



- (51) International Patent Classification:  
*A61K 31/505* (2006.01)
- (21) International Application Number:  
PCT/US2012/030567
- (22) International Filing Date:  
26 March 2012 (26.03.2012)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:  
61/467,289 24 March 2011 (24.03.2011) US
- (71) Applicant (for all designated States except US): **THE SCRIPPS RESEARCH INSTITUTE** [US/US]; 10550 North Torrey Pines Road, Mail TPC-8, La Jolla, California 92037 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **SHULTZ, Peter** [US/US]; La Jolla, California 92037 (US). **BOUCHEZ, Laure** [FR/CH]; Basel (CH).
- (74) Agents: **TRIMBLE, Alexander R.** et al.; Kilpatrick Townsend & Stockton LLP, Two Embarcadero Center, Eighth Floor, San Francisco, CA 94111-3834 (US).

- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report (Rule 48.2(g))

(54) Title: COMPOUNDS AND METHODS FOR INDUCING CHONDROGENESIS

(57) Abstract: The present invention provides compounds and compositions for the amelioration of arthritis and joint injuries by inducing mesenchymal stem cells into chondrocytes.

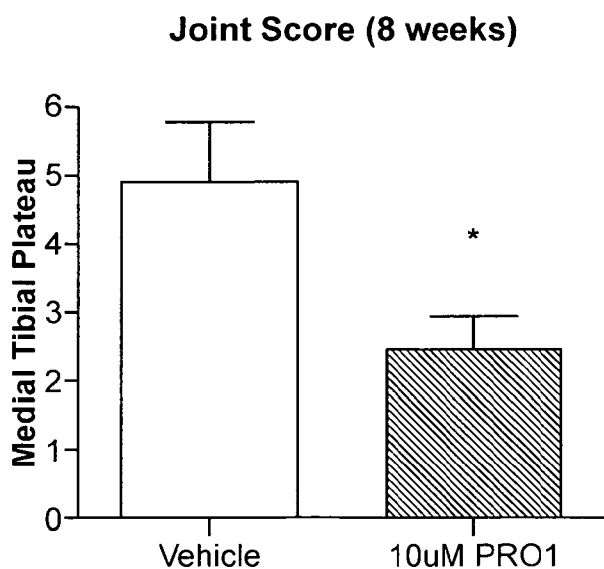


FIG. 3B.

WO 2012/129562 A2

## COMPOUNDS AND METHODS FOR INDUCING CHONDROGENESIS

### CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 61/467,289,  
5 filed March 24, 2011, which is incorporated in its entirety herein for all purposes.

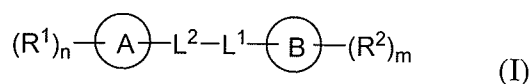
### BACKGROUND OF THE INVENTION

[0002] Osteoarthritis (OA) represents the most common musculoskeletal disorder. Approximately 40 million Americans are currently affected and this number is predicted to  
10 increase to 60 million within the next twenty years as a result of the aging population and an increase in life expectancy, making it the fourth leading cause of disability. OA is characterized by a slow degenerative breakdown of the joint including both the articular cartilage (containing the cells and matrix which produce lubrication and cushioning for the joint) and the subchondral bone underlying the articular cartilage. Current OA therapies  
15 include pain relief with oral NSAIDs or selective cyclooxygenase 2 (COX-2) inhibitors, intra-articular (IA) injection with agents such as corticosteroids and hyaluronan, and surgical approaches.

[0003] Mesenchymal stem cells (MSCs) are present in adult articular cartilage and upon isolation can be programmed *in vitro* to undergo differentiation to chondrocytes and other  
20 mesenchymal cell lineages. In part it is regulated by growth factors (TGFβs, BMPs), serum conditions and cell-cell contact.

### BRIEF SUMMARY OF THE INVENTION

[0004] In one embodiment, the present invention provides a method of ameliorating  
25 arthritis or joint injury in a mammal, the method including administering to a joint of the mammal a composition having a therapeutically effective amount of a compound of formula I:



[0005] In formula I, each of ring A and ring B are independently cycloalkyl, aryl or  
30 heteroaryl.

**[0006]** In formula I, each R<sup>1</sup> and R<sup>2</sup> is independently H, C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, C<sub>1-6</sub> alkoxy, C<sub>1-6</sub> heteroalkyl, halogen, C<sub>1-6</sub> haloalkyl, C<sub>1-6</sub> haloalkoxy, C<sub>1-6</sub> alkyl-CN, C<sub>1-6</sub> alkylhydroxy, -OR<sup>2a</sup>, -NR<sup>2b</sup>R<sup>2d</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>R<sup>2d</sup>, -C(O)R<sup>1a</sup>, -C(O)R<sup>2d</sup>, -C(O)OR<sup>2a</sup>, C<sub>1-6</sub> alkyl-C(O)OR<sup>2b</sup>, -OC(O)R<sup>2b</sup>, -OC(O)OR<sup>2b</sup>, -C(O)NR<sup>2a</sup>R<sup>2b</sup>, -C(O)N(OH)R<sup>2b</sup>,  
 5 -NR<sup>2b</sup>C(O)R<sup>2c</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)R<sup>2c</sup>, -NR<sup>2b</sup>C(O)OR<sup>2c</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)OR<sup>2c</sup>, -OC(O)NR<sup>2b</sup>R<sup>2c</sup>, -NR<sup>2b</sup>C(O)NR<sup>2b</sup>R<sup>2c</sup>, -NR<sup>2b</sup>C(NR<sup>2b</sup>)NR<sup>2b</sup>R<sup>2c</sup>, -C(O)NR<sup>2b</sup>C(O)R<sup>2b</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)NR<sup>2b</sup>R<sup>2c</sup>, -SR<sup>2a</sup>, -SO<sub>2</sub>R<sup>2b</sup>, -SO<sub>2</sub>OR<sup>2b</sup>, -SO<sub>2</sub>NR<sup>2b</sup>R<sup>2d</sup>, -NR<sup>2b</sup>SO<sub>2</sub>R<sup>2b</sup>, -P(O)(OR<sup>2b</sup>)<sub>2</sub>, -B(OR<sup>2b</sup>), -CN, -NO<sub>2</sub>, -N<sub>3</sub>, heterocycloalkyl, aryl, heteroaryl, C<sub>1-6</sub> alkyl-heterocycloalkyl, C<sub>1-6</sub> alkyl-aryl, C<sub>1-6</sub> alkyl-O-aryl, C<sub>1-6</sub> alkyl-heteroaryl, or  
 10 heteroaryl-aryl, and wherein the heterocycloalkyl, aryl and heteroaryl groups are optionally substituted with 1 to 2 R<sup>2a</sup> groups.

**[0007]** In formula I, R<sup>1a</sup> is -OR<sup>1b</sup> or -NR<sup>1b</sup>R<sup>1c</sup>; R<sup>1b</sup> and R<sup>1c</sup> are each independently H, C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, C<sub>1-6</sub> alkyl-aryl, or C<sub>1-6</sub> alkyl-heteroaryl, wherein the cycloalkyl, heterocycloalkyl, aryl and  
 15 heteroaryl groups are optionally substituted with from 1 to 4 R<sup>1d</sup> groups; and each R<sup>1d</sup> is independently H, C<sub>1-6</sub> alkyl, C<sub>1-6</sub> alkoxy, or -NO<sub>2</sub>.

**[0008]** In formula I, each R<sup>2a</sup> is independently H, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, C<sub>1-6</sub> alkyl-cycloalkyl, C<sub>1-6</sub> alkyl-heterocycloalkyl, C<sub>1-6</sub> alkyl-aryl or C<sub>1-6</sub> alkyl-heteroaryl, optionally substituted with 1 to 2 R<sup>2b</sup> groups; each R<sup>2b</sup>  
 20 and R<sup>2c</sup> is independently H, or C<sub>1-6</sub> alkyl; and each R<sup>2d</sup> is independently H, C<sub>1-6</sub> alkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, C<sub>1-6</sub> alkyl-cycloalkyl, C<sub>1-6</sub> alkyl-heterocycloalkyl, C<sub>1-6</sub> alkyl-aryl or C<sub>1-6</sub> alkyl-heteroaryl, each optionally substituted with 1 to 2 R<sup>2b</sup> groups.

**[0009]** In formula I, each of L<sup>1</sup> and L<sup>2</sup> are independently L<sup>1</sup> and L<sup>2</sup> are independently a  
 25 bond, C<sub>1-6</sub> alkylene, C<sub>2-6</sub> alkenylene, C<sub>1-6</sub> alkylene-O-, -O-C<sub>1-6</sub> alkylene, C<sub>1-6</sub> alkylene-NR<sup>3a</sup>-, -NR<sup>3a</sup>-C<sub>1-6</sub> alkylene, -C(O)-, C<sub>1-6</sub> alkylene-C(O)-, -C(O)-C<sub>1-6</sub> alkylene-NH-, -NH-C<sub>1-6</sub> alkylene-C(O)-, -C(O)NH-, -NHC(O)-, C<sub>1-6</sub> alkylene-NHC(O)-, -SO<sub>2</sub>NH-, -NHSO<sub>2</sub>-, -NHC(O)NH-, cycloalkylene, -N=N-, or -C(R<sup>3a</sup>)=N(R<sup>3c</sup>)-, wherein the alkylene group is optionally substituted with from 1-4 R<sup>3b</sup> groups. R<sup>3a</sup> of formula I is H or C<sub>1-6</sub> alkyl.  
 30 Each R<sup>3b</sup> of formula I is independently H, C<sub>1-6</sub> alkyl, halogen, -OR<sup>3a</sup> or -NR<sup>3a</sup>R<sup>3a</sup>. R<sup>3c</sup> of formula I is absent or -OH.

**[0010]** Alternatively, L<sup>2</sup> is combined with R<sup>1</sup>, L<sup>1</sup> is combined with L<sup>2</sup>, L<sup>1</sup> is combined with R<sup>2</sup>, two R<sup>1</sup> groups on adjacent ring atoms, or two R<sup>2</sup> groups on adjacent ring atoms are

combined to form a 5-6 membered heterocycloalkyl with from 1 to 3 heteroatoms selected from N, O and S, or a 5-6 membered heteroaryl with from 1 to 3 heteroatoms selected from N, O and S, and optionally substituted with from 1 to 3 groups of H, C<sub>1-6</sub> alkyl or oxo.

[0011] In formula I, subscripts m and n are each an integer from 1 to 3.

5 [0012] Moreover, the compounds of formula I are those wherein:

- (a) L<sup>1</sup> is a bond, L<sup>2</sup> is -C(O)NH-, ring B is phenyl, and at least one R<sup>2</sup> is -CN or phenyl, or
- (b) at least one R<sup>1</sup> is -C(O)OH, ring A is phenyl, L<sup>2</sup> is -C(O)NH-, and L<sup>1</sup> is a bond or C<sub>1-6</sub> alkylene, or
- 10 (c) each of ring A and ring B is phenyl, at least one R<sup>1</sup> is -C(O)OH or combined with L<sup>2</sup>, and at least one R<sup>2</sup> is H, -CN and -C(O)OH.

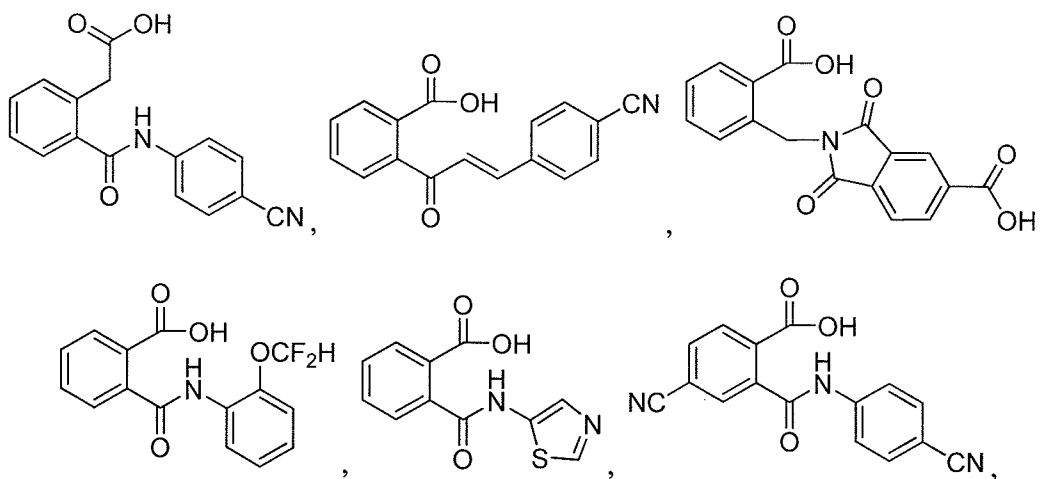
[0013] The compounds of formula I are such that when R<sup>1</sup> is -CO<sub>2</sub>H, subscript n is 1, ring A is phenyl, L<sup>2</sup> is -C(O)NH-, L<sup>1</sup> is a bond, ring B is phenyl, subscript m is 1, and R<sup>2</sup> is phenyl, then the phenyl of R<sup>2</sup> is substituted with C<sub>1-6</sub> alkyl.

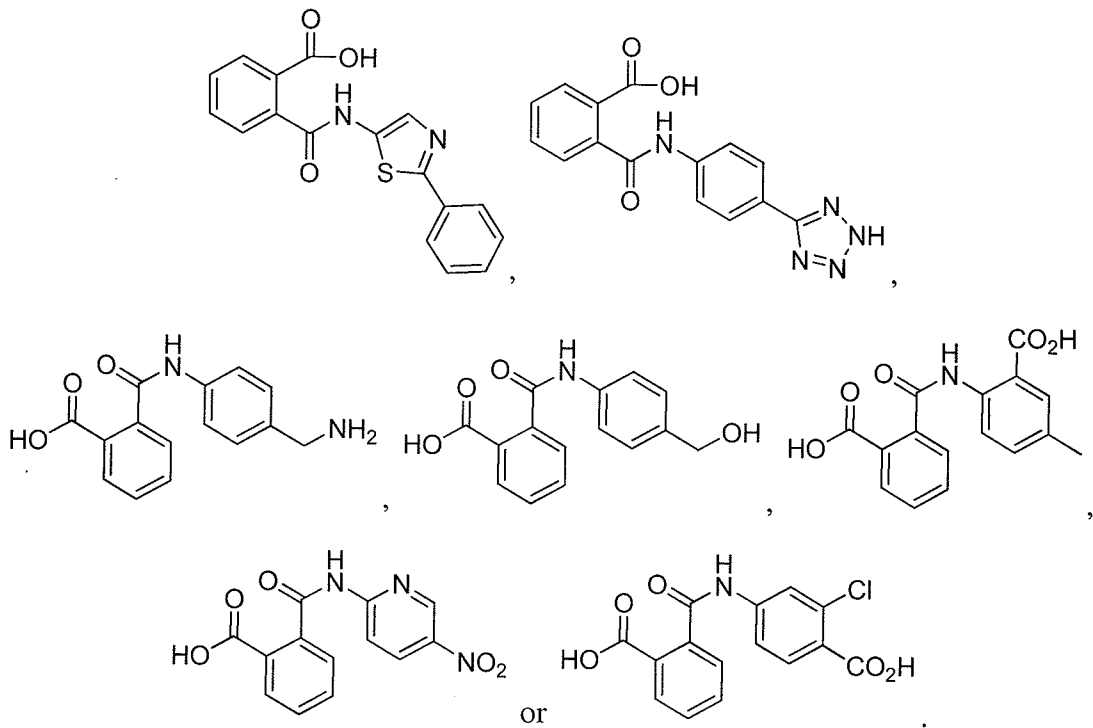
15 [0014] The compounds of formula I include the salts and isomers thereof. In this manner, the arthritis or joint injury in the mammal is ameliorated.

[0015] In some embodiments, the present invention provides a method of inducing differentiation of mesenchymal stem cells into chondrocytes, the method including contacting mesenchymal stem cells with a sufficient amount of a compound of Formula I, thereby

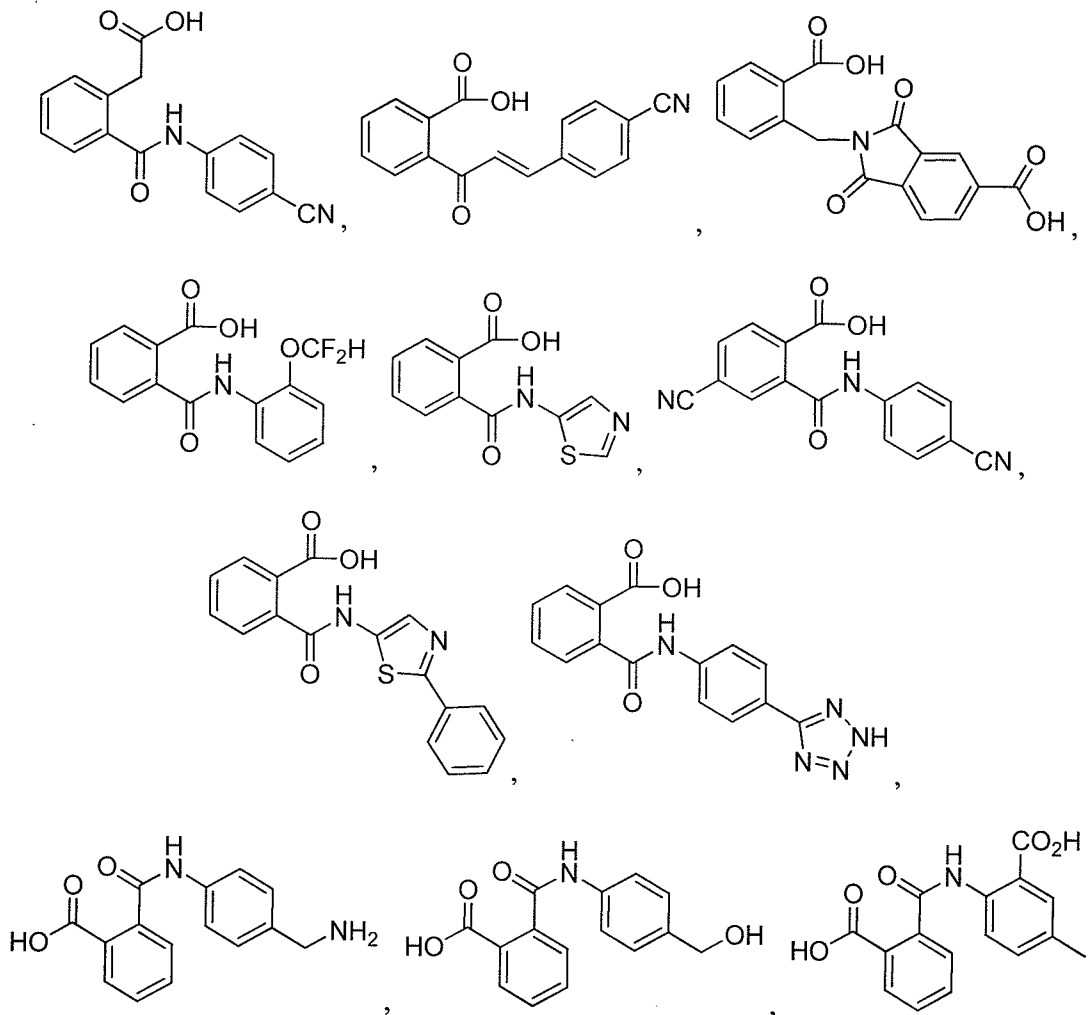
20 inducing differentiation of the stem cells into chondrocytes.

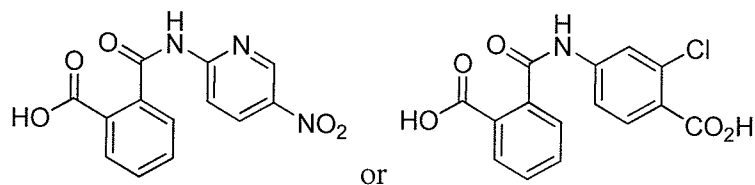
[0016] In some embodiments, the present invention provides a compound having the structure:





[0017] In some embodiments, the present invention provides a pharmaceutical composition including a pharmaceutically acceptable excipient and a compound having the structure:





[0018] In some embodiments, the present invention provides a pharmaceutical composition formulated for intra-articular delivery, the composition including a pharmaceutically effective amount of a compound of Formula I and a pharmaceutically acceptable excipient.

5

#### BRIEF DESCRIPTION OF THE DRAWINGS

[0019] **Figure 1** shows the formation of chondrogenic nodules by human mesenchymal stem cells (hMSCs) upon treatment with inducer PRO1 (AKT), as visualized by rhodamine B-staining of cultured cells.

10 [0020] **Figure 2A** shows chondrocyte differentiation as indicated by immunocytochemical staining of type II collagen in a PRO1-treated hMSC culture.

[0021] **Figure 2B** shows the chondrogenic differentiation of hMSCs, as assessed by quantitation of type II collagen expression, in cell cultures treated with PRO1 (AKT), PRO1-CN (AKT-CN), and known inducers of chondrogenic differentiation.

