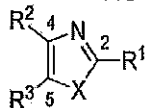


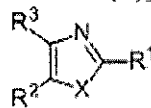
Summary

According to the disclosure, there is provided a method for inducing or enhancing the differentiation of pluripotent stem cells into cardiomyocyte via casein kinase 1 inhibition said method comprising culturing the stem cells in the presence of a medium comprising a casein kinase 1 inhibitor of the formula (I) or (II) or a stereoisomer, tautomer, or a salt thereof:

[Formula (I)]



[Formula (II)]



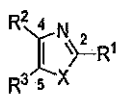
wherein R^1 , R^2 and R^3 independently from another represent hydrogen, optionally substituted alkyl, alkenyl, alkynyl, heterocyclyl, heteroaryl or aryl;

X represents NR^4 , O or S; and

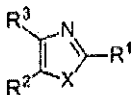
R^4 represents hydrogen, optionally substituted alkyl, alkenyl, alkynyl, heterocyclyl, heteroaryl or aryl.

According to a first aspect, there is provided a method for inducing or enhancing the differentiation of pluripotent stem cells into cardiomyocyte via casein kinase 1 inhibition said method comprising culturing the stem cells in the presence of a medium comprising a casein kinase 1 inhibitor of the formula (I) or (II) or a stereoisomer, tautomer, or a salt thereof

[Formula (I)]



[Formula (II)]



wherein R^1 represents fluorine substituted aryl having 6 to 10 carbon atoms, wherein the aryl is optionally additionally substituted by one or more substituents;

R^2 represents fluorine substituted phenyl, wherein the phenyl is optionally additionally substituted by one or more substituents;

R^3 represents pyridinyl or pyranlyl; and

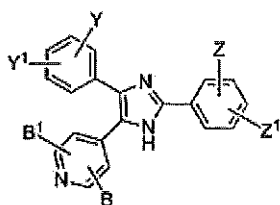
X represents NH.

Advantageously the use of small molecules that inhibit Wnt pathway during cardiomyocyte differentiation of pluripotent stem cells via casein kinase 1 (CK1 or CK 1) inhibition leads to improved differentiation. Differentiation of cardiomyocytes via inhibition of CK1 can be used on human induced pluripotent stem cells and human embryonic stem cells after EB formation, preferably from day 2 onwards. The small molecules according to the invention target CK 1 epsilon and CK 1 delta to give 10-20 % cardiomyocytes when applied as the sole inhibitor which is significantly better compared to other inhibition pathway methods. In the method of the invention not only the Wnt pathway, but also the stress pathway, is addressed. This double inhibition of Wnt and cell stress pathways according to the inventive method is unique. Wnt pathway inhibition is known to inhibit growth in cells. Many protocols compensate for this effect with highly expensive differentiation medium supplements (e.g. B27) that provides

several growth factors to compensate for growth inhibition and boost cell growth. Such compensation is not needed with the use of selected small molecules according to the inventive method.

According to the present disclosure, the compounds of formula (I), (Ia), (Ia)' and (II), especially IM-31 and ZQX-19, can therefore be used in methods to promote cell growth and survival.

According to another aspect, there is provided a method for inducing or enhancing the differentiation of pluripotent stem cells into cardiomyocyte via casein kinase 1 inhibition said method comprising culturing the stem cells in the presence of a medium comprising a casein kinase 1 inhibitor of the formula (Ia) or a stereoisomer, tautomer, or a salt thereof



(Ia)

