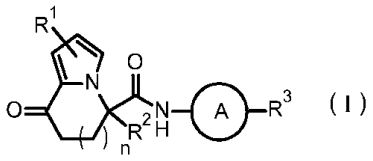
[Document Name] Claims

[Claim 1]

A method for producing insulin-producing cells by differentiating pluripotent stem cells into insulin-producing cells, comprising the step of three-dimensionally culturing cells in a medium containing a compound represented by formula (I):

[Formula 1]



(I)

wherein each substituent is defined as follows:

R^1 represents a hydrogen atom, a halogen atom, or a C1-C6 alkyl group;

R^2 represents a hydrogen atom or a C1-C6 alkyl group;

R^3 represents an aryl group optionally substituted with one to four substituents independently selected from a substituent group α, a C5-C10 cycloalkenyl group optionally substituted with one to four substituents independently selected from the substituent group α, or a heterocyclyl group optionally substituted with one to four substituents independently selected from the substituent group α;

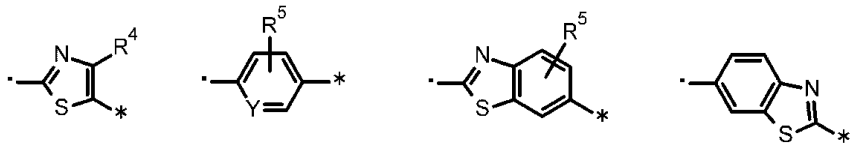
the substituent group α includes a halogen atom, a cyano group, a carboxy group, a C1-C6 alkyl group, a C1-C6 alkoxy group, a halo-C1-C6 alkyl group, a halo-C1-C6 alkoxy group, a hydroxy C1-C6 alkyl group, a C1-C6 alkoxy C1-C6 alkoxy group, a (C1-C6 alkyl)carbonyl group, a (C1-C6 alkoxy)carbonyl group, a (C1-C6 alkoxy)carbonyloxy group, a phenyl C1-C6 alkoxy group, a non-aromatic heterocyclyl group, a carbamoyl group optionally substituted with one or two C1-C6 alkyl groups, a C1-C6 alkoxy group substituted by a carbamoyl group optionally substituted with one or two C1-C6 alkyl groups, a sulfamoyl group substituted with one or two C1-C6 alkyl groups, a phenoxy group optionally substituted with one to four substituents independently selected from a substituent group β, a phenyl group optionally substituted with one to four substituents independently selected from the substituent group β, and a benzoyl group optionally substituted with one to four substituents independently selected from the substituent group β;

the substituent group β includes a halogen atom, a C1-C6 alkyl group, a C1-C6 alkoxy group, a halo-C1-C6 alkyl group, a halo-C1-C6 alkoxy group, and a (C1-C6 alkoxy)carbonyl group;

n represents 0 or 1; and

A represents a group represented by any one of formulae (i) to (iv) below:

[Formula 2]



(i) (ii) (iii) (iv)

wherein each substituent is defined as follows:

· and * each represent a bond, where · is bonded to a nitrogen atom in an amido group of formula (I), and * is bonded to R^3;

R^4 represents a hydrogen atom, a C1-C6 alkyl group, a halo-C1-C6 alkyl group, or a (C1-C6 alkoxy)carbonyl group;

R^5 represents a hydrogen atom, a halogen atom, or a C1-C6 alkyl group; and

Y represents N or CH; or a salt thereof.

