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(54) **Title:** METHOD OF TREATING AND PREVENTING HYPERPARATHYROIDISM WITH VITAMIN D COMPOUNDS

(57) **Abstract:** This invention relates to a method for treating or preventing hyperthyroidism associated with chronic kidney disease by administering a sufficient amount of a vitamin D compound utilizing a variety of effective treatment protocols, the kidney disease being stages 1-4.

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METHOD OF TREATING AND PREVENTING HYPERPARATHYROIDISM WITH VITAMIN D COMPOUNDS

CROSS-REFERENCE TO RELATED APPLICATIONS

This International application is a continuation-in-part of U.S. Patent Application Serial No. 10/385,327, filed March 10, 2003.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Not Applicable

This invention relates to a method for treating or preventing hyperparathyroidism associated with chronic kidney disease by administration of an active vitamin D compound utilizing effective treatment protocols.

Historically, it has long been known that vitamin D plays a critical role in regulating calcium metabolism. The discovery of the active forms of vitamin D in the 1970's, [Holick, M. F. *et al.*, *Proc. Natl. Acad. Sci. USA* 68, 803-804 (1971); Jones, G. *et al.*, *Biochemistry* 14, 1250-1256 (1975)], and active vitamin D analogues, [Holick, M. F. *et al.*, *Science* 180, 190, 191 (1973); Lam, H. Y. *et al.*, *Science* 186, 1038-1040 (1974)], caused much excitement and speculation about the usefulness of these compounds in the treatment of bone depletive disorders.

Animal and early clinical studies examining the effects of these active vitamin D compounds suggested that such agents would be useful in restoring calcium balance. However, the best indicator of the efficacy of vitamin D compounds to prevent or treat depletive bone disorders is bone itself (or, in the case of renal osteodystrophy, serum levels of parathyroid hormone (PTH)) rather than calcium absorption or calcium balance. Certain clinical studies with 1α,25-dihydroxyvitamin D_3 (also known as calcitriol), and 1α-hydroxyvitamin D_3 indicate that the ability of these agents to restore lost bone mass or bone mineral content is dose-related. [See, Ott, S. M. and Chesnut, C. H. , *Annals of Int. Med.*; 110:267-274 (1989); Gallagher, J. C. *et al.*, *Annals of Int. Med.*; 113:649-655 (1990); Aloia, J. *et al.*, *Amer. J. Med.* 84:401-08 (1988); and Shiraki, M. *et al.*, *Endocrinol. Japan* 32, 305-315 (1985)].

These clinical studies also indicate that at the dosage ranges required for these agents to be truly effective, toxicity in the form of hypercalcemia and hypercalciuria becomes a major problem. Attempts to increase the amount of 1α,25-dihydroxyvitamin D_3 above 0.5 μg/day have frequently resulted in toxicity. At dosage levels below 0.5 μg/day, clinically significant effects on bone are rarely observed. [See, Jensen, G. F. *et al.*, *Clin. Endocrinol.* 16, 515-524 (1982); Christiansen, C. *et al.*, *Eur. J. Clin.* Invest. 11, 305-309 (1981)]. Doses of 2 μg/day of 1α-hydroxyvitamin D_3 were found to have efficacy in increasing bone mass in patients exhibiting senile osteoporosis. [Sorensen, O. H. *et al.*, *Clin. Endocrinol.* 7, 169S-175S (1977)]. Data from clinical studies in Japan, a population that has low calcium intake, indicate that efficacy is found with 1α-hydroxyvitamin D_3 when administered at 1 μg/day. [Shiraki, M. *et al.*, *Endocrinol. Japan.* 32:305-315 (1985); Orimo, H. *et al.*, *Bone and Mineral* 3, 47-52 (1987)]. However, at 2 μg/day, toxicity with 1α-hydroxyvitamin D_3 occurs in approximately 67% of the patients, and at 1 μg/day this percentage is approximately 20%.

Thus, due to their toxicity, 1-hydroxylated vitamin D_3 compounds can only be administered at dosages that are, at best, modestly beneficial in preventing or treating loss of bone or bone mineral content. Indeed, Aloia *et al.*, recommend that alternative routes of administration be sought that might avoid the toxicity problems and allow higher dosage levels to be achieved. [Aloia, J. *et al.*, *Amer. J. Med.* 84:401-408 (1988)].

Despite reported toxicities of 1α-hydroxyvitamin D_3 and 1α,25-dihydroxyvitamin D_3, these two compounds remain the drugs of choice for treatment of many bone depletive diseases. Both 1α-hydroxyvitamin D_3 and 1α,25-dihydroxyvitamin D_3 have been studied and are clinically used in certain countries in Asia and Europe to treat osteoporosis. [Gillespie, W.J., *et al.*, Abstract, *The Cochrane Library*, issue 2, 2001; DeChant, K.L. and Goa, K.L., *Drugs & Aging*, 5(4):300-317 (1994); Ikeda, K and Ogata, E., *Mechanisms of Aging & Development* 116:103-111 (2000); Tanizawa, T., *Osteoporos. Int.* 9:163-170 (1999); Civitelli, R., *Calcif. Tissue* 57:409-414 (1995); Parfitt, A.M., *Drugs* 36:513-520 (1988); Thompson, S.P. *et al.*,

Brit. Edit. Soc. Bone Joint Surgery, 72:1053-1056 (1990); Sairanen, S. *et al.*, *Calcif. Tissue Int.* 67:122-127 (2000); Haas, H.G., *Horm. Metab. Res.* 11:168-171 (1979); Tilyard, M.W. *et al.*, *New England J. Med.* 326:357-362 (1992); Aloia, J.F. *et al.*, *Am. J. Med.* 84:401-408 (1988); Avioli, L., *Calcif. Tissue Int.* 65:2392-294 (1999); Orimi, H. *et al.*, *Calcif. Tissue Int.* 54:370-376 (1994); Sorensen, O.H. *et al.*, *Clinical Endocrinol.* 7 (Suppl.): 169S-175S (1997)]. Some studies suggest that active vitamin D, such as 1α-hydroxyvitamin D_3 and 1α,25-dihydroxyvitamin D_3, appears to be more effective than precursors, e.g., vitamin D, in treating, e.g., osteoporosis. These drugs appear to be most effective in those patients that have defective calcium absorption, e.g., in osteoporosis. Active vitamin D also appears to be more effective in treating 1α,25-dihydroxyvitamin D_3 resistance in target organs, decline in responsiveness to PTH inducement of 1α,25-dihydroxyvitamin D_3 synthesis, and lower production of 1α,25-dihydroxyvitamin D_3 especially with aging. [Zerwekh, J.E. *et al.*, *J. Clin. Endocrinol. Metab.* 56:410-413 (1983); Nordin, B.E.C. *et al.*, *Calcif. Tissue Int.* 65:307-310 (1999); Morris, H.A. *et al.*, *Calcif. Tissue Int.* 49:240-243 (1991); Shiraishi, A. *et al.*, *Calcif. Tissue Int.*65:311-316 (1999); Silverberg, S.J. *et al.*, *New England J. Med* . 320(5):277-281 (1989); Francis, R.M., *Calcif. Tissue Int.* 60:111-114 (1997); Francis, R.M. *et al.*, *Osteoporosis Int.* 6:284-290 (1996); Theiler, R. *et al.*, *Int. J. Vit. Nur. Res.* 68:36-41 (1998)].

Both of these drugs, 1α-hydroxyvitamin D_3 and 1α,25-dihydroxyvitamin D_3, are approved for the treating and preventing of secondary hyperparathyroidism in end-stage renal disease, although both drugs are not currently approved in all major pharmaceutical markets.

The disease of hyperparathyroidism is a generalized disorder resulting from excessive secretion of PTH by one or more parathyroid glands. The disease is characterized by elevated blood PTH levels and parathyroid glandular enlargement.

Hyperparathyroidism is subcategorized into primary, secondary and tertiary hyperparathyroidism. In primary hyperparathyroidism, the growth of the parathyroid glands is autonomous in nature, is usually due to tumors, e.g., parathyroid adenomas, and is presumably irreversible. Such adenomas typically do not exhibit vitamin D

receptors and exhibit a resistance to natural hormone form of vitamin D, i.e., 1,25-dihydroxyvitamin D_3. In secondary hyperparathyroidism, associated with, e.g., 1,25-dihydroxyvitamin D_3 deficiency and/or resistance, parathyroid gland hyperplasia is typically adaptive owing to resistance to the metabolic actions of the hormone, and is presumably reversible. Secondary hyperparathyroidism occurs in patients with, e.g., kidney disease, osteomalacia, and intestinal malabsorption syndrome. Tertiary hyperparathyroidism is characterized by an autonomous proliferation state of the parathyroid glands with biological hyperfunction. Tertiary hyperparathyroidism can occur in patients with secondary hyperparathyroidism, wherein the reversible hyperplasia associated with secondary hyperparathyroidism converts to an irreversible growth defect, the enlarged tissue having vitamin D receptors. In all forms of hyperparathyroidism, bone abnormalities, e.g., the loss of bone mass or decreased mineral content, are common and kidney damage is possible. Hyperparathyroidism is thus also characterized by abnormal calcium, phosphorus and bone metabolism.

Secondary (and tertiary) hyperparathyroidism is a significant clinical problem associated with chronic kidney disease. Chronic kidney disease is a worldwide public health problem. In the United States, it is estimated that 11% of the adult population has varying stages of chronic kidney disease, with about 4% of U.S. adults having less than half of the normal kidney function of a young adult. Further, the prevalence of end-stage renal disease (i.e., kidney failure) has more than doubled during the past decade. At present, end-stage renal disease afflicts an estimated 300,000 individuals, and that number is predicted to reach more than 600,000 individuals by 2010.

Chronic kidney disease is defined as either kidney damage or glomerular filtration rate (GFR) of less than 90 mL/min/1.73 m^2 for more than three months. The level of GFR is widely accepted as the best overall measure of kidney function in health and disease. Chronic kidney disease is now classified in stages based on estimated kidney function as measured by GFR. Stage 1 is defined as normal kidney function with some kidney damage and a GFR of $\geq$ 90 mL/min/1.73 m^2; stage 2 involves mildly decreased kidney function with a mild decrease in GFR, i.e., a GFR of 60-89 mL/min/1.73 m^2. Stage 3 is defined as moderately decreased kidney function with a GFR of 30-59 mL/min/1.73 m^2. Stage 4 is defined as severely decreased kidney

function with a GFR of 15-29 mL/min/1.73 m^2. Stage 5 is kidney failure with a GFR of <15 mL/min/1.73 m^2 or dialysis. Stage 5 is also known as end-stage renal disease (ESRD).

As noted above, secondary hyperparathyroidism is a common finding in patients with chronic kidney disease. It is established that the reduction of renal 1,25-dihydroxyvitamin D_3 synthesis is one of the principal mechanisms leading to the secondary hyperparathyroidism in these patients and it has been shown that 1,25-dihydroxyvitamin D_3 possesses direct suppressive action on PTH synthesis. Therefore, administration of 1,25- dihydroxyvitamin D_3 has been recommended for the treatment of secondary hyperparathyroidism in these patients. However, as described below, 1,25- dihydroxyvitamin D_3 has potent hypercalcemic effects giving it a narrow therapeutic window which limits its usage, especially at high doses.

In chronic kidney disease, there is a progressive loss of cells of the proximal nephrons, the primary site for the synthesis of the vitamin D hormones (collectively "1α,25-dihydroxyvitamin D") from 25-hydroxyvitamin D_3 and 25-hydroxyvitamin D_2. In addition, the loss of functioning nephrons leads to retention of excess phosphorus which reduces the activity of the renal 25-hydroxyvitamin D-1α-hydroxylase, the enzyme which catalyzes the reaction to produce the vitamin D hormones. These two events account for the low serum levels of 1α,25-dihydroxyvitamin D commonly found in patients with moderate to severe chronic kidney disease.

Reduced serum levels of 1α,25-dihydroxyvitamin D cause increased, and ultimately excessive, secretion of PTH by direct and indirect mechanisms. The resulting hyperparathyroidism leads to markedly increased bone turnover and its sequela of renal osteodystrophy, which may include a variety of other diseases, such as osteitis fibrosa cystica, osteomalacia, osteoporosis, extraskeletal calcification and related disorders, e.g., bone pain, periarticular inflammation and Mockerberg's sclerosis. Reduced serum levels of 1α,25-dihydroxyvitamin D also can cause muscle weakness and growth retardation with skeletal deformities (most often seen in pediatric patients).

Previous clinical studies utilizing hormonally active vitamin D drugs in end stage renal disease patients, i.e., for the treatment of secondary hyperthyroidism, have focused on compounds derived from vitamin D_3. 1,25-dihydroxyvitamin D_3 and 1α-hydroxyvitamin D_3 (α-calcidiol) are the major approved forms of 1α-hydroxylated vitamin D, although, as noted above, these drugs are not currently approved in all major pharmaceutical markets. Use of 1α,25-dihydroxyvitamin D_3 and 1α-hydroxyvitamin D_3 as replacement therapy seeks to treat or prevent renal osteodystrophy by treating or preventing hyperparathyroidism in end stage renal disease patients. As noted above, 1α,25-dihydroxyvitamin D_3 often causes toxic side effects (hypercalcemia and hyperphosphatemia) at dosages above 0.5 μg, especially when concomitantly administered phosphate binders, such as calcium compounds, are used to control serum phosphorus. The minimum effective dose for preventing hyperparathyroidism is in the range of 0.25 to 0.50 μg/day; most patients respond to oral treatment doses of 0.5 to 1.0 μg/day or intravenous doses between 0.5 and 3.0 μg three times per week. As described above, the other commonly used vitamin D drug is 1α-hydroxyvitamin D_3 which often causes toxic effects at dosages over 1.0 μg/day, especially when used with phosphate binders. The minimum effective dosage for preventing hyperparathyroidism is in the range of 0.25 to 1.0 μg/day, and most patients require treatment dosages of 1.0 μg/day or more. When either drug, 1α,25-dihydroxyvitamin D_3 or 1α-hydroxyvitamin D_3, is administered in higher dosages, both efficacy and toxicity are found to increase. Thus, the hormonally active vitamin D_3 compounds are limited in their therapeutic usefulness in treatment of hyperparathyroidism due to their inherent toxicities.

Attempts to reduce the toxic side effects of active vitamin D_3, in the renal failure setting, have included administration of a low calcium dialysate with an ionized calcium concentration of 1.25 mM. However, it has been found that use of the low calcium dialysate has lead to higher serum PTH and phosphorus levels in patients who do not receive increased doses of oral calcium supplements as phosphate binders. When the dosages of calcium-based phosphate binders are increased, serum levels of phosphorus can be controlled, but the incidence of hypercalcemia rises markedly.

Thus, there are many problems associated with the use of current vitamin D therapies for secondary hyperparathyroidism.

Notwithstanding these known problems with use of the hormonally active vitamin D_3 for hyperparathyroidism, there is a need for vitamin D compounds, derivatives or analogs, and treatment protocols that have low inherent toxicity.

In one aspect, the present invention provides a method of treating, i.e., ameliorating or preventing, hyperparathyroidism associated with chronic kidney disease (i.e., stages 1-4) by lowering elevated or maintaining lowered blood PTH levels in a patient suffering from the disease. The method includes administering to a subject in need thereof an amount of an active vitamin D compound sufficient to lower elevated or maintain lowered blood PTH levels, i.e., sufficient to suppress parathyroid activity.

Specifically, the present invention provides a method of lowering elevated or excessive PTH (i.e., a blood PTH level greater than the normal range of 15-65 pg/mL) or maintaining therapeutically lowered blood PTH in patients suffering from hyperparathyroidism associated with chronic kidney disease (i.e., stages 1-4), which includes administering to these patients an effective amount of a vitamin D analog of formula (I), as described below, to lower elevated or maintain lowered blood PTH level. It is believed that the analogs of formula (I) may be effective in prolonging or slowing the progression in renal patients to stage 5 chronic kidney disease, (i.e. end-stage renal disease). The analog of formula (I) is any active vitamin D compound which has potent biological activity but low calcemic activity relative to the active forms of vitamin D_3. Such compounds include suitably 1α-hydroxyvitamin D_2; 1α,24-dihydroxyvitamin D_2; 1α,24(S)-dihydroxyvitamin D_2; 1α-hydroxy-25-ene-vitamin D_2; 1α,24-dihydroxy-25-ene-vitamin D_2; 1α- hydroxyvitamin D_4; 1α,24-dihydroxyvitamin D_4 and 1α,24(R)-dihydroxyvitamin D_4. The analog of formula (I) is suitably administered in a dosage amount averaging about 0.5 μg/week to about 100 μg/week. As used herein, the term "vitamin D analog" is meant to refer to compounds having vitamin D hormonal bioactivity.