wherein B, B¹, Y¹ and Z¹ are absent or each independently selected from C₁-C₆-alkyl, C₂-C₆-alkenyl, C₂-C₆-alkynyl, thio-C₁-C₆-alkyl, C₃-C₈-cycloalkyl, C₃-C₈-cycloalkenyl, five to six membered heterocycloalkyl, halo, -COOH, -CONH₂, C₁-C₆-carboxyl, halo-C₁-C₆-alkyl, halo-C₂-C₆-alkynyl, hydroxyl, C₁-C₆-alkoxy, thio-C₁-C₆-alkoxy, C₂-C₆-alkenyloxy, halo-C₁-C₆-alkoxy, halo-C₂-C₆-alkenyloxy, nitro, amino, N-hydroxy-imidamide, nitro-C₁-C₆-alkyl, nitro-C₂-C₆-alkenyl, nitro-C₂-C₆-alkynyl, five to six ring membered nitro-heterocyclyl, C₁-C₆-alkylamino, di-C₁-C₆-alkylamino, C₂-C₆-alkenylamine, C₂-C₆-alkynylamino, C₁-C₆-acyl, C₂-C₆-alkenoyl, C₂-C₆-alkynoyl, C₁-C₆-acylamino, di-C₁-C₆-acylamino, C₁-C₆-acyloxy, C₁-C₆-alkylsulfonyloxy, five to six ring membered heterocycloxy, five to six ring membered heterocycloamino, five to six ring membered haloheterocycloalkyl, C₁-C₆-alkylsulfinyl, C₁-C₆-alkylsulfinyl, C₁-C₆-alkylsulfonyl, C₁-C₆-alkylsulfonylamino, C₁-C₆-alkylsulfonylamino, C₁-C₆-alkylcarbonyloxy, C₁-C₆-alkylthio, C₁-C₆-acylthio, phosphorus-containing groups such as phosphono and phosphinyl, aryl having 6 to 10 carbon atoms, five to six ring membered heteroaryl, C₁-C₄-alkylaryl having 6 or 10 carbon atoms in the aryl, five to six ring membered C₁-C₆-alkylheteroaryl, cyano, cyanate, isocyanate, -C(O)NH(C₁-C₆-alkyl), or -C(O)N(C₁-C₆-alkyl)₂ or two substituents forming an -O-C₂-C₆-alkyl-O- bridge,

Y is halo or nitro;

Z is halo or nitro;

provided that at least two of B, B¹, Y, Y¹, Z and Z¹ are not absent.

According to the present disclosure, the casein kinase 1 inhibitor is added about 3 to 4 days after the mesoderm has been established. This allows the use of small molecules which specifically address the CK1 inhibition of the Wnt pathway at this later stage.

Advantageously, small molecules of formula (I) or (II) then even show an action which is not present when they are used earlier (day 0-2). The late application window also improves the results on differentiation as shown by higher percentages of cardiomyocytes obtained. A set time frame between days 3 to 8 allows a wider range of compounds to be tested using the method of the invention without changing the concentration of the small molecules added. This is a great

wherein R^1 , R^2 and R^3 independently from another represent hydrogen, optionally substituted alkyl, alkenyl, alkynyl, heterocyclyl, heteroaryl or aryl;

X represents NR^4 , O or S; and

R^4 represents hydrogen, optionally substituted alkyl, alkenyl, alkynyl, heterocyclyl, heteroaryl or aryl, together with excipients.

According to another aspect, there is provided a kit for use in stem cell differentiation to cardiomyocytes comprising a compound as defined herein or a stereoisomer, tautomer, or a salt thereof together with excipients. There is provided the use of any compound as defined herein for inducing or enhancing the differentiation of pluripotent stem cells into cardiomyocytes.

According to another aspect, there is provided compound as defined herein for use in inducing or enhancing the differentiation of pluripotent stem cells into cardiomyocytes

There is provided the use of any compound of the formula (I) or (II) as defined herein for inducing or enhancing the differentiation of pluripotent stem cells into cardiomyocytes by acting on casein kinase 1.

According to another aspect, there is provided compound of the formula (I) or (II) as defined herein for use in inducing or enhancing the differentiation of pluripotent stem cells into cardiomyocytes by acting on casein kinase 1

Definitions

The following words and terms used herein shall have the meaning indicated:

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features.

As used herein, the term "alkyl group" includes within its meaning monovalent ("alkyl") and divalent ("alkylene") straight chain or branched chain saturated aliphatic groups having from 1 to 10 carbon atoms, eg, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms. For example, the term alkyl includes, but is not limited to, methyl, ethyl, 1-propyl, isopropyl, 1-butyl, 2-butyl, isobutyl, tert-butyl, amyl, 1,2-dimethylpropyl, 1,1-dimethylpropyl, pentyl, isopentyl, hexyl, 4-methylpentyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 1,2,2-trimethylpropyl, 1,1,2-trimethylpropyl, 2-ethylpentyl, 3-ethylpentyl, heptyl, 1-methylhexyl, 2,2-dimethylpentyl, 3,3-dimethylpentyl, 4,4-dimethylpentyl, 1,2-dimethylpentyl, 1,3-dimethylpentyl, 1,4-dimethylpentyl, 1,2,3-trimethylbutyl, 1,1,2-trimethylbutyl, 1,1,3-trimethylbutyl, 5-methylheptyl, 1-methylheptyl, octyl, nonyl, decyl, and the like.

