Abstract:
The use of 2-oxopyrimidine-5-carboxylic acid esters and amides of formula (I) is disclosed. The compounds are agonists at the TSH receptor (TSHR) and are therefore useful in the treatment of patients with thyroid dysfunction and in the management of differentiated thyroid cancer.
2-OXOPYRIMIDINE-5-CARBOXYLATE DERIVATIVES
FOR TREATING THYROID DISEASES

Cross Reference to Related Applications

[001] This application claims priority of US provisional application 62/019,489, filed July 1, 2014, the entire disclosure of which is hereby incorporated herein by reference.

Government Rights Statement

[002] This invention was made with Government support under award numbers DK069713 and DK052464 awarded by the National Institutes of Health. The Government has certain rights in the invention.

Field of the Invention

[003] The invention relates to 2-oxopyrimidine-5-carboxylic esters and amides that are agonists at the TSH receptor (TSHR). The compounds are useful in the treatment of patients with thyroid dysfunction and in the management of differentiated thyroid cancer.

Background of the Invention

[004] Cancer of the thyroid gland is characterized by a high likelihood of relapse (up to 30% of patients) following thyroidectomy. To minimize relapse, thyroid tissue remaining after thyroidectomy is ablated by treatment with radioactive iodine. To induce the thyroid tissue to take up the radioactive iodine, it is necessary to either treat the patient with recombinant TSH or have thyroid hormone treatment withdrawn in order to elevate natural TSH levels. Withdrawal of thyroid hormone has quite unpleasant side effects for the patient; these include fatigue, muscle cramps, puffiness and constipation. At present, recombinant human TSH (rhTSH, Thyrogen®, Genzyme) is used clinically for screening after surgery in patients with well-differentiated thyroid cancer. rhTSH is expensive and must be administered parenterally. Small molecule agonists to the TSHR have potential for clinical use as a cost-effective method of diagnosing and treating thyroid cancer metastases when used as a substitute for expensive recombinant TSH. They also have possible uses in the
treatment of thyroid dysfunction.

[005] Thyroid-stimulating hormone (TSH) is a heterodimeric glycoprotein hormone secreted from the anterior pituitary. Its action is mediated through the TSHR, which is a member of the class A GPCR family. The holoreceptor has been fully characterized. TSHR, in addition to being the major regulator of thyroid gland function, is also expressed in bone. *In vitro* and *in vivo* studies of TSHR regulation in osteoclasts and osteoblasts have defined the importance of TSH and the TSHR in bone remodeling (see Abe et al., Cell. 2003;115(2):151-162 and Baliram et al., J Clin Invest. 2012;122(10):3737-3741). Similarly, there is strong evidence in support of a role for the TSHR in differentiation of retroorbital fibroblasts obtained from patients with Graves’ ophthalmopathy (Bahn et al., J Endocrinol Invest. 2004;27(3):2 16-220). TSHR also happens to be a primary autoantigen in autoimmune thyroid disease, especially Graves’ disease. Modulating the function of the receptor either orthosterically or allosterically, using small molecule ligands (SMLs), therefore has therapeutic potential.

**Summary of the Invention**

[006] In one aspect, the invention relates to method for the treatment of a thyroid disease or condition comprising administering to a mammal a therapeutically effective amount of a compound of formula I:

![Chemical Structure](image)
wherein

\( R^1 \) is selected from hydrogen, (C\( i - C_6 \))alkyl, fluoro(C\( i - C_6 \))alkyl, (C\( i - C_6 \))alkoxy, fluoro(C\( i - C_6 \))alkoxy, (C\( i - C_6 \))alkylthio, fluoro(C\( i - C_6 \))alkylthio, aryl, aryloxy, arylthio, cyano, and alkoxy carbonyl;

\( R^2 \) is selected from O and S;

\( R^3 \) is selected from hydrogen and (C\( i - C_e \))alkyl;

\( R^4 \) is (C\( i - C_io \))hydrocarbyl optionally substituted with one, two, or three substituents independently selected from -OH, halogen, (C\( i - C_e \))alkyl, (C\( i - C_6 \))alkoxy, cyano, and nitro;

\( R^5 \) is selected from -OR\(^{5a}\) and -NR\(^{5b}R^{5c}\);

\( R^{5a} \) is hydrogen or (C\( i - C_io \))hydrocarbyl, optionally substituted with one, two, or three substituents independently selected from -OH, halogen, (C\( i - C_6 \))alkyl, (C\( i - C_6 \))alkoxy, methylenedioxy, ethylenedioxy, cyano, and nitro;

\( R^{5b} \) is (C\( i - C_io \))hydrocarbyl, optionally substituted with one, two, or three substituents independently selected from -OH, halogen, (C\( i - C_e \))alkyl, (C\( i - C_6 \))alkoxy, methylenedioxy, ethylenedioxy, cyano, and nitro; and

\( R^{5c} \) is selected from hydrogen and (C\( i - C_6 \))alkyl.
In another aspect, the invention relates to a method for treating hyperthyroid diseases such as Graves' disease, thyroid cancer, or hyperthyroid as a consequence of pituitary cancer. In these diseases, the compound of formula I is administered in combination with radioactive iodine.

In another aspect, the invention relates to a method for treating a hypothyroid condition, such as Hashimoto's thyroiditis. In this case, the compound of formula I functions directly to stimulate thyroid output.

In another aspect, the invention relates to a method for determining the success of thyroid ablation in a mammal. The method comprises the sequential steps of:

a) obtaining a first measure of thyroid output in a mammal whose thyroid has been ablated;

b) administering a diagnostically effective amount of a compound of formula I to the mammal; and

c) obtaining a second measure of thyroid output in the mammal;

In this case, the observation of an increase between the first and second measurement indicates metastasis or incomplete ablation. In one embodiment, the measure of thyroid output is thyroglobulin concentration.

