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25-hydroxy-16-ene-26,27-bishomo-cholecalciferols

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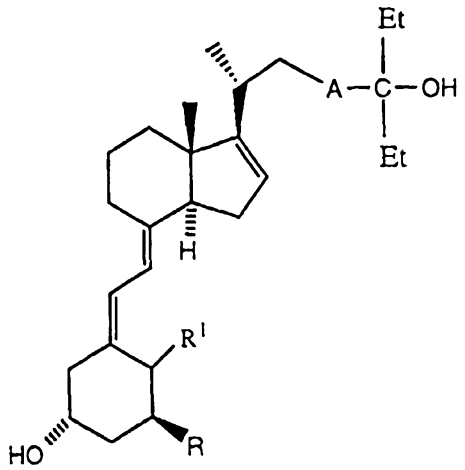


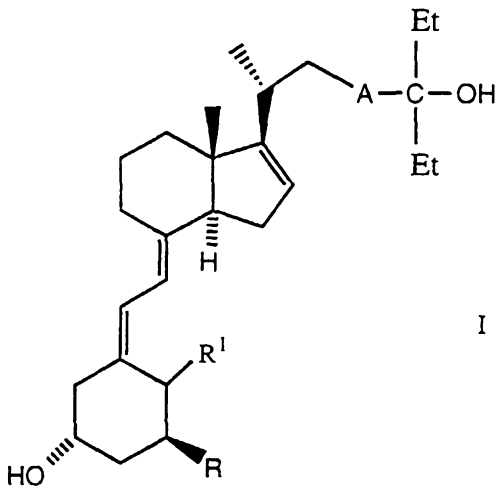
(I)

(57) Abstract

The invention relates to a compound of formula (I) wherein A is a carbon-carbon double bond having the E or Z stereochemical configuration, or A is a carbon-carbon triple bond, Et is ethyl, and R is hydroxy and R^1 is hydrogen or $=CH_2$, or R is hydrogen or fluoro and R^1 is $=CH_2$. They stimulate HL-60 cell differentiation and accordingly, are useful as agents for the treatment of neoplastic diseases, such as leukemia.

25-Hydroxy-16-ene-26,27-bishomo-cholecalciferols

The invention relates to 25-hydroxy-16-ene-26,27-bishomo-cholecalciferol derivatives of the formula



I

wherein A is a carbon-carbon double bond having the E or Z stereochemical configuration, or A is a carbon-carbon triple bond, Et is ethyl, and R is hydroxy and R^1 is hydrogen or $=CH_2$, or R is hydrogen or fluoro and R^1 is $=CH_2$.

Compounds of formula I stimulate HL-60 cell differentiation and accordingly, are useful as agents for the treatment of neoplastic diseases, such as leukemia.

As used herein, the term "lower alkyl" denotes a straight or branched-chain alkyl group containing 1 to 4 carbon atoms, for example, methyl, ethyl, propyl, isopropyl, butyl, t-butyl and the like. The term "ar-lower alkyl" are p-tolyl, benzyl, phenylethyl, phenylpropyl, and the like. The term "aryl" denotes a group derived from an aromatic hydrocarbon which may be unsubstituted or substituted by one or more lower alkyl groups. Exemplary of "aryl" are phenyl and p-methyl phenyl.

The invention further relates to a composition comprising a compound of formula I, or a mixture of two or more compounds of formula I, as well as to the use of these compounds for the manufacture of medicaments for stimulating differentiation of HL-60 cells and for the treatment of neoplastic diseases such as leukemia.

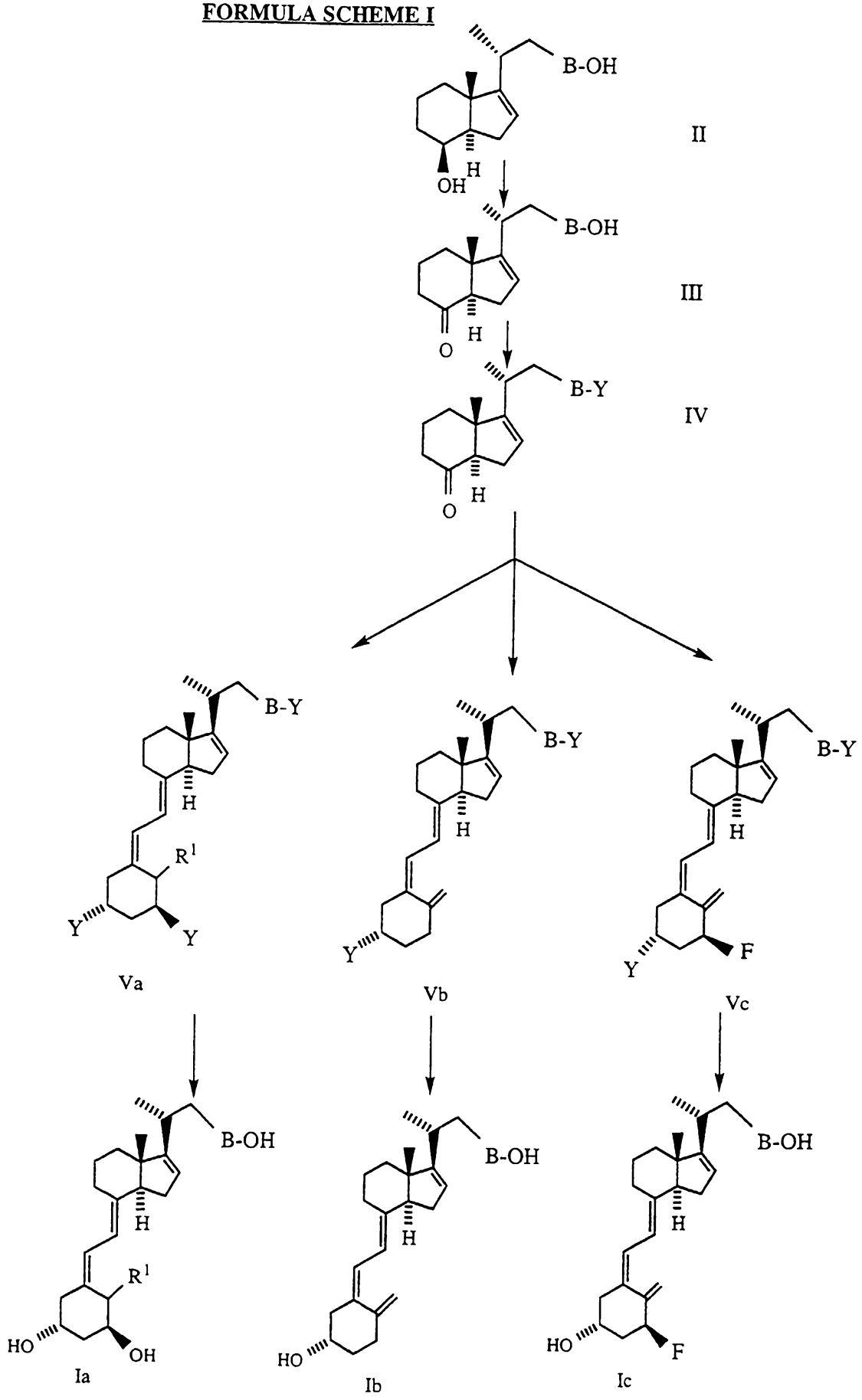
The invention also relates to the compounds of formula I as agents for treating the above-mentioned disease states. The invention also relates to a process for preparing compounds of formula I and to intermediates occurring therein.

