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(71) Applicants: **TRUSTEES OF DARTMOUTH COLLEGE** [US/US]; Technology Transfer Office, 11 Rope Ferry Road, Hanover, NH 03755 (US). **PRESIDENT AND FELLOWS OF HARVARD COLLEGE** [US/US]; 1300 Massachusetts Avenue, Cambridge, MA 02138 (US). **THE UNITED STATES OF AMERICA, as represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES** [US/US]; Office of Technology Transfer, National Institutes of Health, 6011 Execut-

ive Boulevard, Suite 325, MSC 7660, Bethesda, Maryland 20892-7660 (US). **RUTGERS, THE STATE UNIVERSITY** [US/US]; 3 Rutgers Plaza, New Brunswick, NJ 08901 (US).

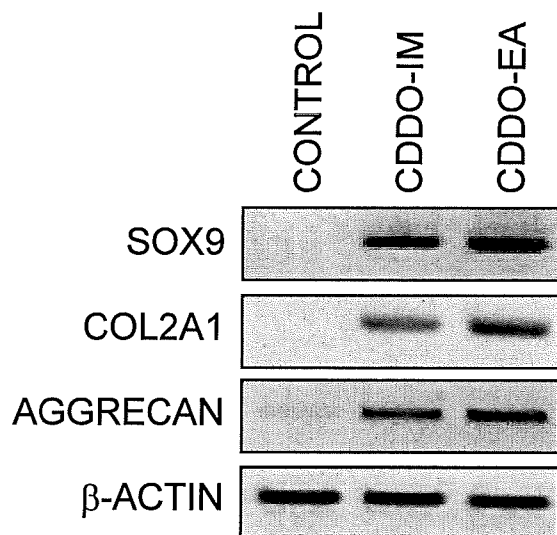
(72) Inventors: **GRIBBLE, Gordon, W.**; 15 Eldridge Street, Lebanon, NH 03755 (US). **MEDICI, Damian**; 25 Shattuck Street, Boston, MA 02115 (US). **ROBEY, Pamela**; 30 Convent Drive, Bethesda, MD 20892 (US). **LIBY, Karen, T.**; 131 North Main Street, West Lebanon, NH 03784 (US). **SPORN, Michael, B.**; 9 Sporn Drive, Tunbridge, VT 05077 (US). **SUH, Nanjoo**; 2303 Johnson Circle, Bridgewater, NJ 08807 (US).

(74) Agents: **LICATA, Jane, Massey et al.**; Licata & Tyrrell P.C., 66 E. Main Street, Marlton, NJ 08053 (US).

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(54) Title: METHODS OF INDUCING CHONDROGENESIS IN MESENCHYMAL STEM CELLS USING SYNTHETIC TRITERPENOID

**FIG. 2**

(57) Abstract: The present invention relates to methods for enhancing differentiation of mesenchymal stem cells into chondrocytes and/or inducing chondrogenesis. The invention also relates to applications in the treatment of diseases which can affect cartilage (chondrodystrophies). The present invention also relates to methods of treatment comprising establishing a population of chondrocytes from a population of mesenchymal stem cells, which have been induced to differentiate with a synthetic triterpenoid, and administering the population of cells to a patient.



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METHODS OF INDUCING CHONDROGENESIS IN MESENCHYMAL STEM CELLS USING SYNTHETIC TRITERPENOIDs

BACKGROUND OF THE INVENTION

5 This invention was made with government support under grant number R01 CA78814 from the National Institutes of Health. The government has certain rights in the invention.

I. Field of the Invention

 The present invention relates generally to the fields of biology and medicine.
10 More particularly, it concerns methods for enhancing differentiation of mesenchymal stem cells into chondrocytes and/or inducing chondrogenesis. The invention also relates to applications in the treatment of diseases which can affect cartilage (chondrodystrophies).

II. Description of Related Art

15 Triterpenoids, biosynthesized in plants by the cyclization of squalene, are used for medicinal purposes in many Asian countries; and some, like ursolic and oleanolic acids, are known to be anti-inflammatory and anti-carcinogenic (Huang *et al.*, 1994; Nishino *et al.*, 1988). However, the biological activity of these naturally-occurring molecules is relatively weak, and therefore the synthesis of new analogs to enhance their
20 potency was undertaken (Honda *et al.*, 1997; Honda *et al.*, 1998). An ongoing effort for the improvement of anti-inflammatory and antiproliferative activity of oleanolic and ursolic acid analogs led to the discovery of 2-cyano-3,12-dioxooleane-1,9(11)-dien-28-oic acid (CDDO) and related compounds (Honda *et al.*, 1997, 1998, 1999, 2000a, 2000b, 2002; Suh *et al.*, 1998; 1999; 2003; Place *et al.*, 2003; Liby *et al.*, 2005). Several potent
25 derivatives of oleanolic acid were identified, including methyl-2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO-Me).

 CDDO-Me suppresses the induction of several important inflammatory mediators, such as iNOS, COX-2, TNF α , and IFN γ , in activated macrophages. CDDO-Me has also been reported to activate the Keap1/Nrf2/ARE signaling pathway
30 resulting in the production of several anti-inflammatory and antioxidant proteins, such as heme oxygenase-1 (HO-1). These properties have made CDDO-Me a candidate for the treatment of neoplastic and proliferative diseases, such as cancer. Moreover, synthetic triterpenoids have been found to induce apoptosis and differentiation and inhibit

proliferation in human leukemia cells (Ikeda *et al.*, 2003; Konopleva *et al.*, 2002; Suh *et al.*, 1999; Ito *et al.*, 2000), induce osteoblastic differentiation in osteosarcoma cells (Ito *et al.*, 2001), enhance neuronal growth factor-induced neuronal differentiation of rat PC12 pheochromocytoma cells, and induce adipogenic differentiation of fibroblasts into adipocytes (Suh *et al.*, 1999). CDDO-Me has also been found an effective drug for improving kidney function in patients suffering for renal/kidney disease using CDDO-Me (U.S. Patent Application Publication 2009/0326063).

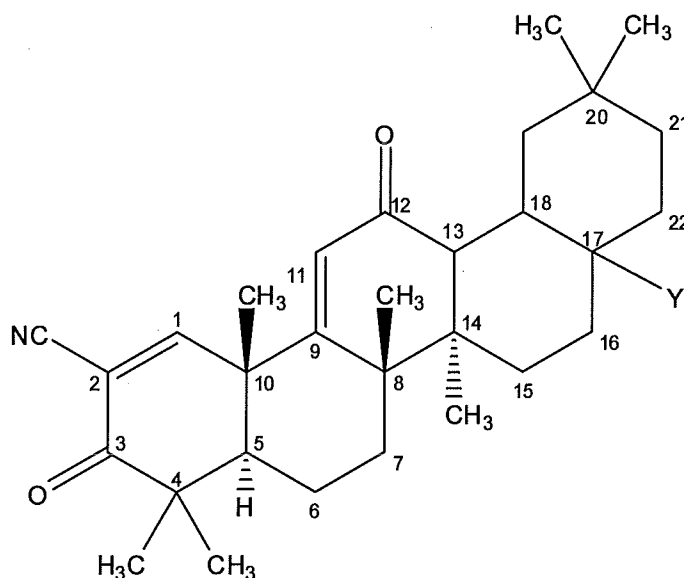
Synthetic triterpenoids may also be used for the treatment of bone and cartilage-related diseases. U.S Patent Application Publication 2008/0233195, which is incorporated by reference herein, describes methods for stimulating a bone- and/or cartilage forming cell comprising: (a) providing a bone- or cartilage-producing cell or precursor thereof; (b) contacting said cell with a synthetic triterpenoid. Chondrocytes isolated from the superficial zone of articular cartilage of femoral condyles were exposed to synthetic triterpenoids, which is shown to up-regulate superficial protein (SZP) secretion from superficial zone chondrocytes at low concentrations. However, as promising as these results are, it remains important to find new ways to induce chondrogenesis, for many practical applications. Chondrogenesis is the process by which cartilage is formed from mesenchymal tissue. During chondrogenesis, such tissue differentiates into chondroblasts and begins secreting the molecules that form the extracellular matrix.

There are several diseases which can affect the cartilage, osteoarthritis, achondroplasia, costochondritis and relapsing polychondritis. Tumors made up of cartilage tissue, either benign or malignant, can also occur. Benign tumors are called chondroma, the malignant ones are called chondrosarcomas.

Cartilage has limited repair capabilities because chondrocytes are bound and cannot typically migrate to damaged areas. Also, some forms of cartilage does not have blood supply, making the deposition of new matrix slow. Accordingly, there is a significant need for new cartilage repair treatments. Thus agents that can be used to induce chondrogenesis and/or enhance the differentiation of cells into chondrocytes would represent a significant advance.

SUMMARY OF THE INVENTION

In some aspects of the present invention, there are provided methods of stimulate mesenchymal stem cells in a manner that enhances their differentiation into chondrocytes and/or induces chondrogenesis. For example, the present invention provides methods of inducing differentiation of a mesenchymal stem cell into a chondrocyte, comprising contacting a mesenchymal stem cell with a sufficient amount of a synthetic triterpenoid, wherein the synthetic triterpenoid is a compound of the formula:



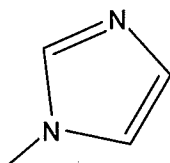
wherein Y is:

10 $-\text{CN}$ or $-\text{C}(\text{O})\text{R}$, wherein R is hydroxy, amino, alkoxy_(C≤8), alkylamino_(C≤8), substituted alkylamino_(C≤8), or heteroaryl_(C≤8);

or a pharmaceutically-acceptable salt or tautomer of the formula.

In some embodiments, the mesenchymal stem cell is a bone marrow-derived mesenchymal stem cell. In some embodiments, the mesenchymal stem cell was isolated prior to contacting with the synthetic triterpenoid.

In some embodiments, R is hydroxy, methoxy, ethyl-amino, or



In some embodiments, the synthetic triterpenoid is CDDO-Im. In others, it is CDDO-EA.

In some embodiments, the method further comprises contacting the mesenchymal stem cell with a differentiating medium. In some embodiments, the method further comprises contacting the mesenchymal stem cell with a co-factor.

5 In some embodiments, the stem cell is contacted with the synthetic triterpenoid *in vitro*. In some embodiments, the stem cell is contacted with the synthetic triterpenoid *in vivo*. In some embodiments, the sufficient amount of the synthetic triterpenoid is from 1 nM to 1 μ M.

10 In some embodiments, the method further comprises incubating the mesenchymal stem cell with a growth factor. In some embodiments, the growth factor is TGF- β 1, TGF- β 2, TGF- β 1.2, VEGF, insulin-like growth factor I or II, BMP2, BMP4, or BMP7. In some embodiments, the growth factor is parathyroid hormone, calcitonin, interleukin-6, or interleukin-11.

15 In some embodiments, the method further comprising culturing the mesenchymal stem cell before or after contacting it with the synthetic triterpenoid. In some embodiments, the method further comprises purifying the mesenchymal stem cell before or after contacting it with the synthetic triterpenoid. In some embodiments, the method further comprises implanting the resulting chondrocyte *in vivo*.