[Claim 2]

A method for producing insulin-producing cells according to claim 1, wherein

R^3 in the compound represented by formula (I) represents a naphthyl group, a 1,3-benzodioxolyl group, a 2,2-dihalo-1,3-benzodioxolyl group, a C5-C10 cycloalkenyl group, a phenyl group optionally substituted with one or two substituents independently selected from a substituent group α1, or a 5- or 6-membered heterocyclyl group optionally substituted with one or two substituents independently selected from the substituent group α1;

the substituent group α1 includes a halogen atom, a cyano group, a carboxy group, a phenoxy group, a benzoyl group, a C1-C6 alkyl group, a C1-C6 alkoxy group, a halo-C1-C6 alkyl group, a halo-C1-C6 alkoxy group, a hydroxy C1-C6 alkyl group, a C1-C6 alkoxy C1-C6 alkoxy group, a (C1-C6 alkyl)carbonyl group, a (C1-C6 alkoxy)carbonyl group, a (C1-C6 alkoxy)carbonyloxy group, a phenyl C1-C6 alkoxy group, a 5- or 6-membered non-aromatic heterocyclyl group, a carbamoyl group optionally substituted with one or two C1-C6 alkyl groups, a C1-C6 alkoxy group substituted by a carbamoyl group optionally substituted with one or two C1-C6 alkyl groups, a sulfamoyl group substituted with one or two C1-C6 alkyl groups, and a phenyl group optionally substituted with one or two substituents independently selected from a substituent group β1; and

the substituent group β1 includes a halogen atom, a C1-C6 alkyl group, a C1-C6 alkoxy group, and a (C1-C6 alkoxy)carbonyl group.

[Claim 3]

A method for producing insulin-producing cells according to claim 1, wherein

R^3 in the compound represented by formula (I) represents a naphthyl group, a 1,3-benzodioxolyl group, a 2,2-dihalo-1,3-benzodioxolyl group, a C5-C10 cycloalkenyl group, a phenyl group optionally substituted with one or two substituents independently selected from a

substituent group α2, or a 5- or 6-membered heterocyclyl group optionally substituted with one or two substituents independently selected from a substituent group γ2;

the substituent group α2 includes a halogen atom, a cyano group, a carboxy group, a phenoxy group, a benzoyl group, a C1-C6 alkyl group, a C1-C6 alkoxy group, a halo-C1-C6 alkyl group, a halo-C1-C6 alkoxy group, a hydroxy C1-C6 alkyl group, a C1-C6 alkoxy C1-C6 alkoxy group, a (C1-C6 alkyl)carbonyl group, a (C1-C6 alkoxy)carbonyl group, a (C1-C6 alkoxy)carbonyloxy group, a phenyl C1-C6 alkoxy group, a 5- or 6-membered non-aromatic heterocyclyl group, a carbamoyl group optionally substituted with one or two C1-C6 alkyl groups, a C1-C6 alkoxy group substituted by a carbamoyl group optionally substituted with one or two C1-C6 alkyl groups, a sulfamoyl group substituted with one or two C1-C6 alkyl groups, and a phenyl group optionally substituted with one or two substituents independently selected from a substituent group β2;

the substituent group β2 includes a halogen atom, a C1-C6 alkyl group, and a C1-C6 alkoxy group; and

the substituent group γ2 includes a halogen atom, a C1-C6 alkyl group, a C1-C6 alkoxy group, a (C1-C6 alkyl)carbonyl group, and a (C1-C6 alkoxy)carbonyl group.

[Claim 4]

A method for producing insulin-producing cells according to claim 1, wherein

R^3 in the compound represented by formula (I) represents a naphthyl group, a 1,3-benzodioxolyl group, a 2,2-difluoro-1,3-benzodioxolyl group, a C5-C8 cycloalken-1-yl group, a phenyl group optionally substituted with one or two substituents independently selected from a substituent group α3, or a 5- or 6-membered heterocyclyl group optionally substituted with one or two substituents independently selected from a substituent group γ3;

