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A B O G A D O S

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2318/P

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES

E. S. D.

SUPERINDUSTRIA Y COMERCIO
Radicación: 00015555 00000001
Fecha (AMD): 2000-05-17 15:35:17
Trámite: 008 PATENTES D. REGISTRO/ 444 RESPUESTA
Dependencia: 3030 DIVISION DE NUEVAS CREACIONES

Re: Expediente No. 000 [REDACTED]
Solicitud de Patente en nombre de
THE PROCTER & GAMBLE COMPANY.

Yo, JIMENA ESCOBAR URIBE, identificada como aparece al pie de mi firma, domiciliada en la Carrera 11 A No. 94-76, of. 305-306, obrando como apoderada de la sociedad The Procter & Gamble Company, domiciliada en Cincinnati, Ohio, Estados Unidos de América, solicitante de la patente de la referencia, en respuesta al auto No. 619 del 4 de mayo de 2000, relacionado con el concepto técnico 626 del 26 de abril de 2000, me permito manifestar lo siguiente:

Anexo al presente memorial copia certificada de la solicitud de Patente Número 60/122,921 presentada en la Oficina de Patentes de los Estados Unidos de América el día 5 de marzo de 1999, con la debida traducción de lo pertinente, incluyendo el capítulo reivindicatorio.

Espero con esto haber dado cabal cumplimiento a lo solicitado por ese despacho. En caso de que la anterior respuesta no se adecue técnica o jurídicamente al requerimiento, solicito atentamente que ese despacho proceda de conformidad con lo indicado en la Circular Interna No. 001 del 13 de febrero de 1997 expedida por la Delegatura para la Propiedad Industrial de la Superintendencia de Industria y Comercio y que de conformidad con lo indicado en los artículos 3º., inciso 3, 35 inciso 2 y 59 inciso 2 del CCA, al analizar este memorial, se traten todas las cuestiones planteadas con motivo del mismo.

Señor Superintendente,



JIMENA ESCOBAR URIBE
C.C.39.779.452 de usaquén
T.P. 68228
Cód. 5376

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ESTADOS UNIDOS DE AMERICA
A TODOS AQUELLOS A QUIENES EL PRESENTE LLEGUE:

DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS
Oficina de Patentes y Marcas de los Estados Unidos

Febrero 14, 2000

La presente es para certificar que el anexo es una copia fiel de los registros de la Oficina de Patentes y Marcas de los Estados Unidos, de los documentos de la solicitud de patente identificada más adelante, que cumplieron con los requerimientos para concederse una fecha de presentación de acuerdo con 35 USC 111.

Número de Solicitud: 60/122,921

Fecha de Presentación: Marzo 05, 1999

Por la autoridad del
Comisionado de Patentes y Marcas

(fdo) T. WALLACE
Oficial Certificador
Sello oficial de la Oficina de Patentes y Marcas de los
Estados Unidos de América.

REIVINDICACION

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1. Un método para incrementar el volumen óseo, que comprende administrar a un sujeto una cantidad terapéuticamente efectiva de un agonista selectivo FP de ocurrencia no natural y un agonista selectivo EP₁ de ocurrencia no natural.



THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

February 14, 2000

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: 60/122,921

FILING DATE: *March 05, 1999*



**By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS**

T. Wallace
T. WALLACE

Certifying Officer

"Express Mail" mailing label number EJ302200904US

Date of Deposit March 5, 1999

I hereby certify that this paper/fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231

James C. Kellerman P43.708
Attorney mailing application Reg No

Signature of Attorney mailing application

PTO/SB/16 (2-98)

Approved for use through 01/31/2001. OMB 0651-0037
Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL PATENT APPLICATION under 37 CFR §1.53(c).

Docket Number		7464P		Type a plus sign (+) inside this box ⇒		+	
INVENTOR(s) / APPLICANT(s)							
FAMILY NAME OR SURNAME		GIVEN NAME (first name)		MIDDLE INITIAL (if any)		RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)	
Hartke Lundy deLong		James Mark Mitchell		R. W. A.		West Chester, OH Cincinnati, OH West Chester, OH	
TITLE OF THE INVENTION (280 characters max)							
Method Of Increasing Bone Volume Using Non-Naturally-Occurring FP Selective Agonists And EP ₁ Selective Agonists							
CORRESPONDENCE ADDRESS							
James C. Kellerman The Procter & Gamble Company Health Care Research Center 8700 Mason Montgomery Road Mason							
STATE		OH		ZIP CODE		45040-8006	
COUNTRY		USA					
ENCLOSED APPLICATION PARTS (check all that apply)							
☒ Specification Number of Pages: 18 + Abstract ☐ Small Entity Statement							
☐ Drawing(s) Number of Sheets: ☐ Other (specify) ☐							
METHOD OF PAYMENT (check one)							
☐ A check or money order is enclosed to cover the Provisional filing fees						PROVISIONAL FILING FEE AMOUNT(S)	
☒ The Commissioner is hereby authorized to charge filing fees specified under 37 CFR §1.19(f) and credit Deposit Account Number: 16-2480						\$150.00	

The invention was made by an agency of the United States Government or under a contract with an agency of United States Government.

☒ No

☐ Yes, the name of the U. S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE

James C. Kellerman

Date March 5, 1999

TYPED or PRINTED NAME James C. Kellerman

REGISTRATION NO. P43.708

(if appropriate)

☐ Additional inventors are being named on separately numbered sheets attached hereto

PROVISIONAL PATENT APPLICATION FILING ONLY

Burden Hour Statement: This form is estimated to take .2 hours to complete. Time will vary depending upon the needs of the individual cases. Any comments on the amount of time you are required to complete this form should be sent to the Office of Assistance Quality and Enhancement Division, Patent and Trademark Office, Washington, DC 20231, and to the Office of Information and Regulatory Affairs, Office of Management and Budget (Project 0651-0037), Washington, DC 20503. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Patent Application; Assistant Commissioner for Patents, Washington, D.C. 20231.