20 In some embodiments, the method further comprises implanting the resulting chondrocyte to a patient as part of a treatment for a cartilage-related disease. In some embodiments, the cartilage-related disease is osteoarthritis, achondrogenesis, chondrodysplasia, SED congenita, Langer-Saldino achondrogenesis, Kniest dysplasia, Stickler syndrome type I, or spondyloepimetaphyseal dysplasia Strudwick type.

25 In some embodiments, the method further comprises inducing within the mesenchymal stem cell the expression of a cellular marker associated with chondrogenesis. In some embodiments, the cellular marker is SOX9, COL2A1, or aggrecan.

30 In some embodiments, the method further comprises increasing or up-regulating within the mesenchymal stem cell the level of TGF- β , BMP2, BMP4, SMAD3, SMAD4, SMAD6, SMAD7, TIMP-1 or TIMP-2. In some embodiments, the method further comprises decreasing or down-regulating within the mesenchymal stem cell the level of MMP-9.

Other objects, features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that

the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description. Note that simply
5 because a particular compound is ascribed to one particular generic formula does not mean that it cannot also belong to another generic formula.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color.
10 Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure. The invention may be better understood by reference to one of these drawings in combination with the detailed
15 description of specific embodiments presented herein.

FIGS. 1a & b – CDDO-Im and CDDO-EA Induce Chondrogenesis in Newborn Mouse Calvaria. FIG. 1a shows the histological analysis where the metachromatic toluidine blue purple staining is indicative of the formation of proteoglycans, such as aggrecan, which are characteristic of cartilage and the toluidine
20 blue staining is indicative of the formation of bone; FIG. 1b shows the immunohistochemical analysis where red fluorescence is indicative of the formation of type II collagen (COL2A1).

FIG. 2 – CDDO-Im and CDDO-EA Induce Expression of Chondrocyte Markers in Bone Marrow-Derived Mesenchymal Stem Cells. Immunoblotting for the
25 antibodies SOX9, COL2A1, Aggrecan, and β -actin show that there is a clear induction of the chondrocyte markers SOX9, COL2A1, and Aggrecan in cells treated with CDDO-Im or CDDO-EA.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

30 The current invention provides methods of using synthetic triterpenoids that may be used, for example, to induce the differentiation of bone-marrow derived mesenchymal stem cells into chondrocytes and/or induces chondrogenesis. For example, CDDO-

Imidazolidine, induces chondrocytic differentiation of bone marrow-derived stem cells. This combination of effects represents a significant advance in the state of the art. These and other aspects of the invention are described in greater detail below.

I. Definitions

Mesenchymal stem cells, or MSC, are multipotent stem cells, derived from different tissues, that can differentiate into a variety of cell types, including: osteoblasts (bone cells), chondrocytes (cartilage cells), adipocytes (fat cells), and myocytes (muscle cells). As used herein the term mesenchymal stem cell encompasses the term adherent stromal cell."

The term "differentiation," as used herein, refers to the developmental process wherein an unspecialized or less specialized cell becomes more specialized for a specific function, such as, for example, the process by which a mesenchymal stem cell becomes a more specialized cell such as a cartilage cell, a bone cell, a muscle cell, or a fat cell. Differentiation can be assessed by identifying lineage-specific markers, such as, for example, aggrecan, a proteoglycan specific for cartilage, or SOX9, a transcription factor specific for cartilage.

When used in the context of a chemical group, "hydrogen" means $-H$; "hydroxy" means $-OH$; "oxo" means $=O$; "halo" means independently $-F$, $-Cl$, $-Br$ or $-I$; "amino" means $-NH_2$ (see below for definitions of groups containing the term amino, *e.g.*, alkylamino); "hydroxyamino" means $-NHOH$; "nitro" means $-NO_2$; imino means $=NH$ (see below for definitions of groups containing the term imino, *e.g.*, alkylimino); "cyano" means $-CN$; "isocyanate" means $-N=C=O$; "azido" means $-N_3$; in a monovalent context "phosphate" means $-OP(O)(OH)_2$ or a deprotonated form thereof; in a divalent context "phosphate" means $-OP(O)(OH)O-$ or a deprotonated form thereof; "mercapto" means $-SH$; "thio" means $=S$; and "sulfamoyl" means $-S(O)_2NH_2$.

In the context of chemical formulas, the symbol $-$ means a single bond, $=$ means a double bond, and $\equiv$ means triple bond. The symbol $----$ represents an optional bond, which if present is either single or double. The symbol $=====$ represents

a single bond or a double bond. Thus, for example, the structure  includes the

structures , , ,  and . As will be understood by a person of skill in the art, no one such ring atom forms part of more than one double bond. The

symbol “”, when drawn perpendicularly across a bond indicates a point of attachment of the group. It is noted that the point of attachment is typically only identified in this manner for larger groups in order to assist the reader in rapidly and unambiguously identifying a point of attachment. The symbol “” means a single bond where the group attached to the thick end of the wedge is “out of the page.” The symbol “” means a single bond where the group attached to the thick end of the wedge is “into the page”. The symbol “” means a single bond where the conformation (*e.g.*, either *R* or *S*) or the geometry is undefined (*e.g.*, either *E* or *Z*).

Any undefined valency on an atom of a structure shown in this application implicitly represents a hydrogen atom bonded to the atom.

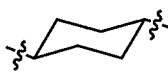
For the groups and classes below, the following parenthetical subscripts further define the group/class as follows: “(C_n)” defines the exact number (*n*) of carbon atoms in the group/class. “(C_{≤n})” defines the maximum number (*n*) of carbon atoms that can be in the group/class, with the minimum number as small as possible for the group in question, *e.g.*, it is understood that the minimum number of carbon atoms in the group “alkenyl_(C_{≤8})” or the class “alkene_(C_{≤8})” is two. For example, “alkoxy_(C_{≤10})” designates those alkoxy groups having from 1 to 10 carbon atoms (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, or any range derivable therein (*e.g.*, 3 to 10 carbon atoms)). (C_n-*n'*) defines both the minimum (*n*) and maximum number (*n'*) of carbon atoms in the group. Similarly, “alkyl_(C₂₋₁₀)” designates those alkyl groups having from 2 to 10 carbon atoms (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10, or any range derivable therein (*e.g.*, 3 to 10 carbon atoms)).

The term “saturated” as used herein means the compound or group so modified has no carbon-carbon double and no carbon-carbon triple bonds, except as noted below. The term does not preclude carbon-heteroatom multiple bonds, for example a carbon oxygen double bond or a carbon nitrogen double bond. Moreover, it does not preclude a carbon-carbon double bond that may occur as part of keto-enol tautomerism or imine/enamine tautomerism.

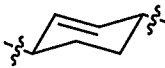
The term “aliphatic” when used without the “substituted” modifier signifies that the compound/group so modified is an acyclic or cyclic, but non-aromatic hydrocarbon compound or group. In aliphatic compounds/groups, the carbon atoms can be joined together in straight chains, branched chains, or non-aromatic rings (alicyclic). Aliphatic compounds/groups can be saturated, that is joined by single bonds (alkanes/alkyl), or unsaturated, with one or more double bonds (alkenes/alkenyl) or with one or more triple

bonds (alkynes/alkynyl). When the term “aliphatic” is used without the “substituted” modifier only carbon and hydrogen atoms are present. When the term is used with the “substituted” modifier one or more hydrogen atom has been independently replaced by $-\text{OH}$, $-\text{F}$, $-\text{Cl}$, $-\text{Br}$, $-\text{I}$, $-\text{NH}_2$, $-\text{NO}_2$, $-\text{CO}_2\text{H}$, $-\text{CO}_2\text{CH}_3$, $-\text{CN}$, $-\text{SH}$, $-\text{OCH}_3$,
 5 $-\text{OCH}_2\text{CH}_3$, $-\text{C}(\text{O})\text{CH}_3$, $-\text{N}(\text{CH}_3)_2$, $-\text{C}(\text{O})\text{NH}_2$, $-\text{OC}(\text{O})\text{CH}_3$, or $-\text{S}(\text{O})_2\text{NH}_2$.

The term “alkyl” when used without the “substituted” modifier refers to a monovalent saturated aliphatic group with a carbon atom as the point of attachment, a linear or branched, cyclo, cyclic or acyclic structure, and no atoms other than carbon and hydrogen. Thus, as used herein cycloalkyl is a subset of alkyl. The groups $-\text{CH}_3$ (Me),
 10 $-\text{CH}_2\text{CH}_3$ (Et), $-\text{CH}_2\text{CH}_2\text{CH}_3$ (*n*-Pr), $-\text{CH}(\text{CH}_3)_2$ (*iso*-Pr), $-\text{CH}(\text{CH}_2)_2$ (cyclopropyl), $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$ (*n*-Bu), $-\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$ (*sec*-butyl), $-\text{CH}_2\text{CH}(\text{CH}_3)_2$ (*iso*-butyl), $-\text{C}(\text{CH}_3)_3$ (*tert*-butyl), $-\text{CH}_2\text{C}(\text{CH}_3)_3$ (*neo*-pentyl), cyclobutyl, cyclopentyl, cyclohexyl, and cyclohexylmethyl are non-limiting examples of alkyl groups. The term “alkanediyl” when used without the “substituted” modifier refers to a divalent saturated aliphatic
 15 group, with one or two saturated carbon atom(s) as the point(s) of attachment, a linear or branched, cyclo, cyclic or acyclic structure, no carbon-carbon double or triple bonds, and no atoms other than carbon and hydrogen. The groups, $-\text{CH}_2-$ (methylene), $-\text{CH}_2\text{CH}_2-$,

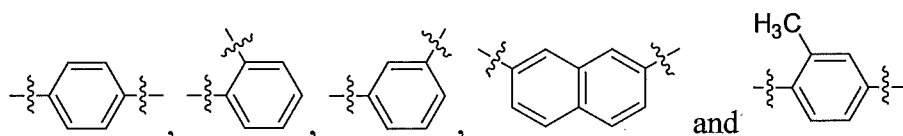
$-\text{CH}_2\text{C}(\text{CH}_3)_2\text{CH}_2-$, $-\text{CH}_2\text{CH}_2\text{CH}_2-$, and , are non-limiting examples of
 alkanediyl groups.

