(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 March 2004 (18.03.2004)

PCT

(10) International Publication Number
WO 2004/022068 A1

(51) International Patent Classification[7]: A61K 31/59

(21) International Application Number:
PCT/US2003/020517

(22) International Filing Date: 26 June 2003 (26.06.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/235,244 5 September 2002 (05.09.2002) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/022068 A1

(54) Title: A METHOD OF EXTENDING THE DOSE RANGE OF VITAMIN D COMPOUNDS

(57) Abstract: Inhibitors of bone calcium resorption are administered to allow high doses of vitamine D compounds or mimetics to be given the intent of treating non-calcium related diseases such as cancer, psoriasis, and autoimmune disease without the dangers of calcification of kidney, heart, and aorta. Inhibitors of bone calcium resorption include the bis-phosphonates, OPG or the soluble RANKL receptor known as sRANK, and function to block the availabilite of calcium from bone thereby preventing hypercalcemia and the resulting calcification of soft tissues. Thus, high doses of 1α,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$), its analogs, prodrugs, or mimetics can be utilized with minimal risk to a patient. Specifically, alendronate is shown to block the bone calcium mobilization activity of both 1,25-$(OH)_2D_3$ and its very potent analog, 2-methylene-19-nor(20S)-1α,25 dihydroxyvitamin D_3.

A METHOD OF EXTENDING THE DOSE RANGE OF VITAMIN D COMPOUNDS

BACKGROUND AND SUMMARY OF THE INVENTION

Vitamin D intoxication has been known since its discovery in 1922. Of the fat-soluble vitamins, vitamins A and D given at super-physiologic doses will cause toxicity. In the case of vitamin D, the toxicity is the result of elevated blood calcium and blood phosphorus levels that result in calcification primarily of the kidney, heart, aorta and other tissues. Death may result from kidney failure or failure of important organs such as the heart and aorta. It is also known that vitamin D must be metabolized *in vivo* first in the liver to 25-hydroxyvitamin D_3 (25-OH-D_3) and then in the kidney to 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) before it can carry out its functions. 1,25-$(OH)_2D_3$ then stimulates intestinal calcium and phosphorus absorption, increases the reabsorption of calcium in the kidney, and most importantly, stimulates the mobilization of calcium from bone in a parathyroid hormone dependent process. Thus, an important and unavoidable, until now, activity of the native vitamin D hormone is to mobilize calcium and phosphorus from bone in direct relationship to dose.

It is also known that 1,25-$(OH)_2D_3$ functions through a receptor that dimerizes with the protein, RXR, on responsive elements of target genes to either stimulate or suppresses transcription. The gene products then carry out the functions attributed to 1,25-$(OH)_2D_3$. With the development of receptor knockout mice, and the discovery that Type II vitamin D-dependent rickets is the result of a mutation or mutations in the vitamin D receptor (VDR), it is very clear that most, if not all, actions of vitamin D are mediated through the VDR. This receptor has been found in tissues not previously considered targets of vitamin D action and certainly not considered as playing a role in its functions to mobilize calcium and phosphorus. Such targets are the parathyroid gland, the keratinocytes of skin, the islet cells of the pancreas, and the lymphocytes. Further, Suda and his colleagues

have clearly shown that the vitamin D hormone, i.e. 1,25-$(OH)_2D_3$, causes the differentiation of promyelocytes to monocytes, an action not considered to be related to calcium. Because of this differentiation and suppression of growth of cancer tissues in culture, the possibility that vitamin D compounds might be used in a differentiative treatment of cancer has emerged in an enthusiastic fashion. Furthermore, the suppression of autoimmune disease by 1,25-$(OH)_2D_3$ and many of its analogs is also known. The use of topical treatment with vitamin D compounds such as 1,25-$(OH)_2D_3$ and several of its analogs for the disease psoriasis is another well-established fact. However, a main limitation in the realization of these therapies via the administration of vitamin D compounds is that the primary effect of vitamin D compounds is to elevate blood plasma calcium and phosphorus usually at the expense of bone. Thus, if vitamin D compounds are administered in too high a dosage, vitamin D intoxication is a distinct possibility. Attempts have been made to synthesize vitamin D analogs that do not raise blood calcium yet will act *in vitro* to suppress cancer cells in culture, but so far many of these analogs are non-calcemic because they are rapidly metabolized and rendered inactive. Although that search continues, the present invention provides an alternative route whereby relatively high doses of vitamin D compounds, their analogs, or vitamin D mimetics can be administered without the attendant vitamin D intoxication. Thus, by co-administering agents that block bone calcium mobilization, the mobilization of calcium from bone can be prohibited or prevented or at least minimized, thereby allowing higher and higher doses of vitamin D compounds or mimetics to be used for the treatment of diseases when raising blood calcium is not required. This invention provides that avenue.

The present invention uses a bis-phosphonate, or a calcitonin, or other osteoclastic-mediated bone resorption inhibitor to block bone calcium mobilization and thus prevent the hypercalcemia caused by vitamin D compounds or vitamin D-like mimetics. As a result, high doses of vitamin D compounds can be administered with minimal danger of vitamin D intoxication or hypercalcemia to

the patient and with the distinct possibility of suppressing cancer, psoriasis or autoimmune disease. More specifically, the present invention provides a method of administering high doses of a vitamin D compound or a vitamin D mimetic without developing hypercalcemia or resulting in vitamin D intoxication comprising administering to a mammal being treated with a vitamin D compound or vitamin D mimetic an effective amount of a bone calcium resorption inhibitor in an appropriate dosage schedule. A method of treating psoriasis is also provided which comprises administering to a patient with psoriasis an effective amount of a bone calcium resorption inhibitor and an effective amount of a vitamin D compound or vitamin D mimetic in an appropriate dosage schedule. Further, a method of treating a cancer selected from the group consisting of leukemia, colon cancer, breast cancer or prostate cancer comprises administering to a patient with said cancer an effective amount of a bone calcium resorption inhibitor and an effective amount of a vitamin D compound or vitamin D mimetic in an appropriate dosage schedule. Yet another aspect of the present invention is a method of treating an autoimmune disease selected from the group consisting of multiple sclerosis, lupis, inflammatory bowel disease, Type I diabetes, host versus graft reaction, and rejection of organ transplants, comprising administering to a patient with said disease an effective amount of a bone calcium resorption inhibitor and an effective amount of a vitamin D compound or vitamin D mimetic in an appropriate dosage schedule.

The finding that 1,25-$(OH)_2D_3$ causes differentiation of the promyelocytes and suppresses growth of the promyelocytes led several investigators to follow the purpose of this differentiation and has led to the discovery that the vitamin D hormone as well as other agents induce the formation of osteoclasts. The vitamin D hormone appears to be involved not only in the differentiation of monocytes but further in the formation of multinuclear cells and the activation of the multinuclear cells to become active osteoclasts. This is mediated by the vitamin D hormone through its receptor stimulating the production of a protein RANKL which binds to

the osteoclast precursors to a RANKL receptor termed RANK located in the membrane surface of osteoclast precursors and mature osteoclasts. It is this signal that then activates both osteoclast development and osteoclast function. A naturally secreted soluble version of RANK called osteoprotegerin (OPG) can block this differentiation or activation process by binding membrane bound or secreted RANKL (See for example PCT Application No. WO 96/26271). Preliminary work has suggested that OPG, or a synthetic recombinant soluble protein comprised of only the extra-cellular domain of RANK (sRANK), will prevent the 1,25-$(OH)_2D_3$-induced increase in serum calcium.

Specifically, this invention utilizes inhibitors of bone calcium mobilization especially the bis-phosphonates, OPG, soluble synthetic RANK, or long-lived chimeric proteins comprised of either OPG or soluble RANK fused to the human Fc (OPG-Fc, sRANK-Fc) to block the availability of calcium from bone thereby preventing hypercalcemia and the resulting calcification of soft tissues. Thus, high doses of 1α,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$), its analogs, prodrugs, or other vitamin D-like compounds (referred to herein as "mimetics") can be utilized with minimal risk of developing hypercalcemia to the patient. Specifically, alendronate is shown to block the bone calcium mobilization activity of both 1,25-$(OH)_2D_3$ and its very potent analog, 2-methylene-19-nor-(20S)-1α,25-dihydroxyvitamin D_3 (referred to herein as 2MD).

In accordance with the preferred method of the present invention, patients are to be first administered a bone calcium resorption inhibitor such as either the bis-phosphonates, calcitonin, OPG, or sRANK or other similar RANKL binder or inhibitor (OPG-Fc, RANK-Fc) to prevent bone calcium mobilization. Thereafter, the vitamin D analog or compound can be administered in much higher doses than previously thought possible without causing hypercalcemia. Alternately, the bone resorption inhibitor and vitamin D compound can be administered at the same time. This, therefore, will extend the therapeutic dose from 0.5 μg/patient/day in the case of 1,25-$(OH)_2D_3$ to as much as 5 or 10 μg/patient/day when the agents that block

bone calcium mobilization are administered. This method will prevent the development of hypercalcemia and will result in achieving concentrations of the vitamin D analogs that can suppress cancer, prevent autoimmune disease, or alleviate psoriasis.

It is expected that the use of this methodology will allow 10-fold or higher increase in dosage level of vitamin D compounds with minimal danger of developing hypercalcemia to the patient.

BRIEF DESCRIPTION OF THE DRAWINGS

The drawings illustrate the best mode presently contemplated of carrying out the invention.

Figure 1 is a graph of the body weight versus time after dose administration of mice treated in accordance with the present method; and

Figure 2 is a bar graph of serum calcium versus time after dose administration of mice treated in accordance with the present invention.

DETAILED DESCRIPTION OF THE INVENTION

As used in the description and in the claims, the term "hydroxy-protecting group" signifies any group commonly used for the temporary protection of hydroxy functions, such as for example, alkoxycarbonyl, acyl, alkylsilyl or alkylarylsilyl groups (hereinafter referred to simply as "silyl" groups), and alkoxyalkyl groups. Preferred hydroxy-protecting groups are those that are base stable but readily removable when desired. Alkoxycarbonyl protecting groups are alkyl-O-CO- groupings such as methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, isopropoxycarbonyl, butoxycarbonyl, isobutoxycarbonyl, tert-butoxycarbonyl, benzyloxycarbonyl or allyloxycarbonyl. The term "acyl" signifies an alkanoyl group of 1 to 6 carbons, in all of its isomeric forms, or a carboxyalkanoyl group of 1 to 6 carbons, such as an oxalyl, malonyl, succinyl, glutaryl group, or an aromatic acyl group such as benzoyl, or a halo, nitro or alkyl substituted benzoyl group. The word "alkyl" as used in the description or

the claims, denotes a straight-chain or branched alkyl radical of 1 to 10 carbons, in all its isomeric forms. Alkoxyalkyl protecting groups are groupings such as methoxymethyl, ethoxymethyl, methoxyethoxymethyl, or tetrahydrofuranyl and tetrahydropyranyl. Preferred silyl-protecting groups are trimethylsilyl, triethylsilyl, t-butyldimethylsilyl, dibutylmethylsilyl, diphenylmethylsilyl, phenyldimethylsilyl, diphenyl-t-butylsilyl and analogous alkylated silyl radicals. The term "aryl" specifies a phenyl-, or an alkyl-, nitro- or halo-substituted phenyl group.

