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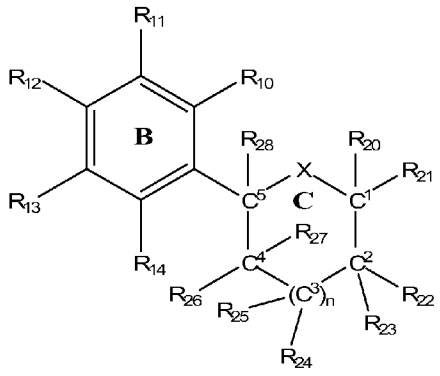
(54) Title: IN VITRO PRESERVATION OF LIVING ANIMAL CELLS AND COMPOUNDS SUITABLE FOR USE IN THE PRESERVATION OF LIVING ANIMAL CELLS



(I)

(57) Abstract: Certain compounds of Formula (I) or salts thereof are suitable for use in the in vitro preservation of living animal cells. The living animal cells may be isolated cells, such as stem cells, or groups of cells such as tissue or an organ.

WO 2009/047568 A2

In vitro preservation of living animal cells and compounds suitable for use in the preservation of living animal cells

This invention is concerned with *in vitro* preservation of living animal cells and compounds suitable for use in the preservation of living animal cells. In particular, though not exclusively, the present invention concerns *in vitro* preservation of stem cells and compounds suitable for use in preserving stem cells.

Background

Once harvested, in order to remain viable and retain their undifferentiated state, stem cells must be preserved prior to use for medical or research purposes.

It is well known that stem cells can be stored indefinitely if stored at the temperature of liquid nitrogen (-196 °C). However, it is well documented that the process of freezing may cause irreparable damage to the cells and as such, various cryopreservative agents can be added to the cell suspension prior to the freezing process. US2005/0106554 discloses methods and compositions for the cryopreservation of pluripotent cells, in particular human embryonic stem cells. The methods disclosed in US2005/0106554 are shown to exhibit an increase in cell viability and a decrease in cell differentiation when compared with conventional methods. The cryopreservation method disclosed in US2005/0106554 comprises encapsulation of the cells between two layers of a solid support matrix, adding a cryopreservative to the matrix-cell-matrix composition, and cooling said composition to a temperature sufficient to cryopreserve the cells. US2005/0106554 discloses a carbohydrate-based medium, preferably trehalose, as a suitable cryopreservative. WO2005/118785 discloses methods for the cryopreservation of stem cells, wherein said method includes performing ice nucleation on a cell suspension prior to the reduction of temperature sufficient to allow long term storage of the stem cells. WO2005/118785 further discloses that the cell suspension may or may not contain any exogenous biological cryoprotectant, such as serum.

A number of non-cryogenic methods of stem cell preservation are also known. In such methods, stem cells are generally stored as a cell dispersion in an aqueous solution containing tissue cell culture growth media. Often a preservative compound is included in such aqueous solutions so as to reduce the rate at which cell viability decreases. In a typical example, US 5,912,174 discloses a method of storing a population of mammalian cells capable of duplication and differentiation by suspending said population of cells in an aqueous mixture containing gelatin. Prefered mixtures contain standard tissue cell culture growth media such as RMPI or Eagle's media. Optionally, cell-specific growth factor may also be added to preserve cell viablility and, for optimum storage life, the storage temperature should be maintained between 0 and 4 °C. Further, EP-A-1057405 discloses the use of an aqueous storage liquid comprising polyphenol and a storage liquid selected from Euro-Collin's solution, UW solution, serum and antibiotic solution for the *in vitro* freeze-free preservation of, inter alia, a stem cell, tissue or organ for transplantation. The polyphenols disclosed in EP-A-1057405 include catechins such as epigallocatechin, tannic acid, proanto-dianisidine, resorcinol, hydroquinone, pyrogallol, phloroglucinol, eugenol and quercetin. There is no suggestion in EP-A-1057405 to the effect that any of the disclosed compounds may preserve the viability of undifferentiated cells.

The potential therapeutic use of certain flavonoid compounds as antioxidants for the treatment of patients having a disease or disorder involving oxidative damage, such as cancer, heart disease, neurological

disorders, auto-immune disorders, ischaemia-reperfusion injury, diabetic complications, septic shock, hepatitis, atherosclorosis and complications arising from HIV or Hepatitis B is known from WO 2004/007475. WO 2004/007475 also discloses the potential application of the flavonoid compounds in sunscreen compositions and skincare compositions. In addition, WO 2004/007475 discloses the potential use of the flavonoid compounds as foodstuff stabilizers, where the ability of the compounds to combat free radicals is considered to be of utility in preventing or delaying the deterioration in food quality during storage. However, whilst WO 2004/007475 discloses the use of the flavonoid compounds for the *in vivo* therapeutic treatment of living matter and for the *in vitro* stabilizing treatment of dead matter, there is no disclosure or suggestion that the flavonoid compounds would be useful for *in vitro* preservation of living matter.

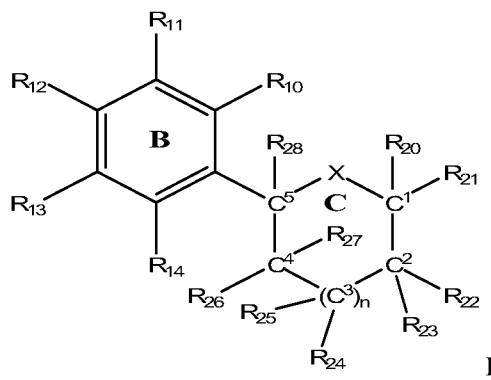
J. Agric. Food Chem., Vol. 48, pages 3876-3884 (Miranda et al.), disclosed test of certain flavanoid compounds to inhibit in vitro oxidation of human low density lipoprotein. British Journal of Cancer, 2003, Vol. 89(2), pages 357-362, and Vol. 89(11), pages 2140-2146, disclose the use of monoHER as providing protection against doxorubicin-induced inflammatory effects in vitro and against doxorubicin-induced cardiotoxicity in vitro, based on protection of human umbilical cord vascular endotheial cells and neonatal rat cardiac mycocytes, respectively. Both cell types are fully differentiated.Free Radical Biology & Medicine, 2002, Vol. 32(7), pages 596-604, disclose the testing of cetain flavenoids for protecting primary cultures of rat cortical cells against oxidative stress. The cell types are fully differentiated. Bio. & Pharm. Bull., 2001, Vol. 24(12) pages 1373-1379, disclose in vitro protective effect of mixtures of extracts from certain plants, including some flavenoids, against oxidative stress on human skin cells. The cell types are fully differentiated.

The object of the present invention is to provide an improved method for the *in vitro* preservation of living animal cells. In particular, it is an object of the present invention to provide an improved method for the *in vitro* preservation of a mammalian cell, tissue or organ for research or medical purposes e.g. transplantation. More preferred, it is an object of the present invention to provide an improved method for the *in vitro* preservation of stem cells.

The Invention

The invention in its various aspects is as set out in the accompanying claims.

In a first aspect, the present invention provides a method for *in vitro* preservation of living animal cells, said method comprising contacting said living animal cells with a compound of Formula I or a salt thereof:



Formula I

wherein:

A) R_{12} and R_{26} each independently represent –OH or a glycosidic functional group; R_{10}, R_{11}, R_{13}, and R_{14} each independently represent H, -OH, nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, a glycosidic functional group, C_{1-6} alkoxy-, hydroxy-C_{1-6} alkyl-, C_{1-6} alkoxy-C_{1-6} alkyl-, or a saturated or unsaturated C_{1-6} hydrocarbon chain which may be substituted with one or more of nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone or aldehyde groups; and wherein ring B comprises no more than one glycosidic functional group;

B) either a):

R_{20} represents H or a C_{2-30} saturated or unsaturated hydrocarbon chain;

R_{21}:

i) represents H;

ii) together with R_{22} provides a second bond between C^1 and C^2; or

iii) when X is $–NR_1$- and R_1 is not H or C_{1-6}alkyl, together with R_1 provides a second bond between C^1 and N;

R_{22}:

i) represents H;

ii) together with R_{23} forms =O; or

iii) together with R_{21} provides a second bond between C^1 and C^2;

R_{23}:

i) represents H or a C_{2-30} saturated or unsaturated hydrocarbon chain; or

ii) together with R_{22} forms =O;

wherein at least one of R_{20} and R_{23} is a C_{2-30} saturated or unsaturated hydrocarbon chain;

or b):

R_{20}, R_{21}, R_{22}, and R_{23} form part of a 5, 6 or 7 membered unsaturated-ring including C^1 and C^2, which ring is substituted with a group which is a C_{2-30} saturated or unsaturated hydrocarbon chain, which ring is optionally and independently further substituted with one or more groups selected from nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde and saturated or unsaturated C_{2-15} hydrocarbon chain, which C_{2-15} hydrocarbon chain may be substituted with one or more of nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde or nitrone groups;

C) n is 0 or 1, wherein when n is 0, either i) R_{27} and R_{28} represent H or ii) R_{27} together with R_{28} provide a second bond between C^4 and C^5; or when n is 1, either i) R_{24} and R_{25} together form =O and R_{27} and R_{28} represent H or R_{27} together with R_{28} provide a second bond between C^4 and C^5, or ii) R_{24} and R_{25} represent H and R_{27} and R_{28} represent H or R_{27} together with R_{28} provide a second bond between C^4 and C^5or iii) R_{24} represents H, R_{25} together with R_{27} provide a second bond between C^3 and C^4, R_{28} represents –OH and X is –O-;

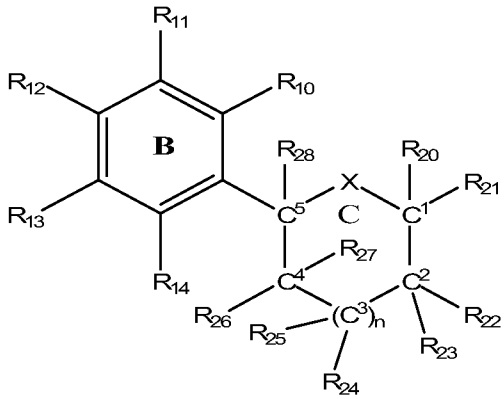
D) X is –O-, -S- or $–NR_1$-, wherein R_1 represents i) H or C_{1-6}alkyl, or ii) together with R_{21} provides a second bond between C^1 and N;

wherein said C_{2-30} saturated or unsaturated hydrocarbon chain of R_{20}, R_{23} and the 5, 6 or 7 membered unsaturated ring is optionally and independently substituted with one or more groups selected from C_{1-6} alkyl, C_{1-6}alkoxy, hydroxy-C_{1-6} alkyl, Cl, F, Br, I, -CN, $-CO_2H$, $-CO_2C_{1-6}$alkyl, $-S(O)_2C_{1-6}$alkyl, $-S(O)_2$phenyl, -

SC_{1-6}alkyl, $-NO_2$, -OH, -CF3, $-N(R_2)(R_3)$, $-NHC(O)NHC_{1-6}$alkyl, $-C(O)N(R_2)(R_3)$, imine and substituted or unsubstituted triphenylphosphonium; and wherein one or more available $-CH_2-$ groups present in the C_{2-30} hydrocarbon chain of R_{20}, R_{23} or the 5, 6 or 7 membered unsaturated ring is optionally and independently substituted by -O-, -C(O)-, $-S(O)_p-$, or $-N(R_2)-$ provided always that no two such substitutions in the resulting chain are consecutive; wherein R_2 and R_3 each independently represent H or C_{1-6}alkyl, and wherein p is 0 to 2; and

wherein the total number of =O on ring C is no greater than 1.