In another aspect, the invention features a pharmaceutical composition having serum (or plasma) PTH lowering activity, which includes, in unit dosage form, one or more of the following suitable vitamin D analogs: 1α-hydroxyvitamin D_2; 1α,24-dihydroxyvitamin D_2; 1α,24(S)-dihydroxyvitamin D_2; 1α-hydroxy-25-ene-vitamin D_2; 1α,24-dihydroxy-25-ene-vitamin D_2; 1α-hydroxyvitamin D_4; 1α,24-dihydroxyvitamin D_4; and 1α,24(R)-dihydroxyvitamin D_4, and a pharmaceutically acceptable excipient. More suitably, the composition includes 1α-hydroxyvitamin D_2; 1α,24-dihydroxyvitamin D_2 or its (S) epimer, 1α,24(S)-dihydroxyvitamin D_2; 1α-hydroxy-25-ene-vitamin D_2; or 1α,24-dihydroxy-25-ene-vitamin D_2, and a pharmaceutically acceptable excipient. The composition is of especial pharmaceutical value in lowering elevated or maintaining lowered serum (or blood) PTH levels in patients with hyperparathyroidism associated with chronic kidney disease.

The treatment method of the present invention is an alternative to conventional therapy with 1α,25-dihydroxyvitamin D_3 or 1α-hydroxyvitamin D_3; the method is characterized by providing an active vitamin D compound having equivalent bioactivity but much lower toxicity than these conventional therapies. This is true especially in the case where oral calcium-based phosphate binders are used concomitantly to control serum phosphorus. As such, the method addresses a long felt need in hyperparathyroidism therapy.

A fuller appreciation of the specific attributes of this invention will be gained upon an examination of the following detailed description of the invention, and appended claims.

The present invention relates to treating, ameliorating or preventing hyperparathyroidism associated with chronic kidney disease by administering an effective amount of an active vitamin D compound utilizing a variety of treatment protocols. An elevated blood PTH level, i.e., hyperparathyroidism, is typically associated with chronic kidney disease. Accordingly, the present invention will now be described in detail with respect to such endeavors; however, those skilled in the art will appreciate that such a description of the invention is meant to be exemplary only and should not be viewed as limitative on the full scope thereof.

More specifically, the present invention relates to therapeutic methods for lowering elevated, including excessively high, blood levels of PTH and/or maintaining lowered, e.g., therapeutically lowered, serum PTH levels associated with chronic kidney disease; particularly, stages 1-4. The method is of value in ameliorating or preventing hyperparathyroidism by administering an active vitamin D compound of formula (I), as described hereinbelow. The method in accordance with the present invention has significantly less resultant hypercalcemia and hyperphosphatemia, especially in patients who use oral calcium as a phosphate binder to control serum phosphorus levels. Furthermore, the active vitamin D compounds can be administered intermittently or episodically in a high dose regimen with high efficacy and reduced toxicity. These attributes are achieved through a novel method of treating patients suffering from hyperparathyroidism associated with chronic kidney disease.

In the following description of the method of the invention, process steps are carried out at room temperature and atmospheric pressure unless otherwise specified. It also is understood that any numerical range recited herein includes all values from the lower value to the upper value. For example, if a concentration range is stated as 1% to 50%, it is intended that values such as 2% to 40%, 10% to 30%, or 1% to 3%, etc., are expressly enumerated in this specification. For further example, if a unit dose of a pharmaceutical composition is stated to be from 0.5 μg to 100 μg, it is intended that values such as 1.0 μg, 2.0 μg, 10 μg and 30 μg are expressly recited. These are only examples of what is specifically intended, and all possible combinations of numerical values between the lowest value and the highest value enumerated are to be considered to be expressly stated in this application.

As used herein, the term "chronic kidney disease" refers to stage 1 through stage 5 of kidney disease as measured by reduced GFR and/or kidney damage. Also, as used herein, the term "hyperparathyroidism" refers to primary, secondary and/or tertiary hyperparathyroidism.

It has been found that when the analogs of formula (I), described hereinbelow, are administered to patients with elevated serum (or plasma, i.e., blood) PTH levels, PTH level is lowered with significantly less hypercalcemia and hyperphosphatemia

than is observed after the same amount of activated vitamin D_3 administered in previously known formulations and dosing regimens. Thus, the compounds of formula (I) have an improved therapeutic index relative to active vitamin D_3 analogs administered using conventional protocols.

It has been shown that 1α-hydroxyvitamin D_2, an analog of formula (I), has the same biopotency as 1α-hydroxyvitamin D_3 and 1α,25-dihydroxyvitamin D_3 but is much less toxic. [See, U.S. Patent 5,403,831 and U.S. Patent 5,104,864]. 1α-hydroxyvitamin D_2 is equally active as 1α-hydroxyvitamin D_3 in the healing of rickets, in the stimulation of intestinal calcium absorption and in the elevation of serum inorganic phosphorous of rachitic rats. [G. Sjoden *et al.*, *J. Nutr.* 114, 2043-2946 (1984)]. In normal rats, 1α-hydroxyvitamin D_2 was found to be 5 to 15 times less toxic than 1α- hydroxyvitamin D_3. [See, also, G. Sjoden *et al.*, *Proc. Soc. Exp. Biol. Med.* 178, 432-436 (1985)]. It has also now been found that, for example, 1α-hydroxyvitamin D_2 may be safely administered for up to two years to human subjects experiencing or having a tendency toward loss of bone mass or bone mineral content at dosages greater than 3 μg/day. Even dosages up to 10 μg/day of 1α-hydroxyvitamin D_2 in women with postmenopausal osteoporosis (in both open label and double blind testing) exhibited only mild hypercalciuria (>300 mg/24 hrs), while marked hypercalcemia (>11.0 mg/dL) solely due to 1α-hydroxyvitamin D_2 was not evident. Additionally, 1α-hydroxyvitamin D_2 did not adversely affect kidney function, as determined by creatinine clearance and BUN; nor did it increase urinary excretion of hydroxyproline, indicating the absence of any stimulatory effect on bone resorption. Administration of 1α-hydroxyvitamin D_2 to healthy adult males in dosages up to 8 μg/day has shown no hypercalcemia or other adverse effects.

It is known that vitamin D_3 must be hydroxylated in the C-1 and C-25 positions before it is activated, i.e., before it will produce a biological response. A similar metabolism appears to be required to activate other forms of vitamin D, e.g., vitamin D_2 and vitamin D_4. Therefore, as used herein, the term "activated vitamin D" or "active vitamin D" is intended to refer to a vitamin D compound or analog that has been hydroxylated in at least one of the C-1, C-24 or C-25 positions of the molecule (i.e., a

hydroxyvitamin D) and either the compound itself, or one of its metabolites in the case of a prodrug, binds to the vitamin D receptor. For example, vitamin D "prodrugs" suitably include compounds that are hydroxylated in the C-1 position. Such compounds undergo further hydroxylation *in vivo* and their metabolites bind the vitamin D receptor.

Also, as used herein, the term "lower" as a modifier for alkyl, alkenyl, acyl, or cycloalkyl is meant to refer to a straight or branched, saturated or unsaturated hydrocarbon radical having 1 to 4 carbon atoms. Specific examples of such hydrocarbon radicals are methyl, ethyl, propyl, isopropyl, butyl, isobutyl, *t*-butyl, ethenyl, propenyl, butenyl, isobutenyl, isopropenyl, formyl, acetyl, propionyl, butyryl or cyclopropyl. The term "aromatic acyl" is meant to refer to an unsubstituted or substituted benzyl group.

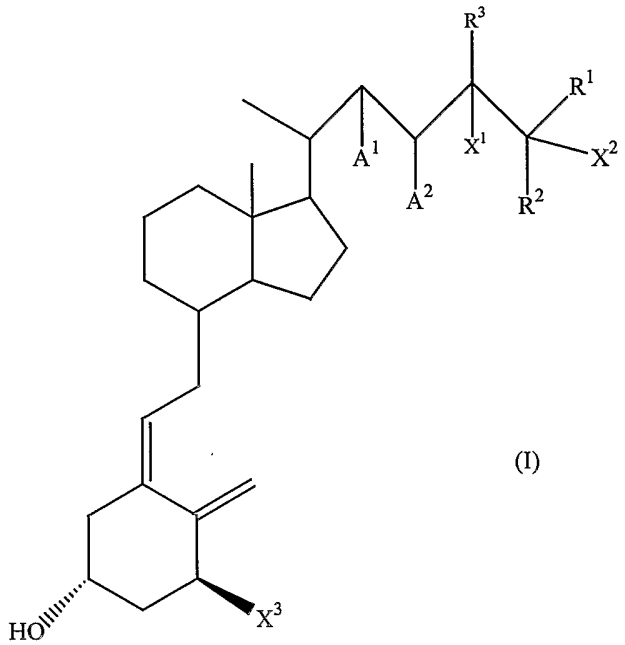
As used herein, the term "hydrocarbon moiety" refers to a lower alkyl, a lower alkenyl, a lower acyl group or a lower cycloalkyl, i.e., a straight or branched, saturated or unsaturated C_1-C_4 hydrocarbon radial. Also, as used herein, the terms "pharmacologic" and "pharmacologically active" are used interchangeably with "biological" and "biologically active".

Further, the active vitamin D of formula (I) may have an unsaturated side chain, i.e., there may be one or more double bonds, e.g., there may suitably be a double bond between C-22 and C-23, between C-25 and C-26 or between C-25 and C-27.

Compounds of this invention are useful in treating diseases that manifest elevated levels of PTH. In one aspect, compounds of the invention are used in treating secondary hyperparathyroidism associated with chronic kidney disease, and concomitantly, with reversing or reducing bone loss associated with this disease. The patients so treated generally have GFRs < 90 mL/min/1.73 m^2, but ≥ 15 mL/min/1.73 m^2. In other words, the compounds in accordance with the present invention are of especial value for patients with chronic kidney disease that are not yet on dialysis. Such patients are also known as pre-dialysis patients. Other aspects of the invention

include the treatment of renal osteodystrophy associated with late stage secondary hyperparathyroidism, and the treatment of primary hyperparathyroidism.

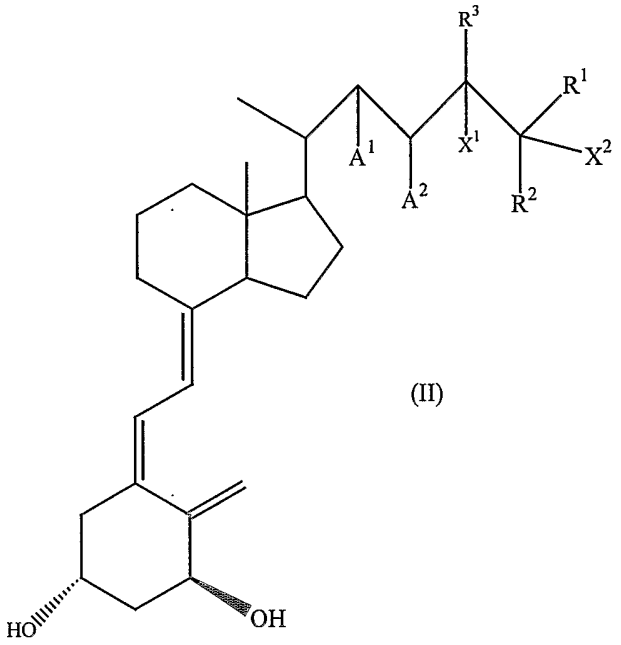
An active vitamin D of the present invention, i.e., a hydroxyvitamin D, has the general formula described in formula (I):



(I)

wherein A^1 and A^2 each are hydrogen or together represent a carbon-carbon bond, thus forming a double bond between C-22 and C-23; R^1 and R^2 are identical or different and are hydrogen, hydroxyl, lower alkyl, lower fluoroalkyl, O-lower alkyl, lower alkenyl, lower fluoroalkenyl, O-lower alkenyl, O-lower acyl, O-aromatic acyl, lower cycloalkyl with the proviso that R^1 and R^2 cannot both be an alkenyl, or taken together with the carbon to which they are bonded, form a C_3-C_8 cyclocarbon ring; R^3 is lower alkyl, lower alkenyl, lower fluoroalkyl, lower fluoroalkenyl, O-lower alkyl, O-lower alkenyl, O-lower acyl, O-aromatic acyl or lower cycloalkyl; X^1 is hydrogen or hydroxyl; X^2 is hydrogen or hydroxyl, or, is taken with R^1 or R^2, to constitute a double bond; X^3 is hydrogen or hydroxyl provided that at least one of X^1, X^2 and X^3 is hydroxyl.

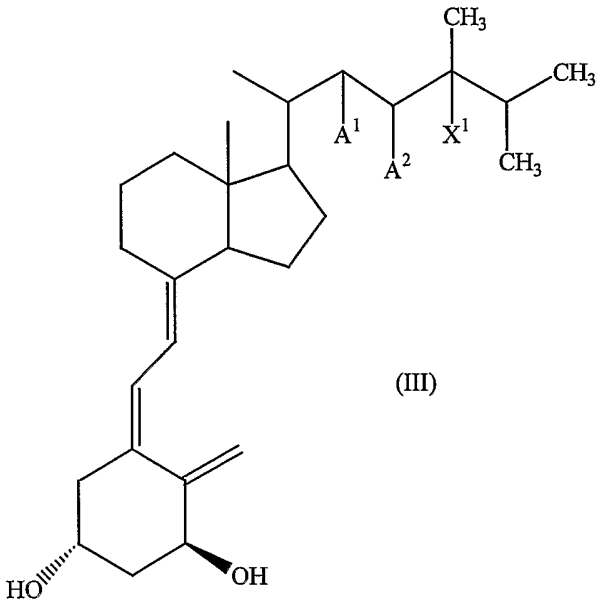
Specific 1α-hydroxyvitamin D compounds are characterized by the general formula (II):



(II)

wherein A^1 and A^2 each are hydrogen or together represent a carbon-carbon bond, thus forming a double bond between C-22 and C-23; R^1 and R^2 are identical or different and are hydrogen, hydroxyl, lower alkyl, lower fluoroalkyl, O-lower alkyl, lower alkenyl, lower fluoroalkenyl, O-lower alkenyl, O-lower acyl, O-aromatic acyl, lower cycloalkyl with the proviso that R^1 and R^2 cannot both be an alkenyl, or taken together with the carbon to which they are bonded, form a C_3-C_8 cyclocarbon ring; R^3 is lower alkyl, lower alkenyl, lower fluoroalkyl; lower fluoroalkenyl, O-lower alkyl, O-lower alkenyl, O-lower acyl, O-aromatic acyl or lower cycloalkyl; X^1 is hydrogen or hydroxyl; and X^2 is hydrogen or hydroxyl, or, may be taken with R^1 or R^2, to constitute a double bond.

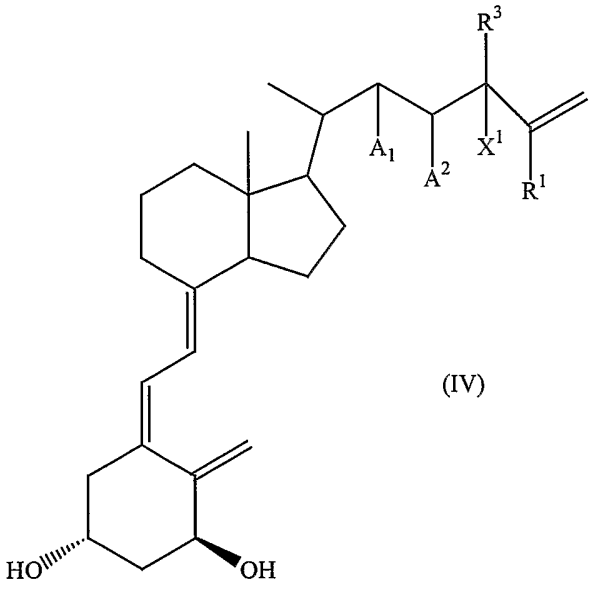
Active 1α-hydroxylated vitamin D analogs wherein R^1, R^2, and R^3 are all methyl groups and X^2 is hydrogen, have the general formula (III):



(III)

wherein A^1 and A^2 are each either hydrogen or together represent a carbon-carbon double bond; and X^1 is either hydrogen or hydroxyl.