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P&G Case No. 7464P

**METHOD OF INCREASING BONE VOLUME USING
NON-NATURALLY-OCCURRING FP SELECTIVE
AGONISTS AND EP₁ SELECTIVE AGONISTS**

James Richard Hartke

Mark Walden Lundy

Mitchell Anthony deLong

TECHNICAL FIELD

The present invention relates to novel methods of increasing bone volume comprising the administration of a non-naturally-occurring selective FP agonist and a non-naturally-occurring selective EP₁ agonist to a subject in need of such treatment. This invention further relates to a method of treating or preventing bone disorders comprising the administration of a non-naturally-occurring selective FP agonist and a non-naturally-occurring selective EP₁ agonist to a subject in need of such treatment.

BACKGROUND OF THE INVENTION

In osteoporotics an imbalance in the bone remodeling process develops in which bone is resorbed at a rate faster than it is being made. Although this imbalance occurs to some extent in most individuals, both male and female, as they age, it is much more severe in osteoporotics, particularly those who develop the post menopausal form of the condition. Accelerated bone loss may also result from drug administration, such as corticosteroids; prolonged bedrest; disuse of a limb; and microgravity. A consequence of this loss of bone is the complete removal of trabeculae and a deterioration of bone architecture such that the remaining bone is disproportionately decreased in strength.

It is thought that to completely return the bone to normal strength, new trabeculae should be formed to restore architecture and increase bone mass. It is further thought that when the restoration of normal architecture is associated not only with an increase in the strength, but also a return to normal stiffness and shock absorbing capability, the bone is less likely to fracture. Subjects suffering from other bone disorders such as rheumatoid arthritis, periodontal disease, and fractures may also benefit from treatments that restore bone mass and normal architecture to bone.

There have been many attempts to treat bone disorders with a variety of pharmacologic agents with the goal being to either slow further bone loss or to

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produce a net gain in bone mass. There are antiresorptive agents, such as bisphosphonates, which slow further bone loss, and there are anabolic agents, such as PTH, fluoride, and prostaglandins, which build bone. But, none of these agents build bone that is substantially similar, i.e. structurally or architecturally, to the type of bone lost.

PTH and prostaglandins, especially the non-selective prostaglandins of the E series (e.g. PGE₂), are known to be potent stimulators of bone resorption and formation. The acceleration in turnover seen with these known bone anabolic agents may be detrimental to an already osteoporotic skeleton since the increased resorption may cause perforation and loss of trabeculae, or may weaken the existing trabecular structure. In addition, increased resorption may occur in cortical bone. These effects may in turn lead to increased fracture incidence at some sites.

Prostaglandins, in addition, have several drawbacks which limit their desirability for systemic administration. For example, although prostaglandins are characterized by activity at a particular prostaglandin receptor, they often bind to and stimulate other prostaglandin receptors. Thus, systemic administration of prostaglandins is known to cause side effects such as inflammation, as well as smooth muscle contraction, bronchoconstriction, and vasoconstriction. Systemic administration of non-selective prostaglandin analogs can likewise cause side effects.

Thus, there is a continuing need to develop methods of replacing bone that result in bone that is substantially similar, structurally and architecturally, to the type of bone lost with a bone-specific agent.

SUMMARY OF THE INVENTION

The present invention relates to novel methods of increasing bone volume comprising the administration of a non-naturally-occurring FP selective agonist and a non-naturally-occurring EP₁ selective agonist to a subject in need of such treatment. This invention further relates to a method of treating or preventing bone disorders comprising the administration of a non-naturally-occurring FP selective agonist and a non-naturally-occurring EP₁ selective agonist to a subject in need of such treatment.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to methods of increasing bone volume, methods of increasing trabecular number, and methods of treating bone disorders

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by administering to a subject a safe and effective amount of a non-naturally-occurring selective FP agonist and a non-naturally-occurring selective EP₁ agonist.

Definitions and Usage of Terms

"Alkyl" is a saturated or unsaturated hydrocarbon chain having 1 to 18 carbon atoms, preferably 1 to 12, more preferably 1 to 6, more preferably still 1 to 4 carbon atoms. Alkyl chains may be straight or branched. Preferred branched alkyl have one or two branches, preferably one branch. Preferred alkyl are saturated. Unsaturated alkyl have one or more double bonds and/or one or more triple bonds. Preferred unsaturated alkyl have one or two double bonds or one triple bond, more preferably one double bond. Alkyl chains may be unsubstituted or substituted with from 1 to 4 substituents. Preferred substituted alkyl are mono-, di-, or trisubstituted. The substituents may be lower alkyl, halo, hydroxy, aryloxy (e.g., phenoxy), acyloxy (e.g., acetoxy), carboxy, monocyclic aromatic ring (e.g., phenyl), monocyclic heteroaromatic ring, monocyclic carbocyclic aliphatic ring, monocyclic heterocyclic aliphatic ring, and amino.

"Lower alkyl" is an alkyl chain comprised of 1 to 6, preferably 1 to 3 carbon atoms.