In another aspect, the invention relates to a method for activating a thyroid stimulating hormone receptor in a mammal comprising administering to the mammal an amount of a compound of formula I.

In another aspect, the invention relates to a method for activating a thyroid stimulating hormone receptor in at least one mammalian cell comprising obtaining a sample of thyroid tissue from a mammal and bringing a compound of formula I into contact with the sample.

In another aspect, the invention relates to a method for treating a bone degenerative disorder, such as osteoporosis, comprising administering to a mammal a therapeutically effective amount of a compound of formula I.

In another aspect, the invention relates to pharmaceutical compositions comprising a pharmaceutically acceptable carrier and a compound of formula I or one of the subgenera
described below.

**Detailed Description of the Invention**

[014] In its most basic aspects, the invention relates to the use of compounds of formula I:

![Chemical Structure](image)

for treating thyroid diseases. In some embodiments, $R^2$ is O, and in some it is S. In some embodiments $R^3$ is hydrogen or methyl. In some embodiments, $R^4$ is selected from methyl and optionally substituted phenyl; usually $R^4$ is methyl. In some embodiments $R^1$ is selected from hydrogen, methyl, ethyl, propyl, butyl, methoxy, ethoxy, -CF$_3$, -CF$_2$CF$_3$, -SCH$_3$, -SCF$_3$, -OCF$_3$, phenyl, phenyloxy, benzyloxy, phenylthio, benzylthio, -CN, and -CO$_2$CH$_3$. In some embodiments $R^1$ is selected from -CF$_3$, ethyl, isopropyl, and -SCH$_3$.

[015] There are two primary subclasses of formula I, the esters and the amides. In the esters, $R^5$ is -OR$^{5a}$. In some ester embodiments, $R^{5a}$ is selected from (Ci-C6)alkyl and benzyl, and benzyl may be optionally substituted with (Ci-C4)alkyl or -OCH$_2$O-. In other ester embodiments, $R^{5a}$ is selected from (Ci-C4)alkyl, benzyl, and methylenedioxybenzyl. In a particular ester embodiment, $R^2$ is O, $R^3$ is hydrogen, and $R^4$ is methyl. In the amides, $R^5$ is -NR$^{5b}$R$^{5c}$. In some amide embodiments, $R^{5b}$ is selected from hydrogen, methyl, phenyl,
and benzyl. In particular amide embodiments, R² is O, R³ is hydrogen, R⁴ is methyl, R⁵b is benzyl or substituted benzyl and R⁵c is hydrogen or methyl.

[016] Throughout this specification the terms and substituents retain their definitions.

[017] Ci to C20 hydrocarbon includes alkyl, cycloalkyl, polycycloalkyl, alkenyl, alkynyl, aryl and combinations thereof. Examples include benzyl, phenethyl, cyclohexylmethyl, adamantyl, camphoryl and naphthylethyl. Hydrocarbyl refers to any substituent comprised of hydrogen and carbon as the only elemental constituents. Aliphatic hydrocarbons are hydrocarbons that are not aromatic; they may be saturated or unsaturated, cyclic, linear or branched. Examples of aliphatic hydrocarbons include isopropyl, 2-butenyl, 2-butynyl, cyclopentyl, norbornyl, etc. Aromatic hydrocarbons include benzene (phenyl), naphthalene (naphthyl), anthracene, etc.

[018] Unless otherwise specified, alkyl (or alkenylene) is intended to include linear or branched saturated hydrocarbon structures and combinations thereof. Alkyl refers to alkyl groups from 1 to 20 carbon atoms, preferably 1 to 10 carbon atoms, more preferably 1 to 6 carbon atoms. Examples of alkyl groups include methyl, ethyl, propyl, isopropyl, n-butyl, s-butyl, t-butyl and the like. The use of the generic term includes all isomers; thus, for example, "butyl" encompasses all four-carbon alkyls: w-butyl, sec-butyl, isobutyl and i-butyl.

[019] Cycloalkyl is a subset of hydrocarbon and includes cyclic hydrocarbon groups of from 3 to 8 carbon atoms. Examples of cycloalkyl groups include cy-propyl, cy-butyl, cy-pentyl, norbornyl and the like.

[020] Unless otherwise specified, the term "carbocycle" is intended to include ring systems in which the ring atoms are all carbon but of any oxidation state. Thus (C3-C10) carbocycle refers to both non-aromatic and aromatic systems, including such systems as cyclopropane, benzene and cyclohexene; (Cs-Ci₂) carbopolycycle refers to such systems as norbornane, decalin, indane and naphthalene. Carbocycle, if not otherwise limited, refers to monocycles, bicycles and polycycles.

[021] Heterocycle means an aliphatic or aromatic carbocycle residue in which from one to four carbons is replaced by a heteroatom selected from the group consisting of N, O, and S. The nitrogen and sulfur heteroatoms may optionally be oxidized, and the nitrogen heteroatom may optionally be quaternized. Unless otherwise specified, a heterocycle may be non-
aromatic (heteroaliphatic) or aromatic (heteroaryl). Examples of heterocycles include pyrrolidine, pyrazole, pyrrole, indole, quinoline, isoquinoline, tetrahydroisoquinoline, benzo furan, benzodioxan, benzodioxole (commonly referred to as methylenedioxyphenyl, when occurring as a substituent), tetrazole, morpholine, thiazole, pyridine, pyridazine, pyrimidine, thiophene, furan, oxazole, oxazoline, isoxazole, dioxane, tetrahydrofuran and the like. Examples of heterocycyl residues include piperazinyl, piperidinyl, pyrazolidinyl, imidazolyl, imidazolinyl, imidazolidinyl, pyrazinyl, oxazolidinyl, isoazolidinyl, thiazolidinyl, isothiazolyl, quinuclidinyl, isothiazolidinyl, benzimidazolyl, thiadiazolyl, benzopyranyl, benzothiazolyl, tetrahydrofuryl, tetrahydropyranyl, thiencyl (also historically called thiophenyl), benzothienyl, thiamorpholinyl, oxadiazolyl, triazolyl and tetrahydroquinolinyl.

