METHODS FOR IDENTIFYING COMPOUNDS USEFUL FOR INHIBITING FARNESYL DIPHOSPHATE SYNTHASE

The present invention relates to methods for identifying compounds useful as inhibitors of farnesyl diphosphate synthase. More particularly, the compounds so identified are useful for inhibiting bone resorption. The present invention also relates to methods for inhibiting bone resorption in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of a farnesyl diphosphate synthase inhibitor.
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TITLE OF THE INVENTION
METHODS FOR IDENTIFYING COMPOUNDS USEFUL FOR INHIBITING
FARNESYL DIPHOSPHATE SYNTHASE

5  BRIEF DESCRIPTION OF THE INVENTION
   The present invention relates to methods for identifying compounds
   useful as inhibitors of farnesyl diphosphate synthase. More particularly, the
   compounds so identified are useful for inhibiting bone resorption. The present
   invention also relates to methods for inhibiting bone resorption in a mammal
   comprising administering to a mammal in need thereof a therapeutically effective
   amount of a farnesyl diphosphate synthase inhibitor.

BACKGROUND OF THE INVENTION
   A variety of disorders in humans and other mammals involve or are
   associated with abnormal bone resorption. Such disorders include, but are not limited
   to, osteoporosis, glucocorticoid induced osteoporosis, Paget’s disease, abnormally
   increased bone turnover, periodontal disease, tooth loss, bone fractures, rheumatoid
   arthritis, periprosthetic osteolysis, osteogenesis imperfecta, metastatic bone disease,
   hypercalcemia of malignancy, and multiple myeloma. One of the most common of
   these disorders is osteoporosis, which in its most frequent manifestation occurs in
   postmenopausal women. Osteoporosis is a systemic skeletal disease characterized by
   a low bone mass and microarchitectural deterioration of bone tissue, with a
   consequent increase in bone fragility and susceptibility to fracture. Osteoporotic
   fractures are a major cause of morbidity and mortality in the elderly population. As
   many as 50% of women and a third of men will experience an osteoporotic fracture.
   A large segment of the older population already has low bone density and a high risk
   of fractures. There is a significant need to both prevent and treat osteoporosis and
   other conditions associated with bone resorption. Because osteoporosis, as well as
   other disorders associated with bone loss, are generally chronic conditions, it is
   believed that appropriate therapy will typically require chronic treatment.

   Normal bone physiology involves a process wherein bone tissue is
   continuously being turned over by the processes of modeling and remodeling. In
   other words, there is normally an appropriate balance between resorption of existing
   bone tissue and the formation of new bone tissue. The exact mechanism underlying
   the coupling between bone resorption and formation is still unknown. However, an
imbalance in these processes is manifested in various disease states and conditions of
the skeleton.

Two different types of cells called osteoblasts and osteoclasts are
involved in the bone formation and resorption processes, respectively. See H. Fleisch,
_Bisphosphonates In Bone Disease, From The Laboratory To The Patient_, 3rd Edition,
Parthenon Publishing (1997), which is incorporated by reference herein in its entirety.

Osteoblasts are cells that are located on the bone surface. These cells
secrete an osseous organic matrix, which then calcifies. Substances such as fluoride,
parathyroid hormone, and certain cytokines such as prostaglandins are known to
provide a stimulatory effect on osseblast cells. However, an aim of current research
is to develop therapeutic agents that will selectively increase or stimulate the bone
formation activity of the osteoblasts.

Osteoclasts are usually large multinucleated cells that are situated
either on the surface of the cortical or trabecular bone or within the cortical bone. The
osteoclasts resorb bone in a closed, sealed-off microenvironment located between the
cell and the bone. The recruitment and activity of osteoclasts is known to be
influenced by a series of cytokines and hormones. It is well known that
bisphosphonates are selective inhibitors of osteoclastic bone resorption, making these
compounds important therapeutic agents in the treatment or prevention of a variety of
systemic or localized bone disorders caused by or associated with abnormal bone
resorption. However, despite the utility of bisphosphonates, there remains the desire
amongst researchers to develop additional therapeutic agents for inhibiting the bone
resorption activity of osteoclasts.

The mevalonate biosynthetic pathway is an important pathway of
osteoclast function. This pathway is involved in the biosynthesis of cholesterol and
of isoprenoids, some of which are used in protein prenylation. The enzyme farnesyl
diphosphate synthase (FPP synthase) mediates the synthesis of farnesyl diphosphate
by catalyzing the sequential condensation of two molecules of isopentenyl
diphosphate (IPP) with one molecule of dimethylallyl diphosphate (DMAPP) to
produce geranyl diphosphate (GPP) and then farnesyl diphosphate (FPP).

Farnesyl diphosphate is essential for the farnesylation of several
proteins required for cytoskeletal organization and vesicular traffic. Interference with
the function of these proteins can also lead to apoptosis, i.e. programmed cell death.
Therefore, farnesyl diphosphate synthase, the enzyme involved in the synthesis of
farnesyl diphosphate, is essential for the proper biological functioning of the osteoclasts.

It would be highly desirable to identify and develop compounds useful as selective inhibitors of farnesyl diphosphate synthase in the osteoclasts. Such inhibitors would be useful for inhibiting ostetoclast function, thereby inhibiting undesired bone resorption and its manifestations.

In the present invention it is surprising found that nitrogen-containing bisphosphonates such as alendronate and risedronate are specific nanomolar inhibitors of farnesyl diphosphate synthase. It is also surprisingly found that it is possible to identify other compounds useful as farnesyl diphosphate synthase inhibitors.

In the present invention it is also found that inhibitors of farnesyl diphosphate synthase are useful for inhibiting bone resorption. Without being limited by theory, it is believed that these inhibitors are responsible for inhibiting the bone resorption activity of the osteoclasts.

It is an object of the present invention to provide methods for identifying compounds useful as farnesyl diphosphate synthase inhibitors.

It is an object of the present invention to provide methods for inhibiting farnesyl diphosphate synthase in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of a farnesyl diphosphate synthase inhibitor having an IC50 value from about 0.01 nanoM to about 1000 nanoM.

It is an object of the present invention to provide methods for inhibiting bone resorption in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of a farnesyl diphosphate synthase inhibitor having an IC50 value from about 0.01 nanoM to about 1000 nanoM.

It is another object of the present invention to provide methods for treating or reducing the risk of contracting a disease state or condition mediated by farnesyl diphosphate synthase in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of a farnesyl diphosphate synthase inhibitor having an IC50 value from about 0.01 nanoM to about 1000 nanoM.

It is another object of the present invention to provide methods for treating or reducing the risk of contracting a disease state or condition involving or affecting bone tissue in a mammal comprising administering to a mammal in need
thereof a therapeutically effective amount of a farnesyl disphosphate synthase inhibitor having an IC50 value from about 0.01 nanoM to about 1000 nanoM.

It is an object of the present invention to provide methods for inhibiting farnesyl diphosphate synthase activity in a mammal comprising administering to a mammal in need thereof comprising administering to a mammal in need thereof a therapeutically effective amount of the combination of: (a) a farnesyl disphosphate synthase inhibitor having an IC50 value from about 0.01 nanoM to about 1000 nanoM, and (b) a bisphosphonate active.