In one embodiment, the present invention provides a method of *in vitro* preservation of living animal cells in a viable non-terminally differentiated state, said method comprising contacting living non-terminally differentiated animal cells with a compound of Formula I or a salt thereof:



Formula I

wherein:

A) R_{12} and R_{26} each independently represent –OH or a glycosidic functional group; R_{10}, R_{11}, R_{13}, and R_{14} each independently represent H, -OH, nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, a glycosidic functional group, C_{1-6} alkoxy-, hydroxy-C_{1-6} alkyl-, C_{1-6} alkoxy-C_{1-6} alkyl-, or a saturated or unsaturated C_{1-6} hydrocarbon chain which may be substituted with one or more of nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone or aldehyde groups; and wherein ring B comprises no more than one glycosidic functional group;

B) either a):

R_{20} represents H or a C_{2-30} saturated or unsaturated hydrocarbon chain;

R_{21}:

i) represents H;

ii) together with R_{22} provides a second bond between C^1 and C^2; or

iii) when X is $-NR_1-$ and R_1 is not H or C_{1-6}alkyl, together with R_1 provides a second bond between C^1 and N;

R_{22}:

i) represents H;

ii) together with R_{23} forms =O; or

iii) together with R_{21} provides a second bond between C^1 and C^2;

R_{23}:

i) represents H or a C_{2-30} saturated or unsaturated hydrocarbon chain;

ii) together with R_{22} forms =O;

wherein at least one of R_{20} and R_{23} is a C_{2-30} saturated or unsaturated hydrocarbon chain;

or b):

R_{20}, R_{21}, R_{22}, and R_{23} form part of a 5, 6 or 7 membered unsaturated-ring including C^1 and C^2, which ring is substituted with a group which is a C_{2-30} saturated or unsaturated hydrocarbon chain, which ring is optionally and independently further substituted with one or more groups selected from nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde and saturated or unsaturated C_{2-15} hydrocarbon chain, which C_{2-15} hydrocarbon chain may be substituted with one or more of nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde or nitrone groups;

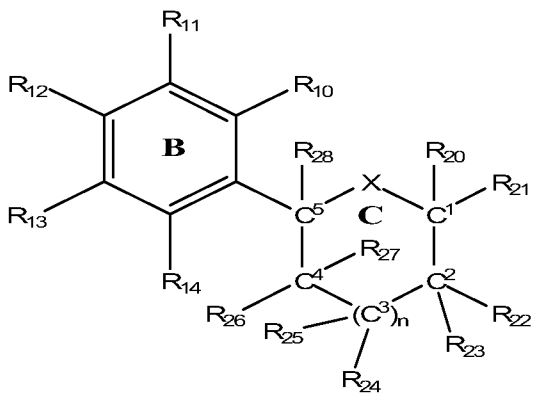
C) n is 0 or 1, wherein when n is 0, either i) R_{27} and R_{28} represent H or ii) R_{27} together with R_{28} provide a second bond between C^4 and C^5; or when n is1, either i) R_{24} and R_{25} together form =O and R_{27} and R_{28} represent H or R_{27} together with R_{28} provide a second bond between C^4 and C^5, or ii) R_{24} and R_{25} represent H and R_{27} and R_{28} represent H or R_{27} together with R_{28} provide a second bond between C^4 and C^5or iii) R_{24} represents H, R_{25} together with R_{27} provide a second bond between C^3 and C^4, R_{28} represents –OH and X is –O-;

D) X is –O-, -S- or $–NR_1$-, wherein R_1 i) represents H or C_{1-6}alkyl, or ii) together with R_{21} provides a second bond between C^1 and N;

wherein said C_{2-30} saturated or unsaturated hydrocarbon chain of R_{20}, R_{23} and the 5, 6 or 7 membered unsaturated ring is optionally and independently substituted with one or more groups selected from C_{1-6} alkyl, C_{1-6}alkoxy, hydroxy-C_{1-6} alkyl, Cl, F, Br, I, -CN, $-CO_2H$, $-CO_2C_{1-6}$alkyl, $-S(O)_2C_{1-6}$alkyl, $-S(O)_2$phenyl, $-SC_{1-6}$alkyl, $-NO_2$, -OH, -CF3, $-N(R_2)(R_3)$, $-NHC(O)NHC_{1-6}$alkyl, $–C(O)N(R_2)(R_3)$, imine and substituted or unsubstituted triphenylphosphonium; and wherein one or more available $-CH_2$- groups present in the C_{2-30} hydrocarbon chain of R_{20}, R_{23} and the 5, 6 or 7 membered unsaturated ring is optionally and independently replaced by -O-, -C(O)-, $-S(O)_p$-, or $-N(R_2)$- provided always that no two such replacements in the resulting chain are consecutive; wherein R_2 and R_3 each independently represent H or C_{1-6}alkyl, and wherein p is 0 to 2; and

wherein the total number of =O on ring C is no greater than 1.

In another embodiment, the present invention provides a method for *in vitro* preservation of living animal cells wherein said living animal cells are in the form of a tissue or an organ, said method comprising contacting said living animal cells with a compound of Formula I or a salt thereof:



Formula I

wherein:

A) R_{12} and R_{26} each independently represent –OH or a glycosidic functional group; R_{10}, R_{11}, R_{13}, and R_{14} each independently represent H, -OH, nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, a glycosidic functional group, C_{1-6} alkoxy-, hydroxy-C_{1-6} alkyl-, C_{1-6} alkoxy-C_{1-6} alkyl-, or a saturated or unsaturated C_{1-6} hydrocarbon chain which may be substituted with one or more of nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone or aldehyde groups; and wherein ring B comprises no more than one glycosidic functional group;

B) either a):

R_{20} represents H or a C_{2-30} saturated or unsaturated hydrocarbon chain;

R_{21}:

i) represents H;

ii) together with R_{22} provides a second bond between C^1 and C^2; or

iii) when X is $–NR_1$- and R_1 is not H or C_{1-6}alkyl, together with R_1 provides a second bond between C^1 and N;

R_{22}:

i) represents H;

ii) together with R_{23} forms =O; or

iii) together with R_{21} provides a second bond between C^1 and C^2;

R_{23}:

i) represents H or a C_{2-30} saturated or unsaturated hydrocarbon chain; or

ii) together with R_{22} forms =O;

wherein at least one of R_{20} and R_{23} is a C_{2-30} saturated or unsaturated hydrocarbon chain;

or b):

R_{20}, R_{21}, R_{22}, and R_{23} form part of a 5, 6 or 7 membered unsaturated-ring including C^1 and C^2, which ring is substituted with a group which is a C_{2-30} saturated or unsaturated hydrocarbon chain, which ring is optionally and independently further substituted with one or more groups selected from nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde and saturated or unsaturated C_{2-15} hydrocarbon chain, which C_{2-15} hydrocarbon chain may be substituted with one or more of nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde or nitrone groups;

C) n is 0 or 1, wherein when n is 0, either i) R_{27} and R_{28} represent H or ii) R_{27} together with R_{28} provide a second bond between C^4 and C^5; or when n is 1, either i) R_{24} and R_{25} together form =O and R_{27} and R_{28} represent H or R_{27} together with R_{28} provide a second bond between C^4 and C^5, or ii) R_{24} and R_{25} represent H and R_{27} and R_{28} represent H or R_{27} together with R_{28} provide a second bond between C^4 and C^5or iii) R_{24} represents H, R_{25} together with R_{27} provide a second bond between C^3 and C^4, R_{28} represents –OH and X is –O-;

D) X is –O-, -S- or $–NR_1$-, wherein R_1 represents i) H or C_{1-6}alkyl, or ii) together with R_{21} provides a second bond between C^1 and N;

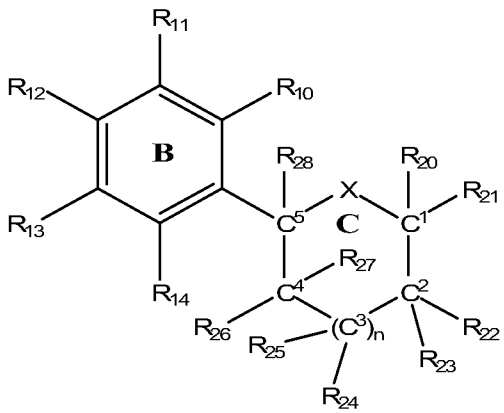
wherein said C_{2-30} saturated or unsaturated hydrocarbon chain of R_{20}, R_{23} and the 5, 6 or 7 membered unsaturated ring is optionally and independently substituted with one or more groups selected from C_{1-6} alkyl, C_{1-6}alkoxy, hydroxy-C_{1-6} alkyl, Cl, F, Br, I, -CN, $-CO_2H$, $-CO_2C_{1-6}$alkyl, $-S(O)_2C_{1-6}$alkyl, $-S(O)_2$phenyl, -

SC_{1-6}alkyl, $-NO_2$, -OH, -CF3, $-N(R_2)(R_3)$, $-NHC(O)NHC_{1-6}$alkyl, $-C(O)N(R_2)(R_3)$, imine and substituted or unsubstituted triphenylphosphonium; and wherein one or more available $-CH_2-$ groups present in the C_{2-30} hydrocarbon chain of R_{20}, R_{23} or? the 5, 6 or 7 membered unsaturated ring is optionally and independently substituted by -O-, -C(O)-, $-S(O)_p-$, or $-N(R_2)-$ provided always that no two such substitutions in the resulting chain are consecutive; wherein R_2 and R_3 each independently represent H or C_{1-6}alkyl, and wherein p is 0 to 2; and

wherein the total number of =O on ring C is no greater than 1.

As well as concerning a method for the *in vitro* preservation of living animal cells, the present invention is also concerned with novel compounds. Surprisingly, these novel compounds have been found to be suitable for use as preservatives for living animal cells.

Accordingly, in another aspect of the present invention, there is provided a compound of Formula Ia or a salt thereof:



Formula Ia

wherein:

A) R_{12} and R_{26} each independently represent –OH or a glycosidic functional group; R_{10}, R_{11}, R_{13}, and R_{14} each independently represent H, -OH, nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, a glycosidic functional group, C_{1-6} alkoxy-, hydroxy-C_{1-6} alkyl-, C_{1-6} alkoxy-C_{1-6} alkyl-, or a saturated or unsaturated C_{1-6} hydrocarbon chain which may be substituted with one or more of nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone or aldehyde groups; and wherein ring B comprises no more than one glycosidic functional group;

B) either a):

R_{20} represents H or a C_{2-30} saturated or unsaturated hydrocarbon chain;

R_{21}:

i) represents H;

ii) together with R_{22} provides a second bond between C^1 and C^2; or

iii) when X is $-NR_1-$ and R_1 is not H or C_{1-6}alkyl, together with R_1 provides a second bond between C^1 and N;

R_{22}:

i) represents H;

ii) together with R_{23} forms =O; or

iii) together with R_{21} provides a second bond between C^1 and C^2;

R_{23}:

i) represents H or a C_{2-30} saturated or unsaturated hydrocarbon chain; or

ii) together with R_{22} forms =O;

wherein at least one of R_{20} and R_{23} is a C_{2-30} saturated or unsaturated hydrocarbon chain;

or b):

R_{20}, R_{21}, R_{22}, and R_{23} form part of a 5, 6 or 7 membered unsaturated-ring including C^1 and C^2, which ring is substituted with a group which is a C_{2-30} saturated or unsaturated hydrocarbon chain, which ring is optionally and independently further substituted with one or more groups selected from nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde and saturated or unsaturated C_{2-15} hydrocarbon chain, which C_{2-30} hydrocarbon chain may be substituted with one or more of nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde or nitrone groups;

C) n is 0 or 1, wherein when n is 0, either i) R_{27} and R_{28} represent H or ii) R_{27} together with R_{28} provide a second bond between C^4 and C^5; or when n is 1, either i) R_{24} and R_{25} together form =O and R_{27} and R_{28} represent H or R_{27} together with R_{28} provide a second bond between C^4 and C^5, or ii) R_{24} and R_{25} represent H and R_{27} and R_{28} represent H or R_{27} together with R_{28} provide a second bond between C^4 and C^5or iii) R_{24} represents H, R_{25} together with R_{27} provide a second bond between C^3 and C^4, R_{28} represents –OH and X is –O-;

D) X is –O-, -S- or $–NR_1$-, wherein R_1 represents i) H or C_{1-6}alkyl, or ii) together with R_{21} provides a second bond between C^1 and N;

wherein said C_{2-30} saturated or unsaturated hydrocarbon chain of R_{20}, R_{23} and the 5, 6 or 7 membered unsaturated ring is optionally and independently substituted with one or more groups selected from C_{1-6} alkyl, C_{1-6}alkoxy, hydroxy-C_{1-6} alkyl, Cl, F, Br, I, -CN, $-CO_2H$, $-CO_2C_{1-6}$alkyl, $-S(O)_2C_{1-6}$alkyl, $-S(O)_2$phenyl, $-SC_{1-6}$alkyl, $-NO_2$, -OH, -CF3, $-N(R_2)(R_3)$, $-NHC(O)NHC_{1-6}$alkyl, $–C(O)N(R_2)(R_3)$, imine and substituted or unsubstituted triphenylphosphonium; and wherein one or more available $-CH_2-$ groups present in the C_{2-30} hydrocarbon chain of R_{20}, R_{23} or the 5, 6 or 7 membered unsaturated ring is optionally and independently replaced by -O-, -C(O)-, $-S(O)_p$-, or $-N(R_2)-$ provided always that the resulting chain includes a $–CH_2-$ group connecting to C^1, C^2 or the 5,6 or 7 membered ring and no two such replacements are consecutive; wherein R_2 and R_3 each independently represent H or C_{1-6}alkyl, and wherein p is 0 to 2;and

