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(71) Applicants (for all designated States except US): **NPS PHARMACEUTICALS, INC.** [US/US]; 383 Colorow Drive, Salt Lake City, UT 84108 (US). **GLAXOSMITHKLINE** [US/US]; 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **MARQUIS, Robert, W.** [US/US]; 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US). **SHCHERBAKOVA, Irina** [US/US]; 383 Colorow Drive, Salt Lake City, UT 84108 (US).

(74) Agent: **LAURENCE, Kevin, B.**; One Utah Center, 201 So. Main Street, Suite 1100, Salt Lake City, UT 84111 (US).

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(54) Title: CHROMENONE COMPOUNDS AS CALCILYTICS

(57) Abstract: Calcilytic compounds and methods of preparing them are disclosed. Methods of using the calcilytic compounds are also provided.



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CHROMENONE COMPOUNDS AS CALCILYTICS

Technical Field

[0001] The present invention relates to chromenone compounds able to inhibit calcium receptor activity, pharmaceutical compositions containing these compounds, and methods for preparing the compounds and compositions. The present invention also relates to the uses of such compounds and compositions, particularly their use in administering to patients to achieve a therapeutic effect.

Background of the Invention

[0002] In mammals, extracellular Ca^{2+} is under rigid homeostatic control and regulates various processes such as blood clotting, nerve and muscle excitability, and proper bone formation. Extracellular Ca^{2+} inhibits the secretion of parathyroid hormone ("PTH") from parathyroid cells, inhibits bone resorption by osteoclasts, and stimulates secretion of calcitonin from C-cells. Calcium receptor proteins enable certain specialized cells to respond to changes in extracellular Ca^{2+} concentration.

[0003] PTH is the principal endocrine factor regulating Ca^{2+} homeostasis in the blood and extracellular fluids. PTH, by acting on bone and kidney cells, increases the level of Ca^{2+} in the blood. This increase in extracellular Ca^{2+} then acts as a negative feedback signal, depressing PTH secretion. The reciprocal relationship between extracellular Ca^{2+} and PTH secretion forms an important mechanism maintaining bodily Ca^{2+} homeostasis.

[0004] Extracellular Ca^{2+} acts directly on parathyroid cells to regulate PTH secretion. The existence of a parathyroid cell surface protein which detects changes

in extracellular Ca^{2+} has been confirmed. See Brown et al., Nature 366:574, 1993. In parathyroid cells, this protein, the calcium receptor, acts as a receptor for extracellular Ca^{2+} , detects changes in the ion concentration of extracellular Ca^{2+} , and initiates a functional cellular response, PTH secretion.

[0005] Extracellular Ca^{2+} influences various cell functions, reviewed in Nemeth et al., Cell Calcium 11:319, 1990. For example, extracellular Ca^{2+} plays a role in parafollicular (C-cells) and parathyroid cells. See Nemeth, Cell Calcium 11:323, 1990. The role of extracellular Ca^{2+} on bone osteoclasts has also been studied. See Zaidi, Bioscience Reports 10:493, 1990.

[0006] Various compounds are known to mimic the effects of extra-cellular Ca^{2+} on a calcium receptor molecule. Calcilytics are compounds able to inhibit calcium receptor activity, thereby causing a decrease in one or more calcium receptor activities evoked by extracellular Ca^{2+} . Calcilytics are useful as lead molecules in the discovery, development, design, modification and/or construction of useful calcium modulators, which are active at Ca^{2+} receptors. Such calcilytics are useful in the treatment of various disease states characterized by abnormal levels of one or more components, e.g., polypeptides such as hormones, enzymes or growth factors, the expression and/or secretion of which is regulated or affected by activity at one or more Ca^{2+} receptors. Target diseases or disorders for calcilytic compounds include diseases involving abnormal bone and mineral homeostasis.

[0007] Abnormal calcium homeostasis is characterized by one or more of the following activities: an abnormal increase or decrease in serum calcium; an abnormal increase or decrease in urinary excretion of calcium; an abnormal increase or decrease in bone calcium levels (for example, as assessed by bone mineral density measurements); an abnormal absorption of dietary calcium; an abnormal increase or decrease in the production and/or release of messengers which affect serum calcium levels such as PTH and calcitonin; and an abnormal change in the response elicited by messengers which affect serum calcium levels.

[0008] Thus, calcium receptor antagonists offer a unique approach towards the pharmacotherapy of diseases associated with abnormal bone or mineral homeostasis, such as hypoparathyroidism, osteosarcoma, periodontal disease, fracture healing, osteoarthritis, joint replacement, rheumatoid arthritis, Paget's

disease, humoral hypercalcemia associated with malignancy and fracture healing, and osteoporosis.

Summary of the Invention

[0009] Chromenone compounds are disclosed herein which are useful as calcium receptor antagonists in the treatment of a variety of diseases associated with abnormal bone or mineral homeostasis, including but not limited to hypoparathyroidism, osteosarcoma, periodontal disease, fracture healing, osteoarthritis, joint replacement, rheumatoid arthritis, Paget's disease, humoral hypercalcemia associated with malignancy and fracture healing, and osteoporosis. The compounds are represented by Formula (I) hereinbelow.