The term "alkenyl group" includes within its meaning monovalent ("alkenyl") and divalent ("alkenylene") straight or branched chain unsaturated aliphatic hydrocarbon groups having

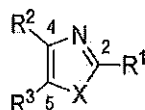
from 2 to 10 carbon atoms, e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms and having at least one double bond, of either E, Z, cis or trans stereochemistry where applicable, anywhere in the alkyl chain. Examples of alkenyl groups include but are not limited to ethenyl, vinyl, allyl, 1-methylvinyl, 1-propenyl, 2-propenyl, 2-methyl-1-propenyl, 2-methyl-1-propenyl, 1-butenyl, 2-butenyl, 3-butenyl, 1,3-butadienyl, 1-pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, 1,3-pentadienyl, 2,4-pentadienyl, 1,4-pentadienyl, 3-methyl-2-butenyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 1,3-hexadienyl, 1,4-hexadienyl, 2-methylpentenyl, 1-heptenyl, 2-heptenyl, 3-heptenyl, 1-octenyl, 1-nonenyl, 1-decenyl, and the like.

The term "alkynyl group" as used herein includes within its meaning monovalent ("alkynyl") and divalent ("alkynylene") straight or branched chain unsaturated aliphatic hydrocarbon groups having from 2 to 10 carbon atoms and having at least one triple bond anywhere in the carbon chain. Examples of alkynyl groups include but are not limited to ethynyl, 1-propynyl, 1-butyne, 2-butyne, 1-methyl-2-butyne, 3-methyl-1-butyne, 1-

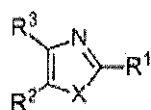
Claims

1. A method for inducing or enhancing the differentiation of pluripotent stem cells into cardiomyocyte via casein kinase 1 inhibition said method comprising culturing the stem cells in the presence of a medium comprising a casein kinase 1 inhibitor of the formula (I) or (II) or a stereoisomer, tautomer, or a salt thereof

[Formula (I)]



[Formula (II)]



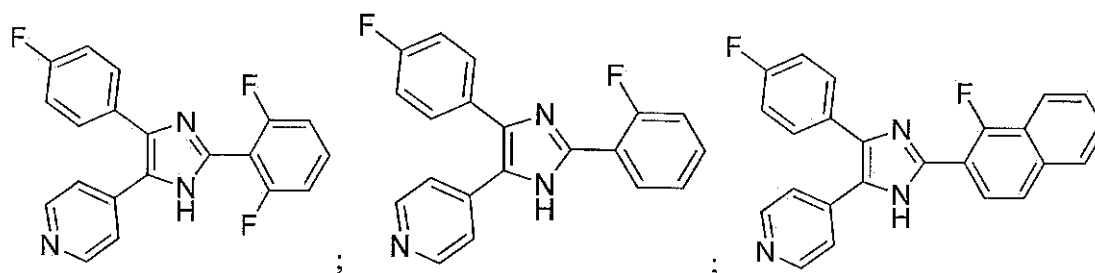
wherein R¹ represents fluorine substituted aryl having 6 to 10 carbon atoms, wherein the aryl is optionally additionally substituted by one or more substituents;

R² represents fluorine substituted phenyl, wherein the phenyl is optionally additionally substituted by one or more substituents;

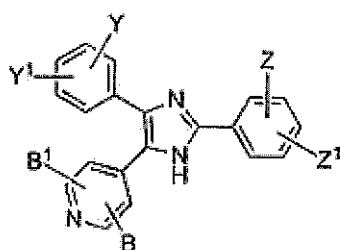
R³ represents pyridinyl or pyran-4-yl; and

X represents NH.

