

DESCRIPTIONCalcium Receptor-Active CompoundsField of the Invention

This invention relates to the design, development, composition and use of compounds able to modulate one or more inorganic ion receptor activities.

5 Background of the Invention

Certain cells in the body respond not only to chemical signals, but also to ions such as extracellular calcium ions (Ca^{2+}). Changes in the concentration of extracellular Ca^{2+} (referred to herein as " $[\text{Ca}^{2+}]$ ") alter
10 the functional responses of these cells. One such specialized cell is the parathyroid cell which secretes parathyroid hormone (PTH). PTH is the principal endocrine factor regulating Ca^{2+} homeostasis in the blood and extracellular fluids.

15 PTH, by acting on bone and kidney cells, increases the level of Ca^{2+} in the blood. This increase in $[\text{Ca}^{2+}]$ then acts as a negative feedback signal, depressing PTH secretion. The reciprocal relationship between $[\text{Ca}^{2+}]$ and PTH secretion forms the essential mechanism maintaining
20 bodily Ca^{2+} homeostasis.

Extracellular Ca^{2+} acts directly on parathyroid cells to regulate PTH secretion. The existence of a parathyroid cell surface protein which detects changes in $[\text{Ca}^{2+}]$ has been confirmed. Brown et al., 366 Nature 574, 1993. In
25 parathyroid cells, this protein acts as a receptor for extracellular Ca^{2+} ("the calcium receptor"), and detects changes in $[\text{Ca}^{2+}]$ and to initiate a functional cellular response, PTH secretion.

Extracellular Ca^{2+} can exert effects on different cell
30 functions, reviewed in Nemeth et al., 11 Cell Calcium 319, 1990. The role of extracellular Ca^{2+} in parafollicular (C-cells) and parathyroid cells is discussed in Nemeth, 11

Cell Calcium 323, 1990. These cells have been shown to express similar Ca^{2+} receptor. Brown et al., 366 Nature 574, 1993; Mithal et al., 9 Suppl. 1 J. Bone and Mineral Res. s282, 1994; Rogers et al., 9 Suppl. 1 J. Bone and
5 Mineral Res. s409, 1994; Garrett et al., 9 Suppl. 1 J. Bone and Mineral Res. s409, 1994. The role of extra-cellular Ca^{2+} on bone osteoclasts is discussed by Zaidi, 10 Bioscience Reports 493, 1990. In addition keratinocytes, juxtaglomerular cells, trophoblasts, pancreatic beta cells
10 and fat/adipose cells all respond to increases in extra-cellular calcium which likely reflects activation of calcium receptors of these cells.

The ability of various compounds to mimic extra-cellular Ca^{2+} in vitro is discussed by Nemeth et al.,
15 (spermine and spermidine) in "Calcium-Binding Proteins in Health and Disease," 1987, Academic Press, Inc., pp. 33-35; Brown et al., (e.g., neomycin) 128 Endocrinology 3047, 1991; Chen et al., (diltiazem and its analog, TA-3090) 5 J. Bone and Mineral Res. 581, 1990; and Zaidi
20 et al., (verapamil) 167 Biochem. Biophys. Res. Commun. 807, 1990. Nemeth et al., PCT/US93/01642, International Publication Number WO 94/18959, and Nemeth et al., PCT/US92/07175, International Publication Number WO 93/04373, describe various compounds which can modulate
25 the effect of an inorganic ion on a cell having an inorganic ion receptor.

The references provided in the background are not admitted to be prior art.

Summary of the Invention

30 The present invention features compounds able to modulate one or more activities of an inorganic ion receptor and methods for treating diseases or disorders by modulating inorganic ion receptor activity. Preferred compounds can mimic or block the effect of extracellular
35 calcium on a cell surface calcium receptor.

Diseases or disorders which can be treated by modulating inorganic ion receptor activity include one or more of the following types: (1) those characterized by abnormal inorganic ion homeostasis, preferably calcium homeostasis; (2) those characterized by an abnormal amount of an extracellular or intracellular messenger whose production can be affected by inorganic ion receptor activity, preferably calcium receptor activity; (3) those characterized by an abnormal effect (e.g., a different effect in kind or magnitude) of an intracellular or extracellular messenger which can itself be ameliorated by inorganic ion receptor activity, preferably calcium receptor activity; and (4) other diseases or disorders in which modulation of inorganic ion receptor activity, preferably calcium receptor activity will exert a beneficial effect, for example, in diseases or disorders where the production of an intracellular or extracellular messenger stimulated by receptor activity compensates for an abnormal amount of a different messenger. Examples of extracellular messengers whose secretion and/or effect can be affected by modulating inorganic ion receptor activity include inorganic ions, hormones, neurotransmitters, growth factors, and chemokines. Examples of intracellular messengers include cAMP, cGMP, IP₃, and diacylglycerol.

Thus, a compound of this invention preferably modulates calcium receptor activity and is used in the treatment of diseases or disorders which can be affected by modulating one or more activities of a calcium receptor. Calcium receptor proteins enable certain specialized cells to respond to changes in extracellular Ca²⁺ concentration. For example, extracellular Ca²⁺ inhibits the secretion of parathyroid hormone from parathyroid cells, inhibits bone resorption by osteoclasts, and stimulates secretion of calcitonin from C-cells.

In a preferred embodiment, the compound is used to treat a disease or disorder characterized by abnormal bone and mineral homeostasis, more preferably calcium homeo-

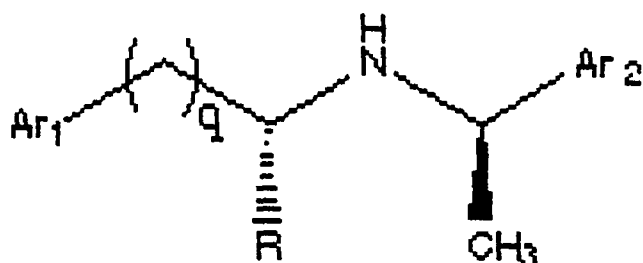
stasis. Extracellular Ca^{2+} is under tight homeostatic control and controls various processes such as blood clotting, nerve and muscle excitability, and proper bone formation. Abnormal calcium homeostasis is characterized by one or more of the following activities: (1) an abnormal increase or decrease in serum calcium; (2) an abnormal increase or decrease in urinary excretion of calcium; (3) an abnormal increase or decrease in bone calcium levels, for example, as assessed by bone mineral density measurements; (4) an abnormal absorption of dietary calcium; (5) an abnormal increase or decrease in the production and/or release of messengers which affect serum calcium levels such as parathyroid hormone and calcitonin; and (6) an abnormal change in the response elicited by messengers which affect serum calcium levels. The abnormal increase or decrease in these different aspects of calcium homeostasis is relative to that occurring in the general population and is generally associated with a disease or disorder.

Diseases and disorders characterized by abnormal calcium homeostasis can be due to different cellular defects such as a defective calcium receptor activity, a defective number of calcium receptors, or a defective intracellular protein acted on by a calcium receptor. For example, in parathyroid cells, the calcium receptor is coupled to the G_i protein which in turn inhibits cyclic AMP production. Defects in G_i protein can affect its ability to inhibit cyclic AMP production.

Thus, a first aspect the invention features an inorganic ion receptor modulating compound having the formula:

5

STRUCTURE I



where Ar₁ is either naphthyl or phenyl optionally substituted with 0 to 5 substituents each independently selected from the group consisting of, lower alkyl, halogen, lower alkoxy, lower thioalkyl, methylene dioxy, lower haloalkyl, lower haloalkoxy, OH, CH₂OH, CONH₂, CN, acetoxy, N(CH₃)₂, phenyl, phenoxy, benzyl, benzyloxy, α,α-dimethylbenzyl, NO₂, CHO, CH₂CH(OH), acetyl, ethylene dioxy;

Ar₂ is either naphthyl or phenyl optionally substituted with 0 to 5 substituents each independently selected from the group consisting of, lower alkyl, halogen, lower alkoxy, lower thioalkyl, methylene dioxy, lower haloalkyl, lower haloalkoxy, OH, CH₂OH, CONH₂, CN, and acetoxy;

q is 0, 1, 2, or 3; and

R is either H, or lower alkyl;

and pharmaceutically salts and complexes thereof.

Compounds of this invention have preferred stereochemistry. The CH₃ shown in Structure I is at a chiral center and provides an α-(R)-methyl structure. When R is CH₃, the R shown in Structure I is also at chiral center which provides an (R)-methyl structure. Thus, when R is CH₃, the Structure I compound has (R,R) stereochemistry.

Inorganic ion receptor activities are those processes brought about as a result of inorganic ion receptor activation. Such processes include the production of molecules which can act as intracellular or extracellular messengers.

Inorganic ion receptor-modulating compound include ionomimetics, ionolytics, calcimimetics, and calcilytics. Ionomimetics are compounds which bind to an inorganic ion receptor and mimic (*i.e.*, evoke or potentiate) the effects of an inorganic ion at an inorganic ion receptor. Preferably, the compound affects one or more calcium receptor activities. Calcimimetics are ionomimetics which effects one or more calcium receptor activities and bind to a calcium receptor.

10 Ionolytics are compounds which bind to an inorganic ion receptor and block (*i.e.*, inhibit or diminish) one or more activities caused by an inorganic ion at an inorganic ion receptor. Preferably, the compound affects one or more calcium receptor activities. Calcilytics are ionolytics which block one or more calcium receptor activities evoked by extracellular calcium and bind to a calcium receptor.

Ionomimetics and ionolytics may bind at the same receptor site as the native inorganic ion ligand binds or can bind at a different site (*e.g.*, allosteric site). For example, NPS R-467 binding to a calcium receptor results in calcium receptor activity and, thus, NPS R-467 is classified as a calcimimetic. However, NPS R-467 binds to the calcium receptor at a different site (*i.e.*, an allosteric site) than extracellular calcium.

A measure of a compounds effectiveness can be determined by calculating the EC_{50} or IC_{50} for that compound. The EC_{50} is the concentration of a compound which causes a half maximal mimicking effect. The IC_{50} is the concentration of compound which causes a half-maximal blocking effect. EC_{50} and IC_{50} for compounds at a calcium receptor can be determined by assaying one or more of the activities of extracellular calcium at a calcium receptor. Examples of assays for measuring EC_{50} , and IC_{50} are described Nemeth et al., PCT/US93/01642, International Publication Number WO 94/18959, and Nemeth et al., PCT/US92/07175, International Publication Number WO

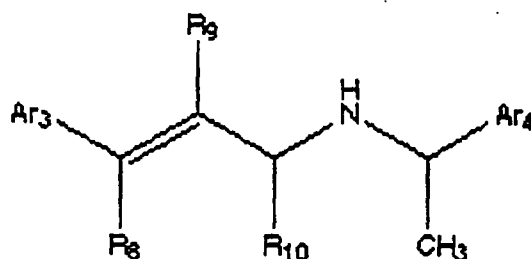
93/04373, (both of these publications are hereby incorporated by reference here) and below. Such assays include oocyte expression assays and measuring increases in intracellular calcium ion concentration ($[Ca^{2+}]_i$) due to calcium
5 receptor activity. Preferably, such assays measure the release or inhibition of a particular hormone associated with activity of a calcium receptor.

An inorganic ion receptor-modulating compound preferably selectively targets inorganic ion receptor activity
10 in a particular cell. For example, selective targeting of a calcium receptor activity is achieved by a compound exerting a greater effect on a calcium receptor activity in one cell type than at another cell type for a given concentration of compound. Preferably, the differential
15 effect is 10-fold or greater as measured *in vivo* or *in vitro*. More preferably, the differential effect is measured *in vivo* and the compound concentration is measured as the plasma concentration or extracellular fluid concentration and the measured effect is the production of
20 extracellular messengers such as plasma calcitonin, parathyroid hormone, or plasma calcium. For example, in a preferred embodiment, the compound selectively targets PTH secretion over calcitonin secretion.

Preferably, the compound is either a calcimimetic or
25 calcilytic having an EC_{50} or IC_{50} at a calcium receptor of less than or equal to 5 μM , and even more preferably less than or equal to 1 μM , 100 nmolar, 10 nmolar, or 1 nmolar using one of the assays described below. More preferably, the assay measures intracellular Ca^{2+} in HEK 293 cells
30 transformed with nucleic acid expressing the human parathyroid calcium receptor and loaded with fura-2. Lower EC_{50} 's or IC_{50} 's are advantageous since they allow lower concentrations of compounds to be used *in vivo* or *in vitro*. The discovery of compounds with low EC_{50} 's and
35 IC_{50} 's enables the design and synthesis of additional compounds having similar or improved potency, effectiveness, and/or selectivity.

Another aspect of the present invention features an inorganic ion receptor modulating compound having the formula:

STRUCTURE II



5 where Ar₃ is either naphthyl or phenyl optionally substituted with 0 to 5 substituents each independently selected from the group consisting of, lower alkyl, halogen, lower alkoxy, lower thioalkyl, methylene dioxy, lower haloalkyl, lower haloalkoxy, OH, CH₂OH, CONH₂, CN,
 10 acetoxy, benzyl, benzyloxy, α,α-dimethylbenzyl, NO₂, CHO, CH₃CH(OH), N(CH₃)₂, acetyl, ethylene dioxy.

Ar₄ is either naphthyl or phenyl optionally substituted with 0 to 5 substituents each independently selected from the group consisting of, lower alkyl, halogen, lower alkoxy, lower thioalkyl, methylene dioxy, lower haloalkyl, lower haloalkoxy, OH, CH₂OH, CONH₂, CN,
 15 and acetoxy;

R₈ is either hydrogen or phenyl;

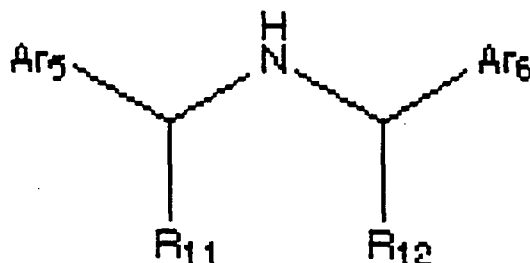
R₉ is either hydrogen or methyl; and

20 R₁₀ is either hydrogen, methyl, or phenyl;

or pharmaceutically acceptable salts and complexes thereof.

Another aspect of the present invention features an inorganic ion receptor modulating compound having the
 25 formula:

STRUCTURE III



where Ar₅ is either naphthyl or phenyl optionally substituted with 0 to 5 substituents each independently selected from the group consisting of, lower alkyl, halogen, lower alkoxy, lower thioalkyl, methylene dioxy, lower haloalkyl, lower haloalkoxy, OH, CH₂OH, CONH₂, CN, acetoxy, benzyl, benzyloxy, α,α-dimethylbenzyl, NO₂, CHO, CH₃CH(OH), acetyl, ethylene dioxy, -CH=CH-phenyl;

Ar₆ is either naphthyl or phenyl optionally substituted with 0 to 5 substituents each independently selected from the group consisting of, acetyl, lower alkyl, halogen, lower alkoxy, lower thioalkyl, methylene dioxy, lower haloalkyl, lower haloalkoxy, OH, CH₂OH, CONH₂, CN, carbomethoxy, OCH₂C(O)C₂H₅ and acetoxy;

R₁₁ is hydrogen or methyl; and

R₁₂ is hydrogen or methyl.

Another aspect of the present invention features a pharmaceutical composition made up of an inorganic ion receptor-modulating compound described herein and a physiologically acceptable carrier. A "pharmacological composition" refers to a composition in a form suitable for administration into a mammal, preferably a human. Preferably, the pharmaceutical composition contains a sufficient amount of a calcium receptor modulating compound in a proper pharmaceutical form to exert a therapeutic effect on a human.

Considerations concerning forms suitable for administration are known in the art and include toxic effects, solubility, route of administration, and maintaining

activity. For example, pharmacological compositions injected into the blood stream should be soluble.

Pharmaceutical compositions can also be formulated as pharmaceutically acceptable salts (e.g., acid addition salts) and complexes thereof. The preparation of such salts can facilitate the pharmacological use of a compound by altering its physical characteristics without preventing it from exerting a physiological effect.

Another aspect the present invention features a method for treating a patient by modulating inorganic ion receptor activity using inorganic ion receptor modulating compounds described herein. The method involves administering to the patient a pharmaceutical composition containing a therapeutically effective amount of an inorganic ion receptor-modulating compound. In a preferred embodiment, the disease or disorder is treated by modulating calcium receptor activity by administering to the patient a therapeutically effective amount of a calcium receptor-modulating compound.

Inorganic ion receptor-modulating compounds, and compositions containing the compounds, can be used to treat patients. A "patient" refers to a mammal in which modulation of an inorganic ion receptor will have a beneficial effect. Patients in need of treatment involving modulation of inorganic ion receptors can be identified using standard techniques known to those in the medical profession.

Preferably, a patient is a human having a disease or disorder characterized by one more of the following: (1) abnormal inorganic ion homeostasis, more preferably abnormal calcium homeostasis; (2) an abnormal level of a messenger whose production or secretion is affected by inorganic ion receptor activity, more preferably affected by calcium receptor activity; and (3) an abnormal level or activity of a messenger whose function is affected by inorganic ion receptor activity, more preferably affected by calcium receptor activity.

Diseases characterized by abnormal calcium homeostasis include hyperparathyroidism, osteoporosis and other bone and mineral-related disorders, and the like (as described, e.g., in standard medical text books, such as "Harrison's Principles of Internal Medicine"). Such diseases are treated using calcium receptor-modulating compounds which mimic or block one or more of the effects of extracellular Ca^{2+} on a calcium receptor and, thereby, directly or indirectly affect the levels of proteins or other compounds in the body of the patient.

By "therapeutically effective amount" is meant an amount of a compound which relieves to some extent one or more symptoms of the disease or disorder in the patient; or returns to normal either partially or completely one or more physiological or biochemical parameters associated with or causative of the disease or disorder.

In a preferred embodiment, the patient has a disease or disorder characterized by an abnormal level of one or more calcium receptor-regulated components and the compound is active on a calcium receptor of a cell selected from the group consisting of: parathyroid cell, bone osteoclast, juxtaglomerular kidney cell, proximal tubule kidney cell, distal tubule kidney cell, central nervous system cell, peripheral nervous system cell, cell of the thick ascending limb of Henle's loop and/or collecting duct, keratinocyte in the epidermis, parafollicular cell in the thyroid (C-cell), intestinal cell, platelet, vascular smooth muscle cell, cardiac atrial cell, gastrin-secreting cell, glucagon-secreting cell, kidney mesangial cell, mammary cell, beta cell, fat/adipose cell, immune cell, GI tract cell, skin cell, adrenal cell, pituitary cell, hypothalamic cell and cell of the subfornical organ.

More preferably, the cells are chosen from the group consisting of: parathyroid cell, central nervous system cell, peripheral nervous system cell, cell of the thick ascending limb of Henle's loop and/or collecting duct in the kidney, parafollicular cell in the thyroid (C-cell),

intestinal cell, GI tract cell, pituitary cell, hypothalamic cell and cell of the subfornical organ.

In a preferred embodiment, the compound is a calcimimetic acting on a parathyroid cell calcium receptor and reduces the level of parathyroid hormone in the serum of the patient. More preferably, the level is reduced to a degree sufficient to cause a decrease in plasma Ca^{2+} . Most preferably, the parathyroid hormone level is reduced to that present in a normal individual.

10 In another preferred embodiment, the compound is a calcilytic acting on a parathyroid cell calcium receptor and increases the level of parathyroid hormone in the serum of the patient. More preferably, the level is increased to a degree sufficient to cause an increase in bone mineral density of a patient.

Patients in need of such treatments can be identified by standard medical techniques, such as blood or urine analysis. For example, by detecting a deficiency of protein whose production or secretion is affected by changes in inorganic ion concentrations, or by detecting abnormal levels of inorganic ions or hormones which effect inorganic ion homeostasis.

Various examples are used throughout the application. These examples are not intended in any way to limit the invention.

Other features and advantages of the invention will be apparent from the following figures, detailed description of the invention, examples, and the claims.

Brief Description of the Drawings

30 Figs. 1a-1r, show the chemical structures of different compounds.

Figs. 2-131 provided physical data for representative compounds herein described.

Description of the Preferred Embodiments

The present invention features compounds able to modulate one or more inorganic ion receptor activities, preferably the compound can mimic or block an effect of an extracellular ion on a cell having an inorganic ion receptor, more preferably the extracellular ion is Ca^{2+} and the effect is on a cell having a calcium receptor. Publications concerned with the calcium activity, calcium receptor and/or calcium receptor modulating compounds include the following: Brown et al., Nature 366: 574, 1993; Nemeth et al., PCT/US93/01642, International Publication Number WO 94/18959; Nemeth et al., PCT/US92/07175, International Publication Number WO 93/04373; Shoback and Chen, J. Bone Mineral Res. 9: 293 (1994); and Racke et al., FEBS Lett. 333: 132, (1993). These publications are not admitted to be prior art to the claimed invention.

I. Calcium Receptors

Calcium receptors are present on different cell types and can have different activities in different cell types. The pharmacological effects of the following cells, in response to calcium, is consistent with the presence of a calcium receptor: parathyroid cell, bone osteoclast, juxtaglomerular kidney cell, proximal tubule kidney cell, distal tubule kidney cell, central nervous system cell, peripheral nervous system cell, cell of the thick ascending limb of Henle's loop and/or collecting duct, keratinocyte in the epidermis, parafollicular cell in the thyroid (C-cell), intestinal cell, platelet, vascular smooth muscle cell, cardiac atrial cell, gastrin-secreting cell, glucagon-secreting cell, kidney mesangial cell, mammary cell, beta cell, fat/adipose cell, immune cell, GI tract cell, skin cell, adrenal cell, pituitary cell, hypothalamic cell and cell of the subfornical organ. In addition, the presence of calcium receptors on parathyroid cell, central nervous system cell, peripheral nervous system

cell, cell of the thick ascending limb of Henle's loop and/or collecting duct in the kidney, parafollicular cell in the thyroid (C-cell), intestinal cell, GI tract cell, pituitary cell, hypothalamic cell and cell of the sub-fornical organ, has been confirmed by physical data.

The calcium receptor on these different cell types may be different. It is also possible that a cell can have more than one type of calcium receptor. Comparison of calcium receptor activities and amino acid sequences from different cells indicate that distinct calcium receptor types exist. For example, calcium receptors can respond to a variety of di- and trivalent cations. The parathyroid calcium receptor responds to calcium and Gd^{3+} , while osteoclasts respond to divalent cations such as calcium, but do not respond to Gd^{3+} . Thus, the parathyroid calcium receptor is pharmacologically distinct from the calcium receptor on the osteoclast.

On the other hand, the nucleic acid sequences encoding calcium receptors present in parathyroid cells and C-cells indicate that these receptors have a very similar amino acid structure. Nevertheless, calcimimetic compounds exhibit differential pharmacology and regulate different activities at parathyroid cells and C-cells. Thus, pharmacological properties of calcium receptors may vary significantly depending upon the cell type or organ in which they are expressed even though the calcium receptors may have similar or even identical structures.

Calcium receptors, in general, have a low affinity for extracellular Ca^{2+} (apparent K_d generally greater than about 0.5 mM). Calcium receptors may include a free or bound effector mechanism as defined by Cooper, Bloom and Roth, "The Biochemical Basis of Neuropharmacology", Ch. 4, and are thus distinct from intracellular calcium receptors, e.g., calmodulin and the troponins.

Calcium receptors respond to changes in extracellular calcium levels. The exact changes depend on the particular receptor and cell line containing the receptor. For

example, the *in vitro* effect of calcium on the calcium receptor in a parathyroid cell includes the following:

1. An increase in internal calcium. The increase is due to the influx of external calcium and/or to mobilization of internal calcium. Characteristics of the increase in internal calcium include the following:

(a) A rapid (time to peak < 5 seconds) and transient increase in $[Ca^{2+}]_i$ that is refractory to inhibition by 1 μM La^{3+} or 1 μM Gd^{3+} and is abolished by pretreatment with ionomycin (in the absence of extracellular Ca^{2+});

(b) The increase is not inhibited by dihydropyridines;

(c) The transient increase is abolished by pretreatment for 10 minutes with 10 mM sodium fluoride;

(d) The transient increase is diminished by pretreatment with an activator of protein kinase C (PKC), such as phorbol myristate acetate (PMA), mezerein or (-)-indolactam V. The overall effect of the protein kinase C activator is to shift the concentration-response curve of calcium to the right without affecting the maximal response; and

(e) Pretreatment with pertussis toxin (100 ng/ml for > 4 hours) does not affect the increase.

2. A rapid (< 30 seconds) increase in the formation of inositol-1,4,5-triphosphate or diacylglycerol. Pretreatment with pertussis toxin (100 ng/ml for > 4 hours) does not affect this increase;

3. The inhibition of dopamine- and isoproterenol-stimulated cyclic AMP formation. This effect is blocked by pretreatment with pertussis toxin (100 ng/ml for > 4 hours); and

4. The inhibition of PTH secretion. Pretreatment with pertussis toxin (100 ng/ml for > 4 hours) does not affect the inhibition in PTH secretion.