It is an object of the present invention to provide methods for inhibiting bone resorption in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of the combination of: (a) a farnesyl disphosphate synthase inhibitor having an IC50 value from about 0.01 nanoM to about 1000 nanoM, and (b) a bisphosphonate active.

It is an object of the present invention to provide methods for treating or reducing the risk of contracting a disease state or condition mediated by farnesyl diphosphate synthase comprising administering to a mammal in need thereof a therapeutically effective amount of the combination of: (a) a farnesyl disphosphate synthase inhibitor having an IC50 value from about 0.01 nanoM to about 1000 nanoM, and (b) a bisphosphonate active.

It is an object of the present invention to provide methods for treating or reducing the risk of contracting a disease state or condition involving or affecting bone tissue in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of the combination of: (a) a farnesyl disphosphate synthase inhibitor having an IC50 value from about 0.01 nanoM to about 1000 nanoM, and (b) a bisphosphonate active.

It is another object of the present invention to provide pharmaceutical compositions comprising a therapeutically effective amount of a farnesyl disphosphate synthase inhibitor having an IC50 value from about 0.01 nanoM to about 1000 nanoM.

It is another object of the present invention to provide pharmaceutical compositions comprising a therapeutically effective amount of the combination of: (a) a farnesyl disphosphate synthase inhibitor having an IC50 value from about 0.01 nanoM to about 1000 nanoM and (b) a bisphosphonate active.

These and other objects will become readily apparent from the detailed description which follows.
SUMMARY OF THE INVENTION

The present invention relates to methods for identifying compounds useful as farnesyl diphotphate synthase inhibitors, comprising:

5 a). contacting a putative farnesyl diphotphate synthase inhibitor with a farnesyl diphotphate synthase solution, and

b). determining the farnesyl diphotphate synthase activity of said solution with a farnesyl diphotphate synthase solution not contacted with said putative inhibitor.

The present invention also relates to methods for inhibiting farnesyl diphotphate synthase in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of a farnesyl diphotphate synthase inhibitor having an IC50 value from about 0.01 nanoM to about 1000 nanoM.

The present invention also relates to methods for inhibiting bone resorption in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of a farnesyl diphotphate synthase inhibitor having an IC50 value from about 0.01 nanoM to about 1000 nanoM.

The present invention also relates to methods for treating or reducing the risk of contracting a disease state or condition mediated by farnesyl diphotphate synthase in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of a farnesyl diphotphate synthase inhibitor having an IC50 value from about 0.01 nanoM to about 1000 nanoM.

The present invention also relates to methods for treating or reducing the risk of contracting a disease state or condition involving or affecting bone tissue in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of a farnesyl diphotphate synthase inhibitor having an IC50 value from about 0.01 nanoM to about 1000 nanoM.

The present invention also relates to methods for inhibiting farnesyl diphotphate synthase activity in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of the combination of: (a) a farnesyl diphotphate synthase inhibitor having an IC50 value from about 0.01 nanoM to about 1000 nanoM, and (b) a bisphosphonate active.

The present invention also relates to methods for inhibiting bone resorption in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of the combination of: (a) a farnesyl diphotphate
synthase inhibitor having an IC\textsubscript{50} value from about 0.01 nanoM to about 1000 nanoM, and (b) a bisphosphonate active.

The present invention also relates to methods for treating or reducing the risk of contracting a disease state or condition mediated by farnesyl diphosphate synthase comprising administering to a mammal in need thereof a therapeutically effective amount of the combination of: (a) a farnesyl diphosphate synthase inhibitor having an IC\textsubscript{50} value from about 0.01 nanoM to about 1000 nanoM, and (b) a bisphosphonate active.

The present invention also relates to methods for treating or reducing the risk of contracting a disease state or condition involving or affecting bone tissue in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of the combination of: (a) a farnesyl diphosphate synthase inhibitor having an IC\textsubscript{50} value from about 0.01 nanoM to about 1000 nanoM, and (b) a bisphosphonate active.

The present invention also relates to pharmaceutical compositions comprising a therapeutically effective amount of a farnesyl diphosphate synthase inhibitor having an IC\textsubscript{50} value from about 0.01 nanoM to about 1000 nanoM.

The present invention also relates to pharmaceutical compositions comprising a therapeutically effective amount of the combination of: (a) a farnesyl diphosphate synthase inhibitor having an IC\textsubscript{50} value from about 0.01 nanoM to about 1000 nanoM and (b) a bisphosphonate active.

The present invention also relates to the use of such compositions in the manufacture of a medicament for the methods disclosed herein.

All percentages and ratios used herein, unless otherwise indicated, are by weight. The invention hereof can comprise, consist of, or consist essentially of the essential as well as optional ingredients, components, and methods described herein.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods for identifying compounds useful as farnesyl diphosphate synthase inhibitors and for inhibiting this enzyme with the compounds so identified.

The mevalonate biosynthetic pathway is an important pathway of osteoclast function. This pathway is involved in the biosynthesis of cholesterol and of isoprenoids, some of which are used in protein prenylation. It would be highly
desirable to identify and develop compounds useful as selective inhibitors of farnesyl diphosphate synthase in the osteoclasts. Such inhibitors would be useful for inhibiting ostetoclast function, thereby inhibiting undesired bone resorption and its manifestations in various disease states and conditions.

Farnesyl diphosphate synthase is also known by the following names: prenyltransferase, dimethylallyltransferase, and dimethylallyl diphosphate:isopentenyl diphosphate dimethylallyltransferase.

Alendronate (4-amino-1-hydroxybutyridene-1,1-bisphosphonate) is a potent inhibitor of bone resorption, used in the treatment and prevention of osteoporosis and other bone diseases. Without being limited by theory, it is believed that alendronate and other bisphosphonates are readily adsorbed onto the bone surface and are selectively taken up by osteoclasts during bone resorption. It is generally accepted that at the cellular level bisphosphonates act by inhibiting osteoclast activity. The effects of alendronate monosodium trihydrate and of the HMG-CoA reductase inhibitor, lovastatin, on osteoclasts in culture is known. Osteoclast formation and bone resorption are inhibited by alendronate monosodium trihydrate and by lovastatin. Mevalonic acid lactone or geranylgeraniol reverse the effects of lovastatin but only geranylgeraniol reverses the effects of alendronate, thereby supporting the hypothesis that alendronate monosodium trihydrate induces apoptosis by inhibiting protein prenylation via inhibition of the mevalonate pathway prior to the formation of geranylgeranyl diphosphate.

