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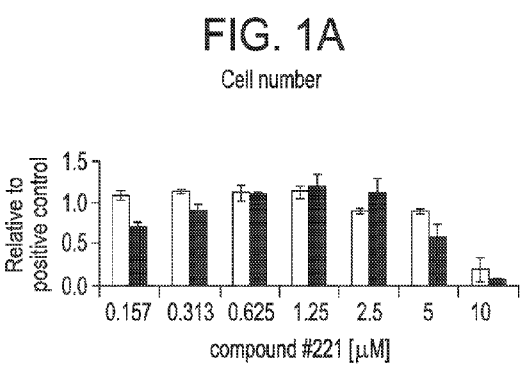
(54) Title: DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS INTO PANCREATIC ENDOCRINE CELLS

FIG. 1A



(57) Abstract: TThe present invention is directed to methods to treat pluripotent cells, whereby the pluripotent cells can be efficiently expanded in culture and differentiated by treating the pluripotent cells with an inhibitor of GSK-3B enzyme activity.

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TREATMENT OF PLURIPOTENT CELLS

CROSS REFERENCE TO RELATED APPLICATION

[0001] The present application claims the benefit of U.S. Provisional Patent Application Serial No. 61/741,776, filed June 14, 2012, which is incorporated herein by reference in its entirety for all purpose.

FIELD OF THE INVENTION

[0002] The present invention is directed to methods to treat pluripotent cells, whereby the pluripotent cells can be efficiently expanded in culture and differentiated by treating the pluripotent cells with an inhibitor of GSK-3B enzyme activity.

BACKGROUND

[0003] Advances in cell-replacement therapy for Type I diabetes mellitus and a shortage of transplantable islets of Langerhans have focused interest on developing sources of insulin-producing cells, or β cells, appropriate for engraftment. One approach is the generation of functional β cells from pluripotent cells, such as, for example, embryonic stem cells.

[0004] In vertebrate embryonic development, a pluripotent cell gives rise to a group of cells comprising three germ layers (ectoderm, mesoderm, and endoderm) in a process known as gastrulation. Tissues such as, for example, thyroid, thymus, pancreas, gut, and liver, will develop from the endoderm, via an intermediate stage. The intermediate stage in this process is the formation of definitive endoderm. Definitive endoderm cells express a number of markers, such as, HNF-3 beta, GATA-4, Mixl1, CXCR4 and SOX-17.

[0005] Formation of the pancreas arises from the differentiation of definitive endoderm into pancreatic endoderm. Cells of the pancreatic endoderm express the pancreatic-duodenal homeobox gene, PDX-1. In the absence of PDX-1, the pancreas fails to develop beyond the formation of ventral and dorsal buds. Thus, PDX-1 expression marks a critical step in pancreatic organogenesis.

The mature pancreas contains, among other cell types, exocrine tissue and endocrine tissue. Exocrine and endocrine tissues arise from the differentiation of pancreatic endoderm.

[0006] The generation of a sufficient amount of cellular material for transplantation requires a source of the cellular material that can be efficiently expanded in culture, and efficiently differentiated into the tissue of interest, for example, functional β cells.

[0007] Current methods to culture human embryonic stem cells are complex; they require the use of exogenous factors, or chemically defined media in order for the cells to proliferate without loosing their pluripotency. Furthermore differentiation of embryonic stem cells often results in a decrease in the cells to expand in culture.

[0008] In one example, Cheon *et al* (BioReprod DOI:10.1095/biolreprod.105.046870, October 19, 2005) disclose a feeder-free, serum-free culture system in which embryonic stem cells are maintained in unconditioned serum replacement (SR) medium supplemented with different growth factors capable of triggering embryonic stem cell self-renewal.

[0009] In another example, US20050233446 discloses a defined media useful in culturing stem cells, including undifferentiated primate primordial stem cells. In solution, the media is substantially isotonic as compared to the stem cells being cultured. In a given culture, the particular medium comprises a base medium and an amount of each of bFGF, insulin, and ascorbic acid necessary to support substantially undifferentiated growth of the primordial stem cells.

[00010] In another example, WO2005086845 discloses a method for maintenance of an undifferentiated stem cell, said method comprising exposing a stem cell to a member of the transforming growth factor-beta (TGFβ) family of proteins, a member of the fibroblast growth factor (FGF) family of proteins, or nicotinamide (NIC) in an amount sufficient to maintain the cell in an undifferentiated state for a sufficient amount of time to achieve a desired result.

[0010] Inhibitors of glycogen synthase kinase-3 (GSK-3) are known to promote proliferation and expansion of adult stem cells. In one example, Tateishi *et al.* (Biochemical and Biophysical Research Communications (2007) 352: 635) show that inhibition of GSK-3 enhances growth and survival of human cardiac stem cells (hCSCs) recovered from the neonatal or adult human heart and having mesenchymal features.

[0011] For example, Rulifson *et al* (PNAS 144, 6247-6252, (2007)) states "Wnt signaling stimulates islet β cell proliferation.

[0012] In another example, WO2007016485 reports that addition of GSK-3 inhibitors to the culture of non-embryonic stem cells, including multipotent adult progenitor cells, leads to the maintenance of a pluripotent phenotype during expansion and results in a more robust differentiation response.

[0013] In another example, US2006030042 uses a method of inhibiting GSK-3, either by addition of Wnt or a small molecule inhibitor of GSK-3 enzyme activity, to maintain embryonic stem cells without the use of a feeder cell layer.

[0014] In another example, WO2006026473 reports the addition of a GSK-3B inhibitor, to stabilize pluripotent cells through transcriptional activation of c-myc and stabilization of c-myc protein.

[0015] In another example, WO2006100490 reports the use of a stem cell culture medium containing a GSK-3 inhibitor and a gp130 agonist to maintain a self-renewing population of pluripotent stem cells, including mouse or human embryonic stem cells.

[0016] In another example, Sato *et al.* (Nature Medicine (2004) 10:55-63) show that inhibition of GSK-3 with a specific pharmacological compound can maintain the undifferentiated phenotype of embryonic stem cells and sustain expression of pluripotent state-specific transcription factors such as Oct-3/4, Rex-1, and Nanog.

[0017] In another example, Maurer *et al* (Journal of Proteome Research (2007) 6:1198-1208) show that adult, neuronal stem cells treated with a GSK-3

inhibitor show enhanced neuronal differentiation, specifically by promoting transcription of β-catenin target genes and decreasing apoptosis.

[0018] In another example, Gregory *et al* (Annals of the New York Academy of Sciences (2005) 1049:97-106) report that inhibitors of GSK-3B enhance *in vitro* osteogenesis.

[0019] In another example, Feng *et al* (Biochemical and Biophysical Research Communcations (2004) 324:1333-1339) show that hematopoietic differentiation from embryonic stem cells is associated with down-regulation of the Wnt/β-catenin pathway, where Wnt is a natural inhibitor of GSK3.

[0020] Therefore, there still remains a significant need to develop methods for treating pluripotent stem cell such that they can be expanded to address the current clinical needs, while retaining the potential to differentiate into pancreatic endocrine cells, pancreatic hormone expressing cells, or pancreatic hormone secreting cells.

SUMMARY

[0021] The present invention provides a method to expand and differentiate pluripotent cells by treating the pluripotent cells with an inhibitor of GSK-3B enzyme activity.

[0022] In one embodiment, the present invention provides a method to expand and differentiate pluripotent cells, comprising the steps of:

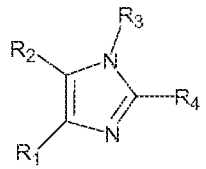
a. Culturing pluripotent cells, and

b. Treating the pluripotent cells with an inhibitor of GSK-3B enzyme activity.

[0023] In one embodiment, the pluripotent cells are differentiated into cells expressing markers characteristic of the definitive endoderm lineage.

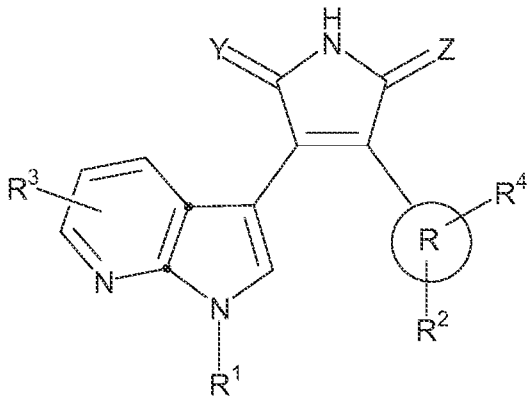
[0024] The pluripotent cells may be human embryonic stem cells, or they may be cells expressing pluripotency markers derived from human embryonic stem cells, according to the methods disclosed in 60/913475.

[0025] In one embodiment, the inhibitor of GSK-3B enzyme activity is a compound of the Formula (I):



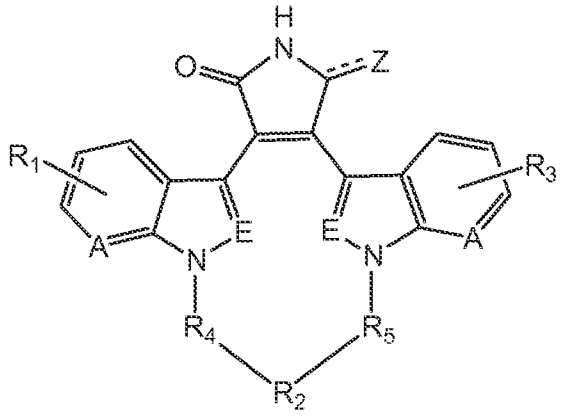
Formula (I)

[0026] In one embodiment, the inhibitor of GSK-3B enzyme activity is a compound of the Formula (II):



Formula (II)

[0027] In one embodiment, the inhibitor of GSK-3B enzyme activity is a compound of the Formula (III):



Formula (III)

BRIEF DESCRIPTION OF THE FIGURES

[0028] **Figure 1** shows the effect of a range of concentrations of the compound #221 on cell number, as determined by the number of nuclei observed (Figure 1A) and Sox-17 expression, as determined by intensity of immunofluorescent staining (Figure 1B). Results were obtained from cells of the human embryonic stem cell line H1 (white bars), or cells of the human embryonic stem cell line H9 (black bars), using the IN Cell Analyzer 1000 (GE Healthcare).

[0029] **Figure 2** shows the effect of a range of concentrations of the compound #206 on cell number, as determined by the number of nuclei observed (Figure 2A) and Sox-17 expression, as determined by intensity of immunofluorescent staining (Figure 2B). Results were obtained from cells of the human embryonic stem cell line H1 (white bars), or cells of the human embryonic stem cell line H9 (black bars), using the IN Cell Analyzer 1000 (GE Healthcare).

[0030] **Figure 3** shows the effect of a range of concentrations of the compound #223 on cell number, as determined by the number of nuclei observed (Figure 3A) and Sox-17 expression, as determined by intensity of immunofluorescent staining (Figure 3B). Results were obtained from cells of the human embryonic stem cell line H1 (white bars), or cells of the human embryonic

stem cell line H9 (black bars), using the IN Cell Analyzer 1000 (GE Healthcare).

[0031] **Figure 4** shows the effect of a range of concentrations of the compound #47 on cell number, as determined by the number of nuclei observed (Figure 4A) and Sox-17 expression, as determined by intensity of immunofluorescent staining (Figure 4B). Results were obtained from cells of the human embryonic stem cell line H1 (white bars), or cells of the human embryonic stem cell line H9 (black bars), using the IN Cell Analyzer 1000 (GE Healthcare).

[0032] **Figure 5** shows the effect of a range of concentrations of the compound #103 on cell number, as determined by the number of nuclei observed (Figure 5A) and Sox-17 expression, as determined by intensity of immunofluorescent staining (Figure 5B). Results were obtained from cells of the human embryonic stem cell line H1 (white bars), or cells of the human embryonic stem cell line H9 (black bars), using the IN Cell Analyzer 1000 (GE Healthcare).

[0033] **Figure 6** shows the effect of a range of concentrations of the compound #133 on cell number, as determined by the number of nuclei observed (Figure 6A) and Sox-17 expression, as determined by intensity of immunofluorescent staining (Figure 6B). Results were obtained from cells of the human embryonic stem cell line H1 (white bars), or cells of the human embryonic stem cell line H9 (black bars), using the IN Cell Analyzer 1000 (GE Healthcare).

[0034] **Figure 7** shows the effect of a range of concentrations of the compound #136 on cell number, as determined by the number of nuclei observed (Figure 7A) and Sox-17 expression, as determined by intensity of immunofluorescent staining (Figure 7B). Results were obtained from cells of the human embryonic stem cell line H1 (white bars), or cells of the human embryonic stem cell line H9 (black bars), using the IN Cell Analyzer 1000 (GE Healthcare).

[0035] **Figure 8** shows the effect of a range of concentrations of the compound #198 on cell number, as determined by the number of nuclei observed (Figure 8A) and Sox-17 expression, as determined by intensity of immunofluorescent staining (Figure 8B). Results were obtained from cells of the human embryonic stem cell line H1 (white bars), or cells of the human embryonic stem cell line H9 (black bars), using the IN Cell Analyzer 1000 (GE Healthcare).

[0036] **Figure 9** shows the expression of CXCR4 on the surface of cells, as determined by immunofluorescent staining and flow cytometric analysis, on cells treated with the compounds shown, according to the methods described in Example 8.

[0037] **Figure 10** shows the expression of CXCR4 (Figure 10A), HNF-3 beta (Figure 10B), and Sox-17 (Figure 10C), as determined by real-time PCR, in cells treated with the compounds shown, according to the methods described in Example 8.

[0038] **Figure 11** shows the effect of a range of concentrations of the compounds shown on cell number, as determined by the number of nuclei observed (Figure 11A) and Pdx-1 expression, as determined by intensity of immunofluorescent staining (Figure 11B), using the IN Cell Analyzer 1000 (GE Healthcare). Cells were treated according to the methods described in Example 9.

[0039] **Figure 12** shows the effect of a range of concentrations of the compounds shown on Pdx-1 expression (white bars) and HNF-6 (black bars), as determined by real-time PCR. Cells were treated according to the methods described in Example 9.

[0040] **Figure 13** shows the effect of a range of concentrations of the compounds shown on cell number, as determined by the number of nuclei observed (Figure 13A) and insulin expression, as determined by intensity of immunofluorescent staining (Figure 13B), using the IN Cell Analyzer 1000 (GE Healthcare). Cells were treated according to the methods described in Example 10.

[0041] **Figure 14** shows effect of a range of concentrations of the compounds shown on Pdx-1 expression (white bars) and insulin (black bars), as determined by real-time PCR. Cells were treated according to the methods described in Example 10.

[0042] **Figure 15** shows the effect of a range of concentrations of the compounds shown on cell number, as determined by the number of nuclei observed (Figure 15A) and insulin expression, as determined by intensity of immunofluorescent staining (Figure 15B), using the IN Cell Analyzer 1000 (GE Healthcare). Cells were treated according to the methods described in Example 11.

DETAILED DESCRIPTION

[0043] For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the following subsections that describe or illustrate certain features, embodiments, or applications of the present invention.

Definitions

[0044] Stem cells are undifferentiated cells defined by their ability at the single cell level to both self-renew and differentiate to produce progeny cells, including self-renewing progenitors, non-renewing progenitors, and terminally differentiated cells. Stem cells are also characterized by their ability to differentiate *in vitro* into functional cells of various cell lineages from multiple germ layers (endoderm, mesoderm and ectoderm), as well as to give rise to tissues of multiple germ layers following transplantation and to contribute substantially to most, if not all, tissues following injection into blastocysts.

[0045] Stem cells are classified by their developmental potential as: (1) totipotent, meaning able to give rise to all embryonic and extraembryonic cell types; (2) pluripotent, meaning able to give rise to all embryonic cell types; (3) multipotent, meaning able to give rise to a subset of cell lineages, but all within a particular tissue, organ, or physiological system (for example, hematopoietic stem cells (HSC) can produce progeny that include HSC (self-

renewal), blood cell restricted oligopotent progenitors and all cell types and elements (e.g., platelets) that are normal components of the blood); (4) oligopotent, meaning able to give rise to a more restricted subset of cell lineages than multipotent stem cells; and (5) unipotent, meaning able to give rise to a single cell lineage (e.g. , spermatogenic stem cells).

[0046] Differentiation is the process by which an unspecialized ("uncommitted") or less specialized cell acquires the features of a specialized cell such as, for example, a nerve cell or a muscle cell. A differentiated or differentiation-induced cell is one that has taken on a more specialized ("committed") position within the lineage of a cell. The term "committed", when applied to the process of differentiation, refers to a cell that has proceeded in the differentiation pathway to a point where, under normal circumstances, it will continue to differentiate into a specific cell type or subset of cell types, and cannot, under normal circumstances, differentiate into a different cell type or revert to a less differentiated cell type. De-differentiation refers to the process by which a cell reverts to a less specialized (or committed) position within the lineage of a cell. As used herein, the lineage of a cell defines the heredity of the cell, i.e., which cells it came from and what cells it can give rise to. The lineage of a cell places the cell within a hereditary scheme of development and differentiation. A lineage-specific marker refers to a characteristic specifically associated with the phenotype of cells of a lineage of interest and can be used to assess the differentiation of an uncommitted cell to the lineage of interest.

[0047] "β-cell lineage" refer to cells with positive gene expression for the transcription factor PDX-1 and at least one of the following transcription factors: NGN-3, Nkx2.2, Nkx6.1, NeuroD, Isl-1, HNF-3 beta, MAFA, Pax4, and Pax6. Cells expressing markers characteristic of the β cell lineage include β cells.

[0048] "Cells expressing markers characteristic of the definitive endoderm lineage" as used herein refer to cells expressing at least one of the following markers: SOX-17, GATA-4, HNF-3 beta, GSC, Cer1, Nodal, FGF8, Brachyury, Mix-like homeobox protein, FGF4 CD48, eomesodermin (EOMES), DKK4, FGF17, GATA-6, CXCR4, C-Kit, CD99, or OTX2. Cells expressing markers

characteristic of the definitive endoderm lineage include primitive streak precursor cells, primitive streak cells, mesendoderm cells and definitive endoderm cells.

[0049] "Cells expressing markers characteristic of the pancreatic endoderm lineage" as used herein refer to cells expressing at least one of the following markers: PDX-1, HNF-1beta, PTF-1 alpha, HNF-6, or HB9. Cells expressing markers characteristic of the pancreatic endoderm lineage include pancreatic endoderm cells.

[0050] "Cells expressing markers characteristic of the pancreatic endocrine lineage" as used herein refer to cells expressing at least one of the following markers: NGN-3, NeuroD, Islet-1, PDX-1, NKX6.1, Pax-4, Ngn-3, or PTF-1 alpha. Cells expressing markers characteristic of the pancreatic endocrine lineage include pancreatic endocrine cells, pancreatic hormone expressing cells, and pancreatic hormone secreting cells, and cells of the β-cell lineage.

[0051] "Definitive endoderm" as used herein refers to cells which bear the characteristics of cells arising from the epiblast during gastrulation and which form the gastrointestinal tract and its derivatives. Definitive endoderm cells express the following markers: HNF-3 beta, GATA-4, SOX-17, Cerberus, OTX2, goosecoid, C-Kit, CD99, and Mix11.

[0052] "Extraembryonic endoderm" as used herein refers to a population of cells expressing at least one of the following markers: SOX-7, AFP, and SPARC.

[0053] "Markers" as used herein, are nucleic acid or polypeptide molecules that are differentially expressed in a cell of interest. In this context, differential expression means an increased level for a positive marker and a decreased level for a negative marker. The detectable level of the marker nucleic acid or polypeptide is sufficiently higher or lower in the cells of interest compared to other cells, such that the cell of interest can be identified and distinguished from other cells using any of a variety of methods known in the art.

[0054] "Mesendoderm cell" as used herein refers to a cell expressing at least one of the following markers: CD48, eomesodermin (EOMES), SOX-17, DKK4, HNF-3 beta, GSC, FGF17, GATA-6.

[0055] "Pancreatic endocrine cell", or "pancreatic hormone expressing cell" as used herein refers to a cell capable of expressing at least one of the following hormones: insulin, glucagon, somatostatin, and pancreatic polypeptide.

[0056] "Pancreatic hormone secreting cell" as used herein refers to a cell capable of secreting at least one of the following hormones: insulin, glucagon, somatostatin, and pancreatic polypeptide.

[0057] "Pre-primitive streak cell" as used herein refers to a cell expressing at least one of the following markers: Nodal, or FGF8

[0058] "Primitive streak cell" as used herein refers to a cell expressing at least one of the following markers: Brachyury, Mix-like homeobox protein, or FGF4.

[0059] In one embodiment, the present invention provides a method for the expansion and differentiation of pluripotent cells comprising treating the pluripotent cells with an inhibitor of GSK-3B enzyme activity.

[0060] In one embodiment, the present invention provides a method to expand and differentiate pluripotent cells, comprising the steps of:

c. Culturing pluripotent cells, and

d. Treating the pluripotent cells with an inhibitor of GSK-3B enzyme activity.

[0061] In one embodiment, the pluripotent cells are differentiated into cells expressing markers characteristic of the definitive endoderm lineage.

[0062] Markers characteristic of the definitive endoderm lineage are selected from the group consisting of SOX17, GATA4, Hnf-3beta, GSC, Cer1, Nodal, FGF8, Brachyury, Mix-like homeobox protein, FGF4 CD48, eomesodermin (EOMES), DKK4, FGF17, GATA6, CXCR4, C-Kit, CD99, and OTX2. Contemplated in the present invention is a cell, derived from a pluripotent cell

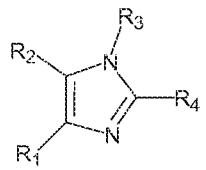
that expresses at least one of the markers characteristic of the definitive endoderm lineage. In one aspect of the present invention, a cell expressing markers characteristic of the definitive endoderm lineage is a primitive streak precursor cell. In an alternate aspect, a cell expressing markers characteristic of the definitive endoderm lineage is a mesendoderm cell. In an alternate aspect, a cell expressing markers characteristic of the definitive endoderm lineage is a definitive endoderm cell.

[0063] The pluripotent cells may be treated with the inhibitor of GSK-3B enzyme activity for about one to about 72 hours. Alternatively, the pluripotent cells may be treated with the inhibitor of GSK-3B enzyme activity for about 12 to about 48 hours. Alternatively, the pluripotent cells may be treated with the inhibitor of GSK-3B enzyme activity for about 48 hours.

[0064] In one embodiment, the inhibitor of GSK-3B enzyme activity is used at a concentration of about 100nM to about 100μM. Alternatively, the inhibitor of GSK-3B enzyme activity is used at a concentration of about 1μM to about 10μM. Alternatively, the inhibitor of GSK-3B enzyme activity is used at a concentration of about 10μM.

Compounds suitable for use in the methods of the present invention

[0065] In one embodiment, the inhibitor of GSK-3B enzyme activity is a compound of the Formula (I):



Formula (I)

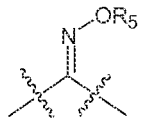
wherein:

[0066] R_1 is phenyl, substituted phenyl wherein the phenyl substituents are selected from the group consisting of C_{1-5}alkyl, halogen, nitro, trifluoromethyl and nitrile, or pyrimidinyl;

[0067] R_2 is phenyl, substituted phenyl wherein the phenyl substituents are selected from the group consisting of C_{1-5}alkyl, halogen, nitro, trifluoromethyl and nitrile, or pyrimidinyl which is optionally C_{1-4}alkyl substituted, and at least one of R_1 and R_2 is pyrimidinyl;

[0068] R_3 is hydrogen, 2-(trimethylsilyl)ethoxymethyl, C_{1-5}alkoxycarbonyl, aryloxycarbonyl, arylC_{1-5}alkyloxycarbonyl, arylC_{1-5}alkyl, substituted arylC_{1-5}alkyl wherein the one or more aryl substituents are independently selected from the group consisting of C_{1-5}alkyl, C_{1-5}alkoxy, halogen, amino, C_{1-5}alkylamino, and diC_{1-5}alkylamino, phthalimidoC_{1-5}alkyl, aminoC_{1-5}alkyl, diaminoC_{1-5}alkyl, succinimidoC_{1-5}alkyl, C_{1-5}alkylcarbonyl, arylcarbonyl, C_{1-5}alkylcarbonylC_{1-5}alkyl and aryloxycarbonylC_{1-5}alkyl;

[0069] R_4 is -(A)-$(CH_2)_q$-X;

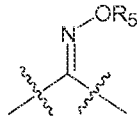
[0070] A is vinylene, ethynylene or 

;

[0071] R_5 is selected from the group consisting of hydrogen, C_{1-5}alkyl, phenyl and phenylC_{1-5}alkyl;

[0072] q is 0-9;

[0073] X is selected from the group consisting of hydrogen, hydroxy, vinyl, substituted vinyl wherein one or more vinyl substituents are each selected from the group consisting of fluorine, bromine, chlorine and iodine, ethynyl, substituted ethynyl wherein the ethynyl substituents are selected from the group consisting of fluorine, bromine chlorine and iodine, C_{1-5}alkyl, substituted C_{1-5}alkyl wherein the one or more alkyl substituents are each selected from the group consisting of C_{1-5}alkoxy, trihaloalkyl, phthalimido and amino, C_{3-7}cycloalkyl, C_{1-5}alkoxy, substituted C_{1-5}alkoxy wherein the alkyl substituents are selected from the group consisting of phthalimido and amino, phthalimidooxy, phenoxy, substituted phenoxy wherein the one or more phenyl substituents are each selected from the group consisting of C_{1-5}alkyl, halogen and C_{1-5}alkoxy, phenyl, substituted phenyl wherein the one

or more phenyl substituents are each selected from the group consisting of C_{1-5}alkyl, halogen and C_{1-5}alkoxy, arylC_{1-5}alkyl, substituted arylC_{1-5}alkyl wherein the one or more aryl substituents are each selected from the group consisting of C_{1-5}alkyl, halogen and C_{1-5}alkoxy, aryloxyC_{1-5}alkylamino, C_{1-5}alkylamino, diC_{1-5}alkylamino, nitrile, oxime, benxyloxyimino, C_{1-5}alkyloxyimino, phthalimido, succinimido, C_{1-5}alkylcarbonyloxy, phenylcarbonyloxy, substituted phenylcarbonyloxy wherein the one or more phenyl substituents are each selected from the group consisting of C_{1-5}alkyl, halogen and C_{1-5}alkoxy, phenylC_{1-5}alkylcarbonyloxy wherein the one or more phenyl substituents are each selected from the group consisting of C_{1-5}alkyl, halogen and C_{1-5}alkoxy, aminocarbonyloxy, C_{1-5}alkylaminocarbonyloxy, diC_{1-5}alkylaminocarbonyloxy, C_{1-5}alkoxycarbonyloxy, substituted C_{1-5}alkoxycarbonyloxy wherein the one or more alkyl substituents are each selected from the group consisting of methyl, ethyl, isopropyl and hexyl, phenoxycarbonyloxy, substituted phenoxycarbonyloxy wherein the one or more phenyl substituents are each selected from the group consisting of C_{1-5}alkyl, C_{1-5}alkoxy and halogen, C_{1-5}alkylthio, substituted C_{1-5}alkylthio wherein the alkyl substituents are selected from the group consisting of hydroxy and phthalimido, C_{1-5}alkylsulfonyl, phenylsulfonyl, substituted phenylsulfonyl wherein the one or more phenyl substituents are each selected from the group consisting of bromine, fluorine, chloride, C_{1-5}alkoxy and trifluoromethyl; with the proviso that if A is 

, q is 0 and X is H, then R_3 may not be 2-(trimethylsilyl)ethoxymethyl; and pharmaceutically acceptable salts thereof.

[0074] An example of the invention includes a compound of Formula (I) wherein R_1 is substituted phenyl and R_2 is pyrimidin-3-yl.

[0075] An example of the invention includes a compound of Formula (I) wherein R_1 is 4-fluorophenyl.

[0076] An example of the invention includes a compound of Formula (I) wherein R_3 is hydrogen, arylC_{1-5}alkyl, or substituted arylC_{1-5}alkyl.

[0077] An example of the invention includes a compound of Formula (I) wherein R_3 is hydrogen or phenylC_{1-5}alkyl.

[0078] An example of the invention includes a compound of Formula (I) wherein A is ethynylene and q is 0-5.

[0079] An example of the invention includes a compound of Formula (I) wherein X is succinimido, hydroxy, methyl, phenyl, C_{1-5}alkylsulfonyl, C_{3-6}cycloalkyl, C_{1-5}alkylcarbonyloxy, C_{1-5}alkoxy, phenylcarbonyloxy, C_{1-5}alkylamino, diC_{1-5}alkylamino or nitrile.

[0080] Compounds of Formula (I) are disclosed in commonly assigned United States Patent Number 6,214,830, the complete disclosure of which is herein incorporated by reference.

[0081] An example of the invention includes a compound of Formula (I) wherein the compound is selected from the group consisting of the compounds listed in Table A, below:

Table A

Compounds of Formula (I)

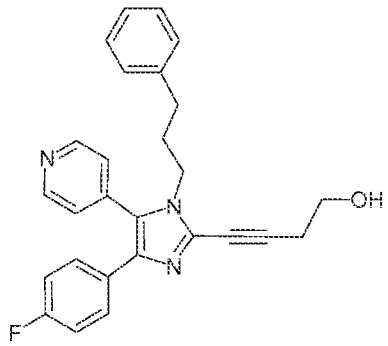
Compound	Name
A-1	4-[5-(4-Fluorophenyl)-1H-imidazol-4-yl]pyridine
A-2	4-[4-(4-Fluorophenyl)-1-(3-phenylpropyl)-1H-imidazol-5-yl]pyridine
A-3	4-[5-(4-Fluorophenyl)-1-(3-phenylpropyl)-1H-imidazol-4-yl]pyridine
A-4	4-[4-(4-Fluorophenyl)-2-iodo-1-(3-phenylpropyl)-1H-imidazol-5-yl]pyridine
A-5	4-[4-(4-Fluorophenyl)-1-(3-phenylpropyl)-5-pyridin-4-yl-1H-imidazol-2-yl]but-3-yn-1-ol
A-6	4-[4-(4-Fluorophenyl)-1-({[2-(trimethylsilyl)ethyl]oxy}methyl)-1H-imidazol-5-yl]pyridine
A-7	4-[5-(4-Fluorophenyl)-1-({[2-(trimethylsilyl)ethyl]oxy}methyl)-1H-imidazol-4-yl]pyridine
A-8	5-(4-fluorophenyl)-2-iodo-4-(4-pyridyl)-1-[2-(trimethylsilyl)ethoxymethyl]-imidazole

Compound	Name
A-9	5-(4-fluorophenyl)-4-(4-pyridyl)-2-(trimethylsilyl)ethinyl-1-[2-(trimethylsilyl)ethoxymethyl]-imidazole
A-10	2-(2-chlorovinyl)-5-(4-fluorophenyl)-4-(4-pyridyl)-imidazole
A-11	5-(4-Fluorophenyl)-4-pyridin-4-yl-1-({[2-(trimethylsilyl)ethyl]oxy}methyl)-1H-imidazole-2-carbaldehyde
A-12	4-[2-(2,2-Dibromoethenyl)-5-(4-fluorophenyl)-1-({[2-(trimethylsilyl)ethyl]oxy}methyl)-1H-imidazol-4-yl]pyridine
A-13	3-[4-(4-Fluorophenyl)-5-pyridin-4-yl-1H-imidazol-2-yl]-1-phenylprop-2-yn-1-ol
A-14	5-(4-Fluorophenyl)-4-pyridin-4-yl-1-{[2-(trimethylsilyl)ethoxy]methyl}-1H-imidazole-2-carbaldehyde oxime
A-15	5-(4-fluorophenyl)-4-(4-pyridyl)-2-imidazole oxime

TABLE A - CONTINUED

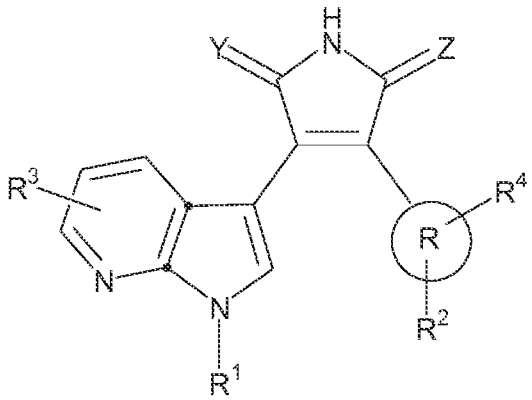
Compound	Name
A-16	4-[2-(5-Chloropent-1-yn-1-yl)-4-(4-fluorophenyl)-1-(3-phenylpropyl)-1H-imidazol-5-yl]pyridine
A-17	4-[4-(4-Fluorophenyl)-1-(3-phenylpropyl)-5-pyridin-4-yl-1H-imidazol-2-yl]but-3-yn-1-yl phenylcarbamate
A-18	4-[2-(4-Chlorobut-1-yn-1-yl)-4-(4-fluorophenyl)-1-(3-phenylpropyl)-1H-imidazol-5-yl]pyridine
A-19	4-[4-(4-Fluorophenyl)-1-(3-phenylpropyl)-5-pyridin-4-yl-1H-imidazol-2-yl]-N,N-dimethylbut-3-yn-1-amine

[0082] An example of the invention includes a compound of Formula (I) wherein the compound is Compound A-5 of the formula:



Compound A-5

[0083] In one embodiment, the inhibitor of GSK-3B enzyme activity is a compound of the Formula (II):



Formula (II)

Wherein:

[0084] R is selected from the group consisting of R_a, -C_{1-8}alkyl-R_a, -C_{2-8}alkenyl-R_a, -C_{2-8}alkynyl-R_a and cyano;

[0085] R_a is selected from the group consisting of cycloalkyl, heterocyclyl, aryl and heteroaryl;

[0086] R^1 is selected from the group consisting of hydrogen, -C_{1-8}alkyl-R^5, -C_{2-8}alkenyl-R^5, -C_{2-8}alkynyl-R^5, -C(O)-(C_{1-8})alkyl-R^9, -C(O)-aryl-R^8, -C(O)-O-(C_{1-8})alkyl-R^9, -C(O)-O-aryl-R^8, -C(O)-NH(C_{1-8}alkyl-R^9),

-C(O)-NH(aryl-R^8), -C(O)-N(C_{1-8}alkyl-R^9)$_2$, -SO_2-(C_{1-8})alkyl-R^9, -SO_2-aryl-R^8, -cycloalkyl-R^6, -heterocyclyl-R^6, -aryl-R^6 and -heteroaryl-R^6; wherein heterocyclyl and heteroaryl are attached to the azaindole nitrogen atom in the one position via a heterocyclyl or heteroaryl ring carbon atom;

[0087] R^5 is 1 to 2 substituents independently selected from the group consisting of hydrogen, -O-(C_{1-8})alkyl, -O-(C_{1-8})alkyl-OH, -O-(C_{1-8})alkyl-O-(C_{1-8})alkyl, -O-(C_{1-8})alkyl-NH_2, -O-(C_{1-8})alkyl-NH(C_{1-8}alkyl), -O-(C_{1-8})alkyl-N(C_{1-8}alkyl)$_2$, -O-(C_{1-8})alkyl-S-(C_{1-8})alkyl, -O-(C_{1-8})alkyl-SO_2-(C_{1-8})alkyl, -O-(C_{1-8})alkyl-SO_2-NH_2, -O-(C_{1-8})alkyl-SO_2-NH(C_{1-8}alkyl), -O-(C_{1-8})alkyl-SO_2-N(C_{1-8}alkyl)$_2$, -O-C(O)H, -O-C(O)-(C_{1-8})alkyl, -O-C(O)-NH_2, -O-C(O)-NH(C_{1-8}alkyl), -O-C(O)-N(C_{1-8}alkyl)$_2$, -O-(C_{1-8})alkyl-C(O)H, -O-(C_{1-8})alkyl-C(O)-(C_{1-8})alkyl, -O-(C_{1-8})alkyl-CO_2H, -O-(C_{1-8})alkyl-C(O)-O-(C_{1-8})alkyl, -O-(C_{1-8})alkyl-C(O)-NH_2, -O-(C_{1-8})alkyl-C(O)-NH(C_{1-8}alkyl), -O-(C_{1-8})alkyl-C(O)-N(C_{1-8}alkyl)$_2$, -C(O)H, -C(O)-(C_{1-8})alkyl, -CO_2H, -C(O)-O-(C_{1-8})alkyl, -C(O)-NH_2, -C(NH)-NH_2, -C(O)-NH(C_{1-8}alkyl), -C(O)-N(C_{1-8}alkyl)$_2$, -SH, -S-(C_{1-8})alkyl, -S-(C_{1-8})alkyl-S-(C_{1-8})alkyl, -S-(C_{1-8})alkyl-O-(C_{1-8})alkyl, -S-(C_{1-8})alkyl-O-(C_{1-8})alkyl-OH, -S-(C_{1-8})alkyl-O-(C_{1-8})alkyl-NH_2, -S-(C_{1-8})alkyl-O-(C_{1-8})alkyl-NH(C_{1-8}alkyl), -S-(C_{1-8})alkyl-O-(C_{1-8})alkyl-N(C_{1-8}alkyl)$_2$, -S-(C_{1-8})alkyl-NH(C_{1-8}alkyl), -SO_2-(C_{1-8})alkyl, -SO_2-NH_2, -SO_2-NH(C_{1-8}alkyl), -SO_2-N(C_{1-8}alkyl)$_2$, -N-R^7, cyano, (halo)$_{1-3}$, hydroxy, nitro, oxo, -cycloalkyl-R^6, -heterocyclyl-R^6, -aryl-R^6 and -heteroaryl-R^6;

[0088] R^6 is 1 to 4 substituents attached to a carbon or nitrogen atom independently selected from the group consisting of hydrogen, -C_{1-8}alkyl, -C_{2-8}alkenyl, -C_{2-8}alkynyl, -C(O)H, -C(O)-(C_{1-8})alkyl, -CO_2H, -C(O)-O-(C_{1-8})alkyl, -C(O)-NH_2, -C(NH)-NH_2, -C(O)-NH(C_{1-8}alkyl), -C(O)-N(C_{1-8})alkyl)$_2$, -SO_2-(C_{1-8})alkyl, -SO_2-NH_2, -SO_2-NH(C_{1-8}alkyl), -SO_2-N(C_{1-8}alkyl)$_2$, -(C_{1-8})alkyl-N-R^7, -(C_{1-8})alkyl-(halo)$_{1-3}$, -(C_{1-8})alkyl-OH, -aryl-R^8, -(C_{1-8})alkyl-aryl-R^8 and -(C_{1-8})alkyl-heteroaryl-R^8; with the proviso that, when R^6 is attached to a carbon atom, R^6 is further selected from the group

consisting of -C_{1-8}alkoxy, -(C_{1-8})alkoxy-(halo)$_{1-3}$, -SH, -S-(C_{1-8})alkyl, -N-R^7, cyano, halo, hydroxy, nitro, oxo and -heteroaryl-R^8;

[0089] R^7 is 2 substituents independently selected from the group consisting of hydrogen, -C_{1-8}alkyl, -C_{2-8}alkenyl, -C_{2-8}alkynyl, -(C_{1-8})alkyl-OH, -(C_{1-8})alkyl-O-(C_{1-8})alkyl, -(C_{1-8})alkyl-NH_2, -(C_{1-8})alkyl-NH(C_{1-8}alkyl), -(C_{1-8})alkyl-N(C_{1-8}alkyl)$_2$, -(C_{1-8})alkyl-S-(C_{1-8})alkyl, -C(O)H, -C(O)-(C_{1-8})alkyl, -C(O)-O-(C_{1-8})alkyl, -C(O)-NH_2, -C(O)-NH(C_{1-8}alkyl), -C(O)-N(C_{1-8}alkyl)$_2$, -SO_2-(C_{1-8})alkyl, -SO_2-NH_2, -SO_2-NH(C_{1-8}alkyl), -SO_2-N(C_{1-8}alkyl)$_2$, -C(N)-NH_2, -cycloalkyl-R^8, -(C_{1-8})alkyl-heterocyclyl-R^8, -aryl-R^8, -(C_{1-8})alkyl-aryl-R^8 and -(C_{1-8})alkyl-heteroaryl-R^8;

[0090] R^8 is 1 to 4 substituents attached to a carbon or nitrogen atom independently selected from the group consisting of hydrogen, -C_{1-8}alkyl, -(C_{1-8})alkyl-(halo)$_{1-3}$ and -(C_{1-8})alkyl-OH; with the proviso that, when R^8 is attached to a carbon atom, R^8 is further selected from the group consisting of -C_{1-8}alkoxy, -NH_2, -NH(C_{1-8}alkyl), -N(C_{1-8}alkyl)$_2$, cyano, halo, -(C_{1-8})alkoxy-(halo)$_{1-3}$, hydroxy and nitro;

[0091] R^9 is 1 to 2 substituents independently selected from the group consisting of hydrogen, -C_{1-8}alkoxy, -NH_2, -NH(C_{1-8}alkyl), -N(C_{1-8}alkyl)$_2$, cyano, (halo)$_{1-3}$, hydroxy and nitro;

[0092] R^2 is one substituent attached to a carbon or nitrogen atom selected from the group consisting of hydrogen, -C_{1-8}alkyl-R^5, -C_{2-8}alkenyl-R^5, -C_{2-8}alkynyl-R^5, -C(O)H, -C(O)-(C_{1-8})alkyl-R^9, -C(O)-NH_2, -C(O)-NH(C_{1-8}alkyl-R^9), -C(O)-N(C_{1-8}alkyl-R^9)$_2$, -C(O)-NH(aryl-R^8), -C(O)-cycloalkyl-R^8, -C(O)-heterocyclyl-R^8, -C(O)-aryl-R^8, -C(O)-heteroaryl-R^8, -CO_2H, -C(O)-O-(C_{1-8})alkyl-R^9, -C(O)-O-aryl-R^8, -SO_2-(C_{1-8})alkyl-R^9, -SO_2-aryl-R^8, -cycloalkyl-R^6, -aryl-R^6 and -(C_{1-8})alkyl-N-R^7; with the proviso that, when R^2 is attached to a carbon atom, R^2 is further selected from the group consisting of -C_{1-8}alkoxy-R^5, -N-R^7, cyano, halogen, hydroxy, nitro, oxo, -heterocyclyl-R^6 and -heteroaryl-R^6;

[0093] R^3 is 1 to 3 substituents attached to a carbon atom independently selected from the group consisting of hydrogen, -C_{1-8}alkyl-R^{10}, -C_{2-8}alkenyl-R^{10},

-C_{2-8}alkynyl-R^{10}, -C_{1-8}alkoxy-R^{10}, -C(O)H, -C(O)-(C_{1-8})alkyl-R^9, -C(O)-NH_2, -C(O)-NH(C_{1-8}alkyl-R^9), -C(O)-N(C_{1-8}alkyl-R^9)$_2$, -C(O)-cycloalkyl-R^8, -C(O)-heterocyclyl-R^8, -C(O)-aryl-R^8, -C(O)-heteroaryl-R^8, -C(NH)-NH_2, -CO_2H, -C(O)-O-(C_{1-8})alkyl-R^9, -C(O)-O-aryl-R^8, -SO_2-(C_{1-8})alkyl-R^9, -SO_2-aryl-R^8, -N-R^7, cyano, halogen, hydroxy, nitro, -cycloalkyl-R^8, -heterocyclyl-R^8, -aryl-R^8 and -heteroaryl-R^8;

[0094] R^4 is 1 to 4 substituents attached to a carbon atom independently selected from the group consisting of hydrogen, -C_{1-8}alkyl-R^{10}, -C_{2-8}alkenyl-R^{10}, -C_{2-8}alkynyl-R^{10}, -C_{1-8}alkoxy-R^{10}, -C(O)H, -C(O)-(C_{1-8})alkyl-R^9, -C(O)-NH_2, -C(O)-NH(C_{1-8}alkyl-R^9), -C(O)-N(C_{1-8}alkyl-R^9)$_2$, -C(O)-cycloalkyl-R^8, -C(O)-heterocyclyl-R^8, -C(O)-aryl-R^8, -C(O)-heteroaryl-R^8, -C(NH)-NH_2, -CO_2H, -C(O)-O-(C_{1-8})alkyl-R^9, -C(O)-O-aryl-R^8, -SH, -S-(C_{1-8})alkyl-R^{10}, -SO_2-(C_{1-8})alkyl-R^9, -SO_2-aryl-R^8, -SO_2-NH_2, -SO_2-NH(C_{1-8}alkyl-R^9), -SO_2-N(C_{1-8}alkyl-R^9)$_2$, -N-R^7, cyano, halogen, hydroxy, nitro, -cycloalkyl-R^8, -heterocyclyl-R^8, -aryl-R^8 and -heteroaryl-R^8;

[0095] R^{10} is 1 to 2 substituents independently selected from the group consisting of hydrogen, -NH_2, -NH(C_{1-8}alkyl), -N(C_{1-8}alkyl)$_2$, cyano, (halo)$_{1-3}$, hydroxy, nitro and oxo; and,

[0096] Y and Z are independently selected from the group consisting of O, S, (H,OH) and (H,H); with the proviso that one of Y and Z is O and the other is selected from the group consisting of O, S, (H,OH) and (H,H); and pharmaceutically acceptable salts thereof.

[0097] Embodiments of the present invention include compounds of Formula (II) wherein, R is selected from the group consisting of R_a, -C_{1-4}alkyl-R_a, -C_{2-4}alkenyl-R_a, -C_{2-4}alkynyl-R_a and cyano.

[0098] Embodiments of the present invention include compounds of Formula (II) wherein, R_a is selected from the group consisting of heterocyclyl, aryl and heteroaryl.

[0099] In one embodiment, R_a is selected from the group consisting of dihydro-pyranyl, phenyl, naphthyl, thienyl, pyrrolyl, imidazolyl, pyrazolyl,

pyridinyl, azaindolyl, indazolyl, benzofuryl, benzothienyl, dibenzofuryl and dibenzothienyl.

[0100] Embodiments of the present invention include compounds of Formula (II) wherein, R^1 is selected from the group consisting of hydrogen, -C_{1-4}alkyl-R^5, -C_{2-4}alkenyl-R^5, -C_{2-4}alkynyl-R^5, -C(O)-(C_{1-4})alkyl-R^9, -C(O)-aryl-R^8, -C(O)-O-(C_{1-4})alkyl-R^9, -C(O)-O-aryl-R^8, -C(O)-NH(C_{1-4}alkyl-R^9), -C(O)-NH(aryl-R^8), -C(O)-N(C_{1-4}alkyl-R^9)$_2$, -SO_2-(C_{1-4})alkyl-R^9, -SO_2-aryl-R^8, -cycloalkyl-R^6, -heterocyclyl-R^6, -aryl-R^6 and -heteroaryl-R^6; wherein heterocyclyl and heteroaryl are attached to the azaindole nitrogen atom in the one position via a heterocyclyl or heteroaryl ring carbon atom.

[0101] In one embodiment, R^1 is selected from the group consisting of hydrogen, -C_{1-4}alkyl-R^5, -aryl-R^6 and -heteroaryl-R^6; wherein heteroaryl is attached to the azaindole nitrogen atom in the one position via a heteroaryl ring carbon atom.

[0102] In one embodiment, R^1 is selected from the group consisting of hydrogen, -C_{1-4}alkyl-R^5 and -naphthyl-R^6.

[0103] Embodiments of the present invention include compounds of Formula (II) wherein, R^5 is 1 to 2 substituents independently selected from the group consisting of hydrogen, -O-(C_{1-4})alkyl, -O-(C_{1-4})alkyl-OH, -O-(C_{1-4})alkyl-O-(C_{1-4})alkyl, -O-(C_{1-4})alkyl-NH_2, -O-(C_{1-4})alkyl-NH(C_{1-4}alkyl), -O-(C_{1-4})alkyl-N(C_{1-4}alkyl)$_2$, -O-(C_{1-4})alkyl-S-(C_{1-4})alkyl, -O-(C_{1-4})alkyl-SO_2-(C_{1-4})alkyl, -O-(C_{1-4})alkyl-SO_2-NH_2, -O-(C_{1-4})alkyl-SO_2-NH(C_{1-4}alkyl), -O-(C_{1-4})alkyl-SO_2-N(C_{1-4}alkyl)$_2$, -O-C(O)H, -O-C(O)-(C_{1-4})alkyl, -O-C(O)-NH_2, -O-C(O)-NH(C_{1-4}alkyl), -O-C(O)-N(C_{1-4}alkyl)$_2$, -O-(C_{1-4})alkyl-C(O)H, -O-(C_{1-4})alkyl-C(O)-(C_{1-4})alkyl, -O-(C_{1-4})alkyl-CO_2H, -O-(C_{1-4})alkyl-C(O)-O-(C_{1-4})alkyl, -O-(C_{1-4})alkyl-C(O)-NH_2, -O-(C_{1-4})alkyl-C(O)-NH(C_{1-4}alkyl), -O-(C_{1-4})alkyl-C(O)-N(C_{1-4}alkyl)$_2$, -C(O)H, -C(O)-(C_{1-4})alkyl, -CO_2H, -C(O)-O-(C_{1-4})alkyl, -C(O)-NH_2, -C(NH)-NH_2, -C(O)-NH(C_{1-4}alkyl), -C(O)-N(C_{1-4}alkyl)$_2$, -SH, -S-(C_{1-4})alkyl, -S-(C_{1-4})alkyl-S-(C_{1-4})alkyl, -S-(C_{1-4})alkyl-O-(C_{1-4})alkyl,

-S-(C_{1-4})alkyl-O-(C_{1-4})alkyl-OH, -S-(C_{1-4})alkyl-O-(C_{1-4})alkyl-NH_2, -S-(C_{1-4})alkyl-O-(C_{1-4})alkyl-NH(C_{1-4}alkyl), -S-(C_{1-4})alkyl-O-(C_{1-4})alkyl-N(C_{1-4}alkyl)$_2$, -S-(C_{1-4})alkyl-NH(C_{1-4}alkyl), -SO_2-(C_{1-4})alkyl, -SO_2-NH_2, -SO_2-NH(C_{1-4}alkyl), -SO_2-N(C_{1-4}alkyl)$_2$, -N-R^7, cyano, (halo)$_{1-3}$, hydroxy, nitro, oxo, -cycloalkyl-R^6, -heterocyclyl-R^6, -aryl-R^6 and -heteroaryl-R^6.