A "protected hydroxy" group is a hydroxy group derivatised or protected by any of the above groups commonly used for the temporary or permanent protection of hydroxy functions, e.g. the silyl, alkoxyalkyl, acyl or alkoxycarbonyl groups, as previously defined. The terms "hydroxyalkyl", "deuteroalkyl" and "fluoroalkyl" refer to an alkyl radical substituted by one or more hydroxy, deuterium or fluoro groups respectively.

The terms "hypercalcemia" and "vitamin D toxicity" as used herein refer to a blood serum calcium concentration that is equal to or greater than 2 mg/100 ml of serum. A "toxic dose" of a vitamin D compound is a dose of the vitamin D compound which when administered to a mammal such as a human results in hypercalcemia or vitamin D toxicity.

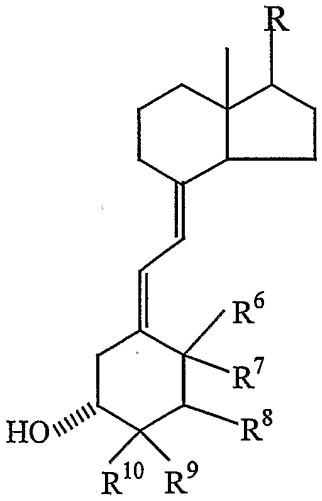
The term "appropriate dosage schedule" refers to a regimen of administering the vitamin D compound and bone calcium resorption inhibitor to a patient at appropriate doses and at appropriate time intervals in order to effectively treat a targeted disease. As is well known in the pharmaceutical arts, such doses and time intervals may be adjusted according to the disease to be treated, its severity, and the response of the subject being treated.

VITAMIN D COMPOUNDS

As used herein the term "vitamin D compound" encompasses compounds which control one or more of the various vitamin D-responsive processes in mammals, i.e. intestinal calcium absorption, bone mobilization, bone

mineralization, and cell differentiation through activation via the VDR. Thus the vitamin D compounds encompassed by this invention include cholecalciferol and ergocalciferol and their metabolites, as well as the synthetic cholecalciferol and ergocalciferol analogs which express calcemic or cell differentiation activity. The term "vitamin D compound" also includes structurally unrelated vitamin D-like compounds, herein referred to as "vitamin D mimetics," which also activate via the VDR. Without limiting the vitamin D compounds encompassed by the present invention, these synthetic cholecalciferol and ergocalciferol analogs comprise such categories of compounds as the 5,6-trans-cholecalciferols and 5, 6-trans-ergocalciferols, the fluorinated cholecalciferols, the side chain homologated cholecalciferols and side chain homologated Δ^{22}-cholecalciferols, the side chain truncated cholecalciferols, the 19-nor cholecalciferols and ergocalciferols, and the 2-substituted cholecalciferols and ergocalciferols.

Structurally, the vitamin D compounds encompassed may be represented by the formula I as follows:



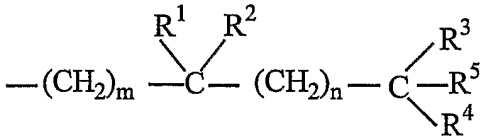
I

where R^6 and R^7 each represent hydrogen or taken together R^6 and R^7 represent a methylene group, R^8 represents hydrogen, hydroxy or a protected hydroxy, R^9 and R^{10} may each independently represent hydrogen, alkyl, hydroxyalkyl, or fluoroalkyl, or R^9 and R^{10} taken together may represent the group $-(CH_2)_x-$ where x is an integer from 2 to 5, the group –OY or $=R^{11}R^{12}$ where R^{11} and R^{12}, which may

be the same are different, are each selected from hydrogen, alkyl, hydroxyalkyl and fluoroalkyl, or when taken together R^{11} and R^{12} represent the group $-(CH_2)x-$ where x is an integer from 2 to 5, and the side chain group R in the above-shown structure, may represent any of the steroid side chain types.

More specifically R can represent a saturated or unsaturated hydrocarbon radical of 1 to 35 carbons, that may be straight-chain, branched or cyclic and that may contain one or more additional substituents, such as hydroxy- or protected-hydroxy groups, fluoro, carbonyl, ester, epoxy, amino or other heteroatomic groups. Preferred side chains of this type are represented by the structure below

where the stereochemical center (corresponding to C-20 in steroid numbering) may have the R or S configuration, (i.e. either the natural configuration about carbon 20 or the 20-epi configuration), and where Z is selected from Y, -OY, $-CH_2OY$, $-C{\equiv}CY$ and -CH=CHY, where the double bond may have the cis or trans geometry, and where Y is selected from hydrogen, methyl, $-COR^5$ and a radical of the structure:

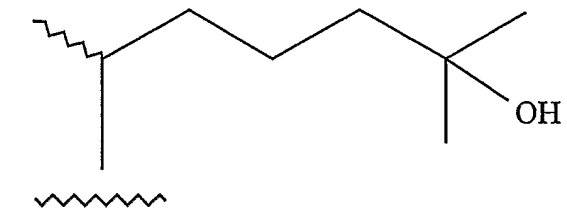


where m and n, independently, represent the integers from 0 to 5, where R^1 is selected from hydrogen, deuterium, hydroxy, protected hydroxy, fluoro, trifluoromethyl, and C_{1-5}-alkyl, which may be straight chain or branched and, optionally, bear a hydroxy or protected-hydroxy substituent, and where each of R^2, R^3, and R^4, independently, is selected from deuterium, deuteroalkyl, hydrogen, fluoro, trifluoromethyl and C_{1-5} alkyl, which may be straight-chain or branched, and optionally, bear a hydroxy or protected-hydroxy substituent, and where R^1 and R^2, taken together, represent an oxo

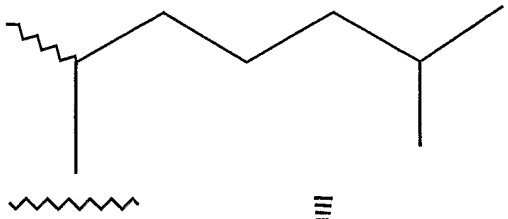
group, or an alkylidene group, $=CR^2R^3$, or the group $-(CH_2)_p-$, where p is an integer from 2 to 5, and where R^3 and R^4, taken together, represent an oxo group, or the group $-(CH_2)_q-$, where q is an integer from 2 to 5, and where R^5 represents hydrogen, hydroxy, protected hydroxy, or C_{1-5} alkyl and wherein any of the CH-groups at positions 20, 22, or 23 in the side chain may be replaced by a nitrogen atom, or where any of the groups $-CH(CH_3)-$, $-(CH_2)_m$, $-(CR_1R_2)-$ or $-(CH_2)_n-$ at positions 20, 22, and 23, respectively, may be replaced by an oxygen or sulfur atom.

The wavy line to the methyl substituent at C-20 indicates that carbon 20 may have either the R or S configuration.

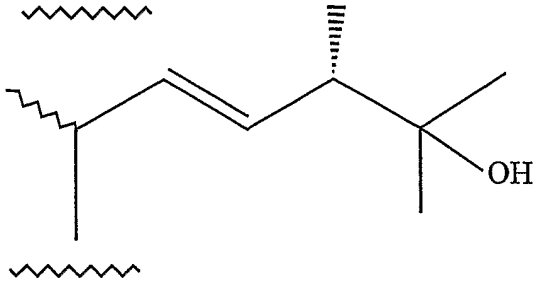
Specific important examples of side chains are the structures represented by formulas (**a**), **b**), (**c**), (**d**) and (**e**)



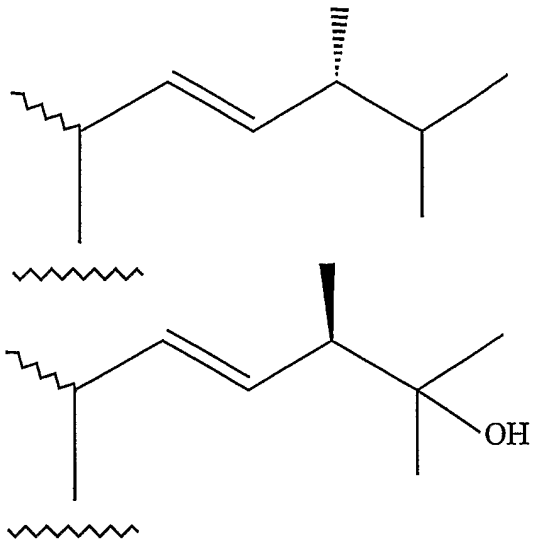
(a)



(b)



(c)



(d)

(e)

Some specific examples of vitamin D compounds useful herein include vitamin D metabolites or analogs such as vitamin D_3, vitamin D_2, 1α-hydroxyvitamin D_3, 1α-hydroxyvitamin D_2, lα,25-dihydroxyvitamin D_3, 1α,25-dihydroxyvitamin D_2, 25 hydroxyvitamin D_3, 25-hydroxyvitamin D_2, 24,24-difluoro-25 hydroxyvitamin D_3, 24,24-difluoro-1α, 25-dihydroxyvitamin D_3, 24-fluoro-25-hydroxyvitamin D_3, 24-fluoro-1α, 25-dihydroxyvitamin D_3, 2β-fluoro-25-hydroxyvitamin D_3, 2β-fluoro- lα-hydroxyvitamin D_3, 2β-fluoro-lα,25-dihydroxyvitamin D_3, 26,26,26,27,27,27-hexafluoro-25-hydroxyvitamin D_3, 26,26,26,27,27,27-hexafluoro-1α,25-dihydroxyvitamin D_3, 24, 25-dihydroxyvitamin D_3, 1α,24,25-trihydroxyvitamin D_3, 25,26-dihydroxyvitamin D_3, lα,25,26-trihydroxyvitamin D_3, 23,25-dihydroxyvitamin D_3, 23,25,26-trihydroxyvitamin D_3, and the corresponding 1α-hydroxylated forms, 25-hydroxyvitamin D_3-26,23-lactone and its lα-hydroxylated derivative, the side chain nor, dinor, trinor and tetranor-analogs of hydroxyvitamin D_3 and of lα,25-dihydroxyvitamin D_3, lα-hydroxypregnacalciferol, and its homo and dihomo derivatives, lα,25-dihydroxy-24-epi-vitamin D_2, 24-homo-1,25-dihydroxyvitamin D_3, 24-dihomo-1,25-dihydroxyvitamin D_3, 24-trihomo-1,25-dihydroxyvitamin D_3

and the corresponding 26- or 26,27-homo, dihomo or trihomo analogs of 1α,25-dihydroxyvitamin D_3 as well as the corresponding 19-nor and 2-substituted compounds of those listed above.

