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(54) Title: METHODS OF MODULATING SPERM MOTILITY

(57) Abstract: The present invention relates to methods for enhancing mammalian sperm motility, mobility and vitality using cal-cimimetics.



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METHODS OF MODULATING SPERM MOTILITY

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims benefit of Provisional Application No. 61/209,678 filed March
5 10, 2009 the entirety of which is incorporated herein by reference.

FIELD OF THE INVENTION

This invention relates generally to the field of medicine and, more specifically, to
methods for enhancing mammalian sperm motility, mobility and vitality. This application
10 claims benefit of Provisional Application No. 61/209,678 filed March 10, 2009 the entirety of
which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

Male infertility is characterized by low sperm count, low motility and low proportion of
15 sperm with normal morphology. It is a disorder with largely unknown causes, probably related
to primary testicular disease or external factors such as trauma or environmental causes. It has
been suggested that the chances of conception with low sperm motility are lower than the
chances of conception with low sperm count because of the differences in causes between the
two. The most common cause of low sperm motility is a genetic problem. In addition, there
20 are fewer treatments that have proven to be effective in increasing the chances of conception
with low sperm motility (asthenozoospermia) than there are for other causes of infertility.
Analysis of sperm motility includes a description of specific motility characteristics such as
speed, straight-line swimming or "hyperactivity" (a wildly gyrating swim pattern).

Research into the causes of male infertility has lagged considerably behind the success
25 in other areas of medicine, leaving approximately 40-70% of patients with disorders described
as "idiopathic" (i.e. the cause of which is unknown). Treatment of male infertility includes
mostly addressing known reversible factors first. For example, discontinuing any medication
known to have an effect on spermatogenesis or ejaculation, as well as decreasing alcohol
intake, and treating thyroid or other endocrine disease.

30 There are currently no available or acceptable pharmaceutical treatments for male
infertility or subfertility.

SUMMARY OF THE INVENTION

The present invention provides methods for enhancing the motility, mobility and viability of a mammalian spermatozoon comprising contacting the mammalian spermatozoon with a therapeutically effective amount of a calcimimetic compound. In one aspect, a plurality of mammalian spermatozoa are identified as having decreased motility. In another aspect, mammalian spermatozoa are identified as having subnormal motility. In a further aspect, mammalian spermatozoa are identified as having normal motility, but the subject is in need of increasing sperm motility to increase chances of fertilization.

In one aspect, the contacting comprises culturing the spermatozoon in a physiological medium comprising a therapeutically effective amount of a calcimimetic compound. Contacting may be, for example, from about 30 seconds to about 120 minutes or from about 1 minute to 20 minutes.

In one aspect of the invention, the spermatozoon can be substantially separated from seminal plasma. In another aspect, the contacting may occur in a mammalian female reproductive tract. In one aspect, the mammalian female can be a human. In other aspect, the mammalian aspect can be a horse, a pig, a goat, a sheep, or a cow.

The invention further provides methods of fertilizing a mammalian egg, the method comprising contacting a mammalian egg with a mammalian spermatozoon, whose motility has been enhanced contacting the mammalian spermatozoon with a therapeutically effective amount of a calcimimetic compound. In one aspect, the contacting of the mammalian egg occurs *in vitro*. In one aspect, the method may be further comprising fertilization of the mammalian egg and placing the fertilized egg in a mammalian uterus. In another aspect, the contacting of the mammalian egg occurs *in vivo*. In a further aspect, the contacting of the mammalian egg occurs in a female reproductive tract. In one aspect, the subject can be mammal. For example, the mammal may be a horse, a pig, a goat, a sheep, or a cow. In one aspect, the subject may be human. In one aspect, the mammalian egg is human. In other aspects, the mammalian egg can be from a horse, a pig, a goat, a sheep, or a cow or any other mammal.

The calcimimetic compounds useful in the methods of the present invention are described in detail in Detailed Description below.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the expression of the calcium sensing receptor (CaSR) in as demonstrated by immunohistochemistry staining in rat testicle. a) Sertoli cells; b)

spermatogonias I and II; c) spermatocyte I and II; d) spermatides; e) spermatozoa; f) Leydig cells. Magnification X40.

Figure 2 demonstrates that the CaSR is expressed both in basal and principal epididymal cells as demonstrated by immunohistochemistry staining of the CaSR. a) Basal epididymal cells; b) principal epididymal cells; c) spermatozoa. Magnification X20.

Figure 3 shows immunohistochemistry staining of the CaSR within the epididymal duct. The staining is more pronounced in the head of spermatozoa. Magnification X40.

Figure 4 demonstrates the effect of calcimimetic Compound A (*N*-((6-(methoxy)-4'-(trifluoromethyl)-1,1'-biphenyl-3-yl)methyl)-1-phenylethanamine) on rat sperm motility after 60 and 120 minutes of incubation. Results are presented as the mean $\pm$ SE of a semiquantitative score (0-4).

Figure 5 illustrates the effect of calcimimetic Compound A on pig sperm motility after 60 and 240 minutes of incubation. Influence of Compound A on the percentage of cells that reach a mean velocity (VAP) $> 45 \mu\text{m/s}$.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. In case of conflict, the present document, including definitions, will control.

As used herein, the term "subject" is intended to mean a human, or an animal, in need of a treatment.

"Treating" or "treatment" of a disease includes: (1) preventing the disease, *i.e.*, causing the clinical symptoms of the disease not to develop in a subject that may be or has been exposed to the disease or conditions that may cause the disease, or predisposed to the disease but does not yet experience or display symptoms of the disease, (2) inhibiting the disease, *i.e.*, arresting or reducing the development of the disease or any of its clinical symptoms, or (3) relieving the disease, *i.e.*, causing regression of the disease or any of its clinical symptoms.

Administration "in combination with" or "together with" one or more further therapeutic agents includes simultaneous or concurrent administration and consecutive administration in any order.

The phrase "therapeutically effective amount" is the amount of the compound of the invention that will achieve the goal of improvement in disorder severity and the frequency of incidence. The improvement in disorder severity includes the reversal of the disease, as well as slowing down the progression of the disease.

As used herein, “calcium sensing receptor” or “CaSR” refers to the G-protein-coupled receptor responding to changes in extracellular calcium and/or magnesium levels. Activation of the CaSR produces rapid, transient increases in cytosolic calcium concentration by mobilizing calcium from thapsigargin-sensitive intracellular stores and by increasing calcium influx through voltage-insensitive calcium channels in the cell membrane (Brown *et al.*, Nature 366: 575-580, 1993; Yamaguchi *et al.*, *Adv Pharmacol* 47: 209-253, 2000).

The terms “spermatozoon” and “spermatozoa” are used interchangeably with the term “sperm”.

Asthenospermia is defined as less than 60% motility, frequently with subnormal sperm velocity.

II. Calcimimetic compounds and pharmaceutical compositions comprising them, administration and dosage

A. *Calcimimetic compounds, definitions*

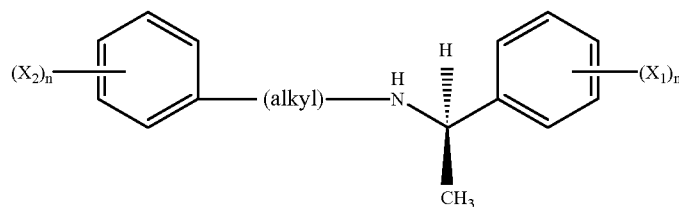
As used herein, the term “calcimimetic compound” or “calcimimetic” refers to a compound that binds to calcium sensing receptors and induces a conformational change that reduces the threshold for calcium sensing receptor activation by the endogenous ligand Ca^{2+} . These calcimimetic compounds can also be considered allosteric modulators of the calcium receptors.

In one aspect, a calcimimetic can have one or more of the following activities: it evokes a transient increase in internal calcium, having a duration of less than 30 seconds (for example, by mobilizing internal calcium); it evokes a rapid increase in $[\text{Ca}^{2+}]_i$, occurring within thirty seconds; it evokes a sustained increase (greater than thirty seconds) in $[\text{Ca}^{2+}]_i$ (for example, by causing an influx of external calcium); evokes an increase in inositol-1,4,5-triphosphate or diacylglycerol levels, usually within less than 60 seconds; and inhibits dopamine- or isoproterenol-stimulated cyclic AMP formation. In one aspect, the transient increase in $[\text{Ca}^{2+}]_i$ can be abolished by pretreatment of the cell for ten minutes with 10 mM sodium fluoride or with an inhibitor of phospholipase C, or the transient increase is diminished by brief pretreatment (not more than ten minutes) of the cell with an activator of protein kinase C, for example, phorbol myristate acetate (PMA), mezerein or (-) indolactam V. In one aspect, a calcimimetic compound can be a small molecule. In another aspect, a calcimimetic can be an agonistic antibody to the CaSR.

Calcimimetic compounds useful in the present invention include those disclosed in, for example, European Patent No. 637,237, 657,029, 724,561, 787,122, 907,631, 933,354, 1,203,761, 1,235,797, 1,258,471, 1,275,635, 1,281,702, 1,284,963, 1,296,142, 1,308,436,

1,509,497, 1,509,518, 1,553,078; International Publication Nos. WO 93/04373, WO 94/18959, WO 95/11221, WO 96/12697, WO 97/41090, WO 01/34562, WO 01/90069, WO 02/14259, WO 02/059102, WO 03/099776, WO 03/099814, WO 04/017908; WO 04/094362, WO 04/106280, WO 06/117211; WO 06/123725; WO 07/060026; WO 08/006625; WO 08/019690; U.S. Patent Nos. 5,688,938, 5,763,569, 5,962,314, 5,981,599, 6,001,884, 6,011,068, 6,031,003, 6,172,091, 6,211,244, 6,313,146, 6,342,532, 6,362,231, 6,432,656, 6,710,088, 6,750,255, 6,908,935, 7,157,498, 7,176,322 and U.S. Patent Application Publication No. 2002/0107406, 2003/0008876, 2003/0144526, 2003/0176485, 2003/0199497, 2004/0006130, 2004/0077619, 2005/0032796, 2005/0107448, 2005/0143426, European patent application PCT/EP2006/004166, French patent application 0511940.

In certain embodiments, the calcimimetic compound is chosen from compounds of Formula I and pharmaceutically acceptable salts thereof:



I

wherein:

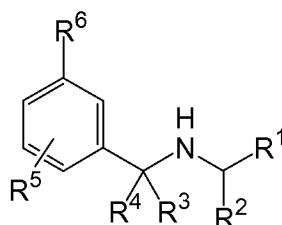
X_1 and X_2 , which may be identical or different, are each a radical chosen from CH_3 , CH_3O , CH_3CH_2O , Br, Cl, F, CF_3 , CHF_2 , CH_2F , CF_3O , CH_3S , OH, CH_2OH , $CONH_2$, CN, NO_2 , CH_3CH_2 , propyl, isopropyl, butyl, isobutyl, t-butyl, acetoxy, and acetyl radicals, or two of X_1 may together form an entity chosen from fused cycloaliphatic rings, fused aromatic rings, and a methylene dioxy radical, or two of X_2 may together form an entity chosen from fused cycloaliphatic rings, fused aromatic rings, and a methylene dioxy radical; provided that X_2 is not a 3-t-butyl radical;

n ranges from 0 to 5;

m ranges from 1 to 5; and

the alkyl radical is chosen from C1-C3 alkyl radicals, which are optionally substituted with at least one group chosen from saturated and unsaturated, linear, branched, and cyclic C1-C9 alkyl groups, dihydroindolyl and thiodihydroindolyl groups, and 2-, 3-, and 4-piperid(in)yl groups.

