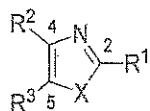


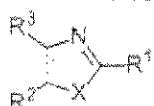
Summary of Invention

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 , 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.

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 ~~invention~~ 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.

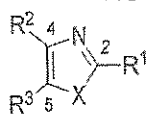
According to a ~~second aspect of the invention~~ present disclosure, the casein kinase 1 inhibitor is added about 3 to 4 days after the mesoderm has been established. This allows the use of small molecules which specifically address the CK1 inhibition of the Wnt pathway at this later stage. Advantageously,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

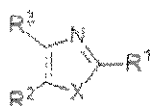
According to ~~a third aspect of the invention the present disclosure~~, there is provided a method wherein the compound CHIR99021 is added to the embryoid bodies after the mesoderm has been established. Advantageously this results in even higher yields of cardiomyocytes of over 50 %.

According to ~~a fourth another aspect of the invention~~ 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;

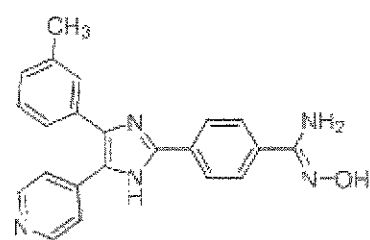
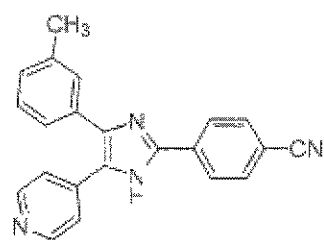
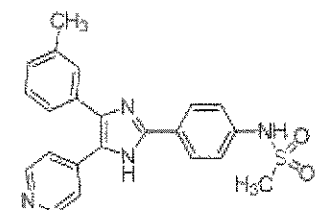
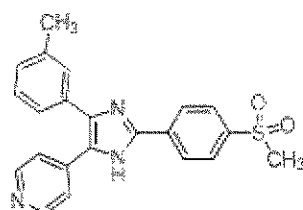
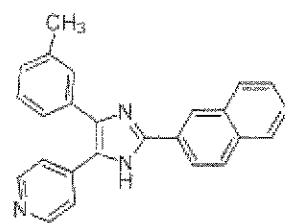
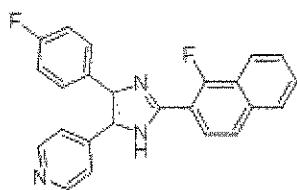
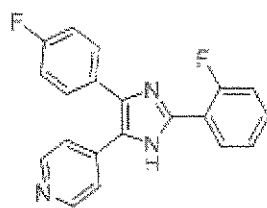
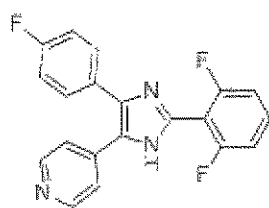
R³ represents saturated 5 to 6 ring membered heterocycloalkyl having 1 to 3 hetero atoms selected from N or O, or an aromatic radical having 5 to 6 ring atoms wherein 1 to 3 atoms are heteroatoms selected from O and N; and