The term “alkylidene” when used without the “substituted” modifier
 20 refers to the divalent group $=\text{CRR}'$ in which R and R' are independently hydrogen, alkyl, or R and R' are taken together to represent an alkanediyl having at least two carbon atoms. Non-limiting examples of alkylidene groups include: $=\text{CH}_2$, $=\text{CH}(\text{CH}_2\text{CH}_3)$, and $=\text{C}(\text{CH}_3)_2$. When any of these terms is used with the “substituted” modifier one or more hydrogen atom has been independently replaced by $-\text{OH}$, $-\text{F}$, $-\text{Cl}$, $-\text{Br}$, $-\text{I}$, $-\text{NH}_2$,
 25 $-\text{NO}_2$, $-\text{CO}_2\text{H}$, $-\text{CO}_2\text{CH}_3$, $-\text{CN}$, $-\text{SH}$, $-\text{OCH}_3$, $-\text{OCH}_2\text{CH}_3$, $-\text{C}(\text{O})\text{CH}_3$, $-\text{N}(\text{CH}_3)_2$, $-\text{C}(\text{O})\text{NH}_2$, $-\text{OC}(\text{O})\text{CH}_3$, or $-\text{S}(\text{O})_2\text{NH}_2$. The following groups are non-limiting examples of substituted alkyl groups: $-\text{CH}_2\text{OH}$, $-\text{CH}_2\text{Cl}$, $-\text{CF}_3$, $-\text{CH}_2\text{CN}$, $-\text{CH}_2\text{C}(\text{O})\text{OH}$, $-\text{CH}_2\text{C}(\text{O})\text{OCH}_3$, $-\text{CH}_2\text{C}(\text{O})\text{NH}_2$, $-\text{CH}_2\text{C}(\text{O})\text{CH}_3$, $-\text{CH}_2\text{OCH}_3$, $-\text{CH}_2\text{OC}(\text{O})\text{CH}_3$, $-\text{CH}_2\text{NH}_2$, $-\text{CH}_2\text{N}(\text{CH}_3)_2$, and $-\text{CH}_2\text{CH}_2\text{Cl}$. The term “fluoroalkyl” is a subset of
 30 substituted alkyl, in which one or more hydrogen has been substituted with a fluoro group and no other atoms aside from carbon, hydrogen and fluorine are present. The groups, $-\text{CH}_2\text{F}$, $-\text{CF}_3$, and $-\text{CH}_2\text{CF}_3$ are non-limiting examples of fluoroalkyl groups. An “alkane” refers to the compound $\text{H}-\text{R}$, wherein R is alkyl.

The term “alkenyl” when used without the “substituted” modifier refers to an monovalent unsaturated aliphatic group with a carbon atom as the point of attachment, a linear or branched, cyclo, cyclic or acyclic structure, at least one nonaromatic carbon-carbon double bond, no carbon-carbon triple bonds, and no atoms other than carbon and hydrogen. Non-limiting examples of alkenyl groups include: $-\text{CH}=\text{CH}_2$ (vinyl), $-\text{CH}=\text{CHCH}_3$, $-\text{CH}=\text{CHCH}_2\text{CH}_3$, $-\text{CH}_2\text{CH}=\text{CH}_2$ (allyl), $-\text{CH}_2\text{CH}=\text{CHCH}_3$, and $-\text{CH}=\text{CH}-\text{C}_6\text{H}_5$. The term “alkenediyl” when used without the “substituted” modifier refers to a divalent unsaturated aliphatic group, with two carbon atoms as points of attachment, a linear or branched, cyclo, cyclic or acyclic structure, at least one nonaromatic carbon-carbon double bond, no carbon-carbon triple bonds, and no atoms other than carbon and hydrogen. The groups, $-\text{CH}=\text{CH}-$, $-\text{CH}=\text{C}(\text{CH}_3)\text{CH}_2-$, $-\text{CH}=\text{CHCH}_2-$, and , are non-limiting examples of alkenediyl groups. When these terms are used with the “substituted” modifier one or more hydrogen atom has been independently replaced by $-\text{OH}$, $-\text{F}$, $-\text{Cl}$, $-\text{Br}$, $-\text{I}$, $-\text{NH}_2$, $-\text{NO}_2$, $-\text{CO}_2\text{H}$, $-\text{CO}_2\text{CH}_3$, $-\text{CN}$, $-\text{SH}$, $-\text{OCH}_3$, $-\text{OCH}_2\text{CH}_3$, $-\text{C}(\text{O})\text{CH}_3$, $-\text{N}(\text{CH}_3)_2$, $-\text{C}(\text{O})\text{NH}_2$, $-\text{OC}(\text{O})\text{CH}_3$, or $-\text{S}(\text{O})_2\text{NH}_2$. The groups, $-\text{CH}=\text{CHF}$, $-\text{CH}=\text{CHCl}$ and $-\text{CH}=\text{CHBr}$, are non-limiting examples of substituted alkenyl groups. An “alkene” refers to the compound $\text{H}-\text{R}$, wherein R is alkenyl.

The term “alkynyl” when used without the “substituted” modifier refers to an monovalent unsaturated aliphatic group with a carbon atom as the point of attachment, a linear or branched, cyclo, cyclic or acyclic structure, at least one carbon-carbon triple bond, and no atoms other than carbon and hydrogen. As used herein, the term alkynyl does not preclude the presence of one or more non-aromatic carbon-carbon double bonds. The groups, $-\text{C}\equiv\text{CH}$, $-\text{C}\equiv\text{CCH}_3$, and $-\text{CH}_2\text{C}\equiv\text{CCH}_3$, are non-limiting examples of alkynyl groups. The term “alkynediyl” when used without the “substituted” modifier refers to a divalent unsaturated aliphatic group, with two carbon atoms as points of attachment, a linear or branched, cyclo, cyclic or acyclic structure, at least one carbon-carbon triple bond, and no atoms other than carbon and hydrogen. When these terms are used with the “substituted” modifier one or more hydrogen atom has been independently replaced by $-\text{OH}$, $-\text{F}$, $-\text{Cl}$, $-\text{Br}$, $-\text{I}$, $-\text{NH}_2$, $-\text{NO}_2$, $-\text{CO}_2\text{H}$, $-\text{CO}_2\text{CH}_3$, $-\text{CN}$, $-\text{SH}$, $-\text{OCH}_3$, $-\text{OCH}_2\text{CH}_3$, $-\text{C}(\text{O})\text{CH}_3$, $-\text{N}(\text{CH}_3)_2$, $-\text{C}(\text{O})\text{NH}_2$, $-\text{OC}(\text{O})\text{CH}_3$, or $-\text{S}(\text{O})_2\text{NH}_2$. An “alkyne” refers to the compound $\text{H}-\text{R}$, wherein R is alkynyl.

The term “aryl” when used without the “substituted” modifier refers to a monovalent unsaturated aromatic group with an aromatic carbon atom as the point of attachment, said carbon atom forming part of a one or more six-membered aromatic ring structure, wherein the ring atoms are all carbon, and wherein the group consists of no atoms other than carbon and hydrogen. If more than one ring is present, the rings may be fused or unfused. As used herein, the term does not preclude the presence of one or more alkyl group (carbon number limitation permitting) attached to the first aromatic ring or any additional aromatic ring present. Non-limiting examples of aryl groups include phenyl (Ph), methylphenyl, (dimethyl)phenyl, $-C_6H_4CH_2CH_3$ (ethylphenyl), naphthyl, and the monovalent group derived from biphenyl. The term “arenediyl” when used without the “substituted” modifier refers to a divalent aromatic group, with two aromatic carbon atoms as points of attachment, said carbon atoms forming part of one or more six-membered aromatic ring structure(s) wherein the ring atoms are all carbon, and wherein the monovalent group consists of no atoms other than carbon and hydrogen. As used herein, the term does not preclude the presence of one or more alkyl group (carbon number limitation permitting) attached to the first aromatic ring or any additional aromatic ring present. If more than one ring is present, the rings may be fused or unfused. Non-limiting examples of arenediyl groups include:

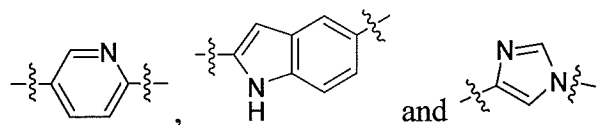


When these terms are used with the “substituted” modifier one or more hydrogen atom has been independently replaced by $-OH$, $-F$, $-Cl$, $-Br$, $-I$, $-NH_2$, $-NO_2$, $-CO_2H$, $-CO_2CH_3$, $-CN$, $-SH$, $-OCH_3$, $-OCH_2CH_3$, $-C(O)CH_3$, $-N(CH_3)_2$, $-C(O)NH_2$, $-OC(O)CH_3$, or $-S(O)_2NH_2$. An “arene” refers to the compound $H-R$, wherein R is aryl.

The term “aralkyl” when used without the “substituted” modifier refers to the monovalent group $-alkanediyl-aryl$, in which the terms alkanediyl and aryl are each used in a manner consistent with the definitions provided above. Non-limiting examples of aralkyls are: phenylmethyl (benzyl, Bn) and 2-phenyl-ethyl. When the term is used with the “substituted” modifier one or more hydrogen atom from the alkanediyl and/or the aryl has been independently replaced by $-OH$, $-F$, $-Cl$, $-Br$, $-I$, $-NH_2$, $-NO_2$, $-CO_2H$, $-CO_2CH_3$, $-CN$, $-SH$, $-OCH_3$, $-OCH_2CH_3$, $-C(O)CH_3$, $-N(CH_3)_2$, $-C(O)NH_2$, $-OC(O)CH_3$, or $-S(O)_2NH_2$. Non-limiting examples of substituted aralkyls are: (3-chlorophenyl)-methyl, and 2-chloro-2-phenyl-eth-1-yl.

The term “heteroaryl” when used without the “substituted” modifier refers to a monovalent aromatic group with an aromatic carbon atom or nitrogen atom as the point of attachment, said carbon atom or nitrogen atom forming part of an aromatic ring structure wherein at least one of the ring atoms is nitrogen, oxygen or sulfur, and wherein the group consists of no atoms other than carbon, hydrogen, aromatic nitrogen, aromatic oxygen and aromatic sulfur. As used herein, the term does not preclude the presence of one or more alkyl group (carbon number limitation permitting) attached to the aromatic ring or any additional aromatic ring present. Non-limiting examples of heteroaryl groups include furanyl, imidazolyl, indolyl, indazolyl (Im), methylpyridyl, oxazolyl, pyridyl, pyrrolyl, pyrimidyl, pyrazinyl, quinolyl, quinazolyl, quinoxaliny, thienyl, and triazinyl.

The term “heteroarenediyl” when used without the “substituted” modifier refers to an divalent aromatic group, with two aromatic carbon atoms, two aromatic nitrogen atoms, or one aromatic carbon atom and one aromatic nitrogen atom as the two points of attachment, said atoms forming part of one or more aromatic ring structure(s) wherein at least one of the ring atoms is nitrogen, oxygen or sulfur, and wherein the divalent group consists of no atoms other than carbon, hydrogen, aromatic nitrogen, aromatic oxygen and aromatic sulfur. As used herein, the term does not preclude the presence of one or more alkyl group (carbon number limitation permitting) attached to the first aromatic ring or any additional aromatic ring present. If more than one ring is present, the rings may be fused or unfused. Non-limiting examples of heteroarenediyl groups include:



When these terms are used with the “substituted” modifier one or more hydrogen atom has been independently replaced by $-\text{OH}$, $-\text{F}$, $-\text{Cl}$, $-\text{Br}$, $-\text{I}$, $-\text{NH}_2$, $-\text{NO}_2$, $-\text{CO}_2\text{H}$, $-\text{CO}_2\text{CH}_3$, $-\text{CN}$, $-\text{SH}$, $-\text{OCH}_3$, $-\text{OCH}_2\text{CH}_3$, $-\text{C}(\text{O})\text{CH}_3$, $-\text{N}(\text{CH}_3)_2$, $-\text{C}(\text{O})\text{NH}_2$, $-\text{OC}(\text{O})\text{CH}_3$, or $-\text{S}(\text{O})_2\text{NH}_2$.