Preferred are compounds of formula I, wherein R is hydroxy and R^1 is $=CH_2$, particularly compounds A, B and C, i.e.:

1,25-dihydroxy-16-ene-23-yne-26,27-bishomo-cholecalciferol;
1,25-dihydroxy-16,23Z-diene-26,27-bishomo-cholecalciferol;
1,25-dihydroxy-16,23E-diene-26,27-bishomo-cholecalciferol,
respectively.

The compounds of formula I wherein A is -C≡C- are prepared as hereafter described, with particular reference to formula Scheme I and the Examples below.

FORMULA SCHEME I

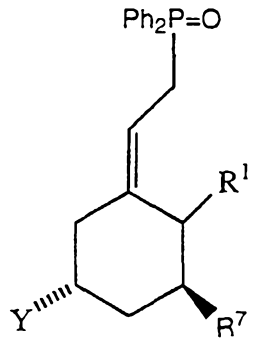


wherein R^1 is as described above, B is $-C\equiv CC(Et)_2-$, Y is $-OSi(R^4,R^5,R^6)$, Et is ethyl, R^4 and R^6 are lower alkyl and R^5 is lower alkyl, aryl or ar-lower-alkyl.

In the above formula Scheme I, the compound of formula II is converted to a compound of formula III by treatment with an oxidizing agent such as 2,2'-bipyridinium chlorochromate or pyridinium dichromate at room temperature, in a aprotic solvent such as dry tetrahydrofuran, or more preferably, dry methylene chloride. The compound of formula III is worked up by conventional means such as extraction followed by chromatography.

The compound of formula III is converted to a compound of formula IV, by reaction with, for example, a (trialkylsilyl)imidazole such as 1-(trimethylsilyl)imidazole in an aprotic, organic solvent such as dry tetrahydrofuran, or more preferably, dry methylene chloride. The compound of formula IV is worked up by conventional means such as extraction followed by chromatography.

The compound of formula IV is converted to a compound of formula Va, Vb or Vc by reaction with the corresponding compound of formula



VI

where Ph is phenyl; R^7 is hydrogen, fluorine or Y; and Y and R^1 are as described above.

The reaction is carried out at -60°C to -90°C, preferably -78°C, in a polar, aprotic, organic solvent, such as dry ether or more preferably dry tetrahydrofuran, in the presence of a strong base such as an alkyl lithium, like butyl lithium. The compound of formula Va, Vb, or Vc is

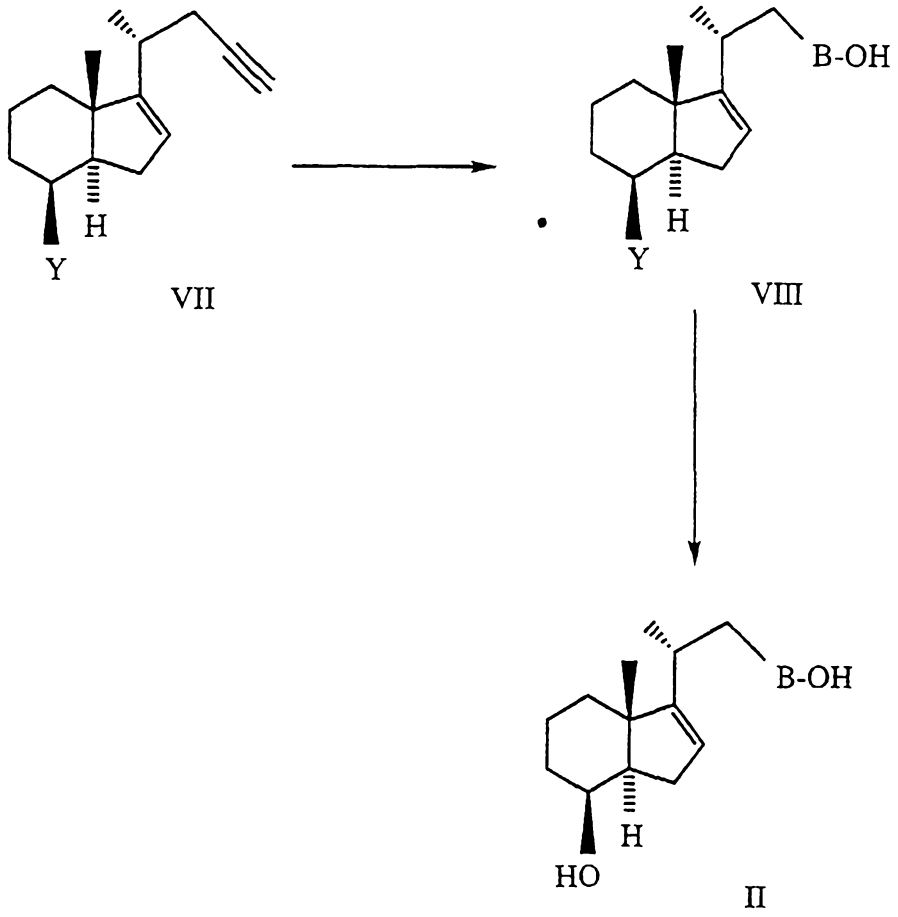
worked up by conventional means such as extraction followed by chromatography.

Compounds of formula VI are known or can be prepared in accordance with known methods.

The protecting groups of the compound of formula Va, Vb or Vc are removed by reaction with a fluorine salt, such as tetrabutyl-ammonium fluoride in a polar, organic solvent such as dry ether, or more preferably dry tetrahydrofuran to yield a corresponding compound of formula Ia, Ib or Ic.

The intermediate of formula II as described above is prepared as hereinafter described with particular reference to formula Scheme II and the Examples below.

FORMULA SCHEME II



wherein Y and B are as described above.