2. The method according to claim 1, wherein the one or more substituents in R¹ is selected from amino, -COOH, hydroxyl, C₁-C₄-alkyl, C₁-C₄-alkoxy, C₁-C₄-alkoxycarbonyl, chlorine, bromine, iodine, nitro or a -O- C₁-C₄-alkyl-O- bridge; and
the one or more substituents in R² is selected from amino, -COOH, hydroxyl, C₁-C₄-alkyl, C₁-C₄-alkoxy, C₁-C₄-alkoxycarbonyl, chlorine, bromine, iodine or nitro.
3. The method according to claim 1, wherein R³ represents pyridin-4-yl or 3,6-dihydro-2H-pyran-4-yl.
4. The method according to any of claims 1 to 3, wherein the compound of formula (I) is selected from the following compounds:



5. A method for inducing or enhancing the differentiation of pluripotent stem cells into cardiomyocyte via casein kinase 1 inhibition said method comprising culturing the stem cells in the presence of a medium comprising a casein kinase 1 inhibitor of the formula (Ia) or a stereoisomer, tautomer, or a salt thereof



(Ia)

wherein B, B¹, Y¹ and Z¹ are absent or each independently selected from C₁-C₆-alkyl, C₂-C₆-alkenyl, C₂-C₆-alkynyl, thio-C₁-C₆-alkyl, C₃-C₈-cycloalkyl, C₃-C₈-cycloalkenyl, five to six membered heterocycloalkyl, halo, -COOH, -CONH₂, C₁-C₆-carboxyl, halo-C₁-C₆-alkyl, halo-C₂-C₆-alkynyl, hydroxyl, C₁-C₆-alkoxy, thio-C₁-C₆-alkoxy, C₂-C₆-alkenyloxy, halo-C₁-C₆-alkoxy, halo-C₂-C₆-alkenyloxy, nitro, amino, N-hydroxy-imidamide, nitro-C₁-C₆-alkyl, nitro-C₂-C₆-alkenyl, nitro-C₂-C₆-alkynyl, five to six ring membered nitro-heterocyclyl, C₁-C₆-alkylamino, di-C₁-C₆-alkylamino, C₂-C₆-alkenylamine, C₂-C₆-alkynylamino, C₁-C₆-acyl, C₂-C₆-alkenoyl, C₂-C₆-alkynoyl, C₁-C₆-acylamino, di-C₁-C₆-acylamino, C₁-C₆-acyloxy, C₁-C₆-alkylsulfonyloxy, five to six ring membered heterocycloxy, five to six ring membered heterocycloamino, five to six ring membered haloheterocycloalkyl, C₁-C₆-alkylsulfonyl, C₁-C₆-alkylsulfinyl, C₁-C₆-alkylsulfonylamino, C₁-C₆-alkylsulfonylamino, C₁-C₆-alkylcarbonyloxy, C₁-C₆-alkylthio, C₁-C₆-acylthio, phosphorus-containing groups such as phosphono and phosphinyl, aryl having 6 to 10 carbon atoms, five to six ring membered heteroaryl, C₁-C₄-alkylaryl having 6 or 10 carbon atoms in the aryl, five to six ring membered C₁-C₆-alkylheteroaryl, cyano, cyanate, isocyanate, -C(O)NH(C₁-C₆-alkyl), or -C(O)N(C₁-C₆-alkyl)₂ or two substituents forming an -O-C₂-C₆-alkyl-O- bridge,