[022] Hydrocarbyloxy refers to groups of from 1 to 20 carbon atoms, preferably 1 to 10 carbon atoms, more preferably 1 to 6 carbon atoms attached to the parent structure through an oxygen. Alkoxy is a subset of hydrocarbyloxy and includes groups of a straight or branched configuration. Examples include methoxy, ethoxy, propoxy, isoproxy and the like. For the purpose of this disclosure, alkoxy (and hydrocarbyloxy) includes methylenedioxy and ethylenedioxy. Lower-alkoxy refers to groups containing one to four carbons. The term "halogen" means fluorine, chlorine, bromine or iodine atoms.

[023] Unless otherwise specified, acyl refers to formyl and to groups of 1, 2, 3, 4, 5, 6, 7 and 8 carbon atoms of a straight, branched, cyclic configuration, saturated, unsaturated and aromatic and combinations thereof, attached to the parent structure through a carbonyl functionality. Examples include acetyl, benzoyl, propionyl, isobutyryl and the like. Lower-acyl refers to groups containing one to four carbons. The double bonded oxygen, when referred to as a substituent itself is called "oxo".

[024] As used herein, the term "optionally substituted" may be used interchangeably with "unsubstituted or substituted". The term "substituted" refers to the replacement of one or more hydrogen atoms in a specified group with a specified radical. For example, substituted alkyl, aryl, cycloalkyl, heterocyclyl etc. refer to alkyl, aryl, cycloalkyl, or heterocyclyl wherein one or more H atoms in each residue are replaced with halogen, haloalkyl, alkyl, acyl, alkoxyalkyl, hydroxy lower alkyl, carbonyl, phenyl, heteroaryl, benzenesulfonyl, hydroxy, lower alkoxy, haloalkoxy, oxaalkyl, carboxy, alkoxycarbonyl [-C(=O)0-alkyl], alkoxycarbamino [ HNC(=O)0-alkyl], aminocarbonyl (also known as carboxamido) [-
C(=0)NH₂, alkylaminocarbonyl [-C(=0)NH-alkyl], cyano, acetoxy, nitro, amino, alkylamino, dialkylamino, (alkyl)(aryl)aminoalkyl, alkylaminoalkyl (including cycloalkylaminoalkyl), dialkylaminoalkyl, dialkylaminoalkoxy, heterocyclylalkoxy, mercapto, alkylthio, sulfoxide, sulfone, sulfonlamino, alkylsulfinyl, alkylsulfonyl, acylaminoalkyl, acylaminoalkoxy, acylamino, amidino, aryl, benzyl, heterocycl, heterocyclylalkyl, phenoxy, benzyl, heteroaryloxy, hydroxyimino, alkoxylimino, oxalkyl, aminosulfonyl, trityl, amidino, guanidino, ureido, benzylxyphenyl, and benzylxy. "Oxo" is also included among the substituents referred to in "optionally substituted"; it will be appreciated by persons of skill in the art that, because oxo is a divalent radical, there are circumstances in which it will not be appropriate as a substituent (e.g. on phenyl). In one embodiment, 1, 2, or 3 hydrogen atoms are replaced with a specified radical. In the case of alkyl and cycloalkyl, more than three hydrogen atoms can be replaced by fluorine; indeed, all available hydrogen atoms could be replaced by fluorine. In preferred embodiments, substituents are halogen, halo(Ci-C4)hydrocarbyl, halo(Ci-C4)hydrocarbyloxy, cyano, thiocyanato, (Ci-C4)hydrocarbylsulfinyl, (Ci-C4)hydrocarbylsulfonol, aminosulfonyl, nitro, acetyl, and acetamido.

[025] Substituents Rⁿ are generally defined when introduced and retain that definition throughout the specification and in all independent claims. The abbreviations Me, Et, Ph, Tf, Ts, Ms and Bn represent methyl, ethyl, phenyl, trifluoromethanesulfonyl, toluensulfonyl, methanesulfonyl, and benzyl respectively. A comprehensive list of abbreviations utilized by organic chemists (i.e. persons of ordinary skill in the art) appears in the first issue of each volume of the Journal of Organic Chemistry. The list, which is typically presented in a table entitled "Standard List of Abbreviations" is incorporated herein by reference.

[026] Preparation of compounds can involve the protection and deprotection of various chemical groups. The need for protection and deprotection, and the selection of appropriate protecting groups, can be readily determined by one skilled in the art. Suitable groups for that purpose are discussed in standard textbooks in the field of chemistry, such as Protective Groups in Organic Synthesis by T.W. Greene and P.G.M. Wuts [John Wiley & Sons, New York, 1999], in Protecting Group Chemistry, 1st Ed., Oxford University Press, 2000; and in March's Advanced Organic chemistry: Reactions, Mechanisms, and Structure, 5th Ed., Wiley-Interscience Publication, 2001.
In general, compounds of formula I can be prepared as shown in Scheme 1. The appropriately substituted starting materials and intermediates used in the preparation of compounds of the invention are either commercially available or readily prepared by methods known in the literature to those skilled in the art. Many species within genus I are known in the art, and some are commercially available. Compounds described herein were purchased from ChemBridge Corporation, San Diego, CA. The compounds are made by the well-known Biginelli reaction. Ethyl acetoacetate, an aryl aldehyde and urea are reacted, usually in the presence of a Lewis acid or Bronsted acid, such as copper(II) trifluoroacetate hydrate or boron trifluoride.