It should be noted in this description that the term "24-homo" refers to the addition of one methylene group and the term "24-dihomo" refers to the addition of two methylene groups at the carbon 24 position in the side chain. Likewise, the term "trihomo" refers to the addition of three methylene groups. Also, the term "26,27-dimethyl" refers to the addition of a methyl group at the carbon 26 and 27 positions so that for example R^3 and R^4 in formula I are ethyl groups. Likewise, the term "26,27-diethyl" refers to the addition of an ethyl group at the 26 and 27 positions so that R^3 and R^4 in formula I are propyl groups.

Specific and preferred examples of the vitamin D compounds of structure **I** when the side chain is unsaturated are:

1α-hydroxy-22-dehydrovitamin D_3;

1α,25-dihydroxy-22-dehydrovitamin D_3;

25-hydroxy-22-dehydrovitamin D_3;

24-homo-1,25-dihydroxy-22-dehydrovitamin D_3;

24-dihomo-1,25-dihydroxy-22-dehydrovitamin D_3;

24-trihomo-1,25-dihydroxy-22-dehydrovitamin D_3;

26,27-dimethyl-24-homo-1,25-dihydroxy-22-dehydrovitamin D_3;

26,27-dimethyl-24-dihomo-1,25-dihydroxy-22-dehydrovitamin D_3;

26,27-dimethyl-24-trihomo-1,25-dihydroxy-22-dehydrovitamin D_3;

26,27-diethyl-24-homo-1,25-dihydroxy-22-dehydrovitamin D_3;

26,27-diethyl-24-dihomo-1,25-dihydroxy-22-dehydrovitamin D_3;

26,27-diethyl-24-trihomo-1,25-dihydroxy-22-dehydrovitamin D_3;

26,27-dipropoyl-24-homo-1,25-dihydroxy-22-dehydrovitamin D_3;

26,27-dipropyl-24-dihomo-1,25-dihydroxy-22-dehydrovitamin D_3; and

26,27-dipropyl-24-trihomo-1,25-dihydroxy-22-dehydrovitamin D_3.

Specific and preferred examples of the vitamin D compounds of structure **I** when the side chain is saturated are:

1α-hydroxyvitamin D_3;

1α,25-dihydroxyvitamin D_3;

25-hydroxyvitamin D_3;

24-homo-1,25-dihydroxyvitamin D_3;

24-dihomo-1,25-dihydroxyvitamin D_3;

24-trihomo-1,25-dihydroxyvitamin D_3;

26,27-dimethyl-24-homo-1,25-dihydroxyvitamin D_3;

26,27-dimethyl-24-dihomo-1,25-dihydroxyvitamin D_3;

26,27-dimethyl-24-trihomo-1,25-dihydroxyvitamin D_3;

26,27-diethyl-24-homo-1,25-dihydroxyvitamin D_3;

26,27-diethyl-24-dihomo-1,25-dihydroxyvitamin D_3;

26,27-diethyl-24-trihomo-1,25-dihydroxyvitamin D_3;

26,27-dipropyl-24-homo-1,25-dihydroxyvitamin D_3;

26,27-dipropyl-24-dihomo-1,25-dihydroxyvitamin D_3; and

26,27-dipropyl-24-trihomo-1,25-dihydroxyvitamin D_3.

In the above lists of vitamin D compounds, if a particular substituent is attached at the carbon 2 position it should be added to the nomenclature. For example, if an alkyl substituent is attached at the carbon 2 position and a methyl group is the alkyl substituent, the term "2-methyl" should precede each of the named compounds. If an ethyl group is the alkyl substituent, the term "2-ethyl" should precede each of the named compounds, and so on. Also, if an alkylidene substituent is attached at the carbon 2 position and a methylene group is the alkylidene substituent, the term "2-

methylene" should proceed each of the named compounds. If an ethylene group is the alkylidene substituent, the term "2-ethylene" should proceed each of the named compounds, and so on. 2-alkyl-19-nor vitamin D compounds are more completely described in U.S. Patent 6,127,559 the disclosure of which is specifically incorporated herein by reference. 2-alkylidene-19-nor vitamin D compounds are more completely described in U.S. Patent 5,843,928 the disclosure of which is specifically incorporated herein by reference. Other vitamin D compounds are disclosed in U.S. Patent 6,369,099 the disclosure of which is specifically incorporated herein by reference. In addition, if the methyl group attached at the carbon 20 position is in its epi or unnatural configuration, the term "20(S)" or "20-epi" should be included in each of the named compounds. The named compounds could also be of the vitamin D_2 type having the side chain of formula (c) or (d) above if desired as well as the 19-nor type where the normal methylene group attached at carbon 10 of the A-ring is replaced with two hydrogen atoms. 19-nor vitamin D compounds are more completely described in U.S. Patent 5,587,497 the disclosure of which is specifically incorporated herein by reference.

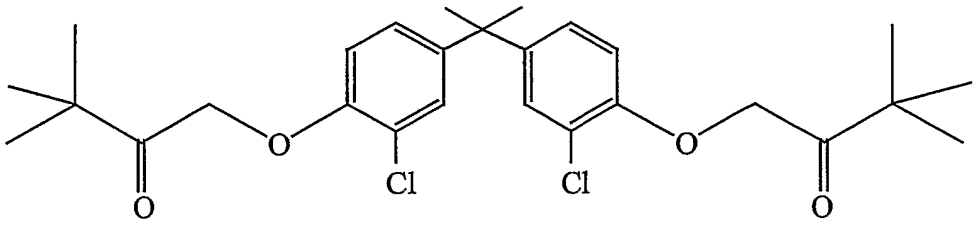
The preferred vitamin D compounds for use in the methods of the present invention are 1α,25-dihydroxyvitamin D_3 and 2-methylene-19-nor-20(S)-1α,25-dihydroxyvitamni D_3 (herein referred to as "2MD").

The preparation of the vitamin D compounds, having the basic structure **I** can be accomplished by a common general method, i.e. the condensation of a bicyclic Windaus-Grundmann type ketone with an allylic phosphine oxide followed by deprotection at C-1 and C-3 in the latter compounds, if desired. This synthesis is well known, and reference is made to U.S. Patents 5,843,928 and 5,945,410 for a more detailed illustration of the technique.

Structurally, vitamin D mimetics may be represented by but not limited to the non-secosteroidal VDR ligand reported by Boehm et al. (Chem. Biol. 6:265-275, 1999) and Polek et al. (The Prostate 49:224-233, 2001), or derivatives thereof, the disclosures of each being specifically incorporated herein by reference. Examples of vitamin D

mimetics that activate the VDR are those identified by Boehm et al. (Chem. Biol. 6:265-275, 1999) and the bile acid lithocholic acid and several of its derivatives (Makishima et al., Science 296:1313-1316, 2002).

Examples of vitamin D mimetics include, but are not limited to, the following five compounds:



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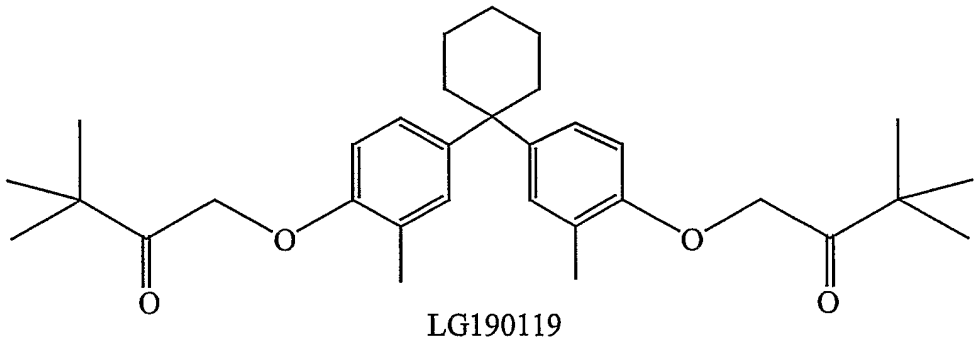

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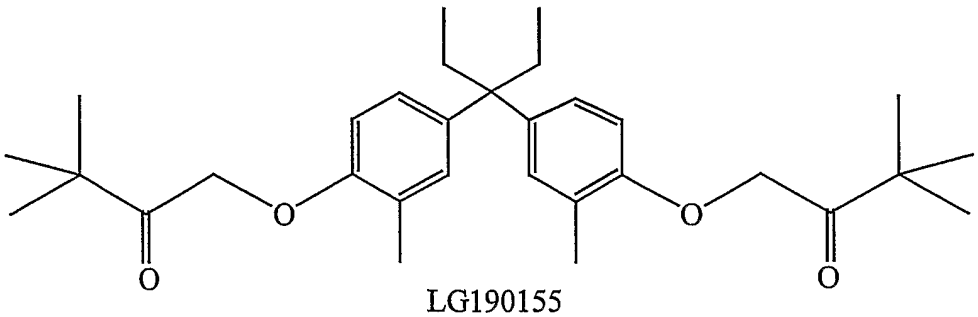

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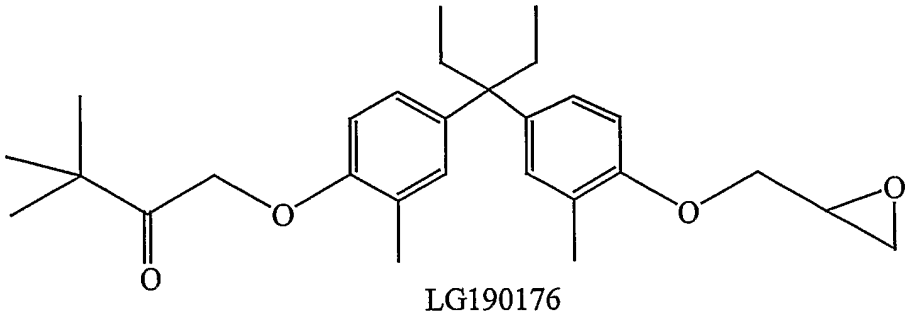

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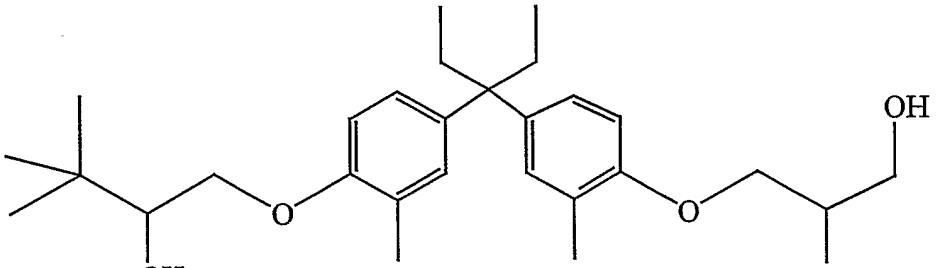