The term “acyl” when used without the “substituted” modifier refers to the group $-\text{C}(\text{O})\text{R}$, in which R is a hydrogen, alkyl, aryl, aralkyl or heteroaryl, as those terms are defined above. The groups, $-\text{CHO}$, $-\text{C}(\text{O})\text{CH}_3$ (acetyl, Ac), $-\text{C}(\text{O})\text{CH}_2\text{CH}_3$, $-\text{C}(\text{O})\text{CH}_2\text{CH}_2\text{CH}_3$, $-\text{C}(\text{O})\text{CH}(\text{CH}_3)_2$, $-\text{C}(\text{O})\text{CH}(\text{CH}_2)_2$, $-\text{C}(\text{O})\text{C}_6\text{H}_5$, $-\text{C}(\text{O})\text{C}_6\text{H}_4\text{CH}_3$, $-\text{C}(\text{O})\text{CH}_2\text{C}_6\text{H}_5$, $-\text{C}(\text{O})(\text{imidazolyl})$ are non-limiting examples of acyl groups. A “thioacyl” is defined in an analogous manner, except that the oxygen atom of the group $-\text{C}(\text{O})\text{R}$ has been replaced with a sulfur atom, $-\text{C}(\text{S})\text{R}$. When either of these terms are

used with the “substituted” modifier one or more hydrogen atom has been independently replaced by $-\text{OH}$, $-\text{F}$, $-\text{Cl}$, $-\text{Br}$, $-\text{I}$, $-\text{NH}_2$, $-\text{NO}_2$, $-\text{CO}_2\text{H}$, $-\text{CO}_2\text{CH}_3$, $-\text{CN}$, $-\text{SH}$, $-\text{OCH}_3$, $-\text{OCH}_2\text{CH}_3$, $-\text{C}(\text{O})\text{CH}_3$, $-\text{N}(\text{CH}_3)_2$, $-\text{C}(\text{O})\text{NH}_2$, $-\text{OC}(\text{O})\text{CH}_3$, or $-\text{S}(\text{O})_2\text{NH}_2$. The groups, $-\text{C}(\text{O})\text{CH}_2\text{CF}_3$, $-\text{CO}_2\text{H}$ (carboxyl), $-\text{CO}_2\text{CH}_3$ (methylcarboxyl),
 5 $-\text{CO}_2\text{CH}_2\text{CH}_3$, $-\text{C}(\text{O})\text{NH}_2$ (carbamoyl), and $-\text{CON}(\text{CH}_3)_2$, are non-limiting examples of substituted acyl groups.

The term “alkoxy” when used without the “substituted” modifier refers to the group $-\text{OR}$, in which R is an alkyl, as that term is defined above. Non-limiting examples of alkoxy groups include: $-\text{OCH}_3$, $-\text{OCH}_2\text{CH}_3$, $-\text{OCH}_2\text{CH}_2\text{CH}_3$, $-\text{OCH}(\text{CH}_3)_2$,
 10 $-\text{OCH}(\text{CH}_2)_2$, $-\text{O}$ -cyclopentyl, and $-\text{O}$ -cyclohexyl. The terms “alkenyloxy”, “alkynyloxy”, “aryloxy”, “aralkoxy”, “heteroaryloxy”, and “acyloxy”, when used without the “substituted” modifier, refers to groups, defined as $-\text{OR}$, in which R is alkenyl, alkynyl, aryl, aralkyl, heteroaryl, and acyl, respectively. Similarly, the term “alkylthio” when used without the “substituted” modifier refers to the group $-\text{SR}$, in
 15 which R is an alkyl, as that term is defined above. When any of these terms is used with the “substituted” modifier one or more hydrogen atom has been independently replaced by $-\text{OH}$, $-\text{F}$, $-\text{Cl}$, $-\text{Br}$, $-\text{I}$, $-\text{NH}_2$, $-\text{NO}_2$, $-\text{CO}_2\text{H}$, $-\text{CO}_2\text{CH}_3$, $-\text{CN}$, $-\text{SH}$, $-\text{OCH}_3$, $-\text{OCH}_2\text{CH}_3$, $-\text{C}(\text{O})\text{CH}_3$, $-\text{N}(\text{CH}_3)_2$, $-\text{C}(\text{O})\text{NH}_2$, $-\text{OC}(\text{O})\text{CH}_3$, or $-\text{S}(\text{O})_2\text{NH}_2$. The term “alcohol” corresponds to an alkane, as defined above, wherein at least one of the
 20 hydrogen atoms has been replaced with a hydroxy group.

The term “alkylamino” when used without the “substituted” modifier refers to the group $-\text{NHR}$, in which R is an alkyl, as that term is defined above. Non-limiting examples of alkylamino groups include: $-\text{NHCH}_3$ and $-\text{NHCH}_2\text{CH}_3$. The term “dialkylamino” when used without the “substituted” modifier refers to the group $-\text{NRR}'$,
 25 in which R and R' can be the same or different alkyl groups, or R and R' can be taken together to represent an alkanediyl. Non-limiting examples of dialkylamino groups include: $-\text{N}(\text{CH}_3)_2$, $-\text{N}(\text{CH}_3)(\text{CH}_2\text{CH}_3)$, and *N*-pyrrolidinyl. The terms “alkoxyamino”, “alkenylamino”, “alkynylamino”, “arylamino”, “aralkylamino”, “heteroarylamino”, and “alkylsulfonylamino” when used without the “substituted” modifier, refers to groups,
 30 defined as $-\text{NHR}$, in which R is alkoxy, alkenyl, alkynyl, aryl, aralkyl, heteroaryl, and alkylsulfonyl, respectively. A non-limiting example of an arylamino group is $-\text{NHC}_6\text{H}_5$. The term “amido” (acylamino), when used without the “substituted” modifier, refers to the group $-\text{NHR}$, in which R is acyl, as that term is defined above. A non-limiting example of an amido group is $-\text{NHC}(\text{O})\text{CH}_3$. The term “alkylimino” when used without

the “substituted” modifier refers to the divalent group =NR, in which R is an alkyl, as that term is defined above. When any of these terms is used with the “substituted” modifier one or more hydrogen atom has been independently replaced by -OH, -F, -Cl, -Br, -I, -NH₂, -NO₂, -CO₂H, -CO₂CH₃, -CN, -SH, -OCH₃, -OCH₂CH₃, -C(O)CH₃,
 5 -N(CH₃)₂, -C(O)NH₂, -OC(O)CH₃, or -S(O)₂NH₂. The groups -NHC(O)OCH₃ and -NHC(O)NHCH₃ are non-limiting examples of substituted amido groups.

The term “alkylphosphate” when used without the “substituted” modifier refers to the group -OP(O)(OH)(OR), in which R is an alkyl, as that term is defined above. Non-limiting examples of alkylphosphate groups include: -OP(O)(OH)(OMe) and
 10 -OP(O)(OH)(OEt). The term “dialkylphosphate” when used without the “substituted” modifier refers to the group -OP(O)(OR)(OR'), in which R and R' can be the same or different alkyl groups, or R and R' can be taken together to represent an alkanediyl. Non-limiting examples of dialkylphosphate groups include: -OP(O)(OMe)₂, -OP(O)(OEt)(OMe) and -OP(O)(OEt)₂. When any of these terms is used with the
 15 “substituted” modifier one or more hydrogen atom has been independently replaced by -OH, -F, -Cl, -Br, -I, -NH₂, -NO₂, -CO₂H, -CO₂CH₃, -CN, -SH, -OCH₃, -OCH₂CH₃, -C(O)CH₃, -N(CH₃)₂, -C(O)NH₂, -OC(O)CH₃, or -S(O)₂NH₂.

The terms “alkylsulfonyl” and “alkylsulfinyl” when used without the “substituted” modifier refers to the groups -S(O)₂R and -S(O)R, respectively, in which
 20 R is an alkyl, as that term is defined above. The terms “alkenylsulfonyl”, “alkynylsulfonyl”, “arylsulfonyl”, “aralkylsulfonyl”, and “heteroarylsulfonyl”, are defined in an analogous manner. When any of these terms is used with the “substituted” modifier one or more hydrogen atom has been independently replaced by -OH, -F, -Cl, -Br, -I, -NH₂, -NO₂, -CO₂H, -CO₂CH₃, -CN, -SH, -OCH₃, -OCH₂CH₃, -C(O)CH₃,
 25 -N(CH₃)₂, -C(O)NH₂, -OC(O)CH₃, or -S(O)₂NH₂.

The use of the word “a” or “an,” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.”

30 Throughout this application, the term “about” is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study patients.

The terms “comprise,” “have” and “include” are open-ended linking verbs. Any forms or tenses of one or more of these verbs, such as “comprises,” “comprising,” “has,”

“having,” “includes” and “including,” are also open-ended. For example, any method that “comprises,” “has” or “includes” one or more steps is not limited to possessing only those one or more steps and also covers other unlisted steps.

5 The term “effective,” as that term is used in the specification and/or claims, means adequate to accomplish a desired, expected, or intended result.

The term “hydrate” when used as a modifier to a compound means that the compound has less than one (*e.g.*, hemihydrate), one (*e.g.*, monohydrate), or more than one (*e.g.*, dihydrate) water molecules associated with each compound molecule, such as in solid forms of the compound.

10 As used herein, the term “IC₅₀” refers to an inhibitory dose which is 50% of the maximum response obtained. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological, biochemical or chemical process (or component of a process, *i.e.* an enzyme, cell, cell receptor or microorganism) by half.

15 An “isomer” of a first compound is a separate compound in which each molecule contains the same constituent atoms as the first compound, but where the configuration of those atoms in three dimensions differs.

As used herein, the term “patient” or “subject” refers to a living mammalian organism, such as a human, monkey, cow, sheep, goat, dog, cat, mouse, rat, guinea pig, 20 or transgenic species thereof. In certain embodiments, the patient or subject is a primate. Non-limiting examples of human patients are adults, juveniles, infants and fetuses.

As generally used herein “pharmaceutically acceptable” refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues, organs, and/or 25 bodily fluids of human beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit/risk ratio.