"Aromatic ring" is an aromatic hydrocarbon ring. Aromatic rings are monocyclic or fused bicyclic ring systems. Monocyclic aromatic rings contain from about 5 to about 10 carbon atoms, preferably from 5 to 7 carbon atoms, and most preferably from 5 to 6 carbon atoms in the ring. Bicyclic aromatic rings contain from 8 to 12 carbon atoms, preferably 9 or 10 carbon atoms in the ring system. Bicyclic aromatic rings include ring systems wherein one ring in the system is aromatic. Preferred bicyclic aromatic rings are ring systems wherein both rings in the system are aromatic. Aromatic rings may be unsubstituted or substituted with from 1 to 4 substituents on the ring. The substituents may be halo, cyano, lower alkyl, heteroalkyl, haloalkyl, or any combination thereof. Preferred substituents include halo and haloalkyl. Preferred aromatic rings include naphthyl and phenyl. The most preferred aromatic ring is phenyl.

"Bone disorder" means the need for bone repair or replacement. Conditions in which the need for bone repair or replacement may arise include: osteoporosis (including post menopausal osteoporosis, male and female senile osteoporosis and corticosteroid induced osteoporosis), rheumatoid arthritis, osteomalacia, multiple myeloma and other forms of cancer, prolonged bed rest, chronic disuse of a limb, anorexia, microgravity, exogenous and endogenous gonadal insufficiency, bone fracture, non-union, defect, prosthesis implantation and the like.

"Bone turnover rate" is the amount of bone resorption and formation per unit time measured or estimated using incorporation of fluorescent labels into bone, fluorescent and bright field microscopy, and histomorphometric techniques or by measurement of bone metabolism markers. For example, a subject may resorb and replace (turn over) approximately 3% of its skeleton over a 3 month period. A further description of histomorphometric techniques can be found in Bone Histomorphometry, 1994, by Eriksen et al., Raven Press.

"Bone volume" is the percentage of the bone occupied by a mineralized matrix. Measurement or estimation of the mineralized matrix volume can be accomplished using histomorphometry, computed tomography, or magnetic resonance imaging. Two dimensional measurements may be used to estimate the three dimensional volume. A further description of histomorphometric techniques can be found in Bone Histomorphometry, 1994, by Eriksen et al., Raven Press.

"Carbocyclic aliphatic ring" is a saturated or unsaturated hydrocarbon ring. Carbocyclic aliphatic rings are not aromatic. Carbocyclic aliphatic rings are monocyclic. Carbocyclic aliphatic rings contain from about 4 to about 10 carbon atoms, preferably from 4 to 7 carbon atoms, and most preferably from 5 to 6 carbon atoms in the ring. Carbocyclic aliphatic rings may be unsubstituted or substituted with from 1 to 4 substituents on the ring. The substituents may be halo, cyano, alkyl, heteroalkyl, haloalkyl, phenyl, phenoxy or any combination thereof.

"Excitatory prostaglandin receptor" means prostanoid receptors which cause contraction of smooth muscle or release of internal calcium stores. Such receptors include but are not limited to EP₁, EP₃, FP, TP₁ and TP₂.

"EP" is an abbreviation for E prostanoid. "EP₁" is an abbreviation for E prostanoid subtype 1.

"EP₁ agonist" is a compound with affinity for the EP₁ receptor that results in measurable biological activity (including but not limited to an elevation in intracellular calcium or the contraction of smooth muscle) in cells, tissues, or organisms which contain the EP₁ receptor. Whole cell, tissue, and organism assays which demonstrate EP₁ activity of compounds are well known in the art. One particularly useful assay is a modified R-SAT™ Assay. The R-SAT™ Assay is described by Brann, et al. in J. Biomole. Screen, Vol. 1, Number 1, 1996. The R-SAT™ Assay may be modified by transfecting the cDNA sequence for the human EP₁ receptor (described in WO 94/28125) as the appropriate nucleic acid sequence. Further modifications to the R-SAT™ Assay may be made to optimize the repeatability of the assay results. All such modifications can readily be carried out by one of ordinary skill in the art.

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"EP₁ receptor" refers to known human EP₁ receptors, their splice variants, and undescribed receptors that preferentially bind PGE₁. A human EP₁ receptor is disclosed in PCT Publication WO 94/28125.

"FP" is an abbreviation for F prostanoid.

"FP agonist" is a compound with affinity for the FP receptor that results in measurable biological activity (including but not limited to an elevation in intracellular calcium or the contraction of smooth muscle) in cells, tissues, or organisms which contain the FP receptor. Whole cell, tissue, and organism assays which demonstrate FP activity of compounds are well known in the art. One particularly useful assay is the R-SAT™ Assay described by Brann, et al. in J. Biomole. Screen., Vol. 1, Number 1, 1996.

"FP receptor" refers to known human FP receptors, their splice variants, and undescribed receptors that preferentially bind PGF_{2α}. A human FP receptor is disclosed in PCT Publication U.S. Patent No. 5,840,847.

"Halo" or "halogen" is fluoro, chloro, bromo or iodo. Preferred halo are fluoro, chloro and bromo; more preferred are chloro and fluoro, especially fluoro.

"Haloalkyl" is a straight, branched, or cyclic hydrocarbon substituted with one or more halo substituents. Preferred haloalkyl are C₁-C₁₂; more preferred are C₁-C₆; more preferred still are C₁-C₃. Preferred halo substituents are fluoro and chloro. The most preferred haloalkyl is trifluoromethyl.