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INHIBITORS OF BONE RESORPTION

As previously stated, inhibitors of bone calcium resorption are administered to prevent the hypercalcemia caused by vitamin D compounds. The term "inhibitor
10 of bone calcium resorption" or "bone calcium resorption inhibitor" encompasses compounds which block or at least substantially block the body's ability to resorb calcium from bone. Such compounds include:

Estrogens,

Androgens,

15 Cytokines that inhibit bone resorption such as interleukin (IL)-4, IL-12, IL-13, IL-18,

Thiazolidinedione class of activators of peroxisome proliferator activated receptor (PPAR)gamma (e.g. rosglitazone, piaglitazone) (Bendixen et al., Proc. Natl. Acad. Sci. USA 98:2443-2448, 2001),

Calcitonins,

Bisphosphonates (e.g. allendronate, risedronate),

Receptor activator of NFkB (RANK) extracellular domain preparations (Childs et al., J. Bone Miner. Res. 17:192-199, 2002),

RANK mimetics,

Soluble RANK-chimeric proteins (RANK-Fc) (Childs et al., J. Bone Miner. Res. 17:192-199, 2002),

Osteoprotegerin (OPG) (Morony et al., J. Bone Miner. Res. 14:1478-1485, 1999),

OPG chimeric proteins (OPG-Fc) (Morony et al., J. Bone Miner. Res. 14:1478-1485, 1999),

OPG mimetics (Takasaki et al., Nature Biotech 15:1266-1270, 1997),

TNF receptor associated factor 6 (Traf6) decoy peptides (Lomaga et al., Genes & Develop. 13:1015-1024, 1999; Ye et al., Nature 418:443-447, 2002),

Chimeric membrane-permeable Traf6 decoy peptides (Ye et al., Nature 418:443-447, 2002),

Traf6 decoy peptide mimetics,

Inhibitors of src (Wong et al., Mol. Cell 4:1041-1049, 1999),

Inhibitors of the extracellular receptor kinase (ERKs), c-Jun N-terminal kinase (JNKs), stress-activated protein kinase (SAPKs) (p38s) (Darnay et al., J. Biol. Chem. 274:7724-7731, 1999; Matsumoto et al., J. Biol. Chem. 275:31155-31161, 2000),

Peptide/small molecule inhibitors of activator protein-I (AP-1),

Peptide/small molecule inhibitors of c-Fos,

Peptide/small molecule inhibitors of nuclear factor kappa B (NFkB) (Franzoso et al., Genes & Develop. 11:3482-3496, 1997),

Peptide/small molecule inhibitors of inhibitor kinase (IK)beta,

Peptide/small molecule inhibitors of the inhibitory kinase (Ikα, Ikβ, IKKs),

Small molecule antagonists of membrane bound RANK,

Small molecule inhibitors of RANK ligand trimerization or activation,

RGD-containing inhibitors of osteoclast-expressed integrins (Nakamura et al., Endocrinology 139:5182-5193, 1998),

Small molecule mimetics of integrin inhibitors (Nakamura et al., Endocrinology 139:5182-5193, 1998),

Cathespin K inhibitors,

Tartrate resistant acid phosphatase inhibitors, and

Vacuolar ATPase inhibitors.

The above compounds can be used alone or together in various combinations depending upon the desired results.

For treatment purposes, the vitamin D compounds defined by formula I or vitamin D mimetics such as that defined by Boehm et al. (Chem. Biol. 6:265-275, 1999) and Polek et al. (The Prostate 49:224-233, 2001), and the inhibitors of bone calcium resorption may each be formulated for pharmaceutical applications as a solution in innocuous solvents, or as an emulsion, suspension or dispersion in suitable solvents or carriers, or as pills, tablets or capsules, together with solid carriers, according to conventional methods known in the art. Any such formulations may also contain other pharmaceutically-acceptable and non-toxic excipients such as stabilizers, anti-oxidants, binders, coloring agents or emulsifying or taste-modifying agents.

The vitamin D compounds or mimetics and the inhibitors of bone calcium resorption may each be administered orally, topically, parenterally or transdermally. The vitamin D compounds or mimetics and/or the inhibitors of bone calcium resorption are advantageously administered by injection or by intravenous infusion or suitable sterile solutions, or in the form of liquid or solid doses via the alimentary canal, or in the form of creams, ointments, patches, or similar vehicles suitable for transdermal applications. Doses of from 0.1μg per day to 100μg per day of the vitamin D compounds and doses of 7.0 mg per day to 700 mg per day of bone calcium

resorption inhibitor are appropriate for treatment purposes, such doses being adjusted according to the disease to be treated, its severity and the response of the subject as is well understood in the art. Typically, a sufficient amount of bone calcium resorption inhibitor is administered so as to provide 0.1mg/kg to 10 mg/kg of body weight. The vitamin D compounds or mimetics and/or the inhibitors of bone calcium resorption each may be suitably administered independently of each other, or they may be administered simultaneously, in an appropriate dosage schedule, or they may be administered together with graded doses of another vitamin D compound or mimetic and/or inhibitor of bone calcium resorption in situations where different degrees of biological activity is found to be advantageous.

Compositions for use in the above-mentioned treatment of psoriasis, cancer and other malignancies or autoimmune diseases comprise an effective amount of one or more vitamin D compound, as defined by the above formula I, or mimetics, together with one or more inhibitor of bone calcium resorption as defined herein, as the active ingredients, and a suitable pharmaceutical carrier for each. The compositions may be administered substantially simultaneously or the preferred method is for the composition containing the bone calcium resorption inhibitor to be administered first followed by the composition containing the vitamin D compound. It is also contemplated that a single composition could contain both the vitamin D compound or mimetic and the bone calcium resorption inhibitor. An effective amount of each of such compounds for use in accordance with this invention is from about 0.1μg to 100μg per gram of composition for vitamin D compounds or mimetics and 7 mg to 700 mg per gram of composition for the bone resorption inhibitors, and may be formulated to be administered topically, transdermally, orally or parenterally.

The compositions may be formulated as creams, lotions, ointments, topical patches, pills, capsules or tablets, or in liquid form as solutions, emulsions, dispersions, or suspensions in pharmaceutically innocuous and acceptable solvent or oils, and such preparations may contain in addition other pharmaceutically innocuous or beneficial

components, such as stabilizers, antioxidants, emulsifiers, coloring agents, binders or taste-modifying agents.

The compositions are advantageously administered in amounts sufficient to result in the desired effect. Dosages as described above are suitable, it being understood that the amounts given are to be adjusted in accordance with the severity of the disease, and the condition and response of the subject as is well understood in the art.

The formulations of the present invention comprise an active ingredient in association with a pharmaceutically acceptable carrier therefore and optionally other therapeutic ingredients. The carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulations and not deleterious to the recipient thereof.

Formulations of the present invention suitable for oral administration may be in the form of discrete units as capsules, sachets, tablets or lozenges, each containing a predetermined amount of the active ingredient; in the form of a powder or granules; in the form of a solution or a suspension in an aqueous liquid or non-aqueous liquid; or in the form of an oil-in-water emulsion or a water-in-oil emulsion.

Formulations for rectal administration may be in the form of a suppository incorporating the active ingredient and carrier such as cocoa butter, or in the form of an enema.

Formulations suitable for parenteral administration conveniently comprise a sterile oily or aqueous preparation of the active ingredient which is preferably isotonic with the blood of the recipient.

Formulations suitable for topical administration include liquid or semi-liquid preparations such as liniments, lotions, applicants, oil-in-water or water-in-oil emulsions such as creams, ointments or pastes; or solutions or suspensions such as drops; or as sprays.

The formulations may conveniently be presented in dosage unit form and may be prepared by any of the methods well known in the art of pharmacy. By the term

"dosage unit" is meant a unitary, i.e. a single dose which is capable of being administered to a patient as a physically and chemically stable unit dose comprising either the active ingredient as such or a mixture of it with solid or liquid pharmaceutical diluents or carriers.

EXAMPLES

Eight-week-old male CD1 mice were obtained from Harlan-Sprague Dawley and fed purified diet 11 containing 0.47% calcium, 0.3% phosphorus, and supplemented with vitamins A,D,E and K as described by Suda et al. (1970). Two days after arrival, the rats were then transferred to the same diet 11 but containing 0.02% calcium, 0.3% phosphorus, and the A,D,E and K supplement. Thus, the animals were on a diet essentially devoid of calcium. Two days following shifting of the animals to the low calcium diet, they were given the following doses: 1.7 μg/kg bw and/or 4.5 μg/kg bw 2MD or 500 μg/kg bw 1,25-(OH)2D3. The mice were first divided into 6/group and provided the vitamin D compounds by oral administration at the dose levels shown. Alendronate which was obtained from Sigma was dissolved in phosphate-buffered saline and given interperitoneally in a volume of 100 μL. Serum was collected on days 2, 3, 4 and 8 following treatment. Total serum calcium was measured by Atomic Absorption Spectrometry.

Animals were weighed periodically throughout the study.

TREATMENT GROUPS

n=6 animals/group

Group 1 – Neobee oil (4 ml/kg bw)
Group 2 – 1X PBS (100 μl)
Group 3 – alendronate (~1.75 mg/kg bw) + Neobee oil

Group 4 – 2MD (4.5 μg/kg bw in Neobee oil) + 1X PBS

Group 5 – 2MD (4.5 μg/kg bw in Neobee oil) + alendronate (~1.75 mg/kg bw)

Group 6 – 1,25$(OH)_2D_3$ (500 μg/kg bw in Neobee oil) + 1X PBS

Group 7 – 1,25$(OH)_2D_3$ (500 μg/kg bw in Neobee oil) + alendronate (~1.75 mg/kg bw)

Group 8 – alendronate (1.75 mg/kg in PBS 24 hr prior to 2MD (4 μg/kg bw)

The oil and vitamin D compounds were administered by oral gavage. The alendronate and PBS were administered intraperitoneally in a volume of 100 μl.