The calcimimetic compound may also be chosen from compounds of Formula II:



II

and pharmaceutically acceptable salts thereof,

5 wherein:

R^1 is aryl, substituted aryl, heterocyclyl, substituted heterocyclyl, cycloalkyl, or substituted cycloalkyl;

R^2 is alkyl or haloalkyl;

R^3 is H, alkyl, or haloalkyl;

10 R^4 is H, alkyl, or haloalkyl;

each R^5 present is independently selected from the group consisting of alkyl, substituted alkyl, alkoxy, substituted alkoxy, halogen, $-C(=O)OH$, $-CN$, $-NR^dS(=O)_mR^d$, $-NR^dC(=O)NR^dR^d$, $-NR^dS(=O)_mNR^dR^d$, or $-NR^dC(=O)R^d$;

15 R^6 is aryl, substituted aryl, heterocyclyl, substituted heterocyclyl, cycloalkyl, or substituted cycloalkyl;

each R^a is, independently, H, alkyl or haloalkyl;

each R^b is, independently, aryl, aralkyl, heterocyclyl, or heterocyclylalkyl, each of which may be unsubstituted or substituted by up to 3 substituents selected from the group consisting of alkyl, halogen, haloalkyl, alkoxy, cyano, and nitro;

20 each R^c is, independently, alkyl, haloalkyl, phenyl or benzyl, each of which may be substituted or unsubstituted;

each R^d is, independently, H, alkyl, aryl, aralkyl, heterocyclyl, or heterocyclylalkyl wherein the alkyl, aryl, aralkyl, heterocyclyl, and heterocyclylalkyl are substituted by 0, 1, 2, 3 or 4 substituents selected from alkyl, halogen, haloalkyl, alkoxy, cyano, nitro, R^b , $-C(=O)R^c$, $-OR^b$, $-NR^aR^a$, $-NR^aR^b$, $-C(=O)OR^c$, $-C(=O)NR^aR^a$, $-OC(=O)R^c$, $-NR^aC(=O)R^c$, $-NR^aS(=O)_nR^c$ and $-S(=O)_nNR^aR^a$;

m is 1 or 2;

n is 0, 1 or 2; and

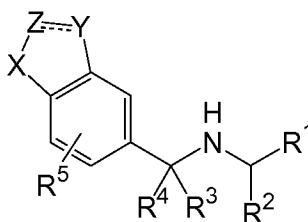
p is 0, 1, 2, 3, or 4;

30 provided that if R^2 is methyl, p is 0, and R^6 is unsubstituted phenyl, then R^1 is not 2,4-dihalophenyl, 2,4-dimethylphenyl, 2,4-diethylphenyl, 2,4,6-trihalophenyl, or 2,3,4-

trihalophenyl. These compounds are described in detail in published US patent application number 20040082625.

In one aspect, the calcimimetic compound can be N-((6-(methyloxy)-4'-(trifluoromethyl)-1,1'-biphenyl-3-yl)methyl)-1-phenylethanamine, or a pharmaceutically acceptable salt thereof. In another aspect, the calcimimetic compound can be (1R)-N-((6-chloro-3'-fluoro-3-biphenyl)methyl)-1-(3-chlorophenyl)ethanamine, or a pharmaceutically acceptable salt thereof. In a further aspect, the calcimimetic compound can be (1R)-1-(6-(methyloxy)-4'-(trifluoromethyl)-3-biphenyl)-N-((1R)-1-phenylethyl)ethanamine, or a pharmaceutically acceptable salt thereof.

In certain embodiments of the invention the calcimimetic compound can be chosen from compounds of Formula III



III

and pharmaceutically acceptable salts thereof, wherein:

----- represents a double or single bond;

R¹ is R^b;

R² is C₁₋₈ alkyl or C₁₋₄ haloalkyl;

R³ is H, C₁₋₄ haloalkyl or C₁₋₈ alkyl;

R⁴ is H, C₁₋₄ haloalkyl or C₁₋₄ alkyl;

R⁵ is, independently, in each instance, H, C₁₋₈alkyl, C₁₋₄haloalkyl, halogen, -OC₁₋₆alkyl, -NR^aR^d or NR^dC(=O)R^d;

X is -CR^d=N-, -N=CR^d-, O, S or -NR^d-;

when ----- is a double bond then Y is =CR⁶- or =N- and Z is -CR⁷= or -N=; and when ----- is a single bond then Y is -CR^aR⁶- or -NR^d- and Z is -CR^aR⁷- or -NR^d-; and

R⁶ is R^d, C₁₋₄haloalkyl, -C(=O)R^c, -OC₁₋₆alkyl, -OR^b, -NR^aR^a, -NR^aR^b, -C(=O)OR^c, -C(=O)NR^aR^a, -OC(=O)R^c, -NR^aC(=O)R^c, cyano, nitro, -NR^aS(=O)_mR^c or -S(=O)_mNR^aR^a;

R⁷ is R^d, C₁₋₄haloalkyl, -C(=O)R^c, -OC₁₋₆alkyl, -OR^b, -NR^aR^a, -NR^aR^b, -C(=O)OR^c, -C(=O)NR^aR^a, -OC(=O)R^c, -NR^aC(=O)R^c, cyano, nitro, -NR^aS(=O)_mR^c or -S(=O)_mNR^aR^a; or R⁶ and R⁷ together form a 3- to 6-atom saturated or unsaturated bridge containing 0, 1, 2 or 3

N atoms and 0, 1 or 2 atoms selected from S and O, wherein the bridge is substituted by 0, 1 or 2 substituents selected from R^5 ; wherein when R^6 and R^7 form a benzo bridge, then the benzo bridge may be additionally substituted by a 3- or 4- atoms bridge containing 1 or 2 atoms selected from N and O, wherein the bridge is substituted by 0 or 1 substituents selected from

5 C_{1-4} alkyl;

R^a is, independently, at each instance, H, C_{1-4} haloalkyl or C_{1-6} alkyl;

R^b is, independently, at each instance, phenyl, benzyl, naphthyl or a saturated or unsaturated 5- or 6-membered ring heterocycle containing 1, 2 or 3 atoms selected from N, O and S, with no more than 2 of the atoms selected from O and S, wherein the phenyl, benzyl or
10 heterocycle are substituted by 0, 1, 2 or 3 substituents selected from C_{1-6} alkyl, halogen, C_{1-4} haloalkyl, $-OC_{1-6}$ alkyl, cyano and nitro;

R^c is, independently, at each instance, C_{1-6} alkyl, C_{1-4} haloalkyl, phenyl or benzyl;

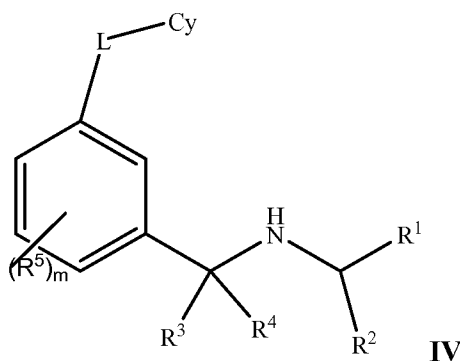
R^d is, independently, at each instance, H, C_{1-6} alkyl, phenyl, benzyl or a saturated or unsaturated 5- or 6-membered ring heterocycle containing 1, 2 or 3 atoms selected from N, O
15 and S, with no more than 2 of the atoms selected from O and S, wherein the C_{1-6} alkyl, phenyl, benzyl, naphthyl and heterocycle are substituted by 0, 1, 2, 3 or 4 substituents selected from C_{1-6} alkyl, halogen, C_{1-4} haloalkyl, $-OC_{1-6}$ alkyl, cyano and nitro, R^b , $-C(=O)R^c$, $-OR^b$, $-NR^aR^a$, $-NR^aR^b$, $-C(=O)OR^c$, $-C(=O)NR^aR^a$, $-OC(=O)R^c$, $-NR^aC(=O)R^c$, $-NR^aS(=O)_mR^c$ and $-S(=O)_mNR^aR^a$; and

20 m is 1 or 2.

Compounds of Formula **III** are described in detail in U.S. patent application 20040077619.

In one aspect, a calcimimetic compound is *N*-(3-[2-chlorophenyl]-propyl)- R - $\blacksquare$ -methyl-3-methoxybenzylamine. In another aspect, a calcimimetic compound is *N*-((6-(methoxy)-4'-(trifluoromethyl)-1,1'-biphenyl-3-yl)methyl)-1-phenylethanamine.
25

In one aspect, the calcimimetic compound of the invention can be chose from compounds of Formula **IV**



wherein:

R^1 is phenyl, benzyl, naphthyl or a saturated or unsaturated 5- or 6-membered heterocyclic ring containing 1, 2 or 3 atoms selected from N, O and S, with no more than 2 of the atoms selected from O and S, wherein the phenyl, benzyl, naphthyl or heterocyclic ring are substituted by 0, 1, 2 or 3 substituents selected from C_{1-6} alkyl, halogen, C_{1-4} haloalkyl,

5 - OC_{1-6} alkyl, cyano and nitro;

R^2 is C_{1-8} alkyl or C_{1-4} haloalkyl;

R^3 is H, C_{1-4} haloalkyl or C_{1-8} alkyl;

R^4 is H, C_{1-4} haloalkyl or C_{1-8} alkyl;

10 R^5 is, independently, in each instance, H, C_{1-8} alkyl, C_{1-4} haloalkyl, halogen, - OC_{1-6} alkyl, - NR^aR^d , $NR^aC(=O)R^d$, substituted or unsubstituted pyrrolidinyl, substituted or unsubstituted azetidiny, or substituted or unsubstituted piperidyl, wherein the substituents can be selected from halogen, - OR^b , - NR^aR^d , - $C(=O)OR^c$, - $C(=O)NR^aR^d$, - $OC(=O)R^c$, - $NR^aC(=O)R^c$, cyano, nitro, - $NR^aS(=O)_nR^c$ or - $S(=O)_nNR^aR^d$;

15 L is -O-, - OC_{1-6} alkyl-, - C_{1-6} alkylO-, - $N(R^a)(R^d)$ -, - $NR^aC(=O)$ -, - $C(=O)$ -, - $C(=O)NR^dC_{1-6}$ alkyl-, - C_{1-6} alkyl-C(=O) NR^d -, - $NR^dC(=O)NR^d$ -, - $NR^dC(=O)NR^dC_{1-6}$ alkyl-, - $NR^aC(=O)R^c$ -, - $NR^aC(=O)OR^c$ -, - OC_{1-6} alkyl-C(=O)O-, - NR^dC_{1-6} alkyl-, - C_{1-6} alkyl NR^d -, -S-, - $S(=O)_n$ -, - $NR^aS(=O)_n$, or - $S(=O)_nN(R^a)$ -;

20 Cy is a partially or fully saturated or unsaturated 5-8 membered monocyclic, 6-12 membered bicyclic, or 7-14 membered tricyclic ring system, the ring system formed of carbon atoms optionally including 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, and wherein each ring of the ring system is optionally substituted independently with one or more substituents of R^6 , C_{1-8} alkyl, C_{1-4} haloalkyl, halogen, cyano, nitro, - OC_{1-6} alkyl, - NR^aR^d , $NR^dC(=O)R^d$, - $C(=O)OR^c$, - $C(=O)NR^aR^d$, - $OC(=O)R^c$, - $NR^aC(=O)R^c$, - $NR^aS(=O)_mR^c$ or - $S(=O)_mNR^aR^d$;

25 R^6 is a partially or fully saturated or unsaturated 5-8 membered monocyclic, 6-12 membered bicyclic, or 7-14 membered tricyclic ring system, the ring system formed of carbon atoms optionally including 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, and wherein each ring of the ring system is optionally substituted independently with one or more substituents of C_{1-8} alkyl, C_{1-4} haloalkyl, halogen, cyano, nitro, 30 - OC_{1-6} alkyl, - NR^aR^d , $NR^dC(=O)R^d$, - $C(=O)OR^c$, - $C(=O)NR^aR^d$, - $OC(=O)R^c$, - $NR^aC(=O)R^c$, - $NR^aS(=O)_mR^c$ or - $S(=O)_mNR^aR^d$;

R^a is, independently, at each instance, H, C_{1-4} haloalkyl, C_{1-6} alkyl, C_{1-6} alkenyl, C_{1-6} alkylaryl or aryl C_{1-6} alkyl;

35 R^b is, independently, at each instance, C_{1-8} alkyl, C_{1-4} haloalkyl, phenyl, benzyl, naphthyl or a saturated or unsaturated 5- or 6-membered heterocyclic ring containing 1, 2 or 3 atoms

selected from N, O and S, with no more than 2 of the atoms selected from O and S, wherein the phenyl, benzyl, naphthyl or heterocyclic ring are substituted by 0, 1, 2 or 3 substituents selected from C₁₋₆alkyl, halogen, C₁₋₄haloalkyl, -OC₁₋₆alkyl, cyano and nitro;

R^c is, independently, at each instance, C₁₋₆alkyl, C₁₋₄haloalkyl, phenyl or benzyl;

5 R^d is, independently, at each instance, H, C₁₋₆alkyl, C₁₋₆alkenyl, phenyl, benzyl, naphthyl or a saturated or unsaturated 5- or 6-membered heterocycle ring containing 1, 2 or 3 atoms selected from N, O and S, with no more than 2 of the atoms selected from O and S, wherein the C₁₋₆alkyl, phenyl, benzyl, naphthyl and heterocycle are substituted by 0, 1, 2, 3 or 4 substituents selected from C₁₋₆alkyl, halogen, C₁₋₄haloalkyl, -OC₁₋₆alkyl, cyano and nitro, R^b,
10 -C(=O)R^c, -OR^b, -NR^aR^b, -C(=O)OR^c, -C(=O)NR^aR^b, -OC(=O)R^c, -NR^aC(=O)R^c,
-NR^aS(=O)_mR^c and -S(=O)_mNR^aR^a;

m is 1 or 2;

n is 1 or 2;

provided that if L is -O- or -OC₁₋₆alkyl-, then Cy is not phenyl.