"Heteroalkyl" is a saturated or unsaturated chain containing carbon and at least one heteroatom, wherein no two heteroatoms are adjacent. Heteroalkyl chains contain from 1 to 18 member atoms (carbon and heteroatoms) in the chain, preferably 1 to 12, more preferably 1 to 6, more preferably still 1 to 4. Heteroalkyl chains may be straight or branched. Preferred branched heteroalkyl have one or two branches, preferably one branch. Preferred heteroalkyl are saturated. Unsaturated heteroalkyl have one or more double bonds and/or one or more triple bonds. Preferred unsaturated heteroalkyl have one or two double bonds or one triple bond, more preferably one double bond. Heteroalkyl chains may be unsubstituted or substituted with from 1 to 4 substituents. Preferred substituted heteroalkyl are mono-, di-, or trisubstituted. The substituents may be lower alkyl, halo, hydroxy, aryloxy (e.g., phenoxy), acyloxy (e.g., acetoxy), carboxy, monocyclic aromatic ring (e.g., phenyl), monocyclic heteroaromatic ring, monocyclic carbocyclic aliphatic ring, monocyclic heterocyclic aliphatic ring, and amino.

"Lower heteroalkyl" is a heteroalkyl chain comprised of 1 to 6, preferably 1 to 3 member atoms.

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"Heteroaromatic ring" is an aromatic ring containing carbon and from 1 to about 4 heteroatoms in the ring. Heteroaromatic rings are monocyclic or fused bicyclic ring systems. Monocyclic heteroaromatic rings contain from about 5 to about 10 member atoms (carbon and heteroatoms), preferably from 5 to 7, and most preferably from 5 to 6 in the ring. Bicyclic heteroaromatic rings include ring systems wherein only one ring in the system is aromatic. Preferred bicyclic heteroaromatic rings are ring systems wherein both rings in the system are aromatic. Bicyclic heteroaromatic rings contain from 8 to 12 member atoms, preferably 9 or 10 in the ring. Heteroaromatic rings may be unsubstituted or substituted with from 1 to 4 substituents on the ring. The substituents may be halo, cyano, alkyl, heteroalkyl, haloalkyl, phenyl, phenoxy or any combination thereof. Preferred substituents include halo, haloalkyl, and phenyl.

"Heteroatom" is a nitrogen, sulfur, or oxygen atom. Groups containing more than one heteroatom may contain different heteroatoms.

"Heterocyclic aliphatic ring" is a saturated or unsaturated ring containing carbon and from 1 to about 4 heteroatoms in the ring, wherein no two heteroatoms are adjacent in the ring and no carbon in the ring that has a heteroatom attached to it also has a hydroxyl, amino, or thiol group attached to it. Heterocyclic aliphatic rings are not aromatic. Heterocyclic aliphatic rings are monocyclic. Heterocyclic aliphatic rings contain from about 4 to about 10 member atoms (carbon and heteroatoms), preferably from 4 to 7 member atoms, and most preferably from 5 to 6 member atoms in the ring. Heterocyclic aliphatic rings may be unsubstituted or substituted with from 1 to 4 substituents on the ring. The substituents may be halo, cyano, alkyl, heteroalkyl, haloalkyl, phenyl, phenoxy or any combination thereof. Preferred substituents include halo and haloalkyl.

"Measurable" means the biologic effect is both reproducible and significantly different from the baseline variability of the assay.

"Non-naturally-occurring" means an agent that is not biologically derived in mammals.

"Prostaglandin analog" is a non-naturally-occurring compound which is structurally similar to a prostaglandin.

"Prostaglandin receptor" or "prostanoid receptor" is a naturally-occurring protein that binds prostaglandins, which when bound alters the function of a cell. Prostaglandin receptors may be characterized as either excitatory or relaxant. Such receptors include but are not limited to EP₁, EP₂, EP₃, EP₄, DP, FP, IP, TP₁ and TP₂. These receptors are further discussed by Coleman et al., in Pharmacological Reviews, 1994, Volume 6, No. 2, pages 205 - 229.

"Selective" means having an activation preference for a specific receptor over other receptors which can be quantified based upon whole cell, tissue, or organism assays which demonstrate receptor activity, such as the R-SAT™ Assay disclosed above. A compound's selectivity is determined from a comparison of its EC₅₀ (or ED₅₀ if using an organism assay) at the relevant receptors. For example, a compound having an EC₅₀ of 8nM at the FP receptor and an EC₅₀ of 80 nM at the EP₁ receptor has a selectivity ratio for the FP receptor over the EP₁ receptor of 1:10. Additionally, a compound having an EC₅₀ of 8nM at the EP₁ receptor and an EC₅₀ of 80 nM at the EP₂ receptor has a selectivity ratio for the EP₁ receptor over the EP₂ receptor of 1:10.

"Subject" is a living vertebrate animal such as a mammal (especially human) in need of treatment.

"Trabecular number" is to the number of individual trabeculae of bone per unit volume of cancellous bone measured or estimated from a two dimensional representation or a three dimensional specimen using histomorphometry, computed tomography, or magnetic resonance imaging.

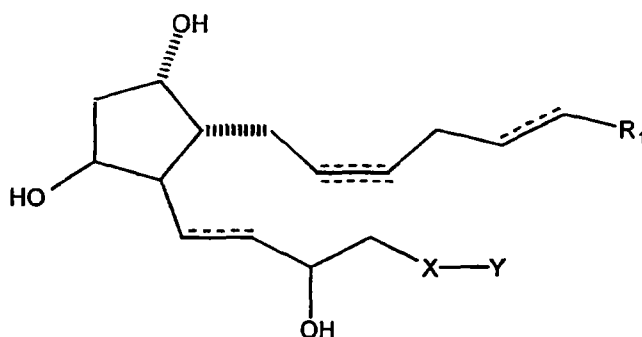
Compounds

The methods of the present invention comprise administering a non-naturally-occurring selective FP agonist and a non-naturally-occurring selective EP₁ agonist. Thus, compounds useful in the present invention are non-naturally-occurring selective FP agonists and non-naturally-occurring selective EP₁ agonists.