The following are examples of species in the genus I:

When it is desired that R² be sulfur, the pyrimidinone above can be treated with P₂S₅ or Lawesson's reagent. The nitrogen may be alkylated to provide compounds in which R³ is other than hydrogen. To produce compounds in which R⁵ is -NR₅R₅', the ester in which R₅⁵ is methyl or ethyl may be saponified and the resulting acid condensed with the appropriate amine by methods well-known in the peptide art.
The compounds described herein may contain an asymmetric center (depending on substitution) and may thus give rise to enantiomers, diastereomers, and other stereoisometric forms which may be defined in terms of absolute stereochemistry as \((R)\)- or \((S)\)-. The present invention is meant to include all such possible diastereomers as well as their racemic and optically pure forms. Optically active \((R)\)- and \((S)\)- isomers may be prepared using homochiral synthons or homo-chiral reagents, or optically resolved using conventional techniques. All tautomeric forms are intended to be included.

As used herein, and as would be understood by the person of skill in the art, the recitation of "a compound" - unless expressly further limited - is intended to include salts of that compound, although most compounds of the invention do not form salts under physiologic conditions.

The term "pharmaceutically acceptable salt" refers to salts prepared from pharmaceutically acceptable non-toxic acids or bases including inorganic acids and bases and organic acids and bases. When the compounds of the present invention are basic, salts may be prepared from pharmaceutically acceptable non-toxic acids including inorganic and organic acids. Suitable pharmaceutically acceptable acid addition salts for the compounds of the present invention include acetic, adipic, alginic, ascorbic, aspartic, benzenesulfonic (besylate), benzoic, boric, butyric, camphoric, camphorsulfonic, carbonic, citric, ethanedisulfonic, ethanesulfonic, ethylenediaminetetraacetic, formic, fumaric, glucoheptonic, gluconic, glutamic, hydrobromic, hydrochloric, hydroiodic, hydroxynaphthoic, isethionic, lactic, lactobionic, laurylsulfonic, maleic, malic, mandelic, methanesulfonic, mucic, naphthylsulfonic, nitric, oleic, pamoic, pantothenic, phosphoric, pivalic, polygalacturonic, salicylic, stearic, succinic, sulfuric, tannic, tartaric acid, teoclatic, p-toluenesulfonic, and the
like. When the compounds contain an acidic side chain, suitable pharmaceutically acceptable base addition salts for the compounds of the present invention include, but are not limited to, metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from lysine, arginine, N,N'-dibenzylethlenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium cations and carboxylate, sulfonate and phosphonate anions attached to alkyl having from 1 to 20 carbon atoms.

[033] Also provided herein is a pharmaceutical composition comprising a compound disclosed above, or a pharmaceutically acceptable salt form thereof, and a pharmaceutically acceptable carrier or diluent.

[034] While it may be possible for the compounds of formula I to be administered as the raw chemical, it is preferable to present them as a pharmaceutical composition. According to a further aspect, the present invention provides a pharmaceutical composition comprising a compound of formula I or a pharmaceutically acceptable salt thereof, together with one or more pharmaceutically carriers thereof and optionally one or more other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

[035] The formulations include those suitable for oral, parenteral (including subcutaneous, intradermal, intramuscular, intravenous and intraarticular), rectal and topical (including dermal, buccal, sublingual and intraocular) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association a compound of formula I with the carrier, which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both and then, if necessary, shaping the product into the desired formulation.

[036] Formulations of the present invention suitable for oral administration maybe presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion.
or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

[037] A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and maybe formulated so as to provide sustained, delayed or controlled release of the active ingredient therein.

[038] Formulations for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient. Formulations for parenteral administration also include aqueous and non-aqueous sterile suspensions, which may include suspending agents and thickening agents. The formulations may be presented in unit-dose of multi-dose containers, for example sealed ampoules and vials, and maybe stored in a freeze-dried (lyophilized) condition requiring only the addition of a sterile liquid carrier, for example saline, phosphate-buffered saline (PBS) or the like, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

[039] In the methods of treatment, wherein the disease or condition is one in which the thyroid is hyperactive, the compound of formula I maybe administered in combination with radioactive iodine. The compound of formula I acts as an agonist (analogously to natural TSH or Thyrogen®) to stimulate the thyroid to take up the radioiodine, resulting in chemical ablation. The initial therapy for most patients with well differentiated thyroid cancer is total or near-total thyroidectomy. Thyroidectomy is followed by radioactive iodine ($^{131}$I) thyroid remnant ablation to destroy residual thyroid tissue. In this procedure, the compound of formula I is given either concurrently with $^{131}$I, or the two are administered separately, usually with the $^{131}$I administered 12- 48 hours after one or more doses of the compound of formula I.
It will be recognized that the compounds of this invention can exist in radiolabeled form, i.e., the compounds may contain one or more atoms containing an atomic mass or mass number different from the atomic mass or mass number most abundant in nature. Radioisotopes of hydrogen, carbon, phosphorous, fluorine, and chlorine include $^3$H, $^3$H, $^{13}$C, $^{14}$C, $^{15}$N, $^{35}$S, $^{18}$F, and $^{36}$Cl, respectively. Compounds that contain those radioisotopes and/or other radioisotopes of other atoms are within the scope of this invention. Tritiated, i.e., $^3$H, and carbon-14, i.e., $^{14}$C, radioisotopes are particularly preferred for their ease in preparation and detectability. Compounds that contain isotopes $^{11}$C, $^{13}$N, $^{15}$O and $^{18}$F are well suited for positron emission tomography. Radiolabeled compounds of formula I of this invention and prodrugs thereof can generally be prepared by methods well known to those skilled in the art. Conveniently, such radiolabeled compounds can be prepared by carrying out the procedures disclosed in the Examples and Schemes by substituting a readily available radiolabeled reagent for a non-radiolabeled reagent.