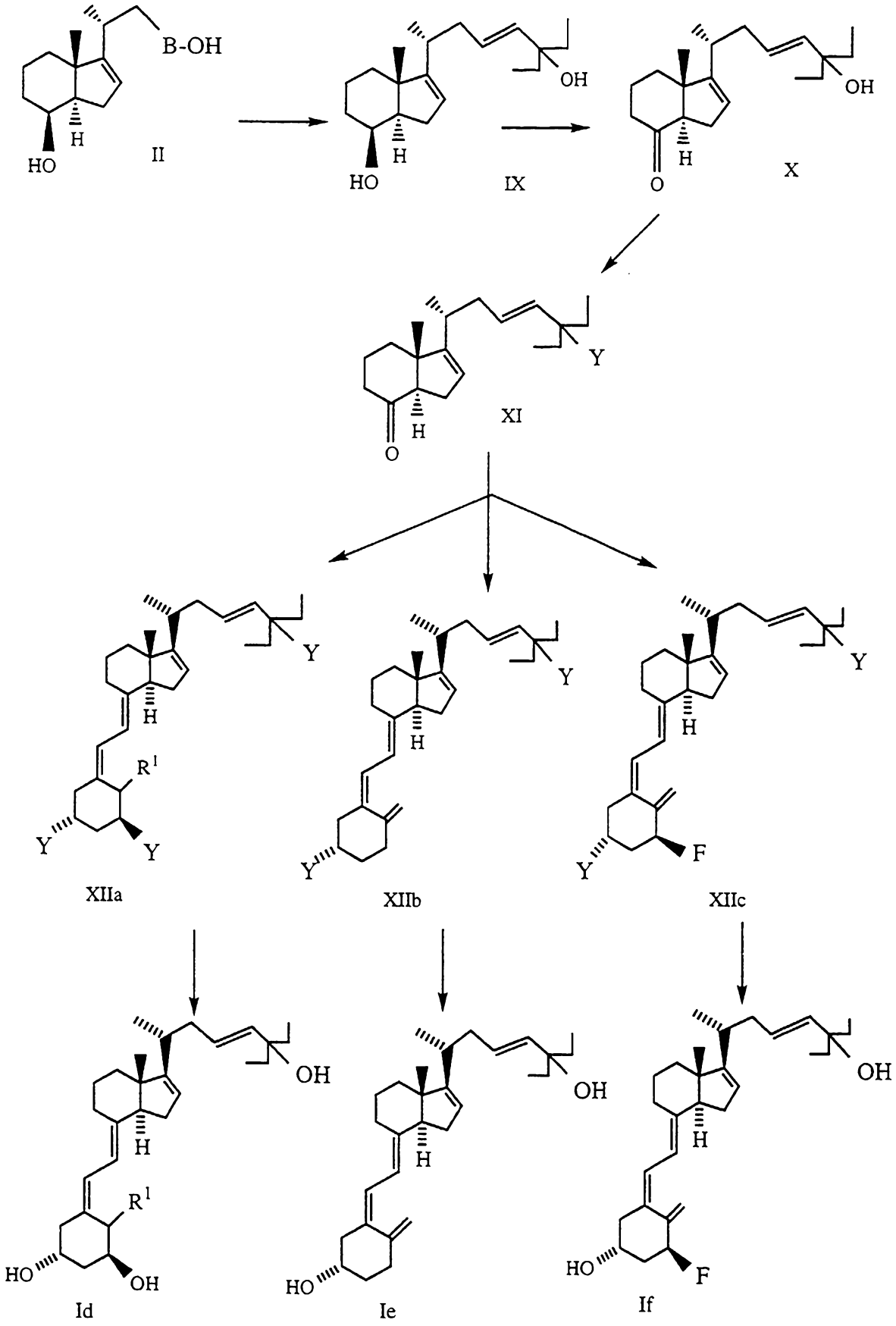
In the above formula Scheme II, the compound of formula VII is converted to a compound of formula VIII by reaction with 3-pentanone. This reaction is carried out at -60°C to -90°C, preferably -78°C, in a polar aprotic, organic solvent such as dry ether or more preferably dry tetrahydrofuran, in the presence of a strong base such as an alkyl lithium, like butyl lithium. The compound of formula VIII is worked up by conventional means such as extraction followed by chromatography.

The compound of formula VII is known U.S. Patent No. 5,087,619 and U.S. Patent No. 5,145,846, both incorporated by reference.

The compound of formula VIII is converted to compound of formula II by reaction with a fluorine salt, such as tetrabutyl-ammonium fluoride in a polar, organic solvent such as dry ether, or more preferably by tetrahydrofuran. The compound of formula II is worked up by conventional means such as extraction followed by chromatography.

The compounds of formula I wherein A is a carbon-carbon double bond with E configuration is prepared as hereinafter described with particular reference to formula Scheme III and the Examples below.

FORMULA SCHEME III



wherein Y, B and R^1 are as described above.

In the above formula Scheme III, the compound of formula II is partially reduced to obtain a compound of formula IX by reaction with a reducing agent, such as lithium aluminum hydride, preferably in the presence of an alkali metal alkoxide, like sodium methoxide, in an aprotic organic solvent, like dry ether, or more preferably dry tetrahydrofuran at reflux temperature (about 80°C for tetrahydrofuran) for about 24 hours, and cooled to about 0°C. The compound of formula IX is worked up by conventional means, such as extraction followed by chromatography.

The resulting compound of formula IX is oxidized to the compound of formula X by treatment with an oxidizing agent such as 2,2'-bipyridinium chlorochromate, or pyridinium dichromate, at room temperature, in an aprotic solvent such as dry tetrahydrofuran, or more preferably, dry methylene chloride. The compound of formula X is worked up by conventional means, such as extraction followed by chromatography.

The compound of formula X is converted to a compound of formula XI by reaction with, for example, a (trialkylsilyl)imidazole such as 1-(trimethylsilyl)imidazole in an aprotic, organic solvent such as dry tetrahydrofuran, or more preferably, dry methylene chloride. The compound of formula XI is worked up by conventional means such as extraction followed by chromatography.

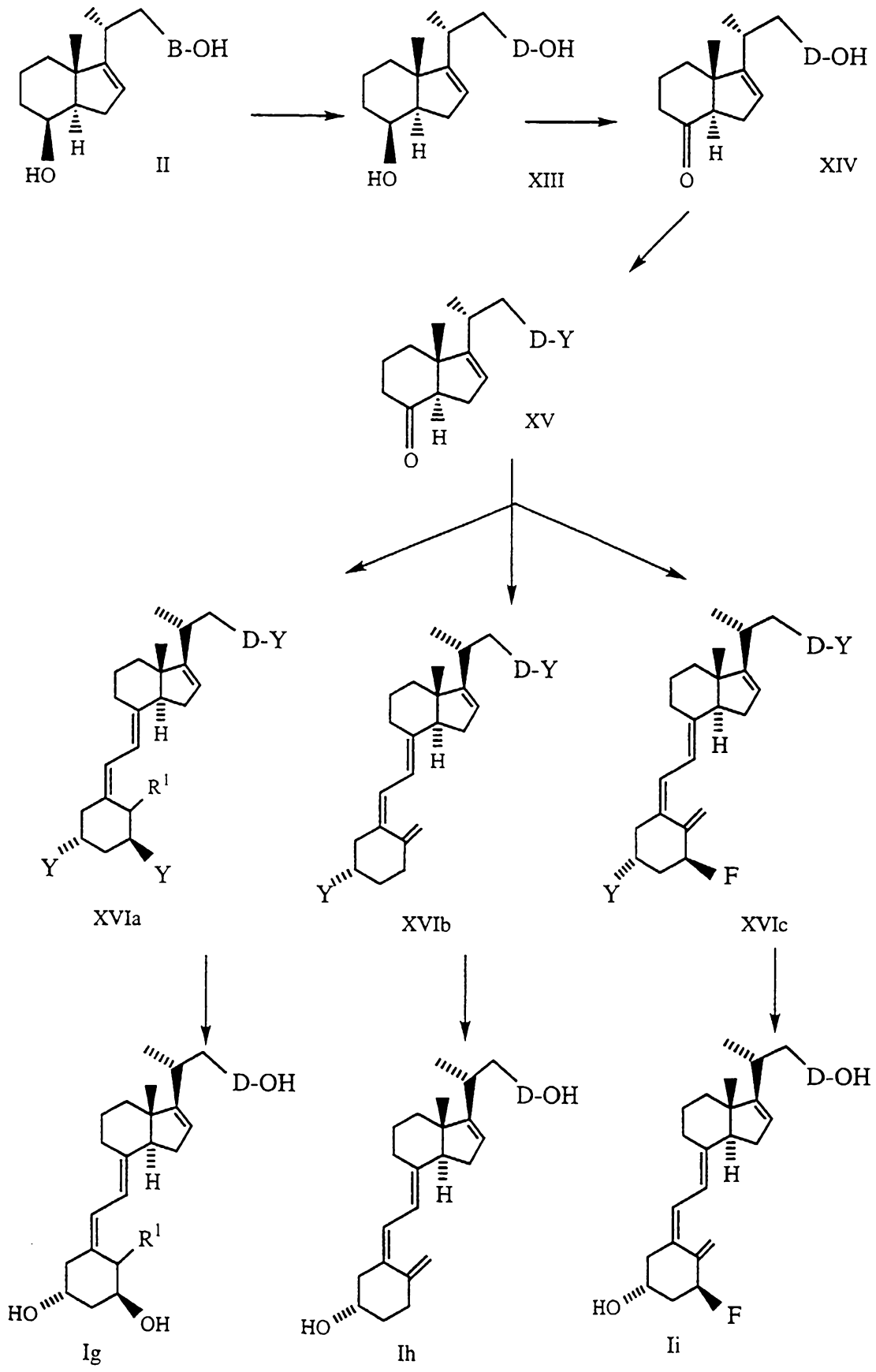
The compound of formula XI is converted to a compound of formula XIIa, XIIb, or XIIc by reaction with the corresponding compound of formula VI. The reaction is carried out at -60°C to -90°C, preferably -78°C, in a polar, aprotic, organic solvent, such as dry ether or more preferably, dry tetrahydrofuran, in the presence of a strong base such as an alkyl lithium, like butyl lithium. The compounds of formula XIIa, XIIb, or XIIc is worked up by conventional means such as extraction followed by chromatography.

