(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 3 827 665 A1

(12) **EUROPEAN PATENT APPLICATION**

(43) Date of publication:
02.06.2021 Bulletin 2021/22

(51) Int Cl.:
A01H 4/00 *(2006.01)*

(21) Application number: **19383042.9**

(22) Date of filing: **26.11.2019**

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR
Designated Extension States:
BA ME
KH MA MD TN

(71) Applicant: **Consejo Superior de Investigaciones Científicas (CSIC)**
28006 Madrid (ES)

(72) Inventors:
- **SÁNCHEZ TESTILLANO, Pilar**
  28040 Madrid (ES)
- **MARTINEZ GIL, Ana**
  28040 Madrid (ES)
- **GIL AYUSO-GONTAN, Carmen**
  28040 Madrid (ES)
- **BERENGUER PEINADO, Eduardo**
  28040 Madrid (ES)
- **CARNEROS GARCÍA, Elena**
  28040 Madrid (ES)
- **PÉREZ PÉREZ, Yolanda**
  28040 Madrid (ES)

(74) Representative: **Pons**
Glorieta Ruben Dario 4
28010 Madrid (ES)

(54) **MAMMAL KINASE INHIBITORS TO PROMOTE IN VITRO EMBRYOGENESIS INDUCTION OF PLANTS**

(57) The present invention relates to the use of mammal kinase inhibitors, preferably human kinase inhibitors, to promote the induction of *in vitro* embryogenesis, a strategy never used in plants systems before. The results obtained indicated that these inhibitors have beneficial effects in both crop and forest plants in *in vitro* systems of microspore and somatic embryogenesis.

EP 3 827 665 A1

Description

[0001] The invention relates to the use of mammal kinase inhibitors to promote *in vitro* induction of plant embryogenesis and plant regeneration. Furthermore, the present invention discloses methods to promote *in vitro* induction of plant embryogenesis and plant regeneration by the use of mammal kinase inhibitors.

BACKGROUND ART

[0002] The ability of many plant cells to regenerate embryos through *in vitro* culture is extensively exploited by companies for regeneration, propagation and selection of high quality/adapted plant material in agroforestry and industrial sectors, a technology that permits the propagation of plants with increased genetic gain, reducing time and cost in breeding and conservation programs. The capacity to regenerate adult fertile plants from *in vitro* cultured explants is well described for many species and through various developmental pathways. Multiple environmental factors have been shown to determine the *in vitro* responses of plant tissues.

[0003] Through *in vitro* embryogenesis, somatic cells from donor plants can be reprogrammed by different treatments (mainly stress and hormonal treatments), giving rise to entire embryos that further germinate and ultimately produce a plant. *In vitro* embryogenesis can also be induced from microspores, precursors cells of pollen grains. Due to the haploid condition of these cells, microspore embryogenesis is a useful biotechnological tool in plant breeding as a source of new genetic variability, fixed in fully homozygous plants in only one generation.

[0004] In the case of woody species, somatic embryogenesis has many advantages since classical genetic breeding programs have important limitations in trees due to their long-life span, and difficulties of seed conservation and vegetative reproduction. Somatic embryogenesis has a great potential for large-scale propagation and cryopreservation of tree elite genotypes, as well as for transformation strategies.

[0005] *In vitro* systems of somatic and microspore embryogenesis have been developed for many plant species belonging to a wide range of families. The primary advantage of *in vitro* plant propagation is the rapid production of high numbers of high quality, disease-free and uniform planting material for agroindustry companies. Despite decades of research, poor *in vitro* regeneration is still a lingering problem with the process still being highly inefficient in many species of economic interest in the fields of agriculture and forestry, a fact that severely affects the application and cost of this technology in plant breeding and conservation programs.

[0006] The yield of somatic and microspore-derived embryo production has several bottlenecks at various stages of the process. One of the major problems is the low proportion of cells that are reprogrammed and initiate embryogenesis, being embryogenesis initiation efficiency a crucial step. Therefore, new strategies are necessary to improve *in vitro* embryogenesis induction in different species of economic interest, such as crops and forest plant species.

DESCRIPTION OF THE INVENTION

[0007] To solve the aforementioned limitations, a general object of the invention is to provide the use of mammal kinase inhibitors, preferably human kinase inhibitors, preferably human **g**lycogen **s**ynthase **k**inase-**3**β (GSK3β) and/or leucine-rich repeat **k**inase **2** (LRRK2) inhibitors compounds and methods for such uses to induce plant embryogenesis.

[0008] In their search for novel strategies to improve the induction of embryogenesis and embryo production of plants, the inventors surprisingly found that mammal kinase inhibitors, preferably human kinase inhibitors, have a positive effect on plant embryogenesis initiation. Moreover, the present disclosure shows that treatments with these inhibitors have been successfully applied to different *in vitro* protocols, in liquid or solid media, and with direct, indirect and secondary/recurrent embryogenesis, providing similar promoting effects over embryogenesis induction. The inventors have demonstrated that mammal kinase inhibitors, preferably human kinase inhibitors, preferably inhibitors of GSK3β and/or LRRK2 lead to an increase in the *in vitro* embryogenesis induction, from both somatic cells and microspores, in crop and forest plant species (Figs. 1-3). Furthermore, the inventors demonstrated that this surprising effect is obtained with several inhibitors for several human kinases, all of them having different molecular structures. (Figs. 4-5). Additionally, the inventors show that the increase in induction of plant embryogenesis is obtained both in liquid and in solid embryogenesis cultures using as a starting material both microspores (microspore embryogenesis) as well as other plant explants (somatic embryogenesis) (Figs. 7-12). Moreover, the inventors confirmed through DNA staining and fluorescence microscopy that the proembryos obtained and quantified from cultures treated with the inhibitors were indeed multicellular microspores (Fig. 6), the first sign of embryogenesis initition. These experiments support the use of such small molecule inhibitors of mammal kinases as new tools to promote the induction and optimization of *in vitro* plant embryogenesis. Finally, these results suggest that common mechanisms may operate in other *in vitro* plant systems and that a similar strategy could be extended to other species to increase embryogenesis induction efficiency and plant cell reprogramming.

[0009] Thus, a first aspect of the present invention relates to the use of at least a mammal kinases inhibitor to improve *in vitro* plant embryogenesis induction.

[0010] The term "mammal" as used herein refers to any animal classified as a mammal including cows, horses, dogs, cats, rats, mice, primates and human beings. In a preferred embodiment of the invention, the mammal is a human.

[0011] The term "kinase" as used herein refers to a member of an enzyme superfamily which functions to phosphorylate one or more proteins, this is, they have protein kinase activity. The terms also relate to a nucleic acid encoding the protein/enzyme.

[0012] In a preferred embodiment of the invention the mammal kinases are human kinases.

[0013] For the purposes of the invention the mammal kinase, preferably human kinase is selected from a list consisting of: CDK1 (UniProt:P06493), CDK10 (UniProt:Q15131), CDK11A (UniProt:Q9UQ88), CDK11B (UniProt:P21127), CDK12 (UniProt:Q9NYV4), CDK13 (UniProt:Q14004), CDK14 (UniProt:O94921), CDK15 (UniProt:Q96Q40), CDK16 (UniProt:Q00536), CDK17 (UniProt:Q00537), CDK18 (UniProt:Q07002), CDK19 (UniProt:Q9BWU1), CDK2 (UniProt:P24941), CDK20 (UniProt:Q8IZL9), CDK3 (UniProt:Q00526), CDK4 (UniProt:P11802), CDK5 (UniProt:Q00535), CDK6 (UniProt:Q00534), CDK7 (UniProt:P50613), CDK8 (UniProt:P49336), CDK9 (UniProt:P50750), CDKL1 (UniProt:Q00532), CDKL2 (UniProt:Q92772), CDKL3 (UniProt:Q8IVW4), CDKL4 (UniProt:Q5MAI5), CDKL5 (UniProt:076039), CLK1 (UniProt:P49759), CLK2 (UniProt:P49760), CLK3 (UniProt:P49761), CLK4 (UniProt:Q9HAZ1), DYRK1A (UniProt:Q13627), DYRK1B (UniProt:Q9Y463), DYRK2 (UniProt:Q92630), DYRK3 (UniProt:O43781), DYRK4 (UniProt:Q9NR20), GSK3A (UniProt:P49840), GSK3B (UniProt:P49841), HIPK1 (UniProt:Q86Z02), HIPK2 (UniProt:Q9H2X6), HIPK3 (UniProt:Q9H422), HIPK4 (UniProt:Q8NE63), ICK (UniProt:Q9UPZ9), MAK (UniProt:P20794), MAPK1 (UniProt:P28482), MAPK10 (UniProt:P53779), MAPK11 (UniProt:Q15759), MAPK12 (UniProt:P53778), MAPK13 (UniProt:O15264), MAPK14 (UniProt:Q16539), MAPK15 (UniProt:Q8TD08), MAPK3 (UniProt:P27361), MAPK4 (UniProt:P31152), MAPK6 (UniProt:Q16659), MAPK7 (UniProt:Q13164), MAPK8 (UniProt:P45983), MAPK9 (UniProt:P45984), MOK (UniProt:Q9UQ07), NLK (UniProt:Q9UBE8), PRPF4B (UniProt:Q13523), SRPK1 (UniProt:Q96SB4), SRPK2 (UniProt:P78362), SRPK3 (UniProt:Q9UPE1), ACVR1 (UniProt:Q04771), ACVR1B (UniProt:P36896), ACVR1C (UniProt:Q8NER5), ACVR2A (UniProt:P27037), ACVR2B (UniProt:Q13705), ACVRL1 (UniProt:P37023), AMHR2 (UniProt:Q16671), ANKK1 (UniProt:Q8NFD2), ARAF (UniProt:P10398), BMPR1A (UniProt:P36894), BMPR1B (UniProt:000238), BMPR2 (UniProt:Q13873), BRAF (UniProt:P15056), ILK (UniProt:Q13418), IRAK1 (UniProt:P51617), IRAK2 (UniProt:043187), IRAK3 (UniProt:Q9Y616), IRAK4 (UniProt:Q9NWZ3), KSR1 (UniProtQ8IVT5), KSR2 (UniProt:Q6VAB6), LIMK1 (UniProt:P53667), LIMK2 (UniProt:P53671), LRRK1 (UniProt:Q38SD2), LRRK2 (UniProt:Q5S007), RAF1 (UniProt:P04049), RIPK1 (UniProt:Q13546), RIPK2 (UniProt:043353), RIPK3 (UniProt:Q9Y572), RIPK4 (UniProt:P57078), TESK1 (UniProt:Q15569), TESK2 (UniProt:Q96S53), TGFBR1 (UniProt:P36897), TGFBR2 (UniProt:P37173), TNNI3K (UniProt:Q59H18), MLKL (UniProt:Q8NB16), - All accession numbers correspond to UniProt release of 16 of October 2019.

[0014] In a further embodiment of the present invention, the mammal kinases, preferably human kinases are selected from GSK3β and/or LRRK2.

[0015] As used herein the term GSK3β refers to the glycogen synthase kinase 3 beta protein (EC:2.7.11.26) set forth by Uniprot Accesion Nos: P49841-1 (SEQ ID NO: 1) and P49841-2 (SEQ ID NO:2), or alternatively by GenBank Accession Nos. NP_002084.2 (SEQ ID NO: 2), NP_001139628.1 (SEQ ID NO: 1), NP_001341525.1 (SEQ ID NO: 3) and/or XP_006713673.1 (SEQ ID NO: 4) having the WNT signalling regulatory activity via its kinase activity.

[0016] In a further embodiment the mammal kinase GSK3β comprises an amino acid sequence with at least 90% identity with any of the SEQ ID NOs.:1 to 4, preferably 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity with any of the SEQ ID NOs.: 1 to 4. In a more preferred embodiment, the mammal kinase GSK3β comprises any of the SEQ ID NOs.: 1 to 4. In a more preferred embodiment, the mammal kinase GSK3β consists of any of the SEQ ID NOs.: 1 to 4.

[0017] The term "identity", as used herein, refers to the proportion of identical amino acids between two compared peptides or proteins or the proportion of identical nucleotides between two compared nucleotide sequences. The methods for comparing sequences are known in the state of the art, and include, but not limited to, the programs BLASTP or BLASTN, ClustalW and FASTA. We can consider that peptides, proteins or nucleotide sequences with percent identities of at least 90% will maintain the same properties as the sequence to which they refer.

[0018] As used herein the term LRRK2 refers to the leucine-rich repeat kinase 2 protein (EC 2.7.11.1) set forth by Uniprot Accession No: Q5S007 (SEQ ID NO: 5), or alternatively by GenBank Accession Nos. AAI17181.1 (SEQ ID NO: 6) and/or AAV63975.1 (SEQ ID NO: 7).

[0019] In a further preferred embodiment, the mammal kinase LRRK2 comprises an amino acid sequence having at least 90% sequence identity with any of the SEQ ID NO.: 5 to 7, preferably 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity with any of SEQ ID NO.: 5 to 7. In a more preferred embodiment, the mammal kinase LRRK2 comprises any of the SEQ ID NO.: 5 to 7. In a more preferred embodiment, the mammal kinase LRRK2 consists of any of the SEQ ID NO.: 5 to 7.

[0020] In a further preferred embodiment of the present invention, the mammal kinase inhibitors to induce *in vitro* plant embryogenesis are selected from GSK3β inhibitors and/or LRRK2 inhibitors.

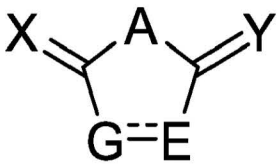
[0021] As used herein, the term "inhibitor" is interchangeably used to denote "antagonist". These terms define compounds or compositions which have the capability of decreasing certain enzyme activity or competing with the activity

or function of a substrate of said enzyme. As used in the present invention, refers to a chemical compound (naturally occurring or non-naturally occurring), such as a biological macromolecule (e.g., polynucleotide, protein or polypeptide, hormone, polysaccharide, lipid), an organic molecule (e.g., a small organic molecule), or an extract made from biological materials such as bacteria, plants, fungi, or animal (particularly mammalian, including human) cells or tissues which has been evaluated to reduce, diminish or inhibit (directly or indirectly) the activity of a kinase.

[0022] As used herein the term "GSK3β inhibitor" or "LRRK2 inhibitor" refers to any molecule as described above, capable of inhibiting the activity of GSK3β or LRRK2 as determined by specifically inhibiting levels of phosphorylated substrates specific for GSK3β or LRRK2 (out of total substrates present in a cell).

[0023] In a further preferred embodiment of the present invention, the GSK3β inhibitors to induce *in vitro* plant embryogenesis are selected from a list consisting of thiadiazolidindiones (Formula I), iminothiadiazoles (Formula II), disubstituted maleimides (Formula III) and disusbtituted carbohydrazides (Formula IV):

Thiadiazolidindiones of Formula (I):



Formula (I),

wherein:

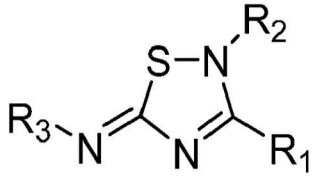
A is $-C(R^1)_2-$, -O- or $-NR^1-$;
E is $-NR^1-$ or $-CR^1R^2-$ and the substituent R^2 is absent if ------ is a second bond between E and G;
G is -S-, $-NR^1-$ or $-CR^1R^2-$ and the substituent R^2 is absent if ------ is a second bond between E and G;
------ may be a second bond between E and G where the nature of E and G permits and E with G optionally then forms a fused aryl group;
R^1 and R^2 are independently selected from hydrogen, (C_1-C_8)alkyl, cycloakyl, haloalkyl, aryl, $-(Z)_n$-aryl, heteroaryl, $-OR^3$, $-C(O)R^3$, $-C(O)OR^3$, $-(Z)_n-C(O)OR^3-$ and-$S(O)_t-$ or as indicated R^2 can be such that E with G then form a fused aryl group; Z is independently selected from $-C(R^3)(R^4)-$, -C(O)-, -O-, $-C(=NR^3)-$, $-S(O)_t-$ and $-N(R^3)-$; n is zero, one or two; t is zero, one or two; R^3 and R^4 are independently selected from hydrogen, (C_1-C_8)alkyl, aryl and heterocyclic; X and Y are independently selected from =O, =S, $=N(R^3)$ and $=C(R^1)(R^2)$.

[0024] In a preferred embodiment of the inhibitor of Formula (I), A is $-NR^1-$, E is $-NR^1-$, G is -S- and X and Y are from =O.

[0025] In a more preferred embodiment of the inhibitor of Formula (I), R^1 is independently selected from hydrogen, (C_1-C_8)alkyl, aryl, and $-(C(R^3)(R^4))$n-aryl; n is zero, one or two, R^3 and R^4 are each independently selected from hydrogen, (C_1-C_8)alkyl, aryl and heterocyclic. More preferably R^1 is independently selected from (C_1-C_8)alkyl or-$(C(R^3)(R^4))$n-aryl.,

[0026] In another preferred embodiment, the inhibitor of Formula (I) is 4-benzyl-2-methyl-1,2,4-thiadozilidine-3,5-dione (TDZD8).

[0027] Iminothiadiazoles of Formula (II):



Formula (II)

wherein: R_1 is selected from H, CN, NO_2, F, Cl, Br, I, or a group X_1-R_1' wherein X_1 is a single bond or a group selected from C_1-C_6 alkylene, C_2-C_6 alkenylene, C_2-C_6 alkynylene, C_3-C_{10} cycloalkylene, C_3-C_{10} heterocycloalkylene, arylene and heteroaryl; being X_1 optionally substituted with at least one or more groups which may be identical or different and are selected from H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_{10} cycloalkyl, C_3-C_{10} heterocycloalkyl, F, Cl, Br, I, -OH, =O, -CN,$-NO_2$, $-CO_2R_4$, $-OR_4$, $-SR_4$, $-SO_2NR_4R_5$, $-C(=O)NR_4$ or $-NR_4R_6$;

R_1' is selected from H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_7 cycloalkyl, C_1-C_6 alkoxy, aryl, heteroaryl, C_3-C_{10} cycloalkyl or C_3-C_{10} heterocycloalkyl; being R_1' optionally substituted with one or more groups X_1'-R_8 which may be identical or different; being R_1' optionally substituted with one or more groups X_1'-R_8 which may be identical or different;

X_1' is a single bond or a group selected from C_1-C_6 alkylene, C_2-C_6 alkenylene, C_2-C_5 alkynylene, arylene, heteroarylene, C_3-C_{10} cycloalkylene and C_3-C_{10} heterocycloalkylene, -C(O)O-, amino, -O-, -S- and -SO_2-; being X_1' optionally substituted with at least one or more groups which may be identical or different and are selected from H, C_1-C_3 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_4-C_7 cycloalkyl, F, Cl, Br, I, =O, -CN, -NO_2, -CO_2R_4, -OR_4, -SR_4, -$SO_2NR_6R_7$, =NR_4 and -NR_6R_7 being R_6 and R_7 independently selected from R_4 and R_5

R_8 is H, -OH, =O, -NO_2, CN, F, Cl, Br, I, C_1-C_4 alkyl, -CO_2R_{6a}, -C(=O)R_{6a}, C(=S)R_{6a}, SO_2R_{6a}, SOR_{6a}, SO_3R_{6a}, SR_{6a}, OR_{6a}, C(=O)$NR_{6a}R_{7a}$, C(=S)$NR_{6a}R_{7a}$, C(=N-CN)$NR_{6a}R_{7a}$, C(=N-SO_2NH_2)$NR_{6a}R_{7a}$, C(=CH-NO_2)$NR_{6a}R_{7a}$, $SO_2NR_{6a}R_{7a}$, C(=NR_{6a})NHR_{7a}, C(=NR_{6a})R_{7a} or $NR_{6a}R_{7a}$, being R_{6a} and R_{7a} independently selected from R_4 and R_5;

R_4 and R_5 are independently selected from: H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_7 X_4-cycloalkyl, X_4-cyclobutyl, X_4-cyclopentyl, X_4-cyclohexyl, X_4-cycloheptyl, X_4-benzyl, X_4-pyridinyl, X_4-pirimidinyl, X_4-pyperidinyl, X_4-pyrrolidinyl, X_4-pyrrolyl, X_4-imidazolyl and X_4-pyranyl saturated or unsaturated; being optionally substituted the groups R_4 and R_5 with one or more groups selected from =O, -NO_2, CN, F, Cl, Br, I, C_1-C_4 alkyl, -CO_2R_{10}, -C(=O)R_{10}, C(=S)R_{10}, SO_2R_{10}, SOR_{10}, SO_3R_{10}, SR_{10}, OR_{10}, C(=O)$NR_{10}R_{11}$, C(=N-SO_2NH_2)$NR_{10}R_{11}$, C(=CH-NO_2)$NR_{10}R_{11}$, $SO_2NR_{10}R_{11}$, C(=NR_{10})NHR_{11}, C(=NR_{10})R_{11} and $NR_{10}R_{11}$;

X_4 is a single bond or a group selected from C_1-C_6 alkylene, C_2-C_6 alkenylene; each one of the groups optionally substituted with one or more groups which may be identical or different and are selected from =O, -NO_2, CN, F, Cl, Br, I, C_1-C_4 alkyl,-CO_2R_{10}, -C(=O)R_{10}, OR_{10}, C(=O)$NR_{10}R_{11}$, -$SO_2NR_{10}R_{11}$ and $NR_{10}R_{11}$;

R_{10} and R_{11} are independently selected from H and C_1-C_6 alkyl;

R_2 is selected from C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, aryl, heteroaryl, C_3-C_{10} cycloalkyl and C_3-C_{10} heterocycloalkyl, CN or amino; being R_2 optionally substituted with at least one or more groups which may be identical or different and are selected from H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, aryl, heteroaryl, C_3-C_{10} cycloalkyl y C_3-C_{10} heterocycloalkyl, =O, -NO_2, CN, F, Cl, Br, I, C_1-C_4 alkyl, -CO_2R_{6b}, -C(=O)R_{6b}, SO_2R_{6b}, SOR_{6b}, SO_3R_{6b}, SR_{6b}, OR_{6b}, C(=O)$NR_{6b}R_{7b}$, $SO_2NR_{6b}R_{7b}$, and $NR_{6b}R_{7b}$, being R_{6b} and R_{7b} independently selected from R_4 and R_5;

R_3 is -CH_2- R_3'; R_3' is selected from heteroaryl, -C(O)OR_{12},

or R_3' is selected from -$(CH_2)_nOR_{6e}$, n being between 1 and 20, with the condition, that R_3' cannot be -$(CH_2)_2$-OH, R_{6e} being selected from R_4 and R_5,

or R_3' is selected from -$(CH_2)_n$-(C_3-C_{10}heterocycloalkyl), with n being 0 to 20

R_{12} is independently selected from the groups defined for R_{10};

regarding that "cycloalkyl" comprises preferably a group C_3-C_{10} cycloalkyl, more particularly a saturated cycloalkyl group saturated with the length indicated in the ring, as for example; cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl, or cyclodecyl and also comprises unsaturated cycloalkyls that contain one or more double bonds in the carbonated chain as for example cycloalkenyl groups C_3-C_{10} such as cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, cycloheptenyl, cyclooctenyl, cyclononenyl, or cyclodecenyl and related to the bonds, for the rest of the molecule, the cycloalkyl group may contain single or double bonds, in other words, it may be saturated or unsaturated and may optionally be substituted with one or more times, independently from the other groups with an alkyl group C_1-C_6 and/or an halogen and/or an OR^f group and/or a $NR^{g1}R^{g2}$ group as for example 2-methylcyclopropyl, 2,2-dimethylcyclopropyl, 2,2-dimethylcyclobutyl, 3-hydroxycyclopentyl, 3-hydroxycyclohexyl, 3-dimethylaminocyclobutyl, 3-dimethylaminocyclopentyl and 4-dimethylaminocyclohexyl groups;

and that the term "heterocycloalkyl" comprises preferably a cycloalkyl group C_3-C_{10}, as defined before, wherein one of the atoms of the rings is an heteroatom like NH, NR^{d3}, O, S or groups like C (O), S (O), $S(O)_2$, or also a group C_n-cyclo alkyl, wherein n is a number selected from 3, 4, 5, 6, 7, 8, 9 and 10, wherein one or more of the carbon atoms are substituted by the heteroatoms or before cited groups in order to be a C_n-cycloheteroalkyl group; they

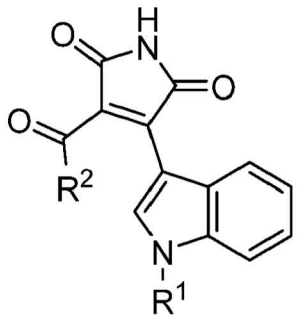
also comprises unsaturated cycloheteroalkyl groups that contain one or more double bonds in the carbonated chain, therefore related to the bonds, for the rest of the molecule, cycloheteroalkyl group may contain single and double bonds, in other words, it may be saturated or unsaturated and may optionally substituted one or more times, independently of the other groups with an alkyl group C_1-C_6 and/or an halogen and/or an OR^f group and/or a group and that the C_n-cycloheteroalkyl group is related for example to heterocycles of three members expressed as C3-heterocycloalkyl named oxyranyles.

[0028] In a preferred embodiment, the inhibitor of Formula of formula (II) is selected from:

2,3-Diphenyl-5-(3-pyridylmethylimino)-2,5-dihydro-1,2,4-thiadiazole
3-(4-Methoxyphenyl)-2-phenyl-5-(3-pyridylmethylimino)-2,5-dihydro-1,2,4-thiadiazole
2-(4-Methoxyphenyl)-3-phenyl-5-(3-pyridylmethylimino)-2,5-dihydro-1,2,4-thiadiazole
2-(4-Nitrophenyl)-3-phenyl-5-(3-pyridylmethylimino)-2,5-dihydro-1,2,4-thiadiazole
2-Phenyl-5-(3-pyridylmethylimino)-3-(4-trifluoromethylphenyl)-2,5-dihydro-1,2,4-thiadiazole
2-(1-Naphthyl)-3-phenyl-5-(3-pyridylmethylimino)-2,5-dihydro-1,2,4-thiadiazole
3-(1-Naphthyl)-2-phenyl-5-(3-pyridylmethylimino)-2,5-dihydro-1,2,4-thiadiazole
3-Methyl-2-phenyl-5-(3-pyridylmethylimino)-2,5-dihydro-1,2,4-thiadiazole
5-Ethoxycarbonylmethylimino-2,3-diphenyl-2,5-dihydro-1,2,4-thiadiazole
5-Ethoxycarbonylmethylimino-3-(4-methoxyphenyl)-2-phenyl-2,5-dihydro-1,2,4-thiadiazole
5-Ethoxycarbonylmethylimino-2-(4-methoxyphenyl)-3-phenyl-2,5-dihydro-1,2,4-thiadiazole
5-Ethoxycarbonylmethylimino-2-(4-nitrophenyl)-3-phenyl-2,5-dihydro-1,2,4-thiadiazole
5-(2-Hydroxyethylimino)-2,3-diphenyl-2,5-dihydro-1,2,4-thiadiazole
5-(2-Hydroxyethylimino)-3-(4-methoxyphenyl)-2-phenyl-2,5-dihydro-1,2,4-thiadiazole
5-(2-Hydroxyethylimino)-2-(4-methoxyphenyl)-3-phenyl-2,5-dihydro-1,2,4-thiadiazole
5-(2-Hydroxyethylimino)-2-(1-naphthyl)-3-phenyl-2,5-dihydro-1,2,4-thiadiazole
5-(2-Hydroxyethylimino)-3-(1-naphthyl)-2-phenyl-2,5-dihydro-1,2,4-thiadiazole, and
5-(2-Morpholinethylimino)-2,3-diphenyl-2,5-dihydro-1,2,4-thiadiazole.

[0029] More preferably 5-(2-Morpholinethylimino)-2,3-diphenyl-2,5-dihydro-1,2,4-thiadiazole (VP3.15).