[0104] In one embodiment, R^5 is 1 to 2 substituents independently selected from the group consisting of hydrogen, -O-(C_{1-4})alkyl, -N-R^7, hydroxy and -heteroaryl-R^6.

[0105] In one embodiment, R^5 is 1 to 2 substituents independently selected from the group consisting of hydrogen, -O-(C_{1-4})alkyl, -N-R^7, hydroxy, -imidazolyl-R^6, -triazolyl-R^6 and -tetrazolyl-R^6.

[0106] Embodiments of the present invention include compounds of Formula (II) wherein, R^6 is 1 to 4 substituents attached to a carbon or nitrogen atom independently selected from the group consisting of hydrogen, -C_{1-4}alkyl, -C_{2-4}alkenyl, -C_{2-4}alkynyl, -C(O)H, -C(O)-(C_{1-4})alkyl, -CO_2H, -C(O)-O-(C_{1-4})alkyl, -C(O)-NH_2, -C(NH)-NH_2, -C(O)-NH(C_{1-4}alkyl), -C(O)-N(C_{1-4})alkyl)$_2$, -SO_2-(C_{1-4})alkyl, -SO_2-NH_2, -SO_2-NH(C_{1-4}alkyl), -SO_2-N(C_{1-4}alkyl)$_2$, -(C_{1-4})alkyl-N-R^7, -(C_{1-4})alkyl-(halo)$_{1-3}$, -(C_{1-4})alkyl-OH, -aryl-R^8, -(C_{1-4})alkyl-aryl-R^8 and -(C_{1-4})alkyl-heteroaryl-R^8; with the proviso that, when R^6 is attached to a carbon atom, R^6 is further selected from the group consisting of -C_{1-4}alkoxy, -(C_{1-4})alkoxy-(halo)$_{1-3}$, -SH, -S-(C_{1-4})alkyl, -N-R^7, cyano, halo, hydroxy, nitro, oxo and -heteroaryl-R^8.

[0107] In one embodiment, R^6 is hydrogen.

[0108] Embodiments of the present invention include compounds of Formula (II) wherein, R^7 is 2 substituents independently selected from the group consisting of hydrogen, -C_{1-4}alkyl, -C_{2-4}alkenyl, -C_{2-4}alkynyl, -(C_{1-4})alkyl-OH, -(C_{1-4})alkyl-O-(C_{1-4})alkyl, -(C_{1-4})alkyl-NH_2, -(C_{1-4})alkyl-NH(C_{1-4}alkyl), -(C_{1-4})alkyl-N(C_{1-4}alkyl)$_2$, -(C_{1-4})alkyl-S-(C_{1-4})alkyl, -C(O)H, -C(O)-(C_{1-4})alkyl, -C(O)-O-(C_{1-4})alkyl, -C(O)-NH_2, -C(O)-NH(C_{1-4}alkyl), -C(O)-N(C_{1-4}alkyl)$_2$, -SO_2-(C_{1-4})alkyl, -SO_2-NH_2, -SO_2-NH(C_{1-4}alkyl),

-SO_2-N(C_{1-4}alkyl)$_2$, -C(N)-NH_2, -cycloalkyl-R^8, -(C_{1-4})alkyl-heterocyclyl-R^8, -aryl-R^8, -(C_{1-4})alkyl-aryl-R^8 and -(C_{1-4})alkyl-heteroaryl-R^8.

[0109] In one embodiment R^7 is 2 substituents independently selected from the group consisting of of hydrogen, -C_{1-4}alkyl, -C(O)H, -C(O)-(C_{1-4})alkyl, -C(O)-O-(C_{1-4})alkyl, -SO_2-NH_2, -SO_2-NH(C_{1-4}alkyl) and -SO_2-N(C_{1-4}alkyl)$_2$.

[0110] Embodiments of the present invention include compounds of Formula (II) wherein, R^8 is 1 to 4 substituents attached to a carbon or nitrogen atom independently selected from the group consisting of hydrogen, -C_{1-4}alkyl, -(C_{1-4})alkyl-(halo)$_{1-3}$ and -(C_{1-4})alkyl-OH; with the proviso that, when R^8 is attached to a carbon atom, R^8 is further selected from the group consisting of -C_{1-4}alkoxy, -NH_2, -NH(C_{1-4}alkyl), -N(C_{1-4}alkyl)$_2$, cyano, halo, -(C_{1-4})alkoxy-(halo)$_{1-3}$, hydroxy and nitro.

[0111] In one embodiment, R^8 is hydrogen.

[0112] Embodiments of the present invention include compounds of Formula (II) wherein, R^9 is 1 to 2 substituents independently selected from the group consisting of hydrogen, -C_{1-4}alkoxy, -NH_2, -NH(C_{1-4}alkyl), -N(C_{1-4}alkyl)$_2$, cyano, (halo)$_{1-3}$, hydroxy and nitro.

[0113] In one embodiment, R^9 is hydrogen.

[0114] Embodiments of the present invention include compounds of Formula (II) wherein, R^2 is one substituent attached to a carbon or nitrogen atom selected from the group consisting of hydrogen, -C_{1-4}alkyl-R^5, -C_{2-4}alkenyl-R^5, -C_{2-4}alkynyl-R^5, -C(O)H, -C(O)-(C_{1-4})alkyl-R^9, -C(O)-NH_2, -C(O)-NH(C_{1-4}alkyl-R^9), -C(O)-N(C_{1-4}alkyl-R^9)$_2$, -C(O)-NH(aryl-R^8), -C(O)-cycloalkyl-R^8, -C(O)-heterocyclyl-R^8, -C(O)-aryl-R^8, -C(O)-heteroaryl-R^8, -CO_2H, -C(O)-O-(C_{1-4})alkyl-R^9, -C(O)-O-aryl-R^8, -SO_2-(C_{1-4})alkyl-R^9, -SO_2-aryl-R^8, -cycloalkyl-R^6, -aryl-R^6 and -(C_{1-4})alkyl-N-R^7; with the proviso that, when R^2 is attached to a carbon atom, R^2 is further selected from the group consisting of -C_{1-4}alkoxy-R^5, -N-R^7, cyano, halogen, hydroxy, nitro, oxo, -heterocyclyl-R^6 and -heteroaryl-R^6.

[0115] In one embodiment, R^2 is one substituent attached to a carbon or nitrogen atom selected from the group consisting of hydrogen, -C_{1-4}alkyl-R^5, -C_{2-4}alkenyl-R^5, -C_{2-4}alkynyl-R^5, -CO_2H, -C(O)-O-(C_{1-4})alkyl-R^9, -cycloalkyl-R^6, -aryl-R^6 and -(C_{1-4})alkyl-N-R^7; with the proviso that, when R^2 is attached to a nitrogen atom, a quaternium salt is not formed; and, with the proviso that, when R^2 is attached to a carbon atom, R^2 is further selected from the group consisting of -C_{1-4}alkoxy-R^5, -N-R^7, cyano, halogen, hydroxy, nitro, oxo, -heterocyclyl-R^6 and -heteroaryl-R^6.

[0116] In one embodiment, R^2 is one substituent attached to a carbon or nitrogen atom selected from the group consisting of hydrogen, -C_{1-4}alkyl-R^5 and -aryl-R^6; with the proviso that, when R^2 is attached to a nitrogen atom, a quaternium salt is not formed; and, with the proviso that when R^2 is attached to a carbon atom, R^2 is further selected from the group consisting of -N-R^7, halogen, hydroxy and -heteroaryl-R^6.

[0117] Embodiments of the present invention include compounds of Formula (II) wherein, R^3 is 1 to 3 substituents attached to a carbon atom independently selected from the group consisting of hydrogen, -C_{1-4}alkyl-R^{10}, -C_{2-4}alkenyl-R^{10}, -C_{2-4}alkynyl-R^{10}, -C_{1-4}alkoxy-R^{10}, -C(O)H, -C(O)-(C_{1-4})alkyl-R^9, -C(O)-NH_2, -C(O)-NH(C_{1-4}alkyl-R^9), -C(O)-N(C_{1-4}alkyl-R^9)$_2$, -C(O)-cycloalkyl-R^8, -C(O)-heterocyclyl-R^8, -C(O)-aryl-R^8, -C(O)-heteroaryl-R^8, -C(NH)-NH_2, -CO_2H, -C(O)-O-(C_{1-4})alkyl-R^9, -C(O)-O-aryl-R^8, -SO_2-(C_{1-8})alkyl-R^9, -SO_2-aryl-R^8, -N-R^7, -(C_{1-4})alkyl-N-R^7, cyano, halogen, hydroxy, nitro, -cycloalkyl-R^8, -heterocyclyl-R^8, -aryl-R^8 and -heteroaryl-R^8.

[0118] In one embodiment, R^3 is one substituent attached to a carbon atom selected from the group consisting of hydrogen, -C_{1-4}alkyl-R^{10}, -C_{2-4}alkenyl-R^{10}, -C_{2-4}alkynyl-R^{10}, -C_{1-4}alkoxy-R^{10}, -C(O)H, -CO_2H, -NH_2, -NH(C_{1-4}alkyl), -N(C_{1-4}alkyl)$_2$, cyano, halogen, hydroxy and nitro.

[0119] In one embodiment, R^3 is one substituent attached to a carbon atom selected from the group consisting of hydrogen, -C_{1-4}alkyl-R^{10}, -NH_2, -NH(C_{1-4}alkyl), -N(C_{1-4}alkyl)$_2$, halogen and hydroxy.

[0120] Embodiments of the present invention include compounds of Formula (II) wherein, R^4 is 1 to 4 substituents attached to a carbon atom independently selected from the group consisting of hydrogen, -C_{1-4}alkyl-R^{10}, -C_{2-4}alkenyl-R^{10}, -C_{2-4}alkynyl-R^{10}, -C_{1-4}alkoxy-R^{10}, -C(O)H, -C(O)-(C_{1-4})alkyl-R^9, -C(O)-NH_2, -C(O)-NH(C_{1-4}alkyl-R^9), -C(O)-N(C_{1-4}alkyl-R^9)$_2$, -C(O)-cycloalkyl-R^8, -C(O)-heterocyclyl-R^8, -C(O)-aryl-R^8, -C(O)-heteroaryl-R^8, -C(NH)-NH_2, -CO_2H, -C(O)-O-(C_{1-4})alkyl-R^9, -C(O)-O-aryl-R^8, -SH, -S-(C_{1-4})alkyl-R^{10}, -SO_2-(C_{1-4})alkyl-R^9, -SO_2-aryl-R^8, -SO_2-NH_2, -SO_2-NH(C_{1-4}alkyl-R^9), -SO_2-N(C_{1-4}alkyl-R^9)$_2$, -N-R^7, cyano, halogen, hydroxy, nitro, -cycloalkyl-R^8, -heterocyclyl-R^8, -aryl-R^8 and -heteroaryl-R^8.

[0121] In one embodiment, R^4 is 1 to 4 substituents attached to a carbon atom independently selected from the group consisting of hydrogen, -C_{1-4}alkyl-R^{10}, -C_{2-4}alkenyl-R^{10}, -C_{2-4}alkynyl-R^{10}, -C_{1-4}alkoxy-R^{10}, -C(O)H, -CO_2H, -NH_2, -NH(C_{1-4}alkyl), -N(C_{1-4}alkyl)$_2$, cyano, halogen, hydroxy, nitro, -cycloalkyl, -heterocyclyl, -aryl and -heteroaryl.

[0122] In one embodiment, R^4 is 1 to 4 substituents attached to a carbon atom independently selected from the group consisting of hydrogen, C_{1-4}alkyl-R^{10}, C_{1-4}alkoxy-R^{10}, -NH_2, -NH(C_{1-4}alkyl), -N(C_{1-4}alkyl)$_2$, halogen and hydroxy.

[0123] In one embodiment, R^4 is 1 to 4 substituents attached to a carbon atom independently selected from the group consisting of hydrogen, C_{1-4}alkyl-R^{10}, C_{1-4}alkoxy-R^{10}, -NH_2, -NH(C_{1-4}alkyl), -N(C_{1-4}alkyl)$_2$, chlorine, fluorine and hydroxy.

[0124] Embodiments of the present invention include compounds of Formula (II) wherein, R^{10} is 1 to 2 substituents independently selected from the group consisting of hydrogen, -NH_2, -NH(C_{1-4}alkyl), -N(C_{1-4}alkyl)$_2$, cyano, (halo)$_{1-3}$, hydroxy, nitro and oxo.

[0125] In one embodiment, R^{10} is 1 to 2 substituents independently selected from the group consisting of hydrogen and (halo)$_{1-3}$.

[0126] In one embodiment, R^{10} is 1 to 2 substituents independently selected from the group consisting of hydrogen and $(fluoro)_3$.

[0127] Embodiments of the present invention include compounds of Formula (II) wherein, Y and Z are independently selected from the group consisting of O, S, (H,OH) and (H,H); with the proviso that one of Y and Z is O and the other is selected from the group consisting of O, S, (H,OH) and (H,H).

[0128] In one embodiment, Y and Z are independently selected from the group consisting of O and (H,H); with the proviso that one of Y and Z is O, and the other is selected from the group consisting of O and (H,H).

[0129] In one embodiment, Y and Z are independently selected from O.

[0130] Compounds of Formula (II) are disclosed in commonly assigned United States Patent Number 7,125,878, the complete disclosure of which is herein incorporated by reference.

[0131] An example of the invention includes a compound of Formula (II) wherein the compound is selected from the group consisting of the compounds listed in Table B, below:

Table B

Compounds of Formula (II)

Compound	Name
B-1	3-(2-Chlorophenyl)-4-[1-(3-hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-1H-pyrrole-2,5-dione
B-2	3-(2-Chlorophenyl)-4-{1-[3-(dimethylamino)propyl]-1H-pyrrolo[2,3-b]pyridin-3-yl}-1H-pyrrole-2,5-dione
B-3	3-[1-(3-Hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-4-naphthalen-1-yl-1H-pyrrole-2,5-dione
B-4	3-{1-[3-(Dimethylamino)propyl]-1H-pyrrolo[2,3-b]pyridin-3-yl}-4-naphthalen-1-yl-1H-pyrrole-2,5-dione
B-5	3-(5-Chloro-1-benzothiophen-3-yl)-4-[1-(3-hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-1H-pyrrole-2,5-dione

B-6	3-[1-(3-Hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-4-(1H-indazol-3-yl)-1H-pyrrole-2,5-dione
B-7	3-(1-Ethyl-1H-pyrrolo[2,3-b]pyridin-3-yl)-4-[1-(3-hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-1H-pyrrole-2,5-dione
B-8	3-[1-(3-Hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-4-(2-methoxyphenyl)-1H-pyrrole-2,5-dione
B-9	3-[1-(3-Hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-4-(3-methoxyphenyl)-1H-pyrrole-2,5-dione
B-10	3-(2-Chloro-4-fluorophenyl)-4-[1-(3-hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-1H-pyrrole-2,5-dione
B-11	3-[1-(3-Hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-4-[2-(trifluoromethyl)phenyl]-1H-pyrrole-2,5-dione

Table B - CONTINUED

Compound	Name
B-12	3-[1-(3-Hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-4-pyridin-2-yl-1H-pyrrole-2,5-dione
B-13	3-[3-Chloro-5-(trifluoromethyl)pyridin-2-yl]-4-[1-(3-hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-1H-pyrrole-2,5-dione
B-14	3-[1-(3-Hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-4-thiophen-2-yl-1H-pyrrole-2,5-dione
B-15	3-(2,5-Dichlorothiophen-3-yl)-4-[1-(3-hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-1H-pyrrole-2,5-dione
B-16	3-[1-(3-Hydroxypropyl)-1H-pyrazol-3-yl]-4-[1-(3-hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-1H-pyrrole-2,5-dione
B-17	3-[1-(3-Hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-4-(1H-imidazol-2-yl)-1H-pyrrole-2,5-dione
B-18	3-[1-(3-Hydroxypropyl)-1H-imidazol-4-yl]-4-[1-(3-hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-1H-pyrrole-2,5-dione
B-19	3-[1-(2-Hydroxyethyl)-1H-imidazol-4-yl]-4-[1-(3-hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-1H-pyrrole-2,5-dione
B-20	3-{1-[3-(Dimethylamino)propyl]-1H-indazol-3-yl}-4-(1-naphthalen-2-yl-1H-pyrrolo[2,3-b]pyridin-3-yl)-1H-pyrrole-2,5-dione

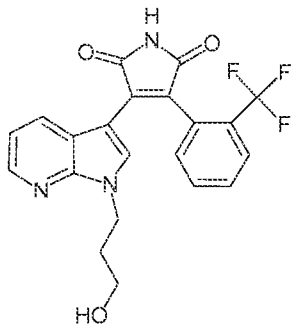
Table B - CONTINUED

Compound	Name
B-21	3-[1-(3-Hydroxypropyl)-1H-indazol-3-yl]-4-(1-naphthalen-2-yl-1H-pyrrolo[2,3-b]pyridin-3-yl)-1H-pyrrole-2,5-dione
B-22	3-[(E)-2-(4-Fluorophenyl)ethenyl]-4-[1-(3-hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-1H-pyrrole-2,5-dione
B-23	3-(3,4-Dihydro-2H-pyran-6-yl)-4-[1-(3-hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-1H-pyrrole-2,5-dione
B-24	4-[1-(3-Hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-1H,1'H-3,3'-bipyrrole-2,5-dione
B-25	3-(1-Benzofuran-2-yl)-4-[1-(3-hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-1H-pyrrole-2,5-dione
B-26	3-[1-(3-Hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-4-(1-methyl-1H-pyrazol-3-yl)-1H-pyrrole-2,5-dione
B-27	4-[1-(3-Hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-2,5-dioxo-2,5-dihydro-1H-pyrrole-3-carbonitrile
B-28	3-Dibenzo[b,d]thien-4-yl-4-[1-(3-hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-1H-pyrrole-2,5-dione
B-29	3-Dibenzo[b,d]furan-4-yl-4-[1-(3-hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-1H-pyrrole-2,5-dione
B-30	3-(2-Hydroxyphenyl)-4-{1-[3-(methyloxy)propyl]-1H-pyrrolo[2,3-b]pyridin-3-yl}-1H-pyrrole-2,5-dione
B-31	3-[3,4-Bis(methyloxy)phenyl]-4-{1-[3-(methyloxy)propyl]-1H-pyrrolo[2,3-b]pyridin-3-yl}-1H-pyrrole-2,5-dione
B-32	3-(3,4-Dihydroxyphenyl)-4-[1-(3-hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-1H-pyrrole-2,5-dione
B-33	3-[2-(Methyloxy)phenyl]-4-(1-naphthalen-2-yl-1H-pyrrolo[2,3-b]pyridin-3-yl)-1H-pyrrole-2,5-dione
B-34	1,1-Dimethylethyl [3-(3-{4-[2-(methyloxy)phenyl]-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl}-1H-pyrrolo[2,3-b]pyridin-1-yl)propyl]carbamate
B-35	3-[1-(3-Aminopropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-4-[2-(methyloxy)phenyl]-1H-pyrrole-2,5-dione
B-36	N-[3-(3-{4-[2-(Methyloxy)phenyl]-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl}-1H-pyrrolo[2,3-b]pyridin-1-yl)propyl]acetamide

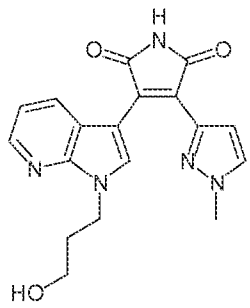
Table B - CONTINUED

Compound	Name
B-37	N-[3-(3-{4-[2-(Methyloxy)phenyl]-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl}-1H-pyrrolo[2,3-b]pyridin-1-yl)propyl]sulfamide
B-38	3-(2-methoxyphenyl)-4-[1-[3-(1*H*-tetrazol-1-yl)propyl]-1*H*-pyrrolo[2,3-*b*]pyridine-3-yl]-1*H*-pyrrole-2,5-dione
B-39	3-(2-methoxyphenyl)-4-[1-[3-(2*H*-tetrazol-2-yl)propyl]-1*H*-pyrrolo[2,3-*b*]pyridine-3-yl]-1*H*-pyrrole-2,5-dione
B-40	3-[1-(3-Hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-4-pyrazin-2-yl-1H-pyrrole-2,5-dione
B-41	3-[2,4-Bis(methyloxy)pyrimidin-5-yl]-4-[1-(3-hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-1H-pyrrole-2,5-dione
B-42	4-(3-{4-[2,4-Bis(methyloxy)pyrimidin-5-yl]-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl}-1H-pyrrolo[2,3-b]pyridin-1-yl)butanenitrile
B-43	4-{3-[4-(1-Methyl-1H-pyrazol-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-1H-pyrrolo[2,3-b]pyridin-1-yl}butanenitrile
B-44	3-[2,4-Bis(methyloxy)pyrimidin-5-yl]-4-[1-(2-phenylethyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-1H-pyrrole-2,5-dione

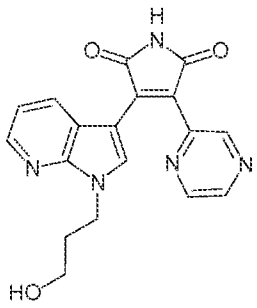
[0132] An example of the invention includes a compound of Formula (II) wherein the compound is selected from the group consisting of:



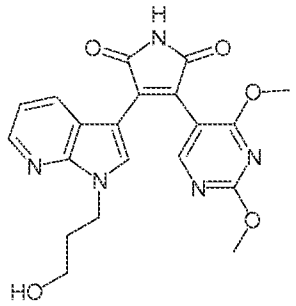
Compound B-11



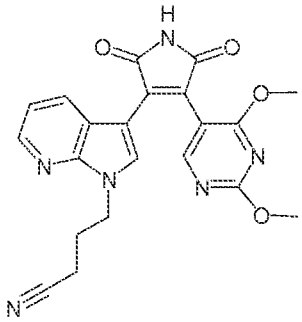
Compound B-26



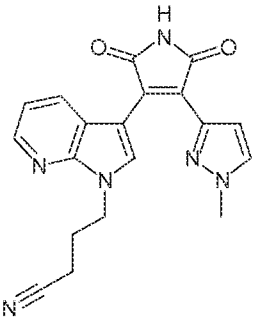
Compound B-40



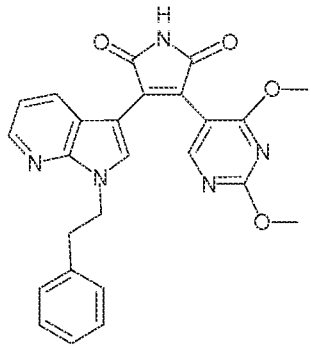
Compound B-41



Compound B-42

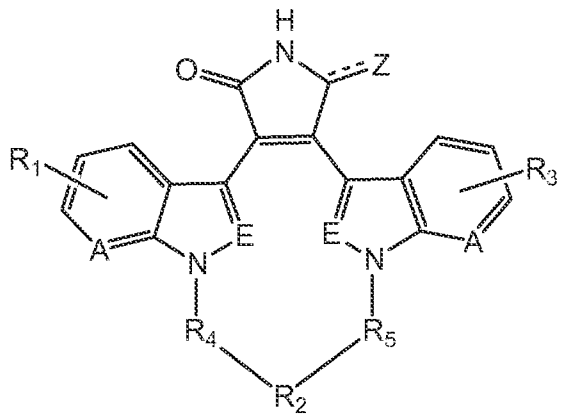


Compound B-43



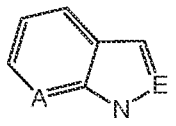
Compound B-44

[0134] In one embodiment, the inhibitor of GSK-3B enzyme activity is a compound of the Formula (III):



Formula (III)

[0135] wherein

[0136] A and E are independently selected from the group consisting of a hydrogen substituted carbon atom and a nitrogen atom; wherein  is independently selected from the group consisting of 1*H*-indole, 1*H*-pyrrolo[2,3-*b*]pyridine, 1*H*-pyrazolo[3,4-*b*]pyridine and 1*H*-indazole;

[0137] Z is selected from O; alternatively, Z is selected from dihydro; wherein each hydrogen atom is attached by a single bond;

[0138] R_4 and R_5 are independently selected from C_{1-8}alkyl, C_{2-8}alkenyl and C_{2-8}alkynyl optionally substituted with oxo;

[0139] R_2 is selected from the group consisting of -C_{1-8}alkyl-, -C_{2-8}alkenyl-, -C_{2-8}alkynyl-, -O-(C_{1-8})alkyl-O-, -O-(C_{2-8})alkenyl-O-, -O-(C_{2-8})alkynyl-O-, -C(O)-(C_{1-8})alkyl-C(O)- (wherein any of the foregoing alkyl, alkenyl and alkynyl linking groups are straight carbon chains optionally substituted with one to four substituents independently selected from the group consisting of C_{1-8}alkyl, C_{1-8}alkoxy, C_{1-8}alkoxy(C_{1-8})alkyl, carboxyl, carboxyl(C_{1-8})alkyl, -C(O)O-(C_{1-8})alkyl, -C_{1-8}alkyl-C(O)O-(C_{1-8})alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), halogen, (halo)$_{1-3}$(C_{1-8})alkyl, (halo)$_{1-3}$(C_{1-8})alkoxy, hydroxy, hydroxy(C_{1-8})alkyl and oxo; and, wherein any of the foregoing alkyl, alkenyl and alkynyl linking groups are optionally substituted with one to two substituents independently selected from the group consisting of heterocyclyl, aryl, heteroaryl, heterocyclyl(C_{1-8})alkyl, aryl(C_{1-8})alkyl, heteroaryl(C_{1-8})alkyl, spirocycloalkyl and spiroheterocyclyl (wherein any of the foregoing cycloalkyl, heterocyclyl, aryl and heteroaryl substituents are optionally substituted with one to four substituents independently selected from the group consisting of C_{1-8}alkyl, C_{1-8}alkoxy, C_{1-8}alkoxy(C_{1-8})alkyl, carboxyl,

carboxyl(C_{1-8})alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), halogen, (halo)$_{1-3}$(C_{1-8})alkyl, (halo)$_{1-3}$(C_{1-8})alkoxy, hydroxy and hydroxy(C_{1-8})alkyl; and, wherein any of the foregoing heterocyclyl substituents are optionally substituted with oxo)), cycloalkyl, heterocyclyl, aryl, heteroaryl (wherein cycloalkyl, heterocyclyl, aryl and heteroaryl are optionally substituted with one to four substituents independently selected from the group consisting of C_{1-8}alkyl, C_{1-8}alkoxy, C_{1-8}alkoxy(C_{1-8})alkyl, carboxyl, carboxyl(C_{1-8})alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), halogen, (halo)$_{1-3}$(C_{1-8})alkyl, (halo)$_{1-3}$(C_{1-8})alkoxy, hydroxy and hydroxy(C_{1-8})alkyl; and, wherein heterocyclyl is optionally substituted with oxo), -(O-(CH_2)$_{1-6}$)$_{0-5}$-O-, -O-(CH_2)$_{1-6}$-O-(CH_2)$_{1-6}$-O-, -O-(CH_2)$_{1-6}$-O-(CH_2)$_{1-6}$-O-(CH_2)$_{1-6}$-O-, -(O-(CH_2)$_{1-6}$)$_{0-5}$-NR_6-, -O-(CH_2)$_{1-6}$-NR_6-(CH_2)$_{1-6}$-O-, -O-(CH_2)$_{1-6}$-O-(CH_2)$_{1-6}$-NR_6-, -(O-(CH_2)$_{1-6}$)$_{0-5}$-S-, -O-(CH_2)$_{1-6}$-S-(CH_2)$_{1-6}$-O-, -O-(CH_2)$_{1-6}$-O-(CH_2)$_{1-6}$-S-, -NR_6-, -NR_6-NR_7-, -NR_6-(CH_2)$_{1-6}$-NR_7-, -NR_6-(CH_2)$_{1-6}$-NR_7-(CH_2)$_{1-6}$-NR_8-, -NR_6-C(O)-, -C(O)-NR_6-, -C(O)-(CH_2)$_{0-6}$-NR_6-(CH_2)$_{0-6}$-C(O)-, -NR_6-(CH_2)$_{0-6}$-C(O)-(CH_2)$_{1-6}$-C(O)-(CH_2)$_{0-6}$-NR_7-, -NR_6-C(O)-NR_7-, -NR_6-C(NR_7)-NR_8-, -O-(CH_2)$_{1-6}$-NR_6-(CH_2)$_{1-6}$-S-, -S-(CH_2)$_{1-6}$-NR_6-(CH_2)$_{1-6}$-O-, -S-(CH_2)$_{1-6}$-NR_6-(CH_2)$_{1-6}$-S-, -NR_6-(CH_2)$_{1-6}$-S-(CH_2)$_{1-6}$-NR_7- and -SO_2- (wherein R_6, R_7 and R_8 are independently selected from the group consisting of hydrogen, C_{1-8}alkyl, C_{1-8}alkoxy(C_{1-8})alkyl, carboxyl(C_{1-8})alkyl, amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), hydroxy(C_{1-8})alkyl, heterocyclyl(C_{1-8})alkyl, aryl(C_{1-8})alkyl and heteroaryl(C_{1-8})alkyl (wherein the foregoing heterocyclyl, aryl and heteroaryl substituents are optionally substituted with one to four substituents independently selected from the group consisting of C_{1-8}alkyl, C_{1-8}alkoxy, C_{1-8}alkoxy(C_{1-8})alkyl, carboxyl, carboxyl(C_{1-8})alkyl, amino

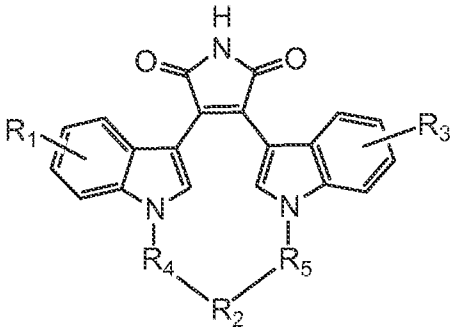
(substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), halogen, (halo)$_{1-3}$(C_{1-8})alkyl, (halo)$_{1-3}$(C_{1-8})alkoxy, hydroxy and hydroxy(C_{1-8})alkyl; and, wherein heterocyclyl is optionally substituted with oxo)); with the proviso that, if A and E are selected from a hydrogen substituted carbon atom, then R_2 is selected from the group consisting of -C_{2-8}alkynyl-, -O-(C_{1-8})alkyl-O-, -O-(C_{2-8})alkenyl-O-, -O-(C_{2-8})alkynyl-O-, -C(O)-(C_{1-8})alkyl-C(O)- (wherein any of the foregoing alkyl, alkenyl and alkynyl linking groups are straight carbon chains optionally substituted with one to four substituents independently selected from the group consisting of C_{1-8}alkyl, C_{1-8}alkoxy, C_{1-8}alkoxy(C_{1-8})alkyl, carboxyl, carboxyl(C_{1-8})alkyl, -C(O)O-(C_{1-8})alkyl, -C_{1-8}alkyl-C(O)O-(C_{1-8})alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), halogen, (halo)$_{1-3}$(C_{1-8})alkyl, (halo)$_{1-3}$(C_{1-8})alkoxy, hydroxy, hydroxy(C_{1-8})alkyl and oxo; and, wherein any of the foregoing alkyl, alkenyl and alkynyl linking groups are optionally substituted with one to two substituents independently selected from the group consisting of heterocyclyl, aryl, heteroaryl, heterocyclyl(C_{1-8})alkyl, aryl(C_{1-8})alkyl, heteroaryl(C_{1-8})alkyl, spirocycloalkyl and spiroheterocyclyl (wherein any of the foregoing cycloalkyl, heterocyclyl, aryl and heteroaryl substituents are optionally substituted with one to four substituents independently selected from the group consisting of C_{1-8}alkyl, C_{1-8}alkoxy, C_{1-8}alkoxy(C_{1-8})alkyl, carboxyl, carboxyl(C_{1-8})alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), halogen, (halo)$_{1-3}$(C_{1-8})alkyl, (halo)$_{1-3}$(C_{1-8})alkoxy, hydroxy and hydroxy(C_{1-8})alkyl; and, wherein any of the foregoing heterocyclyl substituents are optionally substituted with oxo)), cycloalkyl (wherein cycloalkyl is optionally substituted with one to four substituents independently selected from the group consisting of C_{1-8}alkyl, C_{1-8}alkoxy,

C_{1-8}alkoxy(C_{1-8})alkyl, carboxyl, carboxyl(C_{1-8})alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), halogen, (halo)$_{1-3}$(C_{1-8})alkyl, (halo)$_{1-3}$(C_{1-8})alkoxy, hydroxy and hydroxy(C_{1-8})alkyl), -(O-(CH_2)$_{1-6}$)$_{1-5}$-O-, -O-(CH_2)$_{1-6}$-O-(CH_2)$_{1-6}$-O-, -O-(CH_2)$_{1-6}$-O-(CH_2)$_{1-6}$-O-(CH_2)$_{1-6}$-O-, -(O-(CH_2)$_{1-6}$)$_{1-5}$-NR_6-, -O-(CH_2)$_{1-6}$-NR_6-(CH_2)$_{1-6}$-O-, -O-(CH_2)$_{1-6}$-O-(CH_2)$_{1-6}$-NR_6-, -(O-(CH_2)$_{1-6}$)$_{0-5}$-S-, -O-(CH_2)$_{1-6}$-S-(CH_2)$_{1-6}$-O-, -O-(CH_2)$_{1-6}$-O-(CH_2)$_{1-6}$-S-, -NR_6-NR_7-, -NR_6-(CH_2)$_{1-6}$-NR_7-, -NR_6-(CH_2)$_{1-6}$-NR_7-(CH_2)$_{1-6}$-NR_8-, -NR_9-C(O)-, -C(O)-NR_9-, -C(O)-(CH_2)$_{0-6}$-NR_6-(CH_2)$_{0-6}$-C(O)-, -NR_6-(CH_2)$_{0-6}$-C(O)-(CH_2)$_{1-6}$-C(O)-(CH_2)$_{0-6}$-NR_7-, -NR_6-C(O)-NR_7-, -NR_6-C(NR_7)-NR_8-, -O-(CH_2)$_{1-6}$-NR_6-(CH_2)$_{1-6}$-S-, -S-(CH_2)$_{1-6}$-NR_6-(CH_2)$_{1-6}$-O-, -S-(CH_2)$_{1-6}$-NR_6-(CH_2)$_{1-6}$-S- and -NR_6-(CH_2)$_{1-6}$-S-(CH_2)$_{1-6}$-NR_7- (wherein R_6, R_7 and R_8 are independently selected from the group consisting of hydrogen, C_{1-8}alkyl, C_{1-8}alkoxy(C_{1-8})alkyl, carboxyl(C_{1-8})alkyl, amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), hydroxy(C_{1-8})alkyl, heterocyclyl(C_{1-8})alkyl, aryl(C_{1-8})alkyl and heteroaryl(C_{1-8})alkyl (wherein the foregoing heterocyclyl, aryl and heteroaryl substituents are optionally substituted with one to four substituents independently selected from the group consisting of C_{1-8}alkyl, C_{1-8}alkoxy, C_{1-8}alkoxy(C_{1-8})alkyl, carboxyl, carboxyl(C_{1-8})alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), halogen, (halo)$_{1-3}$(C_{1-8})alkyl, (halo)$_{1-3}$(C_{1-8})alkoxy, hydroxy and hydroxy(C_{1-8})alkyl; and, wherein heterocyclyl is optionally substituted with oxo); and, wherein R_9 is selected from the group consisting of C_{1-8}alkyl, C_{1-8}alkoxy(C_{1-8})alkyl, carboxyl(C_{1-8})alkyl, amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), hydroxy(C_{1-8})alkyl, heterocyclyl(C_{1-8})alkyl, aryl(C_{1-8})alkyl and heteroaryl(C_{1-8})alkyl (wherein the foregoing heterocyclyl, aryl and heteroaryl substituents are optionally

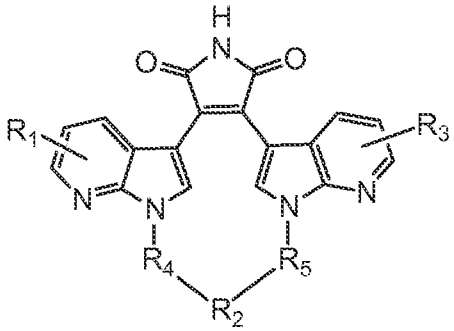
substituted with one to four substituents independently selected from the group consisting of C_{1-8}alkyl, C_{1-8}alkoxy, C_{1-8}alkoxy(C_{1-8})alkyl, carboxyl, carboxyl(C_{1-8})alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), halogen, (halo)$_{1-3}$(C_{1-8})alkyl, (halo)$_{1-3}$(C_{1-8})alkoxy, hydroxy and hydroxy(C_{1-8})alkyl; and, wherein heterocyclyl is optionally substituted with oxo)); and,

[0140] R_1 and R_3 are independently selected from the group consisting of hydrogen, C_{1-8}alkyl, C_{2-8}alkenyl, C_{2-8}alkynyl (wherein alkyl, alkenyl and alkynyl are optionally substituted with a substituent selected from the group consisting of C_{1-8}alkoxy, alkoxy(C_{1-8})alkyl, carboxyl, carboxyl(C_{1-8})alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), (halo)$_{1-3}$, (halo)$_{1-3}$(C_{1-8})alkyl, (halo)$_{1-3}$(C_{1-8})alkoxy, hydroxy, hydroxy(C_{1-8})alkyl and oxo), C_{1-8}alkoxy, C_{1-8}alkoxycarbonyl, (halo)$_{1-3}$(C_{1-8})alkoxy, C_{1-8}alkylthio, aryl, heteroaryl (wherein aryl and heteroaryl are optionally substituted with a substituent selected from the group consisting of C_{1-8}alkyl, C_{1-8}alkoxy, alkoxy(C_{1-8})alkyl, carboxyl, carboxyl(C_{1-8})alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), halogen, (halo)$_{1-3}$(C_{1-8})alkyl, (halo)$_{1-3}$(C_{1-8})alkoxy, hydroxy and hydroxy(C_{1-8})alkyl), amino (substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), cyano, halogen, hydroxy and nitro; and pharmaceutically acceptable salts thereof.

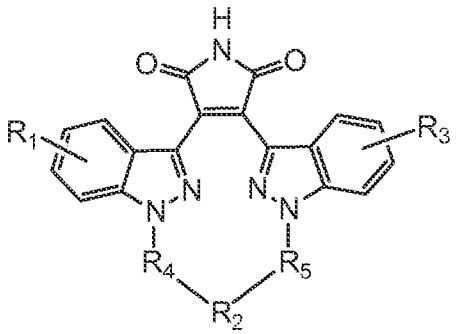
[0141] In one embodiment, a compound of Formula (III) is a compound selected from the group consisting of:



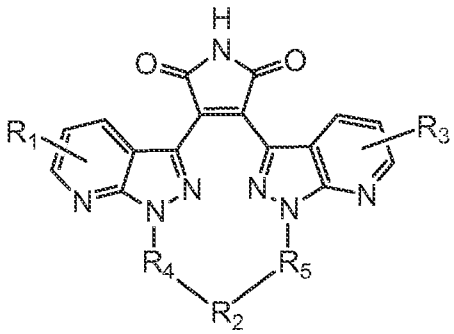
Formula (IIIa)



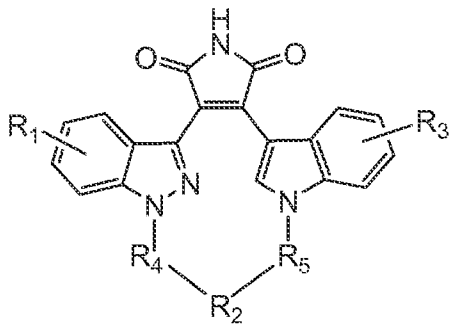
Formula (IIIb)



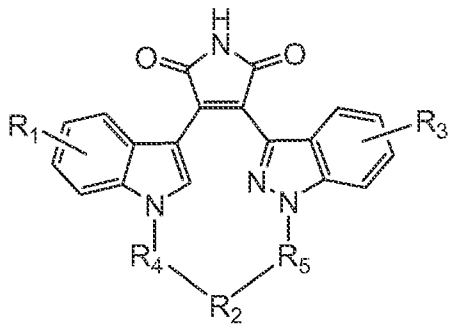
Formula (IIIc)



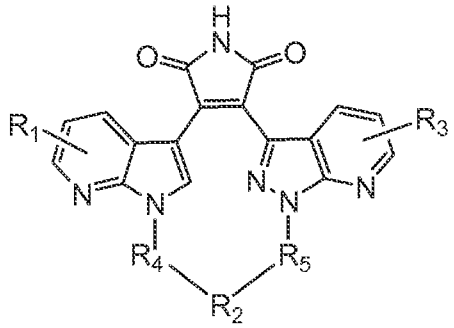
Formula (IIId)



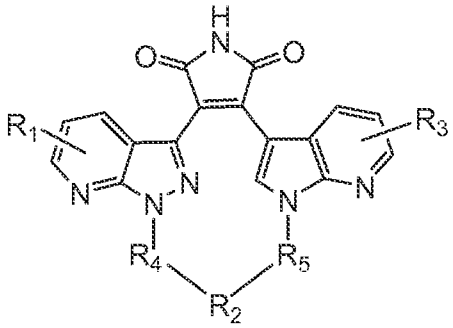
Formula (IIIe)



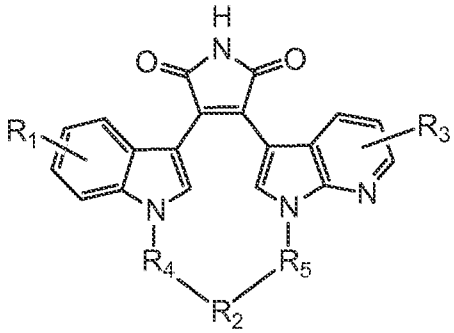
Formula (IIIf)



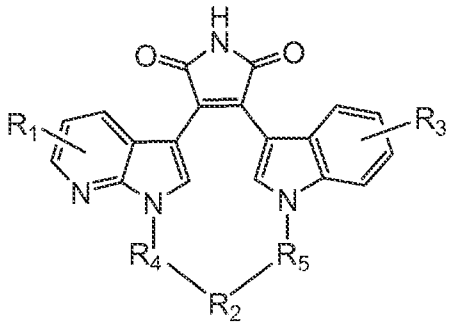
Formula (IIIg)



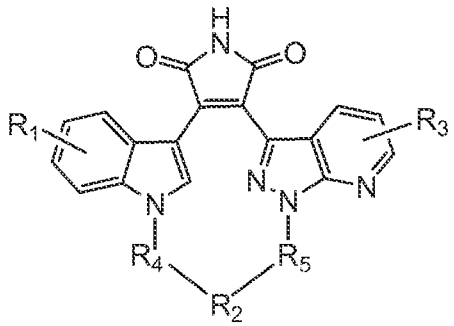
Formula (IIIh)



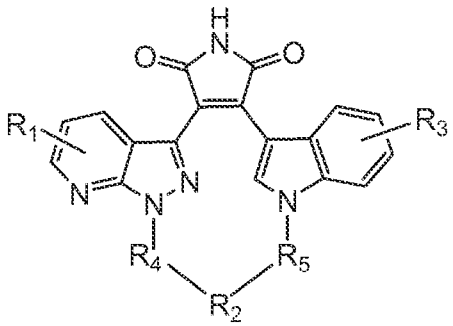
Formula (IIIi)



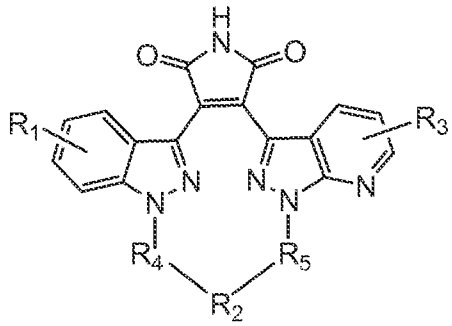
Formula (IIIj)



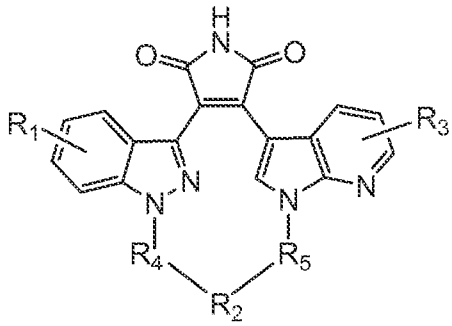
Formula (IIIk)



Formula (IIIi)



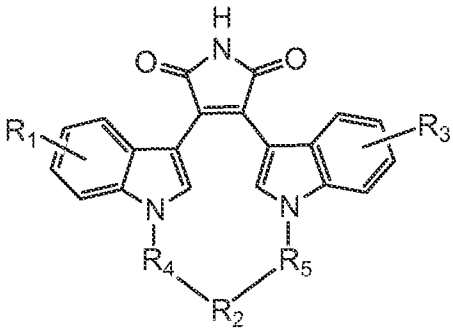
Formula (IIIm)



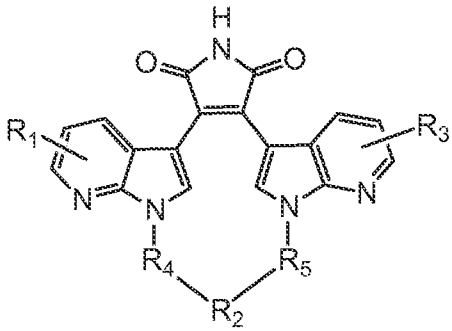
Formula (IIIn)

[0142] wherein all other variables are as previously defined; and, pharmaceutically acceptable salts thereof.

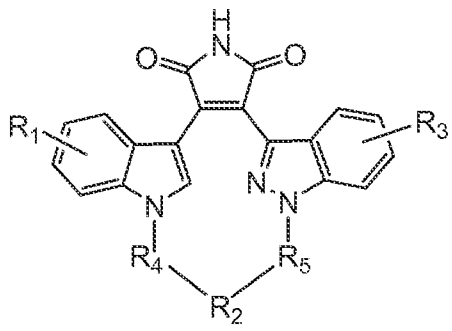
[0143] In one embodiment, a compound of Formula (III) is a compound selected from the group consisting of:



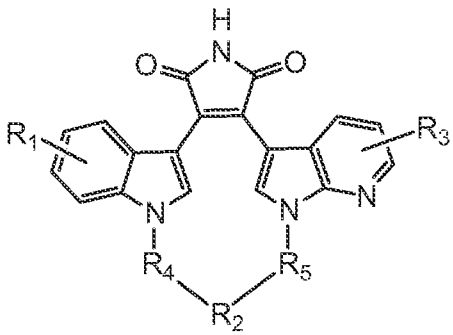
Formula (IIIa)



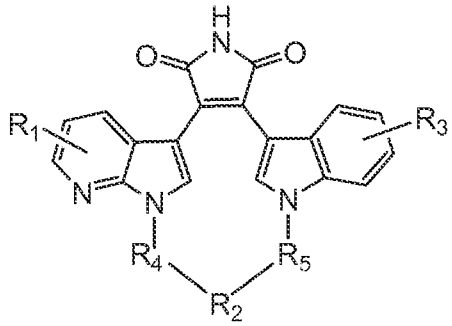
Formula (IIIb)



Formula (IIIf)



Formula (IIIi)



Formula (IIIj)

[0144] wherein all other variables are as previously defined; and, pharmaceutically acceptable salts thereof.

[0145] Compounds of Formula (III) are disclosed in commonly assigned United States Patent Number 6,828,327, the complete disclosure of which is herein incorporated by reference.