15 In one aspect, the calcimimetic compound can be N-(2-chloro-5-(((1R)-1-phenylethyl)amino)methyl)phenyl)-5-methyl-3-isoxazolecarboxamide or a pharmaceutically acceptable salt thereof. In another aspect, the calcimimetic compound can be N-(2-chloro-5-(((1R)-1-phenylethyl)amino)methyl)phenyl)-2-pyridinecarboxamide or a pharmaceutically acceptable salt thereof.

20 Calcimimetic compounds useful in the methods of the invention include the calcimimetic compounds described above, as well as their stereoisomers, enantiomers, polymorphs, hydrates, and pharmaceutically acceptable salts of any of the foregoing.

B. Methods of assessing calcimimetic activity

25 In one aspect, compounds binding at the CaSR-activity modulating site can be identified using, for example, a labeled compound binding to the site in a competition-binding assay format.

Calcimimetic activity of a compound can be determined using techniques such as those described in International Publications WO 93/04373, WO 94/18959 and WO 95/11211.

30 Other methods that can be used to assess compounds calcimimetic activity are described below.

HEK 293 Cell Assay

HEK 293 cells engineered to express human parathyroid CaSR (HEK 293 4.0-7) have been described in detail previously (Nemeth EF *et al.* (1998) Proc. Natl. Acad. Sci. USA
35 95:4040-4045). This clonal cell line has been used extensively to screen for agonists,

allosteric modulators, and antagonists of the CaSR (Nemeth EF *et al.* (2001) J. Pharmacol. Exp. Ther. 299:323-331).

For measurements of cytoplasmic calcium concentration, the cells are recovered from tissue culture flasks by brief treatment with 0.02% ethylenediaminetetraacetic acid (EDTA) in phosphate-buffered saline (PBS) and then washed and resuspended in Buffer A (126 mM NaCl, 4 mM KCl, 1 mM CaCl₂, 1 mM MgSO₄, 0.7 mM K₂HPO₄/KH₂PO₄, 20 mM Na-Hepes, pH 7.4) supplemented with 0.1% bovine serum albumin (BSA) and 1 mg/ml D-glucose. The cells are loaded with fura-2 by incubation for 30 minutes at 37°C in Buffer A and 2 µM fura-2 acetoxymethylester. The cells are washed with Buffer B (Buffer B is Buffer A lacking sulfate and phosphate and containing 5 mM KCl, 1 mM MgCl₂, 0.5 mM CaCl₂ supplemented with 0.5% BSA and 1 mg/ml D-glucose) and resuspended to a density of 4 to 5 x 10⁶ cells/ml at room temperature. For recording fluorescent signals, the cells are diluted five-fold into prewarmed (37°C) Buffer B with constant stirring. Excitation and emission wavelengths are 340 and 510 nm, respectively. The fluorescent signal is recorded in real time using a strip-chart recorder.

For fluorometric imaging plate reader (FLIPR) analysis, HEK 293 cells are maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and 200 µg/ml hygromycin. At 24 hrs prior to analysis, the cells are trypsinized and plated in the above medium at 1.2 x 10⁵ cells/well in black sided, clear-bottom, collagen 1-coated, 96-well plates. The plates are centrifuged at 1,000 rpm for 2 minutes and incubated under 5% CO₂ at 37°C overnight. Cells are then loaded with 6 µM fluo-3 acetoxymethylester for 60 minutes at room temperature. All assays are performed in a buffer containing 126 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 20 mM Na-Hepes, supplemented with 1.0 mg/ml D-glucose and 1.0 mg/ml BSA fraction IV (pH 7.4).

In one aspect, the EC₅₀'s for the CaSR-active compounds can be determined in the presence of 1 mM Ca²⁺. The EC₅₀ for cytoplasmic calcium concentration can be determined starting at an extracellular Ca²⁺ level of 0.5 mM. FLIPR experiments are done using a laser setting of 0.8 W and a 0.4 second CCD camera shutter speed. Cells are challenged with calcium, CaSR-active compound or vehicle (20 µl) and fluorescence monitored at 1 second intervals for 50 seconds. Then a second challenge (50 µl) of calcium, CaSR-active compound, or vehicle can be made and the fluorescent signal monitored. Fluorescent signals are measured as the peak height of the response within the sample period. Each response is then normalized to the maximum peak observed in the plate to determine a percentage maximum fluorescence.

Bovine Parathyroid Cells

The effect of calcimimetic compounds on CaSR-dependent regulation of PTH secretion can be assessed using primary cultures of dissociated bovine parathyroid cells. Dissociated cells can be obtained by collagenase digestion, pooled, then resuspended in Percoll purification buffer and purified by centrifugation at 14,500 x g for 20 minutes at 4°C. The dissociated
5 parathyroid cells are removed and washed in a 1:1 mixture of Ham's F-12 and DMEM (F-12/DMEM) supplemented with 0.5% BSA, 100 U/ml penicillin, 100 µg/ml streptomycin, and 20 µg/ml gentamicin. The cells are finally resuspended in F-12/DMEM containing 10 U/ml penicillin, 10 µg/ml streptomycin, and 4 µg/ml gentamicin, and BSA was substituted with ITS+ (insulin, transferrin, selenous acid, BSA, and linoleic acid; Collaborative Research,
10 Bedford, MA). Cells are incubated in T-75 flasks at 37°C in a humidified atmosphere of 5% CO₂ in air.

Following overnight culture, the cells are removed from flasks by decanting and washed with parathyroid cell buffer (126 mM NaCl, 4 mM KCl, 1 mM MgSO₄, 0.7 mM K₂HPO₄/KH₂PO₄, 20 mM Na-Hepes, 20; pH 7.45 and variable amounts of CaCl₂ as specified)
15 containing 0.1% BSA and 0.5 mM CaCl₂. The cells are resuspended in this same buffer and portions (0.3 ml) are added to polystyrene tubes containing appropriate controls, CaSR-active compound, and/or varying concentrations of CaCl₂. Each experimental condition is performed in triplicate. Incubations at 37°C are for 20 minutes and can be terminated by placing the tubes on ice. Cells are pelleted by centrifugation (1500 x g for 5 minutes at 4°C) and 0.1 ml of
20 supernatant is assayed immediately. A portion of the cells is left on ice during the incubation period and then processed in parallel with other samples. The amount of PTH in the supernatant from tubes maintained on ice is defined as "basal release" and subtracted from other samples. PTH is measured according to the vendor's instructions using rat PTH-(1-34) immunoradiometric assay kit (Immunotopics, San Clemente, CA).

25 MTC 6-23 Cell Calcitonin Release

Rat MTC 6-23 cells (clone 6), purchased from ATCC (Manassas, VA) are maintained in growth media (DMEM high glucose with calcium/15% HIHS) that is replaced every 3 to 4 days. The cultures are passaged weekly at a 1:4 split ratio. Calcium concentration in the formulated growth media is calculated to be 3.2 mM. Cells are incubated in an atmosphere of
30 90% O₂/10% CO₂, at 37°C. Prior to the experiment, cells from sub-confluent cultures are aspirated and rinsed once with trypsin solution. The flasks are aspirated again and incubated at room temperature with fresh trypsin solution for 5-10 minutes to detach the cells. The detached cells are suspended at a density of 3.0 x 10⁵ cells/mL in growth media and seeded at a density of 1.5 x 10⁵ cells/well (0.5 mL cell suspension) in collagen-coated 48 well plates

(Becton Dickinson Labware, Bedford, MA). The cells are allowed to adhere for 56 hours post-seeding, after which the growth media was aspirated and replaced with 0.5 mL of assay media (DMEM high glucose without/2% FBS). The cells are then incubated for 16 hours prior to determination of calcium-stimulated calcitonin release. The actual calcium concentration in this media is calculated to be less than 0.07 mM. To measure calcitonin release, 0.35 mL of test agent in assay media is added to each well and incubated for 4 hours prior to determination of calcitonin content in the media. Calcitonin levels are quantified according to the vendor's instructions using a rat calcitonin immunoradiometric assay kit (Immutopics, San Clemente, CA).

10 Inositol phosphate Assay

The calcimimetic properties of compounds could also be evaluated in a biochemical assay performed on Chinese hamster ovarian (CHO) cells transfected with an expression vector containing cloned CaSR from rat brain [CHO(CaSR)] or not [CHO(WT)] (Ruat M., Snowman AM., J. Biol. Chem 271, 1996, p 5972). CHO(CaSR) has been shown to stimulate tritiated inositol phosphate ($[^3\text{H}]\text{IP}$) accumulation upon activation of the CaSR by Ca^{2+} and other divalent cations and by NPS 568 (Ruat *et al.*, J. Biol. Chem 271, 1996). Thus, $[^3\text{H}]\text{IP}$ accumulation produced by 10 μM of each CaSR-active compound in the presence of 2 mM extracellular calcium can be measured and compared to the effect produced by 10 mM extracellular calcium, a concentration eliciting maximal CaSR activation (Dauban P. *et al.*, Bioorganic & Medicinal Chemistry Letters, 10, 2000, p 2001).

C. *Pharmaceutical compositions and administration*

Calcimimetic compounds useful in the present invention can be used in the form of pharmaceutically acceptable salts derived from inorganic or organic acids. The salts include, but are not limited to, the following: acetate, adipate, alginate, citrate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, camphorate, camphorsulfonate, digluconate, cyclopentanepropionate, dodecylsulfate, ethanesulfonate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, fumarate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxy-ethanesulfonate, lactate, maleate, mandelate, methansulfonate, nicotinate, 2-naphthalenesulfonate, oxalate, palmoate, pectinate, persulfate, 2-phenylpropionate, picrate, pivalate, propionate, salicylate, succinate, sulfate, tartrate, thiocyanate, tosylate, mesylate, and undecanoate. When compounds of the invention include an acidic function such as a carboxy group, then suitable pharmaceutically acceptable salts for the carboxy group are well known to those skilled in the art and include, for example, alkaline, alkaline earth, ammonium, quaternary ammonium cations and the like. For additional examples of "pharmacologically

acceptable salts," see Berge *et al. J. Pharm. Sci.* 66: 1, 1977. In certain embodiments of the invention salts of hydrochloride and salts of methanesulfonic acid can be used.

In some aspects of the present invention, the calcium-receptor active compound can be chosen from cinacalcet, *i.e.*, *N*-(1-(*R*)-(1-naphthyl)ethyl)-3-[3-(trifluoromethyl)phenyl]-1-aminopropane, cinacalcet HCl, and cinacalcet methanesulfonate. The calcimimetic compound, such as cinacalcet HCl and cinacalcet methanesulfonate, can be in various forms such as amorphous powders, crystalline powders, and mixtures thereof. The crystalline powders can be in forms including polymorphs, psuedopolymorphs, crystal habits, micromeretics, and particle morphology.

For administration, the compounds useful in this invention are ordinarily combined with one or more adjuvants appropriate for the indicated route of administration. The compounds may be admixed with lactose, sucrose, starch powder, cellulose esters of alkanolic acids, stearic acid, talc, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulphuric acids, acacia, gelatin, sodium alginate, polyvinyl-pyrrolidone, and/or polyvinyl alcohol, and tableted or encapsulated for conventional administration. Alternatively, the compounds useful in this invention may be dissolved in saline, water, polyethylene glycol, propylene glycol, ethanol, corn oil, peanut oil, cottonseed oil, sesame oil, tragacanth gum, and/or various buffers. Other adjuvants and modes of administration are well known in the pharmaceutical art. The carrier or diluent may include time delay material, such as glyceryl monostearate or glyceryl distearate alone or with a wax, or other materials well known in the art.

The pharmaceutical compositions may be made up in a solid form (including granules, powders or suppositories) or in a liquid form (*e.g.*, solutions, suspensions, or emulsions). The pharmaceutical compositions may be subjected to conventional pharmaceutical operations such as sterilization and/or may contain conventional adjuvants, such as preservatives, stabilizers, wetting agents, emulsifiers, buffers etc.