[0030] Disubstituted maleimides of Formula (III):



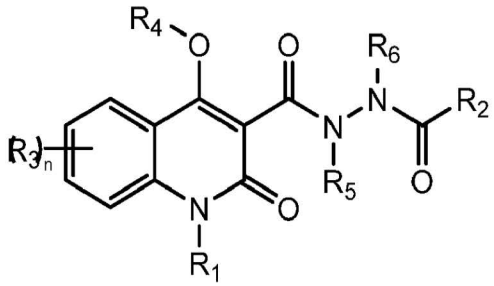
Formula (III)

wherein: R^1 is selected from H or C_1-C_{10} alkyl and R^2 is selected from C_1-C_{10} alkyl or C_2-C_{10} alkenyl; being optionally substituted by halogen.

[0031] In a preferred embodiment of the inhibitor of Formula (III), R^1 is C_1-C_5 alkyl and R^2 is C_1-C_5 alkyl.

[0032] In a more preferred embodiment, the inhibitor of Formula (III) is 3-acetyl-4-(1-methyl-1H-indol-3-yl)-1H-pirrol-2,5-dione (VP3.36).

[0033] Disusbtituted carbohydrazides of Formula (IV):



Formula (IV)

wherein R_1 is selected from H and C_1-C_5 alkyl, optionally substituted, R_2 is C_5-C_{15} alkyl, optionally substituted, R_3 is selected from H, halogen, C_1-C_5 alkyl, optionally substituted, and -(O)- C_1-C_5 alkyl, optionally substituted, n is between 1 and 4, R_4, R_5 y R_6 are each independently selected from H and C_1-C_5 alkyl, optionally substituted.

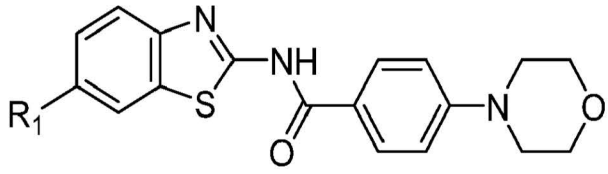
[0034] In a preferred embodiment of the inhibitor of Formula (IV), R_3, R_4, R_5, or R_6 are H.

[0035] In another preferred embodiment of the inhibitor of Formula (IV), R_1 and R_2 are each independently selected from C_9-C_{12} alkyl.

[0036] In a more preferred embodiment, the inhibitor of Formula (IV) is 4-hydroxy-1-ethyl-N'-palmitoyl-2-oxo-1,2-dihydroquinoline-3-carbohydrazide (VP0.7).

[0037] For the purposes of the current invention, preferably the GSK3β inhibitors are selected from: 4-benzyl-2-methyl-1,2,4-thiadozilidine-3,5-dione (TDZD8); 5-(2-morpholinethylimino)-2,3-diphenyl-2,5-di-hydro-1,2,4-thiadiazole (VP3.15); 3-acetyl-4-(1-methyl-1H-indol-3-yl)-1H-pirrol-2,5-dione (VP3.36); and 4-hydroxy-1-ethyl-N'-palmitoyl-2-oxo-1,2-dihydroquinoline-3-carbohydrazide (VP0.7).

[0038] In a further embodiment of the present invention, the LRRK2 inhibitors to induce *in vitro* plant embryogenesis are selected from the substituted N-(benzotiazolil-4-morfolinobenzamide (Formula V) and the (E,Z)-3-(morpholinoimino)indolin-2-one, named as IGS4.75.



Formula (V)

wherein: R_1 is selected from H, C_1-C_6 alkyl, halogen, CF_3, and -O-C_1-C_6.alkyl.

[0039] In a preferred embodiment of the inhibitor of Formula (V), R_1 is H.

[0040] In another preferred embodiment of the inhibitor of Formula (V), R_1 is a C_1-C_4 alkyl. In a more preferred embodiment of the compound (V), R_1 is selected from methyl or isopropyl.

[0041] In another preferred embodiment of the inhibitor of Formula (V), R_1 is selected from F, Cl or Br.

[0042] In another preferred embodiment of the inhibitor of Formula (V), R_1 is a -O-C_1-C_4 alkyl. In a more preferred embodiment of the inhibitor of Formula (V), R_1 is selected from -O-methyl, -O-ethyl and -O-propyl.

[0043] In another preferred embodiment of the inhibitor of Formula (V), R_1 is CF_3.

[0044] In another preferred embodiment, the inhibitor of Formula (V) is selected from the following list:

- N-(benzothiazole-2-yl)-4-morpholinobenzamide,
- N-(6-methoxybenzothiazole-2-yl)-4-morpholinobenzamide,
- N-(6-trifluoromethylbenzothiazole-2-yl)-4-morpholinobenzamide,
- N-(6-methylbenzothiazole-2-yl)-4-morpholinobenzamide (JZ1.3),
- N-(6-chlorobenzothiazole-2-yl)-4-morpholinobenzamide,
- N-(6-fluorobenzothiazole-2-yl)-4-morpholinobenzamide (JZ1.6),
- N-(6-ethoxybenzothiazole-2-yl)-4-morpholinobenzamide,
- N-(6-bromobenzothiazole-2-yl)-4-morpholinobenzamide (JZ1.24),
- N-(6-propoxybenzothiazole-2-yl)-4-morpholinobenzamide,
- N-(6-isopropylbenzothiazole-2-yl)-4-morpholinobenzamide.

[0045] More preferably the inhibitor of Formula (V) is selected from N-(6-methylbenzothiazole-2-yl)-4-morpholinobenzamide (JZ1.3), N-(6-fluorobenzothiazole-2-yl)-4-morpholinobenzamide, (JZ1.6) and N-(6-bromobenzothiazole-2-yl)-4-morpholinobenzamide (JZ1.24).

[0046] For the purposes of the current invention, preferably the LRRK2 inhibitors are selected from: N-(6-metilbenzotiazol-2-il)-4-morfolinobenzamida (JZ1.3), N-(6-flurobenzotiazol-2-il)-4-morfolinobenzamida, (JZ1.6) and N-(6-bromobenzotiazol-2-il)-4-morfolinobenzamida (JZ1.24) and (*E,Z*)-3-(morpholinoimino)indolin-2-one (IGS4.75).

[0047] In another embodiment of the present invention, the induction of *in vitro* plant embryogenesis comprises increased plant embryo growth and/or increased embryo production yield. As it is shown in the examples, the results states that all of the inhibitors tested lead to an increase of embryogenesis induction efficiency in the range of 20-25% for GSK3β inhibitors and 23-30% for LRRK2 inhibitors, when applied at their optimal concentration.

[0048] As used herein, the term "plant embryo growth regulator" refers to any compound capable of inducing plant embryo growth, preferably embryos from agricultural and/or forest plant.

[0049] As used herein, the term "embryo production yield" refers to the number of individual embryos resulting from *in vitro* embryogenesis induction, preferably microspore and/or somatic embryogenesis.

[0050] In another embodiment of the present invention, the mammal kinase inhibitors are used in *in vitro* plant embryogenesis wherein the embryogenesis is somatic and/or by microspores.

[0051] The term "somatic embryogenesis" as used herein refers to a type of plant tissue culture where a piece of a donor plant, composed by somatic cells, is excised, cultured and induced to form multiple embryos, which can further germinate and produce entire plants.

[0052] The term "microspore embryogenesis" as used herein refers to a unique process in which haploid, immature pollen (microspores) are induced by different treatments to form embryos in culture. These microspore-derived embryos can then be germinated and converted to homozygous doubled haploid plants by chromosome doubling agents and/or through spontaneous doubling.

[0053] In a further embodiment of the present invention, the mammal kinase inhibitors are used to induce *in vitro* plant embryogenesis wherein the plants are crops and/or forests plants.

[0054] As used herein the term "plant" refers to a whole plant or parts thereof. The phrase "plant part" refers to isolated plant cells or isolated plant parts (tissues) such as from which plants can be (re)generated, including plant protoplasts, plant cali, plant clumps, and plant cells that are intact in plants, or part of plants, such as seeds, leaves, stems, pollens, roots, root tips, anthers, ovules, petals, flowers, seedlings, embryos and bolls.

[0055] In a preferred embodiment the crop plants as used herein are selected from the list consisting of: *Medicago spp., Prunus spp., Angelica spp., Pimpinella spp., Ceratonia siliqua, Malus spp., Areca spp, Arracacia spp, Maranta spp., Cynara spp., Daucus carota, Anacardium occidentale, Asparagus spp., Persea spp., Pearl spp., Pennisetum spp., Vigna spp., Musa spp., Sechium edule, Jatropha spp., Cocos nucifera, Hordeum spp., Apium graveolens, Cyclamen spp., Atalantia spp. Anethum graveoles, Vigna subterranea, Laurus spp., Phaseolus spp. Ocumum spp., Cinnamomum verum, Paulinia cupana, Areca spp., Annona reticulate, Piper spp., Acacia spp., Rubus spp. Vaccinium spp. Bertholletia excelsa, Sesamum indicum, Artocarpus spp., Vicia spp, Fagopyrum esculentum, Carum carvi, Elettaria cardamomum, Ricinus communis, Castanea sativa, Cicer spp. Cichorium spp, Eugenia aromatica, Syzygium aromaticum, Trifolium spp. Erythroxypum spp., Cola spp., Brassica spp., Valerianella locusta, Gossypium spp., Lepidium sativum, Cucumis spp., Ficus carica, Corylus spp., Furcraea macrophylla, Linum spp., Geranium spp., Zingiber spp., Panax spp., Ribes spp., Vitis vinifera, Lygeum spartum, Dactylis spp., Arachis hypogaea, Corylus avellana, Cannabis sativa, Crotalaria juncea, Lawsonia inermis, Armoracia rusticana, Indigofera tinctoria, Jasminum spp., Helianthus spp., Actinidia deliciosa, Lavandula spp., Citrus spp., Cymbopogon citratus, Lens culinaris, Lespedeza spp., Lactuca spp., Litchi chinensis, Eriobotrya japonica, Lupinus spp., Macadamia spp., Zea mays, Mangifera spp., Secale spp, Setaria italica, Echinochloa esculenta, Pennisetum americanum, Panicum miliaceum, Mentha spp., Morus spp., Sinapis spp., Avena spp., Elaeis guineensis, Abelmoschus esculentus, Hibiscus esculentus, Olea spp, Allium spp., Papaver spp., Borassus flabellifer, Elaeis guineensis, Pastinaca sativa, Pisum sativum, Pyrus communis, Carya illinoensis, Capsicum spp., Cajanus cajan, Ananas comosus, Pistacia vera, Punica granatum, Solamum spp., Ipomoea spp. Cucurbita spp., Chrysanthemum spp., Aspidosperma spp., Cydonia oblonga, Cinchona spp., Chenopodium quinoa, Raphanus sativus, Rubus spp., Agrostis spp., Rheum spp., Oryza spp., Rose spp., Hevea brasiliensis, Lolium spp. Crocus savitus, Vitellaria paradoxa, Butyrospermum parkii, Agave spp.,Glycine spp., Triticum spp., Spinacia oleracea, Fragaria spp., Beta spp., Sorghum spp., Thymus spp., Timothy spp., Phleum pratense, Phleum alpinum, Saccharum officinarum, Nicotiana spp., Bixa spp, Solanum spp., Lotus spp., Triticale (Hybrid of Triticum aestivum and Secale cereale), Curcuma spp., Vanilla planifolia, Juglans spp., Citrullus lanatus, Dioscorea spp., Ilex paraguariensis, Pennisetum glaucum, Setaria italic, Eleusine coracana, Panicum virgatum, Echinochloa frumentacea, Paspalum scrobiculatum, Digitaria exilis, Milium effusum, Phalaris canariensis, Coix lacryma-jobi.* Where so applicable the crop plants species here listed include all different sub-species, varieties and cultivars existent, including, without limitation, regional, seasonal and agricultural varieties.

[0056] In a more preferred embodiment, the crop plants are selected from the list consisting of: *Hordeum spp., Zea mays, Secale spp, Setaria italica, Panicum miliaceum, Avena spp., Oryza spp., Triticum spp, Sorghum spp., Triticale*

(Hybrid of Triticum aestivum and Secale cereale), Pennisetum glaucum, Eleusine coracana, Phalaris canariensis, Cynara spp., Daucus carota, Piper spp, Trifolium spp, Brassica spp, Lactuca spp, Mentha spp, Allium spp., Pisum sativum, Capsicum spp, Solamum spp, Cucurbita spp, Chenopodium quinoa, Rubus spp, Spinacia oleracea, Beta spp, Solanum spp., Helianthus spp., Gossypium spp, Arachis hypogaea, Cannabis sativa, Saccharum officinarum, Linum spp., Glycine spp., Nicotiana spp, Medicago spp., and/or *Agrostis spp.* In a more preferred embodiment, the crop plants belong to *Hordeum spp. and/or Brassica spp.*

[0057] In another preferred embodiments the forest plants are selected from a list consisting of: *Araucaria spp., Cryptomeria japonica, Cupressus spp, Juniperus spp., Sequoia sempervirens, Sequoiadendron giganteum, Thuja spp, Abies spp., Cedrus spp, Larix spp, Picea spp., Pinus spp., Pseudotsuga spp, Taxus spp., Ginkgo biloba, Acer spp., Anacardium occidentale, Mangifera spp, Pistacia spp, Cocos nucifera, Phoenix spp, Betula spp., Corylus spp, Paulownia tomentosa, Adansonia spp, Capparis spp, Sambucus spp., Carica papaya, Euonymus spp, Hevea brasiliensis, Manihot spp., Acacia spp., Robinia spp, Castanea spp, Fagus spp, Quercus spp., Carya spp., Juglans spp., Cinnamomum spp., Laurus spp, Persea spp., Swietenia spp., Artocarpus spp., Ficus spp., Morus spp., Myrtus communis, Psidium spp., Nothofagus spp., Fraxinus spp., Olea europaea, Platanus spp, Dendrocalamus asper, Malus spp., Photinia spp, Photinia × fraser, Prunus spp., Pyrus spp., Coffea spp., Citrus spp., Populus spp., Salix spp., Solanum erianthum; Theobroma cacao, Camellia spp., Tilia spp., Ulmus spp.,* Tamarillo, *(Cyphomandra betacea (Cav.) (Sendtn.); Solanum betaceum Cav.),* Indian olive *(Elaeocarpus robustus L.);* bottle palm *(Hyophorbe lagenicaulis*), Indian rosewood *(Dalbergia sissoo*), canela petrea (*Ocotea catharinensis* Mez.), Sandalwood (*Santalum album*), *Echinacea purpurea* L., longan (*Dimocarpus longan* Lour.), (*Aspidosperma polyneuron* Mull.Arg), rattan (*Calamus* spp.), jojoba (*Simmondsia chiensis*), (*Aegle marmelos* L.), black cohosh (*Actaea racemosa* L.), *Gomortega keule, Cyclamen* spp., Hybrid Aspen (*Populus tremuloides* x *Populus tremula*), Oil palm (*Elaeis guineensis* Jacq.), *Passiflora* spp., Açaí palm (*Euterpe oleracea* Mart.), tree-fern (*Cyathea delgadii* Sternb.), *Eucalyptus* spp., Hybrid Larch (*Larix* x *eurolepis* Henry), neem (*Azadirachta indica*). Where so applicable the forest plants species here listed include all different sub-species, varieties and cultivars existent, including, without limitation, regional, seasonal and agricultural varieties.

[0058] In a more preferred embodiment, the forest plants are selected from the list consisting of: *Araucaria spp., Cupressus spp, Juniperus spp., Abies spp., Cedrus spp, Larix spp, Picea spp., Pinus spp., Pseudotsuga spp, Taxus spp., Ginkgo biloba, Acer spp., Anacardium occidentale, Mangifera spp, Pistacia spp, Cocos nucifera, Phoenix spp, Betula spp., Corylus spp, Carica papaya, Hevea brasiliensis, Acacia spp., Robinia spp, Castanea spp, Fagus spp, Quercus spp., Cinnamomum spp., Laurus spp, Persea spp., Morus spp., Psidium spp., Fraxinus spp., Olea europaea, Platanus spp, Malus spp., Prunus spp., Pyrus spp., Coffea spp., Citrus spp., Populus spp., Salix spp., Theobroma cacao, Camellia spp., Ulmus* spp.,Tamarillo, *(Cyphomandra betacea (Cav.) (Sendtn.); Eucalyptus* spp. In a more preferred embodiment, the forest plants belong to *Quercus spp.*

[0059] In a further aspect, the invention relates to a method, here onwards the method of the invention, to induce *in vitro* plant embryogenesis, where the method comprising:

a. culturing the microspores and/or explants in a culture medium suitable for embryo development; and
b. adding mammal kinase inhibitors to the culture medium of step a); and c. culturing for a period sufficient to obtain embryos.

[0060] The term "culture medium" as used herein is intended to indicate any material either solid or liquid in which plant cells, tissues, organs and whole plants may grow. Additives may be provided to the cells in the form of media, and environmental conditions controlled. There are many types of plant tissue culture media comprised of mixtures of mineral salts containing essential oligoelements plus various additives like amino acids, sugars, growth regulators and vitamins which must therefore be added to the culture medium to allow development of (pro)embryo, explant and/or plant growth. Examples of plant tissue culture medium are, without limitation, Chu (N6) medium (Duchefa, Sigma-Aldrich), Clc/Ipomoea CP medium (Duchefa), CLC/Ipomoea ep medium (Duchefa), DKV/Junglans medum (Duchefa, Sigma-Aldrich), Erikson medium (Duchefa), Gamborg B5 medium (Duchefa, Sigma-Aldrich), Gresshoff and Doy medium (Duchefa), Lindemann orchid medium (Duchefa), NLN medium (Duchefa), Nitsch medium (Duchefa), Woody plant medium (Duchefa, Sigma-Aldrich), Linsmaier and Skoog medium (Duchefa), Litvay medium (Duchefa), Quorin and Lepoivre medium (Duchefa), Rugini olive medium (Duchefa), Schenk and Hildebrant medium (Duchefa, Sigma-Aldrich), White's medium (Duchefa, Sigma-Aldrich), Westvaco WV5 medium (Duchefa), Murashige and Skoog medium (Duchefa, Sigma-Aldrich), Murashige and Skoog medium with B5 vitamins (Duchefa), Murashige and Skoog medium with Nitsch vitamins (Duchefa), Murashige and Skoog medium van der Salm (Duchefa), Hoagland's n°2 basal salt mixture (Sigma-Aldrich), Sommer macronutrients + MS micronutrients and vitamins (Testillano et al. 2018, Plant Cell Culture Protocols, eds. V.M. Loyola-Vargas & N. Ochoa-Alejo. Springer and Bussines Media. pp. 247-256), KBP medium (Kumlehn et al. 2006).

[0061] As used herein, the term "plant embryo" refers to a somatic plant embryo or a microspore plant embryo. Somatic plant embryos may be produced by culturing embryogenic tissue by standard methods under laboratory conditions in which some of the cells comprising the tissue, the responsive ones, are induced to reprogram and develop into complete

embryos. In the same sense, microspore plant embryo may be produced by culturing either anthers containing microspores or isolated microspores in appropriate culture medium under defined conditions in which some microspores, the responsive ones, are induced to reprogram and develop into complete haploid and doubled-haploid embryos.

[0062] As used herein, "plant embryo" includes embryos at various stages of development.

[0063] The term "explant" as used herein refers to a piece of tissue taken from a donor plant for culturing.

[0064] In a preferred embodiment, the method of the invention is a method wherein the embryogenesis is somatic and/or by microspores.

[0065] All the terms and definitions mentioned previously by the use of the mammal's kinase inhibitors to induce *in vitro* plant embryogenesis, apply in the same way to the method to induce *in vitro* plant embryogenesis disclosed herein.

[0066] Thus, in another preferred embodiment, the method of the invention is a method wherein the mammal kinases are human kinases, preferably GSK3β and/or LRRK2, as it has been disclosed previously.

[0067] In a further preferred embodiment, the method of the invention is a method wherein the plants are crops and/or forest plants.

[0068] In yet another preferred embodiment, the method of the invention is a method wherein the crop plants are selected from the list consisting of: *Hordeum spp., Zea mays, Secale spp, Setaria italica, Panicum miliaceum, Avena spp., Oryza spp., Triticum spp, Sorghum spp., Triticale (Hybrid of Triticum aestivum and Secale cereale), Pennisetum glaucum, Eleusine coracana, Phalaris canariensis, Cynara spp., Daucus carota, Piper spp, Trifolium spp, Brassica spp, Lactuca spp, Mentha spp, Allium spp., Pisum sativum, Capsicum spp, Solamum spp, Cucurbita spp, Chenopodium quinoa, Rubus spp, Spinacia oleracea, Beta spp, Solanum spp., Helianthus spp., Gossypium spp, Arachis hypogaea, Cannabis sativa, Saccharum officinarum, Linum spp., Glycine spp., Nicotiana spp, Medicago spp., and/or Agrostis spp.* In a more preferred embodiment, the crop plants belong to *Brassica spp.* and/or *Hordeum spp.*

[0069] In another preferred embodiment, the method of the invention is a method wherein the forest plants are selected from the list consisting of: *Araucaria spp., Cupressus spp, Juniperus spp., Abies spp., Cedrus spp, Larix spp, Picea spp., Pinus spp., Pseudotsuga spp, Taxus spp., Ginkgo biloba, Acer spp., Anacardium occidentale,Mangifera spp, Pistacia spp, Cocos nucifera, Phoenix spp, Betula spp., Corylus spp, Carica papaya, Hevea brasiliensis, Acacia spp., Robinia spp, Castanea spp, Fagus spp, Quercus spp., Cinnamomum spp., Laurus spp, Persea spp., Morus spp., Psidium spp., Fraxinus spp., Olea europaea, Platanus spp, Malus spp., Prunus spp., Pyrus spp., Coffea spp., Citrus spp., Populus spp., Salix spp., Theobroma cacao, Camellia spp., Ulmus spp.,Tamarillo, (Cyphomandra betacea (Cav.) (Sendtn.); Eucalyptus spp.* In a more preferred embodiment, the forest plants belong to *Quercus spp.*

[0070] In a further preferred embodiment, the method of the invention is a method wherein the GSK3β inhibitors inhibitors used are the same as described above. In a more preferred embodiment, the GSK3β inhibitors are selected from a list consisting of: 4-benzyl-2-methyl-1,2,4-thiadozilidine-3,5-dione (TDZD8), 5-(2-Morpholinethylimino)-2,3-diphenyl-2,5-dihydro-1,2,4-thiadiazole (VP3.15), 3-acetyl-4-(1-methyl-1H-indol-3-yl)-1H-pirrol-2,5-dione (VP3.36), 4-hydroxy-1-ethyl-N'-palmitoyl-2-oxo-1,2-dihydroquinoline-3-carbohydrazide (VP0.7).

[0071] In a further preferred embodiment, the method of the invention is a method wherein the LRRK2 inhibitors used are the same as described above. In a more preferred embodiment, are selected from a list consisting of: N-(6-metilbenzotiazol-2-il)-4-morfolinobenzamida (JZ1.3), N-(6-flurobenzotiazol-2-il)-4-morfolinobenzamida (JZ1.6), N-(6-bromobenzotiazol-2-il)-4-morfolinobenzamida (JZ1.24), and (E,Z)-3-(morpholinoimino)indolin-2-one (IGS4.75).

[0072] In a further preferred embodiment, the method of the invention is a method wherein the inhibitor concentration ranges from 0.1 μM to 100 μM inclusive. Preferably the inhibitor concentrations have ranges of 0.5 - 10 μM, 10 - 20 μM, 20 - 30 μM, 30 - 40 μM, 40 - 50 μM, 50 - 60 μM, 70 - 80 μM, 80 - 90 μM, 90 - 100 μM. More preferably the inhibitor concentrations have ranges of 0.1 - 5 μM, 5 - 10 μM, 10 - 15 μM, 15 - 20 μM, 20 - 25 μM, 25 - 30 μM, 30 - 35 μM, 35 - 40 μM, 40 - 45 μM, 45 - 50 μM, 50 - 55 μM, 55 - 60 μM, 60 - 65 μM, 65 - 70 μM, 70 - 75 μM, 75 - 80 μM, 80 - 85 μM, 85 - 90 μM, 90 - 95 μM, 95 - 100 μM.

[0073] In a further preferred embodiment, the method of the invention is a method wherein the culture medium is a liquid medium and the inhibitor concentration ranges from 0.1 μM to 100 μM inclusive, preferably from 0.5 μM to 5 μM inclusive.

[0074] In another preferred embodiment, the method of the invention is a method wherein the culture medium is a solid medium and the inhibitor concentration ranges from 0.1 μM to 100 μM inclusive, preferably from 25 μM to 100 μM, preferably from 25 μM to 50 μM.

[0075] In a more preferred embodiment, wherein the embryogenesis is an embryogenesis from microspores, the inhibitor concentration ranges from 0.1 μM to 100 μM inclusive, preferably from 0.5 μM to 10 μM and more preferably from 0.5 μM to 5 μM wherein the culture medium is a liquid media. In another preferred embodiment, the inhibitor concentration ranges from 20 μM to 100 μM inclusive, preferably from 25 μM to 50 μM, wherein the culture medium is a solid media.

[0076] In a more preferred embodiment, wherein the embryogenesis in a somatic embryogenesis, the inhibitor concentration ranges from 20 μM to 100 μM inclusive, preferably from 25 μM to 50 μM, wherein the culture medium is a solid media.

[0077] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skilled in the art to which this invention belongs. Methods and materials similar or equivalent to those described herein can be used in the practice of the present invention. Throughout the description and claims the word "comprise" and its variations are not intended to exclude other technical features, additives, components, or steps. Additional objects, advantages and features of the invention will become apparent to those skilled in the art upon examination of the description or may be learned by practice of the invention. The following examples and drawings are provided by way of illustration and are not intended to be limiting of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0078]

Fig. 1. *In vitro* microspore embryogenesis in *B. napus.* Cotyledonary embryos developed from isolated microspore culture in liquid medium without mammal's kinase inhibitors.**Fig. 2. *In vitro* microspore embryogenesis in *H. vulgare.*** Cotyleoptilar and leaf-stage embryos developed from isolated microspore culture in liquid medium without mammal's kinase inhibitors.

Fig. 3. *In vitro* somatic embryogenesis in *Q. suber.* Embryos at different developmental stages, cultured in solid medium without mammal's kinase inhibitors, emerging from proembryogenic masses and other embryos, some of them have differentiated fully mature cotyledonary embryos.

Fig. 4. Effects of four different GSK3β inhibitors (TDZD.8, VP3.15, VP3.36 and VP0.7) over embryogenesis induction efficiency in *B. napus* microspore cultures. Columns indicate percent change of proembryos at 4 days and referred to the mean percentage of proembryos in control cultures which has been normalized to 100%. Asterisks on columns indicate statistically significant differences with control cultures, as assessed by Student's *t*-test at $P \leq 0.05$.

Fig. 5. Effects of four different LRRK2 inhibitors (JZ1.3, JZ1.6, JZ1.24 and IGS4.75) over embryogenesis induction efficiency in *B. napus* microspore cultures. Columns indicate percent change of proembryos at 4 days and refer to the mean percentage of proembryos in control cultures, which has been normalized to 100%. Asterisks on columns indicate statistically significant differences with control cultures, as assessed by Student's *t*-test at $P \leq 0.05$.

Fig. 6. Proembryos in TDZD.8-treated cultures of microspore embryogenesis of *B. napus.* After 4 days in culture, proembryos (arrows) coexisted with non-responding and dead microspores (smaller structures); DAPI staining (right panel) reveals that proembryos contain several nuclei (arrows), indicating embryogenesis initiation.

Fig. 7. Effects of TDZD.8 (GSK3β inhibitor) and JZ1.24 (LRRK2 inhibitor) over embryogenesis induction efficiency in *B. napus* microspore cultures. Columns indicate percent change of proembryos at 4 days and refer to the mean percentage of proembryos in control cultures, which has been normalized to 100%. Asterisks on columns indicate statistically significant differences with control cultures, as assessed by Student's *t*-test at $P \leq 0.05$.

Fig. 8. Evaluation of germination capacity of embryos produced in microspore cultures of *B. napus.* Germinating embryos from control (left) and treated (right) cultures, showing well-developed roots and hypocotyls in most embryos, in both conditions.

Fig. 9. Effects of selected GSK3β and LRRK2 inhibitors (TDZD.8 and JZ1.24, respectively) over embryogenesis induction efficiency in *H. vulgare* microspore cultures. Columns indicate percent change of proembryos at 4 days in control (untreated) and treated cultures. Asterisks on columns indicate statistically significant differences with control cultures, as assessed by Student's *t*-test at $P \leq 0.05$.