[0146] An example of the invention includes a compound of Formula (III) wherein the compound is selected from the group consisting of compounds listed in Table C, below:

Table C

Compounds of Formula (III)

Compound	Name
C-1	6,7,9,10,12,13,15,16-Octahydro-23H-5,26:17,22-di(metheno)dipyrido[2,3-k:3',2'-q]pyrrolo[3,4-n][1,4,7,10,19]trioxadiazacyclohenicosine-23,25(24H)-dione
C-2	10,11,13,14,16,17,19,20,22,23-Decahydro-1H-9,4:24,29-di(metheno)dipyrido[2,3-n:3',2'-t]pyrrolo[3,4-q][1,4,7,10,13,22]tetraoxadiazacyclotetracosine-1,3(2H)-dione
C-3	10,11,13,14,16,17,19,20,22,23,25,26-Dodecahydro-1H-9,4:27,32-di(metheno)dipyrido[2,3-q:3',2'-w]pyrrolo[3,4-t][1,4,7,10,13,16,25]pentaoxadiazacycloheptacosine-1,3(2H)-dione

C-4	6,7,9,10,12,13-Hexahydro-20H-5,23:14,19-di(metheno)dibenzo[h,n]pyrrolo[3,4-k][1,4,7,16]dioxadiazacyclooctadecine-20,22(21H)-dione
C-5	6,7,9,10,12,13,15,16-Octahydro-23H-5,26:17,22-di(metheno)dibenzo[k,q]pyrrolo[3,4-n][1,4,7,10,19]trioxadiazacyclohenicosine-23,25(24H)-dione
C-6	10,11,13,14,16,17,19,20,22,23-Decahydro-1H-9,4:24,29-di(metheno)dibenzo[n,t]pyrrolo[3,4-q][1,4,7,10,13,22]tetraoxadiazacyclotetracosine-1,3(2H)-dione
C-7	10,11,13,14,16,17,19,20,22,23,25,26-Dodecahydro-1H-9,4:27,32-di(metheno)dibenzo[q,w]pyrrolo[3,4-t][1,4,7,10,13,16,25]pentaoxadiazacycloheptacosine-1,3(2H)-dione
C-8	4,12,14,22-Tetraazaheptacyclo[20.6.1.1~7,14~.1~16,20~.0~2,6~.0~8,13~.0~23,28~]hentriaconta-1(29),2(6),7(31),8,10,12,16(30),17,19,23,25,27-dodecaene-3,5-dione (non-preferred name)
C-9	4,12,14,22,30-Pentaazaheptacyclo[20.6.1.1~7,14~.1~16,20~.0~2,6~.0~8,13~.0~23,28~]hentriaconta-1(29),2(6),7(31),8,10,12,16(30),17,19,23,25,27-dodecaene-3,5-dione (non-preferred name)
C-10	6,7,9,10,12,13-Hexahydro-20H-5,23:14,19-di(metheno)pyrido[2,3-k]pyrrolo[3,4-n][4,7,1,10]benzodioxadiazacyclooctadecine-20,22(21H)-dione

Table C - CONTINUED

Compound	Name
C-11	6,7,9,10,12,13,15,16-Octahydro-23H-5,26:17,22-di(metheno)pyrido[2,3-n]pyrrolo[3,4-q][4,7,10,1,13]benzotrioxadiazacyclohenicosine-23,25(24H)-dione
C-12	11-Ethyl-6,7,10,11,12,13,15,16-octahydro-9H,23H-5,26:17,22-di(metheno)dibenzo[k,q]pyrrolo[3,4-n][1,7,4,10,19]dioxatriazacyclohenicosine-23,25(24H)-dione
C-13	11-Methyl-6,7,10,11,12,13,15,16-octahydro-9H,23H-5,26:17,22-di(metheno)dibenzo[k,q]pyrrolo[3,4-n][1,7,4,10,19]dioxatriazacyclohenicosine-23,25(24H)-dione
C-14	11-(1-Methylethyl)-6,7,10,11,12,13,15,16-octahydro-9H,23H-5,26:17,22-di(metheno)dibenzo[k,q]pyrrolo[3,4-n][1,7,4,10,19]dioxatriazacyclohenicosine-23,25(24H)-dione

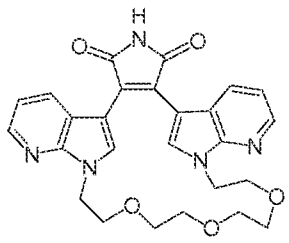
Table C - CONTINUED

Compound	Name
C-15	8,11,14-Trimethyl-7,8,9,10,11,12,13,14,15,16-decahydro-6H,23H-5,26:17,22-di(metheno)dibenzo[n,t]pyrrolo[3,4-q][1,4,7,10,13]pentaazacyclohenicosine-23,25(24H)-dione
C-16	11-Methyl-6,7,10,11,12,13,15,16-octahydro-9H,23H-5,26-(azeno)-17,22-(metheno)dibenzo[k,q]pyrrolo[3,4-n][1,7,4,10,19]dioxatriazacyclohenicosine-23,25(24H)-dione
C-17	11-Ethyl-6,7,10,11,12,13,15,16-octahydro-9H,23H-5,26-(azeno)-17,22-(metheno)dibenzo[k,q]pyrrolo[3,4-n][1,7,4,10,19]dioxatriazacyclohenicosine-23,25(24H)-dione
C-18	11-Ethyl-6,7,10,11,12,13,15,16-octahydro-9H,23H-5,26:17,22-di(metheno)dipyrido[2,3-k:3',2'-q]pyrrolo[3,4-n][1,7,4,10,19]dioxatriazacyclohenicosine-23,25(24H)-dione
C-19	6,7,9,10,12,13,15,16-Octahydro-23H-5,26:17,22-di(metheno)dipyrido[2,3-k:3',2'-q]pyrrolo[3,4-n][1,7,4,10,19]dioxathiadiazacyclohenicosine-23,25(24H)-dione
C-20	7,8,9,10,11,12,13,14,15,16-Decahydro-6H,23H-5,26:17,22-di(metheno)dipyrido[2,3-n:3',2'-t]pyrrolo[3,4-q][1,7,13]triazacyclohenicosine-23,25(24H)-dione
C-21	11-Ethyl-7,8,9,10,11,12,13,14,15,16-decahydro-6H,23H-5,26:17,22-di(metheno)dipyrido[2,3-n:3',2'-t]pyrrolo[3,4-q][1,7,13]triazacyclohenicosine-23,25(24H)-dione
C-22	6,7,8,9,10,11,12,13,14,15-Decahydro-22H-5,25:16,21-di(metheno)dipyrido[2,3-m:3',2'-s]pyrrolo[3,4-p][1,6,12]triazacycloicosine-22,24(23H)-dione
C-23	10-Ethyl-6,7,8,9,10,11,12,13,14,15-decahydro-22H-5,25:16,21-di(metheno)dipyrido[2,3-m:3',2'-s]pyrrolo[3,4-p][1,6,12]triazacycloicosine-22,24(23H)-dione
C-24	6,7,9,10-Tetrahydro-17H-5,20-(azeno)-11,16-(metheno)dibenzo[e,k]pyrrolo[3,4-h][1,4,13]oxadiazacyclopentadecine-17,19(18H)-dione
C-25	8,9,11,12,13,14,15,16-Octahydro-6H,23H-5,26:17,22-di(metheno)dipyrido[2,3-b:3',2'-h]pyrrolo[3,4-e][1,10]diazacyclohenicosine-10,23,25(7H,24H)-trione
C-26	8,9,11,12,13,14-Hexahydro-6H,21H-5,24:15,20-di(metheno)dipyrido[2,3-b:3',2'-h]pyrrolo[3,4-e][1,10]diazacyclononadecine-10,21,23(7H,22H)-trione

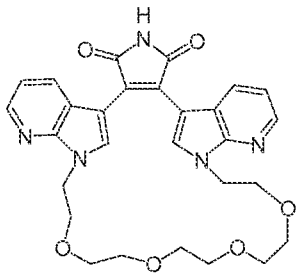
Table C - CONTINUED

Compound	Name
C-27	(7R,14R)-7,14-Dihydroxy-6,7,8,9,10,11,12,13,14,15-decahydro-22H-5,25:16,21-di(metheno)dipyrido[2,3-b:3',2'-h]pyrrolo[3,4-e][1,10]diazacycloicosine-22,24(23H)-dione
C-28	6,7,9,10,12,13-Hexahydro-20H-5,23:14,19-di(metheno)dipyrido[2,3-h:3',2'-n]pyrrolo[3,4-k][1,4,7,16]dioxadiazacyclooctadecine-20,22(21H)-dione
C-29	11-(2-Methoxyethyl)-6,7,10,11,12,13,15,16-octahydro-9H,23H-5,26-(azeno)-17,22-(metheno)dibenzo[k,q]pyrrolo[3,4-n][1,7,4,10,19]dioxatriazacyclohenicosine-23,25(24H)-dione
C-30	11-(2-Hydroxyethyl)-6,7,10,11,12,13,15,16-octahydro-9H,23H-5,26:17,22-di(metheno)dibenzo[k,q]pyrrolo[3,4-n][1,7,4,10,19]dioxatriazacyclohenicosine-23,25(24H)-dione
C-31	14-Methyl-6,7,9,10,12,13,14,15,16,17-decahydro-24H-5,27:18,23-di(metheno)dibenzo[l,r]pyrrolo[3,4-o][1,4,7,11,20]dioxatriazacyclodocosine-24,26(25H)-dione

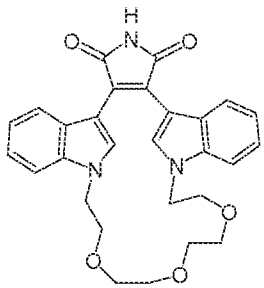
[0147] An example of the invention includes a compound of Formula (III) wherein the compound is selected from the group consisting of:



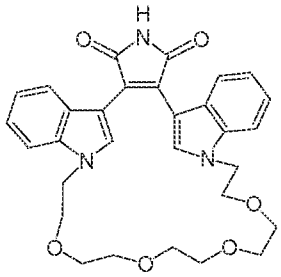
Compound C-1



Compound C-2



Compound C-5



Compound C-6

[0148] Other examples of the invention include a compound selected from the group consisting of the compounds listed in Table D, below:

Table D

Additional Compounds

Compound	Name
D-1a	11-Ethyl-6,7,10,11,12,13,15,16-octahydro-9H,23H-5,26:17,22-di(metheno)dibenzo[k,q]pyrrolo[3,4-n][1,7,4,10,19]dioxatriazacyclohenicosine-23,25(24H)-dione
D-2a	3-(1-{3-[(2-Hydroxyethyl)(methyl)amino]propyl}-1H-indazol-3-yl)-4-(1-pyridin-3-yl-1H-indol-3-yl)-1H-pyrrole-2,5-dione
D-3a	3,5-Dichloro-N-[3-chloro-4-(3,4,12,12a-tetrahydro-1H-[1,4]thiazino[3,4-c][1,4]benzodiazepin-11(6H)-ylcarbonyl)phenyl]benzamide
D-4a	3-[1-(2-Hydroxyethyl)-1H-indol-3-yl]-4-(1-pyridin-3-yl-1H-indol-3-yl)-1H-pyrrole-2,5-dione

Table D - CONTINUED

Compound	Name
D-5a	3-[2-(Methyloxy)phenyl]-4-(1-pyridin-3-yl-1H-indol-3-yl)-1H-pyrrole-2,5-dione
D-6a	6-[(2-{[4-(2,4-Dichlorophenyl)-5-(4-methyl-1H-imidazol-2-yl)pyrimidin-2-yl]amino}ethyl)amino]pyridine-3-carbonitrile
D-7a	3-(5-Chloro-1-methyl-1H-indol-3-yl)-4-{1-[3-(1H-imidazol-1-yl)propyl]-1H-indazol-3-yl}-1H-pyrrole-2,5-dione

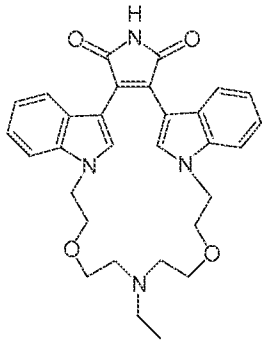
Table D - CONTINUED

Compound	Name
D-8a	3-(5-Chloro-1-methyl-1H-indol-3-yl)-4-{1-[3-(1H-1,2,3-triazol-1-yl)propyl]-1H-indazol-3-yl}-1H-pyrrole-2,5-dione
D-9a	3-[1-(3-Hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-4-(1-methyl-1H-pyrazol-3-yl)-1H-pyrrole-2,5-dione
D-10a	N-[3-(3-{4-[1-(1-Benzothien-3-yl)-1H-indol-3-yl]-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl}-1H-indazol-1-yl)propyl]sulfamide
D-11a	3-[1-(3-Hydroxy-3-methylbutyl)-1H-indazol-3-yl]-4-(1-pyridin-3-yl-1H-indol-3-yl)-1H-pyrrole-2,5-dione
D-12a	3-[1-(2-Hydroxyethyl)-1H-indazol-3-yl]-4-(1-pyrimidin-5-yl-1H-indol-3-yl)-1H-pyrrole-2,5-dione
D-13a	3-[1-(2-Hydroxyethyl)-1H-indol-3-yl]-4-(1-pyrimidin-5-yl-1H-indol-3-yl)-1H-pyrrole-2,5-dione
D-14a	(11Z)-8,9,10,13,14,15-Hexahydro-2,6:17,21-di(metheno)pyrrolo[3,4-h][1,15,7]dioxazacyclotricosine-22,24(1H,23H)-dione
D-15a	3-(5-Chloro-1-pyridin-3-yl-1H-indol-3-yl)-4-[1-(3-hydroxypropyl)-1H-indazol-3-yl]-1H-pyrrole-2,5-dione
D-16a	3-[2-(Methyloxy)phenyl]-4-{1-[3-(methyloxy)propyl]-1H-pyrrolo[3,2-c]pyridin-3-yl}-1H-pyrrole-2,5-dione
D-17a	3-[1-(3-Hydroxypropyl)-1H-indazol-3-yl]-4-[1-(tetrahydro-2H-pyran-4-yl)-1H-indol-3-yl]-1H-pyrrole-2,5-dione
D-18a	2-{3-[4-(5-Chloro-1-methyl-1H-indol-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-1H-indazol-1-yl}-N-(2-hydroxyethyl)acetamide
D-19a	4-(3-Chlorophenyl)-6-[3-(dimethylamino)propyl]-5,6-dihydropyrrolo[3',4':5,6]pyrido[3,4-b]indole-1,3(2H,4H)-dione
D-20a	14-Ethyl-6,7,9,10,13,14,15,16-octahydro-12H,23H-5,26:17,22-di(metheno)dibenzo[k,q]pyrrolo[3,4-n][1,4,7,10,19]dioxatriazacyclohenicosine-23,25(24H)-dione
D-21a	14-(Phenylmethyl)-6,7,9,10,13,14,15,16-octahydro-12H,23H-5,26:17,22-di(metheno)dibenzo[k,q]pyrrolo[3,4-n][1,4,7,10,19]dioxatriazacyclohenicosine-23,25(24H)-dione
D-22a	3-[1-(2-Hydroxyethyl)-1H-indol-3-yl]-4-{1-[2-({2-[(2-hydroxyethyl)oxy]ethyl}oxy)ethyl]-1H-indol-3-yl}-1H-pyrrole-2,5-dione

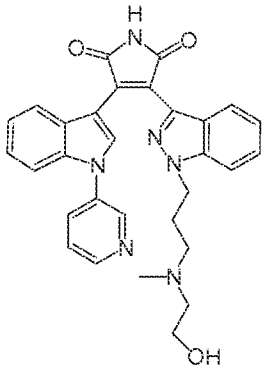
Table D - CONTINUED

Compound	Name
D-23a	8,11-Dimethyl-6,7,8,9,10,11,12,13-octahydro-20H-5,23:14,19-di(metheno)dibenzo[k,q]pyrrolo[3,4-n][1,4,7,10]tetraazacyclooctadecine-20,22(21H)-dione
D-24a	12,21-Dimethyl-11,12,13,14,16,17,20,21,22,23-decahydro-1H,10H,19H-9,4:24,29-di(metheno)dibenzo[k,q]pyrrolo[3,4-n][1,4,7,10,19,22]dioxatetraazacyclotetracosine-1,3(2H)-dione
D-25a	14-(Furan-2-ylmethyl)-6,7,9,10,13,14,15,16-octahydro-12H,23H-5,26:17,22-di(metheno)dibenzo[k,q]pyrrolo[3,4-n][1,4,7,10,19]dioxatriazacyclohenicosine-23,25(24H)-dione
D-26a	14-(2-Thienylmethyl)-6,7,9,10,13,14,15,16-octahydro-12H,23H-5,26:17,22-di(metheno)dibenzo[k,q]pyrrolo[3,4-n][1,4,7,10,19]dioxatriazacyclohenicosine-23,25(24H)-dione
D-27a	14-(Naphthalen-1-ylmethyl)-6,7,9,10,13,14,15,16-octahydro-12H,23H-5,26:17,22-di(metheno)dibenzo[k,q]pyrrolo[3,4-n][1,4,7,10,19]dioxatriazacyclohenicosine-23,25(24H)-dione
D-28a	14-(Pyridin-4-ylmethyl)-6,7,9,10,13,14,15,16-octahydro-12H,23H-5,26:17,22-di(metheno)dibenzo[k,q]pyrrolo[3,4-n][1,4,7,10,19]dioxatriazacyclohenicosine-23,25(24H)-dione
D-29a	3-{1-[2-(1,2,3,4-Tetrahydronaphthalen-1-ylamino)ethyl]-1H-indol-3-yl}-4-(1-{2-[(2-{[2-(1,2,3,4-tetrahydronaphthalen-1-ylamino)ethyl]oxy}ethyl)oxy]ethyl}-1H-indol-3-yl)-1H-pyrrole-2,5-dione
D-30a	3-{1-[3-(Dimethylamino)phenyl]-1H-indol-3-yl}-4-[1-(2-hydroxyethyl)-1H-indazol-3-yl]-1H-pyrrole-2,5-dione
D-31a	3-{5-Chloro-1-[6-(dimethylamino)pyridin-3-yl]-1H-indol-3-yl}-4-[1-(2-hydroxyethyl)-1H-indazol-3-yl]-1H-pyrrole-2,5-dione
D-32a	Methyl 5-(5-chloro-3-{4-[1-(2-hydroxyethyl)-1H-indazol-3-yl]-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl}-1H-indol-1-yl)pyridine-3-carboxylate

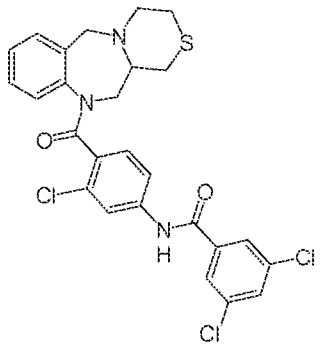
[0149] Other examples of the invention include a compound selected from the group consisting of:



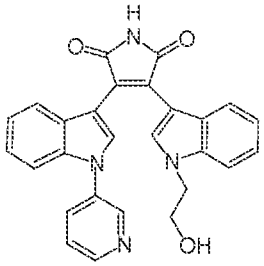
Compound D-1a



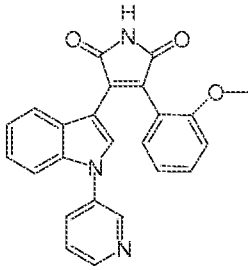
Compound D-2a



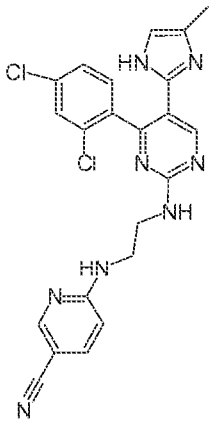
Compound D-3a



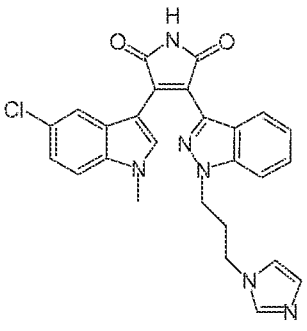
Compound D-4a



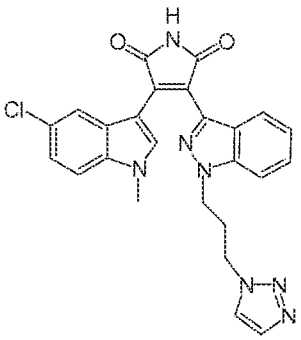
Compound D-5a



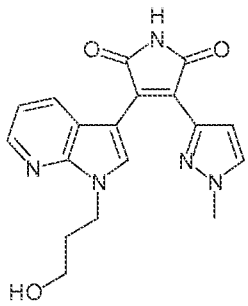
Compound D-6a



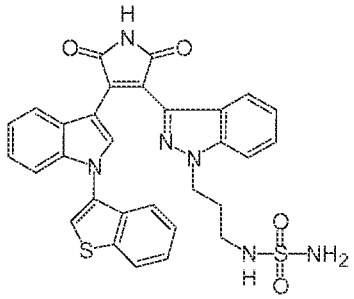
Compound D-7a



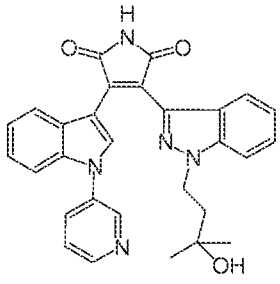
Compound D-8a



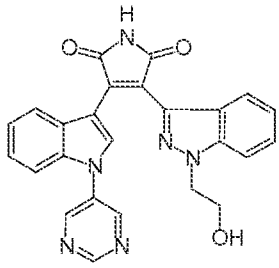
Compound D-9a



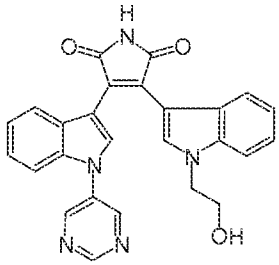
Compound D-10a



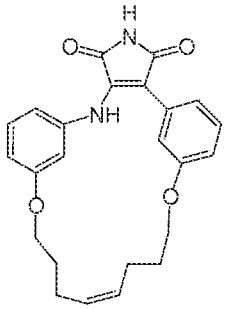
Compound D-11a



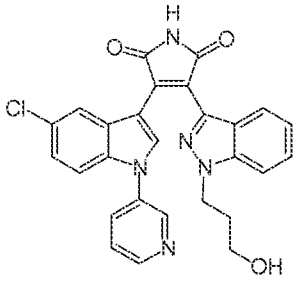
Compound D-12a



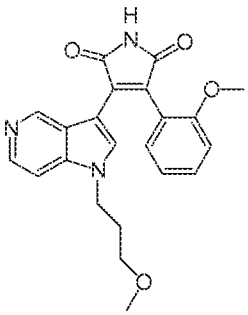
Compound D-13a



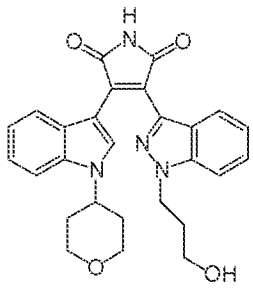
Compound D-14a



Compound D-15a



Compound D-16a



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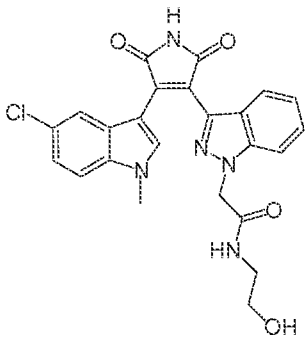

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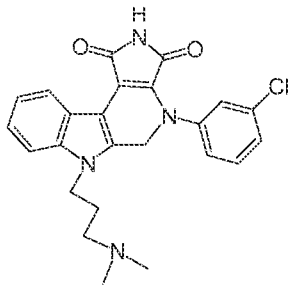

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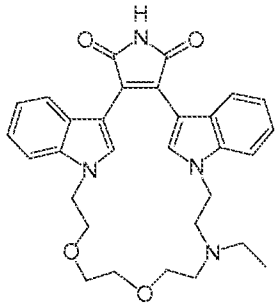

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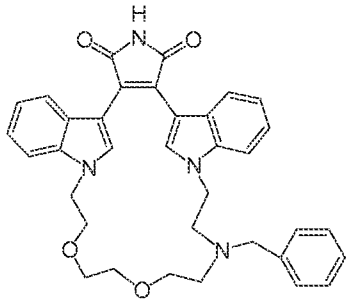

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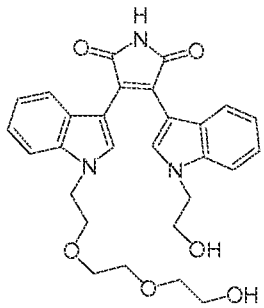

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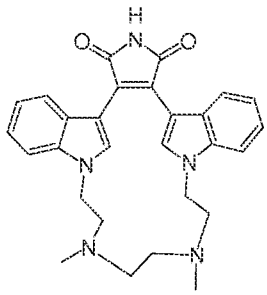

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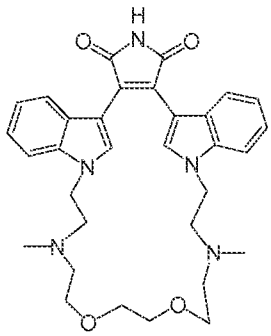

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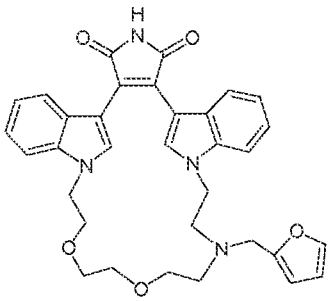

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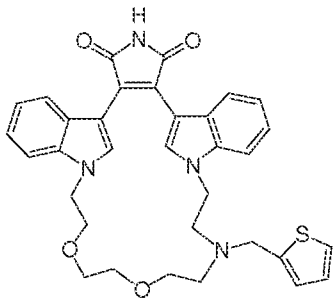

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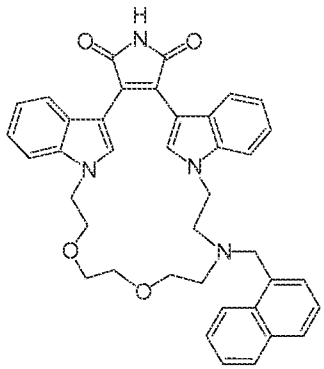

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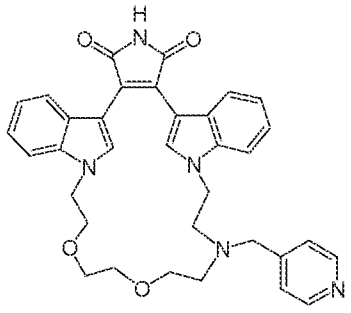

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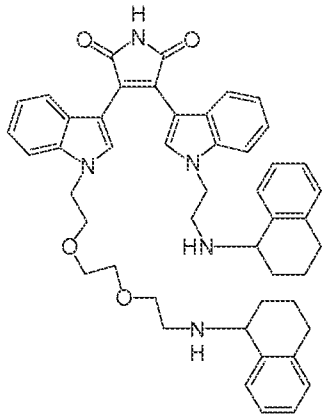

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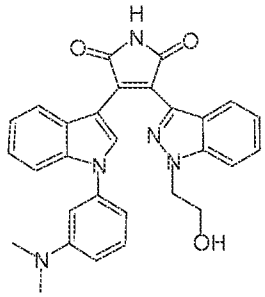

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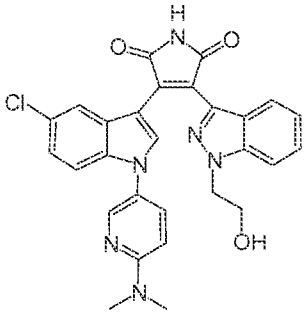
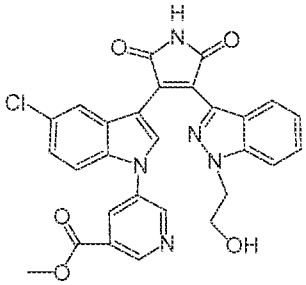


Compound D-31a



Compound D-32a

Cells suitable for treatment according to the methods of the present invention

[0150] Pluripotent cells, suitable for use in the present invention express at least one of the following pluripotency markers selected from the group consisting of: ABCG2, cripto, FoxD3, Connexin43, Connexin45, Oct4, SOX-2, Nanog, hTERT, UTF-1, ZFP42, SSEA-3, SSEA-4, Tra1-60, and Tra1-81.

[0151] In one embodiment, the pluripotent cells are embryonic stem cells. In an alternate embodiment, the pluripotent cells are cells expressing pluripotency markers derived from embryonic stem cells. In one embodiment, the embryonic stem cells are human.

Isolation, expansion and culture of human embryonic stem cells

[0152] *Characterization of human embryonic stem cells:* Human embryonic stem cells may express one or more of the stage-specific embryonic antigens (SSEA) 3 and 4, and markers detectable using antibodies designated Tra-1-60 and Tra-1-81 (Thomson *et al.*, Science 282:1145, 1998). Differentiation of human embryonic stem cells *in vitro* results in the loss of SSEA-4, Tra- 1-60, and Tra-1-81 expression (if present) and increased expression of SSEA-1. Undifferentiated human embryonic stem cells typically have alkaline phosphatase activity, which can be detected by fixing the cells with 4% paraformaldehyde, and then developing with Vector Red as a substrate, as described by the manufacturer (Vector Laboratories, Burlingame Calif.) Undifferentiated pluripotent stem cells also typically express Oct-4 and TERT, as detected by RT-PCR.

[0153] Another desirable phenotype of propagated human embryonic stem cells is a potential to differentiate into cells of all three germinal layers: endoderm, mesoderm, and ectoderm tissues. Pluripotency of human embryonic stem cells can be confirmed, for example, by injecting cells into SCID mice, fixing the teratomas that form using 4% paraformaldehyde, and then examining them histologically for evidence of cell types from the three germ layers. Alternatively, pluripotency may be determined by the creation of embryoid bodies and assessing the embryoid bodies for the presence of markers associated with the three germinal layers.

[0154] Propagated human embryonic stem cell lines may be karyotyped using a standard G-banding technique and compared to published karyotypes of the corresponding primate species. It is desirable to obtain cells that have a "normal karyotype", which means that the cells are euploid, wherein all human chromosomes are present and not noticeably altered.

[0155] *Sources of human embryonic stem cells:* Types of human embryonic stem cells that may be used include established lines of human embryonic cells derived from tissue formed after gestation, including pre-embryonic tissue (such as, for example, a blastocyst), embryonic tissue, or fetal tissue taken any

time during gestation, typically but not necessarily before approximately 10-12 weeks gestation. Non-limiting examples are established lines of human embryonic stem cells or human embryonic germ cells, such as, for example the human embryonic stem cell lines H1, H7, and H9 (WiCell). Also contemplated is use of the compositions of this disclosure during the initial establishment or stabilization of such cells, in which case the source cells would be primary pluripotent cells taken directly from the source tissues. Also suitable are cells taken from a pluripotent stem cell population already cultured in the absence of feeder cells. Also suitable are mutant human embryonic stem cell lines, such as, for example, BG01v (BresaGen, Athens, GA).

[0156] In one embodiment, Human embryonic stem cells are prepared as described by Thomson *et al.* (U.S. Pat. No. 5,843,780; Science 282:1145, 1998; Curr. Top. Dev. Biol. 38:133 ff., 1998; Proc. Natl. Acad. Sci. U.S.A. 92:7844, 1995).

[0157] *Culture of human embryonic stem cells:* In one embodiment, human embryonic stem cells are cultured in a culture system that is essentially free of feeder cells, but nonetheless supports proliferation of human embryonic stem cells without undergoing substantial differentiation. The growth of human embryonic stem cells in feeder-free culture without differentiation is supported using a medium conditioned by culturing previously with another cell type. Alternatively, the growth of human embryonic stem cells in feeder-free culture without differentiation is supported using a chemically defined medium.

[0158] In an alternate embodiment, human embryonic stem cells are initially cultured layer of feeder cells that support the human embryonic stem cells in various ways. The human embryonic are then transferred to a culture system that is essentially free of feeder cells, but nonetheless supports proliferation of human embryonic stem cells without undergoing substantial differentiation.

[0159] Examples of conditioned media suitable for use in the present invention are disclosed in US20020072117, US6642048, WO2005014799, and Xu *et al* (Stem Cells 22: 972-980, 2004).

[0160] An example of a chemically defined medium suitable for use in the present invention may be found in US20070010011.

[0161] Suitable culture media may be made from the following components, such as, for example, Dulbecco's modified Eagle's medium (DMEM), Gibco # 11965-092; Knockout Dulbecco's modified Eagle's medium (KO DMEM), Gibco # 10829-018; Ham's F12/50% DMEM basal medium; 200 mM L-glutamine, Gibco # 15039-027; non-essential amino acid solution, Gibco 11140-050; β-mercaptoethanol, Sigma # M7522; human recombinant basic fibroblast growth factor (bFGF), Gibco # 13256-029.

[0162] In one embodiment, the human embryonic stem cells are plated onto a suitable culture substrate that is treated prior to treatment according to the methods of the present invention. In one embodiment, the treatment is an extracellular matrix component, such as, for example, those derived from basement membrane or that may form part of adhesion molecule receptor-ligand couplings. In one embodiment, a the suitable culture substrate is Matrigel® (Becton Dickenson). Matrigel® is a soluble preparation from Engelbreth-Holm-Swarm tumor cells that gels at room temperature to form a reconstituted basement membrane.

[0163] Other extracellular matrix components and component mixtures are suitable as an alternative. This may include laminin, fibronectin, proteoglycan, entactin, heparan sulfate, and the like, alone or in various combinations.

[0164] The human embryonic stem cells are plated onto the substrate in a suitable distribution and in the presence of a medium that promotes cell survival, propagation, and retention of the desirable characteristics. All these characteristics benefit from careful attention to the seeding distribution and can readily be determined by one of skill in the art.

Isolation, expansion and culture of cells expressing pluripotency markers that are derived from human embryonic stem cells

[0165] In one embodiment, cells expressing pluripotency markers are derived from human embryonic stem cells by a method comprising the steps of:

a. Culturing human embryonic stem cells,

b. Differentiating the human embryonic stem cells into cells expressing markers characteristic of definitive endoderm cells, and

c. Removing the cells, and subsequently culturing them under hypoxic conditions, on a tissue culture substrate that is not pre-treated with a protein or an extracellular matrix prior to culturing the cells.

[0166] In one embodiment, cells expressing pluripotency markers are derived from human embryonic stem cells by a method comprising the steps of:

a. Culturing human embryonic stem cells, and

b. Removing the cells, and subsequently culturing them under hypoxic conditions, on a tissue culture substrate that is not pre-treated with a protein or an extracellular matrix.

Cell culture under hypoxic conditions on a tissue culture substrate that is not pre-treated with a protein or an extracellular matrix

[0167] In one embodiment, the cells are cultured under hypoxic conditions, on a tissue culture substrate that is not coated with an extracellular matrix for about 1 to about 20 days. In an alternate embodiment, the cells are cultured under hypoxic conditions, on a tissue culture substrate that is not coated with an extracellular matrix for about 5 to about 20 days. In an alternate embodiment, the cells are cultured under hypoxic conditions, on a tissue culture substrate that is not coated with an extracellular matrix for about 15 days.

[0168] In one embodiment, the hypoxic condition is about 1% O_2 to about 20% O_2. In an alternate embodiment, the hypoxic condition is about 2% O_2 to about 10% O_2. In an alternate embodiment, the hypoxic condition is about 3% O_2.

[0169] The cells may be cultured, under hypoxic conditions on a tissue culture substrate that is not pre-treated with a protein or an extracellular matrix, in medium containing serum, activin A, and a Wnt ligand. Alternatively, the medium may also contain IGF-1.

[0170] The culture medium may have a serum concentration in the range of about 2% to about 5%. In an alternate embodiment, the serum concentration may be about 2%.

[0171] Activin A may be used at a concentration from about 1pg/ml to about 100µg/ml. In an alternate embodiment, the concentration may be about 1pg/ml to about 1µg/ml. In another alternate embodiment, the concentration may be about 1pg/ml to about 100ng/ml. In another alternate embodiment, the concentration may be about 50ng/ml to about 100ng/ml. In another alternate embodiment, the concentration may be about 100ng/ml.

[0172] The Wnt ligand may be selected from the group consisting of Wnt-1, Wnt-3a, Wnt-5a and Wnt-7a. In one embodiment, the Wnt ligand is Wnt-1. In an alternate embodiment, the Wnt ligand is Wnt-3a.

[0173] The Wnt ligand may be used at a concentration of about 1ng/ml to about 1000ng/ml. In an alternate embodiment, the Wnt ligand may be used at a concentration of about 10ng/ml to about 100ng/ml. In one embodiment, the concentration of the Wnt ligand is about 20ng/ml.

[0174] IGF-1 may be used at a concentration of about 1ng/ml to about 100ng/ml. In an alternate embodiment, the IGF-1may be used at a concentration of about 10ng/ml to about 100ng/ml. In one embodiment, the concentration of IGF-1 is about 50ng/ml.

[0175] The cells expressing pluripotency markers derived by the methods of the present invention are capable of expansion in culture under hypoxic conditions, on tissue culture substrate that is not pre-treated with a protein or an extracellular matrix.

[0176] The cells expressing pluripotency markers derived by the methods of the present invention express at least one of the following pluripotency markers selected from the group consisting of: ABCG2, cripto, FoxD3, Connexin43, Connexin45, Oct4, SOX-2, Nanog, hTERT, UTF-1, ZFP42, SSEA-3, SSEA-4, Tra1-60, and Tra1-81.

Further differentiation of cells expressing markers characteristic of the definitive endoderm lineage

[0177] Cells expressing markers characteristic of the definitive endoderm lineage may be differentiated into cells expressing markers characteristic of the pancreatic endoderm lineage by any method in the art.

[0178] For example, cells expressing markers characteristic of the definitive endoderm lineage may be differentiated into cells expressing markers characteristic of the pancreatic endoderm lineage according to the methods disclosed in D'Amour *et al*, Nature Biotechnology 24, 1392 - 1401 (2006).

[0179] For example, cells expressing markers characteristic of the definitive endoderm lineage are further differentiated into cells expressing markers characteristic of the pancreatic endoderm lineage, by treating the cells expressing markers characteristic of the definitive endoderm lineage with a fibroblast growth factor and KAAD-cyclopamine, then removing the medium containing the fibroblast growth factor and KAAD-cyclopamine and subsequently culturing the cells in medium containing retinoic acid, a fibroblast growth factor and KAAD-cyclopamine. An example of this method is disclosed in D' Amour et al, Nature Biotechnology, 24: 1392-1401, (2006).

[0180] Markers characteristic of the pancreatic endoderm lineage are selected from the group consisting of Pdx1, HNF-1beta, PTF1a, HNF-6, HB9 and PROX1. Suitable for use in the present invention is a cell that expresses at least one of the markers characteristic of the pancreatic endoderm lineage. In one aspect of the present invention, a cell expressing markers characteristic of the pancreatic endoderm lineage is a pancreatic endoderm cell.

Further differentiation of cells expressing markers characteristic of the pancreatic endoderm lineage

[0181] Cells expressing markers characteristic of the pancreatic endoderm lineage may be differentiated into cells expressing markers characteristic of the pancreatic endocrine lineage by any method in the art.

[0182] For example, cells expressing markers characteristic of the pancreatic endoderm lineage may be differentiated into cells expressing markers characteristic of the pancreatic endocrine lineage according to the methods disclosed in D'Amour *et al*, Nature Biotechnology 24, 1392 - 1401 (2006).

[0183] Markers characteristic of the pancreatic endocrine lineage are selected from the group consisting of NGN-3, NeuroD, Islet-1, Pdx-1, NKX6.1, Pax-4, Ngn-3, and PTF-1 alpha. In one embodiment, a pancreatic endocrine cell is capable of expressing at least one of the following hormones: insulin, glucagon, somatostatin, and pancreatic polypeptide. Suitable for use in the present invention is a cell that expresses at least one of the markers characteristic of the pancreatic endocrine lineage. In one aspect of the present invention, a cell expressing markers characteristic of the pancreatic endocrine lineage is a pancreatic endocrine cell. The pancreatic endocrine cell may be a pancreatic hormone expressing cell. Alternatively, the pancreatic endocrine cell may be a pancreatic hormone secreting cell.

[0184] In one aspect of the present invention, the pancreatic endocrine cell is a cell expressing markers characteristic of the β cell lineage. A cell expressing markers characteristic of the β cell lineage expresses Pdx1 and at least one of the following transcription factors: NGN-3, Nkx2.2, Nkx6.1, NeuroD, Isl-1, HNF-3 beta, MAFA, Pax4, and Pax6. In one aspect of the present invention, a cell expressing markers characteristic of the β cell lineage is a β cell.

Detection of cells expressing markers characteristic of the definitive endoderm linage

[0185] Formation of cells expressing markers characteristic of the definitive endoderm lineage may be determined by testing for the presence of the markers before and after following a particular protocol. Pluripotent stem cells typically do not express such markers. Thus, differentiation of pluripotent cells is detected when cells begin to express them.

[0186] The efficiency of differentiation may be determined by exposing a treated cell population to an agent (such as an antibody) that specifically recognizes a

protein marker expressed by cells expressing markers characteristic of the definitive endoderm lineage.

[0187] Methods for assessing expression of protein and nucleic acid markers in cultured or isolated cells are standard in the art. These include quantitative reverse transcriptase polymerase chain reaction (RT-PCR), Northern blots, *in situ* hybridization (see, e.g., Current Protocols in Molecular Biology (Ausubel *et al.*, eds. 2001 supplement)), and immunoassays such as immunohistochemical analysis of sectioned material, Western blotting, and for markers that are accessible in intact cells, flow cytometry analysis (FACS) (see, e.g., Harlow and Lane, Using Antibodies: A Laboratory Manual, New York: Cold Spring Harbor Laboratory Press (1998)).

[0188] Examples of antibodies useful for detecting certain protein markers are listed in **Table IA** and **Table IB.** It should be noted that alternate antibodies directed to the same markers that are recognized by the antibodies listed in **Table IA** and **Table IB** are available, or can be readily developed. Such alternate antibodies can also be employed for assessing expression of markers in the cells isolated in accordance with the present invention.

[0189] For example, characteristics of pluripotent stem cells are well known to those skilled in the art, and additional characteristics of pluripotent stem cells continue to be identified. Pluripotent stem cell markers include, for example, the expression of one or more of the following: ABCG2, cripto, FoxD3, Connexin43, Connexin45, Oct4, Sox2, Nanog, hTERT, UTF-1, ZFP42, SSEA-3, SSEA-4, Tra1-60, Tra1-81.

[0190] After treating pluripotent stem cells with the methods of the present invention, the differentiated cells may be purified by exposing a treated cell population to an agent (such as an antibody) that specifically recognizes a protein marker, such as CXCR4, expressed by cells expressing markers characteristic of the definitive endoderm lineage.

Detection of cells expressing markers characteristic of the pancreatic endoderm linage

[0191] Markers characteristic of the pancreatic endoderm lineage are well known to those skilled in the art, and additional markers characteristic of the pancreatic endoderm lineage continue to be identified. These markers can be used to confirm that the cells treated in accordance with the present invention have differentiated to acquire the properties characteristic of the pancreatic endoderm lineage. Pancreatic endoderm lineage specific markers include the expression of one or more transcription factors such as, for example, Hlxb9, PTF-1a, PDX-1, HNF-6, HNF-1beta.

[0192] The efficiency of differentiation may be determined by exposing a treated cell population to an agent (such as an antibody) that specifically recognizes a protein marker expressed by cells expressing markers characteristic of the pancreatic endoderm lineage.

[0193] Methods for assessing expression of protein and nucleic acid markers in cultured or isolated cells are standard in the art. These include quantitative reverse transcriptase polymerase chain reaction (RT-PCR), Northern blots, *in situ* hybridization (see, e.g., Current Protocols in Molecular Biology (Ausubel *et al.*, eds. 2001 supplement)), and immunoassays such as immunohistochemical analysis of sectioned material, Western blotting, and for markers that are accessible in intact cells, flow cytometry analysis (FACS) (see, e.g., Harlow and Lane, Using Antibodies: A Laboratory Manual, New York: Cold Spring Harbor Laboratory Press (1998)).

[0194] Examples of antibodies useful for detecting certain protein markers are listed in **Table IA** and **Table IB.** It should be noted that alternate antibodies directed to the same markers that are recognized by the antibodies listed in **Table IA** and **Table IB** are available, or can be readily developed. Such alternate antibodies can also be employed for assessing expression of markers in the cells isolated in accordance with the present invention.

Detection of cells expressing markers characteristic of the pancreatic endocrine linage

[0195] Markers characteristic of cells of the pancreatic endocrine lineage are well known to those skilled in the art, and additional markers characteristic of the pancreatic endocrine lineage continue to be identified. These markers can be used to confirm that the cells treated in accordance with the present invention have differentiated to acquire the properties characteristic of the pancreatic endocrine lineage. Pancreatic endocrine lineage specific markers include the expression of one or more transcription factors such as, for example, NGN-3, NeuroD, Islet-1.

[0196] Markers characteristic of cells of the β cell lineage are well known to those skilled in the art, and additional markers characteristic of the β cell lineage continue to be identified. These markers can be used to confirm that the cells treated in accordance with the present invention have differentiated to acquire the properties characteristic of the β-cell lineage. β cell lineage specific characteristics include the expression of one or more transcription factors such as, for example, Pdx1 (pancreatic and duodenal homeobox gene-1), Nkx2.2, Nkx6.1, Isl1, Pax6, Pax4, NeuroD, Hnf1b, Hnf-6, Hnf-3beta, and MafA, among others. These transcription factors are well established in the art for identification of endocrine cells. See, e.g., Edlund (Nature Reviews Genetics 3: 524-632 (2002)).

[0197] The efficiency of differentiation may be determined by exposing a treated cell population to an agent (such as an antibody) that specifically recognizes a protein marker expressed by cells expressing markers characteristic of the pancreatic endocrine lineage. Alternatively, the efficiency of differentiation may be determined by exposing a treated cell population to an agent (such as an antibody) that specifically recognizes a protein marker expressed by cells expressing markers characteristic of the β cell lineage.

[0198] Methods for assessing expression of protein and nucleic acid markers in cultured or isolated cells are standard in the art. These include quantitative reverse transcriptase polymerase chain reaction (RT-PCR), Northern blots, *in*

situ hybridization (see, e.g., Current Protocols in Molecular Biology (Ausubel *et al.*, eds. 2001 supplement)), and immunoassays such as immunohistochemical analysis of sectioned material, Western blotting, and for markers that are accessible in intact cells, flow cytometry analysis (FACS) (see, e.g., Harlow and Lane, Using Antibodies: A Laboratory Manual, New York: Cold Spring Harbor Laboratory Press (1998)).

[0199] Examples of antibodies useful for detecting certain protein markers are listed in **Table IA** and **Table IB.** It should be noted that alternate antibodies directed to the same markers that are recognized by the antibodies listed in **Table IA** and **Table IB** are available, or can be readily developed. Such alternate antibodies can also be employed for assessing expression of markers in the cells isolated in accordance with the present invention.

[0200] The present invention is further illustrated, but not limited by, the following examples.

Example 1

Human Embryonic Stem Cell Culture

[0201] Stem cells are undifferentiated cells defined by their ability at the single cell level to both self-renew and differentiate to produce progeny cells, including self-renewing progenitors, non-renewing progenitors, and terminally differentiated cells. Stem cells are also characterized by their ability to differentiate *in vitro* into functional cells of various cell lineages from multiple germ layers (endoderm, mesoderm and ectoderm), as well as to give rise to tissues of multiple germ layers following transplantation and to contribute substantially to most, if not all, tissues following injection into blastocysts.

[0202] The human embryonic stem cell lines H1, H7 and H9 were obtained from WiCell Research Institute, Inc., (Madison, WI) and cultured according to instructions provided by the source institute. Briefly, cells were cultured on mouse embryonic fibroblast (MEF) feeder cells in ES cell medium consisting of DMEM/F12 (Invitrogen/GIBCO) supplemented with 20% knockout serum replacement, 100 nM MEM nonessential amino acids, 0.5 mM beta-

mercaptoethanol, 2mM L-glutamine with 4ng/ml human basic fibroblast growth factor (bFGF) (all from Invitrogen/GIBCO). MEF cells, derived from E13 to 13.5 mouse embryos, were purchased from Charles River. MEF cells were expanded in DMEM medium supplemented with 10% FBS (Hyclone), 2mM glutamine, and 100 mM MEM nonessential amino acids. Sub-confluent MEF cell cultures were treated with 10μg/ml mitomycin C (Sigma, St. Louis, MO) for 3h to arrest cell division, then trypsinized and plated at $2x10^4/cm^2$ on 0.1% bovine gelatin-coated dishes. MEF cells from passage two through four were used as feeder layers. Human embryonic stem cells plated on MEF cell feeder layers were cultured at 37°C in an atmosphere of 5% CO_2/ within a humidified tissue culture incubator. When confluent (approximately 5-7 days after plating), human embryonic stem cells were treated with 1mg/ml collagenase type IV (Invitrogen/GIBCO) for 5-10 min and then gently scraped off the surface using a 5-ml pipette. Cells were spun at 900 rpm for 5 min, and the pellet was resuspended and re-plated at a 1:3 to 1:4 ratio of cells in fresh culture medium.

[0203] In parallel, H1, H7, and H9 human embryonic stem cells were also seeded on plates coated with a 1:30 dilution of growth factor reduced MATRIGEL™ (BD Biosciences) and cultured in MEF-conditioned media supplemented with 8 ng/ml bFGF. The cells cultured on MATRIGEL™ were routinely passaged with collagenase IV (Invitrogen/GIBCO), Dispase (BD Biosciences) or Liberase enzyme (Source). Some of the human embryonic stem cell cultures were incubated under hypoxic conditions (approximately 3% O_2).

Example 2

Derivation and Culture of Cells Expressing Pluripotency Markers, Derived from Human Embryonic Stem Cells

[0204] Cells from the human embryonic stem cell lines H1 and H9 various passages (Passage 30-54) were cultured under hypoxic conditions (approximately 3% O_2) for at least three passages. The cells were cultured in MEF-CM supplemented with 8 ng/ml of bFGF and plated on MATRIGEL coated plates according to **Example 1.**

[0205] Cells were then treated with DMEM/F12 medium supplemented with 0.5% FBS, 20 ng/ml WNT-3a (Catalog# 1324-WN-002, R&D Systems, MN), and 100 ng/ml Activin-A (R&D Systems, MN) for two days followed by treatment with DMEM/F12 media supplemented with 2% FBS and 100 ng/ml Activin-A (AA) for an additional 3 to 4 days. This protocol resulted in significant upregulation of definitive endoderm markers.

[0206] The cells were then treated with TrypLE™ Express solution (Invitrogen, CA) for 5 mins. Released cells were resuspended in DMEM-F12 + 2% FBS medium, recovered by centrifugation, and counted using a hemocytometer. The released cells were seeded at 1000-10,000 cells/cm^2 on tissue culture polystyrene (TCPS) treated flasks and cultured in DMEM-F12 + 2% FBS + 100 ng/ml activin-A + 20 ng/ml WNT-3A under hypoxic conditions (approximately 3% O_2) at 37 °C in standard tissue culture incubator. The TCPS flaks were not coated with MATRIGEL or other extarcellular matrix proteins. The media was changed daily. In some cultures, the media was further supplemented with 10-50 ng/ml of IGF-I (insulin growth factor-I from R&D Systems, MN) or 1X ITS (Insulin, transferrin, and selenium from Invitrogen, Ca). In some of the culture conditions the basal media (DM-F12 + 2% FBS) was further supplemented with 0.1 mM mercaptoethanol (Invitrogen, CA) and non-essential amino acids (1X, NEAA from Invitrogen, CA).

[0207] Following 5 to 15 days of culturing, distinct cell colonies appeared surrounded by a large number of enlarged cells that appear to be in senescence. At approximately 50 to 60% confluency, the cultures were passaged by exposure to TrypLE™ Express solution for 5 mins at room temperature. The released cells were resuspended in DMEM-F12 + 2% FBS medium, recovered by centrifugation, and seeded at 10,000 cells/cm^2 on tissue culture polystyrene (TCPS) treated flasks in DMEM-F12 + 2%FBS + 100 ng/ml activin-A + 20 ng/ml WNT-3A +/- 50 ng/ml of IGF-I. This media will be further referred to as the "growth media".