It is known that several nitrogen-containing bisphosphonates, including YM 175, EB 1053 and PHPBP, are potent, nanomolar inhibitors of rat liver squalene synthase. See, Amin D, Cornell SA, Gustafson SK, Needle SJ, Ullrich JW, Bilder GE, and Perrone MH (1992) J. Lipid Res. 33: 1657-1663, which is incorporated by reference herein in its entirety. On the other hand, alendronate and pamidronate, two other nitrogen containing bisphosphonates, have comparatively little effect on squalene synthase. Alendronate and pamidronate, however, block sterol synthesis, as measured by the incorporation of C-MVA into sterol in a rat liver-cell free system, with respective IC50's of 168 nM and 420 nM, suggesting that these compounds inhibit another enzyme in the pathway. Without being limited by theory, it is therefore believed that nitrogen-containing bisphosphonates are potent inhibitors of any of several enzymes involved in isoprenoid synthesis.
The synthesis of geranylgeranyl diphosphate from mevalonate involves six enzymes, mevalonate (MVA) kinase (EC 2.7.1.36), phosphomevalonate (MVAP) kinase (EC 2.7.4.2), mevalonate diphosphate (MVAPP) decarboxylase, isopentenyl diphosphate (IPP) isomerase (EC 5.3.3.2), farnesyl diphosphate (FPP) synthase (EC 2.5.1.1), and geranylgeranyl diphosphate (GGPP) synthase. Farnesyl protein transferase (FTase), geranylgeranyl protein transferase I (GGTase I) and geranylgeranyl protein transferase II (GGTase II) are the enzymes responsible for prenylating proteins. These transferases are not inhibited by alendronate. It is found in the present invention that nitrogen-containing bisphosphonates such as alendronate monosodium trihydrate are specific and potent inhibitors of farnesyl diphosphate synthase. This specificity is seen in that high micromolar concentrations of alendronate monosodium trihydrate do not inhibit any other enzyme in the mevalonate pathway.

Alendronate inhibition of osteoclast activity in vitro is prevented by geranylgeraniol, consistent with alendronate inhibition of the mevalonate pathway, resulting in a decrease in cellular GGPP. The surprising findings in the present invention show that alendronate, which is a nitrogen-containing bisphosphonate, is a specific inhibitor of FPP synthase and that it does not inhibit any other enzymes involved in the conversion of MVA to GGPP. The present invention also surprisingly demonstrates that alendronate and other nitrogen-containing bisphosphonates inhibit farnesyl diphosphate synthase and lower the concentration of the isoprenylation substrate farnesyl diphosphate and the downstream product geranylgeranyl diphosphate. These lipids are essential for the prenylation of several proteins including GTP binding proteins of around 20 KDa, including those belonging to the rho, rac, Cdc42 and rab families. These proteins are essential for cytoskeletal organization and vesicular traffic. Inactivation of rhoA, for example, causes osteoclast inactivation, and rab is implicated in vesicular fusion to membranes, which is impaired following alendronate administration. Interference with the function of these proteins also leads to apoptosis. The IC50 for alendronate inhibition of farnesyl diphosphate synthase is 340 nanoM and for pamidronate inhibition of farnesyl diphosphate synthase is 500 nanoM.

Other nitrogen-containing bisphosphonates are found to inhibit prenyl transferases involved in isoprenoid synthesis. It is therefore surprising, in view of the similarity of these enzymatic reactions, that the alendronate inhibition is specific for farnesyl diphosphate synthase to the exclusion of geranylgeranyl diphosphate synthase.
and squalene synthase. The data show that although these enzymes are closely related, their interaction with bisphosphonates is distinctly different.

Without being limited by theory, it is believed that nitrogen-containing bisphosphonates have a different mechanism of action from non-nitrogen-containing bisphosphonates. Three nitrogen-containing bisphosphonates, alendronate, risedronate, and pamidronate effectively inhibit farnesyl diphosphate synthase, whereas the two non-nitrogen-containing bisphosphonate, etidronate and clodronate, have little or no effect on farnesyl diphosphate synthase. Without being limited by theory, these findings are consistent with the notion that the pharmacological action of the nitrogen-containing bisphosphonates is based on a similar mechanism: osteoclast inactivation and/or apoptosis resulting from interference with protein prenylation, due to reduced cellular geranylgeranyl diphosphate levels, caused by farnesyl diphosphate synthase inhibition.

Methods of Identifying Inhibitors of Farnesyl Diphosphate Synthase

The present invention relates to a method for identifying an inhibitor of farnesyl diphosphate synthase comprising:

a). contacting a putative farnesyl diphosphate synthase inhibitor with a farnesyl diphosphate synthase assay solution, and

b). determining, i.e. comparing, the farnesyl diphosphate synthase activity of said assay solution with a farnesyl diphosphate synthase assay solution not contacted with said putative inhibitor, in order to determine the amount of inhibition.

In these methods the farnesyl diphosphatesynthase assay solution is typically an aqueous solution. The inhibition effect is measured with respect to the catalysis of an appropriate reaction that one of ordinary skill in the art can select. Typical substrates include dimethylallyl diphosphate, isopentenyl diphosphate, and geranyl diphosphate. Reaction times, conditions, quantitation methods, and other variables are chosen for convenience to obtain a readily quantitated system for measuring the inhibition of the farnesyl diphosphate synthase.

Additionally, in these methods of identifying inhibitors of farnesyl diphosphate synthase, the enzyme can be used in a crude, unpurified state, from various tissues, e.g., liver. Alternatively, the enzyme can be used in a partially purified state, a purified state, or as an expressed form of the enzyme, e.g., the expressed human enzyme.
Methods Of Inhibiting Bone Resorption

The present invention relates to methods for inhibiting bone resorption in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of a farnesyl diphosphate synthase inhibitor.

The methods and compositions of the present invention are useful for both treating and reducing the risk of contracting disease states or conditions involving or associated with abnormal bone resorption. Such disease states or conditions include, but are not limited to, osteoporosis, glucocorticoid induced osteoporosis, Paget's disease, abnormally increased bone turnover, periodontal disease, tooth loss, bone fractures, rheumatoid arthritis, periprosthetic osteolysis, osteogenesis imperfecta, metastatic bone disease, hypercalcemia of malignancy, and multiple myeloma. The methods and compositions are also useful for both treating and reducing the risk of contracting other disease states or conditions mediated by farnesyl diphosphate synthase.

In further embodiments, the methods comprise administering a therapeutically effective amount of the combination of (a) a farnesyl diphosphate synthase inhibitor, which can itself be a bisphosphonate active, and (b) an additional bisphosphonate active. Both concurrent and sequential administration of the farnesyl diphosphate synthase inhibitor and the additional bisphosphonate active are deemed within the scope of the present invention. With sequential administration, the farnesyl diphosphate synthase inhibitor and the additional bisphosphonate can be administered in either order. In a subclass of sequential administration the farnesyl diphosphate synthase inhibitor and the additional bisphosphonate are typically administered within the same 24 hour period. In yet a further subclass, the farnesyl diphosphate synthase inhibitor and the additional bisphosphonate are typically administered within about 4 hours of each other.

The term "therapeutically effective amount", as used herein, means that amount of the farnesyl diphosphate synthase inhibitor, or other actives of the present invention, that will elicit the desired therapeutic effect or response or provide the desired benefit when administered in accordance with the desired treatment regimen. A preferred therapeutically effective amount is a bone resorption inhibiting amount.

"Pharmaceutically acceptable" as used herein, means generally suitable for administration to a mammal, including humans, from a toxicity or safety standpoint.
In the present invention, the farnesyl diphosphate synthase inhibitor is typically administered for a sufficient period of time until the desired therapeutic effect is achieved. The term "until the desired therapeutic effect is achieved", as used herein, means that the therapeutic agent or agents are continuously administered, according to the dosing schedule chosen, up to the time that the clinical or medical effect sought for the disease or condition being mediated is observed by the clinician or researcher. For methods of treatment of the present invention, the compounds are continuously administered until the desired change in bone mass or structure is observed. In such instances, achieving an increase in bone mass or a replacement of abnormal bone structure with normal bone structure are the desired objectives. For methods of reducing the risk of a disease state or condition, the compounds are continuously administered for as long as necessary to prevent the undesired condition. In such instances, maintenance of bone mass density is often the objective.