Non-naturally-occurring selective FP agonists

Preferred non-naturally-occurring FP agonists are selective for the FP receptor over other excitatory prostaglandin receptors in a ratio of at least about 1:10, more preferably at least about 1:20, and most preferably at least about 1:50. Still more preferred non-naturally-occurring FP agonists are selective for FP receptors over all other prostanoid receptors in a ratio of at least about 1:10, more preferably at least about 1:20, and most preferably at least about 1:50.

Particularly useful non-naturally-occurring FP selective agonists are prostaglandin analogs. Examples of such compounds are prostaglandin analogs having the following general structure:

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Formula I

In the above structure, R_1 is CO_2H , $\text{C}(\text{O})\text{NHOH}$, CO_2R_2 , CH_2OH , $\text{S}(\text{O})_2\text{R}_2$, $\text{C}(\text{O})\text{NHR}_2$, $\text{C}(\text{O})\text{NHS}(\text{O})_2\text{R}_2$, or tetrazole; characterized in that R_2 is alkyl, heteroalkyl, carbocyclic aliphatic ring, heterocyclic aliphatic ring, aromatic ring, or heteroaromatic ring

In the above structure, X is $(\text{CH}_2)_n$, where n is 0 to 3, NH , S , or O .

In the above structure, Y is a cycloalkyl or aromatic moiety, either substituted or unsubstituted.

The above structure includes optical isomers, diastereomers, enantiomers of the above structure or pharmaceutically-acceptable salts, or bio-hydrolyzable amides, esters, or imides thereof. Preferred stereochemistry mimics that of naturally occurring $\text{PGF}_{2\alpha}$.

Prostaglandin analogs of Formula I above include: cloprostenol (Estrumate®), fluprostenol (Equimate®), tiaprost, alfaprostol, delprostenate, froxiprost, latanoprost, 13,14-dihydro-16-((3-trifluoromethyl)phenoxy)-16-tetranor prostaglandin $\text{F}_{1\alpha}$, 17-((3-trifluoromethyl)phenyl)-17-trinor-prostaglandin $\text{F}_{2\alpha}$, 13,14-dihydro-18-thienyl-18-dinor prostaglandin $\text{F}_{1\alpha}$ and their analogs.

Other non-naturally-occurring FP selective agonists that are prostaglandin analogs include 9- α , 11- α , 15- α -trihydroxy-16-(3-chlorophenoxy)- ω -tetranor-prosta-4-cis-13-trans-dienoic acid and its analogs. Additional prostaglandin analogs are also disclosed in CRC Handbook of Eicosanoids: Prostaglandins and Related Lipids, Volume I, Chemical and Biochemical Aspects, Part B. Ed. by Anthony L. Willis, CRC Press (Boca Raton, 1987) Table Four pp. 80-97 (incorporated herein by reference), and references therein.

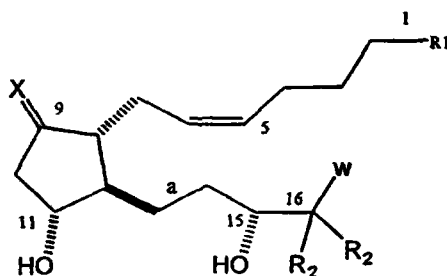
Non-naturally-occurring EP_1 selective agonists

Preferred non-naturally-occurring EP_1 agonists are selective for the EP_1 receptor over other excitatory prostaglandin receptors in a ratio of at least about

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1:10, more preferably at least about 1:20, and most preferably at least about 1:50. Still more preferred non-naturally-occurring EP₁ agonists are selective for EP₁ receptors over all other prostanoid receptors in a ratio of at least about 1:10, more preferably at least about 1:20, and most preferably at least about 1:50.

Particularly useful non-naturally-occurring EP₁ selective agonists are prostaglandin analogs. Examples of such compounds are prostaglandin analogs having the following general structure:



In the above structure, R₁ is CO₂H, C(O)NHOH, CO₂R₃, CH₂OH, S(O)₂R₃, or C(O)NHR₃; characterized in that each R₃ is independently alkyl, heteroalkyl, carbocyclic aliphatic ring, heterocyclic aliphatic ring, aromatic ring, or heteroaromatic ring. Preferred R₁ is CO₂H.

In the above structure, X is CH₂, O, or N-OR₄; wherein R₄ is hydrogen or lower alkyl. Preferred X is CH₂ and N-OR₄. Most preferred X is CH₂.

In the above structure, a is single bond, trans double bond, or triple bond. Preferred a is a trans double bond.

In the above structure, each R₂ is independently hydrogen or lower alkyl. Preferred R₂ is lower alkyl. Most preferred R₂ is methyl.

In the above structure, W is: (a) [C(R₅)(R₅)]_m-Y-[C(R₅)(R₅)]_n-Z; wherein each R₅ is independently hydrogen, lower alkyl, alkoxy, or halo; m is an integer from 0 to about 1, n is an integer from 0 to about 1; Y is, C(R₅)(R₅), O, NH, S or a covalent bond; and Z is phenyl, thienyl, or furanyl, said phenyl, thienyl, or furanyl being unsubstituted or substituted with 1 or 2 halogens; or (b) [C(R₅)(R₅)]_p-U-[C(R₅)(R₅)]_q; wherein R₅ is as defined above; p is an integer from 0 to about 3, q is an integer from about 1 to about 3, and p + q is from about 1 to about 4; and U is C(R₅)(R₅), O, NH, or S. Preferred Z is unsubstituted. Preferred substituted Z is substituted with fluoro.