[0010] A method for antagonizing calcium receptors in an animal, including humans, is also disclosed. The method comprises administering to an animal in need thereof an effective amount of a compound of Formula (I), indicated hereinbelow.

[0011] A method for increasing serum parathyroid levels in an animal, including humans, is additionally disclosed. The method comprises administering to an animal in need thereof an effective amount of a compound of Formula (I), indicated herein below.

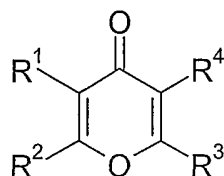
Detailed Description of Preferred Embodiments

[0012] Chromenone compounds are disclosed herein which are useful as calcilytic compounds or calcilytics. "Calcilytics" and "calcilytic compounds" refer to compounds able to inhibit calcium receptor activity. The ability of a compound to "inhibit calcium receptor activity" means that the compound causes a decrease in one or more calcium receptor activities evoked by extracellular Ca^{2+} .

[0013] The use of calcilytic compounds to inhibit calcium receptor activity and/or achieve a beneficial effect in a patient are described below. More specifically, the ability of calcilytic compounds to increase PTH secretion is demonstrated, thereby confirming that the parathyroid gland calcium receptor is a target site for these compounds. Also described below are techniques which can be used to obtain additional calcilytic compounds.

[0014] Examples of the featured calcilytic compounds are provided by the chemical formula depicted in Structure I and the accompanying description.

Structure I



wherein:

R^1 and R^2 are independently one of: H, halogen, CN, CF_3 , lower alkyl, cycloalk, or aryl;

or R^1 and R^2 are together $-(CH_2)_n-$ and n is 5, 4, or 3;

or R^1 and R^2 are together an aromatic ring;

R^3 is an aryl group, which may have 0 to 4 substituents in the aryl ring and each substituent is at least one of: halogen, CN, CF_3 , OCF_3 , lower alkyl, $N(\text{lower alkyl})_2$, lower alkoxy, OH, $OC(O)\text{-lower alkyl}$, $OC(O)\text{-lower alkylamino}$, or $OC(O)\text{-lower alkyl-N}(\text{lower alkyl})_2$;

R^4 is one of H, lower alkyl, and a group of the formula $-(CH_2)_n-R^5$ wherein n is 0, 1, or 2, and R^5 is an aryl group which may have 0 to 3 substituents on the aryl ring and each substituent is at least one of: halogen, CN, CF_3 , OCF_3 , lower alkyl, lower alkoxy, NH-lower alkyl, NH-alkylaryl, $N(\text{lower alkyl})_2$, OH, $OC(O)\text{-lower alk}$, $OC(O)\text{-lower alkylamino}$, and $OC(O)\text{-lower alkyl-N}(\text{lower alk})_2$; or

pharmaceutically acceptable salts, hydrates, tautomers, solvates or complexes thereof.

[0015] As used herein, "alkyl" refers to an optionally substituted hydrocarbon group joined by single carbon-carbon bonds and having 1-20 carbon atoms joined together. The alkyl hydrocarbon group may be linear, branched or cyclic, saturated or unsaturated. Substituents on optionally substituted alkyl may be one of: aryl, CO_2R , CO_2NHR , OH, OR, CO, NH_2 , halo, CF_3 , OCF_3 or NO_2 , wherein R represents H, C_{1-4} alkyl, C_{3-6} cycloalkyl, C_{2-5} alkenyl, C_{2-5} alkynyl, heterocycloalkyl, or aryl.

Additional substituents may be at least one of: F, Cl, Br, I, N, S or O. In one embodiment, no more than three substituents are present. In another embodiment, the alkyl has 1-12 carbon atoms and is unsubstituted. The alkyl group may be linear.

[0016] As used herein "cycloalkyl" refers to optionally substituted 3-7 membered carbocyclic rings wherein any substituents may be at least one of, F, Cl, Br, I, N(R₄)₂, SR₄ or OR₄, unless otherwise indicated.

[0017] As used herein, "aryl" refers to an optionally substituted aromatic group with at least one ring having a conjugated pi-electron system, containing up to two conjugated or fused ring systems. Aryl includes carbocyclic aryl, and biaryl groups, all of which may be optionally substituted. Phenyl and naphthyl are particularly useful aryl. Examples of suitable substituents include at least one of: halogen, C₁₋₄ alkyl, OCF₃, CF₃, OMe, CN, OSO₂R or NO₂, wherein R represents C₁₋₄ alkyl or C₃₋₆ cycloalkyl.

[0018] As used herein, "heteroaryl" refers to an aryl ring containing 1, 2 or 3 heteroatoms such as N, S, or O.