Using techniques known in the art, the effect of calcium on other calcium receptors in different cells can

be readily determined. Such effects may be similar in regard to the increase in internal calcium observed in parathyroid cells. However, the effect is expected to differ in other aspects, such as causing or inhibiting the release of a hormone other than parathyroid hormone.

II. Inorganic Ion Receptor Modulating Compounds

Inorganic ion receptor modulating compounds modulate one or more inorganic ion receptor activities. Preferred calcium receptor modulating compounds are calcimimetics and calcilytics. Inorganic ion receptor modulating compounds can be identified by screening compounds which are modelled after a compound shown to have a particular activity (i.e., a lead compound).

A preferred method of measuring calcium receptor activity is to measure changes in $[Ca^{2+}]_i$. Changes in $[Ca^{2+}]_i$ can be measured using different techniques such by using HEK 293 cells transduced with nucleic acid expressing the human parathyroid calcium receptor and loaded with fura-2; and by measuring an increase in Cl^- current in a *Xenopus* oocyte injected with nucleic acid coding for a calcium receptor. (See Nemeth et al., PCT/US93/01642, International Publication Number WO 94/18959.) For example, poly(A)⁺ mRNA can be obtained from cells expressing a calcium receptor, such as a parathyroid cell, bone osteoclast, juxtaglomerular kidney cell, proximal tubule kidney cell, distal tubule kidney cell, cell of the thick ascending limb of Henle's loop and/or collecting duct, keratinocyte in the epidermis, parafollicular cell in the thyroid (C-cell), intestinal cell, central nervous cell, peripheral nervous system cell, platelet, vascular smooth muscle cell, cardiac atrial cell, gastrin-secreting cell, glucagon-secreting cell, kidney mesangial cell, mammary cell, beta cell, fat/adipose cell, immune cell, and GI tract cell. Preferably, the nucleic acid is from a parathyroid cell, C-cell, or osteoclast. More preferably,

the nucleic acid encodes a calcium receptor and is present on a plasmid or vector.

In preferred embodiments the calcium receptor modulating compound is a calcimimetic which inhibits bone resorption *in vivo* by an osteoclast; inhibits bone resorption *in vitro* by an osteoclast; stimulates calcitonin secretion *in vitro* or *in vivo* from a c-cell; inhibits parathyroid hormone secretion from a parathyroid cell *in vitro* and decreases PTH secretion *in vivo*; elevates calcitonin levels *in vivo*; or blocks osteoclastic bone resorption *in vitro* and inhibits bone resorption *in vivo*.

In another preferred embodiment the calcium receptor modulating compound is a calcilytic which evokes the secretion of parathyroid hormone from parathyroid cells *in vitro* and elevates the level of parathyroid hormone *in vivo*.

Preferably, the compound selectively targets inorganic ion receptor activity, more preferably calcium receptor activity, in a particular cell. By "selectively" is meant that the compound exerts a greater effect on inorganic ion receptor activity in one cell type than at another cell type for a given concentration of compound. Preferably, the differential effect is 10-fold or greater. Preferably, the concentration refers to blood plasma concentration and the measured effect is the production of extracellular messengers such as plasma calcitonin, parathyroid hormone or plasma calcium. For example, in a preferred embodiment, the compound selectively targets PTH secretion over calcitonin secretion.

In another preferred embodiment, the compound has an EC_{50} or IC_{50} less than or equal to 5 μM at one or more, but not all cells chosen from the group consisting of: parathyroid cell, bone osteoclast, juxtaglomerular kidney cell, proximal tubule kidney cell, distal tubule kidney cell, central nervous system cell, peripheral nervous system cell, cell of the thick ascending limb of Henle's loop and/or collecting duct, keratinocyte in the epi-

dermis, parafollicular cell in the thyroid (C-cell), intestinal cell, platelet, vascular smooth muscle cell, cardiac atrial cell, gastrin-secreting cell, glucagon-secreting cell, kidney mesangial cell, mammary cell, beta
5 cell, fat/adipose cell, immune cell, GI tract cell, skin cell, adrenal cell, pituitary cell, hypothalamic cell and cell of the subfornical organ. More preferably, the cells are chosen from the group consisting of parathyroid cell, central nervous system cell, peripheral nervous system
10 cell, cell of the thick ascending limb of Henle's loop and/or collecting duct in the kidney, parafollicular cell in the thyroid (C-cell), intestinal cell, GI tract cell, pituitary cell, hypothalamic cell and cell of the subfornical organ. The presence of a calcium receptor in
15 this group of cells has been confirmed by physical data such as *in situ* hybridization and antibody staining.

Preferably, inorganic ion receptor modulating compounds mimic or block the effects of an extracellular ion on a cell having an inorganic ion receptor, such that the
20 compounds achieve a therapeutic effect. Inorganic ion receptor modulating compounds may have the same, or different, effects on cells having different types of inorganic ion receptor morphology (e.g., such as cells having normal inorganic ion receptors, a normal number of inorganic
25 ion receptor, an abnormal inorganic ion receptor, and an abnormal number of inorganic ion receptors).

Calcium receptor modulating compounds preferably mimic or block all of the effects of extracellular ion in a cell having a calcium receptor. However, calcimimetics
30 need not possess all the biological activities of extracellular Ca^{2+} . Similarly, calcilytics need not block all of the activities caused by extracellular calcium. Additionally, different calcimimetics and different calcilytics do not need to bind to the same site on the calcium
35 receptor as does extracellular Ca^{2+} to exert their effects.

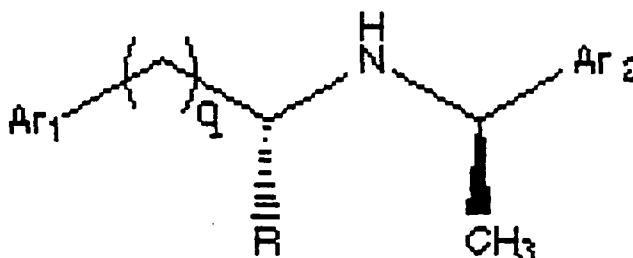
Inorganic modulating compounds need not effect inorganic receptor activity to the same extent or in exactly

the same manner as the natural ligand. For example, a calcimimetic may effect calcium receptor activity to a different extent, to a different duration, by binding to a different binding site, or by having a different affinity, compared to calcium acting at a calcium receptor.

A. Calcimimetics

1. Structure I Compounds

Structure I compounds able to modulate calcium receptor activity have the following formula:



where, Ar₁ is either naphthyl or phenyl optionally substituted with 0 to 5 substituents each independently selected from the group consisting of, lower alkyl, halogen, lower-alkoxy, lower thioalkyl, methylene dioxy, lower haloalkyl, lower haloalkoxy, OH, CH₂OH, CONH₂, CN, acetoxy, N(CH₃)₂, phenyl, phenoxy, benzyl, benzyloxy, α,α-dimethylbenzyl, NO₂, CHO, CH₃CH(OH), acetyl, ethylene dioxy, preferably each substituent is independently selected from the group consisting of, CH₃, CH₃O, CH₃CH₂O, methylene dioxy, Br, Cl, F, I, CF₃, CHF₂, CH₂F, CF₃O, CF₃CH₂O, CH₃S, OH, CH₂OH, CONH₂, CN, NO₂, CH₃CH₂, propyl, isopropyl, butyl, isobutyl, t-butyl, and acetoxy. More preferably, Ar₁ is either a naphthyl or a phenyl having 1-5 substituents each independently selected from the group consisting of isopropyl, CH₃O, CH₃S, CF₃O, I, Cl, F, CF₃, and CH₃, more preferably CF₃O, I, Cl, F, and CF₃;

Ar₂ is either naphthyl or phenyl optionally substituted with 0 to 5 substituents each independently selected from the group consisting of, lower alkyl,

halogen, lower alkoxy, lower thioalkyl, methylene dioxy, lower haloalkyl, lower haloalkoxy, OH, CH₂OH, CONH₂, CN, and acetoxy, preferably each substituent is independently selected from the group consisting of, CH₃, CH₃O, CH₃CH₂O, methylene dioxy, Br, Cl, F, I, CF₃, CHF₂, CH₂F, CF₃O, CF₃CH₂O, CH₃S, OH, CH₂OH, CONH₂, CN, NO₂, CH₃CH₂, propyl, isopropyl, butyl, isobutyl, t-butyl, and acetoxy. More preferably, Ar₂ is either a naphthyl or a phenyl having 1-5 substituents each independently selected from the group consisting of isopropyl, CH₃O, CH₃S, CF₃O, I, Cl, F, CF₃, and CH₃, more preferably CF₃O, I, Cl, F, CH₃O, and CF₃.

q is 0, 1, 2, or 3; and

R is either H, or CH₃;

and pharmaceutically salts and complexes thereof.

"Lower alkyl" refers to a saturated hydrocarbon having 1-4 carbons, preferably 1-3 carbon atoms, which may be straight chain or branched.

"Lower alkoxy" refers to "O-lower alkyl". Where "O" is an oxygen joined to a lower alkyl.

"Lower thioalkyl" refers to "S-lower alkyl". Where "S" is a sulfur joined to a lower alkyl.

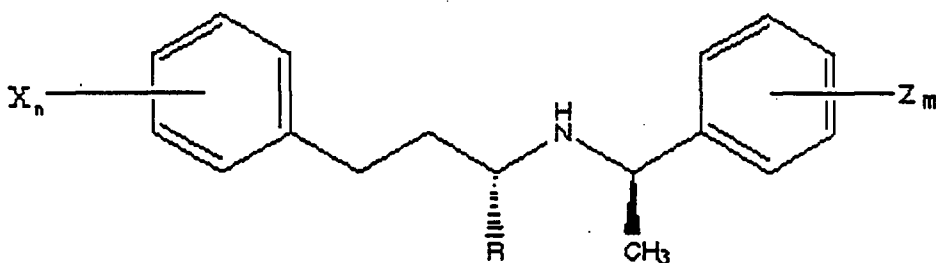
"Lower haloalkyl" refers to a lower alkyl substituted with at least one halogen. Preferably, only the terminal carbon of the lower haloalkyl is substituted with a halogen and 1 to 3 halogens are present. More preferably, the lower haloalkyl contains 1 carbon. Preferably, the halogen substitutions are either Cl or F.

"Lower haloalkoxy" refers to "O-lower haloalkyl". Where "O" is an oxygen joined to a lower haloalkyl.

a. Ar₁ and Ar₂ are Both Optionally Substituted Phenyls

In a preferred embodiment both Ar₁ and Ar₂ are optionally substituted phenyls and the compound has following formula:

21



where R is hydrogen or methyl

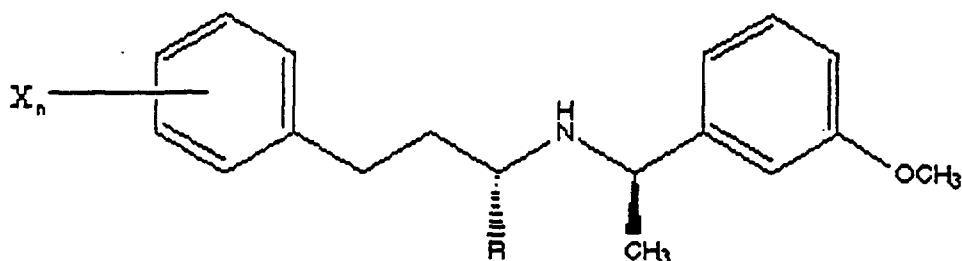
m and n are each independently 0, 1, 2, 3, 4, or 5;

each X is independently selected from the group consisting of, lower alkyl, halogen, lower alkoxy, lower thioalkyl, methylene dioxy, lower haloalkyl, lower haloalkoxy, OH, CH₂OH, CONH₂, CN, acetoxy, N(CH₃)₂, phenyl, phenoxy, benzyl, benzyloxy, α,α-dimethylbenzyl, NO₂, CHO, CH₃CH(OH), acetyl, ethylene dioxy. Preferably each X is independently selected from the group consisting of, CH₃,
 10 CH₃O, CH₃CH₂O, methylene dioxy, Br, Cl, F, I, CF₃, CHF₂, CH₂F, CF₃O, CF₃CH₂O, CH₃S, OH, CH₂OH, CONH₂, CN, NO₂, CH₃CH₂, propyl, isopropyl, butyl, isobutyl, t-butyl, and acetoxy. More preferably, each X is independently selected from the group consisting of isopropyl, CH₃O, CH₃S, CF₃O, I, Cl, F,
 15 CF₃, and CH₃, more preferably CF₃O, I, Cl, F, and CF₃;

each Z is independently selected from the group consisting of, lower alkyl, halogen, lower alkoxy, lower thioalkyl, methylene dioxy, lower haloalkyl, lower haloalkoxy, OH, CH₂OH, CONH₂, CN, and acetoxy. Preferably each
 20 Z is independently selected from the group consisting of, CH₃, CH₃O, CH₃CH₂O, methylene dioxy, Br, Cl, F, I, CF₃, CHF₂, CH₂F, CF₃O, CF₃CH₂O, CH₃S, OH, CH₂OH, CONH₂, CN, CH₃CH₂, propyl, isopropyl, butyl, isobutyl, t-butyl, and acetoxy. More preferably, each Z is independently selected from the
 25 group consisting of, isopropyl, CH₃O, CH₃S, CF₃O, CF₃, I, Cl, F, and CH₃.

In a more preferred embodiment, at least one of the Z substituents is in the meta position. More preferably, the compound has the following formula:

22

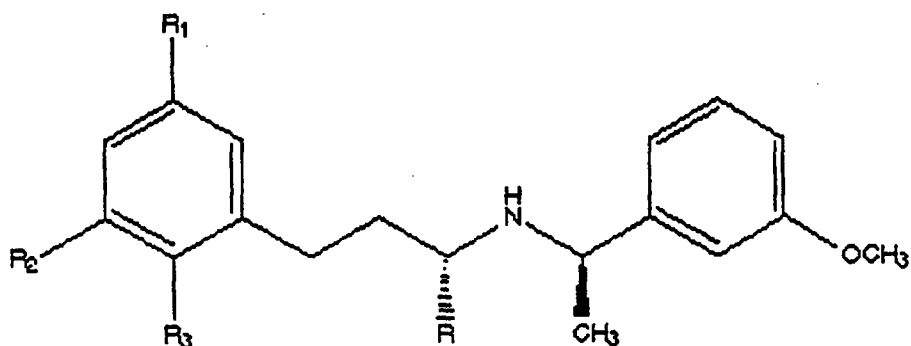


where R is either hydrogen or methyl;

m is 0, 1, 2, 3, 4, or 5, preferably 1 or 2;

and each X is independently selected from the group consisting of, lower alkyl, halogen, lower alkoxy, lower thioalkyl, methylene dioxy, lower haloalkyl, lower haloalkoxy, OH, CH₂OH, CONH₂, CN, acetoxy, N(CH₃)₂, phenyl, phenoxy, benzyl, benzyloxy, α,α-dimethylbenzyl, NO₂, CHO, CH₃CH(OH), acetyl, ethylene dioxy, preferably each substituent is independently selected from the group consisting of, CH₃, CH₃O, CH₃CH₂O, methylene dioxy, Br, Cl, F, I, CF₃, CHF₂, CH₂F, CF₃O, CF₃CH₂O, CH₃S, OH, CH₂OH, CONH₂, CN, NO₂, CH₃CH₂, propyl, isopropyl, butyl, isobutyl, t-butyl, and acetoxy, more preferably, isopropyl, CH₃O, CH₃S, CF₃O, CF₃, I, Cl, F, and CH₃.

More preferably, the compound has the formula:



where R is either hydrogen or methyl;

R₁ is either halogen or hydrogen, preferably R₁ is either F, or hydrogen;

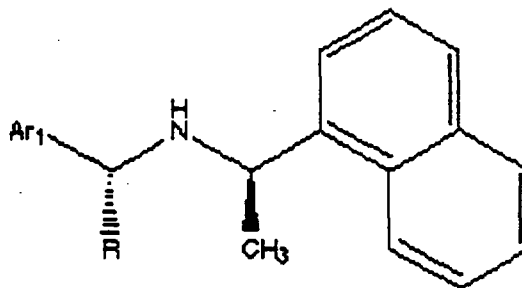
R_2 is either hydrogen, halogen, lower alkyl, lower haloalkyl, or lower haloalkoxy, preferably, R_2 is either hydrogen, CF_3 , CH_3 , OCF_3 , or F, and

R_3 is either hydrogen, halogen, or alkoxy, preferably,
 5 R_3 is either Cl, F, hydrogen, or methoxy, more preferably methoxy.

In alternative more preferred combinations; at least two of R_1 , R_2 , and R_3 is halogen, preferably F and R is hydrogen or CH_3 ; R is hydrogen or CH_3 , R_2 is either lower
 10 haloalkyl, or lower haloalkoxy, preferably OCF_3 or CF_3 , and R_1 and R_3 is hydrogen; and R is CH_3 , R_3 is halogen, preferably Cl, R_1 is either halogen or hydrogen, preferably F or hydrogen, and R_2 is either hydrogen, lower alkyl, lower haloalkyl, or lower haloalkoxy, preferably, hydrogen, CF_3 ,
 15 CH_3 , OCF_3 , or F.

b. Ar_2 is Naphthyl and q is 0

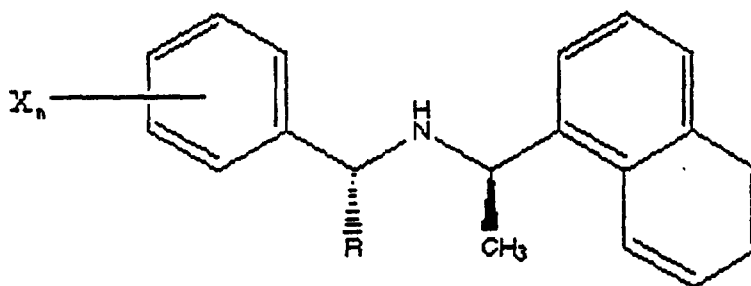
In another preferred embodiment, Ar_2 is naphthyl, q is 0, and the compound has the formula:



where Ar_1 is either naphthyl or phenyl optionally
 20 substituted with 0 to 5 substituents each independently selected from the group consisting of, lower alkyl, halogen, lower alkoxy, lower thioalkyl, methylene dioxy, lower haloalkyl, lower haloalkoxy, OH, CH_2OH , $CONH_2$, CN, acetoxy, $N(CH_3)_2$, phenyl, phenoxy, benzyl, benzyloxy, α, α -
 25 dimethylbenzyl, NO_2 , CHO, $CH_3CH(OH)$, acetyl, ethylene dioxy, preferably each substituent is independently selected from the group consisting of, CH_3 , CH_3O , CH_3CH_2O ,

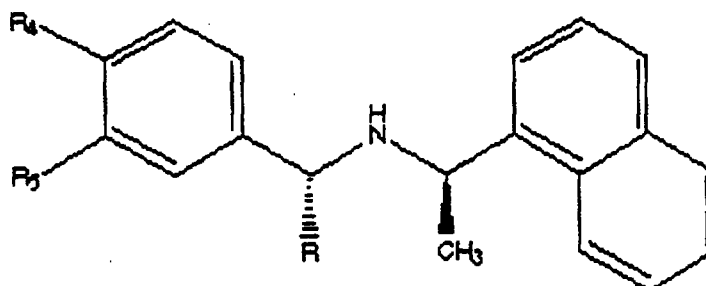
methylene dioxy, Br, Cl, F, I, CF₃, CHF₂, CH₂F, CF₃O, CF₃CH₂O, CH₃S, OH, CH₂OH, CONH₂, CN, NO₂, CH₃CH₂, propyl, isopropyl, butyl, isobutyl, t-butyl, and acetoxy. More preferably, Ar₁ is either a naphthyl or a phenyl having 1-5
 5 substituents each independently selected from the group consisting of isopropyl, CH₃O, CH₃S, CF₃, CF₃O I, Cl, F, and CH₃

More preferably, Ar₁ is an optional substituted phenyl where the compound has the formula:



10 where X_n represents the optional substituents for the optionally substituted phenyl as described above (with the preferred substituents and number of substituents as described above).

Even more preferably the compound has the formula:



15 where R is either CH₃ or hydrogen;

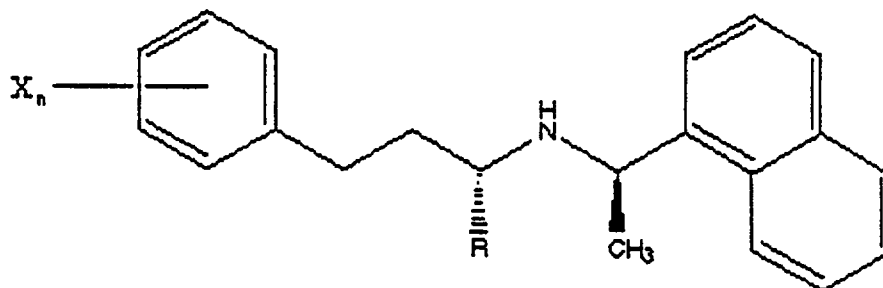
R₄ is either lower alkyl, halogen, or alkoxy, preferably isopropyl, chlorine, or methoxy; and

R₅ is either hydrogen, lower alkyl, or halogen, preferably methyl, CH₃, Br, or Cl.

c. Ar₂ is Naphthyl and q is 2

In another preferred embodiment, Ar₁ is a substituted phenyl, Ar₂ is naphthyl, q is 2 and the compound has the formula:

5



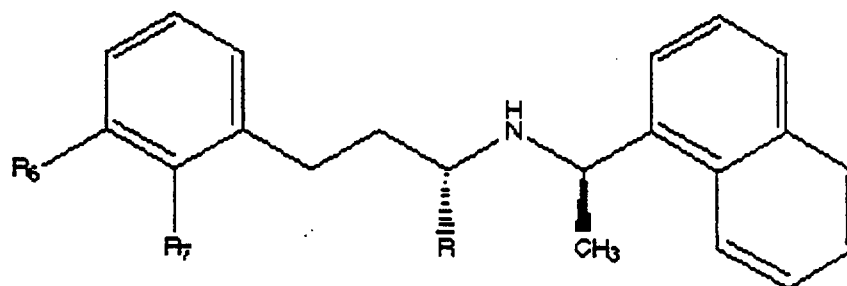
where R is either hydrogen or CH₃;

n is 0, 1, 2, 3, 4, or 5, preferably 1 or 2; and

each X is independently selected from the group consisting of, lower alkyl, halogen, lower alkoxy, lower thioalkyl, methylene dioxy, lower haloalkyl, lower haloalkoxy, OH, CH₂OH, CONH₂, CN, acetoxy, N(CH₃)₂, phenyl, phenoxy, benzyl, benzyloxy, α,α-dimethylbenzyl, NO₂, CHO, CH₃CH(OH), acetyl, ethylene dioxy, preferably each substituent is independently selected from the group consisting of, CH₃, CH₃O, CH₃CH₂O, methylene dioxy, Br, Cl, F, I, CF₃, CHF₂, CH₂F, CF₃O, CF₃CH₂O, CH₃S, OH, CH₂OH, CONH₂, CN, NO₂, CH₃CH₂, propyl, isopropyl, butyl, isobutyl, t-butyl, and acetoxy, more preferably, isopropyl, CH₃O, CH₃S, CF₃O, CF₃, I, Cl, F, and CH₃.

20

More preferably, the compound has the formula:



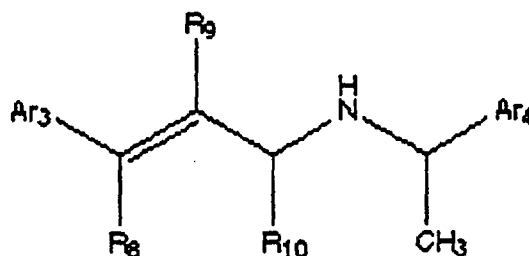
where R_6 is either hydrogen, lower haloalkyl, or lower haloalkoxy, preferably hydrogen, OCF_3 , or CF_3 ; and

R_7 is either halogen or hydrogen, preferably chlorine
5 or hydrogen.

In other embodiments R , R_6 and R_7 are as described above (with the preferred substituents as described above), provided that when both R and R_6 are hydrogen, R_7 is not Cl ; and R is CH_3 , and R_6 and R_7 is as described above
10 (with the preferred substituents as described above).

2. Structure II Compounds

Structure II compounds have the formula:



where Ar_3 is either naphthyl or phenyl optionally substituted with 0 to 5 substituents each independently
15 selected from the group consisting of, lower alkyl, halogen, lower alkoxy, lower thioalkyl, methylene dioxy, lower haloalkyl, lower haloalkoxy, OH , CH_2OH , $CONH_2$, CN , acetoxy, benzyl, benzyloxy, α,α -dimethylbenzyl, NO_2 , CHO , $CH_3CH(OH)$, $N(CH_3)_2$, acetyl, ethylene dioxy, preferably
20 $N(CH_3)_2$, lower alkoxy, or lower alkyl;

Ar_4 is either naphthyl or phenyl optionally substituted with 0 to 5 substituents each independently selected from the group consisting of, lower alkyl, halogen, lower alkoxy, lower thioalkyl, methylene dioxy,
25 lower haloalkyl, lower haloalkoxy, OH , CH_2OH , $CONH_2$, CN , and acetoxy, preferably lower alkoxy, more preferably methoxy;

R_9 is either hydrogen or phenyl, preferably hydrogen;

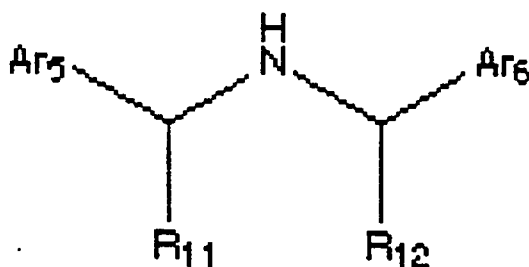
R_9 is either hydrogen or methyl; and

R_{10} is either hydrogen, methyl, or phenyl, more preferably when R_{10} is methyl the chiral carbon it is attached to is the (R) stereoisomer.