15 [0022] **Figure 2C** shows the inhibition of cytokine-induced nitric oxide release in primary bovine chondrocyte cultures after treatment with PRO1 (AKT) and related analogues.

[0023] **Figure 2D** shows the inhibition of cytokine-induced glycosaminoglycan (GAG) release in cartilage explants treated with PRO1 (AKT) *ex vivo*.

20 [0024] **Figure 2E** shows the PRO1-induced promotion of type II collagen and aggrecan expression in a three-dimensional culture environment, as assessed by immunohistochemical staining of treated pellet cultures.

[0025] **Figure 3A** shows the histological analysis of collagenase-induced joint damage in C57BL/10 mice with and without treatment via IA injection of PRO1 (AKT).

25 [0026] **Figure 3B** shows the total medial tibial plateau joint score as determined by two blinded observers using the OARSI scoring system.

[0027] **Figure 3C** shows the ELISA determination of circulating COMP in peripheral blood serum drawn from C57BL/10 mice during and after the course of treatment via IA injection of PRO1 (AKT).

[0028] **Figure 4A** shows the visualization of the medial tibial plateau from representative female 129SVE mice during treatment of surgically-induced cartilage injury via IA injection of PRO1 (AKT).

5 [0029] **Figure 4B** shows the joint severity scores on day 28 after cartilage injury, as determined by histomorphometric analysis and grading by two blinded observers using a modified OARSI scoring system.

[0030] **Figure 4C** shows the ELISA determination of cleaved type II collagen fragments (CTX-II) levels in peripheral blood serum collected 28 days after cartilage injury.

10 [0031] **Figure 5A** shows the redistribution of weight to the hind legs by female 129SVE mice after IA injection of PRO1 (AKT) for treatment of surgically-induced cartilage injury.

[0032] **Figure 5B** shows the joint severity scores of the lateral tibial plateau on day 56 after cartilage injury, as determined by histomorphometric analysis and grading by two blinded observers using a modified OARSI scoring system.

15 [0033] **Figure 5C** shows the immunohistochemical analysis of type II collagen in representative mice following IA injection of PRO1 (AKT) for treatment of surgically-induced cartilage injury.

[0034] **Figure 6A** shows the detection of biotinylated species resulting from reaction of a biotin-PRO1-azide (AKT-azide) analogue with closely associated proteins in cultured cells, as analyzed by Western blotting of fractionated hMSC lysates.

20 [0035] **Figure 6B** shows expression levels of FLNA in hMSCs cultured in the presence of PRO1 (AKT), as assessed by Western blotting.

[0036] **Figure 6C** shows the expression levels of PEBP2 $\beta$  / Runx1 pathway members in hMSCs treated with PRO1 (AKT) in monolayer or pellet cultures, as assessed by Western blotting.

25 [0037] **Figure 7** shows the inhibition of FLNA/PEB2 $\beta$  interactions by PRO1 (AKT), as assessed by immunoprecipitation and Western blotting with the indicated antibodies.

[0038] **Figure 8** shows compounds of the present invention.

## DETAILED DESCRIPTION OF THE INVENTION

**I. General**

[0039] The present invention is based, in part, on the discovery that the compounds of the present invention stimulate chondrocyte differentiation in mesenchymal stem cells.

5 Accordingly, the present invention provides for methods of induction of mesenchymal stem cell differentiation into chondrocytes. Further, the present invention provides for administration of compounds and compositions of the present invention to prevent or ameliorate arthritis or joint injury by administering the compound or composition into a joint, the vertebrae, vertebral disc or systemically.

**10 II. Definitions**

[0040] "Alkyl" refers to a straight or branched, saturated, aliphatic radical having the number of carbon atoms indicated. For example, C<sub>1</sub>-C<sub>6</sub> alkyl includes, but is not limited to, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl, hexyl, etc. Other alkyl groups include, but are not limited to heptyl, octyl, nonyl, decyl, etc. Alkyl can include any number of carbons, such as 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 2-3, 2-4, 2-5, 2-6, 3-4, 3-5, 3-6, 4-5, 4-6 and 5-6. The alkyl group is typically monovalent, but can be divalent, such as when the alkyl group links two moieties together.

[0041] The term "lower" referred to above and hereinafter in connection with organic radicals or compounds respectively defines a compound or radical which can be branched or unbranched with up to and including 7, preferably up to and including 4 and (as unbranched) one or two carbon atoms.

[0042] "Alkylene" refers to an alkyl group, as defined above, linking at least two other groups, *i.e.*, a divalent hydrocarbon radical. The two moieties linked to the alkylene can be linked to the same atom or different atoms of the alkylene. For instance, a straight chain alkylene can be the bivalent radical of -(CH<sub>2</sub>)<sub>n</sub>, where n is 1, 2, 3, 4, 5 or 6. Alkylene groups include, but are not limited to, methylene, ethylene, propylene, isopropylene, butylene, isobutylene, sec-butylene, pentylene and hexylene.

[0043] "Alkoxy" refers to an alkyl group having an oxygen atom that connects the alkyl group to the point of attachment: alkyl-O-. As for alkyl group, alkoxy groups can have any suitable number of carbon atoms, such as C<sub>1-6</sub>. Alkoxy groups include, for example, methoxy, ethoxy, propoxy, iso-propoxy, butoxy, 2-butoxy, iso-butoxy, sec-butoxy,



tert-butoxy, pentoxy, hexoxy, etc. The alkoxy groups can be further substituted with a variety of substituents described within. Alkoxy groups can be substituted or unsubstituted.

[0044] "Alkylhydroxy" refers to an alkyl group, as defined above, where at least one of the hydrogen atoms is replaced with a hydroxy group. As for the alkyl group, hydroxyalkyl groups can have any suitable number of carbon atoms, such as C<sub>1-6</sub>. Exemplary hydroxyalkyl groups include, but are not limited to, hydroxy-methyl, hydroxy-ethyl (where the hydroxy is in the 1- or 2-position), hydroxy-propyl (where the hydroxy is in the 1-, 2- or 3-position), hydroxy-butyl (where the hydroxy is in the 1-, 2-, 3- or 4-position), hydroxy-pentyl (where the hydroxy is in the 1-, 2-, 3-, 4- or 5-position), hydroxy-hexyl (where the hydroxy is in the 1-, 2-, 3-, 4-, 5- or 6-position), 1,2-dihydroxyethyl, and the like.

[0045] "Halogen" refers to fluorine, chlorine, bromine and iodine.

[0046] "Haloalkyl" refers to alkyl as defined above where some or all of the hydrogen atoms are substituted with halogen atoms. Halogen (halo) preferably represents chloro or fluoro, but may also be bromo or iodo. For example, haloalkyl includes trifluoromethyl, fluoromethyl, 1,2,3,4,5-pentafluoro-phenyl, etc. The term "perfluoro" defines a compound or radical which has at least two available hydrogens substituted with fluorine. For example, perfluorophenyl refers to 1,2,3,4,5-pentafluorophenyl, perfluoromethane refers to 1,1,1-trifluoromethyl, and perfluoromethoxy refers to 1,1,1-trifluoromethoxy.

[0047] "Halo-alkoxy" refers to an alkoxy group having at least one halogen. Halo-alkoxy is as defined for alkoxy where some or all of the hydrogen atoms are substituted with halogen atoms. The alkoxy groups can be substituted with 1, 2, 3, or more halogens. When all the hydrogens are replaced with a halogen, for example by fluorine, the compounds are per-substituted, for example, perfluorinated. Halo-alkoxy includes, but is not limited to, trifluoromethoxy, 2,2,2-trifluoroethoxy, perfluoroethoxy, etc.

[0048] "Alkyl amine" refers to an alkyl group as defined within, having one or more amino groups. The amino groups can be primary, secondary or tertiary. The alkyl amine can be further substituted with a hydroxy group. Alkyl amines useful in the present invention include, but are not limited to, ethyl amine, propyl amine, isopropyl amine, ethylene diamine and ethanolamine. The amino group can link the alkyl amine to the point of attachment with the rest of the compound, be at the omega position of the alkyl group, or link together at least two carbon atoms of the alkyl group. One of skill in the art will appreciate that other alkyl amines are useful in the present invention.

[0049] "Heteroalkyl" refers to an alkyl group of any suitable length and having from 1 to 3 heteroatoms such as N, O and S. Additional heteroatoms can also be useful, including, but not limited to, B, Al, Si and P. The heteroatoms can also be oxidized, such as, but not limited to, -S(O)- and -S(O)<sub>2</sub>-. For example, heteroalkyl can include ethers, thioethers and alkyl-amines. The heteroatom portion of the heteroalkyl can replace a hydrogen of the alkyl group to form a hydroxy, thio or amino group. Alternatively, the heteroatom portion can be the connecting atom, or be inserted between two carbon atoms.

[0050] Substituents for the alkyl and heteroalkyl radicals (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) can be one or more of a variety of groups selected from, but not limited to: -OR', =O, =NR', =N-OR', -NR'R'', -SR', -halogen, -SiR'R''R''', -OC(O)R', -C(O)R', -CO<sub>2</sub>R', -CONR'R'', -OC(O)NR'R'', -NR''C(O)R', -NR'-C(O)NR''R''', -NR''C(O)<sub>2</sub>R', -NR-C(NR'R''R''')=NR''', -NR-C(NR'R'')=NR'', -S(O)R', -S(O)<sub>2</sub>R', -S(O)<sub>2</sub>NR'R'', -NRSO<sub>2</sub>R', -CN and -NO<sub>2</sub> in a number ranging from zero to (2m'+1), where m' is the total number of carbon atoms in such radical. R', R'', R''' and R'''' each preferably independently refer to hydrogen, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, *e.g.*, aryl substituted with 1-3 halogens, substituted or unsubstituted alkyl, alkoxy or thioalkoxy groups, or arylalkyl groups. When a compound of the invention includes more than one R group, for example, each of the R groups is independently selected as are each R', R'', R''' and R'''' groups when more than one of these groups is present. When R' and R'' are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 5-, 6-, or 7-membered ring. For example, -NR'R'' is meant to include, but not be limited to, 1-pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents, one of skill in the art will understand that the term "alkyl" is meant to include groups including carbon atoms bound to groups other than hydrogen groups, such as haloalkyl (*e.g.*, -CF<sub>3</sub> and -CH<sub>2</sub>CF<sub>3</sub>) and acyl (*e.g.*, -C(O)CH<sub>3</sub>, -C(O)CF<sub>3</sub>, -C(O)CH<sub>2</sub>OCH<sub>3</sub>, and the like).

[0051] "Alkenyl" refers to either a straight chain or branched hydrocarbon of 2 to 6 carbon atoms, having at least one double bond. Examples of alkenyl groups include, but are not limited to, vinyl, propenyl, isopropenyl, 1-butenyl, 2-butenyl, isobutenyl, butadienyl, 1-pentenyl, 2-pentenyl, isopentenyl, 1,3-pentadienyl, 1,4-pentadienyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 1,3-hexadienyl, 1,4-hexadienyl, 1,5-hexadienyl, 2,4-hexadienyl, or 1,3,5-hexatrienyl. Alkenyl groups can also have from 2 to 3, 2 to 4, 2 to 5, 3 to 4, 3 to 5, 3 to 6, 4

to 5, 4 to 6 and 5 to 6 carbons. The alkenyl groups is typically monovalent, but can be divalent, such as when the alkenyl group links two moieties together.

[0052] "Alkenylene" refers to an alkenyl group, as defined above, linking at least two other groups, *i.e.*, a divalent hydrocarbon radical. The two moieties linked to the alkenylene can be  
5 linked to the same atom or different atoms of the alkenylene. Alkenylene groups include, but are not limited to, ethenylene, propenylene, isopropenylene, butenylene, isobutenylene, sec-butenylene, pentenylene and hexenylene.

[0053] "Alkynyl" refers to either a straight chain or branched hydrocarbon of 2 to 6 carbon atoms, having at least one triple bond. Examples of alkynyl groups include, but are not  
10 limited to, acetylenyl, propynyl, 1-butylyl, 2-butylyl, isobutylyl, sec-butylyl, butadiynyl, 1-pentylyl, 2-pentylyl, isopentylyl, 1,3-pentadiynyl, 1,4-pentadiynyl, 1-hexynyl, 2-hexynyl, 3-hexynyl, 1,3-hexadiynyl, 1,4-hexadiynyl, 1,5-hexadiynyl, 2,4-hexadiynyl, or 1,3,5-hexatriynyl. Alkynyl groups can also have from 2 to 3, 2 to 4, 2 to 5, 3 to 4, 3 to 5, 3 to 6, 4 to 5, 4 to 6 and 5 to 6 carbons. The alkynyl groups is typically monovalent, but can be  
15 divalent, such as when the alkynyl group links two moieties together.

[0054] "Alkynylene" refers to an alkynyl group, as defined above, linking at least two other groups, *i.e.*, a divalent hydrocarbon radical. The two moieties linked to the alkynylene can be linked to the same atom or different atoms of the alkynylene. Alkynylene groups include, but are not limited to, ethynylene, propynylene, isopropynylene, butynylene, sec-butylylene,  
20 pentynylene and hexynylene.

[0055] "Cycloalkyl" refers to a saturated or partially unsaturated, monocyclic, fused bicyclic or bridged polycyclic ring assembly containing from 3 to 12 ring atoms, or the number of atoms indicated. Monocyclic rings include, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cyclooctyl. Bicyclic and polycyclic rings include, for example,  
25 norbornane, decahydronaphthalene and adamantane. For example, C<sub>3-8</sub>cycloalkyl includes cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclooctyl, and norbornane. Partially unsaturated cycloalkyl rings include, but are not limited to, cyclohexene and norbornene.

[0056] "Cycloalkylene" refers to a cycloalkyl group, as defined above, linking at least two other groups, *i.e.*, a divalent hydrocarbon radical. The two moieties linked to the  
30 cycloalkylene can be linked to the same atom or different atoms of the cycloalkylene. Cycloalkylene groups include, but are not limited to, cyclopropylene, cyclobutylene, cyclopentylene, cyclohexylene, and cyclooctylene.

[0057] "Heterocycloalkyl" refers to a ring system having from 3 ring members to about 20 ring members and from 1 to about 5 heteroatoms such as N, O and S. Additional heteroatoms can also be useful, including, but not limited to, B, Al, Si and P. The heteroatoms can also be oxidized, such as, but not limited to, -S(O)- and -S(O)<sub>2</sub>-. Heterocycloalkyl groups can have from 5 to 8 ring members and from 1 to 4 heteroatoms, or from 5 to 8 ring members and from 1 to 3 heteroatoms, or from 5 to 6 ring members and from 1 to 4 heteroatoms, or from 5 to 6 ring members and from 1 to 3 heteroatoms. For example, heterocycloalkyl includes, but is not limited to, tetrahydrofuranyl, tetrahydrothiophenyl, morpholino, pyrrolidinyl, pyrrolinyl, imidazolidinyl, imidazoliny, pyrazolidinyl, pyrazolinyl, piperazinyl, piperidinyl, indolinyl, quinuclidinyl and 1,4-dioxo-8-aza-spiro[4.5]dec-8-yl.

[0058] "Heterocyclalkylene" refers to a heterocyclalkyl group, as defined above, linking at least two other groups. The two moieties linked to the heterocyclalkylene can be linked to the same atom or different atoms of the heterocyclalkylene.

[0059] "Aryl" refers to a monocyclic or fused bicyclic, tricyclic or greater, aromatic ring assembly containing 6 to 16 ring carbon atoms. For example, aryl may be phenyl, benzyl, biphenyl, or naphthyl, preferably phenyl. "Arylene" means a divalent radical derived from an aryl group. Aryl groups can be mono-, di- or tri-substituted by one, two or three radicals selected from alkyl, alkoxy, aryl, hydroxy, halogen, cyano, amino, amino-alkyl, trifluoromethyl, alkylenedioxy and oxy-C<sub>2</sub>-C<sub>3</sub>-alkylene; all of which are optionally further substituted, for instance as hereinbefore defined; or 1- or 2-naphthyl; or 1- or 2-phenanthrenyl. Alkylenedioxy is a divalent substitute attached to two adjacent carbon atoms of phenyl, e.g. methylenedioxy or ethylenedioxy. Oxy-C<sub>2</sub>-C<sub>3</sub>-alkylene is also a divalent substituent attached to two adjacent carbon atoms of phenyl, e.g. oxyethylene or oxypropylene. An example for oxy-C<sub>2</sub>-C<sub>3</sub>-alkylene-phenyl is 2,3-dihydrobenzofuran-5-yl.

[0060] "Arylene" refers to an aryl group, as defined above, linking at least two other groups. The two moieties linked to the arylene are linked to different atoms of the arylene. Arylene groups include, but are not limited to, phenylene.

[0061] "Arylene-oxy" refers to an arylene group, as defined above, where one of the moieties linked to the arylene is linked through an oxygen atom. Arylene-oxy groups include, but are not limited to, phenylene-oxy.

[0062] "Heteroaryl" refers to a monocyclic or fused bicyclic or tricyclic aromatic ring assembly containing 5 to 16 ring atoms, where from 1 to 4 of the ring atoms are a heteroatom each N, O or S. For example, heteroaryl includes pyridyl, indolyl, indazolyl, quinoxaliny, and

quinolinyl, isoquinolinyl, benzothienyl, benzofuranyl, furanyl, pyrrolyl, thiazolyl, benzothiazolyl, oxazolyl, isoxazolyl, triazolyl, tetrazolyl, pyrazolyl, imidazolyl, thienyl, or any other radicals substituted, especially mono- or di-substituted, by e.g. alkyl, nitro or halogen. Pyridyl represents 2-, 3- or 4-pyridyl, advantageously 2- or 3-pyridyl. Thienyl

5 represents 2- or 3-thienyl. Quinolinyl represents preferably 2-, 3- or 4-quinolinyl. Isoquinolinyl represents preferably 1-, 3- or 4-isoquinolinyl. Benzopyranyl, benzothiopyranyl represents preferably 3-benzopyranyl or 3-benzothiopyranyl, respectively. Thiazolyl represents preferably 2- or 4-thiazolyl, and most preferred, 4-thiazolyl. Triazolyl is preferably 1-, 2- or 5-(1,2,4-triazolyl). Tetrazolyl is preferably 5-tetrazolyl.

10 **[0063]** Preferably, heteroaryl is pyridyl, indolyl, quinolinyl, pyrrolyl, thiazolyl, isoxazolyl, triazolyl, tetrazolyl, pyrazolyl, imidazolyl, thienyl, furanyl, benzothiazolyl, benzofuranyl, isoquinolinyl, benzothienyl, oxazolyl, indazolyl, or any of the radicals substituted, especially mono- or di-substituted.

**[0064]** Similarly, substituents for the aryl and heteroaryl groups are varied and are selected from: -halogen, -OR', -OC(O)R', -NR'R'', -SR', -R', -CN, -NO<sub>2</sub>, -CO<sub>2</sub>R', -CONR'R'', -C(O)R', -OC(O)NR'R'', -NR''C(O)R', -NR''C(O)<sub>2</sub>R', -NR'-C(O)NR'R''', -NH-C(NH<sub>2</sub>)=NH, -NR'C(NH<sub>2</sub>)=NH, -NH-C(NH<sub>2</sub>)=NR', -S(O)R', -S(O)<sub>2</sub>R', -S(O)<sub>2</sub>NR'R'', -N<sub>3</sub>, -CH(Ph)<sub>2</sub>, perfluoro(C<sub>1</sub>-C<sub>4</sub>)alkoxy, and perfluoro(C<sub>1</sub>-C<sub>4</sub>)alkyl, in a number ranging from zero to the total number of open valences on the aromatic ring system; and where R', R'' and

20 R''' are independently selected from hydrogen, (C<sub>1</sub>-C<sub>8</sub>)alkyl and heteroalkyl, unsubstituted aryl and heteroaryl, (unsubstituted aryl)-(C<sub>1</sub>-C<sub>4</sub>)alkyl, and (unsubstituted aryl)oxy-(C<sub>1</sub>-C<sub>4</sub>)alkyl.

**[0065]** "Alkyl-aryl" refers to a radical having an alkyl component and an aryl component, where the alkyl component links the aryl component to the point of attachment. The alkyl component is as defined above, except that the alkyl component is at least divalent in order to link to the aryl component and to the point of attachment. In some instances, the alkyl component can be absent. The aryl component is as defined above. Examples of alkyl-aryl groups include, but are not limited to, benzyl.

25

**[0066]** "Alkyl-heteroaryl" refers to a radical having an alkyl component and a heteroaryl component, where the alkyl component links the heteroaryl component to the point of attachment. The alkyl component is as defined above, except that the alkyl component is at least divalent in order to link to the heteroaryl component and to the point of attachment. In

30

some instances, the alkyl component can be absent. The heteroaryl component is as defined within. Examples of alkyl-heteroaryl include methylene-pyridyl, among others.

[0067] "Alkyl-cycloalkyl" refers to a radical having an alkyl component and a cycloalkyl component, where the alkyl component links the cycloalkyl component to the point of attachment. The alkyl component is as defined above, except that the alkyl component is at least divalent in order to link to the cycloalkyl component and to the point of attachment. In some instances, the alkyl component can be absent. The cycloalkyl component is as defined within. Examples of alkyl-cycloalkyl include methylene-cyclohexane, among others.