[0019] As used herein, "alkenyl" refers to an optionally substituted hydrocarbon group containing at least one carbon-carbon double bond and containing up to 5 carbon atoms joined together. The alkenyl hydrocarbon chain may be straight, branched or cyclic. The substituents are at least one of: halogen, C₁₋₄ alkyl, OCF₃, CF₃, OMe, CN, OSO₂R and NO₂, wherein R represents C₁₋₄ alkyl or C₃₋₆ cycloalkyl.

[0020] As used herein, "alkynyl" refers to an optionally substituted hydrocarbon group containing at least one carbon-carbon triple bond between the carbon atoms and containing up to 5 carbon atoms joined together. The alkynyl hydrocarbon group may be straight-chained, branched or cyclic. The substituents are at least one of: halogen, C₁₋₄ alkyl, OCF₃, CF₃, OMe, CN, OSO₂R or NO₂, wherein R represents C₁₋₄ alkyl or C₃₋₆ cycloalkyl.

[0021] The chromenone compound may contain one or more asymmetric carbon atoms and may exist in racemic and optically active forms. All of these compounds and diastereomers are contemplated to be within the scope of the present invention.

[0022] One compound corresponding with Formula (I) is:

2-(2-hydroxyphenyl)-3-(2-phenylethyl)-4*H*-chromen-4-one. Example 1 provides a method for preparing this compound.

[0023] Pharmaceutically acceptable salts are non-toxic salts in the amounts and concentrations at which they are administered.

[0024] Pharmaceutically acceptable salts include acid addition salts such as those containing sulfate, hydrochloride, fumarate, maleate, phosphate, sulfamate, acetate, citrate, lactate, tartrate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, cyclohexylsulfamate and quinate. Hydrochloride is a particularly useful pharmaceutically acceptable salt. 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, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, cyclohexylsulfamic acid, fumaric acid, and quinic acid.

[0025] Pharmaceutically acceptable salts also include basic addition salts such as those containing benzathine, chlorprocaine, choline, diethanolamine, ethylenediamine, meglumine, procaine, aluminum, calcium, lithium, magnesium, potassium, sodium, ammonium, alkylamine, and zinc, when acidic functional groups, such as carboxylic acid or phenol are present.

[0026] The compounds of Formula (I) above may be prepared using standard techniques. An overall strategy for preparing preferred compounds described herein can be carried out as described in this section. The examples, which follow, illustrate the synthesis of specific compounds. Using the protocols described herein as a model, one of ordinary skill in the art can readily produce other compounds of the present invention.

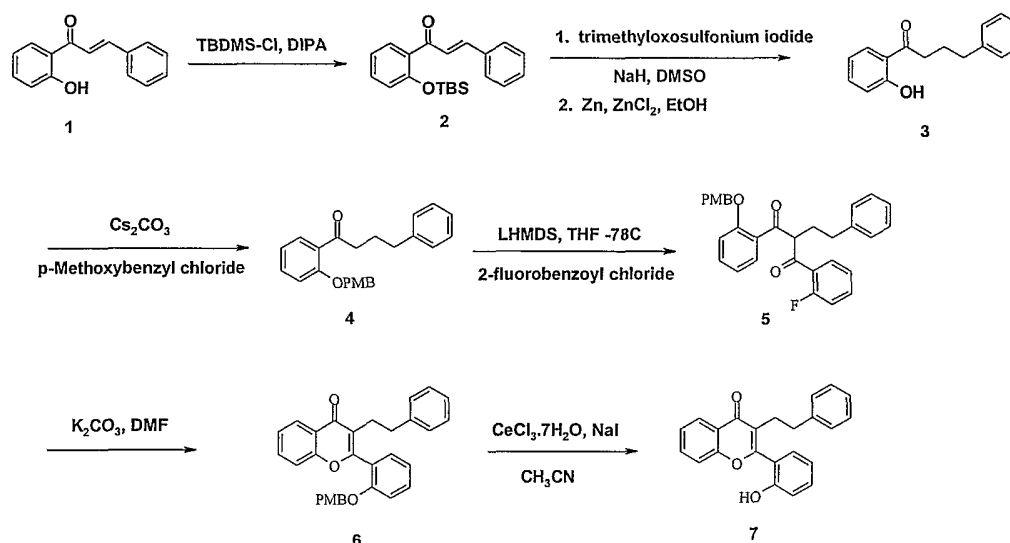
[0027] All reagents and solvents may be obtained from commercial vendors. Starting materials may be synthesized using standard techniques and procedures.

Synthesis Schemes

[0028] The chromenones disclosed herein may be synthesized in the general manner outlined below in Scheme 1 with the starting compound, 2'-hydroxychalcone, identified as compound (1) and the final compound identified as compound (7). 1-(2-Hydroxyphenyl)-4-phenyl-butanone (3) is prepared from 2'-hydroxychalcone (1) following literature procedures (Henke, B.R.; Adkison, K.K.; Blanchard, S.G.; Leesnitzer, L.M.; Mook, R.A.; Plunket, K.D.; Ray, J.A.; Roberson, C.; Unwalla, R.;

Willson, T.M. *Biorg. and Med. Chem. Lett.* **1999**, 9, 3329). Protection of the phenol of (3) with p-methoxybenzyl chloride provides compound (4). Enolization of (4) with LHMDS followed by treatment with 2-fluorobenzoyl chloride provides the β -ketone (5). Treatment of the β -ketone (5) under basic conditions provides the chromenone (6) that is deprotected to provide (7).