Solid dosage forms for oral administration may include capsules, tablets, pills, powders, suppositories, and granules. In such solid dosage forms, the active compound may be admixed with at least one inert diluent such as sucrose, lactose, or starch. Such dosage forms may also comprise, as in normal practice, additional substances other than inert diluents, *e.g.*, lubricating agents such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration may include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used

in the art, such as water. Such compositions may also comprise adjuvants, such as wetting, sweetening, flavoring, and perfuming agents.

The therapeutically effective amount of the calcium receptor-active compound in the compositions useful in the invention can range from about 0.1 mg to about 180 mg, for example from about 5 mg to about 180 mg, or from about 1mg to about 100 mg of the calcimimetic compound per subject. In some aspects, the therapeutically effective amount of calcium receptor-active compound in the composition can be chosen from about 0.1 mg, about 1 mg, 5 mg, about 15 mg, about 20 mg, about 30 mg, about 50 mg, about 60 mg, about 75 mg, about 90 mg, about 120 mg, about 150 mg, about 180 mg.

While it may be possible to administer a calcium receptor-active compound to a subject alone, the compound administered will normally be present as an active ingredient in a pharmaceutical composition. Thus, a pharmaceutical composition of the invention may comprise a therapeutically effective amount of at least one calcimimetic compound, or an effective dosage amount of at least one calcimimetic compound.

As used herein, an “effective dosage amount” is an amount that provides a therapeutically effective amount of the calcium receptor-active compound when provided as a single dose, in multiple doses, or as a partial dose. Thus, an effective dosage amount of the calcium receptor-active compound of the invention includes an amount less than, equal to or greater than an effective amount of the compound; for example, a pharmaceutical composition in which two or more unit dosages, such as in tablets, capsules and the like, are required to administer an effective amount of the compound, or alternatively, a multidose pharmaceutical composition, such as powders, liquids and the like, in which an effective amount of the calcimimetic compound is administered by administering a portion of the composition.

Alternatively, a pharmaceutical composition in which two or more unit dosages, such as in tablets, capsules and the like, are required to administer an effective amount of the calcium receptor-active compound may be administered in less than an effective amount for one or more periods of time (*e.g.*, a once-a-day administration, and a twice-a-day administration), for example to ascertain the effective dose for an individual subject, to desensitize an individual subject to potential side effects, to permit effective dosing readjustment or depletion of one or more other therapeutics administered to an individual subject, and/or the like.

The effective dosage amount of the pharmaceutical composition useful in the invention can range from about 1 mg to about 360 mg from a unit dosage form, for example about 5 mg, about 15 mg, about 30 mg, about 50 mg, about 60 mg, about 75 mg, about 90 mg, about 120

mg, about 150 mg, about 180 mg, about 210 mg, about 240 mg, about 300 mg, or about 360 mg from a unit dosage form.

In some aspects of the present invention, the compositions disclosed herein comprise a therapeutically effective amount of a calcium receptor-active compound for the enhancement of sperm motility. For example, in certain embodiments, the calcimimetic compound such as cinacalcet HCl can be present in an amount ranging from about 1% to about 70%, such as from about 5% to about 40%, from about 10% to about 30%, or from about 15% to about 20%, by weight relative to the total weight of the composition.

The compositions useful in the invention may contain one or more active ingredients in addition to the calcium sensing receptor-active compound. The additional active ingredient may be another calcimimetic compound, or it may be an active ingredient having a different therapeutic activity. When administered as a combination, the therapeutic agents can be formulated as separate compositions that are given at the same time or different times, or the therapeutic agents can be given as a single composition.

In one aspect, the pharmaceutical compositions useful for methods of the invention may include additional compounds as described in more detail below.

In another aspect, the compounds used to practice the methods of the instant invention can be formulated for oral administration that release biologically active ingredients.

III. Methods of treatment

In one aspect, the invention provides methods for enhancing the motility of a mammalian spermatozoon. In one aspect, the methods provide contacting a spermatozoon from a mammalian subject with a calcimimetic compound. The spermatozoon can be derived from a male subject of the relevant species by methods known in the art including as a component of semen ejaculated by the male subject. In other aspect, sperm can be removed by aspiration from the vas deferens, the epididymis, or the testis of a male subject. The semen can be optionally washed by the standard procedures so that the contacting of the spermatozoon with the calcimimetic compounds of the invention occurs in a solution that is substantially free from seminal fluid (*e.g.*, free from seminal fluid-derived components such as proteins, lipids or nucleic acids that occur in the seminal fluid).

The mammalian male subjects can be any mammal including a human, a primate (*e.g.*, a monkey, a gorilla, a chimpanzee), an equine subject (*e.g.*, a horse, a donkey, a zebra), a pig, a goat, a bull, a sheep, a dog, a cat, a rabbit, a guinea pig, a hamster, a gerbil, a rat, or a mouse.

The mammalian subject used in the present invention may be one whose spermatozoa have lower than normal progressive (straight line) velocity. As used herein, "normal straight

line velocity” or “normal progressive velocity” is the straight line velocity exhibited by spermatozoa from a fertile male subject of the same species (*i.e.*, a subject whose spermatozoa have no evident compromised ability to fertilize an egg of the same species). In another aspect, the methods of the invention can be applied to spermatozoa from normal subjects, *e.g.*,
5 subjects whose spermatozoa have no evident compromised ability to fertilize an appropriate egg but whose chances to conceive need to be increased.

Prior to treatment using the methods of the invention, spermatozoa of the subject can be optionally tested for motility. The spermatozoa tested in this way may be obtained from the same semen sample used to obtain spermatozoa to be contacted with the compounds of the
10 invention or from a separate sample of semen from the subject. Spermatozoa can be tested for motility after the treatment to assess the efficacy of the methods of the invention.

The methods of the invention can be practiced *in vitro* or *in vivo*. When used *in vitro*, spermatozoa can be cultured in a physiological medium (*e.g.*, tissue culture medium) at a variety of temperatures (*e.g.*, from about 15°C to 39°C). The physiological medium can be any
15 culture medium in which mammalian spermatozoa can remain viable and retain their fertilizing potential. Examples of appropriate media include BWW medium or Dulbecco’s modified Eagle’s Medium (DMEM). Any of a variety of medium supplements can be added to the medium, for example, bacterial and fungicidal antibiotics (*e.g.*, penicillin, streptomycin, gentamicin, amphotericin B); blood serum from the same species but different individuals as
20 the semen donor (*i.e.*, allogenic blood serum), blood semen from the semen donor (*i.e.*, autologous blood serum), blood serum from one or more individuals of a species other than that of the semen donor (*i.e.*, xenogeneic blood serum). Xenogeneic blood serum can be, for example, fetal bovine serum, equine serum, goat serum, sheep serum, or pig serum. Sera used to supplement tissue culture medium can be allogenic or autologous blood serum. Sera to be
25 used as culture medium supplement can be screened for the presence of anti-spermatozoa antibodies prior to use, and those sera containing detectable levels of such antibodies would be excluded from use. Additional media supplements include various essential or non-essential amino acids (*e.g.*, glutamine), proteins (*e.g.*, human or bovine serum albumin, insulin, and transferrin), nucleic acids, nucleotides, nucleosides, and lipids.

30 In one aspect of the invention, the sperm sample from a subject, optionally washed to substantially remove seminal fluid, is combined with the culture medium. The compounds of the invention can be added to the medium prior to combining with the spermatozoa or it can be added after the mixing. After the spermatozoa, the calcimimetic compounds, and the physiological medium are mixed, the resulting mixture can be incubated for a various periods

of time, e.g., from 1 minute to 180 minutes. After the incubation, this mixture can be used for a number of fertilization procedures.

Examples of fertilization procedures are described in detail in Hall et al (1997) Baillieres Clin. Obstet. Gynaecol. 11:711, Van Steirteghem (1994) Curr. Opin. Obstet. Gynecol. 6: 173, and Van Voorhis (2007) N. Engl. J. Med. 356: 379. In vitro fertilization (IVF) procedures involve incubating an egg from a female subject of any of the species with a spermatozoon of a male subject of the same species which was treated with the methods of the present invention to enhance motility of spermatozoa. In one aspect, the methods of the invention may be used instead of the ICSI procedure. After the incubation of the egg with the sperm that was treated according to the methods of the invention, one or more embryos developed after sperm penetration of one or more eggs are inserted into the uterus of a female subject.

The methods of the invention can be practiced *in vivo* by delivering a composition comprising a calcimimetic compound of the invention and a sperm sample containing a spermatozoon into a female reproductive tract. The composition containing a calcimimetic compound can be delivered into a female reproductive tract prior to (for example, from 1 to 60 minutes) or soon after (for example, from 1 minute to 180 minutes) sexual intercourse between a male and a female subject of a species of interest. In another aspect, a composition comprising a calcimimetic compound and spermatozoa can be artificially inserted or infused into a female reproductive tract. The compositions useful in the present invention are described in more detail *supra*. In one aspect, the compounds of the invention can be used with carriers that protect the compound of the invention against rapid elimination from the body, such as controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Liposomal suspension can also be used. These can be prepared as described, for example, in U.S. Pat. No. 4,522,811.

The following examples are offered to more fully illustrate the invention, but are not to be construed as limiting the scope thereof.

Example 1

This example outlines methods and techniques used in the present invention.

Study of CaSR localization and distribution

Animals

Male Wistar rats weighing 250 g were purchased from the Animal Breeding Facility of the University of Cordoba (Spain). Rats were housed with a 12h/12h light/dark cycle and given ad libitum access to standard diet (calcium: 0.9%, phosphorus: 0.6%). All animals received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research, and the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Science.

Tissue sampling

Rats were sacrificed by aortic puncture and exsanguination under general anesthesia (ip sodium tiopental). Immediately following sacrifice, both testes, epididymis, parathyroid glands and kidneys were removed: one sample was used for measurement of CaSR mRNA and the other for CaSR protein expression.

CaSR mRNA measurement (RNA isolation and real time RT-PCR)

Fresh testicle, epididymus, parathyroid gland and kidney were dry-frozen in liquid nitrogen and stored at -80°C until RNA isolation. For RNA isolation, 1 ml of phenol-guanidine isothiocyanate solution (Tri-Reagent; Sigma, St Louis, MO, USA) was added to the glands. Tissues were ultrasonicated for 5 min at 4°C to allow for complete cell rupture. Thereafter, total RNA was extracted following a modification of the Chomczynski and Sacchi protocol. Extracted total RNA was dissolved in nuclease-free water (Promega, Madison, WI, USA) and heated for 10 min at 60°C . Total RNA was quantified by spectrophotometry. CaSR versus β -actin were amplified with a RT-PCR kit (QuantiTect SYBR green, Qiagen, Hilden, Germany) using specific primers and 100 ng of total RNA per sample. The following primers were used: CaSR (sense) 5'-TGG AGA GAC AGA TGC GAC TG-3', (antisense) 5'-GTC CAC GCC AGA AAC TCA AT-3'; β -Actin (sense) 5'-TGT CAC CAA CTG GGA CGA TAT GGA G-3', (antisense) 5'-ACA ATG CCA GTG GTA CGA CCA GA-3'. DNA amplifications were processed by real time PCR (Lightcycler; Roche, Basel, Switzerland).

CaSR protein expression

Fresh testicle, epididymus, parathyroid gland and kidney were fixed in 10% buffered formalin, embedded in paraffin and sectioned at $3\text{ }\mu\text{m}$. The avidin-biotin-peroxidase (ABC) method (Vector, Burlingame, USA) was used for the immunohistochemical study.

Endogenous peroxidase activity was inhibited by incubation in 3% hydrogen peroxide in methanol for 30 min. After 30 min incubation in 10% normal goat serum in phosphate buffered saline (PBS) pH 7.6, tissue sections were incubated with the primary antibodies overnight at 4°C (Acris antibodies GmbH, Hiddenhausen, Germany). After three-10 min rinses in PBS, tissue sections were incubated with the secondary antibody biotinylated goat anti-rabbit immunoglobulins for 30 min (Dako, Glostrup, Denmark). After two 10 min rinses

in PBS, tissue section were incubated with the ABC diluted in PBS for 1 hour. After three 10 min rinses in PBS, tissue sections were incubated with for several seconds in Vector[®] NovaRed (Vector laboratories, CA, USA), rinsed in tap water, lightly counterstained with Mayer's haematoxylin, dehydrated and mounted.