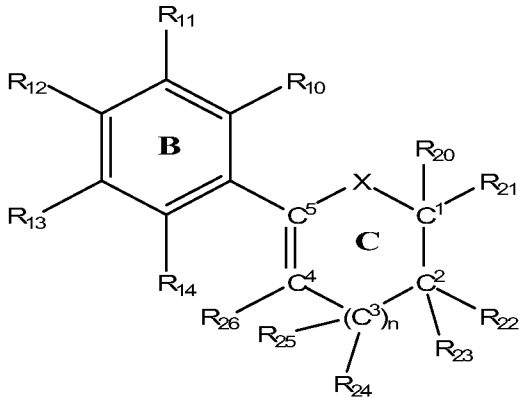
wherein the total number of =O on ring C is no greater than 1;

provided that when i) n=1, ii) X represents –O-, iii) R_{12} represents –OH, iv) R_{24} together with R_{25} represent =O, v) R_{20}, R_{21}, R_{22} and R_{23} form a benzene ring including C^1 and C^2, and vi) said benzene ring is substituted with at least one group which is a C_{2-30} saturated or unsaturated hydrocarbon chain, then:

said C_{2-30} saturated or unsaturated hydrocarbon chain is substituted with one or more groups selected from C_{1-6}alkoxy, hydroxy-C_{1-6} alkyl, Cl, F, Br, I, -CN, $-CO_2H$, sulphonyl, $-CO_2C_{1-6}$alkyl, $-S(O)_2C_{1-6}$alkyl, $-S(O)_2$phenyl, $-SC_{1-6}$alkyl, $-NO_2$, -OH, $-CF_3$, $-N(R_2)(R_3)$, $-NHC(O)NHC_{1-6}$alkyl, $–C(O)N(R_2)(R_3)$, imine and substituted or unsubstituted triphenylphosphonium; and/or wherein one or more available $-CH_2-$ groups present in said C_{2-30} hydrocarbon chain is replaced by -O-, -C(O)-, $-S(O)_p$-, or $-N(R_2)-$; wherein R_2 and R_3 each independently represent H or C_{1-6}alkyl, and wherein p is 0 to 2; and/or

said benzene ring is substituted with one or more groups selected from nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, ketone, aldehyde and saturated or unsaturated C_{1-6} hydrocarbon chain, which C_{1-6} hydrocarbon chain is substituted with one or more of nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde or nitrone groups.

In each aspect of the present invention, the compound of Formula I or Ia or salt thereof may be a compound of Formula II or a salt thereof:



Formula II

wherein:

A) R_{12} and R_{26} each independently represent –OH or a glycosidic functional group; R_{10}, R_{11}, R_{13} and R_{14} each independently represent H, -OH, nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, a glycosidic functional group, C_{1-6} alkoxy-, hydroxyC_{1-6} alkyl-, C_{1-6} alkoxy-C_{1-6} alkyl-, or a saturated or unsaturated C_{1-6} hydrocarbon chain which may be substituted with one or more of nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone or aldehyde groups; and wherein ring B comprises no more than one glycosidic functional group;

B) either a):

R_{20} represents H or a C_{2-30} saturated or unsaturated hydrocarbon chain;

R_{21}:

i) represents H;

ii) together with R_{22} provides a second bond between C^1 and C^2; or

iii) when X is $–NR_1$- and R_1 is not H or C_{1-6} alkyl, together with R_1 provides a second bond between C^1 and N;

R_{22}:

i) represents H;

ii) together with R_{23} forms =O; or

iii) together with R_{21} provides a second bond between C^1 and C^2; and

R_{23}:

i) represents H or a C_{2-30} saturated or unsaturated hydrocarbon chain; or

ii) together with R_{22} forms =O;

wherein at least one of R_{20} and R_{23} is a C_{2-30} saturated or unsaturated hydrocarbon chain;

or b)

R_{20}, R_{21}, R_{22} and R_{23} form part of a 5, 6 or 7 membered unsaturated-ring including C^1 and C^2("A" ring), which ring is substituted with at least one group which is a C_{2-30} saturated or unsaturated hydrocarbon chain, which ring is optionally and independently further substituted with one or more groups selected from nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde and saturated or unsaturated C_{2-15} hydrocarbon chain, which C_{2-15} hydrocarbon chain may be substituted with one or more of nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde or nitrone groups;

C) n is 0 or 1, wherein when n is 1, either i) R_{24} and R_{25} together form =O, or ii) R_{24} and R_{25} represent H;

D) X is –O-, -S- or $-NR_1$-, wherein R_1 represents i) H or C_{1-6}alkyl, or ii) together with R_{21} provides a second bond between C^1 and N;

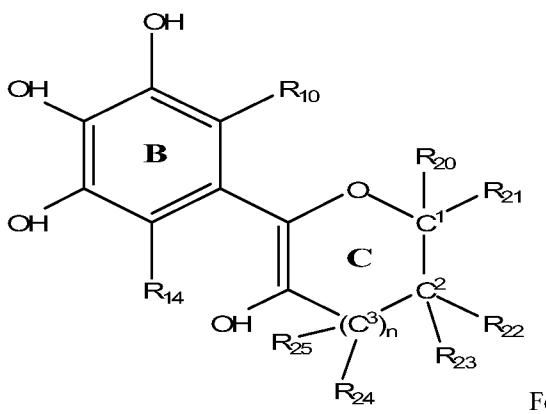
wherein said C_{2-30} saturated or unsaturated hydrocarbon chain of R_{20}, R_{23} or the 5, 6 or 7 membered unsaturated ring is optionally and independently substituted with one or more groups selected from C_{1-6} alkyl, C_{1-6}alkoxy, hydroxy-C_{1-6} alkyl, Cl, F, Br, I, -CN, $-CO_2H$, $-CO_2C_{1-6}$alkyl, $-S(O)_2C_{1-6}$alkyl, $-S(O)_2$phenyl, $-SC_{1-6}$alkyl, $-NO_2$, -OH, $-CF_3$, $-N(R_2)(R_3)$, $-NHC(O)NHC_{1-6}$alkyl, $-C(O)N(R_2)(R_3)$, imine and substituted or unsubstituted triphenylphosphonium; wherein one or more available $-CH_2$- groups present in the C_{2-30} hydrocarbon chain of R_{20}, R_{23} or the 5, 6 or 7 membered unsaturated ring is optionally and independently replaced by -O-, -C(O)-, $-S(O)_p$-, or $-N(R_2)$- provided always that no two such substitutions in the resulting chain are consecutive; wherein R_2 and R_3 each independently represent H or C_{1-6}alkyl, and wherein p is 0 to 2;and

wherein the total number of =O on ring C is no greater than 1.

In each aspect of the present invention, X may be O.

In some embodiments of each aspect of the present invention n=0. In other embodiments of each aspect of the present invention n=1.

In each aspect of the present invention, R_{12} and R_{26} may both represent OH or they may both represent a glycosidic functional group; and/or one but not both of R_{12} and R_{26} may represent a glycosidic functional group, for example R_{12} may be OH when R_{26} is a glycosidic functional group or visa versa; and/or one or both of R_{11} and R_{13} may represent OH; and/or R_{10} and R_{14} each independently represent H, OH or C_{1-6}alkoxy-. An example of such a compound, wherein X=O and R_{27} together with R_{28} provides a second bond between C^4 and C^5 is the compound of Formula III or a salt thereof:



Formula III

wherein:

A) R_{10} and R_{14} each independently represent H, -OH, nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, a glycosidic functional group,C_{1-6} alkoxy-, hydroxyC_{1-6} alkyl-, C_{1-6} alkoxy-C_{1-6} alkyl-, or a saturated or unsaturated C_{1-6} hydrocarbon chain which may be substituted with one or more of nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone or aldehyde groups; and wherein ring B comprises no more than one glycosidic functional group;

B) either a):

R_{20} represents H or a C_{2-30} saturated or unsaturated hydrocarbon chain;

R_{21}:

i) represents H; or

ii) together with R_{22} provides a second bond between C^1 and C^2;

R_{22}:

i) represents H;

ii) together with R_{23} forms =O; or

iii) together with R_{21} provides a second bond between C^1 and C^2; and

R_{23}:

i) represents H or a C_{2-30} saturated or unsaturated hydrocarbon chain; or

ii) together with R_{22} forms =O;

wherein at least one of R_{20} and R_{23} is a C_{2-30} saturated or unsaturated hydrocarbon chain;

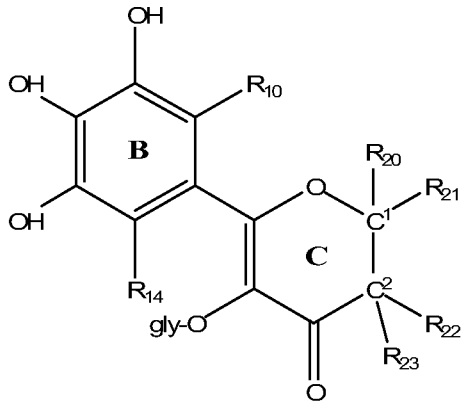
or b)

R_{20}, R_{21}, R_{22} and R_{23} form part of a 5, 6 or 7 membered unsaturated-ring including C^1 and C^2 ("A" ring), which ring is substituted with a group which is a C_{2-30} saturated or unsaturated hydrocarbon chain, which ring is optionally and independently further substituted with one or more groups selected from nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde and saturated or unsaturated C_{2-15} hydrocarbon chain, which C_{2-15} hydrocarbon chain may be substituted with one or more of nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde or nitrone groups;

C) n is 0 or 1, wherein when n is 1, either i) R_{24} and R_{25} together form =O, or ii) R_{24} and R_{25} represent H;

wherein said C_{2-30} saturated or unsaturated hydrocarbon chain of R_{20}, R_{23} or the 5, 6 or 7 membered unsaturated ring is optionally and independently substituted with one or more groups selected from C_{1-6} alkyl, C_{1-6}alkoxy, hydroxy-C_{1-6} alkyl, Cl, F, Br, I, -CN, $-CO_2H$, $-CO_2C_{1-6}$alkyl, $-S(O)_2C_{1-6}$alkyl, $-S(O)_2$phenyl, $-SC_{1-6}$alkyl, $-NO_2$, -OH, -CF3, $-N(R_2)(R_3)$, $-NHC(O)NHC_{1-6}$alkyl, $-C(O)N(R_2)(R_3)$, imine and substituted or unsubstituted triphenylphosphonium; wherein one or more available $-CH_2-$ groups present in the C_{2-30} hydrocarbon chain of R_{20}, R_{23} or the 5, 6 or 7 membered unsaturated ring is optionally and independently replaced by -O-, -C(O)-, $-S(O)_p-$, or $-N(R_2)-$ provided always that no two such replacements in the resulting chain are consecutive; wherein R_2 and R_3 each independently represent H or C_{1-6}alkyl, and wherein p is 0 to 2; and

wherein the total number of =O on ring C is no greater than 1. A further example of such a compound, but wherein X=O, n=1, R_{24} together with R_{25} forms =O and R_{27} together with R_{28} provides a second bond between C^4 and C^5,is the compound of Formula VI or salt thereof:



Formula VI

wherein:

A) R_{10} and R_{14} each independently represent H, -OH, nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, a glycosidic functional group, C_{1-6} alkoxy- hydroxy-C_{1-6} alkyl-, C_{1-6} alkoxy-C_{1-6} alkyl-, or a saturated or unsaturated C_{1-6} hydrocarbon chain which may be substituted with one or more of nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone or aldehyde groups; and wherein ring B comprises no more than one glycosidic functional group; and -O-gly represents a glycosidic functional group;

B) either a):

R_{20} represents H or a C_{2-30} saturated or unsaturated hydrocarbon chain;

R_{21}:

i) represents H; or

ii) together with R_{22} provides a second bond between C^1 and C^2;

R_{22}:

i) represents H;

ii) together with R_{21} provides a second bond between C^1 and C^2; and

R_{23} represents H, or a C_{2-30} saturated or unsaturated hydrocarbon chain;

wherein at least one of R_{20} and R_{23} is a C_{2-30} saturated or unsaturated hydrocarbon chain;

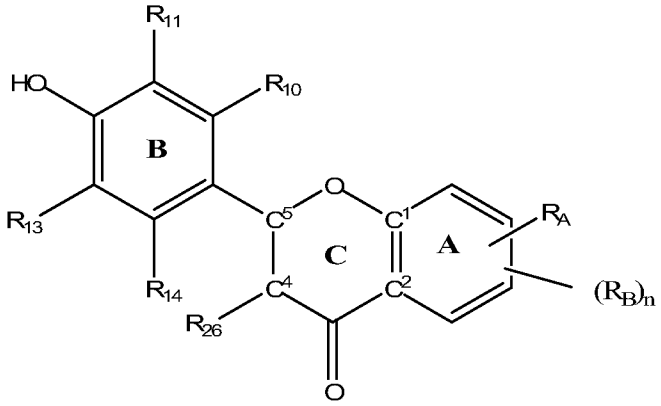
or b)

R_{20}, R_{21}, R_{22} and R_{23} form part of a 5, 6 or 7 membered unsaturated-ring including C^1 and C^2, which ring is substituted with a group which is a C_{2-30} saturated or unsaturated hydrocarbon chain, which ring is optionally and independently further substituted with one or more groups selected from nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde and saturated or unsaturated C_{2-15} hydrocarbon chain, which C_{2-15} hydrocarbon chain may be substituted with one or more of nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde or nitrone groups;

wherein said C_{2-30} saturated or unsaturated hydrocarbon chain of R_{20}, R_{23} or the 5, 6 or 7 membered unsaturated ring is optionally and independently substituted with one or more groups selected from C_{1-6} alkyl, C_{1-6}alkoxy, hydroxy-C_{1-6} alkyl, Cl, F, Br, I, -CN, $-CO_2H$, $-CO_2C_{1-6}$alkyl, $-S(O)_2C_{1-6}$alkyl, $-S(O)_2$phenyl, $-SC_{1-6}$alkyl, $-NO_2$, -OH, $-CF_3$, $-N(R_2)(R_3)$, $-NHC(O)NHC_{1-6}$alkyl, $-C(O)N(R_2)(R_3)$, imine and substituted or

unsubstituted triphenylphosphonium; wherein one or more available -CH_2- groups present in the C_{2-30} hydrocarbon chain of R_{20}, R_{23} or the 5, 6 or 7 membered unsaturated ring is optionally and independently replaced by -O-, -C(O)-, -$S(O)_p$-, or -$N(R_2)$- provided always that no two such replacements in the resulting chain are consecutive; and wherein R_2 and R_3 each independently represent H or C_{1-6}alkyl, and wherein p is 0 to 2.