Fig. 10. Effects of selected GSK3β and LRRK2 inhibitors (TDZD.8 and JZ1.24) over embryo production in *H. vulgare* microspore cultures. Columns indicate mean number of embryos per plate formed at 40 days in control (untreated) and treated cultures. Different letters on columns indicate significant differences according to ANOVA and Tukey's tests at $P \leq 0.05$.

Figure 11: Effects of selected GSK3β and LRRK2 inhibitors (TDZD.8 and JZ1.24) over embryo production in *Q. suber* somatic embryogenesis. Columns indicate mean number of embryos per gram of initial explant, in control (untreated) and treated cultures. Different letters on columns indicate significant differences according to ANOVA and Tukey's tests at $P \leq 0.05$.

Figure 12: Effects of selected GSK3β and LRRK2 inhibitors (TDZD.8 and JZ1.24) over embryo production in *Q. suber* microspore embryogenesis. Columns indicate mean number of embryos per gram of initial explant, in control (untreated) and treated cultures. Asterisks on columns indicate statistically significant differences with control cultures, as assessed by Student's *t*-test at $P \leq 0.05$.

Examples

Methodology

1.1. Microspore embryogenesis of *B. napus*, through isolated microspore culture (protocol without inhibitors)

[0079] *B. napus L.* (rapeseed) cv. 'Topas' line DH407 plants were used as donor plants. Rapeseed seeds were germinated and grew under controlled conditions (relative humidity 60%, 15°C under long-day photoperiod 16 h light and 8 h dark at 10°C) in a growth chamber in pots containing a mixture of organic substrate and vermiculite (2/1, v/v).

[0080] Flower buds containing vacuolated microspores, the most responsive stage for microspore induction, were isolated for microspore culture as previously described (Prem et al., 2012 BMC Plant Biology 12, 127). The selected buds were surface-sterilized in 5.0% (v/v) commercial bleach (5% active chlorine) for 20 min and then rinsed 6-7 times with sterile distilled water. Ten to 15 buds were crushed using a cold mortar and pestle in 5 ml of cold NLN-13 medium containing 13% sucrose (w/v). The suspension was filtered through 48 μm nylon mesh and the filtrate collected in 15-ml falcon centrifuge tubes. The crushed buds were rinsed with 5 ml NLN-13 to make up the volume to 10 ml and the filtrate was then centrifuged at 1100 rpm for 5 min at 4°C. The pellet was re-suspended in 10 ml of cold NLN-13 and centrifuged as mentioned above. This process was repeated three times for washing of the microspores. The final pellet was suspended in the NLN-13, and the cell density was adjusted to 10,000 cells per ml. The cell suspension was then poured into 90-mm Petri dishes (10 ml per Petri dish) and cultured in darkness. For embryogenesis induction, microspore cultures were subjected to an *in vitro* stress treatment of 32°C for 15 days. In response to the inductive treatment, responsive microspores divide and produce multicellular structures or proembryos, still confined within the microspore wall (exine). Such structures are considered to be the first sign of embryogenesis initiation; they can be found after 4-6 days in culture. When globular/heart shaped embryos were observed (around 20 days), cultures were shifted to 25°C on a gyratory shaker at 60 rpm until complete development and maturation of the embryos was observed (Fig. 1), normally around 30 days in culture.

1.2. Microspore embryogenesis of *H. vulgare*, through isolated microspore culture (protocol without inhibitors)

[0081] *H. vulgare* L. cv. Igri plants were used as donor plants. Seeds were vernalized in soil for one month at 4 °C, and then transferred for one month in a plant growth chamber at 18°C for germination and growth. Finally, plants were transferred to a greenhouse under 18°C temperature.

[0082] Spikes containing microspores at the stage of vacuolated microspore, the most responsive stage for embryogenesis induction, were collected and surface sterilized by immersion in 5% bleach for 20 min, followed by 4 washes with sterile distilled water. Isolated microspore culture was settled as previously described (Rodriguez-Serrano et al., 2012, Journal of Experimental Botany 63(5), 2007-2024). The sterilized spikes were pre-treated at 4°C for 21-24 days as stress treatment to induce microspore embryogenesis. Microspore were isolated blending spikes in 20 ml of pre-cooled 0.4 M mannitol at 4°C, using a Waring Blender pre-cooled in a refrigerator at -20°C, and the extract was filtered through a 100 μm nylon mesh into a beaker pre-cooled at -20°C. The collected microspore suspension was transferred into a 50 ml tube and centrifuged at 800 rpm for 10 min at 4 °C. After removing the supernatant, the pellet was resuspended in 4 ml of pre-cooled 0.55 M maltose and transferred in 15ml falcon tube. 1.5 ml of 0.4 M mannitol solution were cautiously added unmixed. After gradient centrifugation at 800 rpm for 10 min at 4 °C, the interphase band consisting of an almost pure population of vacuolated microspores was resuspended in 0.4M mannitol solution giving a final volume of 10 ml. After counting cells in the Neubauer chamber, the pelleted microspores were diluted in an appropriate volume of KBP medium to obtain a cell density of 1.1 X 10^5 cells per ml, and plated in 30 mm Petri dishes, at a volume of 1 ml per plate. Then, microspore cultures were incubated at 25 °C in the dark, and microspores reprogrammed and produced multicellular structures/proembryos that can be found after 4-6 days in culture, as the first sign of embryogenesis initiation. Proembryos further developed and produced coleoptylar and mature embryos (Fig. 2), which were observed after 30 days.

1.3. Microspore embryogenesis of *Q. suber*, through anther culture (protocol without inhibitors)

[0083] Branches with several catkins were cut and collected from *Q. suber* trees in the countryside (El Pardo region, Madrid, Spain), during the flowering period (from early May to early-mid June). Cut tips of branches were immediately covered with moist cotton and aluminium foil, and transferred to the laboratory, where they were kept in the dark at 4 °C for several days, until use for *in vitro* culture. Selected catkins were separated from branches and sterilized by immersion in 70% ethanol for 30-60 s, under vacuum, to aid penetration of the solvent. They were then immersed in 2% sodium hypochlorite with 1% Tween-20 for 20 min, with magnetic stirring. After three washes in sterile distilled water, catkins were prepared for dissection and anther excision.

[0084] Anther culture and microspore embryogenesis induction were performed as previously described (Testillano

et al. 2018, Forestry Sciences Vol. 84. Springer International Publishing AG. pp. 93-105). Anthers containing vacuolated microspores, the most responsive stage for embryogenesis induction, were carefully excised from sterilized catkins under aseptic conditions and plated in Petri dishes of 90 mm diameter on solid induction medium which contained Sommer medium macronutrients, Murashige and Skoog (MS) micronutrients and vitamins, as well as 30 g/L sacarose and activated charcoal. Anthers were placed in linear arrays of 10-12 anthers each, with a gap of around 5 mm between each anther, and up to 100 anthers per Petri dish. Embryogenesis was induced by stress treatment at 33°C in darkness for 5 days. After this inductive treatment, the anther cultures were transferred to 25 °C in darkness. In the following 20-30 days, responsive anthers become swollen and proembryos and small proembryogenic masses were visible as very small white structures emerging from the anther interior, breaking the tissues of the anther wall. After some more days, proembryos and proembryogenic masses grew and formed globular embryos by direct and indirect embryogenesis from individual microspores.

[0085] Microspore-derived embryogenic masses and embryos were transferred to new plates with proliferation medium which has a similar composition to induction medium except that it does not contain activated charcoal and is supplemented with 0.5 g/L glutamine. They were kept at 25 °C in darkness and sub-cultured every month in the same medium, where embryogenic masses can proliferate and spontaneously originate new globular embryos, which further developed heart-shaped, torpedo and cotyledonary embryos. In proliferation medium, some of these embryos produced new embryos by secondary and recurrent embryogenesis.

1.4. Somatic embryogenesis of *Q. suber*, through immature zygotic embryos culture (protocol without inhibitors)

[0086] Immature pollinated acorns were collected from *Q. suber* L. (cork oak) trees in the countryside (El Pardo region, Madrid, Spain) during fruit development period (late August and September), transferred to the laboratory and kept at 4°C for one week before *in vitro* culture initiation. Immature acorns were selected at the most responsive stage to somatic embryogenesis induction; they are those with small size, around 1 cm diameter, and green colour; they contain immature zygotic embryos at the early cotyledonary stage.

[0087] Immature zygotic embryos were carefully excised from the acorns by dissecting the surrounding tissues with the help of scalpel and forceps. After dissection, explants (immature zygotic embryos) were sterilized by immersion in 70% ethanol for 30 s and in 2% sodium hypochlorite for 20 min, followed by three rinses in sterile distilled water of 10 min each. Five explants were placed per plate.

[0088] Somatic embryogenesis was induced as previously described (Testillano et al. 2018, Plant Cell Culture Protocols, eds. V.M. Loyola-Vargas & N. Ochoa-Alejo. Springer and Bussines Media. pp. 247-256). Explants were first cultured in solid induction medium, which contains Sommer macronutrients, MS micronutrients and vitamins, 0.5mg/l Glutamine, 30g/l Sucrose, and 0.5mg/l 2,4-Dichlorophenoxyacetic acid (2,4-D), for one month at 25°C and 16/8h light/darkness. During this induction period, cell reprogramming occurs in some responsive cells which initiated the embryogenesis pathway, producing small proembryogenic masses. Then, the explants were transferred to solid proliferation medium, with the same composition but growth regulator-free (without 2,4-D). During the next weeks of culture in the proliferation medium, proembryogenic masses proliferated and protruded from different parts of the explants; they produce new embryogenic masses and embryos, which in turn give rise to new embryos, that developed to fully developed cotyledonary embryos, by recurrent and secondary embryogenesis (Fig. 3).

1.5. Treatment with mammal kinases inhibitors on microspore embryogenesis cultures of *B. napus* and *H. vulgare* in liquid media

[0089] The compounds were added to the microspore liquid culture media by using stock solutions of 10 mM in DMSO. Appropriate volumes of stock solutions of the drugs were added to the culture media to get the selected working concentrations of the inhibitors, keeping DMSO concentration below 0.2%.

- In *B. napus* microspore cultures: 4-benzyl-2-methyl-1,2,4-thiadozilidine-3,5-dione (TDZD8), 5-(2-Morpholinethylimino)-2,3-diphenyl-2,5-dihydro-1,2,4-thiadiazole (VP3.15), 3-acetyl-4-(1-methyl-1H-indol-3-yl)-1H-pirrol-2,5-dione (VP3.36), 4-hydroxy-1-ethyl-N'-palmitoyl-2-oxo-1,2-dihydroquinoline-3-carbohydrazide (VP0.7), N-(6-methylbenzothiazole-2-yl)-4-morpholinobenzamide (JZ1.3), N-(6-fluorobenzothiazole-2-yl)-4-morpholinobenzamide, (JZ1.6) and N-(6-bromobenzothiazole-2-yl)-4-morpholinobenzamide (JZ1.24). and (E,Z)-3-(morpholinoimino)indolin-2-one (IGS4.75) were tested at 3-4 different concentrations, ranging from 0.5 to 5 μM.

- In *H. vulgare* microspore cultures: 4-benzyl-2-methyl-1,2,4-thiadozilidine-3,5-dione (TDZD8) and N-(6-bromobenzothiazole-2-yl)-4-morpholinobenzamide (JZ1.24) inhibitors at the selected concentrations (2.5 μM and 5μ, respectively) were tested.

[0090] The compounds were added from culture initiation and their effect on embryogenesis efficiency was assessed. Several plates of the same cultures were kept without the inhibitors, as controls.

1.6. Evaluation of the effect of mammal kinases inhibitors over *in vitro* embryogenesis induction in isolated microspore cultures of *B. napus* and *H. vulgare*

[0091] Embryogenesis induction was quantified in control and treated-cultures by the number of proembryos formed (considered the first sign of embryogenesis initiation), as previously described, and by the number of embryos produced after 40 days. Proembryos were easily identified under inverted microscope in 4 day-culture plates as rounded multicellular structures with higher size and density than microspores, still surrounded by the exine (special microspore wall). Embryos produced after 40 days in culture were quantified through images captured under a stereo microscope. Randomly obtained micrographs from inverted and stereo microscopes were collected from untreated and treated microspore culture plates. Mean percentage of proembryos and mean number of embryos per plate were obtained from three independent experiments per each *in vitro* system and treatment. A minimum of 1000 proembryos were counted for each treatment and plant species. Results on proembryos were expressed as percentages (percent change) and referred to the mean percentage of proembryos in control cultures, which has been normalized to 100%.

[0092] In order to evaluate whether proembryo structures of treated cultures, identified under the inverted microscope for quantification, were actually dividing microspores, similar to the same structures in control cultures, a simply staining technique was performed to visualize nuclei inside proembryos. Samples from control and treated-cultures of 4 days, containing proembryos, were stained with 10 μg/mL 4',6-diamidine-2-phenyl indole dihydrochloride (DAPI). Squash preparations were analysed under fluorescence microscopy using UV excitation for observing nuclei.

1.7. Evaluation of quality/germination capacity of embryos produced after treatment with mammal kinases inhibitors

[0093] To evaluate the quality of embryos produced in microspore embryogenesis cultures in the presence of the mammal kinases inhibitors, embryo germination assays were performed. *B. napus* microspore cotyledonary embryos originated from control and treated-cultures were used for *in vitro* embryo germination and conversion to plantlets as previously described (Prem et al., 2012, BMC Plant Biology 12, 127). The 34 - 40 old dicotyledonous embryos, after air desiccation on sterile filter paper were germinated in MS medium containing sucrose 2 % (w/v) and gelled with 7 g/L bacteriological agar (w/v). Microspore derived-embryos were incubated for 15 - 20 days at 18°C in darkness conditions till activation of radicle and plumule, and quantified in terms of percentage of embryos showing normal growth, similar to zygotic embryo germination.

1.8. Treatments with kinases inhibitors on microspore and somatic embryogenesis cultures of *Q. suber* in solid media

[0094] Since the *in vitro* systems of *Q. suber* were two-step processes in solid culture media, a different strategy than in liquid microspore cultures was applied for the treatments with the mammal kinases inhibitors. During *in vitro* embryogenesis of *Q. suber,* after incubation in induction medium, the transfer of explants to proliferating medium involves the multiplication of proembryogenic masses, embryogenesis initiation, by recurrent and secondary embryogenesis, and embryo development. Therefore, treatments with the mammal kinases inhibitors were performed during the first 15-30 days in proliferating media, and afterwards, explants with emerging embryos were transferred to fresh proliferating media without the inhibitor.

[0095] Since solid media involve much less diffusion and availability of compounds to cells in comparison with liquid media, as referred in other *in vitro* systems, the concentration of the mammal kinases inhibitors used in solid media was around 10X higher than in liquid media, in the range of 25 to 100 μM. Appropriate volumes of stock solutions of 10 mM in DMSO of the selected compounds were added to cooled media, before its gelling, keeping DMSO concentration below 0.2%. Mock parallel plates of the same cultures were kept as controls.

1.9. Evaluation of the effect of inhibitors over *in vitro* embryogenesis induction in microspore and somatic embryogenesis cultures of *Q. suber*

[0096] Embryogenesis induction efficiency was quantified in control and treated-cultures by the number of cotyledonary embryos produced by 15-30 days of treatment (culture medium containing the inhibitor) followed by 30 days of recovery (culture medium without inhibitor). Embryo production was estimated as the number of cotyledonary embryos originated per gram of embryogenic masses at culture initiation.

Results

1.1. Effect of kinases inhibitors over microscope embryogenesis cultures *B. napus*

[0097] To evaluate the effect of the kinase inhibitors over *in vitro* embryogenesis induction, we first tested them in *B. napus* microspore embryogenesis, as a model platform to check the mammal kinases inhibitors and different concentrations. After these analyses, one selected mammal kinases inhibitor of each category was tested in other two plant species, *H. vulgare* and *Q. suber,* with different *in vitro* systems. The efficiency of embryogenesis induction was evaluated in control cultures and cultures treated with the mammal kinases inhibitors, at different concentrations. The results for the GSK3β inhibitors and LRRK2 inhibitors tested are shown as the percentage of proembryos (first sign of embryogenesis initiation) in Figs. 4 and 5, respectively. The presence of the inhibitors in the culture media affected the production of proembryos in comparison with control cultures, being the proportion of proembryos different depending on the concentration used. Four inhibitors of GSK3β TDZD8; VP3.15, VP3.36, VP0.7 were tested at 3-4 concentrations in the range of 0.1μM to 5μM. The results of the quantification of the proembryos produced, as first sign of embryogenesis initiation, in control and treated cultures showed that all inhibitors, at least with one or two of the concentrations used, led to an increase of the production of proembryos (Figs. 4 and 5). The concentrations and compounds that provided an improvement of embryogenesis initiation yield were the following: GSK3β inhibitors, 0.5μM and 1μM TDZD-8, 2.5μM VP3.15, 2.5μM VP3.36, and 5μM VP0.7 (Fig. 4); LRRK2 inhibitors, 2.5μM JZ1.24, 5μM JZ1.3, 5μM IGS4.75, and 1μM JZ1.6 (Fig. 5). With the other concentrations, treated cultures showed a proportion of proembryos either similar to or slightly higher than control cultures (Figs. 4 and 5), while they did not show any deleterious/toxic effect.

[0098] The results showed that the increase of embryogenesis induction efficiency provided by the use of the inhibitors was in the range of 20-25% for GSK3β inhibitors and 23-30% for LRRK2 inhibitors.

[0099] To confirm that proembryos quantified in treated cultures were multicellular microspores that have initiated embryogenesis, squash preparations from control and treated cultures at 4 days were stained with DAPI and observed under fluorescence microscopy. Results showed that proembryos from treated cultures contained several nuclei (Fig. 6), as in control cultures, indicating that they were actually dividing microspores that likely initiated embryogenesis.

[0100] Taking into account these results in *B. napus,* the compounds that were selected for testing in other *in vitro* embryogenesis systems were:

- 4-benzyl-2-methyl-1,2,4-thiadozilidine-3,5-dione (TDZD.8) as GSK3β inhibitor and
- N-(6-bromobenzothiazole-2-yl)-4-morpholinobenzamide (JZ1.24) as LRRK2 inhibitor.

[0101] As it is showed in Fig 7, the selected compounds, the GSK3β inhibitor TDZD.8 and the LRRK2 inhibitor JZ1.24 showed an increase of embryogenesis efficiency, i.e. increase in the percentage of proembryos that was of 20% increase in the case of 0.5μM TDZD.8, and 27.5% increase in the case of 2.5 μM JZ1.24. in *B. napus* microspore cultures (Fig. 7).

[0102] The quality of the embryos produced in microspore cultures treated with the mentioned inhibitors was evaluated by germination assays. Fully developed cotyledonary embryos from control and treatedcultures, produced after 30 days were desiccated and cultured under germination conditions. Results showed that embryos from treated cultures germinated very well, producing roots and hypocotyl, similarly and in the same proportion than embryos from control cultures (Fig. 8).

1.2. Effect of kinase inhibitors over microscope embryogenesis cultures of *H. vulgare*

[0103] The selected inhibitors, TDZ.8 and JZ1.24 were tested in microspore embryogenesis cultures of a different crop, *H. vulgare.* The inhibitors were firstly applied at the same concentrations that provided the best results in *B. napus,* 0.5μM TDZD8 and 2.5μM JZ1.24, but the results obtained (percentage of proembryos) in *H. vulgare* treated cultures using these concentrations were similar to control cultures. Therefore, two slightly higher concentrations were tested for both inhibitors (1μM and 2.5μM for TDZD8; and 2.5μM and 5μM for JZ1.24). The results showed that the two inhibitors lead to an increase in the embryogenesis initiation in *H. vulgare,* when used at slightly different concentrations than in *B. napus,* 2.5 μM TDZD8 and 5 μM JZ1.24. This indicates that optimal concentrations of these inhibitors could differ among species, probably due to differences in cell wall and permeability properties, and the specific features of each plant and *in vitro* system. The quantification of the proembryos formed at 4 days showed that treatments with the two inhibitors at the selected concentrations enhanced embryogenesis induction efficiency in *H. vulgare,* being the increase in proembryo formation of 27% in the case of 2.5 μM TDZD8, and 47% in the case of 5 μM JZ1.24-treated cultures (Fig. 9).

[0104] Untreated and treated cultures further developed and total number of embryos produced per plate at 40 days was quantified. Microspore cultures treated with these inhibitors produced more embryos than control cultures, being the increment of 22% for JZ1.24 and 15% for TDZD8 (Fig. 10).

[0105] The results indicated that small molecule inhibitors of mammalian GSK3β and LRRK2 produced a similar

promoting effect in *H. vulgare* than in *B. napus* microspore cultures, an increase of *in vitro* embryogenesis induction efficiency.

1.3. Effect of kinase inhibitors over microspore and somatic embryogenesis cultures of *Q. suber*

[0106] In order to evaluate the possibility to extend the findings from *B. napus* and H. *vulgare* to more distant species and processes, the selected inhibitors, TDZD.8 and JZ1.24, were applied to a forest woody species *Q. suber,* in which two different embryogenesis *in vitro* systems were established, somatic embryogenesis from immature zygotic embryos and microspore embryogenesis, two culture systems that consisted in two-step cultures in solid media.

[0107] Inhibitor treatments were applied at concentrations 10X higher than in liquid media, because of the lower diffusion and availability of compounds in gelled medium. The evaluation of the effects of the compounds over embryogenesis efficiency in the two systems were assessed by the quantification of the embryos produced in control and treated cultures. Results showed that treatments with the two types of inhibitors increased embryogenesis induction efficiency and lead to higher embryo production, in somatic embryogenesis from immature zygotic embryos cultures (Fig. 11), as well as in microspore embryogenesis from anther cultures (Fig. 12).

[0108] The results indicated that also in a woody species and in different *in vitro* embryogenesis systems, involving solid culture media, the small molecule inhibitors of mammalian GSK3β and LRRK2 produced the same effect than in rapeseed and barley systems in liquid media, an increase of *in vitro* embryogenesis induction efficiency.

Conclusions

[0109] The present invention deals with a major challenge of *in vitro* plant propagation techniques, that is to improve the efficiency of embryogenesis induction for rapid production of high numbers of high quality, disease-free and uniform planting material for agroindustry companies, reducing time and costs, in many species of economic interest. The new strategy reported in the present invention uses for the first time in plant *in vitro* systems inhibitors of mammalian protein kinases, specifically inhibitors of GSK3β and LRRK2 families, which have demonstrated capacity to increase embryogenesis induction and embryo production yield in three different crop and forest species. Moreover, treatments with these inhibitors have been successfully applied to different *in vitro* protocols, in liquid or solid media, and with direct, indirect and secondary/recurrent embryogenesis, providing similar promoting effects over embryogenesis. Several inhibitors of each group, with different molecular structure, have shown to be able to enhance embryogenesis efficiency, giving additional support to the use of these type of small molecules as new tools to optimize *in vitro* plant embryogenesis protocols.

2. Synthesis and characterisation of the inibitors of the present invention.

2.1. Inhibitors of Formula (I)

[0110] 4-benzyl-2-methyl-1,2,4-thiadozilidine-3,5-dione (TDZD8) is disclosed in Martinez A *et al.* (Martinez A et al. J Med Chem. 2002; 45(6):1292-9).

2.2. Inhibitors of Formula (II)

[0111] All of inhibotors of Formula (II), including 5-(2-Morpholinethylimino)-2,3-diphenyl-2,5-dihydro-1,2,4-thiadiazole (VP3.15), are disclosed in EP2484670A1.

2.3. Inhibitors of Formula (III)

[0112] 3-acetyl-4-(1-methyl-1H-indol-3-yl)-1H-pirrol-2,5-dione (VP3.36) is disclosed in Perez DI *et al.* (Perez DI et al. J Med Chem. 2011; 54(12):4042-56).

2.4. Inhibitors of Formula (IV)

[0113] 4-hydroxy-1-ethyl-N'-palmitoyl-2-oxo-1,2-dihydroquinoline-3-carbohydrazide (VP0.7) is disclosed in Palomo V *et al.* (Palomo V et al. J Med Chem. 201; 60(12):4983-5001).

2.5. Inhibitors of Formula (V)

[0114] ***N*-(benzothiazole-2-yl)-4-morpholinobenzamide:** 276.0 mg of 4-morpholinobenzoic acid (1.3 mmol), 331.00

mg of EDCI (1.4 mmol), 24.4 mg of DMAP (0.3 mmol) and 335 μL (2.4 mmol) of triethylamine were dissolved in dichloromethane. After stirring for 1 hour at room temperature, 200 mg of 2-aminobenzothiazole (1.3 mmol) were added. The reaction is left under stirring at room temperature during the night. After this time period the crude was washed with saturated solutions of $NaHCO_3$ and NaCl, respectively. Next, the organic phase is dried on anhydrous magnesium sulfate, the solvent is evaporated at low pressure and is purified by chromatography in a flash column using a mixture of eluents CH_2Cl_2/MeOH (20:1) to obtain a yellow solid (72 mg, 16%). HPLC Purity >95%. MS: m/z 340 $[M + 1]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 10.21 (s, 1 H, NH), 7.90 (d, *J* = 9.0 Hz, 2 H), 7.84 (dd, *J* = 8.5, 1.5 Hz, 1 H), 7.62 (dd, *J* = 8.3, 1.2 Hz, 1 H), 7.44 - 7.35 (m, 1 H), 7.35 - 7.27 (m, 1 H), 4.01 - 3.71 (m, 4 H), 3.49 - 3.16 (m, 4 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 164.6, 159.1, 154.3, 148.2, 132.2, 129.4, 126.0, 123.7, 121.3, 121.1, 120.7, 113.8, 66.5, 47.4.

[0115] ***N*-(6-methoxybenzothiazole-2-yl)-4-morpholinobenzamide:** 230.0 mg of 4-morpholinobenzoic acid (1.1 mmol), 276.6 mg of EDCI (1.4 mmol), 24.43 mg of DMAP (0.2 mmol) and 248 μL (1.7 mmol) of triethylamine were dissolved in dichloromethane. After stirring for 1 hour at room temperature, 200 mg of 2-amino-6-methoxybenzothiazole (1.1 mmol) were added. The reaction is left under stirring at room temperature during the night. After this time period the crude was washed with saturated solutions of $NaHCO_3$ and NaCl, respectively. Next, the organic phase is dried on anhydrous magnesium sulfate, the solvent is evaporated at low pressure and is purified by flash column chromatography using a mixture of eluents CH_2Cl_2/MeOH (50:1) to obtain a yellow solid (36 mg, 9%). P.f.: 237.6-240.0 °C. HPLC Purity: 95%. MS: m/z 370 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 9.47 (s, 1H), 7.88 (d, *J* = 9.0 Hz, 2H), 7.64 (d, *J* = 8.8 Hz, 1H), 7.33 (d, *J* = 2.6 Hz, 1H), 7.04 (dd, *J* = 8.8, 2.6 Hz, 1H), 6.94 (d, *J* = 9.0 Hz, 2H), 3.93-3.83 (m, 7H), 3.36-3.31 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 164.8, 156.9, 156.0, 153.8, 142.7, 132.8, 129.8, 120.8, 120.5, 114.80, 113.1, 104.6, 65.8, 55.6, 46.8.