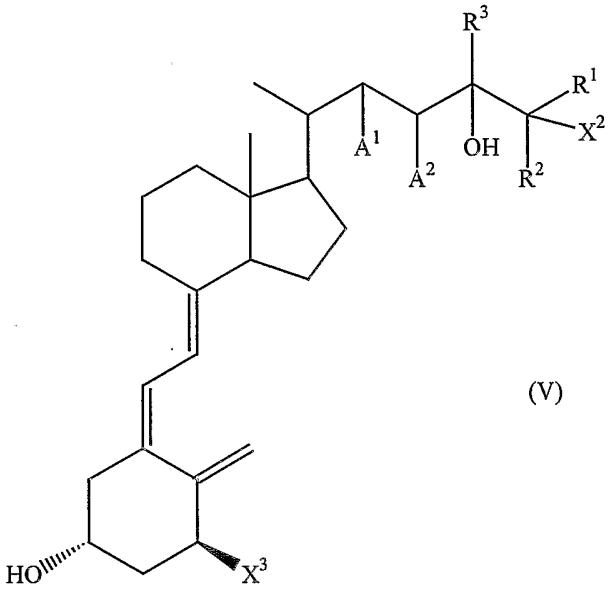
Other active 1α-hydroxylated vitamin D analogs may be represented by formula (IV):



(IV)

wherein A^1 and A^2 are each either hydrogen or, taken together, form a carbon-carbon double bond; X^1 is hydrogen or hydroxyl; and R^1 and R^3 are independently lower alkyl or lower fluoroalkyl. Compounds of formula (IV) include 1α-hydroxy-25-ene-vitamin D and 1α,24-dihydroxy-25-ene-vitamin D.

Specific 24-hydroxyvitamin D compounds in accordance with the present invention are represented by the general formula (V):



(V)

wherein A^1 and A^2 each are hydrogen or together represent a carbon-carbon bond, thus forming a double bond between C-22 and C-23; R^1 and R^2 are identical or different and are hydrogen, hydroxyl, lower alkyl, lower fluoroalkyl, O-lower alkyl, lower alkenyl, lower fluoroalkenyl, O-lower alkenyl, O-lower acyl, O-aromatic acyl, lower cycloalkyl with the proviso that both R^1 and R^2 cannot both be an alkenyl, or taken together with the carbon to which they are bonded, form a C_3-C_8 cyclocarbon ring; R^3 is lower alkyl, lower alkenyl, lower fluoroalkyl, lower fluoroalkenyl, O-lower alkyl, O-lower alkenyl, O-lower acyl, O-aromatic acyl or lower cycloalkyl; X^3 is hydrogen or hydroxyl, and X^2 is hydrogen or hydroxyl, or, may be taken with R^1 or R^2, to constitute a double bond.

Compounds in accordance with formulas (I)-(V) include generally 24-hydroxyvitamin D compounds, 25-hydroxyvitamin D compounds and 1α-hydroxyvitamin D compounds. Specific examples of such compounds of formulas (I)-(V) include, without limitation, 1α,24-dihydroxyvitamin D_2, 1α,24-dihydroxy-25-ene-vitamin D_2, 1α,24-dihydroxyvitamin D_4, 1α,25-dihydroxyvitamin D_4, 1α,25-dihydroxyvitamin D_2, 1α,24,25-trihydroxyvitamin D_2, and also include such pro-drugs or pro-hormones as 1α-hydroxyvitamin D_2, 1α-hydroxy-25-ene-vitamin D_2, 1α-hydroxyvitamin D_4, 24-hydroxyvitamin D_2, 24-hydroxyvitamin D_4, 25-hydroxyvitamin D_2, and 25-hydroxyvitamin D_4.

The compounds in accordance with the present invention are typically hypocalcemic compared to the natural D hormone, 1α,25-dihydroxyvitamin D_3. "Hypocalcemic" is meant to refer to an active vitamin D compound that has reduced calcemic activity compared to that of the natural vitamin D hormone, 1α,25-dihydroxyvitamin D_3; in other words, a calcemic index less than that of 1α,25-dihydroxyvitamin D_3. The calcemic activity of these compounds typically ranges from 0.001 to 0.5 times that of 1α,25-dihydroxyvitamin D_3. "Calcemic index" is a relative measure of the ability of a drug to generate a calcemic response, the calcemic activity of 1α,25-dihydroxyvitamin D_3 being designated as 1. Such hypocalcemic vitamin D compounds provide reduced risk of hypercalcemia even when administered in high doses.

Further, for compounds of formulas (I)-(V) that have a chiral center, such as at the C-24 position, it is understood that all epimers (e.g., R and S) and the epimeric mixture are within the scope of the present invention. Where certain epimeric forms are more suitable, the form is substantially free of its other epimeric form, e.g., 1α,24(S)-dihydroxyvitamin D_2 is suitably substantially free of its (R) epimer, and 1α,24(R)-dihydroxyvitamin D_4 is suitably substantially free of its (S) epimer.

The vitamin D analogs of formulas (I)-(V) are useful as active compounds in pharmaceutical compositions. The active vitamin D compounds of the present invention include vitamin D compounds having a hydroxy group substituted in at least

one of the C_1, C_{24} or C_{25} positions of the molecule, i.e., a hydroxy vitamin D. The analogs of formulas (III), (IV) and (V) are of especial value as they are substantially less toxic than their vitamin D_3 counterparts when administered by conventional protocols to patients experiencing hyperparathyroidism. For example, in patients using oral calcium as a phosphate binder, e.g., calcium carbonate or calcium acetate, administration of the analogs of formulas (III), (IV) and (V) at dosage levels higher than possible with the vitamin D_3 compounds provides greater efficacy than heretofore possible in treating hyperparathyroidism. It is expressly contemplated that analogs of formula (I) may be co-administered with both calcium-based phosphate binders and non-calcium-based phosphate binders, e.g., lanthanum carbonate (Fosrenol™) and sevelamer hydrochloride (Renagel™).

Generally, the pharmacologically active compounds of the present invention can be processed in accordance with conventional methods of pharmacy to produce medicinal agents for administration to patients, e.g., mammals including humans. For example, the active vitamin D compounds of the present invention can be formulated in pharmaceutical compositions in a conventional manner using one or more conventional excipients, which do not deleteriously react with the active compounds, e.g., pharmaceutically acceptable carrier substances suitable for enteral administration (e.g., oral), parenteral, topical, buccal or rectal application, or by administration by inhalation or insufflation (e.g., either through the mouth or the nose).

Acceptable carriers for pharmaceutical formulation generally include, but are not limited to, water, salt solutions, alcohols, gum arabic, vegetable oils (e.g., almond oil, corn oil, cottonseed oil, peanut oil, olive oil, coconut oil), mineral oil, fish liver oils, oily esters such as Polysorbate 80, polyethylene glycols, gelatin, carbohydrates (e.g., lactose, amylose or starch), magnesium stearate, talc, silicic acid, viscous paraffin, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, hydroxy methylcellulose, polyvinylpyrrolidone, etc.

Enteral administration is of especial interest. For enteral application, particularly suitable are tablets, dragees, liquids, drops, suppositories, lozenges, powders, or capsules. Syrup, elixir, or the like can be used if a sweetened vehicle is

desired. For example, for oral administration, the pharmaceutical compositions may take the form of tablets or capsules, e.g., soft or hard gel capsules, prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). Tablets may be coated by methods well known in the art.

Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

Preparations for oral administration may also be suitably formulated to give controlled release of the active compound. Many controlled release systems are known in the art, (see, e.g., U.S. Patent No. 5,529,991.)

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered, for example, by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. The compounds may be formulated with suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, e.g., a sparingly soluble salt. Depots for sustained release delivery are described in detail in U.S. Patent Application Publication No. US2003/0009145 and US 2002/0151876, for example.

An injectable depot is an injectable biodegradable sustained release device that is generally non-containerized and that may act as a reservoir for the active vitamin D, from which the active vitamin D is released. Depots include polymeric and non-polymeric materials, and may be solid, liquid or semi-solid in form. For example, a depot as used in the present invention may be a high viscosity liquid, such as a non-polymeric, non-water-soluble liquid carrier material, e.g., sucrose acetate isobutyrate (SAIB) or another compound described in U.S. Pat. Nos. 5,747,058 and 5,968,542.

The depot may be formulated as an injectable depot gel composition containing a polymer, a solvent that can dissolve the polymer to form a viscous gel,and the compound, and an emulsifying agent in the form of a dispersed droplet phase in the viscous gel, as described in U.S. Patent No. 6,331,311. The injectable depot gel composition can be prepared by mixing the polymer and the solvent so that the solvent dissolves the polymer and forms a viscous gel. The compound is then dissolved or dispersed in the viscous gel and the emulsifying agent is mixed with the compound and viscous gel. The emulsifying agent forms a dispersed droplet phase in the viscous gel to provide the injectable depot gel composition. The injectable depot gel composition can deliver a beneficial agent to a human or animal with a desired release profile.

Biodegradable matrices are useful as because they obviate the need to remove the drug-depleted device. The most common matrix materials for drug delivery are polymers. Polylactic acid and other polymers including, but not limited to, polyanhydrides, polyesters such as polyglycolides and polylactide-co-glycolides, polyamino acids such as polylysine, polymers and copolymers of polyethylene oxide, acrylic terminated polyethylene oxide, polyamides, polyurethanes, polyorthoesters, polyacrylonitriles, and polyphosphazenes are useful as a matrix material for delivery devices.

Degradable materials of biological origin such as crosslinked gelatin or crosslinked hyaluronic acid are useful as degradable swelling polymers for biomedical applications. Biodegradable hydrogels have also been developed for use in controlled drug delivery as carriers of biologically active materials. Proper choice of hydrogel macromers can produce membranes with a range of permeability, pore sizes and degradation rates suitable for a variety of applications.

Dispersion systems (i.e., suspensions or emulsions) can be used as depots for delivery of the compound. Suspensions of solid particles (i.e., microspheres, microcapsules, or nanospheres) dispersed in a liquid medium using suspending agents may be used. Emulsions are defined as dispersions of one liquid in another, stabilized by an emulsifier such as surfactants and lipids. Emulsion formulations include water in oil and oil in water emulsions, multiple emulsions, microemulsions, microdroplets, and liposomes. Micro droplets are unilamellar phospholipid vesicles that consist of a spherical lipid layer with an oil phase inside. Liposomes are phospholipid vesicles prepared by mixing water-insoluble polar lipids with an aqueous solution, which produces an assembly of essentially concentric closed membranes of phospholipid with entrapped aqueous solution.

The depot may be in the form of an implant formed in situ, as described in U.S. Pat. No. 4,938,763, by dissolving a non-reactive, water insoluble thermoplastic polymer in a biocompatible, water soluble solvent to form a liquid, placing the liquid within the body, and upon dissipation of the solvent, producing a solid implant. The polymer solution can be placed in the body, for example, by injection. The implant can assume the shape of its surrounding cavity. The implant may also be formed from reactive, liquid oligomeric polymers which contain no solvent and which cure in place to form solids, usually upon addition of a curing catalyst

The depot preparation may be formed by dissolving the compound in an oily, unsaturated carrier as described in U.S. Patent No. 4181721.

Parenteral e.g., injectable, dosage forms are also of interest. Using the parenteral route of administration allows for bypass of the first pass of active vitamin D compound through the intestine, thus avoiding stimulation of intestinal calcium absorption, and further, reduces the risk of esophageal irritation which may be associated with high dose oral administration. Because an injectable route of administration is typically done by a health care professional, the dosing can be more effectively controlled as to precise amount and timing. Parenteral administration suitably includes subcutaneous, intramuscular, or intravenous injection, nasopharyngeal or mucosal absorption, or transdermal absorption.

Injectable compositions may take such forms as sterile suspensions, solutions, or emulsions in oily vehicles (such as coconut oil, cottonseed oil, sesame oil, peanut oil or soybean oil) or aqueous vehicles, and may contain various formulating agents.

In injectable compositions, the carrier is typically sterile and pyrogen-free, e.g., water, saline, aqueous propylene glycol, or another injectable liquid, e.g., peanut oil for intramuscular injections. Also, various buffering agents, preservatives, suspending, stabilizing or dispensing agents, surface-active agents and the like can be included. Aqueous solutions may be suitably buffered, if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. Aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal injection purposes. In this connection, the sterile aqueous media employed are all readily obtainable by standard techniques well known to those skilled in the art. The oily solutions are especially suitable for intra-articular, intramuscular and subcutaneous injection purposes. The preparation of all these solutions under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art.

Compounds formulated for parenteral administration by injection may be administered by bolus injection or continuous infusion. Formulations for injection may be conveniently presented in unit dosage form, e.g., in ampoules or in multi-use containers, with an added preservative as needed. The dosage of the analogs for parenteral administration generally is about 0.5-30 μg given 1 to 3 times per week. Longer interval-higher dose regimens are also contemplated, e.g., 30 μg – 100 μg biweekly, triweekly or once per month, as further described hereinbelow.

As described hereinbefore, the analogs of formula (I) are suitably administered to the human patients in oral dosage formulation. As an analog of formula (I) is released from the oral dosage formulation, it is absorbed from the intestine into the blood. Generally, for oral administration the analogs of this invention are conveniently dispensed in unit dosage form comprising about 0.25 μg to about 25 μg in a pharmaceutically acceptable carrier per unit dosage. For example, an analog may be presented as 0.25 μg to 5 μg in unit dosage form. The dosage of the analogs generally

is about 0.5 μg per week to about 100 μg per week, suitably about 0.5 μg per week to about 25 μg per week or 3.5 μg per week to 17.5 μg per week. Dosage regimens may vary from daily to longer episodic dosing, e.g., weekly, biweekly or monthly, as described hereinbelow.

For buccal administration, the compositions may take the form of tablets, lozenges or absorption wafers formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g. gelatin, for use in an inhaler or insufflator may be formulated containing a powder mix of the active compound and a suitable powder base such as lactose or starch.

The compounds may also be formulated in rectal or vaginal compositions, such as suppositories containing conventional suppository bases or retention enemas. These compositions can be prepared by mixing the active ingredient with a suitable non-irritating excipient which is solid at room temperature (for example, 10° C to 32° C) but liquid at the rectal or vaginal temperature, and will melt in the rectum or vagina to release the active ingredient. Such materials include polyethylene glycols, cocoa butter, other glycerides and wax. To prolong storage life, the compositions advantageously may include an antioxidant such as ascorbic acid, butylated hydroxyanisole or hydroquinone.

The compositions may, if desired, be presented in a pack or dispenser device that may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device is suitably accompanied by instructions for administration, e.g., a notice associated with the pack or dispenser in a form prescribed by a governmental

agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of manufacture, use or sale of the composition for human or veterinary administration.

For topical application, suitable nonsprayable viscous, semi-solid or solid forms can be employed which include a carrier compatible with topical application and having a dynamic viscosity preferably greater than water, for example, mineral oil, almond oil, self-emulsifying beeswax, vegetable oil, white soft paraffin, and propylene glycol. Suitable formulations include, but are not limited to, creams, jellies, gels, pastes, ointments, lotions, solutions, suspensions, emulsions, powders, liniments, salves, aerosols, transdermal patches, etc., which are, if desired, sterilized or mixed with auxiliary agents, e.g., preservatives, stabilizers, demulsifiers, wetting agents, etc. A cream preparation in accordance with the present invention suitably includes, for example, a mixture of water, almond oil, mineral oil and self-emulsifying beeswax; an ointment preparation suitably includes, for example, almond oil and white soft paraffin; and a lotion preparation suitably includes, for example, dry propylene glycol. For purposes of transdermal administration, dilute sterile, aqueous or partially aqueous solutions (usually in about 0.1% to 5% concentration), otherwise similar to the above parenteral solutions, are prepared.

Dosage forms of the analogs of formula (I) may also contain adjuvants, such as preserving or stabilizing adjuvants. They may also contain other therapeutically valuable substances or may contain more than one of the compounds specified herein and in the claims in admixture.

Episodic dosing is contemplated in the administration of the compounds or analogs in accordance with the present invention for treatment of hyperparathyroidism associated with chronic kidney disease. "Episodic dosing" is meant to refer to intermittent, i.e., non-daily, dosing, for example, once weekly, bi-weekly, tri-weekly, monthly, or twice weekly or thrice weekly. An compound of formula (I) such as 1α-hydroxyvitamin D_2 (also known as doxercalciferol or 1α-hydroxy ergocalciferol) may be administered in a dose, e.g., of 10-30 μg once per week or 3 μg three times per week. An intermittent or episodic dosing regimen may be suitably between once per

week to once every 12 weeks, e.g., once every 3 weeks. As a function of body weight, the effective dose ranges from about 0.2 μg to about 3.0 μg per kilogram of body weight of the patient on a weekly basis.

While not wanting to be bound by any particular theory, it is believed that each single dose at the dosage levels indicated is sufficient to upregulate vitamin D hormone receptors, and that continuous dosing is not required because the binding and upregulation by vitamin D compounds is sufficient to initiate the cascade of intracellular metabolic processes occurring with receptor binding. Intermittent or episodic dosing reduces the risk of hypercalcemia. Episodic dosing also can be of therapeutic value because PTH levels that are therapeutically lowered by administration of active vitamin D have been found to remain suppressed for a period of time after cessation of a therapeutic dose of the active vitamin D. Thus, the method of the present invention can be used to treat hyperparathyroidism by administering any active vitamin D compound. At the same time, it is contemplated, in accordance with the present invention, that the risk of hypercalcemia can be further mitigated if the active vitamin D compound is a hypocalcemic active vitamin D compound.