[0029] Scheme 1. Synthesis of Chromenone Analog 7



[0030] In order to use a compound of Formula (I) or a pharmaceutically acceptable salt thereof for the treatment of humans and other mammals, it is normally formulated in accordance with standard pharmaceutical practice as a pharmaceutical composition.

[0031] The calcilytic compounds can be administered by different routes including intravenous, intraperitoneal, subcutaneous, intramuscular, oral, topical (transdermal), or transmucosal administration. Oral administration is suitable for systemic administration. For oral administration, the compounds can be formulated into conventional oral dosage forms. Examples of suitable oral dosage forms include capsules, tablets, and liquid preparations such as syrups, elixirs, and concentrated drops.

[0032] Injection (parenteral administration) may also be used, e.g., intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the compounds of the invention are formulated in liquid solutions. For example, the compounds may be formulated in physiologically compatible buffers or solutions, such as saline solution,

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.

[0033] Systemic administration can also be achieved by transmucosal or transdermal administration. 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, for example, may be through nasal sprays, rectal suppositories, or vaginal suppositories.

[0034] For topical administration, the compounds of the invention can be formulated into ointments, salves, gels, or creams, as is generally known in the art.

[0035] The amounts of various calcilytic compounds to be administered can be determined by standard procedures taking into account factors such as the compound IC_{50} , EC_{50} , the biological half-life of the compound, the age, size and weight of the patient, and the disease or disorder associated with the patient. The importance of these and other factors to be considered are known to those of ordinary skill in the art.

[0036] Amounts administered also depend on the routes of administration and the degree of oral bioavailability. For example, for compounds with low oral bioavailability, relatively higher doses will have to be administered.

[0037] The composition is typically administered in unit dosage form. For oral application, for example, a tablet, or capsule may be administered, for nasal application, a metered aerosol dose may be administered, for transdermal application, a topical formulation or patch may be administered and for transmucosal delivery, a buccal patch may be administered. In each case, dosing is such that the patient may administer a single dose.

[0038] Each dosage unit for oral administration contains suitably from about 0.01 to about 500 mg/kg of a compound of Formula (I) or a pharmaceutically acceptable salt thereof, calculated as the free base. The dosage unit for oral administration may also be about 0.1 to about 50 mg/kg. The daily dosage for parenteral, nasal, oral inhalation, transmucosal or transdermal routes contains suitably from about 0.01 mg

to about 100 mg/kg, of a compound of Formula (I). A topical formulation contains suitably about 0.01 to about 5.0% of a compound of Formula (I). While a single dose is convenient, multiple doses, such as 2 to 6 times per day may be utilized. As is readily apparent to one skilled in the art, the amount and dosage of the active ingredient may be administered as needed to exhibit the desired activity.

[0039] As used herein, "treatment" of a disease includes, but is not limited to prevention, retardation and prophylaxis of the disease.

[0040] Diseases and disorders which might be treated or prevented, based upon the affected cells, include bone and mineral-related diseases or disorders; hypoparathyroidism; those of the central nervous system such as seizures, stroke, head trauma, spinal cord injury, hypoxia-induced nerve cell damage, such as occurs 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; autoimmune diseases and organ transplant rejection; squamous cell carcinoma; and pancreatitis.

[0041] In one embodiment, the chromenone compounds are used to increase serum parathyroid hormone ("PTH") levels. Increasing serum PTH levels can be helpful in treating diseases such as hypoparathyroidism, osteosarcoma, periodontal disease, fracture, osteoarthritis, rheumatoid arthritis, Paget's disease, humoral hypercalcemia malignancy and osteoporosis.

[0042] The chromenone compounds can be co-administered with an anti-resorptive agent. Such agents include, but are not limited to estrogen, 1, 25 (OH)₂ vitamin D₃, calcitonin, selective estrogen receptor modulators, vitronectin receptor antagonists, V-H⁺-ATPase inhibitors, src SH2 antagonists, bisphosphonates and cathepsin K inhibitors.

[0043] The compounds disclosed herein can be utilized in a method of treating a patient to increase the patient's serum PTH level. The method is carried out by administering to the patient an amount of the compound effective to cause an increase in duration and/or quantity of serum PTH level sufficient to have a therapeutic effect.

[0044] In various embodiments, the compound administered to a patient causes an increase in serum PTH having a duration of up to one hour, about one to about twenty-four hours, about one to about twelve hours, about one to about six hours, about one to about five hours, about one to about four hours, about two to about five hours, about two to about four hours, or about three to about six hours.

[0045] In another embodiment, the compound administered to a patient causes an increase in serum PTH having a duration of more than about twenty four hours provided that it is co-administered with an anti resorptive agent.