Example 3A

Derivation of Cells Expressing Pluripotency Markers from a Single Cell Suspension of Human Embryonic Stem Cells

[0208] Cells from the human embryonic stem cell lines H1 P33 and H9 P45 were cultured under hypoxic conditions (approximately 3% O_2) for at least three passages. The cells were cultured in MEF-CM supplemented with 8 ng/ml of bFGF and plated on MATRIGEL coated plates according to **Example 1.** At approximately 60% confluency, the cultures were exposed to TrypLE™ Express solution (Invitrogen, CA) for 5 minutes. Released cells were resuspended in DMEM-F12 + 2% FBS medium, recovered by centrifugation, and counted using a hemocytometer. The released cells were seeded at 1000 to 10,000 cells/cm^2 on tissue culture polystyrene (TCPS) treated flasks and cultured in DM-F12 + 2% FBS + 100 ng/ml activin-A + 20 ng/ml WNT-3A + 50 ng/ml of IGF-I + 0.1 mM mercaptoethanol (Invitrogen, CA) and non-essential amino acids (1X, NEAA from Invitrogen, CA) under hypoxic conditions (approximately 3% O_2) at 37 °C in standard tissue culture incubator. The TCPS flasks were not coated with MATRIGEL or other extarcellular matrix proteins. The media was changed daily. The first passage cells are referred to as P1.

Example 3B

Various Growth Media Useful for Expansion of Cells Expressing Pluripotency Markers Derived from Human Embryonic Stem Cells

[0209] Cells expressing pluripotency markers derived from human embryonic stem cells have been successfully cultured in the following media compositions for at least 2-30 passages:

1. DM-F12 + 2% FBS + 100 ng/ml AA + 20 ng/ml WNT-3A
2. DM-F12 + 2% FBS + 100 ng/ml AA + 20 ng/ml WNT-3A + 50 ng/ml IGF-I

3. DM-F12 + 2% FBS + 100 ng/ml AA + 20 ng/ml WNT-3A + 10 ng/ml IGF-I

4. DM-F12 + 2% FBS + 50 ng/ml AA + 20 ng/ml WNT-3A + 50 ng/ml IGF-I

5. DM-F12 + 2% FBS + 50 ng/ml AA + 10 ng/ml WNT-3A + 50 ng/ml IGF-I

6. DM-F12 + 2% FBS + 50 ng/ml AA + 20 ng/ml WNT-3A + 10 ng/ml IGF-I

7. DM-F12 + 2% FBS + 100 ng/ml AA + 10 ng/ml WNT-3A + 10 ng/ml IGF-I

8. HEScGRO defined media (Chemicon, CA)

[0210] The basal component of the above listed media may be replaced with similar media such as, RPMI, DMEM, CRML, Knockout ™DMEM, and F12.

Example 4

Effects of Inhibitors of GSK-3β Enzyme Activity on the Viability of Cells Expressing Pluripotency Markers

[0211] Derivation and maintenance of cells expressing pluripotency makers was conducted as has been described in **Example 2**. Cells were grown in DMEM:F12 supplemented with 2% FCS (Invitrogen), 100 ng/ml Activin A, 20 ng/ml Wnt-3a, and 50 ng/ml IGF(R&D Biosystems). Cells were seeded at a density of 10,000 cells/cm^2 on Falcon polystyrene flasks and grown in monolayer culture at 37°C, 5% CO_2, low oxygen. After reaching 60-70% confluence, cells were passed by washing the monolayer with PBS and incubating with TrypLE (Invitrogen) for 3-5 minutes to allow detachment and single cell dispersal.

[0212] Screening was conducted using test compounds from a proprietary library of small molecules selected for their ability to inhibit GSK-3B enzyme activity. Compounds from this library were made available as 1mM stocks, in a 96-well

plate format in 50mM HEPES, 30% DMSO. For assay, cells expressing pluripotency markers were washed, counted, and plated in normal culture medium at a seeding density of 20,000 cells per well in 96-well clear-bottom, dark-well plates (Costar). This seeding density was previously determined to yield optimal monolayer formation in overnight culture. On the following day, culture medium was removed, cell monolayers were rinsed three times with PBS, and test compounds were added to the wells in 80µl aliquots, each diluted into assay medium at a final assay concentration of 10µM. On day 2 of the assay, medium was removed from each well and replaced with a fresh aliquot of test compounds diluted into assay medium. Assay medium on days 1 and 2 of culture consisted of DMEM:F12 supplemented with 0.5% FCS and 100ng/ml Activin A. On days 3 and 4 of culture, medium was removed from each well and replaced with DMEM:F12 supplemented with 2% FCS and 100ng/ml Activin A (no test compound). On day 4 of assay, 15µl of MTS (Promega) was added to each well and plates were incubated at 37°C for 1.5 to 4 hours prior to reading optical density at 490 nm on a SpectraMax (Molecular Devices) instrument. Statistical measures consisting of mean, standard deviation, and coefficient of variation were calculated for each duplicate set. Toxicity was calculated for each test well relative to a positive control (wells treated with Activin A and Wnt3a on days 1 and 2 of culture).

[0213] **Table II** is a compilation of all screening results. Cells expressing pluripotency markers were plated initially as a confluent monolayer in this assay; hence, the results are representative of a toxicity measure over the four-day culture period. Results are expressed as percentage viability of control, and demonstrate variable toxicity for some compounds at the 10µM screening concentration used. A larger proportion of the compounds have minimal or no measurable toxicity in this cell-based assay.

[0214] A small panel of select compounds was repeat tested over a narrow dose titration range, again using cells expressing pluripotency markers in a similar assay as described above. **Table III** is a summary of these results, demonstrating variable dose titration effects for a range of toxic and non-toxic compounds.

Example 5

Effects of Inhibitors of GSK-3β Enzyme Activity on the Differentiation and Proliferation of Human Embryonic Stem Cells Determined using a High Content Screening Assay

[0215] Maintenance of human embryonic stem cells (H9 line) was conducted as described in **Example 1**. Colonies of cells were maintained in an undifferentiated, pluripotent state with passage on average every four days. Passage was performed by exposing cell cultures to a solution of collagenase (1 mg/ml; Sigma-Aldrich) for 10 to 30 minutes at 37°C followed by gentle scraping with a pipette tip to recover cell clusters. Clusters were allowed to sediment by gravity, followed by washing to remove residual collagenase. Cell clusters were split at a 1:3 ratio for routine maintenance culture or a 1:1 ratio for immediate assay. The human embryonic stem cell lines used were maintained at passage numbers less than passage 50 and routinely evaluated for normal karyotypic phenotype and absence of mycoplasma contamination.

[0216] Cell clusters used in the assay were evenly resuspended in normal culture medium and plated onto MATRIGEL-coated 96-well Packard VIEWPLATES (PerkinElmer) in volumes of 100µl/well. MEF conditioned medium supplemented with 8ng/ml bFGF was used for initial plating and recovery. Daily feeding was conducted by aspirating spent culture medium from each well and replacing with an equal volume of fresh medium. Plates were maintained at 37°C, 5% CO_2 in a humidified box throughout the duration of assay.

[0217] Screening was conducted using test compounds from a proprietary library of small molecules selected for their ability to inhibit GSK-3B enzyme activity. Compounds from this library were made available as 1mM stocks, in a 96-well plate format in 50mM HEPES, 30% DMSO. Screening compounds were tested in triplicate or duplicate sets. Primary screening assays were initiated by aspirating culture medium from each well followed by three washes in PBS to remove residual growth factors and serum. Test volumes of 80 to 100µl per well were added back containing DMEM:F12 base medium (Invitrogen)

supplemented with 0.5% FCS (HyClone) and 100ng/ml activin A (R&D Biosystems) plus 10μM test compound. Positive control wells contained the same base medium, substituting 10-20ng/ml Wnt3a (R&D Biosystems) for the test compound. Negative control wells contained base medium with 0.5% FCS and activin A alone (AA only) or alternatively, 0.5% FCS without activin A or Wnt3a (no treatment). Wells were aspirated and fed again with identical solutions on day 2 of assay. On days 3 and 4, all assay wells were aspirated and converted to DMEM:F12 supplemented with 2% FCS and 100ng/ml activin A (without test compound or Wnt3a); parallel negative control wells were maintained in DMEM:F12 base medium with 2% FCS and activin A (AA only) or alternatively, 2% FCS without activin A (no treatment).

[0218] At the end of culture, cells in 96-well plates were fixed with 4% paraformaldehyde at room temperature for 20 minutes, washed three times with PBS, and then permeabilized with 0.5% Triton X-100 for 20 minutes at room temperature. Alternatively, cells were fixed with ice cold 70% ethanol overnight at -20°C, washed three times with PBS, and then permeabilized with Triton X-100 for 5 minutes at 4°C. After fixing and permeabilizing, cells were washed again three times with PBS and then blocked with 4% chicken serum (Invitrogen) in PBS for 30 minutes at room temperature. Primary antibodies (goat anti-human Sox17 and goat anti-human HNF-3beta; R&D Systems) were diluted 1:100 in 4% chicken serum and added to cells for one hour at room temperature. Alexa Fluor 488 conjugated secondary antibody (chicken anti-goat IgG; Molecular Probes) was diluted 1:200 in PBS and added after washing the cells three times with PBS. To counterstain nuclei, 5 mM Draq5 (Alexis Biochemicals) was added for five minutes at room temperature. Cells were washed once with PBS and left in 100 ml/well PBS for imaging.

[0219] Cells were imaged using an IN Cell Analyzer 1000 (GE Healthcare) utilizing the 51008bs dichroic for cells stained with Draq5 and Alexa Fluor 488. Exposure times were optimized using a positive control wells and wells with secondary only for untreated negative controls. Twelve fields per well were obtained to compensate for any cell loss during the treatment and staining procedures. Total cell numbers and total cell intensity for Sox-17 and HNF-

3beta were measured using the IN Cell Developer Toolbox 1.6 (GE Healthcare) software. Segmentation for the nuclei was determined based on grey-scale levels (baseline range 100-300) and nuclear size. Averages and standard deviations were calculated for replicates. Total protein expression was reported as total intensity or integrated intensity, defined as total fluorescence of the cell times area of the cell. Background was eliminated based on acceptance criteria of grey-scale ranges between 300 to 3000 and form factors greater than or equal to 0.4. Total intensity data were normalized by dividing the total intensities for each well by the average total intensity for the Wnt3a/Activin A positive control. Normalized data was calculated for averages and standard deviation for each replicate set.

[0220] **Table IV** is a representative summary of all screening results. **Table V** is a list of hits from this screening. Strong hits are defined as greater than or equal to 120% of control values; moderate hits are defined as falling within the interval of 60-120% of control values. A significant number of compounds induce both a proliferative response in this assay. In parallel, a significant number of compounds induce differentiation in this assay, as measured by the protein expression of Sox17 and Hnf-3b transcription factors.

Example 6

Effects of Inhibitors of GSK-3β Enzyme Activity on the Proliferation of Human Embryonic Stem Cells Determined using a Plate Reader Assay

[0221] Maintenance of human embryonic stem cells (H9 or H1 lines) was conducted as described in **Example 1**. Colonies of cells were maintained in an undifferentiated, pluripotent state with passage on average every four days. Passage was performed by exposing cell cultures to a solution of collagenase (1 mg/ml; Sigma-Aldrich) for 10 to 30 minutes at 37°C followed by gentle scraping with a pipette tip to recover cell clusters. Clusters were allowed to sediment and washed to remove residual collagenase. Cell clusters were split at a ratio of 1:3 monolayer area for routine culture or a 1:1 ratio for immediate assay. The human embryonis stem cell lines used for these examples were

maintained at passage numbers less than 50 and routinely evaluated for normal karyotypic phenotype as well as absence of mycoplasm contamination.

[0222] Cell clusters used in assay were evenly resuspended in normal culture medium and plated into MATRIGEL-coated 96-well Packard VIEWPLATES (PerkinElmer) in volumes of 100µl/well. MEF conditioned medium supplemented with 8ng/ml bFGF) was used for initial plating and recovery. Daily feeding was conducted by aspirating spent culture medium from each well and replacing with an equal volume of fresh medium. Plates were maintained at 37°C in a humidified box, 5% CO_2 throughout the duration of assay.

[0223] Primary screening assays were initiated by aspirating culture medium from each well followed by three washes in PBS to remove residual growth factors and serum. Test volumes of 80-100µl per well were added back containing DMEM:F12 base medium (Invitrogen) supplemented with 0.5% FCS (HyClone) and 100ng/ml activin A (R&D Biosystems) and 10µM test compound. Positive control wells contained the same medium substituting 10-20ng/ml Wnt3a (R&D Biosystems). Negative control wells contained base medium with 0.5% FCS without activin A or Wnt3a. Screening compounds were tested in triplicate. Wells were aspirated and fed again with identical solutions on day 2 of the assay. On days 3 and 4, all assay wells were aspirated and converted to DMEM:F12 supplemented with 2% FCS and 100ng/ml activin A with the exception of negative control wells which were maintained in DMEM:F12 base medium with 2% FCS.

[0224] On day 4 of assay, 15-20µl of MTS (Promega) was added to each well and plates were incubated at 37°C for 1.5 to 4 hours. Densitometric readings at OD490 were determined using a Molecular Devices spectrophotometer plate reader. Average readings for replicate sets were calculated along with standard deviation and coefficient of variation. Experimental wells were compared to the Activin A/Wnt3a positive control to calculate a percent control value as a measure of proliferation.

[0225] **Table VI** is a representative summary of all screening results. **Table VII** is a list of hits from this screening. Strong hits are defined as greater than or equal to 120% of control values; moderate hits are defined as falling within the interval of 60-120% of control values. A significant number of compounds induce a proliferative response in this assay.

Example 7

Effects of GSK-3β Enzyme Inhibitors on the Differentiation and Proliferation of Human Embryonic Stem Cells: Dose Titration of Lead Compounds

[0226] It was important to confirm the activity of hits identified from primary screening and further analyze the range of activity by dose titration. New samples of a selective subset of primary screening hits were obtained as dry powders, solubilized to make fresh stock reagents, and diluted into secondary confirmation assays to evaluate effects on human embryonic stem cells.

[0227] Culture of two human embryonic stem cells (H1 and H9) was conducted as described in **Example 1**. Colonies of cells were maintained in an undifferentiated, pluripotent state on Matrigel™ (Invitrogen)–coated polystyrene plastic, using a 1:30 dilution of Matrigel™ in DMEM:F12 to coat the surface. Cells were split by enzymatic passage every four days on average. Passage was performed by exposing cell monolayers to a solution of collagenase (1 mg/ml; Sigma-Aldrich) for 10 to 60 minutes at 37°C followed by gentle scraping with a pipette tip to recover cell clusters. Clusters were allowed to sediment by gravity, then washed to remove residual collagenase. Cell clusters were split at a 1:3 ratio for maintenance culture or a 1:1 ratio for subsequent assay. The human embryonic stem cell lines were maintained at less than passage 50 and routinely evaluated for normal karyotypic phenotype and absence of mycoplasma contamination.

[0228] *Preparation of cells for assay:* Cell clusters of the H1 or H9 human embryonic stem cell lines used in the assay were evenly resuspended in culture medium and plated onto Matrigel™-coated 96-well Packard VIEWPLATES

(PerkinElmer) in volumes of 100µl/well. MEF conditioned medium supplemented with 8ng/ml bFGF was used for initial plating and expansion. Daily feeding was conducted by aspirating spent culture medium from each well and replacing with an equal volume of fresh medium. Cultures were allowed to expand one to three days after plating prior to initiating assay. Plates were maintained at 37°C, 5% CO_2 in a humidified box for the duration of assay.

[0229] *Preparation of compounds and assay medium:* A subset of hits resulting from primary screening was used for follow-up study and subsequent secondary assays. Twenty compounds available as dry powders were solubilized as 10mM stocks in DMSO and stored dessicated at –20°C until use. Immediately prior to assay, compound stocks were diluted 1:1000 to make 10µM test compound in DMEM:F12 base medium (Invitrogen) supplemented with 0.5% FCS (HyClone) and 100ng/ml Activin A (R&D Biosystems). This was further diluted two-fold in series to make a seven point dilution curve for each compound, also in DMEM:F12 base medium with 0.5% FCS and 100ng/ml Activin A.

[0230] *Secondary screening assay:* Assay was initiated by aspirating culture medium from cell monolayers in each well followed by three washes in PBS to remove residual growth factors and serum. Test volumes of 100µl per well were added back containing medium with 0.5% FCS and different concentrations of inhibitor compounds with 100ng/ml Activin A, without Wnt3a. Positive control wells contained the same base medium with 0.5% FCS and with 20ng/ml Wnt3a (R&D Biosystems) in the absence of test compound. Negative control wells contained the same base medium with 0.5% FCS, in the absence of Activin A, Wnt3a, or test compound. Assay wells were aspirated and fed again with identical concentrations of test compound or control solutions on day 2 of assay. On days 3 and 4, all assay wells were aspirated and fed with DMEM:F12 supplemented with 2% FCS and 100ng/ml Activin A in the absence of both test compound or Wnt3a. Parallel negative control wells were maintained on days 3 and 4 in DMEM:F12 base medium with 2% FCS.

[0231] *Assay evaluation:* At the end of culture, cells in 96-well plates were washed twice with PBS then fixed with 4% paraformaldehyde at room temperature for 20 minutes, washed three times more with PBS, and then permeabilized with 0.5% Triton X-100 for 20 minutes at room temperature. After fixing and permeabilizing, cells were washed again three times with PBS and then blocked with 4% chicken serum (Invitrogen) in PBS for 30 minutes at room temperature. Primary antibodies (goat anti-human Sox17; R&D Systems) were diluted 1:100 in 4% chicken serum and added to the cells for one hour at room temperature. Alexa Fluor 488 conjugated secondary antibody (chicken anti-goat IgG; Molecular Probes) was diluted 1:200 in PBS and added to each well after washing the cells three times with PBS. To counterstain nuclei, 2μg/ml Hoechst 33342 (Invitrogen) was added for ten minutes at room temperature. Cells were washed once with PBS and left in 100 μl/well PBS for imaging.

[0232] Cells were imaged using an IN Cell Analyzer 1000 (GE Healthcare) utilizing the 51008bs dichroic for cells stained with Hoechst 33342 and Alexa Fluor 488. Exposure times were optimized using positive control wells and wells stained with secondary antibody alone as an untreated negative control. Images from 15 fields per well were acquired to compensate for any cell loss during the treatment and staining procedures. Measurements for total cell number and total Sox-17 intensity were obtained for each well using IN Cell Developer Toolbox 1.7 (GE Healthcare) software. Segmentation for the nuclei was determined based on grey-scale levels (baseline range 100-300) and nuclear size. Averages and standard deviations were calculated for each replicate data set. Total Sox17 protein expression was reported as total intensity or integrated intensity, defined as total fluorescence of the cell times area of the cell. Background was eliminated based on acceptance criteria of grey-scale ranges between 300 to 3000 and form factors greater than or equal to 0.4. Total intensity data were normalized by dividing the total intensities for each well by the average total intensity for the Wnt3a/Activin A positive control. Normalized data were calculated for averages and standard deviations for each replicate set.

Results

[0233] Results are shown for eight GSK-3B enzyme inhibitors where activity was confirmed and potency was determined by titration in this secondary assay. Data presented show compound effects on cell number and Sox17 intensity where respective data points were averaged from a duplicate set and mined for each parameter from identical fields and wells. In this example, Sox17 expression is indicative of definitive endoderm differentiation. Results for cell number and Sox17 intensity, respectively, using the H1 human embryonic stem cell line are shown in **Tables VIII** and **IX**. Results for the H9 human embryonic stem cell line are shown in **Tables X** and **XI**. Positive control values were normalized to 1.000 for cell number and Sox17 intensity. Negative control values were less-than 0.388 for cell number and less-than 0.065 for Sox17 intensity with both cell lines. A graphic portrayal of these data, comparing both human embryonic stem cell lines and including a dose titration of each compound, is provided in **Figures 1 to 8**. Cell number is presented in panel A; Sox 17 intensity is shown in panel B. These data confirm that each compound can promote hES cell proliferation and definitive endoderm differentiation and identify an optimal range of activity.

Example 8

Effects of GSK-3β Enzyme Inhibitors on the Expression of Additional Markers Associated with Definitive Endoderm

[0234] It was important to demonstrate that lead compounds could also induce other markers indicative of definitive endoderm differentiation, in addition to the transcription factor Sox17. A select subset of hits was tested for their ability to promote expression of CXCR4, a surface receptor protein, and HNF-3 beta, a transcription factor also associated with definitive endoderm differentiation.

[0235] *Preparation of cells for assay:* Cell clusters from the H1 human embryonis stem cell line used in the assay were evenly resuspended in culture medium and plated onto MATRIGEL™-coated (1:30 dilution) 6-well plates (Corning) in volumes of 2 ml/well. MEF conditioned medium supplemented with 8ng/ml bFGF was used for initial plating and expansion. Daily feeding was

conducted by aspirating spent culture medium from each well and replacing with an equal volume of fresh medium. Cultures were allowed to expand one to three days after plating prior to initiating assay. Plates were maintained at 37°C, 5% CO_2 for the duration of assay.

[0236] *Preparation of compounds and assay medium:* A subset of seven hits resulting from primary screening was used for follow-up study and subsequent secondary assays. Neat compounds were solubilized as 10mM stocks in DMSO and stored dessicated at –20°C until use. Immediately prior to assay, compound stocks were diluted to a final concentration ranging between 1μM and 5μM in DMEM:F12 base medium (Invitrogen) supplemented with 0.5% FCS (HyClone) and 100ng/ml Activin A (R&D Biosystems).

[0237] *Assay:* The assay was initiated by aspirating culture medium from cell monolayers in each well followed by three washes in PBS to remove residual growth factors and serum. Test volumes of 2ml per well were added back containing medium with 0.5% FCS and different concentrations of inhibitor compounds with 100ng/ml Activin A, without Wnt3a. Positive control wells contained the same base medium and 0.5% FCS with 100ng/ml Activin A and 20ng/ml Wnt3a (R&D Biosystems) in the absence of test compound. Negative control wells contained base medium with 0.5% FCS, in the absence of Activin A, Wnt3a, or test compound. Assay wells were aspirated and fed again with identical concentrations of test compound or control solutions on day 2 of assay. On days 3 and 4, all assay wells were aspirated and fed with DMEM:F12 supplemented with 2% FCS and 100ng/ml Activin A in the absence of both test compound or Wnt3a. Parallel negative control wells were maintained on days 3 and 4 in DMEM:F12 base medium with 2% FCS.

[0238] *Assay evaluation:* At the end of culture, cell monolayers were washed with PBS and harvested from culture plates by incubating 5 minutes with TrypLE™ Express solution (Invitrogen, CA). Cells were resuspended in MEF conditioned medium and split into two equal samples. One set of samples was further stained with various fluorescent labeled antibodies and subjected to flow cytometric (FACS) analysis. A second parallel set of samples was subjected to quantitative PCR.

[0239] Cells for FACS analysis were washed into PBS and blocked for 15 minutes at 4°C in 0. 125% human gamma-globulin (Sigma cat# G-4386) diluted in PBS and BD FACS staining buffer. Aliquots of cells (approximately 10^5 cells each) were stained for 30 minutes at 4°C with antibodies directly conjugated to a fluorescent tag and having specificity for CD9 PE (BD#555372), CD99 PE (Caltag#MHCD9904), or CXCR-4 APC (R&D Systems cat# FAB173A). After a series of washes in BD FACS staining buffer, cells were stained with 7-AAD (BD# 559925) to assess viability and analyzed on a BD FACS Array instrument (BD Biosciences), collecting at least 10,000 events. Mouse IgG_1k isotype control antibodies for both PE and APC were used to gate percent positive cells.

[0240] Cells for quantitative PCR were processed for RNA extraction, purification, and cDNA synthesis. RNA samples were purified by binding to a silica-gel membrane (Rneasy Mini Kit, Qiagen, CA) in the presence of an ethanol-containing, high-salt buffer followed by washing to remove contaminants. The RNA was further purified using a TURBO DNA-free kit (Ambion, Inc.), and high-quality RNA was eluted in water. Yield and purity were assessed by A260 and A280 readings on a spectrophotometer. cDNA copies were made from purified RNA using an Applied Biosystems, Inc. (ABI, CA) high capacity cDNA archive kit.

[0241] Unless otherwise stated, all reagents for real-time PCR amplification and quantitation were purchased from ABI. Real-time PCR reactions were performed using the ABI PRISM 7900 Sequence Detection System. TAQMAN UNIVERSAL PCR MASTER MIX (ABI, CA) was used with 20 ng of reverse transcribed RNA in a total reaction volume of 20 µl. Each cDNA sample was run in duplicate to correct for pipetting errors. Primers and FAM-labeled TAQMAN probes were used at concentrations of 200 nM. The level of expression for each target gene was normalized using a human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) endogenous control previously developed by ABI. Primer and probe sets are listed as follows: CXCR4 (Hs00237052), GAPDH (4310884E), HNF3b (Hs00232764), SOX17 (probe part # 450025, forward and reverse part # 4304971).

[0242] After an initial incubation at 50°C for 2 min followed by 95°C for 10 min, samples were cycled 40 times in two stages, a denaturation step at 95°C for 15 sec followed by an annealing/extension step at 60°C for 1 min. Data analysis was carried out using GENEAMP 7000 Sequence Detection System software. For each primer/probe set, a Ct value was determined as the cycle number at which the fluorescence intensity reached a specific value in the middle of the exponential region of amplification. Relative gene expression levels were calculated using the comparative Ct method. Briefly, for each cDNA sample, the endogenous control Ct value was subtracted from the gene of interest Ct to give the delta Ct value (ΔCt). The normalized amount of target was calculated as 2-ΔCt, assuming amplification to be 100% efficiency. Final data were expressed relative to a calibrator sample.

Results

[0243] **Figure 9** displays the FACS analysis of percent positive cells expressing CXCR4 surface receptor after treatment with various GSK3 inhibitors. Two concentrations of each compound, ranging between 1μM and 5μM, are shown relative to an untreated population of cells (negative control) or cells treated with Activin A and Wnt3 (positive control). **Figure 10 panels a, b, and c** show real-time PCR data for CXCR4, Sox17, and HNF3beta, which are also considered to be markers of definitive endoderm. Both FACS and real-time PCR analysis demonstrate a significant increase in each of these markers observed in differentiated cells relative to untreated control cells. Expression levels of these definitive endoderm markers were equivalent in some cases to the positive control, demonstrating that a GSK3 inhibitor can substitute for Wnt3a at this stage of differentiation.

Example 9

Effects of GSK-3β Enzyme Inhibitors on the Formation of Pancreatic Endoderm

[0244] It was important to demonstrate that treatment with GSK3β inhibitors during induction of definitive endoderm did not prevent the subsequent differentiation

of other cell types, such as pancreatic endoderm, for example. A select subset of hits was tested for their ability to promote expression of PDX1 and HNF6, key transcription factors associated with pancreatic endoderm.

[0245] Maintenance of human embryonic stem cells (H1 and H9 lines) was conducted as described in **Example 1**. Colonies of cells were maintained in an undifferentiated, pluripotent state with passage on average every four days. Passage was performed by exposing cell cultures to a solution of collagenase (1 mg/ml; Sigma-Aldrich) for 10 to 30 minutes at 37°C, followed by gentle scraping with a pipette tip to recover cell clusters. Clusters were allowed to sediment by gravity, followed by washing to remove residual collagenase. Cell clusters were split at a 1:3 ratio for routine maintenance culture or a 1:1 ratio for subsequent assay. The human embryonic stem cell lines used were maintained at less than passage 50 and routinely evaluated for normal karyotypic phenotype and absence of mycoplasma contamination.

[0246] *Cell preparation of assay:* Cell clusters of the H1 human embryonis stem cell line used in the assay were evenly resuspended in culture medium and plated onto MATRIGEL™-coated (1:30 dilution) 24-well plates (black well; Arctic White) in volumes of 1 ml/well. MEF conditioned medium supplemented with 8ng/ml bFGF was used for initial plating and expansion. In a second experiment, clusters of hES cells from the H9 line were plated in 96-well plates on mouse embryonic feeder (MEF) layers, previously inactivated by treating with mitomycin C (Sigma Chemical Co). Culture medium for hES cells on MEF monolayers consisted of DMEM:F12 with 20% Knockout Serum Replacer (Invitrogen) supplemented with minimal essential amino acids (Invitrogen), L-glutamine, and 2-mercaptoethanol. Daily feeding was conducted by aspirating spent culture medium from each well and replacing with an equal volume of fresh medium. Cultures were allowed to expand one to three days after plating prior to initiating assay. Plates were maintained at 37°C, 5% CO_2 for the duration of assay.

[0247] *Preparation of compounds and assay medium:* A subset of eight hits resulting from primary screening was used for follow-up study and subsequent secondary assays. Neat compounds were solubilized as 10mM stocks in

DMSO and stored dessicated at –20°C until use. Immediately prior to assay, compound stocks were diluted to a final concentration ranging between 1μM and 5μM in base medium with additives.

[0248] *Assay:* In this assay, GSK3 inhibitors were included only on days 1 and 2 of the definitive endoderm differentiation step, substituting for Wnt3a. Embryonic stem cell cultures on MATRIGEL™ were initiated as described in **Examples 7** and **8** above by aspirating culture medium from cell monolayers in each well followed by three washes in PBS to remove residual growth factors and serum. For differentiation to definitive endoderm, test volumes (0.5 ml per well for 24-well plates, 100 μl per well for 96-well plates) were added containing DMEM:F12 medium with) 0.5% FCS and different concentrations of inhibitor compounds with 100 ng/ml Activin A, without Wnt3a. Positive control wells contained the same base medium with 0.5% FCS and with 100ng/ml Activin A and 20ng/ml Wnt3a (R&D Biosystems) in the absence of test compound. Negative control wells contained the same base medium with 0.5% FCS, in the absence of Activin A, Wnt3a, or test compound. Assay wells were aspirated and fed again with identical concentrations of test compound or control solutions on day 2 of assay. On days 3 and 4, all assay wells were aspirated and fed with DMEM:F12 supplemented with 2% FCS and 100ng/ml Activin A in the absence of both test compound or Wnt3a. Parallel negative control wells were maintained on days 3 and 4 in DMEM:F12 base medium with 2% FCS. For differentiation to pancreatic endoderm, cells were treated for three days, feeding daily with DMEM:F12 base medium containing 2% FCS with 0.25 μM KAAD cyclopamine (EMD Biosciences) and 20 ng/ml FGF7 (R&D Biosystems). Cells were then treated for an additional four days, feeding daily with DMEM:F12 containing 1% B27 (Invitrogen) , 0.25 μM KAAD cyclopamine, 2 μM Retinoic Acid (RA; Sigma-Aldrich) and 20 ng/ml FGF7. Parallel negative control wells were maintained throughout in DMEM:F12 base medium with 2% FCS (stage 2) or 1% B27 (stage 3) and without any other additives.

[0249] Parallel cultures of H9 human embryonic cells were grown on MEF feeder layers, and differentiated to pancreatic endoderm. Definitive endoderm differentiation was achieved by culturing the cells in medium consisting of RPMI-1640 (Invitrogen) containing no serum on day 1 and 0.2% FCS on days 2 and 3 along with different concentrations of inhibitor compounds and 100 ng/ml Activin A. Positive control wells contained the same base medium (with or without serum) with 100ng/ml Activin A and 20ng/ml Wnt3a (R&D Biosystems) in the absence of test compound. Negative control wells contained the same base medium with or without serum, in the absence of Activin A, Wnt3a, or test compound. Assay wells were aspirated and fed again with identical concentrations of test compound or control solutions on day 2 of assay. On day 3, all assay wells were aspirated and fed with RPMI-1640 supplemented with 2% FCS and 100ng/ml Activin A in the absence of both test compound and Wnt3a. Parallel negative control wells were maintained on day 3 in RPMI-1640 base medium with 2% FCS. Cells were differentiated into pancreatic endoderm by treating the cells for four days, feeding daily with RPMI-1640 base medium containing 2% FCS with 0.25 mM KAAD cyclopamine (EMD Biosciences) and 50 ng/ml FGF10 (R&D Biosystems). Subsequently, cells were treated for three days duration, feeding daily with RPMI-1640 containing 1% B27 (Invitrogen), 0.25 mM KAAD cyclopamine, 2 mM Retinoic Acid (RA; Sigma-Aldrich) and 50 ng/ml FGF10. Parallel negative control wells were maintained throughout in RPMI-1640 base medium with 2% FCS (stage 2) or 1% B27 (stage 3) and without any other additives.

[0250] *Assay evaluation:* At the end the differentiation, cells were examined as described in **Example 8** for gene expression by real-time PCR. For high content fluorescence staining, cells in 96-well plates were washed twice with PBS then fixed with 4% paraformaldehyde at room temperature for 20 minutes, washed three times more with PBS, and then permeabilized with 0.5% Triton X-100 for 20 minutes at room temperature. After fixing and permeabilizing, cells were washed again three times with PBS and blocked with 4% chicken serum (Invitrogen) in PBS for 30 minutes at room temperature. Primary antibody (goat anti-human Pdx1; Santa Cruz) was

diluted 1:100 in 4% chicken serum and added to cells for two hours at room temperature. Alexa Fluor 488 conjugated secondary antibody (chicken anti-goat IgG; Molecular Probes) was diluted 1:200 in PBS and added to each well after washing the cells three times with PBS. To counterstain nuclei, 2μg/ml Hoechst 33342 (Invitrogen) was added for ten minutes at room temperature. Cells were washed once with PBS and left in 100 μl/well PBS for imaging.

[0251] Cells were imaged using an IN Cell Analyzer 1000 (GE Healthcare) utilizing the 51008bs dichroic for cells stained with Hoechst 33342 and Alexa Fluor 488. Exposure times were optimized using positive control wells and wells stained with secondary antibody alone. Images from 15 fields per well were acquired to compensate for any cell loss during the treatment and staining procedures. Measurements for total cell number and total Pdx1 intensity were obtained for each well using IN Cell Developer Toolbox 1.7 (GE Healthcare) software. Segmentation for the nuclei was determined based on grey-scale levels (baseline range 100-300) and nuclear size. Averages and standard deviations were calculated for each replicate data set. Total Pdx1 protein expression was reported as total intensity or integrated intensity, defined as total fluorescence of the cell times area of the cell. Background was eliminated based on acceptance criteria of grey-scale ranges between 300 to 3000. Total intensity data were normalized by dividing the total intensities for each well by the average total intensity for the Wnt3a/Activin A positive control. Normalized data were calculated for averages and standard deviations for each replicate set.

[0252] Cells for quantitative PCR were lysed in RLT buffer (Qiagen) and then processed for RNA extraction, purification, and cDNA synthesis. RNA samples were purified by binding to a silica-gel membrane (Rneasy Mini Kit, Qiagen, CA) in the presence of an ethanol-containing, high-salt buffer followed by washing to remove contaminants. The RNA was further purified using a TURBO DNA-free kit (Ambion, Inc.), and high-quality RNA was then eluted in water. Yield and purity were assessed by A260 and A280 readings on a spectrophotometer. cDNA copies were made from purified RNA using an Applied Biosystems, Inc. (ABI, CA) high capacity cDNA archive kit.

[0253] Unless otherwise stated, all reagents for real-time PCR amplification and quantitation were purchased from ABI. Real-time PCR reactions were performed using the ABI PRISM 7900 Sequence Detection System. TAQMAN UNIVERSAL PCR MASTER MIX was used with 20 ng of reverse transcribed RNA in a total reaction volume of 20 µl. Each cDNA sample was run in duplicate to correct for pipetting errors. Primers and FAM-labeled TAQMAN probes were used at concentrations of 200 nM. The level of expression for each target gene was normalized using a human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) endogenous control previously developed by ABI. Primer and probe sets are listed as follows: PDX1 (Hs00236830_m1), GAPDH (4310884E), and HNF6 (Hs00413554_m1).

[0254] After an initial incubation at 50°C for 2 min followed by 95°C for 10 min, samples were cycled 40 times in two stages, a denaturation step at 95°C for 15 sec followed by an annealing/extension step at 60°C for 1 min. Data analysis was carried out using GENEAMPÒ7000 Sequence Detection System software. For each primer/probe set, a Ct value was determined as the cycle number at which the fluorescence intensity reached a specific value in the middle of the exponential region of amplification. Relative gene expression levels were calculated using the comparative Ct method. Briefly, for each cDNA sample, the endogenous control Ct value was subtracted from the gene of interest Ct to give the delta Ct value (ΔCt). The normalized amount of target was calculated as 2-ΔCt, assuming amplification to be 100% efficiency. Final data were expressed relative to a calibrator sample.

Results

[0255] Results are shown for eight GSK-3β enzyme inhibitors. Data presented in **Figure 11** from high content analysis show effects on cell number (panel A) and Pdx1 intensity (panel B) for the H1 hES cell line, where respective data points were averaged from a duplicate sample set and mined for each parameter from identical fields and wells. Data presented in **Figure 12** from real-time PCR show effects of these small molecule inhibitors on induced expression of two transcription factors, Pdx1 and HNF6. In these examples,

Pdx1 and HNF6 expression are indicative of pancreatic endoderm differentiation. GSK3β inhibitor compounds in these assays can substitute for Wnt3a during early stages of cell lineage commitment; resulting cells sustain a capacity to form pancreatic endoderm during later sequential stages of differentiation.

Example 10

Effects of GSK-3β Enzyme Inhibitors on the Formation of Pancreatic Endocrine Cells

[0256] It was important to demonstrate that treatment with GSK3 inhibitors during induction of definitive endoderm did not prevent the subsequent differentiation of other cell types, such as pancreatic endocrine cells, or insulin producing cells, for example. A select subset of hits was tested for their ability to promote expression of pancreatic hormones.

[0257] *Cell preparation for assay:* Pancreatic endoderm cells obtained according to the methods described in **Example 9** (cultured on 96-wellplates and 24-well plates) were subsequently subjected to agents that cause the cells to differentiate into pancreatic hormone expressing cells.

[0258] Assay for cultures of the H1 human embryonic stem cell line on MATRIGEL™ was initiated as described in **Examples 7 - 9** above by aspirating culture medium from cell monolayers in each well followed by three washes in PBS to remove residual growth factors and serum. For differentiation to definitive endoderm, test volumes (0.5 ml per well for 24-well plates, 100 µl per well for 96-well plates) were added containing medium with 0.5% FCS and different concentrations of inhibitor compounds with 100 ng/ml Activin A, without Wnt3a. Positive control wells contained the same base medium and 0.5% FCS with 100ng/ml Activin A and 20ng/ml Wnt3a (R&D Biosystems) in the absence of test compound. Negative control wells contained the same base medium with 0.5% FCS, in the absence of Activin A, Wnt3a, or test compound. Assay wells were aspirated and fed again with identical concentrations of test compound or control solutions on day 2 of

assay. On days 3, 4, and 5, all assay wells were aspirated and fed with DMEM:F12 supplemented with 2% FCS and 100ng/ml Activin A in the absence of both test compound or Wnt3a. Parallel negative control wells were maintained on days 3, 4, and 5 in DMEM:F12 base medium with 2% FCS. For differentiation to pancreatic endoderm, cells were treated for three days, feeding daily with DMEM:F12 base medium containing 2% FCS with 0.25 μM KAAD cyclopamine (EMD Biosciences) and 20 ng/ml FGF7 (R&D Biosystems). Cells were subsequently treated for four days, feeding daily with DMEM:F12 containing 1% B27 (Invitrogen) , 0.25 μM KAAD cyclopamine, 2 μM Retinoic Acid (RA; Sigma-Aldrich) and 20 ng/ml FGF7. Parallel negative control wells during stages 2 and 3 were maintained throughout in DMEM:F12 base medium with 2% FCS or 1% B27 and without any other additives. After formation of pancreatic endoderm, cells were treated further for six days duration, feeding daily with DMEM:F12 base medium containing 1% B27 with 1 μM DAPT (gamma secretase inhibitor: EMD Biosciences) and 50 ng/ml Exendin 4 (Sigma-Aldrich). Cells were then treated for another three days duration, feeding daily with DMEM:F12 base medium containing 1% B27, 50 ng/ml Exendin 4, 50 ng/ml IGF (R&D Biosystems) and 50 ng/ml HGF (R&D Biosystems). Parallel negative control wells were maintained throughout in DMEM:F12 base medium with 1% B27 and without any other additives.

[0259] *Assay evaluation:* At the end of culture, cells were treated as in **Examples 7** and **8** above for evaluation by high content analysis or real-time PCR.

[0260] For high content fluorescence staining, cells in 96-well plates were washed twice with PBS then fixed with 4% paraformaldehyde at room temperature for 20 minutes, washed three times more with PBS, and then permeabilized with 0.5% Triton X-100 for 20 minutes at room temperature. After fixing and permeabilizing, cells were washed again three times with PBS and blocked with 4% chicken serum (Invitrogen) in PBS for 30 minutes at room temperature. Primary antibody (guinea pig anti-swine insulin, cross-reactive with human insulin; DakoCytomation) was diluted 1:500 in 4% goat serum and added to cells for one hour at room temperature. Cells were washed three

times with PBS and then stained with Alexa Fluor 488 conjugated secondary antibody (goat anti-guinea pig IgG; Molecular Probes) diluted 1:100 in 4% goat serum. To counterstain nuclei, 2μg/ml Hoechst 33342 (Invitrogen) was added for ten minutes at room temperature. Cells were washed once with PBS and left in 100 μl/well PBS for imaging.

[0261] Cells were imaged using an IN Cell Analyzer 1000 (GE Healthcare) utilizing the 51008bs dichroic for cells stained with Hoechst 33342 and Alexa Fluor 488. Exposure times were optimized using positive control wells and wells stained with secondary antibody alone. Images from 15 fields per well were acquired to compensate for any cell loss during the treatment and staining procedures. Measurements for total cell number and total insulin intensity were obtained for each well using IN Cell Developer Toolbox 1.7 (GE Healthcare) software. Segmentation for the nuclei was determined based on grey-scale levels (baseline range 100-300) and nuclear size. Averages and standard deviations were calculated for each replicate data set. Total insulin protein expression was reported as total intensity or integrated intensity, defined as total fluorescence of the cell times area of the cell. Background was eliminated based on acceptance criteria of grey-scale ranges between 300 to 3000. Total intensity data were normalized by dividing the total intensities for each well by the average total intensity for the Wnt3a/Activin A positive control. Normalized data were calculated for averages and standard deviations for each triplicate set.

[0262] Cells for quantitative PCR were lysed in RLT buffer (Qiagen) and then processed for RNA extraction, purification, and cDNA synthesis. RNA samples were purified by binding to a silica-gel membrane (Rneasy Mini Kit, Qiagen, CA) in the presence of an ethanol-containing, high-salt buffer followed by washing to remove contaminants. The RNA was further purified using a TURBO DNA-free kit (Ambion, INC), and high-quality RNA was eluted in water. Yield and purity were assessed by A260 and A280 readings on a spectrophotometer. cDNA copies were made from purified RNA using an Applied Biosystems, Inc. (ABI, CA) high capacity cDNA archive kit.

[0263] Unless otherwise stated, all reagents for real-time PCR amplification and quantitation were purchased from ABI. Real-time PCR reactions were performed using the ABI PRISM® 7900 Sequence Detection System. TAQMAN® UNIVERSAL PCR MASTER MIX® (ABI, CA) was used with 20 ng of reverse transcribed RNA in a total reaction volume of 20 µl. Each cDNA sample was run in duplicate to correct for pipetting errors. Primers and FAM-labeled TAQMAN®probes were used at concentrations of 200 nM. The level of expression for each target gene was normalized using a human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) endogenous control previously developed by ABI. Primer and probe sets are listed as follows: PDX1 (Hs00236830_m1), Insulin (Hs00355773), and GAPDH (4310884E).

[0264] After an initial incubation at 50°C for 2 min followed by 95°C for 10 min, samples were cycled 40 times in two stages, a denaturation step at 95°C for 15 sec followed by an annealing/extension step at 60°C for 1 min. Data analysis was carried out using GENEAMP®7000 Sequence Detection System software. For each primer/probe set, a C_t value was determined as the cycle number at which the fluorescence intensity reached a specific value in the middle of the exponential region of amplification. Relative gene expression levels were calculated using the comparative C_t method. Briefly, for each cDNA sample, the endogenous control C_t value was subtracted from the gene of interest C_t to give the delta C_t value (ΔC_t). The normalized amount of target was calculated as $2^{-\Delta Ct}$, assuming amplification to be 100% efficiency. Final data were expressed relative to a calibrator sample.

Results

[0265] Results are shown for eight GSK-3B enzyme inhibitors. Data presented in **Figure 13** from high content analysis show compound effects on cell number (panel A) and insulin intensity (panel B) for the H1 hES cell line where respective data points were averaged from a triplicate set and mined for each parameter from identical fields and wells. Data presented in **Figure 14** from real-time PCR show compound effects for Pdx1 and insulin. In these examples, Pdx1 and insulin expression are indicative of pancreatic endoderm

differentiation and generation of hormonal positive cells. Selective GSK3β inhibitor compounds in these assays can substitute for Wnt3a during early stages of cell lineage commitment and can induce and sustain pancreatic beta cell formation during later sequential stages of differentiation, as evident from both insulin immunostaining and real-time PCR.

Example 11

Additive Effects of GSK-3β Enzyme Inhibitors on the Formation of Pancreatic Endocrine Cells

[0266] It was important to demonstrate that treatment with GSK3β inhibitors could improve pancreatic beta cell differentiation if added during multiple phases of cell fate commitment. A select subset of hits was tested by sequential timed addition to enhance insulin expression associated with pancreatic hormonal positive cells.

[0267] *Preparation of cells for assay: Cell preparation for assay:* Pancreatic endoderm cells obtained according to the methods described in **Example 9** and **10** (cultured on 96-wellplates) were subsequently subjected to agents that cause the cells to differentiate into pancreatic hormone expressing cells.

[0268] Assay for cultures of the H1 human embryonic stem cell line on MATRIGEL™ was initiated as described in **Examples 7 - 9** above by aspirating culture medium from cell monolayers in each well followed by three washes in PBS to remove residual growth factors and serum. For differentiation to definitive endoderm, test volumes (100 µl per well for 96-well plates) were added containing medium with 0.5% FCS and different concentrations of inhibitor compounds with 100 ng/ml Activin A, without Wnt3a. Positive control wells contained the same base medium and 0.5% FCS with 100ng/ml Activin A and 20ng/ml Wnt3a (R&D Biosystems) in the absence of test compound. Negative control wells contained the same base medium with 0.5% FCS, in the absence of Activin A, Wnt3a, or test compound. Assay wells were aspirated and fed again with identical concentrations of test compound or control solutions on day 2 of assay. On

days 3, 4, and 5, all assay wells were aspirated and fed with DMEM:F12 supplemented with 2% FCS and 100ng/ml Activin A in the absence of both test compound or Wnt3a. Parallel negative control wells were maintained on days 3, 4, and 5 in DMEM:F12 base medium with 2% FCS. For differentiation to pancreatic endoderm, cells were treated for three days, feeding daily with DMEM:F12 base medium containing 2% FCS with 0.25 μM KAAD cyclopamine (EMD Biosciences) and 20 ng/ml FGF7 (R&D Biosystems). Cells were subsequently treated for four days, feeding daily with DMEM:F12 containing 1% B27 (Invitrogen) , 0.25 μM KAAD cyclopamine, 2 μM Retinoic Acid (RA; Sigma-Aldrich) and 20 ng/ml FGF7. Parallel negative control wells were maintained throughout in DMEM:F12 base medium with 2% FCS or 1% B27 and without any other additives. After formation of pancreatic endoderm, cells were treated further for six days duration, feeding alternating days with DMEM:F12 base medium containing 1% B27 with 1 μM DAPT (gamma secretase inhibitor: EMD Biosciences) and 50 ng/ml Exendin 4 (Sigma-Aldrich) and 1 μM TGFbeta R1 inhibitor II (ALK5 inhibitor; EMD Biosciences). During this six day period, GSK3β inhibitors were added back to respective wells, using the same concentration as previous treatment at the initiation of differentiation. Cells were then treated for another three days duration, feeding alternating days with DMEM:F12 base medium containing 1% B27, 50 ng/ml Exendin 4, 50 ng/ml IGF (R&D Biosystems) and 50 ng/ml HGF (R&D Biosystems), and 1 μM TGFbeta R1 inhibitor II (ALK5 inhibitor; EMD Biosciences). During this three day period, GSK3β inhibitors were added back to respective wells, using the same concentration as previous treatment at the initiation of differentiation. Parallel sets of positive control wells were treated in the presence or absence of 20ng/ml Wnt3a. Parallel negative control wells were maintained throughout in DMEM:F12 base medium with 1% B27 and without any other additives.

[0269] *Assay evaluation:* At the end of culture, cells were treated as in **Examples 10** above for evaluation by high content analysis.

[0270] For high content fluorescence staining, cells in 96-well plates were washed twice with PBS then fixed with 4% paraformaldehyde at room temperature for 20 minutes, washed three times more with PBS, and then permeabilized with 0.5% Triton X-100 for 20 minutes at room temperature. After fixing and permeabilizing, cells were washed again three times with PBS and blocked with 4% chicken serum (Invitrogen) in PBS for 30 minutes at room temperature. Primary antibody (guinea pig anti-swine insulin, cross-reactive with human insulin; DakoCytomation) was diluted 1:500 in 4% goat serum and added to cells for one hour at room temperature. Cells were washed three times with PBS and then stained with Alexa Fluor 488 conjugated secondary antibody (goat anti-guinea pig IgG; Molecular Probes) diluted 1:100 in 4% goat serum. To counterstain nuclei, 2μg/ml Hoechst 33342 (Invitrogen) was added for ten minutes at room temperature. Cells were washed once with PBS and left in 100 μl/well PBS for imaging.

[0271] Cells were imaged using an IN Cell Analyzer 1000 (GE Healthcare) utilizing the 51008bs dichroic for cells stained with Hoechst 33342 and Alexa Fluor 488. Exposure times were optimized using positive control wells and wells stained with secondary antibody alone. Images from 15 fields per well were acquired to compensate for any cell loss during the treatment and staining procedures. Measurements for total cell number and total insulin intensity were obtained for each well using IN Cell Developer Toolbox 1.7 (GE Healthcare) software. Segmentation for the nuclei was determined based on grey-scale levels (baseline range 100-300) and nuclear size. Averages and standard deviations were calculated for each replicate data set. Total insulin protein expression was reported as total intensity or integrated intensity, defined as total fluorescence of the cell times area of the cell. Background was eliminated based on acceptance criteria of grey-scale ranges between 300 to 3000. Total intensity data were normalized by dividing the total intensities for each well by the average total intensity for the Wnt3a/Activin A positive control. Normalized data were calculated for averages and standard deviations for each triplicate set.