“Pharmaceutically acceptable salts” means salts of compounds of the present invention which are pharmaceutically acceptable, as defined above, and which possess 30 the desired pharmacological activity. Such salts include acid addition salts formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like; or with organic acids such as 1,2-ethanedisulfonic acid, 2-hydroxyethanesulfonic acid, 2-naphthalenesulfonic acid, 3-phenylpropionic acid, 4,4'-methylenebis(3-hydroxy-2-ene-1-carboxylic acid), 4-methylbicyclo[2.2.2]oct-2-ene-

1-carboxylic acid, acetic acid, aliphatic mono- and dicarboxylic acids, aliphatic sulfuric acids, aromatic sulfuric acids, benzenesulfonic acid, benzoic acid, camphorsulfonic acid, carbonic acid, cinnamic acid, citric acid, cyclopentanepropionic acid, ethanesulfonic acid, fumaric acid, glucoheptonic acid, gluconic acid, glutamic acid, glycolic acid, 5 heptanoic acid, hexanoic acid, hydroxynaphthoic acid, lactic acid, laurylsulfuric acid, maleic acid, malic acid, malonic acid, mandelic acid, methanesulfonic acid, muconic acid, *o*-(4-hydroxybenzoyl)benzoic acid, oxalic acid, *p*-chlorobenzenesulfonic acid, phenyl-substituted alkanolic acids, propionic acid, *p*-toluenesulfonic acid, pyruvic acid, salicylic acid, stearic acid, succinic acid, tartaric acid, tertiarybutylacetic acid, 10 trimethylacetic acid, and the like. Pharmaceutically acceptable salts also include base addition salts which may be formed when acidic protons present are capable of reacting with inorganic or organic bases. Acceptable inorganic bases include sodium hydroxide, sodium carbonate, potassium hydroxide, aluminum hydroxide and calcium hydroxide. Acceptable organic bases include ethanolamine, diethanolamine, triethanolamine, 15 tromethamine, *N*-methylglucamine and the like. It should be recognized that the particular anion or cation forming a part of any salt of this invention is not critical, so long as the salt, as a whole, is pharmacologically acceptable. Additional examples of pharmaceutically acceptable salts and their methods of preparation and use are presented in *Handbook of Pharmaceutical Salts: Properties, and Use* (P. H. Stahl & C. G. 20 Wermuth eds., Verlag Helvetica Chimica Acta, 2002).

“Prevention” or “preventing” includes: (1) inhibiting the onset of a disease in a subject or patient which may be at risk and/or predisposed to the disease but does not yet experience or display any or all of the pathology or symptomatology of the disease, and/or (2) slowing the onset of the pathology or symptomatology of a disease in a subject 25 or patient which may be at risk and/or predisposed to the disease but does not yet experience or display any or all of the pathology or symptomatology of the disease.

“Prodrug” means a compound that is convertible *in vivo* metabolically into an inhibitor according to the present invention. The prodrug itself may or may not also have activity with respect to a given target protein. For example, a compound 30 comprising a hydroxy group may be administered as an ester that is converted by hydrolysis *in vivo* to the hydroxy compound. Suitable esters that may be converted *in vivo* into hydroxy compounds include acetates, citrates, lactates, phosphates, tartrates, malonates, oxalates, salicylates, propionates, succinates, fumarates, maleates, methylene-

bis- β -hydroxynaphthoate, gentisates, isethionates, di-*p*-toluoyltartrates, methane-sulfonates, ethanesulfonates, benzenesulfonates, *p*-toluenesulfonates, cyclohexyl-sulfamates, quinate, esters of amino acids, and the like. Similarly, a compound comprising an amine group may be administered as an amide that is converted by
5 hydrolysis *in vivo* to the amine compound.

“Effective amount,” “Therapeutically effective amount” or “pharmaceutically effective amount” means that amount which, when administered to a subject or patient for treating a disease, is sufficient to effect such treatment for the disease.

“Treatment” or “treating” includes (1) inhibiting a disease in a subject or patient
10 experiencing or displaying the pathology or symptomatology of the disease (*e.g.*, arresting further development of the pathology and/or symptomatology), (2) ameliorating a disease in a subject or patient that is experiencing or displaying the pathology or symptomatology of the disease (*e.g.*, reversing the pathology and/or symptomatology), and/or (3) effecting any measurable decrease in a disease in a subject or patient that is
15 experiencing or displaying the pathology or symptomatology of the disease.

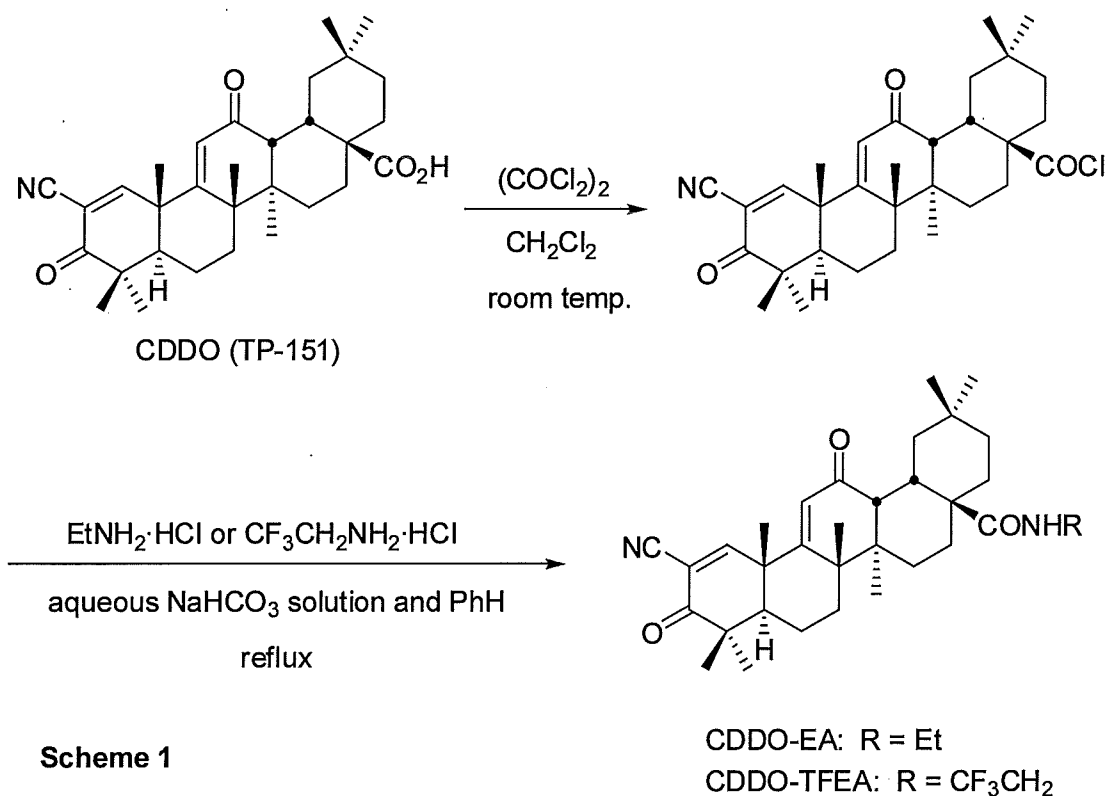
As used herein, the term “water soluble” means that the compound dissolves in water at least to the extent of 0.010 mole/liter or is classified as soluble according to literature precedence.

Other abbreviations used herein are as follows: BMP, bone morphogenic protein;
20 COL2A1, type II collagen ($\alpha 1$); COX-2, cyclooxygenase-2; HO-1, inducible heme oxygenase, IFN γ or IFN- γ , interferon- γ ; iNOS, inducible nitric oxide synthase; MMP, matrix metalloproteinase; MSCs, mesenchymal stem cells; NO, nitric oxide; Nrf2, nuclear factor (erythroid-derived 2)-like 2; SMAD, intracellular proteins that transduce extracellular signals from transforming growth factor beta ligands to the nucleus where they activate downstream TGF- β gene transcription; SOX9, transcription factor SOX-9;
25 TIMP, tissue inhibitor of metalloproteinase; TGF- β , transforming growth factor- β ; TNF α or TNF- α , tumor necrosis factor- α ; VEGF, vascular endothelial growth factor.

The above definitions supersede any conflicting definition in any of the reference that is incorporated by reference herein. The fact that certain terms are defined,
30 however, should not be considered as indicative that any term that is undefined is indefinite. Rather, all terms used are believed to describe the invention in terms such that one of ordinary skill can appreciate the scope and practice the present invention.

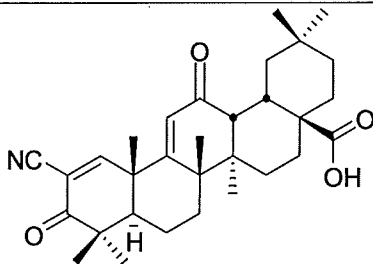
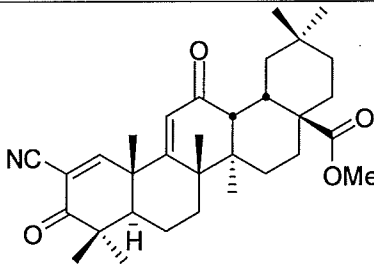
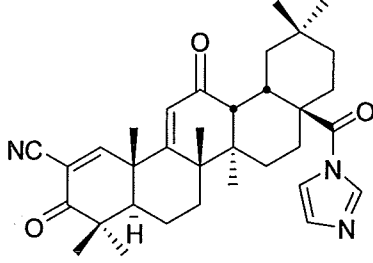
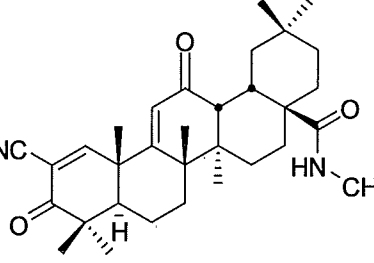
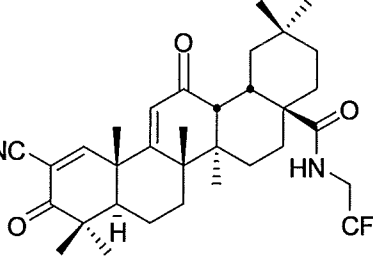
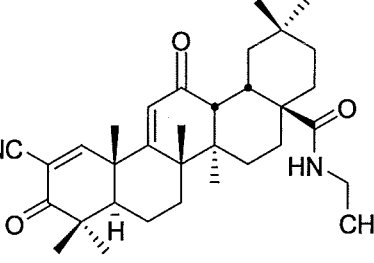
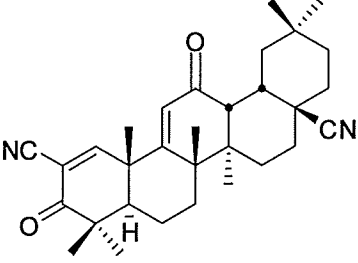
II. Synthesis and Biological Activity of Synthetic Triterpenoids

The compounds of the present invention can be prepared according to the methods taught by Honda *et al.* (1998), Honda *et al.* (2000b), Honda *et al.* (2002), Yates *et al.* (2007), and U.S. Patents 6,326,507 and 6,974,801, which are all incorporated herein by reference. The synthesis of CDDO-MA is discussed in Honda *et al.* (2002), which is incorporated herein by reference. The syntheses of CDDO-EA and CDDO-TFEA are presented in Yates *et al.* (2007), which is incorporated herein by reference, and shown in the Scheme 1 below. These methods can be further modified and optimized using the principles and techniques of organic chemistry as applied by a person skilled in the art. Such principles and techniques are taught, for example, in *March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure* (2007), which is also incorporated by reference herein.