The above structure includes optical isomers, diastereomers, enantiomers of the above structure or pharmaceutically-acceptable salts, or bio-hydrolyzable

amides, esters, or imides thereof. Preferred stereochemistry mimics that of naturally occurring PGE₂.

Prostaglandin analogs of the above structure include: 17-phenyl-17-trinor PGE₂, 9-methylene-9-deoxy PGE₂, and 9-methylene-9-deoxy-16,16-dimethyl PGE₂.

Methods of Use

Administration of the compounds described above results in the following desired effects: (1) an increase in bone volume, (2) an increase in trabecular number, (3) an increase in bone mass without an increase in the bone turnover rate, and/or (4) an increase in formation at the endosteal surface without removing bone from the existing cortex. Additionally, the quality of bone formed by the administration of these compounds is superior to that formed by the administration of other anabolic agents, including non-selective prostaglandins of the E series. Bone quality refers to the combination of bone matrix (inorganic and organic), bone mass or volume, and bone architecture which impart overall strength and fracture resistance to bone. Accordingly, these compounds are further useful in the treatment and prevention of a variety of bone disorders.

The present invention is directed to methods wherein a non-naturally-occurring FP selective agonist and a non-naturally occurring EP₁ selective agonist are administered in combination. "In combination" means that the two agents are dosed in such a manner that the expected plasma levels of each agent are present at the same time. It is not necessary that both agents be administered at the same time or by the same route.

When both a non-naturally-occurring FP selective agonist and a non-naturally occurring EP₁ selective agonist are administered in combination, the dosage of each agent required to achieve one or more of the desired effects stated above is reduced. The dosage of each required to achieve the desired effects is reduced because it is believed that both selective FP agonists and selective EP₁ agonists primarily activate the osteoblastic protein kinase C/ intracellular calcium pathways ("PKC/Ca⁺⁺ pathways"). "Activate a subject's osteoblastic protein kinase C /intracellular calcium pathways" refers to the activation of the Gq family of proteins associated with seven transmembrane receptor proteins which initiates the intracellular messenger cascade containing both protein kinase C (PKC) and Ca⁺⁺. "Primarily" means the agent preferentially activates the osteoblastic PKC/Ca⁺⁺ pathways over the cAMP pathway. Preferential activation of the PKC/Ca⁺⁺ pathways over the cAMP pathway can be measured using a variety of assays. For example, intracellular Ca⁺⁺ concentration can be measured by the use of the

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calcium indicator Fura-2 assay described in "The Molecular Biology of the Cell", edited by Alberts et al., Garland Publishing, 1994, p. 183, and intracellular cAMP can be measured by the assay described in The Principles of Bone Biology edited by J Bilezikian et al., Academic Press, 1996, p 1205. To provide a direct comparison of these two pathways, a ratio of activation of 1 is defined as 100% of the maximum activation of the PKC/Ca⁺⁺ pathways of PGE₂ divided by 100% of the activation of the cAMP pathway by PGE₂. The concentrations of PGE₂ required to reach the maximum activation may differ by up to 2 orders of magnitude. Preferential activation would then represent an increase in this ratio above about 1, preferably above about 2, more preferably above about 3.5, and most preferably above about 5, where the pathway activator could either increase the PKC/Ca⁺⁺ pathway activation (for example to 125% of PGE₂) or decrease the cAMP pathway activation (for example to 75% of PGE₂).

Activation of non-target cells often results in undesirable effects. Because the dosage of each agent is reduced, non-target cells will be exposed to a lower level of each agent versus the level achieved when either agent is given alone. Thus, non-target cells which do not have both an FP receptor and an EP₁ receptor present will be activated to a lesser extent than if either agent is given alone. Administration of both a non-naturally-occurring FP selective agonist and a non-naturally-occurring EP₁ selective agonist (each at a reduced dosage), therefore, enables the practitioner to achieve one or more of the desired effects stated above and to avoid or to minimize undesirable effects.

Dosages

Once the decision regarding which particular non-naturally-occurring FP selective agonist to administer and which non-naturally-occurring EP₁ selective agonist to administer has been made, the appropriate dosage of each must be determined. At a minimum, the appropriate dosage of each agent may be determined from one efficacious dose of each agent. One-half of the efficacious dose of each agent may be administered to produce one or more of the desired effects while avoiding or minimizing undesirable effects.

A more preferred method of dosing each agent is to give the ED₅₀ of each agent. This requires characterization of the dose response curve. Characterization of the dose response curve can be obtained from the literature or from routine pharmacodynamic experimentation including experimentation in animal models or in human clinical trials (See Goodman & Gilman's The Pharmacological Basis Of Therapeutics, Chapter 3 (9th Ed. 1996), incorporated herein by reference, for a

discussion of dose response curve characterization). Such models include, but are not limited to, the intact and ovariectomized rat models of osteoporosis, the ferret, canine, and non human primate models of osteoporosis, as well as disuse models of osteoporosis. The ED_{50} can be readily determined from the dose response curve.

A still more preferred method of dosing each agent is to dose the safer of the two agents above its ED_{50} and the other below its ED_{50} . The "safer of the two agents" is the one less likely to cause serious undesirable effects. This determination is based upon the therapeutic index and the nature of the undesirable effect. "Therapeutic index" is an indication of how selective a drug is in producing its desired effects relative to its toxicity. For a discussion of therapeutic index and methods for determining how it is calculated, see Goodman & Gilman's The Pharmacological Basis Of Therapeutics, Chapter 3 (9th Ed. 1996). In a preferred embodiment, the safer of the two agents is dosed above its ED_{60} and below its ED_{90} .