Results

[0272] Results are shown for eight GSK-3B enzyme inhibitors. Data presented in **Figure 15** from high content analysis show compound effects on cell number (panel A) and insulin intensity (panel B) for the H1 hES cell line, where respective data points were averaged from a triplicate set and mined for each parameter from identical fields and wells. In this example, insulin expression is indicative of differentiation to hormonal positive pancreatic cells. Selective GSK3β inhibitor compounds in these assays can substitute for Wnt3a during early stages of cell lineage commitment and, when added at later stages of differentiation, appear to promote enhanced insulin expression relative to a positive control sample.

[0273] Publications cited throughout this document are hereby incorporated by reference in their entirety. Although the various aspects of the invention have been illustrated above by reference to examples and preferred embodiments, it will be appreciated that the scope of the invention is defined not by the foregoing description but by the following claims properly construed under principles of patent law.

TABLE IA: LIST OF PRIMARY ANTIBODIES USED FOR FACS AND IMMUNOSTAININGANALYSIS.

Antibody	Supplier	Isotype	Clone
SSEA-1	Chemicon (CA)	Mouse IgM	MC-480
SSEA-3	Chemicon (CA)	Mouse IgG3	MC-631
SSEA-4	Chemicon (CA)	Rat IgM	MC-813-70
TRA 1-60	Chemicon (CA)	Mouse IgM	TRA 1-60
TRA 1-81	Chemicon (CA)	Mouse IgM	TRA 1-81
TRA 1-85	Chemicon (CA)	Mouse IgG1	TRA 1-85
AP	R&D Systems	Mouse IgG1	B4-78
HNF3β	R&D Systems	Goat IgG	
PDX1	Santa Cruz Biotechnology, INC	Goat IgG	A-17
GATA4	R&D Systems	Goat IgG	
Sox 17	R&D Systems	Goat IgG	
CD 9	BD	Mouse IgG1	M-L13

TABLE IB: LIST OF SECONDARY CONJUGATED ANTIBODIES USED FOR FACS AND IMMUNOSTAININGANALYSIS.

Secondary Conjugated Antibody	Supplier	Dilution
Goat Anti-Mouse IgG APC conjugated	Jackson ImmunoResearch (PA)	1:200
Goat Anti-Mouse IgG PE conjugated	Jackson ImmunoResearch (PA)	1:200
Donkey anti-rabbit PE or – APC conjugated	Jackson ImmunoResearch (PA)	1:200
Donkey anti-goat PE or – APC conjugated	Jackson ImmunoResearch (PA)	1:200
Goat anti-mouse IgM PE	SouthernBiotech (AL)	1:200
Goat anti-Rat IgM PE	SouthernBiotech (AL)	1:200
Goat anti-mouse IgG3 PE	SouthernBiotech (AL)	1:200

TABLE II: EFFECTS OF INHIBITORS OF GSK-3B ENZYME ACTIVITY ON THE VIABILITY OF CELLS EXPRESSING PLURIPOTENCY MARKERS.

Compound #	Raw data (duplicate)		Average	S.D.	% CV	% Control
2	0.785	0.790	0.788	0.00382	0.48	94.0
12	0.148	0.152	0.150	0.00247	1.65	4.8
20	0.427	0.462	0.444	0.02496	5.62	46.0
28	0.643	0.638	0.641	0.00368	0.57	73.5
1	0.762	0.762	0.762	0.00007	0.01	90.4
46	0.850	0.824	0.837	0.01824	2.18	101.0
52	0.911	0.884	0.898	0.01881	2.10	109.5
61	0.723	0.743	0.733	0.01421	1.94	86.4
3	0.161	0.169	0.165	0.00559	3.39	6.9
13	0.767	0.789	0.778	0.01556	2.00	92.6
20	0.512	0.555	0.533	0.03048	5.72	58.4
28	0.282	0.293	0.288	0.00792	2.75	24.1
37	0.764	0.723	0.743	0.02892	3.89	87.9
47	0.853	0.858	0.855	0.00382	0.45	103.5
53	0.832	0.837	0.834	0.00361	0.43	100.6
62	0.726	0.725	0.725	0.00042	0.06	85.3
4	0.132	0.137	0.134	0.00368	2.74	2.6
14	0.797	0.793	0.795	0.00346	0.44	95.1
21	0.776	0.787	0.782	0.00792	1.01	93.2
29	0.164	0.148	0.156	0.01131	7.24	5.6
38	0.475	0.383	0.429	0.06548	15.26	43.8
47	0.823	0.774	0.798	0.03444	4.31	95.6
54	0.781	0.729	0.755	0.03649	4.83	89.5
63	0.143	0.149	0.146	0.00396	2.72	4.2
5	0.716	0.716	0.716	0.00014	0.02	84.1
14	0.804	0.802	0.803	0.00148	0.18	96.2
22	0.900	0.877	0.888	0.01626	1.83	108.2
30	0.824	0.799	0.812	0.01725	2.13	97.4
39	0.744	0.819	0.781	0.05261	6.73	93.2

TABLE II: CONTINUED

Compound #	Raw data (duplicate)		Average	S.D.	% CV	% Control
48	0.952	0.966	0.959	0.00933	0.97	118.1
55	0.952	0.919	0.935	0.02277	2.43	114.8
64	0.776	0.777	0.777	0.00042	0.05	92.5
5	0.691	0.617	0.654	0.05254	8.03	75.4
15	0.162	0.134	0.148	0.02022	13.66	4.5
9	0.791	0.608	0.700	0.12947	18.50	81.8
31	0.153	0.129	0.141	0.01676	11.87	3.5
40	0.731	0.585	0.658	0.10317	15.68	75.9
DMSO	0.789	0.700	0.744	0.06279	8.44	88.0
56	0.909	0.675	0.792	0.16546	20.88	94.7
65	0.164	0.151	0.157	0.00926	5.89	5.8
6	0.706	0.672	0.689	0.02404	3.49	83.9
16	0.641	0.601	0.621	0.02878	4.63	73.7
23	0.882	0.748	0.815	0.09504	11.66	102.5
32	0.822	0.802	0.812	0.01400	1.72	102.1
41	0.777	0.764	0.771	0.00919	1.19	95.9
DMSO	0.798	0.771	0.785	0.01916	2.44	98.0
57	0.791	0.789	0.790	0.00134	0.17	98.7
66	0.628	0.640	0.634	0.00806	1.27	75.6
7	0.149	0.135	0.142	0.00969	6.81	2.7
17	0.803	0.782	0.792	0.01492	1.88	99.1
24	0.125	0.129	0.127	0.00318	2.51	0.4
33	0.315	0.542	0.428	0.15995	37.34	45.2
42	0.820	0.748	0.784	0.05091	6.49	97.9
48	0.154	0.165	0.160	0.00806	5.05	5.3
58	0.737	0.730	0.734	0.00481	0.66	90.4
67	0.659	0.647	0.653	0.00813	1.25	78.5
8	0.165	0.154	0.159	0.00785	4.93	5.2
18	0.637	0.554	0.595	0.05876	9.87	69.9
25	0.684	0.588	0.636	0.06809	10.71	76.0
34	0.750	0.624	0.687	0.08945	13.02	83.5
43	0.678	0.618	0.648	0.04285	6.61	77.8

TABLE II: CONTINUED

Compound #	Raw data (duplicate)		Average	S.D.	% CV	% Control
49	0.777	0.667	0.722	0.07757	10.74	88.7
DMSO	0.799	0.649	0.724	0.10564	14.59	89.0
68	0.648	0.625	0.636	0.01662	2.61	76.0
10	0.601	0.620	0.611	0.01308	2.14	72.2
19	0.695	0.702	0.698	0.00552	0.79	85.2
26	0.568	0.709	0.639	0.09956	15.59	76.4
35	0.623	0.765	0.694	0.10041	14.46	84.6
44	0.758	0.762	0.760	0.00297	0.39	94.3
50	0.487	0.434	0.461	0.03769	8.18	49.9
59	0.690	0.686	0.688	0.00262	0.38	83.7
69	0.535	0.550	0.543	0.01089	2.01	62.1
11	0.743	0.638	0.691	0.07446	10.78	84.1
19	0.694	0.603	0.649	0.06449	9.94	77.8
27	0.160	0.186	0.173	0.01824	10.56	7.2
36	0.662	0.566	0.614	0.06788	11.05	72.7
45	0.600	0.514	0.557	0.06102	10.96	64.2
51	0.685	0.524	0.605	0.11427	18.90	71.3
60	0.731	0.525	0.628	0.14552	23.18	74.7
74	0.715	0.596	0.655	0.08436	12.87	78.8
74	0.592	0.572	0.582	0.01393	2.39	70.0
80	0.614	0.611	0.613	0.00177	0.29	74.6
90	0.766	0.849	0.807	0.05869	7.27	104.3
99	0.830	0.813	0.822	0.01195	1.45	106.5
108	0.727	0.730	0.728	0.00198	0.27	92.2
117	0.713	0.836	0.774	0.08733	11.28	99.3
126	0.726	0.719	0.722	0.00523	0.72	91.3
138	0.646	0.681	0.663	0.02510	3.78	82.4
71	0.651	0.649	0.650	0.00120	0.19	80.3
81	0.642	0.622	0.632	0.01407	2.23	77.5
91	0.843	0.672	0.758	0.12099	15.97	96.7
100	0.734	0.815	0.774	0.05728	7.40	99.3
109	0.823	0.743	0.783	0.05699	7.28	100.6

TABLE II: CONTINUED

Compound #	Raw data (duplicate)		Average	S.D.	% CV	% Control
118	0.871	0.874	0.872	0.00219	0.25	114.2
152	0.652	0.642	0.647	0.00721	1.12	79.8
134	0.617	0.633	0.625	0.01174	1.88	76.5
72	0.657	0.655	0.656	0.00134	0.20	81.2
82	0.684	0.809	0.746	0.08803	11.80	95.0
92	0.901	0.735	0.818	0.11731	14.34	106.0
101	0.791	0.768	0.779	0.01591	2.04	100.1
110	0.948	0.764	0.856	0.12982	15.17	111.7
119	0.821	0.608	0.714	0.15033	21.05	90.1
127	0.745	0.685	0.715	0.04243	5.94	90.2
135	0.624	0.618	0.621	0.00417	0.67	76.0
73	0.652	0.624	0.638	0.01916	3.00	78.5
83	0.773	0.662	0.718	0.07792	10.86	90.6
93	0.856	0.834	0.845	0.01570	1.86	110.1
102	0.828	0.800	0.814	0.02008	2.47	105.4
111	0.821	0.841	0.831	0.01421	1.71	108.0
120	0.816	0.787	0.802	0.02072	2.58	103.5
127	0.744	0.737	0.741	0.00453	0.61	94.1
136	0.699	0.679	0.689	0.01464	2.12	86.3
76	0.186	0.208	0.197	0.01541	7.83	11.3
84	0.665	0.699	0.682	0.02432	3.57	85.2
94	0.810	0.683	0.746	0.09030	12.10	95.0
103	0.141	0.162	0.151	0.01506	9.95	4.3
DMSO	0.784	0.605	0.695	0.12671	18.25	87.1
121	0.726	0.590	0.658	0.09624	14.63	81.5
128	0.635	0.620	0.628	0.01068	1.70	76.9
136	0.697	0.695	0.696	0.00113	0.16	87.3
75	0.154	0.153	0.154	0.00042	0.28	4.5
85	0.616	0.645	0.630	0.02072	3.29	82.1
70	0.909	0.830	0.869	0.05614	6.46	121.0
104	0.150	0.150	0.150	0.00028	0.19	3.9
112	0.981	1.056	1.018	0.05303	5.21	145.3

TABLE II: CONTINUED

Compound #	Raw data (duplicate)		Average	S.D.	% CV	% Control
122	0.166	0.189	0.177	0.01626	9.19	8.3
129	0.718	0.451	0.584	0.18887	32.34	74.6
137	0.652	0.647	0.649	0.00389	0.60	85.2
77	0.503	0.529	0.516	0.01860	3.61	63.5
86	0.603	0.609	0.606	0.00424	0.70	78.1
95	0.856	0.793	0.824	0.04419	5.36	113.7
154	0.883	0.848	0.866	0.02503	2.89	120.5
113	0.779	0.784	0.781	0.00368	0.47	106.7
123	0.892	0.914	0.903	0.01591	1.76	126.6
130	0.544	0.537	0.540	0.00460	0.85	67.5
139	0.532	0.682	0.607	0.10543	17.37	78.3
77	0.665	0.645	0.655	0.01400	2.14	86.1
87	0.676	0.677	0.677	0.00035	0.05	89.7
96	0.935	0.807	0.871	0.09115	10.47	121.3
105	0.916	0.859	0.887	0.03981	4.49	124.0
114	0.907	0.891	0.899	0.01124	1.25	125.9
124	0.909	0.896	0.902	0.00919	1.02	126.4
131	0.682	0.797	0.740	0.08118	10.98	99.9
140	0.679	0.644	0.661	0.02510	3.80	87.2
78	0.300	0.223	0.261	0.05452	20.88	22.0
88	0.183	0.175	0.179	0.00573	3.20	8.6
97	0.741	0.728	0.734	0.00884	1.20	99.1
106	0.935	0.906	0.921	0.02051	2.23	129.4
115	0.131	0.128	0.129	0.00212	1.64	0.5
125	0.138	0.137	0.138	0.00092	0.67	1.9
132	0.241	0.227	0.234	0.01032	4.41	17.6
155	0.604	0.639	0.622	0.02475	3.98	80.7
79	0.247	0.182	0.215	0.04617	21.52	14.4
89	0.659	0.634	0.647	0.01718	2.66	84.8
98	0.758	0.575	0.667	0.12961	19.44	88.1
107	0.166	0.170	0.168	0.00276	1.64	6.9
116	0.651	0.559	0.605	0.06541	10.81	78.0

TABLE II: CONTINUED

Compound #	Raw data (duplicate)		Average	S.D.	% CV	% Control
126	0.803	0.694	0.748	0.07693	10.28	101.3
133	0.823	0.634	0.728	0.13378	18.37	98.1
141	0.624	0.618	0.621	0.00431	0.69	80.6
161	0.639	0.603	0.621	0.02553	4.11	73.6
171	0.143	0.149	0.146	0.00403	2.76	2.9
251	0.817	0.818	0.818	0.00071	0.09	102.8
188	0.742	0.752	0.747	0.00679	0.91	92.2
198	0.856	0.905	0.881	0.03479	3.95	112.1
207	0.650	0.576	0.613	0.05268	8.59	72.4
216	0.768	0.724	0.746	0.03097	4.15	92.2
225	0.556	0.549	0.553	0.00537	0.97	63.4
162	0.227	0.242	0.235	0.01103	4.70	16.1
172	0.634	0.663	0.649	0.02044	3.15	77.7
180	0.141	0.128	0.135	0.00919	6.83	1.3
189	0.847	0.832	0.840	0.01110	1.32	106.0
199	0.803	0.845	0.824	0.02998	3.64	103.7
208	0.860	0.860	0.860	0.00035	0.04	109.1
217	0.528	0.497	0.513	0.02227	4.34	57.5
226	0.683	0.688	0.686	0.00332	0.48	83.1
180	0.611	0.628	0.620	0.01202	1.94	73.3
173	0.719	0.749	0.734	0.02143	2.92	90.3
181	0.916	0.838	0.877	0.05487	6.26	111.6
190	0.771	0.740	0.755	0.02178	2.88	93.5
200	0.820	0.852	0.836	0.02305	2.76	105.5
209	0.971	0.913	0.942	0.04137	4.39	121.2
221	0.839	0.743	0.791	0.06746	8.53	98.8
227	0.562	0.527	0.544	0.02440	4.48	62.2
163	0.678	0.661	0.670	0.01195	1.78	80.8
174	0.722	0.713	0.717	0.00658	0.92	87.9
182	0.802	0.801	0.802	0.00106	0.13	100.4
191	0.854	0.857	0.855	0.00205	0.24	108.4
201	0.767	0.798	0.782	0.02157	2.76	97.5

TABLE II: CONTINUED

Compound #	Raw data (duplicate)		Average	S.D.	% CV	% Control
210	0.789	0.776	0.782	0.00870	1.11	97.5
218	0.720	0.709	0.714	0.00764	1.07	87.4
228	0.641	0.618	0.630	0.01619	2.57	74.9
164	0.603	0.584	0.593	0.01372	2.31	69.4
175	0.135	0.158	0.146	0.01633	11.18	3.0
183	0.792	0.572	0.682	0.15563	22.83	82.6
192	0.752	0.593	0.673	0.11292	16.79	81.2
202	0.805	0.598	0.702	0.14644	20.87	85.5
211	0.599	0.504	0.552	0.06682	12.11	63.2
219	0.714	0.593	0.654	0.08549	13.08	78.4
229	0.699	0.698	0.698	0.00099	0.14	85.0
165	0.690	0.674	0.682	0.01131	1.66	83.3
176	0.616	0.634	0.625	0.01301	2.08	74.8
184	0.809	0.817	0.813	0.00552	0.68	103.0
193	0.128	0.133	0.131	0.00361	2.76	0.7
203	0.821	0.811	0.816	0.00721	0.88	103.4
212	0.456	0.474	0.465	0.01223	2.63	50.8
220	0.762	0.766	0.764	0.00304	0.40	95.7
230	0.680	0.663	0.671	0.01195	1.78	81.8
166	0.615	0.635	0.625	0.01400	2.24	74.8
169	0.681	0.698	0.689	0.01266	1.84	84.5
185	0.830	0.807	0.818	0.01584	1.94	103.8
194	0.869	0.849	0.859	0.01442	1.68	109.9
204	0.821	0.841	0.831	0.01428	1.72	105.7
213	0.819	0.840	0.830	0.01485	1.79	105.5
221	0.795	0.793	0.794	0.00078	0.10	100.1
231	0.640	0.636	0.638	0.00283	0.44	76.7
168	0.610	0.628	0.619	0.01266	2.05	73.9
177	0.143	0.144	0.144	0.00035	0.25	2.6
167	0.804	0.903	0.853	0.07000	8.20	109.0
195	0.918	0.854	0.886	0.04483	5.06	113.9
205	0.105	1.080	0.593	0.68971	116.37	70.0

TABLE II: CONTINUED

Compound #	Raw data (duplicate)		Average	S.D.	% CV	% Control
214	0.877	0.860	0.868	0.01209	1.39	111.3
222	0.808	0.695	0.751	0.07941	10.57	93.8
232	0.720	0.697	0.709	0.01648	2.33	87.3
169	0.636	0.621	0.629	0.01054	1.68	75.4
178	0.640	0.634	0.637	0.00474	0.74	76.6
186	0.833	0.833	0.833	0.00000	0.00	106.0
196	0.887	0.846	0.866	0.02934	3.39	111.0
206	0.845	0.877	0.861	0.02326	2.70	110.2
214	0.794	0.784	0.789	0.00686	0.87	99.4
223	0.770	0.786	0.778	0.01138	1.46	97.8
158	0.629	0.659	0.644	0.02128	3.30	77.7
170	0.584	0.558	0.571	0.01817	3.18	66.8
179	0.707	0.679	0.693	0.01987	2.87	85.0
187	0.727	0.578	0.652	0.10536	16.15	78.9
197	0.742	0.629	0.685	0.07969	11.63	83.8
DMSO	0.653	0.507	0.580	0.10310	17.78	68.0
215	0.722	0.568	0.645	0.10904	16.90	77.9
224	0.643	0.581	0.612	0.04384	7.16	72.9
233	0.608	0.590	0.599	0.01245	2.08	70.9
142	0.597	0.610	0.603	0.00926	1.54	71.2
143	0.687	0.668	0.677	0.01336	1.97	82.4
144	0.840	0.832	0.836	0.00594	0.71	106.1
145	0.831	0.822	0.826	0.00587	0.71	104.7
146	0.863	0.856	0.860	0.00509	0.59	109.7
147	0.886	0.802	0.844	0.05954	7.05	107.3
148	0.753	0.687	0.720	0.04660	6.47	88.8
149	0.455	0.463	0.459	0.00587	1.28	49.6
150	0.668	0.678	0.673	0.00764	1.13	81.7
151	0.181	0.171	0.176	0.00658	3.74	7.2
152	0.832	0.842	0.837	0.00658	0.79	106.3
153	0.795	0.802	0.798	0.00445	0.56	100.5
70	0.157	0.140	0.148	0.01202	8.11	3.0

TABLE II: CONTINUED

Compound #	Raw data (duplicate)		Average	S.D.	% CV	% Control
154	0.153	0.153	0.153	0.00035	0.23	3.7
155	0.168	0.154	0.161	0.00969	6.02	4.9
156	0.670	0.641	0.655	0.02079	3.17	79.1
159	0.706	0.679	0.693	0.01888	2.73	84.7
234	0.788	0.666	0.727	0.08627	11.86	89.8
235	0.879	0.785	0.832	0.06640	7.98	105.6
236	0.168	0.176	0.172	0.00537	3.13	6.6
237	0.946	0.848	0.897	0.06972	7.77	115.3
238	0.187	0.202	0.194	0.01089	5.61	9.9
239	0.906	0.688	0.797	0.15394	19.31	100.3
240	0.715	0.674	0.694	0.02850	4.10	84.9
241	0.695	0.700	0.697	0.00339	0.49	85.3
241	0.665	0.631	0.648	0.02369	3.66	78.0
242	0.590	0.613	0.601	0.01655	2.75	71.0
243	0.681	0.687	0.684	0.00382	0.56	83.3
244	0.829	0.821	0.825	0.00530	0.64	104.5
245	0.822	0.790	0.806	0.02270	2.82	101.6
246	0.671	0.684	0.677	0.00912	1.35	82.3
247	0.686	0.668	0.677	0.01266	1.87	82.3
248	0.212	0.197	0.204	0.01047	5.12	11.5
249	0.666	0.666	0.666	0.00007	0.01	80.7
250	0.736	0.656	0.696	0.05643	8.11	85.1
160	0.726	0.610	0.668	0.08217	12.30	81.0
157	0.303	0.310	0.306	0.00488	1.59	26.7
DMSO	0.786	0.659	0.722	0.09001	12.46	89.1
DMSO	0.673	0.649	0.661	0.01676	2.53	79.9
DMSO	0.701	0.686	0.693	0.01011	1.46	84.8

TABLE III: EFFECTS OF INHIBITORS OF GSK-3B ENZYME ACTIVITY ON THE VIABILITY OF CELLS EXPRESSING PLURIPOTENCY MARKERS.

Compound #	cmpd conc (μM)	Raw data (duplicate)		Average	S.D.	% CV	% Control
EXPRES 01 medium		0.6379	0.6180	0.6280	0.0141	2.2	74.6
no treatment		0.7412	0.7038	0.7225	0.0264	3.7	88.7
AA only		0.7674	0.8047	0.7861	0.0264	3.4	98.3
AA + Wnt3a		0.7754	0.8200	0.7977	0.0315	4.0	100.0
144	10	0.1412	0.1515	0.1464	0.0073	5.0	2.4
144	5	0.1501	0.1444	0.1473	0.0040	2.7	2.5
144	2.5	0.1541	0.4254	0.2898	0.1918	66.2	23.9
145	10	0.1272	0.1282	0.1277	0.0007	0.6	-0.4
145	5	0.5862	0.5880	0.5871	0.0013	0.2	68.4
145	2.5	0.7613	0.7603	0.7608	0.0007	0.1	94.5
148	10	0.1481	0.1592	0.1537	0.0078	5.1	3.5
148	5	0.1479	0.1639	0.1559	0.0113	7.3	3.8
148	2.5	0.2861	0.2477	0.2669	0.0272	10.2	20.4
150	10	0.2092	0.2426	0.2259	0.0236	10.5	14.3
150	5	0.6815	0.7128	0.6972	0.0221	3.2	84.9
150	2.5	0.7389	0.7870	0.7630	0.0340	4.5	94.8
101	10	0.1381	0.1398	0.1390	0.0012	0.9	1.3
101	5	0.7826	0.7578	0.7702	0.0175	2.3	95.9
101	2.5	0.8231	0.7742	0.7987	0.0346	4.3	100.1
103	10	0.1352	0.1326	0.1339	0.0018	1.4	0.5
103	5	0.2632	0.2604	0.2618	0.0020	0.8	19.7
103	2.5	0.4160	0.5314	0.4737	0.0816	17.2	51.4
198	10	0.4447	0.4791	0.4619	0.0243	5.3	49.7

TABLE III - CONTINUED

Compound #	cmpd conc (μM)	Raw data (duplicate)		Average	S.D.	% CV	% Control
198	5	0.6902	0.6884	0.6893	0.0013	0.2	83.8
198	2.5	0.7476	0.7483	0.7480	0.0005	0.1	92.5
110	10	0.6790	0.6704	0.6747	0.0061	0.9	81.6
110	5	0.7833	0.7924	0.7879	0.0064	0.8	98.5
110	2.5	0.8155	0.8389	0.8272	0.0165	2.0	104.4
111	10	0.6533	0.6884	0.6709	0.0248	3.7	81.0
111	5	0.7697	0.7738	0.7718	0.0029	0.4	96.1
111	2.5	0.8119	0.8219	0.8169	0.0071	0.9	102.9
112	10	0.1242	0.1323	0.1283	0.0057	4.5	-0.4
112	5	0.1263	0.1303	0.1283	0.0028	2.2	-0.3
112	2.5	0.8480	0.7725	0.8103	0.0534	6.6	101.9
206	10	0.1695	0.1890	0.1793	0.0138	7.7	7.3
206	5	0.7217	0.7435	0.7326	0.0154	2.1	90.2
206	2.5	0.7812	0.7221	0.7517	0.0418	5.6	93.1
EXPRES 01medium		0.6294	0.6363	0.6329	0.0049	0.8	70.3
no treatment		0.7156	0.7356	0.7256	0.0141	1.9	83.3
AA only		0.8732	0.9046	0.8889	0.0222	2.5	106.0
AA + Wnt3a		0.8415	0.8500	0.8458	0.0060	0.7	100.0
52	10	0.1403	0.1493	0.1448	0.0064	4.4	2.3
52	5	0.4434	0.3878	0.4156	0.0393	9.5	40.1
52	2.5	0.7734	0.8038	0.7886	0.0215	2.7	92.0
133	10	0.2993	0.3026	0.3010	0.0023	0.8	24.1
133	5	0.7023	0.6299	0.6661	0.0512	7.7	75.0
133	2.5	0.7835	0.8043	0.7939	0.0147	1.9	92.8
223	10	0.7205	0.7369	0.7287	0.0116	1.6	83.7
223	5	0.7769	0.8272	0.8021	0.0356	4.4	93.9
223	2.5	0.8214	0.8640	0.8427	0.0301	3.6	99.6

TABLE III - CONTINUED

Compound #	cmpd conc (μM)	Raw data (duplicate)		Average	S.D.	% CV	% Control
221	10	0.6275	0.5980	0.6128	0.0209	3.4	67.5
221	5	0.7159	0.7222	0.7191	0.0045	0.6	82.3
221	2.5	0.9245	0.9403	0.9324	0.0112	1.2	112.1
226	10	0.7220	0.6670	0.6945	0.0389	5.6	78.9
226	5	0.7526	0.7486	0.7506	0.0028	0.4	86.7
226	2.5	0.7557	0.7390	0.7474	0.0118	1.6	86.3
136	10	0.8214	0.8636	0.8425	0.0298	3.5	99.5
136	5	0.7996	0.7873	0.7935	0.0087	1.1	92.7
136	2.5	0.8669	0.8195	0.8432	0.0335	4.0	99.6
158	10	0.6195	0.5908	0.6052	0.0203	3.4	66.5
158	5	0.8047	0.8319	0.8183	0.0192	2.4	96.2
158	2.5	0.8041	0.7900	0.7971	0.0100	1.3	93.2
233	10	0.1261	0.1520	0.1391	0.0183	13.2	1.5
233	5	0.1303	0.1263	0.1283	0.0028	2.2	0.0
233	2.5	0.4482	0.4051	0.4267	0.0305	7.1	41.6

TABLE IV: EFFECTS OF INHIBITORS OF GSK-3B ENZYME ACTIVITY ON THE DIFFERENTIATION AND PROLIFERATION OF HUMAN EMBRYONIC STEM CELLS.

	Proliferative Response		SOX-17 Expression		Proliferative Response		HNF-3b Expression	
Compound #	Total cells	Fold over Wnt 3a/AA control	Total Intensity	Fold over Wnt 3a/AA control	Total cells	Fold over Wnt 3a/AA control	Total Intensity	Fold over Wnt 3a/AA control
142	1723	0.11244207	68870409	0.0708	1645	0.10460717	50143628	0.0453
143	1110	0.07245904	42978557	0.0442	94	0.00597755	0	0.0000
144	7990	0.52154188	339840000	0.3494	6833	0.43448539	231745000	0.2092
145	4914	0.32074548	238555000	0.2453	2907	0.18485899	82808745	0.0747
146	3056	0.19945819	153145000	0.1575	2643	0.16807097	122246784	0.1103
147	3960	0.25850251	47669463	0.0490	4641	0.29512575	210730000	0.1902
148	12243	0.79917096	699160000	0.7189	6536	0.41559887	248855000	0.2246
149	401	0.02614400	25580022	0.0263	27	0.00168516	0	0.0000
150	7958	0.51948561	351070000	0.3610	6992	0.44459636	288075000	0.2600
151	277	0.01808212	6558563	0.0067	12	0.00073130	535481	0.0005
152	1327	0.08662445	69037756	0.0710	1194	0.07589584	40478497	0.0365
153	791	0.05160259	24732475	0.0254	64	0.00406982	1092011	0.0010
70	0	0.00000000	0	0.0000	3	0.00019077	95784	0.0001
154	2	0.00013056	0	0.0000	0	0.00000000	0	0.0000
155	6	0.00035903	1092432	0.0011	2	0.00009539	150222	0.0001
156	2742	0.17899341	122926199	0.1264	3166	0.20132905	120729987	0.1090
157	33	0.00212155	3855900	0.0040	8	0.00050873	208129	0.0002
213	2000	0.13055682	110080123	0.1132	116	0.00737655	4290889	0.0039

TABLE IV. CONTINUED

Compound #	Proliferative Response		SOX-17 Expression		Proliferative Response		HNF-3b Expression	
	Total cells	Fold over Wnt 3a/AA control	Total Intensity	Fold over Wnt 3a/AA control	Total cells	Fold over Wnt 3a/AA control	Total Intensity	Fold over Wnt 3a/AA control
214	3495	0.22814805	110559816	0.1137	438	0.02782105	24450647	0.0221
214	3107	0.20278739	120998421	0.1244	6177	0.39276971	273965000	0.2473
215	658	0.04295320	37841044	0.0389	646	0.04107977	31352380	0.0283
216	5991	0.39108297	252690000	0.2598	8479	0.53915615	306520000	0.2767
217	1953	0.12745610	88653625	0.0912	641	0.04076182	18162585	0.0164
218	2024	0.13209087	128395000	0.1320	4923	0.31302661	232020000	0.2094
219	2979	0.19446439	93454696	0.0961	3582	0.22775110	137054653	0.1237
220	3703	0.24169332	138180000	0.1421	3980	0.25306032	139550000	0.1260
221	21070	1.37538351	1089750000	1.1205	21203	1.34831961	1281000000	1.1562
222	1297	0.08466610	47445962	0.0488	30	0.00190773	0	0.0000
223	14529	0.94839741	1013360000	1.0419	9871	0.62767480	540725000	0.4881
224	4063	0.26522619	207891758	0.2137	3973	0.25264697	177190000	0.1599
225	1	0.00006528	0	0.0000	7	0.00041334	0	0.0000
226	9716	0.63421242	572520000	0.5887	7650	0.48643922	329425000	0.2973
227	916	0.05979503	0	0.0000	1076	0.06839210	40211776	0.0363
228	738	0.04817547	30943000	0.0318	503	0.03198626	0	0.0000
229	8367	0.54618448	373185000	0.3837	7976	0.50720168	260000000	0.2347
230	20079	1.31069260	1104750000	1.1359	16884	1.07363836	1052345000	0.9499
231	13789	0.90012403	789085000	0.8113	11369	0.72296588	547055000	0.4938
232	16652	1.08698348	1045395000	1.0749	14950	0.95065340	854325000	0.7711
158	6376	0.41618252	324450000	0.3336	6058	0.38523417	269025000	0.2428
233	6470	0.42231869	327055000	0.3363	4357	0.27706591	109160000	0.0985

TABLE IV. CONTINUED

Compound #	Proliferative Response		SOX-17 Expression		Proliferative Response		HNF-3b Expression	
	Total cells	Fold over Wnt 3a/AA control	Total Intensity	Fold over Wnt 3a/AA control	Total cells	Fold over Wnt 3a/AA control	Total Intensity	Fold over Wnt 3a/AA control
No treatment	3891	0.25396566	97657703	0.1004	6091	0.38733268	109336609	0.0987
AA	4348	0.28379790	104735084	0.1077	122	0.00775810	5341271	0.0048
AA/3a	15319	1.00000000	972595000	1.0000	15726	1.00000000	1107900000	1.0000
161	738	0.44211577	0	0.0000	0	0.00000000	0	0.0000
162	0	0.00000000	0	0.0000	0	0.00000000	0	0.0000
DMSO	56	0.03353293	454796	0.0148	211	0.16644754	4455058	0.1626
163	1313	0.78642715	28506437	0.9266	5485	4.32684722	85245671	3.1115
164	12	0.00738523	85949	0.0028	67	0.05259006	1300640	0.0475
165	2899	1.73612774	32703235	1.0630	7460	5.88456482	149772525	5.4668
166	562	0.33632735	11388240	0.3702	787	0.62108861	10743082	0.3921
168	118	0.07045908	2574279	0.0837	57	0.04522745	2584708	0.0943
169	136	0.08163673	410648	0.0133	0	0.00000000	0	0.0000
170	19	0.01137725	0	0.0000	0	0.00000000	0	0.0000
171	3	0.00159681	431883	0.0140	31	0.02419143	847186	0.0309
172	33	0.01976048	0	0.0000	225	0.17749145	5223879	0.1907
173	16	0.00978044	0	0.0000	496	0.39127005	8966327	0.3273
174	26	0.01556886	459801	0.0149	189	0.14935577	1819533	0.0664
175	1	0.00039920	0	0.0000	42	0.03339469	1605538	0.0586
176	22	0.01297405	82062	0.0027	311	0.24506968	5749996	0.2099
177	0	0.00000000	0	0.0000	0	0.00000000	0	0.0000
178	26	0.01556886	0	0.0000	0	0.00000000	0	0.0000
179	202	0.12095808	627280	0.0204	1079	0.85143308	14326715	0.5229

TABLE IV. CONTINUED

Compound #	Proliferative Response		SOX-17 Expression		Proliferative Response		HNF-3b Expression	
	Total cells	Fold over Wnt 3a/AA control	Total Intensity	Fold over Wnt 3a/AA control	Total cells	Fold over Wnt 3a/AA control	Total Intensity	Fold over Wnt 3a/AA control
180	3	0.00179641	0	0.0000	4	0.00315540	101114	0.0037
181	1310	0.78423154	24382455	0.7926	3249	2.56323955	75834631	2.7680
182	20	0.01177645	0	0.0000	425	0.33526164	8880858	0.3242
184	9	0.00538922	37140	0.0012	134	0.10570602	2144545	0.0783
183	7	0.00419162	48154	0.0016	5	0.00420720	170177	0.0062
185	70	0.04191617	589594	0.0192	0	0.00000000	0	0.0000
186	1215	0.72774451	7568849	0.2460	0	0.00000000	0	0.0000
no Treatment	1145	0.68542914	6979814	0.2269	not done			
AA	100	0.05988024	1264807	0.0411	51	0.04049435	923625	0.0337
AA/3a	1670	1.00000000	30764293	1.0000	1268	1.00000000	27396787	1.0000
187	43	0.00510815	706614	0.0055	0	0.00000000	0	0.0000
188	7	0.00079815	102445	0.0008	0	0.00000000	0	0.0000
189	46	0.00546732	0	0.0000	46	0.00548446	818478	0.0044
190	5	0.00059861	284777	0.0022	32	0.00385502	2309043	0.0124
191	258	0.03092825	4009395	0.0312	391	0.04665766	14340307	0.0769
192	62	0.00742278	782261	0.0061	112	0.01335347	2792473	0.0150
193	36	0.00431000	312039	0.0024	2	0.00027820	1731575	0.0093
194	59	0.00702371	397711	0.0031	103	0.01232017	3561761	0.0191
195	22	0.00267380	770128	0.0060	0	0.00000000	0	0.0000
196	77	0.00925852	1631067	0.0127	0	0.00000000	0	0.0000
197	129	0.01540426	997629	0.0078	98	0.01164454	4138261	0.0222
198	2386	0.28565728	20866647	0.1625	2594	0.30931563	61161468	0.3280

TABLE IV. CONTINUED

Compound #	Proliferative Response		SOX-17 Expression		Proliferative Response		HNF-3b Expression	
	Total cells	Fold over Wnt 3a/AA control	Total Intensity	Fold over Wnt 3a/AA control	Total cells	Fold over Wnt 3a/AA control	Total Intensity	Fold over Wnt 3a/AA control
199	172	0.02063213	625299	0.0049	133	0.01589699	3578458	0.0192
200	8	0.00099769	394948	0.0031	530	0.06319053	16678849	0.0894
201	17	0.00207519	0	0.0000	53	0.00627931	2270954	0.0122
202	11	0.00127704	0	0.0000	36	0.00433193	2287281	0.0123
203	2	0.00023944	0	0.0000	0	0.00000000	0	0.0000
204	174	0.02087158	1451727	0.0113	0	0.00000000	0	0.0000
205	80	0.00961769	940367	0.0073	333	0.03970273	5586343	0.0300
206	11886	1.42305850	223646667	1.7415	10331	1.23173834	30900000	1.6618
207	545	0.06524862	5849381	0.0455	404	0.04820761	6738305	0.0361
208	10	0.00115732	315367	0.0025	35	0.00421270	3072013	0.0165
209	2473	0.29603320	80676667	0.6282	4209	0.50182815	143916667	0.7718
210	8	0.00091787	233687	0.0018	6	0.00071536	0	0.0000
211	1	0.00007981	1309298	0.0102	0	0.00000000	0	0.0000
212	0	0.00003991	0	0.0000	0	0.00000000	0	0.0000
No treatment	7653	0.91619443	26272707	0.2046	12050	1.43665050	74453588	0.3993
AA	15	0.00175593	0	0.0000	210	0.02503776	3777945	0.0203
AA/3a	8353	1.00000000	128424304	1.0000	8387	1.00000000	186480000	1.0000
169	7319	0.91843393	387695000	1.0342	5436	1.07644321	43749500	0.9520
185	6620	0.83065629	333205000	0.8889	4767	0.94395485	39743500	0.8649
167	6217	0.78014807	337920000	0.9014	5013	0.99277156	43723500	0.9515
reference compound	5934	0.74463546	363935000	0.9708	4122	0.81621943	34813500	0.7576

TABLE IV. CONTINUED

Compound #	Proliferative Response		SOX-17 Expression		Proliferative Response		HNF-3b Expression	
	Total cells	Fold over Wnt 3a/AA control	Total Intensity	Fold over Wnt 3a/AA control	Total cells	Fold over Wnt 3a/AA control	Total Intensity	Fold over Wnt 3a/AA control
47	10447	1.31089221	382680000	1.0208	6908	1.36805624	560475000	1.2196
2	10963	1.37570586	296920000	0.7921	5679	1.12456679	463525000	1.0087
3	1766	0.22160873	162790000	0.4343	2184	0.43241905	189875000	0.4132
4	2914	0.36566696	230965000	0.6161	2776	0.54975740	125125000	0.2723
5	3600	0.45175053	276080000	0.7365	4121	0.81612041	294665000	0.6412
5	1977	0.24808633	164760000	0.4395	2266	0.44865828	152060000	0.3309
6	9964.5	1.25040783	363855000	0.9706	9728	1.92642836	635655000	1.3832
7	2536.5	0.31829590	179185000	0.4780	2397	0.47460145	150600000	0.3277
8	5706.5	0.71608734	319930000	0.8534	5096	1.00920883	341360000	0.7428
10	4645.5	0.58294642	257295000	0.6864	4507	0.89256362	312605000	0.6803
11	2892.5	0.36296900	213165000	0.5686	3043	0.60253490	269570000	0.5866
12	2460.5	0.30875894	203350000	0.5425	2410	0.47727498	209795000	0.4565
13	4783	0.60020078	306085000	0.8165	4556	0.90226755	326475000	0.7104
14	6916.5	0.86792571	377885000	1.0080	4504	0.89196950	365090000	0.7945
14	7370.5	0.92489647	365075000	0.9739	5300	1.04950985	399265000	0.8688
15	10533	1.32174677	475250000	1.2678	5186	1.02693336	404710000	0.8807
16	3513	0.44083323	242750000	0.6476	2522	0.49945539	214575000	0.4669
No Treatment	not done				not done			
AA	not done				not done			
AA/3a	7969	1.00000000	374870000	1.0000	5050	1.00000000	459540000	1.0000
16	563	0.31250000	57351132	0.3295	1744	0.03386884	165365000	1.1010
17	158	0.08777778	14786632	0.0850	83	0.00161234	14201404	0.0946

TABLE IV. CONTINUED

Compound #	Proliferative Response		SOX-17 Expression		Proliferative Response		HNF-3b Expression	
	Total cells	Fold over Wnt 3a/AA control	Total Intensity	Fold over Wnt 3a/AA control	Total cells	Fold over Wnt 3a/AA control	Total Intensity	Fold over Wnt 3a/AA control
18	3	0.00166667	0	0.0000	4	0.00007770	28439	0.0002
19	5	0.00277778	0	0.0000	10	0.00019426	0	0.0000
19	15	0.00805556	548982	0.0032	0	0.00000000	0	0.0000
20	24	0.01305556	689535	0.0040	11	0.00021368	0	0.0000
20	94	0.05194444	11142426	0.0640	12	0.00022340	1767033	0.0118
21	15	0.00805556	0	0.0000	21	0.00039823	4567590	0.0304
22	33	0.01805556	2188847	0.0126	69	0.00134038	13689421	0.0911
9	4	0.00194444	0	0.0000	3	0.00005828	291660	0.0019
23	88	0.04888889	7121122	0.0409	399	0.00774117	65100086	0.4335
24	11	0.00583333	1073763	0.0062	5	0.00008742	0	0.0000
25	8	0.00444444	0	0.0000	9	0.00016512	0	0.0000
26	109	0.06027778	15714170	0.0903	136	0.00263219	15725984	0.1047
27	5	0.00250000	125443	0.0007	5	0.00009713	0	0.0000
28	20	0.01083333	3135653	0.0180	8	0.00015541	0	0.0000
28	9	0.00472222	72387	0.0004	17	0.00033024	736311	0.0049
29	6	0.00305556	644015	0.0037	4	0.00007770	0	0.0000
30	77	0.04277778	12632849	0.0726	28	0.00054392	9312311	0.0620
31	14	0.00750000	887585	0.0051	1	0.00001943	52047	0.0003
32	23	0.01277778	2117429	0.0122	13	0.00024282	0	0.0000
No Treatment	not done				432	0.00838222	42987388	0.2862
AA	147	0.08138889	20330009	0.1168	8	0.00014569	87206	0.0006
AA/3a	1800	1.00000000	174052346	1.0000	1478	0.02870158	150190000	1.0000

TABLE V: EFFECTS OF INHIBITORS OF GSK-3B ENZYME ACTIVITY ON THE DIFFERENTIATION AND PROLIFERATION OF HUMAN EMBRYONIC STEM CELLS.

Proliferative Response – Strong Hits		SOX 17 Strong Hits		HNF3β Expression - Strong Hits	
Compound #	Fold over Wnt 3a/AA control	Compound #	Fold over Wnt 3a/AA control	Compound #	Fold over Wnt 3a/AA control
165	5.8846	206	1.7415	165	5.4668
163	4.3268	15	1.2678	163	3.1115
181	2.5632	SOX17 Expression -Moderate Hits		181	2.7680
6	1.9264	230	1.1359	206	1.6618
206	1.4231	221	1.1205	6	1.3832
2	1.3757	232	1.0749	47	1.2196
221	1.3754	165	1.0630	HNF3β Expression - Moderate Hits	
47	1.3681	223	1.0419	221	1.1562
15	1.3217	169	1.0342	16	1.1010
230	1.3107	47	1.0208	2	1.0087
Proliferative Response - Moderate Hits		14	1.0080	169	0.9520
2	1.1246	reference cmpd	0.9708	167	0.9515
232	1.0870	6	0.9706	230	0.9499
169	1.0764	163	0.9266	15	0.8807
230	1.0736	167	0.9014	14	0.8688
14	1.0495	185	0.8889	185	0.8649
15	1.0269	8	0.8534	209	0.7718
8	1.0092	13	0.8165	232	0.7711
167	0.9928	231	0.8113	reference cmpd	0.7576
223	0.9484	181	0.7926	8	0.7428

TABLE V: CONTINUED

Proliferative Response – Moderate Hits		SOX 17 Moderate Hits		HNF3β Expression - Moderate Hits	
185	0.9440	2	0.7921	13	0.7104
14	0.9249	5	0.7365	10	0.6803
13	0.9023	148	0.7189	5	0.6412
231	0.9001	10	0.6864	11	0.5866
10	0.8926	16	0.6476		
179	0.8514	209	0.6282		
reference cmpd	0.8162	4	0.6161		
5	0.8161	226	0.5887		
148	0.7992	11	0.5686		
163	0.7864				
181	0.7842				
186	0.7277				
226	0.6342				
166	0.6211				
11	0.6025				

TABLE VI: EFFECTS OF INHIBITORS OF GSK-3B ENZYME ACTIVITY ON THE PROLIFERATION OF HUMAN EMBRYONIC STEM CELLS.