Those of ordinary skill in the art will readily optimize effective doses and co-administration regimens (as described hereinbelow) as determined by good medical practice and the clinical condition of the individual patient. Regardless of the manner of administration, it will be appreciated that actual amounts of active compound in a specific case will vary according to the efficacy of the specific compound employed, the particular formulation and the mode of application. For example, the specific dose for a particular patient depends on age, sex, body weight, general state of health, on diet, on the timing and mode of administration, on the rate of excretion, and on medicaments used in combination and the severity of the particular disorder to which the therapy is applied. Dosages for a given patient can be determined using conventional considerations, e.g., by customary comparison of the differential activities of the subject compounds and of a known agent, such as by means of an appropriate conventional pharmacological protocol. A physician of ordinary skill can readily determine and prescribe the effective amount of the drug required to counter or arrest the progress of the condition. Optimal precision in achieving concentrations of drug

within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the drug's availability. This involves a consideration of the distribution, equilibrium, and elimination of a drug. The dosage of active ingredient in the compositions of this invention may be varied; however, it is necessary that the amount of the active ingredient be such that an efficacious dosage is obtained. The active ingredient is administered to patients (animal and human) in need of treatment in dosages that will provide optimal pharmaceutical efficacy.

Also included within the scope of the present invention is the co-administration of effective dosages of the analogs of formulas (I)-(V) in conjunction with other therapeutic agents such as hormones, e.g., estrogens, which are known to ameliorate bone diseases or disorders typically associated with hyperparathyroidism or to ameliorate abnormal calcium homeostatis, or which lower PTH levels. Such agents may include: other vitamin D compounds; conjugated estrogens or their equivalents; calcitonin; sodium fluoride; bisphosphonates including, but not limited to, zolendronate and pamidronate; calcium supplements; cobalamin; pertussis toxin; boron; calcimimetics; and certain antagonists, antibodies, and oligonucleotides (see, below).

The term "co-administration" is meant to refer to a combination therapy by any administration route in which two or more agents are administered to a patient or subject. Co-administration of agents may be referred to as combination therapy or combination treatment. The agents may be in the same dosage formulations or separate formulations. For combination treatment with more than one active agent, where the active agents are in separate dosage formulations, the active agents can be administered concurrently, or they each can be administered at separately staggered times. The agents may be administered simultaneously or sequentially (i.e., one agent may directly follow administration of the other or the agents may be give episodically, i.e., one can be given at one time followed by the other at a later time, e.g., within a week), as along as they are given in a manner sufficient to allow both agents to achieve effective concentrations in the body.

The agents may also be administered by different routes, e.g., one agent may be administered intravenously while a second agent is administered intramuscularly,

intravenously or orally. In other words, the co-administration of the active vitamin D compound of the present invention with another therapeutic agent is suitably considered a combined pharmaceutical preparation which contains an active vitamin D compound and, e.g., a bone agent, the preparation being adapted for the administration of the active vitamin D compound on a daily or intermittent basis, and the administration of, e.g., a bone agent on a daily or intermittent basis. The agents also may be formulated as an admixture, as, for example, in a single tablet or capsule.

It is anticipated that the vitamin D compounds used in combination with various bone and antihyperparathyroid drugs, such as calcimimetics (see, e.g. U.S. Patent Nos. 5,688,938, 5,763,569, 5,962,314 and 6,001,884), antagonists of PTH and parathyroidhormone related protein (PTHrP), antibodies (monoclonal or polyclonal) to PTH receptor and antisense oligonucleotides to PTH receptor RNA in the case of a genomic component to the hyperparathyroidism (see, e.g., U.S. Published Patent Application No. 2003/10153041), can give rise to a significantly enhanced lowering of excessive parathyroid activity or excessive hormone levels in a patient suffering from hyperparathyroidism, thus providing an increased therapeutic effect. Specifically, as a significantly increased PTH inhibitory or enhanced bone loss inhibitory effect is obtained with the above disclosed combinations utilizing lower concentrations of the drugs compared to the treatment regimes in which the drugs are used alone, there is the potential to provide therapy wherein any adverse side effects associated with the drugs are considerably reduced than normally observed with the drugs used alone in larger doses.

Possible dose ranges for exemplary co-administered agents are provided in Table 1.

TABLE 1

Possible Oral Dose Ranges for Various Agents Co-Administered With Active vitamin D Compounds of Formulas (I)-(V)

Agent	Dose Ranges		
	Broad	Preferred	Most Preferred
Conjugated Estrogens or Equivalent (mg/day)	0.3-5.0	0.4-2.4	0.6-1.2
Sodium Fluoride (mg/day)	5-150	30-75	40-60
Calcitonin (IU/day)	5-800	25-500	50-200
Bisphosphonates (mg/day)	50-2000	100-1500	250-1000
Calcium Supplements (mg/day)	250-2500	500-1500	750-1000
Cobalamin (μg/day)	5-200	20-100	30-50
Pertussis Toxin (mg/day)	0.1-2000	10-1500	100-1000
Boron (mg/day)	0.10-3000	1-250	2-100

Calcimimetics, such as cinacalcet hydrochloride, which modulate calcium-sensing receptors, may be used in possible oral dosage ranges of 4 to 400 mg/dose, co-administered with active vitamin D compounds. Possible dosage ranges for PTH antagonists or antibodies, co-administered with active vitamin D compounds, may be 1 ng to 10 mg/kg of body weight.

Although dosages are given above for some of the agents for oral administration, it is understood that the co-administered agents can also be administered in alternative fashions, including intranasally, transdermally, intrarectally, intravaginally, subcutaneously, intravenously, and intramuscularly, as appropriate for the particular agents. It is also contemplated that some of the co-administered agents may be given on an other-than-daily basis.

For convenience, the active vitamin D compound of the present invention and the co-administered therapeutic agent may be packaged together, e.g., in a blister pack or dispenser device. In other words, the active vitamin D compound and the other therapeutic agent may be contained in a common package, each contained in a separate container or a separate compartment therein, and also having instructions for use of the compound and the agent in the treatment of hyperparathyroidism, e.g., instructions for

administering the active vitamin D compound and the therapeutic agent to a subject having hyperparathyroidism on a daily or episodic basis. Such instructions are suitably a notice in a form prescribed by a governmental regulatory agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the vitamin D compound and the therapeutic agent for human or veterinary administration to treat hyperparathyroidism and for bone loss.

It is understood that all forms of administration, formulation and active ingredients are regulated by a governmental agency, e.g., the United States Food and Drug Administration, and the form of notice or instruction for administration is prescribed by such agency.

Bulk quantities of the vitamin D analogs in accordance with the present invention can be readily obtained in accordance with the many widely known processes, e.g., as described in U.S. Patents Nos. 3,907,843; 4,195,027; 4,202,829; 4,234,495; 4,260,549; 4,555,364; 4,554,106; 4,670,190; 5,488,120 and 5,972,917; WO 94/05630, and Strugnell *et al.*, 310 *Biochem. J.* 233-241 (1995), all of which are herein fully incorporated by reference.

The present invention is further explained by the following examples which should not be construed by way of limiting the scope of the present invention.

A comparison of 1α-hydroxyvitamin D_2 to 1α-hydroxyvitamin D_3 has been conducted. The following examples demonstrate that 1α-hydroxyvitamin D_2 and 1α,24-dihydroxyvitamin D_4 are effective in reducing or preventing elevated blood PTH levels as well as preventing or restoring the loss of bone mass or bone mineral content while being substantially less toxic than 1α,25-dihydroxyvitamin D_3 and 1α-hydroxyvitamin D_3. It is to be understood that although the following examples detail the use of 1α-hydroxyvitamin D_2 and 1α,24-dihydroxyvitamin D_4, compounds of formula (I) may be readily utilized in the treatment of this invention with essentially equivalent results. For example, 1α,24(S)-dihydroxyvitamin D_2 shows activity equivalent to 1α,24(R)-dihydroxyvitamin D_3 and is also significantly less toxic than its vitamin D_3 counterpart.

Example 1: Study Demonstrating Better Safety

The low toxicity of 1α-hydroxyvitamin D_2 in human patients was demonstrated in a clinical study involving 15 postmenopausal osteopenic women. [*J. Bone Min. Res.*; 9:607-614 (1994).] The selected patients were between 55 and 75 years of age, and exhibited L2-L3 vertebral bone mineral density ("BMD") between 0.7 and 1.05 g/cm^2, as determined by measurements with a LUNAR dual-photon absorptiometer. (The mean bone mineral density in women with osteopenia is about 0.85 ± 0.17 g/cm^2, so that these limits correspond to about the 15th to 85th percentiles.)

On admission to the study, all patients received instruction on selecting a daily diet containing 400 to 600 mg of calcium. Compliance to this diet was verified at weekly intervals by 24-hour food records and by interviews with each patient.

All patients completed a one-week baseline period, a five- to seven-week treatment period, and a one-week post-treatment observation period. During the treatment period, patients orally self-administered 1α-hydroxyvitamin D_2 at an initial dose of 0.5 μg/day for the first week, and at successively higher doses of 1.0, 2.0, 4.0, and 5.0 μg/day in each of the following weeks, with some patients receiving succesively higher doses of 8.0 and 10.0 μg/day in weeks six and seven, respectively. All doses were administered before breakfast.

Blood and urine chemistries were monitored on a weekly basis throughout the study. Key blood chemistries included fasting serum levels of calcium, phosphorus, osteocalcin, creatinine and blood urea nitrogen. Key urine chemistries included 24-hour excretion of calcium, phosphorus and creatinine.

Data from the study clearly demonstrated that 1α-hydroxyvitamin D_2 can be safely administered at high dose levels on a daily dosing regimen for periods of several weeks. In particular, the compound did not adversely affect kidney function, as determined by creatinine clearance and blood levels of urea nitrogen; nor did it increase urinary excretion of hydroxyproline, indicating the absence of any stimulatory effect on bone resorption. The compound had no effect on any routinely monitored serum chemistries, indicating the absence of adverse metabolic effects.

A positive effect of 1α-hydroxyvitamin D_2 on calcium homeostasis was evident from dose-related increases observed in 24-hour urinary calcium levels, confirming that the compound increases intestinal calcium absorption, and from dose-related increases in serum osteocalcin, suggesting that the compound directly stimulates bone formation.

Example 2: Study Demonstrating Safety and Efficacy for Human Osteoporosis

The safety and efficacy of 1α-hydroxyvitamin D_2 as an oral treatment for osteoporosis was confirmed in a study involving 60 postmenopausal osteoporotic outpatients. The selected subjects had ages between 60 and 70 years, and exhibited L2-L3 vertebral BMD between 0.7 and 1.05 g/cm^2, as determined by dual-energy x-ray absorptiometry (DEXA). Exclusion criteria encompassed significant medical disorders and recent use of medications known to affect bone or calcium metabolism.

On admission to the study, each subject was assigned at random to one of two treatment groups; one group received up to a 104-week course of therapy with 1α-hydroxyvitamin D_2; the other received only placebo therapy. All subjects received instruction on selecting a daily diet containing 700-900 mg of calcium and were advised to adhere to this diet over the course of the study. Compliance to the diet was verified at regular intervals by 24-hour food records and by interviews with each subject.

During the treatment period, subjects from one group orally self-administered 1α-hydroxyvitamin D_2 at an initial dosage of 1.0 μg/day for one week, and increased the dosage to 2.0, 3.0, 4.0 μg/day in each of the following weeks, to a maximum dosage of 5.0 μg/day. The dosage for any given subject was increased in this way until the rate of urinary calcium excretion was elevated to approximately 275-300 mg/24 hours, at which point the subject held the dosage constant at the highest level attained. Subjects from the second group self-administered a matching placebo medication every day, titrating the apparent dosage upwards in the same manner as subjects being treated with 1α-hydroxyvitamin D_2.

Spinal and femoral neck BMD were measured in all subjects by DEXA at the beginning of the study, and at six-month intervals thereafter. Intestinal calcium

absorption was estimated in all subjects by a single isotope technique at the beginning of the study, and at 12-month intervals. Serum levels of vitamin D metabolites were determined by radioreceptor binding assays at baseline and at six-month intervals. Serum osteocalcin, serum PTH and urine hydroxyproline also were determined at baseline and at six-month intervals.

Other blood and urine chemistries were monitored at regular intervals during the treatment period. These chemistries included serum calcium, serum ionized calcium, urine calcium, blood urea nitrogen, serum creatinine and creatinine clearance. Kidney-ureter-bladder (KUB) x-rays were obtained at baseline and at 12-month intervals thereafter.

The results of the study are summarized below:

Subjects: Sixty subjects enrolled in what was originally intended to be a 52-week study. Of these 60 subjects, 55 completed one year of treatment (28 active; 27 placebo); and 41 subjects completed an optional second year of treatment.

Test Drug Dosages: The average prescribed dosage for subjects who received 1α-hydroxyvitamin D_2 was 4.2 μg/day at 52 weeks and 3.6 μg/day at 104 weeks. The average prescribed dosage for placebo subjects was an apparent 4.8 μg/day at 52 weeks and 4.8 μg/day at 104 weeks.

Exclusions: One subject failed to comply with the prescribed dosage of test drug, as confirmed by an absence of serum 1α,25-dihydroxyvitamin D_2 at any time during the study. Data for this subject were excluded from analysis. Three patients were diagnosed with hyperparathyroidism when the PTH assays were completed (in batch) at the study's conclusion; data for these subjects were excluded from analysis. No subjects were excluded from analysis for noncompliance with the required dietary calcium intake of 700-900 mg/day.

Episodes of Hypercalcemia/Hypercalciuria: Marked hypercalcemia (>10.8 mg/dL) occurred in one subject in association with an intercurrent illness. The prescribed dosage of 1α-hydroxyvitamin D_2 at the time of this episode was 5.0 μg/day.

Moderate hypercalcemia (10.4-10.8 mg/dL) occurred in two subjects over the course of the study at prescribed dosages of 5.0 μg/day. Mild hypercalcemia (10.2-10.4 mg/dL) occurred in four subjects in the first year and in two subjects in the second year. Hypercalciuria was observed occasionally over the two-year study in 17 subjects treated with 1α-hydroxyvitamin D_2.

Serum Calcium/Ionized Calcium: Mean serum calcium was approximately 0.1 to 0.2 mg/dL higher in subjects treated with 1α-hydroxyvitamin D_2 than in subjects treated with placebo. This difference was significant ($P<0.05$) only during the second year of treatment. Mean serum ionized calcium was approximately 0.05 to 0.10 mg/dL higher in subjects treated with 1α-hydroxyvitamin D_2.

Urine Calcium: Mean urine calcium increased during the initial titration period in a dose-response fashion. After titration, mean urine calcium was 50 to 130% higher with 1α-hydroxyvitamin D_2 treatment than with placebo treatment.

Kidney Function: No significant changes were observed with long-term 1α-hydroxyvitamin D_2 treatment in BUN, serum creatinine or creatinine clearance. KUB x-rays revealed no abnormalities in either treatment group throughout the course of the study.

Bone: Bone mineral density (BMD) in the L2-L4 vertebrae progressively increased with 1α-hydroxyvitamin D_2 treatment and decreased with placebo treatment over the two-year study. The difference in spinal BMD between the treatment groups became statistically significant ($P<0.05$) after 24 months of treatment. Similar changes were observed in femoral neck BMD with statistically significant differences observed after 18 months ($P<0.001$) and 24 months ($P<0.05$) of treatment.

Calcium Uptake: Intestinal absorption of orally administered ^{45}Ca increased by 40% ($P<0.001$) after 52 weeks of 1α-hydroxyvitamin D_2 therapy, and by 29% ($P<0.5$) after 104 weeks of 1α-hydroxyvitamin D_2 therapy, relative to placebo control.

Vitamin D Metabolites: Treatment with 1α-hydroxyvitamin D_2 caused progressive increases in mean serum total 1α,25-dihydroxyvitamin D from 21%

($P<0.05$) at six months to 49% ($P<0.01$) at 24 months relative to placebo therapy. This increase resulted from a dramatic rise in serum 1α,25-dihydroxyvitamin D_2 which was partially offset by a 50+% decrease in serum 1α,25-dihydroxyvitamin D_3. No treatment related changes were apparent in serum total 25-hydroxyvitamin D.

Biochemical Parameters:

Serum levels of PTH decreased with 1α-hydroxyvitamin D_2 therapy by 17% at 52 weeks and by 25% at 1-4 weeks, relative to placebo therapy.