- 5 Preferably, the α -methyl in Structure II is an (R)- α -methyl.

3. Structure III Compounds

Structure III compounds have the formula:



- where Ar_5 is either naphthyl or phenyl optionally substituted with 0 to 5 substituents each independently selected from the group consisting of, lower alkyl, halogen, lower alkoxy, lower thioalkyl, methylene dioxy, lower haloalkyl, lower haloalkoxy, OH, CH_2OH , $CONH_2$, CN, acetoxy, benzyl, benzyloxy, α, α -dimethylbenzyl, NO_2 , CHO, $CH_3CH(OH)$, acetyl, ethylene dioxy, $-CH=CH$ -phenyl, preferably, lower alkyl, phenoxy, $-CH=CH$ -phenyl, dimethylbenzyl, methoxy, methylene, or ethylene;
- 10
- 15

- Ar_6 is either naphthyl or phenyl optionally substituted with 0 to 5 substituents each independently selected from the group consisting of, acetyl, lower alkyl, halogen, lower alkoxy, lower thioalkyl, methylene dioxy, lower haloalkyl, lower haloalkoxy, OH, CH_2OH , $CONH_2$, CN, carbomethoxy, $OCH_2C(O)C_2H_5$ and acetoxy, preferably methoxy, lower alkyl, phenyl, halogen, CF_3 , CN, carbomethoxy or, $OCH_2C(O)C_2H_5$;
- 20
- 25

R_{11} is hydrogen or methyl, preferably when R_{11} is methyl the carbon to which it is attached is an (R) stereoisomer; and

R_{12} is hydrogen or methyl, preferably when R_{12} is methyl the carbon to which it is attached is an (R) stereoisomer.

4. Calcimimetic Activity

5 The ability of compounds to mimic the activity of Ca^{2+} at calcium receptors can be determined using procedures known in the art and described by Nemeth et al., PCT/US93/01642, International Publication Number WO 94/18959. For example, calcimimetics possess one or more and preferably
10 all of the following activities when tested on parathyroid cells *in vitro*:

1. The compound causes a rapid (time to peak < 5 seconds) and transient increase in intracellular calcium concentration that is refractory to inhibition by
15 $1 \mu M La^{3+}$ or $1 \mu M Gd^{3+}$. The increase in $[Ca^{2+}]_i$ persists in the absence of extracellular Ca^{2+} , but is abolished by pre-treatment with ionomycin (in the absence of extracellular Ca^{2+});

2. The compound potentiates increases in $[Ca^{2+}]_i$,
20 elicited by submaximal concentrations of extracellular Ca^{2+} ;

3. The increase in $[Ca^{2+}]_i$ elicited by extracellular Ca^{2+} is not inhibited by dihydropyridines;

4. The transient increase in $[Ca^{2+}]_i$ caused by
25 the compound is abolished by pretreatment for 10 minutes with 10 mM sodium fluoride;

5. The transient increase in $[Ca^{2+}]_i$ caused by the compound is diminished by pretreatment with an activator of protein kinase C (PKC), such as phorbol
30 myristate acetate (PMA), mezerein or (-)-indolactam V. The overall effect of the protein kinase C activator is to shift the concentration-response curve of the compound to the right without affecting the maximal response;

6. The compound causes a rapid (< 30 seconds)
35 increase in the formation of inositol-1,4,5-triphosphate and/or diacylglycerol;

7. The compound inhibits dopamine- or isoproterenol-stimulated cyclic AMP formation;
8. The compound inhibits PTH secretion;
9. Pretreatment with pertussis toxin (100
5 ng/ml for > 4 hours) blocks the inhibitory effect of the compound on cyclic AMP formation, but does not effect increases in $[Ca^{2+}]_i$, inositol-1,4,5-triphosphate, or diacylglycerol, nor decreases in PTH secretion;
10. The compound elicits increases in Cl^-
10 current in *Xenopus* oocytes injected with poly(A)⁺-enriched mRNA from bovine or human parathyroid cells, but is without effect in *Xenopus* oocytes injected with water, or liver mRNA; and
11. Similarly, using a cloned calcium receptor
15 from a parathyroid cell, the compound will elicit a response in *Xenopus* oocytes injected with the specific cDNA or mRNA encoding the receptor.

Different calcium activities can be measured using available techniques. (See, Nemeth et al., PCT/US93/01642,
20 International Publication Number WO 94/18959.) Parallel definitions of compounds mimicking Ca^{2+} activity on other calcium responsive cell, preferably at a calcium receptor, are evident from the examples provided herein and Nemeth et al., PCT/US93/01642, International Publication Number
25 WO 94/18959.

Preferably, the compound as measured by the bioassays described herein, or by Nemeth et al., PCT/US93/01642, International Publication Number WO 94/18959, has one or more, more preferably all of the following activities:
30 evokes a transient increase in internal calcium, having a duration of less than 30 seconds (preferably by mobilizing internal calcium); evokes a rapid increase in $[Ca^{2+}]_i$, occurring within thirty seconds; evokes a sustained increase (greater than thirty seconds) in $[Ca^{2+}]_i$ (preferably by causing an influx of external calcium); evokes an
35 increase in inositol-1,4,5-triphosphate or diacylglycerol levels, preferably within less than 60 seconds; and

inhibits dopamine- or isoproterenol-stimulated cyclic AMP formation.

The transient increase in $[Ca^{2+}]_i$ is preferably abolished by pretreatment of the cell for ten minutes with 10 mM sodium fluoride, or the transient increase is diminished by brief pretreatment (not more than ten minutes) of the cell with an activator of protein kinase C, preferably, phorbol myristate acetate (PMA), mezerein or (-) indolactam V.

10 C. Calcilytics

The ability of a compound to block the activity of extracellular calcium at a calcium receptor can be determined using standard techniques based on the present disclosure. (See, also Nemeth et al., PCT/US93/01642, International Publication Number WO 94/18959.) For example, compounds which block the effect of extracellular calcium, when used in reference to a parathyroid cell, possess one or more, and preferably all of the following characteristics when tested on parathyroid cells in vitro:

20 1. The compound blocks, either partially or completely, the ability of increased concentrations of extracellular Ca^{2+} to:

- (a) increase $[Ca^{2+}]_i$,
- (b) mobilize intracellular Ca^{2+} ,
- 25 (c) increase the formation of inositol-1,4,5-triphosphate,
- (d) decrease dopamine- or isoproterenol-stimulated cyclic AMP formation, and
- (e) inhibit PTH secretion;

30 2. The compound blocks increases in Cl^- current in *Xenopus* oocytes injected with poly(A)⁺-mRNA from bovine or human parathyroid cells elicited by extracellular Ca^{2+} or calcimimetic compounds, but not in *Xenopus* oocytes injected with water or liver mRNA;

35 3. Similarly, using a cloned calcium receptor from a parathyroid cell, the compound will block a response in

Xenopus oocytes injected with the specific cDNA, mRNA or cRNA encoding the calcium receptor, elicited by extracellular Ca^{2+} or a calcimimetic compound.

Parallel definitions of compounds blocking Ca^{2+} activity on a calcium responsive cell, preferably at a calcium receptor, are evident from the examples provided herein and Nemeth et al., PCT/US93/01642, International Publication Number WO 94/18959.

III. TREATMENT OF DISEASES OR DISORDERS

10 Diseases or disorders which can be treated by modulating calcium receptor activity are known in the art. For example, diseases or disorders which can be treated by modulating calcium receptor activity can be identified based on the functional responses of cells regulated by calcium receptor activity. Functional responses of cells regulated by calcium receptor are known in the art, including PTH secretion by parathyroid cells, calcitonin secretion by C-cells, and bone resorption by osteoclasts.

Such functional responses are associated with different diseases or disorders. For example, hyperparathyroidism results in elevated levels of PTH in the plasma. Decreasing the plasma levels of PTH offers an effective means of treating hyperparathyroidism. Likewise, increasing plasma levels of calcitonin is associated with an inhibition of bone resorption. Inhibiting bone resorption is an effective treatment for osteoporosis. Thus, modulation of calcium receptor activity can be used to treat diseases such as hyperparathyroidism, and osteoporosis.

Those compounds modulating inorganic ion receptor activity, preferably calcium receptor activity, can be used to confer beneficial effects to patients suffering from a variety of diseases or disorders. For example, osteoporosis is an age-related disorder characterized by loss of bone mass and increased risk of bone fracture. Compounds can be used to block osteoclastic bone resorption either directly (e.g., an osteoclast ionomimetic

compound) or indirectly by increasing endogenous calcitonin levels (e.g., a C-cell calcimimetic). Alternatively, a calcilytic active on the parathyroid cell calcium receptor will increase circulating levels of parathyroid hormone, stimulating bone formation. All three of these approaches will result in beneficial effects to patients suffering from osteoporosis.

In addition, it is known that intermittent low dosing with PTH results in an anabolic effect on bone mass and appropriate bone remodeling. Thus, compounds and dosing regimens evoking transient increases in parathyroid hormone (e.g., intermittent dosing with a parathyroid cell ionolytic) can increase bone mass in patients suffering from osteoporosis.

Additional diseases or disorders can be identified by identifying additional cellular functional responses, associated with a disease or disorder, which are regulated by calcium receptor activity. Diseases or disorder which can be treated by modulating other inorganic ion receptors can be identified in an analogous manner.

The inorganic ion receptor-modulating compounds of the present invention can exert an affect at an inorganic ion receptor causing one or more cellular effects ultimately producing a therapeutic effect. Calcium receptor-modulating compounds of the present invention can exert an effect on calcium receptor causing one or more cellular effects ultimately producing a therapeutic effect. Different diseases can be treated by the present invention by targeting cells having a calcium receptor.

For example, primary hyperparathyroidism (HPT) is characterized by hypercalcemia and abnormal elevated levels of circulating PTH. A defect associated with the major type of HPT is a diminished sensitivity of parathyroid cells to negative feedback regulation by extracellular Ca^{2+} . Thus, in tissue from patients with primary HPT, the "set-point" for extracellular Ca^{2+} is shifted to the right so that higher than normal concentrations of

extracellular Ca^{2+} are required to depress PTH secretion. Moreover, in primary HPT, even high concentrations of extracellular Ca^{2+} often depress PTH secretion only partially. In secondary (uremic) HPT, a similar increase
5 in the set-point for extracellular Ca^{2+} is observed even though the degree to which Ca^{2+} suppresses PTH secretion is normal. The changes in PTH secretion are paralleled by changes in $[\text{Ca}^{2+}]_i$: the set-point for extracellular Ca^{2+} -induced increases in $[\text{Ca}^{2+}]_i$ is shifted to the right and the
10 magnitude of such increases is reduced.

Patients suffering from secondary HPT may also have renal osteodystrophy. Calcimimetics appear to be useful for treating both abnormal PTH secretion and osteodystrophy in such patients.

15 Compounds that mimic the action of extracellular Ca^{2+} are beneficial in the long-term management of both primary and secondary HPT. Such compounds provide the added impetus required to suppress PTH secretion which the hypercalcemic condition alone cannot achieve and, thereby, help to
20 relieve the hypercalcemic condition. Compounds with greater efficacy than extracellular Ca^{2+} may overcome the apparent nonsuppressible component of PTH secretion which is particularly troublesome in the major form of primary HPT caused by adenoma of the parathyroid gland.
25 Alternatively or additionally, such compounds can depress synthesis of PTH, as prolonged hypercalcemia has been shown to depress the levels of preproPTH mRNA in bovine and human adenomatous parathyroid tissue. Prolonged hypercalcemia also depresses parathyroid cell proliferation
30 in vitro, so calcimimetics can also be effective in limiting the parathyroid cell hyperplasia characteristic of secondary HPT.

Cells other than parathyroid cells can respond directly to physiological changes in the concentration of
35 extracellular Ca^{2+} . For example, calcitonin secretion from parafollicular cells in the thyroid (C-cells) is regulated by changes in the concentration of extracellular Ca^{2+} .

Isolated osteoclasts respond to increases in the concentration of extracellular Ca^{2+} with corresponding increases in $[\text{Ca}^{2+}]_i$ that arise partly from the mobilization of intracellular Ca^{2+} . Increases in $[\text{Ca}^{2+}]_i$ in osteoclasts
5 are associated with the inhibition of bone resorption. Release of alkaline phosphatase from bone-forming osteoblasts is directly stimulated by calcium.

Renin secretion from juxtaglomerular cells in the kidney, like PTH secretion, is depressed by increased
10 concentrations of extracellular Ca^{2+} . Extracellular Ca^{2+} causes the mobilization of intracellular Ca^{2+} in these cells. Other kidney cells respond to calcium as follows: elevated Ca^{2+} inhibits formation of $1,25(\text{OH})_2$ -vitamin D by proximal tubule cells, stimulates production of calcium-
15 binding protein in distal tubule cells, and inhibits tubular reabsorption of Ca^{2+} and Mg^{2+} and the action of vasopressin on the thick ascending limb of Henle's loop (MTAL), reduces vasopressin action in the cortical collecting duct cells, and affects vascular smooth muscle
20 cells in blood vessels of the renal glomerulus.

Calcium also promotes the differentiation of intestinal goblet cells, mammary cells, and skin cells; inhibits atrial natriuretic peptide secretion from cardiac atria; reduces cAMP accumulation in platelets; alters
25 gastrin and glucagon secretion; acts on vascular smooth muscle cells to modify cell secretion of vasoactive factors; and affects cells of the central nervous system and peripheral nervous system.

Thus, there are sufficient indications to suggest
30 that Ca^{2+} , in addition to its ubiquitous role as an intracellular signal, also functions as an extracellular signal to regulate the responses of certain specialized cells. Compounds of this invention can be used in the treatment of diseases or disorders associated with
35 disrupted Ca^{2+} responses in these cells.

Specific diseases and disorders which might be treated or prevented, based upon the affected cells, also

include those of the central nervous system such as seizures, stroke, head trauma, spinal cord injury, hypoxia-induced nerve cell damage such as in cardiac arrest or neonatal distress, epilepsy, neurodegenerative diseases such as Alzheimer's disease, Huntington's disease and Parkinson's disease, dementia, muscle tension, depression, anxiety, panic disorder, obsessive-compulsive disorder, post-traumatic stress disorder, schizophrenia, neuroleptic malignant syndrome, and Tourette's syndrome; diseases involving excess water reabsorption by the kidney such as syndrome of inappropriate ADH secretion (SIADH), cirrhosis, congestive heart failure, and nephrosis; hypertension; preventing and/or decreasing renal toxicity from cationic antibiotics (e.g., aminoglycoside antibiotics); gut motility disorders such as diarrhea, and spastic colon; GI ulcer diseases; GI diseases with excessive calcium absorption such as sarcoidosis; and autoimmune diseases and organ transplant rejection.

While calcium receptor-modulating compounds of the present invention will typically be used in therapy for human patients, they may also be used to treat similar or identical diseases in other warm-blooded animal species such as other primates, farm animals such as swine, cattle, and poultry; and sports animals and pets such as horses, dogs and cats.

IV. Administration

The different compounds described by the present invention can be used to treat different diseases or disorders by modulating inorganic ion receptor activity, preferably calcium receptor activity. The compounds of the invention can be formulated for a variety of modes of administration, including systemic and topical or localized administration. Techniques and formulations generally may be found in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA. Administration of ionomimetics and ionolytics is discussed by Nemeth et al.,

PCT/US93/01642, International Publication Number WO 94/18959.

Suitable dosage forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or
5 by injection. Such dosage forms should allow the compound to reach a target cell whether the target cell is present in a multicellular host or in culture. For example, pharmacological compounds or compositions injected into the blood stream should be soluble. Other factors are
10 known in the art, and include considerations such as toxicity and dosage form which retard the compound or composition from exerting its effect.

Compounds can also be formulated as pharmaceutically acceptable salts (e.g., acid addition salts) and complexes
15 thereof. Pharmaceutically acceptable salts are non-toxic salts at the concentration at which they are administered. The preparation of such salts can facilitate the pharmacological use by altering the physical characteristic of the compound without preventing it from exerting its physio-
20 logical effect. Useful alterations in physical properties include lowering the melting point to facilitate transmucosal administration and increasing the solubility to facilitate administering higher concentrations of the drug.

25 Pharmaceutically acceptable salts include acid addition salts such as those containing sulfate, hydrochloride, maleate, phosphate, sulfamate, acetate, citrate, lactate, tartrate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, cyclohexylsulfamate
30 and quinate. (See e.g., PCT/US92/03736, hereby incorporated by reference herein.) Pharmaceutically acceptable salts can be obtained from acids such as hydrochloric acid, maleic acid, sulfuric acid, phosphoric acid, sulfamic acid, acetic acid, citric acid, lactic acid,
35 tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, cyclohexylsulfamic acid, and quinic acid.

Pharmaceutically acceptable salts can be prepared by standard techniques. For example, the free base form of a compound is dissolved in a suitable solvent, such as an aqueous or aqueous-alcohol solution, containing the appropriate acid and then isolated by evaporating the solution. In another example, a salt is prepared by reacting the free base and acid in an organic solvent.

Carriers or excipients can also be used to facilitate administration of the compound. Examples of carriers and excipients include calcium carbonate, calcium phosphate, various sugars such as lactose, glucose, or sucrose, or types of starch, cellulose derivatives, gelatin, vegetable oils, polyethylene glycols and physiologically compatible solvents. The compositions or pharmaceutical composition can be administered by different routes including intravenously, intraperitoneal, subcutaneous, and intramuscular, orally, topically, or transmucosally.

For systemic administration, oral administration is preferred. Alternatively, injection may be used, e.g., intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the compounds of the invention are formulated in liquid solutions, preferably in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the compounds may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms can also be produced.

Systemic administration can also be by transmucosal or transdermal means, or the compounds can be administered orally. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, bile salts and fusidic acid derivatives. In addition, detergents may be used to facilitate permeation. Transmucosal administration may be through nasal sprays, for example, or using suppositories. For

oral administration, the compounds can be formulated into conventional oral administration dosage forms such as capsules, tablets, and liquid preparations.

For topical administration, the compounds of the invention can be formulated into ointments, salves, gels, or creams, as is generally known in the art.

The amounts of various compounds of this invention to be administered can be determined by standard procedures. Generally, a therapeutically effective amount is between about 1 nmole and 3 μ mole of the compound, preferably 0.1 nmole and 1 μ mole depending on its EC_{50} or IC_{50} and on the age and size of the patient, and the disease or disorder associated with the patient. Generally, it is an amount between about 0.1 and 50 mg/kg, preferably 0.01 and 20 mg/kg of the animal to be treated.

V. Examples

Examples are provided below illustrating different aspects and embodiments of the present invention. These examples are not intended to limit the claimed invention.

20 Example 1: Cloning of Human Parathyroid Calcium Receptor From a Human Parathyroid Gland Adenoma Tumor

This example describes the cloning of a human parathyroid calcium receptor from a human parathyroid gland adenoma tumor using pBoPCaR1 as a hybridization probe (See, Nemeth et al., PCT/US93/01642, International Publication Number WO 94/18959). The probe was used to identify nucleic acid encoding human parathyroid gland calcium receptor by cross-hybridization at reduced stringency.

Messenger RNA was prepared from a human parathyroid gland adenoma tumor removed from a 39-year-old Caucasian male diagnosed with primary hyperparathyroidism. Northern blot analysis of this mRNA using pBoPCaR1 as a hybridization probe identified calcium receptor transcripts of about 5 Kb and about 4 Kb. A cDNA library was constructed

from the mRNA. Double-stranded cDNA larger than 3 Kbp were size-selected on an agarose gel and ligated into the cloning vector lambda ZapII. Five hundred thousand primary recombinant phage were screened with the 5.2 Kbp
5 cDNA insert of pBoPCaR1 as a hybridization probe. The pBoPCaR1 insert was labeled by random-primed synthesis using [³²P]-dCTP to a specific activity of 1×10^9 cpm/ μ g.

Library screening was performed at a hybridization stringency of 400 mM Na⁺, 50% formamide at a temperature of
10 38°C. Plaque lift filters were hybridized at a probe concentration of 500,000 cpm/ml for 20 hours. Following hybridization, filters were washed in 1 x SSC at 40°C for 1 hr.

The primary screen identified about 250 positive
15 clones identified by hybridization to pBoPCaR1. Seven of these clones were taken through secondary and tertiary screens to isolate single clones that hybridized to the pBoPCaR1 probe. These seven clones were analyzed by restriction enzyme mapping and Southern blot analysis.
20 Three of the clones contained cDNA inserts of about 5 Kbp and appear to be full-length clones corresponding to the 5 Kb mRNA. Two of the clones contain cDNA inserts of about 4 Kbp and appear to be full-length clones corresponding to the 4 Kb mRNA.

25 Restriction enzyme mapping of the two different sized inserts indicate that they share regions of sequence similarity in their 5' ends, but diverge in their 3' end sequences. DNA sequence analyses indicate that the smaller insert may result from alternative polyadenylation
30 upstream of the polyadenylation site used in the larger insert.

Representative cDNA inserts for both size classes were subcloned into the plasmid vector pBluescript SK. Linearization followed by in vitro transcription using T7
35 RNA polymerase produced cRNA transcripts. The cRNA transcripts were injected into *Xenopus* oocytes (150 ng/ μ l RNA; 50 nl/oocyte) for functional analysis. Following

incubation periods of 2-4 days, the oocytes were assayed for the presence of functional calcium receptors. Both clone types gave rise to functional calcium receptors as assessed by the stimulation of calcium-activated chloride currents upon addition of appropriate calcium receptor agonists. Known calcium receptor agonists, including NPS R-467 and NPS R-568 (see, Nemeth et al., PCT/US93/01642, International Publication Number WO 94/18959), activated the oocyte-expressed receptor at about the same concentrations known to be effective for the native parathyroid cell receptor. Thus, both clones encode a functional, human parathyroid cell calcium receptor.

Plasmids were prepared by subcloning each size class of insert into pBluescript thereby producing pHuPCaR 5.2 and pHuCaR 4.0. The nucleic acid sequence, and amino acid sequence, of the inserts are shown in SEQ. ID. Nos. 1 and 2.

Several differences were observed between the nucleic acid sequences of the two cDNA inserts. Sequence analyses of the two cDNA inserts indicate the existence of at least two sequence variants differing in the 3' untranslated region and which may result from alternative polyadenylation. In addition, sequence variation exists at the 5' end of the inserts. These distinct sequences correspond to untranslated regions and may have arisen due to alternative transcriptional initiation and/or splicing.

Three additional sites of sequence variation are observed within the coding regions of cDNA clones pHuPCaR5.2 and pHuPCaR4.0 (see SEQ. ID. NOS. 1 and 2) demonstrating that these cDNA clones encode distinct proteins. Sequence analysis of the human CaR gene indicates that the additional 30 base pairs of DNA in cDNA clone pHuPCaR5.2, as compared to the pHuPCaR 4.0 cDNA clone, results from alternative mRNA splicing. The alternative mRNA splicing is predicted to insert 10 additional amino acids into the CaR polypeptide encoded by the pHuPCaR5.2 cDNA at a site between aa#536 and aa#537 in

polypeptide encoded by pHuPCaR4.0 cDNA. In addition, pHuPCaR4.0 encodes glutamine (Gln) at aa#925 and glycine (Gly) at position 990 whereas pHuPCaR5.2 encodes arg (Arg) at both equivalent positions. The human CaR gene encodes for Gln and Arg, respectively, at these positions. The difference between the pHuPCaR4.0 cDNA compared to human DNA appears to represent a true sequence polymorphism within the human population while the single base change in pHuPCaR5.2 probably reflects a mutation which occurred during its cloning. Both cDNAs encode functional calcium receptors as demonstrated by the ability of *Xenopus* oocytes injected with cRNA prepared from these cDNA clones to respond to 10 mM extracellular calcium as ascertained by Cl⁻ conductance. However, it is possible that these two receptor isoforms are functionally and/or pharmacologically distinct.