Nonlimiting examples of administration periods can range from about 2 weeks to the remaining lifespan of the mammal. For humans, administration periods can range from about 2 weeks to the remaining lifespan of the human, preferably from about 2 weeks to about 20 years, more preferably from about 1 month to about 20 years, more preferably from about 6 months to about 10 years, and most preferably from about 1 year to about 10 years.

Compositions Of The Present Invention

The pharmaceutical compositions of the present invention comprise a therapeutically effective amount of a farnesyl diphosphate synthase inhibitor. These compositions can further comprise a pharmaceutically-acceptable carrier.

In further embodiments these compositions can also comprise an additional active.

Farnesyl Diphosphate Synthase Inhibitor

The methods and compositions of the present invention comprise a farnesyl diphosphate synthase inhibitor. These inhibitors can in themselves be bisphosphonates.

The farnesyl diphosphate synthase inhibitors useful herein generally have an IC50 value from about 0.01 nM to about 1000 nanoM, although inhibitors with activities outside this range can be useful depending upon the dosage and route
of administration. In a subclass of the present invention, the inhibitors have an IC₅₀ value of from about 0.01 nM to about 100 nM. In a further subclass of the present invention, the inhibitors have an IC₅₀ value of from about 0.01 nM to about 1 nM. IC₅₀ is a common measure of inhibition activity well known to those of ordinary skill in the art and is defined as the concentration of the inhibitor needed to obtain a 50% reduction in the activity of the farnesyl diphosphate synthase.

The combination of two or more farnesyl diphosphate synthase inhibitors are also deemed as within the scope of the present invention.

The precise dosage of the farnesyl diphosphate synthase inhibitor will vary with the dosing schedule, the particular compound chosen, the age, size, sex and condition of the mammal or human, the nature and severity of the disorder to be treated, and other relevant medical and physical factors. Thus, a precise pharmaceutically effective amount cannot be specified in advance and can be readily determined by the caregiver or clinician. Appropriate amounts can be determined by routine experimentation from animal models and human clinical studies. Generally, an appropriate amount is chosen to obtain an inhibition of the farnesyl diphosphate synthase activity so as to obtain a bone resorption inhibiting effect.

For humans, an effective oral dose of the farnesyl diphosphate synthase inhibitor is about 1 µg/kg to about 1000 µg/kg, preferably about 10 µg/kg, for a human subject.

For the farnesyl diphosphate synthase inhibitor, human doses which can be administered are generally in the range of about 0.1 mg/day to about 10 mg/day, preferably from about 0.25 mg/day to about 5 mg/day, and more preferably from about 0.5 mg/day to about 1.5 mg/day, based on a geranylgeraniol active weight basis. A typical nonlimiting dosage amount would be about 0.75 mg/day. The pharmaceutical compositions herein comprise from about 0.1 mg to about 10 mg, preferably from about 0.25 mg to about 5 mg, and more preferably from about 0.5 mg to about 1.5 mg of the farnesyl diphosphate synthase inhibitor. A typical nonlimiting amount is about 0.75 mg.

Bisphosphonates

The methods and compositions of the present invention, can further comprise a bisphosphonate active or a pharmaceutically acceptable salt thereof. These bisphosphonate actives are defined herein to be distinct from and not to
included the farnesyl diphosphate synthase inhibitors of the present invention, because certain nitrogen-containing bisphosphonates, e.g., alendronate are found to have activity as farnesyl diphosphate synthase inhibitors. In other words, the present invention can include the combination of a farnesyl diphosphate synthase inhibitor which happens to have a bisphosphonate structure and an additional bisphosphonate active which does not necessarily have activity as a farnesyl diphosphate synthase inhibitor.

The term “nitrogen-containing” as used herein means that the bisphosphonate compound or pharmaceutically acceptable salt thereof comprises at least one nitrogen atom in the bisphosphonate portion of the molecule. In other words, for a pharmaceutically-acceptable salt of the bisphosphonate, any nitrogen atom contained in the positive counter ion of such a salt, e.g., the nitrogen atom of an ammonium counter ion, would not be considered in meeting the “nitrogen-containing” definition. For example, alendronic acid, i.e. 4-amino-1-hydroxybutyridene-1,1-bisphosphonic acid is an example of a nitrogen-containing bisphosphonate. However, the ammonium salt of the unsubstituted 1-hydroxybutyridene-1,1-bisphosphonic acid would not be a nitrogen-containing bisphosphonate as defined herein.

The bisphosphonates useful in certain embodiments of the present invention correspond to the chemical formula

\[
\text{PO}_3\text{H}_2
\]

\[
\text{A-}(\text{CH}_2)_n\text{C-X}
\]

\[
\text{PO}_3\text{H}_2
\]

wherein \( n \) is an integer from 0 to 7 and wherein A and X are independently selected from the group consisting of H, OH, halogen, NH2, SH, phenyl, C1-C30 alkyl, C3-C30 branched or cycloalkyl, C1-C30 substituted alkyl, C1-C10 alkyl substituted NH2, C3-C10 branched or cycloalkyl substituted NH2, C1-C10 dialkyl substituted NH2, C3-C10 branched or cycloalkyl disubstituted NH2, C1-C10 alkoxy, C1-C10 alkyl substituted thio, thiophenyl, halophenylthio, C1-C10 alkyl substituted phenyl, pyridyl, furanyl, pyrrolidinyl, imidazolyl, imidazopyridinyl, and benzyl, such that both A and
X are not selected from H or OH when n is 0; or A and X are taken together with the carbon atom or atoms to which they are attached to form a C3-C10 ring.

In the foregoing chemical formula, the alkyl groups can be straight, branched, or cyclic, provided that sufficient atoms are selected for the chemical formula. The C1-C30 substituted alkyl can include a wide variety of substituents, nonlimiting examples which include those selected from the group consisting of phenyl, pyridyl, furanyl, pyrrolidinyl, imidazonyl, NH₂, C1-C10 alkyl or dialkyl substituted NH₂, OH, SH, and C1-C10 alkoxy.

The foregoing chemical formula is also intended to encompass complex carbocyclic, aromatic and hetero atom structures for the A and/or X substituents, nonlimiting examples of which include naphthyl, quinolyl, isoquinolyl, adamantyl, and chlorophenylthio.

A non-limiting class of structures useful in the instant invention are those in which A is selected from the group consisting of H. OH, and halogen, X is selected from the group consisting of C1-C30 alkyl, C1-C30 substituted alkyl, halogen, and C1-C10 alkyl or phenyl substituted thio, and n is 0.

A non-limiting subclass of structures useful in the instant invention are those in which A is selected from the group consisting of H, OH, and Cl, X is selected from the group consisting of C1-C30 alkyl, C1-C30 substituted alkyl, Cl, and chlorophenylthio, and n is 0.

