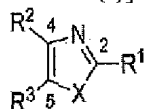


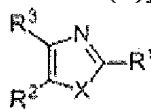
Summary of Invention

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)]



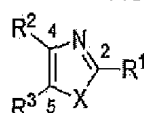
wherein R^1 , R^2 and R^3 independently from another represent hydrogen, optionally substituted alkyl, alkenyl, alkynyl, heterocyclyl, heteroaryl or aryl;

X represents NR^4 , O or S; and

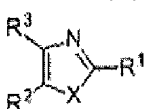
R^4 represents hydrogen, optionally substituted alkyl, alkenyl, alkynyl, heterocyclyl, heteroaryl or aryl.

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;

R^2 represents fluorine substituted phenyl;

R^3 represents pyridinyl or pyranyl; and

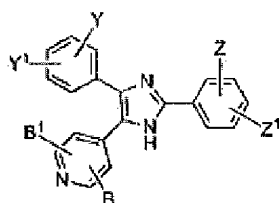
X represents NH.

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-alkylsulfenyl, C₁-C₆-alkylsulfinyl, C₁-C₆-alkylsulfonyl, C₁-C₆-alkylsulfinylamino, 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

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.

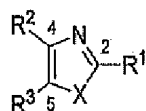
Advantageously, small molecules of formula (I) or (II) then even show an action which is not present when they are used earlier (day 0-2). The late application window also improves the results on differentiation as shown by higher percentages of cardiomyocytes obtained. A set time frame between days 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

advantage, which can reduce the number of experiments to one concentration and one time course in order to investigate the correlations between a compound's *in vitro* effect on target protein(s) and its cardiomyogenic activity. Moreover the effect of the compounds of formula (I) or (II) on cardiomyogenesis in the days 4 to 8 time course indicates a preferred new late stage application time slot.

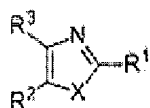
According to the present disclosure, there is provided a method wherein the compound CHIR99021 is added to the embryoid bodies after the mesoderm has been established. Advantageously this results in even higher yields of cardiomyocytes of over 50 %.

According to the present disclosure, there is provided novel compounds. Such compounds include compounds of the formula (I) or (II) or a stereoisomer, tautomer, or salt thereof:

[Formula (I)]



[Formula (II)]



wherein R¹ represents fluorine substituted monovalent and divalent, single, polynuclear, conjugated and fused residues of aromatic hydrocarbons having from 6 to 10 carbon atoms;

R² represents fluorine substituted aromatic hydrocarbons having 6 carbon atoms;

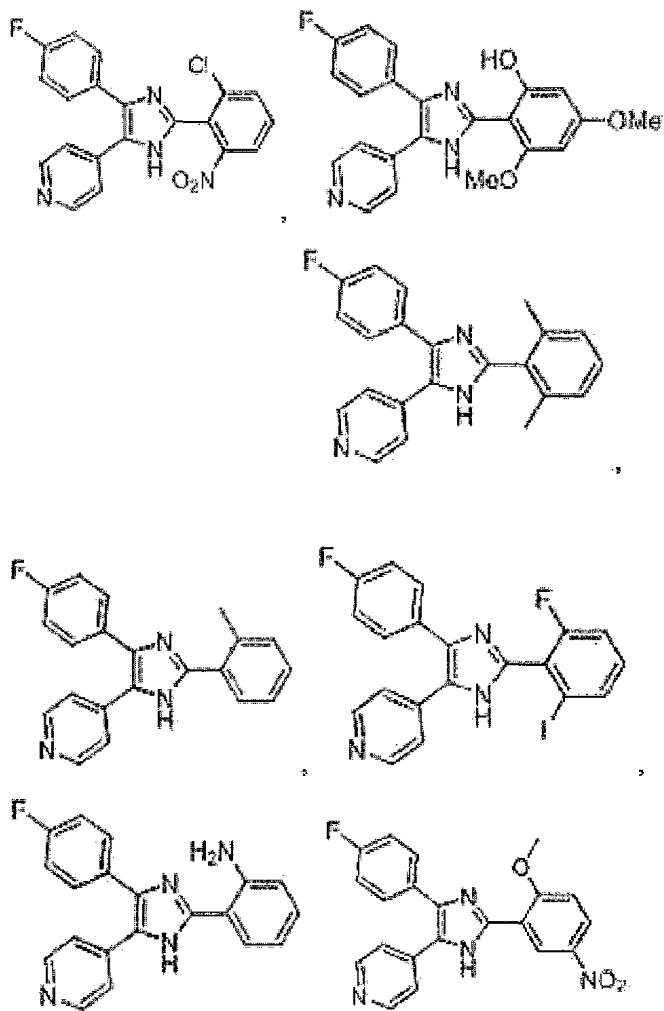
R³ represents saturated 5 to 6 ring membered heterocycloalkyl having 1 to 3 hetero atoms selected from N or O, or an aromatic radical having 5 to 6 ring atoms wherein 1 to 3 atoms are heteroatoms selected from O and N; and