It should be recognized that the particular anion or cation forming a part of any salt of this invention is not critical, so long as the salt, as a whole, is pharmacologically acceptable. Additional examples of pharmaceutically acceptable salts and their methods of preparation and use are presented in *Handbook of Pharmaceutical Salts: Properties, and Use* (2002), which is incorporated herein by reference.

Non-limiting examples of synthetic triterpenoids that may be used in accordance with the methods of this invention are shown here.

 CDDO TP-151 RTA 401	 CDDO-Me bardoxolone methyl (BARD) TP-155 RTA 402
 CDDO-Imidazole CDDO-Im TP-235 RTA 403	 CDDO-Methyl amide CDDO-MA TP-224
 CDDO-Trifluoroethyl amide CDDO-TFEA TP-500 RTA 404	 CDDO-Ethyl amide CDDO-EA TP-319 RTA 405
 TP-225	

Compounds employed in methods of the invention may also exist in prodrug form. Since prodrugs are known to enhance numerous desirable qualities of pharmaceuticals, *e.g.*, solubility, bioavailability, manufacturing, *etc.*, the compounds employed in some methods of the invention may, if desired, be delivered in prodrug form. Thus, the invention contemplates prodrugs of compounds of the present invention

as well as methods of delivering prodrugs. Prodrugs of the compounds employed in the invention may be prepared by modifying functional groups present in the compound in such a way that the modifications are cleaved, either in routine manipulation or *in vivo*, to the parent compound. Accordingly, prodrugs include, for example, compounds
5 described herein in which a hydroxy, amino, or carboxy group is bonded to any group that, when the prodrug is administered to a patient, cleaves to form a hydroxy, amino, or carboxylic acid, respectively.

It should be recognized that the particular anion or cation forming a part of any salt of this invention is not critical, so long as the salt, as a whole, is pharmacologically
10 acceptable. Additional examples of pharmaceutically acceptable salts and their methods of preparation and use are presented in *Handbook of Pharmaceutical Salts: Properties, and Use* (2002), which is incorporated herein by reference.

Compounds employed in methods of the invention may also have the advantage that they may be more efficacious than, be less toxic than, be longer acting than, be more
15 potent than, produce fewer side effects than, be more easily absorbed than, and/or have a better pharmacokinetic profile (*e.g.*, higher oral bioavailability and/or lower clearance) than, and/or have other useful pharmacological, physical, or chemical properties over, compounds known in the prior art for use in the indications stated herein.

III. Cartilage forming Cells

20 In some aspects of the present invention, there are provided *ex vivo* and *in vivo* methods for enhancing differentiation of mesenchymal stem cells into chondrocytes and/or inducing chondrogenesis. The following section describes various sources for these cells, their isolation and characterization. For example, cells can be derived from several different species, including cells of human, bovine, equine, canine, feline and
25 murine origin. Mesenchymal stem cells can generally be derived from humans according to the method taught by Kassem (2004), which is incorporated herein by reference.

Chondrocytes produce and maintain the cartilaginous matrix. From least- to terminally-differentiated, the chondrocytic lineage may be summarized as follows:
30 (a) colony-forming unit-fibroblast (CFU-F), (b) mesenchymal stem cell / marrow stromal cell, (c) chondrocyte, an (d) hypertrophic chondrocyte. When referring to bone or cartilage, mesenchymal stem cells (MSCs) are commonly known as osteochondrogenic (or osteogenic, chondrogenic, osteoprogenitor) cells since a single MSC has been shown

to have the ability to differentiate into chondrocytes or osteoblasts, depending on the medium. *In vivo* differentiation of a MSC in a vascularized area (such as bone) typically yields an osteoblasts, whereas differentiation of a MSC in a non-vascularized area (such as cartilage) typically yields a chondrocyte.

5 The present disclosure provides methods of treating or contacting mesenchymal stem cells with synthetic triterpenoids, including those disclosed herein, *e.g.*, CDDO-Im and CDDO-EA. In some embodiments, these techniques may be used to induce chondrogenic differentiation in mesenchymal stem cells. Induction of chondrogenic differentiation of such cells may be evidenced at least by the induced expression of one
10 or more cellular markers, *e.g.*, SOX9, COL2A1, and aggrecan. For example, the results presented below demonstrate that CDDO-Imidazolide (CDDO-Im) and CDDO-Ethyl amide (CDDO-EA), including at concentrations as low as 200 nM, induce chondrogenesis in organ cultures of newborn mouse calvaria. In some embodiments, concentrations from 1 nM to 100 μ M may be used, including 10 nM, 20 nM, 50 nM,
15 100 nM, 150 nM, 250 nM, 500 nM, 1 μ M, 10 μ M, or 50 μ M.

The cartilage phenotype can be measured histologically, for example, with metachromatic toluidine blue staining for proteoglycans and by immunohistochemical staining for type II collagen. In some embodiments, the methods disclosed herein may be used to up-regulate chondrocyte markers, including SOX9 and type II collagen
20 (alpha1). In some embodiments, the methods described herein may be used to increase one or more of TGF- β ; BMPs 2 and 4; SMADs 3, 4, 6, and 7; and TIMPs-1 and -2. In some embodiments, the methods may be used to down-regulate MMP-9. For example, treatment of human bone marrow-derived mesenchymal stem cells with the synthetic oleanolic triterpenoids, CDDO-Im and CDDO-EA (100 nM), may be used to induced
25 expression of SOX9, COL2A1, and aggrecan.

IV. Cellular Markers

The classic markers of the chondrocytic phenotype are SOX9, collagen II, and aggrecan. See Sive *et al.* (2002), which is incorporated by reference. SOX9 is known to play a major role in chondrocyte differentiation and maintenance of the chondrocytic
30 phenotype. The product of the collagen II gene (*Col2a1*) is an early and practically unique marker of chondrocyte differentiation, and aggrecan is a characteristic proteoglycan produced by chondrocytes.

V. Pharmaceutical Formulations and Routes of Administration

The compounds of the present disclosure may be administered by a variety of methods, *e.g.*, orally or by injection (*e.g.* subcutaneous, intravenous, intraperitoneal, *etc.*). Depending on the route of administration, the active compounds may be coated in a material to protect the compound from the action of acids and other natural conditions which may inactivate the compound. They may also be administered by continuous perfusion/infusion of a disease or wound site.

To administer the therapeutic compound by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation. For example, the therapeutic compound may be administered to a patient in an appropriate carrier, for example, liposomes, or a diluent. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes (Strejan *et al.*, 1984).

The therapeutic compound may also be administered parenterally, intraperitoneally, intraspinally, or intracerebrally. Dispersions can be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

Sterile injectable solutions can be prepared by incorporating the therapeutic compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the therapeutic compound into a sterile carrier which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient (*i.e.*, the therapeutic compound) plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The therapeutic compound can be orally administered, for example, with an inert diluent or an assimilable edible carrier. The therapeutic compound and other ingredients may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the patient's diet. For oral therapeutic administration, the therapeutic compound may be incorporated with excipients and used in the form of

ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. The percentage of the therapeutic compound in the compositions and preparations may, of course, be varied. The amount of the therapeutic compound in such therapeutically useful compositions is such that a suitable dosage will be obtained.

5 It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the patients to be treated; each unit containing a predetermined quantity of therapeutic compound calculated to produce the desired therapeutic effect in association with the required
10 pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the therapeutic compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such a therapeutic compound for the treatment of a selected condition in a patient.

15 The therapeutic compound may also be administered topically to the skin, eye, or mucosa. Alternatively, if local delivery to the lungs is desired the therapeutic compound may be administered by inhalation in a dry-powder or aerosol formulation.

Active compounds are administered at a therapeutically effective dosage sufficient to treat a condition associated with a given patient. For example, the efficacy
20 of a compound can be evaluated in an animal model system that may be predictive of efficacy in treating the disease in humans, such as the model systems shown in the examples and drawings.

The actual dosage amount of a compound of the present disclosure or composition comprising a compound of the present disclosure administered to a patient
25 may be determined by physical and physiological factors such as age, sex, body weight, severity of condition, the type of disease being treated, previous or concurrent therapeutic interventions, idiopathy of the patient and on the route of administration. These factors may be determined by a skilled artisan. The practitioner responsible for administration will typically determine the concentration of active ingredient(s) in a
30 composition and appropriate dose(s) for the individual patient. The dosage may be adjusted by the individual physician in the event of any complication.

An effective amount typically will vary from about 0.001 mg/kg to about 1000 mg/kg, from about 0.01 mg/kg to about 750 mg/kg, from about 100 mg/kg to about 500 mg/kg, from about 1.0 mg/kg to about 250 mg/kg, from about 10.0 mg/kg to about 150

mg/kg in one or more dose administrations daily, for one or several days (depending of course of the mode of administration and the factors discussed above). Other suitable dose ranges include 1 mg to 10000 mg per day, 100 mg to 10000 mg per day, 500 mg to 10000 mg per day, and 500 mg to 1000 mg per day. In some particular embodiments, the amount is less than 10,000 mg per day with a range of 750 mg to 9000 mg per day.

The effective amount may be less than 1 mg/kg/day, less than 500 mg/kg/day, less than 250 mg/kg/day, less than 100 mg/kg/day, less than 50 mg/kg/day, less than 25 mg/kg/day or less than 10 mg/kg/day. It may alternatively be in the range of 1 mg/kg/day to 200 mg/kg/day. For example, regarding treatment of diabetic patients, the unit dosage may be an amount that reduces blood glucose by at least 40% as compared to an untreated patient. In another embodiment, the unit dosage is an amount that reduces blood glucose to a level that is $\pm 10\%$ of the blood glucose level of a non-diabetic patient.

In other non-limiting examples, a dose may also comprise from about 1 microgram/kg/body weight, about 5 microgram/kg/body weight, about 10 microgram/kg/body weight, about 50 microgram/kg/body weight, about 100 microgram/kg/body weight, about 200 microgram/kg/body weight, about 350 microgram/kg/body weight, about 500 microgram/kg/body weight, about 1 milligram/kg/body weight, about 5 milligram/kg/body weight, about 10 milligram/kg/body weight, about 50 milligram/kg/body weight, about 100 milligram/kg/body weight, about 200 milligram/kg/body weight, about 350 milligram/kg/body weight, about 500 milligram/kg/body weight, to about 1000 mg/kg/body weight or more per administration, and any range derivable therein. In non-limiting examples of a derivable range from the numbers listed herein, a range of about 5 mg/kg/body weight to about 100 mg/kg/body weight, about 5 microgram/kg/body weight to about 500 milligram/kg/body weight, *etc.*, can be administered, based on the numbers described above.

