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Synthesis and antiproliferative activity of side-chain unsaturated and homologated analogs of 1,25-dihydroxyvitamin D_2 (24*E*)-(1*S*)-24-Dehydro-24a-homo-1,25-dihydroxyergocalciferol and congeners☆

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Abstract

A series of analogs of 1,25-dihydroxyergocalciferol (**1**–**4**) was synthesized and screened for their antiproliferative activity in vitro. The structure of new analogs was designed based on biological activity of the previously obtained side-chain modified analogs of vitamin D_2 and D_3. The analogs were obtained by the Julia olefination of C_{22}-vitamin D sulfone **11** with side-chain aldehyde **15**. The analogs were tested for their antiproliferative activity against the cells of human breast cancer lines T47D and MCF7 as well as human and mouse leukemia lines, HL-60 and WEHI-3, respectively. Analog **2** (PRI-1907) showed the strongest antiproliferative activity out of the present series of analogs of 1,25-dihydroxyvitamin D_2 with the mono homologated and double unsaturated side chain. The activity of **2** was 3–150 times stronger, depending on the cell line, than that of 1,25-dihydroxycholecalciferol (calcitriol), used as standard.

Keywords: Vitamin D_2 analogs; C_{22}-Vitamin D synthon; Antiproliferative activity; Breast cancer cell lines MCF7 and T47D; Human promyelocytic leukemia cell line HL-60; Mouse leukemia cell line WEHI-3

1. Introduction

Vitamin D hormone (1,25-dihydroxyvitamin D_3, calcitriol, 1,25-$(OH)_2D_3$ [1,2]) was recently found to enhance the antitumor activity of paclitaxel, a microtubule-disrupting agent, in human cancer cells both in vitro and in vivo in a synergistic schedule-independent manner [3]. Combined effects [4,5] of 1,25-$(OH)_2D_3$ and cytostatics (anthracycline antibiotics [6,7], platinum drugs [8]) or antiestrogens [9,10] were also reported. It is now the common thought that combined therapy based on vitamin D might have a clinical significance in the treatment of patients with various solid tumors [11,12].

Ernst Binderup et al. [13] obtained vitamin D_3 analog (seocalcitol, EB 1089) with extended and unsaturated side-chain, using our vitamin D C-22 synthon approach [14]. The analog was 50–200 times more potent than 1,25-$(OH)_2D_3$ in regulation of cell growth and differentiation in a number of cancer cell lines and in animal models [15]. However, the analog still retained [16] substantial calcemic activity at the level of 50% that of 1,25-$(OH)_2D_3$. This might strongly limit the possible therapeutic application of this compound in clinical anticancer treatment, due to development of hypercalcemia in patients [12].

In our continuous search for vitamin D compounds with anticancer potential [17] we have previously obtained [18,19] vitamin D_3 dihomo analogs monounsaturated in the side-chain with lowered calcemic and enhanced cell-differentiating activity [20]. A few years ago we first obtained side-chain homoanalogs [21] of 1,25-dihydrovitamin D_2 [1,25-$(OH)_2D_2$], but its biological potential was not fully explored at that time. Vitamin D_2 and analogs are thought to be generally less toxic than the respective vitamin D_3 compounds.

Based on these findings and with the aid of molecular dynamics modeling [22], we have now designed a

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homologated analog of 1,25-$(OH)_2D_2$ with the conjugated unsaturation in the side-chain.

In the present paper, we described synthesis of this compound, as well as of a series of its 26,27-congeners, from vitamin D C_{22}-synthon by using our previously developed [23,24] convergent strategy. Moreover, we present their antiproliferative activity in vitro against the cells of various human and murine tumor cell lines.

2. Experimental

2.1. Synthesis

Sodium amalgam was prepared by using the method adopted from Organic Syntheses. Tetrahydrofuran (THF) was distilled over sodium benzophenone ketyl. Infrared (IR) spectra were recorded on a Perkin-Elmer Model 1725X FT-IR spectrophotometer as films of oily substances or $CHCl_3$ solutions of crystalline substances. Ultraviolet (UV) spectra were taken on a Shimadzu Model 160A UV–VIS spectrophotometer in the solvents indicated. Nuclear magnetic resonance (^1H and ^{13}C NMR) spectra were recorded at 200 MHz on a Varian Gemini 2000 spectrometer, at 400 MHz on a Bruker AM 400 spectrometer, and at 500 MHz on a Bruker AM 500 spectrometer in the solvents indicated, downfield from internal tetramethylsilane (TMS) as standard. Electron impact mass spectrometry spectra (EIMS) were recorded on a Finnigan MAT Model 8200 spectrometer. Column flash chromatography was performed on silica gel Si 60 (230–400 mesh, Merck) and on LiChroprep RP-18 (25–40 mm, Merck). High-performance liquid chromatography (HPLC) separations were performed using a Knauer Instrument Model 64, Hibar Si 60 column, 5 μm, 4 cm × 25 cm (Merck), Si 100 column, 10 μm, 10 cm × 25 cm and 22 cm × 25 cm.

2.2. (7E)-(1S,3R)-4′-Phenyl-6,9-[1′,2′]epi[1′,2′,4′] triazolo-1,3-dihydroxy-22,23-dinor-9,10-secochola-5(10),7-dien-24-oxy-3′,5′-dion (6)

A gentle stream of ozone was purged with stirring through the solution of adduct **5** (3.2 g, 5.1 mmol) in 50 ml of methylene chloride and 0.5 ml of pyridine at −79 °C for 17 min. The mixture was flushed with nitrogen. Zinc powder (2.8 g) and acetic acid were added and the reaction mixture was allowed to warm up to RT. The mixture was heated at 40 °C for 10 min. After extraction and solvent removal, 2.67 g of yellow foam was obtained. A silica gel chromatography separation using a solvent system of methylene chloride/methanol (96/4, v/v) resulted in aldehyde **6** as a light yellow foam (1.53 g, 55%). A sample for analytical purposes was crystallized from a mixture of *n*-hexane and ethyl acetate to give fine colorless needles: m.p. 162–164 °C. ^1H NMR ($CDCl_3$) δ: 0.84 (3H, s, 18-CH_3), 1.16 (3H, d, 21-CH_3), 1.93 (3H, s, 19-CH_3), 2.64 (1H, m, 14-H), 3.17 (1H, m, 11-H), 4.00 (1H, m, 3-H), 4.17 (1H, m, 1-H), 4.48 (1H, m, 9-H), 5.28 (2H, m, 6-H and 7-H), 7.4 (5H, m, Ar–H), 9.6 (1H, s, 22-H).