RESULTS

As shown in Figure 1, weights did not change except for the group receiving the 2MD. Thus, a loss of body weight indicative of hypercalcemia and intoxication was clearly evident in mice receiving 2MD. All other groups maintained their weight during the test period. The lower graph demonstrates that 1,25-$(OH)_2D_3$ in 2 days caused a significant rise in serum calcium as did the 2MD. After 3 days, 2MD showed further hypercalcemia, while the effect of 1,25-$(OH)_2D_3$ had subsided. By day 4, 1,25-$(OH)_2D_3$ showed no hypercalcemia, whereas the 2MD still showed hypercalcemic values of 12.5 mg/100 ml. The administration of alendronate clearly blocked the rise in serum calcium caused by either 1,25-$(OH)_2D_3$ or 2MD, while alendronate by itself did not change serum calcium concentration. These results demonstrate that the hypercalcemia caused by the mobilization of calcium from bone following treatment with a potent vitamin D analog, 2MD or 1,25-$(OH)_2D_3$ itself can be completely prevented by the simultaneous administration of the bis-phosphonate alendronate. Thus, it would be possible to continue treatment of mice with the high level of 2MD safely in the presence of the alendronate and, therefore, can be used to determine efficacy of 2MD against a malignancy or some other disease where calcium is not involved. It

is anticipated that calcitonin can be used similarly as can OPG, sRANK, OPG-Fc, or RANK-Fc in preventing the rise in serum calcium at the expense of bone or to prevent hypercalcemia of bone origin.

CLAIMS

We claim:

1. A method of administering a toxic dose of a vitamin D compound to a mammal without developing hypercalcemia comprising administering to said mammal in an appropriate dosage schedule an effective amount of a bone calcium resorption inhibitor.

2. The method of claim 1 wherein the bone calcium resorption inhibitor is administered orally.

3. The method of claim 1 wherein the bone calcium resorption inhibitor is administered parenterally.

4. The method of claim 1 wherein the bone calcium resorption inhibitor is administered transdermally.

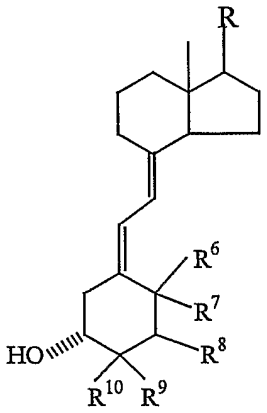
5. The method of claim 1 wherein the bone calcium resorption inhibitor is administered topically.

6. The method of claim 1 wherein the bone calcium resorption inhibitor is administered in a dosage of from about 0.1 mg/kg to 100 mg/kg of body weight.

7. The method of claim 1 wherein the bone calcium resorption inhibitor is administered before the vitamin D compound.

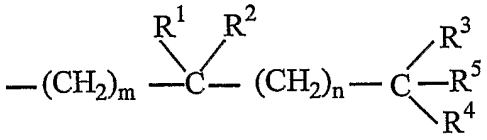
8. The method of claim 1 wherein the bone calcium resorption inhibitor is administered substantially simultaneously with the vitamin D compound.

9. The method of claim 1 wherein the vitamin D compound is selected from a compound having the formula



where R^6 and R^7 each represent hydrogen or taken together R^6 and R^7 represent a methylene group, R^8 represents hydrogen, hydroxy or a protected hydroxy, R^9 and R^{10} may each independently represent hydrogen, alkyl, hydroxyalkyl, or fluoroalkyl, or R^9 and R^{10} taken together may represent the group $-(CH_2)_x-$ where x is an integer from 2 to 5, the group –OY or $=R^{11}R^{12}$ where R^{11} and R^{12}, which may be the same are different, are each selected from hydrogen, alkyl, hydroxyalkyl and fluoroalkyl, or when taken together R^{11} and R^{12} represent the group $-(CH_2)x-$ where x is an integer from 2 to 5 and the group R is represented by the structure

where the stereochemical center at carbon 20 may have the R or S configuration, and where Z is selected from Y, -OY, $-CH_2OY$, -C≡CY and -CH=CHY, where the double bond may have the cis or trans geometry, and where Y is selected from hydrogen, methyl, $-COR^5$ and a radical of the structure:



where m and n, independently, represent the integers from 0 to 5, where R^1 is selected from hydrogen, deuterium, hydroxy, protected hydroxy, fluoro, trifluoromethyl, and C_{1-5}-alkyl, which may be straight chain or branched and, optionally, bear a hydroxy or protected-hydroxy substituent, and where each of R^2, R^3, and R^4, independently, is selected from deuterium, deuteroalkyl, hydrogen, fluoro, trifluoromethyl and C_{1-5} alkyl, which may be straight-chain or branched, and optionally, bear a hydroxy or protected-hydroxy substituent, and where R^1 and R^2, taken together, represent an oxo group, or an alkylidene group, $=CR^2R^3$, or the group $-(CH_2)_p-$, where p is an integer from 2 to 5, and where R^3 and R^4, taken

together, represent an oxo group, or the group -$(CH_2)_q$-, where q is an integer from 2 to 5, and where R^5 represents hydrogen, hydroxy, protected hydroxy, or C_{1-5} alkyl and wherein any of the CH-groups at positions 20, 22, or 23 in the side chain may be replaced by a nitrogen atom, or where any of the groups -$CH(CH_3)$-, -$(CH_2)_m$-, -(CR_1R_2)- or –$(CH_2)_n$- at positions 20, 22, and 23, respectively, may be replaced by an oxygen or sulfur atom.

10. The method of claim 1 wherein the vitamin D compound is 2-methylene-19-nor-20(S)-1α,25-dihydroxyvitamin D_3.

11. The method of claim 1 wherein the vitamin D compound is 1α,25-dihydroxyvitamin D_3.

12. The method of claim 1 wherein the vitamin D compound is a vitamin D mimetic selected from any group of compounds that bind to the VDR and activate its transcriptional capability.

13. The method of claim 1 wherein the bone calcium resorption inhibitor is selected from the group consisting of:

Estrogens,

Androgens,

Cytokines that inhibit bone resorption,

Thiazolidinedione class of activators of peroxisome proliferator activated receptor (PPAR)gamma,

Calcitonins,

Bisphosphonates,

Receptor activator of NFkB (RANK) extracellular domain preparations,

RANK mimetics,

Soluble RANK-chimeric proteins (RANK-Fc),

Osteoprotegerin (OPG),

OPG chimeric proteins (OPG-Fc),

OPG mimetics,

TNF receptor associated factor 6 (Traf6) decoy peptides,

Chimeric membrane-permeable Traf6 decoy peptides,

Traf6 decoy peptide mimetics,

Inhibitors of src,

Inhibitors of the extracellular receptor kinase (ERKs), c-Jun N-terminal kinase (JNKs), or stress-activated protein kinase (SAPKs),

Peptide/small molecule inhibitors of activator protein-I (AP-1),

Peptide/small molecule inhibitors of c-Fos,

Peptide/small molecule inhibitors of nuclear factor kappa B (NFkB),

Peptide/small molecule inhibitors of inhibitor kinase (IK)beta,

Peptide/small molecule inhibitors of the inhibitory kinase (Ikα, Ikβ, IKKs),

Small molecule antagonists of membrane bound RANK,

Small molecule inhibitors of RANK ligand trimerization or activation,

RGD-containing inhibitors of osteoclast-expressed integrins,

Small molecule mimetics of integrin inhibitors,

Cathespin K inhibitors,

Tartrate resistant acid phosphatase inhibitors, and

Vacuolar ATPase inhibitors.

14. The method of claim 1 wherein the bone calcium resorption inhibitor is alendronate.

15. The method of claim 1 wherein the mammal is a human.

16. A method of treating psoriasis comprising administering to a patient with psoriasis in an appropriate dosage schedule an effective amount of a bone calcium resorption inhibitor and an effective amount of a vitamin D compound.

17. The method of claim 16 wherein the bone calcium resorption inhibitor is administered before the vitamin D compound.

18. The method of claim 16 wherein the bone calcium resorption inhibitor is administered substantially simultaneously with the vitamin D compound or mimetic.

19. The method of claim 16 wherein either or both of the bone calcium resorption inhibitor and the vitamin D compound is administered orally.

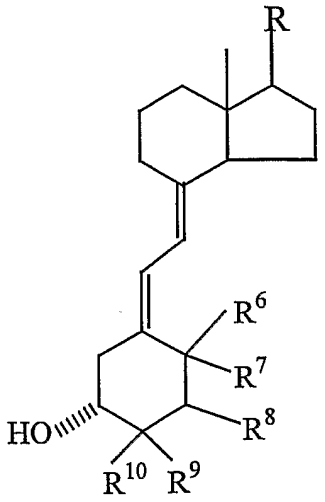
20. The method of claim 16 wherein either or both of the bone calcium resorption inhibitor and the vitamin D compound is administered parenterally.

21. The method of claim 16 wherein either or both of the bone calcium resorption inhibitor and the vitamin D compound is administered transdermally.

22. The method of claim 16 wherein either or both of the bone calcium resorption inhibitor and the vitamin D compound is administered topically.

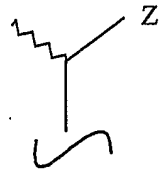
23. The method of claim 16 wherein the bone calcium resorption inhibitor is administered in a dosage of from about 7 mg/day to 700 mg/day and the vitamin D compound is administered in a dosage of from about 0.1 μg/day to 100 μg/day.

24. The method of claim 16 wherein the vitamin D compound is selected from a compound having the formula

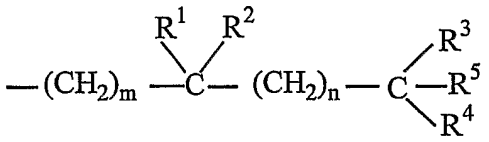


where R^6 and R^7 each represent hydrogen or taken together R^6 and R^7 represent a methylene group, R^8 represents hydrogen, hydroxy or a protected hydroxy, R^9 and R^{10} may each independently represent hydrogen, alkyl, hydroxyalkyl, or

fluoroalkyl, or R^9 and R^{10} taken together may represent the group $-(CH_2)_x-$ where x is an integer from 2 to 5, the group –OY or $=R^{11}R^{12}$ where R^{11} and R^{12}, which may be the same are different, are each selected from hydrogen, alkyl, hydroxyalkyl and fluoroalkyl, or when taken together R^{11} and R^{12} represent the group $-(CH_2)x-$ where x is an integer from 2 to 5 and the group R is represented by the structure



where the stereochemical center at carbon 20 may have the R or S configuration, and where Z is selected from Y, -OY, $-CH_2OY$, -C≡CY and -CH=CHY, where the double bond may have the cis or trans geometry, and where Y is selected from hydrogen, methyl, $-COR^5$ and a radical of the structure:



where m and n, independently, represent the integers from 0 to 5, where R^1 is selected from hydrogen, deuterium, hydroxy, protected hydroxy, fluoro, trifluoromethyl, and C_{1-5}-alkyl, which may be straight chain or branched and, optionally, bear a hydroxy or protected-hydroxy substituent, and where each of R^2, R^3, and R^4, independently, is selected from deuterium, deuteroalkyl, hydrogen, fluoro, trifluoromethyl and C_{1-5} alkyl, which may be straight-chain or branched, and optionally, bear a hydroxy or protected-hydroxy substituent, and where R^1 and R^2, taken together, represent an oxo group, or an alkylidene group, $=CR^2R^3$, or the group $-(CH_2)_p-$, where p is an integer from 2 to 5, and where R^3 and R^4, taken together, represent an oxo group, or the group $-(CH_2)_q-$, where q is an integer from 2 to 5, and where R^5 represents hydrogen, hydroxy, protected hydroxy, or C_{1-5} alkyl

and wherein any of the CH-groups at positions 20, 22, or 23 in the side chain may be replaced by a nitrogen atom, or where any of the groups -$CH(CH_3)$-, -$(CH_2)_m$-, -(CR_1R_2)- or –$(CH_2)_n$- at positions 20, 22, and 23, respectively, may be replaced by an oxygen or sulfur atom.