In certain embodiments, a pharmaceutical composition of the present disclosure may comprise, for example, at least about 0.1% of a compound of the present disclosure. In other embodiments, the compound of the present disclosure may comprise between about 2% to about 75% of the weight of the unit, or between about 25% to about 60%, for example, and any range derivable therein.

Single or multiple doses of the agents are contemplated. Desired time intervals for delivery of multiple doses can be determined by one of ordinary skill in the art employing no more than routine experimentation. As an example, patients may be

administered two doses daily at approximately 12 hour intervals. In some embodiments, the agent is administered once a day.

The agent(s) may be administered on a routine schedule. As used herein a routine schedule refers to a predetermined designated period of time. The routine schedule may encompass periods of time which are identical or which differ in length, as long as the schedule is predetermined. For instance, the routine schedule may involve administration twice a day, every day, every two days, every three days, every four days, every five days, every six days, a weekly basis, a monthly basis or any set number of days or weeks there-between. Alternatively, the predetermined routine schedule may involve administration on a twice daily basis for the first week, followed by a daily basis for several months, *etc.* In other embodiments, the invention provides that the agent(s) may be taken orally and that the timing of which is or is not dependent upon food intake. Thus, for example, the agent can be taken every morning and/or every evening, regardless of when the patient has eaten or will eat.

VI. Examples

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1 – CDDO-Im and CDDO-EA Induce Chondrogenesis in Newborn Mouse Calvarial Organ Cultures

The synthetic triterpenoids, CDDO-Imidazolide (CDDO-Im) and CDDO-Ethyl amide (CDDO-EA), added to newborn mouse calvarial organ cultures, were found to induce chondrogenesis, which was measured histologically and with the expression of classic markers of a chondrocytic phenotype (Sive *et al.*, 2002; Lefebvre and de Crombrughe, 1998; Hardingham *et al.*, 2006).

All chemicals were from Sigma-Aldrich. Primers for RT-PCR were from Applied Biosystems. The isolation of calvaria was essentially performed as described previously

(Garrett, 2003; Garret *et al.*, 2003). The calvaria were cultured in Biggers, Gwatkins, Judah medium supplemented with 1 mg/mL of bovine serum albumin (Cohn fraction V), 100 U/mL each of penicillin/streptomycin, and 0.292 mg/mL of glutamine. On Day 1, the calvaria were treated with CDDO-Im or CDDO-EA. On Day 4, the medium was replaced with fresh medium, again containing either CDDO-Im or CDDO-EA. On Day 7, the calvaria were collected and either stored at -80°C for further RNA analysis or fixed in 10% buffered formalin for 24 h and transferred to 80% ethanol for histological analysis.

Histological Analysis

After fixation for 24 h, the calvaria were decalcified in EDTA, embedded in paraffin, and cut into 4 μm sections. The sections were stained either with modified hematoxylin and eosin or with toluidine blue (1% in 70% ethanol for 20 min, followed by destaining in 70%, 90%, and 100% ethanol for 15 seconds), placed in xylene twice, and then mounted (Garret, 2003). Procedures for immunofluorescence were performed essentially as previously described (Medici *et al.*, 2010). Primary antibodies against collagen type II (AB746P, Millipore, Billerica, MA) were used at 1:100 dilution, and AlexFluor secondary antibodies (Invitrogen, Carlsbad, CA) were used at 1:200 dilution.

Both CDDO-Im and CDDO-EA have marked ability to induce chondrogenesis in the calvaria as evidenced by the metachromatic toluidine blue purple staining, which is indicative of the formation of proteoglycans, such as aggrecan, which are characteristic of cartilage (FIG. 1a). No new cartilage was seen on control sections stained with either H&E or toluidine blue. Results shown in FIG. 1a with 200 nM CDDO-Im and CDDO-EA were obtained in at least 3 sets of replicate experiments. Immunohistochemical analysis demonstrates that CDDO-Im and CDDO-EA at 200 nM both induce the formation of collagen type II (COL2A1), where red fluorescence is indicative of the formation of COL2A1 (FIG. 1b).

RNA Analysis

After 7 days of culture, RNA was isolated from the calvaria and quantitative RT-PCR analysis was performed for more than 15 different markers including: SOX9 (a transcription factor specific for cartilage), collagen type II ($\alpha 1$), all three isoforms of TGF- β , BMPs 2 and 4, BMP receptor II, Smads 3, 4, 6, and 7, tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2), and matrix metalloproteinase-9 (MMP-9). Table 1 (see below) shows that essentially all of these markers (except MMP-9) are significantly up-regulated by both triterpenoids, when the calvaria were treated at either

the 200 or the 500 nM dose. Both triterpenoids are strong inhibitors of the expression of MMP-9; CDDO-EA (200 nM), caused almost 80% inhibition of the expression of this metalloproteinase, which is known to be involved in the degradation of cartilage (Shinoda *et al.*, 2008).

5 **Example 2 – CDDO-Im and CDDO-EA Induce Chondrogenesis in Bone Marrow-Derived Mesenchymal Stem Cells**

The synthetic triterpenoids, CDDO-Imidazolide (CDDO-Im) and CDDO-Ethyl amide (CDDO-EA), added to bone marrow-derived mesenchymal stem cells (MSCs), were found to induce the expression of type II collagen (COL2A1), SOX9 (a
10 transcription factor specific for cartilage), and aggrecan (a proteoglycan specific for cartilage), which are widely accepted markers of a chondrocytic phenotype (Sive *et al.*, 2002; Lefebvre and de Crombrughe, 1998; Hardingham *et al.*, 2006).

Human bone marrow-derived stromal cells (ScienCell Research Laboratories), which contain a population of responsive mesenchymal stem cells, were grown in
15 mesenchymal stem cell medium (ScienCell Research Laboratories). MSCs were serum-starved 24 hours prior to all experimental conditions. The synthesis of the synthetic triterpenoids, CDDO-Im and CDDO-EA, have been previously described (Sporn *et al.*, 2011).

For directed chondrocytic differentiation, MSCs were grown in StemPro
20 chondrogenic culture medium (Invitrogen) prepared by the technique of Kassem (2004), which is incorporated herein by reference. See also Medici *et al.* (2010), which is also incorporated herein by reference. CDDO-Im and CDDO-EA were added to the cultures at a concentration of 100 nM for 7 days. Western blot analysis was performed using the following antibodies at concentrations (and using protocols) recommended by the
25 respective manufacturers: SOX9, COL2A1 (Santa Cruz Biotechnology), Aggrecan (Abcam), β -actin (Sigma-Aldrich). β -actin was used as a loading control. HRP-conjugated IgG TrueBlot reagents (eBioscience) were used at a dilution of 1:1000. The chondrocyte markers, SOX9, COL2A1, and Aggrecan, were not detectable in the control MSCs, but there was a clear induction of all three markers in the MSCs treated with
30 either CDDO-Im or CDDO-EA (FIG. 2). It was also found that both triterpenoids were cytotoxic to the MSCs in culture at the 200 nM dosage.

Table 1. Quantitative RT-PCR Analysis on Calvaria

Gene	CDDO-Im			CDDO-EA		
	50 nM	200 nM	500 nM	50 nM	200 nM	500 nM
SOX-9	1.26 ± 0.08	1.33 ± 0.05	1.83 ± 0.13**	1.64 ± 0.17	2.62 ± 0.33**	3.12 ± 0.38**
Col II alpha1	1.45 ± 0.09	1.32 ± 0.23	1.72 ± 0.39	4.12 ± 1.78*	5.45 ± 1.13**	3.18 ± 0.71
TGF-β1	0.96 ± 0.07	1.08 ± 0.12	1.46 ± 0.12*	1.17 ± 0.09	1.47 ± 0.24*	1.74 ± 0.17**
TGF-β2	1.17 ± 0.05	1.15 ± 0.09	1.17 ± 0.10	1.36 ± 0.13*	1.61 ± 0.11**	1.88 ± 0.09**
TGF-β3	1.30 ± 0.07	1.52 ± 0.16**	1.66 ± 0.13**	1.39 ± 0.07*	1.56 ± 0.13**	1.44 ± 0.11**
BMP-2	1.17 ± 0.07	1.60 ± 0.19	3.14 ± 0.53**	1.53 ± 0.20	2.52 ± 0.29**	5.66 ± 0.67**
BMP-4	0.97 ± 0.05	0.98 ± 0.06	1.35 ± 0.22	1.28 ± 0.12	1.47 ± 0.13	2.49 ± 0.35**
BMPRII	1.09 ± 0.06	1.22 ± 0.11	1.46 ± 0.14*	1.50 ± 0.14*	1.71 ± 0.19**	2.24 ± 0.22**
Smad3	0.97 ± 0.07	0.90 ± 0.05	1.35 ± 0.19	1.14 ± 0.12	1.35 ± 0.07	1.93 ± 0.16**
Smad4	0.96 ± 0.06	1.03 ± 0.09	1.26 ± 0.09	1.06 ± 0.03	1.37 ± 0.12**	1.89 ± 0.12**
Smad6	0.95 ± 0.05	1.23 ± 0.14	1.77 ± 0.19**	1.09 ± 0.04	1.77 ± 0.24**	2.85 ± 0.31**
Smad7	1.05 ± 0.07	1.32 ± 0.14	1.78 ± 0.18**	1.28 ± 0.10	1.86 ± 0.19**	3.06 ± 0.28**
TIMP-1	1.43 ± 0.19	1.83 ± 0.18	3.00 ± 0.65**	1.88 ± 0.34	2.85 ± 0.38**	4.65 ± 0.38**
TIMP-2	1.24 ± 0.10	1.67 ± 0.19	2.62 ± 0.29**	1.78 ± 0.18*	2.44 ± 0.31**	4.09 ± 0.36**
MMP-9	0.73 ± 0.16	0.44 ± 0.12**	0.26 ± 0.03**	0.49 ± 0.09**	0.22 ± 0.03**	0.19 ± 0.06**

N = 20 calvaria for control group and N = 12 calvaria per treatment group. Statistical significance was performed with ANOVA followed by Dunnett's multiple comparison test, * p<0.05, ** p<0.01. Methods for isolation of RNA and RT-PCR are described in Lee *et al.* (2006), which is incorporated herein by reference. All values have been normalized to the 20 control cultures, assigned a value of 1.00

* * * * *

All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

REFERENCES

The following references to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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U.S. Patent 6,974,801

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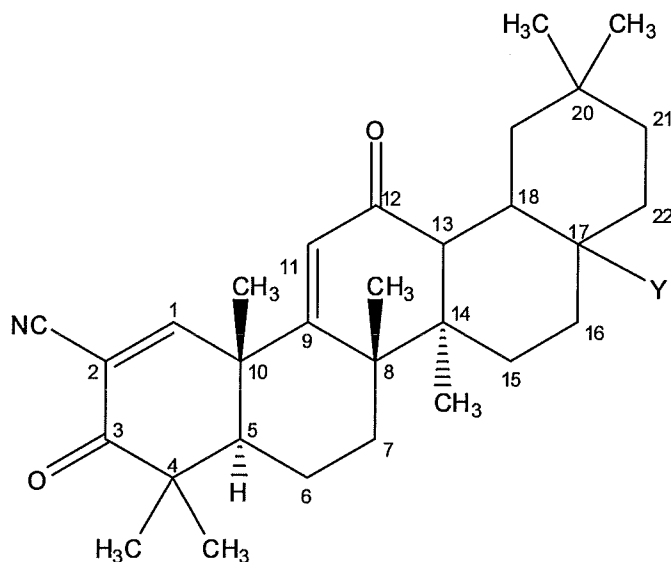
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CLAIMS**What is claimed is:**

1. A method of inducing differentiation of a mesenchymal stem cell into a chondrocyte, comprising contacting a mesenchymal stem cell with a sufficient amount of a synthetic triterpenoid to induce chondrogenesis, wherein the synthetic triterpenoid is a compound of the formula:



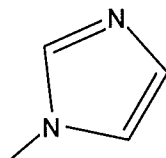
wherein Y is:

–CN or –C(O)R, wherein R is hydroxy, amino, alkoxy_(C≤8), alkylamino_(C≤8), substituted alkylamino_(C≤8), or heteroaryl_(C≤8);

or a pharmaceutically-acceptable salt or tautomer of the formula.