[0116] ***N*-(6-trifluoromethylbenzothiazole-2-yl)-4-morpholinobenzamide:** 189.9 mg of 4-morpholinobenzoic acid (0.9 mmol), 228.53 mg of EDCI (1.2 mmol), 22.41 mg of DMAP (0.2 mmol) and 223 μL (1.5 mmol) of triethylamine were dissolved in dichloromethane. After stirring for 1 hour at room temperature, 200 mg of 2-amino-6-trifluorobenzothiazole (0.9 mmol) were added. The reaction is left under stirring at room temperature during the night. After this time period the crude was washed with saturated solutions of $NaHCO_3$ and NaCl, respectively. Next, the organic phase is dried on anhydrous magnesium sulfate, the solvent is evaporated at low pressure and is purified by automatic flash column chromatography (Biotage®Isolera One) using a mixture of eluents hexane/AcOEt to obtain a yellow solid (79 mg, 26%). P.f.: 218.5-218.5 °C. HPLC Purity: 95%. MS: m/z 408 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 10.85 (s, 1H), 8.13 (s, 1H), 7.89 (d, *J* = 9.0 Hz, 1H), 7.57-7.53 (m, 2H), 6.84 (d, *J* = 9.0 Hz, 2H), 3.87-3.83 (m, 4H), 3.31-3.26 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.8, 160.8, 153.4, 149.4, 131.2, 128.5, 124.9 (d, *J* = 32.5 Hz), 124.3, 122.1 (d, *J* = 3.4 Hz), 119.6 (d, *J* = 32.2 Hz), 118.0 (d, *J* = 4.2 Hz), 112.7, 65.4, 46.3, 28.6. $C_{19}H_{16}F_3N_3O_2S$: Theoretical (%) C, 56.01; H, 3.96; N, 10.31; S, 7.87. Found (%) C, 56.13; H, 3.98; N, 10.38; S, 7.59.

[0117] ***N*-(6-methylbenzothiazole-2-yl)-4-morpholinobenzamide** (JZ1.3): 252.4 mg of 4-morpholinobenzoic acid (1.2 mmol), 303.5 mg of EDCI (1.58 mmol), 20.06 mg of DMAP (0.2 mmol) and 272 μL (1.9 mmol) of triethylamine were dissolved in dichloromethane. After stirring for 1 hour at room temperature, 200 mg of 2-amino-6-methylbenzothiazole (1.2 mmol) were added. The reaction is left under stirring at room temperature during the night. After this time period the crude was washed with solutions of HCl (0.1M), saturated $NaHCO_3$ and saturated NaCl, respectively. Next, the organic phase is dried on anhydrous magnesium sulfate, the solvent is evaporated at low pressure and is purified by automatic flash column chromatography (Biotage®Isolera One) using a mixture of eluents hexane/AcOEt to obtain a yellow solid (43 mg, 10%). P.f.: 287.7-288.8 °C. MS (ESI+): m/z 354 [M+H]+. ^1H NMR (300 MHz, $CDCl_3$) δ 10.56 (s, 1H), 7.89 (d, J= 8.9 Hz, 2H), 7.63 (s, 1H), 7.42 (d, *J* = 8.3 Hz, 1H), 7.16 (dd, *J* = 8.3, 1.7 Hz, 1H), 6.85 (d, *J* = 8.9 Hz, 1H), 3.87-3.83 (m, 4H), 3.29-3.26 (m, 4H), 2.46 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.7, 158.5, 154.2, 146.1, 133.7, 132.3, 129.4, 127.5, 121.3, 121.1, 120.3, 113.8, 66.5, 47.5, 21.4. $C_{19}H_{19}N_3O_2S$: Theoretical (%) C, 64.57; H, 5.42; N, 11.89; S, 9.07. Found (%) C, 64.33; H, 5.38; N, 11.85; S, 8.96.

[0118] ***N*-(6-chlorobenzothiazole-2-yl)-4-morpholinobenzamide:** 224.4 mg of 4-morpholinobenzoic acid (1.1 mmol), 269.89 mg of EDCI (1.4 mmol), 26.4 mg of DMAP (0.2 mmol) and 242 μL (1.7 mmol) of triethylamine were dissolved in dichloromethane. After stirring for 1 hour at room temperature, 200 mg of 2-amino-6-chlorobenzothiazole (1.1 mmol) were added. The reaction is left under stirring at room temperature during the night. After this time period the crude was washed with solutions of HCl (0.1M), saturated $NaHCO_3$ and saturated NaCl, respectively. Next, the organic phase is dried on anhydrous magnesium sulfate, the solvent is evaporated at low pressure and is purified by automatic flash column chromatography (Biotage®Isolera One) using a mixture of eluents hexane/AcOEt to obtain a white solid (96 mg, 24%). P.f.: 245.4-246.4 °C. HPLC Purity: 97%. MS: m/z 374 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 10.25 (s, 1H), 7.89 (d, *J* = 8.9 Hz, 2H), 7.81 (d, *J* = 2.1 Hz, 1H), 7.52 (d, *J* = 8.7 Hz, 1H), 7.33 (dd, *J* = 8.7, 2.1 Hz, 1H), 6.89 (d, *J* = 9.0 Hz, 2H), 3.92-3.82 (m, 4H), 3.33-3.30 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.5, 159.3, 154.4, 146.8, 139.7, 133.5, 129.4, 126.7, 121.5, 121.0, 120.8, 113.7, 66.5, 47.4. $C_{18}H_{16}ClN_3O_2S$: Theoretical (%) C, 57.83; H, 4.31; N, 11.24; S, 8.58. Found (%) C, 57.56; H, 4.09; N, 11.43; S, 8.40.

[0119] ***N*-(6-fluorobenzothiazole-2-yl)-4-morpholinobenzamide** (JZ1.6): 168.20 mg of 4-morpholinobenzoic acid (1.2 mmol), 296.3 mg of EDCI (1.5 mmol), 29.05 mg of DMAP (0.2 mmol) and 265 μL (1.9 mmol) of triethylamine were

dissolved in dichloromethane. After stirring for 1 hour at room temperature, 200 mg of 2-amino-6-fluorobenzothiazole (1.2 mmol) were added. The reaction is left under stirring at room temperature during the night. After this time period the crude was washed with solutions of HCl (0.1M), saturated $NaHCO_3$ and saturated NaCl, respectively. Next, the organic phase is dried on anhydrous magnesium sulfate, the solvent is evaporated at low pressure and is purified by automatic flash column chromatography (Biotage®Isolera One) using a mixture of eluents hexane/AcOEt to obtain a white solid (79 mg, 19%). P.f.: 228.3-229.3 °C. HPLC Purity: 98%. MS: m/z 358 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 9.96 (s, 1H), 7.81 (d, *J* = 8.9 Hz, 2H), 7.74 (d, *J* = 2.1 Hz, 1H), 7.49 (d, *J* = 8.6 Hz, 1H), 7.28 (dd, *J* = 8.7, 2.1 Hz, 1H), 6.84 (d, *J* = 8.7 Hz, 2H), 3.87-3.85 (m, 4H), 3.34-3.30 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.4, 159.2, 154.4, 147.0, 138.7, 133.6, 129.3, 126.8, 121.6, 121.0, 120.8, 113.8, 66.5, 47.4. $C_{18}H_{16}FN_3O_2S$: Theoretical (%) C, 60.49; H, 4.51; N, 11.76; S, 8.97. Found (%) C, 60.68; H, 4.50; N, 11.55; S, 8.72.

[0120] ***N*-(6-ethoxybenzothiazole-2-yl)-4-morpholinobenzamide:** 213.1 mg of 4-morpholinobenzoic acid (1.0 mmol), 256.2 mg of EDCI (1.3 mmol), 25.12 mg of DMAP (0.2 mmol) were dissolved in dichloromethane. After stirring for 6 hours at room temperature, 200 mg of 2-amino-6-ethoxybenzothiazole (1.0 mmol) and 229 μL of triethylamine (1.9 mmol) were added. The reaction is left under stirring at room temperature during the night. After this time period the crude was washed with solutions of HCl (0.1M), saturated $NaHCO_3$ and saturated NaCl, respectively. Next, the organic phase is dried on anhydrous magnesium sulfate, the solvent is evaporated at low pressure and is purified by automatic flash column chromatography (Biotage®Isolera One) using a mixture of eluents hexane/AcOEt to obtain a yellow solid (20 mg, 5%). P.f.: 222.8-223.8 °C. HPLC Purity: 95%. MS: m/z 384 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 8.07 (d, *J* = 8.6 Hz, 2H), 7.54 (d, *J* = 8.9 Hz, 1H), 7.35-7.18 (m, 1H), 7.04 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.89 (d, *J* = 8.6 Hz, 2H), 4.07 (q, *J* = 6.9 Hz, 2H), 3.89-3.69 (m, 4H), 3.38-3.25 (m, 4H), 1.43 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.4, 157.1, 156.0, 154.2, 142.3, 133.3, 129.3, 121.3, 121.2, 119.7, 115.5, 114.2, 113.8, 106.0, 104.9, 99.5, 66.5, 64.1, 64.1, 47.5, 14.8.

[0121] ***N*-(6-bromobenzothiazole-2-yl)-4-morpholinobenzamide** (JZ1.24): 180.9 mg of 4-morpholinobenzoic acid (0.9 mmol), 217.6 mg of EDCI (1.1 mmol), 21.33 mg of DMAP (0.2 mmol) were dissolved in dichloromethane. After stirring for 6 hours at room temperature, 200 mg of 2-amino-6-bromobenzothiazole (0.9 mmol) and 195 μl of triethylamine (1.4 mmol) were added. The reaction is left under stirring at room temperature during the night. After this time period the crude was washed with solutions of HCl (0.1M), saturated $NaHCO_3$ and saturated NaCl, respectively. Next, the organic phase is dried on anhydrous magnesium sulfate, the solvent is evaporated at low pressure and is purified by automatic flash column chromatography (Biotage®Isolera One) using a mixture of eluents hexane/AcOEt to obtain a yellow solid (41 mg, 11%). P.f.: 237.5-238.5 °C. HPLC Purity: 98%. MS: m/z 418 [M+H] +. ^1H NMR (300 MHz, $CDCl_3$) δ 10.51 (s, 1H), 7.96 (s, 1H), 7.87 (d, *J* = 9.0 Hz, 3H), 7.44 (dd, *J* = 8.6, 1.9 Hz, 2H), 7.38 (d, *J* = 8.6 Hz, 2H), 6.86 (d, *J* = 9.0 Hz, 3H), 3.89-3.83 (m, 11H), 3.33-3.26 (m, 11H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.2, 160.4, 154.8, 147.1, 134.1, 130.0, 129.9, 124.3, 122.1, 121.1, 117.2, 114.1, 66.9, 47.8.

[0122] ***N*-(6-propoxybenzothiazole-2-yl)-4-morpholinobenzamide:** 248.8 mg of 4-morpholinobenzoic acid (1.2 mmol), 299.00 mg of EDCI (1.6 mmol), 29.3 mg of DMAP (0.2 mmol) were dissolved in dichloromethane. After stirring for 6 hours at room temperature, 250 mg of 2-amino-6-propoxybenzothiazole (1.2 mmol) and 267.6 μL (1.9 mmol) of triethylamine were added. The reaction is left under stirring at room temperature during the night. After this time period the crude was washed with a HCl solution (0.1M). Next, the organic phase is dried on anhydrous magnesium sulfate, the solvent is evaporated at low pressure and is purified by flash column chromatography using a mixture of eluents CH_2Cl_2/MeOH (50:1) to obtain a yellow solid (127 mg, 27%). HPLC Purity>95%. MS: m/z 398 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 7.88 (d, *J* = 9.0 Hz, 2H), 7.46 (d, *J* = 8.9 Hz, 1H), 7.31 (d, *J* = 2.5 Hz, 1H), 6.96 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.88 (d, *J* = 9.0 Hz, 2H), 3.98 (t, *J* = 6.6 Hz, 2H), 3.89 - 3.83 (m, 4H), 3.32 - 3.27 (m, 4H), 1.85 (h, *J* = 7.3 Hz, 2H), 1.06 (t, J = 7.4 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.6, 156.3, 155.2, 153.3, 141.27, 132.2, 128.4, 120.4, 120.3, 114.5, 112.8, 103.9, 69.2, 65.5, 46.5, 21.6, 9.5. $C_{21}H_{23}N_3O_3S$: Theoretical (%) C, 63.46; H, 5.83; N, 10.57; S, 8.07. Found (%) C, 63.73; H, 5.74, N, 10.09; S, 7.71.

[0123] ***N*-(6-isopropylbenzothiazole-2-yl)-4-morpholinobenzamide:** 269.4 mg of 4-morpholinobenzoic acid (1.3 mmol), 324.00 mg of EDCI (1.7 mmol), 32.00 mg of DMAP (0.3 mmol) were dissolved in dichloromethane. After stirring for 6 hours at room temperature, 250 mg of 2-amino-6-isopropylbenzothiazole (1.3 mmol) and 290.0 μL (2.1 mmol) of triethylamine were added. The reaction is left under stirring at room temperature during the night. After this time period the crude was washed with a HCl solution (0.1M). Next, the organic phase is dried on anhydrous magnesium sulfate, the solvent is evaporated at low pressure and is purified by flash column chromatography using a mixture of eluents CH_2Cl_2/MeOH (50:1) to obtain a yellow solid (218.4 mg, 44%). HPLC Purity>95%. MS: m/z 382 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 10.35 (s, 1H), 7.89 (d, *J* = 9.0 Hz, 2H), 7.68 (d, *J* = 1.7 Hz, 1H), 7.49 (d, *J* = 8.4 Hz, 1H), 7.26 - 7.22 (m, 1H), 6.88 (d, *J* = 9.0 Hz, 2H), 3.89 - 3.81 (m, 4H), 3.33 - 3.25 (m, 4H), 3.03 (p, *J* = 6.9 Hz, 1H), 1.31 (d, *J* = 6.9 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.7, 157.7, 153.2, 145.3, 143.9, 131.3, 128.4, 124.0, 120.4, 119.4, 119.1, 117.5, 112.8, 65.5, 46.5, 33.2, 23.3. $C_{21}H_{23}N_3O_2S$: Theoretical (%) C, 66.12; H, 6.08; N, 11.00; S, 8.40. Found (%) C, 66.09; H, 6.13; N, 10.69; S, 8.54.

[0124] **2.6. Inhibitor of formula (E,Z)-3-(morpholinoimino)indolin-2-one (IGS4.75):** is disclosed in Salado IG. *et al.,* (Salado IG. et al., Eur J Med Chem. 2017 Sep 29;138:328-342).

SEQUENCE LISTING

<110> CSIC - Consejo Superior de Investigaciones Científicas

<120> Mammal kinase inhibitors for the induction of the in vitro embryogenesis of plants

<130> 1641.1479

<160> 7

<170> PatentIn version 3.5

<210> 1
<211> 420
<212> PRT
<213> Homo sapiens

<400> 1

```
Met Ser Gly Arg Pro Arg Thr Thr Ser Phe Ala Glu Ser Cys Lys Pro
1               5                  10                  15

Val Gln Gln Pro Ser Ala Phe Gly Ser Met Lys Val Ser Arg Asp Lys
            20                  25                  30

Asp Gly Ser Lys Val Thr Thr Val Val Ala Thr Pro Gly Gln Gly Pro
        35                  40                  45

Asp Arg Pro Gln Glu Val Ser Tyr Thr Asp Thr Lys Val Ile Gly Asn
    50                  55                  60

Gly Ser Phe Gly Val Val Tyr Gln Ala Lys Leu Cys Asp Ser Gly Glu
65                  70                  75                  80

Leu Val Ala Ile Lys Lys Val Leu Gln Asp Lys Arg Phe Lys Asn Arg
                85                  90                  95

Glu Leu Gln Ile Met Arg Lys Leu Asp His Cys Asn Ile Val Arg Leu
            100                 105                 110

Arg Tyr Phe Phe Tyr Ser Ser Gly Glu Lys Lys Asp Glu Val Tyr Leu
        115                 120                 125

Asn Leu Val Leu Asp Tyr Val Pro Glu Thr Val Tyr Arg Val Ala Arg
    130                 135                 140

His Tyr Ser Arg Ala Lys Gln Thr Leu Pro Val Ile Tyr Val Lys Leu
145                 150                 155                 160

Tyr Met Tyr Gln Leu Phe Arg Ser Leu Ala Tyr Ile His Ser Phe Gly
                165                 170                 175
```

```
Ile Cys His Arg Asp Ile Lys Pro Gln Asn Leu Leu Leu Asp Pro Asp
            180                 185                 190

Thr Ala Val Leu Lys Leu Cys Asp Phe Gly Ser Ala Lys Gln Leu Val
        195                 200                 205

Arg Gly Glu Pro Asn Val Ser Tyr Ile Cys Ser Arg Tyr Tyr Arg Ala
    210                 215                 220

Pro Glu Leu Ile Phe Gly Ala Thr Asp Tyr Thr Ser Ser Ile Asp Val
225                 230                 235                 240

Trp Ser Ala Gly Cys Val Leu Ala Glu Leu Leu Leu Gly Gln Pro Ile
                245                 250                 255

Phe Pro Gly Asp Ser Gly Val Asp Gln Leu Val Glu Ile Ile Lys Val
            260                 265                 270

Leu Gly Thr Pro Thr Arg Glu Gln Ile Arg Glu Met Asn Pro Asn Tyr
        275                 280                 285

Thr Glu Phe Lys Phe Pro Gln Ile Lys Ala His Pro Trp Thr Lys Val
    290                 295                 300

Phe Arg Pro Arg Thr Pro Pro Glu Ala Ile Ala Leu Cys Ser Arg Leu
305                 310                 315                 320

Leu Glu Tyr Thr Pro Thr Ala Arg Leu Thr Pro Leu Glu Ala Cys Ala
                325                 330                 335

His Ser Phe Phe Asp Glu Leu Arg Asp Pro Asn Val Lys Leu Pro Asn
            340                 345                 350

Gly Arg Asp Thr Pro Ala Leu Phe Asn Phe Thr Thr Gln Glu Leu Ser
        355                 360                 365

Ser Asn Pro Pro Leu Ala Thr Ile Leu Ile Pro Pro His Ala Arg Ile
    370                 375                 380

Gln Ala Ala Ala Ser Thr Pro Thr Asn Ala Thr Ala Ala Ser Asp Ala
385                 390                 395                 400

Asn Thr Gly Asp Arg Gly Gln Thr Asn Asn Ala Ala Ser Ala Ser Ala
                405                 410                 415

Ser Asn Ser Thr
            420
```

<210> 2
<211> 433
<212> PRT
<213> Homo sapiens

<400> 2

```
Met Ser Gly Arg Pro Arg Thr Thr Ser Phe Ala Glu Ser Cys Lys Pro
1               5                  10                  15

Val Gln Gln Pro Ser Ala Phe Gly Ser Met Lys Val Ser Arg Asp Lys
            20                  25                  30

Asp Gly Ser Lys Val Thr Thr Val Val Ala Thr Pro Gly Gln Gly Pro
        35                  40                  45

Asp Arg Pro Gln Glu Val Ser Tyr Thr Asp Thr Lys Val Ile Gly Asn
    50                  55                  60

Gly Ser Phe Gly Val Val Tyr Gln Ala Lys Leu Cys Asp Ser Gly Glu
65                  70                  75                  80

Leu Val Ala Ile Lys Lys Val Leu Gln Asp Lys Arg Phe Lys Asn Arg
                85                  90                  95

Glu Leu Gln Ile Met Arg Lys Leu Asp His Cys Asn Ile Val Arg Leu
            100                 105                 110

Arg Tyr Phe Phe Tyr Ser Ser Gly Glu Lys Lys Asp Glu Val Tyr Leu
        115                 120                 125

Asn Leu Val Leu Asp Tyr Val Pro Glu Thr Val Tyr Arg Val Ala Arg
    130                 135                 140

His Tyr Ser Arg Ala Lys Gln Thr Leu Pro Val Ile Tyr Val Lys Leu
145                 150                 155                 160

Tyr Met Tyr Gln Leu Phe Arg Ser Leu Ala Tyr Ile His Ser Phe Gly
                165                 170                 175

Ile Cys His Arg Asp Ile Lys Pro Gln Asn Leu Leu Leu Asp Pro Asp
            180                 185                 190

Thr Ala Val Leu Lys Leu Cys Asp Phe Gly Ser Ala Lys Gln Leu Val
        195                 200                 205

Arg Gly Glu Pro Asn Val Ser Tyr Ile Cys Ser Arg Tyr Tyr Arg Ala
    210                 215                 220
```

```
Pro Glu Leu Ile Phe Gly Ala Thr Asp Tyr Thr Ser Ser Ile Asp Val
225                 230                 235                 240

Trp Ser Ala Gly Cys Val Leu Ala Glu Leu Leu Leu Gly Gln Pro Ile
                245                 250                 255

Phe Pro Gly Asp Ser Gly Val Asp Gln Leu Val Glu Ile Ile Lys Val
            260                 265                 270

Leu Gly Thr Pro Thr Arg Glu Gln Ile Arg Glu Met Asn Pro Asn Tyr
        275                 280                 285

Thr Glu Phe Lys Phe Pro Gln Ile Lys Ala His Pro Trp Thr Lys Asp
    290                 295                 300

Ser Ser Gly Thr Gly His Phe Thr Ser Gly Val Arg Val Phe Arg Pro
305                 310                 315                 320

Arg Thr Pro Pro Glu Ala Ile Ala Leu Cys Ser Arg Leu Leu Glu Tyr
                325                 330                 335

Thr Pro Thr Ala Arg Leu Thr Pro Leu Glu Ala Cys Ala His Ser Phe
            340                 345                 350

Phe Asp Glu Leu Arg Asp Pro Asn Val Lys Leu Pro Asn Gly Arg Asp
        355                 360                 365

Thr Pro Ala Leu Phe Asn Phe Thr Thr Gln Glu Leu Ser Ser Asn Pro
    370                 375                 380

Pro Leu Ala Thr Ile Leu Ile Pro Pro His Ala Arg Ile Gln Ala Ala
385                 390                 395                 400

Ala Ser Thr Pro Thr Asn Ala Thr Ala Ala Ser Asp Ala Asn Thr Gly
                405                 410                 415

Asp Arg Gly Gln Thr Asn Asn Ala Ala Ser Ala Ser Ala Ser Asn Ser
            420                 425                 430

Thr
```

<210> 3
<211> 387
<212> PRT
<213> Homo sapiens

<400> 3

```
Met Ser Gly Arg Pro Arg Thr Thr Ser Phe Ala Glu Ser Cys Lys Pro
1               5                  10                  15

Val Gln Gln Pro Ser Ala Phe Gly Ser Met Lys Val Ser Arg Asp Lys
            20                  25                  30

Asp Gly Ser Lys Val Thr Thr Val Val Ala Thr Pro Gly Gln Gly Pro
        35                  40                  45

Asp Arg Pro Gln Glu Val Ser Tyr Thr Asp Thr Lys Val Ile Gly Asn
    50                  55                  60

Gly Ser Phe Gly Val Val Tyr Gln Ala Lys Leu Cys Asp Ser Gly Glu
65                  70                  75                  80

Leu Val Ala Ile Lys Lys Val Leu Gln Asp Lys Arg Phe Lys Asn Arg
                85                  90                  95

Glu Leu Gln Ile Met Arg Lys Leu Asp His Cys Asn Ile Val Arg Leu
            100                 105                 110

Arg Tyr Phe Phe Tyr Ser Ser Gly Glu Lys Lys Asp Glu Val Tyr Leu
        115                 120                 125

Asn Leu Val Leu Asp Tyr Val Pro Glu Thr Val Tyr Arg Val Ala Arg
    130                 135                 140

His Tyr Ser Arg Ala Lys Gln Thr Leu Pro Val Ile Tyr Val Lys Leu
145                 150                 155                 160

Tyr Met Tyr Gln Leu Phe Arg Ser Leu Ala Tyr Ile His Ser Phe Gly
                165                 170                 175

Ile Cys His Arg Asp Ile Lys Pro Gln Asn Leu Leu Leu Asp Pro Asp
            180                 185                 190

Thr Ala Val Leu Lys Leu Cys Asp Phe Gly Ser Ala Lys Gln Leu Val
        195                 200                 205

Arg Gly Glu Pro Asn Val Ser Tyr Ile Cys Ser Arg Tyr Tyr Arg Ala
    210                 215                 220

Pro Glu Leu Ile Phe Gly Ala Thr Asp Tyr Thr Ser Ser Ile Asp Val
225                 230                 235                 240

Trp Ser Ala Gly Cys Val Leu Ala Glu Leu Leu Leu Gly Gln Pro Ile
```

245 250 255

Phe Pro Gly Asp Ser Gly Val Asp Gln Leu Val Glu Ile Ile Lys Val
260 265 270

Leu Gly Thr Pro Thr Arg Glu Gln Ile Arg Glu Met Asn Pro Asn Tyr
275 280 285

Thr Glu Phe Lys Phe Pro Gln Ile Lys Ala His Pro Trp Thr Lys Val
290 295 300

Phe Arg Pro Arg Thr Pro Pro Glu Ala Ile Ala Leu Cys Ser Arg Leu
305 310 315 320

Leu Glu Tyr Thr Pro Thr Ala Arg Leu Thr Pro Leu Glu Ala Cys Ala
325 330 335

His Ser Phe Phe Asp Glu Leu Arg Asp Pro Asn Val Lys Leu Pro Asn
340 345 350

Gly Arg Asp Thr Pro Ala Leu Phe Asn Phe Thr Thr Gln Asp Ala Asn
355 360 365

Thr Gly Asp Arg Gly Gln Thr Asn Asn Ala Ala Ser Ala Ser Ala Ser
370 375 380

Asn Ser Thr
385

<210> 4
<211> 400
<212> PRT
<213> Homo sapiens

<400> 4

Met Ser Gly Arg Pro Arg Thr Thr Ser Phe Ala Glu Ser Cys Lys Pro
1 5 10 15

Val Gln Gln Pro Ser Ala Phe Gly Ser Met Lys Val Ser Arg Asp Lys
20 25 30

Asp Gly Ser Lys Val Thr Thr Val Val Ala Thr Pro Gly Gln Gly Pro
35 40 45

Asp Arg Pro Gln Glu Val Ser Tyr Thr Asp Thr Lys Val Ile Gly Asn
50 55 60

Gly Ser Phe Gly Val Val Tyr Gln Ala Lys Leu Cys Asp Ser Gly Glu

65 70 75 80

Leu Val Ala Ile Lys Lys Val Leu Gln Asp Lys Arg Phe Lys Asn Arg
85 90 95

Glu Leu Gln Ile Met Arg Lys Leu Asp His Cys Asn Ile Val Arg Leu
100 105 110

Arg Tyr Phe Phe Tyr Ser Ser Gly Glu Lys Lys Asp Glu Val Tyr Leu
115 120 125

Asn Leu Val Leu Asp Tyr Val Pro Glu Thr Val Tyr Arg Val Ala Arg
130 135 140

His Tyr Ser Arg Ala Lys Gln Thr Leu Pro Val Ile Tyr Val Lys Leu
145 150 155 160

Tyr Met Tyr Gln Leu Phe Arg Ser Leu Ala Tyr Ile His Ser Phe Gly
165 170 175

Ile Cys His Arg Asp Ile Lys Pro Gln Asn Leu Leu Leu Asp Pro Asp
180 185 190

Thr Ala Val Leu Lys Leu Cys Asp Phe Gly Ser Ala Lys Gln Leu Val
195 200 205

Arg Gly Glu Pro Asn Val Ser Tyr Ile Cys Ser Arg Tyr Tyr Arg Ala
210 215 220

Pro Glu Leu Ile Phe Gly Ala Thr Asp Tyr Thr Ser Ser Ile Asp Val
225 230 235 240

Trp Ser Ala Gly Cys Val Leu Ala Glu Leu Leu Leu Gly Gln Pro Ile
245 250 255

Phe Pro Gly Asp Ser Gly Val Asp Gln Leu Val Glu Ile Ile Lys Val
260 265 270

Leu Gly Thr Pro Thr Arg Glu Gln Ile Arg Glu Met Asn Pro Asn Tyr
275 280 285

Thr Glu Phe Lys Phe Pro Gln Ile Lys Ala His Pro Trp Thr Lys Asp
290 295 300

Ser Ser Gly Thr Gly His Phe Thr Ser Gly Val Arg Val Phe Arg Pro
305 310 315 320

Arg Thr Pro Pro Glu Ala Ile Ala Leu Cys Ser Arg Leu Leu Glu Tyr
325 330 335

Thr Pro Thr Ala Arg Leu Thr Pro Leu Glu Ala Cys Ala His Ser Phe
340 345 350

Phe Asp Glu Leu Arg Asp Pro Asn Val Lys Leu Pro Asn Gly Arg Asp
355 360 365

Thr Pro Ala Leu Phe Asn Phe Thr Thr Gln Asp Ala Asn Thr Gly Asp
370 375 380

Arg Gly Gln Thr Asn Asn Ala Ala Ser Ala Ser Ala Ser Asn Ser Thr
385 390 395 400

<210> 5
<211> 2527
<212> PRT
<213> Homo sapiens

<400> 5

Met Ala Ser Gly Ser Cys Gln Gly Cys Glu Glu Asp Glu Glu Thr Leu
1 5 10 15

Lys Lys Leu Ile Val Arg Leu Asn Asn Val Gln Glu Gly Lys Gln Ile
20 25 30

Glu Thr Leu Val Gln Ile Leu Glu Asp Leu Leu Val Phe Thr Tyr Ser
35 40 45

Glu Arg Ala Ser Lys Leu Phe Gln Gly Lys Asn Ile His Val Pro Leu
50 55 60

Leu Ile Val Leu Asp Ser Tyr Met Arg Val Ala Ser Val Gln Gln Val
65 70 75 80

Gly Trp Ser Leu Leu Cys Lys Leu Ile Glu Val Cys Pro Gly Thr Met
85 90 95

Gln Ser Leu Met Gly Pro Gln Asp Val Gly Asn Asp Trp Glu Val Leu
100 105 110

Gly Val His Gln Leu Ile Leu Lys Met Leu Thr Val His Asn Ala Ser
115 120 125

Val Asn Leu Ser Val Ile Gly Leu Lys Thr Leu Asp Leu Leu Leu Thr
130 135 140

```
Ser Gly Lys Ile Thr Leu Leu Ile Leu Asp Glu Glu Ser Asp Ile Phe
145                 150                 155                 160

Met Leu Ile Phe Asp Ala Met His Ser Phe Pro Ala Asn Asp Glu Val
                165                 170                 175

Gln Lys Leu Gly Cys Lys Ala Leu His Val Leu Phe Glu Arg Val Ser
            180                 185                 190

Glu Glu Gln Leu Thr Glu Phe Val Glu Asn Lys Asp Tyr Met Ile Leu
        195                 200                 205

Leu Ser Ala Leu Thr Asn Phe Lys Asp Glu Glu Glu Ile Val Leu His
    210                 215                 220

Val Leu His Cys Leu His Ser Leu Ala Ile Pro Cys Asn Asn Val Glu
225                 230                 235                 240

Val Leu Met Ser Gly Asn Val Arg Cys Tyr Asn Ile Val Val Glu Ala
                245                 250                 255

Met Lys Ala Phe Pro Met Ser Glu Arg Ile Gln Glu Val Ser Cys Cys
            260                 265                 270

Leu Leu His Arg Leu Thr Leu Gly Asn Phe Phe Asn Ile Leu Val Leu
        275                 280                 285

Asn Glu Val His Glu Phe Val Val Lys Ala Val Gln Gln Tyr Pro Glu
    290                 295                 300

Asn Ala Ala Leu Gln Ile Ser Ala Leu Ser Cys Leu Ala Leu Leu Thr
305                 310                 315                 320

Glu Thr Ile Phe Leu Asn Gln Asp Leu Glu Glu Lys Asn Glu Asn Gln
                325                 330                 335

Glu Asn Asp Asp Glu Gly Glu Glu Asp Lys Leu Phe Trp Leu Glu Ala
            340                 345                 350

Cys Tyr Lys Ala Leu Thr Trp His Arg Lys Asn Lys His Val Gln Glu
        355                 360                 365

Ala Ala Cys Trp Ala Leu Asn Asn Leu Leu Met Tyr Gln Asn Ser Leu
    370                 375                 380

His Glu Lys Ile Gly Asp Glu Asp Gly His Phe Pro Ala His Arg Glu
385                 390                 395                 400
```