Compound #	Raw Data			Average	S.D.	% CV	% Control
conditioned medium	1.1348	1.0099	1.1092	1.0846	0.0660	6.1	116.5
no treatment	0.9344	0.5977	0.8454	0.7925	0.1745	22.0	85.2
AA/DMSO	0.3878	0.2434	0.2252	0.2855	0.0891	31.2	30.7
AA/Wnt3a/DMSO	0.6098	1.0804	0.7635	0.8179	0.2403	25.8	100.0
161	0.3418	0.4276	0.5751	0.4482	0.1180	26.3	48.2
162	0.1362	0.1531	0.1532	0.1475	0.0098	6.6	15.8
163	1.3764	1.2753	1.3208	1.3242	0.0506	3.8	142.3
164	0.6923	0.5994	0.6134	0.6350	0.0501	7.9	68.2
165	1.7896	1.4721	2.1908	1.8175	0.3602	19.8	195.3
166	1.7591	1.6274	1.6518	1.6794	0.0701	4.2	180.4
168	0.3702	0.3193	0.3368	0.3421	0.0259	7.6	36.8
169	0.5876	0.6384	0.9154	0.7138	0.1764	24.7	76.7
170	0.3074	0.2328	0.2920	0.2774	0.0394	14.2	29.8
171	0.1311	0.1245	0.1288	0.1281	0.0034	2.6	13.8
172	0.1270	0.2778	0.1916	0.1988	0.0757	38.1	21.4
173	0.2166	0.3062	0.2915	0.2714	0.0481	17.7	29.2
174	0.4362	0.3728	0.2481	0.3524	0.0957	27.2	37.9
175	0.1560	0.1481	0.1359	0.1467	0.0101	6.9	15.8
176	0.2932	0.3883	0.6258	0.4358	0.1713	39.3	46.8
177	0.1362	0.1479	0.1298	0.1380	0.0092	6.7	14.8
178	0.2198	0.2159	0.2300	0.2219	0.0073	3.3	23.8
179	0.7624	0.2705	0.2478	0.4269	0.2908	68.1	45.9
180	0.1239	0.1233	0.1269	0.1247	0.0019	1.5	13.4
181	0.1277	0.1254	0.6980	0.3170	0.3299	104.1	34.1
182	0.2665	0.3215	0.2605	0.2828	0.0336	11.9	30.4
183	0.2395	0.3235	0.1333	0.2321	0.0953	41.1	24.9
184	0.2646	0.1873	0.1293	0.1937	0.0679	35.0	20.8
185	0.3590	0.2790	0.1515	0.2632	0.1047	39.8	28.3
186	0.4690	0.5805	0.3349	0.4615	0.1230	26.6	49.6
Compound #	**Raw Data**			**Average**	**S.D.**	**% CV**	**% Control**
conditioned medium	1.1525	1.1269	1.1140	1.1311	0.0196	1.7	71.0
no treatment	1.2057	1.2358	1.3132	1.2516	0.0555	4.4	78.6
AA/DMSO	0.2622	0.2073	0.2830	0.2508	0.0391	15.6	15.8

Table VI: - CONTINUED

Compound #	Raw Data			Average	S.D.	% CV	% Control
AA/Wnt3a/DMSO	1.3943	1.7976	1.8000	1.5922	0.2136	13.4	100.0
187	0.1930	0.2223	0.2167	0.2107	0.0156	7.4	13.2
188	0.1757	0.1813	0.1835	0.1802	0.0040	2.2	11.3
189	0.1473	0.1880	0.1732	0.1695	0.0206	12.2	10.6
190	0.1330	0.1362	0.1867	0.1520	0.0301	19.8	9.5
191	0.8191	0.5493	0.6526	0.6737	0.1361	20.2	42.3
192	0.4008	0.2779	0.3869	0.3552	0.0673	18.9	22.3
193	0.1220	0.1248	0.1251	0.1240	0.0017	1.4	7.8
194	0.2883	0.3308	0.5503	0.3898	0.1406	36.1	24.5
195	0.2835	0.4024	0.5698	0.4186	0.1438	34.4	26.3
196	0.3704	0.6073	0.5280	0.5019	0.1206	24.0	31.5
197	0.2266	0.1815	0.2289	0.2123	0.0267	12.6	13.3
198	1.0820	1.1862	1.1076	1.1253	0.0543	4.8	70.7
199	0.3590	0.5457	0.6123	0.5057	0.1313	26.0	31.8
200	0.2198	0.3564	0.3202	0.2988	0.0708	23.7	18.8
201	0.2928	0.2920	0.3659	0.3169	0.0424	13.4	19.9
202	0.3349	0.3013	0.3507	0.3290	0.0252	7.7	20.7
203	0.1852	0.1924	0.2349	0.2042	0.0269	13.2	12.8
204	0.2170	0.3003	0.1877	0.2350	0.0584	24.9	14.8
205	0.3094	0.2515	0.1881	0.2497	0.0607	24.3	15.7
206	1.8452	1.7710	1.5591	1.7251	0.1485	8.6	108.3
207	0.7305	0.7067	0.6250	0.6874	0.0553	8.0	43.2
208	0.2113	0.1800	0.1547	0.1820	0.0284	15.6	11.4
209	1.5225	1.5912	0.1081	1.0739	0.8371	78.0	67.4
210	0.4006	1.2807	0.1162	0.5992	0.6071	101.3	37.6
211	0.1972	0.1839	0.1162	0.1658	0.0434	26.2	10.4
212	0.1351	0.1318	0.1169	0.1279	0.0097	7.6	8.0

Compound #	Raw Data		Average	S.D.	% CV	% Control
conditioned medium	1.0568	1.0604	1.0586	0.0025	0.2	71.9
no treatment	1.1544	0.9576	1.0560	0.1392	13.2	71.7
AA only + DMSO	0.6329	0.8434	0.7382	0.1488	20.2	47.1
AA + Wnt3a + DMSO	1.2704	1.8669	1.4229	0.2960	20.8	100.0
213	0.5617	0.2098	0.3858	0.2488	64.5	19.9
214	0.6850	0.5853	0.6352	0.0705	11.1	39.2
214	0.7496	0.9187	0.8342	0.1196	14.3	54.5

Table VI: - CONTINUED

Compound #	Raw Data		Average	S.D.	% CV	% Control
215	0.2320	0.2124	0.2222	0.0139	6.2	7.3
216	0.8079	1.4391	1.1235	0.4463	39.7	76.9
217	0.8310	0.7318	0.7814	0.0701	9.0	50.5
218	1.0646	1.1384	1.1015	0.0522	4.7	75.2
219	0.6344	1.0400	0.8372	0.2868	34.3	54.8
no cells	0.1335	0.2070	0.1703	0.0520	30.5	3.3
220	0.8643	0.4060	0.6352	0.3241	51.0	39.2
221	1.7922	1.8533	1.8228	0.0432	2.4	130.9
222	0.1914	0.2371	0.2143	0.0323	15.1	6.7
223	1.8401	1.7563	1.7982	0.0593	3.3	129.0
224	1.0301	1.0356	1.0329	0.0039	0.4	69.9
225	0.1306	0.1338	0.1322	0.0023	1.7	0.3
226	1.7143	1.6506	1.6825	0.0450	2.7	120.0
227	0.4170	0.4956	0.4563	0.0556	12.2	25.4
228	0.1772	0.2348	0.2060	0.0407	19.8	6.0
229	1.0231	1.2392	1.1312	0.1528	13.5	77.5
230	1.9718	2.0997	2.0358	0.0904	4.4	147.3
231	1.5168	1.6872	1.6020	0.1205	7.5	113.8
232	1.6935	1.9710	1.8323	0.1962	10.7	131.6
158	1.2655	1.1829	1.2242	0.0584	4.8	84.7
233	1.3481	1.3168	1.3325	0.0221	1.7	93.0
142	0.6444	0.7239	0.6842	0.0562	8.2	43.0
143	0.2046	0.3076	0.2561	0.0728	28.4	9.9
144	1.3627	1.0693	1.2160	0.2075	17.1	84.0
145	0.8722	0.9660	0.9191	0.0663	7.2	61.1
146	1.0332	0.4554	0.7443	0.4086	54.9	47.6
147	0.8775	0.7347	0.8061	0.1010	12.5	52.4
148	1.7865	1.2008	1.4937	0.4142	27.7	105.5
149	0.2396	0.1584	0.1990	0.0574	28.9	5.5
150	0.8122	1.0827	0.9475	0.1913	20.2	63.3
151	0.1342	0.1363	0.1353	0.0015	1.1	0.6
152	1.0462	0.5838	0.8150	0.3270	40.1	53.1
153	0.4586	0.2903	0.3745	0.1190	31.8	19.0
70	0.1277	0.1402	0.1340	0.0088	6.6	0.5
154	0.1258	0.1324	0.1291	0.0047	3.6	0.1
155	0.1219	0.1216	0.1218	0.0002	0.2	-0.5
156	0.4223	0.4721	0.4472	0.0352	7.9	24.7

Table VI: - CONTINUED

Compound #	Raw Data		Average	S.D.	% CV	% Control
157	0.1514	0.1396	0.1455	0.0083	5.7	1.4
Compound #	**Raw Data**		**Average**	**S.D.**	**% CV**	**% Control**
conditioned medium	0.7423	0.7081	0.7252	0.0242	3.3	87.7
no treatment	0.4936	0.5689	0.5313	0.0532	10.0	59.8
AA only + DMSO	0.1433	0.1939	0.1686	0.0358	21.2	7.6
AA + Wnt3a + DMSO	0.6808	0.9406	0.8107	0.1837	22.7	100.0
33	0.2447	0.1331	0.1889	0.0789	41.8	10.6
34	0.1537	0.1302	0.1420	0.0166	11.7	3.8
no cells	0.1163	0.1147	0.1155	0.0011	1.0	0.0
35	0.2994	0.2592	0.2793	0.0284	10.2	23.6
36	0.1353	0.2121	0.1737	0.0543	31.3	8.4
1	0.1267	0.1419	0.1343	0.0107	8.0	2.7
37	0.1376	0.1676	0.1526	0.0212	13.9	5.3
38	0.1134	0.1103	0.1119	0.0022	2.0	-0.5
39	0.1318	0.1478	0.1398	0.0113	8.1	3.5
40	0.2569	0.2124	0.2347	0.0315	13.4	17.1
41	0.2674	0.2636	0.2655	0.0027	1.0	21.6
42	0.4357	0.3467	0.3912	0.0629	16.1	39.7
43	0.1265	0.1588	0.1427	0.0228	16.0	3.9
44	0.1662	0.2521	0.2092	0.0607	29.0	13.5
45	0.1596	0.1566	0.1581	0.0021	1.3	6.1
46	0.2725	0.1636	0.2181	0.0770	35.3	14.8
48	1.2256	1.0636	1.1446	0.1146	10.0	148.0
48	0.1134	0.1070	0.1102	0.0045	4.1	-0.8
49	0.1469	0.1495	0.1482	0.0018	1.2	4.7
50	0.1169	0.1122	0.1146	0.0033	2.9	-0.1
51	0.1595	0.1422	0.1509	0.0122	8.1	5.1
52	1.0484	1.0749	1.0617	0.0187	1.8	136.1
53	0.3012	0.2347	0.2680	0.0470	17.5	21.9
54	0.1267	0.1510	0.1389	0.0172	12.4	3.4
55	1.1902	1.1487	1.1695	0.0293	2.5	151.6
56	0.6400	0.7076	0.6738	0.0478	7.1	80.3
57	0.1701	0.1752	0.1727	0.0036	2.1	8.2
58	0.3435	0.3488	0.3462	0.0037	1.1	33.2
59	0.4032	0.3548	0.3790	0.0342	9.0	37.9
60	0.1602	0.1502	0.1552	0.0071	4.6	5.7

Table VI: - CONTINUED

Compound #	Raw Data		Average	S.D.	% CV	% Control
61	0.1604	0.2079	0.1842	0.0336	18.2	9.9
62	0.1646	0.1592	0.1619	0.0038	2.4	6.7
63	0.1779	0.2273	0.2026	0.0349	17.2	12.5
64	0.1225	0.1443	0.1334	0.0154	11.6	2.6
65	0.1300	0.1291	0.1296	0.0006	0.5	2.0
66	0.1263	0.1336	0.1300	0.0052	4.0	2.1
67	0.2778	0.1326	0.2052	0.1027	50.0	12.9
68	0.2569	0.1219	0.1894	0.0955	50.4	10.6
69	0.1640	0.1158	0.1399	0.0341	24.4	3.5
74	1.1486	0.8970	1.0228	0.1779	17.4	130.5
74	0.1358	0.1201	0.1280	0.0111	8.7	1.8
71	0.1257	0.1257	0.1257	0.0000	0.0	1.5
72	0.4676	0.4803	0.4740	0.0090	1.9	51.6
Compound #	**Raw Data**		**Average**	**S.D.**	**% CV**	**% Control**
conditioned medium	0.6935	0.7803	0.7369	0.0614	8.3	104.8
no treatment	0.4735	0.6069	0.5402	0.0943	17.5	71.5
AA only + DMSO	0.1428	0.1656	0.1542	0.0161	10.5	6.3
AA + Wnt3a + DMSO	0.5702	0.8468	0.7085	0.1956	27.6	100.0
73	0.1599	0.2380	0.1990	0.0552	27.8	13.8
76	0.1287	0.1244	0.1266	0.0030	2.4	1.6
no cells	0.1241	0.1100	0.1171	0.0100	8.5	0.0
75	0.1235	0.1152	0.1194	0.0059	4.9	0.4
77	0.1199	0.1278	0.1239	0.0056	4.5	1.1
77	0.1174	0.1162	0.1168	0.0008	0.7	-0.1
78	1.1100	0.9464	1.0282	0.1157	11.3	154.1
79	0.1247	0.1115	0.1181	0.0093	7.9	0.2
80	0.2640	0.1688	0.2164	0.0673	31.1	16.8
81	0.2313	0.1307	0.1810	0.0711	39.3	10.8
82	0.8639	0.9218	0.8929	0.0409	4.6	131.2
83	0.2540	0.2320	0.2430	0.0156	6.4	21.3
84	0.1809	0.3077	0.2443	0.0897	36.7	21.5
85	0.1892	0.1872	0.1882	0.0014	0.8	12.0
86	0.1967	0.2492	0.2230	0.0371	16.7	17.9
87	0.3346	0.1619	0.2483	0.1221	49.2	22.2
88	0.1106	0.1138	0.1122	0.0023	2.0	-0.8
89	0.1224	0.1445	0.1335	0.0156	11.7	2.8

Table VI: - CONTINUED

Compound #	Raw Data		Average	S.D.	% CV	% Control
90	0.1312	0.1270	0.1291	0.0030	2.3	2.0
91	0.1653	0.2114	0.1884	0.0326	17.3	12.0
92	0.1732	0.1467	0.1600	0.0187	11.7	7.2
93	0.1618	0.2754	0.2186	0.0803	36.7	17.2
94	1.0006	0.9631	0.9819	0.0265	2.7	146.2
95	0.6472	0.4319	0.5396	0.1522	28.2	71.4
96	0.1539	0.1469	0.1504	0.0049	3.3	5.6
97	0.1127	0.1309	0.1218	0.0129	10.6	0.8
98	0.6887	0.5860	0.6374	0.0726	11.4	88.0
99	0.1141	0.1094	0.1118	0.0033	3.0	-0.9
100	0.2774	0.1690	0.2232	0.0767	34.3	17.9
101	0.9482	1.1150	1.0316	0.1179	11.4	154.6
102	0.7687	0.6804	0.7246	0.0624	8.6	102.7
103	0.7125	0.3347	0.5236	0.2671	51.0	68.7
104	0.1446	0.1221	0.1334	0.0159	11.9	2.7
105	1.0968	1.3108	1.2038	0.1513	12.6	183.8
106	0.3167	0.3415	0.3291	0.0175	5.3	35.8
107	0.1261	0.1144	0.1203	0.0083	6.9	0.5
108	0.2223	0.2930	0.2577	0.0500	19.4	23.8
109	0.1265	0.1236	0.1251	0.0021	1.6	1.3
110	1.1940	0.9431	1.0686	0.1774	16.6	160.9
111	1.0689	0.6879	0.8784	0.2694	30.7	128.7
112	1.0444	0.7603	0.9024	0.2009	22.3	132.8
113	0.1443	0.1209	0.1326	0.0165	12.5	2.6
114	0.1152	0.1309	0.1231	0.0111	9.0	1.0
Compound #	**Raw Data**		**Average**	**S.D.**	**% CV**	**% Control**
conditioned medium	0.7590	0.7451	0.7521	0.0098	1.3	98.0
no treatment	0.5687	0.4490	0.5089	0.0846	16.6	60.4
AA only + DMSO	0.1988	0.1522	0.1755	0.0330	18.8	8.9
AA + Wnt3a + DMSO	0.6837	0.8460	0.7649	0.1148	15.0	100.0
115	0.1911	0.1101	0.1506	0.0573	38.0	5.0
116	0.2772	0.1151	0.1962	0.1146	58.4	12.1
no cells	0.1278	0.1084	0.1181	0.0137	11.6	0.0
117	0.1443	0.2120	0.1782	0.0479	26.9	9.3
118	0.4413	0.2238	0.3326	0.1538	46.2	33.2
119	0.1098	0.1085	0.1092	0.0009	0.8	-1.4

Table VI: - CONTINUED

Compound #	Raw Data		Average	S.D.	% CV	% Control
120	0.1389	0.2147	0.1768	0.0536	30.3	9.1
121	0.1852	0.1342	0.1597	0.0361	22.6	6.4
122	0.1114	0.1295	0.1205	0.0128	10.6	0.4
123	0.5375	0.6158	0.5767	0.0554	9.6	70.9
124	0.1259	0.1441	0.1350	0.0129	9.5	2.6
125	0.1206	0.1312	0.1259	0.0075	6.0	1.2
126	0.2269	0.2857	0.2563	0.0416	16.2	21.4
126	0.1140	0.1079	0.1110	0.0043	3.9	-1.1
127	0.9589	0.8868	0.9229	0.0510	5.5	124.4
127	1.0442	0.9622	1.0032	0.0580	5.8	136.8
128	0.1961	0.1735	0.1848	0.0160	8.6	10.3
129	0.5732	0.5216	0.5474	0.0365	6.7	66.4
130	0.1273	0.1217	0.1245	0.0040	3.2	1.0
131	0.5932	0.6671	0.6302	0.0523	8.3	79.2
132	0.1444	0.1368	0.1406	0.0054	3.8	3.5
133	1.0786	1.0891	1.0839	0.0074	0.7	149.3
138	0.5418	0.2338	0.3878	0.2178	56.2	41.7
134	0.1268	0.2052	0.1660	0.0554	33.4	7.4
135	0.1169	0.1184	0.1177	0.0011	0.9	-0.1
136	0.8618	1.0400	0.9509	0.1260	13.3	128.8
136	0.8430	1.0187	0.9309	0.1242	13.3	125.7
137	0.3659	0.3168	0.3414	0.0347	10.2	34.5
139	0.9184	0.8116	0.8650	0.0755	8.7	115.5
140	0.2384	0.3156	0.2770	0.0546	19.7	24.6
141	0.2297	0.1469	0.1883	0.0585	31.1	10.9
159	0.1955	0.1256	0.1606	0.0494	30.8	6.6
234	0.1658	0.1704	0.1681	0.0033	1.9	7.7
235	0.1399	0.1303	0.1351	0.0068	5.0	2.6
236	0.1234	0.1236	0.1235	0.0001	0.1	0.8
237	0.1397	0.2147	0.1772	0.0530	29.9	9.1
238	0.1218	0.1310	0.1264	0.0065	5.1	1.3
239	0.1456	0.1981	0.1719	0.0371	21.6	8.3
240	0.5412	0.1898	0.3655	0.2485	68.0	38.2
241	0.1996	0.1245	0.1621	0.0531	32.8	6.8
241	0.1418	0.2014	0.1716	0.0421	24.6	8.3
242	0.1106	0.1197	0.1152	0.0064	5.6	-0.5
243	0.1159	0.1272	0.1216	0.0080	6.6	0.5

Table VI: - CONTINUED

Compound #	Raw Data		Average	S.D.	% CV	% Control
Compound #	Raw Data		Average	S.D.	% CV	% Control
conditioned medium	0.8077	0.7210	0.7644	0.0613	8.0	74.7
no treatment + DMSO	0.4638	0.4073	0.4356	0.0400	9.2	36.7
AA/Wnt3a	0.8466	0.9935	0.9830	0.2592	26.4	100.0
16	0.8095	0.9055	0.8575	0.0679	7.9	85.5
17	0.3519	0.4708	0.4114	0.0841	20.4	33.9
18	0.1609	0.1275	0.1442	0.0236	16.4	3.1
19	0.5020	0.2733	0.3877	0.1617	41.7	31.2
19	0.3413	0.4146	0.3780	0.0518	13.7	30.1
20	0.1176	0.1174	0.1175	0.0001	0.1	0.0
20	0.1148	0.1410	0.1279	0.0185	14.5	1.2
21	0.2394	0.2450	0.2422	0.0040	1.6	14.4
22	0.3672	0.3098	0.3385	0.0406	12.0	25.5
9	0.2722	0.1593	0.2158	0.0798	37.0	11.3
23	0.5079	0.4349	0.4714	0.0516	11.0	40.9
24	0.1076	0.1168	0.1122	0.0065	5.8	-0.6
25	0.2569	0.2151	0.2360	0.0296	12.5	13.7
26	0.2846	0.4376	0.3611	0.1082	30.0	28.1
27	0.1168	0.1136	0.1152	0.0023	2.0	-0.3
28	0.1168	0.1152	0.1160	0.0011	1.0	-0.2
28	0.1137	0.1195	0.1166	0.0041	3.5	-0.1
29	0.1154	0.1152	0.1153	0.0001	0.1	-0.3
30	0.2188	0.2353	0.2271	0.0117	5.1	12.6
31	0.4588	0.2521	0.3555	0.1462	41.1	27.5
32	0.3081	0.1961	0.2521	0.0792	31.4	15.5
Compound #	**Raw Data**		**Average**	**S.D.**	**% CV**	**% Control**
conditioned medium	0.7914	1.1189	0.9552	0.2316	24.2	93.3
no treatment	0.4215	0.5259	0.4737	0.0738	15.6	39.8
no cells	0.1152	0.1160	0.1156	0.0006	0.5	0.0
AA/Wnt3a	0.7168	0.8836	1.0151	0.2016	19.9	100.0
244	0.2882	0.2308	0.2844	0.0499	17.6	18.8
245	0.3049	0.2845	0.3127	0.0282	9.0	21.9
246	0.5403	0.2570	0.3855	0.1332	34.6	30.0
247	0.7323	0.3034	0.4388	0.2041	46.5	35.9
248	0.1185	0.1216	0.1199	0.0018	1.5	0.5
249	0.2496	0.2683	0.2302	0.0376	16.3	12.7

Table VI: - CONTINUED

Compound #	Raw Data		Average	S.D.	% CV	% Control
250	0.1548	0.1356	0.1513	0.0134	8.8	4.0
160	0.1555	0.1450	0.1581	0.0161	10.2	4.7
251	0.2347	0.1920	0.3785	0.2589	68.4	29.2
180	0.1842	0.2093	0.3793	0.2585	68.2	29.3
221	0.7223	0.8707	0.4291	0.2452	57.2	34.8
169	0.6268	0.3192	0.3354	0.1667	49.7	24.4

TABLE VII: EFFECTS OF INHIBITORS OF GSK-3B ENZYME ACTIVITY ON THE PROLIFERATION OF HUMAN EMBRYONIC STEM CELLS.

List Strong Hits	
>=120% control	
Compound #	% Control Value
165	195.3
105	183.8
166	180.4
110	160.9
101	154.6
78	154.1
55	151.6
133	149.3
48	148.0
230	147.3
94	146.2
163	142.3
127	136.8
52	136.1
112	132.8
232	131.6
82	131.2
221	130.9
74	130.5
223	129.0
136	128.8
111	128.7
136	125.7
127	124.4
226	120.0

List Moderate Hits	
60-120% control	
Compound #	% Control Value
139	115.5
231	113.8
206	108.3
148	105.5
102	102.7
233	93.0
98	88.0
16	85.5
158	84.7
144	84.0
56	80.3
131	79.2
229	77.5
216	76.9
169	76.7
218	75.2
95	71.4
123	70.9
198	70.7
224	69.9
103	68.7
164	68.2
209	67.4
129	66.4
150	63.3
145	61.1

TABLE VIII: DOSE-DEPENDANT EFFECTS OF INHIBITORS OF GSK-3B ENZYME ACTIVITY ON THE PROLIFERATION OF CELLS OF THE HUMAN EMBRYONIC STEM CELL LINE H1.

Conc	compound # 198		compound # 206		compound # 221		compound # 223		compound # 47	
[µM]	Cell number	SD	Cell number	SD	Cell number	SD	Cell number	SD	Cell number	SD
10	1.006	0.051	0.039	0.049	0.193	0.147	1.280	0.014	1.049	0.062
5	1.058	0.047	1.164	0.018	0.889	0.035	1.348	0.007	1.104	0.014
2.5	1.031	0.054	1.022	0.023	0.896	0.035	1.318	0.028	0.932	0.087
1.25	0.899	0.040	1.121	0.023	1.120	0.072	1.159	0.041	1.006	0.023
0.625	0.742	0.095	1.092	0.044	1.107	0.093	1.029	0.018	0.832	0.026
0.313	0.754	0.010	0.931	0.056	1.132	0.018	1.018	0.044	0.742	0.127
0.156	0.822	0.074	0.804	0.002	1.082	0.041	0.776	0.054	0.712	0.020
Conc	compound # 103		compound # 133		compound # 136		compound # 226		compound # 233	
[µM]	Cell number	SD	Cell number	SD	Cell number	[uM]	Cell number	SD	Cell number	SD
10	0.001	0.001	0.096	0.103	0.058	0.074	0.290	0.307	0.000	0.000
5	0.034	0.035	0.262	0.268	0.173	0.207	0.458	0.263	0.089	0.067
2.5	0.566	0.461	0.592	0.019	0.428	0.326	0.640	0.104	0.438	0.050
1.25	0.897	0.103	1.124	0.101	0.850	0.238	0.739	0.129	0.636	0.016
0.625	0.921	0.122	1.106	0.056	0.910	0.061	0.805	0.036	0.736	0.025
0.313	1.028	0.069	0.888	0.213	0.868	0.131	0.785	0.094	0.791	0.038
0.156	1.027	0.067	0.890	0.079	0.742	0.051	0.774	0.027	0.832	0.005
Conc	compound # 52		compound # 101		compound # 110		compound # 111		compound # 112	
[µM]	Cell number	SD	Cell number	SD	Cell number	[uM]	Cell number	SD	Cell number	SD
10	0.000	0.000	0.496	0.690	0.129	0.170	0.412	0.081	0.996	0.246
5	0.024	0.034	0.768	0.490	0.530	0.080	1.128	0.026	0.908	0.179
2.5	1.097	0.294	1.001	0.129	1.174	0.016	1.031	0.217	1.005	0.086
1.25	1.446	0.076	1.158	0.043	1.113	0.057	0.914	0.100	1.200	0.085
0.625	1.296	0.183	0.699	0.248	1.188	0.041	0.801	0.136	1.111	0.300
0.313	1.034	0.197	0.617	0.232	1.158	0.102	0.785	0.121	0.959	0.094
0.156	0.826	0.030	0.812	0.120	0.974	0.065	0.659	0.068	0.912	0.059
Conc	compound # 144		compound # 145		compound # 148		compound # 150		compound # 158	
[µM]	Cell number	SD	Cell number	SD	Cell number	[uM]	Cell number	SD	Cell number	SD
10	0.000	0.000	0.021	0.027	0.002	0.002	0.052	0.067	0.053	0.024
5	0.000	0.000	0.339	0.254	1.011	0.499	1.161	0.134	0.905	0.036
2.5	0.192	0.233	1.350	0.170	1.724	0.042	1.293	0.020	1.019	0.015
1.25	0.552	0.458	1.277	0.101	1.652	0.032	1.213	0.087	1.163	0.062
0.625	0.895	0.054	0.713	0.151	1.357	0.023	1.025	0.045	1.231	0.152
0.313	0.734	0.075	0.665	0.207	1.213	0.177	1.241	0.031	1.216	0.007
0.156	0.594	0.078	0.469	0.465	1.206	0.142	1.041	0.007	1.103	0.065

TABLE IX: DOSE-DEPENDANT EFFECTS OF INHIBITORS OF GSK-3B ENZYME ACTIVITY ON THE DIFFERENTIATION OF CELLS OF THE HUMAN EMBRYONIC STEM CELL LINE H1.

Conc.	compound # 198		compound # 206		compound # 221		compound # 223		compound # 47	
[μM]	Sox17 Intensity	SD	Sox17 Intensity	SD	Sox17 Intensity	SD	Sox17 Intensity	SD	Sox17 Intensity	SD
10	0.889	0.144	0.029	0.034	0.140	0.095	1.183	0.044	0.969	0.040
5	1.004	0.021	0.824	0.035	0.785	0.077	1.171	0.010	1.013	0.002
2.5	1.023	0.092	0.849	0.003	0.842	0.032	1.169	0.031	0.838	0.068
1.25	0.954	0.100	0.985	0.082	1.028	0.043	1.106	0.006	0.940	0.071
0.625	0.793	0.135	0.986	0.059	1.016	0.000	0.931	0.033	0.767	0.014
0.313	0.803	0.048	0.916	0.028	1.058	0.017	0.943	0.056	0.692	0.167
0.156	0.941	0.106	0.822	0.036	1.039	0.015	0.789	0.074	0.651	0.032
Conc.	compound # 103		compound # 133		compound # 136		compound # 226		compound # 233	
[μM]	Sox17 Intensity	SD	Sox17 Intensity	SD	Sox17 Intensity	SD	Sox17 Intensity	SD	Sox17 Intensity	SD
10	0.001	0.001	0.034	0.027	0.054	0.063	0.267	0.280	0.000	0.001
5	0.017	0.020	0.071	0.054	0.141	0.169	0.402	0.229	0.056	0.035
2.5	0.200	0.157	0.497	0.076	0.373	0.326	0.605	0.041	0.286	0.034
1.25	0.792	0.066	0.993	0.144	0.783	0.282	0.686	0.185	0.587	0.023
0.625	0.824	0.118	1.061	0.066	0.887	0.062	0.786	0.061	0.695	0.001
0.313	0.934	0.127	0.937	0.136	0.859	0.176	0.780	0.132	0.753	0.098
0.156	0.986	0.055	0.888	0.062	0.666	0.015	0.782	0.061	0.816	0.043
Conc.	compound # 52		compound # 101		compound # 110		compound # 111		compound # 112	
[μM]	Sox17 Intensity	SD	Sox17 Intensity	SD	Sox17 Intensity	SD	Sox17 Intensity	SD	Sox17 Intensity	SD
10	0.000	0.000	0.491	0.681	0.281	0.358	0.330	0.059	0.701	0.307
5	0.035	0.049	0.158	0.224	0.460	0.189	0.846	0.036	0.728	0.146
2.5	1.336	0.192	0.800	0.201	1.018	0.139	0.887	0.191	0.928	0.019
1.25	1.238	0.030	0.910	0.045	0.960	0.106	0.819	0.179	1.159	0.093
0.625	0.997	0.095	0.567	0.190	1.050	0.038	0.755	0.126	1.136	0.186
0.313	0.791	0.172	0.515	0.276	1.032	0.063	0.667	0.125	1.006	0.009
0.156	0.669	0.037	0.708	0.148	0.950	0.087	0.628	0.053	0.922	0.096
Conc.	compound # 144		compound # 145		compound # 148		compound # 150		compound # 158	
[μM]	Sox17 Intensity	SD	Sox17 Intensity	SD	Sox17 Intensity	SD	Sox17 Intensity	SD	Sox17 Intensity	SD
10	0.000	0.000	0.018	0.021	0.002	0.001	0.054	0.062	0.074	0.048
5	0.000	0.000	0.235	0.174	1.052	0.281	1.250	0.177	1.006	0.070
2.5	0.270	0.382	1.153	0.223	1.459	0.074	1.186	0.069	1.120	0.038
1.25	0.678	0.434	1.055	0.046	1.322	0.078	1.112	0.038	1.122	0.009
0.625	0.978	0.021	0.569	0.124	1.173	0.015	0.913	0.005	1.241	0.230
0.313	0.742	0.048	0.555	0.118	1.102	0.165	1.140	0.036	1.231	0.012
0.156	0.508	0.049	0.451	0.443	1.060	0.126	0.998	0.006	1.034	0.008

TABLE X: DOSE-DEPENDANT EFFECTS OF INHIBITORS OF GSK-3B ENZYME ACTIVITY ON THE PROLIFERATION OF CELLS OF THE HUMAN EMBRYONIC STEM CELL LINE H9.

Conc.	compound # 198		compound # 206		compound # 221		compound # 223		compound # 47	
[μM]	Cell number	SD	Cell number	SD	Cell number	SD	Cell number	SD	Cell number	SD
10	0.164	0.209	0.001	0.000	0.049	0.028	0.123	0.106	0.770	0.077
5	0.147	0.141	0.616	0.497	0.583	0.155	0.954	0.146	0.496	0.011
2.5	0.140	0.112	1.295	0.402	1.108	0.170	0.795	0.101	0.384	0.247
1.25	0.307	0.198	1.233	0.058	1.195	0.147	0.541	0.051	0.395	0.002
0.625	0.138	0.071	0.606	0.121	1.100	0.014	0.332	0.049	0.221	0.009
0.313	0.063	0.008	0.397	0.020	0.887	0.078	0.206	0.085	0.172	0.071
0.156	0.069	0.001	0.214	0.025	0.699	0.109	0.142	0.039	0.138	0.048
Conc.	compound # 103		compound # 133		compound # 136		compound # 226		compound # 233	
[μM]	Cell number	SD	Cell number	SD	Cell number	SD	Cell number	SD	Cell number	SD
10	0.001	0.000	0.785	0.192	0.208	0.134	0.377	0.040	0.000	0.000
5	0.023	0.024	1.067	0.236	0.320	0.087	0.336	0.081	0.052	0.009
2.5	0.681	0.223	1.368	0.025	0.388	0.019	0.296	0.016	0.089	0.003
1.25	1.011	0.461	1.477	0.147	0.334	0.113	0.222	0.035	0.106	0.003
0.625	0.927	0.108	0.899	0.108	0.267	0.148	0.282	0.096	0.169	0.041
0.313	0.686	0.022	0.540	0.094	0.192	0.056	0.208	0.003	0.119	0.026
0.156	0.458	0.001	0.206	0.089	0.147	0.067	0.174	0.051	0.067	0.015
Conc.	compound # 52		compound # 101		compound # 110		compound # 111		compound # 112	
[μM]	Cell number	SD	Cell number	SD	Cell number	SD	Cell number	SD	Cell number	SD
10	0.000	0.000	0.452	0.094	0.002	0.001	1.117	0.043	1.022	0.422
5	0.002	0.000	0.433	0.050	1.325	0.015	0.793	0.030	1.281	0.109
2.5	0.668	0.059	0.521	0.229	1.355	0.026	0.600	0.122	1.197	0.068
1.25	0.988	0.032	0.293	0.038	1.182	0.076	0.442	0.018	1.039	0.213
0.625	0.390	0.032	0.200	0.122	0.928	0.127	0.371	0.072	0.686	0.014
0.313	0.250	0.090	0.072	0.025	0.772	0.050	0.100	0.008	0.437	0.066
0.156	0.095	0.020	0.057	0.044	0.336	0.056	0.072	0.015	0.276	0.043
Conc.	compound # 144		compound # 145		compound # 148		compound # 150		compound # 158	
[μM]	Cell number	SD	Cell number	SD	Cell number	SD	Cell number	SD	Cell number	SD
10	0.007	0.002	0.000	0.000	0.000	0.000	0.044	0.038	0.004	0.001
5	0.002	0.001	0.127	0.069	0.415	0.023	0.382	0.110	0.017	0.003
2.5	0.001	0.001	0.151	0.059	0.425	0.082	0.345	0.001	0.033	0.037
1.25	0.090	0.097	0.108	0.051	0.325	0.042	0.284	0.076	0.044	0.028
0.625	0.248	0.058	0.230	0.168	0.314	0.062	0.266	0.021	0.100	0.099
0.313	0.264	0.048	0.086	0.033	0.267	0.098	0.347	0.084	0.057	0.032
0.156	0.133	0.069	0.063	0.004	0.218	0.012	0.192	0.014	0.070	0.048

TABLE XI: DOSE-DEPENDANT EFFECTS OF INHIBITORS OF GSK-3B ENZYME ACTIVITY ON THE DIFFERENTIATION OF CELLS OF THE HUMAN EMBRYONIC STEM CELL LINE H9.

Conc.	compound # 198		compound # 206		compound # 221		compound # 223		compound # 47	
[μM]	Sox17 Intensity	SD	Sox17 Intensity	SD	Sox17 Intensity	SD	Sox17 Intensity	SD	Sox17 Intensity	SD
10	0.121	0.141	0.002	0.002	0.022	0.005	0.140	0.110	0.694	0.123
5	0.105	0.089	0.480	0.423	0.432	0.111	1.114	0.066	0.353	0.080
2.5	0.100	0.062	0.986	0.269	0.869	0.158	0.726	0.079	0.297	0.235
1.25	0.312	0.255	1.012	0.051	1.042	0.134	0.459	0.066	0.317	0.062
0.625	0.103	0.058	0.453	0.076	1.160	0.013	0.277	0.061	0.154	0.013
0.313	0.052	0.008	0.311	0.005	0.951	0.010	0.155	0.071	0.110	0.030
0.156	0.051	0.003	0.132	0.003	0.678	0.093	0.116	0.047	0.095	0.025
Conc.	compound # 103		compound # 133		compound # 136		compound # 226		compound # 233	
[μM]	Sox17 Intensity	SD	Sox17 Intensity	SD	Sox17 Intensity	SD	Sox17 Intensity	SD	Sox17 Intensity	SD
10	0.001	0.001	0.129	0.037	0.129	0.067	0.200	0.022	0.000	0.000
5	0.019	0.019	0.194	0.007	0.154	0.023	0.174	0.070	0.038	0.001
2.5	0.559	0.238	0.857	0.012	0.209	0.045	0.177	0.030	0.053	0.005
1.25	0.943	0.419	1.110	0.042	0.202	0.103	0.129	0.029	0.075	0.017
0.625	0.985	0.072	0.678	0.197	0.212	0.134	0.196	0.084	0.137	0.049
0.313	0.577	0.062	0.398	0.166	0.129	0.018	0.146	0.005	0.070	0.027
0.156	0.364	0.044	0.149	0.058	0.125	0.051	0.132	0.063	0.039	0.010
Conc.	compound # 52		compound # 101		compound # 110		compound # 111		compound # 112	
[μM]	Sox17 Intensity	SD	Sox17 Intensity	SD	Sox17 Intensity	SD	Sox17 Intensity	SD	Sox17 Intensity	SD
10	0.000	0.000	0.262	0.068	0.000	0.000	0.822	0.024	0.759	0.328
5	0.001	0.001	0.251	0.092	1.185	0.012	0.543	0.004	1.127	0.121
2.5	0.914	0.038	0.408	0.279	1.305	0.066	0.432	0.154	1.146	0.137
1.25	0.981	0.075	0.155	0.010	1.119	0.045	0.332	0.006	0.936	0.186
0.625	0.246	0.036	0.150	0.095	0.941	0.111	0.268	0.050	0.563	0.019
0.313	0.170	0.046	0.051	0.016	0.746	0.088	0.080	0.006	0.342	0.068
0.156	0.074	0.024	0.040	0.030	0.291	0.086	0.054	0.014	0.186	0.040
Conc.	compound # 144		compound # 145		compound # 148		compound # 150		compound # 158	
[μM]	Sox17 Intensity	SD	Sox17 Intensity	SD	Sox17 Intensity	SD	Sox17 Intensity	SD	Sox17 Intensity	SD
10	0.009	0.003	0.000	0.000	0.000	0.000	0.042	0.028	0.004	0.003
5	0.001	0.001	0.087	0.036	0.300	0.095	0.234	0.078	0.016	0.001
2.5	0.001	0.001	0.120	0.066	0.299	0.019	0.205	0.002	0.042	0.049
1.25	0.114	0.134	0.076	0.034	0.202	0.002	0.165	0.030	0.053	0.035
0.625	0.165	0.043	0.222	0.201	0.220	0.070	0.202	0.013	0.073	0.066
0.313	0.240	0.030	0.068	0.010	0.203	0.061	0.282	0.135	0.054	0.040
0.156	0.085	0.041	0.049	0.011	0.173	0.009	0.146	0.041	0.059	0.051

TABLE XII-RELATIONSHIP BETWEEN COMPOUNDS ON TABLES AND COMPOUNDS

COMPOUND NO.	TABLE-COMPOUND
15	C-12
230	D-13a
221	D-6a
232	D-8a
165	C-6
223	D-11a
47	B-40
6	C-2
163	C-5
185	B-16
231	D-7a
2	C-1
148	D-31a
10	C-29
16	B-11
226	D-12a
11	C-26
165	C-6
163	C-5
206	D-4a
6	C-2
47	B-40
2	C-1
230	D-13a
15	C-12
185	B-16
232	D-8a
10	C-29
11	C-26
3	C-31
11	C-26
12	B-2
17	B-14
18	B-15
19	B-21
22	B-30
23	B-33
26	B-34
27	B-36
28	D-9a

TABLE XII CONTINUED

COMPOUND NO.	TABLE-COMPOUND
33	B-29
34	B-28
48	B-41
52	D-15a
55	D-16a
74	B-42
78	D-17a
82	D-18a
94	B-43
98	B-44
101	D-19a
103	D-20a
105	D-21a
110	D-22a
111	D-23a
112	D-24a
127	D-25a
133	D-26a
136	D-27a
139	D-28a
144	D-29a
145	D-30a
150	D32a
158	A-5
164	C-4
168	C-3
175	B-4
180	C-28
182	B-18
183	B-19
198	D-2A
216	D-5A
233	D-14a
241	B-25
242	B-24
2	C-1
6	C-2
10	C-29
11	C-26
15	C-12
16	B-11
47	B-40
148	D-31A

TABLE XII CONTINUED

COMPOUND NO.	TABLE-COMPOUND
163	C-5
165	C-6
166	D-3a
185	B-16
206	D-4A
221	D-6a
223	D-11a
226	D-12a
230	D-13a
231	D-7a
232	D-8a

TABLE XIII-CHEMICAL FORMULAS OF OTHER COMPOUNDS TESTED

COMPOUND NO.	CHEMICAL FORMULA
206	3-[1-(2-Hydroxyethyl)-1H-indol-3-yl]-4-(1-pyridin-3-yl-1H-indol-3-yl)-1H-pyrrole-2,5-dione
8	3-{1-[3-(Dimethylamino)propyl]-1H-indazol-3-yl}-4-(1-naphthalen-1-yl-1H-indol-3-yl)-1H-pyrrole-2,5-dione
181	**blocked**
209	3-[1-(3-Hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-4-(1-methyl-1H-pyrazol-3-yl)-1H-pyrrole-2,5-dione
4	3-[1-(3-Aminopropyl)-1H-indazol-3-yl]-4-[1-(1-benzothiophen-3-yl)-1H-indol-3-yl]-1H-pyrrole-2,5-dione
221	6-[(2-{[4-(2,4-Dichlorophenyl)-5-(4-methyl-1H-imidazol-2-yl)pyrimidin-2-yl]amino}ethyl)amino]pyridine-3-carbonitrile
16	3-[1-(3-Hydroxypropyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-4-[2-(trifluoromethyl)phenyl]-1H-pyrrole-2,5-dione
169	10,11,13,14,16,17,19,20,22,23-Decahydro-1H-9,4:24,29-di(metheno)dipyrido[2,3-n:3',2'-t]pyrrolo[3,4-q][1,4,7,10,13,22]tetraoxadiazacyclotetracosine-1,3(2H)-dione
14	3-(1H-Indazol-3-yl)-4-(1-pyridin-3-yl-1H-indol-3-yl)-1H-pyrrole-2,5-dione
167	6,7,9,10,12,13,15,16-Octahydro-23H-5,26:17,22-di(metheno)dipyrido[2,3-k:3',2'-q]pyrrolo[3,4-n][1,4,7,10,19]trioxadiazacyclohenicosine-23,25(24H)-dione
13	3-[1-(3-Hydroxypropyl)-1H-indazol-3-yl]-4-(1-naphthalen-2-yl-1H-indol-3-yl)-1H-pyrrole-2,5-dione
5	3-[1-(1-Benzothiophen-3-yl)-1H-indol-3-yl]-4-[1-(3-hydroxypropyl)-1H-indol-3-yl]-1H-pyrrole-2,5-dione

What is claimed is:

1. A method to expand and differentiate pluripotent cells, comprising the steps of:

    a. Culturing pluripotent cells, and

    b. Treating the pluripotent cells with an inhibitor of GSK-3B enzyme activity.

2. The method of claim 1, wherein the pluripotent cells are embryonic stem cells.

3. The method of claim 1, wherein the pluripotent cells are cells expressing pluripotency markers derived from embryonic stem cells.

4. The method of claim 3, wherein the cells expressing pluripotency markers express at least one of the following pluripotency markers selected from the group consisting of: ABCG2, cripto, FoxD3, Connexin43, Connexin45, Oct4, SOX-2, Nanog, hTERT, UTF-1, ZFP42, SSEA-3, SSEA-4, Tra1-60, and Tra1-81.

5. The method of claim 1, wherein the pluripotent cells are differentiated into cells expressing markers characteristic of the definitive endoderm lineage.

6. The method of claim 1, wherein the pluripotent cells are treated with the inhibitor of GSK-3B enzyme activity for about one to about 72 hours.

7. The method of claim 1, wherein the pluripotent cells are treated with the inhibitor of GSK-3B enzyme activity for about 12 to about 48 hours.

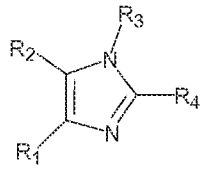
8. The method of claim 1, wherein the pluripotent cells are treated with the inhibitor of GSK-3B enzyme activity for about 48 hours.

9. The method of claim 1, wherein the inhibitor of GSK-3B enzyme activity is used at a concentration of about 100nM to about 100μM.

10. The method of claim 1, wherein the inhibitor of GSK-3B enzyme activity is used at a concentration of about 1μM to about 10μM.

11. The method of claim 1, wherein the inhibitor of GSK-3B enzyme activity is used at a concentration of about 10μM.

12. The method of claim 1, wherein the inhibitor of GSK-3B enzyme activity is a compound of the Formula (I):



Formula (I)

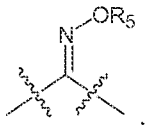
13. The method of claim 12, wherein R_1 is phenyl, substituted phenyl wherein the phenyl substituents are selected from the group consisting of C_{1-5}alkyl, halogen, nitro, trifluoromethyl and nitrile, or pyrimidinyl.

14. The method of claim 12, wherein R_2 is phenyl, substituted phenyl wherein the phenyl substituents are selected from the group consisting of C_{1-5}alkyl, halogen, nitro, trifluoromethyl and nitrile, or pyrimidinyl which is optionally C_{1-4}alkyl substituted, and at least one of R_1 and R_2 is pyrimidinyl.

15. The method of claim 12, wherein R_3 is hydrogen, 2-(trimethylsilyl)ethoxymethyl, C_{1-5}alkoxycarbonyl, aryloxycarbonyl, arylC_{1-5}alkyloxycarbonyl, arylC_{1-5}alkyl, substituted arylC_{1-5}alkyl wherein the one or more aryl substituents are independently selected from the group consisting of C_{1-5}alkyl, C_{1-5}alkoxy, halogen, amino, C_{1-5}alkylamino, and diC_{1-5}alkylamino, phthalimidoC_{1-5}alkyl, aminoC_{1-5}alkyl, diaminoC_{1-5}alkyl, succinimidoC_{1-5}alkyl, C_{1-5}alkylcarbonyl, arylcarbonyl, C_{1-5}alkylcarbonylC_{1-5}alkyl and aryloxycarbonylC_{1-5}alkyl.

16. The method of claim 12, wherein R_4 is -(A)-$(CH_2)_q$-X..

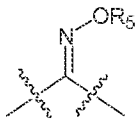
17. The method of claim 16, wherein A is vinylene, ethynylene or



18. The method of claim 17, wherein R_5 is selected from the group consisting of hydrogen, C_{1-5}alkyl, phenyl and phenylC_{1-5}alkyl.

19. The method of claim 16, wherein q is 0-9.

20. The method of claim 16, wherein X is selected from the group consisting of hydrogen, hydroxy, vinyl, substituted vinyl wherein one or more vinyl substituents are each selected from the group consisting of fluorine, bromine, chlorine and iodine, ethynyl, substituted ethynyl wherein the ethynyl substituents are selected from the group consisting of fluorine, bromine chlorine and iodine, C_{1-5}alkyl, substituted C_{1-5}alkyl wherein the one or more alkyl substituents are each selected from the group consisting of C_{1-5}alkoxy, trihaloalkyl, phthalimido and amino, C_{3-7}cycloalkyl, C_{1-5}alkoxy, substituted C_{1-5}alkoxy wherein the alkyl substituents are selected from the group consisting of phthalimido and amino, phthalimidooxy, phenoxy, substituted phenoxy wherein the one or more phenyl substituents are each selected from the group consisting of C_{1-5}alkyl, halogen and C_{1-5}alkoxy, phenyl, substituted phenyl wherein the one or more phenyl substituents are each selected from the group consisting of C_{1-5}alkyl, halogen and C_{1-5}alkoxy, arylC_{1-5}alkyl, substituted arylC_{1-5}alkyl wherein the one or more aryl substituents are each selected from the group consisting of C_{1-5}alkyl, halogen and C_{1-5}alkoxy, aryloxyC_{1-5}alkylamino, C_{1-5}alkylamino, diC_{1-5}alkylamino, nitrile, oxime, benxyloxyimino, C_{1-5}alkyloxyimino, phthalimido, succinimido, C_{1-5}alkylcarbonyloxy, phenylcarbonyloxy, substituted phenylcarbonyloxy wherein the one or more phenyl substituents are each selected from the group consisting of C_{1-5}alkyl, halogen and C_{1-5}alkoxy, phenylC_{1-5}alkylcarbonyloxy wherein the one

or more phenyl substituents are each selected from the group consisting of C_{1-5}alkyl, halogen and C_{1-5}alkoxy, aminocarbonyloxy, C_{1-5}alkylaminocarbonyloxy, diC_{1-5}alkylaminocarbonyloxy, C_{1-5}alkoxycarbonyloxy, substituted C_{1-5}alkoxycarbonyloxy wherein the one or more alkyl substituents are each selected from the group consisting of methyl, ethyl, isopropyl and hexyl, phenoxycarbonyloxy, substituted phenoxycarbonyloxy wherein the one or more phenyl substituents are each selected from the group consisting of C_{1-5}alkyl, C_{1-5}alkoxy and halogen, C_{1-5}alkylthio, substituted C_{1-5}alkylthio wherein the alkyl substituents are selected from the group consisting of hydroxy and phthalimido, C_{1-5}alkylsulfonyl, phenylsulfonyl, substituted phenylsulfonyl wherein the one or more phenyl substituents are each selected from the group consisting of bromine, fluorine, chloride, C_{1-5}alkoxy and trifluoromethyl; with the proviso that if A is  , q is 0 and X is H, then R_3 may not be 2-(trimethylsilyl)ethoxymethyl; and pharmaceutically acceptable salts thereof.

21. The method of claim 12, wherein R_1 is substituted phenyl and R_2 is pyrimidin-3-yl.

22. The method of claim 12, wherein R_1 is 4-fluorophenyl.

23. The method of claim 12, wherein R_3 is hydrogen, arylC_{1-5}alkyl, or substituted arylC_{1-5}alkyl.

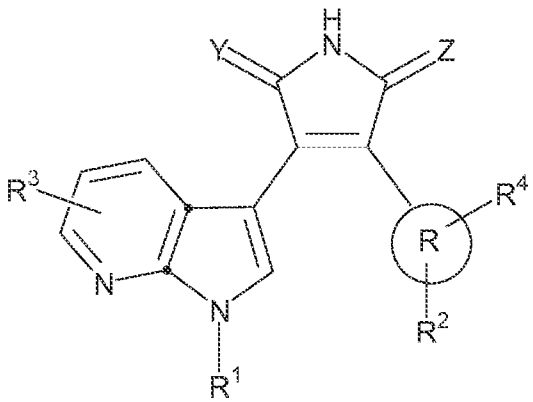
24. The method of claim 12, wherein R_3 is hydrogen or phenylC_{1-5}alkyl.

25. The method of claim 16, wherein A is ethynylene and q is 0-5.

26. The method of claim 16, wherein X is succinimido, hydroxy, methyl, phenyl, C_{1-5}alkylsulfonyl, C_{3-6}cycloalkyl, C_{1-5}alkylcarbonyloxy, C_{1-5}alkoxy, phenylcarbonyloxy, C_{1-5}alkylamino, diC_{1-5}alkylamino or nitrile.

27. The method of claim 12, wherein the compound of the Formula I is 4-(4-fluorophenyl)-2-(4-hydroxybutyn-1-yl)-1-(3-phenylpropyl)-5-(4-pyridyl)imidazole.

28. The method of claim 1, wherein the inhibitor of GSK-3B enzyme activity is a compound of the Formula (II):



Formula (II)

29. The method of claim 28, wherein R is selected from the group consisting of R_a, -C_{1-8}alkyl-R_a, -C_{2-8}alkenyl-R_a, -C_{2-8}alkynyl-R_a and cyano.

30. The method of claim 29, wherein R_a is selected from the group consisting of cycloalkyl, heterocyclyl, aryl and heteroaryl.

31. The method of claim 28, wherein R^1 is selected from the group consisting of hydrogen, -C_{1-8}alkyl-R^5, -C_{2-8}alkenyl-R^5, -C_{2-8}alkynyl-R^5, -C(O)-(C_{1-8})alkyl-R^9, -C(O)-aryl-R^8, -C(O)-O-(C_{1-8})alkyl-R^9, -C(O)-O-aryl-R^8, -C(O)-NH(C_{1-8}alkyl-R^9), -C(O)-NH(aryl-R^8), -C(O)-N(C_{1-8}alkyl-R^9)$_2$, -SO_2-(C_{1-8})alkyl-R^9, -SO_2-aryl-R^8, -cycloalkyl-R^6, -heterocyclyl-R^6, -aryl-R^6 and -heteroaryl-R^6; wherein heterocyclyl and heteroaryl are attached to the azaindole nitrogen atom in the one position via a heterocyclyl or heteroaryl ring carbon atom.