2.3. (7E)-(1S,3R)-4′-Phenyl-6,9-[1′,2′]epi[1′,2′,4′] triazolo-1,3-bis[(t-butyldimethyl-silyl)oxy]-22,23-dinor-9,10-secochola-5(10),7-dien-24-oxy-3′,5′-dion (7)

Imidazole (0.64 g, 9.40 mmol) and *t*-butyldimethylsilyl chloride (1.28 g, 8.49 mmol) were added to a solution of diol **6** (1.96 g, 3.77 mmol) in 20 ml of DMF. This mixture was stirred under nitrogen at RT for 24 h. Extraction with a mixture of PE40-65/ethyl acetate (4/1, v/v) resulted in disilylated aldehyde **7** (2.89 g, 98% yield) as a colorless oil. ^1H NMR ($CDCl_3$) δ: 0.04 (6H, s, Si–CH_3), 0.10 (6H, s, Si–CH_3), 0.83 (3H, s, 18-CH_3), 0.87 (9H, s, Si–C–CH_3), 0.90 (9H, s, Si–C–CH_3), 1.15 (3H, d, 21-CH_3), 1.83 (3H, s, 19-CH_3), 2.64 (1H, m, 14-H), 3.17 (1H, m, 11-H), 4.00 (1H, m, 3-H), 4.17 (1H, m, 1-H), 4.48 (1H, m, 9-H), 5.28 (2H, m, 6-H and 7-H), 7.4 (5H, m, Ar–H), 9.6 (1H, s, 22-H).

2.4. (7E)-(1S,3R)-4′-Phenyl-6,9-[1′,2′]epi[1′,2′,4′] triazolo-1,3-bis[(t-butyldimethylsilyl)oxy]-22,23-dinor-9,10-secochola-5(10),7-diene-24-ol-3′,5′-dion (8)

Sodium borohydride (0.26 g, 6.9 mmol) was added to a solution of aldehyde **7** (4.13 g, 5.5 mmol) in a mixture of 50 ml of methylene chloride and 5 ml of MeOH. This mixture was stirred under nitrogen at RT for 15 min and washed with water. Extraction and removal of the solvents resulted in alcohol **8** (4.04 g, 97% yield) as a colorless foam. ^1H NMR ($CDCl_3$) δ: 0.04 (6H, s, Si–CH_3), 0.10 (6H, s, Si–CH_3), 0.80 (3H, s, 18-CH_3), 0.87 (9H, s, Si–C–CH_3), 0.90 (9H, s, Si–C–CH_3), 1.05 (3H, d, 21-CH_3), 1.83 (3H, m, 19-CH_3), 2.61 (1H, m, 14-H), 3.15 (1H, m, 11-H), 3.41 (1H, m, 22-H), 3.66 (1H, m, 22-H), 4.00 (1H, m, 3-H), 4.17 (1H, m, 1-H), 4.47 (1H, m, 9-H), 5.26 (2H, m, 6-H and 7-H), 7.4 (5H, m, Ar–H).

2.5. (5Z,7E)-(1S,3R,20S)-1,3-[bis(t-Butyldimethylsilyl)oxy]-22,23-dinor-9,10-secochola-5,7,10(19)-triene-24-ol (9)

A solution of KOH (2.3 g, 41 mmol) in 10 ml MeOH was added to a solution of alcohol **8** (1.9 g, 2.5 mmol) in 5 ml MeOH. The mixture was stirred under nitrogen in a pressurized reaction vessel at 95 °C for 48 h. Next, the mixture was cooled down to RT and diluted with 15 ml of MeOH. Then, the mixture was purged with oxygen at 5 °C for 24 h. Finally, the reaction mixture was concentrated under reduced pressure and extracted with methylene chloride. A silica gel column chromatography separation using a mixture PE 40-65/EtOAc (93/7, v/v) resulted in alcohol **9** (0.86 g, 60% yield) as colorless oil. Additional flash chromatography on silica gel resulted in analytical sample of alcohol **9** as a colorless oil. IR (film): 3463, 2951, 2856, 1603, 1471, 1360, 1257, 1075, 991, 907, 836 cm^{-1}; UV (EtOH) λ_{max}:

264.6 nm; ^{1}H NMR ($CDCl_3$) δ: 0.48 (3H, s, 18-CH_3), 0.83 (18H, s, Si–C–CH_3), 1.06 (3H, d, 21-CH_3), 4.19 (1H, m, 3-H), 4.37 (1H, m, 1-H), 4.86 (1H, d, 19*Z*-H), 5.18 (1H, d, 19*E*-H), 6.05 (1H, d, 7-H), 6.24 (1H, d, 6-H); MS (relative intensity) *m*/*z*: 574 (M^+, 65), 559 (12), 442 (95), 427 (24), 248 (100), 192 (22), 73 (91), 57 (86), 43 (96).

2.6. (5Z,7E)-(1S,3R)-1,3-bis[(t-Butyldimethylsilyl)oxy]-24-(p-toluenesulfonyloxy)-22,23-dinor-9,10-secochola-5,7,10(19)-triene (**10**)

p-Toluenesulfonyl chloride (382 mg, 2 mmol) was added to a solution of 520 mg (0.905 mmol) of alcohol **9** in 20 ml of methylene chloride. Triethylamine (4 ml) and catalytic amount of 4,4-dimethylaminopyridine (DMAP) were added and the solution was stirred at 20 °C for 28 h. Saturated aqueous solution of $NaHCO_3$ was added (5 ml) and the mixture was vigorously stirred for 1 h. A silica gel flash chromatography separation resulted in tosylate **10** (540 mg, 90%) as a colorless oil. IR (film): 2952, 2856, 1605, 1471, 1360, 1257, 1175, 1075, 948, 908, 807 cm^{-1}; UV (EtOH) λ_{max}: 263.2 nm; ^{1}H NMR ($CDCl_3$) δ: 0.41 (3H, s, 18-CH_3), 0.84 (18H, s, Si–C–CH_3), 0.91 (3H, d, 21-CH_3), 2.42 (3H, s, Ar–CH_3), 4.22 (1H, m, 3-H), 4.38 (1H, m, 1-H), 4.76 (1H, d, 19*Z*-H), 5.18 (1H, d, 19*E*-H), 5.92 (1H, d, 7-H), 6.26 (1H, d, 6-H), 7.48 and 7.78 (2H, d, Ar–H); MS (relative intensity) *m*/*z*: 728 (M^+, 38), 713 (8), 596 (74), 464 (55), 248 (85), 155 (61), 105 (65), 91 (69), 75 (100).