A non-limiting example of the subclass of structures useful in the instant invention is when A is OH and X is a 3-aminopropyl moiety, and n is 0, so that the resulting compound is a 4-amino-1,1-hydroxybutylidene-1,1-bisphosphonate, i.e. alendronate.

Pharmaceutically acceptable salts and derivatives of the bisphosphonates are also useful herein. Nonlimiting examples of salts include those selected from the group consisting alkali metal, alkaline metal, ammonium, and mono-, di-, tri-, or tetra-C1-C30-alkyl-substituted ammonium. Preferred salts are those selected from the group consisting of sodium, potassium, calcium, magnesium, and ammonium salts. Nonlimiting examples of derivatives include those selected from the group consisting of esters, hydrates, and amides.

It should be noted that the terms "bisphosphonate" and "bisphosphonates", as used herein in referring to the therapeutic agents of the present invention are meant to also encompass diphosphonates, biphosphonic acids, and diphosphonic acids, as well as salts and derivatives of these materials. The use of a
specific nomenclature in referring to the bisphosphonate or bisphosphonates is not meant to limit the scope of the present invention, unless specifically indicated. Because of the mixed nomenclature currently in use by those or ordinary skill in the art, reference to a specific weight or percentage of a bisphosphonate compound in the present invention is on an acid active weight basis, unless indicated otherwise herein. For example, the phrase "about 5 mg of a bisphosphonate selected from the group consisting of alendronate, pharmaceutically acceptable salts thereof, and mixtures thereof, on an alendronic acid active weight basis" means that the amount of the bisphosphonate compound selected is calculated based on 5 mg of alendronic acid. For other bisphosphonates, the amount of bisphosphonate is calculated based on the corresponding bisphosphonic acid.

Nonlimiting examples of bisphosphonates useful herein include the following:

Alendronic acid, 4-amino-1-hydroxybutylidene-1,1-bisphosphonic acid.

Alendronate (also known as alendronate sodium or alendronate monosodium trihydrate), 4-amino-1-hydroxybutylidene-1,1-bisphosphonic acid monosodium trihydrate.

Alendronic acid and alendronate are described in U.S. Patents 4,922,007, to Kieczykowski et al., issued May 1, 1990; 5,019,651, to Kieczykowski et al., issued May 28, 1991; 5,510,517, to Dauer et al., issued April 23, 1996; 5,648,491, to Dauer et al., issued July 15, 1997, all of which are incorporated by reference herein in their entirety.

Cycloheptylaminomethylene-1,1-bisphosphonic acid, YM 175, Yamanouchi (cimadronate), as described in U.S. Patent 4,970,335, to Isomura et al., issued November 13, 1990, which is incorporated by reference herein in its entirety.

1,1-dichloromethylene-1,1-diphosphonic acid (clodronic acid), and the disodium salt (clodronate, Procter and Gamble), are described in Belgium Patent 672,205 (1966) and J. Org. Chem. 32, 4111 (1967), both of which are incorporated by reference herein in their entirety.

1-hydroxy-3-(1-pyrrolidinyl)-propylidene-1,1-bisphosphonic acid (EB-1053).

1-hydroxyethane-1,1-diphosphonic acid (etidronic acid).
1-hydroxy-3-(N-methyl-N-pentylamino)propylidene-1,1-
bisphosphonic acid, also known as BM-210955, Boehringer-Mannheim
(ibandronate), is described in U.S. Patent No. 4,927,814, issued May 22, 1990,
which is incorporated by reference herein in its entirety.

6-amino-1-hydroxyhexylidene-1,1-bisphosphonic acid
(neridronate).

3-(dimethylamino)-1-hydroxypropylidene-1,1-bisphosphonic acid
(olpadronate).

3-amino-1-hydroxypropylidene-1,1-bisphosphonic acid
(pamidronate).

[2-(2-pyridinyl)ethylidene]-1,1-bisphosphonic acid (piridronate) is
described in U.S. Patent No. 4,761,406, which is incorporated by reference in its
entirety.

1-hydroxy-2-(3-pyridinyl)-ethylidene-1,1-bisphosphonic acid
(risedronate).

(4-chlorophenyl)thiomethane-1,1-disphosphonic acid (tiludronate)
as described in U.S. Patent 4,876,248, to Breliere et al., October 24, 1989, which
is incorporated by reference herein in its entirety.

1-hydroxy-2-(1' -imidazol-1-yl)ethylidene-1,1-bisphosphonic acid
(zolendronate).

A non-limiting class of bisphosphonates useful in the instant invention
are selected from the group consisting of alendronate, cimadronate, clodronate,
tiludronate, etidronate, ibandronate, neridronate, olpadronate, risedronate,
piridronate, pamidronate, zolendronate, pharmaceutically acceptable salts thereof, and
mixtures thereof.

A non-limiting subclass of the above-mentioned class in the instant
case is selected from the group consisting of alendronate, pharmaceutically acceptable
salts thereof, and mixtures thereof.

A non-limiting example of the subclass is alendronate monosodium
trihydrate.

It is recognized that mixtures of two or more of the bisphosphonate
actives can be utilized.

The precise dosage of the bisphosphonate will vary with the dosing
schedule, the particular bisphosphonate chosen, the age, size, sex and condition of the
mammal or human, the nature and severity of the disorder to be treated, and other relevant medical and physical factors. Thus, a precise therapeutically effective amount cannot be specified in advance and can be readily determined by the caregiver or clinician. Appropriate amounts can be determined by routine experimentation from animal models and human clinical studies. Generally, an appropriate amount of bisphosphonate is chosen to obtain a bone resorption inhibiting effect, i.e. a bone resorption inhibiting amount of the nitrogen-containing bisphosphonate is administered. For humans, an effective oral dose of nitrogen-containing bisphosphonate is typically from about 1.5 to about 6000 µg/kg body weight and preferably about 10 to about 2000 µg/kg of body weight.

For the bisphosphonate, alendronate monosodium trihydrate, common human doses which are administered are generally in the range of about 2 mg/day to about 40 mg/day, preferably about 5 mg/day to about 40 mg/day. In the U.S. presently approved dosages for alendronate monosodium trihydrate are 5 mg/day for preventing osteoporosis, 10 mg/day for treating osteoporosis, and 40 mg/day for treating Paget’s disease.

In alternative dosing regimens, the bisphosphonate can be administered at intervals other than daily, for example once-weekly dosing, twice-weekly dosing, biweekly dosing, and twice-monthly dosing. In such dosing regimens, appropriate multiples of the bisphosphonate dosage would be administered. For example, in a once weekly dosing regimen, alendronate monosodium trihydrate would be administered at dosages of 35 mg/week or 70 mg/week in lieu of seven consecutive daily dosages of 5 mg or 10 mg.

The pharmaceutical compositions herein comprise from about 1 mg to about 100 mg of bisphosphonate, preferably from about 2 mg to 70 mg, and more preferably from about 5 mg to about 70, on a bisphosphonic acid basis. For the bisphosphonate alendronate monosodium trihydrate, the pharmaceutical compositions useful herein comprise about 2.5 mg, 5 mg, 10 mg, 35 mg, 40 mg, or 70 mg of the active on an alendronic acid active weight basis.