The discussion immediately above presumes that the practitioner desires a maximally efficacious therapy. When less than a maximally efficacious therapy is desirable, the dose of each agent can be adjusted accordingly. For example, the ED_{25} of each agent may be administered. Additionally, it may be desirable, based upon safety, to dose one agent at its ED_{40} and the other agent at its ED_{20} .

The dosage range for systemic administration of the non-naturally-occurring FP selective agonists of the present invention is from about 0.005 to about 500 $\mu\text{g/kg}$ body weight per day, preferably from about 0.01 to about 100 $\mu\text{g/kg}$ body weight per day, most preferably from about 0.1 to about 50 $\mu\text{g/kg}$ body weight per day. Plasma levels are expected to be in the range of about 0.01 to about 200 ng/ml, more preferably from about 0.05 to 100 ng/ml, and most preferably from about 0.1 to 50 ng/ml.

The dosage range for systemic administration of the non-naturally-occurring EP_1 selective agonists of the present invention is from about 0.05 to about 5000 $\mu\text{g/kg}$ body weight per day, preferably from about 0.1 to about 1000 $\mu\text{g/kg}$ per body weight per day, most preferably from about 0.1 to about 100 $\mu\text{g/kg}$ body weight per day. Plasma levels are expected to be in the range of about 0.01 to about 200 ng/ml, more preferably from about 0.05 to 100 ng/ml, and most preferably from about 0.1 to 50 ng/ml.

While the dosages for both the non-naturally-occurring selective FP and EP_1 agonists are based upon a daily administration rate, weekly or monthly accumulated dosages may also be used to calculate the clinical requirements. The

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non-naturally-occurring selective FP and EP₁ agonists of the present invention may be administered, based on a weekly dosage, more frequently than once daily. The non-naturally-occurring selective FP and EP₁ agonists of the present invention may also be administered, based on a weekly dosage, less frequently than once daily. Hence, the weekly dosage may be divided into 3, 4, 5, 6, or 7 daily dosages, preferably 5, 6, or 7 daily dosages.

Dosages may be varied based on the patient being treated, the condition being treated, the severity of the condition being treated, and the route of administration to achieve the desired effect.

It is expected that prolonged delivery (also referred to as "prolonged administration") of both the non-naturally-occurring FP selective agonist and the non-naturally-occurring EP₁ selective agonist results in improved dose separation between side effects and the desired bone effect. As used herein, "prolonged delivery" or "prolonged administration" means that the total daily dosage is delivered into the subject's circulation over a period of at least about 6 hours and up to 24 hours. Preferred prolonged delivery periods are for at least about 12 hours and up to 24 hours. Examples of prolonged delivery include administration of either the non-naturally-occurring FP selective agonist or the non-naturally-occurring EP₁ selective agonist via a transdermal patch or a subcutaneous pump that delivers the total daily dosage over a twenty-four hour period.

It is believed that the flattening of the plasma concentration curve resulting from prolonged delivery mitigates side effects while maintaining bone efficacy. It is further believed that the administration of agents with extended half-lives will likewise result in a flattening of the plasma concentration curve without prolonging the administration.

The preferred routes of administration for increasing bone volume and treating bone disorders are transdermal and subcutaneous, e.g. injection or pellet. Other preferred routes of administration include oral, sublingual, and intranasal.

The following non-limiting examples serve to further illustrate the use of the agents of the present invention.

Example I

The FP agonist, fluprostenol, and the EP₁ agonist 17-phenyl-17-trinor PGE₂ are administered to a 65 year old woman who has decreased bone mass and has been diagnosed with osteoporosis by her physician. She is treated daily with a transdermal patch that delivers 5 µg/kg fluprostenol over a 24 hour period and a

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second transdermal patch that delivers 50 ug/kg 17-phenyl-17-trinor PGE₂ over a 24 hour period. This treatment is continued for 24 months, at which time, vertebral bone mass is substantially increased compared to her vertebral bone mass at the onset of therapy as measured by dual energy X-ray absorptiometry (DXA).

Example II

The FP agonist, fluprostenol, and the EP1 agonist 17-phenyl-17-trinor PGE₂ are administered to a 63 year old woman who has decreased bone mass and has been diagnosed with osteoporosis by her physician. She is treated with an implantable subcutaneous pump that delivers 7.5 µg/kg fluprostenol and a transdermal patch that delivers 25 ug/kg 17-phenyl-17-trinor PGE₂ over a 24 hour period. This treatment is continued for 12 months, at which time, vertebral bone mass is substantially increased compared to her vertebral bone mass at the onset of therapy as measured by dual energy X-ray absorptiometry (DXA).

Example III

The FP agonist, fluprostenol, and the EP₁ agonist 17-phenyl-17-trinor PGE₂ are administered to a 65 year old woman who has decreased bone mass and has been diagnosed with osteoporosis by her physician. She is treated daily with a single transdermal patch that delivers 5 µg/kg fluprostenol and 50 ug/kg 17-phenyl-17-trinor PGE₂ over a 24 hour period. This treatment is continued for 24 months, at which time, vertebral bone mass is substantially increased compared to her vertebral bone mass at the onset of therapy as measured by dual energy X-ray absorptiometry (DXA).

Pharmaceutical Formulations

Pharmaceutical formulations of the present invention comprise a safe and effective amount of the non-naturally-occurring FP selective agonist or the non-naturally-occurring EP₁ selective agonist and a pharmaceutically acceptable carrier.