the substituent group α3 includes a halogen atom, a cyano group, a carboxy group, a phenoxy group, a benzoyl group, a C1-C4 alkyl group, a C1-C4 alkoxy group, a halo-C1-C2 alkyl group, a halo-C1-C2 alkoxy group, a hydroxy C1-C4 alkyl group, a C1-C2 alkoxy C1-C2 alkoxy group, a (C1-C4 alkyl)carbonyl group, a (C1-C4 alkoxy)carbonyl group, a (C1-C4 alkoxy)carbonyloxy group, a phenyl C1-C4 alkoxy group, a morpholin-1-yl group, a carbamoyl group optionally substituted with one or two C1-C4 alkyl groups, a C1-C2 alkoxy group substituted by a carbamoyl group optionally substituted with one or two C1-C4 alkyl groups, a sulfamoyl group substituted with one or two C1-C4 alkyl groups, and a phenyl group optionally substituted with one or two substituents independently selected from a substituent group β3;

the substituent group β3 includes a fluorine atom, a chlorine atom, a C1-C4 alkyl group, and a C1-C4 alkoxy group; and

the substituent group γ3 includes a halogen atom, a C1-C4 alkyl group, a C1-C4 alkoxy group, a (C1-C4 alkyl)carbonyl group, and a (C1-C4 alkoxy)carbonyl group.

[Claim 5]

A method for producing insulin-producing cells according to any one of claims 1 to 4, wherein R^1 in the compound represented by formula (I) represents a hydrogen atom, a chlorine atom, or a methyl group.

[Claim 6]

A method for producing insulin-producing cells according to any one of claims 1 to 5, wherein R^2 in the compound represented by formula (I) represents a hydrogen atom or a methyl group.

[Claim 7]

A method for producing insulin-producing cells according to any one of claims 1 to 6, wherein A in the compound represented by formula (I) represents a group represented by formula (i), and R^4 represents a hydrogen atom, a C1-C6 alkyl group, a halo-C1-C6 alkyl group, or a (C1-C6 alkoxy)carbonyl group.

[Claim 8]

A method for producing insulin-producing cells according to claim 7, wherein R^4 in the compound represented by formula (I) represents a hydrogen atom, a methyl group, or a trifluoromethyl group.

[Claim 9]

A method for producing insulin-producing cells according to any one of claims 1 to 6, wherein A in the compound represented by formula (I) represents a group represented by formula (ii), and R^5 represents a hydrogen atom, a halogen atom, or a C1-C6 alkyl group.

[Claim 10]

A method for producing insulin-producing cells according to claim 9, wherein R^5 in the compound represented by formula (I) represents a hydrogen atom, a fluorine atom, or a methyl group.

[Claim 11]

A method for producing insulin-producing cells according to any one of claims 1 to 6, wherein A in the compound represented by formula (I) represents a group represented by formula (iii), and R^5 represents a hydrogen atom, a fluorine atom, or a methyl group.

[Claim 12]

A method for producing insulin-producing cells according to any one of claims 1 to 6, wherein A in the compound represented by formula (I) represents a group represented by formula (iv).

[Claim 13]

A method for producing insulin-producing cells according to any one of claims 1 to 12, wherein n in the compound represented by formula (I) represents 1.

[Claim 14]

A method for producing insulin-producing cells according to any one of claims 1 to 13, wherein R^3 in the compound represented by formula (I) represents a 2,2-difluoro-1,3-benzodioxolyl group, a 1-tert-butoxycarbonyl-3,6-dihydro-2H-pyridin-4-yl group, or a phenyl group optionally substituted with one or two substituents independently selected from the group consisting of a fluorine atom, a chlorine atom, a trifluoromethyl group, a tert-butoxy group, a trifluoromethoxy group, a 2,2,2-trifluoroethoxy group, a benzyloxy group, and a phenoxy group.

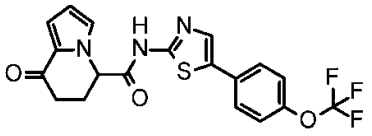
[Claim 15]

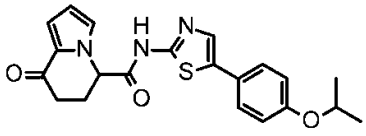
A method for producing insulin-producing cells according to any one of claims 1 to 13, wherein R^3 in the compound represented by formula (I) represents a phenyl group, or a phenyl group substituted at m position or p position with any one substituent selected from the group consisting of a fluorine atom, a chlorine atom, a trifluoromethyl group, a tert-butoxy group, a trifluoromethoxy group, a 2,2,2-trifluoroethoxy group, a benzyloxy group, and a phenoxy group.