See also, U.S. Patent 4,610,077, to Rosini et al., issued November 4, 1986; U.S. Patent 5,358,941, to Bechard et al., issued October 25, 1994; and PCT application number WO 99/04773, to Daifotis et al., published February 4, 1999; all of which are incorporated by reference herein in their entirety.

Other Bone Agents
Further embodiments of the methods and compositions of the present invention can comprise additional bone agents useful for inhibiting bone resorption and providing the desired therapeutic benefits of the invention. Examples of such agents include those selected from the group consisting of calcitonin, estrogens, progesterone, androgens, calcium supplements, fluoride, growth hormone secretagogues, vitamin D analogues, and selective estrogen receptor modulators. The calcitonins useful herein can be from human or nonhuman sources, e.g., salmon calcitonin. Nonlimiting examples of estrogens include estradiol. Nonlimiting examples of selective estrogen receptor modulators include raloxifene, idoxifene, and tamoxifen. Growth hormone secretagogues are described in U.S. Patent No. 5,536,716, to Chen et al., issued July 16, 1996, which is incorporated by reference herein in its entirety.

Other Components Of The Pharmaceutical Compositions

The farenysyl diphosphate synthase inhibitors, and in further embodiments the bisphosphonate actives and any other additional actives, are typically administered in admixture with suitable pharmaceutically acceptable diluents, excipients, or carriers, collectively referred to herein as "carrier materials", suitably selected with respect to the mode of administration. Nonlimiting examples of product forms include tablets, capsules, elixirs, syrups, powders, suppositories, nasal sprays, liquids for ocular administration, formulations for transdermal administration, and the like, consistent with conventional pharmaceutical practices. For example, for oral administration in the form of a tablet, capsule, or powder, the active ingredient can be combined with an oral, non-toxic, pharmaceutically acceptable inert carrier such as lactose, starch, sucrose, glucose, methyl cellulose, magnesium stearate, mannitol, sorbitol, croscarmellose sodium and the like. For oral administration in liquid form, e.g., elixirs and syrups, the oral drug components are combined with any oral, non-toxic, pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents and coloring agents can also be incorporated. Suitable binders can include starch, gelatin, natural sugars such a glucose, anhydrous lactose, free-flow lactose, beta-lactose, and corn sweeteners, natural and synthetic gums, such as acacia, guar, tragacanth or sodium alginate, carboxymethyl cellulose, polyethylene glycol, waxes, and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium
chloride and the like. An example of a tablet formulation is that described in U.S. Patent No. 5,358,941, to Bechard et al, issued October 25, 1994, which is incorporated by reference herein in its entirety. The compounds used in the present method can also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxypropylmethacrylamide, and the like.

The following Examples are presented to better illustrate the invention.

EXAMPLE 1

Isopentenyl diphosphate (IPP), dimethylallyl-diphosphate (DMAPP), farnesyl diphosphate (FPP), and geranylgeranyl diphosphate (GPP) are obtained from Echelon, Salt Lake City, Utah. $^{14}\text{C}}$IPP (55 mCi/mmol), and $^{3}\text{H}\text{MVL}$ (50 Ci/mmol) are obtained from ARC (St. Louis, MO).

Human recombinant farnesyl diphosphate synthase is expressed and purified as described by Ding et al., *Biochem. J.*, 275, pp. 61-65 (1991), which. Alternatively, the crude expressed enzyme in an *E. coli* S100 fraction can be used.

EXAMPLE 2

The farnesyl diphosphate synthase assay is based on the method of Rilling HS, (1985) Methods in Enzymology 110: 145-152, which is incorporated by reference herein in its entirety. Geranyl diphosphate is used as the allylic substrate with $^{14}\text{C}}$ isoprenyl isopentenyl diphosphate as the second substrate. Hepes is used at 100 mM, geranylgeranyl diphosphate is used at 40 µM, isopentenyl diphosphate is used at 20 µM, and heptane is used for extractions. Alternatively, DMAPP is used as the allylic substrate. Control assays are run with $^{14}\text{C}}$IPP without allylic substrate, to correct for IPP isomerase activity. For assays using the human recombinant FPP synthase, 1% BSA is added to stabilize the enzymatic activity.

EXAMPLE 3

Labeling of osteoclasts with $^{3}\text{H}}\text{MVL}$: Osteoclast formation, murine co-cultures of osteoblasts and marrow cells are prepared using the methods of Wesolowski et al., *Exp. Cell Res.*, 219:679-686 (1995), which is incorporated by reference herein in its entirety. Cells are harvested from the bone marrow of 6-week-
old male Balb/C mice and suspended in: α-MEM supplemented with fetal calf serum (10% v/v) and 1,25-(Ovitamin D$_3$ (10 nM). Bone marrow cells are then added to sub-confluent monolayers of osteoblastic MB 1.8 cells and cultured for 7 days at 37°C in the presence of 5% CO$_2$. Co-cultures are treated (1 hr at 37°C) with type 1 collagenase (Wako Pure Chemical Industries, Osaka, Japan) at a concentration of 1 mg/ml in phosphate buffered saline. Suspended osteoblasts are gently aspirated, leaving an enriched mixture of prefusion osteoclasts and remaining MB1.8 osteoblasts. These are released with EDTA (0.2 g/l in PBS) for 20 min at 37°C. Cells are then re-plated in 6-well dishes using α-MEM supplemented with fetal calf serum (10% v/v) and 1,25-(OH)$_2$ vitamin D$_3$ (10 nM) and cultured for an additional three days. Osteoclast co-cultures generated in 6-well plates are treated with Type XI Collagenase (Sigma) in PBS to remove all osteoclasts, followed by EDTA to remove prefusion osteoclasts. Osteoclasts (≥95% purity) are then maintained in α-MEM supplemented with fetal calf serum (10% v/v) and 1,25-(OH)$_2$ vitamin D$_3$ (10 nM) and M-CSF (5 ng/ml).

For the labeling of the non-saponifiable lipids, osteoclasts are treated with alendronate (0-60 µM) for 2 hours before the addition of 200 µCi of R,S-[5-3H]MVL per dish. After 3 hours of labeling, media are removed, cells are washed twice with PBS, and then the cells are scraped into 2 ml of 1 M NaOH. Wells are rinsed with an additional equal volume of 1 M NaOH. Two volumes of 40% NaOH and one volume of methanol are added to the pooled NaOH extracts and are heated at 65°C for 3 hours to saponify the lipids. Non-saponifiable lipids are extracted with heptane and backwashed with 1 M NaOH. The radioactive content of the non-saponifiable lipid extract is determined by scintillation counting. TLC of these lipids is performed on LK6D silica gel 60 A° plates (Whatman, Fairfield, NJ) using hexane:Et$_2$O:acetic acid (70:30:3). After developing the chromatograph, the dried plate is sprayed with En Hance (NEN, Boston, MA) and exposed to XAR2 film (Sigma, St. Louis, MO).

For studying the labeling of prenylated proteins, the osteoclasts are treated with 15 µM lovastatin and alendronate (0-60 µM) for 2 hours before the addition of 200 µCi of R,S-[5-3H]MVL per dish. After 3 hours of labeling, media are removed, cells are washed twice with PBS, and then scraped into 200 µl of SDS sample buffer. SDS gel electrophoresis is performed on 15% gels (Gel electrophoresis system, gels, and buffers from Bio Rad, Hercules, CA). The gel is
fixed in 12% acetic acid/50% MeOH and soaked in Enlightenment (NEN), dried and put under XAR2 film for 10 days before developing.