The phrase "safe and effective amount", as used herein means an amount of a compound or composition high enough to significantly positively modify the symptoms and/or condition to be treated, but low enough to avoid serious side effects (at a reasonable benefit/risk ratio), within the scope of sound medical judgment. The safe and effective amount of an agent for use in the method of the invention herein will vary with the particular condition being treated, the age and physical condition of the patient being treated, the severity of the condition, the duration of the treatment, the nature of concurrent therapy, the particular agent

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being employed, the particular pharmaceutically-acceptable excipients utilized, and like factors within the knowledge and expertise of the attending physician.

In addition to the compound, the compositions of the present invention contain a pharmaceutically-acceptable carrier. The term "pharmaceutically-acceptable carrier", as used herein, means one or more compatible solid or liquid filler diluents or encapsulating substances which are suitable for administration to a subject. The term "compatible", as used herein, means that the components of the composition are capable of being commingled with the compound, and with each other, in a manner such that there is no interaction which would substantially reduce the pharmaceutical efficacy of the composition under ordinary use situations. Pharmaceutically-acceptable carriers must, of course, be of sufficiently high purity and sufficiently low toxicity to render them suitable for administration to the subject being treated.

Some examples of substances which can serve as pharmaceutically-acceptable carriers or components thereof are sugars, such as lactose, glucose and sucrose; starches, such as cornstarch and potato starch; cellulose and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose, cellulose acetate; powdered tragacanth; malt; gelatin; talc; solid lubricants, such as stearic acid, magnesium stearate; calcium sulfate; vegetable oils, such as peanut oil, cottonseed oil, sesame oil, olive oil, corn oil and oil of theobroma; polyols such as propylene glycol, glycerin, sorbitol, mannitol, and polyethylene glycol; alginic acid; emulsifiers, such as the Tweens®; wetting agents such as sodium lauryl sulfate; coloring agents; flavoring agents, excipients; tableting agents; stabilizers; antioxidants; preservatives; pyrogen-free water; isotonic saline; and phosphate buffer solutions.

The choice of a pharmaceutically-acceptable carrier to be used in conjunction with a compound is basically determined by the way the compound is to be administered. The non-naturally-occurring FP selective agonists and the non-naturally-occurring EP₁ selective agonists of the present invention may be administered systemically, including transdermally, orally and/or parenterally, including subcutaneous or intravenous injection, and/or intranasally.

A preferred method of administering non-naturally-occurring FP selective agonists and non-naturally-occurring EP₁ selective agonists is via transdermal delivery. Preferred transdermal dosage forms include transdermal patches, creams, ointments, gels and the like. Another preferred method of administering non-naturally-occurring FP selective agonists and non-naturally-occurring EP₁ selective agonists is via subcutaneous injection in a unit dosage form. Preferred

unit dosage forms for injection include sterile solutions of water, physiological saline, or mixtures thereof. The pH of said solutions should be adjusted to about 7.4. Yet another preferred method of administering non-naturally-occurring FP selective agonists and non-naturally-occurring EP₁ selective agonists is via subcutaneous implant or other subcutaneous slow release dosage forms.

Other preferred dose forms include nasal, rectal, sublingual, and oral. Suitable carriers for injection or surgical implants include hydrogels, controlled- or sustained-release devices, polylactic acid, and collagen matrices. Implant devices may be coated with the non-naturally-occurring FP selective agonist or the non-naturally-occurring EP₁ selective agonist. The non-naturally-occurring FP selective agonist and the non-naturally-occurring prostaglandin EP₁ selective agonist may be dissolved in a buffer and may be mixed with a collagen gel which is then coated onto the porous end of the implant device.

Preferred oral forms include, for example liposomes, lipid emulsions, proteinaceous cages and pharmaceutically-acceptable excipients.

The term "pharmaceutically-acceptable excipients" as used herein includes any physiologically inert, pharmacologically inactive material known to one skilled in the art, which is compatible with the physical and chemical characteristics of the particular active ingredient selected for use. Pharmaceutically-acceptable excipients include, but are not limited to, polymers, resins, plasticizers, fillers, lubricants, binders, disintegrants, solvents, co-solvents, buffer systems, surfactants, preservatives, sweetening agents, flavoring agents, pharmaceutical grade dyes and pigments.

The following non-limiting example illustrates formulations of the subject invention.

Example IV

Pharmaceutical formulations (compositions) in the form of tablets are prepared by conventional methods, such as mixing and direct compaction. A tablet is formulated as follows:

<u>Ingredient</u>	<u>Quantity (mg per tablet)</u>
Fluprostenol	5
Microcrystalline Cellulose	100
Sodium Starch Glycollate	30
Magnesium Stearate	3

A pharmaceutical composition in liquid form is prepared by conventional methods, formulated as follows:

<u>Ingredient</u>	<u>Quantity</u>
17-phenyl-17-trinor PGE ₂	5mg
Phosphate buffered physiologic saline	10 ml
Methyl paraben	0.05 ml

The above tablet is administered orally once daily and 1.0 ml of the above liquid composition is administered subcutaneously once daily for six months to a patient afflicted with osteoporosis. The bone patient's bone volume is substantially increased.

While particular embodiments of the subject invention have been described, it would be obvious to those skilled in the art that various changes and modifications to the compositions disclosed herein can be made without departing from the spirit and scope of the invention.

What is claimed is:

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1. A method of increasing bone volume, comprising administering to a subject a therapeutically effective amount of a non-naturally-occurring FP selective agonist and a non-naturally-occurring EP₁ selective agonist.

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