An example of a compound useful in the first aspect of the present invention where R_{20}, R_{21}, R_{22} and R_{23} form part of a 5, 6 or 7 membered ring is the compound of Formula VII or a salt thereof:



Formula VII

wherein

R_A is a C_2 to C_{30} saturated or unsaturated hydrocarbon chain;

R_{10}, R_{11}, R_{13}, R_{14} and R_{26} each independently represent H, OH, a C_{1-6} alkoxy, or a saturated or unsaturated C_{1-6} hydrocarbon chain which may be substituted with one or more of nitro, halogen, amino, hydroxyl, ketone or aldehyde group;

optionally there is a double bond between C^4 and C^5 of the C ring; and

n represents 0 or 1; and

R_B is a C_2 to C_{15} saturated or unsaturated hydrocarbon chain, where when R_B is present R_A and R_B are both C_2 to C_{12} aliphatic alkyl chains.

The R_A group is preferably substituted on ring A at the para position with respect to C^2. The R_A group is preferably a C_{6-15} saturated or unsaturated hydrocarbon chain.

Compounds of Formula VII are disclosed in WO 2004/007475 and as such are specifically excluded from the scope of the second aspect of the present invention.

In both aspects of the present invention, the compound of Formula I or Ia or salt thereof may be an anthocyanin. Anthocyanins are generally known to exist in equilibrium between their hydrated hemiketal form and their flavylium cation form, both of which forms are considered to fall within the scope of the present invention. Anthocyanins within the scope of the present invention are compounds of Formula I or salts thereof wherein:

R_{12} represents OH

R_{26} represents a glycosidic functional group

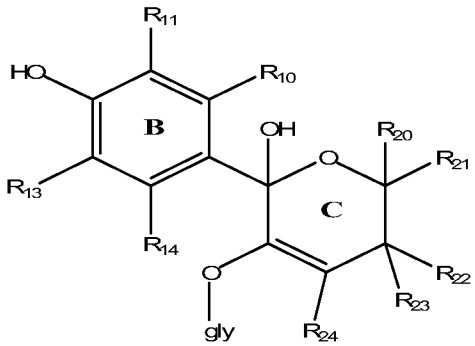
R_{25} together with R_{27} provide a second bond between C^3 and C^4

R_{28} represents OH

X = O,

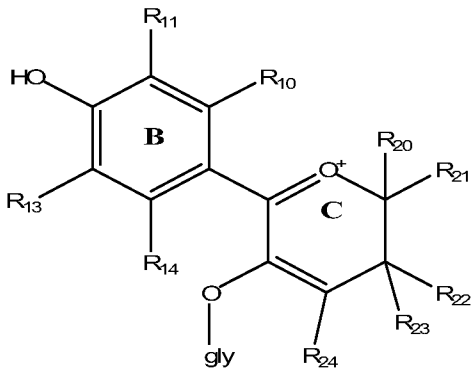
n = 1

and R_{20}, R_{21}, R_{22}, and R_{23} form part of a 6 membered unsaturated-ring including C^1 and C^2, which ring is substituted with at least one group which is a C_{2-30} saturated or unsaturated hydrocarbon chain, which ring is optionally and independently further substituted with one or more groups selected from nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde and saturated or unsaturated C_{1-6} hydrocarbon chain, which C_{1-6} hydrocarbon chain may be substituted with one or more of nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde or nitrone groups. Anthocyanins within the scope of the present invention can be represented by Formula VIIIHH, which is the structural formula of the compound in its hydrated hemiketal form, and Formula VIIIFC, which is the structural formula of the compound in its flavylium cation form. The flavylium cation form is also in equilibrium with the nonionic flavylium form represented by Formula VIIIFH:

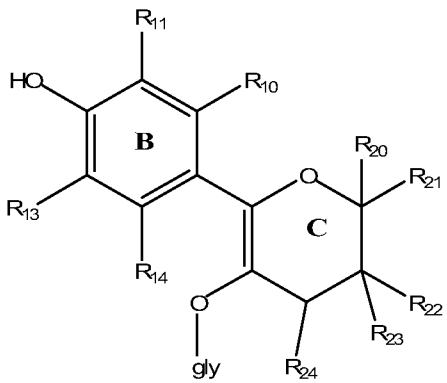


Formula VIIIHH.

$\pm H_2O$



Formula VIIIFC.



Formula VIIIFH.

In each aspect of the present invention, when R_{20}, R_{21}, R_{22}, and R_{23} in the compounds of Formula I or Ia or salts thereof form part of a 5, 6 or 7 membered unsaturated-ring including C^1 and C^2, the ring is substituted with a C_{2-30} saturated or unsaturated hydrocarbon chain, as defined above, at either of the otho, meta or para positions. The ring may be independently further substituted with one or more groups selected from nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde and saturated or unsaturated C_{1-6} hydrocarbon chain, which C_{1-6} hydrocarbon chain may be substituted with one or more of nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde or nitrone groups. By substitution at the ortho position we mean substitution on a carbon next to the C^1 on the ring. By substitution at the meta position we mean substitution on the carbon next to the ortho position remote from C^1. By substitution at the para position we mean substitution on the carbon next to the meta position remote from C^1. It will be appreciated by those skilled in the art that in the case of 5 membered rings, the para position may also be defined as the meta position. In one embodiment, the compound of Formula I or salt thereof comprises a 5, 6 or 7 membered ring having the C_{2-30} hydrocarbon chain substituted at the meta or para position. For example, the compound of Formula I or salt thereof may comporise a 6 membered ring having the C2-30 hydrocarbon substituted at the meta or para position.

The term "glycosidic functional group" is well known in the art, and is represented in the structural formulae herein as –O-gly. For avoidance of any doubt, however, we mean a carbohydrate group linked to the main structure via a glycosidic bond. Preferably, the carbohydrate is a sugar. Preferably the sugar is glucose, rhamnose or rutinose.

In each embodiment of the present invention, the C_{2-30} saturated or unsaturated hydrocarbon chain of R_{20}, R_{23} and the 5, 6 or 7 membered unsaturated ring may have from two to twenty carbon atoms, preferably from six to fifteen carbon atoms. Suitably the hydrocarbon chain has a backbone having two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen or eighteen consecutive carbon atoms.

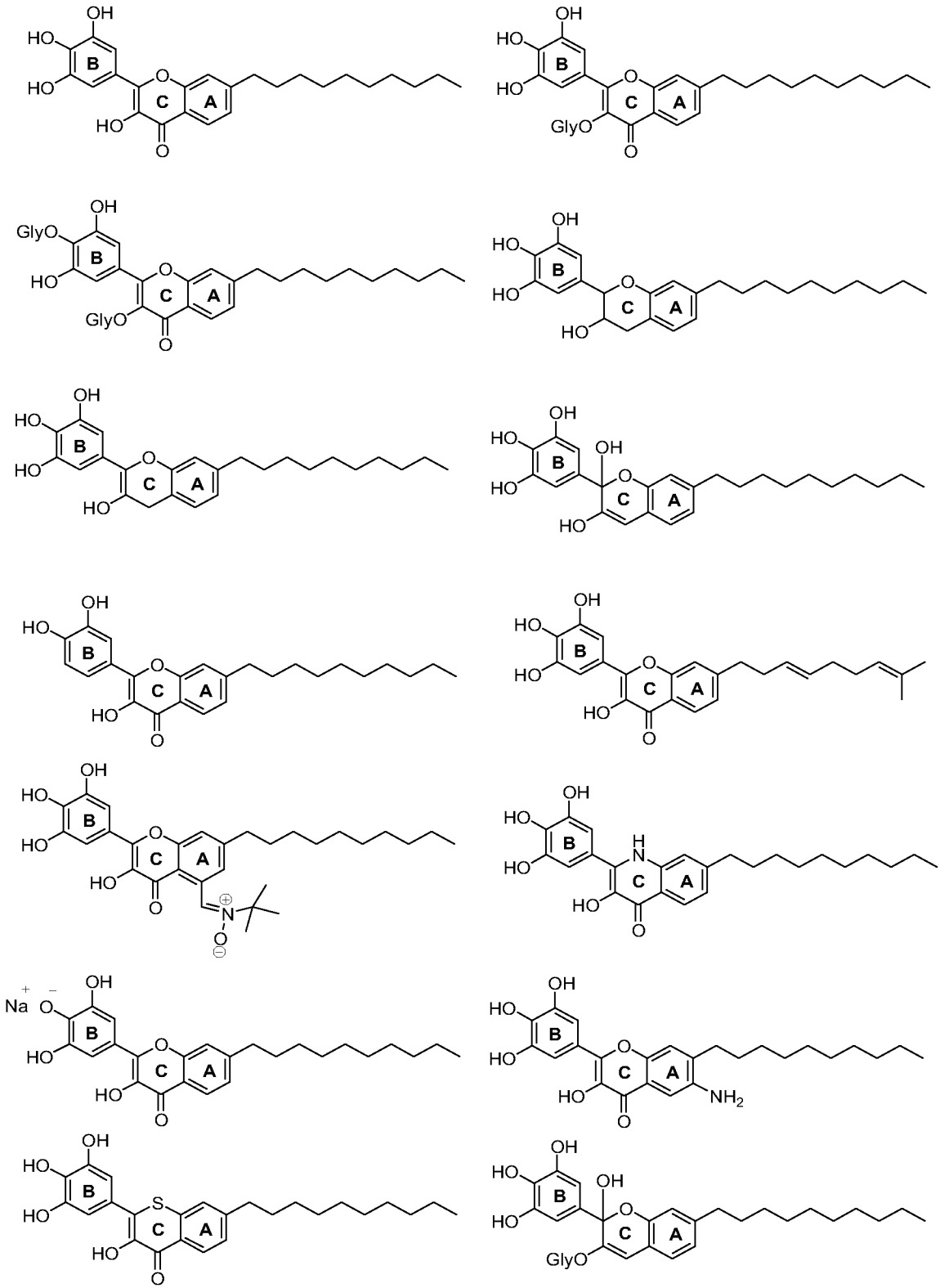
The C_{2-30} saturated or unsaturated hydrocarbon chain of R_{20}, R_{23} and the 5, 6 or 7 membered unsaturated ring may include a $–CH_2-$ group connecting to C^1, C^2 or the 5,6 or 7 membered ring. This means, for example, that the C_{2-30} hydrocarbon chain may not be an alkoxy group, though one or more carbon atoms within the C_{2-30} hydrocarbon chain may be substituted with an alkoxy group.

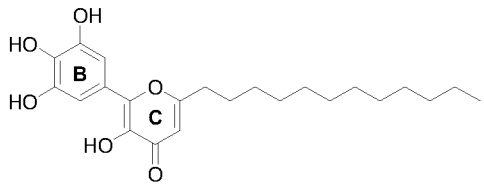
The C_{2-30} saturated or unsaturated hydrocarbon chain of R_{20}, R_{23} and the 5, 6 or 7 membered unsaturated ring may be unsubstituted and is preferably saturated. The C_{2-30} saturated or unsaturated hydrocarbon chain of R_{20}, R_{23} and the 5, 6 or 7 membered unsaturated ring is preferably a straight hydrocarbon chain preferably comprising 6 to 15 carbon atoms.