Serum levels of osteocalcin were unchanged with long-term 1α-hydroxyvitamin D_2 therapy.

Fasting urine hydroxyproline:creatinine ratio tended to decrease with long-term 1α-hydroxyvitamin D_2 treatment but the observed differences between the 1α-hydroxyvitamin D_2 and placebo treatment groups were not significantly different.

The results of this study clearly indicated that 1α-hydroxyvitamin D_2 can be tolerated in higher long-term daily dosages than the commonly used vitamin D_3 analogues. They also showed that 1α-hydroxyvitamin D_2 is well tolerated in postmenopausal women at long-term dosages in the range of 2.0 to 3.0 μg/day, provided that individuals exhibiting abnormally high urine calcium levels (when not receiving vitamin D therapy) are excluded from treatment. Long-term administration of such high dosages of 1α-hydroxyvitamin D_2 significantly reduced bone loss at the spine and femoral neck, the most frequent sites of osteoporotic fractures. These positive effects on bone were accompanied by a sustained increase in intestinal calcium absorption and a sustained decrease in serum PTH. They were not accompanied by clear long-term trends in serum osteocalcin and urine hydroxyproline. Taken together, the results of this study demonstrate that 1α-hydroxyvitamin D_2 is safe and effective in the treatment of postmenopausal or senile osteoporosis.

Example 3: Open Label Study in End Stage Renal Disease Patients Exhibiting Secondary Hyperparathyroidism

Five end-stage renal disease patients were enrolled in an open label study. The selected patients had ages between 36 and 72 years and had been on hemodialysis for at least 4 months prior to enrollment. The patients each had an average serum phosphorus in the range of 3.0 to less than or equal to 6.9 mg/dL during the two months prior to enrollment (often controlled by oral calcium as a phosphate binder e.g., calcium carbonate or calcium acetate), and had a history of elevated serum PTH values of greater than 400 pg/mL when not receiving 1α,25-dihydroxyvitamin D_3 therapy.

Each patient had been receiving 1α,25-dihydroxyvitamin D_3 prior to enrollment, and discontinued the 1α,25-dihydroxyvitamin D_3 therapy for eight weeks prior to receiving 1α-hydroxyvitamin D_2. After 8 weeks, the patients received treatment of 1α-hydroxyvitamin D_2 at a dosage of 4 μg three times per week for 6 weeks. Throughout the eight-week washout period and the treatment period, patients were monitored weekly or biweekly for serum intact PTH level and weekly for excessive elevation in serum levels of calcium and phosphorus.

Throughout the washout period and treatment period, patients underwent routine hemodialysis (3 times per week) using a 1.25 mM calcium dialysate. They also ingested significant amounts of calcium as a phosphate binder (1-10 g elemental Ca) to keep serum phosphorus levels below 6.9 mg/dL.

Average baseline values were as follows: serum PTH - 480±21 pg/mL; serum Ca - 8±0.3 mg/dL and serum phosphorus - 5.1±0.2 mg/dL. In three patients, serum PTH decreased by 68%, 74% and 87% after two weeks. In the other two patients, serum PTH declined by 33% in one and 3% in the other after four weeks. Overall, serum PTH decreased by 49 ± 17% and 33 ± 9% after two and four weeks of 1α-hydroxyvitamin D_2, respectively, ($p<0.05$). Serum calcium (mg/dL) was 10.2 ± 0.4 ($p<0.05$) and 9.8 ± 0.2 (NS) and serum phosphorus (mg/dL) was 5.4 ± 0.5 and 5.5 ± 0.8 at two and four weeks, respectively (NS). A rise in serum PTH from the second to fourth weeks of 1α-hydroxyvitamin D_2 treatment occurred when 1α-hydroxyvitamin D_2 was withheld in three patients with serum PTH < 130 picograms/ml; they developed

mild hypercalcemia (serum calcium, 10.3-11.4 mg/dL) that reversed after stopping 1α-hydroxyvitamin D_2. No other adverse effects occurred. At 4-6 weeks of 1α-hydroxyvitamin D_2 treatment of 4 μg, thrice weekly, four of five patients were in the target range of serum PTH; serum calcium was 10.0 ± 0.2 mg/dL and serum phosphorus, 5.3 ± 0.2 mg/dL. The patient who failed to respond to six weeks of 1α-hydroxyvitamin D_2 treatment had a delayed response to both intravenous and oral 1,25-dihydroxyvitamin D_3 earlier, requiring several months of treatment before serum PTH fell. Serum PTH values in this patient fell by 38% after eight weeks of 1α-hydroxyvitamin D_2 treatment. These data show that 1α-hydroxyvitamin D_2 is efficacious and safe for the control of secondary hyperparathyroidism in end stage renal disease patients.

Example 4: Double Blind Study of Bone in End Stage Renal Disease Patients

A twelve-month double-blind placebo-controlled clinical trial is conducted with thirty-five men and women with renal disease who are undergoing chronic hemodialysis. All patients enter an eight-week control period during which time they receive a maintenance dose of vitamin D_3 (400 IU/day). After this control period, the patients are randomized into two treatment groups: one group receives a constant dosage of 1α-hydroxyvitamin D_2 (u.i.d.; a dosage greater than 3.0 μg/day) and the other group receives a matching placebo. Both treatment groups receive a maintenance dosage of vitamin D_3, maintain a normal intake of dietary calcium, and refrain from using calcium supplements. Oral calcium-based phosphate binders are used as necessary to maintain serum levels of phosphorus below 7.0 mg/dL. Efficacy is evaluated by pre- and post-treatment comparisons of the two patient groups with regard to (a) direct measurements of intestinal calcium absorption, (b) total body calcium retention, (c) radial and spinal bone mineral density, and (d) determinations of serum calcium and osteocalcin. Safety is evaluated by regular monitoring of serum calcium.

Analysis of the clinical data shows that 1α-hydroxyvitamin D_2 significantly increases serum osteocalcin levels and intestinal calcium absorption, as determined by direct measurement using a double-isotope technique. Patients who are treated with 1α-hydroxyvitamin D_2 show normalized serum calcium levels, stable values for total

body calcium, and stable radial and spinal bone densities relative to baseline values. In contrast, patients who are treated with placebo show frequent hypocalcemia, significant reductions in total body calcium and radial and spinal bone density. An insignificant incidence of hypercalcemia is observed in the treated group.

Example 5: Double-blind Study in End Stage Renal Disease (ESRD) Patients Exhibiting Secondary Hyperparathyroidism

Up to 120 ESRD patients undergoing chronic hemodialysis are studied in a multicenter, double-blind, placebo-controlled study. The selected patients reside in two major metropolitan areas within the continental U.S., have ages between 20 and 75 years and have a history of secondary hyperparathyroidism. They have been on hemodialysis for at least four months, have a normal (or near normal) serum albumin, and have controlled serum phosphorus (often by using oral calcium-based phosphate binders).

On admission to the study, each patient is assigned at random to one of two treatment groups. One of these groups receives two consecutive 12-week courses of therapy with 1α-hydroxyvitamin D_2; the other receives a 12-week course of therapy with 1α-hydroxyvitamin D_2 followed, without interruption, by a 12-week course of placebo therapy. Each patient discontinues any 1α,25-dihydroxyvitamin D_3 therapy for eight weeks prior to initiating 1α-hydroxyvitamin D_2 therapy 4 μg three times per week. Throughout this eight-week washout (or control) period and the two subsequent 12-week treatment periods, patients are monitored weekly for serum calcium and phosphorus. Serum intact PTH is monitored weekly or biweekly, and bone-specific serum markers, serum vitamin D metabolites, serum albumin, blood chemistries, hemoglobin and hematocrit are monitored at selected intervals.

During the study, patients undergo routine hemodialysis (three times per week) using a 1.25 mM calcium dialysate and ingest calcium-based phosphate binders (such as calcium carbonate or calcium acetate) in an amount sufficient to keep serum phosphorous controlled (≤6.9 mg/dL). Patients who develop persistent mild hypercalcemia or mild hyperphosphatemia during the treatment periods reduce their 1α-hydroxyvitamin D_2 dosage to 4 μg three times per week (or lower). Patients who

develop marked hypercalcemia or marked hyperphosphatemia immediately suspend treatment. Such patients are monitored at twice weekly intervals until the serum calcium or phosphorus is normalized, and resume 1α-hydroxyvitamin D_2 dosing at a rate which is 4 μg three times per week (or lower).

During the eight-week washout period, the mean serum level of PTH increases progressively and significantly. After initiation of 1α-hydroxyvitamin D_2 dosing, mean serum PTH decreases significantly to less than 50% of pretreatment levels. Due to this drop in serum PTH, some patients need to reduce their dosage of 1α-hydroxyvitamin D_2 below 4 μg three times per week (or to even lower levels) to prevent excessive suppression of serum PTH. In such patients, exhibiting excessive suppression of serum PTH, transient mild hypercalcemia is observed, which is corrected by appropriate reductions in 1α-hydroxyvitamin D_2 dosages.

At the end of the first 12-week treatment period, mean serum PTH is in the desired range of 130 to 240 pg/mL and serum levels of calcium and phosphorus are normal or near normal for end stage renal disease patients. For the placebo group, at the end of the second 12-week treatment period (during which time 1α-hydroxyvitamin D_2 treatment is suspended and replaced by placebo therapy), mean serum PTH values markedly increase, reaching pretreatment levels. This study demonstrates that: (1) 1α-hydroxyvitamin D_2 is effective in reducing serum PTH levels, and (2) 1α-hydroxyvitamin D_2 is safer than currently used therapies, despite its higher dosages and concurrent use of high levels of oral calcium-based phosphate binder.

Example 6: Open Label Study of Elderly Subjects with Elevated Blood PTH from Secondary Hyperparathyroidism

Thirty elderly subjects with secondary hyperparathyroidism are enrolled in an open label study. The selected subjects have ages between 60 and 100 years and have elevated serum PTH levels (greater than the upper limit of young normal range). Subjects also have femoral neck osteopenia (femoral neck bone mineral density of $\leq$ 0.70 g/cm^2).

Subjects are requested to keep a diet providing approximately 500 mg calcium per day without the use of calcium supplements. For a twelve week treatment period, subjects self-administer orally 2.5 μg/day 1α-hydroxyvitamin D_2. At regular intervals throughout the treatment period, subjects are monitored for serum PTH levels, serum calcium and phosphorus, and urine calcium and phosphorus levels. Efficacy is evaluated by pre- and post-treatment comparisons of serum PTH levels. Safety is evaluated by serum and urine calcium and phosphorus values.

The administration of 1α-hydroxyvitamin D_2 is shown to significantly reduce PTH levels with an insignificant incidence of hypercalcemia, hyperphosphatemia, hypercalciuria and hyperphosphaturia.

Example 7: Double Blind Study of Elderly Subjects with Elevated Blood PTH from Secondary Hyperparathyroidism

A twelve month double-blind placebo-controlled clinical trial is conducted with forty subjects with secondary hyperparathyroidism. The selected subjects have ages between 60 and 100 years and have a history of secondary hyperparathyroidism. Subjects also have femoral neck osteopenia (femoral neck bone mineral density of $\leq$ 0.70 g/cm^2).

All subjects enter a six-week control period after which the subjects are randomized into two treatment groups: one group receives a constant dosage of 15 μg/day 1α,24-dihydroxyvitamin D_4; a dosage greater than 7.5 μg/day), and the other group receives a matching placebo. Both groups maintain a normal intake of dietary calcium without the use of calcium supplements. Efficacy is evaluated by pre- and post-treatment comparisons of the two patient groups with regard to (a) intact PTH (iPTH); (b) radial, femoral and spinal bone mineral density; and (c) bone-specific urine markers (e.g., pyridinium crosslinks). Safety is evaluated by (a) serum calcium and phosphorus, and (b) urine calcium and phosphorus.

Analysis of the clinical data shows that 1α,24-dihydroxyvitamin D_4 significantly decreases iPTH and bone specific urine markers. Subjects treated with this compound show normal serum calcium levels and stable radial and spinal bone

densities relative to baseline values. In contrast, patients treated with placebo show no reduction in iPTH and bone-specific urine markers. An insignificant incidence of hypercalcemia is observed in the treatment group.

Example 8: Open Label Study of Renal Patients with Elevated Blood PTH from Secondary and Tertiary Hyperparathyroidism

Fourteen renal patients enrolled in a clinical trial to study secondary hyperparathyroidism showed baseline iPTH levels greater than 1000 pg/mL (range: 1015-4706 pg/mL). These greatly elevated levels indicated a component of the disease as tertiary (i.e., glandular enlargement but continued presence of vitamin D receptors) to the gland as well as a component secondary to the loss of renal function. The initial dose of 1α-hydroxyvitamin D_2 (10 μg - 3 times/week) was increased (maximum, 20 μg - 3 times/ week) or decreased as necessary to attain and maintain iPTH in the range of 150-300 pg/mL. After 11-12 weeks of treatment, the iPTH levels of all but two of the patients had decreased to below 1000 pg/mL, and the iPTH levels in nine of the patients had decreased to below 510 pg/mL. There were no episodes of hypercalcemia with the patients during the study.

Example 9: Placebo-Controlled Study of Elderly Subjects with Elevated Blood PTH from 1,25dihydroxyvitamin D_3 Deficiency Associated with Age-Related Vitamin D Deficiency Syndrome

Sixty elderly subjects with elevated PTH from 1,25dihydroxyvitamin D_3 deficiency associated with age-related vitamin D deficiency (ARVDD) syndrome are enrolled in a blind placebo-controlled study. The selected subjects have ages between 50 and 80 years and have elevated serum PTH levels (greater than the upper limit of normal range) and depressed serum 1,25 dihydroxyvitamin D_3 levels (below the lower limit of normal range). Subjects also have femoral neck osteopenia (femoral neck bone mineral density of ≤ 0.70 g/cm^2).

Subjects are requested to keep a diet providing approximately 500 mg of calcium per day and are not to use calcium supplements. For a twelve month treatment period, thirty subjects self-administer orally 20 μg of 1α-hydroxyvitamin D_2 once per week; the other thirty subjects self-administer placebo capsules once per week. At

regular intervals throughout the treatment period, subjects are monitored for femoral bone mineral density; serum PTH levels, calcium, phosphorus and osteocalcin; and urine calcium, phosphorus and hydroxyproline levels. Other safety parameters monitored include blood urea nitrogen, serum creatinine and creatinine clearance. Efficacy is evaluated by pre- and post-treatment comparisons of serum PTH levels and femoral neck bone mineral density. Safety is evaluated by serum and urine calcium and phosphorus values.

The administration of 1α-hydroxyvitamin D_2 is shown to significantly reduce PTH levels and stabilize or increase femoral neck bone mineral density with an insignificant incidence of hypercalcemia and hyperphosphatemia, and to have no effect on kidney function parameters.

Example 10: Placebo-Controlled Study of Subjects with Elevated Blood PTH from Chronic Kidney Disease

The safety and efficacy of 1α-hydroxyvitamin D_2 (doxercalciferol) as a treatment for hyperparathyroidism associated with chronic kidney disease, specifically stages 1-4, was confirmed in a study involving 55 adults, ages 18-85 years, with mild to moderate chronic kidney disease. The subjects had plasma iPTH levels above 85 pg/mL and completed an eight-week baseline period and then 24 weeks of therapy with either orally administered doxercalciferol or placebo.

The initial dose of test drug was 2 capsules daily (totaling 1.0 μg for subjects randomized to doxercalciferol treatment), with increases in steps of one capsule per day permitted after four weeks. The maximum dosage was limited to 10 capsules per day (5.0 μg/day of doxercalciferol). Subjects were monitored at regular intervals for plasma iPTH, serum calcium and phosphorus, 24-hour and fasting urinary calcium, bone-specific serum markers, plasma total 1α,25-dihydroxyvitamin D, and routine blood chemistries and hematologies. The GFRs were measured prior to beginning the treatment and at study termination. No physical or biochemical differences were detectable between the two treatment groups prior to starting treatment.

During doxercalciferol treatment, mean plasma iPTH progressively decreased from baseline levels, reaching maximum suppression of 45.6% after 24 weeks ($p<0.001$). No corresponding changes in mean iPTH were observed during placebo treatment. Mean iPTH was lower in subjects receiving doxercalciferol versus placebo at all treatment weeks ($p<0.001$). No clinically significant differences in mean serum calcium, serum phosphorus and urine calcium or in rates of hypercalcemia, hyperphosphatemia and hypercalciuria were observed between treatment groups. Serum C- and N-telopeptides and bone-specific alkaline phosphate decreased with doxercalciferol treatment relative to baseline and placebo treatment ($p<0.01$). No differences between treatment groups were observed with regard to renal function and rates of adverse events. These data confirm that doxercalciferol can be used safely and effectively to control secondary hyperparathyroidism in chronic kidney disease patients.