25. The method of claim 16 wherein the vitamin D compound is 2-methylene-19-nor-20(S)-1α,25-dihydroxyvitamin D_3.

26. The method of claim 16 wherein the vitamin D compound is 1α,25-dihydroxyvitamin D_3.

27. The method of claim 16 wherein the vitamin D compound is a vitamin D mimetic selected from any group of compounds that bind to the VDR and activate its transcriptional capability.

28. The method of claim 16 wherein the bone calcium resorption inhibitor is selected from the group consisting of:

Estrogens,

Androgens,

Cytokines that inhibit bone resorption,

Thiazolidinedione class of activators of peroxisome proliferator activated receptor (PPAR)gamma,

Calcitonins,

Bisphosphonates,

Receptor activator of NFkB (RANK) extracellular domain preparations,

RANK mimetics,

Soluble RANK-chimeric proteins (RANK-Fc),

Osteoprotegerin (OPG),

OPG chimeric proteins (OPG-Fc),

OPG mimetics,

TNF receptor associated factor 6 (Traf6) decoy peptides,

Chimeric membrane-permeable Traf6 decoy,

Traf6 decoy peptide mimetics,

Inhibitors of src,

Inhibitors of the extracellular receptor kinase (ERKs), c-Jun N-terminal kinase (JNKs), or stress-activated protein kinase (SAPKs),

Peptide/small molecule inhibitors of activator protein-I (AP-1),

Peptide/small molecule inhibitors of c-Fos,

Peptide/small molecule inhibitors of nuclear factor kappa B (NFkB),

Peptide/small molecule inhibitors of inhibitor kinase (IK)beta,

Peptide/small molecule inhibitors of the inhibitory kinase (Ikα, Ikβ, IKKs),

Small molecule antagonists of membrane bound RANK,

Small molecule inhibitors of RANK ligand trimerization or activation,

RGD-containing inhibitors of osteoclast-expressed integrins,

Small molecule mimetics of integrin inhibitors,

Cathespin K inhibitors,

Tartrate resistant acid phosphatase inhibitors, and

Vacuolar ATPase inhibitors.

29. The method of claim 16 wherein the bone calcium resorption inhibitor is alendronate.

30. A method of treating a disease selected from the group consisting of leukemia, colon cancer, breast cancer or prostate cancer comprising administering to a patient with said disease in an appropriate dosage schedule an effective amount of a bone calcium resorption inhibitor and an effective amount of a vitamin D compound.

31. The method of claim 30 wherein the bone calcium resorption inhibitor is administered before the vitamin D compound.

32. The method of claim 30 wherein the bone calcium resorption inhibitor is administered substantially simultaneously with the vitamin D compound.

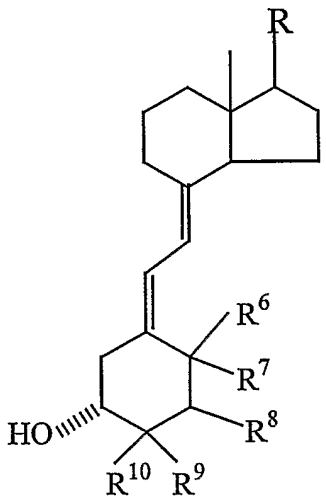
33. The method of claim 30 wherein either or both of the bone calcium resorption inhibitor and vitamin D compound is administered orally.

34. The method of claim 30 wherein either or both of the bone calcium resorption inhibitor and vitamin D compound is administered parenterally.

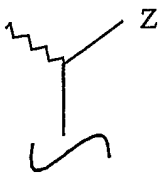
35. The method of claim 30 wherein either or both of the bone calcium resorption inhibitor and vitamin D compound is administered transdermally.

36. The method of claim 30 wherein the vitamin D compound is administered in a dosage of from about 0.1 μg/day to about 100 μg/day and the bone calcium resorption inhibitor is administered in a dosage of from about 7 mg/day to 700 mg/day.

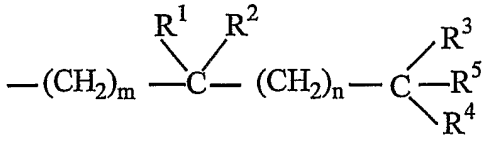
37. The method of claim 30 wherein the vitamin D compound is selected from a compound having the formula



where R^6 and R^7 each represent hydrogen or taken together R^6 and R^7 represent a methylene group, R^8 represents hydrogen, hydroxy or a protected hydroxy, R^9 and R^{10} may each independently represent hydrogen, alkyl, hydroxyalkyl, or fluoroalkyl, or R^9 and R^{10} taken together may represent the group $-(CH_2)_x-$ where x is an integer from 2 to 5, the group –OY or $=R^{11}R^{12}$ where R^{11} and R^{12}, which may be the same are different, are each selected from hydrogen, alkyl, hydroxyalkyl and fluoroalkyl, or when taken together R^{11} and R^{12} represent the group $-(CH_2)x-$ where x is an integer from 2 to 5 and the group R is represented by the structure



where the stereochemical center at carbon 20 may have the R or S configuration, and where Z is selected from Y, -OY, -CH_2OY, -C≡CY and -CH=CHY, where the double bond may have the cis or trans geometry, and where Y is selected from hydrogen, methyl, -COR^5 and a radical of the structure:



where m and n, independently, represent the integers from 0 to 5, where R^1 is selected from hydrogen, deuterium, hydroxy, protected hydroxy, fluoro, trifluoromethyl, and C_{1-5}-alkyl, which may be straight chain or branched and, optionally, bear a hydroxy or protected-hydroxy substituent, and where each of R^2, R^3, and R^4, independently, is selected from deuterium, deuteroalkyl, hydrogen, fluoro, trifluoromethyl and C_{1-5} alkyl, which may be straight-chain or branched, and optionally, bear a hydroxy or protected-hydroxy substituent, and where R^1 and R^2, taken together, represent an oxo group, or an alkylidene group, $=CR^2R^3$, or the group $-(CH_2)_p-$, where p is an integer from 2 to 5, and where R^3 and R^4, taken together, represent an oxo group, or the group $-(CH_2)_q-$, where q is an integer from 2 to 5, and where R^5 represents hydrogen, hydroxy, protected hydroxy, or C_{1-5} alkyl and wherein any of the CH-groups at positions 20, 22, or 23 in the side chain may be replaced by a nitrogen atom, or where any of the groups $-CH(CH_3)-$, $-(CH_2)_m-$, $-(CR_1R_2)-$ or $-(CH_2)_n-$ at positions 20, 22, and 23, respectively, may be replaced by an oxygen or sulfur atom.

38. The method of claim 30 wherein the vitamin D compound is 2-methylene-19-nor-20(S)-1α,25-dihydroxyvitamin D_3.

39. The method of claim 30 wherein the vitamin D compound is 1α,25-dihydroxyvitamin D_3.

40. The method of claim 30 wherein the vitamin D compound is a vitamin D mimetic selected from any group of compounds that bind to the VDR and activate its transcriptional capability.

41. The method of claim 30 wherein the bone calcium resorption inhibitor is selected from the group consisting of:

Estrogens,

Androgens,

Cytokines that inhibit bone resorption,

Thiazolidinedione class of activators of peroxisome proliferator activated receptor (PPAR)gamma,

Calcitonins,

Bisphosphonates,

Receptor activator of NFkB (RANK) extracellular domain preparations,

RANK mimetics,

Soluble RANK-chimeric proteins (RANK-Fc),

Osteoprotegerin (OPG),

OPG chimeric proteins (OPG-Fc),

OPG mimetics,

TNF receptor associated factor 6 (Traf6) decoy peptides,

Chimeric membrane-permeable Traf6 decoy peptides,

Traf6 decoy peptide mimetics,

Inhibitors of src,

Inhibitors of the extracellular receptor kinase (ERKs), c-Jun N-terminal kinase (JNKs), or stress-activated protein kinase (SAPKs),

Peptide/small molecule inhibitors of activator protein-I (AP-1),

Peptide/small molecule inhibitors of c-Fos,

Peptide/small molecule inhibitors of nuclear factor kappa B (NFkB),

Peptide/small molecule inhibitors of inhibitor kinase (IK)beta,

Peptide/small molecule inhibitors of the inhibitory kinase (Ikα, Ikβ, IKKs),

Small molecule antagonists of membrane bound RANK,

Small molecule inhibitors of RANK ligand trimerization or activation,

RGD-containing inhibitors of osteoclast-expressed integrins,

Small molecule mimetics of integrin inhibitors,

Cathespin K inhibitors,

Tartrate resistant acid phosphatase inhibitors, and

Vacuolar ATPase inhibitors.

42. The method of claim 30 wherein the bone calcium resorption inhibitor is alendronate.

43. A method of treating an autoimmune disease selected from the group consisting of multiple sclerosis, lupis, inflammatory bowel disease, Type I diabetes, host versus graft reaction, and rejection of organ transplants, comprising administering to a patient with said disease in an appropriate dosage schedule an effective amount of a bone calcium resorption inhibitor and an effective amount of a vitamin D compound.

44. The method of claim 43 wherein the bone calcium resorption inhibitor is administered before the vitamin D compound.

45. The method of claim 43 wherein the bone calcium resorption inhibitor is administered substantially simultaneously with the vitamin D compound.

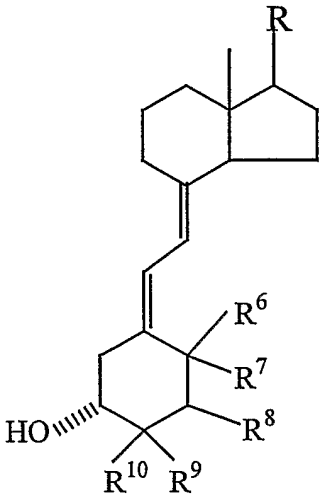
46. The method of claim 43 wherein either or both of the bone calcium resorption inhibitor and vitamin D compound is administered orally.

47. The method of claim 43 wherein either or both of the bone calcium resorption inhibitor and vitamin D compound is administered parenterally.