[0068] "Alkyl-heterocycloalkyl" refers to a radical having an alkyl component and a heterocycloalkyl component, where the alkyl component links the heterocycloalkyl component to the point of attachment. The alkyl component is as defined above, except that the alkyl component is at least divalent in order to link to the heterocycloalkyl component and to the point of attachment. In some instances, the alkyl component can be absent. The heterocycloalkyl component is as defined above. Examples of alkyl-heterocycloalkyl include methylene-piperidinyl, among others.

[0069] "Heteroaryl-aryl" refers to a radical having a heteroaryl component and an aryl component, where the heteroaryl component links the aryl component to the point of attachment on the compound of the present invention. The heteroaryl component is as defined above, except that the heteroaryl component is at least divalent to link to the aryl component and to the point of attachment on the compound of the present invention. The aryl component is as defined above.

[0070] "Linker" refers to a chemical moiety that links the compound of the present invention to a biological material that targets a specific type of cell, such as a cancer cell, other type of diseased cell, or a normal cell type. Linkers useful in the present invention can be up to 30 carbon atoms in length. The types of bonds used to link the linker to the compound and biological molecule of the present invention include, but are not limited to, amides, amines, esters, carbamates, ureas, thioethers, thiocarbamates, thiocarbonate and thioureas. One of skill in the art will appreciate that other types of bonds are useful in the present invention.

[0071] "Pharmaceutically acceptable excipient" refers to a substance that aids the administration of an active agent to and absorption by a subject. Pharmaceutical excipients useful in the present invention include, but are not limited to, binders, fillers, disintegrants, lubricants, coatings, sweeteners, flavors and colors. One of skill in the art will recognize that other pharmaceutical excipients are useful in the present invention.

[0072] “Contacting” refers to the process of bringing into contact at least two distinct species such that they can react. It should be appreciated, however, the resulting reaction product can be produced directly from a reaction between the added reagents or from an intermediate from one or more of the added reagents which can be produced in the reaction mixture.

5 [0073] “Therapeutically effective amount or dose” or “therapeutically sufficient amount or dose” or “effective or sufficient amount or dose” refer to a dose that produces therapeutic effects for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (*see, e.g.,* Lieberman, *Pharmaceutical Dosage Forms* (vols. 1-3, 1992); Lloyd, *The Art, Science and Technology of Pharmaceutical Compounding* (1999); Pickar, *Dosage Calculations* (1999); and *Remington: The Science and Practice of Pharmacy*, 20th Edition, 2003, Gennaro, Ed., Lippincott, Williams & Wilkins).

[0074] “Subject” refers to animals such as mammals, including, but not limited to, primates (*e.g.,* humans), domestic and farm animals, and zoo, sports or pet animals, such as cattle (*e.g.* 15 cows), horses, dogs, sheep, pigs, rabbits, goats, cats, mice, etc. In certain embodiments, the subject is a human.

[0075] “Administering” refers to administration to a specific joint.

[0076] “Treat”, “treating”, “treatment” plus “ameliorate” and “ameliorating” refer to any indicia of success in the treatment or amelioration of an injury, pathology, condition, or 20 symptom (*e.g.,* pain), including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the symptom, injury, pathology or condition more tolerable to the patient; decreasing the frequency or duration of the symptom or condition; or, in some situations, preventing the onset of the symptom or condition. The treatment or amelioration of symptoms can be based on any objective or subjective 25 parameter; including, *e.g.,* the result of a physical examination.

[0077] “At increased risk for” refers to a patient having an above average risk for a particular disease or condition, wherein the increased risk is a result of existing health conditions, genetic or family history, existing or prior injuries, repetitive motion actions or conditions.

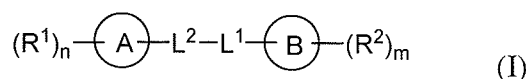
30 [0078] “Chondrocytes” refers to cartilage cells. Chondrocytes produce and maintain the cartilaginous matrix which is composed of collagen and proteoglycans. Chondrocytes are derived from the differentiation of mesenchymal stem cells (MSCs). MSCs are multipotent

stem cells that can differentiate into several different types of cells including, but not limited to, osteoblasts, chondrocytes and adipocytes. Differentiation is the process a specialized cell type is formed from a less specialized cell type, for example, a chondrocyte from a MSC.

[0079] "Hyaluronic acid" refers to derivatives of hyaluronic acid that include esters of hyaluronic acid, salts of hyaluronic acid and also includes the term hyaluronan. The designation also includes both low and high molecular weight forms of hyaluronans and crosslinked hyaluronans or hylans. Examples of such hyaluronans are Synvisc™ (Genzyme Corp. Cambridge, Mass.), ORTHOVISC™ (Anika Therapeutics, Woburn, Mass.), and HYALGAN™ (Sanofi-Synthelabo Inc., Malvern, Pa.).

### 10 III. Compounds

[0080] In some embodiments, the present invention provides compounds of formula I:



[0081] In formula I, each of ring A and ring B are independently cycloalkyl, aryl or heteroaryl.

15 [0082] In formula I, each  $R^1$  and  $R^2$  is independently H,  $C_{1-6}$  alkyl,  $C_{2-6}$  alkenyl,  $C_{2-6}$  alkynyl,  $C_{1-6}$  alkoxy,  $C_{1-6}$  heteroalkyl, halogen,  $C_{1-6}$  haloalkyl,  $C_{1-6}$  haloalkoxy,  $C_{1-6}$  alkyl-CN,  $C_{1-6}$  alkylhydroxy,  $-OR^{2a}$ ,  $-NR^{2b}R^{2d}$ ,  $C_{1-6}$  alkyl- $NR^{2b}R^{2d}$ ,  $-C(O)R^{1a}$ ,  $-C(O)R^{2d}$ ,  $-C(O)OR^{2a}$ ,  $C_{1-6}$  alkyl- $C(O)OR^{2b}$ ,  $-OC(O)R^{2b}$ ,  $-OC(O)OR^{2b}$ ,  $-C(O)NR^{2a}R^{2b}$ ,  $-C(O)N(OH)R^{2b}$ ,  $-NR^{2b}C(O)R^{2c}$ ,  $C_{1-6}$  alkyl- $NR^{2b}C(O)R^{2c}$ ,  $-NR^{2b}C(O)OR^{2c}$ ,  $C_{1-6}$  alkyl- $NR^{2b}C(O)OR^{2c}$ ,  $-OC(O)NR^{2b}R^{2c}$ ,  $-NR^{2b}C(O)NR^{2b}R^{2c}$ ,  $-NR^{2b}C(NR^{2b})NR^{2b}R^{2c}$ ,  $-C(O)NR^{2b}C(O)R^{2b}$ ,  $C_{1-6}$  alkyl- $NR^{2b}C(O)NR^{2b}R^{2c}$ ,  $-SR^{2a}$ ,  $-SO_2R^{2b}$ ,  $-SO_2OR^{2b}$ ,  $-SO_2NR^{2b}R^{2d}$ ,  $-NR^{2b}SO_2R^{2b}$ ,  $-P(O)(OR^{2b})_2$ ,  $-B(OR^{2b})$ ,  $-CN$ ,  $-NO_2$ ,  $-N_3$ , heterocycloalkyl, aryl, heteroaryl,  $C_{1-6}$  alkyl-heterocycloalkyl,  $C_{1-6}$  alkyl-aryl,  $C_{1-6}$  alkyl-O-aryl,  $C_{1-6}$  alkyl-heteroaryl, or heteroaryl-aryl, and wherein the heterocycloalkyl, aryl and heteroaryl groups are optionally substituted with 1 to 2  $R^{2a}$  groups.

20 [0083] In formula I,  $R^{1a}$  is  $-OR^{1b}$  or  $-NR^{1b}R^{1c}$ ;  $R^{1b}$  and  $R^{1c}$  are each independently H,  $C_{1-6}$  alkyl,  $C_{2-6}$  alkenyl,  $C_{2-6}$  alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl,  $C_{1-6}$  alkyl-aryl, or  $C_{1-6}$  alkyl-heteroaryl, wherein the cycloalkyl, heterocycloalkyl, aryl and heteroaryl groups are optionally substituted with from 1 to 4  $R^{1d}$  groups; and each  $R^{1d}$  is  
30 independently H,  $C_{1-6}$  alkyl,  $C_{1-6}$  alkoxy, or  $-NO_2$ .



[0084] In formula I, each  $R^{2a}$  is independently H,  $C_{2-6}$  alkenyl,  $C_{2-6}$  alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl,  $C_{1-6}$  alkyl-cycloalkyl,  $C_{1-6}$  alkyl-heterocycloalkyl,  $C_{1-6}$  alkyl-aryl or  $C_{1-6}$  alkyl-heteroaryl, optionally substituted with 1 to 2  $R^{2b}$  groups; each  $R^{2b}$  and  $R^{2c}$  is independently H, or  $C_{1-6}$  alkyl; and each  $R^{2d}$  is independently H,  $C_{1-6}$  alkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl,  $C_{1-6}$  alkyl-cycloalkyl,  $C_{1-6}$  alkyl-heterocycloalkyl,  $C_{1-6}$  alkyl-aryl or  $C_{1-6}$  alkyl-heteroaryl, each optionally substituted with 1 to 2  $R^{2b}$  groups.

[0085] In formula I, each of  $L^1$  and  $L^2$  are independently  $L^1$  and  $L^2$  are independently a bond,  $C_{1-6}$  alkylene,  $C_{2-6}$  alkenylene,  $C_{1-6}$  alkylene-O-, -O- $C_{1-6}$  alkylene,  $C_{1-6}$  alkylene- $NR^{3a}$ -,  
 10 - $NR^{3a}$ - $C_{1-6}$  alkylene, -C(O)-,  $C_{1-6}$  alkylene-C(O)-, -C(O)- $C_{1-6}$  alkylene-NH-,  
 -NH- $C_{1-6}$  alkylene-C(O)-, -C(O)NH-, -NHC(O)-,  $C_{1-6}$  alkylene-NHC(O)-, -SO<sub>2</sub>NH-,  
 -NHSO<sub>2</sub>-, -NHC(O)NH-, cycloalkylene, -N=N-, or -C( $R^{3a}$ )=N( $R^{3c}$ )-, wherein the alkylene group is optionally substituted with from 1-4  $R^{3b}$  groups.  $R^{3a}$  of formula I is H or  $C_{1-6}$  alkyl. Each  $R^{3b}$  of formula I is independently H,  $C_{1-6}$  alkyl, halogen, -OR<sup>3a</sup> or -NR<sup>3a</sup>R<sup>3a</sup>.  $R^{3c}$  of  
 15 formula I is absent or -OH.

[0086] Alternatively,  $L^2$  is combined with  $R^1$ ,  $L^1$  is combined with  $L^2$ ,  $L^1$  is combined with  $R^2$ , two  $R^1$  groups on adjacent ring atoms, or two  $R^2$  groups on adjacent ring atoms are combined to form a 5-6 membered heterocycloalkyl with from 1 to 3 heteroatoms selected from N, O and S, or a 5-6 membered heteroaryl with from 1 to 3 heteroatoms selected from  
 20 N, O and S, and optionally substituted with from 1 to 3 groups of H,  $C_{1-6}$  alkyl or oxo.

[0087] In formula I, subscripts m and n are each an integer from 1 to 3.

[0088] Moreover, the compounds of formula I are those wherein:

- (a)  $L^1$  is a bond,  $L^2$  is -C(O)NH-, ring B is phenyl, and at least one  $R^2$  is -CN or phenyl, or
- 25 (b) at least one  $R^1$  is -C(O)OH, ring A is phenyl,  $L^2$  is -C(O)NH-, and  $L^1$  is a bond or  $C_{1-6}$  alkylene, or
- (c) each of ring A and ring B is phenyl, at least one  $R^1$  is -C(O)OH or combined with  $L^2$ , and at least one  $R^2$  is H, -CN and -C(O)OH.

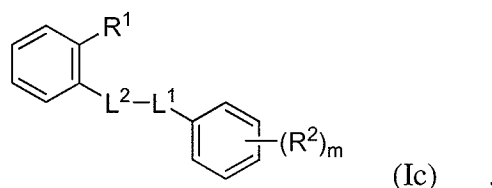
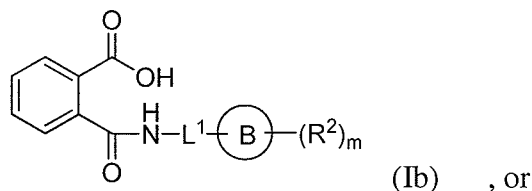
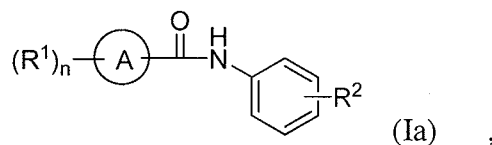
[0089] The compounds of formula I are such that when  $R^1$  is -CO<sub>2</sub>H, subscript n is 1, ring  
 30 A is phenyl,  $L^2$  is -C(O)NH-,  $L^1$  is a bond, ring B is phenyl, subscript m is 1, and  $R^2$  is phenyl, then the phenyl of  $R^2$  is substituted with  $C_{1-6}$  alkyl.

[0090] The compounds of formula I include the salts and isomers thereof.

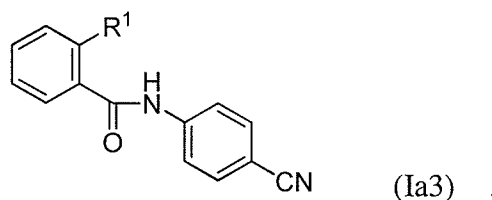
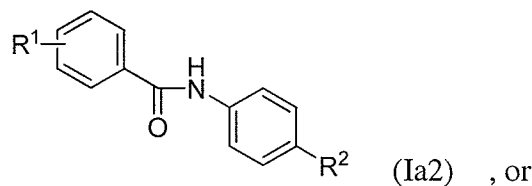
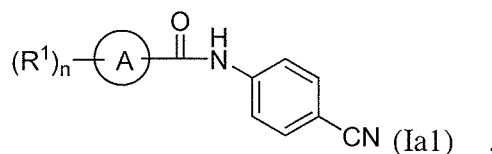
[0091] In some embodiments, the compounds of formula I are those wherein:

- (a)  $L^1$  is a bond,  $L^2$  is  $-C(O)NH-$ , ring B is phenyl,  $R^2$  is  $-CN$  or phenyl, and subscript  $m$  is 1, or
- (b)  $R^1$  is  $-C(O)OH$ , subscript  $n$  is 1, ring A is phenyl,  $L^2$  is  $-C(O)NH-$ , and  $L^1$  is a bond or  $-CH_2-$ , or
- (c) each of ring A and ring B is phenyl,  $R^1$  is  $-C(O)OH$  or combined with  $L^2$ , subscript  $n$  is 1, and at least one  $R^2$  is selected from the group consisting of H,  $-CN$  and  $-CO_2H$ .

[0092] In some embodiments, the compounds of the present invention have the structure:



[0093] The compounds of the present invention can also have any of the following structures:



[0094] In some embodiments, the compounds of formulas I, Ia, Ia1, Ia2, Ia3 and Ic are those wherein each R<sup>1</sup> is independently C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, C<sub>1-6</sub> alkoxy, C<sub>1-6</sub> heteroalkyl, halogen, C<sub>1-6</sub> haloalkyl, C<sub>1-6</sub> haloalkoxy, C<sub>1-6</sub> alkyl-CN, C<sub>1-6</sub> alkylhydroxy, -OR<sup>2a</sup>, -NR<sup>2b</sup>R<sup>2d</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>R<sup>2d</sup>, -C(O)R<sup>1a</sup>, -C(O)R<sup>2d</sup>, -C(O)OR<sup>2a</sup>, C<sub>1-6</sub> alkyl-C(O)OR<sup>2b</sup>,  
 5 -OC(O)R<sup>2b</sup>, -OC(O)OR<sup>2b</sup>, -C(O)NR<sup>2a</sup>R<sup>2b</sup>, -C(O)N(OH)R<sup>2b</sup>, -NR<sup>2b</sup>C(O)R<sup>2c</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)R<sup>2c</sup>, -NR<sup>2b</sup>C(O)OR<sup>2c</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)OR<sup>2c</sup>, -OC(O)NR<sup>2b</sup>R<sup>2c</sup>, -NR<sup>2b</sup>C(O)NR<sup>2b</sup>R<sup>2c</sup>, -NR<sup>2b</sup>C(NR<sup>2b</sup>)NR<sup>2b</sup>R<sup>2c</sup>, -C(O)NR<sup>2b</sup>C(O)R<sup>2b</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)NR<sup>2b</sup>R<sup>2c</sup>, -SR<sup>2a</sup>, -SO<sub>2</sub>R<sup>2b</sup>, -SO<sub>2</sub>OR<sup>2b</sup>, -SO<sub>2</sub>NR<sup>2b</sup>R<sup>2d</sup>, -NR<sup>2b</sup>SO<sub>2</sub>R<sup>2b</sup>, -P(O)(OR<sup>2b</sup>)<sub>2</sub>, -B(OR<sup>2b</sup>), -CN, -NO<sub>2</sub>, -N<sub>3</sub>, heterocycloalkyl, aryl, heteroaryl,  
 10 C<sub>1-6</sub> alkyl-heterocycloalkyl, C<sub>1-6</sub> alkyl-aryl, C<sub>1-6</sub> alkyl-O-aryl, C<sub>1-6</sub> alkyl-heteroaryl, or heteroaryl-aryl, and wherein the heterocycloalkyl, aryl and heteroaryl groups are optionally substituted with 1 to 2 R<sup>2a</sup> groups. In some embodiments, the compounds of formulas I, Ia, Ia1, Ia2, Ia3 and Ic are those wherein each R<sup>1</sup> is independently -C(O)R<sup>2d</sup>, -C(O)OR<sup>2b</sup>, C<sub>1-6</sub> alkyl-C(O)OR<sup>2b</sup>, -NR<sup>2b</sup>C(O)OR<sup>2c</sup>, -NR<sup>2b</sup>C(O)NR<sup>2b</sup>R<sup>2c</sup>, -SO<sub>2</sub>OR<sup>2b</sup>, -SO<sub>2</sub>NR<sup>2b</sup>R<sup>2d</sup>,  
 15 -NR<sup>2b</sup>SO<sub>2</sub>R<sup>2b</sup>, or -CN. In some embodiments, the compounds of formulas I, Ia, Ia1, Ia2, Ia3 and Ic are those wherein each R<sup>1</sup> is independently -CH<sub>2</sub>C(O)OH, -C(O)Me, -NHC(O)NH<sub>2</sub>, -NHC(O)OMe, -NH<sub>2</sub>SO<sub>2</sub>Me, -SO<sub>2</sub>NH<sub>2</sub>, -SO<sub>2</sub>NHMe, -SO<sub>3</sub>H, -C(O)OH, or -CN. Moreover, each of R<sup>2a</sup>, R<sup>2b</sup>, R<sup>2c</sup> and R<sup>2d</sup> are independently H or C<sub>1-6</sub> alkyl. In the compounds of formulas I, Ia, Ia1, Ia2, Ia3 and Ic, the compounds are those wherein when ring A is phenyl and at least one R<sup>1</sup> is -C(O)OH, then subscript n is 2 or 3.

[0095] In some embodiments, the compounds of formulas I, Ia, Ia2, Ib and Ic are those wherein each R<sup>2</sup> is independently H, C<sub>1-6</sub> haloalkoxy, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>R<sup>2d</sup>, -C(O)OR<sup>2b</sup>, -C(O)N(OH)R<sup>2b</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)OR<sup>2c</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)NR<sup>2b</sup>R<sup>2c</sup>, -SO<sub>2</sub>OR<sup>2b</sup>, -PO<sub>3</sub>H, -CN, aryl, heteroaryl, or C<sub>1-6</sub> alkyl-O-aryl. In some embodiments, the compounds of  
 25 formulas I, Ia, Ia2, Ib and Ic are those wherein each R<sup>2</sup> is independently H, -CH<sub>2</sub>NHCONH<sub>2</sub>, -CH<sub>2</sub>NHCOOMe, -CH<sub>2</sub>NHMe, -CH<sub>2</sub>OPh, 2-CN, 4-CN, -C(O)OH, -CONHOH, -OCF<sub>2</sub>H, -PO<sub>3</sub>H, -SO<sub>3</sub>H, phenyl, pyridyl, imidazole or tetrazole. In some embodiments, the compounds of formulas I, Ia, Ia2, Ib and Ic are those wherein each R<sup>2</sup> is independently C<sub>1-6</sub> alkyl, halogen, C<sub>1-6</sub> alkylhydroxy, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>R<sup>2d</sup>, -C(O)R<sup>2d</sup>, -C(O)OR<sup>2b</sup>,  
 30 -C(O)NR<sup>2b</sup>R<sup>2c</sup>, -SO<sub>2</sub>NR<sup>2b</sup>R<sup>2d</sup>, -CN, - heterocycloalkyl, or aryl, wherein the aryl groups are optionally substituted with halogen. In some embodiments, the compounds of formulas I, Ia, Ia2, Ib and Ic are those wherein each R<sup>2</sup> is H, Me, -Cl, -CH<sub>2</sub>OH, -CH<sub>2</sub>CH<sub>2</sub>OH, -CH<sub>2</sub>NH<sub>2</sub>, -C(O)Me, -C(O)OH, -C(O)NH<sub>2</sub>, -CN, morpholine, 3,4-difluorophenyl or -SO<sub>2</sub>NH<sub>2</sub>. Alternatively, in the compounds of formulas I, Ia2, Ib and Ic, two R<sup>2</sup> groups on adjacent ring

atoms can be combined to form a 5-membered heterocycloalkyl. Alternatively, the compounds of formulas I, Ia, Ia2, Ib and Ic are those where two R<sup>2</sup> groups on adjacent ring atoms are combined to form a 1,3-dioxole or 1-methylpyrrolidine-2,5-dione.