5 **Assessment of CaSR function**

Media

The sperm was diluted in basal Tyrode's medium (TBM) (13), which consisted of 96 mM NaCl, 4.7 mM KCl, 0.4 mM MgSO₄, 0.3 mM NaH₂PO₄, 5.5 mM glucose, 1 mM sodium pyruvate, 21.6 mM sodium lactate, 20 mM Hepes and 3 mg/ml BSA. The complete Tyrode's
10 medium (TCM), which was prepared by adding 0.5 mM CaCl₂ and 10 mM NaHCO₃ to TBM and was equilibrated with 5% CO₂, was also used as a positive control for sperm motility and to determine cell viability. All media were prepared on the day of use and maintained at an osmolality of 290–310 mOsm/kg and pH 7.45 at 39°C.

Sperm collection and washing

15 *Rat sperm.*

Sperm was obtained from Male Wistar rats under anesthesia (i.p. sodium tiopental). Rat spermatozoa were retrieved from the distal cauda epididymis. Sperm cells were dispersed in 3 ml of PBS. After culturing (37°C, 5% CO₂) for 15 min, the concentration of spermatozoa was determined by haemocytometer. Pooled semen from 3 rats was used in each experiment.
20 Samples were diluted with the corresponding incubation medium to give a final concentration of about 3x10⁴ spermatozoa per ml.

Pig sperm.

Sperm function was also assessed in preserved pig sperm. Commercial artificial insemination (AI) doses, from Duroc boars of proven fertility and routinely used for AI,
25 diluted to 30x10⁶ sperm cells/ml, in 80 ml of a commercial extender (MR-A; Kubus, Madrid, Spain), and stored for 12 h at 17°C were obtained commercially (Semen Porcino SL, Sevilla, Spain). In order to minimize individual boar variation samples were pooled, using semen from up five boars. Semen was centrifuged once (3 min, 1200 g) and washed twice with TBM. Samples were diluted with the corresponding incubation medium to give a final concentration
30 of about 30x10⁶ spermatozoa per ml.

Motility analysis

Rat sperm

Rat sperm motility was visually evaluated after 1 and 2 h of incubation. 7 µl of TBM diluted rat sperm were placed on a slide-cover and the sperm motility was video-recorded
35 using a microscope (Zeiss Axiophot, Carl Zeiss S.A, Germany) and a videocamara (DS

Camera Head DS-5M, Nikon Instruments Europe, Netherlands). Ten high-power fields (x 450) were recorded in each sample. All materials were thermostated at 37°C. Video recordings were blindly evaluated for motility (number of motile cells and spermatozoa speed) by two independent observers. A semiquantitative score (graded 0 to 4), with the higher number reflecting higher sperm motility was assigned to each sample.

Pig sperm

Aliquots of sperm suspension were used to analyze motility patterns after 1 and 4 h of incubation in a dark chamber. Analysis was based on the examination of 25 consecutive digitalized images obtained from a single field using a x20 negative-phase contrast objective. The image capture speed was therefore one every 40 ms and images were taken throughout 1 second. The number of objects incorrectly identified as spermatozoa was minimized on the monitor by using the playback function. The setting parameters for the CASA program were as follows: an object with an average path velocity (VAP) <10 µm/s was considered immobile, while objects with a velocity >11 µm/s were considered motile. Objects with velocities between 11 and 45 µm/s were considered as medium speed objects; those with a velocity > 45 µm/s were considered rapid objects. Spermatozoa deviating <10% from a straight line were designated linear motile. The sperm motility descriptors obtained by CASA analysis were: VCL, curvilinear velocity; VSL, linear velocity; VAP, mean velocity; LIN, linearity coefficient; STR, straightness coefficient; WOB, Wobble coefficient; ALH, mean lateral head displacement and BCF, frequency of head displacement (18). 5 µl of the sperm suspension were placed on prewarmed (38°C) glass slides. All slides were covered with a 22 x 22 coverslip. Analyses were then immediately performed.

The role of CaSR in sperm function was evaluated. Sperm motility analysis was carried out on both rat and pig sperm (suspended in TBM) treated with calcium and with increasing doses (range 1-1000 nM) of calcimimetic Compound A (*N*-((6-(methoxy)-4'-(trifluoromethyl)-1,1'-biphenyl-3-yl)methyl)-1-phenylethanamine). Sperm on TCM (with and without Compound A) was also used as a positive control for sperm motility.

Cell viability

Flow cytometry was performed to assess pig sperm viability after 4 h of incubation with calcium and with increasing doses (range 1-1000 nM) of calcimimetic Compound A. Briefly, 5 ml of SYBR 14 (100 nM) were added to 1 ml of a dilute semen sample (containing 150x10⁶ spermatozoa per ml) in HEPES-buffered saline solution (10 mM HEPES, 150 mM NaCl, pH 7.4) with 10% BSA and incubated at 38°C. After 10 min incubation, 5 ml of propidium iodide (final concentration 12 mM) were added and incubated for 8 additional min

at 38°C. The cells were immediately acquired by the flow cytometer (FACScan, Becton-Dickinson, San Jose, CA, USA).

Statistics

Values of motility are expressed as the mean $\pm$ SE. Cell viability is expressed as percentage (%). The difference between means for two groups was determined by unpaired t-tests; the difference between means for three or more groups was assessed by ANOVA followed by a Fisher LSD test. $P < 0.05$ was considered significant.

Example 2

This experiment demonstrates CaSR localization and distribution.

CaSR protein expression

Immunostaining for CaSR was observed in testicles, epididymis and sperm cells (Figure 1). Cells present in testicular tissue expressed CaSR with the exception of Leydig cells and fibroblasts. CaSR increased from the base to the lumen of the tubulus seminiferus. Thus CaSR expression was less evident in Sertoli cells, intermediate in spermatogonias, high in spermatocytes and even higher in spermatids. CaSR was distributed in the periphery of spermatogonias and spermatocytes, while spermatids expressed CaSR all over the cell surface (Fig 2A). In the epididymus, CaSR was also expressed both in basal and principal epidymal cells. CaSR immunostaining was more marked in principal epididymal cells in which a high staining was noted in their apical pole (Figure 2). The mature sperm cells present inside the epididymis also expressed CaSR. Immunostaining for CaSR was present mostly in the head of the spermatozoa (Figure 3). CaSR was also detected by immunohistochemistry in parathyroid and renal tissue for comparison (data not shown).

Example 3

This experiment demonstrates the effect of the calcimimetics on the sperm motility.

Addition of Ca (1 mM) to rat sperm in TBM medium had a very minor effect on the semiquantitative assessment of rat sperm motility. By contrast addition of calcimimetic Compound A (*N*-((6-(methyloxy)-4'-(trifluoromethyl)-1,1'-biphenyl-3-yl)methyl)-1-phenylethylamine) resulted in significant increases in sperm motility, both at 1h (from a score of 1.0 ± 0.1 to 3.8 ± 0.3 with Compound A 100 nM, $p < 0.05$) and at 2h (from a score of 0.8 ± 0.3 to 3.5 ± 0.1 with Compound A 50 nM, $p < 0.05$). Although the stimulatory effect of Compound A on spermatozoid motility was noticeable at all doses of Compound A (1-1000 nM) it was more evident in the dose range of 50-100 nM (Figure 4).

The sperm motility descriptors obtained by CASA analysis are shown in Table 1 (pig sperm). Values obtained at 1h in Table 1 and values obtained at 4 h in Table 2. In line with the results obtained in rat sperm, addition of Ca 1mM to pig sperm on TBM medium had an insignificant effect on motility parameters and only seemed to be influential on parameters related with lateral displacement (VCL, AHL). Treatment with calcimimetic Compound A resulted in significant modifications in all motility parameters except STR. The effect of Compound A was more noticeable in the range 10-300 nM. Table 1 summarizes sperm motility parameters obtained by CASA analysis at 60 min. VAP: mean velocity, VCL: curvilinear velocity, VSL: linear velocity, LIN: linearity coefficient, STR: straightness coefficient, WOB: wobble coefficient, ALH: mean lateral head displacement, BCF: frequency of head displacement. ^ap<0.05 vs TBM; ^bp<0.05 TBM+Ca 1mM; ^cp<0.05 TBM+AMG 10nM; ^dp<0.05 TBM+AMG 50nM; ^ep<0.05 TBM+AMG 100nM.

Table 1

60 min	VAP (µm/s)	VCL (µm/s)	VSL (µm/s)	LIN (%)	STR (%)	WOB (%)	ALH (µm)	BCF (Hz)
TBM	29.58±0.6	42.87±0.8	25.59±0.7	60.44±1.2	83.46±1.1	70.24±0.9	1.83±0.0	8.91±0.2
					1		4	
TBM + Ca 1 mM	28.01±1.9	38.46±1.2 ^a	24.63±1.1	64.17±1.5 ^a	85.54±1.1	73.85±1.2 ^a	1.63±0.0	7.80±0.3 ^a
					1		4 ^a	
TBM + comp A 1 nM	32.84±0.4 ^{a,b,c,e}	43.01±0.5 ^{b,c,e}	29.19±0.5 ^a	67.89±0.7 ^{a,b,c}	86.55±0.6 ^{a,c}	77.14±0.5 ^a	1.73±0.0	8.25±0.1 ^a
					6		2 ^{a,b,c}	
TBM + comp A 10 nM	34.39±0.5 ^{a,b}	46.25±0.6 ^{a,b}	29.88±0.5 ^a	65.33±0.8 ^a	84.47±0.7	75.52±0.6 ^a	1.86±0.0	8.12±0.1 ^a
					7		2 ^b	
TBM + comp A 50 nM	33.55±0.5 ^{a,b}	44.73±0.6 ^b	29.57±0.5 ^a	65.95±0.7 ^a	85.68±0.6 ^a	75.56±0.5 ^a	1.78±0.0	8.24±0.1 ^a
					5 ^a		2 ^{b,c}	
TBM + comp A 100 nM	35.14±0.5 ^{a,b,d}	45.20±0.6 ^b	31.47±0.5 ^a	69.08±0.7 ^{a,b}	87.07±0.6 ^{a,c}	78.18±0.5 ^a	1.75±0.0	8.12±0.1 ^a
					5 ^{a,c}		2 ^{a,b,c}	
TBM + comp A 300 nM	31.75±0.5 ^{a,b,c,d,e}	42.30±0.7 ^{b,c,d,e}	28.29±0.6 ^a	66.86±0.8 ^{a,e}	86.70±0.6 ^{a,c}	75.90±0.6 ^a	1.71±0.0	8.54±0.1 ^{b,c,e}
					7 ^{a,c}		2 ^{a,c,d}	

TBM + comp A 1000 nM	31.96±0.5 ^{a,b} c,d,e	43.41±0.6 ^{b,c} ,e	27.44±0.5 ^b ,c,d,e	64.04±0.8 ^{a,e}	83.32±0. 7 ^{d,e}	74.71±0.6 ^a ,e	1.79±0.0 2 ^{b,c}	7.88±0.1 ^{a,d}
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Table 2 summarizes sperm motility parameters obtained by CASA analysis at 240 min.

VAP: mean velocity, VCL: curvilinear velocity, VSL: linear velocity, LIN: linearity

coefficient, STR: straightness coefficient, WOB: wobble coefficient, ALH: mean lateral head

5 displacement, BCF: frequency of head displacement. ^ap<0.05 vs TBM; ^bp<0.05 TBM+Ca

1mM; ^cp<0.05 TBM+AMG 10nM; ^dp<0.05 TBM+AMG 50nM; ^ep<0.05 TBM+AMG 100nM.