Example 2: Selection of Stable Recombinant Cells Expressing the Calcium Receptor

Clonal cell lines that stably express the two human and the bovine calcium receptors have been isolated. Calcium receptor cDNAs were subcloned in two different, commercially available expression vectors; pMSG (obtained from Pharmacia) and Cep4B (obtained from Invitrogen). The first vector contains the selectable marker gene for xanthine-guanine phosphoribosyltransferase (gpt) allowing stably transfected cells to overcome the blockade of the purine biosynthetic pathway imposed by addition of 2 µg/ml aminopterin and 25 µg/ml mycophenolic acid. The second vector encodes a gene conferring resistance to the antibiotic hygromycin (used at 200 µg/ml). HuPCaR 5.2 and HuPCaR 4.0 cDNAs (SEQ. ID. NOs. 1 and 2, respectively) were removed from the parent bluescript plasmid with Not I and Hind III restriction enzymes and then either ligated directly into Not I + Hind III digested Cep4B or treated with the klenow fragment of DNA polymerase prior to blunt-end ligation into Sma I digested pMSG.

The pMSG subclone containing the HuPCaR 5.2 insert was transfected into CHO cells as discussed above. Selection has resulted in 20 resistant clones which are being characterized. The Cep4B subclone containing the HuPCaR 5.2 insert was transfected into HEK 293 cells as described above. Selection with hygromycin resulted in a pool of stable clones. Clones expressing the HuPCaR 4.0 receptor isoform were prepared similarly.

Cells obtained from the pool of hygromycin selected HEK 293 cells transfected with Cep4B containing the HuPCaR 5.2 insert were plated on collagen coated Aklar squares which had been placed into individual wells of 12-well tissue culture plates. Two to six days later, medium was removed and the cells washed with balanced salt solution and 1 ml of buffer containing 1 μ M fura2-AM, 1 mM CaCl_2 , and 0.1% BSA and 1 mM CaCl_2 . Measurements of fluorescence in response to calcium receptor agonists were performed at 37°C in a spectrofluorimeter using excitation and emission wavelengths of 340 and 510 nm, respectively. For signal calibration, F_{max} was determined after addition of ionomycin (40 μ M) and the apparent F_{min} was determined by addition of 0.3 M EGTA, 2.5 M Tris-HCl; pH 10. Robust increases in $[\text{Ca}^{2+}]_i$ were observed in response to the addition of the following calcium receptor agonists: Ca^{2+} (10 mM), Mg^{2+} (20 mM) and NPS R-467. Control cells expressing functional substance K receptors did not respond to these calcimimetic compounds.

Additional clonal isolates of HEK 293 cells transfected with pHuPCaR4.0 sequence were obtained. These were tested for responsiveness to calcimimetics as described above except that the cells were tested while in suspension.

Example 3: Using Fura-2 Loaded Parathyroid cells To Measure to Calcium Receptor Activity

This section describes procedures used to obtain parathyroid cells from calves and humans, and to use the
5 parathyroid cells to measure calcium receptor activity.

Parathyroid glands were obtained from freshly slaughtered calves (12-15 weeks old) at a local abattoir and transported to the laboratory in ice-cold parathyroid cell buffer (PCB) which contains (mM): NaCl, 126; KCl, 4;
10 MgCl₂, 1; Na-HEPES, 20; pH 7.4; glucose, 5.6, and variable amounts of CaCl₂, e.g., 1.25 mM. Human parathyroid glands, were obtained from patients undergoing surgical removal of parathyroid tissue for primary or uremic hyperparathyroidism (uremic HPT), and were treated similarly to bovine
15 tissue.

Glands were trimmed of excess fat and connective tissue and then minced with fine scissors into cubes approximately 2-3 mm on a side. Dissociated parathyroid cells were prepared by collagenase digestion and then
20 purified by centrifugation in Percoll buffer. The resultant parathyroid cell preparation was essentially devoid of red blood cells, adipocytes, and capillary tissue as assessed by phase contrast microscopy and Sudan black B staining. Dissociated and purified parathyroid
25 cells were present as small clusters containing 5 to 20 cells. Cellular viability, as indexed by exclusion of trypan blue or ethidium bromide, was routinely 95%.

Although cells can be used for experimental purposes at this point, physiological responses (e.g., suppressi-
30 bility of PTH secretion and resting levels of $[Ca^{2+}]_i$) should be determined after culturing the cells overnight. Primary culture also has the advantage that cells can be labeled with isotopes to near isotopic equilibrium, as is necessary for studies involving measurements of inositol
35 phosphate metabolism.

After purification on Percoll gradients, cells were washed several times in a 1:1 mixture of Ham's F12-

Dulbecco's modified Eagle's medium (GIBCO) supplemented with 50 $\mu\text{g/ml}$ streptomycin, 100 U/ml penicillin, 5 $\mu\text{g/ml}$ gentamicin and ITS*. ITS* is a premixed solution containing insulin, transferrin, selenium, and bovine serum albumin (BSA)-linolenic acid (Collaborative Research, Bedford, MA). The cells were then transferred to plastic flasks (75 or 150 cm^2 ; Falcon) and incubated overnight at 37°C in a humid atmosphere of 5% CO_2 . No serum is added to these overnight cultures, since its presence allows the cells to attach to the plastic, undergo proliferation, and dedifferentiate. Cells cultured under the above conditions were readily removed from the flasks by decanting, and show the same viability as freshly prepared cells.

Purified parathyroid cells were resuspended in 1.25 mM CaCl_2 -2% BSA-PCB containing 1 μM fura-2-acetoxymethyl-ester and incubated at 37°C for 20 minutes. The cells were then pelleted, resuspended in the same buffer, but lacking the ester, and incubated a further 15 minutes at 37°C. The cells were subsequently washed twice with PCB containing 0.5 mM CaCl_2 and 0.5% BSA and maintained at room temperature (about 20°C). Immediately before use, the cells were diluted five-fold with prewarmed 0.5 mM CaCl_2 -PCB to obtain a final BSA concentration of 0.1%. The concentration of cells in the cuvette used for fluorescence recording was $1-2 \times 10^6/\text{ml}$.

The fluorescence of indicator-loaded cells was measured at 37°C in a spectrofluorimeter (Biomedical Instrumentation Group, University of Pennsylvania, Philadelphia, PA) equipped with a thermostated cuvette holder and magnetic stirrer using excitation and emission wavelengths of 340 and 510 nm, respectively. This fluorescence indicates the level of cytosolic Ca^{2+} . Fluorescence signals were calibrated using digitonin (50 $\mu\text{g/ml}$, final) to obtain maximum fluorescence (F_{max}), and EGTA (10 mM, pH 8.3, final) to obtain minimal fluorescence (F_{min}), and a dissociation constant of 224 nM. Leakage of dye is dependent on temperature and most occurs within the

first 2 minutes after warming the cells in the cuvette. Dye leakage increases only very slowly thereafter. To correct the calibration for dye leakage, cells were placed in the cuvette and stirred at 37°C for 2-3 minutes. The cell suspension was then removed, the cells pelleted, and the supernatant returned to a clean cuvette. The supernatant was then treated with digitonin and EGTA to estimate dye leakage, which is typically 10-15% of the total Ca^{2+} -dependent fluorescent signal. This estimate was subtracted from the apparent $F_{\min}$.

Example 4: Using Fura-2 Loaded HEK 293/pHuPCaR4.0 Cells To Measure to Calcium Receptor Activity

This section describes procedures used to assay calcium receptor activity using fura-2 loaded HEK 293/pHuPCaR4.0 cells. HEK 293 cells transfected with pHuPCaR4.0 were loaded with fura-2 by incubating the cells in Dulbecco's modified Eagle's media buffered with 20 mM HEPES containing about 5 μM fluo-3/AM for one hour at room temperature. Cells were then rinsed with Hank's balanced salt solution buffered with 20 mM HEPES containing 1 mM CaCl_2 and 1 mM MgCl_2 . Compounds to be tested were then added to the cells and fluorescence was measured (excitation and emission wavelengths of 340 and 510 nm, respectively).

Example 5: Measuring the Ability of Compounds to Modulate Calcium Receptor Activity

The ability of different compounds to modulate calcium receptor activity was assayed by measuring increases in $[\text{Ca}^{2+}]_i$ in HEK 293 cells transfected with nucleic acid encoding pHuPCaR4.0 using fura-2 loaded cells or using parathyroid cells loaded with using fura-2 loaded cells. Results of different experiments are summarized in Tables 1.a, 1.b.1, 1.b.2, 1.c., and 2. Tables 1.a, 1.b.1, 1.b.2, and 1.c summarizes the effects of compounds, at different concentrations, on calcium receptor activity assayed as

described in Example 4 (i.e., using HEK 293 cells transfected with nucleic acid encoding pHuPCaR4.0, which were loaded with fura-2).

Table 2, summarizes the results of different experiments where the EC_{50} was calculated either parathyroid cells, or HEK 293/pHuPCaR4.0, loaded with fura-2. Cells were loaded with fura-2 and assayed as described in Example 2 (for parathyroid cells) or Example 3 (for HEK 293/pHuPCaR4.0 cells).

10 Table 1.a. Calcimimetic compounds which produce greater than 40% response at 3.3 ng/mL in HEK-293 cells expressing the human calcium receptor.

	Compound Code	% activity at four concentrations (ng/mL)			
		3300	330	33	3.3
15	Reference compounds				
	R-568		95	69	24
	17P		101	86	54
	17X		105	93	51
	24X	126	109	124	109
20	24Y	119	120	127	102
	17J	116	118	122	102
	25A	122	120	114	92
	17E	116	110	110	92
	24Z	138	138	135	90
25	14S	116	106	105	88
	25E	132	129	122	85
	17G	125	128	119	77
	14T	126	125	117	77
	17H	126	124	111	74
30	14O	119	119	102	74
	25I	119	113	114	74
	12J	131	130	113	68

47

Compound Code		% activity at four concentrations (ng/mL)			
		3300	330	33	3.3
	12I	115	111	93	68
	25G	130	115	99	66
	9R		108	101	64
	12F	118	110	101	63
5	12O	110	117	94	62
	23Z	129	126	100	61
	17M		115	99	59
	16V		114	102	58
	25O	126	115	96	57
10	25J	119	123	105	56
	16L	146	138	98	56
	12N	115	106	102	55
	16T		97	88	55
	25U	107	107	95	55
15	17P		101	86	54
	16Q		110	88	53
	23E	137	113	102	53
	17C	113	120	99	52
	25L	97	97	85	52
20	8Z		101	97	52
	17X		105	93	51
	13R		132	98	51
	17O		112	96	51
	23Q	122	114	98	51
25	16X		111	96	51
	24V	127	98	71	50
	13O		115	94	50
	17N		108	86	49
	21V	122	116	99	48
30	24M	132	134	99	48
	13U		108	79	47

48

	Compound Code	% activity at four concentrations (ng/mL)			
		3300	330	33	3.3
	24P	140	138	110	46
	17Y	109	94	79	46
	11X		100	76	45
	25H	115	107	89	45
5	22J		99	71	45
	9C		104	82	45
	13S		102	87	45
	10Q	103	100	84	44
	13P		110	83	44
10	8K		98	81	44
	13N		114	88	43
	10N	106	97	77	43
	12H	114	115	94	43
	25P	90	81	75	41
15	18A		111	88	40
	14L		109	78	40

Table 1.b.1. Calcimimetic compounds which produce greater than 40% response at 33 ng/mL in HEK-293 cells expressing the human calcium receptor

20	Compound Code	% activity at four concentrations (ng/mL)			
		3300	330	33	3.3
	Reference compounds				
	R-568		95	69	24
	17P		101	86	54
25	17X		105	93	51
	12C	134	125	98	39
	16I	121	117	96	36

49

		Compound Code	% activity at four concentrations (ng/mL)			
			3300	330	33	3.3
		17D		108	91	38
		17F		111	90	28
		24C	116	113	87	32
		25K	124	107	86	35
5		13F	125	122	85	38
		21F		109	85	36
		21S	132	131	85	34
		10F		96	84	27
		14R	106	107	84	37
10		13G	111	128	82	29
		14Z	118	103	82	20
		16N	122	159	82	8
		8U	123	129	82	11
		23W	117	97	81	25
15		12G	139	139	81	35
		15G		113	80	32
		25M	118	100	79	25
		13V		110	79	33
		14P	112	103	78	30
20		6T	123	129	78	15
		14Q		101	78	35
		17L	111	104	78	31
		24K		106	78	30
		24U	106	106	78	25
25		25Q	116	95	77	20
		8J		104	77	39
		23H	121	114	77	28
		21C=4U	134	114	76	17
		25F	97	85	76	28
30		16R		100	76	25
		17I	118	97	76	18

50

Compound Code		% activity at four concentrations (ng/mL)			
		3300	330	33	3.3
	24J		103	75	31
	21O		109	75	37
	24G	109	94	75	22
	15I	111	93	75	24
5	21D		104	75	17
	20Y	117	95	74	24
	10P		102	74	8
	23M	113	97	74	26
	14Y		109	73	17
10	17K	98	97	73	37
	12E	117	121	73	23
	17Z		99	73	37
	16W		102	73	4
	23K	106	107	72	24
15	25X	96	94	72	22
	13W		109	71	12
	23P	125	99	70	22
	18B	111	96	69	26
	21Y		100	68	36
20	17W		92	67	13
	23A		103	67	24
	23G	127	93	67	13
	13M		92	66	15
	21U	104	104	66	18
25	21R		100	66	15
	10S/10T		86	65	13
	17R		98	65	13
	13X		102	65	13
	4N		100	65	13
30	21E		94	64	4
	15J	80	75	64	13

51

	Compound Code	% activity at four concentrations (ng/mL)			
		3300	330	33	3.3
	22Y		114	64	28
	21G		88	63	18
	24L		105	62	10
	10V		99	62	8
5	10W/10X		98	61	9
	17B		92	61	19
	23Y	106	87	61	16
	11Y		103	61	20

10 Table 1.b.2 Calcimimetic compounds which produce greater than 40% response at 33 ng/mL in HEK-293 cells expressing the human calcium receptor

	Compound Code	% activity at four concentrations (ng/mL)			
		3300	330	33	3.3
	reference compounds				
	R568		95	69	24
15	17P		101	86	54
	17X		105	93	51
	18C	99	87	60	18
	23T	102	74	60	31
	4V		93	59	
20	8G		84	59	6
	23I		102	58	3
	21M		102	58	17
	24O	137	114	58	8
	3U		89	57	
25	9A		82	56	6
	12M	98	86	56	11
	12B	130	110	56	4

52

Compound Code		% activity at four concentrations (ng/mL)			
		3300	330	33	3.3
	21P		92	56	13
	8T		85	55	13
	10L/10M		99	55	4
	24I	109	84	55	11
5	14N		89	55	15
	23R	104	86	54	13
	23S		97	53	3
	21T	133	112	53	3
	10W/10X		81	53	4
10	13T		90	53	6
	6R		94	52	7
	20I		87	52	12
	24A	122	85	52	9
	12D	128	109	52	5
15	6X		84	52	10
	18T	99	74	52	14
	21X	119	101	51	2
	23J	102	61	51	29
	10Z		96	51	5
20	16Z		88	51	9
	23N		96	50	2
	16U		85	50	4
	11D		96	50	4
	23X		94	49	1
25	17A		88	49	7
	20J		80	48	8
	22X		86	48	10
	23U		87	48	3
	9Z		74	48	4
30	16J	92	76	47	31
	25N	94	73	46	8
	4P		81	46	8

53

Compound Code		% activity at four concentrations (ng/mL)			
		3300	330	33	3.3
	23O	111	79	46	13
	13Q		95	46	5
	4G		83	46	
	12Y		80	46	10
5	12L		88	45	10
	23F		82	45	5
	11W		81	44	2
	8H		88	44	7
	25V	89	59	43	26
10	25W	95	69	42	8
	10R		82	42	7
	21N	124	98	42	4
	8S		73	42	7
	8X		75	40	19
15	13E	123	94	40	2

Table 1.c. Calcimimetic compounds which produce greater than 40% response at 330 ng/mL in HEK-293 cells expressing the human calcium receptor

Compound Code		% activity at four concentrations (ng/mL)			
		3300	330	33	3.3
20	reference compounds				
	R568		95	69	24
	17P		101	86	54
	17X		105	93	51
	7X		85		
25	3H		84		
	3L		81	28	
	16O	129	81	21	2
	8O/8Q	124	80	14	0

54

Compound Code		% activity at four concentrations (ng/mL)			
		3300	330	33	3.3
	14A	98	78	10	7
	23L	107	77	37	9
	1T		76		
	7W		76		
5	4H		77	37	
	8D		75		
	5M		73	21	
	4U		72		
	24E	94	71	35	6
10	16M	130	68	11	4
	4M		68	34	
	2S		67	29	
	17V	91	66	27	-1
	2X		66	15	
15	23D	91	66	35	13
	4P		65	32	
	5B/5C		65	20	
	3M		64	19	
	16K	78	62	36	8
20	5D		62	18	
	4D		61	13	
	24B	76	61	34	11
	24H	81	60	32	13
	5L		60	16	
25	2Y		59	10	
	5G		58	16	
	3V		56	14	
	2Q		56	4	
	14B	75	55	11	4
30	13Z	93	54	22	5
	8A		54		
	24D	87	53	34	39

55

	Compound Code	% activity at four concentrations (ng/mL)			
		3300	330	33	3.3
	1D		53		
	13I	85	52	3	1
	3B		52	15	
	8C		51		
5	14H	112	49	5	5
	7U		49		
	5E		48	7	
	13H	88	48	36	12
	13Y	106	47	2	4
10	4J		47	8	
	14I	80	45	11	7
	4B		45	8	
	3D		45	4	
	3R		45	2	
15	3A		41	7	
	14J	55	41	6	5
	4I		40	9	

TABLE 2

20 Arylalkylamine Calcimimetics from Figure 1 Active at the
Parathyroid Cell Calcium Receptor In Vitro ($EC_{50} \leq 5 \mu M$)

	Compound Code (from Fig. 1)		EC ₅₀ (μM)	
	Compound Code (from Fig. 1)		EC ₅₀ (μM)	
	NPS R-467	2.0	11X	0.83
	NPS R-568	0.60	11Y	2.8
25	3U	0.64	12L	1.7
	3V	1.8	12U	1.2
	4A	1.4	12V	0.42
	4B	2.0	12W	3.2

	4C	2.0	12Y	2.0
	4D	4.4	12Z	0.11
	4G	1.8	13Q	ca. 0.8
	4H	≥ 3.0	13R	0.25
5	4J	2.2	13S	<0.13
	4M	2.1	13U	0.19
	4N	0.8	13X	<0.75
	4P	1.6	14L	0.26
	4R/6V	4.2	14Q	0.47
10	4S	3.3	14U	0.13
	4T/4U	1.6	14V	1.7
	4V	2.5	14Y	0.38
	4W	2.3	15G	ca. 0.5
	4Y	1.3	16Q	0.04
15	4Z/5A	4.4	16R	0.36
	5B/5C	2.8	16T	0.04
	5W/5Y	3.6	16V	<0.13
	6E	2.7	16W	0.59
	6F (R, R-)	0.83	16X	0.10
20	6R	3.4	17M	0.15
	6T	2.9	17O	0.04
	6X	2.5	17P	0.04
	7W	3.2	17R	0.39
	7X	1.1	17W	0.43
25	8D	2.5	17X	0.02
	8J	0.78	20F	<1.0
	8K	1.3	20I	>1.0
	8R	2.6	20J	>3.0
	8S	1.7	20R	2.4
30	8T	1.8	20S	4.2
	8U	0.44	21D	3.0
	8X	0.76	21F	0.38
	8Z	0.40	21G	1.1

5

9C	0.60	21O	0.26
9D	1.4	21P	0.43
9R	0.25	21Q	1.4
9S	4.8	21R	0.37
10F	0.89	25C	> 2
11D	1.8	25D	0.019

Examples 6-17: Synthesis of Compounds

The compounds described herein can be synthesized using standard techniques such as those described by Nemeth et al., PCT/US93/01642, International Publication Number WO 94/18959. Examples describing representative syntheses of compounds described in the text are provided below.

Synthesis of compounds 9R, 14U, and 17P were prepared by reductive amination of a commercially available aldehyde or ketone with a primary amine in the presence of sodium cyanoborohydride or sodium triacetoxyborohydride. Compounds 11Y, 12H, 12K, 12M, 14S, 14T, 16L-O, 17E, 17G, 17J, 24X, 24Y, 25A, 25E-25K, and 25O were prepared in a similar manner.

It was found for the syntheses of these three compounds (9R, 14U, and 16P) that sodium triacetoxyborohydride afforded the desired diastereoisomers with greater diastereoselectivity than using sodium cyanoborohydride. The enriched mixtures were further purified to a single diastereomer by normal-phase HPLC or by recrystallization from organic solvents.

Compounds 8J, 8U, 11X, 17M, and 25Y were prepared from the condensation of a primary amine with an aldehyde or ketone in the presence of titanium(IV) isopropoxide. The resulting intermediate imines were then reduced in situ by the action of sodium cyanoborohydride, sodium borohydride, or sodium triacetoxyborohydride. The intermediate enamine for the synthesis of compound 8U was

catalytically reduced using or palladium dihydroxide on carbon.

Compounds 12U, 12V and 12Z were prepared by a diisobutylaluminum hydride (DIBAL-H) mediated condensation of an amine with a nitrile. The resulting intermediate imine is reduced *in situ* by the action of sodium cyanoborohydride or sodium borohydride. The intermediate alkenes (compounds 12U and 12V) were reduced by catalytic hydrogenation in EtOH using palladium on carbon. Compounds which were converted to their corresponding hydrochloride were done so by treatment of the free base with ethereal HCl to afford white solids.

The amines in these syntheses were purchased from Aldrich Chemical Co., Milwaukee, WI, or from Celgene Corp., Warren, NJ, or were prepared synthetically using standard techniques. All other reagent chemicals were purchased from Aldrich Chemical Co.

Example 6: Synthesis of Compound 25Y

N-(3-(2-Phenyl)propyl)-1-(1-naphthyl)ethylamine

A mixture of 3-phenyl-1-propylamine (135 mg, 1 mmol), 1'-acetonaphthone (170 mg, 1 mmol), and titanium (IV) isopropoxide (355 mg, 1.3 mmol) was stirred at room temperature for 1 hour. The reaction was treated with 1 M ethanolic sodium cyanoborohydride (1 mL) and stirred at room temperature for 16 hours. The reaction was diluted with ether and treated with water (0.1 mL). The reaction was centrifuged and the ether layer removed and concentrated to a milky oil. A small portion of this material (10 mg) was purified by HPLC (Phenomenex, 1.0 x 25 cm, 5 μ M silica) using a gradient of dichloromethane to 10% methanol in dichloromethane containing 0.1% isopropylamine. This afforded the product (free base) as a single component by GC/EL-MS (R_t = 10.48 min) m/z (rel. int.) 289 (M^+ , 11), 274 (63), 184 (5), 162 (5), 155 (100), 141 (18), 115 (8), 91 (45), 77 (5).

Example 7: Synthesis of Compound 8J*N*-(3-phenylpropyl)-1-(3-thiomethylphenyl)ethylamine hydrochloride

3'-Aminoacetophenone (2.7 g, 20 mmol) was dissolved
5 in 4 mL of concentrated HCl, 4 g of ice and 8 mL of water.
The solution was cooled to 0°C, and sodium nitrite (1.45
g, 21 mmol) dissolved in 3-5 mL of water was added over 5
minutes while maintaining the temperature below 6°C.
Sodium thiomethoxide (1.75 g, 25 mmol) was dissolved in 5
10 mL of water and cooled to 0°C. To this solution was added
the diazonium salt over 10 minutes while maintaining the
temperature below 10°C. The reaction was stirred for an
additional hour while allowing the temperature to rise to
ambient. The reaction mixture was partitioned between
15 ether and water. The ether layer was separated and washed
with sodium bicarbonate and sodium chloride, and dried
over sodium sulfate. The ether was evaporated to give a
74% yield of 3'-thiomethylacetophenone. The crude
material was purified by distillation at reduced pressure.
20 3-Phenylpropylamine (0.13 g, 1 mmol), 3'-
thiomethylacetophenone (0.17 g, 1 mmol), and titanium (IV)
isopropoxide (0.36 g, 1.25 mmol) were mixed together and
allowed to stand for 4 hours. Ethanol (1 mL) and sodium
cyanoborohydride (0.063 g, 1 mmol) were added and the
25 reaction was stirred overnight. The reaction was worked
up by the addition of 4 mL of ether and 200 µL of water.
The mixture was vortexed and then spun in a centrifuge to
separate the solids. The ether layer was separated from
the precipitate, and the solvent removed in vacuo. The
30 oil was redissolved in dichloromethane and the compound
purified by preparative TLC on silica gel eluted with 3%
methanol/dichloromethane to yield the title compound as a
pure oil: GC/EI-MS (R_t =7.64 min) m/z (rel. int.) 285 (M^+ , 18),
270 (90), 180 (17), 151 (100), 136 (32), 104 (17), 91 (54),
35 77 (13).