The protecting groups of the compound of formula XIIa, XIIb, or XIIc are removed by reaction with a fluorine salt, such as tetrabutyl

ammonium fluoride in an organic solvent such as dry ether, or more preferably by tetrahydrofuran to yield a corresponding compound of formula Id, Ie, or If.

The compounds of formula I wherein A is a carbon-carbon double bond with Z configuration, is prepared as hereinafter described with particular reference to formula Scheme IV and the Examples below.

FORMULA SCHEME IV



wherein Y, B, and R^1 are as described above and D is the group $-CH=CHC(Et)_2-$ with Z configuration, and Et is ethyl.

In the above formula Scheme IV, the compound of formula II is hydrogenated to a compound of formula XIII by reaction with hydrogen and a Lindlar catalyst in an organic solvent, such as a combination of ethyl acetate, hexane and ethanol in the presence of quinoline. The compound of formula XIII is worked up by conventional means, such as extraction followed by chromatography.

The resulting compound of formula XIII is oxidized to the compound of formula XIV by treatment with an oxidizing agent such as 2,2'-bipyridinium chlorochromate, or pyridinium dichromate, at room temperature, in an aprotic solvent such as dry tetrahydrofuran, or more preferably, dry methylene chloride. The compound of formula XIV is worked up by conventional means, such as extraction followed by chromatography.

The compound of formula XIV is converted to a compound of formula XV by reaction with, for example, a (trialkylsilyl)imidazole such as 1-(trimethylsilyl)imidazole in an aprotic, organic solvent such as dry tetrahydrofuran, or more preferably, dry methylene chloride. The compound of formula XV is worked up by conventional means such as extraction followed by chromatography.

The compound of formula XV is converted to a compound of formula XVIa, XVIb, or XVIc by reaction with the corresponding compound of formula VI. The reaction is carried out at -60°C to -90°C, preferably -78°C, in a polar, aprotic, organic solvent, such as dry ether or more preferably, dry tetrahydrofuran, in the presence of a strong base such as an alkyl lithium like butyl lithium. The compounds of formula XVIa, XVIb, or XVIc is worked up by conventional means such as extraction followed by chromatography.

The protecting groups of the compound of formula XVIIa, XVIIb, XVIIc are removed by reaction with a fluorine salt, such as tetrabutyl ammonium fluoride in an organic solvent such as dry ether, or more

preferably by tetrahydrofuran to yield a corresponding compound of formula Ig, Ih, or Ii.

The compounds of formula I as described above can be administered orally, for the treatment of neoplastic diseases such as leukemia, to warmblooded animals which need such treatment. More specifically, the compounds of formula I as described above can be administered orally to an adult human in dosages that are in the range of about .05 to 50 µg per day for the treatment of neoplastic diseases such as leukemia.

The useful activity of compounds of formula I as agents for the treatment of neoplastic diseases can be demonstrated by the following test procedures.

HL-60 Cell Differentiation

The induction of differentiation of HL-60 cells was assayed by measuring their oxidative burst potential via the reduction of nitrobluetetrazolium (NBT).

HL-60 cells were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 2mM L-glutamine, 1mM sodium pyruvate, 1% non-essential amino acids, 50 U/ml penicillin, and 50 µg/ml streptomycin. HL-60 cells (30,000 cells in 90 µl of supplemented RPMI medium) were seeded into flat-bottomed microliter wells. Immediately after seeding, 10 µl of test compounds listed below in Table I diluted in supplemented RPMI medium were added to the wells to yield final concentrations of between 10^{-11} and 10^{-6} M (starting from stock solutions of 10^{-2} M in ethanol, stored at -20°C and protected from light). After 3 days, medium was removed from the wells with a multichannel pipette and replaced with 100 µl of NBT solution (1 mg/ml in phosphate buffered saline with 200 nM phorbol myristate acetate). Following an additional hour incubation at 37°C the NBT solution was removed and 100 µl of 10% sodium dodecyl sulfate in 0.01 N HCl was added. The amount of the reduced NBT was quantified photometrically at 540 nm using an automated plate reader. The mean of 3 wells was calculated. S.E.M. were between 5

and 10%. Values were expressed as percent of maximal differentiation achieved with 100-1000 nM calcitriol (compound X) in the same experiment. The concentration (nM) leading to 50% of this maximal value is determined graphically and given in the Table below as ED_{50}.

Compound	ED_{50} (nM)	HTD µg/kg
X	6.0	0.5
A	2.5	4.0
B	6.2	2.5
C	3.8	2.0

From the above results, it can be seen that compounds of formula I induce differentiation of HL-60 cells and thereby stop these tumor cells from growing. Accordingly, compounds of formula I are useful in the treatment of neoplastic diseases, such as leukemia.

Calcium tolerance test in mice

Profound changes in calcium homeostasis strongly affect the weight development of mice.

Mice (25-30 g body weight) received daily subcutaneous administrations of the test compound for 4 consecutive days. Body weight was registered just before and at the end of a 5 day treatment period. The "highest tolerated dose" (HTD) is the dose which results in zero weight gain during this treatment period. The results are set forth in the Table above.

From the above results, it can be seen that the compounds of formula I are better tolerated than 1,25-dihydroxycholecalciferol.

Oral dosage forms comprising compounds of formula I of the invention may be incorporated in capsules, tablets and the like with pharmaceutically acceptable carrier materials. Illustrative of the pharmaceutically acceptable carrier materials which may be

incorporated into capsules, and the like are the following: a binder, such as gum tragacanth, acacia, corn starch, or gelatin; an excipient, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, algenic acid, and the like; a lubricant, such as magnesium stearate, a sweetening agent, such as sucrose, lactose, or saccharin; a flavoring agent, such as peppermint, oil of wintergreen or cherry. Various other materials may be present as coating or to otherwise modify the physical form of the dosage unit. For instance, tablets may be coated with shellac, sugar, or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propyl parabens as preservatives, a dye, and a flavoring such as cherry or orange flavor.

The following Examples are provided to further describe the invention and are not intended to limit it in any way.