Table 2

240 min	VAP (µm/s)	VCL (µm/s)	VSL (µm/s)	LIN (%)	STR (%)	WOB (%)	ALH (µm)	BCF (Hz)
TBM	32.01±0.4	54.04± 0.6	27.47± 0.4	51.42± 0.5	84.21± 0.5	60.26± 0.4	2.28± 0.02	10.91±0.1
TBM + Ca 1mM	25.52±1.1 ^a	44.43± 1.5 ^a	20.64± 1.2 ^a	46.13± 1.9 ^a	77.46± 2.1 ^a	58.01± 1.5	2.03± 0.06 ^a	8.53±0.4 ^a
TBM + comp A 1nM	31.89±0.4 ^{b,c} d,e	51.93± 0.7 ^{a,b,cd,e}	27.04± 0.4 ^{b,cd,e}	52.96± 0.6 ^{b,c,e}	82.50± 0.6 ^{b,c,d}	63.07± 0.5 ^{a,b,c,e}	2.22± 0.03 ^{b,d,e}	9.84±0.1 ^{a,b,c,e}
TBM + comp A 10nM	36.18±0.4 ^{a,b}	55.98± 0.6 ^{a,b}	31.41± 0.5 ^{a,b}	56.15± 0.6 ^{a,b}	84.69± 0.6 ^b	65.37± 0.4 ^{a,b}	2.27± 0.02 ^b	10.61±0.1 ^b
TBM + comp A 50 nM	35.21±0.4 ^{a,b}	57.46± 0.7 ^{a,b}	29.27± 0.5 ^{a,b,c}	51.95± 0.7 ^{b,c}	80.33± 0.7 ^{a,c}	62.69± 0.5 ^{a,b,c}	2.43± 0.03 ^{a,b,c}	10.01±0.1 ^{a,b,c}
TBM + comp A 100 nM	35.82±0.4 ^{a,b}	56.30± 0.6 ^{a,b}	31.01± 0.4 ^{a,b,d}	55.28± 0.6 ^{a,b,d}	83.69± 0.6 ^{b,d}	64.51± 0.4 ^{a,b,d}	2.33± 0.02 ^{b,d}	10.38±0.1 ^{a,b,d}
TBM + comp A 300 nM	34.54±0.4 ^{a,b} c,e	55.20± 0.7 ^{b,d}	29.50± 0.5 ^{a,b,c,e}	53.92± 0.6 ^{a,b,c,d}	83.33± 0.6 ^{b,d}	63.58± 0.4 ^{a,b,c}	2.33± 0.03 ^{b,d}	10.17±0.1 ^{a,b,c}
TBM+	34.46±0.4 ^{a,b}	55.57±	29.53±	53.01±	82.95±	62.76±	2.31±	10.61±0.1 ^{b,d}

compA 1000 nM	c,e	0.6 ^{b,d}	0.4 ^{a,b,c,e}	0.5 ^{a,b,c,e}	0.6 ^{b,d}	0.4 ^{a,b,c,e}	0.02 ^{b,d}	
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Figure 4 depicts the percentage of sperm cells that reached VAP > 45 $\mu\text{m/s}$ in rat sperm motility after 60 and 120 minutes of incubation. Results are presented as the mean $\pm$ SE of a semiquantitative score (0-4). Although addition of calcium 1mM did not influence this parameter, calcimimetic Compound A resulted in increases (up to 12%) which, again, were more accentuated in the range 10-300 nM. Figure 5 illustrates the effect of calcimimetic Compound A on pig sperm motility after 60 and 240 minutes of incubation. Influence of Compound A on the percentage of cells that reach a mean velocity (VAP) > 45 $\mu\text{m/s}$.

All the experiments on sperm motility were also carried out on TCM medium to assure the capacity of the sperm to acquire full motility. The results obtained with TCM indicated that the sperm cells were able to obtain the full range of motility: VAP > 80 m/s at 60 minutes. Addition of Compound A to sperm in TCM medium did not result in a significant modification of motility parameters.

Cell viability of sperm cells was not modified by addition of calcimimetic Compound A in a dose range 1-1000 nM. Percent of viable sperm cells without calcimimetic was 78.3 $\pm$ 0.7 % (TBM medium) and 55.6 $\pm$ 3.4 % (TCM medium), addition of calcimimetic yielded a percent of viable cells in a range 80.1-80.7 % (TBM medium) and 54.8-55.7 % (TCM medium).

All publications, patents and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method of enhancing the motility of a mammalian spermatozoon comprising
contacting the mammalian spermatozoon with a therapeutically effective amount of a
5 calcimimetic compound.
2. The method of claim 1, wherein a plurality of mammalian spermatozoa are identified as
having decreased motility.
- 10 3. The method of claim 1, wherein the contacting comprises culturing the spermatozoon
in a physiological medium comprising a therapeutically effective amount of a
calcimimetic compound.
- 15 4. The method of claim 3, wherein contacting is from about 30 seconds to about 120
minutes.
5. The method of claim 4, wherein contacting is from about 1 minute to 20 minutes.
- 20 6. The method of claim 1, wherein prior to the contacting, the spermatozoon is
substantially separated from seminal plasma.
7. The method of claim 1, wherein the contacting occurs in a mammalian female
reproductive tract.
- 25 8. The method of claim 7, wherein the mammalian female is a human.
9. A method of fertilizing a mammalian egg, the method comprising contacting a
mammalian egg with a mammalian spermatozoon, whose motility has been enhanced
according to claim 1.
- 30 10. The method of claim 9, wherein the contacting of the mammalian egg occurs *in vitro*.
11. The method of claim 10, further comprising fertilization of the mammalian egg and
placing the fertilized egg in a mammalian uterus.
- 35

12. The method of claim 9, wherein the contacting of the mammalian egg occurs *in vivo*.

13. The method of claim 12, wherein the contacting of the mammalian egg occurs in a female reproductive tract.

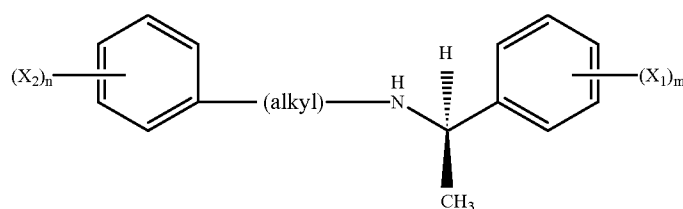
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14. The method of claim 13, wherein the mammalian egg is human.

15. The method of claim 1, wherein the calcimimetic compound is a compound of Formula

I

10



I

wherein:

15 X_1 and X_2 , which may be identical or different, are each a radical chosen from CH_3 , CH_3O , CH_3CH_2O , Br , Cl , F , CF_3 , CHF_2 , CH_2F , CF_3O , CH_3S , OH , CH_2OH , $CONH_2$, CN , NO_2 , CH_3CH_2 , propyl, isopropyl, butyl, isobutyl, t-butyl, acetoxy, and acetyl radicals, or two of X_1 may together form an entity chosen from fused cycloaliphatic rings, fused aromatic rings, and a methylene dioxy radical, or two of X_2 may together form an entity chosen from fused
20 cycloaliphatic rings, fused aromatic rings, and a methylene dioxy radical; provided that X_2 is not a 3-t-butyl radical;

n ranges from 0 to 5;

m ranges from 1 to 5; and

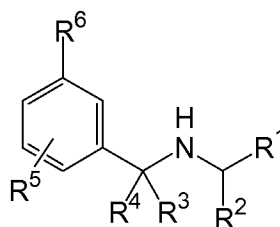
the alkyl radical is chosen from C_1 - C_3 alkyl radicals, which are optionally substituted
25 with at least one group chosen from saturated and unsaturated, linear, branched, and cyclic C_1 - C_9 alkyl groups, dihydroindolyl and thiodihydroindolyl groups, and 2-, 3-, and 4-piperidinyl groups;

or a pharmaceutically acceptable salt thereof.

16. The method of claim 15, wherein the calcimimetic compound is N-(3-[2-chlorophenyl]-propyl)-R- α -methyl-3-methoxybenzylamine or a pharmaceutically acceptable salt thereof.

5 17. The method of claim 1, wherein the calcimimetic compounds is cinacalcet HCl.

18. The method of claim 1, wherein the calcimimetic compound is a compound of the Formula II



II

wherein:

R^1 is aryl, substituted aryl, heterocyclyl, substituted heterocyclyl, cycloalkyl, or substituted cycloalkyl;

R^2 is alkyl or haloalkyl;

R^3 is H, alkyl, or haloalkyl;

R^4 is H, alkyl, or haloalkyl;

each R^5 present is independently selected from the group consisting of alkyl, substituted alkyl, alkoxy, substituted alkoxy, halogen, $-C(=O)OH$, $-CN$, $-NR^dS(=O)_mR^d$, $-NR^dC(=O)NR^dR^d$, $-NR^dS(=O)_mNR^dR^d$, or $-NR^dC(=O)R^d$;

R^6 is aryl, substituted aryl, heterocyclyl, substituted heterocyclyl, cycloalkyl, or substituted cycloalkyl;

each R^a is, independently, H, alkyl or haloalkyl;

each R^b is, independently, aryl, aralkyl, heterocyclyl, or heterocyclylalkyl, each of which may be unsubstituted or substituted by up to 3 substituents selected from the group consisting of alkyl, halogen, haloalkyl, alkoxy, cyano, and nitro;

each R^c is, independently, alkyl, haloalkyl, phenyl or benzyl, each of which may be substituted or unsubstituted;

each R^d is, independently, H, alkyl, aryl, aralkyl, heterocyclyl, or heterocyclylalkyl wherein the alkyl, aryl, aralkyl, heterocyclyl, and heterocyclylalkyl are substituted by 0, 1, 2,

3 or 4 substituents selected from alkyl, halogen, haloalkyl, alkoxy, cyano, nitro, R^b , $-C(=O)R^c$, $-OR^b$, $-NR^aR^a$, $-NR^aR^b$, $-C(=O)OR^c$, $-C(=O)NR^aR^a$, $-OC(=O)R^c$, $-NR^aC(=O)R^c$, $-NR^aS(=O)_nR^c$ and $-S(=O)_nNR^aR^a$;

m is 1 or 2;

5 n is 0, 1 or 2; and

p is 0, 1, 2, 3, or 4;

provided that if R^2 is methyl, p is 0, and R^6 is unsubstituted phenyl, then R^1 is not 2,4-dihalophenyl, 2,4-dimethylphenyl, 2,4-diethylphenyl, 2,4,6-trihalophenyl, or 2,3,4-trihalophenyl;

10 or a pharmaceutically acceptable salt thereof.

19. The method of claim 18, wherein the calcimimetic compound is (1R)-N-((6-(methoxy)-4'-(trifluoromethyl)-3-biphenyl)methyl)-1-phenylethanamine, or a pharmaceutically acceptable salt thereof.

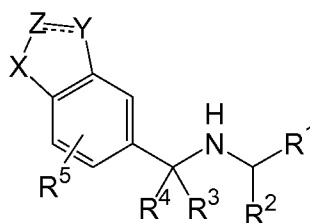
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20. The method of claim 18, wherein the calcimimetic compounds is (1R)-N-((6-chloro-3'-fluoro-3-biphenyl)methyl)-1-(3-chlorophenyl)ethanamine, or a pharmaceutically acceptable salt thereof.

20

21. The method of claim 18, wherein the calcimimetic compounds is (1R)-1-(6-(methoxy)-4'-(trifluoromethyl)-3-biphenyl)-N-((1R)-1-phenylethyl)ethanamine, or a pharmaceutically acceptable salt thereof.

22. The method of claim 1, wherein the calcimimetic compound is a compound of the
25 Formula III



III

30 and pharmaceutically acceptable salts thereof, wherein:

----- represents a double or single bond;

R^1 is R^b ;

R^2 is C_{1-8} alkyl or C_{1-4} haloalkyl;

R^3 is H, C_{1-4} haloalkyl or C_{1-8} alkyl;

R^4 is H, C_{1-4} haloalkyl or C_{1-4} alkyl;

5 R^5 is, independently, in each instance, H, C_{1-8} alkyl, C_{1-4} haloalkyl, halogen, $-OC_{1-6}$ alkyl, $-NR^aR^d$ or $NR^dC(=O)R^d$;

X is $-CR^d=N-$, $-N=CR^d-$, O, S or $-NR^d-$;

when $-----$ is a double bond then Y is $=CR^6-$ or $=N-$ and Z is $-CR^7=$ or $-N=$; and when $-----$ is a single bond then Y is $-CR^aR^6-$ or $-NR^d-$ and Z is $-CR^aR^7-$ or $-NR^d-$; and

10 R^6 is R^d , C_{1-4} haloalkyl, $-C(=O)R^c$, $-OC_{1-6}$ alkyl, $-OR^b$, $-NR^aR^a$, $-NR^aR^b$, $-C(=O)OR^c$, $-C(=O)NR^aR^a$, $-OC(=O)R^c$, $-NR^aC(=O)R^c$, cyano, nitro, $-NR^aS(=O)_mR^c$ or $-S(=O)_mNR^aR^a$;

R^7 is R^d , C_{1-4} haloalkyl, $-C(=O)R^c$, $-OC_{1-6}$ alkyl, $-OR^b$, $-NR^aR^a$, $-NR^aR^b$, $-C(=O)OR^c$, $-C(=O)NR^aR^a$, $-OC(=O)R^c$, $-NR^aC(=O)R^c$, cyano, nitro, $-NR^aS(=O)_mR^c$ or $-S(=O)_mNR^aR^a$; or R^6 and R^7 together form a 3- to 6-atom saturated or unsaturated bridge containing 0, 1, 2 or 3