Example 8: Synthesis of Compound 8U

N-3-(2-methoxyphenyl)-1-propyl-(*R*)-3-methoxy- α -methylbenzylamine hydrochloride

A mixture of (*R*)-(+)-3-methoxy- α -methylbenzylamine
5 (3.02 g, 20 mmol), 2-methoxycinnamaldehyde (3.24 g, 20 mmol), and titanium (IV) isopropoxide (8.53 g, 30 mmol, 1.5 Eq.) was stirred 2 hours at room temperature and treated with 1 M (20 mL) ethanolic sodium cyanoborohydride. The reaction was stirred overnight (16 hours),
10 diluted with diethylether, and treated with water (1.44 mL, 80 mmol, 4 Eq.). After mixing for 1 hour the reaction mixture was centrifuged and the ether layer removed and concentrated to an oil. This material was dissolved in glacial acetic acid, shaken with palladium hydroxide and
15 hydrogenated under 60 p.s.i. hydrogen for 2 hours at room temperature. The catalyst was removed by filtration and the resulting solution concentrated to a thick oil. This material was dissolved in dichloromethane and neutralized with 1 N NaOH. The dichloromethane solution was separated
20 from the aqueous phase, dried over anhydrous potassium carbonate and concentrated to an oil. This material was dissolved in ether and treated with 1 M HCl in diethylether. The resulting precipitate (white solid) was collected, washed with diethylether, and air dried.
25 GC/El-MS (R_t = 9.69 min) of this material (free base) showed a single component: m/z (rel. int.) 299 (M^+ , 21), 284 (100), 164 (17), 150 (8), 135 (81), 121 (40), 102 (17), 91 (43), 77 (18).

Example 9: Synthesis of Compound 9R

30 (*R*)-*N*-(1-(2-naphthyl)ethyl)-(*R*)-1-(1-naphthyl)ethylamine hydrochloride

A mixture of (*R*)-(+)-1-(1-naphthyl)ethylamine (10.0 g, 58 mmol), 2'-acetonaphthone (9.4 g, 56 mmol), titanium (IV) isopropoxide (20.7 g, 73.0 mmol), and EtOH (abs.)
35 (100 mL) was heated to 60°C for 3 hours. Sodium cyanoborohydride (NaCNBH_3) (3.67 g, 58.4 mmol) was then added.

The reaction mixture was stirred at room temperature for 18 hours. Ether (1 L) and H₂O (10 mL) were added to the reaction mixture and the resulting precipitate was then removed by centrifugation. The supernatant was evaporated
5 under vacuum and the crude product was recrystallized four times from hot hexane, to provide 1.5 g of pure (98+%) diastereomer. The free base was dissolved in hexane, filtered, and then ethereal HCl was added to precipitate the product as a white solid (1.1 g, 6 % yield), m.p.:
10 softens 200-240°C (dec.).

Example 10: Synthesis of Compound 11X

N-(4-Isopropylbenzyl)-(R)-1-(1-naphthyl)ethylamine hydrochloride

A mixture of (R)-(+)-1-(1-naphthyl)ethylamine (1.06
15 g, 6.2 mmol), 4-isopropylbenzaldehyde (0.92 g, 6.2 mmol), and titanium (IV) isopropoxide (2.2 g, 7.7 mmol) was heated to 100°C for 5 min then allowed to stir at room temperature for 4 hours. Sodium cyanoborohydride (NaCNBH₃) (0.39 g, 6.2 mmol) was then added followed by EtOH (1 mL).
20 The reaction mixture was stirred at room temperature for 18 hours. Ether (100 mL) and H₂O (1 mL) were added to the reaction mixture and the resulting precipitate was then removed by centrifugation. The supernatant was evaporated under vacuum and the crude product was chromatographed on
25 silica gel (50 mm X 30 cm column) (elution with 1% MeOH/CHCl₃). The chromatographed material was then dissolved in hexane and ethereal HCl was added to precipitate the product as a white solid (0.67 g, 35 % yield), m.p.; 257-259°C.

30 Example 11: Synthesis of Compound 12U

N-3-(2-methylphenyl)-1-propyl-(R)-3-methoxy- α -methylbenzylamine hydrochloride

A solution of 2-methylcinnamionitrile (1.43 g, 10 mmol) in dichloromethane (10 mL) was cooled to 0°C and
35 treated dropwise (15 minutes) with 1 M diisobutylaluminum

hydride (10 mL, dichloromethane). The reaction was stirred at 0°C for 15 minutes and treated dropwise (15 minutes) with a 1 M solution of (R)-(+)-3-methoxy- α -methylbenzylamine (1.51 g, 10 mmol) in dichloromethane (10 mL). The reaction was stirred 1 hours at 0°C and poured into a solution of ethanol (100 mL) containing sodium cyanoborohydride (1 g, 16 mmol). The reaction mixture was stirred 48 hour at room temperature. The reaction was diluted with ether and neutralized with 1 N NaOH. The ether layer was removed, dried over anhydrous potassium carbonate and concentrated to an oil. This material was chromatographed through silica using a gradient of dichloromethane to 5% methanol in dichloromethane to afford the unsaturated intermediate, a single component by GC/EI-MS (R_t =10.06 min) m/z (rel. int.) 281 (M+, 17), 266 (59), 176 (19), 146 (65), 135 (73), 131 (100), 91 (21), 77 (13).

The unsaturated intermediate in ethanol was hydrogenated (1 atm H₂) in the presence of palladium on carbon for 16 hours at room temperature. The product from this reaction was converted to the hydrochloride salt by treatment with 1 M HCl in diethylether. GC/EI-MS (R_t = 9.31 min) of this material (free base) showed a single component: m/z (rel. int.) 283 (M+, 21), 268 (100), 164 (12), 148 (8), 135 (85), 121 (12), 105 (49), 91 (23), 77 (21).

Example 12: Synthesis of Compound 12V

N-3-(3-methylphenyl)-1-propyl-(R)-3-methoxy- α -methylbenzylamine hydrochloride

The compound was prepared following the procedure described in Example 11, but using 2-methylcinnamionitrile. The unsaturated intermediate was a single component by GC/EI-MS (R_t = 10.21 min) m/z (rel. int.) 281 (M+, 57), 266 (86), 146 (98), 135 (88), 131 (100), 115 (43), 102 (26), 91 (43), 77 (18). Reduction of this material and hydrochloride formation using the procedure described Example

11 afforded the product. GC/EI-MS (R_t = 9.18 min) of this material (free base) showed a single component; m/z (rel. int.) 283 (M^+ , 19), 268 (100), 164 (11), 148 (8), 135 (76), 121 (16), 105 (45), 91 (23), 77 (21).

5 Example 13: Synthesis of Compound 12Z

N-3-(2-chlorophenyl)-1-propyl-(*R*)-1-(1-naphthyl)ethylamine hydrochloride

The compound was prepared following the procedures described in Example 11, but using 2-chlorohydrocinnam-
10 nitrile and (*R*)-(+)-1-(1-naphthyl)ethylamine on a 10 mmol scale. Chromatography through silica using a gradient of dichloromethane to 5% methanol in dichloromethane afforded the product as a single component by TLC analysis (5% methanol in dichloromethane). The hydrochloride was
15 prepared by treatment with 1 M HCl in diethylether.

Example 14: Synthesis of Compound 14U

(*R*)-*N*-(1-(4-methoxyphenyl)ethyl)-(*R*)-1-(1-naphthyl)ethylamine hydrochloride

A mixture of (*R*)-(+)-1-(1-naphthyl)ethylamine (1.1 g,
20 6.2 mmol), 4'-methoxyacetophenone (0.93 g, 6.2 mmol), titanium (IV) isopropoxide (2.2 g, 7.7 mmol), and EtOH (abs.) (1 mL) was heated to 60°C for 3 hours. Sodium cyanoborohydride (NaCNBH_3) (0.39 g, 6.2 mmol) was then added, and the reaction mixture was stirred at room
25 temperature for 18 hours. Ether (200 mL) and H_2O (2 mL) were added to the reaction mixture and the resulting precipitate was then removed by centrifugation. The supernatant was evaporated under vacuum and the crude product was chromatographed on silica gel (25 mm X 25 cm
30 column) (elution with 1% MeOH/ CHCl_3). A portion of this material was HPLC chromatographed [Selectosil, 5 μM silica gel; 25 cm x 10.0 mm (Phenomenex, Torrance, CA), 4 mL per minute; UV det. 275 nm; 12% ethyl acetate-88% hexane (elution time 12.0 min)]. The HPLC purified diastereomer
35 was then dissolved in hexanes and ethereal HCl was added

to precipitate the product as a white solid (20 mg), m.p.: 209-210°C(dec.).

Example 15: Synthesis of Compound 17M

N-(3-chloro-4-methoxybenzyl)-(R)-1-(1-naphthyl)ethylamine
5 hydrochloride

A mixture of (R)-(+)-1-(1-naphthyl)ethylamine (6.6 g, 39 mmol), 3'-chloro-4'-methoxybenzaldehyde (6.6 g, 39 mmol), and titanium (IV) isopropoxide (13.8 g, 48.8 mmol), and EtOH (abs.) (30 mL) was heated to 80°C for 30 minutes
10 then allowed to stir at room temperature for 3 hours. Sodium cyanoborohydride (NaCNBH₃) (2.45 g, 39 mmol) was then added. The reaction mixture was stirred at room temperature for 18 hours. Ether (100 mL) and H₂O (2 mL) were added to the reaction mixture and the resulting
15 precipitate was then removed by centrifugation. The supernatant was evaporated under vacuum and the crude product was chromatographed on silica gel (50 mm X 30 cm column) (elution with CH₂Cl₂). The chromatographed material was then dissolved in hexane (500 mL), decolor-
20 ized with Norit® filtered (0.2 μM), and then ethereal HCl was added to precipitate the product as a white solid (10.2 g, 56 % yield), m.p.: 241-242°C (dec.).

Example 16: Synthesis of Compound 17P

4-Methoxy-3-methylacetophenone [17P Precursor]

25 A mixture of 4'-hydroxy-3'-methylacetophenone (5.0 g, 33.3 mmol), iodomethane (5.7 g, 40.0 mmol), K₂CO₃ (granular, anhydrous) (23.0 g, 167 mmol), and acetone (250 mL) was refluxed for 3 hours. The reaction mixture was then cooled to room temperature, filtered to remove the
30 inorganic salts, and evaporated under vacuum. The crude product was dissolved in ether (100 mL) and washed with H₂O (2 x 20 mL). The organic layer was dried (Na₂SO₄) and evaporated to yield 4.5 g, 82.4% yield. The ketone was used in the following reaction without further
35 purification.

(R)-N-(1-(4-Methoxy-3-methylphenyl)ethyl)-(R)-1-(1-naphthyl)ethylamine hydrochloride [Compound 17P]

A mixture of (R)-(+)-1-(1-naphthyl)ethylamine (4.24 g, 24.8 mmol), 4'-methoxy-3'-methylacetophenone (4.06 g, 24.8 mmol), and titanium (IV) isopropoxide (8.8 g, 30.9 mmol), and EtOH (abs.) (1 mL) was heated to 100°C for 2 hours. Isopropanol (45 mL) was added and the reaction was then cooled to 10°C in an ice bath. Sodium triacetoxyborohydride ($\text{NaHB}(\text{O}_2\text{CCH}_3)_3$) (10.5 g, 49.5 mmol) was then added in portions over 15 minutes. The reaction mixture was then heated to 70°C for 18 hours. The mixture was cooled to room temperature and poured into ether (400 mL). The suspension was centrifuged, the supernatant was collected and the pellet was washed with ether (400 mL). The combined organic washings were evaporated under vacuum. The residue was dissolved in ether (400 mL) and washed with 1 N NaOH (4 x 50 mL) and H_2O (2 x 50 mL). The organic layer was dried (Na_2SO_4), filtered and evaporated under vacuum. EtOH (abs.) was added to the wet residue which was then dried thoroughly on a rotary evaporator to provide an oil. The mixture was then chromatographed on silica gel (50 mm x 30 cm) [elution with (1% MeOH:1% IPA:CHCl₃) to give 4.8 g of an oil].

The desired diastereomer was further purified by HPLC chromatography [SUPELCOSIL™ PLC-Si, 18 μM silica gel; 25 cm x 21.2 mm (Supelco, Inc., Bellefonte, PA), 7 mL per minute; UV det. 275 nm; 20% EtOAc-80% hexane (elution time 9.5 - 11.0 min)]. Injections (800 μL aliquots) of the mixture (100 mg/mL solution in eluent) provided 65 mg of the desired isomer. Multiple HPLC injections provided 1.0 g of purified material. The HPLC chromatographed material was dissolved in hexane (50 mL) and the hydrochloride salt was precipitated with ethereal HCl. The salt was collected on fritted glass and washed with hexane to provide 1.0 g of a white solid, mp 204-205°C.

Example 17: Synthesis of Compound 17X3-Chloro-4-methoxybenzaldehyde

A mixture of 3-chloro-4-hydroxybenzaldehyde (25 g, 160 mmol), iodomethane (27.25 g, 192 mmol), K₂CO₃ (granular, anhydrous) (110.6 g, 800 mmol), and acetone (300 mL) was refluxed for 3 hours. The reaction mixture was then cooled to room temperature. Diethyl ether (500 mL) was added and the mixture was filtered through paper to remove the inorganic solids. The filtrate was evaporated under reduced pressure, dissolved in diethyl ether (800 mL), and washed with 0.1 N NaOH (3 x 100 mL). The organic layer was dried (Na₂SO₄) and evaporated under vacuum to yield 24 g, 92% yield of crude product. This material was further purified by chromatography on silica gel (50 mm x 30 cm) (elution with hexane-EtOAc, 5:1) to give 15.02 g, 56% yield of a white solid: TLC (hexane-EtOAc, 5:1) R_f=0.24; GC R_t=4.75 min; MS (EI) m/z 170(M⁺), 172(M+2).

1-Methyl-(3'-chloro-4'-methoxybenzyl) alcohol

A mixture of 3-chloro-4-methoxybenzaldehyde (13 g, 76.5 mmol), methylmagnesium chloride (52 g, 153 mmol), and THF (300 mL) was refluxed for 3 hours. The reaction mixture was cooled to room temperature. NH₄Cl (satd. soln., 6 mL) was added dropwise followed by diethyl ether (500 mL) and the mixture was filtered through paper to remove the inorganic solids. The filtrate was evaporated under reduced pressure and the resulting solid was dissolved in diethyl ether (300 mL) and washed with water (4 x 25 mL). The organic layer was dried (Na₂SO₄) and evaporated under vacuum to yield 11.3 g, 80% yield of crude product. This material was further purified by chromatography on silica gel (50 mm x 30 cm) (elution with CH₂Cl₂) to yield 11.3 g, 63% yield of an oil; TLC (CH₂Cl₂) R_f=0.25; GC R_t=5.30 min; MS (EI) m/z 186(M⁺), 188(M+2).

3'-Chloro-4'-methoxyacetophenone

A mixture of 1-methyl-(3'-Chloro-4'-methoxybenzyl) alcohol (7.6 g, 41 mmol), pyridinium chlorochromate (PCC) (13.16 g, 61.5 mmol), and CH₂Cl₂ (300 mL) was allowed to stir at room temperature for 2 hours. Diethyl ether (1000 mL) was added and the resulting mixture was placed on a chromatography column of silica gel (50 mm x 30 cm) (elution with diethyl ether) to yield 7.3 g, 97% yield of crude solid product. GC analysis of this material showed it to be 99% pure and it was used in the following reaction without further purification. TLC (diethyl ether) R_f=1.0; GC R_t=5.3 min; MS (EI) m/z 184(M⁺), 184(M+2).

(R,R)-N-(1-Ethyl-4'-methoxy-3'-chlorophenyl)-1-(1-naphthylethyl)amine

A mixture of 3'-chloro-4'-methoxyacetophenone (5.3 g, 29 mmol), (R)-(+)-1-(1-naphthyl)ethylamine (4.98 g, 29 mmol), titanium (IV) isopropoxide (10.2 g, 36 mmol), and isopropanol (20 mL) was heated to 100°C for 3 hours. Sodium triacetoxyborohydride (NaB(O₂CCH₃)₃; 12.29 g, 58 mmol) was added in portions over 10 minutes. The reaction mixture was heated to reflux for 30 minutes and was then allowed to stir at room temperature for 18 hours. The mixture was then poured into diethyl ether (500 mL); H₂O (2 mL) was added and the suspension was centrifuged to remove the fine precipitate of titanium salts. The supernatant was collected and the pellet was washed with ether (500 mL). The combined organic layers were dried (Na₂SO₄) and evaporated under vacuum to yield 6.81 g, 70% of crude product.

This material was further purified by chromatography on silica gel (50 mm x 30 cm) (elution with 3% MeOH-97% CH₂Cl₂) to give 2.01 g of an oil. The diastereomer was further purified by recrystallization. The free base (1.98 g) was converted to its HCl salt with ethereal HCl. This salt was dissolved in hot isopropanol (65 mL) and the solution was filtered through paper. The filtrate was

evaporated under vacuum and the resulting solid dissolved in isopropanol (30 mL). After standing at room temperature for 18 hours, the crystalline solid was collected, washed with cold isopropanol (20 mL), and dried to yield
5 0.87 g, 40% (from free base) of the diastereomerically pure hydrochloride salt: mp 236-237°C (dec); TLC (MeOH-CH₂Cl₂ [99:1]) R_f=0.25; GC R_t=11.06 min; FTIR (KBr pellet, cm⁻¹) 3433, 2950, 2931, 2853, 2803, 2659, 2608, 2497, 1604, 1595, 1504, 1461, 1444, 1268, 1260, 1067, 1021, 802, 781,
10 733; MS (EI) m/z 339 (M⁺), 341 (M+2).

Example 18: Additional Synthesis Protocol

Preparation of 22Z and 23A

A stirred solution of sodium hydride (2.173 g, 60% in oil, 54.325 mmol) in dimethylformamide (100ml) was treated
15 dropwise with triethyl phosphonoacetate (12.47 g, 55.65 mmol) and stirred 30 min at rt. After this time, a solution of *m*-trifluoromethoxy benzaldehyde (10.0 g, 52.6 mmol) in dimethylformamide (50 ml) was added dropwise and the solution stirred 30 min at rt and 30 min at 100°C.
20 The reaction was quenched by the addition of water and transferred to a separatory funnel using diethyl ether (500 ml). The ether solution was washed with saturated ammonium chloride (4 x 500 ml), dried over anhydrous magnesium sulfate, filtered and concentrated to afford ethyl
25 *m*-trifluoromethoxycinnamate as an oil; m/z (rel. int.) 260 (M⁺, 19), 232 (16), 215 (100), 187 (21), 101 (28).

The ethyl ester in ethanol (100 ml) was reduced under 60 p.s.i. hydrogen using a catalytic amount (10% by weight) palladium hydroxide. After reduction (2 hr, rt)
30 the reaction was filtered and concentrated to afford ethyl *m*-trifluoromethoxyhydrocinnamate as an oil; m/z (rel. int.) 262 (M⁺, 16), 217 (7), 188 (100), 175 (28), 103 (31), 91 (18), 77 (23).

The saturated ethyl ester was hydrolyzed in a
35 solution of ethanol-10 M sodium hydroxide (1:1) for 16 hr at rt. After this time the solution was acidified and the

product extracted into diethyl ether. The ether solution was dried over anhydrous magnesium sulfate and concentrated to afford *m*-trifluoromethoxyhydrocinnamic acid as a solid; *m/z* (rel. int.) 234 (*M*⁺, 46), 188 (100), 174 (65), 103 (27), 91 (12), 77 (17).

The acid was stirred in excess thionyl chloride for 4 hr at rt. The excess thionyl chloride was evaporated at reduced pressure (100°C) to afford *m*-trifluoromethoxyhydrocinnamyl chloride as an oil. The product was used without further purification.

A solution of *m*-trifluoromethoxyhydrocinnamyl chloride (9.8 g, 39 mmol) in tetrahydrofuran was cooled to -78°C and treated dropwise with a solution (13 ml of 3 M in tetrahydrofuran) of methylmagnesium bromide (39 mmol). The reaction was stirred 4 hr at -78°C, 8 hr at rt, and quenched with dilute HCl. The reaction mixture was extracted with diethyl ether. The ether was dried over anhydrous magnesium sulfate, filtered and concentrated to an oil. Chromatography of this material through silica using a gradient of hexane to acetone afforded 4-(3-trifluoromethoxyphenyl)-2-butanone as an oil; *m/z* (rel. int.) 232 (*M*⁺, 68), 217 (7), 189 (59), 175 (31), 103 (28), 43 (100).

A solution of 4-(3-trifluoromethoxyphenyl)-2-butanone (2.32 g, 10 mmol), (*R*)-1-(3-methoxyphenyl)ethylamine (1.51 g, 10 mmol), and titanium (IV) isopropoxide (3.55 g, 12.5 mmol) were stirred 4 hr at rt. The reaction mixture was then treated with a solution (10 ml of 1 M) of ethanolic sodium cyanoborohydride (10 mmol) and stirred 16 hr at rt. The reaction was diluted with diethyl ether (50 ml) and treated with water (0.72 ml, 40 mmol). After mixing thoroughly the solution was centrifuged and the ether layer decanted and concentrated to an oily solid. The solid was suspended in diethyl ether, filtered through 0.45 µm CR PTFE Acrodisc and concentrated to give a clear oil. Repetitive preparative thin-layer chromatography using 5% methanol in chloroform afforded the two

diastereomers, (S,R)-N-[4-(3-trifluoromethoxyphenyl)-2-butyl]-1-(3-methoxyphenyl)ethylamine, 22Z [m/z (rel. int.) 367 (M⁺,3), 352 (20), 232 (4), 178 (47), 135 (100), 105 (14), 91 (10), 77 (11)] and (R,R)-N-[4-(3-trifluoromethoxyphenyl)-2-butyl]-1-(3-methoxyphenyl)ethylamine, 23A; m/z (rel. int.) 367 (M⁺, 3), 352 (19), 232 (7), 178 (43), 135 (100), 105 (19), 91 (10), 77 (11).

Preparation of 22X and 22Y

In a similar fashion an equal molar amount of 4-(3-trifluoromethoxyphenyl)-2-butanone, (R)-1-(1-naphthyl)ethylamine and 1.25 equivalents titanium (IV) isopropoxide were mixed and the intermediate imine reduced with ethanolic sodium cyanoborohydride. Work-up and repetitive preparative thin-layer chromatography using 5% methanol in chloroform afforded (S,R)-N-[4-(3-trifluoromethoxyphenyl)-2-butyl]-1-(1-naphthyl)ethylamine, 22X; m/z (rel. int.) 387 (M⁺,3), 372 (15), 198 (15), 176 (12), 155 (100), 128 (8), 115 (6), 109 (4), 103 (5), 77 (8) and (R,R)-N-[4-(3-trifluoromethoxyphenyl)-2-butyl]-1-(1-naphthyl)ethylamine, 22Y; m/z (rel. int.) 387 (M⁺,2), 372 (12), 198 (16), 176 (11), 155 (100), 128 (8), 115 (6), 109 (4), 103 (5), 77 (8).

Preparation of 4T

In a similar fashion an equal molar amount of 4-(2-chlorophenyl)-2-butanone, prepared from o-chlorobenzaldehyde, (R)-1-(3-methoxyphenyl)ethylamine and 1.25 equivalents titanium (IV) isopropoxide were mixed and the intermediate imine reduced with ethanolic sodium cyanoborohydride. Work-up and repetitive preparative thin-layer chromatography using 5% methanol in chloroform afforded (R,R)-N-[4-(2-chlorophenyl)-2-butyl]-1-(3-methoxyphenyl)ethylamine, 4T; m/z (rel. int.) 317 (M⁺,3), 302 (16), 178 (62), 178 (62), 135 (100), 125 (15), 105 (10), 91 (6), 77 (8).

Preparation of 21Y

In a similar fashion an equal molar amount of 4-(3-trifluoromethylphenyl)-2-butanone, prepared from *m*-trifluoromethylbenzaldehyde, (R)-1-(3-methoxyphenyl) ethylamine and 1.25 equivalents titanium (IV) isopropoxide were mixed and the intermediate imine reduced with ethanolic sodium cyanoborohydride. Work-up and repetitive preparative thin-layer chromatography using 5% methanol in chloroform afforded (R,R)-N-[4-(3-trifluoromethylphenyl)-2-butyl]-1-(3-methoxyphenyl) ethylamine, 21Y [m/z (rel. int.) 351 (M⁺, 2), 336 (18), 216 (4), 202 (3), 178 (45), 135 (100), 105 (13), 91 (9), 77 (8)] and (S,R)-N-[4-(3-trifluoromethylphenyl)-2-butyl]-1-(3-methoxyphenyl) ethylamine, 21X.

Preparation of 25C and 25D

In a similar fashion an equal molar amount of 4-(3-trifluoromethylphenyl)-2-butanone, (R)-1-(1-naphthyl) ethylamine and 1.25 equivalents titanium (IV) isopropoxide were mixed and the intermediate imine reduced with ethanolic sodium cyanoborohydride. Work-up and repetitive preparative thin-layer chromatography using 5% methanol in chloroform afforded (S,R)-N-[4-(3-trifluoromethylphenyl)-2-butyl]-1-(1-naphthyl) ethylamine, 25C [m/z (rel. int.) 371 (M⁺, 3), 356 (16), 198 (15), 155 (100), 129 (8), 115 (5), 109 (3), 77 (2)] and (R,R)-N-[4-(3-trifluoromethylphenyl)-2-butyl]-1-(1-naphthyl) ethylamine, 25D; m/z (rel. int.) 371 (M⁺, 3), 356 (16), 198 (15), 155 (100), 129 (8), 115 (5), 109 (3), 77 (2).