[0046] In additional different embodiments, the compound administered to a patient causes an increase in serum PTH of up to two fold, two to five fold, five to ten fold, and at least 10 fold, greater than peak serum PTH in the patient. The peak serum level is measured with respect to a patient not undergoing treatment.

[0047] As indicated above, compounds of Formula (I) and their pharmaceutically acceptable salts, which are active when given orally, can be formulated as syrups, tablets, capsules and lozenges. A syrup formulation generally comprises a suspension or solution of the compound or salt in a liquid carrier. Examples of suitable liquid carriers include ethanol, peanut oil, olive oil, glycerine or water with a flavoring or coloring agent. In a composition provided in the form of a tablet, any pharmaceutical carrier routinely used for preparing solid formulations may be used. Examples of such carriers include magnesium stearate, terra alba, talc, gelatin, acacia, stearic acid, starch, lactose and sucrose. For a compound provided in a capsule, any routine encapsulation is suitable. For example, the aforementioned carriers used in preparing tablets may be utilized to form a hard gelatin capsule shell. For compositions in a soft gelatin shell capsule, any pharmaceutical carrier routinely used for preparing dispersions or suspensions may be considered. Examples of suitable materials for forming a soft gelatin capsule shell include aqueous gums, celluloses, silicates and oils.

[0048] Typical parenteral compositions comprise a solution or suspension of a compound or salt in a sterile aqueous or non-aqueous carrier optionally containing parenterally acceptable oil, for example polyethylene glycol, polyvinylpyrrolidone, lecithin, arachis oil or sesame oil.

[0049] Typical compositions for inhalation are in the form of a solution, suspension or emulsion that may be administered as a dry powder or in the form of an aerosol using a conventional propellant such as dichlorodifluoromethane or trichlorofluoromethane.

[0050] A typical suppository formulation comprises a compound of Formula (I) or a pharmaceutically acceptable salt thereof which is active when administered in this way, with a binding and/or lubricating agent, for example polymeric glycols, gelatins, cocoa-butter or other low melting vegetable waxes or fats or their synthetic analogs.

[0051] Typical dermal and transdermal formulations comprise a conventional aqueous or non-aqueous vehicle, for example a cream, ointment, lotion or paste or are in the form of a medicated plaster, patch or membrane.

[0052] The composition is conveniently provided in unit dosage form, for example a tablet, capsule or metered aerosol dose, so that the patient may administer a single dose.

[0053] Additional information about standard pharmaceutical practice for formulating pharmaceutical compositions such as conventional techniques for making tablets and pills containing active ingredients are described in the standard reference, "Remington: the Science and Practice of Pharmacy," (21st ed. 2005). This standard reference is incorporated herein.

[0054] No unacceptable toxicological effects are expected when chromenone compounds are administered in accordance with the understanding of one of ordinary skill in the art.

Examples

[0055] The following specific examples are included for illustrative purposes only and are not to be considered as limiting to this disclosure. The reagents and intermediates used in the following examples are either commercially available or

can be prepared according to standard literature procedures by those skilled in the art of organic synthesis.

Example 1

Preparation of 2-(2-hydroxyphenyl)-3-(2-phenylethyl)-4H-chromen-4-one

[0056] A. 1-{2-[(4-methoxybenzyl)oxy]phenyl}-4-phenylbutan-1-one

[0057] To a solution of 1-(2-hydroxyphenyl)-4-phenylbutan-1-one (1.37 g, 5.71 mmol) in DMF (60 mL) under Ar was added Cs₂CO₃ (4.67 g, 14.3 mmol). *p*-Methoxybenzyl chloride (5.8 mL, 42.8 mmol) was added to the yellow solution and the reaction mixture stirred for 4 hours. A catalytic amount of tetra-butylammonium iodide was then added and the reaction mixture was stirred for an additional 3 days. The reaction was cooled to 0°C and 14 mL of 6N NaOH was added. The reaction was warmed to room temperature and stirred for 45 minutes. The reaction was diluted with water and extracted three times with diethyl ether. The combined organic layers were washed three times with water and then dried over MgSO₄, filtered, and concentrated to yield 1.69 g (82%) of 1-{2-[(4-methoxybenzyl)oxy]phenyl}-4-phenylbutan-1-one as a white solid: ¹H NMR⁺ (400 MHz, CDCl₃) δ 7.70-6.80 (m, 13H), 5.07 (s, 2H), 3.85 (s, 3H), 2.98 (t, 2H), 2.55 (t, 2H), 1.97 (m, 2H); MS(ESI) 361.2 (M + H)⁺.