EXAMPLE 4

Alendronate effects on FPP synthase: FPP synthase catalyzes the sequential condensation of two molecules of IPP with one molecule of DMAPP to produce GPP and then FPP. The FPP synthase assay, is run with a 15 minute preincubation and shows that alendronate inhibits FPP synthase with an IC$_{50}$ of 460 nM (0.15 µg/ml). Because the inhibition of FPP synthase by alendronate is time-dependent, IC$_{50}$ varies with preincubation time and assay length.

EXAMPLE 5

Inhibition of FPP synthase by other bisphosphonates: four other bisphosphonates are tested for their inhibitory effect of FPP synthase. All three nitrogen-containing bisphosphonates examined (alendronate, pamidronate, and risedronate) inhibit FPP synthase. Pamidronate has an (IC$_{50}$ = 500 nM), risedronate has (IC$_{50}$ = 3.9 nanoM). For the non-nitrogen containing bisphosphonate, etidronate the IC$_{50}$ values is 80 µM. For the non-nitrogen containing bisphosphonate clodronate, no inhibition observed.

EXAMPLE 6

Effect of alendronate on protein prenylation and the synthesis of mevalonate-derived lipids in osteoclasts: alendronate inhibition of the mevalonate pathway and of protein prenylation is demonstrated in the osteoclasts. A major branch point in isoprenoid metabolism occurs at FPP, which is used for sterol synthesis via squalene synthase, for prenylation of proteins via farnesyl protein transferase, for GGPP synthesis, and for the synthesis of dolichol and ubiquinone, via cis and trans prenyl transferases, respectively. Osteoclasts are labeled with $^3$H-MVL, and the effects of alendronate on the incorporation of the label into non-saponifiable lipids and into prenylated proteins are examined.

The effects of alendronate on the incorporation of label from $^3$H-MVL into prenylated proteins extracted from osteoclasts is studied. In the absence of alendronate, a series of proteins between 18-25 kD and another one of 44 kD, are labeled. With increasing alendronate concentrations, labeling decreases and
essentially disappears at 60 μM, with an IC₅₀ of approximately 15 μM. A band of about 18 kDa is not affected by alendronate.

The incorporation of label from MVL into the non-saponifiable lipids is lowered by up to 80% by alendronate with an IC₅₀ of around 15 μM. Analysis of these non-saponifiable lipids by TLC shows three major and at least five minor bands labeled. The incorporation into all bands is lowered by alendronate without bias. Individual bands co-migrated with squalene, lanosterol, and sterols (including cholesterol, desmosterol and 7-dehydrocholesterol). A diffuse light area of labeling just under the putative lanosterol band, where farnesol, geranylgeraniol, and dolichols migrate, is also observed.

The incorporation of label from MVL both into prenylated proteins and into non-saponifiable lipids is inhibited by 50% at 15 μM, consistent with FPP synthase being the target for the action of alendronate.

EXAMPLE 7

Pharmaceutical tablets: the tablets are prepared using standard mixing and formation techniques.

Tablets containing about 1 to 100 mg of a farnesyl diphosphate synthase inhibitor are prepared using the following relative weights of ingredients.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Per Tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farnesyl Diphosphate Synthase Inhibitor</td>
<td>0.10 to 10 mg</td>
</tr>
<tr>
<td>Anhydrous Lactose, NF</td>
<td>71.32 mg</td>
</tr>
<tr>
<td>Magnesium Stearate, NF</td>
<td>1.0 mg</td>
</tr>
<tr>
<td>Croscarmellose Sodium, NF</td>
<td>2.0 mg</td>
</tr>
<tr>
<td>Microcrystalline Cellulose, NF</td>
<td>QS 200 mg</td>
</tr>
</tbody>
</table>

The resulting tablets are useful for administration in accordance with the methods of the present invention for inhibiting bone resorption.

In further embodiments, tablets are prepared that also contain 5 or 10 mg of a bisphosphonate active, on a bisphosphonic acid active basis, of a bisphosphonate selected from the group consisting of alendronate cimadronate,
clodronate, tiludronate, etidronate, ibandronate, neridronate, olpadronate,
osedronate, piridronate, pamidronate, zolendronate, and pharmaceutically acceptable
salts thereof.

EXAMPLE 8

Liquid formulation: liquid formulations are prepared using standard
mixing techniques.

A liquid formulation containing about 1 to about 100 mg of a farnesyl
diphosphate synthase inhibitor is prepared using the following relative weights of
ingredients.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farnesyl Diphosphate Synthase Inhibitor</td>
<td>0.10 to 10 mg</td>
</tr>
<tr>
<td>Sodium Propylparaben</td>
<td>22.5 mg</td>
</tr>
<tr>
<td>Sodium Butylparaben</td>
<td>7.5 mg</td>
</tr>
<tr>
<td>Sodium Citrate Dihydrate</td>
<td>1500 mg</td>
</tr>
<tr>
<td>Citric Acid Anhydrous</td>
<td>56.25 mg</td>
</tr>
<tr>
<td>Sodium Saccharin</td>
<td>7.5 mg</td>
</tr>
<tr>
<td>Water</td>
<td>qs 75 mL</td>
</tr>
<tr>
<td>1 N Sodium Hydroxide (aq)</td>
<td>qs pH 6.75</td>
</tr>
</tbody>
</table>

The resulting liquid formulation is useful for administration for
inhibiting bone resorption.

In further embodiments solutions are prepared also containing 5 or 10
mg of a bisphosphonate active, on a bisphosphonic acid active basis, of a
bisphosphonate selected from the group consisting of alendronate cimadronate,
clodronate, tiludronate, etidronate, ibandronate, neridronate, olpadronate,
osedronate, piridronate, pamidronate, zolendronate, and pharmaceutically acceptable
salts thereof.
WHAT IS CLAIMED IS:

1. A method for identifying an inhibitor of farnesyl diphosphate synthase comprising:
   a). contacting a putative farnesyl diphosphate synthase inhibitor with a farnesyl diphosphate synthase solution, and
   b). determining the farnesyl diphosphate synthase activity of said solution with a farnesyl diphosphate synthase solution not contacted with said putative inhibitor.

2. A method according to claim 1 wherein said farnesyl diphosphate synthase is an expressed human farnesyl diphosphate synthase protein.

3. A method for inhibiting farnesyl diphosphate synthase activity in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of a farnesyl diphosphate synthase inhibitor having an IC50 value from about 0.01 nanoM to about 100 0 nanoM.

4. A method according to Claim 3 wherein said mammal is a human.

5. A method for treating or reducing the risk of contracting a disease state or condition involving bone tissue in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of a farnesyl diphosphate synthase inhibitor having an IC50 value from about 0.01 nanoM to about 100 0 nanoM.

6. A method according to Claim 5 wherein said mammal is a human.

7. A method according to Claim 6 wherein said disease state or condition is selected from the group consisting of osteoporosis, glucocorticoid induced osteoporosis, Paget’s disease, abnormally increased bone turnover, periodontal disease, tooth loss, bone fractures, rheumatoid arthritis, periprosthetic
osteolysis, osteogenesis imperfecta, metastatic bone disease, hypercalcemia of malignancy, and multiple myeloma.

