140 Hz), 23.6, 21.5, 20.4, 16.9, 16.5; CIMS *m/z* (relative intensity) 493 ($MH^+$, 10), 475 ($MH^+$-$H_2O$, 24), 457 ($MH^+$-$2H_2O$, 100); HRMS (for $MH^+$, $C_{28}H_{46}O_5P$) calcd 493.3083, found 493.3080. (-)-**9b**: $[\alpha]_D^{20}$ =(-)-6.0° (c=0.7, EtOH); $\lambda_{max}$=261 nm, ε=11,330; IR (film) 3304, 2925, 1227 $cm^{-1}$; $^1$H NMR (400 MHz/$CDCl_3$) δ 6.38 (d, *J*=11.2 Hz, 1H), 6.10 (d, *J*=11.2 Hz, 1H), 5.30 (d, *J*= 10.0 Hz, 2H), 5. 01 (s, 1H), 4.45 (m, 1H), 4.21 (m, 1H), 4.07 (m, 4H), 3.64 (s, 1H), 3.38 (m, 1H), 2.81 (d, *J*=11.6 Hz, 1H), 2.62 (d, *J*=16.8 Hz, 1H), 2.33 (m, 2H), 2.19-1.40 (m, 13H) 1.31 (t, *J*=7.2 Hz, 6H), 1.02 (d, *J*=7.2 Hz, 3H), 0.67 (s, 3H); $^{13}$C NMR (100 MHz/$CDCl_3$) δ 159.3, 147.1, 142.5, 132.9, 124.9, 120.5, 116.9, 112.8, 71.5, 66.7, 61.4, 58.3, 50.0, 45.5, 42.8, 37.3 (d, *J*=16 Hz), 35.3, 32.4, 29.3, 28.7, 25.8 (d, *J*=139 Hz), 23.6, 21.5, 20.4, 16.9, 16.5; CIMS *m/z* (relative intensity) 493 ($MH^+$, 31), 475 ($MH^+$-$H_2O$, 70), 457 ($MH^+$-$2H_2O$, 100); HRMS (for $MH^+$, $C_{28}H_{46}O_5P$) calcd 493.3083, found493.3093.

## RESULTS AND DISCUSSION

The only phosphorus containing side analogues, 25-phosphine oxides **10a** and 25-phosphonates **10b**, showed very low antiproliferatrive activity (Fig. 3, Dauben *et al.* 1991). In order to increase desirable activity, we prepared unsaturated 16-ene-24-phosphonate analogs (**8a,b**) which have either of natural or unnatural A-ring (Uskokovic *et al.*, 1997). We also prepared the corresponding homo-analogs, 16-ene-25-phosphonates (**9a,b**), to evaluate side chain length effect. The synthesis of these analogs is quite straightforward (Scheme 1). Diethoxyphosphide anion was added to the known aldehydes **3a,b** and followed by Barton dehydroxylation to give phosphonates **4a,b** in 43-56% yield (Posner *et al.*, 1999). Triethylsilyl (TES) protecting group was removed by tetrabutylammonium fluoride and pyridinium dichromate oxidation of the corresponding alcohol gave ketones **6a, b** in 68-75% yield. Construction of vitamin D like skeleton could be achieved by coupling with racemic phosphosphine oxide **7** and following deprotection of *t*-butyldimethylsilyl group by tetrabutylammonium fluoride, which gave the final phosphonate analogs **8** and **9** in 30-58% yield (Dai *et al.*, 1994).

Fig. 4 shows *in vitro* antiproliferative activities of four phosphonate analogs (**8a, b** and **9a, b**) in murine keratinocytes, which demonstrates reduction in cell number over concentration as compared to control plates and 1α, 25-




3a R=Me, 3b R=OMe

**Fig. 3.** Known phosphorous analogues

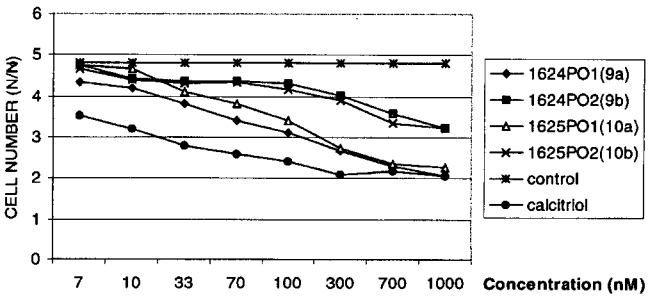



**Fig. 4.** Dose response effects on keratinocyte proliferation (96h)

dihydroxyvitamin $D_3$ (Posner *et al.*, 1992). In both 24-phosphonate and 25-phosphonate series, natural A-ring analogs which have (1α, 3β) stereochemistry at C1 and C3 are more potent than unnatural counterparts (1β, 3α). Antiproliferative activities between 24- and 25- series are very comparable and both of their natural A-ring analogues have very similar activities compared to 1α,25-dihydroxyvitamin $D_3$ at 1 μM level. Even though theses are not as potent as 1α,25-dihydroxyvitamin $D_3$ in nanomolar levels, theses unsaturated 16-ene-phosphonate analogs show much higher activities compared the reported saturated phosphine oxide and phosphonate analogues.

In conclusion, four close phosphonate analogs to 1α,25-dihydroxy-24-oxo-vitamin $D_3$ (**2**) have been synthesized and evaluated. Natural A-ring analogs show higher potency than unnatural A-ring counterparts and are very comparable to the original lead 1α,25-dihydroxyvitamin $D_3$ (**1**) at 1 μM level. Further attempts toward the alkoxy moiety of phosphonate group are now in progress.

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