Preparation of 21D

In a similar fashion an equal molar amount of 4-phenyl-2-butanone (Aldrich Chemical Co.), (R)-1-(3-methoxyphenyl) ethylamine and 1.25 equivalents titanium (IV) isopropoxide were mixed and the intermediate imine reduced with ethanolic sodium cyanoborohydride. Work-up and repetitive preparative thin-layer chromatography using 5%

methanol in chloroform afforded (R,R)-N-(4-phenyl-2-butyl)-1-(3-methoxyphenyl)ethylamine, 21D [m/z (rel. int.) 283 (M⁺, 4), 268 (13), 178 (45), 135 (100), 105 (15), 91 (43), 77 (11)] and (S,R)-N-(4-phenyl-2-butyl)-1-(3-methoxyphenyl)ethylamine, 21E.

Preparation of 21F

In a similar fashion an equal molar amount of 4-phenyl-2-butanone (Aldrich Chemical Co.), (R)-1-(1-naphthyl)ethylamine and 1.25 equivalents titanium (IV) isopropoxide were mixed and the intermediate imine reduced with ethanolic sodium cyanoborohydride. Work-up and repetitive preparative thin-layer chromatography using 5% methanol in chloroform afforded (R,R)-N-(4-phenyl-2-butyl)-1-(1-naphthyl)ethylamine, 21F; m/z (rel. int.) 303 (M⁺, 6), 288 (14), 198 (22), 155 (100), 129 (8), 115 (5), 91 (19), 77 (4).

Preparation of 12Z

A stirred solution of 2-chlorohydrocinnamionitrile (Aldrich Chemical Co., 1.66 g, 10 mmol) in dichloromethane (100 ml) was cooled to -78°C and treated dropwise with diisobutylaluminum hydride (1.42 g, 10 mmol). The reaction was stirred 1 hr at rt, cooled to -78 °C and treated with a solution of 1-(1-naphthyl)ethylamine (1.71 g, 10 mmol) in dichloromethane (25 ml). The reaction was transferred to an ice bath and stirred 2 hr. After this time the reaction was poured directly into a stirred solution of ethanolic sodium borohydride (50 ml of 0.2 M, 10 mmol). The mixture was stirred 30 min at rt and the excess sodium borohydride quenched by the addition of 10% HCl. The solution was then made basic by the addition of 10 N NaOH and transferred to a separatory funnel washing with diethyl ether (300 ml). The aqueous phase was removed and the remaining organic layer washed with 1 N NaOH (3 x 100 ml). The organic layer was dried over anhydrous magnesium sulfate, and concentrated to an oil. Chromatography of

this material through silica gel using a gradient of chloroform to 10% methanol-chloroform afforded 2.34g (72% yield) of (R)-N-[3-(2-chlorophenyl)propyl]-1-(1-naphthyl)ethylamine, 12Z, as a clear oil; m/z (rel. int.)
5 323 (M+, 2), 308 (63), 288 (7), 196 (5), 184 (5), 155 (100), 125 (24), 115 (8), 103 (4), 91 (3), 77 (7).

Preparation of 12B

In a similar fashion, 4-methylcinnamionitrile was treated with diisobutyl aluminum hydride and the
10 intermediate aluminum-imine complex treated with (R)-1-(3-methoxyphenyl)ethylamine. The intermediate imine was treated with ethanolic sodium borohydride. Work-up and chromatography yielded (R)-N-[3-(4-methylphenyl)prop-2-enyl]-1-(3-methoxyphenyl)ethylamine, 12B, as a clear,
15 colorless oil; m/z (rel. int.) 281 (M+, 6), 266 (5), 176 (27), 146 (75), 135 (63), 131 (100), 115 (25), 105 (21), 91 (21), 77 (21).

Preparation of 12C

In a similar fashion, 2-methylcinnamionitrile was
20 treated with diisobutyl aluminum hydride and the intermediate aluminum-imine complex treated with (R)-1-(3-methoxyphenyl)ethylamine. The intermediate imine was treated with ethanolic sodium borohydride. Work-up and chromatography yielded (R)-N-[3-(2-methylphenyl)prop-2-enyl]-1-(3-methoxyphenyl)ethylamine, 12C, as a clear,
25 colorless oil; m/z (rel. int.) 281 (M+, 4), 266 (15), 176 (18), 146 (62), 135 (58), 131 (100), 115 (23), 105 (19), 91 (38), 77 (17).

Preparation of 12D

30 In a similar fashion, 2,4,6-trimethylcinnamionitrile was treated with diisobutyl aluminum hydride and the intermediate aluminum-imine complex treated with (R)-1-(3-methoxyphenyl)ethylamine. The intermediate imine was treated with ethanolic sodium borohydride. Work-up and

chromatography yielded (R)-N-[3-(2,4,6-trimethylphenyl)prop-2-enyl]-1-(3-methoxyphenyl)ethylamine, 12D, as a clear, colorless oil; m/z (rel. int.) 309 (M⁺, 8), 294 (25), 174 (82), 159 (100), 135 (52), 129 (29), 105 (21),
5 91 (17), 77 (14).

Preparation of 12E

In a similar fashion, 4-isopropylcinnamionitrile was treated with diisobutyl aluminum hydride and the intermediate aluminum-imine complex treated with (R)-1-(3-methoxyphenyl)ethylamine. The intermediate imine was
10 treated with ethanolic sodium borohydride. Work-up and chromatography yielded (R)-N-[3-(4-isopropylphenyl)prop-2-enyl]-1-(3-methoxyphenyl)ethylamine, 12E, as a clear, colorless oil; m/z (rel. int.) 309 (M⁺, 9), 294 (7), 174
15 (98), 159 (22), 135 (80), 117 (100), 105 (35), 91 (37), 77 (19).

Preparation of 12F

In a similar fashion, 2,4-dimethylcinnamionitrile was treated with diisobutyl aluminum hydride and the intermediate aluminum-imine complex treated with (R)-1-(3-methoxyphenyl)ethylamine. The intermediate imine was
20 treated with ethanolic sodium borohydride. Work-up and chromatography yielded (R)-N-[3-(2,4-dimethylphenyl)prop-2-enyl]-1-(3-methoxyphenyl)ethylamine, 12F, as a clear, colorless oil; m/z (rel. int.) 295 (M⁺, 8), 294 (15), 174
25 (29), 160 (75), 145 (100), 135 (68), 117 (21), 105 (30), 91 (26), 77 (19).

Preparation of 12G

In a similar fashion, 3-methylcinnamionitrile was
30 treated with diisobutyl aluminum hydride and the intermediate aluminum-imine complex treated with (R)-1-(3-methoxyphenyl)ethylamine. The intermediate imine was treated with ethanolic sodium borohydride. Work-up and chromatography yielded (R)-N-[3-(3-methylphenyl)prop-2-

enyl]-1-(3-methoxyphenyl)ethylamine, 12G, as a clear, colorless oil; m/z (rel. int.) 281 (M⁺, 5), 266 (9), 176 (24), 146 (71), 135 (62), 131 (100), 115 (23), 105 (19), 91 (41), 77 (18).

5 Preparation of 25E

In a similar fashion, cinnamionitrile was treated with diisobutyl aluminum hydride and the intermediate aluminum-imine complex treated with (R)-1-(3-methoxyphenyl)ethylamine. The intermediate imine was treated with ethanolic sodium borohydride. Work-up and chromatography yielded (R)-N-(3-phenylprop-2-enyl)-1-(3-methoxyphenyl)ethylamine, 25E, as a clear colorless oil; m/z (rel. int.) 267 (M⁺, 3), 252 (14), 176 (17), 135 (62), 117 (100), 105 (28), 91 (56), 77 (33).

15 Preparation of 25G

In a similar fashion, α -methylcinnamionitrile was treated with diisobutyl aluminum hydride and the intermediate aluminum-imine complex treated with (R)-1-(3-methoxyphenyl)ethylamine. The intermediate imine was treated with ethanolic sodium borohydride. Work-up and chromatography yielded (R)-N-(2-methyl-3-phenylprop-2-enyl)-1-(3-methoxyphenyl)ethylamine, 25G, as a clear, colorless oil; m/z (rel. int.) 281 (M⁺, 5), 266 (18), 190 (12), 146 (78), 135 (82), 131 (100), 115 (21), 105 (21), 91 (62), 77 (19).

Preparation of 6X

A stirred solution of sodium hydride (1.8 g, 75 mmol) in dimethylformamide (150 ml) was treated with a solution of diethylcyanomethyl phosphonate (13.3 g, 75 mmol) in dimethylformamide (50 ml). The reaction was stirred 30 min at rt. After this time the reaction was treated with 3-chlorobenzaldehyde (10.54 g, 75 mmol) and stirred 1 hr at rt and 30 min at 60°C. The reaction was then quenched by the addition of water (200 ml). The reaction mixture

was transferred to a separatory funnel using diethyl ether (300 ml) and the resulting organic phase washed with water (5 x 300 ml) and brine. The organic layer was dried over anhydrous potassium carbonate and concentrated to yield 3-chlorocinnamionitrile (11.06 g) as a solid. The solid was dissolved in tetrahydrofuran (50 ml) and treated with excess diborane and stirred 30 min at rt. The reaction was poured over ice/10% HCl. The acidic aqueous phase was washed with diethyl ether (2 x 200 ml). The aqueous phase was made basic by the addition of 10 N NaOH and extracted with diethyl ether (200 ml). The ether extract was dried over anhydrous potassium carbonate and concentrated to afford 3-(3-chlorophenyl)propylamine as an oil (0.6 g, 3.54 mmol). The 3-(3-chlorophenyl)propylamine (0.60 g, 3.54 mmol), 3'-methoxyacetophenone (0.53 g, 3.54 mmol) and 1.25 molar equivalents titanium (IV) isopropoxide (1.26 g, 4.43 mmol) were mixed 4 hr at rt and the intermediate imine treated with an ethanolic sodium cyanoborohydride (5 ml of 1 M, 5 mmol). The reaction was stirred 16 hr at rt, diluted with diethyl ether (50 ml) and treated with water (0.32 ml, 17.7 mmol). After mixing thoroughly the solution was centrifuged and the ether layer concentrated to a milky solid. This material was suspended in diethyl ether and filtered through a 0.45 μ M CR PTFE Acrodisc. The ether wash was concentrated to an oil. Chromatography of this material (silica, preparative thin-layer chromatography) using 3% methanol-dichloromethane (containing 0.1% isopropylamine) afforded N-[3-(3-chlorophenyl)propyl]-1-(3-methoxyphenyl)ethylamine, 6X; m/z (rel. int.) 303 (M+, 3), 288 (40), 196 (3), 164 (8), 135 (100), 125 (46), 103 (26), 91 (29), 77 (29).

Preparation of 6V

An equal molar amount of 3-(4-chlorophenyl)propylamine (prepared in a similar fashion from 4-chlorobenzaldehyde as above) 3'-methoxyacetophenone and 1.25 molar equivalents titanium (IV) isopropoxide were

mixed 4 hr at rt and the intermediate imine treated with an ethanolic sodium cyanoborohydride (5 ml of 1M, 5 mmol). Work-up and chromatography afforded *N*-[3-(4-chlorophenyl)propyl]-1-(3-methoxyphenyl)ethylamine, 6V, as an oil; *m/z* (rel. int.) 303 (*M*⁺, 8), 288 (91), 196 (4), 164 (10), 135 (100), 125 (61), 103 (21), 91 (21), 77 (18).

Preparation of 20A

In a similar fashion, an equal molar amount of 1-(1-methoxyphenyl)ethylamine, 4-*t*-butylacetophenone and 1.25 molar equivalents titanium (IV) isopropoxide were mixed 4 hr at rt and the intermediate imine treated with an ethanolic sodium cyanoborohydride (5 ml of 1M, 5 mmol). Work-up and chromatography afforded (*R*)-*N*-[1-(4-*t*-butylphenyl)ethyl]-1-(1-naphthyl)ethylamine, 20A, as an oil; *m/z* (rel. int.) 331 (*M*⁺, 12), 316 (29), 161 (70), 155 (100), 131 (14), 127 (13), 115 (10), 105 (6), 91 (10), 77 (7).

Preparation of 25H and 25I

In a similar fashion, an equal molar amount of (*R*)-1-(3-methoxyphenyl)ethylamine, *trans*-4-phenyl-3-butene-2-one and 1.25 molar equivalents titanium (IV) isopropoxide were mixed 4 hr at rt and the intermediate imine treated with an ethanolic sodium cyanoborohydride (5 ml of 1 M, 5 mmol). Work-up and chromatography afforded (*R,R*)-*N*-(2-methyl-4-phenylbut-3-enyl)-1-(3-methoxyphenyl)ethylamine, 25H, as an oil; *m/z* (rel. int.) 283 (*M*⁺, 4), 268 (13), 178 (40), 135 (100), 105 (15), 91 (47), 77 (13) and (*S,R*)-*N*-(2-methyl-4-phenylbut-3-enyl)-1-(3-methoxyphenyl)ethylamine, 25I, as an oil; *m/z* (rel. int.) 283 (*M*⁺, 4), 268 (13), 178 (40), 135 (100), 105 (15), 91 (47), 77 (13).

Preparation of 16L and 16M

In a similar fashion, an equal molar amount of (*R*)-1-(3-methoxyphenyl)ethylamine, 3-methoxyacetophenone and 1.25 molar equivalents titanium (IV) isopropoxide were mixed 4 hr at rt and the intermediate imine treated with

an ethanolic sodium cyanoborohydride (5 ml of 1 M, 5 mmol). Work-up and chromatography afforded (R,R)-N-[1-(4-methoxyphenyl)ethyl]-1-(3-methoxyphenyl)ethylamine, 16L, as an oil; m/z (rel. int.) 284 (M-1, 1), 270 (85), 150 (83), 135 (100), 120 (12), 105 (28), 91 (25), 77 (23) and (S,R)-N-[1-(4-methoxyphenyl)ethyl]-1-(3-methoxyphenyl)ethylamine, 16M, as an oil; m/z (rel. int.) 284 (M-1, 1), 270 (53), 150 (98), 135 (100), 120 (11), 105 (33), 91 (25), 77 (23).

10 Preparation of 5B/5C

In a similar fashion, 4-chloroacetophenone was used to prepare 3-methyl-3-(4-chlorophenyl)cinnamionitrile. The nitrile was catalytically reduced (palladium hydroxide, acetic acid, 60 p.s.i. hydrogen 2 hr) to generate 3-methyl-3-(4-chlorophenyl)propylamine. An equal molar amount of the amine, 3'-methoxyacetophenone and 1.25 molar equivalents titanium (IV) isopropoxide were mixed 4 hr at rt and the intermediate imine treated with an ethanolic sodium cyanoborohydride (5 ml of 1 M, 5 mmol). Work-up and chromatography afforded N-(3-methyl-3-(4-chlorophenyl)propyl)-1-(3-methoxyphenyl)ethylamine, 5B/5C as an oil; m/z (rel. int.) 317 (M+, 12), 302 (74), 210 (2), 182 (4), 164 (12), 135 (100), 121 (25), 103 (40), 91 (19), 77 (28).

25 Preparation of 4Z/5A

In a similar fashion, 3-chloroacetophenone was used to prepare 3-methyl-3-(3-chlorophenyl)cinnamionitrile. The nitrile was catalytically reduced (palladium hydroxide, acetic acid, 60 p.s.i. hydrogen 2 hr) to generate 3-methyl-3-(3-chlorophenyl)propylamine. An equal molar amount of the amine, 3'-methoxyacetophenone and 1.25 molar equivalents titanium (IV) isopropoxide were mixed 4 hr at rt and the intermediate imine treated with an ethanolic sodium cyanoborohydride (5 ml of 1 M, 5 mmol). Work-up and chromatography afforded N-[3-methyl-3-(3-chlorophenyl)

propyl]-1-(3-methoxyphenyl)ethylamine, 4Z/5A, as an oil; m/z (rel. int.) 283 (M⁺, 17), 268 (71), 164 (13), 135 (100), 121 (21), 105 (27), 91 (26), 77 (14).

Preparation of 4Y

5 In a similar fashion, 2-chloroacetophenone was used to prepare 3-methyl-3-(2-chlorophenyl)cinnamionitrile. The nitrile was catalytically reduced (palladium hydroxide, acetic acid, 60 p.s.i. hydrogen 2 hr) to generate 3-methyl-3-(2-chlorophenyl)propylamine. An equal molar
10 amount of the amine, 3'-methoxyacetophenone and 1.25 molar equivalents titanium (IV) isopropoxide were mixed 4 hr at rt and the intermediate imine treated with an ethanolic sodium cyanoborohydride (5 ml of 1 M, 5 mmol). Work-up and chromatography afforded N-[3-methyl-3-(2-chloro-
15 phenyl)propyl]-1-(3-methoxyphenyl)ethylamine, 4Y, as an oil; m/z (rel. int.) 283 (M⁺, 17) 268 (71), 164 (13), 135 (100), 121 (21), 105 (27), 91 (26), 77 (14).

Preparation of 6T

A solution of NPS R-568 (30.3 g 100 mmol) in
20 dichloromethane at -78°C was treated dropwise with boron-tribromide (50 g, 200 mmol). The reaction was stirred 1 hr at rt and poured over ice. The hydrobromide was extracted from the aqueous phase with chloroform. The chloroform solubles were then washed (4 x 100 ml) with 50%
25 HCl. The chloroform wash was dried over anhydrous magnesium sulfate and concentrated to afford (R)-N-[3-(2-chlorophenyl)propyl]-1-(3-hydroxyphenyl)ethylamine hydrochloride as a solid. A solution of sodium hydride (0.48 g, 20 mmol) in dimethylformamide was treated with (R)-N-
30 [3-(2-chlorophenyl)propyl]-1-(3-hydroxyphenyl)ethylamine hydrochloride (3.25 g, 10 mmol) and the reaction stirred 1 hr at rt. The reaction was treated with iodoethane (1.71 g, 11 mmol) and stirred 16 hr at rt. Work-up and chromatography through silica using 3% methanol in
35 chloroform afforded (R)-N-[3-(2-chlorophenyl)propyl]-1-(3-

ethoxyphenyl)ethylamine, 6T, as an oil; m/z (rel. int.) 316 (M+, 1), 302 (100), 282 (11), 196 (5), 178 (7), 149 (74), 121 (34), 103 (25), 91 (28), 77 (29).

Preparation of 6R

- 5 NPS R-467 was used in a similar fashion to prepare (R)-N-(3-phenylpropyl)-1-(3-ethoxyphenyl)ethylamine, 6R, as an oil; m/z (rel. int.) 283 (M+, 10), 268 (74), 178 (11), 162 (8), 149 (100), 121 (30), 103 (16), 91 (86), 77 (29).

10 Preparation of 3U

- An equal molar mixture of 3,3-diphenylpropylamine (2.11 g, 10 mmol), 1'-acetonaphthone (1.70 g, 10 mmol) and 1.25 equivalents of titanium (IV) isopropoxide (3.55 g, 12.5 mmol) were stirred 4 hr at rt. The reaction mixture
15 was then treated with a 1 M solution of ethanolic sodium cyanoborohydride (12.5 ml, 12.5 mmol) and stirred 16 hr at rt. The reaction was diluted with diethyl ether (50 ml) and treated with water (0.72 ml, 40 mmol). After mixing thoroughly the mixture was centrifuged and the ether layer
20 decanted and concentrated to a milky oil. The oil was suspended in diethyl ether and filtered through a 0.45 μ M CR PTFE Acrodisc. The diethyl ether filtrate was concentrated to afford N-(3,3-diphenylpropyl)-1-(1-naphthyl)ethylamine, 3U, as a clear, colorless oil; m/z (rel. int.)
25 365 (M+, 17), 350 (19), 181 (23), 155 (100), 141 (25), 115 (11), 91 (13), 77 (6).

Preparation of 6F

- In a similar fashion equal molar amounts 1-(3-methoxyphenyl)ethylamine (1.51 g, 10 mmol), 2'-acetonaph-
30 thone (1.70 g, 10 mmol) and 1.25 equivalents of titanium (IV) isopropoxide (3.55 g, 12.5 mmol) were treated as above. Work-up yielded N-[1-(2-naphthyl)ethyl]-1-(3-methoxyphenyl)ethylamine, 6F, as a clear, colorless oil;

m/z (rel. int.) 305 (M+,1), 290 (35), 170 (49), 155 (100), 135 (55), 115 (8), 105 (10), 91 (9), 77 (10).

Preparation of 4G

In a similar fashion equal molar amounts of (R)-1-phenylethylamine,, 1'-acetonaphthone and 1.25 equivalents of titanium (IV) isopropoxide were mixed and the resulting intermediate imine was reduced with ethanolic sodium cyanoborohydride. Work-up and chromatography yielded N-[1-(1-naphthyl)ethyl]-1-phenylethylamine, 4G, as a clear, colorless oil; m/z (rel. int.) 275 (M+,16), 260 (79), 155 (100), 127 (27), 105 (70), 77 (32).

Preparation of 4H

In a similar fashion equal molar amounts of (R)-1-phenylethylamine, 2'-acetonaphthone and 1.25 equivalents of titanium (IV) isopropoxide were mixed and the resulting intermediate imine was reduced with ethanolic sodium cyanoborohydride. Work-up and chromatography yielded N-[1-(2-naphthyl)ethyl]-1-phenylethylamine, 4H, as a clear, colorless oil; m/z (rel. int.) 275 (M+,1), 260 (61), 155 (100), 120 (36), 105 (55), 77 (15).

Preparation of 6E

In a similar fashion equal molar amounts of 1-(3-methoxyphenyl)ethylamine, 1'-acetonaphthone and 1.25 equivalents of titanium (IV) isopropoxide were mixed and the resulting intermediate imine was reduced with ethanolic sodium cyanoborohydride. Work-up and chromatography yielded N-1-(1-naphthyl)ethyl-1-(3-methoxyphenyl)ethylamine, 6E, as a clear, colorless oil; m/z (rel. int.) 305 (M+,10), 290 (30), 170 (43), 155 (100), 135 (69), 115 (9), 105 (15), 91 (14), 77 (18).

Example 19: Pharmaceutical Formulation

Preparation of a pharmaceutical formulation suitable for administering a calcimimetic into a human patient is shown in Table 3.

5

TABLE 3

	Ingredient	mg/capsule	g/representative batch of 5,000 capsules
	NPS R-568	56.0	280.0
	Pregelatinized Starch NF	134.0	670.0
10	Microcrystalline Cellulose NF	34.0	170.0
	Colloidal Silicon Dioxide	1.0	5.0
	Total	225 mg	1125 g

15 Other examples of NPS (R)-568 hydrochloride formulations and dosage forms include those suitable for sustained or extended release, using standard techniques.

Proper dosing can also be carried out using standard techniques. For example, in one set of experiments, 10 -
20 400 mg oral doses of NPS (R)-568 hydrochloride showed pharmacological activity in human subjects. Significant levels of the O-glucuronide conjugate of 17Q, a principal metabolite of NPS (R)-568, was observed in human plasma following oral administration of NPS (R)-568 hydro-
25 chloride. Thus, the glucuronide conjugate of 17Q may be exerting some beneficial effect.

Using standard techniques other suitable dosage ranges for NPS (R)-568 can be determined.

Suitable dosage ranges, formulations, and dosage
30 forms for other compounds described herein can also be determined by one skilled in art based on the teachings provided in the application.

Other embodiments are within the following claims.
Thus, while several embodiments have been shown and described, various modifications may be made, without departing from the spirit and scope of the present invention.
5 tion.