8. A method according to Claim 7 wherein said disease state or condition is selected from the group consisting of osteoporosis, glucocorticoid induced osteoporosis, and Paget’s disease.

9. A method for inhibiting farnesyl diphosphate synthase activity in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of the combination of:
   (a) a farnesyl diphosphate synthase inhibitor having an IC50 value from about 0.01 nanoM to about 1000 nanoM, and
   (b) a bisphosphonate active.

10. A method according to Claim 9 wherein said mammal is a human.

11. A method for inhibiting bone resorption in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of the combination of:
   (a) a farnesyl diphosphate synthase inhibitor having an IC50 value from about 0.01 nanoM to about 1000 nanoM, and
   (b) a bisphosphonate active.

12. A method according to Claim 11 wherein said mammal is a human.

13. A method for treating or reducing the risk of contracting a disease state or condition involving bone tissue in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of the combination of:
   (a) a farnesyl diphosphate synthase inhibitor having an IC50 value from about 0.01 nanoM to about 1000 nanoM, and
   (b) a bisphosphonate active.
14. A method according to Claim 13 wherein said mammal is a human.

15. A method according to Claim 14 wherein said disease state or condition is selected from the group consisting of osteoporosis, glucocorticoid induced osteoporosis, Paget's disease, abnormally increased bone turnover, periodontal disease, tooth loss, bone fractures, rheumatoid arthritis, periprosthetic osteolysis, osteogenesis imperfecta, metastatic bone disease, hypercalcemia of malignancy, and multiple myeloma.

16. A method according to Claim 15 wherein said disease state or condition is selected from the group consisting of osteoporosis, glucocorticoid induced osteoporosis, and Paget's disease.

17. A method according to Claim 16 wherein said bisphosphonate active corresponds to the chemical structure

\[
\begin{align*}
& \text{PO}_3\text{H}_2 \\
& \text{A-(CH}_2\text{)}_n\text{-C-X} \\
& \text{PO}_3\text{H}_2
\end{align*}
\]

wherein \( n \) is an integer from 0 to 7 and wherein A and X are independently selected from the group consisting of H, OH, halogen, NH\(_2\), SH, phenyl, C1-C30 alkyl, C3-C30 branched or cycloalkyl, C1-C30 substituted alkyl, C1-C10 alkyl substituted NH\(_2\), C3-C10 branched or cycloalkyl substituted NH\(_2\), C1-C10 dialkyl substituted NH\(_2\), C1-C10 alkoxy, C1-C10 alkyl substituted thio, thiophenyl, halophenylthio, C1-C10 alkyl substituted phenyl, pyridyl, furanyl, pyrrolidinyl, imidazolyl, imidazopyridinyl, and benzyl; or A and X are taken together with the carbon atom or atoms to which they are attached to form a C3-C10 ring; and provided that when \( n \) is 0, A and X are not selected from the group consisting of H and OH; and the pharmaceutically acceptable salts thereof.
18. A method according to Claim 17 wherein said bisphosphonate active is selected from the group consisting of alendronate, cimadronate, clopidronate, tiludronate, etidronate, ibandronate, neridronate, olpandronate, risedronate, piridronate, pamidronate, zolendronate, pharmaceutically acceptable salts thereof, and mixtures thereof.

19. A method according to Claim 18 wherein said bisphosphonate active is alendronate, pharmaceutically acceptable salts thereof, and mixtures thereof.

20. A method according to Claim 19 wherein said bisphosphonate active is alendronate monosodium trihydrate.

21. A pharmaceutical composition comprising a therapeutically effective amount of a farnesyl diphosphate synthase inhibitor having an IC₅₀ value from about 0.01 nanoM to about 1000 nanoM.

22. A pharmaceutical composition comprising a therapeutically effective amount of the combination of:

(a) a farnesyl diphosphate synthase inhibitor having an IC₅₀ value from about 0.01 nanoM to about 1000 nanoM, and

(b) a bisphosphonate active.

23. A pharmaceutical composition according to Claim 22 wherein said bisphosphonate active corresponds to the chemical structure

\[
\text{PO}_3\text{H}_2 \\
\mid \\
A-(\text{CH}_2)_n\text{-C-X} \\
\mid \\
\text{PO}_3\text{H}_2
\]
wherein n is an integer from 0 to 7 and wherein A and X are independently selected from the group consisting of H, OH, halogen, NH₂, SH, phenyl, C₁-C₃₀ alkyl, C₃-C₃₀ branched or cycloalkyl, C₁-C₃₀ substituted alkyl, C₁-C₁₀ alkyl substituted NH₂, C₃-C₁₀ branched or cycloalkyl substituted NH₂, C₁-C₁₀ dialkyl substituted NH₂, C₁-C₁₀ alkoxy, C₁-C₁₀ alkyl substituted thio, thiophenyl, halophenylthio, C₁-C₁₀ alkyl substituted phenyl, pyridyl, furanyl, pyrrolidinyl, imidazolyl, imidazopyridinyl, and benzyl; or A and X are taken together with the carbon atom or atoms to which they are attached to form a C₃-C₁₀ ring; and provided that when n is 0, A and X are not selected from the group consisting of H and OH; and the pharmaceutically acceptable salts thereof.

24. A pharmaceutical composition according to Claim 23 wherein said bisphosphonate active is selected from the group consisting of alendronate, cimadronate, clodronate, tiludronate, etidronate, ibandronate, neridronate, olpandronate, risedronate, piridronate, pamidronate, zolendronate, pharmaceutically acceptable salts thereof, and mixtures thereof.

25. A pharmaceutical composition according to Claim 24 wherein said bisphosphonate active is alendronate, pharmaceutically acceptable salts thereof, and mixtures thereof.

26. A pharmaceutical composition according to Claim 25 wherein said bisphosphonate active is alendronate monosodium trihydrate.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12Q 1/48; C12N 9/10
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)


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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST 1.2

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tbody>
<tr>
<td>Y</td>
<td>HAMY, N. Role of Bisphosphonates in Metabolic Bone Diseases. Trends in Endocrinol. Metab. 1993, Vol. 4, No. 5, pages 19-25, see entire document.</td>
<td>5-8 and 11-26</td>
</tr>
<tr>
<td>Y</td>
<td>US 5,157,027 A (BILLER et al) 20 October 1992 (20.10.92), see entire document, especially col. 21, lines 26-49.</td>
<td>1-26</td>
</tr>
<tr>
<td>Y</td>
<td>US 5,103,036 A (MAGNIN et al) 07 April 1992 (07.04.92), see entire document, especially col. 5, line 62 - col. 6, line 16.</td>
<td>1-26</td>
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*O* document referring to an oral disclosure, use, exhibition or other means
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Date of the actual completion of the international search
25 APRIL 2000

Date of mailing of the international search report
30 MAY 2000

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/05338

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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<tr>
<td>Y</td>
<td>US 5,547,685 A (CULLINAN) 20 August 1996 (20.08.96), see entire document, especially col. 2, lines 47-59.</td>
<td>5-8 and 11-26</td>
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