2.7. (5Z,7E)-(1S,3R)-1,3-bis[(t-Butyldimethylsilyl)oxy]-24-phenylsulfonyl-22,23-dinor-9,10-secochola-5,7,10(19)-triene (**11**)

Lithium bromide dihydrate (500 mg, 4 mmol) and lithium carbonate (300 mg, 4 mmol) were added to a solution of tosylate **10** (920 mg, 1.26 mmol) in 10 ml of DMF. The mixture was stirred at 80 °C for 20 min. Sodium benzenesulfonate (1.4 g, 8.5 mmol) was added and stirring was continued for 2.5 h. Ethyl acetate extraction and flash chromatography separation on a silica gel resulted in sulfone **11** (740 mg, 88%) as a colorless oil. IR (film): 2953, 2856, 1471, 1447, 1305, 1257, 1144, 1085, 989, 908, 836 cm^{-1}; UV (EtOH) λ_{max}: 264.4 nm; ^{1}H NMR ($CDCl_3$) δ: 0.05 [12H, br s, 2Si (CH_3)$_2$], 0.51 (3H, s, 18-CH_3), 0.88 (18H, br s, 2*t*-BuSi), 1.20 (3H, d, J = 6.4 Hz, 21-CH_3), 2.88 and 3.16 (2H, dd, J = 4.3 Hz, 24-CH_2), 4.19 (1H, m, 3-H), 4.37 (1H, m, 1-H), 4.83 (1H, d, J = 2.4 Hz, 19*Z*-H), 5.16 (1H, d, J = 2.4 Hz, 19*E*-H), 5.97 (1H, d, J = 11.7 Hz, 7-H), 6.21 (1H, d, J = 11.7 Hz, 6-H), 7.58 and 7.91 (5H, m, Ar–H), 0.39 (3H, s, 18-CH_3), 0.85 (18H, s, Si–C–CH_3), 1.10 (3H, d, 21-CH_3), 4.11 (1H, m, 3-H), 4.34 (1H, m, 1-H), 4.72 (1H, d, 19*Z*-H), 5.29 (1H, d, 19*E*-H), 5.91 (1H, d, 7-H), 6.27 (1H, d, 6-H), 7.69 and 7.92 (5H, m, Ar–H); MS (relative intensity) *m*/*z*: 698 (M^+, 51), 683 (11), 566 (95), 551 (17), 248 (100), 192 (18), 73 (82); HRMS calculated for $C_{40}H_{66}O_4SSi_2$: 698.4221; found: 698.4279.

2.8. Ethyl 3-formyl-2E-butenoate (**15**)

Triethyl phosphonoacetate (**12**, 670 mg, 3 mmol) was added to NaH (150 mg, 3.6 mmol) in dry DMF and stirred at 0 °C for 1 h. Pyruvic aldehyde dimethyl acetal **13** (444 mg, 3.6 mmol) was added and stirring was continued for 24 h at RT. Extraction with ethyl acetate resulted in acetal **14** as a colorless oil (395 mg, 70%). IR ($CHCl_3$): 2984, 2937, 1719, 1661, 1446, 1367, 1324, 1220, 1157, 1106, 1061, 969, 875 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 1.29 (3H, t, J = 7 Hz, CH_2CH_3), 1.88 (3H, d, J = 2.0 Hz, 3-C$\underline{H}_3$, *cis*-isomer), 2.11 (3H, d, J = 1.5 Hz, 3-C$\underline{H}_3$, *trans*-isomer), 3.32 [6H, s, 4-H, *trans*-isomer], 3.42 [6H, s, 4-H, *cis*-isomer], 4.18 (2H, q, J = 7 Hz, CH_2CH_3), 4.61 [1H, s, $(CH_3O)_2CH$], 6.02 (1H, d, J = 1.5 Hz, 2-H). This oil was stirred for 3 h in CH_2Cl_2/HCl 3 N. The organic layer was concentrated to afford crude **15** (280 mg). A silica gel flash chromatography separation using CH_2Cl_2/hexane (1/1, v/v) provided pure **15** (200 mg, 67%) as a colorless oil. IR ($CHCl_3$): 2987, 2835, 1719, 1702, 1396, 1352, 1297, 1164, 1041, 878, 828 cm^{-1}; UV (EtOH) λ_{max}: 228.2 nm; ^{1}H NMR ($CDCl_3$) δ: 1.36 (3H, t, J = 7 Hz, CH_2CH_3), 2.18 (3H, d, J = 1.5 Hz, =CCH_3), 4.28 (2H, q, J = 7 Hz, CH_2CH_3), 6.49 (1H, d, J = 1.5 Hz, =CH), 9.56 (1H, s, CHO).

2.9. Ethyl (5Z,7E,23aE)-(1S,3R)-1,3-bis[(t-butyldimethylsilyl)oxy]-22-phenylsulfonyl-23-hydroxy-23a-methyl-23a,23b-dihomo-9,10-secochola-5,7,10(19),23-tetraene-24-oate (**16**)

A solution of *n*-BuLi (130 μl, 1.6 M, 0.210 mmol) was added with stirring to a solution of sulfone **11** (135 mg, 0.193 mmol) in 1 ml of THF at −70 °C. After 15 min a solution of 50 μl (0.380 mmol) of aldehyde **15**. [IR ($CHCl_3$): 2987, 2835, 1720, 1702, 1369, 1352, 1298, 1165, 1041, 878 cm^{-1}; UV (EtOH) λ_{max}: 228.2 nm; ^{1}H NMR ($CDCl_3$) δ: 1.38 (3H, t, J = 7 Hz, OEt), 2.18 (3H, s, 4-CH_3), 4.29 (2H, q, J = 7 Hz, OEt), 6.52 (1H, s, 2-H), 9.58 (1H, s, CHO)] was added. The mixture was stirred for 15 min at −70 °C. A THF extraction and silica gel chromatography separation resulted in ester **16** (145 mg, 88%) as colorless oil. For analytical purposes ester **16** (mixture of isomers) was isolated by silica gel chromatography as a colorless oil. IR ($CHCl_3$): 3484, 3068, 2952, 2857, 1716, 1654, 1472, 1447, 1386, 1300, 1215, 1143, 1082, 836 cm^{-1}; UV (EtOH) λ_{max}: 264.6, 218.8 nm.

2.10. (5Z,7E,24E)-(1S,3R)-1,3-bis[(t-Butyldimethylsilyl)oxy]-22-phenylsulfonyl-24-methyl-24a-homo-9,10-secocholesta-5,7,10(19),24-tetraen-23,25-diol (**17**)

A solution of CH_3MgBr in ethyl ether (150 μl, 3 M, 0.45 mmol) was added to a solution of ester **16** (145 mg, 0.17 mmol) in 2 ml of THF. The mixture was stirred for 2 h at 20 °C. A THF extraction and silica gel chromatography

separation resulted in dihydroxysulfone **17** (99 mg, 70%) as a colorless oil. IR ($CHCl_3$): 3484, 3068, 2953, 2856, 1645, 1471, 1447, 1384, 1302, 1255, 1141, 1082, 909, 835 cm^{-1}; UV (EtOH) λ_{max}: 265.4, 215.2 nm.