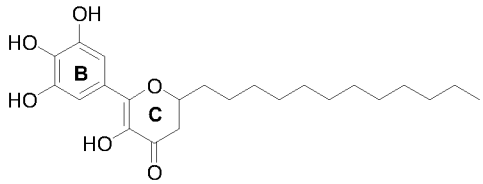
When the C_{2-30} saturated or unsaturated hydrocarbon chain is on a 5, 6 or 7 membered unsaturated ring, the ring is optionally and independently further substituted with one or more groups selected from nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, ketone, aldehyde and saturated or unsaturated C_{2-15} hydrocarbon chain, which C_{2-15} hydrocarbon chain may be substituted with one or more of nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde or nitrone groups. In one embodiment, the ring is unsubstituted except for the C_{2-30} hydrocarbon chain. In another embodiment, the ring may be substituted with one or more groups selected from –NH2 and saturated or unsaturated C_{2-15} hydrocarbon

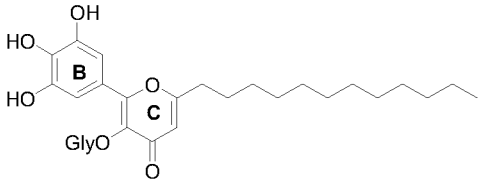
chain, which C_{2-15} hydrocarbon chain may be substituted with one or more of nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde or nitrone groups.

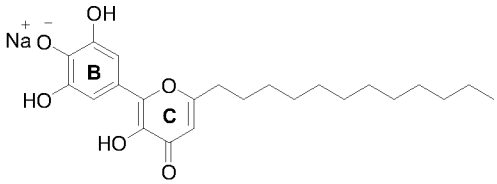
Examples of specific compounds or salts thereof which are capable of preserving living animal cells include:

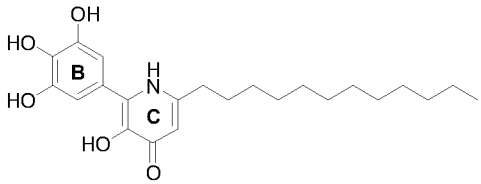


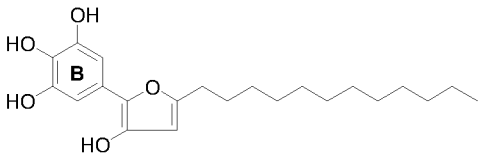


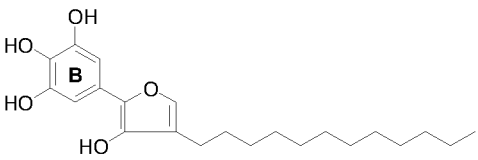


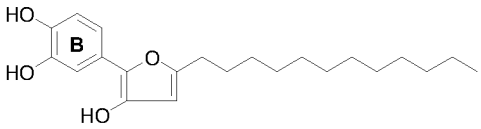


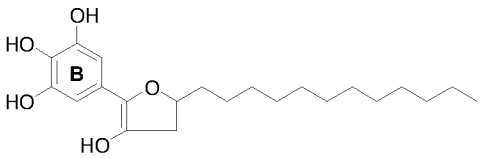


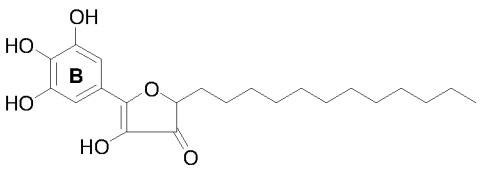


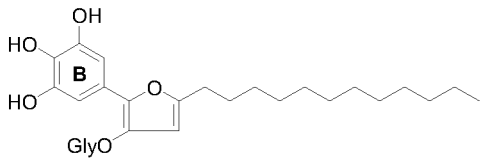


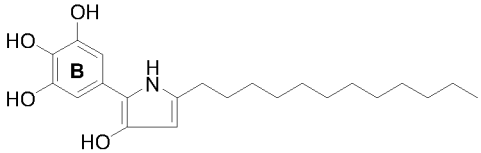


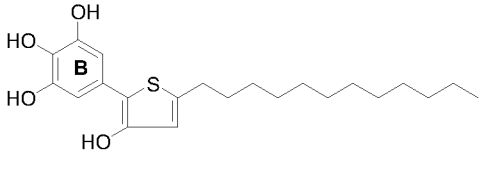


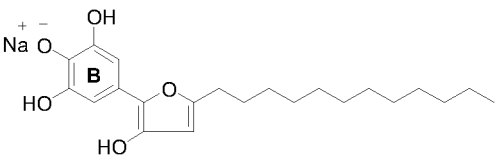


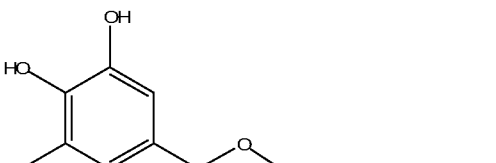


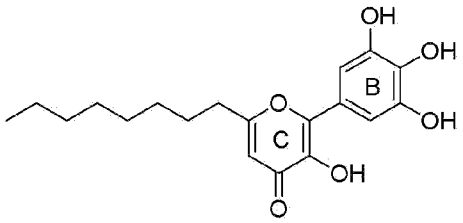












The compounds of the present invention are useful for *in vitro* preservation of living animal cells. It will be appreciated by those skilled in the art that in some circumstances preservation may not be a permanent state, but here preservation is intended to include a reduction in the rate of deterioration of the viability of individual cells or groups of cells. The expression "*in vitro* preservation of living animal cells" will be well understood by those skilled in the art but, should any doubt arise, it is intended to mean the *in vitro* preservation of individual cells or groups of cells, including tissues and organs. As well as being useful for *in vitro* research purposes, it will be appreciated by persons skilled in the art that such *in vitro* preservation of the cells or groups of cells may be for eventual autologous, isogenic, allogenic or xenogenic transplantation.

For example, the compounds of the invention may be used in the *in vitro* preservation of viable terminally differentiated cells e.g. red blood cells and lymphocyte cells such as t-cells and b-cells. Said one or more terminally differentiated cells can be in the form of individual unipotent stem cells or in the form of tissue i.e. a collection of interconnected cells that can perform a similar function *in vivo*. Tissues suitable for preservation include, but are not limited to, epithelium, connective tissue, muscle tissue and nervous tissue. Furthermore, said one or more fully differentiated cells can form or be part of an organ i.e. a group of tissues that can perform a specific function or group of functions *in vivo*. Organs suitable for preservation include, but are not limited to, heart, lungs, brain, eyes, stomach, spleen, bone, pancreas, kidney, liver, intestines, skin and bladder.

The method of the invention is used to preserve living animal cells in a viable non-terminally differentiated state. The expression "preservation of living animal cells in a viable non-terminally differentiated state" will be well understood by those skilled in the art but, should any doubt arise, it is intended to include the preservation of living non-terminally differentiated animal cells such that the cells retain the ability to divide and produce at least one non-terminally differentiated cell type.

Differentiation is the process by which an undifferentiated cell becomes differentiated, which in practice means that the undifferentiated cell becomes committed to a particular cell lineage and/or loses its original capacity for differentiating into particular cell types.

Cells that are non-terminally differentiated give rise to various different cell types and can alternatively be described as partially differentiated cells. Such cells include the cells of the three embryonic germ cell layers: endoderm, mesoderm and ectoderm. Cells that are preserved using the methods of the invention also include undifferentiated cells, which have the capacity to differentiate into a multitude of cell types. Undifferentiated cells that are preserved using the methods of the invention are typically stem cells. Cells that are preserved using the methods of the invention also include de-differentiated cells, which are produced when a partially or terminally differentiated cell reverts to an earlier developmental stage. De-differentiated cells can therefore be undifferentiated or partially differentiated. In this aspect, the methods of

the invention are particularly useful for the preservation of de-differentiated neuronal cells. In addition, the methods of the invention are useful for the preservation of cells in cell culture which have de-differentiated.

Cells that are non-terminally differentiated include cells that are arrested at a particular stage of the differentiation process or the de-differentiation process. Cells that are non-terminally differentiated therefore include cells that are arrested at a particular stage whilst becoming committed to a particular cell lineage, cells that are arrested at a particular stage whilst reverting to an earlier developmental stage after being non-terminally or terminally differentiated and cells that are re-differentiated after becoming de-differentiated.

In one embodiment, the present invention finds use in the preservation of cells that are differentiated to a non-terminal state of differentiation, i.e. partially differentiated cells, before being transported or stored. The cells can then be induced to a terminally differentiated state when required.

In this aspect of the invention, the cells that are preserved by the methods of the invention can be used for transplantation. Typically, such cells are partially differentiated, and retain the capacity to differentiate into a number of different cell types.

Undifferentiated animal cells which can be preserved using the methods of the invention are typically stem cells. Stem cells are unspecialised cells which are capable of differentiating into various different types of cells and which are capable of self-renewal. Stem cells which can be preserved using a method of the present invention include totipotent stem cells (capable of differentiating into embryonic and extraembryonic cell types), pluripotent stem cells (capable of differentiating into endoderm, mesoderm and ectoderm germ layers), and multipotent stem cells (capable of differentiating into a plurality of closely related cells).

Types of stem cells which can be preserved using the methods of the present invention include embryonic stem (ES) cells (ESCs), adult stem cells and induced pluripotent stem (iPS) cells. Embryonic stem cells are derived from the blastocyst of a mammalian embryo and are totipotent. Embryonic stem cells were originally described by Evans and Kaufman (Nature, 292(5819): 154-156, 1981). Adult stem cells are pluripotent, and include hematopoietic stem cells and mesenchymal stem cells. Induced pluripotent cells are artificially derived from a non-pluripotent cell such as an adult somatic cell by the insertion of certain genes and are very similar to embryonic stem cells (Takahashi *et al,* Cell 131(5): 861-872, 2007; and Yu *et al,* Science 318(5858), 1917-1920, 2007). Stem cells are also found in the blood of the umbilical cord, and such umbilical cord blood stem cells can also be preserved using the methods of the present invention.

Stem cells which can be preserved using the methods of present invention can be human or non-human. Typically, the stem cell is a mouse embryonic stem cell or a human embryonic stem cell. Stem cells which can be preserved using the methods of present invention include transgenic stem cells. Stem cells which can be preserved using the methods of present invention also include those produced from hybrid embryos or cytoplasmic hybrid (cybrid) embryos.

Other types of undifferentiated cells which can be preserved using the methods of the present invention include embryonic germ (EG) cells and embryonic carcinoma (EC) cells.

Undifferentiated cells can be identified, for example, by expression of particular marker genes. For example, stem cells can be identified by the expression of the marker genes Oct3/4 and Nanog. Expression of such marker genes can be determined by any suitable method known in the art, for example qPCR.

The undifferentiated animal cells which can be preserved using a method of the present invention are typically mammalian cells. Such mammalian cells include human and non-human cells. For example, non-human cells can be from rodents, such as mice, rats and guinea pigs; ungulates, such as cattle, sheep, goats and pigs; or other mammals such as cats, dogs, horses or rabbits. The animal cells can alternatively be bird cells, for example from chickens or turkeys, or fish cells, for example from zebra fish or salmon.

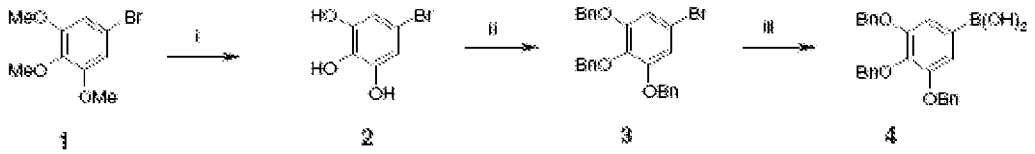
The compounds of Formula I or salts thereof may be used for preserving living animal cells by any of the relevant methods disclosed in EP-A-1057405.

Representative compound synthesis

Compounds of Formula I and Ia wherein R_{20}, R_{21}, R_{22}, and R_{23} form part of a 6 membered unsaturated ring including C^1 and C^2 may be formed by the process decribed in WO 2004/007475. A person skilled in the art would readily be able to adapt this process for the manufacture of compounds of Formula I or I a including a 5 or 7 membered ring.

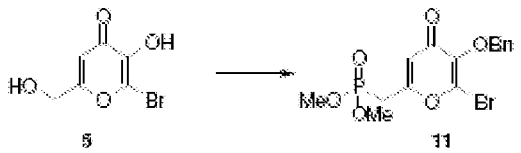
Compounds of Formula I or Ia wherein R_{20}, R_{21}, R_{22}, and R_{23} do not form part of a 6 membered unsaturated ring including C^1 and C^2 may be formed by the following process.