X represents NH or O.

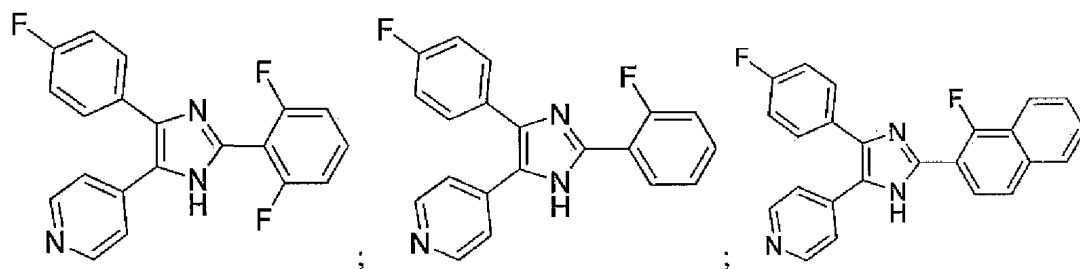
Advantageously these novel group of compounds and the other compounds described in the specification have been proven to be especially well suited to use in the method of the invention and to achieve the abovementioned benefits of improved differentiation.

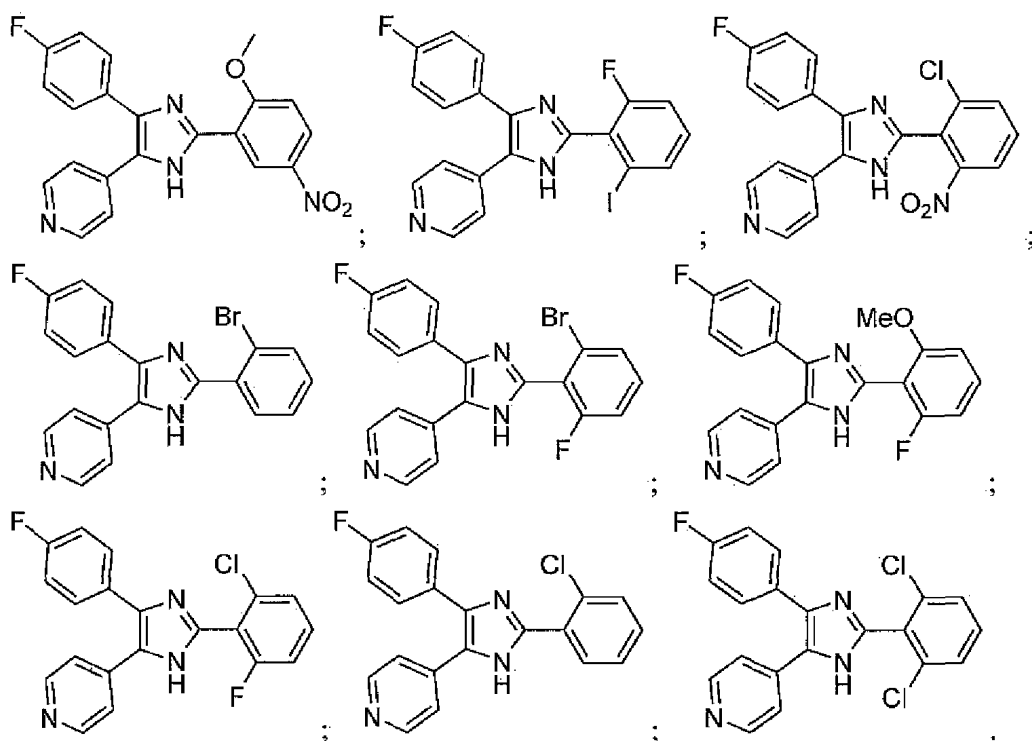
According to the disclosure, there is provided a compound selected from the following compounds, or a stereoisomer, tautomer, or a salt thereof:

3e



According to another aspect, there is provided a compound selected from the following compounds, or a stereoisomer, tautomer, or a salt thereof:

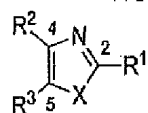




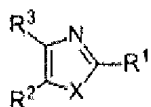
According to another aspect, there is provided a kit for use in stem cell differentiation to cardiomyocytes via inhibition of casein kinase 1 comprising a compound of the formula (I) or (II) as defined herein, or a stereoisomer, tautomer, or a salt thereof, together with excipients.

According to the disclosure, there is provided 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), or a stereoisomer, tautomer, or a salt thereof:

[Formula (I)]



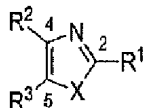
[Formula (II)]



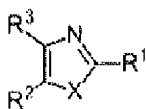
Claims

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)]



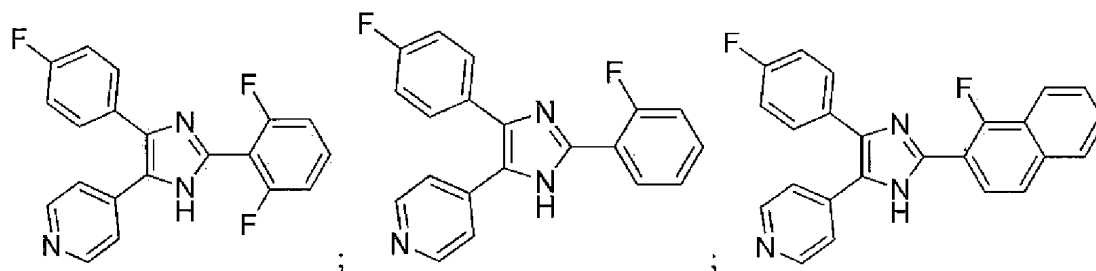
wherein R¹ represents fluorine substituted aryl having 6 to 10 carbon atoms;

R² represents fluorine substituted phenyl;

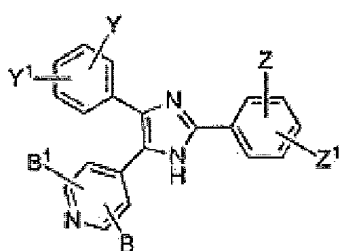
R³ represents pyridinyl or pyranyl; and

X represents NH.

2. The method according to claim 1, wherein
R¹ represents fluorine substituted C₆ to C₁₀ aryl which is optionally additionally substituted by one or more substituents 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
R² represents fluorine substituted phenyl which is optionally additionally substituted by one or more substituents 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)