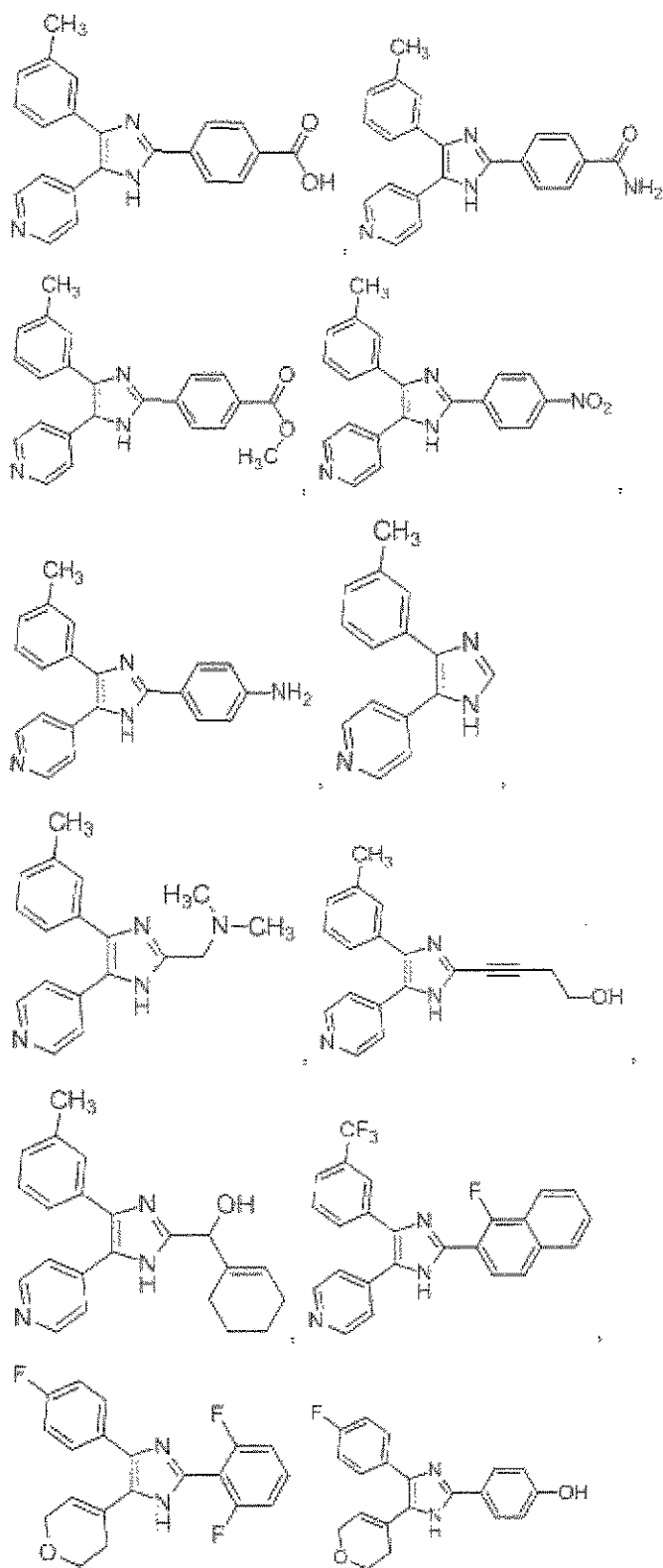
X represents NH or O.

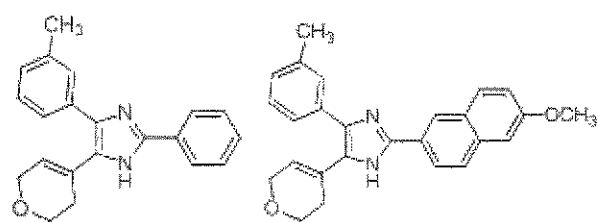
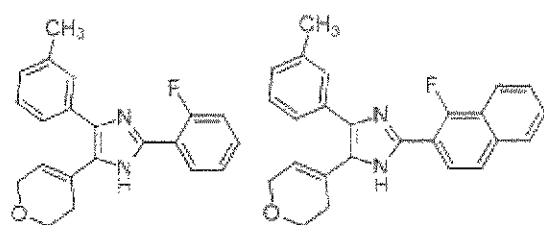
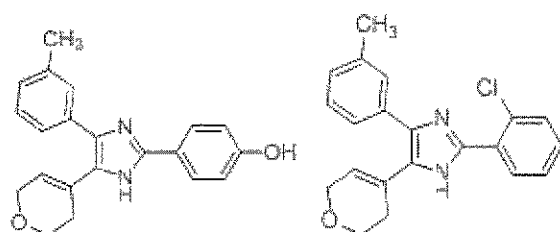
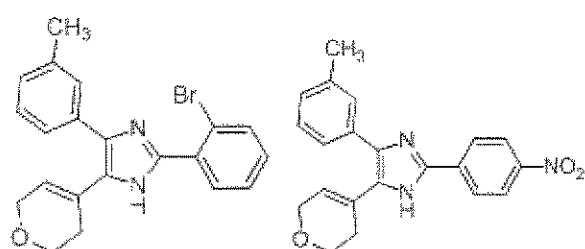
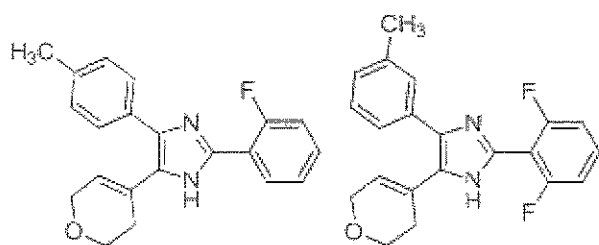
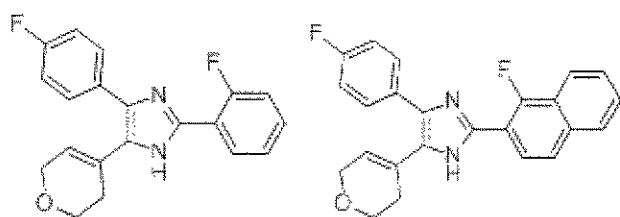
Advantageously these novel group of compounds and the other compounds described in the specification have been proven to be especially well suited to use in the method of the invention and to achieve the abovementioned benefits of improved differentiation.