The compounds of the invention were tested in the following screens:

Stable cell lines used in the study: CHO-HA - TSHR luciferase cells for primary screening were generated by transfecting pGL4.29 [luc2P/CRE/Hygro] construct into a highly selected stable line of HATSHR CHO cells as described by Nagayama et al., [J Clin Invest. 1991;88(1):336-340] and selecting them with hygromycin. TSHR/LHR chimeric luciferase cells were prepared using a construct pSV2-neo-ECE-TSH-LHR-1 1 (Kindly provided us by Dr. Basil Rapoport, Cedars-Sinai Research institute and University of California, Los Angeles, CA). In these cells, a 367 amino acid insert containing the homologous regions of the rat LH/CG receptor sequence replaced the TSHR ectodomain, which was then co-transfected with the pGL4.29 [luc2P/CRE/Hygro] construct in CHO cells and further selected for double transfectants with optimal concentrations of neomycin sulphate and hygromycin. Parent CHO luciferase cells were generated by transfecting pGL4.29 [luc2P/CRE/Hygro] construct into CHO PSVL cells (JP02) and selecting with hygromycin for stable transformants. The best stable clone was selected based on different concentrations of forskolin and unresponsiveness to TSH. All of these above mentioned stable cell lines were cultured in Ham's F-12 medium with 10% fetal bovine serum (FBS) and 100IU/ml of penicillin and streptomycin and 50ug/ml of hygromycin.

For specificity studies against FSH receptor, we used primary Sertoli cells (TM4) obtained from ATCC (CRL-1715) and cultured in DMEM: F12 medium (cat # 30-2006) with
2.5% FBS and 5% horse serum (ATCC; cat #30-2040). The specificity against the LH/hCG receptor was tested using stable line of rat hCGR in HEK 293 cells that we obtained from Dr
K.M.J Menon, University of Michigan, Ann Arbor, Michigan.

[044] For studying response of the test compounds via various G proteins as described by Cheng et al. [Curr Chem Genomics. 2010;4:84-91], we generated double transfected stable lines of CHO-HA: TSHR with pGL4.34 [luc2P/SRF-RE/Hygro],
pGL4.33[luc2P/SRE/Hygro] and pGL4.30 [luc2P/NFAT-RE/Hygro] respectively. These double transfected stable lines were also maintained in complete HamL2 medium with appropriate concentrations of hygromycin.

[045] To develop the screening assay, a high expressing stable line of CHO-HA: TSHR cells carrying an amino terminus HA tagged TSHR was selected. These stable CHO cells were transfected with the construct pGL4.29 [CRE/minP/luc2P] carrying a minimal promoter driving a CREB response element (CRE) tagged to a modified form of luciferase reporter gene luc2P. Luc2P is a synthetically derived luciferase sequence with humanized codon optimization that is designed for high expression and reduced anomalous transcription. In addition the luc2P gene contains hPEST, a protein destabilization sequence, which further reduces background, transcribed protein. Activation of the TSH receptor by TSH or an agonist results in Gsa-adenylate cyclase coupling and increase in intracellular cAMP, which binds to the CRE element and results in the transcription of luciferase gene and accumulation of luciferase enzyme within the activated cells. Luciferase in these cells was detected after lysing the cells using the commercial substrate Bright Glow (Promega Corporation, Madison, WI). For screening, we seeded 15,000 cells of HATSHR luci cells per well in a 384 opaque white bottom poxi-plate (PerkinElmer- ProxiPlate cat # 6008230) using Combi well dispenser in 10uL of Ham's F12 complete medium and incubated overnight at 37°C in a CO2 incubator with relative humidity of > 85%. The plates were then pinned with 17uL of each compound from the library and positives and negative controls on either ends of the plate and the plates were then incubated for 4hrs at 37°C. At the end of 4hrs the cells were lysed by adding 8uL of Bright Glow reagent and incubated for 2 minutes before measuring luciferase activity using the EnVision Multilabel Reader (PerkinElmer, Branford, CT). Throughout the screen, the signal to background ratio was linear and the mean CV was 12 % and Z' score was in the range of 0.7-0.8. Dose responses of the test compounds were done using Tecan HP dispenser by following a similar protocol. Data points of the dose - response curves were
fitted using Prism 5.0.

[046] Results of testing of examples of compounds of the invention in the foregoing TSHR luciferase screen are shown in Table 1:

Table 1

<table>
<thead>
<tr>
<th>Example #</th>
<th>Relative activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>++++</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>++++</td>
</tr>
</tbody>
</table>

[047] RNA isolation: Total RNA was isolated from FRTL5 untreated with 1µM and 10µM of the compound of example 1 for 4hrs using TRizol reagent (Invitrogen, Life Technologies, Carlsbad, CA, USA) and chromosomal DNA from this was removed in accordance with the manufacturer's instructions. The RNA concentration was determined on the basis of absorbance at 260 nm, and its purity was evaluated by the ratio of absorbance at 260/280 nm (>1.9). RNAs were kept frozen at -70°C until analyzed. After digestion of genomic DNA by treatment with Ambion's TURBO DNA-freeTM DNase I (Ambion, Austin, TX), Total RNA (1 µg) was reverse-transcribed into cDNA with random hexamers using Advantage RT-for-PCR kit (Clontech).

[048] Quantitative Reverse Transcription-PCRs (qRT-PCR): The qRT-PCRs were performed using the Applied Biosystem StepOnePlus Real-time PCR system. The reactions were established with 10 µL of SYBR Green master mix (Applied Biosystems, Foster City,
CA), 0.4 µ (2 µM) of sense/anti-sense gene-specific primers, 2 µ of cDNA and DEPC-treated water to a final volume of 20 µL. The PCR reaction mix was denatured at 95 °C for 60 s before the first PCR cycle. The thermal cycle profile was: denaturizing for 30 s at 95 °C; annealing for 30 s at 57-60 °C (dependent on primers); and extension for 60 s at 72°C. A total of 40 PCR cycles were used. An average Ct (threshold cycle) from duplicate or triplicate assays was used for further calculation. For each target gene, the relative gene expression was normalized to that of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping gene by use of Applied Biosystem StepOnePlus Real-time PCR systems software. Sample sets were analyzed in duplicates.