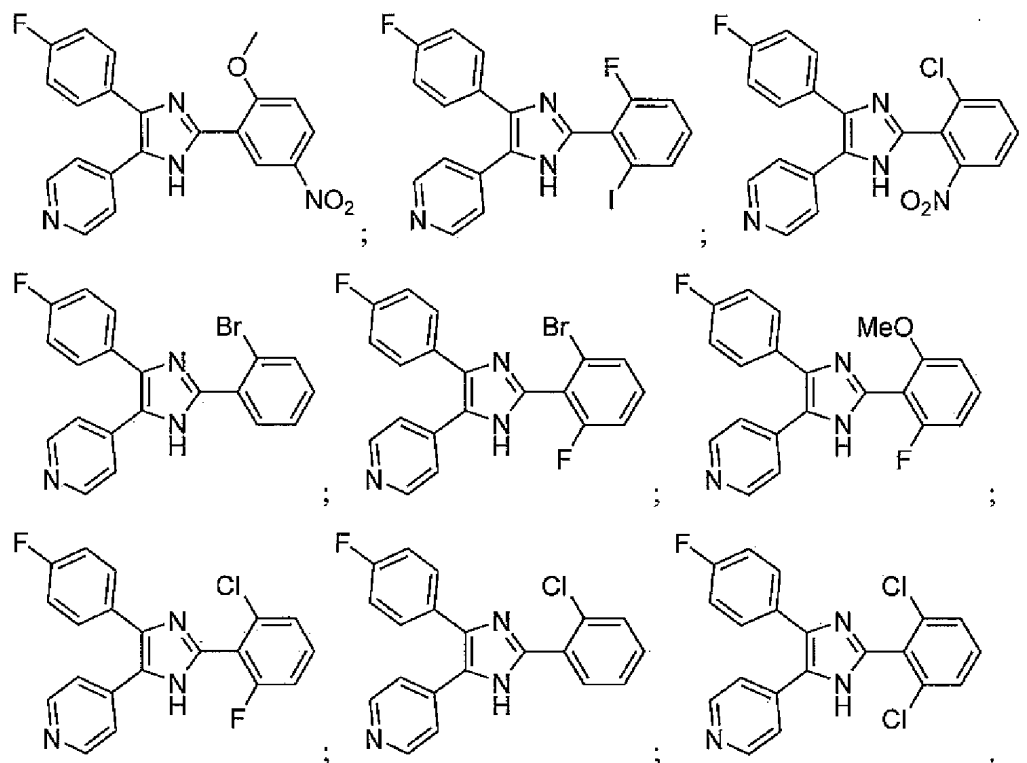
wherein B, B¹, Y¹ and Z¹ are absent or each independently selected from C₁-C₆-alkyl, C₂-C₆-alkenyl, C₂-C₆-alkynyl, thio-C₁-C₆-alkyl, C₃-C₈-cycloalkyl, C₃-C₈-cycloalkenyl, five to six membered heterocycloalkyl, halo, -COOH, -CONH₂, C₁-C₆-carboxyl, halo-C₁-C₆-alkyl, halo-C₂-C₆-alkynyl, hydroxyl, C₁-C₆-alkoxy, 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-alkylsulfenyl, C₁-C₆-alkylsulfinyl, C₁-C₆-alkylsulfonyl, C₁-C₆-alkylsulfinylamino, 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

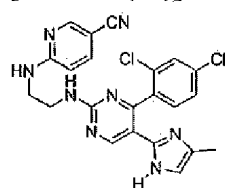
provided that at least two of B, B¹, Y, Y¹, Z and Z¹ are not absent.

6. The method according to claim 5, wherein the compound of formula (I) is selected from the following compounds:



7. The method according to claim 5, wherein at least three of B, B¹, Y, Y¹, Z and Z¹ are not absent.
8. A method according to any of claims 1 to 7, wherein the casein kinase 1 inhibitor is added about 3 to 4 days after the mesoderm has been established.
9. A method according to any of claims 1 to 7, wherein the casein kinase inhibitor is added from about day 3 to 8 after the mesoderm has been established.
10. A method according to any of claims 1 to 7, wherein the casein kinase 1 inhibitor is present from day 3 to 8 of the differentiation.
11. A method according to any of claims 1 to 7, wherein the compound of formula (IV)