[0058] B. 1-(2-fluorophenyl)-3-{2-[(4-methoxybenzyl)oxy]phenyl}-2-(2-phenylethyl)propane-1,3-dione LiHMDS (6.5 mL, 1.0M in THF) was added dropwise via syringe to a cooled (-78°C) solution of 1-{2-[(4-methoxybenzyl)oxy]phenyl}-4-phenylbutan-1-one (0.705 g, 1.96 mmol) in THF (4.0 mL). The reaction was then stirred at -78°C for 45 minutes. 2-Fluorobenzoyl chloride (0.24 mL, 2.01 mmol) was added dropwise and the reaction mixture stirred at -78°C for 1.5 hours. The reaction was quenched with saturated NH₄Cl and ethyl acetate and warmed to room temperature. The layers were separated and the ethyl acetate layer was washed with brine, dried over MgSO₄, filtered, and concentrated in vacuo. Column

chromatography (75:25 to 0:100 Hexanes/CH₂Cl₂) yielded 0.450 g (48%) of 1-(2-fluorophenyl)-3-{2-[(4-methoxybenzyl)oxy]phenyl}-2-(2-phenylethyl)propane-1,3-dione as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 7.80-6.75 (m, 17H), 5.41 (t, 1H), 4.90 (d, 2H), 3.73 (s, 3H), 2.50 (m, 2H), 2.25 (m, 1H), 2.15 (m, 1H); MS(ESI) 483.2 (M + H)⁺.

[0059] C. 2-{2-[(4-methoxybenzyl)oxy]phenyl}-3-(2-phenylethyl)-4*H*-chromen-4-one K₂CO₃ (0.154 g, 1.11 mmol) was added to 1-(2-fluorophenyl)-3-{2-[(4-methoxybenzyl)oxy]phenyl}-2-(2-phenylethyl)propane-1,3-dione (0.450 g, 0.933 mmol) in DMF (5.0 mL). The yellow reaction mixture was stirred at room temperature for 23 hours. The reaction was quenched with brine and extracted with diethyl ether. The organic layer was washed two times with brine and then dried over MgSO₄, filtered, and concentrated to yield 0.378 g (88%) of 2-{2-[(4-methoxybenzyl)oxy]phenyl}-3-(2-phenylethyl)-4*H*-chromen-4-one as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 8.13 (d, 1H), 7.51-6.59 (m, 16 H), 4.87 (s, 2H), 3.57 (s, 3H), 2.64-2.58 (m, 4H); MS(ESI) 463.4 (M + H)⁺.

[0060] D. 2-(2-hydroxyphenyl)-3-(2-phenylethyl)-4*H*-chromen-4-one CeCl₃·7H₂O (0.407 g, 1.09 mmol) and NaI (0.109 g, 0.727 mmol) were added to a solution of 2-{2-[(4-methoxybenzyl)oxy]phenyl}-3-(2-phenylethyl)-4*H*-chromen-4-one (0.337 g, 0.729 mmol) in CH₃CN (7.5 mL) and heated at reflux in a sealed flask for 22 hours. The reaction was cooled and quenched with 0.5 M HCl. The aqueous layer was extracted four times with diethyl ether. The combined organic layers were washed two times with sat. NaHCO₃ and brine. The organic layer was dried over Na₂SO₄, filtered, and concentrated. Column chromatography (3:1 to 1:1 Hexane: Ethyl acetate) provided 0.030 g of pure 2-(2-hydroxyphenyl)-3-(2-phenylethyl)-4*H*-chromen-4-one: ¹H NMR (400 MHz, CDCl₃) δ 8.17 (d, 1H), 7.59-6.87 (m, 12H), 2.75-2.65 (m, 4H); MS(ESI) 343.2 (M + H)⁺.

[0061] A solution of acetic acid 2-[(*Z*)-2-(4-isopropyl-phenylcarbamoyl)-1-methyl-vinylcarbamoyl-phenylester (0.4 g, 0.1 mmol) in EtOH (30 mL) and 85% KOH (5 mL) was heated to reflux for 5 hours. After cooling to RT, the reaction mixture was adjusted to pH 1 with 2N HCl and extracted three times with CH₂Cl₂. The organic portions were combined, dried (Na₂SO₄), filtered, and concentrated. Flash column chromatography of the residue (3% CH₃OH/CH₂Cl₂) provided the title compound as

a white solid: ^1H NMR (400 MHz, CDCl_3): δ 7.28-7.26(m, 2H), 7.13-7.11(m, 3H), 7.10(d, 1H), 6.64(d, 1H), 6.38(t, 1H), 3.00-2.90(m, 1H), 2.64-2.62(q, 2H), 2.44(s, 3H), 1.27-1.26(d, 6H), 1.21-1.17(t, 3H). MS(m/z): 349.2 (M+H).

Examples of the Biological Activity of Reversed Pyrimidinones

[0062] The biological activity of the compounds of Formula (I) are demonstrated by the following tests:

(I) Calcium Receptor Inhibitor Assay

[0063] Calcilytic activity was measured by determining the IC_{50} of the test compound, 2-(2-hydroxyphenyl)-3-(2-phenylethyl)-4*H*-chromen-4-one, for blocking increases of intracellular Ca^{2+} elicited by extracellular Ca^{2+} in HEK 293 4.0-7 cells stably expressing the human calcium receptor. HEK 293 4.0-7 cells were constructed as described by Rogers et al., J. Bone Miner. Res. 10 Suppl. 1:S483, 1995 (hereby incorporated by reference herein). Intracellular Ca^{2+} increases were elicited by increasing extracellular Ca^{2+} from 1 to 1.75 mM. Intracellular Ca^{2+} was measured using fluo-3, a fluorescent calcium indicator.