The use of an *in situ* quench protocol gave the desired boronic acid which would eventually be used to form the B-ring of the target compound (**Scheme 1**).



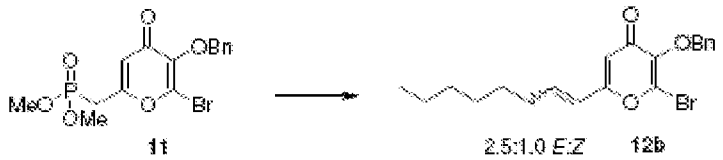
Scheme 1. *Reagents and conditions:* i. BBr3 (1.0 M in CH2Cl2, 10 eq.), CH2Cl2, 0 ^{o}C to rt, 16 hrs (97%); ii. BnBr (3.30 eq.), K2CO3 (4.00 eq.), DMF, 100 ^{o}C, 18 hrs. (57%); iii. *t*-BuLi (2.20eq.), B(O-^{i}Pr)3, THF, -78 ^{o}C to rt, 16 hrs, (60%).

Phosphonate ester (**11**) was prepared as follows:

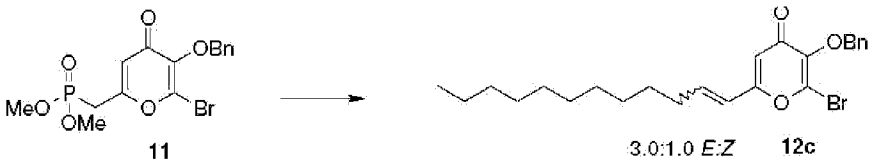


To a stirred solution of **5** (1.88 g, 8.52 mmol, 1.00 eq.) in THF (50 mL) was added benzyl alcohol (4.40 mL, 4.60 g, 42.6 mmol, 5.00 eq.), then triphenylphosphine (2.23 g, 8.52 mmol, 1.00 eq.). The reaction was cooled to 0 ^{o}C and DIAD (1.7 mL, 1.72 g, 8.52 mmol, 1.00 eq.) was added dropwise. The reaction was warmed to room temperature and stirred for 4 hours. The reaction mixture was concentrated *in vacuo* and purified by flash chromatography (10% EtOAc:90% petrol → 20% EtOAc:80% petrol → 30% EtOAc:70% petrol → 50% EtOAc:50% petrol) to yield the benzylated compound as a yellow oil containing about 30% reduced DIAD (1.01 g, 38% including impurity). The crude benzylated compound (535 mg, 1.72 mmol, 1.00

eq.) was diluted with CH2Cl2 (5 mL) and SOCl2 (188 µL, 2.58 mmol, 1.50 eq.) was added at 0 °C. The reaction was stirred, with warming to room temperature, for 2.5 hours, when complete by HPLC. The reaction mixture was concentrated *in vacuo* and purified by flash chromatography (10% EtOAc:90% petrol → 20% EtOAc:80% petrol) to yield a brown oil (276 mg, 49%). The chloride (261 mg, 0.79 mmol, 1.00 eq.) was taken into trimethylphosphite (2 mL) and the reaction was heated to reflux and stirred for 16 hours. The reaction was cooled to room temperature and concentrated *in vacuo*. Purification by flash chromatography (70% EtOAc:30% petrol → 100% EtOAc) gave the title phosphonate ester **11** as a brown oil (233 mg, 73%) ^{1}H-NMR (400 MHz, CDCl3) 7.47-7.43 (2H, m, 2 x Ar*H*), 7.38 7.31 (3H, m, 3 x Ar*H*), 6.35 (1H, d, *J* 3.0, vinylic *H*), 5.25 (2H, s, OC*H2*Ar), 3.80 (6H, d, *J* 11.1, 2 x OC*H3*), 3.07 (2H, d, *J* 21.7, C*H2*P).

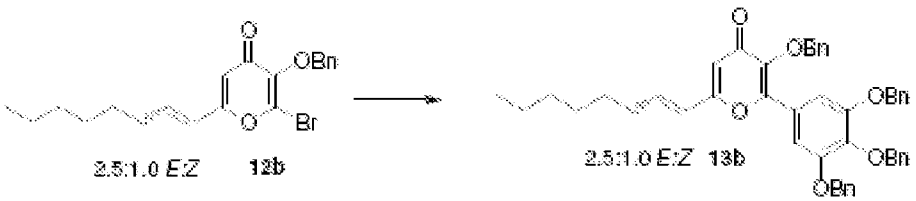


To **11** (400 mg, 0.99 mmol, 1.00 eq.) in 1:1 DMF/THF (8 mL) at –78 °C was added LHMDS (1.0 M in THF; 1.10 mL, 1.09 mmol, 1.10 eq.) and the reaction was stirred at that temperature for 10 mins (yellow/orange colour). Heptaldehyde (113 mg, 0.99 mmol, 1.00 eq.) in DMF (4 mL) was added in one portion at –78 °C and the reaction was stirred at that temperature for 45 mins. The reaction was warmed to 0 °C and stirred for 10 mins until complete by HPLC. The reaction was quenched with saturated aqueous ammonium chloride solution (5 mL) and extracted into EtOAc (3 x 10 mL). The combined organic extracts were washed with water (10 mL) and brine (10 mL), dried (MgSO4) and concentrated *in vacuo*. Purification by flash chromatography (10% EtOAc:90% petrol) gave the alkene **12b** as a colourless oil and a 2.5:1.0 mixture of *E* and *Z* isomers respectively (251 mg, 65%) ^{1}H-NMR (400 MHz, CDCl3) 7.50-7.45 (2H, m, 2 x Ar*H*), 7.40-7.30 (3H, m, 3 x Ar*H*), 6.62 (0.7H, dt, *J* 15.7 and 7.1, vinylic *H*), 6.24 (0.3H, s, *H*C=CO); 6.16 (0.7H, s, *H*C=CO), 6.08-5.98 (0.3 H, m, vinylic *H*), 6.01 (0.7H, dt, *J* 15.7 and 1.5, vinylic *H*), 5.92 (0.3 H, dt, *J* 12.1 and 1.52, vinylic *H*), 5.28 (0.6 H, s, OC*H2*Ar), 5.26 (1.5H, s, OC*H2*Ar), 2.46 (0.6H, ddd, *J* 13.1, 7.5 and 1.5, allylic C*H2*), 2.24 (1.5 H, ddd, *J* 13.1, 7.5 and 1.5, allylic C*H2*), 1.52-1.44 (2H, m, C*H2*), 1.40-1.24 (6H, m, C*H2*), 0.93-0.88 (3H, m, C*H3*).

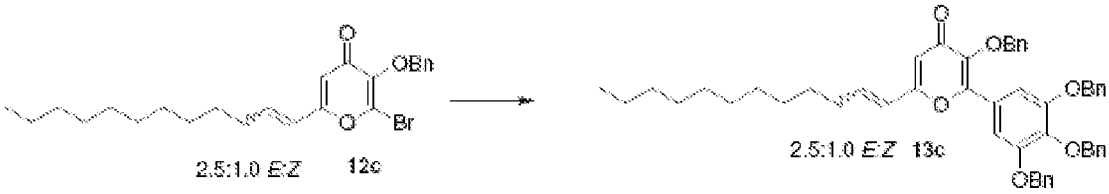


To **11** (400 mg, 0.99 mmol, 1.00 eq.) in 1:1 DMF/THF (8 mL) at –78 °C was added LHMDS (1.0 M in THF; 1.10 mL, 1.09 mmol, 1.10 eq.) and the reaction was stirred at that temperature for 10 mins (yellow/orange colour). Undecanal (168 mg, 0.99 mmol, 1.00 eq.) in DMF (4 mL) was added in one portion at –78 °C and the reaction was stirred at that temperature for 45 mins. The reaction was warmed to 0 °C and stirred for 10 mins until complete by HPLC. The reaction was quenched with saturated aqueous ammonium

chloride solution (5 mL) and extracted into EtOAc (3 x 10 mL). The combined organic extracts were washed with water (10 mL) and brine (10 mL), dried (MgSO4) and concentrated *in vacuo*. Purification by flash chromatography (5% EtOAc:95% petrol → 10% EtOAc:petrol) gave the alkene **12c** as a colourless oil and an approximate 3:1 mixture of *E* and *Z* isomers respectively (336 mg, 76%) ^{1}H-NMR (400 MHz, CDCl3) 7.50-7.45 (2H, m, 2 x Ar*H*), 7.40-7.30 (3H, m, 3 x Ar*H*), 6.61 (0.7 H, dt, *J* 15.7 and 7.1, vinylic *H*), 6.24 (0.3H, s, *H*C=CO), 6.16 (0.7 H, s, *H*C=CO), 6.07- 5.99 (0.3H, m, vinylic *H*), 6.00 (0.7H, dt, *J* 16.1 and 1.5, vinylic *H*), 5.92 (0.3H, dt, *J* 11.6 and 1.5, vinylic *H*), 5.28 (0.6H, s, OC*H2*Ar), 5.25 (1.4H, s, OC*H2*Ar), 2.46 (0.5H, ddd, *J* 15.2, 7.6 and 1.5, allylic *H*), 2.24 (1.5H, ddd, *J* 15.2, 7.6 and 1.5, allylic *H*), 1.52-1.42 (2H, m, C*H2*), 1.40-1.22 (14H, m, 7 x C*H2*), 0.90 (3H, t, *J* 7.1, C*H3*).



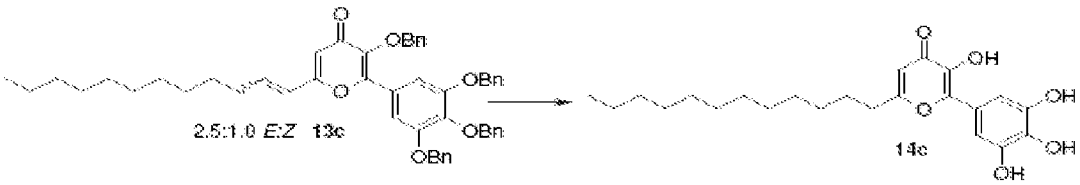
A suspension of **12b** (251 mg, 0.64 mmol, 1.00 eq.), Pd(PPh3)4 (69 mg, 0.06 mmol, 10mol%) and K2CO3 (283 mg, 2.05 mmol, 3.20 eq.) in DMF/H2O (2:1, 5.1 mL) was degassed for 5 mins, then a degassed solution of boronic acid (310 mg, 0.71 mmol, 1.10 eq.) in DMF (1.8 mL) and the reaction was heated to 60 ^{o}C and stirred for 4 hours. The reaction was cooled to room temperature, diluted with water (20 mL) and extracted with EtOAc (2 x 20 mL). The combined organic extracts were dried (MgSO4) and concentrated *in vacuo*. Purification by flash chromatography (10% EtOAc:90% petrol → 20% EtOAc:80% petrol → 30% EtOAc:70% petrol → 40% EtOAc:60% petrol) gave the title compound as a yellow oil (332 mg, 74%) ^{1}H-NMR (400 MHz, CDCl3) 7.48-7.20 (22H, m, 22 x Ar*H*), 6.52-5.90 (3H, m, vinylic *H* and pyranone *H*), 5.32-4.92 (8H, m, 4 x OC*H2*Ar), 2.60-2.20 (2H, m, C*H2*),1.58-1.40 (2H, m, C*H2*), 1.40-1.20 (6H, m, 3 x C*H2*), 1.00-0.80 (3H, m, C*H3*).



A suspension of **12c** (204 mg, 0.46 mmol, 1.00 eq.), Pd(PPh3)4 (53 mg, 0.05 mmol, 10mol%) and K2CO3 (203 mg, 1.47 mmol, 3.20 eq.) in DMF/H2O (2:1, 5.1 mL) was degassed for 5 mins, then a degassed solution of boronic acid 221 mg, 0.50 mmol, 1.10 eq.) in DMF (1.8 mL) and the reaction was heated to 60 ^{o}C and stirred for 4 hours. The reaction was cooled to room temperature, diluted with water (20 mL) and extracted with EtOAc (2 x 20 mL). The combined organic extracts were dried (MgSO4) and concentrated *in vacuo*. Purification by flash chromatography (10% EtOAc:90% petrol → 20% EtOAc:80% petrol → 30% EtOAc:70% petrol → 40% EtOAc:60% petrol) gave the title compound as a yellow oil (260 mg, 74%) ^{1}H-

NMR (400 MHz, CDCl3) 7.50-7.20 (22H, m, 22 x Ar*H*), 6.50-6.00 (3H, m, 3 x vinylic *H*), 5.30-4.90 (6H, m, OC*H2*Ar), 2.56-2.48 (0.4 H, m, C*H2*), 2.28-2.20 (1.4H, m, C*H2*), 1.57-1.40 (2H, m, C*H2*), 1.40-1.20 (14H, m, 7 x C*H2*), 0.92-0.84 (3H, m, C*H3*).