[0096] In some embodiments, the compounds of the present invention have the structure:



[0097] In the compounds of formula Ia1, each R<sup>1</sup> is independently C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, C<sub>1-6</sub> alkoxy, C<sub>1-6</sub> heteroalkyl, halogen, C<sub>1-6</sub> haloalkyl, C<sub>1-6</sub> haloalkoxy, C<sub>1-6</sub> alkyl-CN, C<sub>1-6</sub> alkylhydroxy, -OR<sup>2a</sup>, -NR<sup>2b</sup>R<sup>2d</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>R<sup>2d</sup>, -C(O)R<sup>1a</sup>, -C(O)R<sup>2d</sup>, -C(O)OR<sup>2a</sup>, C<sub>1-6</sub> alkyl-C(O)OR<sup>2b</sup>, -OC(O)R<sup>2b</sup>, -OC(O)OR<sup>2b</sup>, -C(O)NR<sup>2a</sup>R<sup>2b</sup>, -C(O)N(OH)R<sup>2b</sup>,  
 10 -NR<sup>2b</sup>C(O)R<sup>2c</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)R<sup>2c</sup>, -NR<sup>2b</sup>C(O)OR<sup>2c</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)OR<sup>2c</sup>, -OC(O)NR<sup>2b</sup>R<sup>2c</sup>, -NR<sup>2b</sup>C(O)NR<sup>2b</sup>R<sup>2c</sup>, -NR<sup>2b</sup>C(NR<sup>2b</sup>)NR<sup>2b</sup>R<sup>2c</sup>, -C(O)NR<sup>2b</sup>C(O)R<sup>2b</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)NR<sup>2b</sup>R<sup>2c</sup>, -SR<sup>2a</sup>, -SO<sub>2</sub>R<sup>2b</sup>, -SO<sub>2</sub>OR<sup>2b</sup>, -SO<sub>2</sub>NR<sup>2b</sup>R<sup>2d</sup>, -NR<sup>2b</sup>SO<sub>2</sub>R<sup>2b</sup>, -P(O)(OR<sup>2b</sup>)<sub>2</sub>, -B(OR<sup>2b</sup>), -CN, -NO<sub>2</sub>, -N<sub>3</sub>, heterocycloalkyl, aryl, heteroaryl, C<sub>1-6</sub> alkyl-heterocycloalkyl, C<sub>1-6</sub> alkyl-aryl, C<sub>1-6</sub> alkyl-O-aryl, C<sub>1-6</sub> alkyl-heteroaryl, or  
 15 heteroaryl-aryl, and wherein the heterocycloalkyl, aryl and heteroaryl groups are optionally substituted with 1 to 2 R<sup>2a</sup> groups. Moreover, each of R<sup>2a</sup>, R<sup>2b</sup>, R<sup>2c</sup> and R<sup>2d</sup> are independently H or C<sub>1-6</sub> alkyl.

[0098] In the compounds of formula Ia1, ring A is phenyl, biphenyl or pyridyl.

[0099] In formula Ia1, the compounds are those wherein when ring A is phenyl and at least  
 20 one R<sup>1</sup> is -C(O)OH, then subscript n is 2 or 3.

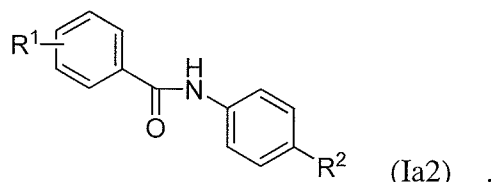
[0100] In some embodiments, the compounds of formula Ia1 are those wherein each R<sup>1</sup> is independently -C(O)R<sup>2d</sup>, -C(O)OR<sup>2b</sup>, C<sub>1-6</sub> alkyl-C(O)OR<sup>2b</sup>, -NR<sup>2b</sup>C(O)OR<sup>2c</sup>, -NR<sup>2b</sup>C(O)NR<sup>2b</sup>R<sup>2c</sup>, -SO<sub>2</sub>OR<sup>2b</sup>, -SO<sub>2</sub>NR<sup>2b</sup>R<sup>2d</sup>, -NR<sup>2b</sup>SO<sub>2</sub>R<sup>2b</sup>, or -CN.

[0101] In some embodiments, the compounds of formula Ia1 are those wherein each R<sup>1</sup> is  
 25 independently -CH<sub>2</sub>C(O)OH, -C(O)Me, -NHC(O)NH<sub>2</sub>, -NHC(O)OMe, -NHSO<sub>2</sub>Me, -SO<sub>2</sub>NH<sub>2</sub>, -SO<sub>2</sub>NHMe, -SO<sub>3</sub>H, -C(O)OH, or -CN.

[0102] In some embodiments, the compounds of formula Ia1 are those wherein ring A is phenyl, and subscript n is 1. In other embodiments, the compounds of formula Ia1 are those wherein ring A is biphenyl or pyridyl, or subscript n is 2. In some other embodiments, the

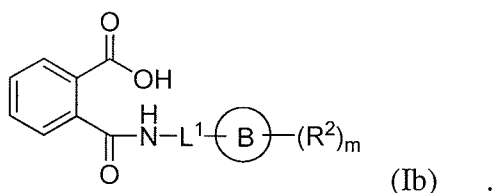
compounds of formula Ia1 are those wherein ring A is biphenyl or pyridyl. In still other embodiments, the compounds of formula Ia1 are those wherein subscript n is 2.

[0103] In some embodiments, the compounds of the present invention have the structure:



5 [0104] In the compounds of formula Ia2, R<sup>1</sup> is C<sub>1-6</sub> alkyl or -C(O)OR<sup>2b</sup>, and R<sup>2</sup> is -CN or Ph.

[0105] In some embodiments, the compounds of the present invention have the structure:



10 [0106] In the compounds of formula Ib, each R<sup>2</sup> is independently H, C<sub>1-6</sub> haloalkoxy, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>R<sup>2d</sup>, -C(O)OR<sup>2b</sup>, -C(O)N(OH)R<sup>2b</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)OR<sup>2c</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)NR<sup>2b</sup>R<sup>2c</sup>, -SO<sub>2</sub>OR<sup>2b</sup>, -PO<sub>3</sub>H, -CN, aryl, heteroaryl, or C<sub>1-6</sub> alkyl-O-aryl. Ring B of formula Ib is cyclohexyl, phenyl, imidazole, oxazole, thiazole, pyrimidine, or pyrazine. L<sup>1</sup> of formula Ib is a bond or -CH<sub>2</sub>-.

15 [0107] The compounds of formula Ib are those wherein when R<sup>2</sup> is C<sub>1-6</sub> alkyl-NR<sup>2b</sup>R<sup>2d</sup>, then one of R<sup>2b</sup> and R<sup>2d</sup> is C<sub>1-6</sub> alkyl; when R<sup>2</sup> is -C(O)OH, then L<sup>1</sup> is -CH<sub>2</sub>- or ring B is cyclohexyl, or both; when R<sup>2</sup> is -CN, then L<sup>1</sup> is -CH<sub>2</sub>- or ring B is cyclohexyl, or both; and when ring B is 2-thiazole, R<sup>2</sup> is unsubstituted phenyl.

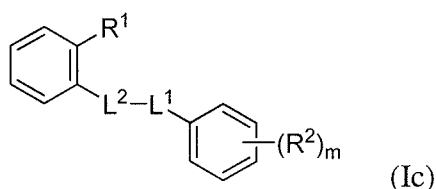
20 [0108] In some embodiments, the compounds of formula Ib are those wherein each R<sup>2</sup> is independently H, -CH<sub>2</sub>NHCONH<sub>2</sub>, -CH<sub>2</sub>NHCOOMe, -CH<sub>2</sub>NHMe, -CH<sub>2</sub>OPh, 2-CN, 4-CN, -C(O)OH, -CONHOH, -OCF<sub>2</sub>H, -PO<sub>3</sub>H, -SO<sub>3</sub>H, phenyl, pyridyl, imidazole or tetrazole.

25 [0109] In some embodiments, the compounds of formula Ib are those wherein each R<sup>2</sup> is independently C<sub>1-6</sub> alkyl, halogen, C<sub>1-6</sub> alkylhydroxy, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>R<sup>2d</sup>, -C(O)R<sup>2d</sup>, -C(O)OR<sup>2b</sup>, -C(O)NR<sup>2b</sup>R<sup>2c</sup>, -SO<sub>2</sub>NR<sup>2b</sup>R<sup>2d</sup>, -CN, - heterocycloalkyl, or aryl, wherein the aryl groups are optionally substituted with halogen. Alternatively, two R<sup>2</sup> groups on adjacent ring atoms of formula Ib can be combined to form a 5-membered heterocycloalkyl. Ring B of formula Ib is phenyl, thiazole or pyridyl. L<sup>1</sup> of formula Ib is a bond or -CH<sub>2</sub>-. Moreover, the

compounds of formula Ib are those wherein when  $R^2$  is  $C_{1-6}$  alkyl- $NR^{2b}R^{2d}$ , then both of  $R^{2b}$  and  $R^{2d}$  are H; when  $R^2$  is  $-C(O)OH$ , then  $L^1$  is a bond and ring B is phenyl; when  $R^2$  is  $-CN$ , then  $L^1$  is a bond and ring B is phenyl; and when ring B is 2-thiazole, then  $R^2$  is substituted phenyl.

- 5 [0110] In some embodiments, the compounds of formula Ib are those wherein each  $R^2$  is H, Me,  $-Cl$ ,  $-CH_2OH$ ,  $-CH_2CH_2OH$ ,  $-CH_2NH_2$ ,  $-C(O)Me$ ,  $-C(O)OH$ ,  $-C(O)NH_2$ ,  $-CN$ , morpholine, 3,4-difluorophenyl or  $-SO_2NH_2$ . Alternatively, two  $R^2$  groups on adjacent ring atoms are combined to form a 1,3-dioxole or 1-methylpyrrolidine-2,5-dione.

[0111] In some embodiments, the compounds of the present invention have the structure:

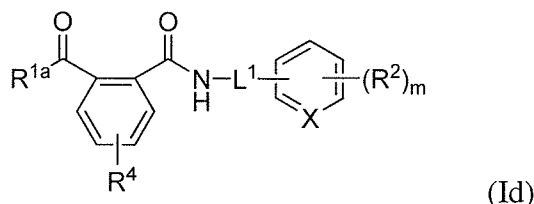


10

[0112] In the compounds of formula Ic,  $R^1$  is  $-C(O)OH$ . Each  $R^2$  of formula Ic is independently  $-CN$  or  $-C(O)OH$ . Each of  $L^1$  and  $L^2$  in formula IC are independently a bond,  $C_{1-6}$  alkylene,  $C_{2-6}$  alkenylene,  $-C(O)-$ ,  $C_{1-6}$  alkylene- $C(O)-$ ,  $-C(O)-C_{1-6}$  alkylene- $NH-$ ,  $-NH-C_{1-6}$  alkylene- $C(O)-$ ,  $-NHC(O)-$ ,  $-SO_2NH-$ ,  $-NHSO_2-$ , or  $-NHC(O)NH-$ , wherein at least one of  $L^1$  and  $L^2$  is  $-C(O)-$ ,  $C_{1-6}$  alkylene- $C(O)-$ ,  $-C(O)-C_{1-6}$  alkylene- $NH-$ ,  $-NH-C_{1-6}$  alkylene- $C(O)-$ ,  $-NHC(O)-$ ,  $-SO_2NH-$ ,  $-NHSO_2-$ , or  $-NHC(O)NH-$ . Alternatively,  $L^2$  is combined with  $R^1$ , or  $L^1$  is combined with  $R^2$ , to form a 5-6 membered heterocycloalkyl with from 1 to 3 heteroatoms selected from N, O and S, or a 5-6 membered heteroaryl with from 1 to 3 heteroatoms selected from N, O and S.

15

20 [0113] In some embodiments, the compounds of the present invention have the structure:



[0114] In the compounds of formula Id,  $R^{1a}$  is  $-OR^{1b}$  or  $-NR^{1b}R^{1c}$ .  $R^{1b}$  and  $R^{1c}$  of formula Id are each independently H,  $C_{1-6}$  alkyl,  $C_{2-6}$  alkenyl,  $C_{2-6}$  alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl,  $C_{1-6}$  alkyl-aryl, and  $C_{1-6}$  alkyl-heteroaryl, wherein the cycloalkyl, heterocycloalkyl, aryl or heteroaryl groups are optionally substituted with from 1 to 4  $R^{1d}$  groups. Each  $R^{1d}$  of formula ID is independently H,  $C_{1-6}$  alkyl,  $C_{1-6}$  alkoxy, or  $-NO_2$ .

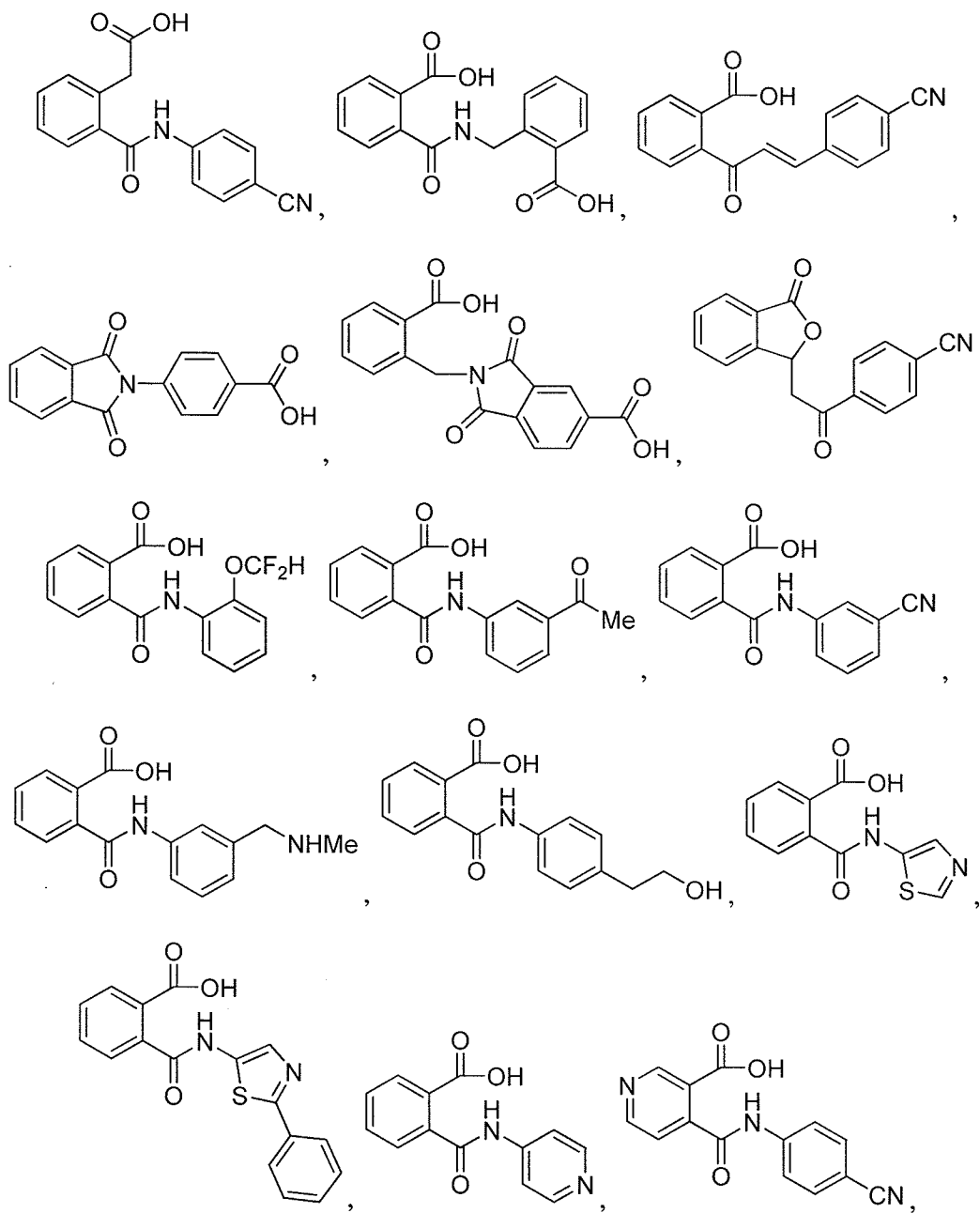
25

- [0115] In the compounds of formula Id, each  $R^2$  is independently  $C_{1-6}$  alkyl,  $C_{1-6}$  alkoxy, halogen,  $C_{1-6}$  haloalkyl,  $C_{1-6}$  haloalkoxy,  $C_{1-6}$  alkylamine,  $C_{1-6}$  alkyl-CN,  $C_{1-6}$  alkyl-OH, heterocycloalkyl, aryl, heteroaryl,  $C_{1-6}$  alkyl-aryl,  $C_{1-6}$  alkyl-heteroaryl, heteroaryl-aryl,  $-OR^{2a}$ ,  $-NR^{2b}R^{2d}$ ,  $-C(O)R^{2d}$ ,  $-C(O)OR^{2b}$ ,  $-OC(O)R^{2b}$ ,  $-C(O)NR^{2b}R^{2c}$ ,  $-NR^{2b}C(O)R^{2c}$ ,  
5  $-NR^{2b}C(O)OR^{2c}$ ,  $-OC(O)NR^{2b}R^{2c}$ ,  $-SO_2R^{2a}$ ,  $-SO_2NR^{2b}R^{2d}$ ,  $-CN$ ,  $-NO_2$ , or  $-N_3$ , wherein the heterocycloalkyl, aryl and heteroaryl groups are optionally substituted with 1 to 2  $R^{2b}$  groups. Alternatively, two  $R^2$  groups on adjacent ring atoms are combined to form a 5 to 6 membered heterocyclic ring having from 1 to 3 heteroatoms each independently N, O and S, and optionally substituted with from 1 to 3 groups of H,  $C_{1-6}$  alkyl or oxo.
- 10 [0116] In the compounds of formula Id, each  $R^{2a}$  is independently H,  $C_{2-6}$  alkenyl,  $C_{2-6}$  alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl,  $C_{1-6}$  alkyl-cycloalkyl,  $C_{1-6}$  alkyl-heterocycloalkyl,  $C_{1-6}$  alkyl-aryl or  $C_{1-6}$  alkyl-heteroaryl, optionally substituted with 1 to 2  $R^{2b}$  groups. Each  $R^{2b}$  and  $R^{2c}$  of formula Id is independently H or  $C_{1-6}$  alkyl. Each  $R^{2d}$  of formula Id is independently H,  $C_{1-6}$  alkyl, cycloalkyl, heterocycloalkyl, aryl,  
15 heteroaryl,  $C_{1-6}$  alkyl-cycloalkyl,  $C_{1-6}$  alkyl-heterocycloalkyl,  $C_{1-6}$  alkyl-aryl or  $C_{1-6}$  alkyl-heteroaryl, each optionally substituted with 1 to 2  $R^{2b}$  groups.
- [0117] In the compounds of formula Id,  $R^4$  is independently  $C_{1-6}$  alkyl, halogen,  $C_{1-6}$  haloalkyl,  $C_{1-6}$  alkoxy,  $C_{1-6}$  haloalkoxy, OH,  $-CO_2H$ , or  $-NO_2$ .
- [0118] In the compounds of formula Id,  $L^1$  is a bond,  $C_{1-6}$  alkylene,  $-C(O)-$ ,  
20  $-C_{1-6}$  alkylene-NH-,  $-C_{1-6}$  alkylene-NHC(O)-, or heteroarylene.
- [0119] In the compounds of formula Id, X is  $-CH-$  or  $-N-$ .
- [0120] In some embodiments, the compounds of formula Id are those wherein each  $R^2$  is independently  $C_{1-6}$  alkyl,  $C_{1-6}$  alkoxy, halogen,  $C_{1-6}$  haloalkyl,  $C_{1-6}$  alkyl-CN,  $C_{1-6}$  alkyl-OH, heterocycloalkyl, aryl, heteroaryl,  $C_{1-6}$  alkyl-aryl,  $C_{1-6}$  alkyl-heteroaryl, heteroaryl-aryl,  
25  $-OR^{2a}$ ,  $-NR^{2b}R^{2d}$ ,  $-C(O)R^{2d}$ ,  $-OC(O)R^{2b}$ ,  $-C(O)NR^{2b}R^{2c}$ ,  $-NR^{2b}C(O)R^{2c}$ ,  $-NR^{2b}C(O)OR^{2c}$ ,  $-OC(O)NR^{2b}R^{2c}$ ,  $-SO_2R^{2a}$ ,  $-SO_2NR^{2b}R^{2d}$ ,  $-CN$ ,  $-NO_2$ , or  $-N_3$ , wherein the heterocycloalkyl, and aryl groups are optionally substituted with 1 to 2  $R^{2b}$  groups, such that when  $R^2$  is  $-CN$ , then subscript m is 1,  $R^{1a}$  is OH,  $R^4$  is H, and  $L^1$  is a bond.
- [0121] In some embodiments, the compounds of formula Id are those wherein each  $R^2$  is  
30 independently  $C_{1-6}$  alkyl, halogen,  $C_{1-6}$  haloalkyl,  $C_{1-6}$  alkyl-CN,  $C_{1-6}$  alkyl-OH, heterocycloalkyl, aryl, heteroaryl,  $C_{1-6}$  alkyl-aryl,  $C_{1-6}$  alkyl-heteroaryl, heteroaryl-aryl,  $-OR^{2a}$ ,  $-NR^{2b}R^{2d}$ ,  $-C(O)R^{2d}$ ,  $-OC(O)R^{2b}$ ,  $-C(O)NR^{2b}R^{2c}$ ,  $-NR^{2b}C(O)R^{2c}$ ,  $-NR^{2b}C(O)OR^{2c}$ ,

-OC(O)NR<sup>2b</sup>R<sup>2c</sup>, -SO<sub>2</sub>R<sup>2a</sup>, -SO<sub>2</sub>NR<sup>2b</sup>R<sup>2d</sup>, -CN, -NO<sub>2</sub>, and -N<sub>3</sub>, wherein the heterocycloalkyl, and aryl groups are optionally substituted with 1 to 2 R<sup>2b</sup> groups; and L<sup>1</sup> is a bond. The compounds of formula Id are those wherein when R<sup>2</sup> is -CN, then subscript m is 1, R<sup>1a</sup> is OH, R<sup>4</sup> is H, and L<sup>1</sup> is a bond. In other embodiments, when two R<sup>2</sup> groups are present on adjacent ring atoms, the R<sup>2</sup> groups are not combined.