[049] Mouse Thyroid Function Testing: Female C57BL/6 mice (Jackson Laboratory) of 6-8 weeks old with mean body weight of 20 g maintained on standard diet were injected intraperitoneal (IP) with 100 µg/mouse of the compound of example 1 for three consecutive days in a fluid volume of 60-90 µL containing a final concentration of ~25% DMSO. The control animals received diluted vehicle (DMSO) or bovine TSH 30 µg/mouse by the same route. Thyroid hormone (T4) levels were estimated in serum from blood collected by submandibular bleeding prior to treatment (pre bleed) and 72 hours post treatment (post bleed). Total T4 was measured with Neonatal free T4 RIA kit (Coat-A-Count, Siemens Medical Solutions Diagnostics, CA) according to the manufacturer's protocol. All experiments involving animals were carried out according to the institutional animal care committee guidelines.

[050] G protein Signaling studies: As outlined above, before for studying the activation of various G-proteins, we developed stable CHO-HA: TSHR cell lines expressing various reporter vectors (CRE-, NFAT-RE, SRE-, SRF-RE-). These stable lines were characterized and optimized for responses using positive (TSH, inomycin, PMA) and negative controls. Prior to measurement of signaling, 20,000 cells were seeded in square bottom white plates (Nunc cat # 164610) in 20 µL of Ham's F12 complete medium and incubated overnight at 37°C. Following the complete medium was replaced with serum free medium for 2hrs and then treated with 10 µM of compound and the appropriate controls for 4hrs. At the end of incubation period the cells were lysed using 10 µL of Bright Glow reagent and incubated for 2 minutes at RT and the plates were read using BMG Pherastar microplate reader.

[051] Pharmacokinetic studies were carried out. Briefly, a group of eighteen mice was used for testing each compound. The animals were weighed before the dose administration and
divided into two groups. Group I was dosed intravenously and Group II was dosed intraperitoneally with solution at a dose of 20mg/kg body weight. Blood samples were collected at pre-dose 2, 4, 6, 12 and 24 hrs (i.v & i.p). Blood was collected from a set of three mice under light isoflurane anesthesia from retro orbital plexus at each time point in tubes containing K2EDTA as anticoagulant. Plasma samples were processed for analysis by protein precipitation using acetonitrile. Glipizide was used as internal standard and analyzed with LC-MS/MS method. Pharmacokinetic parameters were calculated using the non-compartmental analysis tool of Phoenix WinNonlin Enterprise software (version 6.3).

[052] All curve fitting and EC50 calculations were done using GraphPad Prism version 5.02 and statistical differences for P values were calculated using one-way ANOVA build into Prism.

[053] To identify allosteric modulators to the TSHR, compounds were screened at a single concentration of 10µM in duplicate plates and considered preliminarily active if a significant response was obtained in both plates and with the selection criteria being greater than +3SD (standard deviation) above the basal stimulation. False positives are commonly found in any cell-based signaling assays, therefore to identify true agonist compounds the test compound is subjected to a second confirmatory testing using TSHR-containing CHO cells and also CHO cells containing an empty vector (parent cells). Based on this second round of testing, the compound of example 1 showed >10 fold responses above the baseline and no activity on parent CHO luciferase cells. Example 1 exhibited an EC50 of 5.3 x10^-9 M.

[054] To analyze the specificity of compounds of formula I, Example 1 was tested against cells that expressed the LH receptor and FSH receptor. For the LH receptor cells we used HEK 293 transfected with rat hCG receptor and for FSH receptor cells we used primary murine Sertoli cells (line TM4) that express the FSHR and that respond to FSH in a dose dependent manner. Intracellular cAMP was measured in these cells after stimulation with 0.1, 1 and 10µM of the test compound and corresponding positive and negative controls. The compound of example 1 did not show any activity against the LH-receptor- nor the FSH-receptor-expressing cells, even at the highest concentration used (10µM) and in contrast to the response of the cells against their respective ligands hCG and FSH incorporated as positive controls.

[055] Small molecules are known to often activate GPCRs in an allosteric manner by
binding to the transmembrane domain of the receptor. To examine if compounds of formula I bind to the transmembrane domain of the TSHR we used a chimeric construct in which the TSHR ectodomain is replaced with the LH receptor ectodomain but retains the complete TSH receptor transmembrane domain. Stable cells co-transfected with this chimeric receptor and luciferase construct responded to hCG (1000mU/mL) but not to recombinant human TSH, indicating the specificity of the ligand binding ectodomain. On exposure to 10μM of example 1, the cells showed equivalent or greater responses than hCG and forskolin, indicating that the molecule bound to the serpentine portion of the TSHR. The failure of blocking TSHR antibodies that bind to the large ectodomain to dampen the response to example 1 while effectively blocking the TSH response by more than 50% further suggests an allosteric mode of action for compounds of formula I.

[056] Classical and Non-classical G-protein signaling studies: The TSHR has been reported to activate members of all four G protein families (Gas, Gq/11, Gi/o and G12/13). We studied the signaling potential of Example 1 using a quantitative technique via the tagged response elements for CRE, SRE, SF-SRE and NFAT. Based on these constructs, the major pathway that appears to be activated by example 1 was the classical Gas pathway. Similarly, examining the other G-proteins for non-classical pathway responses, it appears that example 1 was able to activate Gq/11 by increasing NFAT. Example 1 showed no significant activation of RhoA kinase via SRF luciferase nor ERK1/2 by SRE luciferase, indicating that it did not engage Gβγ nor Gal2. However, the activation of Gs and Gq by example 1, similar to TSH, would strongly suggest that compounds of formula I are able to initiate iodine organification and thyroid hormone secretion and promote thyroid growth by their ability to engage in Gq activation in the same manner as TSH itself.