[0064] The procedure was as follows:

[0065] 1. Cells were maintained in T-150 flasks in selection media (DMEM supplemented with 10% fetal bovine serum and 200 ug/mL hygromycin B), under 5% CO_2 :95% air at 37°C and were grown up to 90% confluency.

[0066] 2. The medium was decanted and the cell monolayer was washed twice with phosphate-buffered saline (PBS) kept at 37°C. After the second wash, 6 mL of 0.02% EDTA in PBS was added and incubated for 4 minutes at 37°C. Following the incubation, cells were dispersed by gentle agitation.

[0067] 3. Cells from 2 or 3 flasks were pooled and pelleted (100 x g). The cellular pellet was resuspended in 10-15 mL of SPF-PCB+ and pelleted again by centrifugation. This washing was done twice.

[0068] Sulfate- and phosphate-free parathyroid cell buffer (SPF-PCB) contains 20 mM Na-Hepes, pH 7.4, 126 mM NaCl, 5 mM KCl, and 1 mM MgCl_2 . SPF-PCB was

made up and stored at 4°C. On the day of use, SPF-PCB was supplemented with 1 mg/mL of D-glucose and 1 mM CaCl₂ and then split into two fractions. To one fraction, bovine serum albumin (BSA; fraction V, ICN) was added at 5 mg/mL (SPF-PCB+). This buffer was used for washing, loading and maintaining the cells. The BSA-free fraction was used for diluting the cells in the cuvette for measurements of fluorescence.

[0069] 4. The pellet was resuspended in 10 mL of SPF-PCB+ containing 2.2 uM fluo-3 (Molecular Probes) and incubated at room temperature for 35 minutes.

[0070] 5. Following the incubation period, the cells were pelleted by centrifugation. The resulting pellet was washed with SPF-PCB+. After this washing, cells were resuspended in SPF-PCB+ at a density of 1-2 x 10⁶ cells/mL.

[0071] 6. For recording fluorescent signals, 300 uL of cell suspension were diluted in 1.2 mL of SPF buffer containing 1 mM CaCl₂ and 1 mg/mL of D-glucose. Measurements of fluorescence were performed at 37°C with constant stirring using a spectrofluorimeter. Excitation and emission wavelengths were measured at 485 and 535 nm, respectively. To calibrate fluorescence signals, digitonin (5 mg/mL in ethanol) was added to obtain F_{max}, and the apparent F_{min} was determined by adding Tris-EGTA (2.5 M Tris-Base, 0.3 M EGTA). The concentration of intracellular calcium was calculated using the following equation:

[0072] Intracellular calcium = $(F - F_{\min} / F_{\max}) \times K_d$; where $K_d = 400 \text{ nM}$.

[0073] 7. To determine the potential calcilytic activity of test compounds, cells were incubated with test compound (or vehicle as a control) for 90 seconds before increasing the concentration of extracellular Ca²⁺ from 1 to 2mM. Calcilytic compounds were detected by their ability to block, in a concentration-dependent manner, increases in the concentration of intracellular Ca²⁺ elicited by extracellular Ca²⁺.

[0074] Compounds having an IC₅₀ value in the Calcium Receptor Inhibitor which are greater than 50 uM were considered to be inactive. Note that it is desirable for compounds to have lower IC₅₀ values in the Calcium Receptor Inhibitor Assay. For example, it is desirable for the compounds to have an IC₅₀ of 10uM or lower, an IC₅₀ of 1uM, and an IC₅₀ of 0.1uM or lower.

(II) Calcium Receptor Binding Assay

[0075] HEK 293 4.0-7 cells stably transfected with the Human Parathyroid Calcium Receptor ("HuPCaR") were scaled up in T180 tissue culture flasks. Plasma membrane is obtained by polytron homogenization or glass douncing in buffer (50mM Tris-HCl pH 7.4, 1mM EDTA, 3mM MgCl₂) in the presence of a protease inhibitor cocktail containing 1uM Leupeptin, 0.04 uM Pepstatin, and 1 mM PMSF. Aliquoted membrane was snap frozen and stored at -80°C. ³H labeled compound was radiolabeled to a radiospecific activity of 44Ci/mmol and was aliquoted and stored in liquid nitrogen for radiochemical stability.