15 N atoms and 0, 1 or 2 atoms selected from S and O, wherein the bridge is substituted by 0, 1 or 2 substituents selected from R^5 ; wherein when R^6 and R^7 form a benzo bridge, then the benzo bridge may be additionally substituted by a 3- or 4- atoms bridge containing 1 or 2 atoms selected from N and O, wherein the bridge is substituted by 0 or 1 substituents selected from C_{1-4} alkyl;

20 R^a is, independently, at each instance, H, C_{1-4} haloalkyl or C_{1-6} alkyl;

R^b is, independently, at each instance, phenyl, benzyl, naphthyl or a saturated or unsaturated 5- or 6-membered ring heterocycle containing 1, 2 or 3 atoms selected from N, O and S, with no more than 2 of the atoms selected from O and S, wherein the phenyl, benzyl or heterocycle are substituted by 0, 1, 2 or 3 substituents selected from C_{1-6} alkyl, halogen,

25 C_{1-4} haloalkyl, $-OC_{1-6}$ alkyl, cyano and nitro;

R^c is, independently, at each instance, C_{1-6} alkyl, C_{1-4} haloalkyl, phenyl or benzyl;

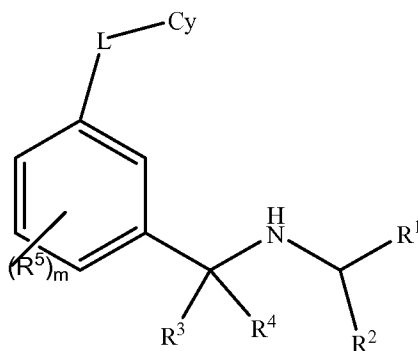
R^d is, independently, at each instance, H, C_{1-6} alkyl, phenyl, benzyl or a saturated or unsaturated 5- or 6-membered ring heterocycle containing 1, 2 or 3 atoms selected from N, O and S, with no more than 2 of the atoms selected from O and S, wherein the C_{1-6} alkyl, phenyl, benzyl, naphthyl and heterocycle are substituted by 0, 1, 2, 3 or 4 substituents selected from C_{1-6} alkyl, halogen, C_{1-4} haloalkyl, $-OC_{1-6}$ alkyl, cyano and nitro, R^b , $-C(=O)R^c$, $-OR^b$, $-NR^aR^a$, $-NR^aR^b$, $-C(=O)OR^c$, $-C(=O)NR^aR^a$, $-OC(=O)R^c$, $-NR^aC(=O)R^c$, $-NR^aS(=O)_mR^c$ and $-S(=O)_mNR^aR^a$; and

m is 1 or 2,

35 or a pharmaceutically acceptable salt thereof.

23. The method of claim 1, wherein the calcimimetic compound is a compound of
Formula IV

5



IV

wherein:

R^1 is phenyl, benzyl, naphthyl or a saturated or unsaturated 5- or 6-membered
heterocyclic ring containing 1, 2 or 3 atoms selected from N, O and S, with no more than 2 of
the atoms selected from O and S, wherein the phenyl, benzyl, naphthyl or heterocyclic ring are
substituted by 0, 1, 2 or 3 substituents selected from C_{1-6} alkyl, halogen, C_{1-4} haloalkyl,
-OC₁₋₆alkyl, cyano and nitro;

R^2 is C_{1-8} alkyl or C_{1-4} haloalkyl;

R^3 is H, C_{1-4} haloalkyl or C_{1-8} alkyl;

R^4 is H, C_{1-4} haloalkyl or C_{1-8} alkyl;

R^5 is, independently, in each instance, H, C_{1-8} alkyl, C_{1-4} haloalkyl, halogen, -OC₁₋₆alkyl,
-NR^aR^d, NR^aC(=O)R^d, substituted or unsubstituted pyrrolidinyl, substituted or unsubstituted
azetidiny, or substituted or unsubstituted piperidyl, wherein the substituents can be selected
from halogen, -OR^b, -NR^aR^d, -C(=O)OR^c, -C(=O)NR^aR^d, -OC(=O)R^c, -NR^aC(=O)R^c, cyano,
nitro, -NR^aS(=O)_nR^c or -S(=O)_nNR^aR^d;

L is -O-, -OC₁₋₆alkyl-, -C₁₋₆alkylO-, -N(R^a)(R^d)-, -NR^aC(=O)-, -C(=O)-, -
C(=O)NR^dC₁₋₆alkyl-, -C₁₋₆alkyl-C(=O)NR^d-, -NR^dC(=O)NR^d-, -NR^dC(=O)NR^dC₁₋₆alkyl-,
-NR^aC(=O)R^c-, -NR^aC(=O)OR^c-, -OC₁₋₆alkyl-C(=O)O-, -NR^dC₁₋₆alkyl-, -C₁₋₆alkylNR^d-, -S-, -
S(=O)_n-, -NR^aS(=O)_n-, or -S(=O)_nN(R^a)-;

Cy is a partially or fully saturated or unsaturated 5-8 membered monocyclic, 6-12
membered bicyclic, or 7-14 membered tricyclic ring system, the ring system formed of carbon
atoms optionally including 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9
heteroatoms if tricyclic, and wherein each ring of the ring system is optionally substituted

independently with one or more substituents of R^6 , C_{1-8} alkyl, C_{1-4} haloalkyl, halogen, cyano, nitro, $-OC_{1-6}$ alkyl, $-NR^aR^d$, $NR^dC(=O)R^d$, $-C(=O)OR^c$, $-C(=O)NR^aR^d$, $-OC(=O)R^c$, $-NR^aC(=O)R^c$, $-NR^aS(=O)_mR^c$ or $-S(=O)_mNR^aR^d$;

R^6 is a partially or fully saturated or unsaturated 5-8 membered monocyclic, 6-12
 5 membered bicyclic, or 7-14 membered tricyclic ring system, the ring system formed of carbon atoms optionally including 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, and wherein each ring of the ring system is optionally substituted independently with one or more substituents of C_{1-8} alkyl, C_{1-4} haloalkyl, halogen, cyano, nitro, $-OC_{1-6}$ alkyl, $-NR^aR^d$, $NR^dC(=O)R^d$, $-C(=O)OR^c$, $-C(=O)NR^aR^d$, $-OC(=O)R^c$, $-NR^aC(=O)R^c$,
 10 $-NR^aS(=O)_mR^c$ or $-S(=O)_mNR^aR^d$;

R^a is, independently, at each instance, H, C_{1-4} haloalkyl, C_{1-6} alkyl, C_{1-6} alkenyl, C_{1-6} alkylaryl or aryl C_{1-6} alkyl;

R^b is, independently, at each instance, C_{1-8} alkyl, C_{1-4} haloalkyl, phenyl, benzyl, naphthyl or a saturated or unsaturated 5- or 6-membered heterocyclic ring containing 1, 2 or 3 atoms
 15 selected from N, O and S, with no more than 2 of the atoms selected from O and S, wherein the phenyl, benzyl, naphthyl or heterocyclic ring are substituted by 0, 1, 2 or 3 substituents selected from C_{1-6} alkyl, halogen, C_{1-4} haloalkyl, $-OC_{1-6}$ alkyl, cyano and nitro;

R^c is, independently, at each instance, C_{1-6} alkyl, C_{1-4} haloalkyl, phenyl or benzyl;

R^d is, independently, at each instance, H, C_{1-6} alkyl, C_{1-6} alkenyl, phenyl, benzyl,
 20 naphthyl or a saturated or unsaturated 5- or 6-membered heterocycle ring containing 1, 2 or 3 atoms selected from N, O and S, with no more than 2 of the atoms selected from O and S, wherein the C_{1-6} alkyl, phenyl, benzyl, naphthyl and heterocycle are substituted by 0, 1, 2, 3 or 4 substituents selected from C_{1-6} alkyl, halogen, C_{1-4} haloalkyl, $-OC_{1-6}$ alkyl, cyano and nitro, R^b , $-C(=O)R^c$, $-OR^b$, $-NR^aR^b$, $-C(=O)OR^c$, $-C(=O)NR^aR^b$, $-OC(=O)R^c$, $-NR^aC(=O)R^c$,
 25 $-NR^aS(=O)_mR^c$ and $-S(=O)_mNR^aR^a$;

m is 1 or 2;

n is 1 or 2;

provided that if L is $-O-$ or $-OC_{1-6}$ alkyl-, then Cy is not phenyl;

or a pharmaceutically acceptable salt thereof.

30

24. The method of claim 23, wherein the calcimimetic compound is N-(2-chloro-5-((((1R)-1-phenylethyl)amino)methyl)phenyl)-5-methyl-3-isoxazolecarboxamide or a pharmaceutically acceptable salt thereof.

25. The method of claim 23, wherein the calcimimetic compound is N-(2-chloro-5-((((1R)-1-phenylethyl)amino)methyl)phenyl)-2-pyridinecarboxamide or a pharmaceutically acceptable salt thereof.
- 5 26. The method of claim 1, wherein the subject is human.