Val Met Leu Ser Met Leu Met His Ser Ser Ser Lys Glu Val Phe Gln
405 410 415

Ala Ser Ala Asn Ala Leu Ser Thr Leu Leu Glu Gln Asn Val Asn Phe
420 425 430

Arg Lys Ile Leu Leu Ser Lys Gly Ile His Leu Asn Val Leu Glu Leu
435 440 445

Met Gln Lys His Ile His Ser Pro Glu Val Ala Glu Ser Gly Cys Lys
450 455 460

Met Leu Asn His Leu Phe Glu Gly Ser Asn Thr Ser Leu Asp Ile Met
465 470 475 480

Ala Ala Val Val Pro Lys Ile Leu Thr Val Met Lys Arg His Glu Thr
485 490 495

Ser Leu Pro Val Gln Leu Glu Ala Leu Arg Ala Ile Leu His Phe Ile
500 505 510

Val Pro Gly Met Pro Glu Glu Ser Arg Glu Asp Thr Glu Phe His His
515 520 525

Lys Leu Asn Met Val Lys Lys Gln Cys Phe Lys Asn Asp Ile His Lys
530 535 540

Leu Val Leu Ala Ala Leu Asn Arg Phe Ile Gly Asn Pro Gly Ile Gln
545 550 555 560

Lys Cys Gly Leu Lys Val Ile Ser Ser Ile Val His Phe Pro Asp Ala
565 570 575

Leu Glu Met Leu Ser Leu Glu Gly Ala Met Asp Ser Val Leu His Thr
580 585 590

Leu Gln Met Tyr Pro Asp Asp Gln Glu Ile Gln Cys Leu Gly Leu Ser
595 600 605

Leu Ile Gly Tyr Leu Ile Thr Lys Lys Asn Val Phe Ile Gly Thr Gly
610 615 620

His Leu Leu Ala Lys Ile Leu Val Ser Ser Leu Tyr Arg Phe Lys Asp
625 630 635 640

Val Ala Glu Ile Gln Thr Lys Gly Phe Gln Thr Ile Leu Ala Ile Leu
645 650 655

```
Lys Leu Ser Ala Ser Phe Ser Lys Leu Leu Val His His Ser Phe Asp
            660                 665                 670

Leu Val Ile Phe His Gln Met Ser Ser Asn Ile Met Glu Gln Lys Asp
        675                 680                 685

Gln Gln Phe Leu Asn Leu Cys Cys Lys Cys Phe Ala Lys Val Ala Met
    690                 695                 700

Asp Asp Tyr Leu Lys Asn Val Met Leu Glu Arg Ala Cys Asp Gln Asn
705                 710                 715                 720

Asn Ser Ile Met Val Glu Cys Leu Leu Leu Leu Gly Ala Asp Ala Asn
                725                 730                 735

Gln Ala Lys Glu Gly Ser Ser Leu Ile Cys Gln Val Cys Glu Lys Glu
            740                 745                 750

Ser Ser Pro Lys Leu Val Glu Leu Leu Leu Asn Ser Gly Ser Arg Glu
        755                 760                 765

Gln Asp Val Arg Lys Ala Leu Thr Ile Ser Ile Gly Lys Gly Asp Ser
    770                 775                 780

Gln Ile Ile Ser Leu Leu Leu Arg Arg Leu Ala Leu Asp Val Ala Asn
785                 790                 795                 800

Asn Ser Ile Cys Leu Gly Gly Phe Cys Ile Gly Lys Val Glu Pro Ser
                805                 810                 815

Trp Leu Gly Pro Leu Phe Pro Asp Lys Thr Ser Asn Leu Arg Lys Gln
            820                 825                 830

Thr Asn Ile Ala Ser Thr Leu Ala Arg Met Val Ile Arg Tyr Gln Met
        835                 840                 845

Lys Ser Ala Val Glu Glu Gly Thr Ala Ser Gly Ser Asp Gly Asn Phe
    850                 855                 860

Ser Glu Asp Val Leu Ser Lys Phe Asp Glu Trp Thr Phe Ile Pro Asp
865                 870                 875                 880

Ser Ser Met Asp Ser Val Phe Ala Gln Ser Asp Asp Leu Asp Ser Glu
                885                 890                 895

Gly Ser Glu Gly Ser Phe Leu Val Lys Lys Lys Ser Asn Ser Ile Ser
```

900 905 910

Val Gly Glu Phe Tyr Arg Asp Ala Val Leu Gln Arg Cys Ser Pro Asn
915 920 925

Leu Gln Arg His Ser Asn Ser Leu Gly Pro Ile Phe Asp His Glu Asp
930 935 940

Leu Leu Lys Arg Lys Arg Lys Ile Leu Ser Ser Asp Asp Ser Leu Arg
945 950 955 960

Ser Ser Lys Leu Gln Ser His Met Arg His Ser Asp Ser Ile Ser Ser
965 970 975

Leu Ala Ser Glu Arg Glu Tyr Ile Thr Ser Leu Asp Leu Ser Ala Asn
980 985 990

Glu Leu Arg Asp Ile Asp Ala Leu Ser Gln Lys Cys Cys Ile Ser Val
995 1000 1005

His Leu Glu His Leu Glu Lys Leu Glu Leu His Gln Asn Ala Leu
1010 1015 1020

Thr Ser Phe Pro Gln Gln Leu Cys Glu Thr Leu Lys Ser Leu Thr
1025 1030 1035

His Leu Asp Leu His Ser Asn Lys Phe Thr Ser Phe Pro Ser Tyr
1040 1045 1050

Leu Leu Lys Met Ser Cys Ile Ala Asn Leu Asp Val Ser Arg Asn
1055 1060 1065

Asp Ile Gly Pro Ser Val Val Leu Asp Pro Thr Val Lys Cys Pro
1070 1075 1080

Thr Leu Lys Gln Phe Asn Leu Ser Tyr Asn Gln Leu Ser Phe Val
1085 1090 1095

Pro Glu Asn Leu Thr Asp Val Val Glu Lys Leu Glu Gln Leu Ile
1100 1105 1110

Leu Glu Gly Asn Lys Ile Ser Gly Ile Cys Ser Pro Leu Arg Leu
1115 1120 1125

Lys Glu Leu Lys Ile Leu Asn Leu Ser Lys Asn His Ile Ser Ser
1130 1135 1140

Leu Ser Glu Asn Phe Leu Glu Ala Cys Pro Lys Val Glu Ser Phe
1145 1150 1155

Ser Ala Arg Met Asn Phe Leu Ala Ala Met Pro Phe Leu Pro Pro
1160 1165 1170

Ser Met Thr Ile Leu Lys Leu Ser Gln Asn Lys Phe Ser Cys Ile
1175 1180 1185

Pro Glu Ala Ile Leu Asn Leu Pro His Leu Arg Ser Leu Asp Met
1190 1195 1200

Ser Ser Asn Asp Ile Gln Tyr Leu Pro Gly Pro Ala His Trp Lys
1205 1210 1215

Ser Leu Asn Leu Arg Glu Leu Leu Phe Ser His Asn Gln Ile Ser
1220 1225 1230

Ile Leu Asp Leu Ser Glu Lys Ala Tyr Leu Trp Ser Arg Val Glu
1235 1240 1245

Lys Leu His Leu Ser His Asn Lys Leu Lys Glu Ile Pro Pro Glu
1250 1255 1260

Ile Gly Cys Leu Glu Asn Leu Thr Ser Leu Asp Val Ser Tyr Asn
1265 1270 1275

Leu Glu Leu Arg Ser Phe Pro Asn Glu Met Gly Lys Leu Ser Lys
1280 1285 1290

Ile Trp Asp Leu Pro Leu Asp Glu Leu His Leu Asn Phe Asp Phe
1295 1300 1305

Lys His Ile Gly Cys Lys Ala Lys Asp Ile Ile Arg Phe Leu Gln
1310 1315 1320

Gln Arg Leu Lys Lys Ala Val Pro Tyr Asn Arg Met Lys Leu Met
1325 1330 1335

Ile Val Gly Asn Thr Gly Ser Gly Lys Thr Thr Leu Leu Gln Gln
1340 1345 1350

Leu Met Lys Thr Lys Lys Ser Asp Leu Gly Met Gln Ser Ala Thr
1355 1360 1365

Val Gly Ile Asp Val Lys Asp Trp Pro Ile Gln Ile Arg Asp Lys
1370 1375 1380

```
Arg Lys  Arg Asp Leu Val Leu  Asn Val Trp Asp Phe  Ala Gly Arg
    1385                 1390                 1395

Glu Glu  Phe Tyr Ser Thr His  Pro His Phe Met Thr  Gln Arg Ala
    1400                 1405                 1410

Leu Tyr  Leu Ala Val Tyr Asp  Leu Ser Lys Gly Gln  Ala Glu Val
    1415                 1420                 1425

Asp Ala  Met Lys Pro Trp Leu  Phe Asn Ile Lys Ala  Arg Ala Ser
    1430                 1435                 1440

Ser Ser  Pro Val Ile Leu Val  Gly Thr His Leu Asp  Val Ser Asp
    1445                 1450                 1455

Glu Lys  Gln Arg Lys Ala Cys  Met Ser Lys Ile Thr  Lys Glu Leu
    1460                 1465                 1470

Leu Asn  Lys Arg Gly Phe Pro  Ala Ile Arg Asp Tyr  His Phe Val
    1475                 1480                 1485

Asn Ala  Thr Glu Glu Ser Asp  Ala Leu Ala Lys Leu  Arg Lys Thr
    1490                 1495                 1500

Ile Ile  Asn Glu Ser Leu Asn  Phe Lys Ile Arg Asp  Gln Leu Val
    1505                 1510                 1515

Val Gly  Gln Leu Ile Pro Asp  Cys Tyr Val Glu Leu  Glu Lys Ile
    1520                 1525                 1530

Ile Leu  Ser Glu Arg Lys Asn  Val Pro Ile Glu Phe  Pro Val Ile
    1535                 1540                 1545

Asp Arg  Lys Arg Leu Leu Gln  Leu Val Arg Glu Asn  Gln Leu Gln
    1550                 1555                 1560

Leu Asp  Glu Asn Glu Leu Pro  His Ala Val His Phe  Leu Asn Glu
    1565                 1570                 1575

Ser Gly  Val Leu Leu His Phe  Gln Asp Pro Ala Leu  Gln Leu Ser
    1580                 1585                 1590

Asp Leu  Tyr Phe Val Glu Pro  Lys Trp Leu Cys Lys  Ile Met Ala
    1595                 1600                 1605

Gln Ile  Leu Thr Val Lys Val  Glu Gly Cys Pro Lys  His Pro Lys
    1610                 1615                 1620
```

Gly Ile Ile Ser Arg Arg Asp Val Glu Lys Phe Leu Ser Lys Lys
1625 1630 1635

Arg Lys Phe Pro Lys Asn Tyr Met Ser Gln Tyr Phe Lys Leu Leu
1640 1645 1650

Glu Lys Phe Gln Ile Ala Leu Pro Ile Gly Glu Glu Tyr Leu Leu
1655 1660 1665

Val Pro Ser Ser Leu Ser Asp His Arg Pro Val Ile Glu Leu Pro
1670 1675 1680

His Cys Glu Asn Ser Glu Ile Ile Ile Arg Leu Tyr Glu Met Pro
1685 1690 1695

Tyr Phe Pro Met Gly Phe Trp Ser Arg Leu Ile Asn Arg Leu Leu
1700 1705 1710

Glu Ile Ser Pro Tyr Met Leu Ser Gly Arg Glu Arg Ala Leu Arg
1715 1720 1725

Pro Asn Arg Met Tyr Trp Arg Gln Gly Ile Tyr Leu Asn Trp Ser
1730 1735 1740

Pro Glu Ala Tyr Cys Leu Val Gly Ser Glu Val Leu Asp Asn His
1745 1750 1755

Pro Glu Ser Phe Leu Lys Ile Thr Val Pro Ser Cys Arg Lys Gly
1760 1765 1770

Cys Ile Leu Leu Gly Gln Val Val Asp His Ile Asp Ser Leu Met
1775 1780 1785

Glu Glu Trp Phe Pro Gly Leu Leu Glu Ile Asp Ile Cys Gly Glu
1790 1795 1800

Gly Glu Thr Leu Leu Lys Lys Trp Ala Leu Tyr Ser Phe Asn Asp
1805 1810 1815

Gly Glu Glu His Gln Lys Ile Leu Leu Asp Asp Leu Met Lys Lys
1820 1825 1830

Ala Glu Glu Gly Asp Leu Leu Val Asn Pro Asp Gln Pro Arg Leu
1835 1840 1845

Thr Ile Pro Ile Ser Gln Ile Ala Pro Asp Leu Ile Leu Ala Asp

1850 1855 1860

Leu Pro Arg Asn Ile Met Leu Asn Asn Asp Glu Leu Glu Phe Glu
1865 1870 1875

Gln Ala Pro Glu Phe Leu Leu Gly Asp Gly Ser Phe Gly Ser Val
1880 1885 1890

Tyr Arg Ala Ala Tyr Glu Gly Glu Glu Val Ala Val Lys Ile Phe
1895 1900 1905

Asn Lys His Thr Ser Leu Arg Leu Leu Arg Gln Glu Leu Val Val
1910 1915 1920

Leu Cys His Leu His His Pro Ser Leu Ile Ser Leu Leu Ala Ala
1925 1930 1935

Gly Ile Arg Pro Arg Met Leu Val Met Glu Leu Ala Ser Lys Gly
1940 1945 1950

Ser Leu Asp Arg Leu Leu Gln Gln Asp Lys Ala Ser Leu Thr Arg
1955 1960 1965

Thr Leu Gln His Arg Ile Ala Leu His Val Ala Asp Gly Leu Arg
1970 1975 1980

Tyr Leu His Ser Ala Met Ile Ile Tyr Arg Asp Leu Lys Pro His
1985 1990 1995

Asn Val Leu Leu Phe Thr Leu Tyr Pro Asn Ala Ala Ile Ile Ala
2000 2005 2010

Lys Ile Ala Asp Tyr Gly Ile Ala Gln Tyr Cys Cys Arg Met Gly
2015 2020 2025

Ile Lys Thr Ser Glu Gly Thr Pro Gly Phe Arg Ala Pro Glu Val
2030 2035 2040

Ala Arg Gly Asn Val Ile Tyr Asn Gln Gln Ala Asp Val Tyr Ser
2045 2050 2055

Phe Gly Leu Leu Leu Tyr Asp Ile Leu Thr Thr Gly Gly Arg Ile
2060 2065 2070

Val Glu Gly Leu Lys Phe Pro Asn Glu Phe Asp Glu Leu Glu Ile
2075 2080 2085

```
Gln Gly  Lys Leu Pro Asp Pro  Val Lys Glu Tyr Gly  Cys Ala Pro
    2090                 2095                 2100

Trp Pro  Met Val Glu Lys Leu  Ile Lys Gln Cys Leu  Lys Glu Asn
    2105                 2110                 2115

Pro Gln  Glu Arg Pro Thr Ser  Ala Gln Val Phe Asp  Ile Leu Asn
    2120                 2125                 2130

Ser Ala  Glu Leu Val Cys Leu  Thr Arg Arg Ile Leu  Leu Pro Lys
    2135                 2140                 2145

Asn Val  Ile Val Glu Cys Met  Val Ala Thr His His  Asn Ser Arg
    2150                 2155                 2160

Asn Ala  Ser Ile Trp Leu Gly  Cys Gly His Thr Asp  Arg Gly Gln
    2165                 2170                 2175

Leu Ser  Phe Leu Asp Leu Asn  Thr Glu Gly Tyr Thr  Ser Glu Glu
    2180                 2185                 2190

Val Ala  Asp Ser Arg Ile Leu  Cys Leu Ala Leu Val  His Leu Pro
    2195                 2200                 2205

Val Glu  Lys Glu Ser Trp Ile  Val Ser Gly Thr Gln  Ser Gly Thr
    2210                 2215                 2220

Leu Leu  Val Ile Asn Thr Glu  Asp Gly Lys Lys Arg  His Thr Leu
    2225                 2230                 2235

Glu Lys  Met Thr Asp Ser Val  Thr Cys Leu Tyr Cys  Asn Ser Phe
    2240                 2245                 2250

Ser Lys  Gln Ser Lys Gln Lys  Asn Phe Leu Leu Val  Gly Thr Ala
    2255                 2260                 2265

Asp Gly  Lys Leu Ala Ile Phe  Glu Asp Lys Thr Val  Lys Leu Lys
    2270                 2275                 2280

Gly Ala  Ala Pro Leu Lys Ile  Leu Asn Ile Gly Asn  Val Ser Thr
    2285                 2290                 2295

Pro Leu  Met Cys Leu Ser Glu  Ser Thr Asn Ser Thr  Glu Arg Asn
    2300                 2305                 2310

Val Met  Trp Gly Gly Cys Gly  Thr Lys Ile Phe Ser  Phe Ser Asn
    2315                 2320                 2325
```

```
Asp Phe  Thr Ile Gln Lys Leu  Ile Glu Thr Arg Thr  Ser Gln Leu
    2330                 2335                 2340

Phe Ser  Tyr Ala Ala Phe Ser  Asp Ser Asn Ile Ile  Thr Val Val
    2345                 2350                 2355

Val Asp  Thr Ala Leu Tyr Ile  Ala Lys Gln Asn Ser  Pro Val Val
    2360                 2365                 2370

Glu Val  Trp Asp Lys Lys Thr  Glu Lys Leu Cys Gly  Leu Ile Asp
    2375                 2380                 2385

Cys Val  His Phe Leu Arg Glu  Val Met Val Lys Glu  Asn Lys Glu
    2390                 2395                 2400

Ser Lys  His Lys Met Ser Tyr  Ser Gly Arg Val Lys  Thr Leu Cys
    2405                 2410                 2415

Leu Gln  Lys Asn Thr Ala Leu  Trp Ile Gly Thr Gly  Gly Gly His
    2420                 2425                 2430

Ile Leu  Leu Leu Asp Leu Ser  Thr Arg Arg Leu Ile  Arg Val Ile
    2435                 2440                 2445

Tyr Asn  Phe Cys Asn Ser Val  Arg Val Met Met Thr  Ala Gln Leu
    2450                 2455                 2460

Gly Ser  Leu Lys Asn Val Met  Leu Val Leu Gly Tyr  Asn Arg Lys
    2465                 2470                 2475

Asn Thr  Glu Gly Thr Gln Lys  Gln Lys Glu Ile Gln  Ser Cys Leu
    2480                 2485                 2490

Thr Val  Trp Asp Ile Asn Leu  Pro His Glu Val Gln  Asn Leu Glu
    2495                 2500                 2505

Lys His  Ile Glu Val Arg Lys  Glu Leu Ala Glu Lys  Met Arg Arg
    2510                 2515                 2520

Thr Ser  Val Glu
    2525
```

<210> 6
<211> 2527
<212> PRT
<213> Homo sapiens

<400> 6

```
Met Ala Ser Gly Ser Cys Gln Gly Cys Glu Glu Asp Glu Glu Thr Leu
1               5                  10                  15

Lys Lys Leu Ile Val Arg Leu Asn Asn Val Gln Glu Gly Lys Gln Ile
            20                  25                  30

Glu Thr Leu Val Gln Ile Leu Glu Asp Leu Leu Val Phe Thr Tyr Ser
        35                  40                  45

Glu His Ala Ser Lys Leu Phe Gln Gly Lys Asn Ile His Val Pro Leu
    50                  55                  60

Leu Ile Val Leu Asp Ser Tyr Met Arg Val Ala Ser Val Gln Gln Val
65                  70                  75                  80

Gly Trp Ser Leu Leu Cys Lys Leu Ile Glu Val Cys Pro Gly Thr Met
                85                  90                  95

Gln Ser Leu Met Gly Pro Gln Asp Val Gly Asn Asp Trp Glu Val Leu
            100                 105                 110

Gly Val His Gln Leu Ile Leu Lys Met Leu Thr Val His Asn Ala Ser
        115                 120                 125

Val Asn Leu Ser Val Ile Gly Leu Lys Thr Leu Asp Leu Leu Leu Thr
    130                 135                 140

Ser Gly Lys Ile Thr Leu Leu Ile Leu Asp Glu Glu Ser Asp Ile Phe
145                 150                 155                 160

Met Leu Ile Phe Asp Ala Met His Ser Phe Pro Ala Asn Asp Glu Val
                165                 170                 175

Gln Lys Leu Gly Cys Lys Ala Leu His Val Leu Phe Glu Arg Val Ser
            180                 185                 190

Glu Glu Gln Leu Thr Glu Phe Val Glu Asn Lys Asp Tyr Met Ile Leu
        195                 200                 205

Leu Ser Ala Leu Thr Asn Phe Lys Asp Glu Glu Glu Ile Val Leu His
    210                 215                 220

Val Leu His Cys Leu His Ser Leu Ala Ile Pro Cys Asn Asn Val Glu
225                 230                 235                 240

Val Leu Met Ser Gly Asn Val Arg Cys Tyr Asn Ile Val Val Glu Ala
                245                 250                 255
```

```
Met Lys Ala Phe Pro Met Ser Glu Arg Ile Gln Glu Val Ser Cys Cys
            260                 265                 270

Leu Leu His Arg Leu Thr Leu Gly Asn Phe Phe Asn Ile Leu Val Leu
        275                 280                 285

Asn Glu Val His Glu Phe Val Val Lys Ala Val Gln Gln Tyr Pro Glu
    290                 295                 300

Asn Ala Ala Leu Gln Ile Ser Ala Leu Ser Cys Leu Ala Leu Leu Thr
305                 310                 315                 320

Glu Thr Ile Phe Leu Asn Gln Asp Leu Glu Glu Lys Asn Glu Asn Gln
                325                 330                 335

Glu Asn Asp Asp Glu Gly Glu Glu Asp Lys Leu Phe Trp Leu Glu Ala
            340                 345                 350

Cys Tyr Lys Ala Leu Thr Trp His Arg Lys Asn Lys His Val Gln Glu
        355                 360                 365

Ala Ala Cys Trp Ala Leu Asn Asn Leu Leu Met Tyr Gln Asn Ser Leu
    370                 375                 380

His Glu Lys Ile Gly Asp Glu Asp Gly His Phe Pro Ala His Arg Glu
385                 390                 395                 400

Val Met Leu Ser Met Leu Met His Ser Ser Ser Lys Glu Val Phe Gln
                405                 410                 415

Ala Ser Ala Asn Ala Leu Ser Thr Leu Leu Glu Gln Asn Val Asn Phe
            420                 425                 430

Arg Lys Ile Leu Leu Ser Lys Gly Ile His Leu Asn Val Leu Glu Leu
        435                 440                 445

Met Gln Lys His Ile His Ser Pro Glu Val Ala Glu Ser Gly Cys Lys
    450                 455                 460

Met Leu Asn His Leu Phe Glu Gly Ser Asn Thr Ser Leu Asp Ile Met
465                 470                 475                 480

Ala Ala Val Val Pro Lys Ile Leu Thr Val Met Lys Arg His Glu Thr
                485                 490                 495

Ser Leu Pro Val Gln Leu Glu Ala Leu Arg Ala Ile Leu His Phe Ile
```