[0076] A typical reaction mixture contains 2 nM ³H compound ((R,R)-N-4'-Methoxy-t-3-3'-methyl-1'-ethylphenyl-1-(1-naphthyl)ethylamine), or ³H compound (R)-N-[2-Hydroxy-3-(3-chloro-2-cyanophenoxy)propyl]-1,1-dimethyl-2-(4-methoxyphenyl)ethylamine 4-10 ug membrane in homogenization buffer containing 0.1% gelatin and 10% EtOH in a reaction volume of 0.5 mL. Incubation is performed in 12 x 75 polyethylene tubes in an ice water bath. To each tube 25 uL of test sample in 100% EtOH is added, followed by 400 uL of cold incubation buffer, and 25 uL of 40 nM ³H-compound in 100% EtOH for a final concentration of 2nM. The binding reaction is initiated by the addition of 50 uL of 80-200 ug/mL HEK 293 4.0-7 membrane diluted in incubation buffer, and allowed to incubate at 4°C for 30 min. Wash buffer is 50 mM Tris-HCl containing 0.1% PEI. Nonspecific binding is determined by the addition of 100-fold excess of unlabeled homologous ligand, and is generally 20% of total binding. The binding reaction is terminated by rapid filtration onto 1% PEI pretreated GF/C filters using a Brandel Harvester. Filters are placed in scintillation fluid and radioactivity assessed by liquid scintillation counting.

[0077] All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

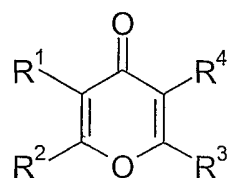
[0078] The above description fully discloses the invention including preferred embodiments thereof. Without further elaboration, it is believed that one skilled in the art can use the preceding description to utilize the invention to its fullest extent. Therefore the Examples herein are to be construed as merely illustrative and not a limitation of the scope of the present invention in any way.

[0079] It will be apparent to those having skill in the art that changes may be made to the details of the above-described embodiments without departing from the underlying principles of the invention. Embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows.

Claims

1. A compound according to formula (I) hereinbelow:

Structure I



wherein:

R^1 and R^2 are independently one of: H, halogen, CN, CF_3 , lower alkyl, cycloalk, or aryl;

or R^1 and R^2 are together $-(CH_2)_n-$ and n is 5, 4, or 3;

or R^1 and R^2 are together an aromatic ring;

R^3 is an aryl group, which may have 0 to 4 substituents in the aryl ring and each substituent is at least one of: halogen, CN, CF_3 , OCF_3 , lower alkyl, $N(\text{lower alkyl})_2$, lower alkoxy, OH, $OC(O)$ -lower alkyl, $OC(O)$ -lower alkylamino, or $OC(O)$ -lower alkyl- $N(\text{lower alkyl})_2$;

R^4 is one of H, lower alkyl, or a group of the formula $-(CH_2)_n-R^5$ wherein n is 0, 1, or 2, and R^5 is an aryl group which may have 0 to 3 substituents on the aryl ring and each substituent is at least one of: halogen, CN, CF_3 , OCF_3 , lower alkyl, lower alkoxy, NH-lower alkyl, NH-alkylaryl, $N(\text{lower alkyl})_2$, OH, $OC(O)$ -lower alk, $OC(O)$ -lower alkylamino, or $OC(O)$ -lower alkyl- $N(\text{lower alk})_2$;

or

pharmaceutically acceptable salts, hydrates, tautomers, solvates or complexes thereof.

2. The compound according to claim 1, comprising 2-(2-hydroxyphenyl)-3-(2-phenylethyl)-4*H*-chromen-4-one.

3. A method of antagonizing a calcium receptor, which comprises administering to a subject in need thereof, an effective amount of a compound according to claim 1.

4. A method of treating a disease or disorder characterized by an abnormal bone or mineral homeostasis which comprises administering to a subject in need of treatment thereof an effective amount of a compound according to claim 1.

5. A method according to claim 4, wherein the bone or mineral disease or disorder is at least one of osteosarcoma, periodontal disease, fracture, healing, osteoarthritis, joint replacement, rheumatoid arthritis, Paget's disease, humoral hypercalcemia, malignancy, or osteoporosis.

6. A method according to claim 4, wherein the bone or mineral disease or disorder is osteoporosis.

7. A method according to claim 6, wherein the compound is co-administered with an anti-resorptive agent.

8. A method according to claim 7, wherein the anti-resorptive agent is at least one of: estrogen, 1, 25 (OH)₂ vitamin D3, calcitonin, selective estrogen receptor modulators, vitronectin receptor antagonists, V-H⁺-ATPase inhibitors, src SH2 antagonists, bisphosphonates or cathepsin K inhibitors.

9. A method of increasing serum parathyroid levels which comprises administering to a subject in need of treatment an effective amount of a compound of claim 1.

10. A method according to claim 9, wherein the compound is co-administered with an anti-resorptive agent.

11. A method according to claim 10, wherein the anti-resorptive agent is at least one of: estrogen, 1, 25 (OH)₂ vitamin D3, calcitonin, selective estrogen receptor modulators, vitronectin receptor antagonists, V-H⁺-ATPase inhibitors, src SH2 antagonists, bisphosphonates or cathepsin K inhibitors.

12. A pharmaceutical composition comprising a compound according to claim 1.