EXAMPLE 1

[1(R*),3aR*(3aα,4β,7aβ)]-3a,4,5,6,7,7a-Hexahydro-1-(1-methyl-5-ethyl-5-hydroxy-3-heptynyl)-7a-methyl-4-[(trimethylsilyl)oxy]-3H-indene

To a solution of 1.02 g (3.51 mmole) of [3aS-[1(R*), 3aβ,7β,7aα]-3α,4,5,6,7,7a-hexahydro-3-[1-methyl-3-butynyl]-3a-methyl-7-[(trimethylsilyl)oxy]-1H-indene in 10 ml anhydrous tetrahydrofuran at -78°C was added slowly 2.5 ml (3.861 mmole) of 1.6M n-butyllithium in hexane. After stirring at -78°C for one hour, 2.5 ml of 3-pentanone was added and the stirring was continued for additional 15 minutes. The reaction mixture was diluted with water and extracted with 4 x 50 ml of hexane. The combined extracts were washed with water and brine, dried over sodium sulfate and evaporated to dryness. The crude product was purified by FLASH chromatography to give 273 mg of recovered starting material and 1 g (75%) of the title compound. ^{1}H-NMR ($CDCl_3$): δ 0.06 (s, 9H, $3CH_3$), 1.02 (t, 6H, J = 7.5 Hz, $2CH_3$), 1.02 (s, 3H, CH_3), 1.10 (d, 3H, J = 6Hz, CH_3), 1.62 (m, 4H, $2CH_2$), 2.08-2.57 (m, 4H, CH, CH_2, CH of CH_2), 4.08 (brs, 1H, CH), 5.32 (brs, 1H, CH).

EXAMPLE 2

[1(R*),3aR*(3aα,4β,7aβ)]-3a,4,5,6,7,7a-Hexahydro-1-(1-methyl-5-ethyl-5-hydroxy-3-heptynyl)-7a-methyl-3H-inden-4-ol

To a stirred solution of 1.178 g (3.12 mmole) of [1(R*),3aR*(3aα,4β,7aβ)]-3a,4,5,6,7,7a-hexahydro-1-[1-methyl-5-ethyl-5-hydroxy-3-heptynyl)-7a-methyl-4-[(trimethylsilyl)oxy]-3H-indene in 15 ml anhydrous tetrahydrofuran was added 6 ml (6 mmole) of 1M tetrabutyl ammonium fluoride. The reaction mixture was stirred at room temperature overnight in an argon atmosphere. It was then diluted with 150 ml of water-brine 1:1 and extracted thoroughly with ethyl acetate. The combined extracts were washed with 2 x 30 ml water, dried over sodium sulfate and evaporated to dryness. The crude product was purified by FLASH chromatography to give 900 mg (94.5%) of the title compound, as a white crystalline solid. $[\alpha]_D^{25}$ -20.5° (c 0.2, EtOH); ^{1}H-NMR ($CDCl_3$): δ 1.01 (t, 6H, J = 7.3 Hz, $2CH_3$), 1.07 (s, 3H, CH_3), 1.10 (d,3H,J = 6Hz, CH_3), 140(dt,1H,Jvic = 3.5 and 12.5 Hz, Jgem = 12.5 Hz, CH of CH_2), 1.63 (m, 4H, 2 CH_2), 1.98 (ddd, 1H, Jvic = 3.5 and 5.5 Hz, Jgem = 15 Hz, CH of CH_2), 2.20 - 2.45 (m, 4H, CH_2, 2CH of CH_2), 4.19 (brs, 1H, CH), 5.39 (s, 1H, CH). Analysis Calcd. for $C_{20}H_{32}O_2$: C 78.90, H 10.59. Found: C 78.92, H 10.30.

EXAMPLE 3

[1(R*),3aR*(3aα,7aβ)]-3,3a,5,6,7,7a-Hexahydro-1-(1-methyl-5-ethyl-5-hydroxy-3-heptynyl)-7a-methyl-4H-inden-4-one

To a stirred solution of 253 mg (0.83 mmole) of [1(R*),3aR*(3aα,4β,7aβ)]-3a,4,5,6,7,7a-hexahydro-1(1-methyl-5-ethyl-5-hydroxy-3-heptynyl)-7a-methyl-3H-inden-4-ol in 15 ml anhydrous methylene chloride at room temperature in argon atmosphere was added 1 g of (2.65 mmole) of pyridinium dichromate. The reaction mixture was stirred for two hours, 0.5 g of 3A molecular sieves was added, and the stirring was continued for one hour. After addition of 25 ml of ether and stirring for 15 minutes, it was filtered through a celite pad. The pad was washed with 3 x 50 ml ethyl acetate. The combined filtrates were washed with 2N potassium bicarbonate, water

and brine, dried over sodium sulfate and evaporated to dryness. The crude product was purified by FLASH chromatography to give 157 mg (62%) of the title compound.

EXAMPLE 4

[1(R*),3aR*(3aα,7aβ)]-3,3a,5,6,7,7a-Hexahydro-1-(1-methyl-5-ethyl-5-[(trimethylsilyl)oxy]-3-heptynyl)-7a-methyl-4H-inden-4-one

To a solution of 223 mg (0.737 mmole) of [1(R*),3aR*-(3aα,7aβ)]-3,3a,4,5,6,7,7a-hexahydro-1(1-methyl-5-ethyl-5-hydroxy-3-heptynyl)-7a-methyl-4H-inden-4-one in 10 ml anhydrous methylene chloride was added 0.67 ml (4.42 mmole) of 1-(trimethylsilyl)-imidazole. The reaction mixture was stirred at room temperature in an argon atmosphere overnight. The reaction was quenched with water, and extracted with hexane. The hexane extracts were washed with water and brine, dried over sodium sulfate and evaporated to dryness. The crude product was purified by FLASH chromatography with hexane-ethyl acetate 10:1 to give 272 mg (98%) of the title compound. ^{1}H-NMR ($CDCl_3$): δ 0.15 (s, 9H, $3CH_3$), 0.83 (s, 3H, CH_3), 0.91 (t, 6H, J = 7.5 Hz, $2CH_3$), 1.16 (m, 3H, CH_3), 1.57 (q, 4H, J = 7.5, $2CH_2$), 2.85 (dd, 1H, Jvic = 7 Hz, Jgem = 10 Hz, CH of CH_2), 5.37 (brs, 1H, CH).

EXAMPLE 5

1,25-Dihydroxy-16-ene-23-yne-26,27-bishomo-cholecalciferol

To a stirred solution of 694 mg (1.19 mmole) of [3S-(1Z,3α,5β)-[2-[3,5-bis[[1,1-dimethylethyl)dimethylsilyl]oxy]-2-methylene-cyclohexylidene]ethyl]diphenylphosphine oxide in 10 ml anhydrous tetrahydrofuran at -78°C was added 0.75 ml (1.2 mmole) of 1.6 M n-butyllithium in hexane. The reaction mixture turned red immediately and the color persisted during the addition of 270 mg (0.72 mmole) of [1(R*),3aR*(3aα,7aβ)]-3,3a,5,6,7,7a-hexahydro-1-(1-methyl-5-ethyl-5-[(trimethylsilyl)oxy]-3-heptynyl)-7a-methyl-4H-inden-4-one in 8 ml of anhydrous tetrahydrofuran. The reaction mixture was stirred at -78°C for 90 min, and then quenched with brine and extracted with

ethyl acetate. The combined extracts were washed with water and brine, dried over sodium sulfate and evaporated to dryness. The crude intermediate was purified by FLASH chromatography with hexane-ethyl acetate 40:1 to give 498 mg of trisilylated intermediate.