[057] In order to confirm the activity of these molecules on more physiologically relevant cells, we examined thyroid specific gene expression using rat thyrocytes (FRTL5). Example 1 was tested for its effect on expression of mRNA for thyroglobulin (TG), sodium-iodide symporter (NIS) thyroid peroxidase (TPO) and TSH receptor expression using FRTL5. Prior to exposure the cells were deprived of TSH for 48hrs and starved in serum free medium for another 2hrs. Single-dose treatment of 1μM of Example 1 for 4hrs showed a 2.8 fold increase in thyroid specific gene expression (Tg, NIS and TSHR) when measured by qPCR. These data show that compounds of formula I have the ability to exert their effects on thyrocytes that express relatively low levels of the TSHR compared to the earlier transfected
cell lines.

[058] In vivo potency of Example 1: T4 levels were measured at different time points in Balb/c mice that received a single ip or iv injection of 20mg/kg body weight of example 1. T4 release was observed at 2hrs after the compounds reached peak levels in the blood but with no sustained action during the course of the study. T4 levels measured after 3 ip injections of 10IC^g/mouse of example 1 dissolved in DMSO showed a sustained two-fold increase serum T4 levels. These in vivo studies clearly indicate the effectiveness of compounds of formula I as agonists to the TSHR.

[059] The compound of example 1 was also subjected to standard pharmacokinetic studies. A single injection of example 1 at 20mg/kg was given to Balb/c mice intravenously and intraperitoneally and their plasma was analyzed by mass spectrometry at different points after reaching Tmax. The half-life (T1/2) was 1 hour by both routes of administration. Example 1 showed moderate plasma clearance of 29.63mL/min/kg and a high volume of distribution (4.6 fold higher than total body water) indicating extravascular distribution.

[060] Cytotoxicity studies in vitro showed no cytotoxic effects at the highest working concentration for example 1.

[061] The effect of compounds of formula I on osteoblast differentiation was studied using a Human osteoblast precursor line (hFOBl. 19). Alkaline phosphatase (ALP) and collagen were used as osteoblast differentiation markers. ALP, measured after treating the cells with 10μM of Example 1 in the presence of osteogenic differentiation factor (ODF) for 10 days, increased in a statistically significant manner. H89 (N-[2-((p-bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide), a known inhibitor of PKA/cAMP pathway, suppressed this response. In the same experiment, gene expression for collagen, another osteoblast marker, was measured and a similar enhancement of osteoblast differentiation was observed. Normally stimulation of cAMP can lead to proliferation of cells, so we measured proliferation colorimetrically on these cells at 72 hrs and observed that Example 1 indeed induced proliferation of osteoblast cells in a manner similar to TSH.
1. A method for treating a thyroid disease or condition comprising administering to a mammal a therapeutically effective amount of a compound of formula I:

\[
R^1 \text{ is selected from hydrogen, (C}_1\text{-C}_6\text{)alkyl, fluoro(C}_1\text{-C}_6\text{)alkyl, (C}_1\text{-C}_6\text{)alkoxy, fluoro(C}_1\text{-C}_6\text{)alkoxy, (C}_1\text{-C}_6\text{)alkythio, fluoro(C}_1\text{-C}_6\text{)alkylthio, aryl, aryloxy, arylthio, cyano, and alkoxy carbonyl;} \\
R^2 \text{ is selected from O and S;} \\
R^3 \text{ is selected from hydrogen and (C}_1\text{-C}_6\text{)alkyl;} \\
R^4 \text{ is (C}_1\text{-C}_6\text{)hydrocarbyl optionally substituted with one, two, or three substituents independently selected from -OH, halogen, (C}_1\text{-C}_6\text{)alkyl, (C}_1\text{-C}_6\text{)alkoxy, cyano, and nitro;} \\
R^5 \text{ is selected from -OR}^a \text{ and -NR}^b \text{R}^c; \\
R^5a \text{ is hydrogen or (C}_1\text{-C}_6\text{)hydrocarbyl, optionally substituted with one, two, or three substituents independently selected from -OH, halogen, (C}_1\text{-C}_6\text{)alkyl, (C}_1\text{-C}_6\text{)alkoxy, methylenedioxy, ethylenedioxy, cyano, and nitro;} \\
R^5b \text{ is (C}_1\text{-C}_6\text{)hydrocarbyl, optionally substituted with one, two, or three substituents independently selected from -OH, halogen, (C}_1\text{-C}_6\text{)alkyl, (C}_1\text{-C}_6\text{)alkoxy, methylenedioxy, ethylenedioxy, cyano, and nitro; and} \\
R^5c \text{ is selected from hydrogen and (C}_1\text{-C}_6\text{)alkyl.}
2. The method according to claim 1, wherein $R^2$ is O.

3. The method according to claim 1, wherein $R^2$ is S.

4. The method according to claim 1, wherein $R^3$ is selected from hydrogen and methyl.

5. The method according to claim 1, wherein $R^4$ is selected from methyl and optionally substituted phenyl.

6. The method according to claim 1, wherein $R^4$ is methyl.

7. The method according to claim 1, wherein $R^5$ is-OR $^5a$.

8. The method according to claim 7, wherein $R^5a$ is selected from (Ci-C6)alkyl and benzyl, wherein said benzyl is optionally substituted with (Ci-C4)alkyl or -OCH2O-.

9. The method according to claim 8, wherein $R^5a$ is selected from (Ci-C4)alkyl, benzyl, and methylenedioxybenzyl.

10. The method according to claim 1, wherein $R^5$ is -NR $^5b$$^5c$.

11. The method according to claim 1, wherein $R^5b$ is selected from hydrogen, methyl, phenyl, and benzyl.

12. The method according to claim 1, wherein $R^1$ is selected from hydrogen, methyl, ethyl, propyl, butyl, methoxy, ethoxy, -CF3, -CF2CF3, -SCH3, -SCF3, -OCF3, phenyl, phenyloxy, benzylxoy, phenylthio, benzylthio, -CN, and -CO2CH3.