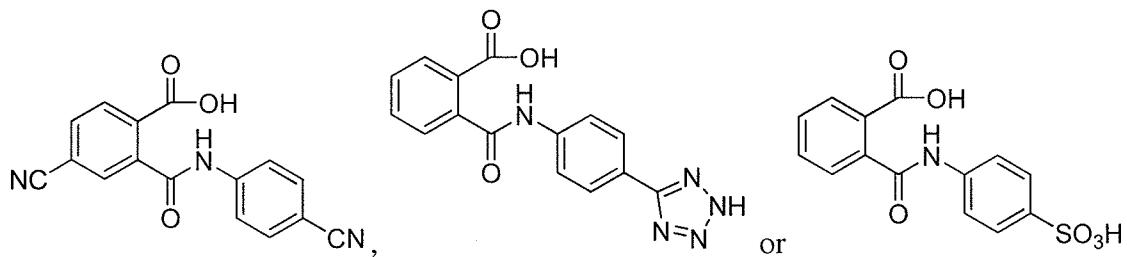
5

[0122] In some embodiments, the compound of the present invention has the structure:

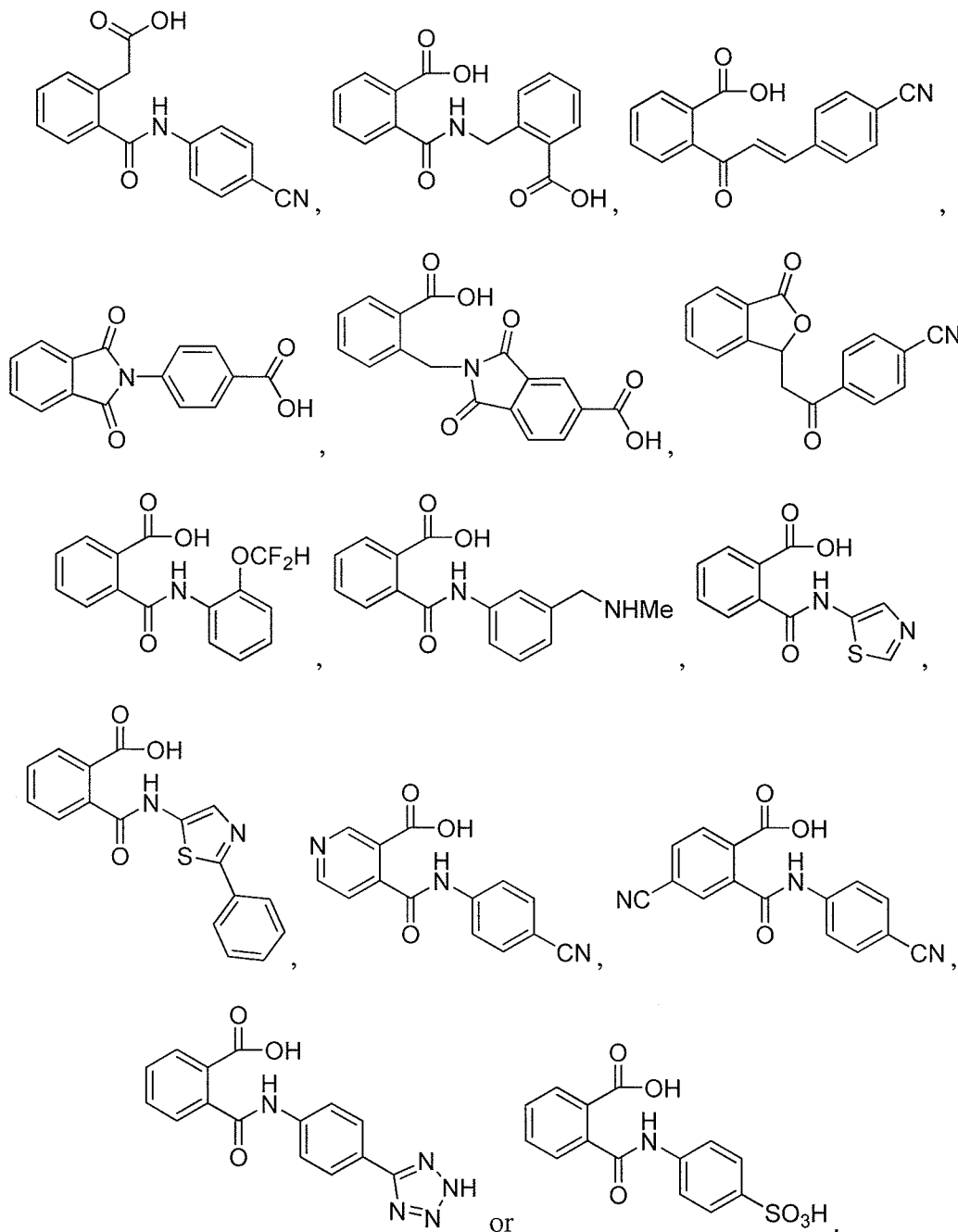


10

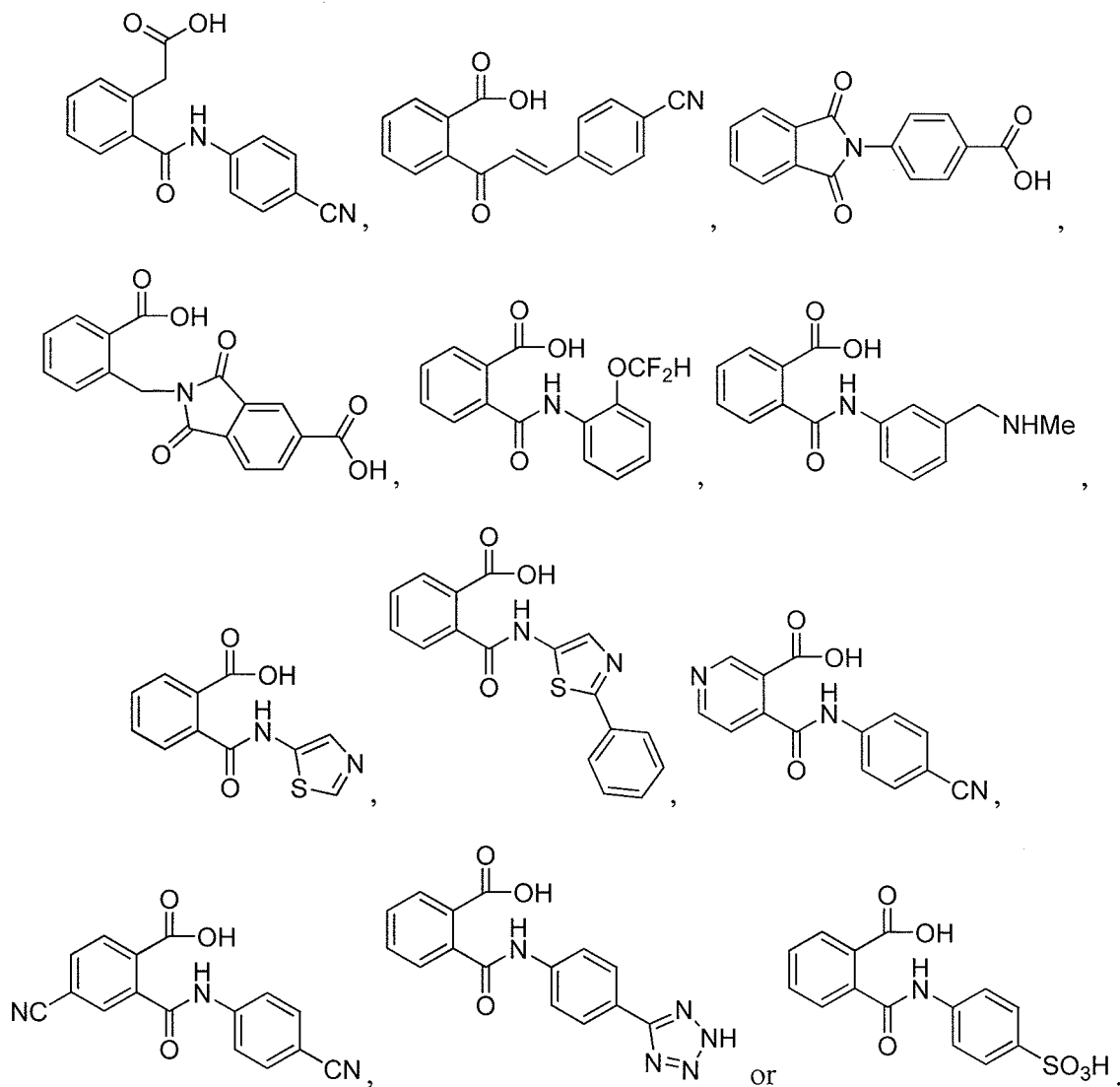




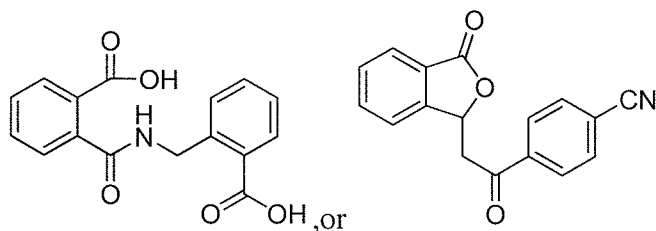
[0123] In some embodiments, the compound of the present invention has the structure:



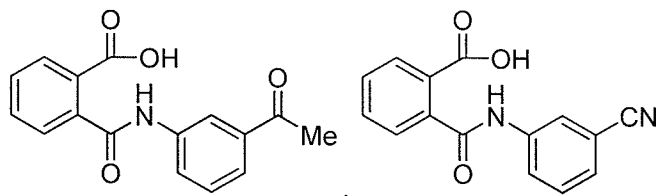
[0124] In some embodiments, the compound of the present invention has the structure:

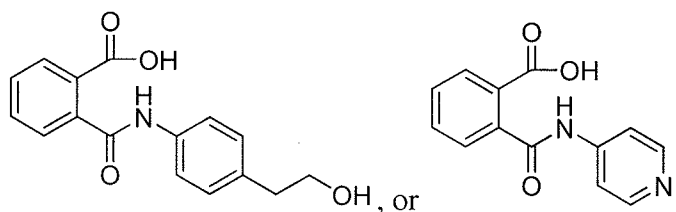


[0125] In some embodiments, the compound of the present invention has the structure:

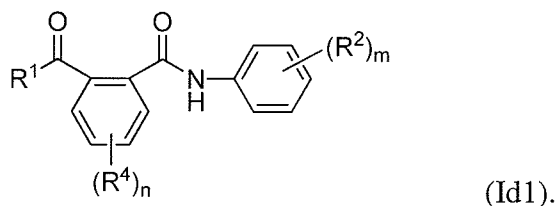


[0126] In some embodiments, the compound of the present invention has the structure:

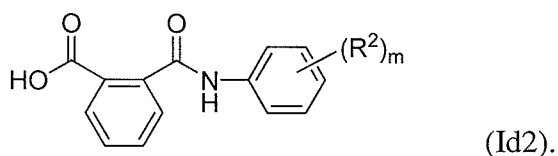




[0127] In some embodiments, the compound of the present invention has the structure:



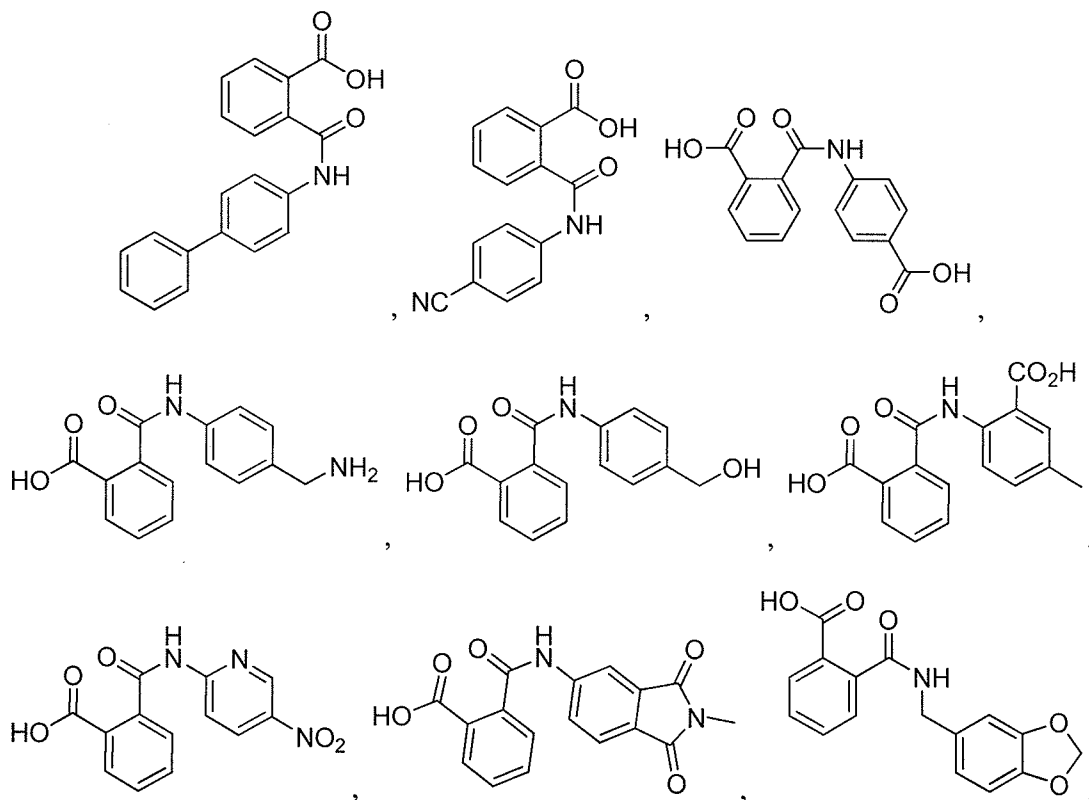
[0128] In some embodiments, the compound of the present invention has the structure:



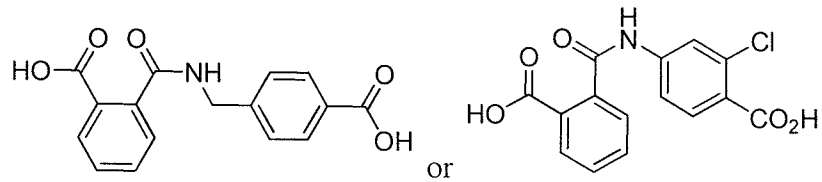
5

[0129] In some embodiments, each  $R^2$  is  $C_{1-6}$  alkyl,  $C_{1-6}$  alkyl-OH,  $C_{1-6}$  alkylamine, halogen, phenyl,  $-NO_2$ ,  $-CO_2H$ , or  $-CN$ .

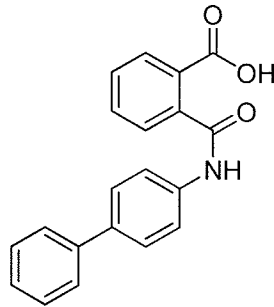
[0130] In some embodiments, the compound of the present invention is:



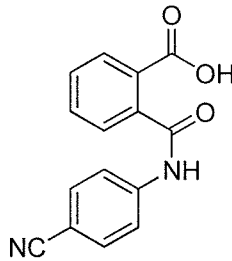
10



[0131] In some embodiments, the compound of the present invention is:

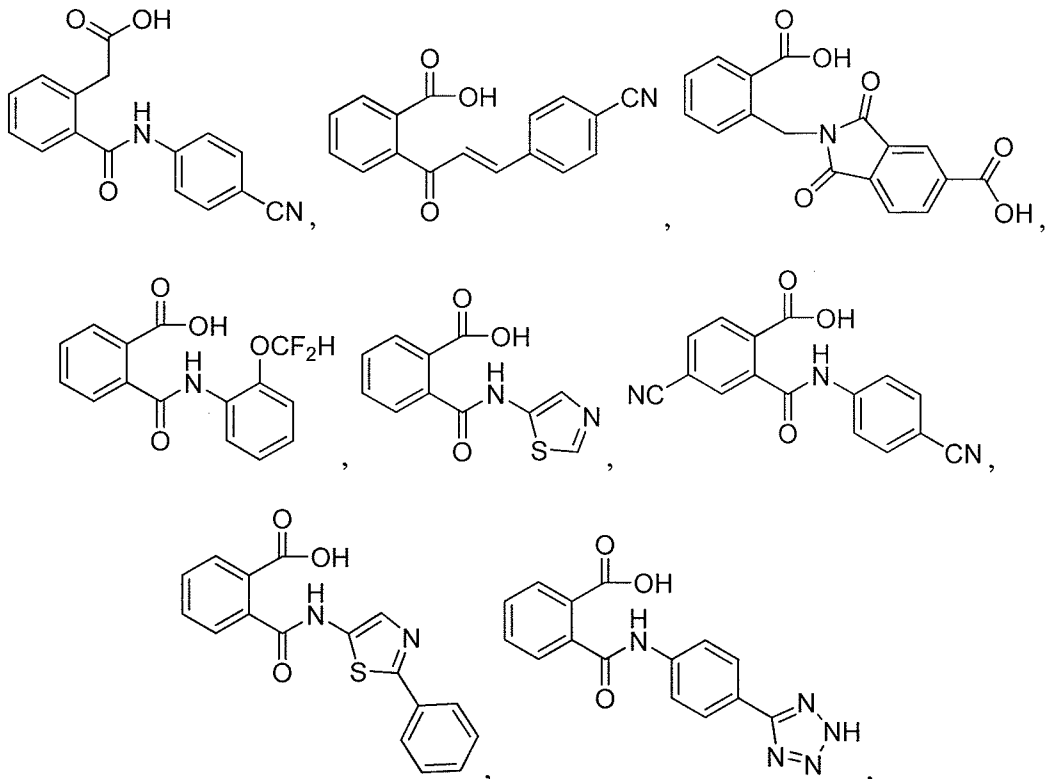


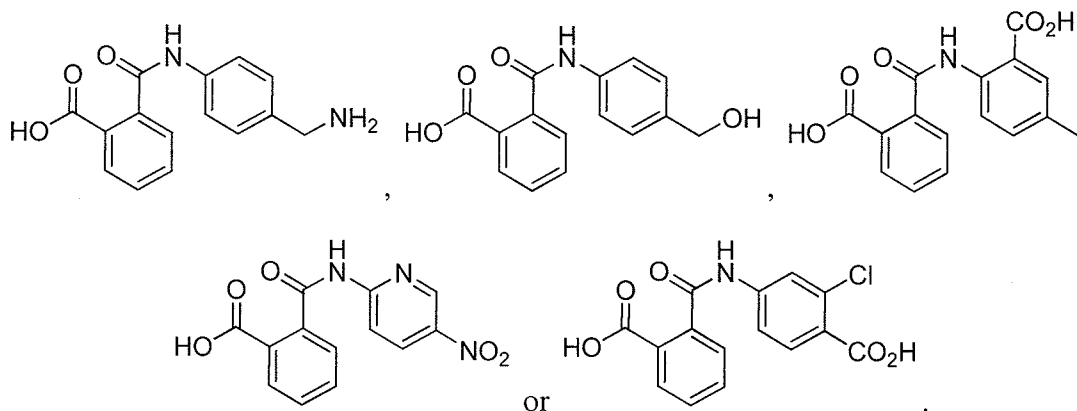
[0132] In some embodiments, the compound of the present invention is:



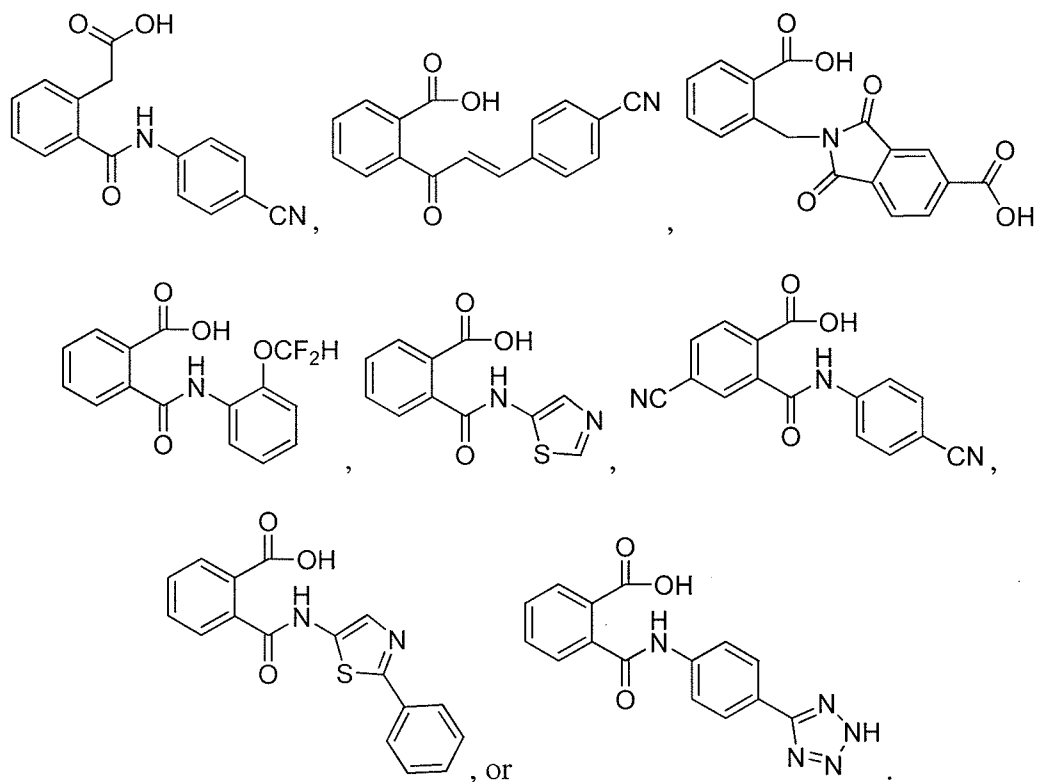
5

[0133] In some embodiments, the present invention provides compounds of the structure:



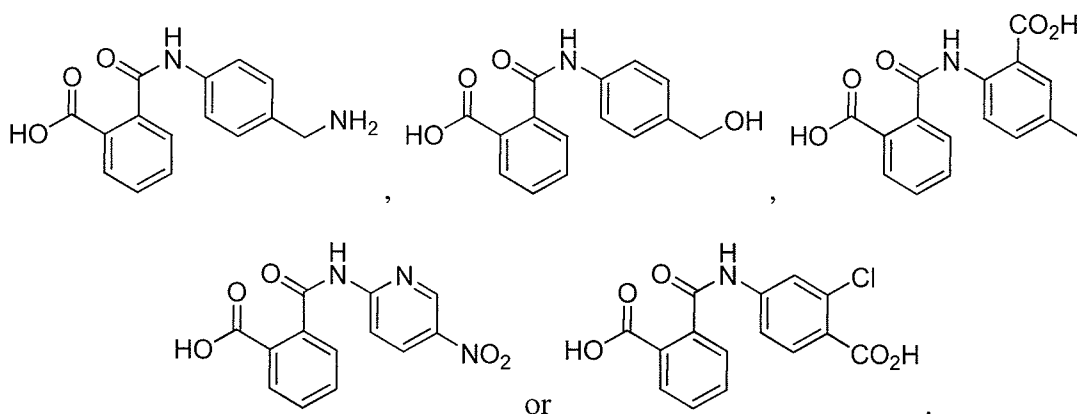


[0134] In some embodiments, the present invention provides compounds of the structure:



5

[0135] In some embodiments, the compound of the present invention is:



10 [0136] As used herein, the term “salt” refers to acid or base salts of the compounds used in the methods of the present invention. Illustrative examples of pharmaceutically acceptable

salts are mineral acid (hydrochloric acid, hydrobromic acid, phosphoric acid, and the like) salts, organic acid (acetic acid, propionic acid, glutamic acid, citric acid and the like) salts, quaternary ammonium (methyl iodide, ethyl iodide, and the like) salts. It is understood that the pharmaceutically acceptable salts are non-toxic. Additional information on suitable

5 pharmaceutically acceptable salts can be found in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, Pa., 1985, which is incorporated herein by reference.

[0137] Pharmaceutically acceptable salts of the acidic compounds of the present invention are salts formed with bases, namely cationic salts such as alkali and alkaline earth metal salts, such as sodium, lithium, potassium, calcium, magnesium, as well as ammonium salts, such as  
10 ammonium, trimethyl-ammonium, diethylammonium, and tris-(hydroxymethyl)-methyl-ammonium salts.

[0138] Similarly acid addition salts, such as of mineral acids, organic carboxylic and organic sulfonic acids, e.g., hydrochloric acid, methanesulfonic acid, maleic acid, are also possible provided a basic group, such as pyridyl, constitutes part of the structure.

15 [0139] The neutral forms of the compounds may be regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but otherwise the salts are equivalent to the parent form of the compound for the purposes of the present invention.

20 [0140] Certain compounds of the present invention possess asymmetric carbon atoms (optical centers) or double bonds; the racemates, diastereomers, geometric isomers and individual isomers are all intended to be encompassed within the scope of the present invention.

[0141] The present invention also includes isotopically-labeled compounds of the present invention, wherein one or more atoms are replaced by one or more atoms having specific  
25 atomic mass or mass numbers. Examples of isotopes that can be incorporated into compounds of the invention include, but are not limited to, isotopes of hydrogen, carbon, nitrogen, oxygen, fluorine, sulfur, and chlorine (such as  $^2\text{H}$ ,  $^3\text{H}$ ,  $^{13}\text{C}$ ,  $^{14}\text{C}$ ,  $^{15}\text{N}$ ,  $^{18}\text{O}$ ,  $^{17}\text{O}$ ,  $^{18}\text{F}$ ,  $^{35}\text{S}$  and  $^{36}\text{Cl}$ ). Isotopically-labeled compounds of the present invention are useful in assays of the tissue distribution of the compounds and their prodrugs and metabolites; preferred  
30 isotopes for such assays include  $^3\text{H}$  and  $^{14}\text{C}$ . In addition, in certain circumstances substitution with heavier isotopes, such as deuterium ( $^2\text{H}$  or D), can provide increased metabolic stability, which offers therapeutic advantages such as increased in vivo half-life or reduced dosage requirements. Isotopically-labeled compounds of this invention can generally be prepared

according to the methods described herein by substituting an isotopically-labeled reagent for a non-isotopically labeled reagent.

5 [0142] The compounds of the present invention can be prepared by a variety of methods known to one of skill in the art. Exemplary methods of making the compounds are described in Example 1. The methods of Example 1 involve first forming an amide by reaction of phthalic anhydride and a suitable amine, such as a substituted aniline. The available carboxylic acid can then be converted to an amide or ester using methods known to one of skill in the art, such as peptide coupling chemistry or carbodiimide chemistry, respectively.

10 [0143] The compounds of the present invention can also be obtained from the Maybridge library from The Scripps Research Institute.

#### IV. Assay for identifying compounds

[0144] The compounds of the present invention were identified using a variety of assays. The initial screen identified compounds that stimulated human mesenchymal stem cells (hMSCs) to develop into chondrocyte nodules. Additional assays were performed to  
15 determine toxicity and specificity of chondrocyte differentiation.

#### V. Method of ameliorating arthritis or joint injury

[0145] The present invention provides a method of ameliorating arthritis or joint injury in a mammal, the method including administering to a joint of the mammal a composition having a therapeutically effective amount of a compound of the present invention.

20 [0146] In some embodiments, the mammal does not have, but is at increased risk for, arthritis or joint injury.

[0147] It is contemplated that the compounds, compositions, and methods of the present invention may be used to ameliorate any type of arthritis or joint injury. It is further contemplated that the compounds, compositions, and methods of the present invention may  
25 be used to ameliorate various cartilagenous disorders. In some embodiments, the compounds and compositions of the present invention are administered to prevent arthritis or joint injury, for example where there is a genetic or family history of arthritis or joint injury or prior or during joint surgery or other circumstances where there is an increased risk of arthritis or joint injury. Exemplary conditions or disorders to be treated or prevented with the  
30 compounds, compositions, and methods of the invention, include, but are not limited to

systemic rheumatoid arthritis, juvenile chronic arthritis, osteoarthritis, degenerative disc disease, spondyloarthropathies, and systemic sclerosis (scleroderma). In some embodiments of the invention, the compounds, compositions, and methods of the present invention may be used to treat osteoarthritis. In some embodiments, the arthritis can be osteoarthritis, trauma  
5 arthritis, degenerative disc disease, dupuytren disease, or tendon disease.

[0148] In some embodiments, the compounds, compositions, and methods of the present invention provide a method for stimulating chondrocyte proliferation and cartilage production in cartilagenous tissues that have been damaged due to traumatic injury or chondropathy. Traumatic injury can include, but is not limited to, blunt trauma to the joint, or damage to  
10 ligaments such as tearing the anterior cruciate ligament, medial collateral ligament, or a miniscal tear. Examples of tissues that exhibit articulated surfaces, and thus are particularly susceptible to treatment include, but are not limited to, spine, shoulder, elbow, wrist, joints of the fingers, hip, knee, ankle, and the joints of the feet. Examples of diseases that may benefit from treatment include osteoarthritis, rheumatoid arthritis, other autoimmune diseases, or  
15 osteochondritis dessicans. In addition, cartilage malformation is often seen in forms of dwarfism in humans suggesting that the compounds, compositions, and methods would be useful in these patients.

[0149] It is contemplated that the compounds, compositions, and methods of the present invention may be used to treat a mammal. As used herein a “mammal” refers to any mammal  
20 classified as a mammal, including humans, domestic and farm animals, and zoo, sports or pet animals, such as cattle (e.g. cows), horses, dogs, sheep, pigs, rabbits, goats, cats, etc. In some embodiments of the invention, the mammal is a human. In some embodiments, the mammal can be a human, a dog, a horse or a cat.

[0150] The compounds and compositions of the present invention can be used in  
25 combination with other components suitable for ameliorating arthritis or joint injury. In some embodiments, the composition can also include an angiopoietin-like 3 protein (ANGPTL3) or chondrogenic variant thereof, oral salmon calcitonin, SD-6010 (iNOS inhibitor), vitamin D3 (cholecalciferol), collagen hydrolyzate, FGF18, BMP7, avocado soy unsaponifiables (ASU) or hyaluronic acid. ANGPTL3 is described in more detail in WO2011/008773 (incorporated  
30 herein in its entirety).



**VI. Method of inducing differentiation of MSCs into chondrocytes**

[0151] The compounds of the present invention are also useful for inducing differentiation of mesenchymal stem cells (MSCs) into chondrocytes. In some embodiments, the present invention provides a method of inducing differentiation of mesenchymal stem cells into  
5 chondrocytes, the method including contacting mesenchymal stem cells with a sufficient amount of a compound of the present invention, thereby inducing differentiation of the stem cells into chondrocytes.

[0152] MSCs are multipotent stem cells that can differentiate into several different types of cells including, but not limited to, osteoblasts, chondrocytes and adipocytes. Differentiation  
10 is the process by which a specialized cell type is formed from a less specialized cell type, for example, a chondrocyte from a MSC. In some embodiments, the method is performed *in vitro*. In some embodiments, the method is performed *in vivo* in a mammal and the stem cells are present in the mammal. In some embodiments, the mammal is a human, a dog, a horse or a cat.

[0153] Inducing differentiation of MSCs into chondrocytes can be accomplished using any  
15 suitable amount of a compound of the present invention. In some embodiments, the compound of the present invention can be present in an amount from about 0.1 mg to about 10000 mg, e.g., 1.0 mg to 1000 mg, e.g., 10 mg to 500 mg, according to the particular application and potency of the active component. In some embodiments, the compound of  
20 the present invention can be present in a concentration of 0.1  $\mu\text{M}$  – 100 $\mu\text{M}$  in an intra-articular injection to the knee.

**VII. Pharmaceutical compositions**

[0154] In some embodiments, the present invention provides a pharmaceutical composition  
25 having a pharmaceutically effective amount of a compound of the present invention and a pharmaceutically acceptable excipient.

[0155] In some embodiments, the present invention provides a pharmaceutical composition formulated for intra-articular delivery, the composition having a pharmaceutically effective amount of a compound of the present invention and a pharmaceutically acceptable excipient.

[0156] The compounds and compositions of the present invention can be used in  
30 combination with other components suitable for formulation for intra-articular delivery. In some embodiments, the composition can also include an angiopoietin-like 3 protein

(ANGPTL3) or chondrogenic variant thereof, oral salmon calcitonin, SD-6010 (iNOS inhibitor), vitamin D3 (cholecalciferol), collagen hydrolyzate, FGF18, BMP7, avocado soy unsaponifiables (ASU) or hyaluronic acid. ANGPTL3 is described in more detail in WO/2011/008773 (incorporated herein in its entirety).

5 [0157] Liquid form preparations include solutions, suspensions, and emulsions, for example, water or water/propylene glycol solutions. For injection, liquid preparations can be formulated in solution in aqueous polyethylene glycol solution.

[0158] Aqueous solutions suitable for use can be prepared by dissolving the active component in water and adding suitable colorants, stabilizers, and thickening agents as  
10 desired. Aqueous suspensions suitable for use can be made by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents such as a naturally occurring phosphatide (e.g., lecithin), a condensation product of an  
15 alkylene oxide with a fatty acid (e.g., polyoxyethylene stearate), a condensation product of ethylene oxide with a long chain aliphatic alcohol (e.g., heptadecaethylene oxycetanol), a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol (e.g., polyoxyethylene sorbitol mono-oleate), or a condensation product of ethylene oxide with a partial ester derived from fatty acid and a hexitol anhydride (e.g.,  
20 polyoxyethylene sorbitan mono-oleate). The aqueous suspension can also contain one or more preservatives such as ethyl or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents and one or more sweetening agents, such as sucrose, aspartame or saccharin. Formulations can be adjusted for osmolarity.

[0159] The compounds and compositions of the present invention can be applied by direct  
25 injection into the synovial fluid of the joint, systemic administration (oral or intravenously) or directly into the cartilage defect, either alone or complexed with a suitable carrier for extended release of protein. The compounds, compositions, and methods of the present invention can also be used to expand chondrocyte populations in culture for autogenous or allogenic chondrocyte transplantation. The transplantation can be optionally administered  
30 with concurrent treatment consisting of administration of the compounds and compositions of the present invention. In these procedures, for example, chondrocytes can be harvested arthroscopically from an uninjured minor load-bearing area of the damaged joint, and can be cultured in the presence of the compounds and compositions of the present invention to increase the number of cells prior to transplantation. The expanded cultures will then be

admixed with the compounds and compositions of the present invention, and placed in the joint space or directly into the defect. The compounds and compositions of the present invention can be used in combination with periosteal or perichondrial grafts that contain cells that can form cartilage and/or help to hold the transplanted chondrocytes or their precursor cells in place. The compounds and compositions of the present invention can be used to repair cartilage damage in conjunction with lavage of the joint, stimulation of bone marrow, abrasion arthroplasty, subchondral drilling, or microfracture of the subchondral bone. Additionally, after the growth of cartilage due to the administration of the compounds and compositions of the present invention, additional surgical treatment may be necessary to suitably contour the newly formed cartilage surface. In some embodiments, the pharmaceutical composition can be formulated for intra-articular delivery.

[0160] The pharmaceutical formulations of the invention can be provided as a salt and can be formed with many acids, including but not limited to hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents that are the corresponding free base forms.

[0161] The pharmaceutical preparation is preferably in unit dosage form. In such form the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules.

[0162] The quantity of active component in a unit dose preparation can be varied or adjusted into an effective dosage. In some embodiments, the dosage range can be from 0.1 mg to 10000 mg, e.g., 1.0 mg to 1000 mg, e.g., 10 mg to 500 mg, according to the particular application and the potency of the active component. The composition can, if desired, also contain other compatible therapeutic agents.

[0163] In some embodiments, co-administration includes administering one active agent within 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20, or 24 hours of a second active agent. Co-administration includes administering two active agents simultaneously, approximately simultaneously (e.g., within about 1, 5, 10, 15, 20, or 30 minutes of each other), or sequentially in any order. In some embodiments, co-administration can be accomplished by co-formulation, i.e., preparing a single pharmaceutical composition including both active agents. In other embodiments, the active agents can be formulated separately. In another embodiment, the active and/or adjunctive agents may be linked or conjugated to one another.

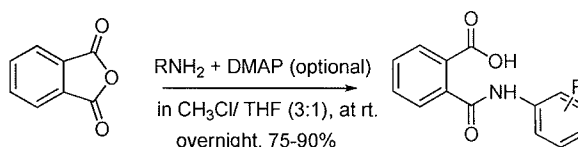
## VIII. Examples

**Example 1. Compound Preparation**

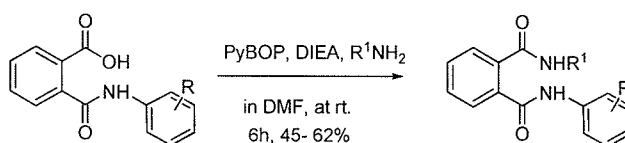
[0164] NMR spectra were recorded with a Bruker AV 400 spectrometer in the solvents indicated CDCl<sub>3</sub> and DMSO-d<sub>6</sub>; chemical shifts ( $\delta$ ) are given in ppm relative to TMS, coupling constants ( $J$ ) in Hertz. The solvent signals were used as references and the chemical shifts converted to the TMS scale (CDCl<sub>3</sub>:  $\delta$ C = 77.0 ppm; residual CHCl<sub>3</sub> in CDCl<sub>3</sub>:  $\delta$ H = 7.24 ppm; DMSO-d<sub>6</sub>:  $\delta$ C = 39.5 ppm; residual DMSO in DMSO-d<sub>6</sub>:  $\delta$ H = 2.5 ppm). The data are reported as follows: chemical shift, integration, multiplicity (s for singlet, d for doublet, t for triplet and m for multiplet). The assignments are based upon 1D and 2D spectra recorded using the following pulse sequences from the Bruker standard pulse program library: DEPT; COSY (*cosygs* and *cosydqtp*); HSQC (*invietgssi*) optimized for  $^1J(\text{C,H}) = 145$  Hz; HMBC (*inv4gslplrnd*) for correlations via  $^nJ(\text{C,H})$ ; HSQC-TOCSY (*invietgsm*) using an MLEV17 mixing time of 120 ms. Finnigan MAT 8200 (70 eV), ESI-MS: Finnigan MAT 95, accurate mass determination. Unless stated otherwise, all commercially available compounds (Fluka, Lancaster, Aldrich) were used as received. Solvents were purchased from VWR and Fisher. Reactions were carried out in oven-dried glassware under argon atmosphere, unless otherwise noted. Flash chromatography: Merck silica gel 60 (230-400 mesh).

General Methods

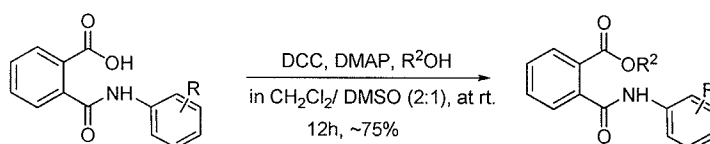
[0165] The following methods can be used to prepare the compounds of the present invention. Additional methods for making selected compounds of the present invention are provided below.



[0166] **General protocol starting from phthalic anhydride.** To a solution of phthalic anhydride (1.05 equiv) in a 3:1 mixture of chloroform / THF (0.05 M concentration) was added the corresponding amine (R/ArNH<sub>2</sub>, 1 equiv). The mixture was stirred overnight at room temperature. The reaction mixture was washed with brine (x 2). The aqueous solution was then extracted with ethyl acetate (x 3) and dried over sodium sulfate. Combined organic phases were concentrated under reduced pressure and adsorbed on silica gel to be purified by flash chromatography to afford the desired amide.