84

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: NPS Pharmaceuticals, Inc.
- (ii) TITLE OF INVENTION: CALCIUM RECEPTOR-ACTIVE COMPOUNDS
- (iii) NUMBER OF SEQUENCES: 2
- (iv) CORRESPONDENCE ADDRESS:
- (A) ADDRESSEE: Lyon & Lyon
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633 West Fifth Street
(C) CITY: Los Angeles
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 90017
- (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb storage
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: FastSeq
- (vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
- Prior applications total,
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(B) FILING DATE: 8 December, 1994
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(B) FILING DATE: 21 October, 1994

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85

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(ix) TELECOMMUNICATION INFORMATION:

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(B) TELEFAX: (213) 955-0440
(C) TELEX: 67-3510

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5006 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 436..3699
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GCTGCTGTGG CCGGACCCGA AGGCGGGCGC CGGGAGCGCA	40
GCGAGCCAGA CGCGCCTCTC CAAGACCGTG ACCTTGGCAT	80
AGGGAGCGGG GCTGCGCGCA GTCCTGAGAT CAGACCAGAG	120
CTCATCCTCG TGGAGACCCA CGGCCGAGGG GCCGGAGCTG	160
CCTCTGTGCG AGGGAGCCCT GGCCGCGGCG CAGAAGGCAT	200
CACAGGAGGC CTCTGCATGA TGTGGCTTCC AAAGACTCAA	240
GGACCACCCA CATTACAAGT CTGGATTGAG GAAGGCAGAA	280
ATGGAGATTC AAACACCACG TCTTCTATTA TTTTATTAAT	320
CAATCTGTAG ACATGTGTCC CCACTGCAGG GAGTGAAGTG	360
CTCCAAGGGA GAAACTTCTG GGAGCCTCCA AACTCCTAGC	400
TGTCTCATCC CTTGCCCTGG AGAGACGGCA GAACC	435
ATG GCA TTT TAT AGC TGC TGC TGG GTC CTC TTG GCA	471
Met Ala Phe Tyr Ser Cys Cys Trp Val Leu Leu Ala	
1 5 10	

86

CTC	ACC	TGG	CAC	ACC	TCT	GCC	TAC	GGG	CCA	GAC	CAG	507
Leu	Thr	Trp	His	Thr	Ser	Ala	Tyr	Gly	Pro	Asp	Gln	
		15					20					
CGA	GCC	CAA	AAG	AAG	GGG	GAC	ATT	ATC	CTT	GGG	GGG	543
Arg	Ala	Gln	Lys	Lys	Gly	Asp	Ile	Ile	Leu	Gly	Gly	
25					30					35		
CTC	TTT	CCT	ATT	CAT	TTT	GGA	GTA	GCA	GCT	AAA	GAT	579
Leu	Phe	Pro	Ile	His	Phe	Gly	Val	Ala	Ala	Lys	Asp	
			40					45				
CAA	GAT	CTC	AAA	TCA	AGG	CCG	GAG	TCT	GTG	GAA	TGT	615
Gln	Asp	Leu	Lys	Ser	Arg	Pro	Glu	Ser	Val	Glu	Cys	
	50					55					60	
ATC	AGG	TAT	AAT	TTC	CGT	GGG	TTT	CGC	TGG	TTA	CAG	651
Ile	Arg	Tyr	Asn	Phe	Arg	Gly	Phe	Arg	Trp	Leu	Gln	
				65					70			
GCT	ATG	ATA	TTT	GCC	ATA	GAG	GAG	ATA	AAC	AGC	AGC	687
Ala	Met	Ile	Phe	Ala	Ile	Glu	Glu	Ile	Asn	Ser	Ser	
		75					80					
CCA	GCC	CTT	CTT	CCC	AAC	TTG	ACG	CTG	GGA	TAC	AGG	723
Pro	Ala	Leu	Leu	Pro	Asn	Leu	Thr	Leu	Gly	Tyr	Arg	
85					90					95		
ATA	TTT	GAC	ACT	TGC	AAC	ACC	GTT	TCT	AAG	GCC	TTG	759
Ile	Phe	Asp	Thr	Cys	Asn	Thr	Val	Ser	Lys	Ala	Leu	
			100					105				
GAA	GCC	ACC	CTG	AGT	TTT	GTT	GCT	CAA	AAC	AAA	ATT	795
Glu	Ala	Thr	Leu	Ser	Phe	Val	Ala	Gln	Asn	Lys	Ile	
	110					115					120	
GAT	TCT	TTG	AAC	CTT	GAT	GAG	TTC	TGC	AAC	TGC	TCA	831
Asp	Ser	Leu	Asn	Leu	Asp	Glu	Phe	Cys	Asn	Cys	Ser	
				125					130			
GAG	CAC	ATT	CCC	TCT	ACG	ATT	GCT	GTG	GTG	GGA	GCA	867
Glu	His	Ile	Pro	Ser	Thr	Ile	Ala	Val	Val	Gly	Ala	
		135					140					
ACT	GGC	TCA	GGC	GTC	TCC	ACG	GCA	GTG	GCA	AAT	CTG	903
Thr	Gly	Ser	Gly	Val	Ser	Thr	Ala	Val	Ala	Asn	Leu	
145					150					155		
CTG	GGG	CTC	TTC	TAC	ATT	CCC	CAG	GTC	AGT	TAT	GCC	939
Leu	Gly	Leu	Phe	Tyr	Ile	Pro	Gln	Val	Ser	Tyr	Ala	
			160					165				
TCC	TCC	AGC	AGA	CTC	CTC	AGC	AAC	AAG	AAT	CAA	TTC	975
Ser	Ser	Ser	Arg	Leu	Leu	Ser	Asn	Lys	Asn	Gln	Phe	
	170					175					180	

87

AAG	TCT	TTC	CTC	CGA	ACC	ATC	CCC	AAT	GAT	GAG	CAC	1011
Lys	Ser	Phe	Leu	Arg	Thr	Ile	Pro	Asn	Asp	Glu	His	
				185					190			
CAG	GCC	ACT	GCC	ATG	GCA	GAC	ATC	ATC	GAG	TAT	TTC	1047
Gln	Ala	Thr	Ala	Met	Ala	Asp	Ile	Ile	Glu	Tyr	Phe	
		195					200					
CGC	TGG	AAC	TGG	GTG	GGC	ACA	ATT	GCA	GCT	GAT	GAC	1083
Arg	Trp	Asn	Trp	Val	Gly	Thr	Ile	Ala	Ala	Asp	Asp	
205					210					215		
GAC	TAT	GGG	CGG	CCG	GGG	ATT	GAG	AAA	TTC	CGA	GAG	1119
Asp	Tyr	Gly	Arg	Pro	Gly	Ile	Glu	Lys	Phe	Arg	Glu	
			220					225				
GAA	GCT	GAG	GAA	AGG	GAT	ATC	TGC	ATC	GAC	TTC	AGT	1155
Glu	Ala	Glu	Glu	Arg	Asp	Ile	Cys	Ile	Asp	Phe	Ser	
	230					235					240	
GAA	CTC	ATC	TCC	CAG	TAC	TCT	GAT	GAG	GAA	GAG	ATC	1191
Glu	Leu	Ile	Ser	Gln	Tyr	Ser	Asp	Glu	Glu	Glu	Ile	
				245					250			
CAG	CAT	GTG	GTA	GAG	GTG	ATT	CAA	AAT	TCC	ACG	GCC	1227
Gln	His	Val	Val	Glu	Val	Ile	Gln	Asn	Ser	Thr	Ala	
		255					260					
AAA	GTC	ATC	GTG	GTT	TTC	TCC	AGT	GGC	CCA	GAT	CTT	1263
Lys	Val	Ile	Val	Val	Phe	Ser	Ser	Gly	Pro	Asp	Leu	
265					270					275		
GAG	CCC	CTC	ATC	AAG	GAG	ATT	GTC	CGG	CGC	AAT	ATC	1299
Glu	Pro	Leu	Ile	Lys	Glu	Ile	Val	Arg	Arg	Asn	Ile	
			280					285				
ACG	GGC	AAG	ATC	TGG	CTG	GCC	AGC	GAG	GCC	TGG	GCC	1335
Thr	Gly	Lys	Ile	Trp	Leu	Ala	Ser	Glu	Ala	Trp	Ala	
	290					295					300	
AGC	TCC	TCC	CTG	ATC	GCC	ATG	CCT	CAG	TAC	TTC	CAC	1371
Ser	Ser	Ser	Leu	Ile	Ala	Met	Pro	Gln	Tyr	Phe	His	
				305					310			
GTG	GTT	GGC	GGC	ACC	ATT	GGA	TTC	GCT	CTG	AAG	GCT	1407
Val	Val	Gly	Gly	Thr	Ile	Gly	Phe	Ala	Leu	Lys	Ala	
		315					320					
GGG	CAG	ATC	CCA	GGC	TTC	CGG	GAA	TTC	CTG	AAG	AAG	1443
Gly	Gln	Ile	Pro	Gly	Phe	Arg	Glu	Phe	Leu	Lys	Lys	
325					330					335		
GTC	CAT	CCC	AGG	AAG	TCT	GTC	CAC	AAT	GGT	TTT	GCC	1479
Val	His	Pro	Arg	Lys	Ser	Val	His	Asn	Gly	Phe	Ala	
			340					345				

88

AAG	GAG	TTT	TGG	GAA	GAA	ACA	TTT	AAC	TGC	CAC	CTC	1515
Lys	Glu	Phe	Trp	Glu	Glu	Thr	Phe	Asn	Cys	His	Leu	
350						355					360	
CAA	GAA	GGT	GCA	AAA	GGA	CCT	TTA	CCT	GTG	GAC	ACC	1551
Gln	Glu	Gly	Ala	Lys	Gly	Pro	Leu	Pro	Val	Asp	Thr	
				365					370			
TTT	CTG	AGA	GGT	CAC	GAA	GAA	AGT	GGC	GAC	AGG	TTT	1587
Phe	Leu	Arg	Gly	His	Glu	Glu	Ser	Gly	Asp	Arg	Phe	
		375					380					
AGC	AAC	AGC	TCG	ACA	GCC	TTC	CGA	CCC	CTC	TGT	ACA	1623
Ser	Asn	Ser	Ser	Thr	Ala	Phe	Arg	Pro	Leu	Cys	Thr	
385					390					395		
GGG	GAT	GAG	AAC	ATC	AGC	AGT	GTC	GAG	ACC	CCT	TAC	1659
Gly	Asp	Glu	Asn	Ile	Ser	Ser	Val	Glu	Thr	Pro	Tyr	
			400					405				
ATA	GAT	TAC	ACG	CAT	TTA	CGG	ATA	TCC	TAC	AAT	GTG	1695
Ile	Asp	Tyr	Thr	His	Leu	Arg	Ile	Ser	Tyr	Asn	Val	
	410					415					420	
TAC	TTA	GCA	GTC	TAC	TCC	ATT	GCC	CAC	GCC	TTG	CAA	1731
Tyr	Leu	Ala	Val	Tyr	Ser	Ile	Ala	His	Ala	Leu	Gln	
				425					430			
GAT	ATA	TAT	ACC	TGC	TTA	CCT	GGG	AGA	GGG	CTC	TTC	1767
Asp	Ile	Tyr	Thr	Cys	Leu	Pro	Gly	Arg	Gly	Leu	Phe	
		435					440					
ACC	AAT	GGC	TCC	TGT	GCA	GAC	ATC	AAG	AAA	GTT	GAG	1803
Thr	Asn	Gly	Ser	Cys	Ala	Asp	Ile	Lys	Lys	Val	Glu	
445					450					455		
GCG	TGG	CAG	GTC	CTG	AAG	CAC	CTA	CGG	CAT	CTA	AAC	1839
Ala	Trp	Gln	Val	Leu	Lys	His	Leu	Arg	His	Leu	Asn	
			460					465				
TTT	ACA	AAC	AAT	ATG	GGG	GAG	CAG	GTG	ACC	TTT	GAT	1875
Phe	Thr	Asn	Asn	Met	Gly	Glu	Gln	Val	Thr	Phe	Asp	
	470					475					480	
GAG	TGT	GGT	GAC	CTG	GTG	GGG	AAC	TAT	TCC	ATC	ATC	1911
Glu	Cys	Gly	Asp	Leu	Val	Gly	Asn	Tyr	Ser	Ile	Ile	
				485					490			
AAC	TGG	CAC	CTC	TCC	CCA	GAG	GAT	GGC	TCC	ATC	GTG	1947
Asn	Trp	His	Leu	Ser	Pro	Glu	Asp	Gly	Ser	Ile	Val	
		495					500					
TTT	AAG	GAA	GTC	GGG	TAT	TAC	AAC	GTC	TAT	GCC	AAG	1983
Phe	Lys	Glu	Val	Gly	Tyr	Tyr	Asn	Val	Tyr	Ala	Lys	
505					510					515		

89

AAG Lys	GGA Gly	GAA Glu	AGA Arg 520	CTC Leu	TTC Phe	ATC Ile	AAC Asn	GAG Glu 525	GAG Glu	AAA Lys	ATC Ile	2019
CTG Leu	TGG Trp 530	AGT Ser	GGG Gly	TTC Phe	TCC Ser	AGG Arg 535	GAG Glu	CCA Pro	CTC Leu	ACC Thr	TTT Phe 540	2055
GTG Val	CTG Leu	TCT Ser	GTC Val	CTC Leu 545	CAG Gln	GTG Val	CCC Pro	TTC Phe	TCC Ser 550	AAC Asn	TGC Cys	2091
AGC Ser	CGA Arg	GAC Asp 555	TGC Cys	CTG Leu	GCA Ala	GGG Gly	ACC Thr 560	AGG Arg	AAA Lys	GGG Gly	ATC Ile	2127
ATT Ile 565	GAG Glu	GGG Gly	GAG Glu	CCC Pro	ACC Thr 570	TGC Cys	TGC Cys	TTT Phe	GAG Glu	TGT Cys 575	GTG Val	2163
GAG Glu	TGT Cys	CCT Pro	GAT Asp 580	GGG Gly	GAG Glu	TAT Tyr	AGT Ser	GAT Asp 585	GAG Glu	ACA Thr	GAT Asp	2199
GCC Ala 590	AGT Ser	GCC Ala	TGT Cys	AAC Asn	AAG Lys	TGC Cys 595	CCA Pro	GAT Asp	GAC Asp	TTC Phe	TGG Trp 600	2235
TCC Ser	AAT Asn	GAG Glu	AAC Asn	CAC His 605	ACC Thr	TCC Ser	TGC Cys	ATT Ile	GCC Ala 610	AAG Lys	GAG Glu	2271
ATC Ile	GAG Glu	TTT Phe 615	CTG Leu	TCG Ser	TGG Trp	ACG Thr	GAG Glu 620	CCC Pro	TTT Phe	GGG Gly	ATC Ile	2307
GCA Ala 625	CTC Leu	ACC Thr	CTC Leu	TTT Phe	GCC Ala 630	GTG Val	CTG Leu	GGC Gly	ATT Ile	TTC Phe 635	CTG Leu	2343
ACA Thr	GCC Ala	TTT Phe	GTG Val	CTG Leu	GGT Gly	GTG Val	TTT Phe	ATC Ile 645	AAG Lys	TTC Phe	CGC Arg	2379
AAC Asn 650	ACA Thr	CCC Pro	ATT Ile	GTC Val	AAG Lys	GCC Ala 655	ACC Thr	AAC Asn	CGA Arg	GAG Glu	CTC Leu 660	2415
TCC Ser	TAC Tyr	CTC Leu	CTC Leu	CTC Leu 665	TTC Phe	TCC Ser	CTG Leu	CTC Leu	TGC Cys 670	TGC Cys	TTT Phe	2451
TCC Ser	AGC Ser	TCC Ser 675	CTG Leu	TTC Phe	TTC Phe	ATC Ile	GGG Gly 680	GAG Glu	CCC Pro	CAG Gln	GAC Asp	2487

90

TGG Trp 685	ACG Thr	TGC Cys	CGC Arg	CTG Leu	CGC Arg 690	CAG Gln	CCG Pro	GCC Ala	TTT Phe	GGC Gly 695	ATC Ile	2523
AGC Ser	TTC Phe	GTG Val	CTC Leu 700	TGC Cys	ATC Ile	TCA Ser	TGC Cys	ATC Ile 705	CTG Leu	GTG Val	AAA Lys	2559
ACC Thr 710	AAC Asn	CGT Arg	GTC Val	CTC Leu	CTG Leu 715	GTG Val	TTT Phe	GAG Glu	GCC Ala	AAG Lys	ATC Ile 720	2595
CCC Pro	ACC Thr	AGC Ser	TTC Phe	CAC His 725	CGC Arg	AAG Lys	TGG Trp	TGG Trp	GGG Gly 730	CTC Leu	AAC Asn	2631
CTG Leu	CAG Gln	TTC Phe 735	CTG Leu	CTG Leu	GTT Val	TTC Phe	CTC Leu 740	TGC Cys	ACC Thr	TTC Phe	ATG Met	2667
CAG Gln 745	ATT Ile	GTC Val	ATC Ile	TGT Cys	GTG Val 750	ATC Ile	TGG Trp	CTC Leu	TAC Tyr	ACC Thr 755	GCG Ala	2703
CCC Pro	CCC Pro	TCA Ser	AGC Ser 760	TAC Tyr	CGC Arg	AAC Asn	CAG Gln	GAG Glu 765	CTG Leu	GAG Glu	GAT Asp	2739
GAG Glu 770	ATC Ile	ATC Ile	TTC Phe	ATC Ile	ACG Thr	TGC Cys 775	CAC His	GAG Glu	GGC Gly	TCC Ser	CTC Leu 780	2775
ATG Met	GCC Ala	CTG Leu	GGC Gly	TTC Phe 785	CTG Leu	ATC Ile	GGC Gly	TAC Tyr	ACC Thr 790	TGC Cys	CTG Leu	2811
CTG Leu	GCT Ala	GCC Ala	ATC Ile 795	TGC Cys	TTC Phe	TTC Phe	TTT Phe 800	GCC Ala	TTC Phe	AAG Lys	TCC Ser	2847
CGG Arg 805	AAG Lys	CTG Leu	CCG Pro	GAG Glu	AAC Asn 810	TTC Phe	AAT Asn	GAA Glu	GCC Ala	AAG Lys 815	TTC Phe	2883
ATC Ile	ACC Thr	TTC Phe	AGC Ser 820	ATG Met	CTC Leu	ATC Ile	TTC Phe	TTC Phe 825	ATC Ile	GTC Val	TGG Trp	2919
ATC Ile 830	TCC Ser	TTC Phe	ATT Ile	CCA Pro	GCC Ala	TAT Tyr 835	GCC Ala	AGC Ser	ACC Thr	TAT Tyr	GGC Gly 840	2955
AAG Lys	TTT Phe	GTC Val	TCT Ser	GCC Ala 845	GTA Val	GAG Glu	GTG Val	ATT Ile	GCC Ala 850	ATC Ile	CTG Leu	2991

91

GCA	GCC	AGC	TTT	GGC	TTG	CTG	GCG	TGC	ATC	TTC	TTC	3027
Ala	Ala	Ser	Phe	Gly	Leu	Leu	Ala	Cys	Ile	Phe	Phe	
		855					860					
AAC	AAG	ATC	TAC	ATC	ATT	CTC	TTC	AAG	CCA	TCC	CGC	3063
Asn	Lys	Ile	Tyr	Ile	Ile	Leu	Phe	Lys	Pro	Ser	Arg	
865					870					875		
AAC	ACC	ATC	GAG	GAG	GTG	CGT	TGC	AGC	ACC	GCA	GCT	3099
Asn	Thr	Ile	Glu	Glu	Val	Arg	Cys	Ser	Thr	Ala	Ala	
			880					885				
CAC	GCT	TTC	AAG	GTG	GCT	GCC	CGG	GCC	ACG	CTG	CGC	3135
His	Ala	Phe	Lys	Val	Ala	Ala	Arg	Ala	Thr	Leu	Arg	
	890					895					900	
CGC	AGC	AAC	GTC	TCC	CGC	AAG	CGG	TCC	AGC	AGC	CTT	3171
Arg	Ser	Asn	Val	Ser	Arg	Lys	Arg	Ser	Ser	Ser	Leu	
				905						910		
GGA	GGC	TCC	ACG	GGA	TCC	ACC	CCC	TCC	TCC	TCC	ATC	3207
Gly	Gly	Ser	Thr	Gly	Ser	Thr	Pro	Ser	Ser	Ser	Ile	
		915					920					
AGC	AGC	AAG	AGC	AAC	AGC	GAA	GAC	CCA	TTC	CCA	CGG	3243
Ser	Ser	Lys	Ser	Asn	Ser	Glu	Asp	Pro	Phe	Pro	Arg	
925					930					935		
CCC	GAG	AGG	CAG	AAG	CAG	CAG	CAG	CCG	CTG	GCC	CTA	3279
Pro	Glu	Arg	Gln	Lys	Gln	Gln	Gln	Pro	Leu	Ala	Leu	
			940					945				
ACC	CAG	CAA	GAG	CAG	CAG	CAG	CAG	CCC	CTG	ACC	CTC	3315
Thr	Gln	Gln	Glu	Gln	Gln	Gln	Gln	Pro	Leu	Thr	Leu	
	950					955					960	
CCA	CAG	CAG	CAA	CGA	TCT	CAG	CAG	CAG	CCC	AGA	TGC	3351
Pro	Gln	Gln	Gln	Arg	Ser	Gln	Gln	Gln	Pro	Arg	Cys	
				965						970		
AAG	CAG	AAG	GTC	ATC	TTT	GGC	AGC	GGC	ACG	GTC	ACC	3387
Lys	Gln	Lys	Val	Ile	Phe	Gly	Ser	Gly	Thr	Val	Thr	
		975					980					
TTC	TCA	CTG	AGC	TTT	GAT	GAG	CCT	CAG	AAG	AAC	GCC	3423
Phe	Ser	Leu	Ser	Phe	Asp	Glu	Pro	Gln	Lys	Asn	Ala	
985					990					995		
ATG	GCC	CAC	AGG	AAT	TCT	ACG	CAC	CAG	AAC	TCC	CTG	3459
Met	Ala	His	Arg	Asn	Ser	Thr	His	Gln	Asn	Ser	Leu	
			1000					1005				
GAG	GCC	CAG	AAA	AGC	AGC	GAT	ACG	CTG	ACC	CGA	CAC	3495
Glu	Ala	Gln	Lys	Ser	Ser	Asp	Thr	Leu	Thr	Arg	His	
	1010					1015					1020	

92

CAG CCA TTA CTC CCG CTG CAG TGC GGG GAA ACG GAC Gln Pro Leu Leu Pro Leu Gln Cys Gly Glu Thr Asp 1025 1030	3531
TTA GAT CTG ACC GTC CAG GAA ACA GGT CTG CAA GGA Leu Asp Leu Thr Val Gln Glu Thr Gly Leu Gln Gly 1035 1040	3567
CCT GTG GGT GGA GAC CAG CGG CCA GAG GTG GAG GAC Pro Val Gly Gly Asp Gln Arg Pro Glu Val Glu Asp 1045 1050 1055	3603
CCT GAA GAG TTG TCC CCA GCA CTT GTA GTG TCC AGT Pro Glu Glu Leu Ser Pro Ala Leu Val Val Ser Ser 1060 1065	3639
TCA CAG AGC TTT GTC ATC AGT GGT GGA GGC AGC ACT Ser Gln Ser Phe Val Ile Ser Gly Gly Gly Ser Thr 1070 1075 1080	3675
GTT ACA GAA AAC GTA GTG AAT TCA TAAAATGGAA Val Thr Glu Asn Val Val Asn Ser 1085	3709
GGAGAAGACT GGGCTAGGGA GAATGCAGAG AGGTTTCTTG	3749
GGGTCCCAGG GATGAGGAAT CGCCCCAGAC TCCTTTCCTC	3789
TGAGGAAGAA GGGATAATAG ACACATCAAA TGCCCCGAAT	3829
TTAGTCACAC CATCTTAAAT GACAGTGAAT TGACCCATGT	3869
TCCCTTTAAA ATTAAAAAAA AGAAGAGCCT TGTGTTTCTG	3909
TGGTTGCATT TGTCAAAGCA TTGAGATCTC CACGGTCAGA	3949
TTTGCTGTTT ACCCACATCT AATGTCTCTT CCTCTGTTCT	3989
ATCCCACCCA ACAGCTCAGA GATGAAACTA TGGCTTTAAA	4029
CTACCCTCCA GAGTGTGCAG ACTGATGGGA CATCAAATTT	4069
GCCACCACTA GAGCTGAGAG TCTGAAAGAC AGAATGTCAC	4109
CAGTCCTGCC CAATGCCTTG ACAACAGACT GAATTTTAAA	4149
TGTTCAACAAC ATAAGGAGAA TGTATCTCCT CCTATTTATG	4189
AAAACCATAT GATATTTTGT CTCCTACCTG CTGCTGCTAT	4229
TATGTAACAT CCAGAAGGTT TGCACCCCTC CTATACCATA	4269
TGTCTGGTTC TGTCCAGGAC ATGATACTGA TGCCATGTTT	4309
AGATTCCAGG ATCACAAGAA TCACCTCAAA TTGTTAGGAA	4349

GGGACTGCAT AAACCAATGA GCTGTATCTG TAATTAATAT	4389
TCCTATATGT AGCTTTATCC TTAGGAAAAT GCTTCTGTTG	4429
TAATAGTCCA TGGACAATAT AAAGTAAAA ATGTCAGTCT	4469
GGTTTATATA AGGCAGTATT ATTGAGCTCT ATTTCCCCAC	4509
CCCACTATCC TCACTCCCAT AAGCTAAGCC TTATGTGAGC	4549
CCCTTCAGGG ACTCAAGGGT CCAGAAGTCC CTCCCATCTC	4589
TACCCCAAAG AATTCCTGAA GCCAGATCCA CCCTATCCCT	4629
GTACAGAGTA AGTTCTCAAT TATTGGCCTG CTAATAGCTG	4669
CTAGGGTAGG AAAGCGTGGT TCCAAGAAAG ATCCACCCTC	4709
AAATGTCGGA GCTATGTTCC CTCCAGCAGT GGTATTAATA	4749
CTGCCGGTCA CCCAGGCTCT GGAGCCAGAG AGACAGACCG	4789
GGGTTCAAGC CATGGCTTCG TCATTTGCAA GCTGAGTGAC	4829
TGTAGGCAGG GAACCTTAAC CTCTCTAAGC CACAGCTTCT	4869
TCATCTTTAA AATAAGGATA ATAATCATTC CTTCCCCTCA	4909
GAGCTCTTAT GTGGATTAAA CGAGATAATG TATATAAAGT	4949
ACTTTAGCCT GGTACCTAGC ACACAATAAG CATTCAATAA	4989
ATATTAGTTA ATATTAT	5006