1/5

FIG. 1



2/5

FIG. 2

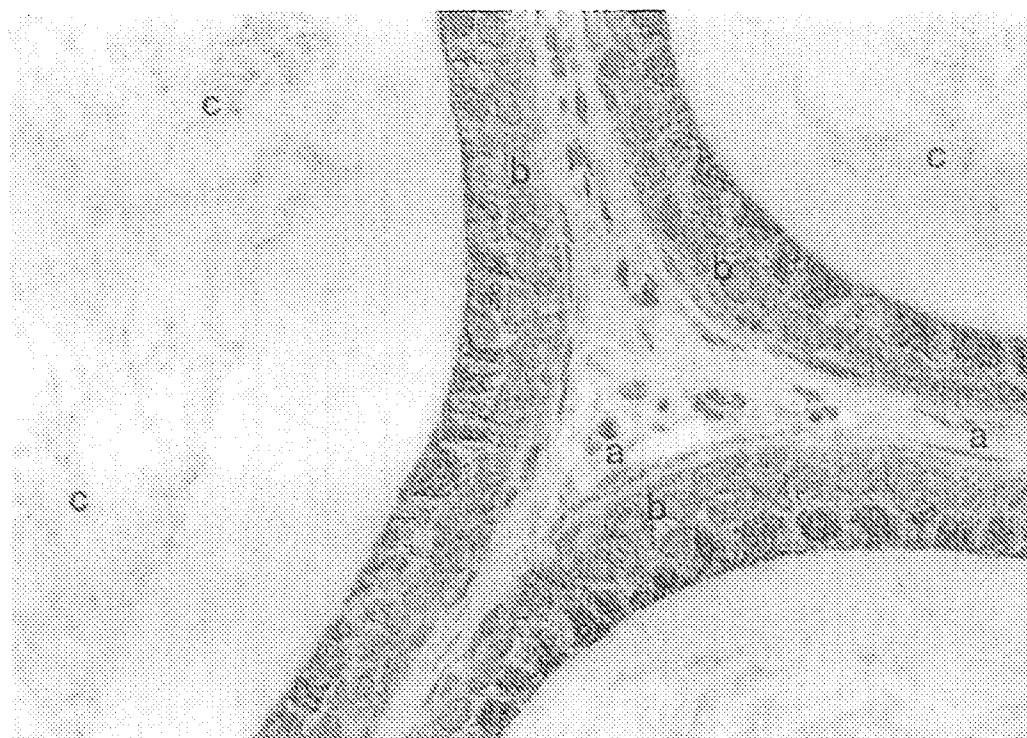


FIG. 3

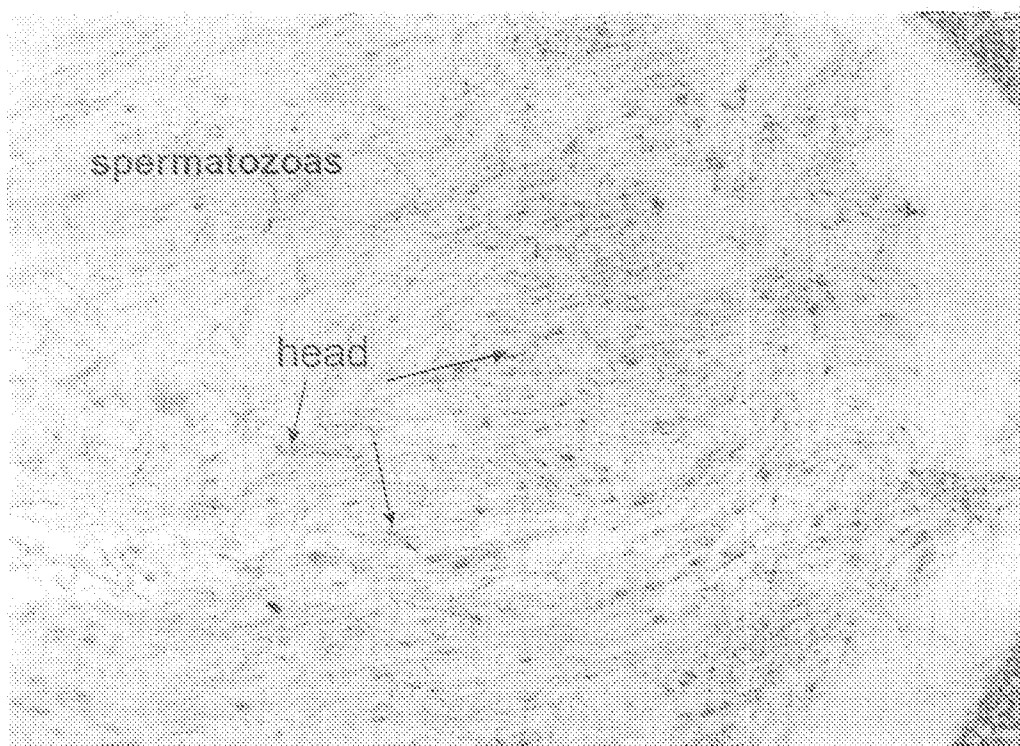
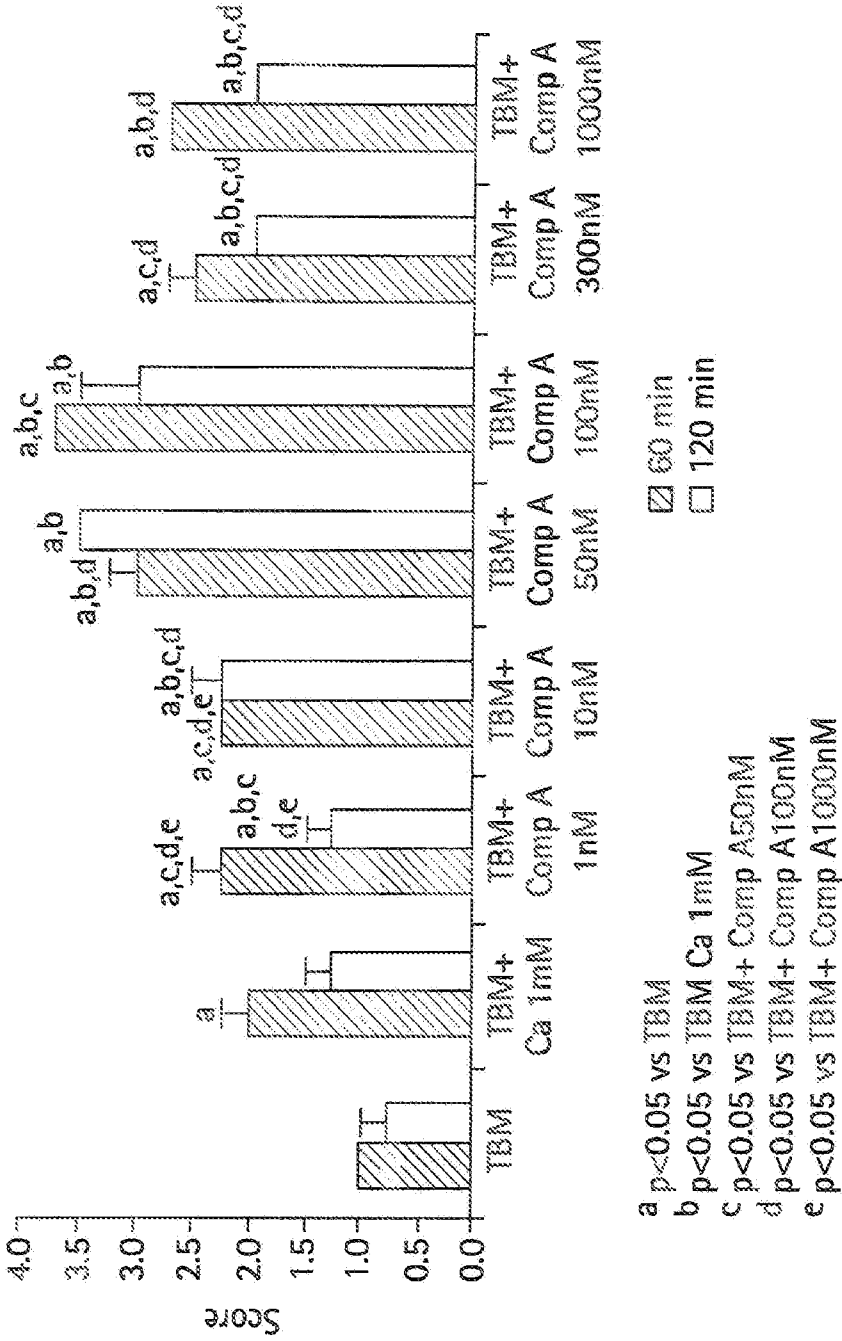


FIG. 4



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FIG. 5A

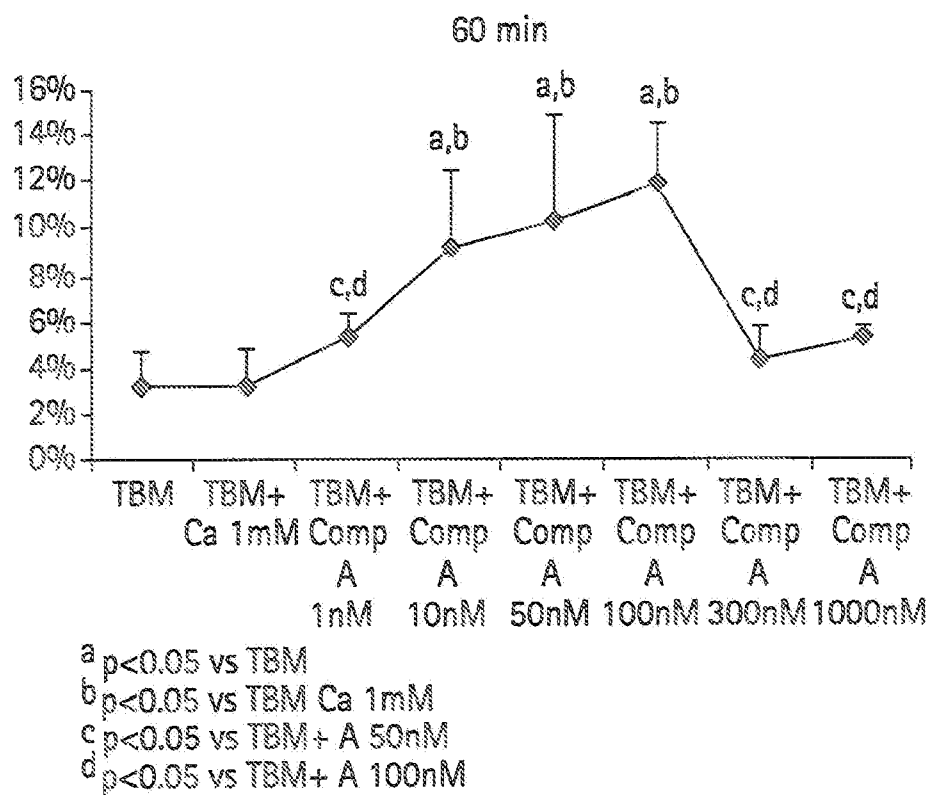
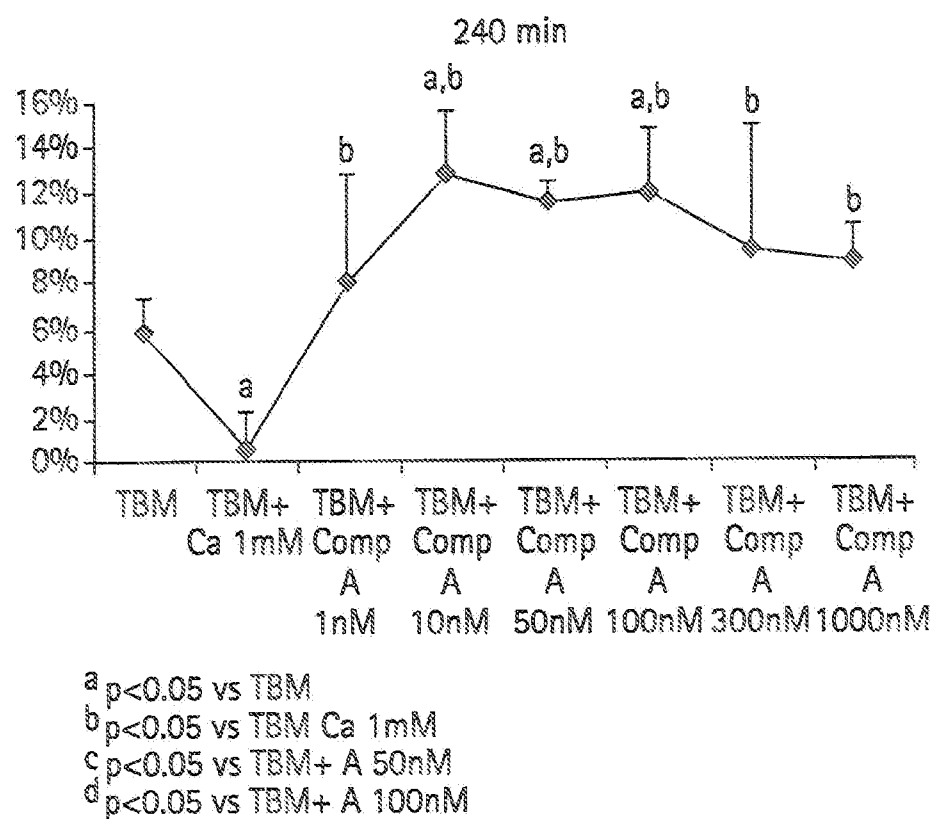


FIG. 5B



INTERNATIONAL SEARCH REPORT

International application No

PCT/US2010/026705

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/137 A61K31/42 A61K31/4402 A61P15/08
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K

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

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

EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE MEDLINE [Online] US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US; June 2003 (2003-06), MORALES MARÍA ESTHER ET AL: "[Progressive motility increase caused by L-arginine and polyamines in sperm from patients with idiopathic and diabetic asthenozoospermia]" XP002590069 Database accession no. NLM14515660	1-14,26
Y	* abstract ----- -/--	1-26

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

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Date of the actual completion of the international search

5 July 2010

Date of mailing of the international search report

16/07/2010

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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2010/026705

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JENSEN ANDERS A ET AL: "Allosteric modulation of the calcium-sensing receptor" CURRENT NEUROPHARMACOLOGY, vol. 5, no. 3, September 2007 (2007-09), pages 180-186, XP002590070 ISSN: 1570-159X page 182 - left-hand column -----	1-14,26
A	QUINN STEPHEN J ET AL: "The Ca ²⁺ -sensing receptor: A target for polyamines" AMERICAN JOURNAL OF PHYSIOLOGY, vol. 273, no. 4 PART 1, October 1997 (1997-10), pages C1315-C1323, XP002590071 ISSN: 0002-9513 * abstract -----	1-14,26
X	WO 2004/039332 A2 (ATHERSYS INC [US]) 13 May 2004 (2004-05-13) -----	1-14,26
Y	page 2	1-26
Y	US 2006/292689 A1 (POZNANSKY MARK C [US] ET AL) 28 December 2006 (2006-12-28) claims -----	1-26
A	JIMENEZ-GONZALEZ C ET AL: "Calcium signalling in human spermatozoa: a specialized 'toolkit' of channels, transporters and stores" HUMAN REPRODUCTION UPDATE, vol. 12, no. 3, May 2006 (2006-05), pages 253-267, XP002590072 ISSN: 1355-4786 * abstract -----	1-26
A	WO 2008/121386 A2 (AMGEN INC [US]; GEIBEL JOHN P [US]; HEBERT STEVEN C [US]; MARTIN DAVID) 9 October 2008 (2008-10-09) claims -----	1-26

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2010/026705

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2004039332 A2	13-05-2004	AU 2003294241 A1	25-05-2004
US 2006292689 A1	28-12-2006	NONE	
WO 2008121386 A2	09-10-2008	AU 2008233088 A1	09-10-2008
		CA 2681582 A1	09-10-2008
		EP 2139462 A2	06-01-2010
		US 2009018135 A1	15-01-2009