The specific design of the study is summarized below.

Study Design: Pre-dialysis patients exhibiting secondary hyperparathyroidism associated with mild to moderate chronic kidney disease were recruited to participate in two multicenter, double-blinded, placebo-controlled studies conducted according to a common protocol. On enrollment, each subject was assigned, at random, in double-blinded fashion, to one of two treatment groups. Both treatment groups completed an 8-week Baseline Period (Weeks -8 to 0) and then underwent therapy with either orally administered doxercalciferol or placebo for a 24-week Treatment Period (Weeks 1 to 24). Irrespective of treatment group assignment, each subject discontinued any 1α,25-dihydroxyvitamin D_3 (1α,25dihydroxyvitamin D_3) therapy for the duration of the study. Throughout the Baseline Period and the subsequent Treatment Period, subjects were monitored at regular intervals for plasma iPTH, serum calcium, serum phosphorus, and 24-hour and fasting urinary calcium, phosphorus and creatinine. Routine blood chemistries and hematologies, bone-specific serum markers, and plasma total 1α,25dihydroxyvitamin D were also monitored at selected intervals. The GFRs were measured prior to beginning treatment and at termination.

Subjects: Subjects qualified for inclusion in the Baseline Period if they were aged 18 to 85 years, had mild to moderate chronic kidney disease, i.e., stages 1-4, with serum creatinine between 1.8 to 5.0/mg/dL (for men) or 1.6 to 4.0 mg/dL (for women), and had elevated plasma iPTH values (> 85 pg/mL). Subjects receiving ongoing treatment with estrogen were required to maintain the same estrogen dosing regimen throughout the study. Subjects who began dialysis treatment or underwent renal transplantation were required to prematurely terminate participation. Screened patients were excluded if they had a current history of alcohol or drug abuse, were pregnant, possibly pregnant, or nursing, had a history of idiopathic urinary calcium stone disease, had undergone renal transplant surgery, or had received treatment in the past year with anticonvulsants, oral steroids, bisphosphonates, fluoride, or lithium. Patients were also excluded who had hypercalcemia, hyperthyroidism, sarcoidosis, malignancy requiring chemotherapy, hormonal therapy and/or radiation treatment, chronic gastrointestinal disease (i.e., malabsorption, surgery affecting absorption, and chronic ulcerative colitis), hepatic impairment, or any other condition which may have put the patient at undue risk. Qualified, enrolled subjects were precluded from entering the Treatment Period and prematurely terminated participation if they exhibited, during the Baseline Period, a urinary protein $\geq$ 4 grams/24 hours associated with a serum albumin $\leq$ 3.5 grams/dL, a urine calcium level (at Week -4) above 150 mg/24 hours, or a markedly elevated serum creatinine value (> 5.0 mg/dL for men or > 4.0 mg/dL for women).

Randomization: The two studies were conducted under double-blind conditions in each geographical region. Assignments of subjects to the two treatment groups were made randomly, by geographical region, in order of enrollment. The randomization was accomplished in subgroups of size 10, 5 subjects assigned to each of the two treatment groups. The randomization was performed by an independent statistician using the Statistical Analysis System (SAS).

Test Products: 1α-hydroxyvitamin D_2 (available as doxercalciferol from Bone Care International) was formulated for oral administration as soft elastic gelatin capsules in units of 0.5 μg/capsule. Matching placebo capsules contained no doxercalciferol and were formulated from the same inactive ingredients in identical proportions. The inactive ingredients, in order of decreasing weight, were as follows:

fractionated coconut oil, gelatin, glycerin, titaninum dioxide, FD&C Red #40, D&C Yellow #10, ethanol and butylated hydroxyanisole (BHA). Both active and placebo capsules were orange in appearance, imprinted with the logo "BCI," and packaged in high-density polyethylene bottles, 50 capsules per bottle. The bottles were sealed with heat-induction tamper-evident seals and reusable child-resistant closures.

Dosing: The *initial* dose of test drug (doxercalciferol or placebo) was 2 capsules (totaling a 1.0 μg dose for subjects receiving doxercalciferol) every day before breakfast. This dosage was increased as necessary at monthly intervals, to suppress plasma iPTH levels by at least 30% from baseline. Dosage increases in steps of one capsule (0.5 μg) per day were permitted only if serum calcium was ≤ 9.6 mg/dL, serum phosphorus was ≤ 5.0 mg/dL, urine calcium was ≤ 200 mg/24 hours, and fasting urine calcium/urine creatinine ratio (urine Ca/Cr) was ≤ 0.25. The maximum dosage was limited to 10 capsules/day (5.0 μg/day of doxercalciferol or 35.0 μg/week).

Subjects suspended treatment if they developed moderate hypercalcemia (serum calcium >10.7 mg/dL corrected for serum albumin) and/or hypercalciuria (urine calcium >200 mg/24 hours or fasting urine Ca/Cr >0.25) during the Treatment Period. Such subjects were monitored weekly until the serum or urine calcium was normalized (≤10.2 mg/dL and/or ≤150 mg/24 hours or <0.25, respectively) and then resumed test drug dosing at a reduced rate with adjustment in their consumption of calcium-based phosphate binder, as appropriate. Subjects who developed mild hypercalcemia (serum calcium of 10.3 to 10.7 mg/dL) or hyperphosphatemia (serum phosphorus > 5.0 mg/dL) during the Treatment Period adjusted their consumption of calcium-based phosphate binder and/or reduced their test drug dosage. At the discretion of the site Investigator(s), the dosage of calcium-based phosphate binder was increased for subjects who presented with hypocalcemia (≤9.0 mg/dL).

If one of the dosage levels was not optimum for a given subject (i.e., maintaining plasma iPTH suppression ≤30% from baseline and > 15 pg/mL), the site Investigator(s) could vary the daily dosage administered according to a defined schedule (e.g., alternating dose of 1.0 μg with 0.5 μg) so that the total weekly dosage was optimized to the subject's needs.

Laboratory Procedures: Blood samples for analysis of serum chemistries, hematology and plasma iPTH were taken. Plasma iPTH samples were analyzed using a two-site immunoradiometric assay (IRMA).

The 24-hour urine samples for total protein and the 24-hour and spot urine samples for calcium, phosphorus, and creatinine were processed at the clinical sites. Urine samples for calcium, phosphorus and creatinine were acidified to a pH <2.0 using 6M HCL. Duplicate 4-mL aliquots of each urine sample were analyzed.

Blood samples for serum osteocalcin, bone-specific alkaline phosphatase, serum C-telopeptide (sCTx) and serum N-telopeptide (sNTx) were collected at the clinical sites. Triplicate 1 -mL aliquots of serum from each sample were analyzed. All samples obtained from each subject for a given parameter were analyzed together in the same batch.

Blood samples for serum total lα,25-dihydroxyvitamin D were analyzed. Serum samples from each subject were analyzed batchwise by means of radioreceptor assay following high-performance liquid chromatography.

GFR was determined at baseline and at termination by the Technetium or lothalamate (Glofil®) method. Each site used the same standardized method among all subjects at that study site. Serial blood and urine samples collected for GFR determination were analyzed on site or were sent on ice to the Cleveland Clinic in Cleveland, OH for analysis.

Data Treatment: Baseline values for all parameters were defined as the mean of the data collected during Weeks -4 and 0 of the Baseline Period. A positive response was defined as a reduction in mean plasma iPTH at Weeks 20 and 24 of $\chi \geq 30\%$ from baseline. At each time point, descriptive statistics were calculated, including *n*, mean, standard deviation, and standard error.

Also, the significance of the mean difference from baseline at each time point was assessed by paired t-test. This assessment was performed separately for each

treatment group, with missing values being replaced by the last observation carried forward (LOCF).

The treatment groups were compared at baseline and at each subsequent time point, and the significance of differences in means was assessed via two-sample t-test. For certain parameters, the data were recalculated as a percent of baseline and the analyses performed on these percentages instead of on the absolute data values.

All adverse events, whether observed by staff or offered by subjects, were recorded, stating the type, onset, duration, severity, relationship to the study medication, and required treatment, and their frequency determined for each treatment group. For each type of serious, unexpected adverse event (SAE) or drug-related adverse experience, the treatment groups were compared with respect to the percent of subjects experiencing the adverse effect, by Fisher's exact test.

The results of the study are summarized below:

Patients Ineligible at Screening: One hundred thirty-three subjects were screened and 72 subjects (54%) entered the Baseline Period. The 61 screen failures were comprised of 28 patients with insufficiently elevated plasma iPTH levels (≤ 85 pg/mL), 9 patients with serum creatinine levels which were outside of the allowed range, 12 patients with both plasma iPTH levels ≤ 85 pg/mL and serum creatinine levels which were outside of the allowed range, three patients due to treatment with oral steroids, one patient due to treatment with anticonvulsants in the preceding year, one patient with a history of idiopathic renal stone disease, one patient who died prior to enrollment, five patients who declined to participate, and one patient who resided too far outside of the local area for 6 months during the year.

Discontinued Subjects: Seventy-two subjects were enrolled into the Baseline Period. Of the 72 enrolled subjects, 55 (76%) were admitted into the Treatment Period of the study. Seventeen subjects (24%) terminated or were disqualified during the Baseline Period and were precluded from entering the Treatment Period. Of these, eight subjects exhibited urine total protein levels ≥ 4 grams/24 hours associated with a serum albumin ≤ 3.5 grams/dL, three subjects had a markedly elevated serum creatinine

(> 5.0 mg/dL for men or > 4.0 mg/dL for women) at either of the first two washout visits (Weeks -8 or -4), one subject demonstrated a serum creatinine level lower than that allowed by the inclusion criterion, three subjects declined to continue participating for personal reasons, and two experienced SAEs and were discontinued prematurely.

Nine subjects discontinued after entering and before completing the Treatment Period. One of the subjects relocated out of the area where the study was being conducted, one was found to have an intestinal malabsorption disorder, six experienced SAEs leading to discontinuation, and one experienced a non-serious adverse event leading to discontinuation.

Enrollment Demographics: The 55 subjects enrolled into the Treatment Period had physical and biochemical characteristics within the specified acceptable ranges and were otherwise qualified to participate in the study. These subjects had ages between 36 and 84 years (mean (± SE) = 64.6 ± 8.7 years). Forty-five subjects were men and 10 were women; 22 were African-Americans, 28 were Caucasians, four were Hispanics, and one was self-designated as "Other".

Dosing Compliance: Dosing compliance was above 80% in 52 of the 55 treated subjects. Dosing compliance was 71% in one subject randomized to placebo treatment and 79% in another subject randomized to active treatment. A third subject (active group) achieved only a 67% dosing compliance due to an adverse event unrelated to the drug. This subject discontinued participation in the study at Week 5.

Prescribed Dosages: The average (± SE) weekly prescribed dosages of test medication remained at the initial level of 2.0 capsules per day (1.0 μg for subjects receiving doxercalciferol) for the first month, as required by the study protocol. Thereafter, the mean dose in the active group increased, reaching 3.28 ± 0.39 capsules per day (1.61 ± 0.20 μg/day) by Week 24 (range: 1.0 to 3.5 μg/day). The mean dose in the placebo group also increased, reaching 5.13 ± 0.49 capsules per day by Week 24 (range: 2.0 to 10.0 capsules/day). The mean weekly prescribed dose trended higher in the placebo group from Week 6 through Week 24, with the difference reaching statistical significance at Weeks 20 and 24.

Decreases in test drug dosage occurred in some subjects. The primary reason for a decrease in prescribed dose was suppression of plasma iPTH by more than 30% from baseline level. In a few cases, dosing with test medication was suspended for intercurrent illness and restarted, when possible, at the same level.

Clinical Laboratory Assessments: Laboratory data included in this report are limited to those specified in the protocol. In some cases, additional laboratory data were obtained in order to monitor adverse events or confirm previous determinations. There was significant variation in subject laboratory measurements during the Baseline Period as well as during the Treatment Period within and outside the laboratory normal reference ranges. Such variation is expected in the subjects who have chronic kidney disease, since concomitant illness and complications related to renal disease are common. Laboratory abnormalities in individual subjects are not specifically discussed within this report unless attributed to the use of test medication or related to a serious adverse event.

Plasma iPTH: At baseline, mean (± SE) plasma PTH was 219.1 ± 22.3 pg/mL in the active group, with a range from 57 to 583 pg/mL and 171 ± 14 pg/mL in the placebo group, with a range from 63 to 330 pg/mL. There was no difference in baseline iPTH levels between treatment groups ($p = 0.07$). With initiation of doxercalciferol treatment, mean iPTH decreased to 165 ± 15 pg/mL at Week 4 ($p=0.001$ vs. baseline) and continued to decrease through Week 24, at which time the mean iPTH was 118 ± 17 pg/mL ($p < 0.001$ vs. baseline). In contrast, mean iPTH remained unchanged from baseline levels in the placebo group throughout the entire Treatment Period ($p \geq 0.17$), ending at 167 ± 15 at Week 24. Mean iPTH was significantly lower in subjects receiving doxercalciferol at Weeks 16-24 ($p < 0.05$ vs. placebo).

At the end of treatment, 20 (74%) of 27 subjects in the active group had achieved plasma iPTH suppression of ≥ 30 % from baseline. This positive end-point response was based on the mean of plasma iPTH determinations at Weeks 20 and 24. Three of the other seven subjects had iPTH reductions of 24.0%, 24.2%, and 19.6%, respectively, and one subject had an increase in iPTH of 3.9%. The remaining three subjects showed the following responses: one discontinued participation in Week 17, at

which time plasma iPTH was suppressed by 44.4%; another discontinued doxercalciferol treatment in Week 8, at which time plasma iPTH was suppressed by 27.9% from baseline; the third subject discontinued treatment in Week 5, at which time iPTH was increased by 22.8%. Only two (7.1%) of the 28 subjects treated with placebo achieved iPTH suppression of ≥30%.

Subjects randomized to doxercalciferol treatment exhibited progressively greater reductions in mean plasma iPTH during the course of the treatment period. Mean reduction of iPTH was 26.3% from baseline at Week 8, and 45.6% at Week 24. Mean iPTH reductions were significant ($p < 0.05$ vs. baseline) from Week 2 through Week 24. Subjects randomized to placebo treatment exhibited no changes in mean plasma iPTH expressed as a percentage of baseline ($p > 0.17$). Mean iPTH reduction was significantly greater in the active group at all Weeks except Week 6 ($p < 0.05$).

Serum Calcium and Phosphorus: Baseline mean (±SE) serum calcium level was 8.74 ± 0.12 mg/dL in the active group and 8.82 ± 0.13 mg/dL in the placebo group (p = NS). At Week 24, mean serum calcium was 9.14 ± 0.11 mg/dL in the active group and 8.95 ±0.13 mg/dL in the placebo group (p = NS). The increase in mean serum calcium from baseline was significant ($p < 0.05$) at Week 4 and at Weeks 12-24 in subjects treated with doxercalciferol, but not in subjects treated with placebo. Mean serum calcium differed between the treatment groups only at Week 20 ($p < 0.04$).

At baseline, mean (±SE) serum phosphorus level was 4.02 ± 0.15 mg/dL in the active group and 3.89 ± 0.13 mg/dL in the placebo group (p = NS). At Week 24, mean serum phosphorus was 4.27 ± 0.13 mg/dL in the active group and 3.92 ± 0.12 mg/dL in the placebo group (p=NS). The increases in mean serum phosphorus relative to baseline were not statistically significant in either treatment group, and mean serum phosphorus differed between groups only at Weeks 2 and 24 ($p < 0.05$).

Two episodes of hypercalcemia (determined as corrected serum calcium > 10.7 mg/dL) occurred in one subject receiving doxercalciferol treatment, with onsets in Week 4 and Week 16, respectively. The maximum serum calcium recorded during each of these episodes was 10.9 and 11.0 mg/dL, respectively, and the duration of each

episode was 5 and 8 weeks, respectively. This subject had a serum calcium of 10.4 mg/dL at baseline and had exhibited serum calcium as high as 10.7 mg/dL during the Baseline Period. One episode of hypercalcemia (defined as corrected serum calcium > 10.7 mg/dL) occurred in one subject receiving placebo treatment with onset in Week 12. The maximum serum calcium recorded during this episode was 10.9 mg/dL, and the duration of the episode was approximately 8 weeks. There were 9 episodes of hyperphosphatemia (defined as serum phosphorus > 5.0 mg/dL) in 9 subjects during the Baseline Period. During the Treatment Period, there were 15 episodes of hyperphosphatemia in 10 subjects receiving active treatment and 9 episodes in 8 subjects receiving placebo treatment. Only one episode of Ca X P > 65 occurred during the Treatment Period in one subject receiving placebo treatment.