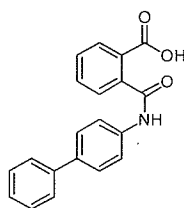


**[0167] General protocol for the amide bond formation.** To a solution of acid and diisopropylethylamine (DIEA, 2.2 equiv) in DMF (0.1 M concentration) at 0°C was added the corresponding amine (R<sup>1</sup>NH<sub>2</sub>, 1.2 equiv). The mixture was stirred for 30 min and PyBOP (1.1 equiv) was added and stirred at room temperature for 6 h. The slurry reaction mixture was washed with brine (x 5 to assure complete removal of DMSO; volume of brine ~10 times volume of DMSO). The aqueous solution was then extracted with ethyl acetate (x 3) and dried over sodium sulfate. Combined organic phases were concentrated under reduced pressure and adsorbed on silica gel to be purified by flash chromatography to afford the desired amide.

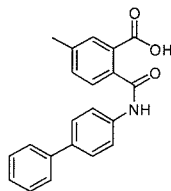


**[0168] General protocol for the ester bond formation.** DCC (0.9 equiv) and DMAP (0.2 equiv) were added to a solution of the selected alcohol (1.2 equiv) in CH<sub>2</sub>Cl<sub>2</sub> / DMSO (2:1; 0.1 M) at 0°C. The mixture was stirred for 30 min before acid (1 equiv) was introduced and stirring continued for 12 h at ambient temperature. For work up, all volatile materials were evaporated, the product adsorbed on silica and purified by flash chromatography (hexanes/ethyl acetate) to give the desired product (yield ~75%).

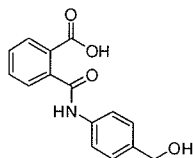
### AKT



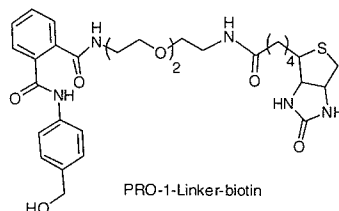
**[0169] AKT-** AKT was recrystallized from of CHCl<sub>3</sub> / MeOH (2:1). <sup>1</sup>H NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO) δ 7.36 (tt, *J* = 7.4, 1.2 Hz, 1H), 7.47 (tt, *J* = 7.5, 1.2 Hz, 2H), 7.59 (m, 2H), 7.66 (m, 5H), 7.81 (dt, *J* = 6.7, 1.7 Hz, 2H), 7.91 (dd, *J* = 7.3, 1.7 Hz, 1H), 10.45 (*brs*, 1H); <sup>13</sup>C NMR (100 MHz, (CD<sub>3</sub>)<sub>2</sub>SO) δ 163.9, 139.0, 136.2, 133.1, 132.4, 129.1, 128.5, 120.5, 118.3, 113.8, 20.5; **HRMS** (ESI): 317.1130 [M + H<sup>+</sup>]; calcd for [C<sub>20</sub>H<sub>15</sub>NO<sub>3</sub> + H<sup>+</sup>] 318.1125.

AKT-Me

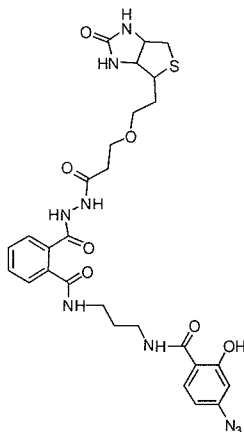
[0170] **AKT-Me.** *Rf* (CHCl<sub>3</sub>/MeOH 3/1): 0.4. The desired **AKT-Me** derivative was obtained as light yellow solid. <sup>1</sup>H NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO) δ 2.66 (s, 3H), 7.49 (t, *J* = 7.4 Hz, 2H), 7.61 (m, 5H), 7.83 (dm, *J* = 8.3 Hz, 2H), 7.92 (dm, *J* = 8.7 Hz, 2H), 8.13 (dd, *J* = 8.7, 5.6 Hz, 1H), 10.71 (*brs*, 1H) **HRMS** (ESI): 332.1277 [M + H<sup>+</sup>]; calcd for [C<sub>21</sub>H<sub>18</sub>NO<sub>3</sub> + H<sup>+</sup>] 332.1281.

AKT-CH<sub>2</sub>OH

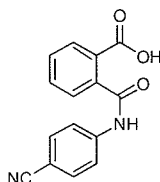
10 [0171] **2-((4-(hydroxymethyl)phenyl)carbamoyl)benzoic acid.** The desired derivative was obtained as brown solid after purification on silica. *Rf* (CHCl<sub>3</sub>/MeOH: 9/1): 0.3. <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 4.32 (d, *J* = 1.6 Hz, 2H), 6.6 (d, *J* = 8.6 Hz, 1H), 7.26 (dd, *J* = 8.6, 2.5 Hz, 1H), 7.39 (d, *J* = 2.3 Hz, 1H), 7.54 (dt, *J* = 6.1, 1.3 Hz, 1H), 7.56 (dt, *J* = 7.6, 1.3 Hz, 1H), 7.63 (dt, *J* = 7.5, 1.3 Hz, 1H), 7.83 (dd, *J* = 7.6, 1.0 Hz, 1H), 8.32 (m, 1H), 9.08 (m, 15 1H), 9.99 (*brs*, 1H). **HRMS** (ESI): 272.0931 [M + H<sup>+</sup>]; calcd for [C<sub>14</sub>H<sub>11</sub>NO<sub>4</sub> + H<sup>+</sup>] 272.0923.

AKT-Biotin

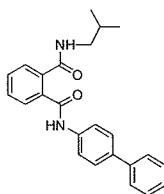
20 [0172] **AKT-Biotine.** *Rf* (CHCl<sub>3</sub>/MeOH 10/1): 0.25. The desired **AKT-Biotine** derivative was obtained white yellow solid. <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 1.22 (m, 2H), 1.53 (m, 2H), 2.60 (dm, *J* = 6.9 Hz, 2H), 2.74 (d, *J* = 2.1 Hz, 2H), 2.85 (m, 2H), 3.03 (m, 1H), 3.21 (m, 1H), 3.30 (d, *J* = 6.8 Hz, 2H), 3.51 (m, 8H), 3.98 (m, 2H), 4.12 (ddm, *J* = 8.7, 2.1 Hz, 2H), 6.2 (*brs*, 2H), 6.78 (dd, *J* = 8.7, 2.3 Hz, 2H), 7.05 (m, 1H), 7.50 (m, 4H), 7.73 (m, 1H), 7.82 (m, 1H), 8.1 (*brs*, 1H-NH), 8.2 (*brs*, 1H-NH), 9.2 (*brs*, 1H-NH). **HRMS** (ESI): 627.2754  
25 [M + H<sup>+</sup>]; calcd for [C<sub>31</sub>H<sub>41</sub>N<sub>5</sub>O<sub>7</sub>S + H<sup>+</sup>] 627.2757.

AKT-Azide

[0173] **AKT-Azide.** *R<sub>f</sub>* (CHCl<sub>3</sub>/MeOH 20/1): 0.33. The desired **AKT-azide** derivative was obtained as light yellow oil. <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 1.76 (m, 3H), 1.84 (m, 2H), 2.14 (m, 2H), 2.20 (t, *J* = 6.9 Hz, 2H), 2.46 (m, 1H), 2.59 (d, *J* = 12.5 Hz, 1H), 2.82 (dd, *J* = 12.5, 5.1 Hz, 1H), 3.03 (m, 2H), 3.12 (m, 2H), 3.41 (m, 1H), 4.13 (ddd, *J* = 7.4, 6.1, 1.1 Hz, 1H), 4.32 (dd, *J* = 7.5, 5.2 Hz, 1H), 6.36 (*brs*, 1H-NH), 6.42 (*brs*, 1H-NH), 7.43 (s, 1H), 7.48 (m, 2H), 7.75 (m, 3H), 7.95 (m, 1H), 7.96 (d, *J* = 8.6 Hz, 1H), 8.17 (*brs*, 1H-NH), 8.18 (*brs*, 1H-NH), 8.29 (*brs*, 1H-NH), 8.3 (*brs*, 1H-NH). **HRMS** (ESI): 640.2293 [M + H<sup>+</sup>]; calcd for [C<sub>28</sub>H<sub>33</sub>N<sub>9</sub>O<sub>7</sub>S + H<sup>+</sup>] 640.2296.

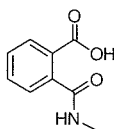
AKT-CN; LK3

[0174] **AKT-CN; LK3.** *R<sub>f</sub>* (CHCl<sub>3</sub>/MeOH 9/1): 0.4. The desired **AKT-CN** derivative was obtained as brown solid. <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 7.57 (m, 2H), 7.59 (m, 1H), 7.61 (dt, *J* = 7.6, 1.2 Hz, 1H), 7.69 (dt, *J* = 7.5, 1.2 Hz, 1H), 7.92 (m, 1H), 8.17 (m, 1H), 10.73 (*brs*, 1H). **HRMS** (ESI): 266.0695 [M + H<sup>+</sup>]; calcd for [C<sub>15</sub>H<sub>10</sub>N<sub>2</sub>O<sub>3</sub> + H<sup>+</sup>] 266.0691.

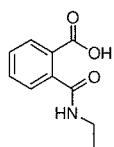
LK-14b

[0175] **LK-14b, N-([1,1'-biphenyl]-4-yl)-N-isobutylphthalamide.** *R<sub>f</sub>* (CHCl<sub>3</sub>/MeOH 15/1): 0.3. The desired **AKT-isopropylamine** derivative was obtained as brown solid. <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 0.93 (d, *J* = 6.8 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H), 1.77 (m,

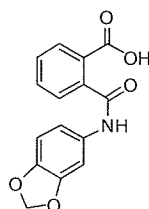
1H), 3.04 (dd,  $J = 6.65, 5.59$  Hz, 2H), 7.31 (dt,  $J = 7.3, 1.59$  Hz, 1H), 7.35 (dt,  $J = 7.3, 1.48$  Hz, 1H), 7.43 (dd,  $J = 7.51, 1.09$  Hz, 1H), 7.49 (t,  $J = 7.4$  Hz, 2H), 7.61 (m, 2H), 7.67 (dd,  $J = 7.51, 1.18$  Hz, 1H), 7.83 (dm,  $J = 8.3$  Hz, 2H), 7.92 (dm,  $J = 8.7$  Hz, 2H), 8.13 (dd,  $J = 8.7, 5.6$  Hz, 1H). **HRMS** (ESI): 373.1928 [M + H<sup>+</sup>]; calcd for [C<sub>24</sub>H<sub>24</sub>N<sub>2</sub>O<sub>2</sub> + H<sup>+</sup>] 373.1916.

5 LK-23

[0176] **LK-23, 2-(methylcarbamoyl)benzoic acid.**  $R_f$  (CHCl<sub>3</sub>/MeOH 15/1): 0.3. The desired derivative was obtained as brown solid. <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO):  $\delta$  2.97 (s, 3H), 7.63 (m, 2H), 7.98 (m, 2H), 9.58 (brs, 1H). **HRMS** (ESI): 189.0672 [M + H<sup>+</sup>]; calcd for [C<sub>9</sub>H<sub>9</sub>NO<sub>3</sub> + H<sup>+</sup>] 180.0661.

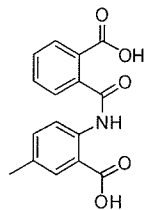
10 LK-19b

[0177] **LK-19b, 2-(ethylcarbamoyl)benzoic acid.**  $R_f$  (CHCl<sub>3</sub>/MeOH 15/1): 0.3. The desired derivative was obtained as brown solid. <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO):  $\delta$  1.07 (t,  $J = 6.8$  Hz, 3H), 3.19 (q,  $J = 6.8$  Hz, 2H), 7.39 (m, 2H), 7.52 (m, 2H), 9.58 (brs, 1H). **HRMS** (ESI): 194.0825 [M + H<sup>+</sup>]; calcd for [C<sub>10</sub>H<sub>11</sub>NO<sub>3</sub> + H<sup>+</sup>] 194.0817.

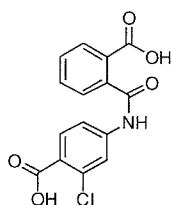
LK-62

[0178] **LK-62, 2-(benzo[1,3]dioxol-5-ylcarbamoyl)benzoic acid.**  $R_f$  (CHCl<sub>3</sub>/MeOH 12/1): 0.5. The desired derivative was obtained as yellow powder. <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO):  $\delta$  6.87 (m, 1H), 6.9 (m, 1H), 7.02 (d,  $J = 1.3$  Hz, 1H), 7.36 (m, 2H), 7.52 (dd,  $J = 7.6, 1.3$  Hz, 1H), 7.65 (dd,  $J = 7.2, 1.3$  Hz, 1H), 10.56 (brs, 1H). **HRMS** (ESI): 286.0725 [M + H<sup>+</sup>]; calcd for [C<sub>15</sub>H<sub>11</sub>NO<sub>5</sub> + H<sup>+</sup>] 286.0715.

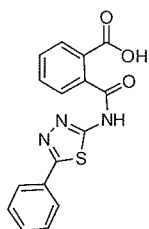


LK-60

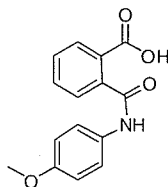
- [0179] **LK-60, 2-(2-carboxybenzamido)-5-methylbenzoic acid.** The desired derivative was obtained as white powder, recrystallized out from CHCl<sub>3</sub>/ MeOH (1:1). <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 2.40 (s, 3H), 7.03 (dd, *J* = 8.1, 1.02 Hz, 1H), 7.66 (m, 3H), 7.86 (dd, *J* = 6.38, 1.02 Hz, 1H), 7.92 (d, *J* = 8.1 Hz, 1H), 8.51 (s, 1H), 11.59 (*brs*, 1H). **HRMS** (ESI): 300.0881 [M + H<sup>+</sup>]; calcd for [C<sub>16</sub>H<sub>13</sub>NO<sub>5</sub> + H<sup>+</sup>] 300.0872.

LK-63

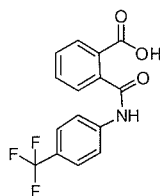
- 10 [0180] **LK-63, 4-(2-carboxybenzamido)-2-chlorobenzoic acid.** The desired derivative was obtained as white powder. <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 7.46 (m, 3H), 7.75 (d, *J* = 8.9 Hz, 1H), 7.92 (d, *J* = 9.03 Hz, 1H), 8.30 (d, *J* = 8.9 Hz, 1H), 8.42 (d, *J* = 1.1 Hz, 1H), 9.15 (*brs*, -NH, 1H), 10.59 (*brs*, 1H), 11.0 (*brs*, 1H). **HRMS** (ESI): 320.0323 [M + H<sup>+</sup>]; calcd for [C<sub>15</sub>H<sub>10</sub>ClNO<sub>5</sub> + H<sup>+</sup>] 320.0326.

15 LK-57

- [0181] **LK-57, 2-((5-phenyl-1,3,4-thiadiazol-2-yl)carbamoyl)benzoic acid.** The desired derivative was obtained as yellow powder, recrystallized out from CHCl<sub>3</sub>/ MeOH (1:1). <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 7.52-7.54 (m, 2H), 7.57 (m, 1H), 7.96 (dm, *J* = 1.6 Hz, 1H), 7.97 (m, 1H), 7.98 (d, *J* = 2.8 Hz, 1H), 7.99 (m, 1H), 8.01 (dd, *J* = 5.6, 2.8 Hz, 2H), 10.81 (*brs*, 1H). **HRMS** (ESI): 326.0594 [M + H<sup>+</sup>]; calcd for [C<sub>16</sub>H<sub>11</sub>N<sub>3</sub>O<sub>3</sub>S + H<sup>+</sup>] 326.0599.

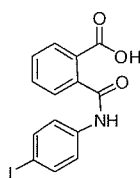
L2

- 5 [0182] **L2, 2-((4-methoxyphenyl)carbamoyl)benzoic acid.** The desired derivative was obtained as colorless oil, *R<sub>f</sub>* (CHCl<sub>3</sub>/MeOH 9/1): 0.3. <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 3.34 (s, 2H), 7.54 (m, 1H), 7.6 (m, 3H), 7.64 (dt, *J* = 7.5, 1.3 Hz, 1H), 7.85 (dd, *J* = 7.6, 1.1 Hz, 1H), 10.2 (*brs*, 1H). **HRMS** (ESI): 272.0929 [M + H<sup>+</sup>]; calcd for [C<sub>15</sub>H<sub>13</sub>NO<sub>4</sub> + H<sup>+</sup>] 272.0923.

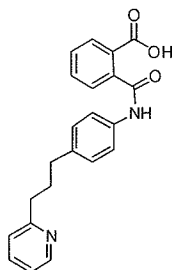
L4

- 10 [0183] **L4, 2-((4-(trifluoromethyl)phenyl)carbamoyl)benzoic acid.** The desired derivative was obtained as yellowish oil, *R<sub>f</sub>* (CHCl<sub>3</sub>/MeOH 9/1): 0.3. <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 7.45 (d, *J* = 7.3 Hz, 1H), 7.51 (t, *J* = 7.6 Hz, 1H), 7.61 (m, 2H), 7.69 (dd, *J* = 14.8, 11.6 Hz, 1H), 7.76 (m, 2H), 7.91 (dd, *J* = 7.7, 0.9 Hz, 1H), 10.12 (*brs*, 1H). **HRMS** (ESI): 310.0687 [M + H<sup>+</sup>]; calcd for [C<sub>15</sub>H<sub>16</sub>FNO<sub>3</sub> + H<sup>+</sup>] 310.0691.

- 15 L11a

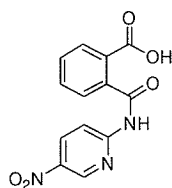


- 20 [0184] **L11a, 2-((4-iodophenyl)carbamoyl)benzoic acid.** The desired derivative was obtained as bright yellow solid after purification on silica. *R<sub>f</sub>* (CHCl<sub>3</sub>/MeOH 11/1): 0.3. <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 7.53 (m, 5H), 7.66 (dt, *J* = 11.6, 2.4 Hz, 2H), 7.80 (m, 1H), 11.2 (*brs*, 1H). <sup>13</sup>C-NMR (100 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): 86.3, 121.5, 128.4, 129.4, 129.9, 130.1, 134.3, 139.7, 167.8, 169.05. **HRMS** (ESI): 367.8798 [M + H<sup>+</sup>]; calcd for [C<sub>14</sub>H<sub>10</sub>INO<sub>3</sub> + H<sup>+</sup>] 367.8794.

L15

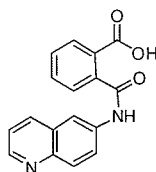
[0185] **LK15, 2-((4-(3-(pyridin-2-yl)propyl)phenyl)carbamoyl)benzoic acid.** The desired derivative was obtained as brown powder after purification on silica. *Rf*

- 5 (CHCl<sub>3</sub>/MeOH 15/1): 0.2. <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 2.82 (t, *J* = 7.2 Hz, 2H), 2.99 (t, *J* = 6.5 Hz, 2H), 3.44 (tt, *J* = 7.2, 6.5 Hz, 2H), 7.32 (m, 2H), 7.55 (dd, *J* = 16.5, 7.3 Hz, 2H), 7.70 (m, 2H), 8.41 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.45 (dd, *J* = 4.4, 1.6 Hz, 1H), 8.47 (*brs*, NH-, 1H). <sup>13</sup>C-NMR (100 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): 30, 32.1, 41.3, 123.8, 127.5, 128.7, 130.0, 131.3, 134.4, 136.3, 145, 148.8, 165.1, 167.2. **HRMS** (ESI): 361.1559 [M + H<sup>+</sup>]; calcd for
- 10 [C<sub>22</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub> + H<sup>+</sup>] 361.1552.

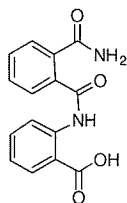
L59

[0186] **LK59, 2-((5-nitropyridin-2-yl)carbamoyl)benzoic acid.** The desired derivative was obtained as light yellow powder after purification on silica. *Rf* (CHCl<sub>3</sub>/MeOH 9/1): 0.3.

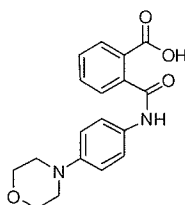
- 15 <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 7.04 (d, *J* = 4.4 Hz, 1H), 7.95 (m, 2H), 7.96 (dd, *J* = 16.1, 8.3 Hz, 1H), 8.49 (m, 2H), 8.71 (d, *J* = 1.8 Hz, 1H), 10.92 (*brs*, 1H). **HRMS** (ESI): 288.0616 [M + H<sup>+</sup>]; calcd for [C<sub>13</sub>H<sub>9</sub>NO<sub>5</sub> + H<sup>+</sup>] 288.0620.