*2.11. (5Z,7E,24E)-(1S,3R)-1,3-bis[(t-Butyldimethylsilyl)oxy]-22-phenylsulfonyl-24-methyl-24a,26,27-trihomo-9,10-secocholesta-5,7,10(19),24-tetraen-23,25-diol (**18**)*

Dihydroxysulfone **18** was obtained from ester **16** (100 mg, 0.12 mmol) by the method described for **17** by using ethylmagnesium bromide (200 μl, 3 M, 0.6 mmol). A THF extraction and silica gel filtration resulted in a mixture **18** (32 mg, 32% yield) as colorless oil. UV (EtOH) λ_{max}: 265.6, 215.6 nm.

*2.12. (5Z,7E,24E)-(1S,3R)-1,3-bis[(t-Butyldimethylsilyl)oxy]-22-phenylsulfonyl-24-methyl-24a-homo-26,27-diethyl-9,10-secocholesta-5,7,10(19),24-tetraen-23,25-diol (**19**)*

Dihydroxysulfone **19** was obtained from ester **16** by using the method described for **17**. Ester **16** (105 mg, 0.15 mmol) was dissolved in *n*-propylmagnesium bromide (0.5 ml, 2 M, 1.0 mmol) and stirred at ambient temperature for 2 h. A THF extraction and silica gel filtration resulted in a mixture **19** (70 mg, 53% yield) as colorless oil. UV (EtOH) λ_{max}: 265, 215 nm.

*2.13. (5Z,7E,24E)-(1S,3R)-1,3-bis[(t-Butyldimethylsilyl)oxy]-22-phenylsulfonyl-24-methyl-24a-homo-26,27-di-n-propyl-9,10-secocholesta-5,7,10(19),24-tetraen-23,25-diol (**20**)*

Dihydroxysulfone **20** was obtained from ester **16** by using the method described for **17**. *n*-Butyl lithium (370 μl, 1.6 M in hexane, 0.59 mmol) was added, with stirring, to a solution of ester **16** (98 mg, 0.12 mmol) in 0.5 ml THF at −20 °C. The mixture was stirred at 0 °C for 1 h. A THF extraction and silica gel filtration resulted in a mixture **20** (43 mg, 41% yield) as colorless oil. UV (EtOH) λ_{max}: 265, 215 nm.

*2.14. (5Z,7E,22E,24E)-(1S,3R)-1,3-bis[(t-Butyldimethylsilyl)oxy]-24a-homo-9,10-seco-ergosta-5,7,10(19),24-tetraen-25-ol (**21**)*

Powdered Na_2HPO_4 (10 mg) and 1 ml of a saturated solution of Na_2HPO_4 in methanol were added under argon to a solution of dihydroxysulfone **17** (99 mg, 0.12 mmol) in 0.5 ml of methanol, followed by sodium amalgam (5%, 0.6 g). The mixture was stirred under argon at RT for 2 h. A hexane/toluene (1/1, v/v) extraction and silica gel chromatography separation resulted in alcohol **21** (40 mg, 50%) as a colorless oil. IR ($CHCl_3$): 3360, 2952, 1643, 1472, 1471, 1254, 1076, 835 cm^{-1}; UV (EtOH) λ_{max}: 264.6, 237.8 nm.

Alcohols **22**, **23** and **24** were prepared by the method described for the synthesis of **21**, in yields 50, 67 and 52%, respectively.

*2.15. (24E)-(1S)-24-Dehydro-24a-homo-1,25-dihydroxyergocalciferol (**1**)*

A solution of tetrabutylammonium fluoride in THF (250 μl, 1 M, 0.25 mmol) was added to a solution of alcohol **21** (40 mg, 0.06 mmol) in 0.5 ml of THF. The mixture was stirred under argon at 60 °C for 1.5 h. A THF extraction, silica gel chromatography filtration and preparative HPLC separation in reversed phases (a Hibar RP-18 column, 5 μm, Merck, H_2O/CH_3CN, 45/55 v/v) resulted in triol **1** (20 mg, 74%) as a colorless oil. IR ($CHCl_3$): 3344, 2947, 2927, 2869, 1636, 1445, 1382, 1216, 1149, 1054, 963, 756 cm^{-1}; UV (EtOH) λ_{max}: 264.2, 237.8 nm; ^{1}H NMR ($CDCl_3$) δ: 0.57 (3H, s, 18-CH_3), 1.05 (3H, d, J = 6.6 Hz, 21-CH_3), 1.40 (6H, s, 26,27-CH_3), 1.97 (3H, s, 28-CH_3), 4.23 (1H, m, 3-H), 4.43 (1H, m, 1-H), 5.00 (1H, bs, 19*Z*-H), 5.33 (1H, bs, 19*E*-H), 5.51 (1H, s, 24a-H), 5.52 (1H, dd, J_1 = 15.6 Hz, J_2 = 8.6 Hz, 22-H), 5.93 (1H, d, J = 15.8 Hz, 23-H), 6.01 (1H, d, J = 11.3 Hz, 7-H), 6.38 (1H, d, J = 11.3 Hz, 6-H); ^{13}C NMR ($CDCl_3$) δ: 12.251 (18), 13.253 (28), 20.754 (21), 22.227 (15), 23.578 (11), 27.769 (16), 29.074 (9), 31.276 and 31.413 (26 and 27), 40.386 (12), 40.462 (20), 42.861 (2), 45.260 (4), 45.958 (13), 56.405 (17), 56.511 (14), 66.897 (3), 70.829 (1), 71.118 (25), 111.916 (19), 117.215 (7), 125.050 (6), 133.006 (23), 133.143 (5), 135.451 (24a), 135.633 (24), 136.847 (22), 143.164 (8), 147.764 (10); MS (relative intensity) *m/z*: M^+ 440 (6), 422 (11), 404 (21), 386 (9), 285 (15), 269 (13), 251 (14), 135 (56), 107 (100), 59 (6); HRMS calculated for $C_{29}H_{44}O_3$: 440.3290; found: 440.3284 and calculated for $C_{29}H_{44}O_3$–H_2O: 422.3185; found: 422.3180.