To the solution of trisilylated intermediate (498 mg) in 10 mg anhydrous tetrahydrofuran was added 5.04 ml (5.04 mmole) of a 1M tetrabutyl ammonium fluoride. The reaction mixture was stirred at room temperature under argon for 48 hours, and then diluted with water and extracted with ethyl acetate. The combined extracts were washed with water and brine, over sodium sulfate and evaporated to dryness. The crude product was purified by FLASH chromatography with hexane-ethyl acetate 1:3 and preparative HPLC with hexane-ethyl acetate 1:4 to give 267 mg (84.5%) of the title compound, $[\alpha]_D^{25}$ + 6° (c 0.2, EtOH); UV λmax:262/3nm (ε 19,100); 1H-NMR ($CDCl_3$): δ 0.72 (s, 3H, CH_3), 1.01 (t,6H,J = 6.8 Hz, 2 CH_3), 1.14 (d,3H,J = 6.2 Hz, CH_3), 1.62 (m,4H, $2CH_2$), 1.92 (ddd, 1H, Jvic = 3.5 and 8.5 Hz, Jgem = 12.5 Hz, CH of CH_2), 2.61 (br dd, 1H, Jvic = 3.5 Hz, Jgem = 12.5 Hz, CH of CH_2), 2.82 (dd, 1H, Jvic = 4Hz, Jgem = 12 Hz, CH of CH_2), 4.24 (brm, 1H, CH), 4.45 (brm, 1H, CH), 5.02, 5.34 (2s, 2H, CH_2), 5.38 (s, 1H, CH), 6.11, 6.38 (AB;2H, J = 11.4 Hz, CH CH).

EXAMPLE 6

[1(R*),3aR*(3aα,4β,7aβ)]-3a,5,6,7,7a-Hexahydro-1-(1-methyl-5-ethyl-5-hydroxy-3E-heptenyl)-7a-methyl-3H-inden-4-ol

To a stirred suspension of 190 mg (5 mmole) of lithium aluminum hydride in 15 ml anhydrous tetrahydrofuran cooled in an ice-bath was added carefully 270 mg (5 mmole) of solid sodium methoxide first, followed by addition of 252 mg (0.852 mmole) of [1(R*),3aR*-(3aα,4β,7aβ)]-3a,4,5,6,7,7a-hexahydro-1-(1-methyl-5-ethyl-5-hydroxy-3-heptynyl)-7a-methyl-3H-inden-4-ol and the reaction mixture was heated at reflux for 24 hours. After cooling in ice-bath, the reaction was quenched by careful addition of 1 ml water, followed by addition of 1 ml of 2N NaOH. After addition of 20 ml ether, it was stirred for 0.5 hr; 2.2 g Mg SO_4 was added and stirred for

another 0.5 hr. It was then filtered, washed with ether, and the combined ether filtrates were evaporated to dryness. The crude product was purified by FLASH chromatography and preparative HPLC with hexane-ethyl acetate 2:1 to give 133 mg (53%) of the title compound. ^{1}H-NMR ($CDCl_3$): δ 0.84 (t, 6H, J = 7.5 Hz, $2CH_3$), 0.99 (d, 3H, J = 6Hz, CH_3), 1.03 (s, 3H, CH_3), 1.51 (q, 4H, J = 7.5 Hz, $2CH_2$), 4.17 (brs, 1H, CH), 5.32 (brs, 1H, CH), 5.38 (d, 1H, Jtrans = 16.5 Hz, CH), 5.52 (dt, 1H, Jvic = 6.5 Hz, Jtrans = 16.5 Hz, CH).

EXAMPLE 7

[1(R*),3aR*(3aα,7aβ)]-3,3a,5,6,7,7a-Hexahydro-1-(1-methyl-5-ethyl-5-hydroxy-3E-heptenyl)-7a-methyl-4H-inden-4-one

To a stirred of 133 mg (0.434 mmole) of [1(R*),3aR*(3aα,4β,7aβ)]-3a,4,5,6,7,7a-hexahydro-1-(1-methyl-5-ethyl-5-hydroxy-3E-heptenyl)-7a-methyl-3H-inden-4-ol in 4 ml anhydrous methylene chloride was added 950 mg (2.527 mmole) of pyridinium dichromate at room temperature in an argon atmosphere. The reaction mixture was stirred for 5 hours. Then, 25 ml of ether was added and stirred for 15 minutes, filtered through celite pad, and the pad was washed with 3 x 40 ml of ethyl acetate. The combined filtrates were evaporated to dryness. The crude product was purified by FLASH chromatography with hexane-ethyl acetate 5:2 to give 111 mg (84%) of the title compound.

EXAMPLE 8

[1(R*),3aR*(3aα,7aβ)]-3,3a,5,6,7,7a-Hexahydro-1-(1-methyl-5-ethyl-5-[(trimethylsilyl)oxy]-3E-heptenyl)-7a-methyl-4H-inden-4-one

To a solution of 111 mg (0.365 mmole) of [1(R*),3aR*-(3aα,7aβ)]-3,3a,5,6,7,7a-hexahydro-1-(1-methyl-5-ethyl-5-hydroxy-3E-heptenyl)-7a-methyl-4H-inden-4-one in 4 ml anhydrous methylene chloride was added 0.375 ml (2.56 mmole) of 1-(trimethylsilyl)-imidazole. The reaction mixture was stirred in an argon atmosphere for 23 hours. It was then quenched by addition of 10 ml water, stirring for 15 minutes, addition of 20 ml of brine and extraction with

3 x 90 ml of ethyl acetate. The organic layers were washed five times with water-brine 1:1, dried over sodium sulfate and evaporated to dryness. The crude product was purified by FLASH chromatography with hexane-ethyl acetate 10:1 to give 131 mg (95%) of the title compound.

EXAMPLE 9

1,25-Dihydroxy-16,23E-diene-26,27-bishomo-cholecalciferol

To a stirred solution of 405 mg (0.695 mmole) of [3S-(1Z,3α,5β)]-[2-[3,5-bis[[1,1-dimethylethyl)dimethylsilyl]oxy]-2-methylene-cyclohexylidene]ethyl]diphenylphosphine oxide in 5 ml anhydrous tetrahydrofuran at -78°C was added 0.434 ml (0.694 mmole) of 1.6 M n-butyllithium in hexane dropwise in an argon atmosphere. After 5 minutes of stirring, to the thus obtained red solution was added dropwise over a 10 minute period a solution of 131 mg (0.348 mmole) of [1(R*),3aR*(3aα,7aβ)]-3,3a,5,6,7,7a-hexahydro-1-(1-methyl-5-ethyl-5-[(trimethylsilyl)oxy]-3E-heptenyl)-7a-methyl-4H-inden-4-one in 4 ml of anhydrous tetrahydrofuran. The reaction mixture was then stirred at -78°C for 2 hours. It was quenched by addition of 10 ml 2N Rochelle salt solution and warming up to room temperature, then extracted with 3 x 90 ml of ethyl acetate. The combined extracts were washed three times with brine, dried over sodium sulfate and evaporated to dryness. The residue was purified by FLASH chromatography with hexane-ethyl acetate 30:1 to give 220 mg of the trisilylated intermediate.