500 505 510

Val Pro Gly Met Pro Glu Glu Ser Arg Glu Asp Thr Glu Phe His His
515 520 525

Lys Leu Asn Met Val Lys Lys Gln Cys Phe Lys Asn Asp Ile His Lys
530 535 540

Leu Val Leu Ala Ala Leu Asn Arg Phe Ile Gly Asn Pro Gly Ile Gln
545 550 555 560

Lys Cys Gly Leu Lys Val Ile Ser Ser Ile Val His Phe Pro Asp Ala
565 570 575

Leu Glu Met Leu Ser Leu Glu Gly Ala Met Asp Ser Val Leu His Thr
580 585 590

Leu Gln Met Tyr Pro Asp Asp Gln Glu Ile Gln Cys Leu Gly Leu Ser
595 600 605

Leu Ile Gly Tyr Leu Ile Thr Lys Lys Asn Val Phe Ile Gly Thr Gly
610 615 620

His Leu Leu Ala Lys Ile Leu Val Ser Ser Leu Tyr Arg Phe Lys Asp
625 630 635 640

Val Ala Glu Ile Gln Thr Lys Gly Phe Gln Thr Ile Leu Ala Ile Leu
645 650 655

Lys Leu Ser Ala Ser Phe Ser Lys Leu Leu Val His His Ser Phe Asp
660 665 670

Leu Val Ile Phe His Gln Met Ser Ser Asn Ile Met Glu Gln Lys Asp
675 680 685

Gln Gln Phe Leu Asn Leu Cys Cys Lys Cys Phe Ala Lys Val Ala Met
690 695 700

Asp Asp Tyr Leu Lys Asn Val Met Leu Glu Arg Ala Cys Asp Gln Asn
705 710 715 720

Asn Ser Ile Met Val Glu Cys Leu Leu Leu Leu Gly Ala Asp Ala Asn
725 730 735

Gln Ala Lys Glu Gly Ser Ser Leu Ile Cys Gln Val Cys Glu Lys Glu
740 745 750

```
Ser Ser Pro Lys Leu Val Glu Leu Leu Leu Asn Ser Gly Ser Arg Glu
        755                 760                 765

Gln Asp Val Arg Lys Ala Leu Thr Ile Ser Ile Gly Lys Gly Asp Ser
    770                 775                 780

Gln Ile Ile Ser Leu Leu Leu Arg Arg Leu Ala Leu Asp Val Ala Asn
785                 790                 795                 800

Asn Ser Ile Cys Leu Gly Gly Phe Cys Ile Gly Lys Val Glu Pro Ser
                805                 810                 815

Trp Leu Gly Pro Leu Phe Pro Asp Lys Thr Ser Asn Leu Arg Lys Gln
            820                 825                 830

Thr Asn Ile Ala Ser Thr Leu Ala Arg Met Val Ile Arg Tyr Gln Met
        835                 840                 845

Lys Ser Ala Val Glu Glu Gly Thr Ala Ser Gly Ser Asp Gly Asn Phe
    850                 855                 860

Ser Glu Asp Val Leu Ser Lys Phe Asp Glu Trp Thr Phe Ile Pro Asp
865                 870                 875                 880

Ser Ser Met Asp Ser Val Phe Ala Gln Ser Asp Asp Leu Asp Ser Glu
                885                 890                 895

Gly Ser Glu Gly Ser Phe Leu Val Lys Lys Lys Ser Asn Ser Ile Ser
            900                 905                 910

Val Gly Glu Phe Tyr Arg Asp Ala Val Leu Gln Arg Cys Ser Pro Asn
        915                 920                 925

Leu Gln Arg His Ser Asn Ser Leu Gly Pro Ile Phe Asp His Glu Asp
    930                 935                 940

Leu Leu Lys Arg Lys Arg Lys Ile Leu Ser Ser Asp Asp Ser Leu Arg
945                 950                 955                 960

Ser Ser Lys Leu Gln Ser His Met Arg His Ser Asp Ser Ile Ser Ser
                965                 970                 975

Leu Ala Ser Glu Arg Glu Tyr Ile Thr Ser Leu Asp Leu Ser Ala Asn
            980                 985                 990

Glu Leu Arg Asp Ile Asp Ala Leu  Ser Gln Lys Cys Cys  Ile Ser Val
        995                 1000                1005
```

```
His Leu  Glu His Leu Glu Lys  Leu Glu Leu His Gln  Asn Ala Leu
    1010                 1015                 1020

Thr Ser  Phe Pro Gln Gln Leu  Cys Glu Thr Leu Lys  Ser Leu Thr
    1025                 1030                 1035

His Leu  Asp Leu His Ser Asn  Lys Phe Thr Ser Phe  Pro Ser Tyr
    1040                 1045                 1050

Leu Leu  Lys Met Ser Cys Ile  Ala Asn Leu Asp Val  Ser Arg Asn
    1055                 1060                 1065

Asp Ile  Gly Pro Ser Val Val  Leu Asp Pro Thr Val  Lys Cys Pro
    1070                 1075                 1080

Thr Leu  Lys Gln Phe Asn Leu  Ser Tyr Asn Gln Leu  Ser Phe Val
    1085                 1090                 1095

Pro Glu  Asn Leu Thr Asp Val  Val Glu Lys Leu Glu  Gln Leu Ile
    1100                 1105                 1110

Leu Glu  Gly Asn Lys Ile Ser  Gly Ile Cys Ser Pro  Leu Arg Leu
    1115                 1120                 1125

Lys Glu  Leu Lys Ile Leu Asn  Leu Ser Lys Asn His  Ile Ser Ser
    1130                 1135                 1140

Leu Ser  Glu Asn Phe Leu Glu  Ala Cys Pro Lys Val  Glu Ser Phe
    1145                 1150                 1155

Ser Ala  Arg Met Asn Phe Leu  Ala Ala Met Pro Phe  Leu Pro Pro
    1160                 1165                 1170

Ser Met  Thr Ile Leu Lys Leu  Ser Gln Asn Lys Phe  Ser Cys Ile
    1175                 1180                 1185

Pro Glu  Ala Ile Leu Asn Leu  Pro His Leu Arg Ser  Leu Asp Met
    1190                 1195                 1200

Ser Ser  Asn Asp Ile Gln Tyr  Leu Pro Gly Pro Ala  His Trp Lys
    1205                 1210                 1215

Ser Leu  Asn Leu Arg Glu Leu  Leu Phe Ser His Asn  Gln Ile Ser
    1220                 1225                 1230

Ile Leu  Asp Leu Ser Glu Lys  Ala Tyr Leu Trp Ser  Arg Val Glu
    1235                 1240                 1245
```

```
Lys Leu  His Leu Ser His Asn  Lys Leu Lys Glu Ile  Pro Pro Glu
    1250                 1255                 1260

Ile Gly  Cys Leu Glu Asn Leu  Thr Ser Leu Asp Val  Ser Tyr Asn
    1265                 1270                 1275

Leu Glu  Leu Arg Ser Phe Pro  Asn Glu Met Gly Lys  Leu Ser Lys
    1280                 1285                 1290

Ile Trp  Asp Leu Pro Leu Asp  Glu Leu His Leu Asn  Phe Asp Phe
    1295                 1300                 1305

Lys His  Ile Gly Cys Lys Ala  Lys Asp Ile Ile Arg  Phe Leu Gln
    1310                 1315                 1320

Gln Arg  Leu Lys Lys Ala Val  Pro Tyr Asn Arg Met  Lys Leu Met
    1325                 1330                 1335

Ile Val  Gly Asn Thr Gly Ser  Gly Lys Thr Thr Leu  Leu Gln Gln
    1340                 1345                 1350

Leu Met  Lys Thr Lys Lys Ser  Asp Leu Gly Met Gln  Ser Ala Thr
    1355                 1360                 1365

Val Gly  Ile Asp Val Lys Asp  Trp Pro Ile Gln Ile  Arg Asp Lys
    1370                 1375                 1380

Arg Lys  Arg Asp Leu Val Leu  Asn Val Trp Asp Phe  Ala Gly Arg
    1385                 1390                 1395

Glu Glu  Phe Tyr Ser Thr His  Pro His Phe Met Thr  Gln Arg Ala
    1400                 1405                 1410

Leu Tyr  Leu Ala Val Tyr Asp  Leu Ser Lys Gly Gln  Ala Glu Val
    1415                 1420                 1425

Asp Ala  Met Lys Pro Trp Leu  Phe Asn Ile Lys Ala  Arg Ala Ser
    1430                 1435                 1440

Ser Ser  Pro Val Ile Leu Val  Gly Thr His Leu Asp  Val Ser Asp
    1445                 1450                 1455

Glu Lys  Gln Arg Lys Ala Cys  Met Ser Lys Ile Thr  Lys Glu Leu
    1460                 1465                 1470

Leu Asn  Lys Arg Gly Phe Pro  Ala Ile Arg Asp Tyr  His Phe Val
```

1475 1480 1485

Asn Ala Thr Glu Glu Ser Asp Ala Leu Ala Lys Leu Arg Lys Thr
1490 1495 1500

Ile Ile Asn Glu Ser Leu Asn Phe Lys Ile Arg Asp Gln Leu Val
1505 1510 1515

Val Gly Gln Leu Ile Pro Asp Cys Tyr Val Glu Leu Glu Lys Ile
1520 1525 1530

Ile Leu Ser Glu Arg Lys Asn Val Pro Ile Glu Phe Pro Val Ile
1535 1540 1545

Asp Arg Lys Arg Leu Leu Gln Leu Val Arg Glu Asn Gln Leu Gln
1550 1555 1560

Leu Asp Glu Asn Glu Leu Pro His Ala Val His Phe Leu Asn Glu
1565 1570 1575

Ser Gly Val Leu Leu His Phe Gln Asp Pro Ala Leu Gln Leu Ser
1580 1585 1590

Asp Leu Tyr Phe Val Glu Pro Lys Trp Leu Cys Lys Ile Met Ala
1595 1600 1605

Gln Ile Leu Thr Val Lys Val Glu Gly Cys Pro Lys His Pro Lys
1610 1615 1620

Gly Ile Ile Ser Arg Arg Asp Val Glu Lys Phe Leu Ser Lys Lys
1625 1630 1635

Arg Lys Phe Pro Lys Asn Tyr Met Thr Gln Tyr Phe Lys Leu Leu
1640 1645 1650

Glu Lys Phe Gln Ile Ala Leu Pro Ile Gly Glu Glu Tyr Leu Leu
1655 1660 1665

Val Pro Ser Ser Leu Ser Asp His Arg Pro Val Ile Glu Leu Pro
1670 1675 1680

His Cys Glu Asn Ser Glu Ile Ile Ile Arg Leu Tyr Glu Met Pro
1685 1690 1695

Tyr Phe Pro Met Gly Phe Trp Ser Arg Leu Ile Asn Arg Leu Leu
1700 1705 1710

```
Glu Ile  Ser Pro Tyr Met Leu  Ser Gly Arg Glu Arg  Ala Leu Arg
    1715                 1720                 1725

Pro Asn  Arg Met Tyr Trp Arg  Gln Gly Ile Tyr Leu  Asn Trp Ser
    1730                 1735                 1740

Pro Glu  Ala Tyr Cys Leu Val  Gly Ser Glu Val Leu  Asp Asn His
    1745                 1750                 1755

Pro Glu  Ser Phe Leu Lys Ile  Thr Val Pro Ser Cys  Arg Lys Gly
    1760                 1765                 1770

Cys Ile  Leu Leu Gly Gln Val  Val Asp His Ile Asp  Ser Leu Met
    1775                 1780                 1785

Glu Glu  Trp Phe Pro Gly Leu  Leu Glu Ile Asp Ile  Cys Gly Glu
    1790                 1795                 1800

Gly Glu  Thr Leu Leu Lys Lys  Trp Ala Leu Tyr Ser  Phe Asn Asp
    1805                 1810                 1815

Gly Glu  Glu His Gln Lys Ile  Leu Leu Asp Asp Leu  Met Lys Lys
    1820                 1825                 1830

Ala Glu  Glu Gly Asp Leu Leu  Val Asn Pro Asp Gln  Pro Arg Leu
    1835                 1840                 1845

Thr Ile  Pro Ile Ser Gln Ile  Ala Pro Asp Leu Ile  Leu Ala Asp
    1850                 1855                 1860

Leu Pro  Arg Asn Ile Met Leu  Asn Asn Asp Glu Leu  Glu Phe Glu
    1865                 1870                 1875

Gln Ala  Pro Glu Phe Leu Leu  Gly Asp Gly Ser Phe  Gly Ser Val
    1880                 1885                 1890

Tyr Arg  Ala Ala Tyr Glu Gly  Glu Glu Val Ala Val  Lys Ile Phe
    1895                 1900                 1905

Asn Lys  His Thr Ser Leu Arg  Leu Leu Arg Gln Glu  Leu Val Val
    1910                 1915                 1920

Leu Cys  His Leu His His Pro  Ser Leu Ile Ser Leu  Leu Ala Ala
    1925                 1930                 1935

Gly Ile  Arg Pro Arg Met Leu  Val Met Glu Leu Ala  Ser Lys Gly
    1940                 1945                 1950
```

Ser Leu Asp Arg Leu Leu Gln Gln Asp Lys Ala Ser Leu Thr Arg
1955 1960 1965

Thr Leu Gln His Arg Ile Ala Leu His Val Ala Asp Gly Leu Arg
1970 1975 1980

Tyr Leu His Ser Ala Met Ile Ile Tyr Arg Asp Leu Lys Pro His
1985 1990 1995

Asn Val Leu Leu Phe Thr Leu Tyr Pro Asn Ala Ala Ile Ile Ala
2000 2005 2010

Lys Ile Ala Asp Tyr Gly Ile Ala Gln Tyr Cys Cys Arg Met Gly
2015 2020 2025

Ile Lys Thr Ser Glu Gly Thr Pro Gly Phe Arg Ala Pro Glu Val
2030 2035 2040

Ala Arg Gly Asn Val Ile Tyr Asn Gln Gln Ala Asp Val Tyr Ser
2045 2050 2055

Phe Gly Leu Leu Leu Tyr Asp Ile Leu Thr Thr Gly Gly Arg Ile
2060 2065 2070

Val Glu Gly Leu Lys Phe Pro Asn Glu Phe Asp Glu Leu Glu Ile
2075 2080 2085

Gln Gly Lys Leu Pro Asp Pro Val Lys Glu Tyr Gly Cys Ala Pro
2090 2095 2100

Trp Pro Met Val Glu Lys Leu Ile Lys Gln Cys Leu Lys Glu Asn
2105 2110 2115

Pro Gln Glu Arg Pro Thr Ser Ala Gln Val Phe Asp Ile Leu Asn
2120 2125 2130

Ser Ala Glu Leu Val Cys Leu Thr Arg Arg Ile Leu Leu Pro Lys
2135 2140 2145

Asn Val Ile Val Glu Cys Met Val Ala Thr His His Asn Ser Arg
2150 2155 2160

Asn Ala Ser Ile Trp Leu Gly Cys Gly His Thr Asp Arg Gly Gln
2165 2170 2175

Leu Ser Phe Leu Asp Leu Asn Thr Glu Gly Tyr Thr Ser Glu Glu
2180 2185 2190

```
Val Ala  Asp Ser Arg Ile Leu  Cys Leu Ala Leu Val  His Leu Pro
    2195                 2200                 2205

Val Glu  Lys Glu Ser Trp Ile  Val Ser Gly Thr Gln  Ser Gly Thr
    2210                 2215                 2220

Leu Leu  Val Ile Asn Thr Glu  Asp Gly Lys Lys Arg  His Thr Leu
    2225                 2230                 2235

Glu Lys  Met Thr Asp Ser Val  Thr Cys Leu Tyr Cys  Asn Ser Phe
    2240                 2245                 2250

Ser Lys  Gln Ser Lys Gln Lys  Asn Phe Leu Leu Val  Gly Thr Ala
    2255                 2260                 2265

Asp Gly  Lys Leu Ala Ile Phe  Glu Asp Lys Thr Val  Lys Leu Lys
    2270                 2275                 2280

Gly Ala  Ala Pro Leu Lys Ile  Leu Asn Ile Gly Asn  Val Ser Thr
    2285                 2290                 2295

Pro Leu  Met Cys Leu Ser Glu  Ser Thr Asn Ser Thr  Glu Arg Asn
    2300                 2305                 2310

Val Met  Trp Gly Gly Cys Gly  Thr Lys Ile Phe Ser  Phe Ser Asn
    2315                 2320                 2325

Asp Phe  Thr Ile Gln Lys Leu  Ile Glu Thr Arg Thr  Ser Gln Leu
    2330                 2335                 2340

Phe Ser  Tyr Ala Ala Phe Ser  Asp Ser Asn Ile Ile  Thr Val Val
    2345                 2350                 2355

Val Asp  Thr Ala Leu Tyr Ile  Ala Lys Gln Asn Ser  Pro Val Val
    2360                 2365                 2370

Glu Val  Trp Asp Lys Lys Thr  Glu Lys Leu Cys Gly  Leu Ile Asp
    2375                 2380                 2385

Cys Val  His Phe Leu Arg Glu  Val Thr Val Lys Glu  Asn Lys Glu
    2390                 2395                 2400

Ser Lys  His Lys Met Ser Tyr  Ser Gly Arg Val Lys  Thr Leu Cys
    2405                 2410                 2415

Leu Gln  Lys Asn Thr Ala Leu  Trp Ile Gly Thr Gly  Gly Gly His
```

2420 2425 2430

Ile Leu Leu Leu Asp Leu Ser Thr Arg Arg Leu Ile Arg Val Ile
2435 2440 2445

Tyr Asn Phe Cys Asn Ser Val Arg Val Met Met Thr Ala Gln Leu
2450 2455 2460

Gly Ser Leu Lys Asn Val Met Leu Val Leu Gly Tyr Asn Arg Lys
2465 2470 2475

Asn Thr Glu Gly Thr Gln Lys Gln Lys Glu Ile Gln Ser Cys Leu
2480 2485 2490

Thr Val Trp Asp Ile Asn Leu Pro His Glu Val Gln Asn Leu Glu
2495 2500 2505

Lys His Ile Glu Val Arg Lys Glu Leu Ala Glu Lys Met Arg Arg
2510 2515 2520

Thr Ser Val Glu
2525

<210> 7
<211> 2527
<212> PRT
<213> Homo sapiens

<400> 7

Met Ala Ser Gly Ser Cys Gln Gly Cys Glu Glu Asp Glu Glu Thr Leu
1 5 10 15

Lys Lys Leu Ile Val Arg Leu Asn Asn Val Gln Glu Gly Lys Gln Ile
20 25 30

Glu Thr Leu Val Gln Ile Leu Glu Asp Leu Leu Val Phe Thr Tyr Ser
35 40 45

Glu His Ala Ser Lys Leu Phe Gln Gly Lys Asn Ile His Val Pro Leu
50 55 60

Leu Ile Val Leu Asp Ser Tyr Met Arg Val Ala Ser Val Gln Gln Val
65 70 75 80

Gly Trp Ser Leu Leu Cys Lys Leu Ile Glu Val Cys Pro Gly Thr Met
85 90 95

Gln Ser Leu Met Gly Pro Gln Asp Val Gly Asn Asp Trp Glu Val Leu

100 105 110

Gly Val His Gln Leu Ile Leu Lys Met Leu Thr Val His Asn Ala Ser
115 120 125

Val Asn Leu Ser Val Ile Gly Leu Lys Thr Leu Asp Leu Leu Leu Thr
130 135 140

Ser Gly Lys Ile Thr Leu Leu Ile Leu Asp Glu Glu Ser Asp Ile Phe
145 150 155 160

Met Leu Ile Phe Asp Ala Met His Ser Phe Pro Ala Asn Asp Glu Val
165 170 175

Gln Lys Leu Gly Cys Lys Ala Leu His Val Leu Phe Glu Arg Val Ser
180 185 190

Glu Glu Gln Leu Thr Glu Phe Val Glu Asn Lys Asp Tyr Met Ile Leu
195 200 205

Leu Ser Ala Ser Thr Asn Phe Lys Asp Glu Glu Glu Ile Val Leu His
210 215 220

Val Leu His Cys Leu His Ser Leu Ala Ile Pro Cys Asn Asn Val Glu
225 230 235 240

Val Leu Met Ser Gly Asn Val Arg Cys Tyr Asn Ile Val Val Glu Ala
245 250 255

Met Lys Ala Phe Pro Met Ser Glu Arg Ile Gln Glu Val Ser Cys Cys
260 265 270

Leu Leu His Arg Leu Thr Leu Gly Asn Phe Phe Asn Ile Leu Val Leu
275 280 285

Asn Glu Val His Glu Phe Val Val Lys Ala Val Gln Gln Tyr Pro Glu
290 295 300

Asn Ala Ala Leu Gln Ile Ser Ala Leu Ser Cys Leu Ala Leu Leu Thr
305 310 315 320

Glu Thr Ile Phe Leu Asn Gln Asp Leu Glu Glu Lys Asn Glu Asn Gln
325 330 335

Glu Asn Asp Asp Glu Gly Glu Glu Asp Lys Leu Phe Trp Leu Glu Ala
340 345 350

```
Cys Tyr Lys Ala Leu Thr Trp His Arg Lys Asn Lys His Val Gln Glu
        355                 360                 365

Ala Ala Cys Trp Ala Leu Asn Asn Leu Leu Met Tyr Gln Asn Ser Leu
    370                 375                 380

His Glu Lys Ile Gly Asp Glu Asp Gly His Phe Pro Ala His Arg Glu
385                 390                 395                 400

Val Met Leu Ser Met Leu Met His Ser Ser Ser Lys Glu Val Phe Gln
                405                 410                 415

Ala Ser Ala Asn Ala Leu Ser Thr Leu Leu Glu Gln Asn Val Asn Phe
            420                 425                 430

Arg Lys Ile Leu Leu Ser Lys Gly Ile His Leu Asn Val Leu Glu Leu
        435                 440                 445

Met Gln Lys His Ile His Ser Pro Glu Val Ala Glu Ser Gly Cys Lys
    450                 455                 460

Met Leu Asn His Leu Phe Glu Gly Ser Asn Thr Ser Leu Asp Ile Met
465                 470                 475                 480

Ala Ala Val Val Pro Lys Ile Leu Thr Val Met Lys Arg His Glu Thr
                485                 490                 495

Ser Leu Pro Val Gln Leu Glu Ala Leu Arg Ala Ile Leu His Phe Ile
            500                 505                 510

Val Pro Gly Met Pro Glu Glu Ser Arg Glu Asp Thr Glu Phe His His
        515                 520                 525

Lys Leu Asn Met Val Lys Lys Gln Cys Phe Lys Asn Asp Ile His Lys
    530                 535                 540

Leu Val Leu Ala Ala Leu Asn Arg Phe Ile Gly Asn Pro Gly Ile Gln
545                 550                 555                 560

Lys Cys Gly Leu Lys Val Ile Ser Ser Ile Val His Phe Pro Asp Ala
                565                 570                 575

Leu Glu Met Leu Ser Leu Glu Gly Ala Met Asp Ser Val Leu His Thr
            580                 585                 590

Leu Gln Met Tyr Pro Asp Asp Gln Glu Ile Gln Cys Leu Gly Leu Ser
        595                 600                 605
```

```
Leu Ile Gly Tyr Leu Ile Thr Lys Lys Asn Val Phe Ile Gly Thr Gly
    610                 615                 620

His Leu Leu Ala Lys Ile Leu Val Ser Ser Leu Tyr Arg Phe Lys Asp
625                 630                 635                 640

Val Ala Glu Ile Gln Thr Lys Gly Phe Gln Thr Ile Leu Ala Ile Leu
                645                 650                 655

Lys Leu Ser Ala Ser Phe Ser Lys Leu Leu Val His His Ser Phe Asp
            660                 665                 670

Leu Val Ile Phe His Gln Met Ser Ser Asn Ile Met Glu Gln Lys Asp
        675                 680                 685

Gln Gln Phe Leu Asn Leu Cys Cys Lys Cys Phe Ala Lys Val Ala Met
    690                 695                 700

Asp Asp Tyr Leu Lys Asn Val Met Leu Glu Arg Ala Cys Asp Gln Asn
705                 710                 715                 720

Asn Ser Ile Met Val Glu Cys Leu Leu Leu Leu Gly Ala Asp Ala Asn
                725                 730                 735

Gln Ala Lys Glu Gly Ser Ser Leu Ile Cys Gln Val Cys Glu Lys Glu
            740                 745                 750

Ser Ser Pro Lys Leu Val Glu Leu Leu Leu Asn Ser Gly Ser Arg Glu
        755                 760                 765

Gln Asp Val Arg Lys Ala Leu Thr Ile Ser Ile Gly Lys Gly Asp Ser
    770                 775                 780

Gln Ile Ile Ser Leu Leu Leu Arg Arg Leu Ala Leu Asp Val Ala Asn
785                 790                 795                 800

Asn Ser Ile Cys Leu Gly Gly Phe Cys Ile Gly Lys Val Glu Pro Ser
                805                 810                 815

Trp Leu Gly Pro Leu Phe Pro Asp Lys Thr Ser Asn Leu Arg Lys Gln
            820                 825                 830

Thr Asn Ile Ala Ser Thr Leu Ala Arg Met Val Ile Arg Tyr Gln Met
        835                 840                 845

Lys Ser Ala Val Glu Glu Gly Thr Ala Ser Gly Ser Asp Gly Asn Phe
    850                 855                 860
```

```
Ser Glu Asp Val Leu Ser Lys Phe Asp Glu Trp Thr Phe Ile Pro Asp
865                 870                 875                 880

Ser Ser Met Asp Ser Val Phe Ala Gln Ser Asp Asp Leu Asp Ser Glu
                885                 890                 895

Gly Ser Glu Gly Ser Phe Leu Val Lys Lys Lys Ser Asn Ser Ile Ser
            900                 905                 910

Val Gly Glu Phe Tyr Arg Asp Ala Val Leu Gln Arg Cys Ser Pro Asn
        915                 920                 925

Leu Gln Arg His Ser Asn Ser Leu Gly Pro Ile Phe Asp His Glu Asp
    930                 935                 940

Leu Leu Lys Arg Lys Arg Lys Ile Leu Ser Ser Asp Asp Ser Leu Arg
945                 950                 955                 960

Ser Ser Lys Leu Gln Ser His Met Arg His Ser Asp Ser Ile Ser Ser
                965                 970                 975

Leu Ala Ser Glu Arg Glu Tyr Ile Thr Ser Leu Asp Leu Ser Ala Asn
            980                 985                 990

Glu Leu Arg Asp Ile Asp Ala Leu  Ser Gln Lys Cys Cys  Ile Ser Val
        995                 1000                1005

His Leu  Glu His Leu Glu Lys  Leu Glu Leu His Gln  Asn Ala Leu
    1010                1015                1020

Thr Ser  Phe Pro Gln Gln Leu  Cys Glu Thr Leu Lys  Ser Leu Thr
    1025                1030                1035

His Leu  Asp Leu His Ser Asn  Lys Phe Thr Ser Phe  Pro Ser Tyr
    1040                1045                1050

Leu Leu  Lys Met Ser Cys Ile  Ala Asn Leu Asp Val  Ser Arg Asn
    1055                1060                1065

Asp Ile  Gly Pro Ser Val Val  Leu Asp Pro Thr Val  Lys Cys Pro
    1070                1075                1080

Thr Leu  Lys Gln Phe Asn Leu  Ser Tyr Asn Gln Leu  Ser Phe Val
    1085                1090                1095

Pro Glu  Asn Leu Thr Asp Val  Val Glu Lys Leu Glu  Gln Leu Ile
```