*2.16. (24E)-(1S)-24-Dehydro-24a,26,27-trihomo-1,25-dihydroxyergocalciferol (**2**)*

Triol **2** (5 mg, 57% yield) was obtained from **22** (13 mg, 0.019 mmol) by using tetrabutylammonium fluoride (50 μl, 1 M, 0.050 mmol) in analogy to the method described for the preparation of **1** from **21** in the form of a colorless oil. IR ($CHCl_3$): 3358, 2947, 2870, 1646, 1440, 1384, 1216, 1148, 1055, 964, 758 cm^{-1}; UV (EtOH) λ_{max}: 264.4, 238.2 nm, ^{1}H NMR ($CDCl_3$) δ: 0.57 (3H, s, 18-CH_3), 0.89 (6H, t, J = 7.3 Hz, 26′,27′-CH_3), 1.06 (3H, d, J = 6.6 Hz, 21-CH_3), 1.63 (4H, t, J = 7.3 Hz, 26,27-CH_2), 1.95 (3H, s, 28-CH_3), 4.23 (1H, m, 3-H), 4.43 (1H, m, 1-H), 5.00 (1H, bs, 19*Z*-H), 5.31 (1H, s, 24a-H), 5.33 (1H, bs, 19*E*-H), 5.49 (1H, dd, J_1 = 8.4 Hz, J_2 = 15.7 Hz, 22-H), 5.96 (1H, d, J = 15.7 Hz, 23-H), 6.02 (1H, d, J = 11.4 Hz, 7-H), 6.39 (1H, d, J = 11.4 Hz, 6-H); MS (relative intensity) *m/z*: 468 (M^+, 4), 450 (10), 432 (7), 287 (19), 269 (17), 225 (7), 209 (9), 175 (11), 155 (21), 135 (100), 107 (35), 91 (32), 81 (21),

57 (76); HRMS calculated for $C_{31}H_{48}O_3$: 468.3604; found: 468.3601.

2.17. (24E)-(1S)-24-Dehydro-24a-homo-26,27-diethyl-1,25-dihydroxyergocalciferol (3)

Triol **3** was obtained from **23** (40 mg, 0.055 mmol) by using tetrabutylammonium fluoride (150 μl, 1 M, 0.150 mmol) with the method described for the preparation of **1** from **21**. Lyophylization of the HPLC eluent resulted in triol **3** as a white powder (12 mg, 44% yield). IR ($CHCl_3$): 3370, 2956, 2872, 1644, 1455, 1378, 1216, 1054, 964, 758 cm^{-1}; UV (EtOH) λ_{max}: 264.0, 237.8 nm; ^1H NMR ($CDCl_3$) δ: 0.57 (3H, s, 18-CH_3), 0.91 (6H, t, $J = 7.3$ Hz, 26″-CH_3, 27″-CH_3), 1.06 (3H, d, $J = 6.6$ Hz, 21-CH_3), 1.94 (3H, bs, 28-CH_3), 4.23 (1H, m, 3-H), 4.43 (1H, m, 1-H), 5.00 (1H, bs, 19*Z*-H), 5.32 (2H, bs, 24a-H, 19*E*-H), 5.48 (1H, dd, $J_1 = 8.4$ Hz, $J_2 = 15.5$ Hz, 22-H), 5.93 (1H, d, $J = 15.5$ Hz, 23-H), 6.00 (1H, d, $J = 11.4$ Hz, 7-H), 6.38 (1H, d, $J = 11.4$ Hz, 6-H); MS (relative intensity) *m*/*z*: 496 (M^+, 5), 478 (13), 460 (10), 435 (14), 301 (26), 269 (24), 251 (22), 175 (12), 135 (64), 107 (25), 95 (35), 81 (22), 71 (100); HRMS calculated for $C_{33}H_{52}O_3$: 496.3917; found: 496.3881.

2.18. (24E)-(1S)-24-Dehydro-24a-homo-26,27-di-n-propyl-1,25-dihydroxyergocalciferol (4)

Triol **4** was obtained from **24** (17 mg, 0.023 mmol) by using tetrabutylammonium fluoride (60 μl, 1 M, 0.060 mmol) with the method described for the preparation of **1** from **21**. Lyophilization of the HPLC eluent resulted in triol **4** as a white powder (4.8 mg, 41% yield). IR ($CHCl_3$): 3358, 2954, 2870, 1643, 1456, 1378, 1215, 1054, 964 cm^{-1}; UV (EtOH) λ_{max}: 264.0, 238.2 nm; ^1H NMR ($CDCl_3$) δ: 0.57 (3H, s, 18-CH_3), 0.91 (6H, t, $J = 6.3$ Hz, 26‴-CH_3, 27‴-CH_3), 1.05 (3H, d, $J = 6.6$ Hz, 21-CH_3), 1.95 (3H, bs, 28-CH_3), 4.23 (1H, m, 3-H), 4.43 (1H, m, 1-H), 5.00 (1H, bs, 19*Z*-H), 5.33 (2H, bs, 24a-H, 19*E*-H), 5.49 (1H, dd, $J_1 = 8.4$ Hz, $J_2 = 15.4$ Hz, 22-H), 5.94 (1H, d, $J = 15.4$ Hz, 23-H), 6.02 (1H, d, $J = 11.4$ Hz, 7-H), 6.38 (1H, d, $J = 11.4$ Hz, 6-H); MS (relative intensity) *m*/*z*: 524 (M^+, 26), 506 (29), 488 (12), 467 (33), 449 (56), 431 (48), 315 (89), 285 (35), 269 (89), 251 (71), 191 (28), 179 (53), 151 (52), 135 (100), 107 (25), 85 (94); HRMS calculated for $C_{35}H_{56}O_3$: 524.4230; found: 524.4220.

2.19. Antiproliferative assays in vitro

2.19.1. Cells

The human breast cancer cell lines T47D and MCF-7 as well as the mouse leukemia cell line WEHI-3 were obtained from the American Type Culture Collection (Rockville, MD) and were maintained in the Cell Culture Collection of the Institute of Immunology and Experimental Therapy, Wrocław, Poland. The human promyelocytic leukemia HL-60 cell line was obtained from the European Type Culture Collection by courtesy of Professor Spik and Dr Mazurier (Laboratory of Biological Chemistry USTL, Lille, France). The cells were plated in 96-well plates (Costar, USA) at a density of 5×10^3 cells per well and cultured in an opti-MEM or in RPMI-1640 medium supplemented with 2 mM glutamine (Gibco, Warsaw, Poland), streptomycin (50 μg/ml) (Jelfa, Jelenia Góra, Poland), penicillin (50 U/ml) (Jelfa, Jelenia Góra, Poland) and 5% fetal calf serum (Gibco, Grand Island, USA) 24 h before addition of the tested compounds. Then, the cells were cultured, in 37 °C, in humid atmosphere saturated with 5% CO_2.

2.19.2. Analogs

Samples of analogs **1**–**4** were stored in the amber ampoules under argon at −20 °C. The amount of each analog in the ampoule was determined by the UV spectrophotometry (Carl Zeiss spectrophotometer, Jena, Germany) at 265 nm. Analogs were dissolved in absolute ethanol to the concentration of 10^{-4} M and, subsequently, diluted in the culture medium to reach the required concentrations (ranging from 1000 to 0.01 nM).