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	3809 base pairs
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) MOLECULE TYPE: cDNA to mRNA

(ix) FEATURE:

(A) NAME/KEY:	CDS
(B) LOCATION:	373..3606
(D) OTHER INFORMATION:	

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

CAACAGGCAC CTGGCTGCAG CCAGGAAGGA CCGCACGCCC	40
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96

CGG	CGC	AAT	ATC	ACG	GGC	AAG	ATC	TGG	CTG	GCC	AGC	1260
Arg	Arg	Asn	Ile	Thr	Gly	Lys	Ile	Trp	Leu	Ala	Ser	
285					290					295		
GAG	GCC	TGG	GCC	AGC	TCC	TCC	CTG	ATC	GCC	ATG	CCT	1296
Glu	Ala	Trp	Ala	Ser	Ser	Ser	Leu	Ile	Ala	Met	Pro	
			300					305				
CAG	TAC	TTC	CAC	GTG	GTT	GGC	GGC	ACC	ATT	GGA	TTC	1332
Gln	Tyr	Phe	His	Val	Val	Gly	Gly	Thr	Ile	Gly	Phe	
310						315					320	
GCT	CTG	AAG	GCT	GGG	CAG	ATC	CCA	GGC	TTC	CGG	GAA	1368
Ala	Leu	Lys	Ala	Gly	Gln	Ile	Pro	Gly	Phe	Arg	Glu	
				325					330			
TTC	CTG	AAG	AAG	GTC	CAT	CCC	AGG	AAG	TCT	GTC	CAC	1404
Phe	Leu	Lys	Lys	Val	His	Pro	Arg	Lys	Ser	Val	His	
		335					340					
AAT	GGT	TTT	GCC	AAG	GAG	TTT	TGG	GAA	GAA	ACA	TTT	1440
Asn	Gly	Phe	Ala	Lys	Glu	Phe	Trp	Glu	Glu	Thr	Phe	
345					350					355		
AAC	TGC	CAC	CTC	CAA	GAA	GGT	GCA	AAA	GGA	CCT	TTA	1476
Asn	Cys	His	Leu	Gln	Glu	Gly	Ala	Lys	Gly	Pro	Leu	
			360					365				
CCT	GTG	GAC	ACC	TTT	CTG	AGA	GGT	CAC	GAA	GAA	AGT	1512
Pro	Val	Asp	Thr	Phe	Leu	Arg	Gly	His	Glu	Glu	Ser	
	370					375					380	
GGC	GAC	AGG	TTT	AGC	AAC	AGC	TCG	ACA	GCC	TTC	CGA	1548
Gly	Asp	Arg	Phe	Ser	Asn	Ser	Ser	Thr	Ala	Phe	Arg	
				385					390			
CCC	CTC	TGT	ACA	GGG	GAT	GAG	AAC	ATC	AGC	AGT	GTC	1584
Pro	Leu	Cys	Thr	Gly	Asp	Glu	Asn	Ile	Ser	Ser	Val	
		395					400					
GAG	ACC	CCT	TAC	ATA	GAT	TAC	ACG	CAT	TTA	CGG	ATA	1620
Glu	Thr	Pro	Tyr	Ile	Asp	Tyr	Thr	His	Leu	Arg	Ile	
405					410					415		
TCC	TAC	AAT	GTG	TAC	TTA	GCA	GTC	TAC	TCC	ATT	GCC	1656
Ser	Tyr	Asn	Val	Tyr	Leu	Ala	Val	Tyr	Ser	Ile	Ala	
			420					425				
CAC	GCC	TTG	CAA	GAT	ATA	TAT	ACC	TGC	TTA	CCT	GGG	1692
His	Ala	Leu	Gln	Asp	Ile	Tyr	Thr	Cys	Leu	Pro	Gly	
	430					435					440	
AGA	GGG	CTC	TTC	ACC	AAT	GGC	TCC	TGT	GCA	GAC	ATC	1728
Arg	Gly	Leu	Phe	Thr	Asn	Gly	Ser	Cys	Ala	Asp	Ile	
				445					450			

97

AAG Lys	AAA Lys	GTT Val 455	GAG Glu	GCG Ala	TGG Trp	CAG Gln	GTC Val 460	CTG Leu	AAG Lys	CAC His	CTA Leu	1764
CGG Arg 465	CAT His	CTA Leu	AAC Asn	TTT Phe	ACA Thr 470	AAC Asn	AAT Asn	ATG Met	GGG Gly	GAG Glu 475	CAG Gln	1800
GTG Val	ACC Thr	TTT Phe	GAT Asp 480	GAG Glu	TGT Cys	GGT Gly	GAC Asp 485	CTG Leu	GTG Val	GGG Gly	AAC Asn	1836
TAT Tyr 490	TCC Ser	ATC Ile	ATC Ile	AAC Asn	TGG Trp	CAC His 495	CTC Leu	TCC Ser	CCA Pro	GAG Glu	GAT Asp 500	1872
GGC Gly	TCC Ser	ATC Ile	GTG Val	TTT Phe 505	AAG Lys	GAA Glu	GTC Val	GGG Gly	TAT Tyr 510	TAC Tyr	AAC Asn	1908
GTC Val	TAT Tyr	GCC Ala 515	AAG Lys	AAG Lys	GGA Gly	GAA Glu	AGA Arg 520	CTC Leu	TTC Phe	ATC Ile	AAC Asn	1944
GAG Glu 525	GAG Glu	AAA Lys	ATC Ile	CTG Leu	TGG Trp 530	AGT Ser	GGG Gly	TTC Phe	TCC Ser	AGG Arg 535	GAG Glu	1980
GTG Val	CCC Pro	TTC Phe	TCC Ser 540	AAC Asn	TGC Cys	AGC Ser	CGA Arg	GAC Asp 545	TGC Cys	CTG Leu	GCA Ala	2016
GGG Gly 550	ACC Thr	AGG Arg	AAA Lys	GGG Gly	ATC Ile	ATT Ile 555	GAG Glu	GGG Gly	GAG Glu	CCC Pro	ACC Thr 560	2052
TGC Cys	TGC Cys	TTT Phe	GAG Glu	TGT Cys 565	GTG Val	GAG Glu	TGT Cys	CCT Pro	GAT Asp 570	GGG Gly	GAG Glu	2088
TAT Tyr 575	AGT Ser	GAT Asp 575	GAG Glu	ACA Thr	GAT Asp	GCC Ala	AGT Ser 580	GCC Ala	TGT Cys	AAC Asn	AAG Lys	2124
TGC Cys 585	CCA Pro	GAT Asp	GAC Asp	TTC Phe	TGG Trp 590	TCC Ser	AAT Asn	GAG Glu	AAC Asn	CAC His 595	ACC Thr	2160
TCC Ser	TGC Cys	ATT Ile	GCC Ala 600	AAG Lys	GAG Glu	ATC Ile	GAG Glu	TTT Phe 605	CTG Leu	TCG Ser	TGG Trp	2196
ACG Thr 610	GAG Glu	CCC Pro	TTT Phe	GGG Gly	ATC Ile	GCA Ala 615	CTC Leu	ACC Thr	CTC Leu	TTT Phe	GCC Ala 620	2232

98

GTG	CTG	GGC	ATT	TTC	CTG	ACA	GCC	TTT	GTG	CTG	GGT	2268
Val	Leu	Gly	Ile	Phe	Leu	Thr	Ala	Phe	Val	Leu	Gly	
				625					630			
GTG	TTT	ATC	AAG	TTC	CGC	AAC	ACA	CCC	ATT	GTC	AAG	2304
Val	Phe	Ile	Lys	Phe	Arg	Asn	Thr	Pro	Ile	Val	Lys	
		635					640					
GCC	ACC	AAC	CGA	GAG	CTC	TCC	TAC	CTC	CTC	CTC	TTC	2340
Ala	Thr	Asn	Arg	Glu	Leu	Ser	Tyr	Leu	Leu	Leu	Phe	
645					650					655		
TCC	CTG	CTC	TGC	TGC	TTC	TCC	AGC	TCC	CTG	TTC	TTC	2376
Ser	Leu	Leu	Cys	Cys	Phe	Ser	Ser	Ser	Leu	Phe	Phe	
			660					665				
ATC	GGG	GAG	CCC	CAG	GAC	TGG	ACG	TGC	CGC	CTG	CGC	2412
Ile	Gly	Glu	Pro	Gln	Asp	Trp	Thr	Cys	Arg	Leu	Arg	
	670					675					680	
CAG	CCG	GCC	TTT	GGC	ATC	AGC	TTC	GTG	CTC	TGC	ATC	2448
Gln	Pro	Ala	Phe	Gly	Ile	Ser	Phe	Val	Leu	Cys	Ile	
				685					690			
TCA	TGC	ATC	CTG	GTG	AAA	ACC	AAC	CGT	GTC	CTC	CTG	2484
Ser	Cys	Ile	Leu	Val	Lys	Thr	Asn	Arg	Val	Leu	Leu	
		695					700					
GTG	TTT	GAG	GCC	AAG	ATC	CCC	ACC	AGC	TTC	CAC	CGC	2520
Val	Phe	Glu	Ala	Lys	Ile	Pro	Thr	Ser	Phe	His	Arg	
705					710					715		
AAG	TGG	TGG	GGG	CTC	AAC	CTG	CAG	TTC	CTG	CTG	GTT	2556
Lys	Trp	Trp	Gly	Leu	Asn	Leu	Gln	Phe	Leu	Leu	Val	
			720					725				
TTC	CTC	TGC	ACC	TTC	ATG	CAG	ATT	GTC	ATC	TGT	GTG	2592
Phe	Leu	Cys	Thr	Phe	Met	Gln	Ile	Val	Ile	Cys	Val	
	730					735					740	
ATC	TGG	CTC	TAC	ACC	GCG	CCC	CCC	TCA	AGC	TAC	CGC	2628
Ile	Trp	Leu	Tyr	Thr	Ala	Pro	Pro	Ser	Ser	Tyr	Arg	
				745					750			
AAC	CAG	GAG	CTG	GAG	GAT	GAG	ATC	ATC	TTC	ATC	ACG	2664
Asn	Gln	Glu	Leu	Glu	Asp	Glu	Ile	Ile	Phe	Ile	Thr	
		755					760					
TGC	CAC	GAG	GGC	TCC	CTC	ATG	GCC	CTG	GGC	TTC	CTG	2700
Cys	His	Glu	Gly	Ser	Leu	Met	Ala	Leu	Gly	Phe	Leu	
765					770					775		
ATC	GGC	TAC	ACC	TGC	CTG	CTG	GCT	GCC	ATC	TGC	TTC	2736
Ile	Gly	Tyr	Thr	Cys	Leu	Leu	Ala	Ala	Ile	Cys	Phe	
			780					785				

99

TTC	TTT	GCC	TTC	AAG	TCC	CGG	AAG	CTG	CCG	GAG	AAC	2772
Phe	Phe	Ala	Phe	Lys	Ser	Arg	Lys	Leu	Pro	Glu	Asn	
	790					795					800	
TTC	AAT	GAA	GCC	AAG	TTC	ATC	ACC	TTC	AGC	ATG	CTC	2808
Phe	Asn	Glu	Ala	Lys	Phe	Ile	Thr	Phe	Ser	Met	Leu	
				805					810			
ATC	TTC	TTC	ATC	GTC	TGG	ATC	TCC	TTC	ATT	CCA	GCC	2844
Ile	Phe	Phe	Ile	Val	Trp	Ile	Ser	Phe	Ile	Pro	Ala	
		815					820					
TAT	GCC	AGC	ACC	TAT	GGC	AAG	TTT	GTC	TCT	GCC	GTA	2880
Tyr	Ala	Ser	Thr	Tyr	Gly	Lys	Phe	Val	Ser	Ala	Val	
825					830					835		
GAG	GTG	ATT	GCC	ATC	CTG	GCA	GCC	AGC	TTT	GGC	TTG	2916
Glu	Val	Ile	Ala	Ile	Leu	Ala	Ala	Ser	Phe	Gly	Leu	
			840					845				
CTG	GCG	TGC	ATC	TTC	TTC	AAC	AAG	ATC	TAC	ATC	ATT	2952
Leu	Ala	Cys	Ile	Phe	Phe	Asn	Lys	Ile	Tyr	Ile	Ile	
	850					855					860	
CTC	TTC	AAG	CCA	TCC	CGC	AAC	ACC	ATC	GAG	GAG	GTG	2988
Leu	Phe	Lys	Pro	Ser	Arg	Asn	Thr	Ile	Glu	Glu	Val	
				865					870			
CGT	TGC	AGC	ACC	GCA	GCT	CAC	GCT	TTC	AAG	GTG	GCT	3024
Arg	Cys	Ser	Thr	Ala	Ala	His	Ala	Phe	Lys	Val	Ala	
		875					880					
GCC	CGG	GCC	ACG	CTG	CGC	CGC	AGC	AAC	GTC	TCC	CGC	3060
Ala	Arg	Ala	Thr	Leu	Arg	Arg	Ser	Asn	Val	Ser	Arg	
885					890					895		
AAG	CGG	TCC	AGC	AGC	CTT	GGA	GGC	TCC	ACG	GGA	TCC	3096
Lys	Arg	Ser	Ser	Ser	Leu	Gly	Gly	Ser	Thr	Gly	Ser	
			900					905				
ACC	CCC	TCC	TCC	TCC	ATC	AGC	AGC	AAG	AGC	AAC	AGC	3132
Thr	Pro	Ser	Ser	Ser	Ile	Ser	Ser	Lys	Ser	Asn	Ser	
	910					915					920	
GAA	GAC	CCA	TTC	CCA	CAG	CCC	GAG	AGG	CAG	AAG	CAG	3168
Glu	Asp	Pro	Phe	Pro	Gln	Pro	Glu	Arg	Gln	Lys	Gln	
				925					930			
CAG	CAG	CCG	CTG	GCC	CTA	ACC	CAG	CAA	GAG	CAG	CAG	3204
Gln	Gln	Pro	Leu	Ala	Leu	Thr	Gln	Gln	Glu	Gln	Gln	
		935					940					
CAG	CAG	CCC	CTG	ACC	CTC	CCA	CAG	CAG	CAA	CGA	TCT	3240
Gln	Gln	Pro	Leu	Thr	Leu	Pro	Gln	Gln	Gln	Arg	Ser	
945					950					955		

100

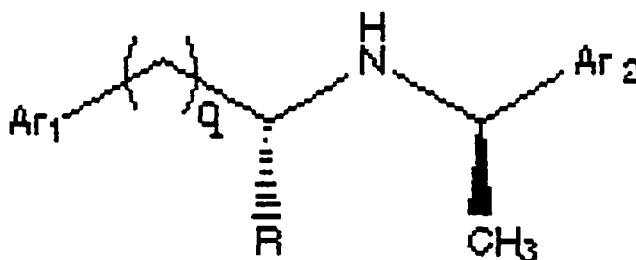
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GGC AGC GGC ACG GTC ACC TTC TCA CTG AGC TTT GAT Gly Ser Gly Thr Val Thr Phe Ser Leu Ser Phe Asp 970 975 980	3312
GAG CCT CAG AAG AAC GCC ATG GCC CAC GGG AAT TCT Glu Pro Gln Lys Asn Ala Met Ala His Gly Asn Ser 985 990	3348
ACG CAC CAG AAC TCC CTG GAG GCC CAG AAA AGC AGC Thr His Gln Asn Ser Leu Glu Ala Gln Lys Ser Ser 995 1000	3384
GAT ACG CTG ACC CGA CAC CAG CCA TTA CTC CCG CTG Asp Thr Leu Thr Arg His Gln Pro Leu Leu Pro Leu 1005 1010 1015	3420
CAG TGC GGG GAA ACG GAC TTA GAT CTG ACC GTC CAG Gln Cys Gly Glu Thr Asp Leu Asp Leu Thr Val Gln 1020 1025	3456
GAA ACA GGT CTG CAA GGA CCT GTG GGT GGA GAC CAG Glu Thr Gly Leu Gln Gly Pro Val Gly Gly Asp Gln 1030 1035 1040	3492
CGG CCA GAG GTG GAG GAC CCT GAA GAG TTG TCC CCA Arg Pro Glu Val Glu Asp Pro Glu Glu Leu Ser Pro 1045 1050	3528
GCA CTT GTA GTG TCC AGT TCA CAG AGC TTT GTC ATC Ala Leu Val Val Ser Ser Ser Gln Ser Phe Val Ile 1055 1060	3564
AGT GGT GGA GGC AGC ACT GTT ACA GAA AAC GTA GTG Ser Gly Gly Gly Ser Thr Val Thr Glu Asn Val Val 1065 1070 1075	3600
AAT TCA TAAATGGAA GGAGAAGACT GGGCTAGGGA Asn Ser	3636
GAATGCAGAG AGGTTTCTTG GGGTCCCAGG GATGAGGAAT	3676
CGCCCCAGAC TCCTTTCCTC TGAGGAAGAA GGGATAATAG	3716
ACACATCAAA TGCCCCGAAT TTAGTCACAC CATCTTAAAT	3756
GACAGTGAAT TGACCCATGT TCCCTTTAAA AAAAAAAAAA	3796
AAAAAGCGGC CGC	3809

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101

Claims

1. An inorganic ion receptor modulating compound having the formula:



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wherein Ar_1 is either naphthyl or phenyl optionally substituted with 0 to 5 substituents each independently selected from the group consisting of, lower alkyl, halogen, lower alkoxy, lower thioalkyl, methylene dioxy, lower haloalkyl, lower haloalkoxy, OH, CH_2OH , $CONH_2$, CN, acetoxy, $N(CH_3)_2$, phenyl, phenoxy, benzyl, benzyloxy, α, α -dimethylbenzyl, NO_2 , CHO, $CH_3CH(OH)$, acetyl, ethylene dioxy;

Ar_2 is either naphthyl or phenyl optionally substituted with 0 to 5 substituents each independently selected from the group consisting of, lower alkyl, halogen, lower alkoxy, lower thioalkyl, methylene dioxy, lower haloalkyl, lower haloalkoxy, OH, CH_2OH , $CONH_2$, CN, and acetoxy;

q is 0, 1, 2, or 3; and

R is either H, lower alkyl;

and pharmaceutically salts and complexes thereof;

wherein said compound modulates one or more inorganic ion receptor activities.

2. The compound of claim 1, said Ar_1 phenyl, if present, has 1 to 5 substituents each independently selected from the group consisting of, isopropyl, CH_3O , CF_3 , CH_3S , CF_3O , I, Cl, F, and CH_3 ;



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102

said Ar₂ phenyl, if present, has 1 to 5 substituents each independently selected from the group consisting of, isopropyl, CH₃O, CH₃S, CF₃O, I, Cl, F, CF₃, and CH₃;

said compound is a calcimimetic; and

- 5 said inorganic ion receptor activity is calcium receptor activity.

3. The compound of claim 2, wherein q is 2, said Ar₁ phenyl having 1 to 5 substituents is present, and said Ar₂ phenyl having 1 to 5 substituents is present.

- 10 4. Compound of claim 3, said Ar₂ phenyl is a meta-methoxy phenyl.

5. The compound of claim 2, wherein q is 0 and said Ar₂ naphthyl is present.

- 15 6. The compound of claim 5, wherein said Ar₁ phenyl having 1 to 5 substituents is present.

7. The compound of claim 2, wherein q is 2, said Ar₁ phenyl having 1 to 5 substituents is present, and said Ar₂ naphthyl.

- 20 8. The compound of claim 2, wherein said Ar₁ phenyl, if present, has 1 to 5 substituents each independently selected from the group consisting of, CF₃O, I, Cl, F, and CF₃; and

25 said Ar₂ phenyl, if present, has 1 to 5 substituents each independently selected from the group consisting of, CF₃O, I, Cl, F, CH₃O, and CF₃.

9. The compound of claim 3, wherein said Ar₁ phenyl has 1 to 5 substituents each independently selected from the group consisting of, CF₃O, I, Cl, F, and CF₃; and



103

said Ar₂ phenyl has 1 to 5 substituents each independently selected from the group consisting of, CF₃O, I, Cl, F, CH₃O, and CF₃.

10. The compound of claim 9, wherein said Ar₂ phenyl
5 is a meta-methoxy phenyl.

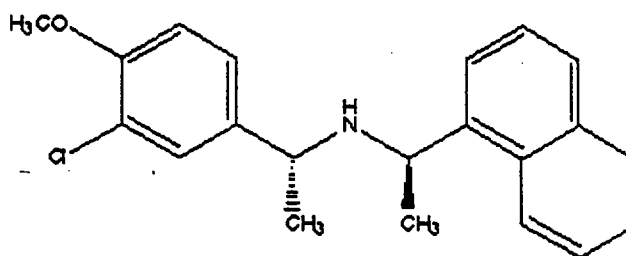
11. The compound of claim 2, wherein R is CH₃.

12. The compound of claim 3, wherein R is CH₃.

13. The compound of claim 4, wherein R is CH₃.

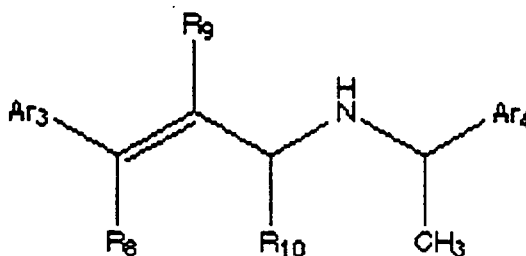
14. The compound of claim 7, wherein R is CH₃.

10 15. The compound of claim 11, wherein said compound has the formula:



or pharmaceutically acceptable salts and complexes thereof.

16. An inorganic ion receptor modulating compound
15 having the formula:



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104

wherein Ar₃ is either naphthyl or phenyl optionally substituted with 0 to 5 substituents each independently selected from the group consisting of, lower alkyl, halogen, lower alkoxy, lower thioalkyl, methylene dioxy, lower haloalkyl, lower haloalkoxy, OH, CH₂OH, CONH₂, CN, acetoxyl, benzyl, benzyloxy, dimethylbenzyl, NO₂, CHO, CH₃CH(OH), N(CH₃)₂, acetyl, ethylene dioxy;

Ar₄ is either naphthyl or phenyl optionally substituted with 0 to 5 substituents each independently selected from the group consisting of, lower alkyl, halogen, lower alkoxy, lower thioalkyl, methylene dioxy, lower haloalkyl, lower haloalkoxy, OH, CH₂OH, CONH₂, CN, and acetoxyl;

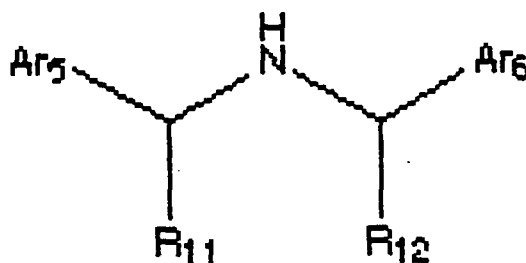
R₈ is either hydrogen or phenyl;

R₉ is either hydrogen or methyl; and

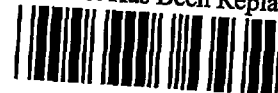
R₁₀ is either hydrogen, methyl, or phenyl;

or pharmaceutically acceptable salts and complexes thereof.

17. An inorganic ion receptor modulating compound having the formula:



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wherein Ar₅ is either naphthyl or phenyl optionally substituted with 0 to 5 substituents each independently selected from the group consisting of, lower alkyl, halogen, lower alkoxy, lower thioalkyl, methylene dioxy, lower haloalkyl, lower haloalkoxy, OH, CH₂OH, CONH₂, CN, acetoxyl, benzyl, benzyloxy, α,α-dimethylbenzyl, NO₂, CHO, CH₃CH(OH), acetyl, ethylene dioxy, -CH=CH-phenyl;

Ar₆ is either naphthyl or phenyl optionally substituted with 0 to 5 substituents each independently



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105

selected from the group consisting of, acetyl, lower alkyl, halogen, lower alkoxy, lower thioalkyl, methylene dioxy, lower haloalkyl, lower haloalkoxy, OH, CH₂OH, CONH₂, CN, carbomethoxy, OCH₂C(O)C₂H₅ and acetoxy;

- 5 R₁₁ is hydrogen or methyl; and
 R₁₂ is hydrogen or methyl.

18. A pharmaceutical composition comprising a compound of any of claims 1-17 and a pharmaceutical acceptable carrier.

- 10 19. A method for treating a patient in need of such treatment comprising the step of administering to said patient a therapeutically effective amount of the pharmaceutical composition of claim 18.

- 15 20. The method of claim 19, wherein said patient is a human, said disease is characterized by either, or both, of: (1) abnormal calcium homeostasis, and (2) an abnormal amount of an extracellular or intracellular messenger whose production can be affected by calcium receptor activity; and said compound is a calcimimetic.

- 20 21. The method of claim 19, wherein said patient is a human and said disease selected from the group consisting of primary and secondary hyperparathyroidism, Paget's disease, hypercalcemia malignancy, osteoporosis, hypertension, and renal osteodystrophy.



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