13. The method according to claim 12, wherein $R^1$ is selected from -CF3, ethyl, isopropyl, and -SCH3.

14. The method according to claim 1, wherein
   
   $R^2$ is O;
   
   $R^3$ is hydrogen;
R4 is -CH3; and
R5 is -OR5a.

15. The method according to claim 14, wherein R5a is selected from (Ci-C6)alkyl and benzyl, and wherein said benzyl is optionally substituted with (Ci-C4)alkyl or -OCH2O-.

16. A method according to claim 1 wherein said disease or condition is chosen from Graves’ disease, thyroid cancer, and pituitary cancer, and wherein said compound of formula I is administered in combination with radioactive iodine.

17. A method according to claim 1 wherein said disease or condition is a hypothyroid condition.

18. A method according to claim 17 wherein said hypothyroid condition is Hashimoto’s thyroiditis.

19. A method for determining the success of thyroid ablation in a mammal comprising:
   a) obtaining a first measure of thyroid output in a mammal whose thyroid has been ablated;
   b) administering a diagnostically effective amount of a compound of formula I to said mammal; and
   c) obtaining a second measurement of thyroid output in said mammal; wherein an increase between said first and said second measurement indicates metastasis or incomplete ablation.

20. A method according to claim 19 wherein said measure of thyroid output is thyroglobulin concentration.

21. A method for activating a thyroid stimulating hormone receptor in a mammal comprising administering to the mammal an amount of a compound of formula I, as defined in claim 1.
22. A method for activating a thyroid stimulating hormone receptor in at least one mammalian cell comprising obtaining a sample of thyroid tissue from a mammal and bringing a compound of formula I, as defined in claim 1, into contact with said sample.

23. A method for treating a bone degenerative disorder comprising administering to a mammal a therapeutically effective amount of a compound of formula I as defined in claim 1.

24. A method according to claim 23 wherein said bone degenerative disorder is osteoporosis.

25. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound of formula I:

![Chemical Structure](attachment:structure.png)

wherein

R₁ is selected from hydrogen, (Ci-C6)alkyl, fluoro(Ci-C6)alkyl, (Ci-C6)alkoxy, fluoro(Ci-C6)alkoxy, (Ci-C6)alkylthio, fluoro(Ci-C6)alkylthio, aryl, aryloxy, arylthio, cyano, and alkoxy carbonyl;

R₂ is selected from O and S;

R₃ is selected from hydrogen and (Ci-Ce)alkyl;

R₄ is (Ci-Cio)hydrocarbyl optionally substituted with one, two, or three substituents independently selected from -OH, halogen, (Ci-Ce)alkyl, (Ci-C6)alkoxy, cyano, and nitro;

R₅ is selected from -OR₅ and -NR₅R₅c;
R$^a$ is hydrogen or (Ci-Cio)hydrocarbyl, optionally substituted with one, two, or three substituents independently selected from -OH, halogen, (Ci-C6)alkyl, (Ci-C6)alkoxy, methylenedioxy, ethylenedioxy, cyano, and nitro;

R$^b$ is (Ci-Cio)hydrocarbyl, optionally substituted with one, two, or three substituents independently selected from -OH, halogen, (Ci-C6)alkyl, (Ci-C6)alkoxy, methylenedioxy, ethylenedioxy, cyano, and nitro; and

R$^c$ is selected from hydrogen and (Ci-C6)alkyl.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01N 43/04 (2015.01)
CPC - C07H 19/06; A61K 31/70; A61K 31/7068

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A01N 43/04
CPC: C07H 19/06; A61K 31/70; A61K 31/7068

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 514/49; 514/50

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase. Keyword limited: Graves' disease, thyroid cancer, pituitary cancer, radioactive iodine, hypothyroid condition, Hashimoto's thyroiditis, thyroid ablation, thyroid output, thyroglobulin concentration, metastasis/incomplete ablation, thyroid stimulating hormone receptor, bone degenerative disorder, osteoporosis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>US 2008/0145453 A1 (LOPEZ, R et al.) 19 Jun 2008 (19.06.2008), entire document, especially: para [0035]; Compound 1; para [0044]; para [0045]; para [0050]</td>
<td>1, 3-9, 12, and 25</td>
</tr>
<tr>
<td>Y</td>
<td>LACOTTE, P et al. Synthesis, evaluation and absolute configuration assignment of novel dihydropyrimidin-2-ones as picomolar sodium iodide symporter inhibitors, European Journal of Medicinal Chemistry. 2013. Vol 62, pp 722-727, entire document, especially: pg 722, col 1, para 1; pg 723, Figure 1; pg 723, Figure 2; pg 723, Figure 2B; pg 723, col 2, para 2; pg 724, Table 1</td>
<td>2, 10-1, 11, 13-24</td>
</tr>
<tr>
<td>Y</td>
<td>WO 2007/101213 A2 (Roppe et al.) 7 September 2007 (07.09.2007), entire document, especially: pg 4, ln 25 to ln 27; pg 24, ln 21 to ln 28; pg 47, Example 42</td>
<td>2, 14, 15, 17, 21, 22</td>
</tr>
<tr>
<td>Y</td>
<td>MA, C et al. &quot;HTSH-aided low-activity versus high-activity regimens of radioiodine in residual ablation for differentiated thyroid cancer: a meta-analysis&quot;, Nuclear Medicine Communications. 2013. Vol 34(12), pp 1150-1156, entire document, especially: abstract; pg 1150, col 1, para 1; pg 1150, col 2; pg 1151, col 2, para 4; pg 1152, col 2, para 3; pg 1152, Table 1</td>
<td>10, 11-13</td>
</tr>
</tbody>
</table>

D. Date of the actual completion of the international search
2 September 2015 (02.09.2015)

E. Date of mailing of the international search report
29 SEP 2015

F. Authorized officer
Lee W. Young

G. PCT Hendisk: 971-272-4300
PCT ODP: 971-272-7774

Form PCT/ISA/2 10 (second sheet) (January 2015)