32. The method of claim 31, wherein R^5 is 1 to 2 substituents independently selected from the group consisting of hydrogen, -O-(C_{1-8})alkyl, -O-(C_{1-8})alkyl-OH, -O-(C_{1-8})alkyl-O-(C_{1-8})alkyl,

-O-(C_{1-8})alkyl-NH_2, -O-(C_{1-8})alkyl-NH(C_{1-8}alkyl), -O-(C_{1-8})alkyl-N(C_{1-8}alkyl)$_2$, -O-(C_{1-8})alkyl-S-(C_{1-8})alkyl, -O-(C_{1-8})alkyl-SO_2-(C_{1-8})alkyl, -O-(C_{1-8})alkyl-SO_2-NH_2, -O-(C_{1-8})alkyl-SO_2-NH(C_{1-8}alkyl), -O-(C_{1-8})alkyl-SO_2-N(C_{1-8}alkyl)$_2$, -O-C(O)H, -O-C(O)-(C_{1-8})alkyl, -O-C(O)-NH_2, -O-C(O)-NH(C_{1-8}alkyl), -O-C(O)-N(C_{1-8}alkyl)$_2$, -O-(C_{1-8})alkyl-C(O)H, -O-(C_{1-8})alkyl-C(O)-(C_{1-8})alkyl, -O-(C_{1-8})alkyl-CO_2H, -O-(C_{1-8})alkyl-C(O)-O-(C_{1-8})alkyl, -O-(C_{1-8})alkyl-C(O)-NH_2, -O-(C_{1-8})alkyl-C(O)-NH(C_{1-8}alkyl), -O-(C_{1-8})alkyl-C(O)-N(C_{1-8}alkyl)$_2$, -C(O)H, -C(O)-(C_{1-8})alkyl, -CO_2H, -C(O)-O-(C_{1-8})alkyl, -C(O)-NH_2, -C(NH)-NH_2, -C(O)-NH(C_{1-8}alkyl), -C(O)-N(C_{1-8}alkyl)$_2$, -SH, -S-(C_{1-8})alkyl, -S-(C_{1-8})alkyl-S-(C_{1-8})alkyl, -S-(C_{1-8})alkyl-O-(C_{1-8})alkyl, -S-(C_{1-8})alkyl-O-(C_{1-8})alkyl-OH, -S-(C_{1-8})alkyl-O-(C_{1-8})alkyl-NH_2, -S-(C_{1-8})alkyl-O-(C_{1-8})alkyl-NH(C_{1-8}alkyl), -S-(C_{1-8})alkyl-O-(C_{1-8})alkyl-N(C_{1-8}alkyl)$_2$, -S-(C_{1-8})alkyl-NH(C_{1-8}alkyl), -SO_2-(C_{1-8})alkyl, -SO_2-NH_2, -SO_2-NH(C_{1-8}alkyl), -SO_2-N(C_{1-8}alkyl)$_2$, -N-R^7, cyano, (halo)$_{1-3}$, hydroxy, nitro, oxo, -cycloalkyl-R^6, -heterocyclyl-R^6, -aryl-R^6 and -heteroaryl-R^6.

33. The method of claim 31, wherein R^6 is 1 to 4 substituents attached to a carbon or nitrogen atom independently selected from the group consisting of hydrogen, -C_{1-8}alkyl, -C_{2-8}alkenyl, -C_{2-8}alkynyl, -C(O)H, -C(O)-(C_{1-8})alkyl, -CO_2H, -C(O)-O-(C_{1-8})alkyl, -C(O)-NH_2, -C(NH)-NH_2, -C(O)-NH(C_{1-8}alkyl), -C(O)-N(C_{1-8})alkyl$_2$, -SO_2-(C_{1-8})alkyl, -SO_2-NH_2, -SO_2-NH(C_{1-8}alkyl), -SO_2-N(C_{1-8}alkyl)$_2$, -(C_{1-8})alkyl-N-R^7, -(C_{1-8})alkyl-(halo)$_{1-3}$, -(C_{1-8})alkyl-OH, -aryl-R^8, -(C_{1-8})alkyl-aryl-R^8 and -(C_{1-8})alkyl-heteroaryl-R^8; with the proviso that, when R^6 is attached to a carbon atom, R^6 is further selected from the group consisting of -C_{1-8}alkoxy, -(C_{1-8})alkoxy-(halo)$_{1-3}$, -SH, -S-(C_{1-8})alkyl, -N-R^7, cyano, halo, hydroxy, nitro, oxo and -heteroaryl-R^8.

34. The method of claim 33, wherein R^7 is 2 substituents independently selected from the group consisting of hydrogen, -C_{1-8}alkyl, -C_{2-8}alkenyl, -C_{2-8}alkynyl, -(C_{1-8})alkyl-OH, -(C_{1-8})alkyl-O-(C_{1-8})alkyl, -(C_{1-8})alkyl-NH_2, -(C_{1-8})alkyl-NH(C_{1-8}alkyl), -(C_{1-8})alkyl-N(C_{1-8}alkyl)$_2$, -(C_{1-8})alkyl-S-(C_{1-8})alkyl, -C(O)H, -C(O)-(C_{1-8})alkyl, -C(O)-O-(C_{1-8})alkyl, -C(O)-NH_2, -C(O)-NH(C_{1-8}alkyl), -C(O)-N(C_{1-8}alkyl)$_2$, -SO_2-(C_{1-8})alkyl, -SO_2-NH_2, -SO_2-NH(C_{1-8}alkyl), -SO_2-N(C_{1-8}alkyl)$_2$, -C(N)-NH_2, -cycloalkyl-R^8, -(C_{1-8})alkyl-heterocyclyl-R^8, -aryl-R^8, -(C_{1-8})alkyl-aryl-R^8 and -(C_{1-8})alkyl-heteroaryl-R^8.

35. The method of claim 31, wherein R^8 is 1 to 4 substituents attached to a carbon or nitrogen atom independently selected from the group consisting of hydrogen, -C_{1-8}alkyl, -(C_{1-8})alkyl-(halo)$_{1-3}$ and -(C_{1-8})alkyl-OH; with the proviso that, when R^8 is attached to a carbon atom, R^8 is further selected from the group consisting of -C_{1-8}alkoxy, -NH_2, -NH(C_{1-8}alkyl), -N(C_{1-8}alkyl)$_2$, cyano, halo, -(C_{1-8})alkoxy-(halo)$_{1-3}$, hydroxy and nitro.

36. The method of claim 31, wherein R^9 is 1 to 2 substituents independently selected from the group consisting of hydrogen, -C_{1-8}alkoxy, -NH_2, -NH(C_{1-8}alkyl), -N(C_{1-8}alkyl)$_2$, cyano, (halo)$_{1-3}$, hydroxy and nitro.

37. The method of claim 28, wherein R^2 is one substituent attached to a carbon or nitrogen atom selected from the group consisting of hydrogen, -C_{1-8}alkyl-R^5, -C_{2-8}alkenyl-R^5, -C_{2-8}alkynyl-R^5, -C(O)H, -C(O)-(C_{1-8})alkyl-R^9, -C(O)-NH_2, -C(O)-NH(C_{1-8}alkyl-R^9), -C(O)-N(C_{1-8}alkyl-R^9)$_2$, -C(O)-NH(aryl-R^8), -C(O)-cycloalkyl-R^8, -C(O)-heterocyclyl-R^8, -C(O)-aryl-R^8, -C(O)-heteroaryl-R^8, -CO_2H, -C(O)-O-(C_{1-8})alkyl-R^9, -C(O)-O-aryl-R^8, -SO_2-(C_{1-8})alkyl-R^9, -SO_2-aryl-R^8, -cycloalkyl-R^6, -aryl-R^6 and -(C_{1-8})alkyl-N-R^7; with the proviso that, when R^2 is attached to a carbon atom, R^2 is further selected from the group consisting of -C_{1-8}alkoxy-R^5, -N-R^7, cyano, halogen, hydroxy, nitro, oxo, -heterocyclyl-R^6 and -heteroaryl-R^6.

38. The method of claim 28, wherein R^3 is 1 to 3 substituents attached to a carbon atom independently selected from the group consisting of hydrogen, -C_{1-8}alkyl-R^{10}, -C_{2-8}alkenyl-R^{10}, -C_{2-8}alkynyl-R^{10}, -C_{1-8}alkoxy-R^{10}, -C(O)H, -C(O)-(C_{1-8})alkyl-R^9, -C(O)-NH_2, -C(O)-NH(C_{1-8}alkyl-R^9), -C(O)-N(C_{1-8}alkyl-R^9)$_2$, -C(O)-cycloalkyl-R^8, -C(O)-heterocyclyl-R^8, -C(O)-aryl-R^8, -C(O)-heteroaryl-R^8, -C(NH)-NH_2, -CO_2H, -C(O)-O-(C_{1-8})alkyl-R^9, -C(O)-O-aryl-R^8, -SO_2-(C_{1-8})alkyl-R^9, -SO_2-aryl-R^8, -N-R^7, cyano, halogen, hydroxy, nitro, -cycloalkyl-R^8, -heterocyclyl-R^8, -aryl-R^8 and -heteroaryl-R^8.

39. The method of claim 38, wherein R^{10} is 1 to 2 substituents independently selected from the group consisting of hydrogen, -NH_2, -NH(C_{1-8}alkyl), -N(C_{1-8}alkyl)$_2$, cyano, (halo)$_{1-3}$, hydroxy, nitro and oxo.

40. The method of claim 28, wherein R^4 is 1 to 4 substituents attached to a carbon atom independently selected from the group consisting of hydrogen, -C_{1-8}alkyl-R^{10}, -C_{2-8}alkenyl-R^{10}, -C_{2-8}alkynyl-R^{10}, -C_{1-8}alkoxy-R^{10}, -C(O)H, -C(O)-(C_{1-8})alkyl-R^9, -C(O)-NH_2, -C(O)-NH(C_{1-8}alkyl-R^9), -C(O)-N(C_{1-8}alkyl-R^9)$_2$, -C(O)-cycloalkyl-R^8, -C(O)-heterocyclyl-R^8, -C(O)-aryl-R^8, -C(O)-heteroaryl-R^8, -C(NH)-NH_2, -CO_2H, -C(O)-O-(C_{1-8})alkyl-R^9, -C(O)-O-aryl-R^8, -SH, -S-(C_{1-8})alkyl-R^{10}, -SO_2-(C_{1-8})alkyl-R^9, -SO_2-aryl-R^8, -SO_2-NH_2, -SO_2-NH(C_{1-8}alkyl-R^9), -SO_2-N(C_{1-8}alkyl-R^9)$_2$, -N-R^7, cyano, halogen, hydroxy, nitro, -cycloalkyl-R^8, -heterocyclyl-R^8, -aryl-R^8 and -heteroaryl-R^8.

41. The method of claim 40, wherein R^{10} is 1 to 2 substituents independently selected from the group consisting of hydrogen, -NH_2, -NH(C_{1-8}alkyl), -N(C_{1-8}alkyl)$_2$, cyano, (halo)$_{1-3}$, hydroxy, nitro and oxo.

42. The method of claim 28, wherein Y and Z are independently selected from the group consisting of O, S, (H,OH) and (H,H); with the proviso that one of Y and Z is O and the other is selected from the group

consisting of O, S, (H,OH) and (H,H); and pharmaceutically acceptable salts thereof.

43. The method of claim 28, wherein R is selected from the group consisting of R_a, -C_{1-4}alkyl-R_a, -C_{2-4}alkenyl-R_a, -C_{2-4}alkynyl-R_a and cyano.

44. The method of claim 29, wherein R_a is selected from the group consisting of heterocyclyl, aryl and heteroaryl.

45. The method of claim 29, R_a is selected from the group consisting of dihydro-pyranyl, phenyl, naphthyl, thienyl, pyrrolyl, imidazolyl, pyrazolyl, pyridinyl, azaindolyl, indazolyl, benzofuryl, benzothienyl, dibenzofuryl and dibenzothienyl.

46. The method of claim 28, wherein R^1 is selected from the group consisting of hydrogen, -C_{1-4}alkyl-R^5, -C_{2-4}alkenyl-R^5, -C_{2-4}alkynyl-R^5, -C(O)-(C_{1-4})alkyl-R^9, -C(O)-aryl-R^8, -C(O)-O-(C_{1-4})alkyl-R^9, -C(O)-O-aryl-R^8, -C(O)-NH(C_{1-4}alkyl-R^9), -C(O)-NH(aryl-R^8), -C(O)-N(C_{1-4}alkyl-R^9)$_2$, -SO_2-(C_{1-4})alkyl-R^9, -SO_2-aryl-R^8, -cycloalkyl-R^6, -heterocyclyl-R^6, -aryl-R^6 and -heteroaryl-R^6; wherein heterocyclyl and heteroaryl are attached to the azaindole nitrogen atom in the one position via a heterocyclyl or heteroaryl ring carbon atom.

47. The method of claim 28, wherein R^1 is selected from the group consisting of hydrogen, -C_{1-4}alkyl-R^5, -aryl-R^6 and -heteroaryl-R^6; wherein heteroaryl is attached to the azaindole nitrogen atom in the one position via a heteroaryl ring carbon atom.

48. The method of claim 28, wherein R^1 is selected from the group consisting of hydrogen, -C_{1-4}alkyl-R^5 and -naphthyl-R^6.

49. The method of claim 31, wherein R^5 is 1 to 2 substituents independently selected from the group consisting of hydrogen, -O-(C_{1-4})alkyl, -O-(C_{1-4})alkyl-OH, -O-(C_{1-4})alkyl-O-(C_{1-4})alkyl, -O-(C_{1-4})alkyl-NH_2, -O-(C_{1-4})alkyl-NH(C_{1-4}alkyl),

-O-(C_{1-4})alkyl-N(C_{1-4}alkyl)$_2$, -O-(C_{1-4})alkyl-S-(C_{1-4})alkyl, -O-(C_{1-4})alkyl-SO_2-(C_{1-4})alkyl, -O-(C_{1-4})alkyl-SO_2-NH_2, -O-(C_{1-4})alkyl-SO_2-NH(C_{1-4}alkyl), -O-(C_{1-4})alkyl-SO_2-N(C_{1-4}alkyl)$_2$, -O-C(O)H, -O-C(O)-(C_{1-4})alkyl, -O-C(O)-NH_2, -O-C(O)-NH(C_{1-4}alkyl), -O-C(O)-N(C_{1-4}alkyl)$_2$, -O-(C_{1-4})alkyl-C(O)H, -O-(C_{1-4})alkyl-C(O)-(C_{1-4})alkyl, -O-(C_{1-4})alkyl-CO_2H, -O-(C_{1-4})alkyl-C(O)-O-(C_{1-4})alkyl, -O-(C_{1-4})alkyl-C(O)-NH_2, -O-(C_{1-4})alkyl-C(O)-NH(C_{1-4}alkyl), -O-(C_{1-4})alkyl-C(O)-N(C_{1-4}alkyl)$_2$, -C(O)H, -C(O)-(C_{1-4})alkyl, -CO_2H, -C(O)-O-(C_{1-4})alkyl, -C(O)-NH_2, -C(NH)-NH_2, -C(O)-NH(C_{1-4}alkyl), -C(O)-N(C_{1-4}alkyl)$_2$, -SH, -S-(C_{1-4})alkyl, -S-(C_{1-4})alkyl-S-(C_{1-4})alkyl, -S-(C_{1-4})alkyl-O-(C_{1-4})alkyl, -S-(C_{1-4})alkyl-O-(C_{1-4})alkyl-OH, -S-(C_{1-4})alkyl-O-(C_{1-4})alkyl-NH_2, -S-(C_{1-4})alkyl-O-(C_{1-4})alkyl-NH(C_{1-4}alkyl), -S-(C_{1-4})alkyl-O-(C_{1-4})alkyl-N(C_{1-4}alkyl)$_2$, -S-(C_{1-4})alkyl-NH(C_{1-4}alkyl), -SO_2-(C_{1-4})alkyl, -SO_2-NH_2, -SO_2-NH(C_{1-4}alkyl), -SO_2-N(C_{1-4}alkyl)$_2$, -N-R^7, cyano, (halo)$_{1-3}$, hydroxy, nitro, oxo, -cycloalkyl-R^6, -heterocyclyl-R^6, -aryl-R^6 and -heteroaryl-R^6.

50. The method of claim 31, wherein R^5 is 1 to 2 substituents independently selected from the group consisting of hydrogen, -O-(C_{1-4})alkyl, -N-R^7, hydroxy and -heteroaryl-R^6.

51. The method of claim 31, wherein R^5 is 1 to 2 substituents independently selected from the group consisting of hydrogen, -O-(C_{1-4})alkyl, -N-R^7, hydroxy, -imidazolyl-R^6, -triazolyl-R^6 and -tetrazolyl-R^6.

52. The method of claim 31, wherein R^6 is 1 to 4 substituents attached to a carbon or nitrogen atom independently selected from the group consisting of hydrogen, -C_{1-4}alkyl, -C_{2-4}alkenyl, -C_{2-4}alkynyl, -C(O)H, -C(O)-(C_{1-4})alkyl, -CO_2H, -C(O)-O-(C_{1-4})alkyl, -C(O)-NH_2, -C(NH)-NH_2, -C(O)-NH(C_{1-4}alkyl), -C(O)-N(C_{1-4})alkyl)$_2$, -SO_2-(C_{1-4})alkyl, -SO_2-NH_2, -SO_2-NH(C_{1-4}alkyl), -SO_2-N(C_{1-4}alkyl)$_2$, -(C_{1-4})alkyl-N-R^7, -(C_{1-4})alkyl-(halo)$_{1-3}$, -(C_{1-4})alkyl-OH, -aryl-R^8,

-(C_{1-4})alkyl-aryl-R^8 and -(C_{1-4})alkyl-heteroaryl-R^8; with the proviso that, when R^6 is attached to a carbon atom, R^6 is further selected from the group consisting of -C_{1-4}alkoxy, -(C_{1-4})alkoxy-(halo)$_{1-3}$, -SH, -S-(C_{1-4})alkyl, -N-R^7, cyano, halo, hydroxy, nitro, oxo and -heteroaryl-R^8.

53. The method of claim 31, wherein R^6 is hydrogen.

54. The method of claim 33, wherein R^7 is 2 substituents independently selected from the group consisting of hydrogen, -C_{1-4}alkyl, -C_{2-4}alkenyl, -C_{2-4}alkynyl, -(C_{1-4})alkyl-OH, -(C_{1-4})alkyl-O-(C_{1-4})alkyl, -(C_{1-4})alkyl-NH_2, -(C_{1-4})alkyl-NH(C_{1-4}alkyl), -(C_{1-4})alkyl-N(C_{1-4}alkyl)$_2$, -(C_{1-4})alkyl-S-(C_{1-4})alkyl, -C(O)H, -C(O)-(C_{1-4})alkyl, -C(O)-O-(C_{1-4})alkyl, -C(O)-NH_2, -C(O)-NH(C_{1-4}alkyl), -C(O)-N(C_{1-4}alkyl)$_2$, -SO_2-(C_{1-4})alkyl, -SO_2-NH_2, -SO_2-NH(C_{1-4}alkyl), -SO_2-N(C_{1-4}alkyl)$_2$, -C(N)-NH_2, -cycloalkyl-R^8, -(C_{1-4})alkyl-heterocyclyl-R^8, -aryl-R^8, -(C_{1-4})alkyl-aryl-R^8 and -(C_{1-4})alkyl-heteroaryl-R^8.

55. The method of claim 33, wherein R^7 is 2 substituents independently selected from the group consisting of hydrogen, -C_{1-4}alkyl, -C(O)H, -C(O)-(C_{1-4})alkyl, -C(O)-O-(C_{1-4})alkyl, -SO_2-NH_2, -SO_2-NH(C_{1-4}alkyl) and -SO_2-N(C_{1-4}alkyl)$_2$.

56. The method of claim 31, wherein R^8 is 1 to 4 substituents attached to a carbon or nitrogen atom independently selected from the group consisting of hydrogen, -C_{1-4}alkyl, -(C_{1-4})alkyl-(halo)$_{1-3}$ and -(C_{1-4})alkyl-OH; with the proviso that, when R^8 is attached to a carbon atom, R^8 is further selected from the group consisting of -C_{1-4}alkoxy, -NH_2, -NH(C_{1-4}alkyl), -N(C_{1-4}alkyl)$_2$, cyano, halo, -(C_{1-4})alkoxy-(halo)$_{1-3}$, hydroxy and nitro.

57. The method of claim 31, wherein R^8 is hydrogen.

58. The method of claim 31, wherein R^9 is 1 to 2 substituents independently selected from the group consisting of hydrogen,

-C_{1-4}alkoxy, -NH_2, -NH(C_{1-4}alkyl), -N(C_{1-4}alkyl)$_2$, cyano, (halo)$_{1-3}$, hydroxy and nitro.

59. The method of claim 31, wherein R^9 is hydrogen.

60. The method of claim 28, wherein R^2 is one substituent attached to a carbon or nitrogen atom selected from the group consisting of hydrogen, -C_{1-4}alkyl-R^5, -C_{2-4}alkenyl-R^5, -C_{2-4}alkynyl-R^5, -C(O)H, -C(O)-(C_{1-4})alkyl-R^9, -C(O)-NH_2, -C(O)-NH(C_{1-4}alkyl-R^9), -C(O)-N(C_{1-4}alkyl-R^9)$_2$, -C(O)-NH(aryl-R^8), -C(O)-cycloalkyl-R^8, -C(O)-heterocyclyl-R^8, -C(O)-aryl-R^8, -C(O)-heteroaryl-R^8, -CO_2H, -C(O)-O-(C_{1-4})alkyl-R^9, -C(O)-O-aryl-R^8, -SO_2-(C_{1-4})alkyl-R^9, -SO_2-aryl-R^8, -cycloalkyl-R^6, -aryl-R^6 and -(C_{1-4})alkyl-N-R^7; with the proviso that, when R^2 is attached to a carbon atom, R^2 is further selected from the group consisting of -C_{1-4}alkoxy-R^5, -N-R^7, cyano, halogen, hydroxy, nitro, oxo, -heterocyclyl-R^6 and -heteroaryl-R^6.

61. The method of claim 28, wherein R^2 is one substituent attached to a carbon or nitrogen atom selected from the group consisting of hydrogen, -C_{1-4}alkyl-R^5, -C_{2-4}alkenyl-R^5, -C_{2-4}alkynyl-R^5, -CO_2H, -C(O)-O-(C_{1-4})alkyl-R^9, -cycloalkyl-R^6, -aryl-R^6 and -(C_{1-4})alkyl-N-R^7; with the proviso that, when R^2 is attached to a nitrogen atom, a quaternium salt is not formed; and, with the proviso that, when R^2 is attached to a carbon atom, R^2 is further selected from the group consisting of -C_{1-4}alkoxy-R^5, -N-R^7, cyano, halogen, hydroxy, nitro, oxo, -heterocyclyl-R^6 and -heteroaryl-R^6.

62. The method of claim 28, wherein R^2 is one substituent attached to a carbon or nitrogen atom selected from the group consisting of hydrogen, -C_{1-4}alkyl-R^5 and -aryl-R^6; with the proviso that, when R^2 is attached to a nitrogen atom, a quaternium salt is not formed; and, with the proviso that when R^2 is attached to a carbon atom, R^2 is further selected from the group consisting of -N-R^7, halogen, hydroxy and -heteroaryl-R^6.

63. The method of claim 28, wherein R^3 is 1 to 3 substituents attached to a carbon atom independently selected from the group consisting of hydrogen, -C_{1-4}alkyl-R^{10}, -C_{2-4}alkenyl-R^{10}, -C_{2-4}alkynyl-R^{10}, -C_{1-4}alkoxy-R^{10}, -C(O)H, -C(O)-(C_{1-4})alkyl-R^9, -C(O)-NH_2, -C(O)-NH(C_{1-4}alkyl-R^9), -C(O)-N(C_{1-4}alkyl-R^9)$_2$, -C(O)-cycloalkyl-R^8, -C(O)-heterocyclyl-R^8, -C(O)-aryl-R^8, -C(O)-heteroaryl-R^8, -C(NH)-NH_2, -CO_2H, -C(O)-O-(C_{1-4})alkyl-R^9, -C(O)-O-aryl-R^8, -SO_2-(C_{1-8})alkyl-R^9, -SO_2-aryl-R^8, -N-R^7, -(C_{1-4})alkyl-N-R^7, cyano, halogen, hydroxy, nitro, -cycloalkyl-R^8, -heterocyclyl-R^8, -aryl-R^8 and -heteroaryl-R^8.

64. The method of claim 28, wherein R^3 is one substituent attached to a carbon atom selected from the group consisting of hydrogen, -C_{1-4}alkyl-R^{10}, -C_{2-4}alkenyl-R^{10}, -C_{2-4}alkynyl-R^{10}, -C_{1-4}alkoxy-R^{10}, -C(O)H, -CO_2H, -NH_2, -NH(C_{1-4}alkyl), -N(C_{1-4}alkyl)$_2$, cyano, halogen, hydroxy and nitro.

65. The method of claim 28, wherein R^3 is one substituent attached to a carbon atom selected from the group consisting of hydrogen, -C_{1-4}alkyl-R^{10}, -NH_2, -NH(C_{1-4}alkyl), -N(C_{1-4}alkyl)$_2$, halogen and hydroxy.

66. The method of claim 28, wherein R^4 is 1 to 4 substituents attached to a carbon atom independently selected from the group consisting of hydrogen, -C_{1-4}alkyl-R^{10}, -C_{2-4}alkenyl-R^{10}, -C_{2-4}alkynyl-R^{10}, -C_{1-4}alkoxy-R^{10}, -C(O)H, -C(O)-(C_{1-4})alkyl-R^9, -C(O)-NH_2, -C(O)-NH(C_{1-4}alkyl-R^9), -C(O)-N(C_{1-4}alkyl-R^9)$_2$, -C(O)-cycloalkyl-R^8, -C(O)-heterocyclyl-R^8, -C(O)-aryl-R^8, -C(O)-heteroaryl-R^8, -C(NH)-NH_2, -CO_2H, -C(O)-O-(C_{1-4})alkyl-R^9, -C(O)-O-aryl-R^8, -SH, -S-(C_{1-4})alkyl-R^{10}, -SO_2-(C_{1-4})alkyl-R^9, -SO_2-aryl-R^8, -SO_2-NH_2, -SO_2-NH(C_{1-4}alkyl-R^9), -SO_2-N(C_{1-4}alkyl-R^9)$_2$, -N-R^7, cyano, halogen, hydroxy, nitro, -cycloalkyl-R^8, -heterocyclyl-R^8, -aryl-R^8 and -heteroaryl-R^8.

67. The method of claim 28, wherein R^4 is 1 to 4 substituents attached to a carbon atom independently selected from the group consisting of hydrogen, -C_{1-4}alkyl-R^{10}, -C_{2-4}alkenyl-R^{10}, -C_{2-4}alkynyl-R^{10}, -C_{1-4}alkoxy-R^{10}, -C(O)H, -CO_2H, -NH_2, -NH(C_{1-4}alkyl), -N(C_{1-4}alkyl)$_2$, cyano, halogen, hydroxy, nitro, -cycloalkyl, -heterocyclyl, -aryl and -heteroaryl.

68. The method of claim 28, wherein R^4 is 1 to 4 substituents attached to a carbon atom independently selected from the group consisting of hydrogen, C_{1-4}alkyl-R^{10}, C_{1-4}alkoxy-R^{10}, -NH_2, -NH(C_{1-4}alkyl), -N(C_{1-4}alkyl)$_2$, halogen and hydroxy.

69. The method of claim 28, wherein R^4 is 1 to 4 substituents attached to a carbon atom independently selected from the group consisting of hydrogen, C_{1-4}alkyl-R^{10}, C_{1-4}alkoxy-R^{10}, -NH_2, -NH(C_{1-4}alkyl), -N(C_{1-4}alkyl)$_2$, chlorine, fluorine and hydroxy.

70. The method of claims 38 and 41, wherein R^{10} is 1 to 2 substituents independently selected from the group consisting of hydrogen, -NH_2, -NH(C_{1-4}alkyl), -N(C_{1-4}alkyl)$_2$, cyano, (halo)$_{1-3}$, hydroxy, nitro and oxo.

71. The method of claims 38 and 41, wherein R^{10} is 1 to 2 substituents independently selected from the group consisting of hydrogen and (halo)$_{1-3}$.

72. The method of claims 38 and 41, wherein R^{10} is 1 to 2 substituents independently selected from the group consisting of hydrogen and (fluoro)$_3$.

73. The method of claim 28, wherein Y and Z are independently selected from the group consisting of O, S, (H,OH) and (H,H); with the proviso that one of Y and Z is O and the other is selected from the group consisting of O, S, (H,OH) and (H,H).

74. The method of claim 28, wherein Y and Z are independently selected from the group consisting of O and (H,H); with the proviso that one of Y and Z is O, and the other is selected from the group consisting of O and (H,H).

75. The method of claim 28, wherein Y and Z are independently selected from O.

76. The method of claim 28, where the compound of the Formula II is 3-[1-(3-hydroxypropyl)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl]-4-[2-(trifluoromethyl)phenyl]-1*H*-pyrrole-2,5-dione.

77. The method of claim 28, where the compound of the Formula II is 3-[1-(3-hydroxypropyl)-1*H*-pyrrolo[2,3-*b*]pyridin-3-yl]-4-(1-methyl-1*H*-pyrazol-3-yl)-1*H*-pyrrole-2,5-dione.

78. The method of claim 28, where the compound of the Formula II is 3-[1-(3-hydroxy-propyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-4-pyrazin-2-yl-pyrrole-2,5-dione.

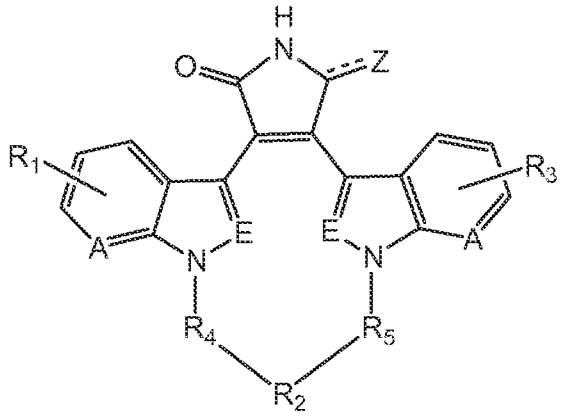
79. The method of claim 28, where the compound of the Formula II is 3-(2,4-dimethoxy-pyrimidin-5-yl)-4-[1-(3-hydroxy-propyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-pyrrole-2,5-dione.

80. The method of claim 28, where the compound of the Formula II is 4-{3-[4-(2,4-dimethoxy-pyrimidin-5-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-pyrrolo[2,3-b]pyridin-1-yl}-butyronitrile.

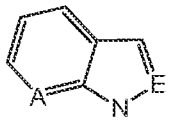
81. The method of claim 28, where the compound of the Formula II is 4-{3-[4-(1-methyl-1H-pyrazol-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-pyrrolo[2,3-b]pyridin-1-yl}-butyronitrile.

82. The method of claim 28, where the compound of the Formula II is 3-(2,4-dimethoxy-pyrimidin-5-yl)-4-(1-phenethyl-1H-pyrrolo[2,3-b]pyridine-3-yl)-pyrrole-2,5-dione.

83. The method of claim 1, wherein the inhibitor of GSK-3B enzyme activity is a compound of the Formula(III):



Formula (III)

84. The method of claim 83, wherein A and E are independently selected from the group consisting of a hydrogen substituted carbon atom and a nitrogen atom; wherein  is independently selected from the group consisting of 1*H*-indole, 1*H*-pyrrolo[2,3-*b*]pyridine, 1*H*-pyrazolo[3,4-*b*]pyridine and 1*H*-indazole.

85. The method of claim 83, wherein Z is selected from O; alternatively, Z is selected from dihydro; wherein each hydrogen atom is attached by a single bond.

86. The method of claim 83, wherein R_4 and R_5 are independently selected from $C_{1\text{-}8}$alkyl, $C_{2\text{-}8}$alkenyl and $C_{2\text{-}8}$alkynyl optionally substituted with oxo.

87. The method of claim 83, wherein R_2 is selected from the group consisting of -$C_{1\text{-}8}$alkyl-, -$C_{2\text{-}8}$alkenyl-, -$C_{2\text{-}8}$alkynyl-, -O-($C_{1\text{-}8}$)alkyl-O-, -O-($C_{2\text{-}8}$)alkenyl-O-, -O-($C_{2\text{-}8}$)alkynyl-O-, -C(O)-($C_{1\text{-}8}$)alkyl-C(O)- (wherein any of the foregoing alkyl, alkenyl and alkynyl linking groups are straight carbon chains optionally substituted with one to four substituents independently selected from the group consisting of $C_{1\text{-}8}$alkyl, $C_{1\text{-}8}$alkoxy, $C_{1\text{-}8}$alkoxy($C_{1\text{-}8}$)alkyl,

carboxyl, carboxyl(C_{1-8})alkyl, -C(O)O-(C_{1-8})alkyl, -C_{1-8}alkyl-C(O)O-(C_{1-8})alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), halogen, (halo)$_{1-3}$(C_{1-8})alkyl, (halo)$_{1-3}$(C_{1-8})alkoxy, hydroxy, hydroxy(C_{1-8})alkyl and oxo; and, wherein any of the foregoing alkyl, alkenyl and alkynyl linking groups are optionally substituted with one to two substituents independently selected from the group consisting of heterocyclyl, aryl, heteroaryl, heterocyclyl(C_{1-8})alkyl, aryl(C_{1-8})alkyl, heteroaryl(C_{1-8})alkyl, spirocycloalkyl and spiroheterocyclyl (wherein any of the foregoing cycloalkyl, heterocyclyl, aryl and heteroaryl substituents are optionally substituted with one to four substituents independently selected from the group consisting of C_{1-8}alkyl, C_{1-8}alkoxy, C_{1-8}alkoxy(C_{1-8})alkyl, carboxyl, carboxyl(C_{1-8})alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), halogen, (halo)$_{1-3}$(C_{1-8})alkyl, (halo)$_{1-3}$(C_{1-8})alkoxy, hydroxy and hydroxy(C_{1-8})alkyl; and, wherein any of the foregoing heterocyclyl substituents are optionally substituted with oxo)), cycloalkyl, heterocyclyl, aryl, heteroaryl (wherein cycloalkyl, heterocyclyl, aryl and heteroaryl are optionally substituted with one to four substituents independently selected from the group consisting of C_{1-8}alkyl, C_{1-8}alkoxy, C_{1-8}alkoxy(C_{1-8})alkyl, carboxyl, carboxyl(C_{1-8})alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), halogen, (halo)$_{1-3}$(C_{1-8})alkyl, (halo)$_{1-3}$(C_{1-8})alkoxy, hydroxy and hydroxy(C_{1-8})alkyl; and, wherein heterocyclyl is optionally substituted with oxo), -(O-$(CH_2)_{1-6}$)$_{0-5}$-O-, -O-$(CH_2)_{1-6}$-O-$(CH_2)_{1-6}$-O-, -O-$(CH_2)_{1-6}$-O-$(CH_2)_{1-6}$-O-$(CH_2)_{1-6}$-O-,

-(O-$(CH_2)_{1-6})_{0-5}$-NR_6-, -O-$(CH_2)_{1-6}$-NR_6-$(CH_2)_{1-6}$-O-, -O-$(CH_2)_{1-6}$-O-$(CH_2)_{1-6}$-NR_6-, -(O-$(CH_2)_{1-6})_{0-5}$-S-, -O-$(CH_2)_{1-6}$-S-$(CH_2)_{1-6}$-O-, -O-$(CH_2)_{1-6}$-O-$(CH_2)_{1-6}$-S-, -NR_6-, -NR_6-NR_7-, -NR_6-$(CH_2)_{1-6}$-NR_7-, -NR_6-$(CH_2)_{1-6}$-NR_7-$(CH_2)_{1-6}$-NR_8-, -NR_6-C(O)-, -C(O)-NR_6-, -C(O)-$(CH_2)_{0-6}$-NR_6-$(CH_2)_{0-6}$-C(O)-, -NR_6-$(CH_2)_{0-6}$-C(O)-$(CH_2)_{1-6}$-C(O)-$(CH_2)_{0-6}$-NR_7-, -NR_6-C(O)-NR_7-, -NR_6-C(NR_7)-NR_8-, -O-$(CH_2)_{1-6}$-NR_6-$(CH_2)_{1-6}$-S-, -S-$(CH_2)_{1-6}$-NR_6-$(CH_2)_{1-6}$-O-, -S-$(CH_2)_{1-6}$-NR_6-$(CH_2)_{1-6}$-S-, -NR_6-$(CH_2)_{1-6}$-S-$(CH_2)_{1-6}$-NR_7- and -SO_2- (wherein R_6, R_7 and R_8 are independently selected from the group consisting of hydrogen, C_{1-8}alkyl, C_{1-8}alkoxy(C_{1-8})alkyl, carboxyl(C_{1-8})alkyl, amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), hydroxy(C_{1-8})alkyl, heterocyclyl(C_{1-8})alkyl, aryl(C_{1-8})alkyl and heteroaryl(C_{1-8})alkyl (wherein the foregoing heterocyclyl, aryl and heteroaryl substituents are optionally substituted with one to four substituents independently selected from the group consisting of C_{1-8}alkyl, C_{1-8}alkoxy, C_{1-8}alkoxy(C_{1-8})alkyl, carboxyl, carboxyl(C_{1-8})alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), halogen, (halo)$_{1-3}$(C_{1-8})alkyl, (halo)$_{1-3}$(C_{1-8})alkoxy, hydroxy and hydroxy(C_{1-8})alkyl; and, wherein heterocyclyl is optionally substituted with oxo)); with the proviso that, if A and E are selected from a hydrogen substituted carbon atom, then R_2 is selected from the group consisting of -C_{2-8}alkynyl-, -O-(C_{1-8})alkyl-O-, -O-(C_{2-8})alkenyl-O-, -O-(C_{2-8})alkynyl-O-, -C(O)-(C_{1-8})alkyl-C(O)- (wherein any of the foregoing alkyl, alkenyl and alkynyl linking groups are straight carbon chains optionally substituted with one to four substituents independently selected from the group consisting of C_{1-8}alkyl, C_{1-8}alkoxy, C_{1-8}alkoxy(C_{1-8})alkyl, carboxyl, carboxyl(C_{1-8})alkyl, -C(O)O-(C_{1-8})alkyl, -C_{1-8}alkyl-C(O)O-(C_{1-8})alkyl, amino (substituted with a substituent

independently selected from the group consisting of hydrogen and C_{1-4}alkyl), amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), halogen, (halo)$_{1-3}$(C_{1-8})alkyl, (halo)$_{1-3}$(C_{1-8})alkoxy, hydroxy, hydroxy(C_{1-8})alkyl and oxo; and, wherein any of the foregoing alkyl, alkenyl and alkynyl linking groups are optionally substituted with one to two substituents independently selected from the group consisting of heterocyclyl, aryl, heteroaryl, heterocyclyl(C_{1-8})alkyl, aryl(C_{1-8})alkyl, heteroaryl(C_{1-8})alkyl, spirocycloalkyl and spiroheterocyclyl (wherein any of the foregoing cycloalkyl, heterocyclyl, aryl and heteroaryl substituents are optionally substituted with one to four substituents independently selected from the group consisting of C_{1-8}alkyl, C_{1-8}alkoxy, C_{1-8}alkoxy(C_{1-8})alkyl, carboxyl, carboxyl(C_{1-8})alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), halogen, (halo)$_{1-3}$(C_{1-8})alkyl, (halo)$_{1-3}$(C_{1-8})alkoxy, hydroxy and hydroxy(C_{1-8})alkyl; and, wherein any of the foregoing heterocyclyl substituents are optionally substituted with oxo)), cycloalkyl (wherein cycloalkyl is optionally substituted with one to four substituents independently selected from the group consisting of C_{1-8}alkyl, C_{1-8}alkoxy, C_{1-8}alkoxy(C_{1-8})alkyl, carboxyl, carboxyl(C_{1-8})alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), halogen, (halo)$_{1-3}$(C_{1-8})alkyl, (halo)$_{1-3}$(C_{1-8})alkoxy, hydroxy and hydroxy(C_{1-8})alkyl), -(O-(CH_2)$_{1-6}$)$_{1-5}$-O-, -O-(CH_2)$_{1-6}$-O-(CH_2)$_{1-6}$-O-, -O-(CH_2)$_{1-6}$-O-(CH_2)$_{1-6}$-O-(CH_2)$_{1-6}$-O-, -(O-(CH_2)$_{1-6}$)$_{1-5}$-NR_6-, -O-(CH_2)$_{1-6}$-NR_6-(CH_2)$_{1-6}$-O-, -O-(CH_2)$_{1-6}$-O-(CH_2)$_{1-6}$-NR_6-, -(O-(CH_2)$_{1-6}$)$_{0-5}$-S-, -O-(CH_2)$_{1-6}$-S-(CH_2)$_{1-6}$-O-, -O-(CH_2)$_{1-6}$-O-(CH_2)$_{1-6}$-S-, -NR_6-NR_7-, -NR_6-(CH_2)$_{1-6}$-NR_7-,

-NR_6-$(CH_2)_{1-6}$-NR_7-$(CH_2)_{1-6}$-NR_8-, -NR_9-C(O)-, -C(O)-NR_9-, -C(O)-$(CH_2)_{0-6}$-NR_6-$(CH_2)_{0-6}$-C(O)-, -NR_6-$(CH_2)_{0-6}$-C(O)-$(CH_2)_{1-6}$-C(O)-$(CH_2)_{0-6}$-NR_7-, -NR_6-C(O)-NR_7-, -NR_6-C(NR_7)-NR_8-, -O-$(CH_2)_{1-6}$-NR_6-$(CH_2)_{1-6}$-S-, -S-$(CH_2)_{1-6}$-NR_6-$(CH_2)_{1-6}$-O-, -S-$(CH_2)_{1-6}$-NR_6-$(CH_2)_{1-6}$-S- and -NR_6-$(CH_2)_{1-6}$-S-$(CH_2)_{1-6}$-NR_7- (wherein R_6, R_7 and R_8 are independently selected from the group consisting of hydrogen, C_{1-8}alkyl, C_{1-8}alkoxy(C_{1-8})alkyl, carboxyl(C_{1-8})alkyl, amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), hydroxy(C_{1-8})alkyl, heterocyclyl(C_{1-8})alkyl, aryl(C_{1-8})alkyl and heteroaryl(C_{1-8})alkyl (wherein the foregoing heterocyclyl, aryl and heteroaryl substituents are optionally substituted with one to four substituents independently selected from the group consisting of C_{1-8}alkyl, C_{1-8}alkoxy, C_{1-8}alkoxy(C_{1-8})alkyl, carboxyl, carboxyl(C_{1-8})alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), halogen, (halo)$_{1-3}$(C_{1-8})alkyl, (halo)$_{1-3}$(C_{1-8})alkoxy, hydroxy and hydroxy(C_{1-8})alkyl; and, wherein heterocyclyl is optionally substituted with oxo); and, wherein R_9 is selected from the group consisting of C_{1-8}alkyl, C_{1-8}alkoxy(C_{1-8})alkyl, carboxyl(C_{1-8})alkyl, amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), hydroxy(C_{1-8})alkyl, heterocyclyl(C_{1-8})alkyl, aryl(C_{1-8})alkyl and heteroaryl(C_{1-8})alkyl (wherein the foregoing heterocyclyl, aryl and heteroaryl substituents are optionally substituted with one to four substituents independently selected from the group consisting of C_{1-8}alkyl, C_{1-8}alkoxy, C_{1-8}alkoxy(C_{1-8})alkyl, carboxyl, carboxyl(C_{1-8})alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of

hydrogen and C_{1-4}alkyl), halogen, (halo)$_{1-3}$(C_{1-8})alkyl, (halo)$_{1-3}$(C_{1-8})alkoxy, hydroxy and hydroxy(C_{1-8})alkyl; and, wherein heterocyclyl is optionally substituted with oxo)).

88. The method of claim 83, wherein R_1 and R_3 are independently selected from the group consisting of hydrogen, C_{1-8}alkyl, C_{2-8}alkenyl, C_{2-8}alkynyl (wherein alkyl, alkenyl and alkynyl are optionally substituted with a substituent selected from the group consisting of C_{1-8}alkoxy, alkoxy(C_{1-8})alkyl, carboxyl, carboxyl(C_{1-8})alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), (halo)$_{1-3}$, (halo)$_{1-3}$(C_{1-8})alkyl, (halo)$_{1-3}$(C_{1-8})alkoxy, hydroxy, hydroxy(C_{1-8})alkyl and oxo), C_{1-8}alkoxy, C_{1-8}alkoxycarbonyl, (halo)$_{1-3}$(C_{1-8})alkoxy, C_{1-8}alkylthio, aryl, heteroaryl (wherein aryl and heteroaryl are optionally substituted with a substituent selected from the group consisting of C_{1-8}alkyl, C_{1-8}alkoxy, alkoxy(C_{1-8})alkyl, carboxyl, carboxyl(C_{1-8})alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), amino(C_{1-8})alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), halogen, (halo)$_{1-3}$(C_{1-8})alkyl, (halo)$_{1-3}$(C_{1-8})alkoxy, hydroxy and hydroxy(C_{1-8})alkyl), amino (substituted with a substituent independently selected from the group consisting of hydrogen and C_{1-4}alkyl), cyano, halogen, hydroxy and nitro; and pharmaceutically acceptable salts thereof.

89. The method of claim 83, wherein the compound of the Formula(III) is 6,7,9,10,12,13,15,16-octahydro-23H-5,26:17,22-dimetheno-5H-dipyrido[2,3-k:3',2'-q]pyrrolo[3,4-n][1,4,7,10,19]trioxadiazacyclohenicosine-23,25(24H)-dione.

90. The method of claim 83, wherein the compound of the Formula(III) is 10,11,13,14,16,17,19,20,22,23-decahydro-9,4:24,29-dimetheno-1H-

dipyrido[2,3-n:3',2'-t]pyrrolo[3,4-q][1,4,7,10,13,22]tetraoxadiazacyclotetracosine-1,3(2H)-dione.

91. The method of claim 83, wherein the compound of the Formula(III) is 10,11,13,14,16,17,19,20,22,23,25,26-dodecahydro-9,4:27,32-dimetheno-1H-dipyrido[2,3-q:3',2'-w]pyrrolo[3,4-t][1,4,7,10,13,16,25]pentaoxadiazacycloheptacosine-1,3(2H)-dione.

92. The method of claim 83, wherein the compound of the Formula(III) is 6,7,9,10,12,13-hexahydro-20H-5,23:14,19-dimetheno-5H-dibenzo[h,n]pyrrolo[3,4-k][1,4,7,16]dioxadiazacyclooctadecine-20,22(21H)-dione.

93. The method of claim 83, wherein the compound of the Formula(III) is 6,7,9,10,12,13,15,16-octahydro-23H-5,26:17,22-dimetheno-5H-dibenzo[k,q]pyrrolo[3,4-n][1,4,7,10,19]trioxadiazacycloheneicosine-23,25(24H)-dione.

94. The method of claim 83, wherein the compound of the Formula(III) is 10,11,13,14,16,17,19,20,22,23-decahydro-9,4:24,29-dimetheno-1H-dibenzo[n,t]pyrrolo[3,4-q][1,4,7,10,13,22]tetraoxadiazacyclotetracosine-1,3(2H)-dione.

95. The method of claim 83, wherein the compound of the Formula(III) is Compound 1a.

96. The method of claim 83, wherein the compound of the Formula(III) is 3-[1-[3-[(2-hydroxyethyl)methylamino]propyl]-1H-indazol-3-yl]-4-[1-(3-pyridinyl)-1H-indol-3-yl]-1H-pyrrole-2,5-dione.

97. The method of claim 83, wherein the compound of the Formula (III) is 3,5-dichloro-N-[3-chloro-4-[(3,4,12,12a-tetrahydro-1H-[1,4]thiazino[3,4-c][1,4]benzodiazepin-11(6H)-yl)carbonyl]phenyl]-benzamide.

98. The method of claim 83, wherein the compound of the Formula (III) is 3-[1-(2-hydroxy-ethyl)-1H-indol-3-yl]-4-(1-pyridin-3-yl-1H-indol-3-yl)-pyrrole-2,5-dione.

99. The method of claim 83, wherein the compound of the Formula (III) is 3-(2-methoxy-phenyl)-4-(1-pyridin-3-yl-1H-indol-3-yl)-pyrrole-2,5-dione.

100. The method of claim 83, wherein the compound of the Formula (III) is 6-[[2-[[4-(2,4-dichlorophenyl)-5-(4-methyl-1H-imidazol-2-yl)-2-pyrimidinyl]amino]ethyl]amino]-3-pyridinecarbonitrile.

101. The method of claim 83, wherein the compound of the Formula (III) is 3-(5-chloro-1-methyl-1H-indol-3-yl)-4-[1-(3-imidazol-1-yl-propyl)-1H-indazol-3-yl]-pyrrole-2,5-dione.

102. The method of claim 83, wherein the compound of the Formula (III) is 3-(5-chloro-1-methyl-1H-indol-3-yl)-4-[1-(3-[1,2,3]triazol-1-yl-propyl)-1H-indazol-3-yl]-pyrrole-2,5-dione.

103. The method of claim 83, wherein the compound of the Formula (III) is 3-[1-(3-hydroxy-propyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]-4-(1-methyl-1H-pyrazol-3-yl)-pyrrole-2,5-dione.

104. The method of claim 83, wherein the compound of the Formula (III) is Compound 10a.

105. The method of claim 83, wherein the compound of the Formula (III) is 3-[1-(3-hydroxy-3-methyl-butyl)-1H-indazol-3-yl]-4-(1-pyridin-3-yl-1H-indol-3-yl)-pyrrole-2,5-dione.

106. The method of claim 83, wherein the compound of the Formula (III) is 3-[1-(2-hydroxy-ethyl)-1H-indazol-3-yl]-4-(1-pyrimidin-5-yl-1H-indol-3-yl)-pyrrole-2,5-dione.

107. The method of claim 83, wherein the compound of the Formula (III) is 3-[1-(2-hydroxy-ethyl)-1H-indol-3-yl]-4-(1-pyrimidin-5-yl-1H-indol-3-yl)-pyrrole-2,5-dione.

108. The method of claim 83, wherein the compound of the Formula (III) is (11Z)-8,9,10,13,14,15-hexahydro-2,6:17,21-di(metheno)pyrrolo[3,4-h][1,15,7]dioxazacyclotricosine-22,24(1H,23H)-dione.

109. The method of claim 83, wherein the compound of the Formula (III) is 3-(5-chloro-1-pyridin-3-yl-1H-indol-3-yl)-4-[1-(3-hydroxy-propyl)-1H-indazol-3-yl]-pyrrole-2,5-dione.