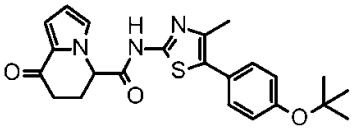
[Claim 16]

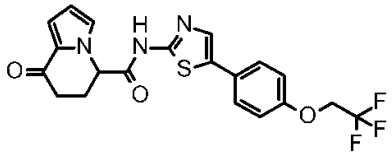
A method for producing insulin-producing cells according to claim 1, wherein the compound represented by formula (I) is any one selected from the compound group shown below:

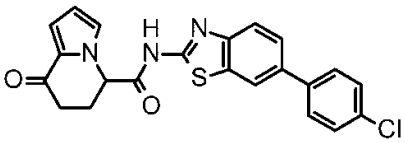
[Formula 3]











[Claim 17]

A method for producing insulin-producing cells according to claim 1, wherein the pluripotent stem cells are human ES cells or human iPS cells.

[Claim 18]

A method for producing insulin-producing cells according to claim 1, wherein the three-dimensional culture is performed in a low-adhesive or non-adhesive culture container.

[Claim 19]

A method for producing insulin-producing cells according to any one of claims 1 to 18, wherein the differentiation process from pluripotent stem cells into insulin-producing cells comprises steps 1 to 5 below, and at least one step selected from the group consisting of step 3, step 4 and step 5 comprises culturing cells in a

medium containing the compound represented by formula (I) or a salt thereof:
step 1 of inducing definitive endoderm cells from pluripotent stem cells;
step 2 of inducing primitive gut tube cells from the definitive endoderm cells;
step 3 of inducing pancreatic progenitor cells from the primitive gut tube cells;
step 4 of inducing pancreatic endocrine progenitor cells from the pancreatic progenitor cells; and
step 5 of inducing insulin-producing cells from the pancreatic endocrine progenitor cells.

[Claim 20]

A method for producing insulin-producing cells according to claim 19, wherein in step 3, step 4 and step 5, cells are cultured in a medium containing the compound represented by formula (I) or a salt thereof.

[Claim 21]

A method for producing insulin-producing cells according to claim 19, wherein in step 1, pluripotent stem cells pretreated with a methionine-depleted medium is used.

[Claim 22]

Insulin-producing cells derived from pluripotent stem cells, the insulin-producing cells being produced by a method according to any one of claims 1 to 21, wherein

the insulin-producing cells are cells enhanced functional maturation as pancreatic β cells.

[Claim 23]

A therapeutic drug for a disease caused by abnormal insulin secretion or insulin secretory disorder, comprising insulin-producing cells according to claim 22.~~derived from pluripotent stem cells, the insulin-producing cells being produced by a method according to any one of claims 1 to 21.~~

[Claim 24]

The therapeutic drug according to claim 23, wherein the disease caused by abnormal insulin secretion or insulin secretory disorder is type 1 diabetes or type 2 diabetes.

[Claim 25]

Insulin-producing cell population derived from pluripotent stem cells, the insulin-producing cell population being produced by a method according to any one of claims 1 to 21, wherein the insulin-producing cell population is population enhanced functional maturation as pancreatic cells.

[Claim 26]

A therapeutic drug for a disease caused by abnormal insulin secretion or insulin secretory disorder, comprising insulin-producing cell population derived from pluripotent stem cells, the insulin-producing cell

population being produced by a method according to claim 25.

[Claim 27]

The therapeutic drug according to claim 26, wherein the disease caused by abnormal insulin secretion or insulin secretory disorder is type 1 diabetes or type 2 diabetes.