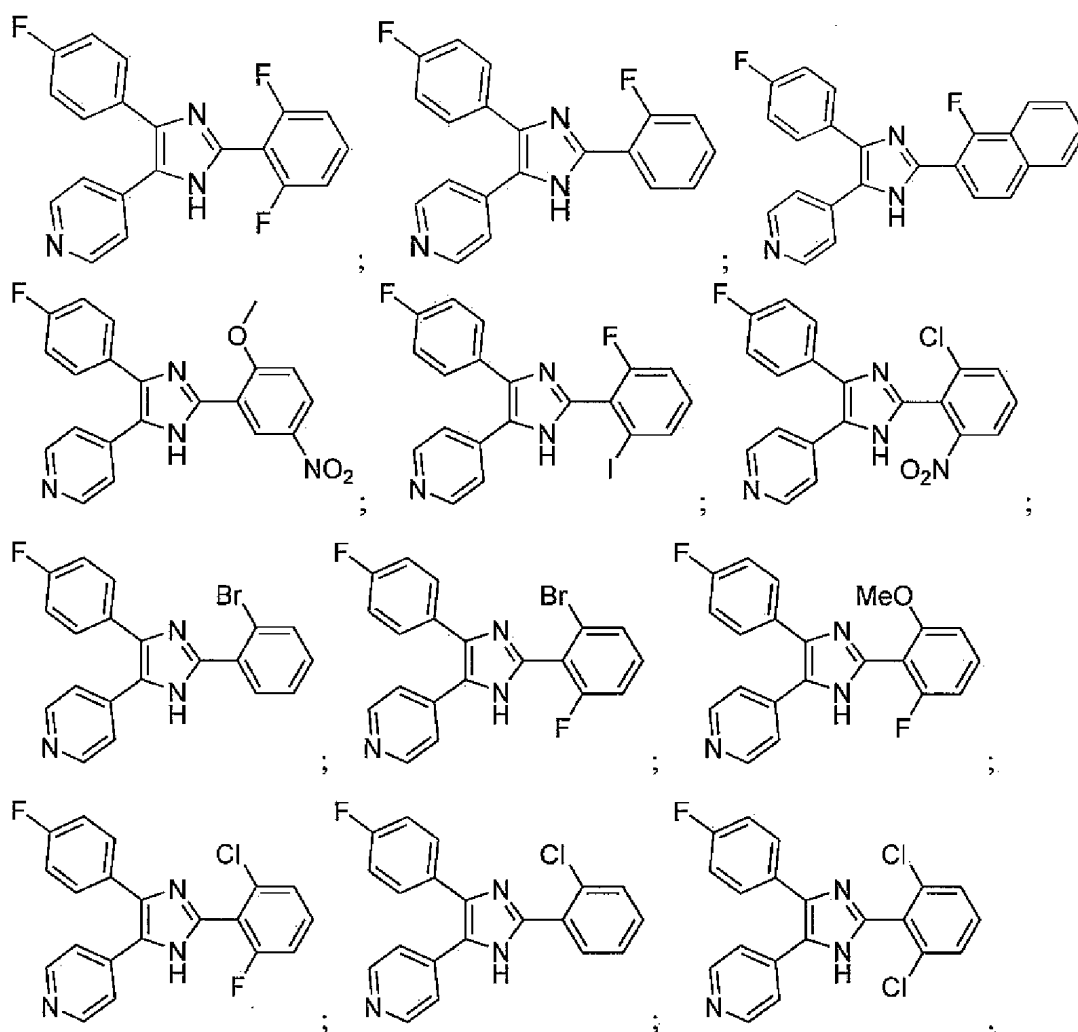
[Formula (IV)]



is added to the embryoid bodies before the mesoderm has been established.

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. The use of any compound as defined in claim 15 for inducing or enhancing the differentiation of pluripotent stem cells into cardiomyocytes.

17. The use of any compound of the formula (I) or (II) as defined in any one of claims 1 to 4 or 6 for 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.