110. The method of claim 83, wherein the compound of the Formula (III) is 3-(2-methoxy-phenyl)-4-[1-(3-methoxy-propyl)-1H-pyrrolo[3,2-c]pyridin-3-yl]-pyrrole-2,5-dione.

111. The method of claim 83, wherein the compound of the Formula (III) is 3-[1-(3-hydroxy-propyl)-1H-indazol-3-yl]-4-[1-(tetrahydro-pyran-4-yl)-1H-indol-3-yl]-pyrrole-2,5-dione.

112. The method of claim 83, wherein the compound of the Formula (III) is 2-{3-[4-(5-chloro-1-methyl-1H-indol-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-indazol-1-yl}-N-(2-hydroxy-ethyl)-acetamide.

113. The method of claim 83, wherein the compound of the Formula (III) is 4-(3-chloro-phenyl)-6-(3-dimethylamino-propyl)-5,6-dihydro-4H-2,4,6-triaza-cyclopenta[c]fluorine-1,3-dione.

114. The method of claim 83, wherein the compound of the Formula (III) is 14-ethyl-6,7,9,10,13,14,15,16-octahydro-12H,23H-5,26:17,22-dimethenodibenzo[k,q]pyrrolo[3,4-n][1,4,7,10,19]dioxatriazacycloheneicosine-23,25(24H)-dione.

115. The method of claim 83, wherein the compound of the Formula (III) is 14-benzyl-6,7,9,10,13,14,15,16-octahydro-12H,23H-5,26:17,22-

di(metheno)dibenzo[k,q]pyrrolo[3,4-n][1,4,7,10,19]dioxatriazacyclohenicosine-23,25(24H)-dione.

116. The method of claim 83, wherein the compound of the Formula (III) is 3-(1-{2-[2-(2-hydroxy-ethoxy)-ethoxy]-ethyl}-1H-indol-3-yl)-4-[1-(2-hydroxy-ethyl)-1H-indol-3-yl]-pyrrole-2,5-dione.

117. The method of claim 83, wherein the compound of the Formula (III) is 6,7,8,9,10,11,12,13-octahydro-8,11-dimethyl-5,23:14,19-dimetheno-20H-dibenzo[k,q]pyrrolo[3,4-n][1,4,7,10]tetraazacyclooctadecine-20,22(21H)-dione.

118. The method of claim 83, wherein the compound of the Formula (III) is 7,8,9,10,12,13,16,17,18,19-decahydro-8,17-dimethyl-15H,26H-5,29:20,25-dimetheno-6H-dibenzo[k,q]pyrrolo[3,4-n][1,4,7,10,19,22]dioxatetraazacyclotetracosine-26,28(27H)-dione.

119. The method of claim 83, wherein the compound of the Formula (III) is 14-(2-furylmethyl)-6,7,9,10,13,14,15,16-octahydro-12H,23H-5,26:17,22-di(metheno)dibenzo[k,q]pyrrolo[3,4-n][1,4,7,10,19]dioxatriazacyclohenicosine-23,25(24H)-dione.

120. The method of claim 83, wherein the compound of the Formula (III) is 14-(2-thienylmethyl)-6,7,9,10,13,14,15,16-octahydro-12H,23H-5,26:17,22-di(metheno)dibenzo[k,q]pyrrolo[3,4-n][1,4,7,10,19]dioxatriazacyclohenicosine-23,25(24H)-dione.

121. The method of claim 83, wherein the compound of the Formula (III) is 14-(1-naphthylmethyl)-6,7,9,10,13,14,15,16-octahydro-12H,23H-5,26:17,22-di(metheno)dibenzo[k,q]pyrrolo[3,4-n][1,4,7,10,19]dioxatriazacyclohenicosine-23,25(24H)-dione.

122. The method of claim 83, wherein the compound of the Formula (III) is 14-(pyridin-4-ylmethyl)-6,7,9,10,13,14,15,16-octahydro-12H,23H-5,26:17,22-di(metheno)dibenzo[k,q]pyrrolo[3,4-n][1,4,7,10,19]dioxatriazacyclohenicosine-23,25(24H)-dione.

123. The method of claim 83, wherein the compound of the Formula (III) is 3-[1-(2-{2-[2-(1,2,3,4-tetrahydro-naphthalen-1-ylamino)-ethoxy]-ethoxy}-ethyl)-1H-indol-3-yl]-4-{1-[2-(1,2,3,4-tetrahydro-naphthalen-1-ylamino)-ethyl]-1H-indol-3-yl}-pyrrole-2,5-dione.

124. The method of claim 83, wherein the compound of the Formula (III) is 3-[1-(3-dimethylamino-phenyl)-1H-indol-3-yl]-4-[1-(2-hydroxy-ethyl)-1H-indazol-3-yl]-pyrrole-2,5-dione.

125. The method of claim 83, wherein the compound of the Formula (III) is 3-[5-chloro-1-(6-dimethylamino-pyridin-3-yl)-1H-indol-3-yl]-4-[1-(2-hydroxy-ethyl)-1H-indazol-3-yl]-pyrrole-2,5-dione.

126. The method of claim 83, wherein the compound of the Formula (III) is 5-(5-chloro-3-{4-[1-(2-hydroxy-ethyl)-1H-indazol-3-yl]-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl}-indol-1-yl)-nicotinic acid methyl ester.

FIG. 1A

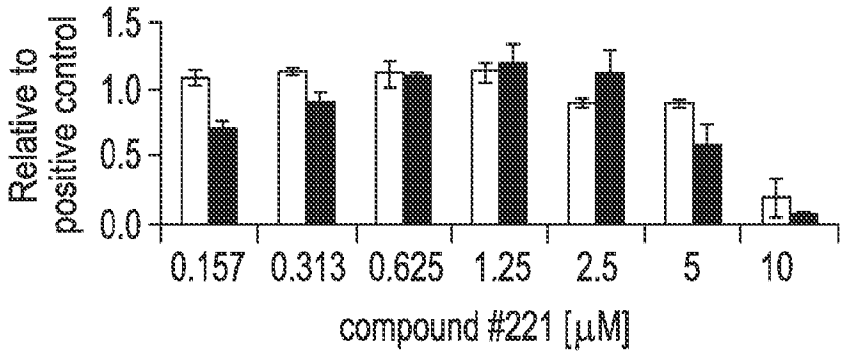

Cell number
Relative to positive control
1.5
1.0
0.5
0.0
0.157
0.313
0.625
1.25
2.5
5
10
compound #221 [μM]

FIG. 1B

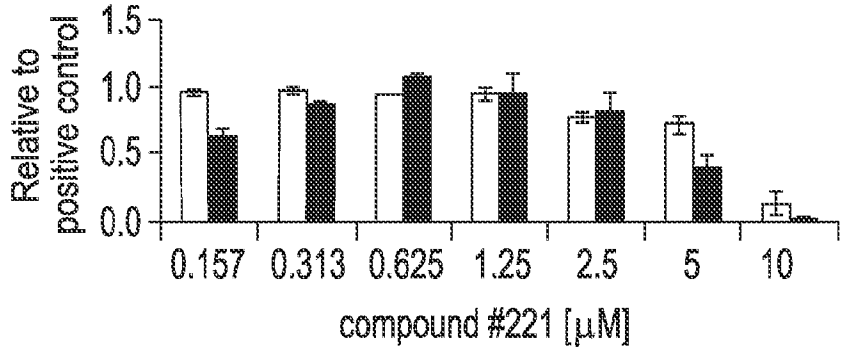

Sox17 Expression
Relative to positive control
1.5
1.0
0.5
0.0
0.157
0.313
0.625
1.25
2.5
5
10
compound #221 [μM]

FIG. 2A

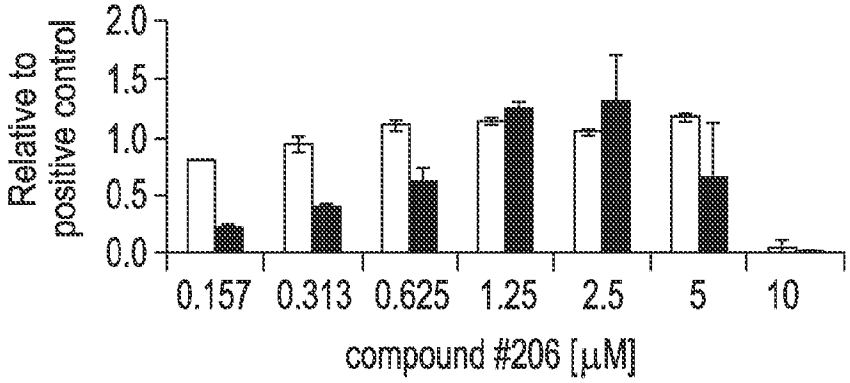

Cell number
Relative to positive control
2.0
1.5
1.0
0.5
0.0
0.157
0.313
0.625
1.25
2.5
5
10
compound #206 [μM]

FIG. 2B

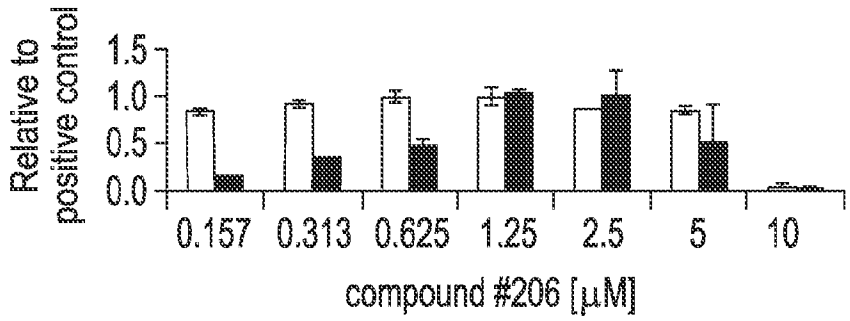

Sox17 Expression
Relative to positive control
1.5
1.0
0.5
0.0
0.157
0.313
0.625
1.25
2.5
5
10
compound #206 [μM]

FIG. 3A

Cell number

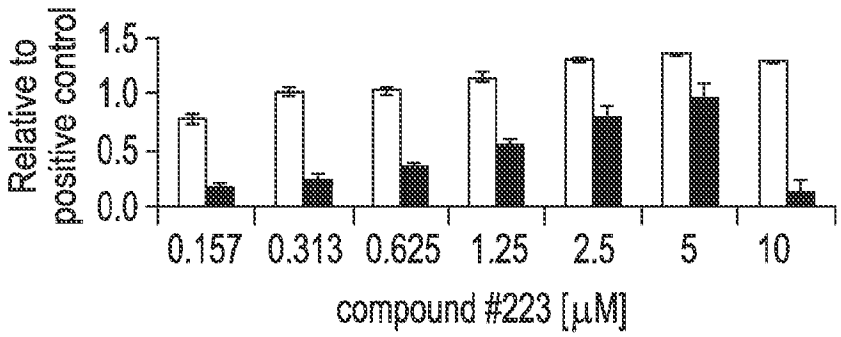


FIG. 3B

Sox17 Expression

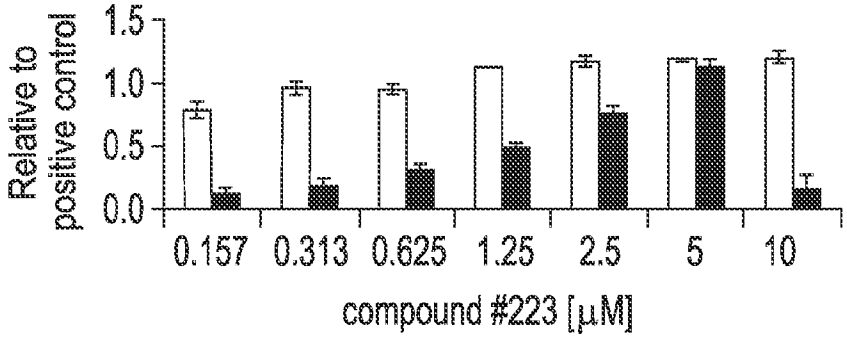


FIG. 4A

Cell number

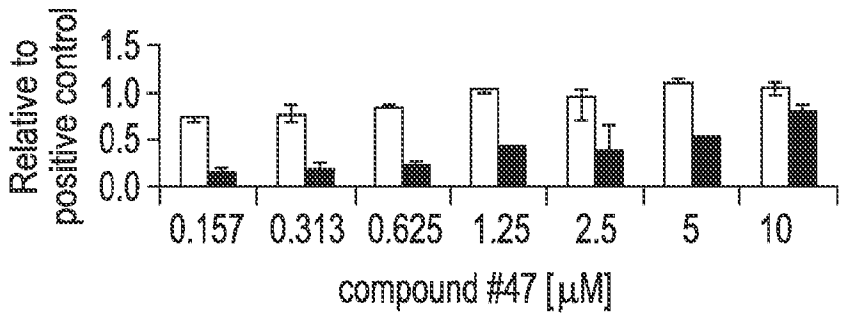


FIG. 4B

Sox17 Expression

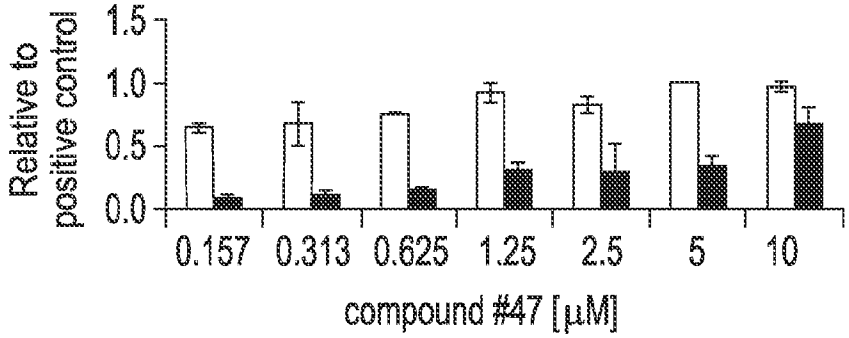


FIG. 5A

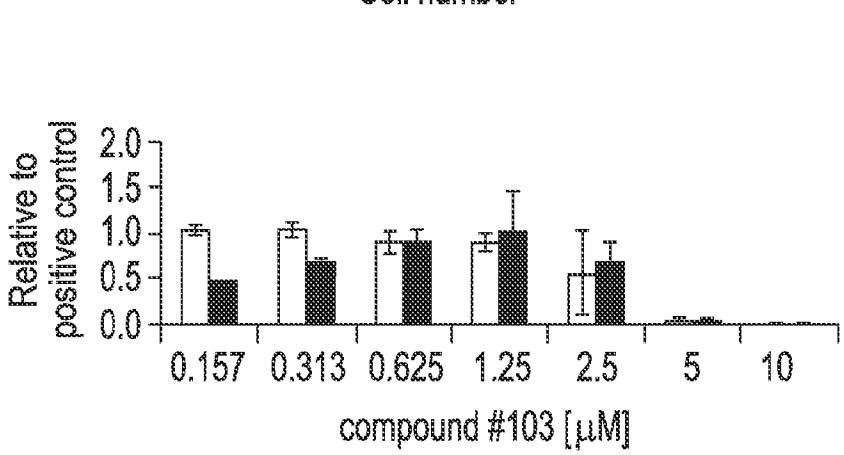

Cell number
Relative to positive control
2.0
1.5
1.0
0.5
0.0
0.157
0.313
0.625
1.25
2.5
5
10
compound #103 [μM]

FIG. 5B

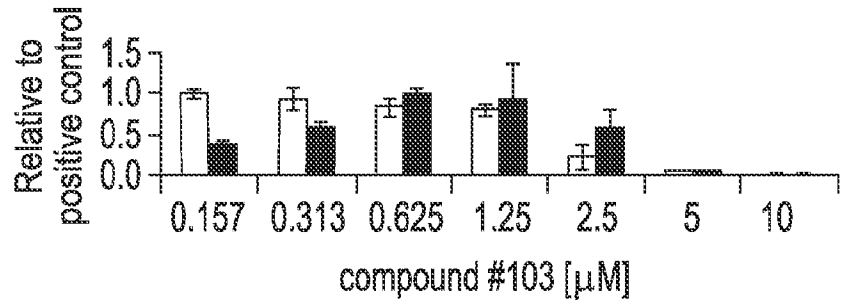

Sox17 Expression
Relative to positive control
1.5
1.0
0.5
0.0
0.157
0.313
0.625
1.25
2.5
5
10
compound #103 [μM]

FIG. 6A

Cell number

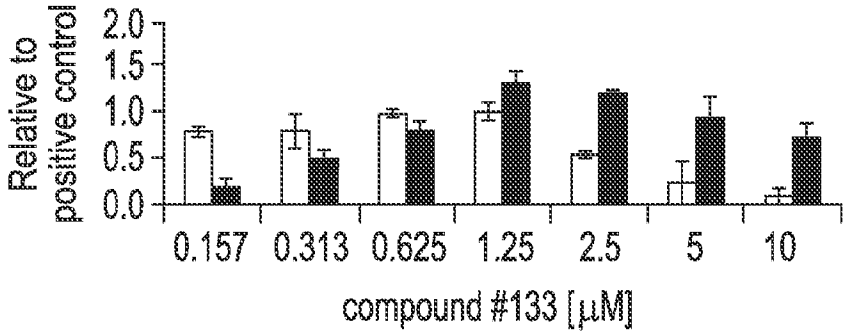


FIG. 6B

Sox17 Expression

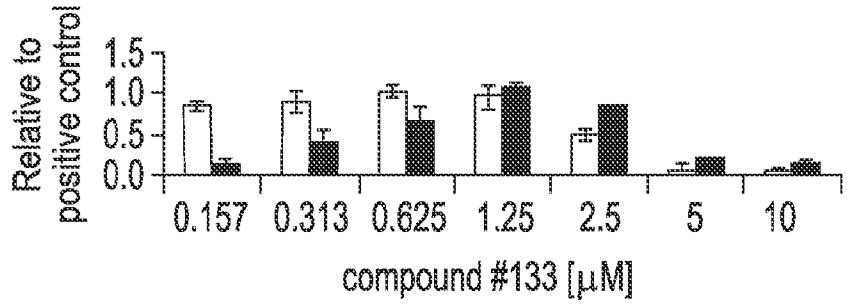


FIG. 7A

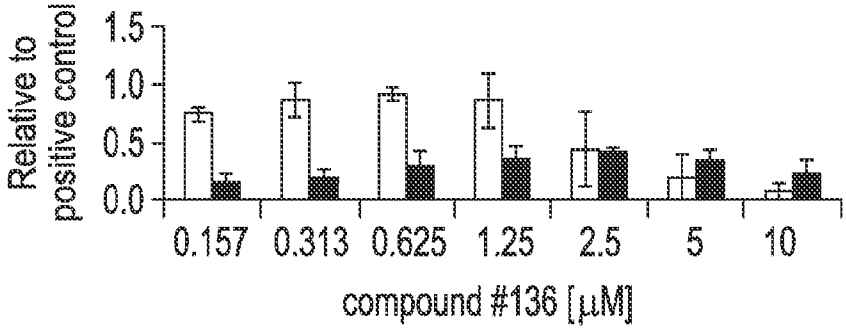

Cell number
Relative to positive control
1.5
1.0
0.5
0.0
0.157
0.313
0.625
1.25
2.5
5
10
compound #136 [μM]

FIG. 7B

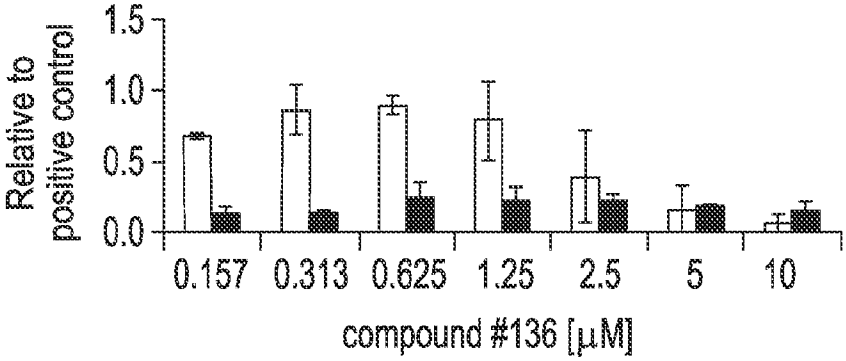

Sox17 Expression
Relative to positive control
1.5
1.0
0.5
0.0
0.157
0.313
0.625
1.25
2.5
5
10
compound #136 [μM]

FIG. 8A

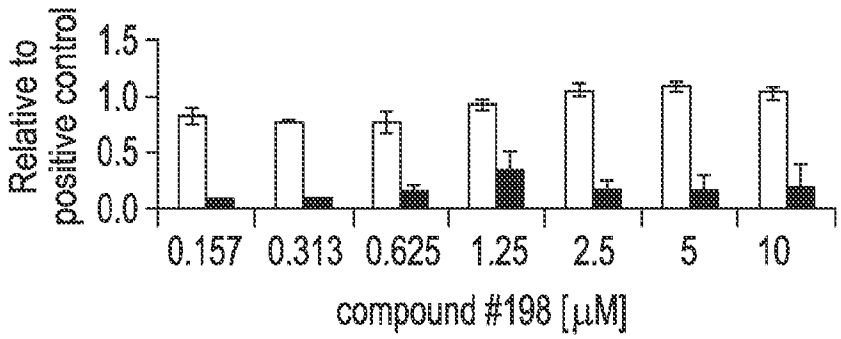

Cell number
Relative to positive control
1.5
1.0
0.5
0.0
0.157
0.313
0.625
1.25
2.5
5
10
compound #198 [μM]

FIG. 8B

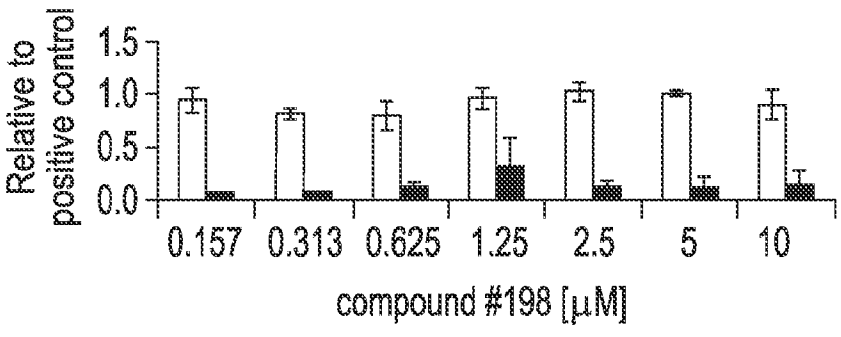

Sox17 Expression
Relative to positive control
1.5
1.0
0.5
0.0
0.157
0.313
0.625
1.25
2.5
5
10
compound #198 [μM]

FIG. 9

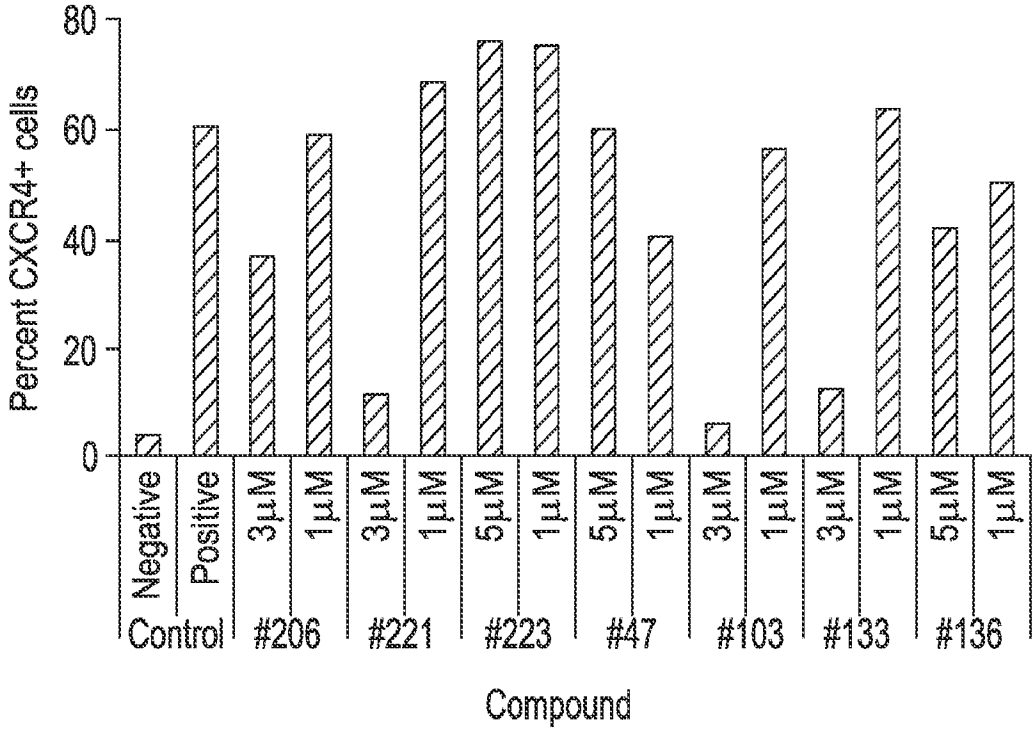

CXCR4 Expression
Percent CXCR4+ cells
80
60
40
20
0
Negative
Positive
3µM
1µM
3µM
1µM
5µM
1µM
5µM
1µM
3µM
1µM
3µM
1µM
5µM
1µM
Control
#206
#221
#223
#47
#103
#133
#136
Compound

FIG. 10A

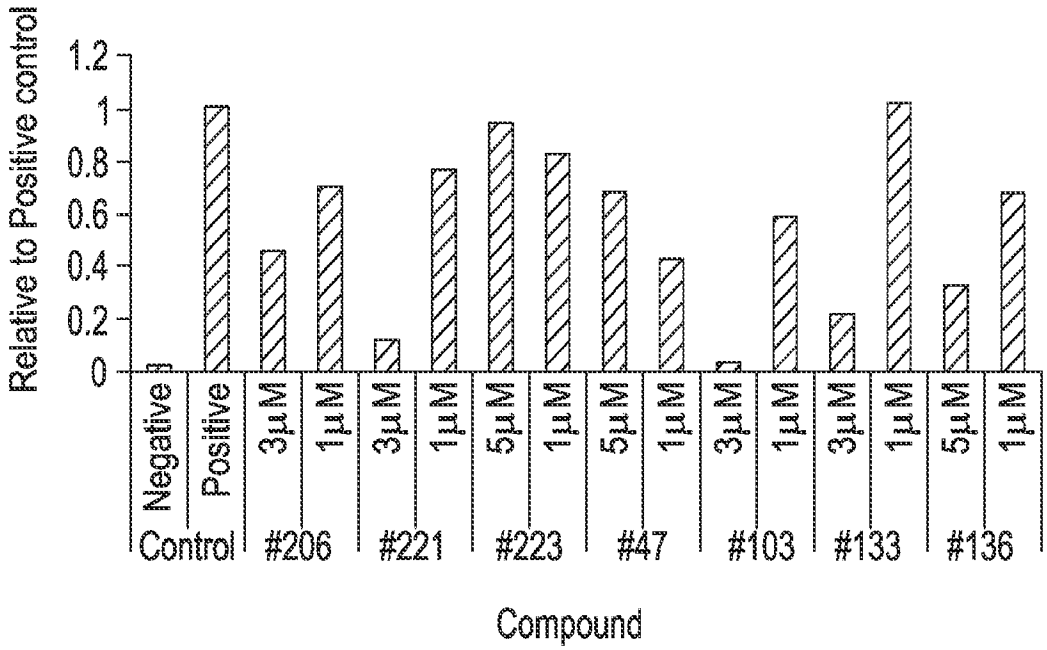

CXCR4 mRNA level
Relative to Positive control
1.2
1
0.8
0.6
0.4
0.2
0
Negative
Positive
3µM
1µM
3µM
1µM
5µM
1µM
5µM
1µM
3µM
1µM
3µM
1µM
5µM
1µM
Control
#206
#221
#223
#47
#103
#133
#136
Compound

FIG. 10B

HNF3b mRNA level

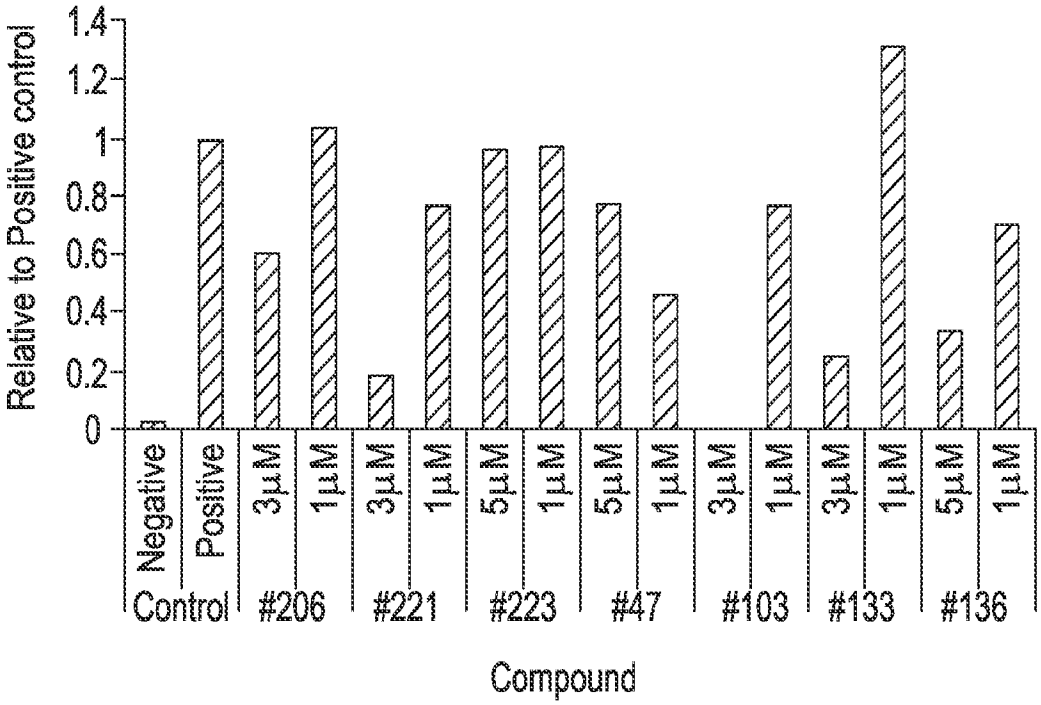


FIG. 10C

Sox17 mRNA level

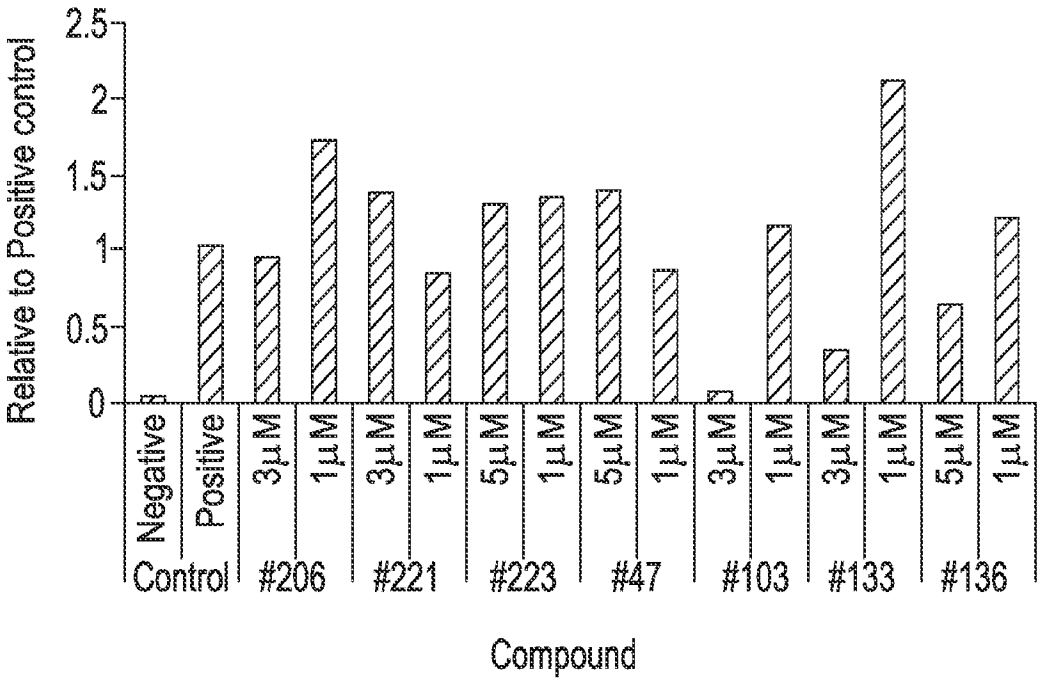


FIG. 11A

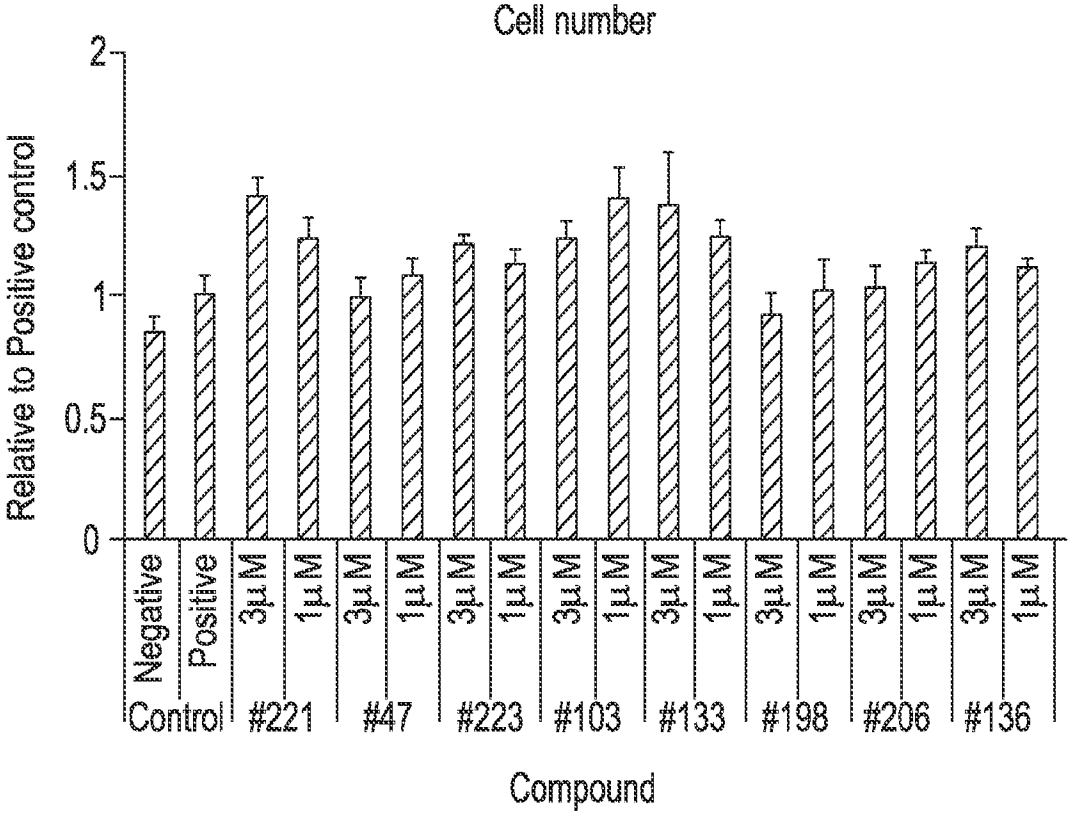


FIG. 11B

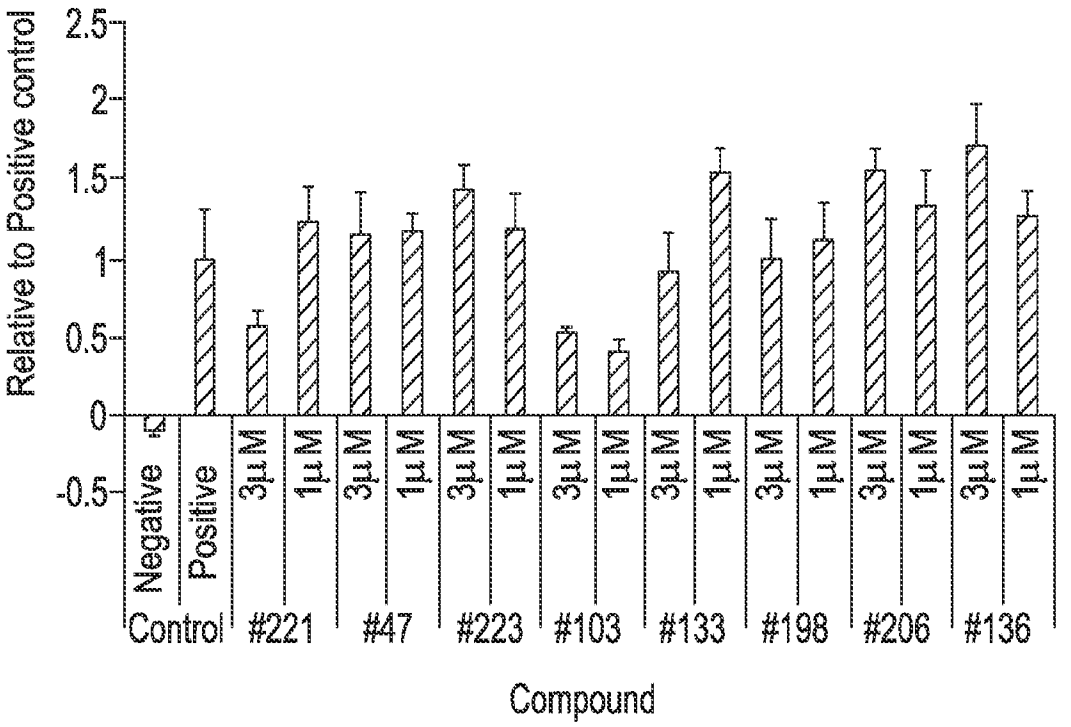


FIG. 12

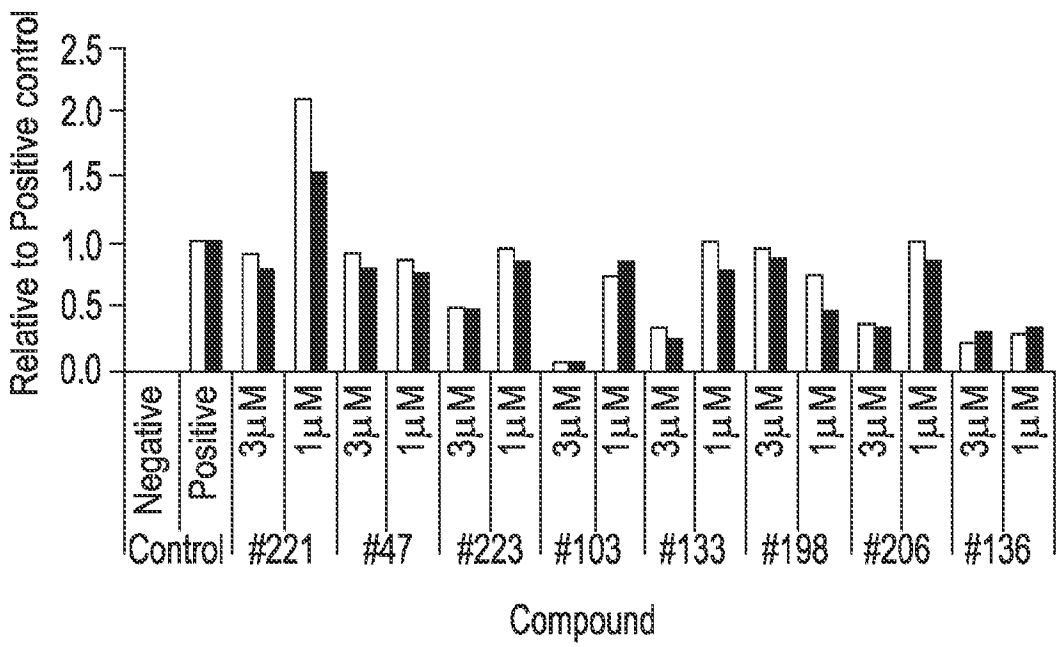


FIG. 13A

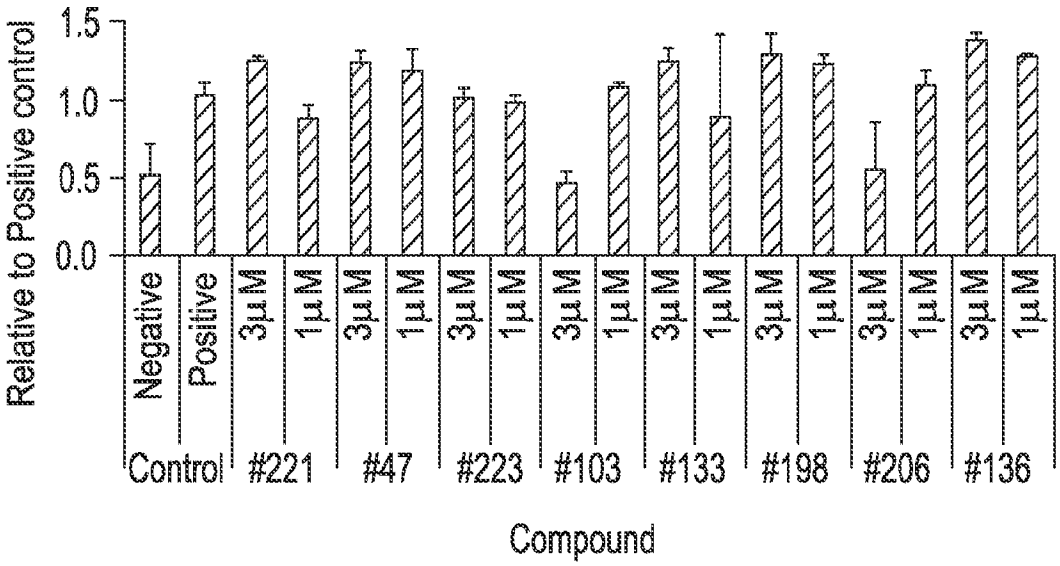


FIG. 13B

Insulin Expression

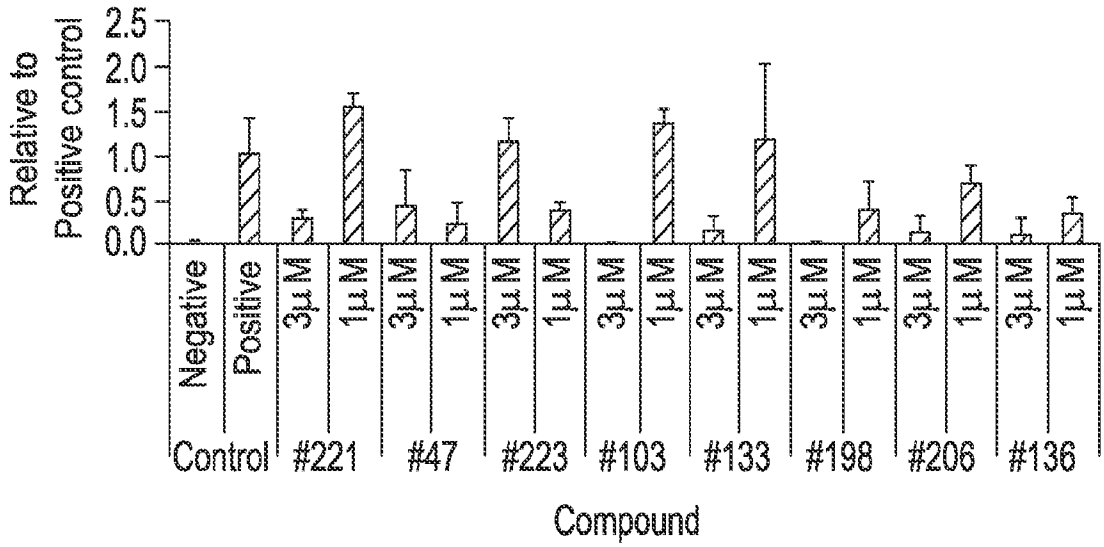


FIG. 14

mRNA level

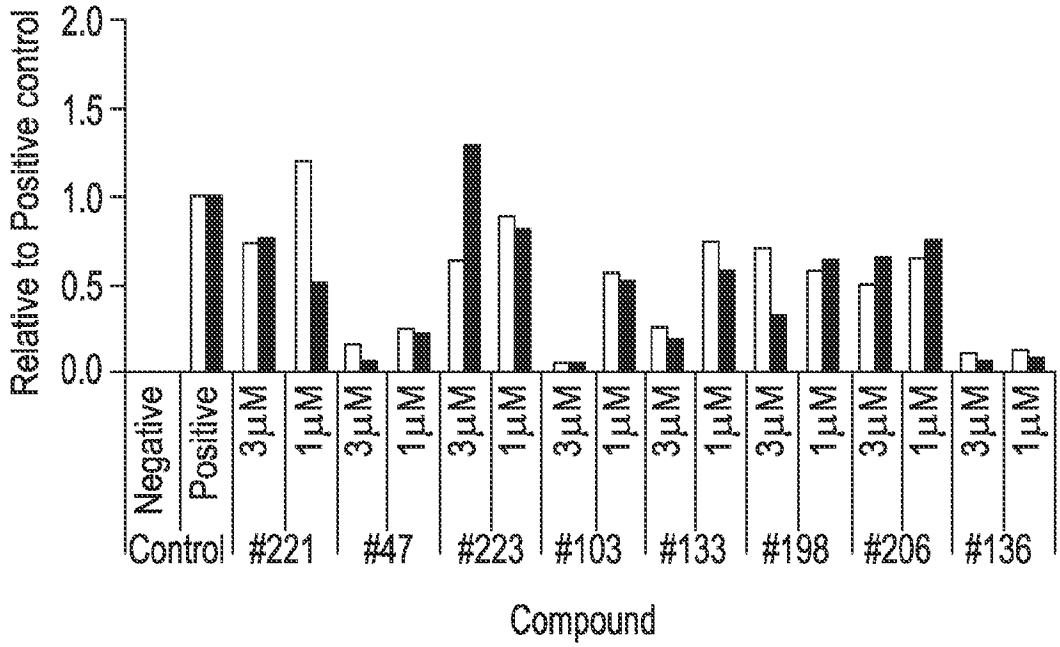


FIG. 15A

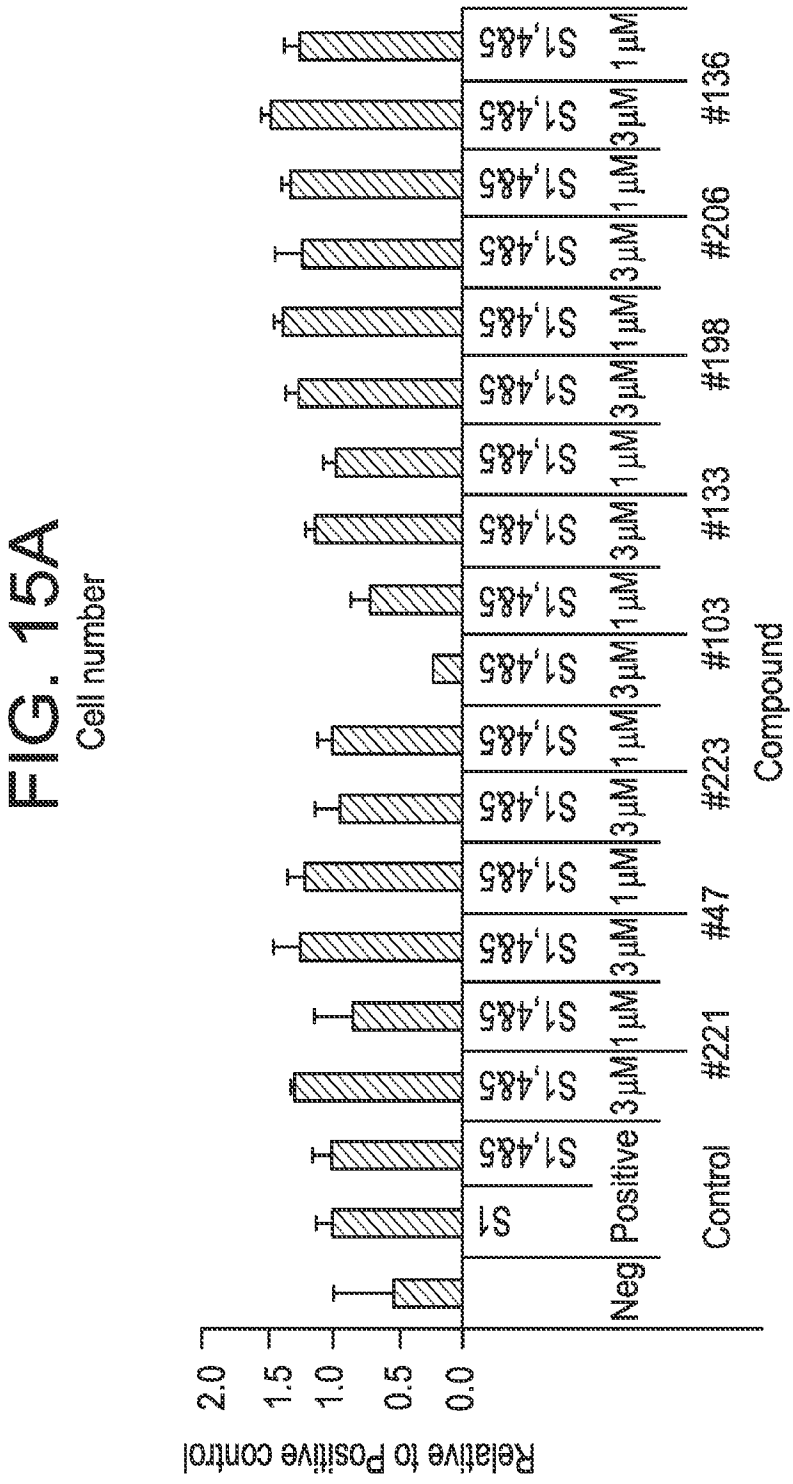

Cell number
Relative to Positive control
2.0
1.5
1.0
0.5
0.0
Neg
Positive
Control
S1
S1,485
3 μM
1 μM
#221
#47
#223
#103
#133
#198
#206
#136
Compound

FIG. 15B

Insulin expression