2.19.3. MTT assay

The MTT assay was performed after exposure of cells to varying concentrations of analogs tested for 120 h. The MTT solution (20 μl) was added to each well for the last 3–4 h of incubation [MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Sigma, St. Louis, MO; stock solution: 5 mg/ml]. The viable cells reduced in their mitochondria a pale yellow MTT to a navy blue formazan. After the incubation time was completed, a sample of 80 μl of the lysing mixture was added to each well (lysing mixture: 225 ml dimethylformamide, 67.5 g sodium dodecyl sulphate, both from Sigma, St. Louis, MO, USA, and 275 ml of distilled water). After the formazan crystals were dissolved (24 h), the optical densities of the samples were measured on a Multiskan RC photometer (Labsystems, Helsinki, Finland) at 570 nm.

2.19.4. SRB assay

The details of the SRB assay were described by Skehan et al. [25]. The cells attached to the plastic were fixed by gentle layering of cold 50% TCA (trichloroacetic acid, Aldrich–Chemie, Germany) on the top of the culture medium in each well. The plates were incubated at 4 °C for 1 h and, then, washed five times with a tap water. The background optical density was measured in the wells filled with the culture medium, without the cells. The cellular material fixed with TCA was stained with 0.4% sulforhodamine B (SRB, Sigma, Germany) dissolved in 1% acetic acid (POCh, Gliwice, Poland) for 30 min. The unbound dye was removed by rinsing four times with 1% acetic acid. The protein-bound dye was extracted with 10 mM unbuffered Tris base (POCh, Gliwice, Poland) for determination of optical density (at 540 nm) in a computer-interfaced, 96-well microtiter plate reader Multiskan RC photometer (Labsystems,

Helsinki, Finland). The results were calculated as an $ID_{30,40,50}$ (inhibitory dose 30, 40, 50)—the dose of tested agent, which inhibits proliferation of cancer cells by 30, 40 and 50%. The ID values were calculated separately for each experiment and the mean values ± S.D. were presented in the tables. Each compound in every concentration was tested in triplicates in a single experiment, which was repeated 3–7 times. A percent of the cell growth inhibition for all experiments was calculated and presented in figures with the respective standard deviations.

2.19.5. Statistical evaluation

Student's *t*-test for independent samples was applied.

3. Results and discussion

The synthesis of analogs **1**–**4** (Fig. 1) followed our general convergent strategy for the side-chain modified analogs of vitamin D. In this strategy, a vitamin D analog is constructed from vitamin D synthon and the respective side-chain fragment. A C_{22}-vitamin D synthon **11** (Scheme 1) was used for our synthesis of analogs **1**–**4**. Previously [23], synthon **11** was obtained from a steroid precursor, 22,23-bisnor-5-cholenic acid, by the classical construction of the vitamin D triene system, followed by Paaren–DeLuca hydroxylation at C-1. In the present paper, an alternative method is first described for the synthesis of this advanced intermediate (Scheme 1). This practical synthesis of synthon **11** started from phenyltriazolinedione adduct **5** [26,27]. The ergosterol side chain of adduct **5** was cleaved by ozonolysis and subsequent reductive cleavage of the resulting ozonide in 55% overall yield. Silylation and sodium borohydride reduction of resulting aldehyde **6** resulted in C_{22}-alcohol in over 95% yield. The sulfone moiety was introduced at C-22 by the usual method. The intermediate tosylate **10** was converted into C_{22}-bromide (not shown) and reacted with sodium benzenesulfonate to give sulfone **11** in 88% yield.

For preparation of a side-chain fragment **15** (Scheme 2), we used a Wittig–Horner methodology, applied by Curley and Ticoras [28,29] for the total synthesis of retinoic acid and its metabolites. Conjugated aldehyde–ester **15** was conveniently prepared in two steps from commercially available components, without tedious distillation of the final product. Thus, the Wittig reagent **12** was condensed with acetal **13** to give unsaturated ester **14** as a mixture of the geometric isomers. The biphasic acid hydrolysis of this ester resulted in **15** in 67% overall yield. *trans*-Stereochemistry of the product was confirmed by a chemical shift of aldehyde proton in ^{1}H NMR ($\delta = 9.56$ ppm) compared to the known shift of the *cis*-isomer ($\delta = 10.12$ ppm). The Julia olefination of deprotonated sulfone **11** with aldehyde **15** resulted in hydroxysulfones **16** in 88% yield (Scheme 3). Attempted dehydroxy-desulfonylation of **16** resulted in a complicated mixture of products. For this reason sulfone **16** was first reacted with Grignard or organolithium reagent to give a series

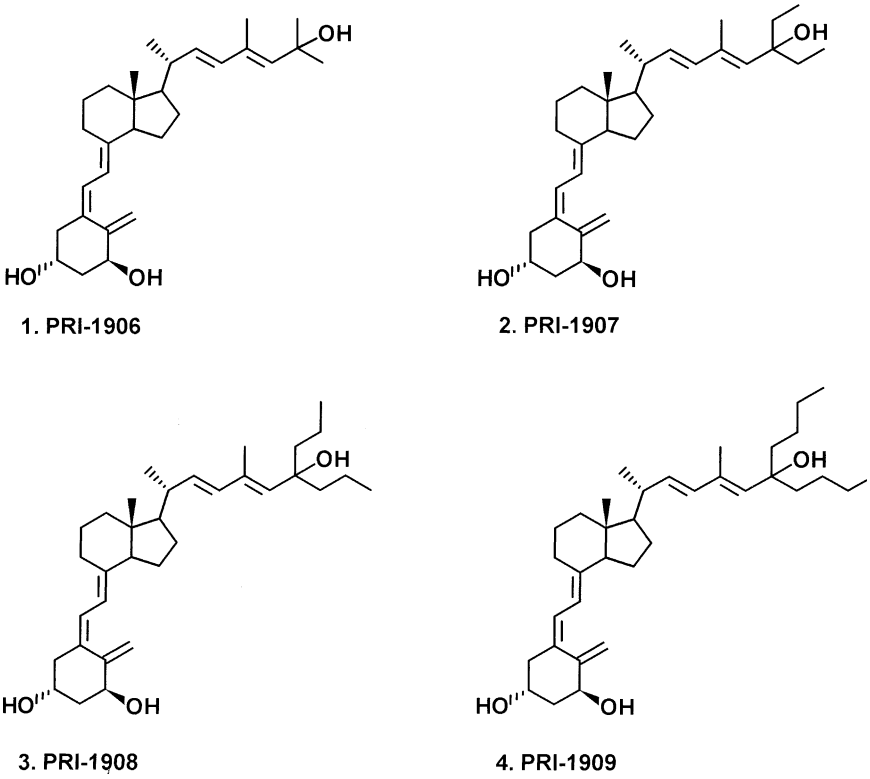
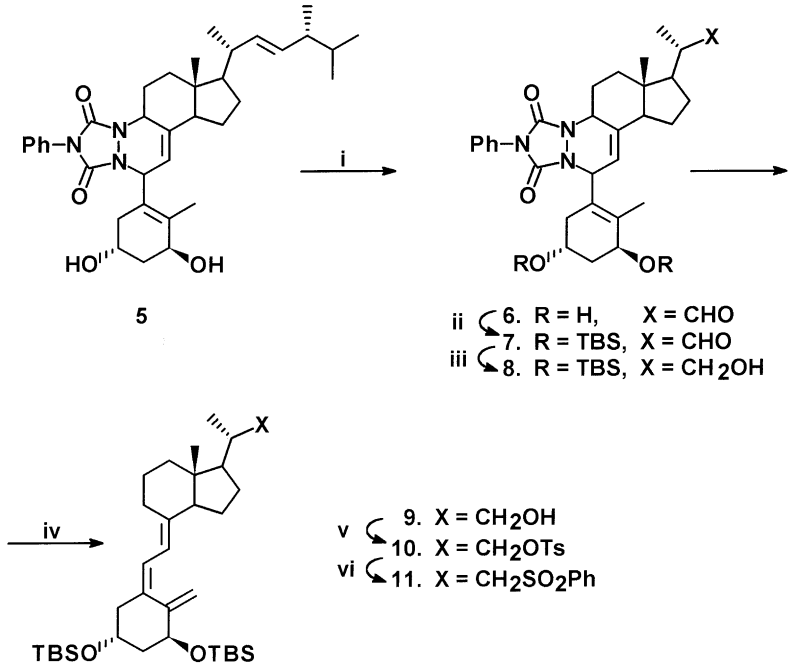