1100 1105 1110

Leu Glu Gly Asn Lys Ile Ser Gly Ile Cys Ser Pro Leu Arg Leu
1115 1120 1125

Lys Glu Leu Lys Ile Leu Asn Leu Ser Lys Asn His Ile Ser Ser
1130 1135 1140

Leu Ser Glu Asn Phe Leu Glu Ala Cys Pro Lys Val Glu Ser Phe
1145 1150 1155

Ser Ala Arg Met Asn Phe Leu Ala Ala Met Pro Phe Leu Pro Pro
1160 1165 1170

Ser Met Thr Ile Leu Lys Leu Ser Gln Asn Lys Phe Ser Cys Ile
1175 1180 1185

Pro Glu Ala Ile Leu Asn Leu Pro His Leu Arg Ser Leu Asp Met
1190 1195 1200

Ser Ser Asn Asp Ile Gln Tyr Leu Pro Gly Pro Ala His Trp Lys
1205 1210 1215

Ser Leu Asn Leu Arg Glu Leu Leu Phe Ser His Asn Gln Ile Ser
1220 1225 1230

Ile Leu Asp Leu Ser Glu Lys Ala Tyr Leu Trp Ser Arg Val Glu
1235 1240 1245

Lys Leu His Leu Ser His Asn Lys Leu Lys Glu Ile Pro Pro Glu
1250 1255 1260

Ile Gly Cys Leu Glu Asn Leu Thr Ser Leu Asp Val Ser Tyr Asn
1265 1270 1275

Leu Glu Leu Arg Ser Phe Pro Asn Glu Met Gly Lys Leu Ser Lys
1280 1285 1290

Ile Trp Asp Leu Pro Leu Asp Glu Leu His Leu Asn Phe Asp Phe
1295 1300 1305

Lys His Ile Gly Cys Lys Ala Lys Asp Ile Ile Arg Phe Leu Gln
1310 1315 1320

Gln Arg Leu Lys Lys Ala Val Pro Tyr Asn Arg Met Lys Leu Met
1325 1330 1335

```
Ile Val  Gly Asn Thr Gly Ser  Gly Lys Thr Thr Leu  Leu Gln Gln
    1340                 1345                 1350

Leu Met  Lys Thr Lys Lys Ser  Asp Leu Gly Met Gln  Ser Ala Thr
    1355                 1360                 1365

Val Gly  Ile Asp Val Lys Asp  Trp Pro Ile Gln Ile  Arg Asp Lys
    1370                 1375                 1380

Arg Lys  Arg Asp Leu Val Leu  Asn Val Trp Asp Phe  Ala Gly Arg
    1385                 1390                 1395

Glu Glu  Phe Tyr Ser Thr His  Pro His Phe Met Thr  Gln Arg Ala
    1400                 1405                 1410

Leu Tyr  Leu Ala Val Tyr Asp  Leu Ser Lys Gly Gln  Ala Glu Val
    1415                 1420                 1425

Asp Ala  Met Lys Pro Trp Leu  Phe Asn Ile Lys Ala  Arg Ala Ser
    1430                 1435                 1440

Ser Ser  Pro Val Ile Leu Val  Gly Thr His Leu Asp  Val Ser Asp
    1445                 1450                 1455

Glu Lys  Gln Arg Lys Ala Cys  Met Ser Lys Ile Thr  Lys Glu Leu
    1460                 1465                 1470

Leu Asn  Lys Arg Gly Phe Pro  Ala Ile Arg Asp Tyr  His Phe Val
    1475                 1480                 1485

Asn Ala  Thr Glu Glu Ser Asp  Ala Leu Ala Lys Leu  Arg Lys Thr
    1490                 1495                 1500

Ile Ile  Asn Glu Ser Leu Asn  Phe Lys Ile Arg Asp  Gln Leu Val
    1505                 1510                 1515

Val Gly  Gln Leu Ile Pro Asp  Cys Tyr Val Glu Leu  Glu Lys Ile
    1520                 1525                 1530

Ile Leu  Ser Glu Arg Lys Asn  Val Pro Ile Glu Phe  Pro Val Ile
    1535                 1540                 1545

Asp Arg  Lys Arg Leu Leu Gln  Leu Val Arg Glu Asn  Gln Leu Gln
    1550                 1555                 1560

Leu Asp  Glu Asn Glu Leu Pro  His Ala Val His Phe  Leu Asn Glu
    1565                 1570                 1575
```

```
Ser Gly  Val Leu Leu His Phe  Gln Asp Pro Ala Leu  Gln Leu Ser
    1580                 1585                 1590

Asp Leu  Tyr Phe Val Glu Pro  Lys Trp Leu Cys Lys  Ile Met Ala
    1595                 1600                 1605

Gln Ile  Leu Thr Val Lys Val  Glu Gly Cys Pro Lys  His Pro Lys
    1610                 1615                 1620

Gly Ile  Ile Ser Arg Arg Asp  Val Glu Lys Phe Leu  Ser Lys Lys
    1625                 1630                 1635

Arg Lys  Phe Pro Lys Asn Tyr  Met Ser Gln Tyr Phe  Lys Leu Leu
    1640                 1645                 1650

Glu Lys  Phe Gln Ile Ala Leu  Pro Ile Gly Glu Glu  Tyr Leu Leu
    1655                 1660                 1665

Val Pro  Ser Ser Leu Ser Asp  His Arg Pro Val Ile  Glu Leu Pro
    1670                 1675                 1680

His Cys  Glu Asn Ser Glu Ile  Ile Ile Arg Leu Tyr  Glu Met Pro
    1685                 1690                 1695

Tyr Phe  Pro Met Gly Phe Trp  Ser Arg Leu Ile Asn  Arg Leu Leu
    1700                 1705                 1710

Glu Ile  Ser Pro Tyr Met Leu  Ser Gly Arg Glu Arg  Ala Leu Arg
    1715                 1720                 1725

Pro Asn  Arg Met Tyr Trp Arg  Gln Gly Ile Tyr Leu  Asn Trp Ser
    1730                 1735                 1740

Pro Glu  Ala Tyr Cys Leu Val  Gly Ser Glu Val Leu  Asp Asn His
    1745                 1750                 1755

Pro Glu  Ser Phe Leu Lys Ile  Thr Val Pro Ser Cys  Arg Lys Gly
    1760                 1765                 1770

Cys Ile  Leu Leu Gly Gln Val  Val Asp His Ile Asp  Ser Leu Met
    1775                 1780                 1785

Glu Glu  Trp Phe Pro Gly Leu  Leu Glu Ile Asp Ile  Cys Gly Glu
    1790                 1795                 1800

Gly Glu  Thr Leu Leu Lys Lys  Trp Ala Leu Tyr Ser  Phe Asn Asp
    1805                 1810                 1815
```

Gly Glu Glu His Gln Lys Ile Leu Leu Asp Asp Leu Met Lys Lys
1820 1825 1830

Ala Glu Glu Gly Asp Leu Leu Val Asn Pro Asp Gln Pro Arg Leu
1835 1840 1845

Thr Ile Pro Ile Ser Gln Ile Ala Pro Asp Leu Ile Leu Ala Asp
1850 1855 1860

Leu Pro Arg Asn Ile Met Leu Asn Asn Asp Glu Leu Glu Phe Glu
1865 1870 1875

Gln Ala Pro Glu Phe Leu Leu Gly Asp Gly Ser Phe Gly Ser Val
1880 1885 1890

Tyr Arg Ala Ala Tyr Glu Gly Glu Glu Val Ala Val Lys Ile Phe
1895 1900 1905

Asn Lys His Thr Ser Leu Arg Leu Leu Arg Gln Glu Leu Val Val
1910 1915 1920

Leu Cys His Leu His His Pro Ser Leu Ile Ser Leu Leu Ala Ala
1925 1930 1935

Gly Ile Arg Pro Arg Met Leu Val Met Glu Leu Ala Ser Lys Gly
1940 1945 1950

Ser Leu Asp Arg Leu Leu Gln Gln Asp Lys Ala Ser Leu Thr Arg
1955 1960 1965

Thr Leu Gln His Arg Ile Ala Leu His Val Ala Asp Gly Leu Arg
1970 1975 1980

Tyr Leu His Ser Ala Met Ile Ile Tyr Arg Asp Leu Lys Pro His
1985 1990 1995

Asn Val Leu Leu Phe Thr Leu Tyr Pro Asn Ala Ala Ile Ile Ala
2000 2005 2010

Lys Ile Ala Asp Tyr Gly Ile Ala Gln Tyr Cys Cys Arg Met Gly
2015 2020 2025

Ile Lys Thr Ser Glu Gly Thr Pro Gly Phe Arg Ala Pro Glu Val
2030 2035 2040

Ala Arg Gly Asn Val Ile Tyr Asn Gln Gln Ala Asp Val Tyr Ser

2045 2050 2055

Phe Gly Leu Leu Leu Tyr Asp Ile Leu Thr Thr Gly Gly Arg Ile
2060 2065 2070

Val Glu Gly Leu Lys Phe Pro Asn Glu Phe Asp Glu Leu Glu Ile
2075 2080 2085

Gln Gly Lys Leu Pro Asp Pro Val Lys Glu Tyr Gly Cys Ala Pro
2090 2095 2100

Trp Pro Met Val Glu Lys Leu Ile Lys Gln Cys Leu Lys Glu Asn
2105 2110 2115

Pro Gln Glu Arg Pro Thr Ser Ala Gln Val Phe Asp Ile Leu Asn
2120 2125 2130

Ser Ala Glu Leu Val Cys Leu Thr Arg Arg Ile Leu Leu Pro Lys
2135 2140 2145

Asn Val Ile Val Glu Cys Met Val Ala Thr His His Asn Ser Arg
2150 2155 2160

Asn Ala Ser Ile Trp Leu Gly Cys Gly His Thr Asp Arg Gly Gln
2165 2170 2175

Leu Ser Phe Leu Asp Leu Asn Thr Glu Gly Tyr Thr Ser Glu Glu
2180 2185 2190

Val Ala Asp Ser Arg Ile Leu Cys Leu Ala Leu Val His Leu Pro
2195 2200 2205

Val Glu Lys Glu Ser Trp Ile Val Ser Gly Thr Gln Ser Gly Thr
2210 2215 2220

Leu Leu Val Ile Asn Thr Glu Asp Gly Lys Lys Arg His Thr Leu
2225 2230 2235

Glu Lys Met Thr Asp Ser Val Thr Cys Leu Tyr Cys Asn Ser Phe
2240 2245 2250

Ser Lys Gln Ser Lys Gln Lys Asn Phe Leu Leu Val Gly Thr Ala
2255 2260 2265

Asp Gly Lys Leu Ala Ile Phe Glu Asp Lys Thr Val Lys Leu Lys
2270 2275 2280

```
Gly Ala  Ala Pro Leu Lys Ile  Leu Asn Ile Gly Asn  Val Ser Thr
    2285                 2290                 2295

Pro Leu  Met Cys Leu Ser Glu  Ser Thr Asn Ser Thr  Glu Arg Asn
    2300                 2305                 2310

Val Met  Trp Gly Gly Cys Gly  Thr Lys Ile Phe Ser  Phe Ser Asn
    2315                 2320                 2325

Asp Phe  Thr Ile Gln Lys Leu  Ile Glu Thr Arg Thr  Ser Gln Leu
    2330                 2335                 2340

Phe Ser  Tyr Ala Ala Phe Ser  Asp Ser Asn Ile Ile  Thr Val Val
    2345                 2350                 2355

Val Asp  Thr Ala Leu Tyr Ile  Ala Lys Gln Asn Ser  Pro Val Val
    2360                 2365                 2370

Glu Val  Trp Asp Lys Lys Thr  Glu Lys Leu Cys Gly  Leu Ile Asp
    2375                 2380                 2385

Cys Val  His Phe Leu Arg Glu  Val Met Val Lys Glu  Asn Lys Glu
    2390                 2395                 2400

Ser Lys  His Lys Met Ser Tyr  Ser Gly Arg Val Lys  Thr Leu Cys
    2405                 2410                 2415

Leu Gln  Lys Asn Thr Ala Leu  Trp Ile Gly Thr Gly  Gly Gly His
    2420                 2425                 2430

Ile Leu  Leu Leu Asp Leu Ser  Thr Arg Arg Leu Ile  Arg Val Ile
    2435                 2440                 2445

Tyr Asn  Phe Cys Asn Ser Val  Arg Val Met Met Thr  Ala Gln Leu
    2450                 2455                 2460

Gly Ser  Leu Lys Asn Val Met  Leu Val Leu Gly Tyr  Asn Arg Lys
    2465                 2470                 2475

Asn Thr  Glu Gly Thr Gln Lys  Gln Lys Glu Ile Gln  Ser Cys Leu
    2480                 2485                 2490

Thr Val  Trp Asp Ile Asn Leu  Pro His Glu Val Gln  Asn Leu Glu
    2495                 2500                 2505

Lys His  Ile Glu Val Arg Lys  Glu Leu Ala Glu Lys  Met Arg Arg
    2510                 2515                 2520
```

Thr Ser Val Glu
2525

Claims

1. Use of at least a mammal kinases inhibitor to improve in *vitro* plant embryogenesis induction.

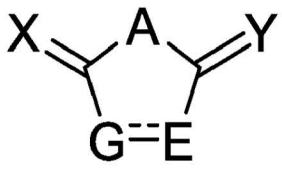
2. Use according to claim 1 wherein the mammal kinases are human kinases, preferably the kinases GSK3β and/or LRRK2.

3. Use according to any of claims 1 to 2 wherein the kinase GSK3β comprises the sequence selected from the list consisting of SEQ ID NO: 1 to 4, and the kinase LRRK2 comprises the sequence selected from the list consisting of SEQ ID NO: 5 to 7.

4. Use according to any of claims 1 to 3 wherein the embryogenesis is somatic and/or by microspores.

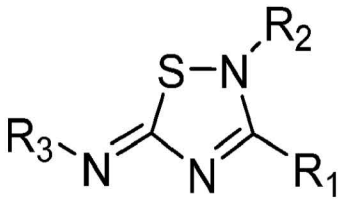
5. Use according to any of claims 1 to 4 wherein the plants are crops plants, preferably *Brassica spp.* and/or *Hordeum spp,* or wherein the plants are forest plants, preferably *Quercus spp.*

6. Use according to any of claims 1 to 5, wherein the mammal kinases inhibitor is selected from a compound of Formula (I) or a salt thereof:



Formula (I)

wherein:
A is $-C(R^1)_2-$, -O- or $-NR^1-$; E is $-NR^1-$ or $-CR^1R^2-$ and the substituent R^2 is absent if ------ is a second bond between E and G; G is -S-, $-NR^1-$ or $-CR^1R^2-$ and the substituent R^2 is absent if ------ is a second bond between E and G; ------ may be a second bond between E and G where the nature of E and G permits and E with G optionally then forms a fused aryl group; R^1 and R^2 are independently selected from hydrogen, (C_1-C_8)alkyl, cycloakyl, haloalkyl, aryl,$-(Z)_n$-aryl, heteroaryl, $-OR^3$, $-C(O)R^3$, $-C(O)OR^3$, $-(Z)_n-C(O)OR^3-$ and $-S(O)_t-$ or as indicated R^2 can be such that E with G then form a fused aryl group; Z is independently selected from $-C(R^3)(R^4)-$, -C(O)-, -O-, $-C(=NR^3)-$, $-S(O)_t-$ and-$N(R^3)-$; n is zero, one or two; t is zero, one or two; R^3 and R^4 are independently selected from hydrogen, (C_1-C_8)alkyl, aryl and heterocyclic; X and Y are independently selected from =O, =S, $=N(R^3)$ and $=C(R^1)(R^2)$;
a compound of Formula (II) or a salt thereof:



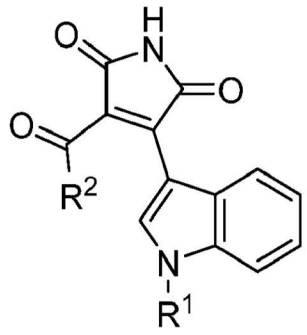
Formula (II),

wherein:

R_1 is selected from H, CN, NO_2, F, Cl, Br, I, or a group X_1-R_1' wherein X_1 is a single bond or a group selected from C_1-C_6 alkylene, C_2-C_6 alkenylene, C_2-C_6 alkynylene, C_3-C_{10} cycloalkylene, C_3-C_{10} heterocycloalkylene,

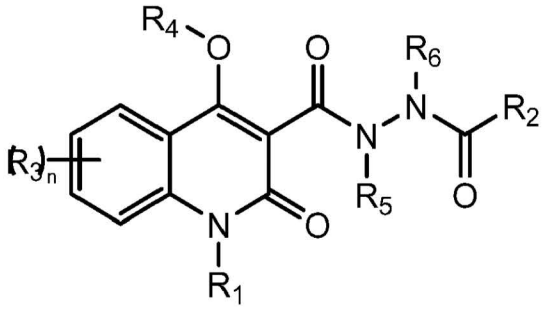
arylene and heteroaryl; being X_1 optionally substituted;
R_1' is selected from H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_7 cycloalkyl, C_1-C_6 alkoxy, aryl, heteroaryl, C_3-C_{10} cycloalkyl or C_3-C_{10} heterocycloalkyl; being R_1' optionally substituted;
R_2 is selected from C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, aryl, heteroaryl, C_3-C_{10} cycloalkyl and C_3-C_{10} heterocycloalkyl, CN or amino; being R_2 optionally substituted;
R_3 is -CH_2- R_3'; R_3' is selected from heteroaryl, -C(O)OR_{12},
or R_3' is selected from -$(CH_2)_n OR_{6e}$, n being between 1 and 20, with the condition, that R_3' cannot be -$(CH_2)_2$-OH, R_{6e} being selected from R_4 and R_5,
or R_3' is selected from -$(CH_2)_n$-(C_3-C_{10}heterocycloalkyl), with n being 0 to 20; and R_{12} is independently selected from H and C_1-C_6 alkyl;
R_4 and R_5 are independently selected from: H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_7 X_4-cycloalkyl, X_4-cyclobutyl, X_4-cyclopentyl, X_4-cyclohexyl, X_4-cycloheptyl, X_4-benzyl, X_4-pyridinyl, X_4-pirimidinyl, X_4-pyperidinyl, X_4-pyrrolidinyl, X_4-pyrrolyl, X_4-imidazolyl and X_4-pyranyl saturated or unsaturated; X_4 is a single bond or a group selected from C_1-C_6 alkylene, C_2-C_6 alkenylene; being R_4 and R_5 optionally substituted;

a compound of Formula (III) or a salt thereof:



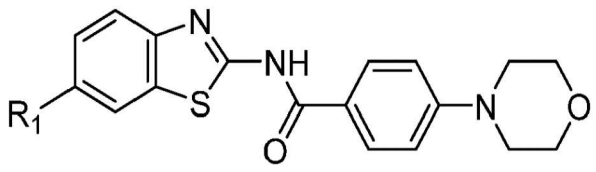
Formula (III)

wherein:
R_1 is selected from H or C_1-C_{10} alkyl and R^2 is selected from C_1-C_{10} alkyl or C_2-C_{10} alkenyl; being optionally substituted by halogen;
a compound of Formula (IV) or a salt thereof:



Formula (IV)

wherein:
R_1 is selected from H and C_1-C_5 alkyl, optionally substituted,, R_2 is C_5-C_{15} alkyl, optionally substituted, R_3 is selected from H, halogen, C_1-C_5 alkyl, optionally substituted, and -(O)- C_1-C_5 alkyl, optionally substituted, n is between 1 and 4, R_4, R_5 y R_6 are each independently selected from H and C_1-C_5 alkyl, optionally substituted;
a compound of Formula (V) or a salt thereof



Formula (V),

wherein:

R_1 is selected from H, C_1-C_6 alkyl, halogen, CF_3, and -O-C_1-C_6.alkyl; and
(E,Z)-3-(morpholinoimino)indolin-2-one or a salt thereof.

7. Use according to any of claims 1 to 6, wherein the mammal kinases inhibitor is selecting from a list consisting of:

4-benzyl-2-methyl-1,2,4-thiadozilidine-3,5-dione (TDZD8),
5-(2-Morpholinethylimino)-2,3-diphenyl-2,5-dihydro-1,2,4-thiadiazole (VP3.15),
3-acetyl-4-(1-methyl-1 H-indol-3-yl)-1 H-pirrol-2,5-dione (VP3.36),
4-hydroxy-1-ethyl-N'-palmitoyl-2-oxo-1,2-dihydroquinoline-3-carbohydrazide (VP0.7),
N-(6-methylbenzothiazole-2-yl)-4-morpholinobenzamide (JZ1.3),
N-(6-fluorobenzothiazole-2-yl)-4-morpholinobenzamide, (JZ1.6),
N-(6-bromobenzothiazole-2-yl)-4-morpholinobenzamide (JZ1.24), and
(*E,Z*)-3-(morpholinoimino)indolin-2-one (IGS4.75).

8. Method to induce *in vitro* plant embryogenesis, comprising:

a. culturing the microspores and/or explants in a culture medium suitable for embryo development; and
b. adding mammal kinase inhibitors to the culture medium of a); and
c. culturing for a period sufficient to obtain embryos.

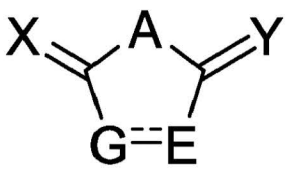
9. Method according to claim 8 wherein the mammal kinases are human kinases, preferably GSK3β and/or LRRK2.

10. Method according to any of claims 8 to 9 wherein the kinase GSK3β comprises the sequence selected from the list consisting of SEQ ID NO: 1 to 4, and the kinase LRRK2 comprises the sequence selected from the list consisting of SEQ ID NO: 5 to 7.

11. Method according to any of claims 8 to 10 wherein the embryogenesis is somatic and/or by microspores.

12. Method according to any of claims 8 to 11 wherein the plants are crops plants, preferably *Brassica spp.* and/or *Hordeum spp,* or wherein the plants are forest plants, preferably *Quercus spp.*

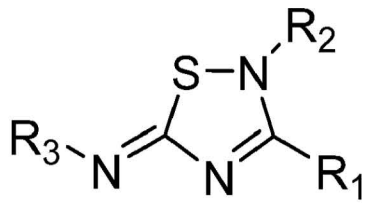
13. Method according to any of claims 8 to 12, wherein the mammal kinases inhibitor is selecting from a compound of Formula (I) or a salt thereof:



Formula (I)

wherein:
A is -C(R^1)$_2$-, -O- or -NR^1-; E is -NR^1- or -CR^1R^2- and the substituent R^2 is absent if ------ is a second bond between E and G; G is -S-, -NR^1- or -CR^1R^2- and the substituent R^2 is absent if ------ is a second bond between E and G;may be a second bond between E and G where the nature of E and G permits and E with G optionally then forms a fused

aryl group; R^1 and R^2 are independently selected from hydrogen, $(C_1\text{-}C_8)$alkyl, cycloakyl, haloalkyl, aryl,-$(Z)_n$-aryl, heteroaryl, -OR^3, -$C(O)R^3$, -$C(O)OR^3$, -$(Z)_n$-$C(O)OR^3$- and -$S(O)_t$- or as indicated R^2 can be such that E with G then form a fused aryl group; Z is independently selected from -$C(R^3)(R^4)$-, -C(O)-, -O-, -$C(=NR^3)$-, -$S(O)_t$- and-$N(R^3)$-; n is zero, one or two; t is zero, one or two; R^3 and R^4 are independently selected from hydrogen, $(C_1\text{-}C_8)$alkyl, aryl and heterocyclic; X and Y are independently selected from =O, =S, $=N(R^3)$ and $=C(R^1)(R^2)$;
a compound of Formula (II) or a salt thereof:

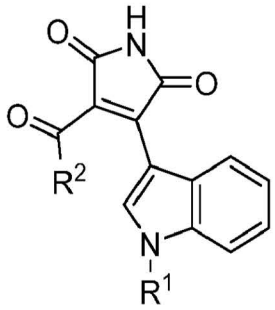


Formula (II)

wherein:

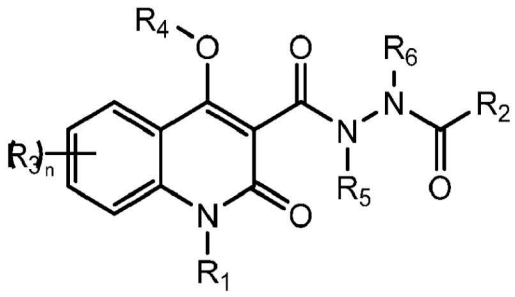
R_1 is selected from H, CN, NO_2, F, Cl, Br, I, or a group X_1-R_1' wherein X_1 is a single bond or a group selected from C_1-C_6 alkylene, C_2-C_6 alkenylene, C_2-C_6 alkynylene, C_3-C_{10} cycloalkylene, C_3-C_{10} heterocycloalkylene, arylene and heteroaryl; being X_1 optionally substituted;
R_1' is selected from H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_7 cycloalkyl, C_1-C_6 alkoxy, aryl, heteroaryl, C_3-C_{10} cycloalkyl or C_3-C_{10} heterocycloalkyl; being R_1' optionally substituted;
R_2 is selected from C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, aryl, heteroaryl, C_3-C_{10} cycloalkyl and C_3-C_{10} heterocycloalkyl, CN or amino; being R_2 optionally substituted;
R_3 is -CH_2- R_3'; R_3' is selected from heteroaryl, -$C(O)OR_{12}$,
or R_3' is selected from -$(CH_2)_nOR_{6e}$, n being between 1 and 20, with the condition, that R_3' cannot be -$(CH_2)_2$-OH, R_{6e} being selected from R_4 and R_5,
or R_3' is selected from -$(CH_2)_n$-(C_3-C_{10}heterocycloalkyl), with n being 0 to 20; and R_{12} is independently selected from H and C_1-C_6 alkyl;
R_4 and R_5 are independently selected from: H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_7 X_4-cycloalkyl, X_4-cyclobutyl, X_4-cyclopentyl, X_4-cyclohexyl, X_4-cycloheptyl, X_4-benzyl, X_4-pyridinyl, X_4-pirimidinyl, X_4-pyperidinyl, X_4-pyrrolidinyl, X_4-pyrrolyl, X_4-imidazolyl and X_4-pyranyl saturated or unsaturated; X_4 is a single bond or a group selected from C_1-C_6 alkylene, C_2-C_6 alkenylene; being R_4 and R_5 optionally substituted;

a compound of Formula (III) or a salt thereof:



Formula (III)

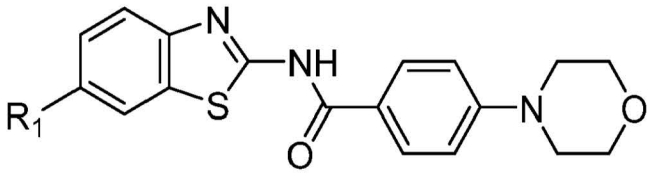
wherein:
R_1 is selected from H or C_1-C_{10} alkyl and R^2 is selected from C_1-C_{10} alkyl or C_2-C_{10} alkenyl; being optionally substituted by halogen;
a compound of Formula (IV) or a salt thereof:



Formula (IV)

wherein:

R_1 is selected from H and C_1-C_5 alkyl, optionally substituted,, R_2 is C_5-C_{15} alkyl, optionally substituted, R_3 is selected from H, halogen, C_1-C_5 alkyl, optionally substituted, and -(O)- C_1-C_5 alkyl, optionally substituted, n is between 1 and 4, R_4, R_5 y R_6 are each independently selected from H and C_1-C_5 alkyl, optionally substituted;
a compound of Formula (V) or a salt thereof:



Formula (V)

wherein:

R_1 is selected from H, C_1-C_6 alkyl, halogen, CF_3, and -O-C_1-C_6.alkyl; and
(E,Z)-3-(morpholinoimino)indolin-2-one or a salt thereof.

14. Method according to any of claims 8 to 13, wherein the mammal kinase inhibitor is selecting from a list consisting of:

4-benzyl-2-methyl-1,2,4-thiadozilidine-3,5-dione (TDZD8),
5-(2-Morpholinethylimino)-2,3-diphenyl-2,5-dihydro-1,2,4-thiadiazole (VP3.15),
3-acetyl-4-(1-methyl-1 H-indol-3-yl)-1 H-pirrol-2,5-dione (VP3.36),
4-hydroxy-1-ethyl-N'-palmitoyl-2-oxo-1,2-dihydroquinoline-3-carbohydrazide (VP0.7),
N-(6-methylbenzothiazole-2-yl)-4-morpholinobenzamide (JZ1.3),
N-(6-fluorobenzothiazole-2-yl)-4-morpholinobenzamide, (JZ1.6),
N-(6-bromobenzothiazole-2-yl)-4-morpholinobenzamide (JZ1.24) and
(E,Z)-3-(morpholinoimino)indolin-2-one (IGS4.75).

15. Method according to any of claims 8 to 14 wherein the mammal kinase inhibitor concentration ranges from 0.5 μM to 100 μM.