To the solution of trisilylated intermediate (220 mg) in 3 ml anhydrous tetrahydrofuran was added 3.2 ml (3.2 mmole) of 1M tetrabutyl ammonium fluoride in tetrahydrofuran, and this reaction mixture was stirred at room temperature in an argon atmosphere for 19 hours. 5 ml of water was then added, stirred for 15 minutes, diluted with 20 ml brine and extracted with 3 x 90 ml ethyl acetate. The combined extracts were washed with water-brine 1:1, dried over sodium sulfate and evaporated to dryness. The crude product was purified by FLASH chromatography and by preparative HPLC with hexane-ethyl acetate 1:5 to give 126 mg (82%) of crystalline title

compound; m.p. 133-135°C (from 4:6 tetrahydrofuran-methyl formate). $[\alpha]_D^{25}$ + 26° (c0.2, EtOH); UV λmax (EtOH): 263 nm (ε 18,200); ^{1}H-NMR ($CDCl_3$): δ 0.69 (s, 3H,CH_3), 0.85 (t, 6H, J = 7.5 Hz, 2CH_3), 1.02 (d,3H, J = 6,7 Hz, CH_3), 1.52 (m, 4H, 2CH_2), 1.68 (m, 1H, CH of CH_2), 1.91 (ddd, 1H, Jvic = 3.5 and 8.5 Hz, Jgem = 12.5 Hz, CH of CH_2), 2.60 (dd, 1H, Jvic = 3.5 Hz, Jgem = 12.5 Hz, CH of CH_2), 2.82 (m, 1H, CH of CH_2), 4.24 (m, 1H, CH), 4.45 (m, 1H, CH), 5.02, 5.34 (2s, 2H, CH_2), 5.32 (brs, 1H, CH), 5.38 (d, 1H, Jtrans = 15.5 Hz, CH), 5.52 (dt, 1H, Jvic = 7Hz, Jtrans = 15.5 Hz, CH), 6.11, 6.38 (AB, 2H, J = 11.5 Hz, CH CH); Analysis: Calcd for $C_{29}H_{44}O_3$:C 79.04, H 10.06; Found: C 78.78, H 10.21.

EXAMPLE 10

[1(R*),3aR*-(3aα,4β,7aβ)]-3a,4,5,6,7,7a-Hexahydro-1-(1-methyl-5-ethyl-5-hydroxy-3Z-heptenyl)-7a-methyl-3H-inden-4-ol

A mixture of 215 mg (0.71 mmole) of [1(R*),3aR*-(3aα,4β,7aβ)]-3a,4,5,6,7,7a-hexahydro-1-(1-methyl-5-ethyl-5-hydroxy-3-heptynyl)-7a-methyl-3-inden-4-ol, 5 ml ethyl acetate, 12.5 ml hexane, 0.35 ml absolute ethanol, 0.0175 ml quinoline and 35 mg of Lindlar catalyst was hydrogenated at room temperature and normal pressure for 1.5 hours. The reaction mixture was filtered through a Celite pad, and the pad was washed with ethyl acetate. The combined filtrates were washed with 1N HCl, water, 2N potassium bicarbonate, water and brine, dried over sodium sulfate and evaporated to dryness. The crude product was purified by preparative HPLC (YMC column) to give 200 mg (92.5% of the title compound). ^{1}H-NMR ($CDCl_3$): δ 0.90 (t,6H,J = 7.5 Hz, 2CH_3, 1.01 (d,3H, J = 6.5 Hz, CH_3), 1.05 (s, 3H, CH_3), 1.57(q, 4H, J = 7.5 Hz, 2CH_2), 4.17 (brs, 1H, CH), 5.22 (br dt, 1H, Jvic = 1.5 Hz, Jcis = 12 Hz, CH), 5.38 (dt, 1H, Jvic = 7 Hz, Jcis = 12 Hz, CH).

EXAMPLE 11

[1(R*),3aR*-(3aα,7aβ)]-3,3a,5,6,7,7a-Hexahydro-1-(1-methyl-5-ethyl-5-hydroxy-3Z-heptenyl)-7a-methyl-4H-inden-4-one

To a stirred solution of 200 mg (0.652 mmole) of [1(R*),3aR*-(3aα,4β,7aβ)]-3a,4,5,6,7,7a-hexahydro-1-(1-methyl-5-ethyl-5-hydroxy-3Z-heptenyl)-7a-methyl-3H-inden-4-ol in 10 ml anhydrous methylene chloride was added 1.286 g (3.301 mmole) of pyridinium dichromate at room temperature in an argon atmosphere. The reaction mixture was stirred for 6 hours. 25 ml of ether was added and stirred for 15 minutes, filtered through a Celite pad, and the pad was washed with 3 x 25 ml ethyl acetate. The combined filtrates were evaporated to dryness. The crude product was purified by FLASH chromatography with hexane-ethyl acetate 3:1, to give 182 mg (91.5%) of the title compound. ^{1}H-NMR ($CDCl_3$): 0.81 (s, 3H, CH_3), 0.88 (t, 6H, J = 7.5 Hz, $2CH_3$), 1.06 (d, 3H, J = 6.5 Hz, CH_3), 1.56 (q, 4H, J = 7.5 Hz, $2CH_2$), 2.84 (dd, 1H, Jvic = 6.5 Hz, Jgem = 10 Hz, CH of CH_2), 5.22 (d, 1H, Jcis = 12 Hz, CH), 5.33 (s, 1H, CH), 5.35 (dt, 1H, Jvic = 6.5 Hz, Jcis = 12 Hz, CH).

EXAMPLE 12

[1(R*),3aR*-(3aα,7aβ)]-3,3a,5,6,7,7a-Hexahydro-1-(1-methyl-5-ethyl-5-[(trimethylsilyl)oxy-3Z-heptenyl)-7a-methyl-4H-inden-4-one

To a solution of 182 mg (0.597 mmole) of [1(R*),3aR*-(3aα,7aβ)]-3,3a,5,6,7,7a-hexahydro-1-(1-methyl-5-ethyl-5-hydroxy-3Z-heptenyl)-7a-methyl-4H-inden-4-one in 10 ml anhydrous methylene chloride was added 0.542 ml (3.58 mmole) of 1-(trimethylsilyl)-imidazole. The reaction mixture was stirred at room temperature overnight. The reaction mixture was then diluted with hexane, washed with water and brine, dried over sodium sulfate and evaporated to dryness. The crude product was purified by FLASH chromatography with hexane-ethyl acetate 10:1 to give 218 mg (97%) of the title compound. ^{1}H-NMR ($CDCl_3$): δ 0.12 (s, 9H, $3CH_3$), 0.80 (s,

3H, CH_3), 0.86(t, 6H, J = 7.5 Hz, $2CH_3$), 1.05 (d, 3H, J = 6.5 Hz, CH_3), 1.55 (q, 4H, J = 7.5 Hz, $2CH_2$), 2.84 (dd, 1H, Jvic = 6.5 Hz, Jgem = 10 Hz, CH of CH_2), 5.08 (br dt, 1H, Jvic = 1.5 Hz, Jcis =11.5 Hz, CH), 5.25 (dt, 1H, Jvic = 6.5 Hz, Jcis = 11.5 Hz, CH), 5.31 (brs, 1H, CH).

EXAMPLE 13

1,25-Dihydroxy-16,23Z-diene-26,27-bishomo-cholecalciferol

To a stirred solution of 578 mg (0.992 mmole) of [3S-(1Z,3α,5β)]-[2-[3,5-bis[[(1,1-dimethylethyl)dimethylsilyl]oxy]-2-methylene-cyclohexylidene]ethyl]diphenylphosphine oxide in 8 ml of anhydrous tetrahydrofuran at -78°C was added 0.62 ml (0.992 mmole) of 1.6M n-butyllithium in hexane dropwise in an argon atmosphere. The reaction mixture turned red and the color persisted during the addition of 218 mg (0.578 mmole) of [1(R*),3aR*(3aα,7aβ)]-3,3a,5,6,7,7a-hexahydro-1-(1-methyl-5-ethyl-5[(trimethylsilyl)oxy]-3Z-heptenyl)-7a-methyl-4H-inden-4-one in 8 ml anhydrous tetrahydrofuran. The reaction mixture was stirred at -78°C for 2 hours, then quenched with water and extracted thoroughly with ethyl acetate. The combined extracts were washed with water and brine, dried over sodium sulfate and evaporated to dryness. The crude intermediate was purified by FLASH chromatography with hexane-ethyl acetate 20:1 to give 328 mg of the trisilylated intermediate.