Urine Calcium: No statistically significant changes relative to baseline in mean 24-hour urine calcium or in mean fasting urine (Ca/Cr) were observed in either the active or placebo group throughout the Treatment Period. No differences between treatment groups reached statistical significance during the Treatment Period.

No episodes of hypercalciuria (defined as 24-hour urine calcium excretion greater than 200 mg or fasting urine Ca/Cr ratio above 0.25) occurred during the Treatment Period in either the active or placebo groups.

Renal Function: A rising trend in mean BUN and in mean serum creatinine relative to baseline was noted in both treatment groups, but changes from baseline were occasionally significant ($p < 0.05$) only for the active group. However, no significant difference were observed between the groups during the Treatment Period.

GFR was measured at baseline and at the end of the study to compare the effects, if any, of active and placebo treatments on renal disease progression. Five subjects (18.5%) in the active treatment group and 8 subjects (28.6%) in the placebo group did not have a GFR measurement upon discontinuation or completion of the study. At baseline, mean GFR level was 33.5 ± 3.0 mL/min in the active group and 36.9 ± 3.3 mL/min in the placebo group. At Week 24, mean GFR was 29.7 ± 3.0 mL/min in the active group and 35.1 ± 3.3 mL/min in the placebo group. The

difference in GFR between groups at Week 24 was not statistically significant ($p = 0.24$).

Routine Chemistries and Hematologies: Mean alkaline phosphatase was reduced significantly from baseline in the active group at Weeks 16 and 24 ($p < 0.05$) but was not lowered in the placebo group during the Treatment Period. No other changes of clinical importance were observed from baseline or between groups for other routine laboratory parameters or in hematologies.

Serum Bone-Specific Markers and 1α,25-dihydroxyvitamin D: Subjects treated with doxercalciferol showed mean reductions in serum bone-specific alkaline phosphatase (BSAP) from baseline of 19.7 ± 3.7% by Week 16 ($p<0.01$) and 27.9 ± 4.6% by Week 24 ($p<0.01$). Subjects treated with placebo showed no change in BSAP relative to baseline at any treatment week. Mean BSAP reductions differed significantly between treatment groups from Weeks 8 to 24 ($p \leq 0.01$). Similar reductions were observed in serum N- and C-telopeptides with doxercalciferol treatment. Mean serum osteocalcin trended upward from baseline with doxercalciferol treatment by nearly 10% at Week 4 and then progressively declined from baseline by about 20% at Week 24. Mean serum total 1α,25-dihydroxyvitamin D levels increased significantly from baseline in the active group at all treatment weeks but did not differ significantly between groups at any treatment week.

Adverse Events (SAE): Twenty-seven SAEs occurred in 17 subjects during the conduct of the studies. All of the SAEs were determined to be unrelated to the test medication. Eighteen SAEs (67%) occurred when subjects were not being administered doxercalciferol. Three hundred fourteen (314) non-serious adverse events occurred during the conduct of both studies with 113 (36%) events occurring in subjects randomized to active treatment. One non-serious adverse event (0.3%), nausea of mild severity, reported in a subject who received doxercalciferol, was determined to be "possibly related" to the test medication. The remaining 313 non-serious events were determined to be "not related" to the test medication (95.6%), "probably not related" (3.5%), or "possibly related to another medicine" (0.6%). An analysis of the

incidence rates for serious and non-serious adverse events by treatment group showed no significant differences.

Concomitant Medications: The most commonly prescribed medications, prescribed to more than 50% of the study subjects, included furosemide, calcium carbonate, warfarin, insulin (all types) and epoetin alfa. Thirty of the 55 subjects (54.5%) who entered the treatment period received a calcium-based phosphate-binding product.

Thus, the results demonstrated that during doxercalciferol treatment, mean plasma iPTH progressively decreased from baseline levels, reaching maximum suppression of 45.6% after 24 weeks ($p<0.001$), while no corresponding changes in mean iPTH were observed during placebo treatment. Mean iPTH was lower in subjects receiving doxercalciferol versus placebo at all treatment weeks ($p<0.0001$). No clinically significant differences in mean serum calcium, serum phosphorus and urine calcium or in rates of hypercalcemia, hyperphosphatemia and hypercalciuria were observed between treatment groups. Serum C- and N-telopeptides and bone-specific alkaline phosphate decreased with doxercalciferol treatment relative to baseline and placebo treatment ($p<0.01$). No differences between treatment groups were observed with regard to renal function and rates of adverse events. These results of this study demonstrate that doxercalciferol is safe and effective in the treatment of secondary hyperparathyroidism in chronic kidney disease patients.

In summary, the present invention provides therapeutic methods for treating hyperparathyroidism associated chronic kidney disease, in particular stages 1-4. The methods are suitable for lowering elevated blood parathyroid hormone levels, or maintaining lowered, e.g., therapeutically lowered, blood PTH levels in subjects with hyperparathyroidism. The methods include administering an effective amount of an active vitamin D compound utilizing a variety of treatment protocols. The method in accordance with the present invention has significantly less resultant hypercalcemia and hyperphosphatemia.

In summary, the present invention provides therapeutic methods for treating hyperparathyroidism associated chronic kidney disease, in particular stages 1-4. The methods are suitable for lowering elevated blood PTH levels, or maintaining lowered, e.g., therapeutically lowered, blood PTH levels in subjects with hyperparathyroidism. The methods include administering an effective amount of an active vitamin D compound utilizing a variety of treatment protocols. The method in accordance with the present invention has significantly less resultant hypercalcemia and hyperphosphatemia.

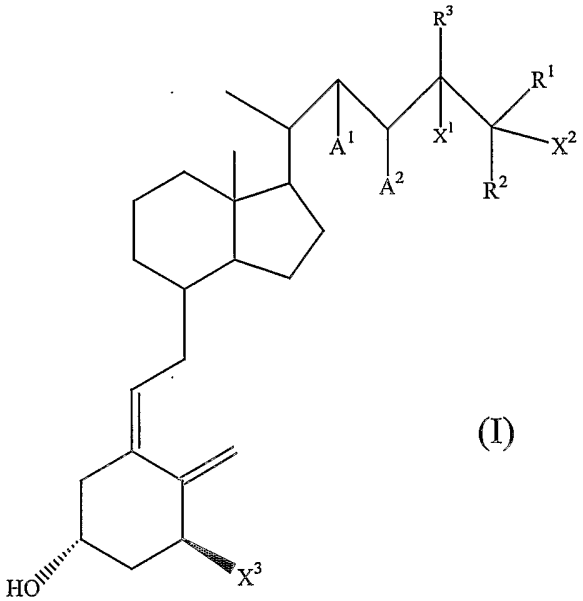
While the present invention has now been described and exemplified with some specificity, those skilled in the art will appreciate the various modifications, including variations, additions, and omissions that may be made in what has been described. Accordingly, it is intended that these modifications also be encompassed by the present invention and that the scope of the present invention be limited solely by the broadest interpretation that lawfully can be accorded the appended claims.

All patents, publications and references cited herein are hereby fully incorporated by reference. In case of conflict between the present disclosure and incorporated patents, publications and references, the present disclosure should control.

CLAIMS

1. A method of treating hyperparathyroidism associated with chronic kidney disease, comprising administering to a subject suffering therefrom an amount of a vitamin D compound sufficient to lower elevated or maintain lowered blood parathyroid hormone (PTH) levels, the subject having stage 1-4 chronic kidney disease.

2. A method in accordance with claim 1, wherein the vitamin D compound is a hydroxyvitamin D compound of formula (I):



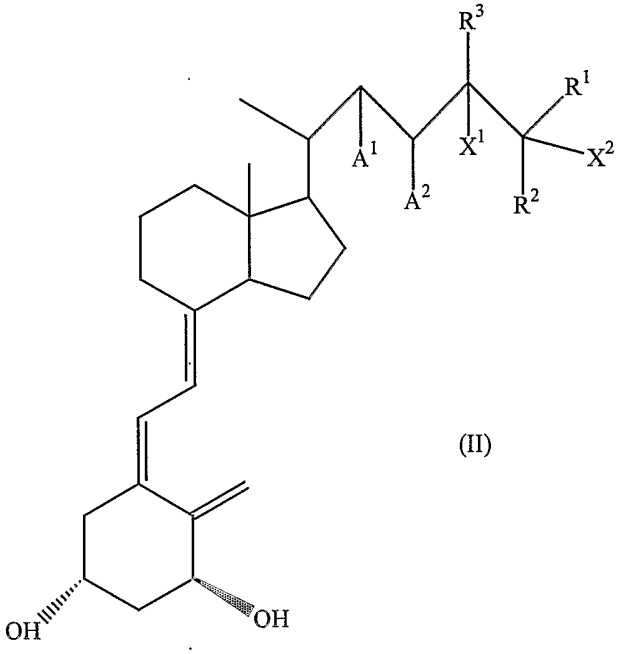
(I)

wherein A^1 and A^2 each are hydrogen or together represent a carbon-carbon bond, thus forming a double bond between C-22 and C-23; R^1 and R^2 are identical or different and are hydrogen, hydroxyl, lower alkyl, lower fluoroalkyl, O-lower alkyl, lower alkenyl, lower fluoroalkenyl, O-lower alkenyl, O-lower acyl, O-aromatic acyl, lower cycloalkyl with the proviso that both R^1 and R^2 cannot both be an alkenyl, or taken together with the carbon to which they are bonded, form a C_3-C_8 cyclocarbon ring; R^3 is lower alkyl, lower alkenyl, lower fluoroalkyl, lower fluoroalkenyl, O-lower alkyl, O-lower alkenyl, O-lower acyl, O-aromatic acyl or lower cycloalkyl; X^1 is hydrogen or hydroxyl; X^2 is

hydrogen or hydroxyl, or, is taken with R^1 or R^2, to constitute a double bond; X^3 is hydrogen or hydroxyl provided that at least one of X^1, X^2 and X^3 is hydroxyl.

3. A method in accordance with claim 2, wherein the compound of formula (I) is a hypocalcemic hydroxyvitamin D compound.

4. A method in accordance with claim 2, wherein the vitamin D compound is a 1α-hydroxyvitamin D compound of formula (II):



(II)

wherein A^1 and A^2 each are hydrogen or together represent a carbon-carbon bond, thus forming a double bond between C-22 and C-23; R^1 and R^2 are identical or different and are hydrogen, hydroxyl, lower alkyl, lower fluoroalkyl, O-lower alkyl, lower alkenyl, lower fluoroalkenyl, O-lower alkenyl, O-lower acyl, O-aromatic acyl, lower cycloalkyl with the proviso that both R^1 and R^2 cannot both be an alkenyl, or taken together with the carbon to which they are bonded, form a C_3-C_8 cyclocarbon ring; R^3 is lower alkyl, lower alkenyl, lower fluoroalkyl, lower fluoroalkenyl, O-lower alkyl, O-lower alkenyl, O-lower acyl, O-aromatic acyl or lower cycloalkyl; X^1 is hydrogen or hydroxyl, and X^2 is hydrogen or hydroxyl, or, is taken with R^1 or R^2, to constitute a double bond.

5. A method in accordance with claim 4, wherein X^2 is hydrogen and wherein R^1, R^2, and R^3 are each CH_3.

6. A method in accordance with claim 5 wherein the vitamin D compound is 1α hydroxyvitamin D_2, 1α,24-dihydroxyvitamin D_2 or 1α,24(S)- dihydroxyvitamin D_2.

7. A method in accordance with claim 6 wherein the vitamin D compound is 1α-hydroxyvitamin D_2.

8. A method in accordance with claim 6, wherein the vitamin D compound is 1α,24-dihydroxyvitamin D_2.

9. A method in accordance with claim 6, wherein the vitamin D compound is 1α,24(S)-dihydroxyvitamin D_2.

10. A method of treating hyperparathyroidism associated with chronic kidney disease, comprising administering to a subject suffering therefrom an amount of a vitamin D compound selected from the group consisting of 1α hydroxyvitamin D_2, 1α,24-dihydroxyvitamin D_2, 1α,24(S)-dihydroxyvitamin D_2, and combinations thereof sufficient to lower elevated or maintain lowered blood parathyroid hormone (PTH) levels.

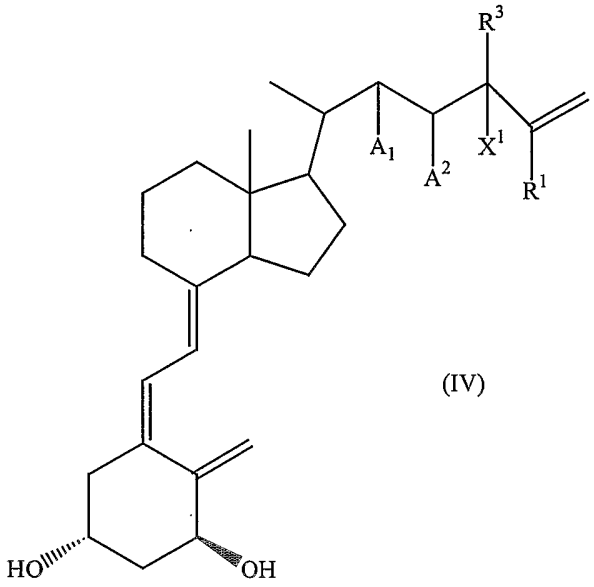
11. A method in accordance with claim 10, wherein the vitamin D compound is 1α-hydroxyvitamin D_2.

12. A method in accordance with claim 10, wherein the vitamin D compound is 1α,24-dihydroxyvitamin D_2.

13. A method in accordance with claim 10, wherein the vitamin D compound is 1α,24(S)-dihydroxyvitamin D_2.

14. A method of treating hyperparathyroidism secondary to chronic kidney disease, comprising administering to a patient suffering therefrom an amount of 1α-hydroxyvitamin D_2 sufficient to lower elevated or maintain lowered blood parathyroid hormone (PTH) levels.

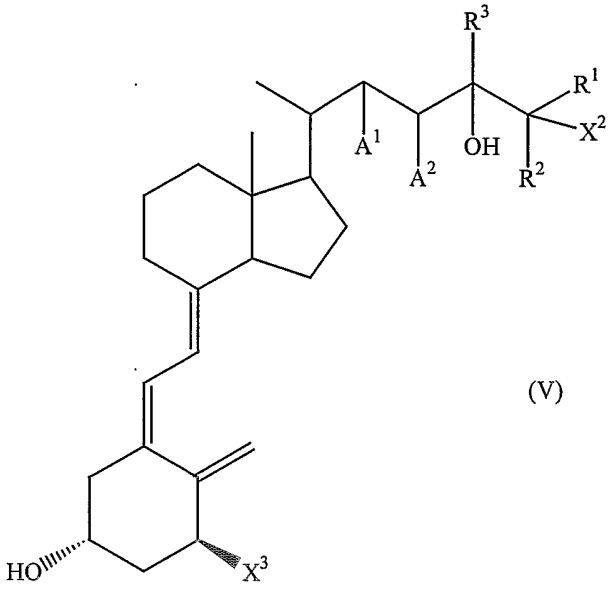
15. A method in accordance with claim 2, wherein the vitamin D compound is a 1α-hydroxy-25-ene-vitamin D_2 compound of formula (IV):



(IV)

wherein A^1 and A^2 are each either hydrogen or taken form a carbon-carbon double bond; X^1 is hydrogen or hydroxyl; and R^1 and R^3 are independently lower alkyl or lower fluoroalkyl.

16. A method in accordance with claim 2, wherein the vitamin D compound is a 24-hydroxyvitamin D compound of formula (V):



(V)

wherein A^1 and A^2 each are hydrogen or together represent a carbon-carbon bond, thus forming a double bond between C-22 and C-23; R^1 and R^2 are identical or different and are hydrogen, hydroxyl, lower alkyl, lower fluoroalkyl, O-lower alkyl, lower alkenyl, lower fluoroalkenyl, O-lower alkenyl, O-lower acyl, O-aromatic acyl, lower cycloalkyl with the proviso that both R^1 and R^2 cannot both be an alkenyl group, or taken together with the carbon to which they are bonded, form a C_3-C_8 cyclocarbon ring; R^3 is lower alkyl, lower alkenyl, lower fluoroalkyl, lower fluoroalkenyl, O-lower alkyl, O-lower alkenyl, O-lower acyl, O-aromatic acyl or lower cycloalkyl; X^3 is hydrogen or hydroxyl; X^2 is hydrogen or hydroxyl; or, is taken with R^1 or R^2, to constitute a double bond.

