Claims

1. A method for differentiating pluripotent stem cells into definitive endoderm cells comprising treating the pluripotent stem cells with medium supplemented with an inhibitor of glycogen synthase kinase 3β enzyme activity selected from the group consisting of:

a. 6-[[2-[[4-(2,4-dichlorophenyl)-5-(4-methyl-1H-imidazol-2-yl)-2-pyrimidinyl]amino]ethyl]amino]-3-pyridinecarbonitrile;

b. 3-[1-[3-[(2-hydroxyethyl)methylamino]propyl]-1H-indazol-3-yl]-4-[1-(3-pyridinyl)-1H-indol-3-yl]-1H-pyrrole-2,5-dione;

c. 3-[1-(3-hydroxy-3-methyl-butyl)-1H-indazol-3-yl]-4-(1-pyridin-3-yl-1H-indol-3-yl)-pyrrole-2,5-dione;

d. 3-[1-(3-hydroxypropyl)-1H-pyrrolo[2,3-b]pyridine-3-yl]-4-pyrazin-2-y1-1H-pyrrole-2,5-dione;

e. 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;

f. 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; and

g. 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.

2. The method of claim 1, wherein the method further comprises culturing the pluripotent stem cells prior to treating the cells with the inhibitor of glycogen synthase kinase 3β enzyme activity.

3. The method of claim 1, wherein the pluripotent stem cells are treated with the inhibitor of glycogen synthase kinase 3β enzyme activity for 12 to 48 hours.

4. The method of claim 1, wherein the pluripotent stem cells are treated with the inhibitor of glycogen synthase kinase 3β enzyme activity for 1 to 72 hours.

5. The method of claim 1, wherein the inhibitor of glycogen synthase kinase 3β enzyme activity is used at a concentration of 100 nM to 100 μM.

6. The method of claim 1, wherein the inhibitor of glycogen synthase kinase 3β enzyme activity is used at a concentration of 1 μM to 10 μM.

7. The method of claim 1, wherein the pluripotent stem cells are human stem cells.

8. The method of claim 7, wherein the human stem cells are human embryonic stem cells.

9. The method of any one of claims 1 to 8, wherein the inhibitor of glycogen synthase kinase 3β enzyme activity is 6-[[2-[[4-(2,4-dichlorophenyl)-5-(4-methyl-1H-imidazol-2-yl)-2-pyrimidinyl]amino]ethyl]amino]-3-pyridinecarbonitrile.

10. The method of any one of claims 1 to 8, wherein the inhibitor of glycogen synthase kinase 3β enzyme activity 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.

11. The method of any one of claims 1 to 8, wherein the inhibitor of glycogen synthase kinase 3β enzyme activity 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.

12. The method of any one of claims 1 to 8, wherein the inhibitor of glycogen synthase kinase 3β enzyme activity is 3-[1-(3-hydroxypropyl)-1H-pyrrolo[2,3-b]pyridine-3-yl]-4-pyrazin-2-y1-1H-pyrrole-2,5-dione.

13. The method of any one of claims 1 to 8, wherein the inhibitor of glycogen synthase kinase 3β enzyme activity 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.

14. The method of any one of claims 1 to 8, wherein the inhibitor of glycogen synthase kinase 3β enzyme activity 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.

15. The method of any one of claims 1 to 8, wherein the inhibitor of glycogen synthase kinase 3β enzyme activity 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.

16. The method of any one of claims 1 to 8, wherein the medium is further supplemented with activin A.

17. The method of claim 15, wherein the medium is supplemented with 1pg/ml to 100μg/ml of activin A.

18. The method of claim 15, wherein the medium is further supplemented with serum.

19. The method of claim 17, wherein the medium is supplemented with 2% to 5% of serum.

20. A method of differentiating pluripotent stem cells into pancreatic endoderm cells or pancreatic endocrine cells comprising differentiating pluripotent stem cells into definitive endoderm cells using the method of claims 1 to 8 and differentiating the definitive endoderm cells into pancreatic endoderm cells or pancreatic endocrine cells.

21. The method of claim 20, wherein the method comprises differentiating pluripotent stem cells into pancreatic endocrine cells and wherein the method comprises:

a. differentiating pluripotent stem cells into definitive endoderm cells using the method of claims 1 to 8;

b. differentiating the definitive endoderm cells into pancreatic endoderm cells; and

c. differentiating pancreatic endoderm cells into pancreatic endocrine cells.