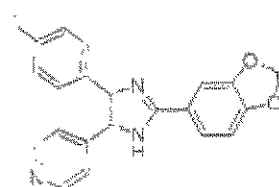
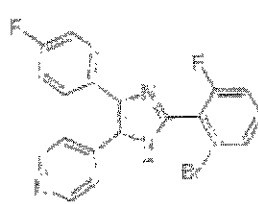
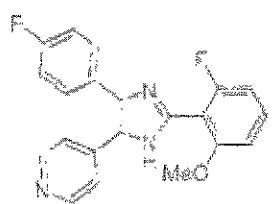
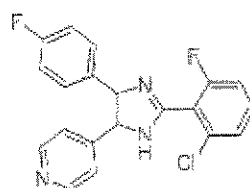
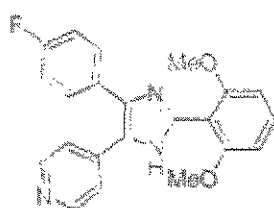
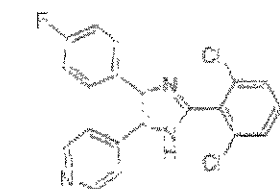
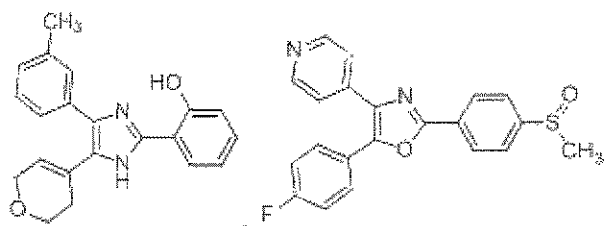
According to another aspect, there is provided a compound selected from the following compounds, or a stereoisomer, tautomer, or a salt thereof:

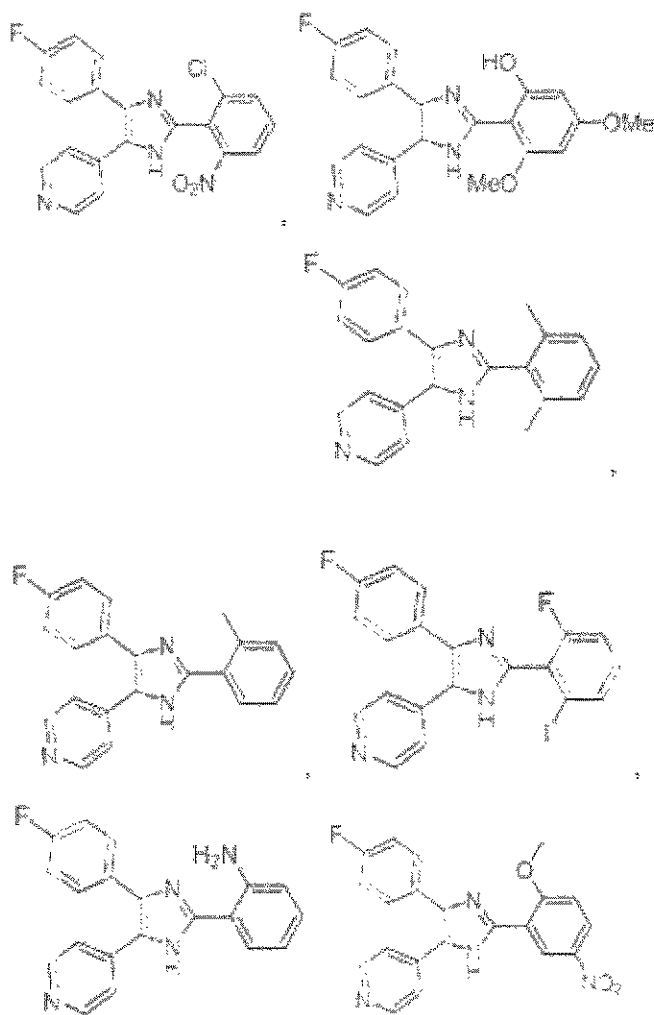
3a





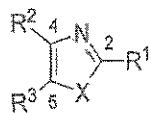




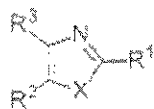


According to a ~~fifth~~^{another} aspect, of the invention there is provided a kit for use in stem cell differentiation to cardiomyocytes via inhibition of casein kinase 1 comprising a compound of the formula (I) or (II), or a stereoisomer, tautomer, or a salt thereof:

[Formula (I)]



[Formula (II)]



wherein R¹, R² and R³ independently from another represent hydrogen, optionally substituted alkyl, alkenyl, alkynyl, heterocyclyl, heteroaryl or aryl;

X represents NR⁴, O or S; and

R⁴ 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.

According to another aspect, 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 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.

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.

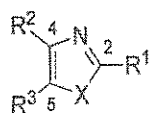
As used herein, the term "alkyl group" includes within its meaning monovalent ("alkyl") and divalent ("alkylene") straight chain or branched chain saturated aliphatic groups having from 1 to 10 carbon atoms, eg, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms. For example, the term alkyl includes, but 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,

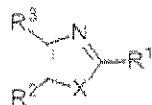
2-hexenyl, 3-hexenyl, 1,3-hexadienyl, 1,4-hexadienyl, 2-methylpentenyl, 1-heptenyl, 2-heptenyl, 3-heptenyl, 1-octenyl, 1-nonenyl, 1-decenyl, and the like.

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-butynyl, 2-butynyl, 1-methyl-2-butynyl, 3-methyl-1-butynyl, 1-

12. A method according to any of claims 1 to 9, wherein the casein kinase 1 inhibitor is present from day 3 to 8 of the differentiation.
13. A method according to any of claims 1 to 9, wherein the compound CHIR99021 is added to the embryoid bodies before the mesoderm has been established.
14. A method according to claim 13, wherein the incubation with the compound CHIR99021 lasts for about 24 hours.
15. A method according to claim 13 or 14, wherein the concentration of the casein kinase inhibitor is 0.5 to 20 μM and the concentration of CHIR99021 is 2 to 20 μM .
16. The method according to claim 15, wherein the concentration of the casein kinase inhibitor is 0.5 to 5 μM .
17. A compound of the formula (I) or (II) or a stereoisomer, tautomer, or a salt thereof:
[Formula (I)]



[Formula (II)]



wherein R^1 represents fluorine substituted monovalent and divalent, single, polynuclear, conjugated and fused residues of aromatic hydrocarbons having from 6 to 10 carbon atoms;

R^2 represents fluorine substituted aromatic hydrocarbons having 6 carbon atoms;

R^3 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.

18. A compound according to ~~statement~~ claim 17 wherein
 R^1 represents fluorine substituted C_6 to C_{10} aryl;
 R^2 represents fluorine substituted phenyl;
 R^3 represents pyridinyl or pyranyl; and
X represents NH.
19. A compound according to claim 18 wherein
 R^1 represents fluorine substituted C_6 to C_{10} aryl which is optionally additionally substituted by one or more substituents selected from amino, -COOH, hydroxyl, C_1 - C_4 -alkyl, C_1 - C_4 -alkoxy, C_1 - C_4 -alkoxycarbonyl, chlorine, bromine, iodine, nitro