17. A method in accordance with claim 2 wherein the vitamin D compound is 1α-hydroxyvitamin D_4; 1α,25-dihydroxyvitamin D_2; 1α,24,25-trihydroxyvitamin D_2; 1α-hydroxy-25-ene-vitamin D_2; 1α-hydroxy-25-ene-vitamin D_4; 1α,24-dihydroxy-25-ene-vitamin D_2; 1α,24-dihydroxy-25-ene-vitamin D_4; 1α,25-dihydroxyvitamin D_4; 1α,24,25-trihydroxyvitamin D_4; 24-hydroxyvitamin D_2; or 24-hydroxyvitamin D_4.

18. A method in accordance with claim 2 wherein the vitamin D compound is 1α-hydroxy-25-ene-vitamin D_2 or 1α,24-dihydroxy-25-ene-vitamin D_2.

19. A method in accordance with claim 2 wherein the vitamin D compound is 1α-hydroxy-25-ene-vitamin D_2.

20. A method in accordance with claim 1, wherein the patients have a GFR of < 90 mL/min/1.73 m^2.

21. A method in accordance with claim 1, wherein the patients have a GFR of < 60 mL/min/1.73 m^2 but < 90 mL/min/1.73 m^2.

22. A method in accordance with claim 1, wherein the patients have a GFR of < 30 mL/min/1.73 m^2 but < 60 mL/min/1.73 m^2.

23. A method in accordance with claim 1, wherein the subject has a GFR of > 15 mL/min/1.73 m^2 but < 30 mL/min/1.73 m^2 .

24. A method in accordance with claim 1, wherein the subject has a GFR of 60-89 mL/min/1.73 m^2.

25. A method in accordance with claim 1, wherein the subject has a GFR of 30-59 mL/min/1.73 m^2.

26. A method in accordance with claim 1, wherein the subject has a GFR of 15-29 mL/min/1.73 m^2.

27. A method in accordance with claim 1, wherein the chronic kidney disease is stage 2 or stage 3.

28. A method in accordance with claim 1 wherein the amount of the vitamin D compound is administered parenterally or orally in combination with a pharmaceutically acceptable carrier.

29. A method in accordance with claim 28 wherein the amount of vitamin D compound is administered parenterally.

30. A method in accordance with claim 29 wherein the amount of vitamin D compound is administered intravenously.

31. A method in accordance with claim 28 wherein the amount of vitamin D compound is administered orally.

32. A method in accordance with claim 1 wherein the vitamin D compound is co-administered with a phosphate binder.

33. A method in accordance with claim 32 wherein the phosphate binder is a calcium-based binder.

34. A method in accordance with claim 32 wherein the phosphate binder is a non-calcium-based binder.

35. A method in accordance with claim 28 wherein the vitamin D compound is administered by intravenous injection, nasopharyngeal or mucosal absorption, or transdermal absorption.

36. A method in accordance with claim 2 wherein the vitamin D compound is administered in a weekly dose of about 0.5 μg to about 100 μg.

37. A method in accordance with claim 2 wherein the vitamin D compound is administered in a weekly dose of about 0.5 μg to about 25 μg.

38. A method in accordance with claim 36, wherein the vitamin D compound is in a 0.5 μg per unit dosage form.

39. A method in accordance with claim 36, wherein the vitamin D compound is in a 2.5 μg per unit dosage form.

40. A method in accordance with claim 36, wherein the vitamin D compound is in a 1 μg per unit dosage form.

41. The method of claim 2 wherein the vitamin D compound is administered in a weekly dose of about 1 μg to about 300 μg.

42. The method of claim 2 wherein the vitamin D compound is administered in a weekly dose of about 30 μg to about 200 μg.

43. A method in accordance with claim 2 wherein the vitamin D compound is administered in a weekly dose of about 30 μg to about 100 μg.

44. A method in accordance with claim 2 wherein the vitamin D compound is administered in a bi-weekly dose of about 30 μg to about 100 μg.

45. A method in accordance with claim 2 wherein the vitamin D compound is administered in a tri-weekly dose of about 30 μg to about 100 μg.

46. A method in accordance with claim 2 wherein the vitamin D compound is administered in a monthly dose of about 30 μg to about 100 μg.

47. A method in accordance with claim 2 wherein the vitamin D compound is co-administered with at least one agent characterized by said agent's ability to reduce loss of bone mass, to reduce loss of bone mineral content, to modulate calcium-sensing receptor, or to suppress parathyroid activity in the subject.

48. A method in accordance with claim 50 wherein the agent is a second vitamin D compound, a conjugated estrogen, sodium fluoride, a bisphosphonate, cobalamin, pertussin toxin, boron, a calcimimetic, a PTH antagonist, or a PTH antibody.

49. A method in accordance with claim 50 wherein the agent is a calcimimetic.

50. A method in accordance with claim 50 wherein the vitamin D compound is administered before, after or concurrently with the other agent.

51. A method in accordance with claim 2 wherein the amount of the vitamin D compound is administered parenterally or orally in combination with a pharmaceutically acceptable carrier.

52. A method in accordance with claim 51 wherein the amount of vitamin D compound is administered parenterally.

53. A method in accordance with claim 52 wherein the vitamin D compound is administered in depot form.

54. A method in accordance with claim 51 wherein the amount of vitamin D compound is administered intravenously.

55. A method in accordance with claim 50 wherein the vitamin D compound is administered orally.

56. A method in accordance with claim 50 wherein the vitamin D compound is co-administered with a phosphate binder.

57. A method in accordance with claim 56 wherein the phosphate binder is a calcium-based binder.

58. A method in accordance with claim 56 wherein the phosphate binder is a non-calcium-based binder.

59. The method of claim 2 wherein the vitamin D compound is administered is by intravenous injection, nasopharyngeal or mucosal absorption, or transdermal absorption.

60. A combined pharmaceutical preparation, comprising a vitamin D compound and another therapeutic agent, the preparation being adapted for the administration of the vitamin D on an episodic basis, and the administration of the the other therapeutic agent on a daily or episodic basis, to a subject having hyperparathyroidism secondary to chronic kidney disease, the therapeutic agent being a bone agent, a calcimimetic, a PTH or PTHrP antogonist, or a PTH receptor antibody or a combination thereof.

61. A pharmaceutical preparation in accordance with claim 60, wherein the vitamin D compound is 1α-hydroxyvitamin D_2, 1α,24-dihydroxyvitamin D_2, 1α-hydroxy-25-ene-vitamin D_2, or 1α,24 -dihydroxy-25-ene-vitamin D.

62. A pharmaceutical preparation in accordance with claim 61 wherein the vitamin D is 1α-hydroxyvitamin D_2.

63. A pharmaceutical preparation in accordance with claim 61 wherein the vitamin D is 1α,24-dihydroxyvitamin D_2.

64. A pharmaceutical preparation in accordance with claim 61 wherein the vitamin D is 1α-hydroxy-25-ene-vitamin D_2 or 1α,24-dihydroxy-25-ene-vitamin D.

65. A pharmaceutical product, comprising (i) a plurality of containers therein, at least one of the containers containing a vitamin D compound, and at least one of the containers containing another therapeutic agent, and (ii) instructions for co-administering the vitamin D compound and the other therapeutic agent to a subject having hyperparathyroidism secondary to chronic kidney disease, the other therapeutic agent being a bone agent, a calcimimetic, a PTH antagonist or antibody or combination thereof.

66. A pharmaceutical product in accordance with claim 65, wherein the instructions comprise a notice in a form prescribed by a governmental regulatory agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the vitamin D compound for human or veterinary administration to treat hyperparathyroidism.

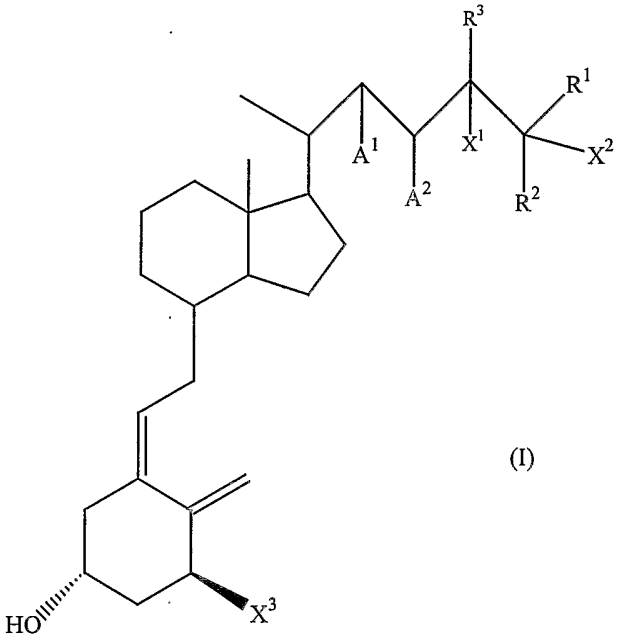
67. The pharmaceutical product in accordance with claim 65, wherein the vitamin D compound is 1α-hydroxyvitamin D_2, 1α,24-dihydroxyvitamin D_2, 1α-hydroxy-25-ene-vitamin D or 1α,24-dihydroxy-25-ene-vitamin D.

68. The pharmaceutical packaging in accordance with claim 65 wherein the vitamin D compound is 1α-hydroxyvitamin D_2.

69. The pharmaceutical packaging in accordance with claim 65 wherein the vitamin D compound is 1α-hydroxy-25-ene-vitamin D.

70. The pharmaceutical packaging in accordance with claim 65 wherein the vitamin D compound is 1α,24-dihydroxyvitamin D_2.

71. A packaged composition, comprising a vitamin D compound having the formula (I) as follows:



(I)

wherein A^1 and A^2 each are hydrogen or together represent a carbon-carbon bond, thus forming a double bond between C-22 and C-23; R^1 and R^2 are identical or different and are hydrogen, hydroxyl, lower alkyl, lower fluoroalkyl, O-lower alkyl, lower alkenyl, lower fluoroalkenyl, O-lower alkenyl, O-lower acyl, O-aromatic acyl, lower cycloalkyl with the proviso that both R^1 and R^2 cannot both be an alkenyl, or taken together with the carbon to which they are bonded, form a C_3-C_8 cyclocarbon ring; R^3 is lower alkyl, lower alkenyl, lower fluoroalkyl, lower fluoroalkenyl, O-lower alkyl, O-lower alkenyl, O-lower acyl, O-aromatic acyl or lower cycloalkyl; X^1 is hydrogen or hydroxyl; X^2 is

hydrogen or hydroxyl, or, is taken with R^1 or R^2, to constitute a double bond; X^3 is hydrogen or hydroxyl provided that at least one of X^1, X^2 and X^3 is hydroxyl; and

instructions for use of the composition for treating and preventing hyperparathyroidism secondary to chronic kidney disease, the kidney disease being stages 1-4.

72. A packaged composition in accordance with claim 55, wherein the vitamin D compound is 1α-hydroxy-25-ene-vitamin D, 1α,24-dihydroxy-25-ene-vitamin D, 1α-dihydroxyvitamin D_2, 1α,24-dihydroxyvitamin D_2, or 1α,24(S)-dihydroxyvitamin D_2.

73. A packaged composition in accordance with claim 72 wherein the vitamin D compound is 1α-hydroxy-25-ene-vitamin D.

74. A packaged composition in accordance with claim 72 wherein the vitamin D compound is 1α-hydroxyvitamin D_2

75. A packaged composition in accordance with claim 72 wherein the vitamin D compound is 1α,24-dihydroxyvitamin D_2.

76. A packaged composition in accordance with claim 72 wherein the vitamin D compound is 1α,24(S)-dihydroxyvitamin D

77. A method for reducing an excessive PTH secretion from parathyroid cells and treating hyperparathyroidism comprising administering the packaged composition of claim 55 to a patient in need of such reduction.

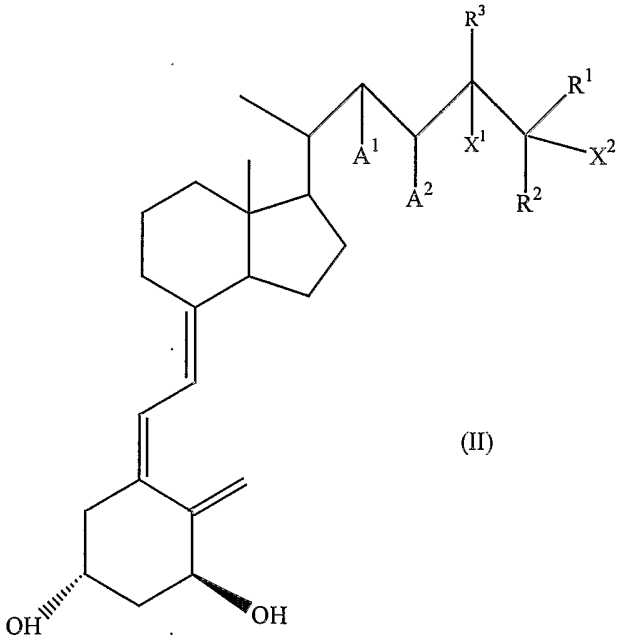
78. A method for inhibiting PTH secretion from parathyroid cells, comprising administering the packaged composition of claim 71.

79. A method of lowering elevated or maintaining lowered blood PTH level comprising, parenterally administering to a subject suffering therefrom the packaged composition of claim 71.

80. A method as set forth in claim 79, wherein the elevated blood PTH level is due to secondary hyperparathyroidism.

81. A method of treating hyperparathyroidism secondary to chronic kidney disease, the kidney disease being stages 1-4, comprising administering to a subject suffering therefrom an amount of a vitamin D compound which includes at least one of 1α,24-dihydroxy-25-ene-vitamin D, 1α-hydroxy-25-ene-vitamin D, 1α-hydroxyvitamin D_2, 1α,24-dihydroxyvitamin D_2, and 1α,24(S)-dihydroxyvitamin D_2 sufficient to lower elevated or maintain lowered blood PTH levels.

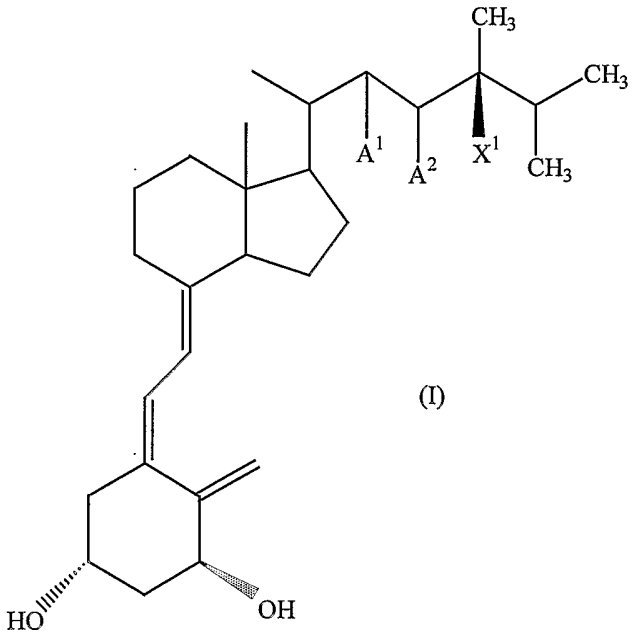
82. A method of treating hyperparathyroidism secondary to chronic kidney disease, the kidney disease being stages 1-4, comprising administering to a subject suffering therefrom an amount of a 1α-hydroxyvitamin D compound of formula (II):



(II)

wherein A^1 and A^2 each are hydrogen or together represent a carbon-carbon bond, thus forming a double bond between C-22 and C-23; R^1 and R^2 are identical or different and are hydrogen, hydroxyl, lower alkyl, lower fluoroalkyl, O-lower alkyl, lower alkenyl, lower fluoroalkenyl, O-lower alkenyl, O-lower acyl, O-aromatic acyl, lower cycloalkyl with the proviso that both R^1 and R^2 cannot both be an alkenyl, or taken together with the carbon to which they are bonded, form a C_3-C_8 cyclocarbon ring; R^3 is lower alkyl, lower alkenyl, lower fluoroalkyl, lower fluoroalkenyl, O-lower alkyl, O-lower alkenyl, O-lower acyl, O-aromatic acyl or lower cycloalkyl; X^1 is hydrogen or hydroxyl, and X^2 is hydrogen or hydroxyl, or, is taken with R^1 or R^2, to constitute a double bond.

83. A method of lowering or maintaining lowered serum parathyroid hormone level in a subject suffering from hyperparathyroidism secondary to chronic kidney disease wherein the subject has a glomerular filtration rate (GFR) of < 90 mL/min/1.73 m^2 but ≥ 15 mL/min/1.73 m^2, comprising administering to the subject an effective amount of a vitamin D analog to lower elevated and maintain lowered serum parathyroid hormone levels, the analog comprising a compound of formula (I):



(I)

wherein A^1 and A^2 are each either hydrogen, or together represent a carbon-carbon double bond; and X^1 is either hydrogen or hydroxyl.