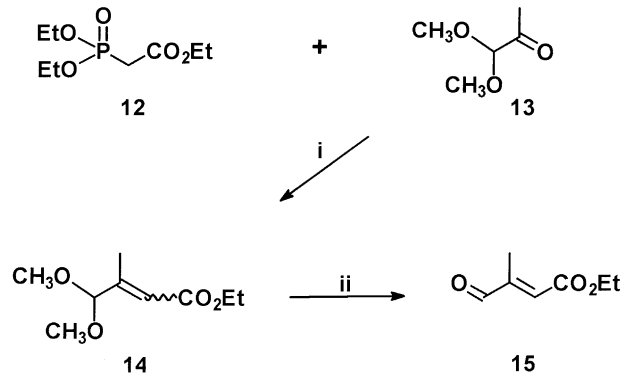
Fig. 1. (24*E*)-(1*S*)-24-Dehydro-24a-homo-1,25-dihydroxyergocalciferol (**1**) and its side-chain unsaturated homo analogs **2**, **3**, and **4**.



Scheme 1. Synthesis of a C_{22}-vitamin D synthon **11**. Reagents and conditions: (i) O_3, CH_2Cl_2, pyridine, −79 °C, 17 min; (ii) TBDMSCl, Im, DMF, RT, 24 h; (iii) $NaBH_4$, MeOH, RT, 15 min; (iv) KOH, MeOH, 95 °C, 48 h; (v) *p*-TsCl, TEA, DMAP, CH_2Cl_2, 20 °C, 28 h; (vi) LiBr, Li_2CO_3, DMF, 80 °C, 20 min; $C_6H_5SO_3Na$, 80 °C, 2.5 h.

of products **17**–**20** and then reacted with sodium amalgam in buffered methanol giving vinyl alcohols **21**–**24**. A fluoride ion promoted desilylation of these alcohols resulted in a series of analogs **1**–**4**. The all-*trans* geometry of the side chain, resulting from the Julia olefination protocol, was confirmed by the values of coupling constants $J_{22,23}$ of side-chain protons in the ^{1}H NMR spectra.

Analogs **1**–**4** were examined for their antiproliferative activity in vitro against cells of the human breast cancer lines and human and mouse leukemia lines. The analogs revealed antiproliferative activity against the human breast carcinoma cell lines T47D and MCF-7 with maximal growth inhibition of 51 and 48%, respectively. This inhibitory effect was dose dependent. The values ID_{40} and ID_{30} were calculated



Scheme 2. Synthesis of a side-chain fragment **15**. Reagents and conditions: (i) NaH, DMF, 0 °C, 1 h; (ii) 3 M HCl/CH_2Cl_2, RT, 3 h.

for both the T47D and MCF-7 cell line in order to compare the effect of analogs with that of calcitriol (Table 1). The analogs were also screened against cells of the human HL-60 and mouse WEHI-3 leukemia lines (Table 1). All the analogs revealed strong antiproliferative activity against these two targets with maximal growth inhibition of 76% for HL-60 and 92% for WEHI-3 cells. The ID_{50} value was estimated for both cell lines with the exception of analog **4** against WEHI-3 cells (Table 1). Analog **2** revealed the strongest antiproliferative activity against all target cells applied. This effect was about 3, 8, and 150 times stronger than that of calcitriol, for both WEHI-3, and MCF-7 as well as for T47D and HL-60 cell lines, respectively. The observed differences between inhibitory doses of calcitriol and **2** were statistically significant. Thus, **2** is the most active analog out of the series of side-chain modified analogues of vitamin D tested. Analog **1** revealed activity weaker than that of **2** but generally stronger than that of calcitriol. Analog **4** did not exhibit any antiproliferative activity in our experimental protocol, with the exception of a weak growth inhibition of the WEHI-3 cells (Table 1 and Fig. 2).

Antiproliferative activity of the most active vitamin D_2 analog **2** was comparable to that of the known vitamin D_3 analog, EB 1089, of similar structure [12]. Based on the known lower toxicity of vitamins D_2, as compared to the respective vitamins D_3, it is anticipated that **2** might be less calcemic in humans than EB 1089. Calcemic activity of **2** and related new vitamin D_2 analogs, both in vitro in caco-2 cell lines and in animal models, will be reported elsewhere.