13b (320 mg, 0.45 mmol, 1.00 eq.) was dissolved in the minimal amount of ethyl acetate and taken into MeOH (10 mL). 10% Pd/C (180 mg) was slurried with MeOH (1 mL) and added to the solution. The solution was evacuated and backfilled with H2 3 times and stirred for 1 hour. The suspension was filtered through Celite® and concentrated *in vacuo* to yield **14b** as a tan solid (105 mg, 67%) ^{1}H-NMR (400 MHz, d^{6}-DMSO/D2O shake) 7.06 (2H, s, 2 x Ar*H*), 6.24 (1H, s, vinylic *H*), 2.59 (2H, t, *J* 7.1, C*H2*), 1.64 (2H, quintet, *J* 7.1, C*H2*), 1.30-1.10 (10H, m, 5xC*H2*), 0.81 (3H, t, *J* 7.1, C*H3*).



13c (250 mg, 0.33 mmol, 1.00 eq.) was dissolved in the minimal amount of ethyl acetate and taken into MeOH (10 mL). 10% Pd/C (150 mg) was slurried with MeOH (1 mL) and added to the solution. The solution was evacuated and backfilled with H2 3 times and stirred for 1 hour. The suspension was filtered through Celite® and concentrated *in vacuo* to yield **14c** as a tan solid (84 mg, 63%) ^{1}H-NMR (400 MHz, d^{6}-DMSO/D2O shake) 7.08 (2H, s, 2 x Ar*H*), 6.24 (1H, s, vinylic *H*), 2.59 (2H, t, *J* 7.1, C*H2*), 1.64 (2H, quintet, *J* 7.1, C*H2*), 1.37-1.00 (18H, m, 9xC*H2*), 0.81 (3H, t, *J* 7.1, C*H3*).

Experimental

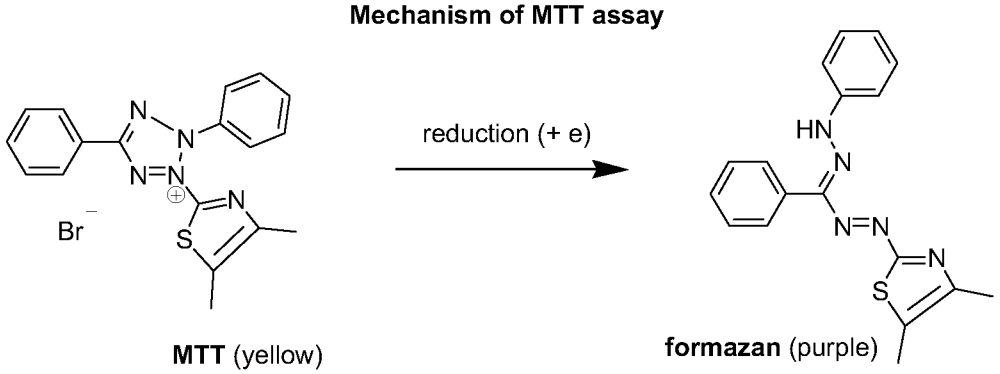
The following experiments were conducted to demonstrate the protective effective of certain antioxidant compounds in relation to oxidative stress-induced cytotoxicity in mouse embryonic stem cells:

Oxidative stress and related free radical damage can be induced in cells, including stem cells, by exposing the cells to *tert*-butyl hydroperoxide in the incubation medium. Decomposition of the peroxide over a period of time leads to a steady generation of reactive oxygen species. This in turn triggers a cascade of free radical-mediated events that eventually leads to irreversible damage and ultimately cell death as the natural antioxidant defences are overwhelmed.

The extent of cytotoxicity induced by peroxide exposure can be quantified using a number of well-documented assays, including the MTT protocol. This is a colorometric assay that measures the reduction of MTT to formazan, a process that can only occur when cells are viable. Thus, by comparing the amount of formazan produced in control cells, against cells treated with a cytotoxic agent, such as *tert*-butyl hydroperoxide, the reduction in cell viability induced by the agent can be measured.

The assay can be further extended to determine the effectiveness of compounds to provide protection against cytotoxic agents. In the case of the novel antioxidants, the subject of this patent, this can be achieved by pre-incubation of the cells with the antioxidant agent prior to induction of oxidative stress by *tert*-butyl hydroperoxide. Thus a comparison of the amount of formazan produced by control cells against cells with the cytotoxic peroxide present, plus or minus pre-incubation with the novel antioxidant, can determine the extent by which the compound can retain cell viability.

Mechanism of MTT assay



MTT (yellow) **formazan** (purple)

(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)

A 96-well plate was seeded with approximately 20,000 mouse embryonic stem cells per well and left to grow at 37°C (5% CO_2) overnight. Antioxidants, denoted as Exp.1, Exp.2 and Exp.3, were dissolved in DMSO and added to the wells in triplicate 30 min before induction of oxidative stress with *tert*-butyl hydroperoxide. The resultant concentrations of the tested antioxidants in the wells were 0.1, 0.5 and 1 μM. Controls were also initiated using cell medium ($DMEM^+$) / DMSO. After the 30 min pre-incubation period, *tert*-butyl peroxide was added to the wells to provide a final concentration of 400 μM. The plated cells were returned to the incubator (37 °C) for 90 min. After this period, 20μL of the MTT reagent solution was added to all of the wells. The plate was then further incubated for a further 3 hours. The supernatant was removed from the wells and 200μl DMSO added to all wells to solubilise reduced MTT (formazan). The plate was protected from light and shaken for 10 min. The supernatant was removed from the wells and aliquoted into a new 96-well plate for the colorometric measurements. Absorbance was determined at 540 nm.

Results and discussion

The average corrected absorbance (0.737) for the $DMEM^+$ control (which contained no antioxidant compounds or *tert*-butyl hydroperoxide) represented the 100% viability benchmark. Comparison of the corrected absorbances in the various well treatment regimes relative to that of the $DMEM^+$ control enabled the resultant effects on cell viability to be calculated. tBHP = *tert*-butyl hydroperoxide.

From Table 1, exposure to *tert*-butyl hydroperoxide reduces cell viability to 37% of the control. At a concentration of 1 μM in the incubation medium without the peroxide present, neither Exp.1 nor Exp.2 had a negative impact on viability indicating that neither compound induced cytotoxicity at that level of exposure.

Exp.1, Exp.2 and Exp.3 all exhibited a strong protective effect from the oxidative stress-induced cytotoxicity of *tert*-butyl hydroperoxide. Exp.2 was of particular note in this respect and was able to almost fully reverse the loss of cell viability induced by *tert*-butyl hydroperoxide (96.9% +/- 3.9 sd). A concentration dependence effect was observed.

In respect of structure activity relationships, it is evident that the dodecyl chain of Exp.2 imparts greater efficacy than the octyl chain of Exp.3. A key finding of these results is in relation to the literature on

natural flavonoids in which a number of authors have alluded to the importance of the A-ring of the flavonoid as being an integral component of the antioxidant activity of the molecule (as exemplified in "Structure-Antioxidant Activity Relationships Of Flavonoids And Phenolic Acids; Rice-Evans et al.; Free Radical Biology & Medicine, Vol. 20, No. 7, pp 933-956, 1996")

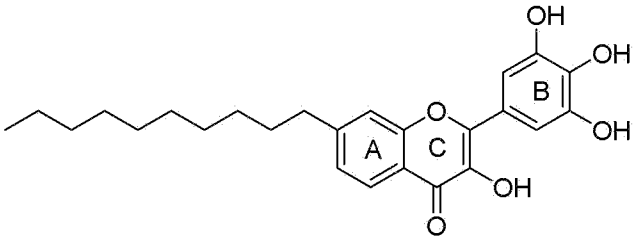
The data presented here unequivocally demonstrates that the A-ring constituent is not pre-requisite and can be completely removed from the molecule with retention of potent antioxidant bio-activity. Comparison of Exp.1 relative to Exp.2 in this cell viability oxidative stress assay indicates that the activity may not just be preserved by removal of the A-ring, but enhanced.

Table 1

Treatments	Ave. Absorbance	Ave. Corrected Absorbance	Std Dev. (±)	Ave. Cell Viability (%)	Std Dev. (±)
Controls					
DMEM$^+$	0.811	0.737	0.027	100.0	3.6
400µM tBHP	0.347	0.273	0.010	37.0	1.3
1µM Exp.1 (No tBHP)	0.866	0.791	0.015	107.4	2.0
1 µM Exp.2 (No tBHP)	0.841	0.767	0.044	104.1	5.9
Exp.1 (µM) 400µM tBHP					
0.1	0.494	0.420	0.042	56.9	5.7
0.5	0.712	0.638	0.041	86.5	5.5
1	0.700	0.625	0.026	84.8	3.5
Exp.2 (µM) 400 µM tBHP					
0.1	0.520	0.446	0.013	60.5	1.7
0.5	0.773	0.699	0.047	94.8	6.4
1	0.788	0.714	0.029	96.9	3.9
Exp.3 (µM) 400 µM tBHP					
0.1	0.328	0.254	0.020	34.4	2.7
0.5	0.483	0.409	0.031	55.5	4.2
1	0.593	0.519	0.026	70.4	3.5

Compounds Tested

Exp.1:



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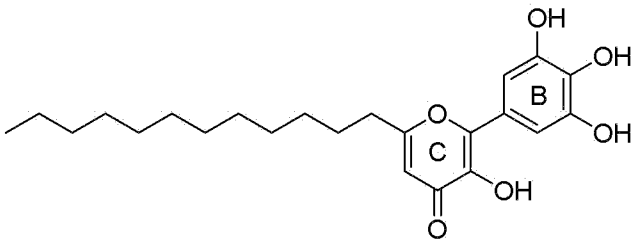
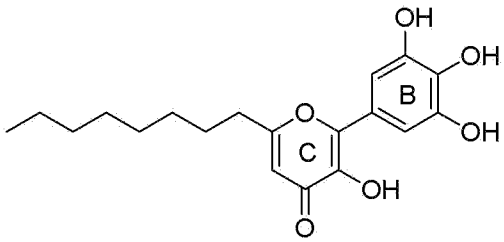
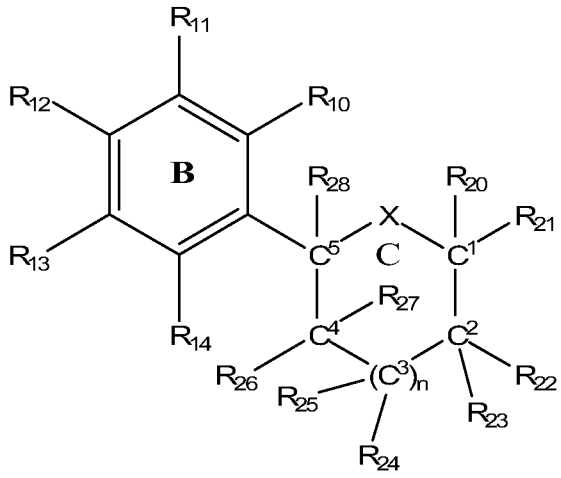


Exp.3:



Claims

1. A method of *in vitro* preservation of living animal cells in a viable non-terminally differentiated state, said method comprising contacting living non-terminally differentiated animal cells with a compound of Formula I or a salt thereof:



Formula I

wherein:

A) R_{12} and R_{26} each independently represent –OH or a glycosidic functional group; R_{10}, R_{11}, R_{13}, and R_{14} each independently represent H, -OH, nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, a glycosidic functional group, C_{1-6} alkoxy-, hydroxy-C_{1-6} alkyl-, C_{1-6} alkoxy-C_{1-6} alkyl-, or a saturated or unsaturated C_{1-6} hydrocarbon chain which may be substituted with one or more of nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone or aldehyde groups; and wherein ring B comprises no more than one glycosidic functional group;

B) either a):

R_{20} represents H or a C_{2-30} saturated or unsaturated hydrocarbon chain;

R_{21}:

i) represents H;

ii) together with R_{22} provides a second bond between C^1 and C^2; or

iii) when X is $–NR_1$- and R_1 is not H or C_{1-6}alkyl, together with R_1 provides a second bond between C^1 and N;

R_{22}:

i) represents H;

ii) together with R_{23} forms =O; or

iii) together with R_{21} provides a second bond between C^1 and C^2;

R_{23}:

i) represents H or a C_{2-30} saturated or unsaturated hydrocarbon chain;

ii) together with R_{22} forms =O;

wherein at least one of R_{20} and R_{23} is a C_{2-30} saturated or unsaturated hydrocarbon chain;

or b):