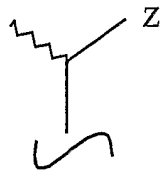
48. The method of claim 43 wherein either or both of the bone calcium resorption inhibitor and vitamin D compound is administered transdermally.

49. The method of claim 43 wherein the vitamin D compound is administered in a dosage of from about 0.1 μg/day to about 100 μg/day and the bone calcium resorption inhibitor is administered in a dosage of from about 7 mg/day to 700 mg/day.

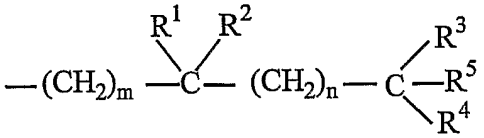
50. The method of claim 43 wherein the vitamin D compound is selected from a compound having the formula



where R^6 and R^7 each represent hydrogen or taken together R^6 and R^7 represent a methylene group, R^8 represents hydrogen, hydroxy or a protected hydroxy, R^9 and R^{10} may each independently represent hydrogen, alkyl, hydroxyalkyl, or fluoroalkyl, or R^9 and R^{10} taken together may represent the group $-(CH_2)_x-$ where x is an integer from 2 to 5, the group –OY or $=R^{11}R^{12}$ where R^{11} and R^{12}, which may be the same are different, are each selected from hydrogen, alkyl, hydroxyalkyl and fluoroalkyl, or when taken together R^{11} and R^{12} represent the group $-(CH_2)x-$ where x is an integer from 2 to 5 and the group R is represented by the structure



where the stereochemical center at carbon 20 may have the R or S configuration, and where Z is selected from Y, -OY, -CH_2OY, -C≡CY and -CH=CHY, where the double bond may have the cis or trans geometry, and where Y is selected from hydrogen, methyl, -COR^5 and a radical of the structure:



where m and n, independently, represent the integers from 0 to 5, where R^1 is selected from hydrogen, deuterium, hydroxy, protected hydroxy, fluoro, trifluoromethyl, and C_{1-5}-alkyl, which may be straight chain or branched and, optionally, bear a hydroxy or protected-hydroxy substituent, and where each of R^2, R^3, and R^4, independently, is selected from deuterium, deuteroalkyl, hydrogen, fluoro, trifluoromethyl and C_{1-5} alkyl, which may be straight-chain or branched, and optionally, bear a hydroxy or protected-hydroxy substituent, and where R^1 and R^2, taken together, represent an oxo group, or an alkylidene group, $=CR^2R^3$, or the group -$(CH_2)_p$-, where p is an integer from 2 to 5, and where R^3 and R^4, taken together, represent an oxo group, or the group -$(CH_2)_q$-, where q is an integer from 2 to 5, and where R^5 represents hydrogen, hydroxy, protected hydroxy, or C_{1-5} alkyl and wherein any of the CH-groups at positions 20, 22, or 23 in the side chain may be replaced by a nitrogen atom, or where any of the groups -$CH(CH_3)$-, -$(CH_2)_m$-, -(CR_1R_2)- or –$(CH_2)_n$- at positions 20, 22, and 23, respectively, may be replaced by an oxygen or sulfur atom.

51. The method of claim 43 wherein the vitamin D compound is 2-methylene-19-snor-20(S)-1α,25-dihydroxyvitamin D_3.

52. The method of claim 43 wherein the vitamin D compound is 1α,25-dihydroxyvitamin D_3.

53. The method of claim 43 wherein the vitamin D compound is a vitamin D mimetic selected from any group of compounds that bind to the VDR and activate its transcriptional capability.

54. The method of claim 43 wherein the bone calcium resorption inhibitor is selected from the group consisting of:

Estrogens,

Androgens,

Cytokines that inhibit bone resorption,

Thiazolidinedione class of activators of peroxisome proliferator activated receptor (PPAR)gamma,

Calcitonins,

Bisphosphonates,

Receptor activator of NFkB (RANK) extracellular domain preparations,

RANK mimetics,

Soluble RANK-chimeric proteins (RANK-Fc),

Osteoprotegerin (OPG),

OPG chimeric proteins (OPG-Fc),

OPG mimetics,

TNF receptor associated factor 6 (Traf6) decoy peptides,

Chimeric membrane-permeable Traf6 decoy peptides,

Traf6 decoy peptide mimetics,

Inhibitors of src,

Inhibitors of the extracellular receptor kinase (ERKs), c-Jun N-terminal kinase (JNKs), or stress-activated protein kinase (SAPKs),

Peptide/small molecule inhibitors of activator protein-I (AP-1),

Peptide/small molecule inhibitors of c-Fos,

Peptide/small molecule inhibitors of nuclear factor kappa B (NFkB),

Peptide/small molecule inhibitors of inhibitor kinase (IK)beta,

Peptide/small molecule inhibitors of the inhibitory kinase (Ikα, Ikβ, IKKs),

Small molecule antagonists of membrane bound RANK,

Small molecule inhibitors of RANK ligand trimerization or activation,

RGD-containing inhibitors of osteoclast-expressed integrins,

Small molecule mimetics of integrin inhibitors,

Cathespin K inhibitors,

Tartrate resistant acid phosphatase inhibitors, and

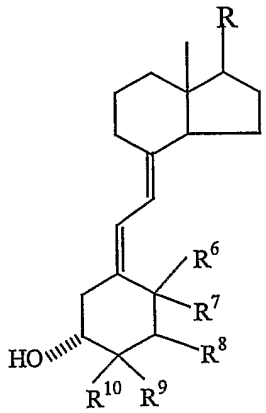
Vacuolar ATPase inhibitors.

55. The method of claim 43 wherein the bone calcium resorption inhibitor is alendronate.

56. A pharmaceutical composition containing a vitamin D compound and a bone calcium resorption inhibitor together with a pharmaceutically acceptable excipient.

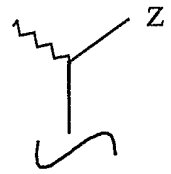
57. The pharmaceutical composition of claim 56 containing from about 0.1 μg to about 100 μg/gram of composition of the vitamin D compound.

58. The pharmaceutical composition of claim 56 wherein the vitamin D compound is selected from a compound having the formula

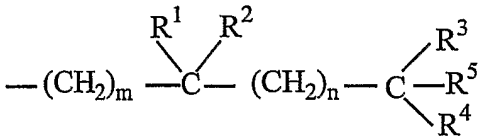


where R^6 and R^7 each represent hydrogen or taken together R^6 and R^7 represent a methylene group, R^8 represents hydrogen, hydroxy or a protected hydroxy, R^9 and R^{10} may each independently represent hydrogen, alkyl, hydroxyalkyl, or

fluoroalkyl, or R^9 and R^{10} taken together may represent the group $-(CH_2)_x-$ where x is an integer from 2 to 5, the group –OY or $=R^{11}R^{12}$ where R^{11} and R^{12}, which may be the same are different, are each selected from hydrogen, alkyl, hydroxyalkyl and fluoroalkyl, or when taken together R^{11} and R^{12} represent the group $-(CH_2)x-$ where x is an integer from 2 to 5 and the group R is represented by the structure



where the stereochemical center at carbon 20 may have the R or S configuration, and where Z is selected from Y, -OY, $-CH_2OY$, -C≡CY and -CH=CHY, where the double bond may have the cis or trans geometry, and where Y is selected from hydrogen, methyl, $-COR^5$ and a radical of the structure:



where m and n, independently, represent the integers from 0 to 5, where R^1 is selected from hydrogen, deuterium, hydroxy, protected hydroxy, fluoro, trifluoromethyl, and C_{1-5}-alkyl, which may be straight chain or branched and, optionally, bear a hydroxy or protected-hydroxy substituent, and where each of R^2, R^3, and R^4, independently, is selected from deuterium, deuteroalkyl, hydrogen, fluoro, trifluoromethyl and C_{1-5} alkyl, which may be straight-chain or branched, and optionally, bear a hydroxy or protected-hydroxy substituent, and where R^1 and R^2, taken together, represent an oxo group, or an alkylidene group, $=CR^2R^3$, or the group $-(CH_2)_p-$, where p is an integer from 2 to 5, and where R^3 and R^4, taken together, represent an oxo group, or the group $-(CH_2)_q-$, where q is an integer from 2 to 5, and where R^5 represents hydrogen, hydroxy, protected hydroxy, or C_{1-5}

alkyl and wherein any of the CH-groups at positions 20, 22, or 23 in the side chain may be replaced by a nitrogen atom, or where any of the groups -$CH(CH_3)$-, -$(CH_2)_m$-, -(CR_1R_2)- or –$(CH_2)_n$- at positions 20, 22, and 23, respectively, may be replaced by an oxygen or sulfur atom.

59. The pharmaceutical composition of claim 56 wherein the vitamin D compound is 2-methylene-19-nor-20(S)-1α,25-dihydroxyvitamin D_3.

60. The pharmaceutical composition of claim 56 wherein the vitamin D compound is 1α,25-dihydroxyvitamin D_3.

61. The pharmaceutical composition of claim 56 wherein the vitamin D compound is a vitamin D mimetic selected from any group of compounds that bind to the VDR and activate its transcriptional capability.

62. The pharmaceutical composition of claim 56 containing from about 7 mg to about 700 mg/gram of composition of the bone calcium resorption inhibitor.

63. The pharmaceutical composition of claim 56 wherein the bone calcium resorption inhibitor is selected from the group consisting of

Estrogens,

Androgens,

Cytokines that inhibit bone resorption,

Thiazolidinedione class of activators of peroxisome proliferator activated receptor (PPAR)gamma,

Calcitonins,

Bisphosphonates,

Receptor activator of NFkB (RANK) extracellular domain preparations,

RANK mimetics,

Soluble RANK-chimeric proteins (RANK-Fc),

Osteoprotegerin (OPG),

OPG chimeric proteins (OPG-Fc),

OPG mimetics,

TNF receptor associated factor 6 (Traf6) decoy peptides,

Chimeric membrane-permeable Traf6 decoy peptides,

Traf6 decoy peptide mimetics,

Inhibitors of src,

Inhibitors of the extracellular receptor kinase (ERKs), c-Jun N-terminal kinase (JNKs), or stress-activated protein kinase (SAPKs),

Peptide/small molecule inhibitors of activator protein-I (AP-1),

Peptide/small molecule inhibitors of c-Fos,

Peptide/small molecule inhibitors of nuclear factor kappa B (NFkB),

Peptide/small molecule inhibitors of inhibitor kinase (IK)beta,

Peptide/small molecule inhibitors of the inhibitory kinase (Ikα, Ikβ, IKKs),

Small molecule antagonists of membrane bound RANK,

Small molecule inhibitors of RANK ligand trimerization or activation,

RGD-containing inhibitors of osteoclast-expressed integrins,

Small molecule mimetics of integrin inhibitors,

Cathespin K inhibitors,

Tartrate resistant acid phosphatase inhibitors, and

Vacuolar ATPase inhibitors.