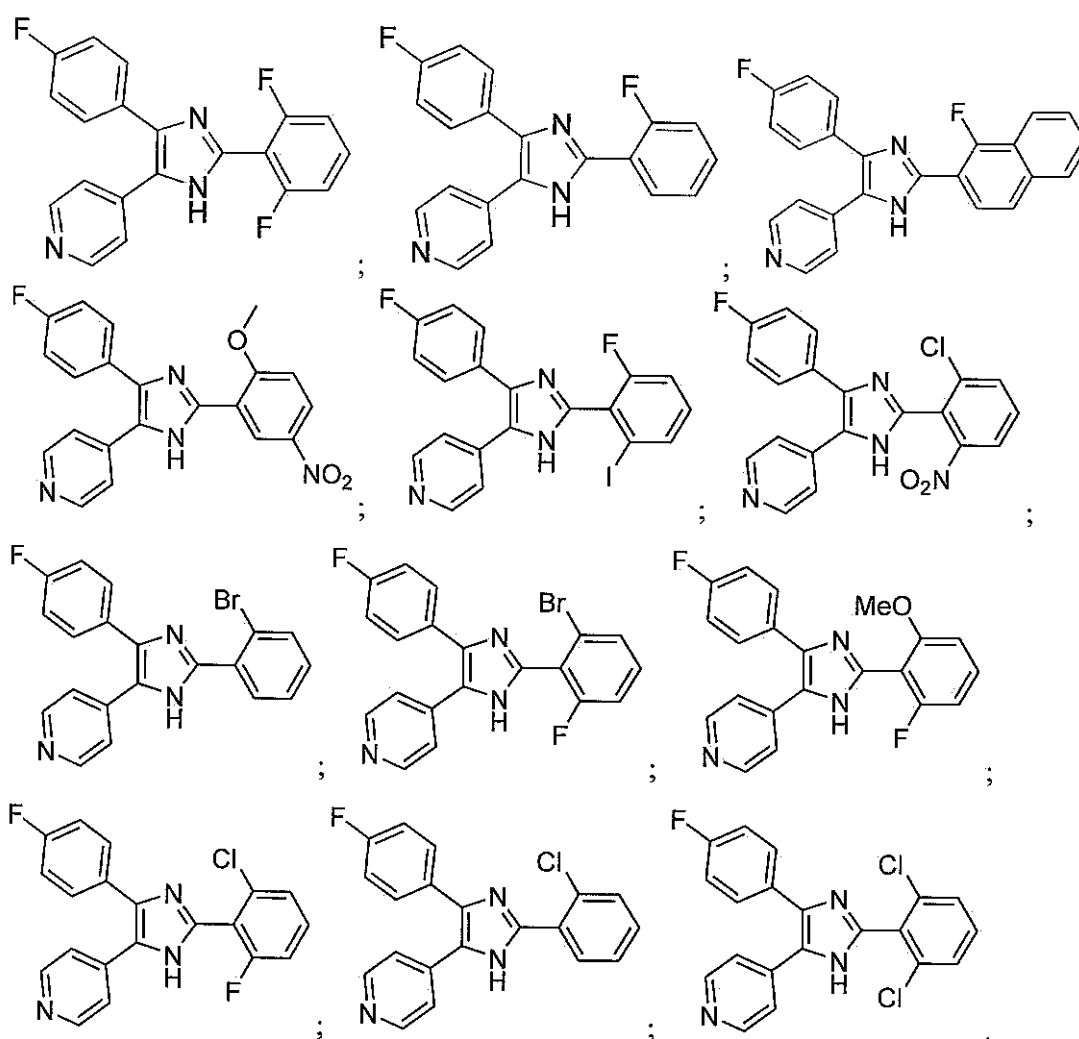
Y is halo or nitro;

Z is halo or nitro;

provided that at least two of B, B¹, Y, Y¹, Z and Z¹ are not absent.

12. A method according to claim 11, wherein the incubation with the compound of formula (IV) lasts for about 24 hours.
13. A method according to claim 11 or 12, wherein the concentration of the casein kinase inhibitor is 0.5 to 20 μM and the concentration of the compound of formula (IV) is 2 to 20 μM .
14. The method according to claim 13, wherein the concentration of the casein kinase inhibitor is 0.5 to 5 μM .

15. A compound selected from the following compounds, or a stereoisomer, tautomer, or a salt thereof:



16. Compound as defined in claim 15 for use in inducing or enhancing the differentiation of pluripotent stem cells into cardiomyocytes.

17. Compound of the formula (I) or (II) as defined in any one of claims 1 to 4 or 6 for use in inducing or enhancing the differentiation of pluripotent stem cells into cardiomyocytes by acting on casein kinase 1.
18. A kit for use in stem cell differentiation to cardiomyocytes via inhibition of casein kinase 1 comprising a compound of the formula (I) or (II) as defined in any one of claims 1 to 4 or 6, or a stereoisomer, tautomer, or a salt thereof, together with excipients.
19. A kit for use in stem cell differentiation to cardiomyocytes comprising a compound as defined in claim 15 or a stereoisomer, tautomer, or a salt thereof together with excipients.