R_{20}, R_{21}, R_{22}, and R_{23} form part of a 5, 6 or 7 membered unsaturated-ring including C^1 and C^2, which ring is substituted with a group which is a C_{2-30} saturated or unsaturated hydrocarbon chain, which ring is optionally and independently further substituted with one or more groups selected from nitro,

halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde and saturated or unsaturated C_{2-15} hydrocarbon chain, which C_{2-15} hydrocarbon chain may be substituted with one or more of nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde or nitrone groups;

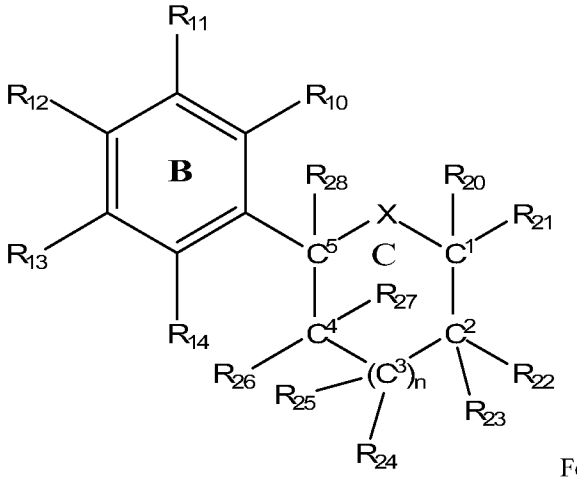
C) n is 0 or 1, wherein when n is 0, either i) R_{27} and R_{28} represent H or ii) R_{27} together with R_{28} provide a second bond between C^4 and C^5; or when n is1, either i) R_{24} and R_{25} together form =O and R_{27} and R_{28} represent H or R_{27} together with R_{28} provide a second bond between C^4 and C^5, or ii) R_{24} and R_{25} represent H and R_{27} and R_{28} represent H or R_{27} together with R_{28} provide a second bond between C^4 and C^5or iii) R_{24} represents H, R_{25} together with R_{27} provide a second bond between C^3 and C^4, R_{28} represents –OH and X is –O-;

D) X is –O-, -S- or $–NR_1$-, wherein R_1 i) represents H or C_{1-6}alkyl, or ii) together with R_{21} provides a second bond between C^1 and N;

wherein said C_{2-30} saturated or unsaturated hydrocarbon chain of R_{20}, R_{23} and the 5, 6 or 7 membered unsaturated ring is optionally and independently substituted with one or more groups selected from C_{1-6} alkyl, C_{1-6}alkoxy, hydroxy-C_{1-6} alkyl, Cl, F, Br, I, -CN, $-CO_2H$, $-CO_2C_{1-6}$alkyl, $-S(O)_2C_{1-6}$alkyl, $-S(O)_2$phenyl, $-SC_{1-6}$alkyl, $-NO_2$, -OH, -CF3, $-N(R_2)(R_3)$, $-NHC(O)NHC_{1-6}$alkyl, $–C(O)N(R_2)(R_3)$, imine and substituted or unsubstituted triphenylphosphonium; and wherein one or more available $-CH_2$- groups present in the C_{2-30} hydrocarbon chain of R_{20}, R_{23} and the 5, 6 or 7 membered unsaturated ring is optionally and independently replaced by -O-, -C(O)-, $-S(O)_p$-, or $-N(R_2)$- provided always that no two such replacements in the resulting chain are consecutive; wherein R_2 and R_3 each independently represent H or C_{1-6}alkyl, and wherein p is 0 to 2;

and wherein the total number of =O on ring C is no greater than 1.

2. A compound of Formula Ia or a salt thereof:



Formula Ia

wherein:

A) R_{12} and R_{26} each independently represent –OH or a glycosidic functional group; R_{10}, R_{11}, R_{13}, and R_{14} each independently represent H, -OH, nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, a glycosidic functional group, C_{1-6} alkoxy-, hydroxy-C_{1-6} alkyl-, C_{1-6} alkoxy-C_{1-6} alkyl-, or a saturated or unsaturated C_{1-6} hydrocarbon chain which may be substituted with one or more of nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone or aldehyde groups; and wherein ring B comprises no more than one glycosidic functional group;

B) either a):

R_{20} represents H or a C_{2-30} saturated or unsaturated hydrocarbon chain;

R_{21}:

i) represents H;

ii) together with R_{22} provides a second bond between C^1 and C^2; or

iii) when X is $-NR_1-$ and R_1 is not H or C_{1-6}alkyl, together with R_1 provides a second bond between C^1 and N;

R_{22}:

i) represents H;

ii) together with R_{23} forms =O; or

iii) together with R_{21} provides a second bond between C^1 and C^2;

R_{23}:

i) represents H or a C_{2-30} saturated or unsaturated hydrocarbon chain;

ii) together with R_{22} forms =O;

wherein at least one of R_{20} and R_{23} is a C_{2-30} saturated or unsaturated hydrocarbon chain;

or b):

R_{20}, R_{21}, R_{22}, and R_{23} form part of a 5, 6 or 7 membered unsaturated-ring including C^1 and C^2, which ring is substituted with a group which is a C_{2-30} saturated or unsaturated hydrocarbon chain, which ring is optionally and independently further substituted with one or more groups selected from nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde and saturated or unsaturated C_{2-15} hydrocarbon chain, which C_{2-15} hydrocarbon chain may be substituted with one or more of nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde or nitrone groups;

C) n is 0 or 1, wherein when n is 0, either i) R_{27} and R_{28} represent H or ii) R_{27} together with R_{28} provide a second bond between C^4 and C^5; or when n is1, either i) R_{24} and R_{25} together form =O and R_{27} and R_{28} represent H or R_{27} together with R_{28} provide a second bond between C^4 and C^5, or ii) R_{24} and R_{25} represent H and R_{27} and R_{28} represent H or R_{27} together with R_{28} provide a second bond between C^4 and C^5or iii) R_{24} represents H, R_{25} together with R_{27} provide a second bond between C^3 and C^4, R_{28} represents –OH and X is –O-;

D) X is –O-, -S- or $-NR_1-$, wherein R_1 i) represents H or C_{1-6}alkyl, or ii) together with R_{21} provides a second bond between C^1 and N;

wherein said C_{2-30} saturated or unsaturated hydrocarbon chain of R_{20}, R_{23} and the 5, 6 or 7 membered unsaturated ring is optionally and independently substituted with one or more groups selected from C_{1-6} alkyl, C_{1-6}alkoxy, hydroxy-C_{1-6} alkyl, Cl, F, Br, I, -CN, $-CO_2H$, $-CO_2C_{1-6}$alkyl, $-S(O)_2C_{1-6}$alkyl, $-S(O)_2$phenyl, $-SC_{1-6}$alkyl, $-NO_2$, -OH, -CF3, $-N(R_2)(R_3)$, $-NHC(O)NHC_{1-6}$alkyl, $-C(O)N(R_2)(R_3)$, imine and substituted or unsubstituted triphenylphosphonium; and wherein one or more available $-CH_2-$ groups present in the C_{2-30} hydrocarbon chain of R_{20}, R_{23} and the 5, 6 or 7 membered unsaturated ring is optionally and independently replaced by -O-, -C(O)-, $-S(O)_p-$, or $-N(R_2)-$ provided always that the resulting chain includes a $-CH_2-$ group connecting to C^1, C^2 or the 5,6 or 7 membered ring and no two such replacements are consecutive; wherein R_2 and R_3 each independently represent H or C_{1-6}alkyl, and wherein p is 0 to 2;

and wherein the total number of =O on ring C is no greater than 1;

provided that when i) n=1, ii) X represents –O-, iii) R_{12} represents –OH, iv) R_{24} together with R_{25} represent =O, v) R_{20}, R_{21}, R_{22} and R_{23} form a benzene ring including C^1 and C^2, and vi) said benzene ring is substituted with at least one group which is a C_{2-30} saturated or unsaturated hydrocarbon chain, then:

said C_{2-30} saturated or unsaturated hydrocarbon chain is substituted with one or more groups selected from C_{1-6}alkoxy, hydroxy-C_{1-6} alkyl, Cl, F, Br, I, -CN, $-CO_2H$, sulphonyl, $-CO_2C_{1-6}$alkyl, $-S(O)_2C_{1-6}$alkyl, $-S(O)_2$phenyl, $-SC_{1-6}$alkyl, $-NO_2$, -OH, $-CF_3$, $-N(R_2)(R_3)$, $-NHC(O)NHC_{1-6}$alkyl, $–C(O)N(R_2)(R_3)$, imine and substituted or unsubstituted triphenylphosphonium; and/or wherein one or more available $-CH_2-$ groups present in said C_{2-30} hydrocarbon chain is replaced by -O-, -C(O)-, $-S(O)_p-$, or $-N(R_2)-$; wherein R_2 and R_3 each independently represent H or C_{1-6}alkyl, and wherein p is 0 to 2; and/or

said benzene ring is substituted with one or more groups selected from nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, ketone, aldehyde and saturated or unsaturated C_{1-6} hydrocarbon chain, which C_{1-6} hydrocarbon chain is substituted with one or more of nitro, halogen, amino, amido, cyano, carboxyl, sulphonyl, hydroxyl, ketone, aldehyde or nitrone groups.

3. A method as claimed in claim 1 or a compound as claimed in claim 2, wherein X represents –O-.

4. A method as claimed in any one of claims 1 or 3, or a compound as claimed in any one of claims 2 or 3, wherein R_{12} and R_{26} both represent –OH.

5. A method as claimed in any one of claims 1 or 3,or a compound as claimed in any one of claims 2 or 3, wherein one of R_{12} and R_{26} represents -OH and the other of R_{12} and R_{26} represents a glycosidic functional group.

6. A method as claimed in any one of claims 1 or 3 to 5, or a compound as claimed in any of claims 2 to 5, wherein n = 1.

7. A method as claimed in any one of claims 1 or 3 to 5, or a compound as claimed in any one of claims 2 to 5, wherein n=0.

8. A method as claimed in any one of claims 1 or 3 to 7, or a compound as claimed in any one of claims 2 to 7, wherein:

R_{20} represents H or a C_{2-30} saturated or unsaturated hydrocarbon chain;

R_{21}:

i) represents H; or

ii) together with R_{22} provides a second bond between C^1 and C^2;

R_{22}:

i) represents H;

ii) together with R_{23} forms =O; or

iii) together with R_{21} provides a second bond between C^1 and C^2; and

R_{23}:

i) represents H or a C_{2-30} saturated or unsaturated hydrocarbon chain; or

ii) together with R_{22} forms =O.

9. A method as claimed in any one of claims 1 or 3 to 7, or a compound as claimed in any one of claims 2 to 7, wherein R_{20}, R_{21}, R_{22} and R_{23} form part of a 5, 6 or 7 membered unsaturated-ring including C^1 and C^2.

10. A method or compound as claimed in claim 9, wherein said unsaturated-ring is substituted with a C_{2-30} saturated or unsaturated hydrocarbon chain at the meta or para position relative to C^1 or wherein said unsaturated ring is substituted with C_{2-15} saturated or unsaturated hydrocarbon chains at two of the ortho, meta and para positions relative to C^1.

11. A method as claimed in claim 1 or in any one of claims 3 to 10 or a compound as claimed in any one of claims 2 to 9, wherein said C_{2-30} saturated or unsaturated hydrocarbon chain of R_{20}, R_{23} and the 5, 6 or 7 membered unsaturated ring includes a $-CH_2-$ group connecting to C^1, C^2 or the 5,6 or 7 membered ring.

12. A method or compound as claimed in claim 11, wherein said C_{2-30} saturated or unsaturated hydrocarbon chain of R_{20}, R_{23} and the 5, 6 or 7 membered unsaturated ring is unsubstituted.

13. A method or compound as claimed in claim 12, wherein said C_{2-30} saturated or unsaturated hydrocarbon chain of R_{20}, R_{23} and the 5, 6 or 7 membered unsaturated ring is saturated.

14. A method or compound as claimed in claim 12 or claim 13, wherein said C_{2-30} saturated or unsaturated hydrocarbon chain of R_{20}, R_{23} and the 5, 6 or 7 membered unsaturated ring is a straight hydrocarbon chain comprising 6 to 15 carbon atoms.

15. A method as claimed in claim 1 or in any one of claim 3 to 14, wherein the living animal cells are stem cells.

16. A method as claimed in claim 15, wherein the stem cells are selected from the group consisting of embryonic stem cells (ESCs), adult stem cells and induced pluripotent stem (iPS) cells.

17. A method as claimed in claim 16, wherein the embryonic stem cells are human embryonic stem cells or mouse embryonic stem cells.

18. A method as claimed in claim 16, wherein the stem cells are induced pluripotent stem (iPS) cells.

19. A method as claimed in claim 1 or any one of claim 3 to 14, wherein the living animal cells are de-differentiated cells.