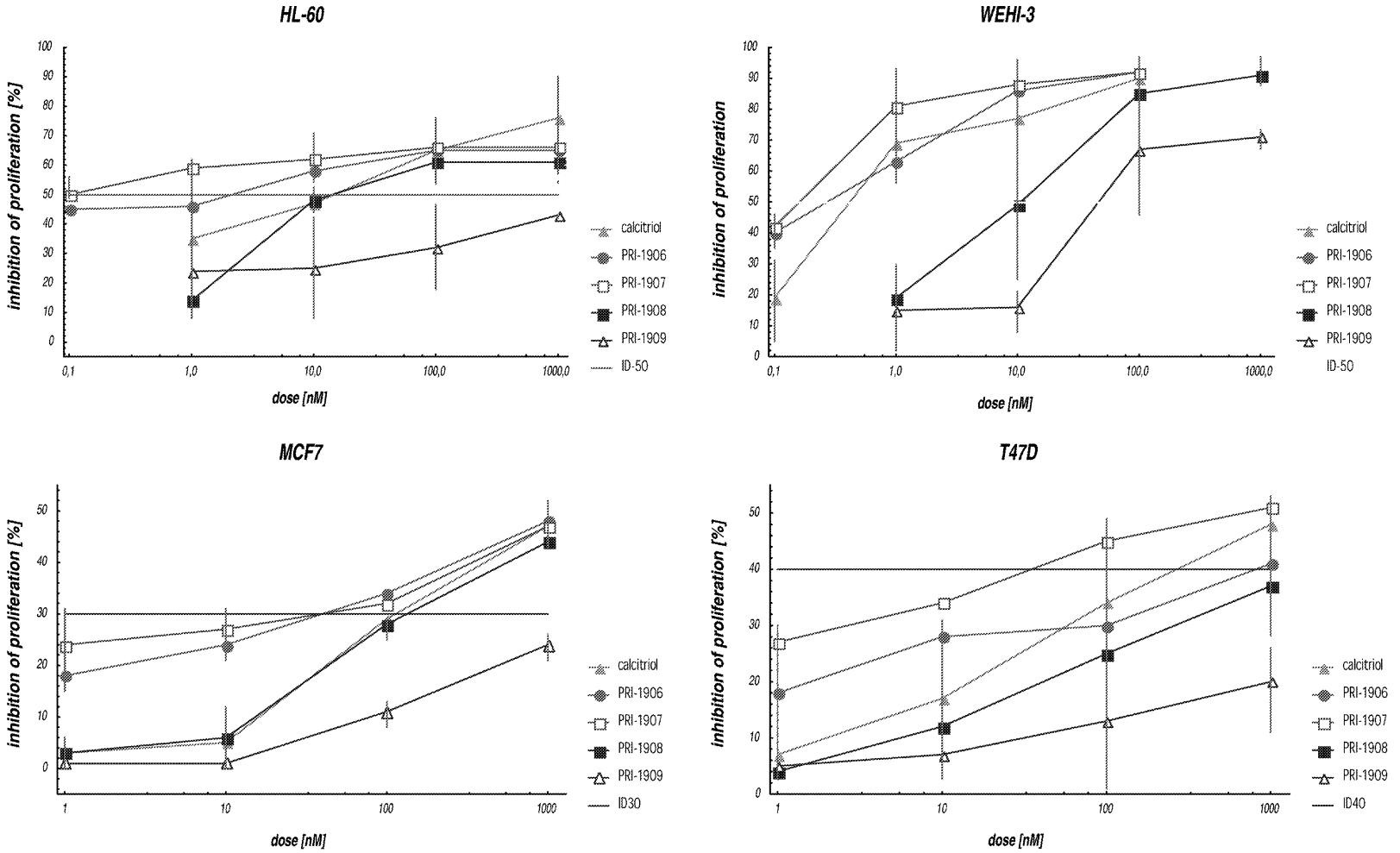
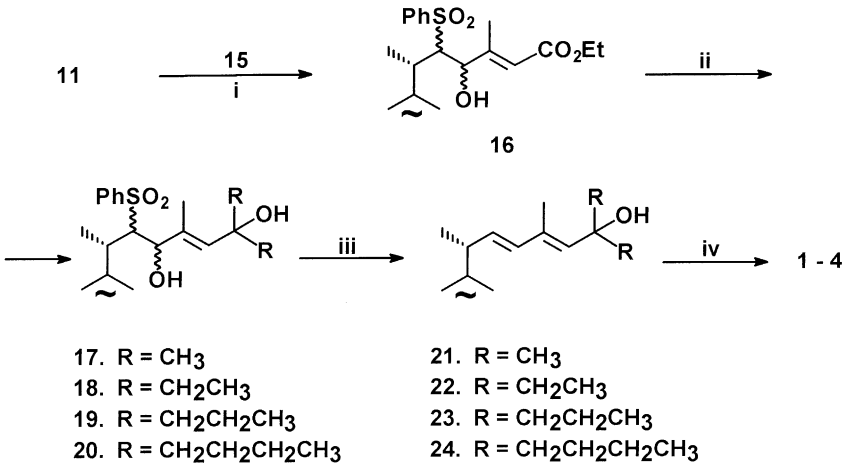


Fig. 2. Antiproliferative activity in vitro of vitamin D analogue **1** (PRI-1906), **2** (PRI-1907), **3** (PRI-1908), and **4** (PRI-1909) against the human and mouse leukemia (HL-60 and WEHI-3) and human breast carcinoma (T47D and MCF-7) cell lines.



Scheme 3. Synthesis of (24*E*)-(1*S*)-24-dehydro-24a-homo-1,25-dihydroxyergocalciferol (**1**) and analogs **2**, **3**, and **4**. Reagents and conditions: (i) *n*-BuLi, THF, −70 °C, 15 min; (ii) CH_3MgBr, THF, 20 °C, 2 h; (iii) Na/Hg, Na_2HPO_4, MeOH, RT, 2 h; (iv) TBAF, THF, 60 °C, 1.5 h.

Table 1
Inhibitory doses in vitro of vitamin D analogues **1**–**4** against the human and mouse leukemia (HL-60 and WEHI-3), and human breast carcinoma (T47D and MCF-7) cell lines

Analog	Inhibitory dose (nM)			
	ID_{50}		ID_{40}, T47D	ID_{30}, MCF-7
	HL-60	WEHI-3		
1,25-$(OH)_2D_3$	15.0 ± 1.14	0.43 ±0.2	275.0 ± 1.1	114.0 ± 1.1
1	2.2 ± 0.50*	0.28 ± 0.3	819.0 ± 3.1	39.0 ± 1.4*
2	0.1 ± 0.02*	0.16 ± 0.1	35.0 ± 2.1*	39.0 ± 1.3*
3	15.0 ± 3.00	11.0 ± 2.3	–[b]	137.0 ± 1.2
4	–[a]	47.0 ± 1.8	–[c]	–[d]

The symbol (∗) indicates statistically significant differences between the analogue and calcitriol ($P < 0.05$).
[a] Maximal growth inhibition, 43%.
[b] Maximal growth inhibition, 37%.
[c] Maximal growth inhibition, 20%.
[d] Maximal growth inhibition, 25%.

The mechanism of the antiproliferative effect in vitro of the vitamin D analogs is not yet fully understood. It might be related, however, to the effect in inducing the cell differentiation [19,30]. Induction of apoptosis by the vitamin D compounds has been also reported for breast cancer cells [31]. Inhibition of the cell cycle in G1 phase is well documented for leukemic cells [32]. Deregulation of intracellular signal transduction might also be considered [19,33,34].

Acknowledgments

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