2. The method of claim 1, wherein the mesenchymal stem cell is a bone marrow-derived mesenchymal stem cell.
3. The method of claim 1, wherein the mesenchymal stem cell was isolated prior to contacting with the synthetic triterpenoid.

4. The method of claim 1, wherein R is hydroxy, methoxy, ethyl-amino, or



5. The method of claim 4, wherein the synthetic triterpenoid is CDDO-Im.
6. The method of claim 4, wherein the synthetic triterpenoid is CDDO-EA.
7. The method of claim 1, further comprising contacting the mesenchymal stem cell with a differentiating medium.
8. The method of claim 1, further comprising contacting the mesenchymal stem cell with a co-factor.
9. The method of claim 1, wherein the stem cell is contacted with the synthetic triterpenoid *in vitro*.
10. The method of claim 1, wherein the stem cell is contacted with the synthetic triterpenoid *in vivo*.
11. The method of claim 1, wherein the sufficient amount of the synthetic triterpenoid is from 1 nM to 1 μ M.
12. The method of claim 1, further comprising incubating the mesenchymal stem cell with a growth factor.
13. The method of claim 12, wherein the growth factor is TGF- β 1, TGF- β 2, TGF- β 1.2, VEGF, insulin-like growth factor I or II, BMP2, BMP4, or BMP7.
14. The method of claim 12, wherein the growth factor is parathyroid hormone, calcitonin, interleukin-6, or interleukin-11.
15. The method of claim 1, further comprising culturing the mesenchymal stem cell before or after contacting it with the synthetic triterpenoid.

16. The method of claim 1, further comprising purifying the mesenchymal stem cell before or after contacting it with the synthetic triterpenoid.
17. The method of claim 1, further comprising implanting the resulting chondrocyte *in vivo*.
18. The method of claim 1, further comprising implanting the resulting chondrocyte to a patient as part of a treatment for a cartilage-related disease.
19. The method of claim 18, wherein the cartilage-related disease is osteoarthritis, achondrogenesis, chondrodysplasia, SED congenita, Langer-Saldino achondrogenesis, Kniest dysplasia, Stickler syndrome type I, or spondyloepimetaphyseal dysplasia Strudwick type..
20. The method of claim 1, further comprising inducing within the mesenchymal stem cell the expression of a cellular marker associated with chondrogenesis.
21. The method of claim 20, wherein the cellular marker is SOX9, COL2A1, or aggrecan.
22. The method of claim 1, further comprising increasing or up-regulating within the mesenchymal stem cell the level of TGF- β , BMP2, BMP4, SMAD3, SMAD4, SMAD6, SMAD7, TIMP-1 or TIMP-2.
23. The method of claim 1, further comprising decreasing or down-regulating within the mesenchymal stem cell the level of MMP-9.

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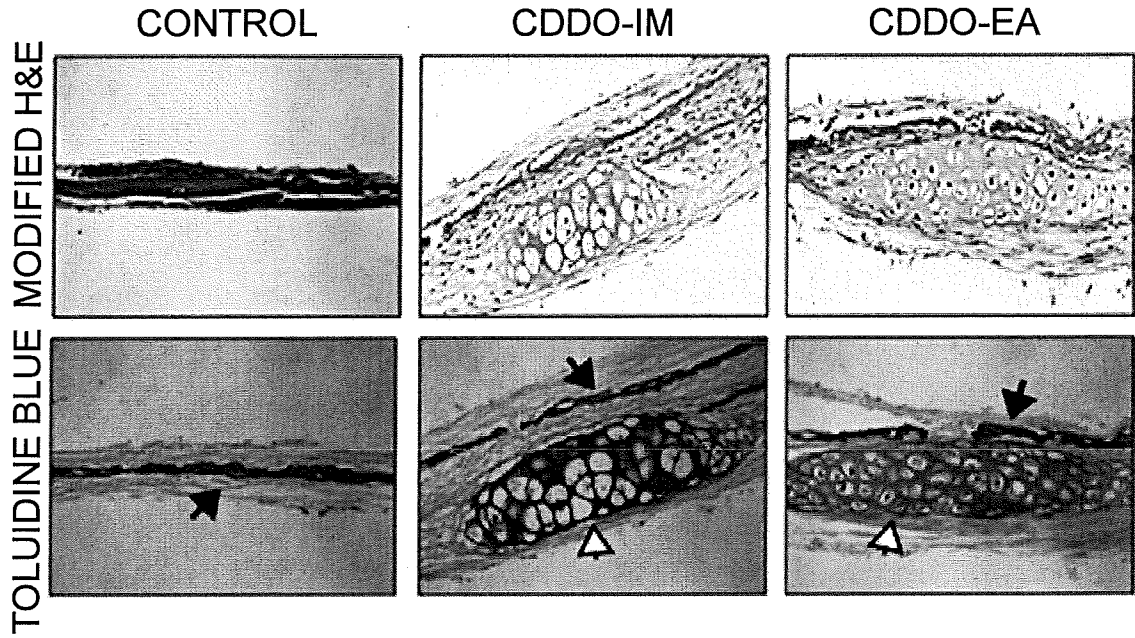


FIG. 1A

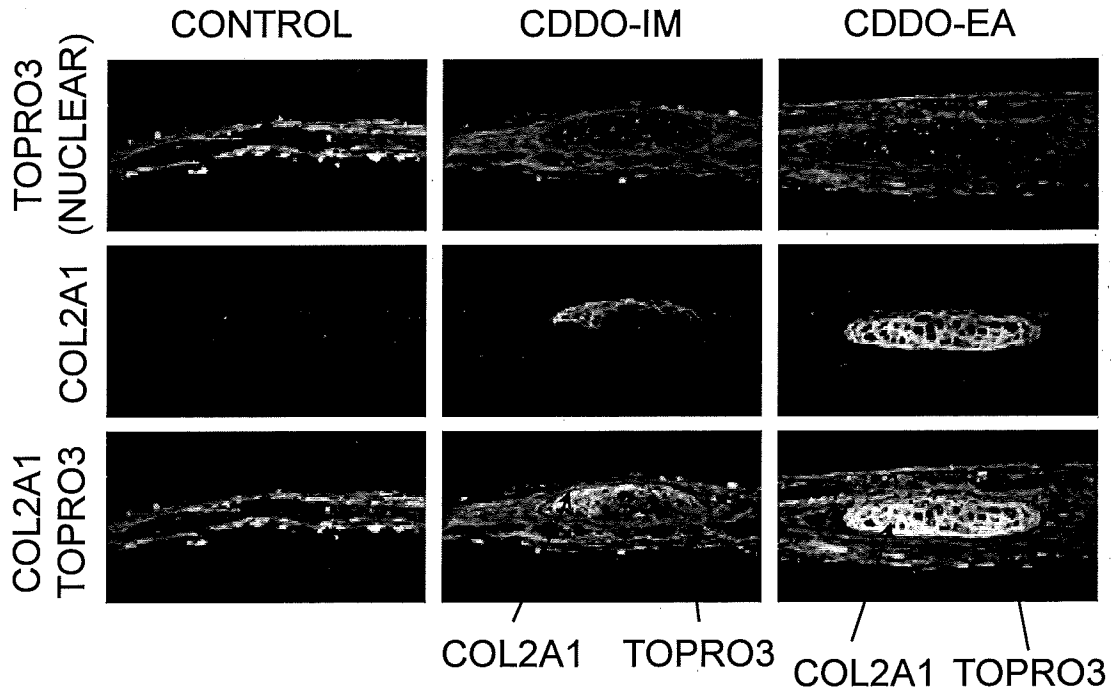
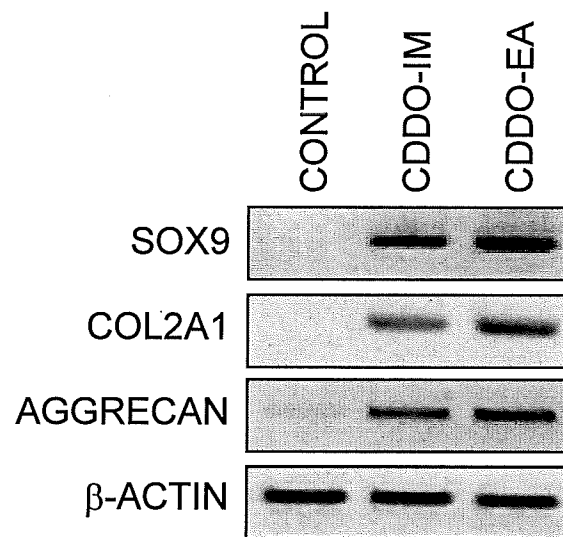


FIG. 1B

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*FIG. 2*

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US12/64909

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 05/0775; A61K 31/122, 35/12; A61P 19/00 (2013.01)

USPC - 435/377; 424/93.7; 514/691

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): C12N 5/00, 5/02, 5/077, 5/0775; A61K 31/122, 35/12, 38/18; A61P 19/00 (2013.01)

USPC: 435/375, 377, 325; 424/93.7, 93.1, 198.1; 514/688, 678, 675, 691, 1; 530/399

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A), induce, promote, chondrocyte, chondrogenesis, differentiation, mesenchymal, stem cell, msc, synthetic, triterpenoid, CDDO; Google/Google Scholar; DialogPRO; ACS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2008/0233195 A1 (SPOORN, MB et al.) September 25, 2008; abstract; figure 12;	1-22
---	paragraphs [0008], [0010]-[0012], [0017], [0020], [0030], [0117], [0167], [0171], [0181], [0192],	-----
Y	[0200], [0204], [0300]-[0303]	23
Y	US 7892799 B2 (SMITH, RL et al.), February 22, 2011; abstract; column 6, lines 21-24; column 6, lines 48-60	23

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Shane Thomas

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774