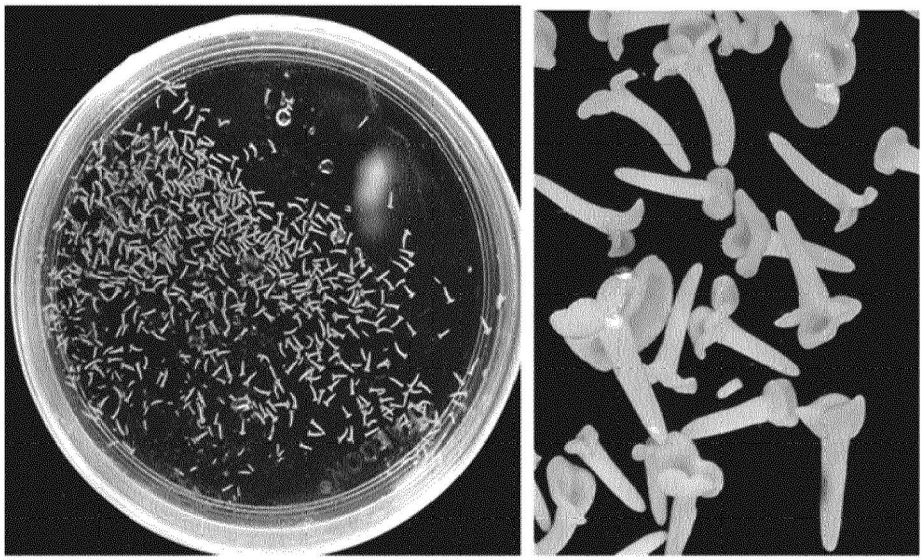


Fig. 1

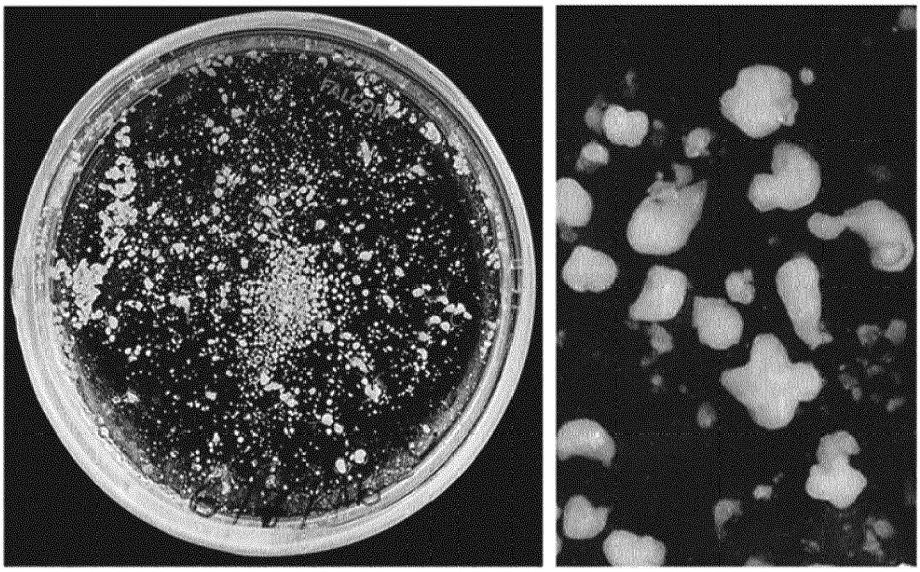


Fig. 2

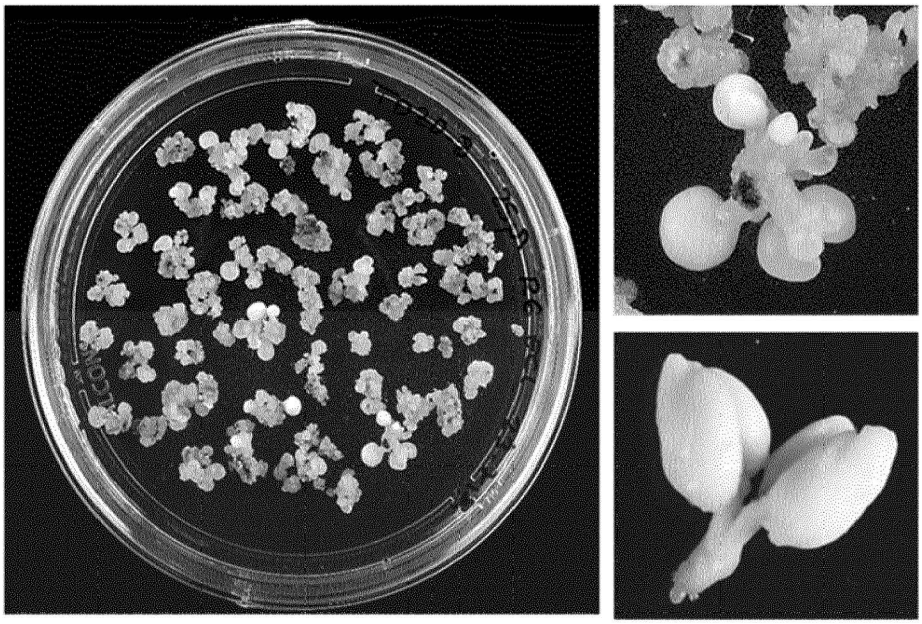


Fig. 3

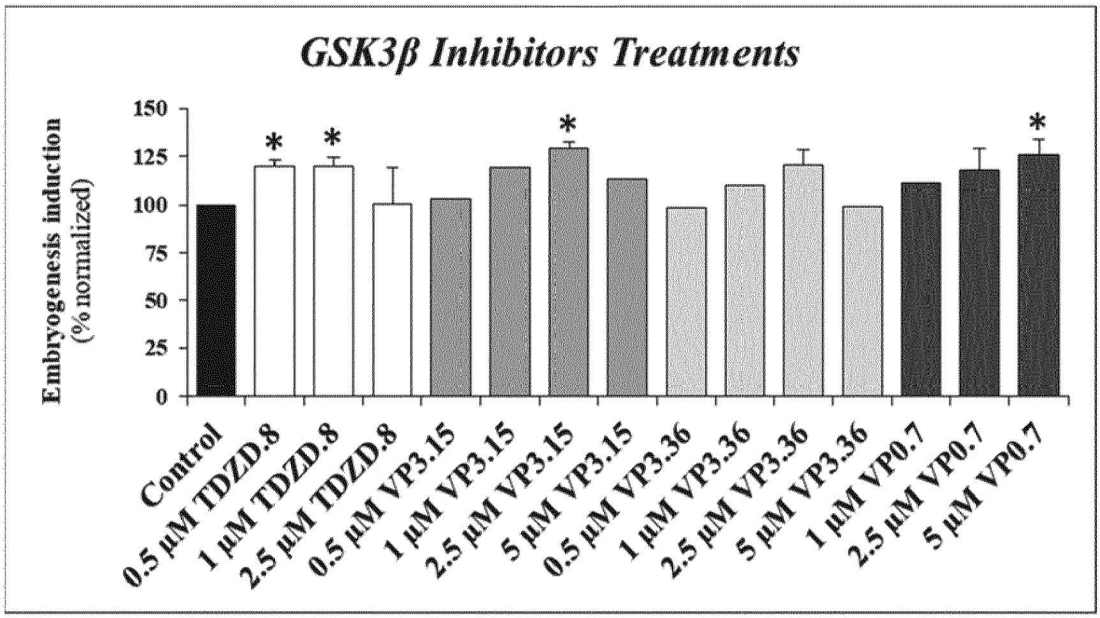

GSK3β Inhibitors Treatments
Embryogenesis induction
(% normalized)
150
125
100
75
50
25
0
Control
0.5 µM TDZD.8
1 µM TDZD.8
2.5 µM TDZD.8
0.5 µM VP3.15
1 µM VP3.15
2.5 µM VP3.15
5 µM VP3.15
0.5 µM VP3.36
1 µM VP3.36
2.5 µM VP3.36
5 µM VP3.36
1 µM VP0.7
2.5 µM VP0.7
5 µM VP0.7

Fig. 4

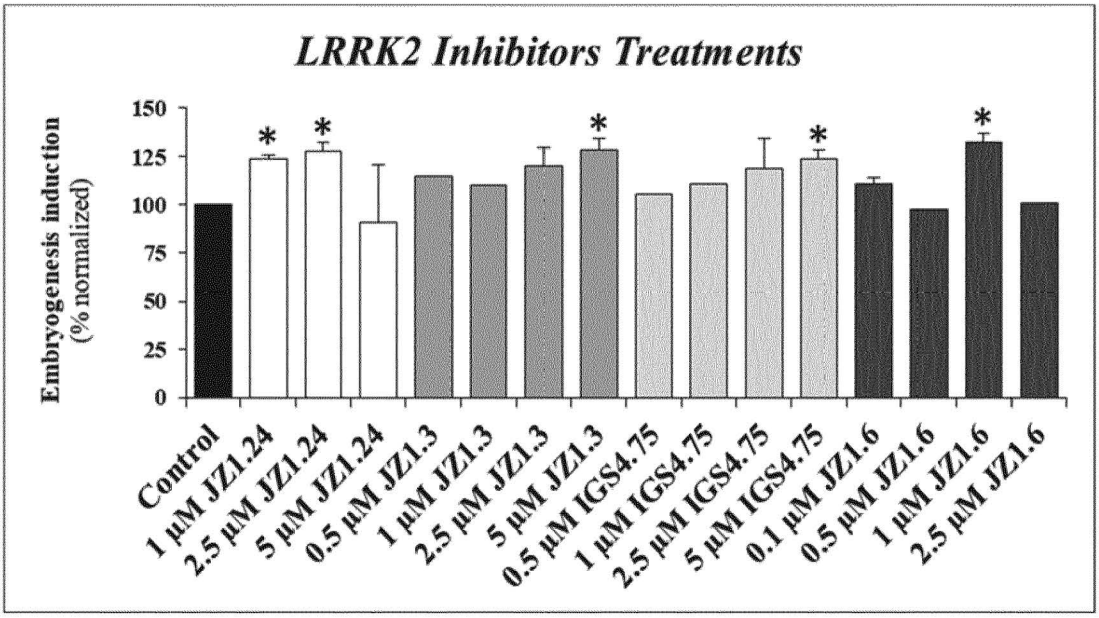

LRRK2 Inhibitors Treatments
Embryogenesis induction
(% normalized)
150
125
100
75
50
25
0
Control
1 µM JZ1.24
2.5 µM JZ1.24
5 µM JZ1.24
0.5 µM JZ1.3
1 µM JZ1.3
2.5 µM JZ1.3
5 µM JZ1.3
0.5 µM IGS4.75
1 µM IGS4.75
2.5 µM IGS4.75
5 µM IGS4.75
0.1 µM JZ1.6
0.5 µM JZ1.6
1 µM JZ1.6
2.5 µM JZ1.6

Fig. 5

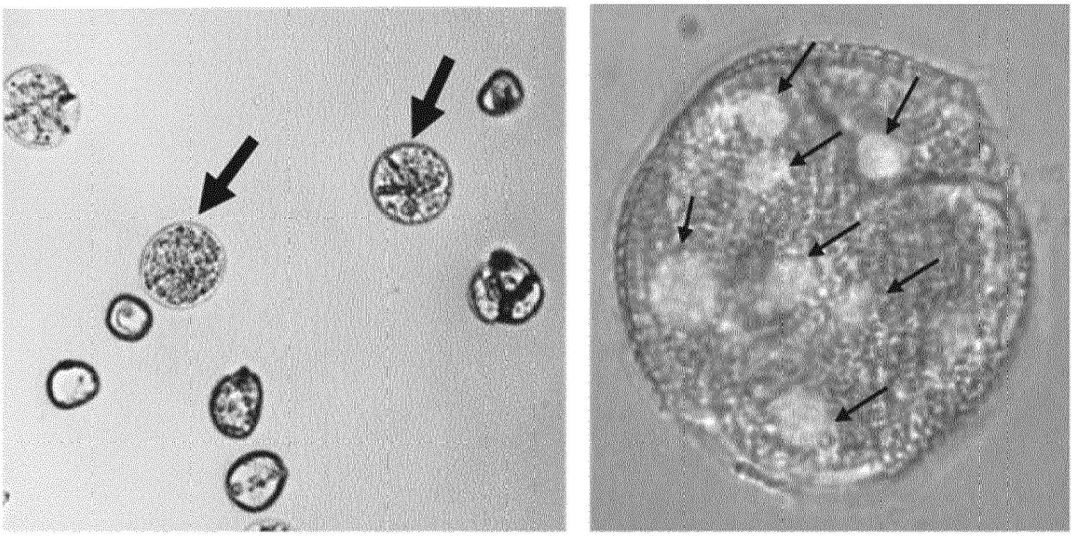


Fig. 6

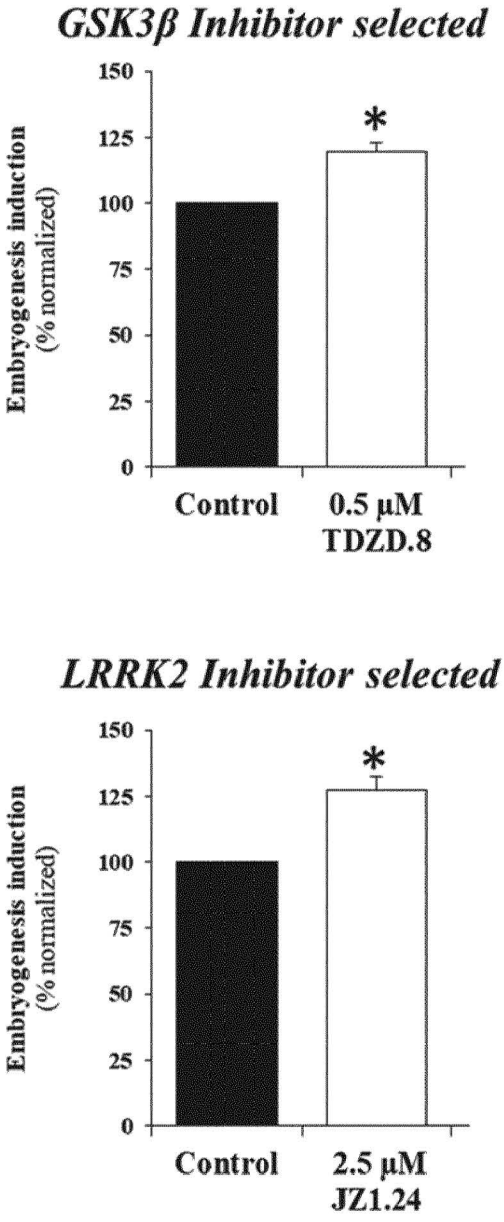

GSK3β Inhibitor selected
Embryogenesis induction
(% normalized)
150
125
100
75
50
25
0
*
Control
0.5 µM
TDZD.8
LRRK2 Inhibitor selected
Embryogenesis induction
(% normalized)
150
125
100
75
50
25
0
*
Control
2.5 µM
JZ1.24

Fig. 7

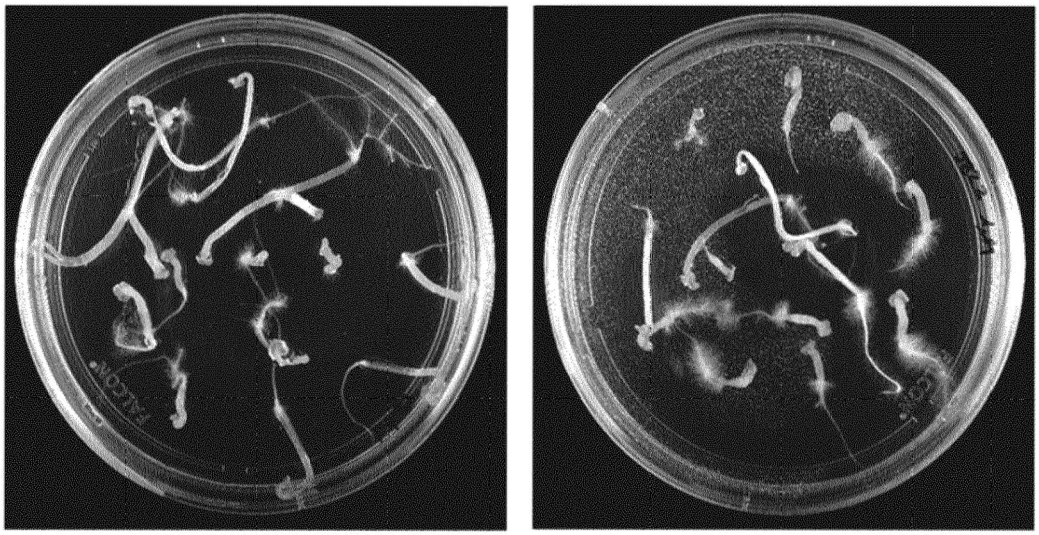
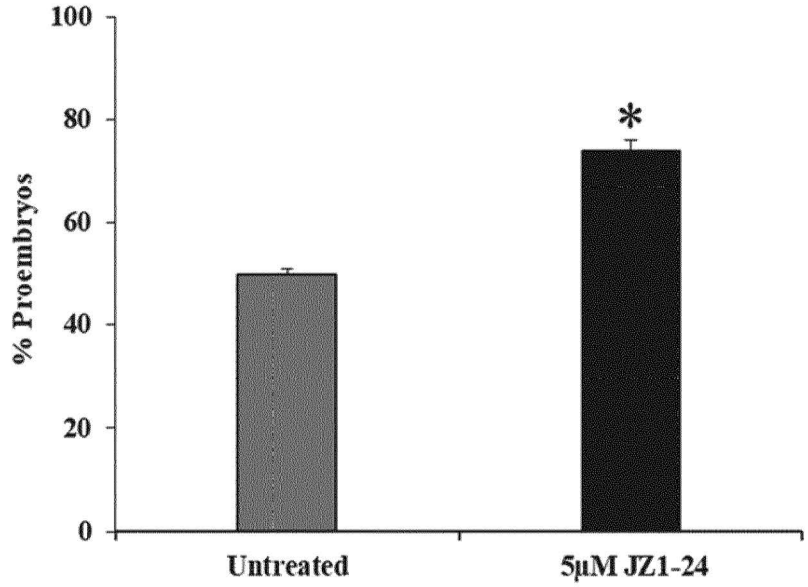


Fig. 8


100
80
60
40
20
0
% Proembryos
*
Untreated
5μM JZ1-24

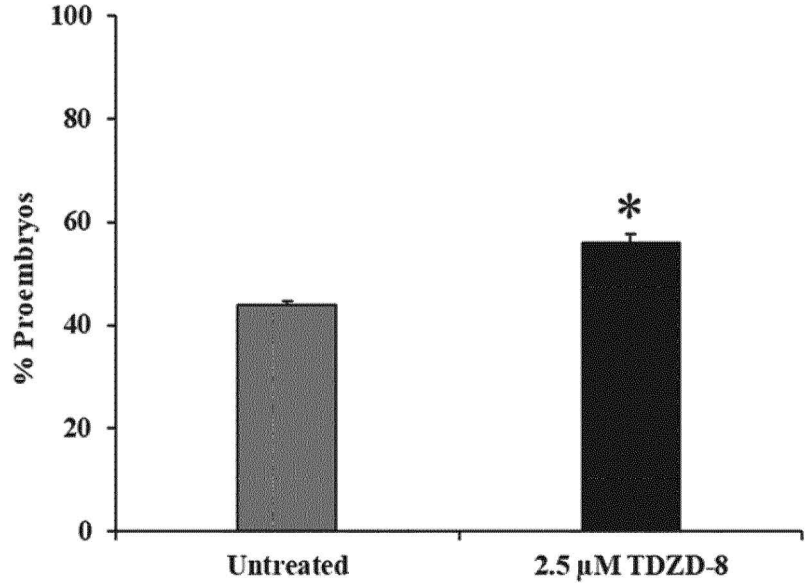

100
80
60
40
20
0
% Proembryos
*
Untreated
2.5 μM TDZD-8

Fig. 9

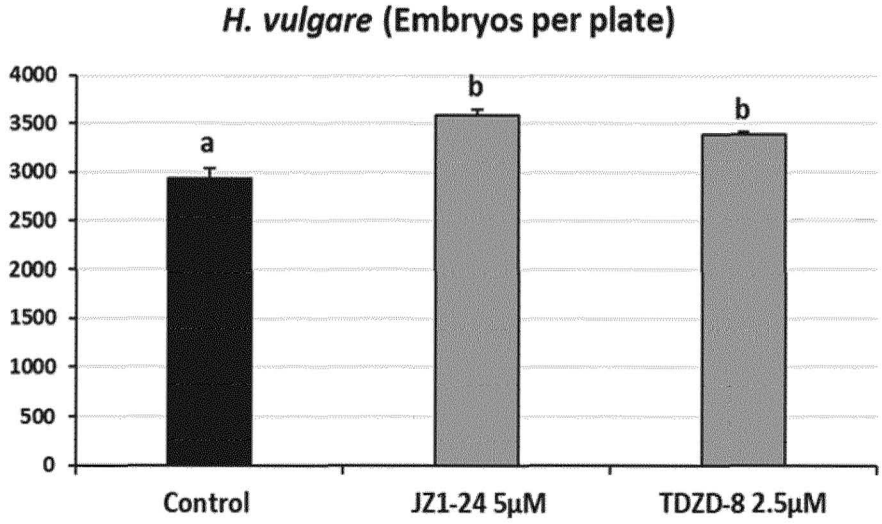

H. vulgare (Embryos per plate)
4000
3500
3000
2500
2000
1500
1000
500
0
a
b
b
Control
JZ1-24 5µM
TDZD-8 2.5µM

Fig. 10

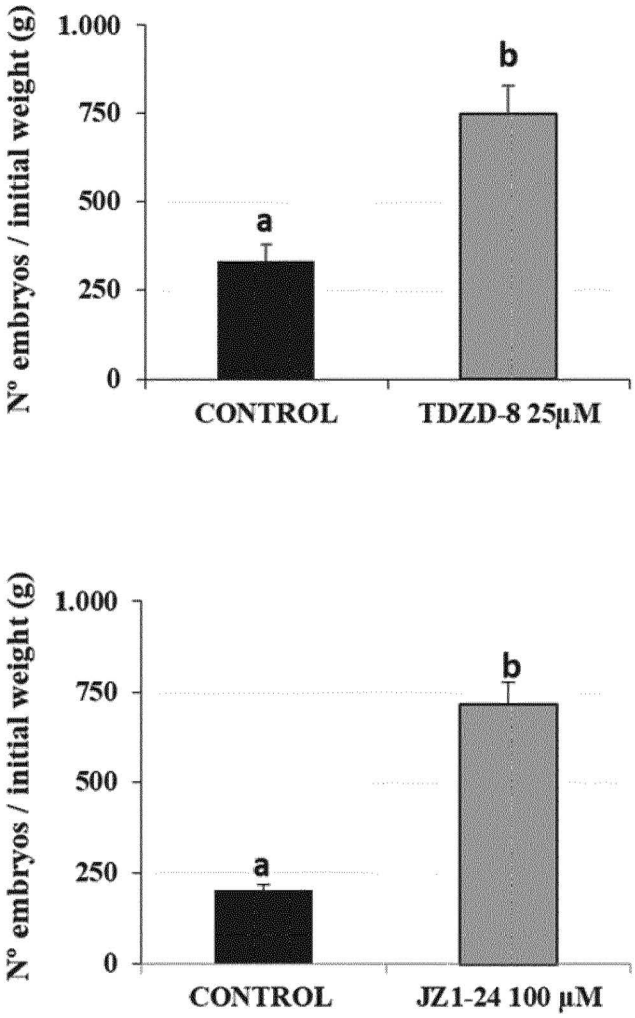

N° embryos / initial weight (g)
1.000
750
500
250
0
a
b
CONTROL
TDZD-8 25µM
N° embryos / initial weight (g)
1.000
750
500
250
0
a
b
CONTROL
JZ1-24 100 µM

Fig. 11

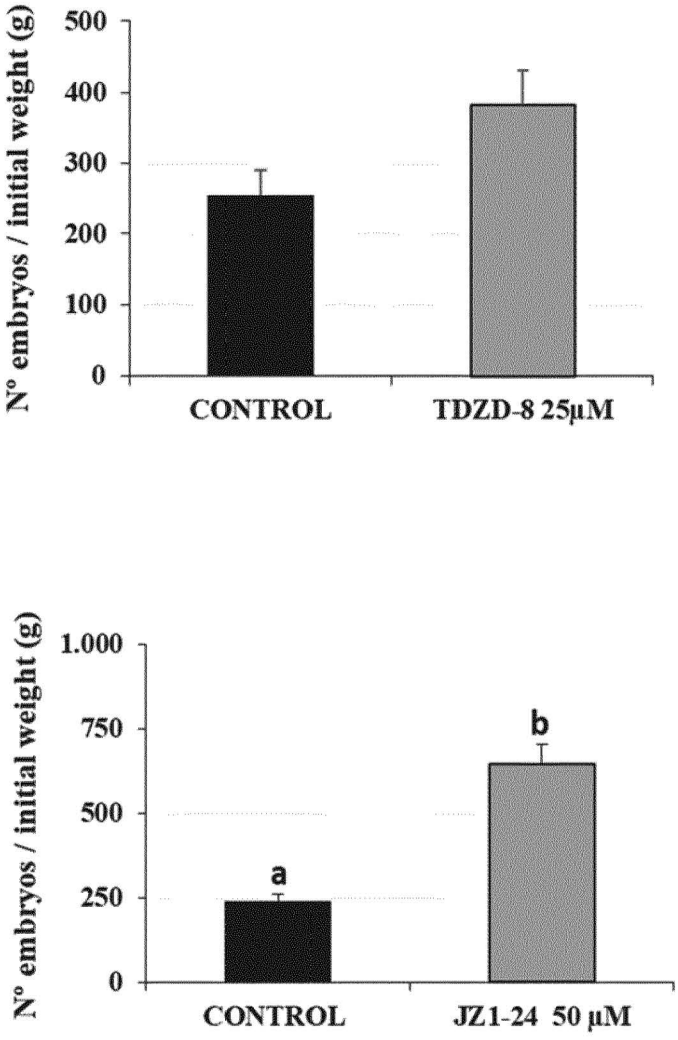

N° embryos / initial weight (g)
500
400
300
200
100
0
CONTROL
TDZD-8 25µM
N° embryos / initial weight (g)
1.000
750
500
250
0
a
b
CONTROL
JZ1-24 50 µM

Fig. 12

Europäisches Patentamt
European Patent Office
Office européen des brevets

EUROPEAN SEARCH REPORT

Application Number
EP 19 38 3042

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	WO 2019/075295 A1 (PIONEER HI BRED INT [US]) 18 April 2019 (2019-04-18) * the whole document *	1-15	INV. A01H4/00
T	WO 2016/016894 A1 (YEDA RES & DEV [IL]) 4 February 2016 (2016-02-04) * the whole document *		
A	WO 03/037072 A2 (UNIV ROCKEFELLER [US]; ZUO JIANRU [US] ET AL.) 8 May 2003 (2003-05-08) * the whole document *	1-15	

TECHNICAL FIELDS SEARCHED (IPC)
A01H

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
Munich	20 May 2020	Keller, Yves

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 19 38 3042

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

20-05-2020

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2019075295 A1	18-04-2019	AU 2018347545 A1	16-04-2020
		WO 2019075295 A1	18-04-2019
WO 2016016894 A1	04-02-2016	CN 108064274 A	22-05-2018
		EP 3194572 A1	26-07-2017
		JP 2017525351 A	07-09-2017
		US 2017275593 A1	28-09-2017
		WO 2016016894 A1	04-02-2016
WO 03037072 A2	08-05-2003	AT 360060 T	15-05-2007
		AT 452966 T	15-01-2010
		CA 2464147 A1	08-05-2003
		CA 2821597 A1	08-05-2003
		DE 60219673 T2	16-08-2007
		EP 1451301 A2	01-09-2004
		EP 1785481 A1	16-05-2007
		ES 2286290 T3	01-12-2007
		MX PA04004003 A	29-10-2004
		US 2003082813 A1	01-05-2003
		US 2005071898 A1	31-03-2005
		US 2010169997 A1	01-07-2010
		US 2011078823 A1	31-03-2011
		US 2011252506 A1	13-10-2011
		US 2012102594 A1	26-04-2012
		US 2013205442 A1	08-08-2013
		WO 03037072 A2	08-05-2003

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- EP 2484670 A1 **[0111]**

Non-patent literature cited in the description

- **TESTILLANO et al.** Plant Cell Culture Protocols. Springer, 2018, 247-256 **[0060] [0088]**
- **PREM et al.** *BMC Plant Biology,* 2012, vol. 12, 127 **[0080] [0093]**
- **RODRIGUEZ-SERRANO et al.** *Journal of Experimental Botany,* 2012, vol. 63 (5), 2007-2024 **[0082]**
- **TESTILLANO et al.** Forestry Sciences. Springer International Publishing, 2018, vol. 84, 93-105 **[0084]**
- **MARTINEZ A et al.** *J Med Chem.,* 2002, vol. 45 (6), 1292-9 **[0110]**
- **PEREZ DI et al.** *J Med Chem.,* 2011, vol. 54 (12), 4042-56 **[0112]**
- **PALOMO V et al.** *J Med Chem.,* vol. 60 (12), 4983-5001 **[0113]**
- **SALADO IG. et al.** *Eur J Med Chem.,* 29 September 2017, vol. 138, 328-342 **[0124]**