To the solution of the trisilylated intermediate (328 mg) in 10 ml anhydrous tetrahydrofuran was added 4 ml (4 mmole) of 1M tetrabutyl ammonium fluoride in tetrahydrofuran, and the reaction mixture was stirred at room temperature overnight. It was then diluted with water and extracted with ethyl acetate. The combined extracts were washed with water and brine, dried over sodium sulfate and evaporated to dryness. The crude product was purified by preparative HPLC (YMC column) with hexane-ethyl acetate 1:4 to give 191 mg (75%) of the title compound as white foam. $[\alpha]_D^{25}$ + 20.5° (c 0.2, EtOH); UVλ (EtOH): 262-263 nm (ε = 14450); ^{1}H-NMR ($CDCl_3$); δ 0.7 (s, 3H, CH_3), 0.9 (t,6H, J = 7.3 Hz, 2 CH_3), 1.05 (d, 3H, J = 6.8 Hz, CH_3), 1.57 (m, 4H, $2CH_2$), 1.91 (ddd, 1H, Jvic = 3.5 and 8.5 Hz, Jgem = 12.5 Hz, CH

of CH_2), 2.48 (m, 2H, CH_2), 2.61 (br d, 1H, Jgem = 12.5 Hz, CH of CH_2), 2.82 (br m, 1H, CH of CH_2), 4.24 (br m, 1 H, CH), 4.45 (br m, 1H, CH), 5.02, 5.34 (2s, 2H, CH_2), 5.23 (d, 1H, Jcis = 12.3 Hz, CH), 5.35 (s, 1H, CH), 5.37 (dt, 1H, Jvic = 7Hz, Jcis = 12.3 Hz, CH), 6.11, 6.38 (AB, 2H, J = 11.3 Hz, CH CH).

EXAMPLE a

Oral Dosage Form Soft Gelatin Capsule

	mg/capsule
Compound C	0.0005 - 0.050
Butylated Hydroxytoluene (BHT)	0.016
Butylated Hydroxyanisole (BHA)	0.016
Myglyol®-812 qs	160

EXAMPLE b

Oral Dosage Form Soft Gelatin Capsule

	mg/capsule
Compound C	0.0005 - 0.050
α-Tocopherol	0.016
Myglyol®-812 qs	160

1. Suspend BHT and BHA (Example a) or α-Tocopherol (Example b) in Myglyol®-812. Warm to about 50° C, and stir until dissolved.

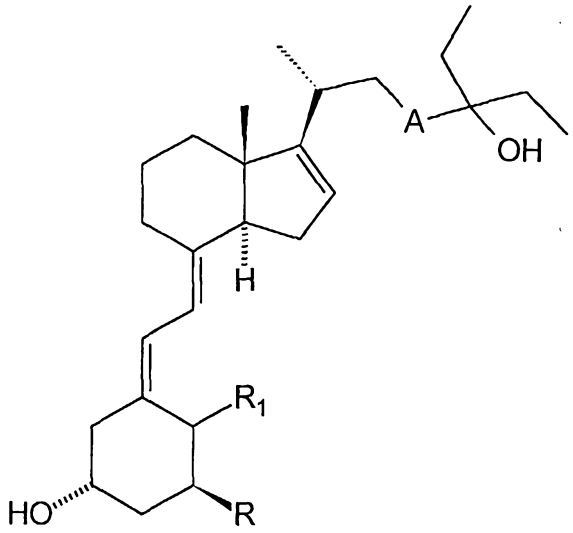
2. Dissolve Compound C in the solution from Step 1.

3. Fill the solution from Step 2 in a soft gelatin cap.

All steps are performed under a nitrogen atmosphere and protected from light.

The claims defining the invention are as follows:

1. 25-Hydroxy- 16-ene-26,27-bishomo-cholecalciferol derivatives of the formula



wherein A is a carbon-carbon double bond having the E or Z stereochemical configuration, or A is a carbon-carbon triple bond, R is hydroxy and R_1 is hydrogen or $=CH_2$, or R is hydrogen or fluoro and R_1 is $=CH_2$.

2. A compound in accordance with claim 1, wherein A is a carbon carbon double bond with E configuration.

3. A compound in accordance with claim 2 wherein R is hydroxy.

4. A compound in accordance with claim 2 or claim 3, wherein R_1 is $=CH_2$

5. A compound in accordance with claim 1, wherein A is a carbon carbon double bond with Z configuration.

6. A compound in accordance with claim 5, wherein R is hydroxy.

7. A compound in accordance with claim 5 or claim 6, wherein R_1 is $=CH_2$.

8. A compound in accordance with claim 1, wherein A is a carbon carbon triple bond.

9. A compound in accordance with claim 8, wherein R is hydroxy.

10. A compound in accordance with claim 8 or claim 9, wherein R_1 is $=CH_2$.

11. 1,25-dihydroxy16,23E-diene-26,27-bishomo-cholecalciferol.

12. 1,25-dihydroxy-16,23Z-diene-26,27-bishomo-cholecalciferol.

13. 1,25-dihydroxy-16-ene-23-yne-26,27-bishomo-cholecalciferol.

14. 1α-fluoro-25-hydroxy-16,23E-diene-26,27-bishomo-cholecalciferol.

15. A 25-hydroxy- 16-ene-26,27-bishomo-cholecalciferol derivative, substantially as hereinbefore described with reference to any one of examples 1 to 13.

16. A pharmaceutical composition comprising an effective amount of at least one compound as in any one of claims 1, to 15 and an inert carrier.

17. A pharmaceutical composition, substantially as hereinbefore described with reference to any one of examples a or b.

18. A method for the treatment or prophylaxis of neoplastic diseases in a mammal requiring said treatment or prophylaxis, which method includes or consists of administering to said mammal an effective amount of at least one compound according to any one of claims 1 to 15, or of a composition according to claim 16.

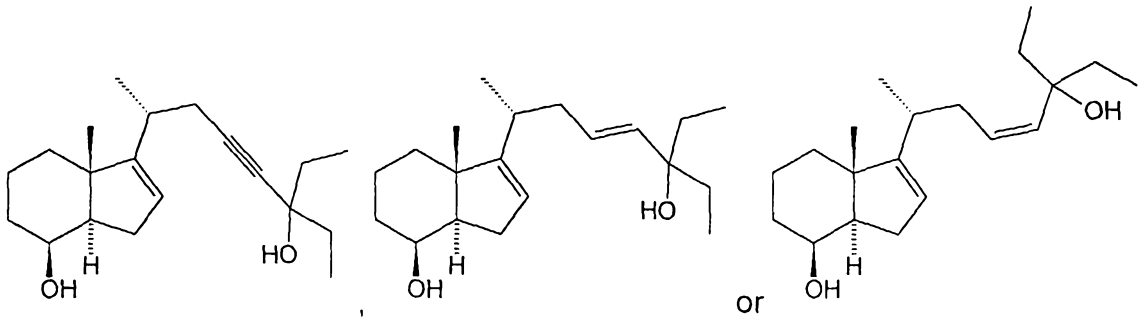
19. A method in accordance with claim 18, wherein the disease is leukemia.

20. A process for the preparation of a 25-hydroxy- 16-ene-26,27-bishomo-cholecalciferol derivative, substantially as hereinbefore described with reference to any one of examples 1 to 13.

21. The compounds as in any one of claims 1 to 15 for use as HL-60 cell differentiation stimulating agents, particularly for the treatment of neoplastic diseases, such as leukemia.

22. The use of the compounds as in any one of claims 1 to 15 for the manufacture of a medicament for the treatment of neoplastic diseases, such as leukemia.

23. A compound of the formulae



Dated 29 May 1998

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