64. The pharmaceutical composition of claim 56 wherein the bone calcium resorption inhibitor is alendronate.

65. The pharmaceutical composition of claim 56 formulated for oral administration.

66. The pharmaceutical composition of claim 56 formulated for parenteral administration.

67. The pharmaceutical composition of claim 56 formulated for transdermal administration.

68. The pharmaceutical composition of claim 56 formulated for topical administration.

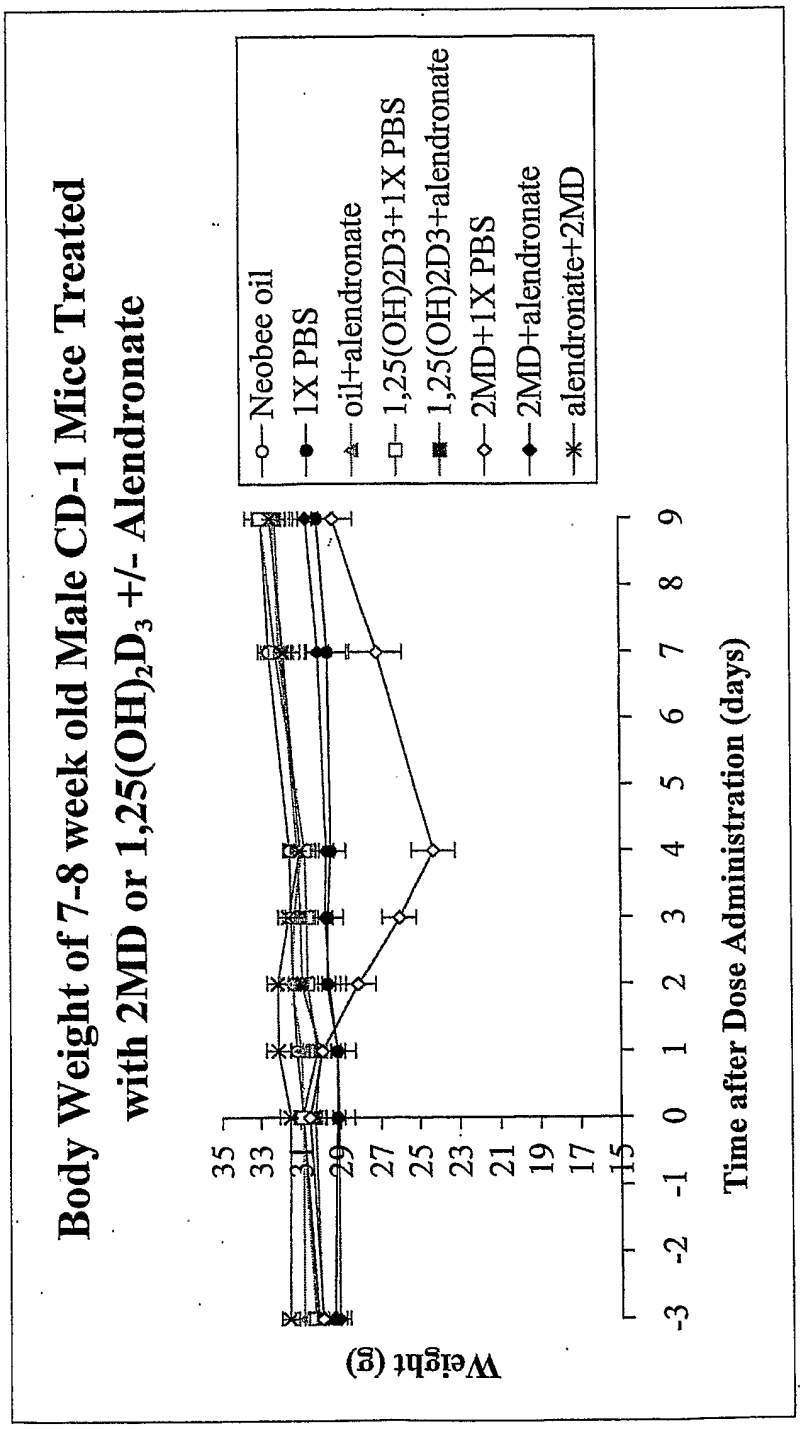

Body Weight of 7-8 week old Male CD-1 Mice Treated with 2MD or 1,25(OH)2D3 +/- Alendronate
Neobee oil
1X PBS
oil+alendronate
1,25(OH)2D3+1X PBS
1,25(OH)2D3+alendronate
2MD+1X PBS
2MD+alendronate
alendronate+2MD
Weight (g)
35
33
31
29
27
25
23
21
19
17
15
-3 -2 -1 0 1 2 3 4 5 6 7 8 9
Time after Dose Administration (days)

FIGURE 1

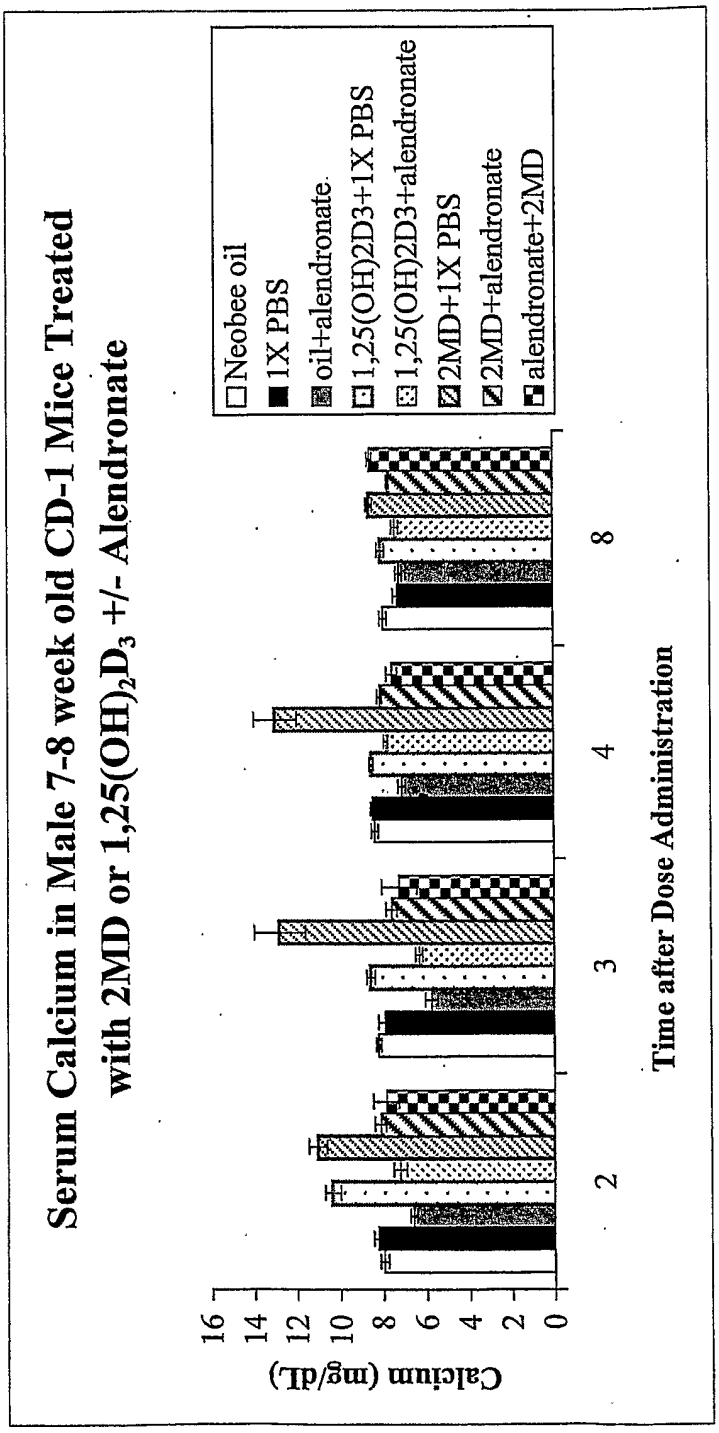

Serum Calcium in Male 7-8 week old CD-1 Mice Treated with 2MD or 1,25(OH)2D3 +/- Alendronate
Neobee oil
1X PBS
oil+alendronate
1,25(OH)2D3+1X PBS
1,25(OH)2D3+alendronate
2MD+1X PBS
2MD+alendronate
alendronate+2MD
Calcium (mg/dL)
0
2
4
6
8
10
12
14
16
2
3
4
8
Time after Dose Administration

FIGURE 2

INTERNATIONAL SEARCH REPORT

Inte ›nal Application No
PCT/US 03/20517

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/59

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal, PAJ, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01 49295 A (UNIV CALIFORNIA) 12 July 2001 (2001-07-12) page 5, line 8-28; figure 1 ---	1-68
X	DATABASE WPI Derwent Publications Ltd., London, GB; AN 1988-108789 XP002256099 YAMAOTO: "Method for preventing adsorption of drug" & JP 63 057527 A (TOYO JOZO KK), 12 March 1988 (1988-03-12) See whole document --- -/--	1-68

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 September 2003	14/10/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Cattell, James

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int onal Application No
PCT/US 03/20517

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	PRICE ET AL: "Osteoprotegerin Inhibits Artery Calcification Induced by Warfarin and by Vitamin D. " ARTERIOSCLER, THROMB. VASC. BIOL., vol. 21, 2001, page 1610-1616 XP002256098 see "Abstract	1-68

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 03/20517

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: -
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1 and 13 relate to a compound defined by reference to a desirable characteristic or property, namely "calcium resorption inhibitor" or (for example in claim 13), "inhibitors of extracellular receptor kine" or Traf6 decoy peptides" or "peptide/small molecule inhibitors of activator protein -I".

The claims cover all compounds having this characteristic or property. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

The problem addressed by the application is the reduction in vitamin D toxicity.

From the cited prior art it is apparent that calcium resorption inhibitors can reduce such toxicity.

Hence each of the proposed solutions to the problem in claim 13, i.e. each of the calcium resorption inhibitors, represents a different solution to the problem. There is no novel link between the greatly differing compounds under Rule 13,1 PCT.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-12, 14-27, 29 to 41, 43 to 54, 55 to 62, 65 to 68 with partially claims 13, 28, 42, 54, 63.

INTERNATIONAL SEARCH REPORT

information on patent family members

Int onal Application No
PCT/US 03/20517

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0149295 A	12-07-2001	AU 2756701 A	16-07-2001
		CA 2396393 A1	12-07-2001
		EP 1267888 A1	02-01-2003
		JP 2003519183 T	17-06-2003
		WO 0149295 A1	12-07-2001
		US 2003027211 A1	06-02-2003
JP 63057527 A	12-03-1988	NONE	