L9

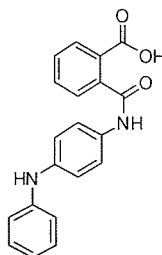
- 20 [0187] **L9, 2-(quinolin-6-ylcarbamoyl)benzoic acid.** The desired derivative was obtained as light brown powder after purification on silica. *Rf* (CHCl<sub>3</sub>/MeOH 8/1): 0.3. <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 7.55 (dd, *J* = 8.5, 4.1 Hz, 1H), 7.62 (m, 1H), 7.72 (m, 2H), 7.80 (m, 2H), 7.94 (m, 2H), 8.65 (d, *J* = 8.5 Hz, 1H), 8.93 (dd, *J* = 4.1, 1.5 Hz, 1H), 10.54 (*brs*, 1H). **HRMS** (ESI): 293.0929 [M + H<sup>+</sup>]; calcd for [C<sub>17</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub> + H<sup>+</sup>] 293.0926.

L7

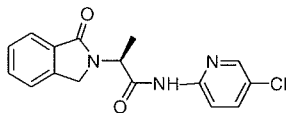
- [0188] **L7, 2-(2-carbamoylbenzamido)benzoic acid.** The desired derivative was obtained as light white solid after purification on silica. *R<sub>f</sub>* (CHCl<sub>3</sub>/MeOH 10/1): 0.25. <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 7.57 (m, 1H), 7.59 (m, 1H), 7.62 (m, 2H), 7.68 (m, 1H), 7.73 (*brs*, 2H), 7.85 (m, 1H), 8.33 (d, *J* = 8.2 Hz, 1H), 8.59 (d, *J* = 8.2 Hz, 1H), 12.22 (*brs*, 1H). **HRMS** (ESI): 285.0882 [M + H<sup>+</sup>]; calcd for [C<sub>15</sub>H<sub>12</sub>N<sub>2</sub>O<sub>4</sub> + H<sup>+</sup>] 285.0875.

LK6

- 10 [0189] **LK6, 2-((4-morpholinophenyl)carbamoyl)benzoic acid.** The desired derivative was obtained as light blue solid after purification on silica. *R<sub>f</sub>* (CHCl<sub>3</sub>/MeOH 6/1): 0.4. <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 3.05 (dd, *J* = 4.8, 4.7 Hz, 2H), 3.74 (dd, *J* = 5.1, 4.5 Hz, 2H), 6.92 (dm, *J* = 9.09 Hz, 2H), 7.51-7.57 (m, 4H), 7.64 (dt, *J* = 7.5, 1.4 Hz, 1H), 7.85 (dd, *J* = 7.6 Hz, 1H), 10.12 (*brs*, 1H). **HRMS** (ESI): 327.1351 [M + H<sup>+</sup>]; calcd for [C<sub>18</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub> + H<sup>+</sup>] 327.1345.

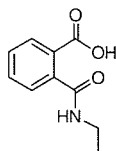
LK17b

- 20 [0190] **LK17b, 2-((4-phenylamino)phenyl)carbamoyl)benzoic acid.** The desired derivative was obtained as white powder after purification on silica. *R<sub>f</sub>* (CHCl<sub>3</sub>/MeOH 9/1): 0.3. <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 6.77 (tt, *J* = 7.3, 1.1 Hz, 2H), 7.02 (ddm, *J* = 15.8, 8.8 Hz, 2H), 7.20 (tt, *J* = 8.2, 1.2 Hz, 2H), 7.54 (dt, *J* = 7.5, 1.4 Hz, 1H), 7.56 (m, 4H), 7.65 (dt, *J* = 7.5, 1.4 Hz, 1H), 7.86 (dd, *J* = 1.2 Hz, 1H), 8.07 (*brs*, NH-, 1H), 10.2 (*brs*, 1H). **HRMS** (ESI): 333.1241 [M + H<sup>+</sup>]; calcd for [C<sub>20</sub>H<sub>16</sub>N<sub>3</sub>O<sub>2</sub> + H<sup>+</sup>] 333.1239.

L11b

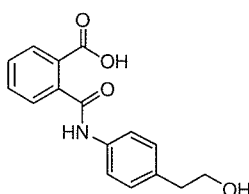
[0191] **L11b, (S)-N-(5-chloropyridin-2-yl)-2-(1-oxoisoindolin-2-yl)propanamide.** The desired derivative was obtained as yellow powder after purification on silica. *R<sub>f</sub>*

- 5 (AcOEt/Hex.: 3/2): 0.2. <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 2.14 (d, *J* = 7.3 Hz, 3H), 4.63 (d, *J* = 17 Hz, 1H), 4.75 (d, *J* = 17 Hz, 1H), 5.10 (dd, *J* = 7.3, 7.3 Hz, 1H), 7.50 (m, 1H), 7.62 (m, 2H), 7.7 (d, *J* = 7.5 Hz, 1H), 7.9 (dd, *J* = 8.9, 2.6 Hz, 1H), 8.05 (d, *J* = 8.9 Hz, 1H), 8.4 (d, *J* = 8.9 Hz, 1H), 10.96 (*brs*, NH-, 1H). **HRMS** (ESI): 316.0859 [M + H<sup>+</sup>]; calcd for [C<sub>16</sub>H<sub>14</sub>ClN<sub>3</sub>O<sub>2</sub> + H<sup>+</sup>] 316.0853.

10 LK19b

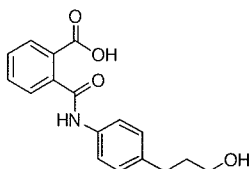
[0192] **LK19b, 2-(ethylcarbamoyl)benzoic acid.** The desired derivative was obtained as bright yellow oil after purification on silica. *R<sub>f</sub>* (CHCl<sub>3</sub>/MeOH: 12/1): 0.3. <sup>1</sup>H-NMR (400

- 15 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 1.09 (t, *J* = 7.2 Hz, 3H), 3.35 (*brs*, 1H), 3.2 (dq, *J* = 15.4, 7.2 Hz, 2H), 7.30 (m, 2H), 7.58 (m, 2H). **HRMS** (ESI): 194.0821 [M + H<sup>+</sup>]; calcd for [C<sub>10</sub>H<sub>11</sub>NO<sub>3</sub> + H<sup>+</sup>] 194.0817.

L11c

[0193] **L11c, 2-((4-(2-hydroxyethyl)phenyl)carbamoyl)benzoic acid.** The desired

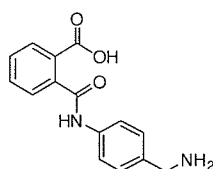
- 20 derivative was obtained as bright yellow oil after purification on silica. *R<sub>f</sub>* (CHCl<sub>3</sub>/MeOH: 12/1): 0.3. <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 2.79 (dd, *J* = 7.0, 6.9 Hz, 2H), 3.65 (dd, *J* = 7.0, 6.9 Hz, 2H), 7.27 (dt, *J* = 9.1, 2.2 Hz, 2H), 7.53 (dd, *J* = 7.5, 1.2 Hz, 2H), 7.56- 7.6 (m, 3H), 7.64 (dt, *J* = 7.5, 1.3 Hz, 1H), 7.86 (dd, *J* = 7.62, 1.05 Hz, 1H), 10.18 (*brs*, 1H). **HRMS** (ESI): 286.1073 [M + H<sup>+</sup>]; calcd for [C<sub>16</sub>H<sub>15</sub>NO<sub>4</sub> + H<sup>+</sup>] 286.1079.

2-((4-(3-hydroxypropyl)phenyl)carbamoyl)benzoic acid

[0194] The desired derivative was obtained as bright yellow oil after purification on silica.

*Rf* (CHCl<sub>3</sub>/MeOH: 12/1): 0.3. <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 0.98 (t, *J* = 6.6 Hz, 2H),

5 1.72 (*sext.*, *J* = 6.6 Hz, 2H), 3.9 (d, *J* = 6.6 Hz, 2H), 6.8 (dt, *J* = 9.0, 2.1 Hz, 2H), 7.52 (dd, *J* = 7.5, 1.14 Hz, 2H), 7.56- 7.59 (m, 3H), 7.65 (td, *J* = 7.5, 1.3 Hz, 1H), 7.86 (dd, *J* = 7.6, 1.2 Hz, 1H), 10.17 (*brs*, 1H). **HRMS** (ESI): 300.1241 [M + H<sup>+</sup>]; calcd for [C<sub>17</sub>H<sub>17</sub>NO<sub>4</sub> + H<sup>+</sup>] 300.1236.

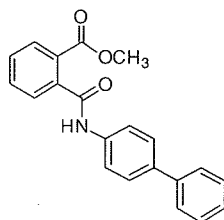
LK55

10

[0195] 2-((4-(3-(aminomethyl)phenyl)carbamoyl)benzoic acid. The desired derivative

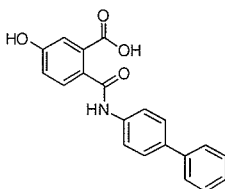
was obtained as light yellow oil after purification on silica. *Rf* (CHCl<sub>3</sub>/MeOH: 15/1): 0.3. <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 2.51 (*brt*, *J* = 1.2 Hz, 2H), 6.87 (d, *J* = 7.6 Hz, 1H), 7.19 (dd, *J* = 8.0, 7.6 Hz, 1H), 7.50 (m, 3H), 7.56 (m, 2H), 7.77 (dd, *J* = 7.2, 1.6 Hz, 1H), 10.2 (*brs*,

15 1H), 11.16 (*brs*, NH<sub>2</sub>, 2H). **HRMS** (ESI): 271.1080 [M + H<sup>+</sup>]; calcd for [C<sub>15</sub>H<sub>14</sub>N<sub>2</sub>O<sub>3</sub> + H<sup>+</sup>] 271.1083.

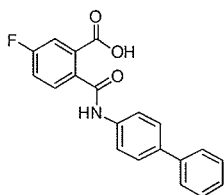
LK55b

[0196] Methyl 2-((1,1'-biphenyl)-4-ylcarbamoyl)benzoate. The desired derivative was

20 obtained as light yellow oil after purification on silica. *Rf* CHCl<sub>3</sub>/MeOH: 8/1): 0.3. <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 2.33 (s, 3H), 6.58 (d, *J* = 7.5 Hz, 1H), 7.19 (t, *J* = 7.8 Hz, 2H), 7.47-7.53 (m, 4H), 7.54 (m, 2H), 7.63 (dm, *J* = 8.5 Hz, 2H), 7.80 (dd, *J* = 8.5, 1.4 Hz, 2H). **HRMS** (ESI): 332.1781 [M + H<sup>+</sup>]; calcd for [C<sub>21</sub>H<sub>17</sub>NO<sub>3</sub> + H<sup>+</sup>] 332.1787.

**LK22b**

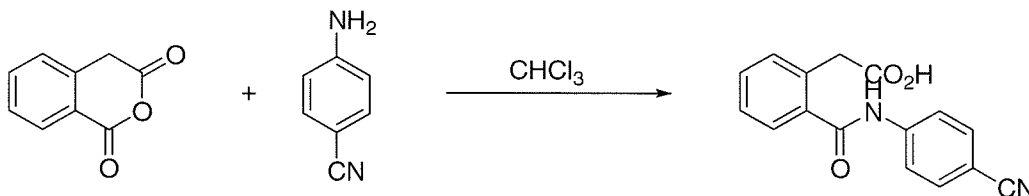
- [0197] **2-([1,1'-biphenyl]-4-ylcarbamoyl)-5-hydroxybenzoic acid.** The desired derivative was obtained as a grey solid after purification on silica. *R<sub>f</sub>* (CHCl<sub>3</sub>/MeOH: 9/1): 0.2. <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 7.29 (d, *J* = 8.2 Hz, 1H), 7.42 (m, 2H), 7.51 (m, 3H), 7.68 (m, 1H), 7.70 (m, 2H), 7.80 (dt, *J* = 8.7, 2.1 Hz, 2H), 7.98 (d, *J* = 8.7 Hz, 1H). **HRMS** (ESI): 334.1082 [M + H<sup>+</sup>]; calcd for [C<sub>20</sub>H<sub>15</sub>NO<sub>4</sub> + H<sup>+</sup>] 334.1079.

**LK5**

- 10 [0198] **2-([1,1'-biphenyl]-4-ylcarbamoyl)-5-fluorobenzoic acid.** The desired derivative was obtained as a grey solid after purification on silica. *R<sub>f</sub>* (CHCl<sub>3</sub>/MeOH: 9/1): 0.4. <sup>1</sup>H-NMR (400 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 7.34 (tt, *J* = 7.3, 1.2 Hz, 1H), 7.41-7.49 (m, 4H), 7.77 (dm, *J* = 8.7 Hz, 2H), 7.99 (dd, *J* = 8.7, 5.6 Hz, 1H), 10.49 (*brs*, 1H). **HRMS** (ESI): 336.1041 [M + H<sup>+</sup>]; calcd for [C<sub>20</sub>H<sub>14</sub>FNO<sub>3</sub> + H<sup>+</sup>] 336.1036.

15 **A01**

- [0199] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 11.08 (s, 1H), 8.04 (d, *J* = 7.5 Hz, 1H), 7.87-7.99 (m, 6H), 7.82 (t, *J* = 7.5 Hz, 1H). **MS** (ESI): 247.90 [M + H<sup>+</sup>]; calcd for [C<sub>15</sub>H<sub>9</sub>N<sub>3</sub>O + H<sup>+</sup>] 248.08.

**A06**

20

- [0200] Prepared from Homophthalic anhydride + 4-aminobenzonitrile under standard conditions. <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 10.55 (s, 1H), 7.91 (d, *J* = 6.6 Hz, 1H), 7.77 (m, 4H), 7.55 (t, *J* = 7.2 Hz, 1H), 7.40 (m, 2H), 4.14 (s, 2H). **MS** (ESI): 280.90 [M + H<sup>+</sup>]; calcd for [C<sub>16</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub> + H<sup>+</sup>] 281.09.

A09

[0201] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 8.00 (m, 2H), 7.83 (t, *J* = 7.2 Hz, 1H), 7.72 (t, *J* = 7.5 Hz, 1H), 7.65 (d, *J* = 7.5 Hz, 1H), 7.44 (m, 2H), 6.41 (d, *J* = 8.7 Hz, 2H), 1.95 (s, 3H). MS (ESI): 265.10 [M + H<sup>+</sup>]; calcd for [C<sub>16</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub> + H<sup>+</sup>] 265.10.

5 A11

[0202] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 10.79 (s, 1H), 9.89 (s, 1H), 7.93 (m, 3H), 7.83 (d, *J* = 6.6 Hz, 2H), 7.76 (d, *J* = 5.7 Hz, 1H), 7.56 (t, *J* = 6.0 Hz, 1H), 7.22 (t, *J* = 5.4 Hz, 1H), 3.64 (s, 3H). MS (ESI): 317.95 [M + Na<sup>+</sup>]; calcd for [C<sub>16</sub>H<sub>13</sub>N<sub>3</sub>O<sub>3</sub> + Na<sup>+</sup>] 318.09.

A12

10 [0203] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 10.78 (s, 1H), 9.04 (s, 1H), 8.17 (d, *J* = 8.4 Hz, 1H), 7.82-7.95 (m, 4H), 7.67 (d, *J* = 6.9 Hz, 1H), 7.45 (t, *J* = 7.8 Hz, 1H), 7.07 (t, *J* = 7.2 Hz, 1H), 6.41 (brs, 2H). MS (ESI): 303.05 [M + Na<sup>+</sup>]; calcd for [C<sub>15</sub>H<sub>12</sub>N<sub>4</sub>O<sub>2</sub> + Na<sup>+</sup>] 303.09.

A13

15 [0204] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 10.91 (brs, 1H), 9.88 (brs, 1H), 7.82-7.94 (m, 5H), 7.52 (m, 2H), 7.26 (brs, 1H), 3.07 (s, 3H). HRMS (ESI): 316.0750 [M + H<sup>+</sup>]; calcd for [C<sub>15</sub>H<sub>13</sub>N<sub>3</sub>O<sub>3</sub> + H<sup>+</sup>] 316.0768.

A22

[0205] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 7.82-7.89 (m, 5H), 7.66 (m, 1H), 7.56 (m, 2H). MS (ESI): 303.05 [M + H<sup>+</sup>]; calcd for [C<sub>14</sub>H<sub>10</sub>N<sub>2</sub>O<sub>4</sub>S + H<sup>+</sup>] 303.04.

20 A23

[0206] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 11.03 (s, 1H), 8.00 (m, 1H), 7.90 (m, 4H), 7.75 (m, 3H), 7.18 (s, 2H). MS (ESI): 323.90 [M + Na<sup>+</sup>]; calcd for [C<sub>14</sub>H<sub>11</sub>N<sub>3</sub>O<sub>3</sub>S + Na<sup>+</sup>] 324.04.

A24

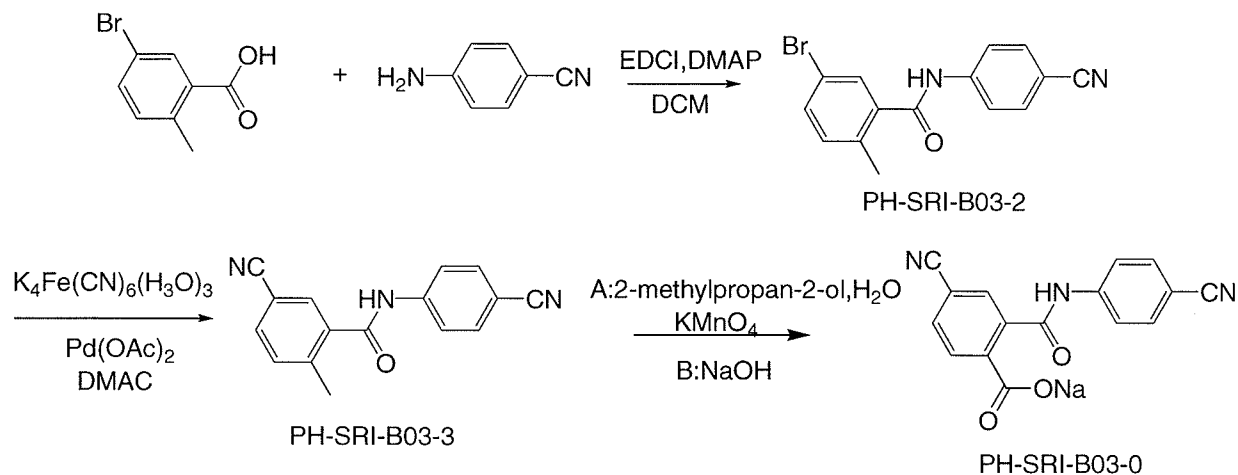
25 [0207] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 10.96 (s, 1H), 7.68-7.92 (m, 8H), 6.99 (m, 1H). MS (ESI): 338.06 [M + Na<sup>+</sup>]; calcd for [C<sub>15</sub>H<sub>13</sub>N<sub>3</sub>O<sub>3</sub>S + Na<sup>+</sup>] 337.95.

B02

[0208] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>OD): δ 7.99 (s, 1H), 7.85-7.92 (m, 4H), 7.70 (d, *J* = 6.3 Hz, 2H). MS (ESI): 290.00 [M - 1]; calcd for [C<sub>14</sub>H<sub>10</sub>N<sub>2</sub>O<sub>4</sub>S - 1] 290.06.



B03



[0209] Into a 50-mL round-bottom flask, was placed a solution of 5-bromo-2-  
 5 methylbenzoic acid (1.5 g, 6.98 mmol, 1.00 equiv) in dichloromethane (15 mL), 4-  
 aminobenzonitrile (820 mg, 6.94 mmol, 1.00 equiv), EDCI (2 g, 10.43 mmol, 1.50 equiv), 4-  
 dimethylaminopyridine (1.28 g, 10.48 mmol, 1.50 equiv). The resulting solution was stirred  
 overnight at room temperature. The resulting mixture was concentrated under vacuum. The  
 residue was dissolved in 100 mL of ethyl acetate. The resulting mixture was washed with  
 10 3x30 mL of hydrogen chloride (2.4 mol/L) and 2x30 mL of water. The resulting mixture was  
 washed with 2x30 mL of sodium bicarbonate (aq.) and 2x30 mL of brine. The mixture was  
 dried over anhydrous sodium sulfate and concentrated under vacuum. This resulted in 1.93 g  
 (88%) of 5-bromo-N-(4-cyanophenyl)-2-methylbenzamide as a light yellow solid.

[0210] Into a 100-mL round-bottom flask purged and maintained with an inert atmosphere  
 15 of nitrogen, was placed a solution of 5-bromo-N-(4-cyanophenyl)-2-methylbenzamide (1 g,  
 3.17 mmol, 1.00 equiv) in DMAC (5 mL), bis(dipotassium) ironhexacarbonitrile trihydrate  
 (337 mg, 0.80 mmol, 0.25 equiv), sodium carbonate (336 mg, 3.17 mmol, 1.00 equiv),  
 $Pd(OAc)_2$  (23 mg, 0.10 mmol, 0.03 equiv). The resulting solution was stirred for 4 h at 120°C  
 in an oil bath. The reaction mixture was cooled. The resulting solution was diluted with 60  
 20 mL of ethyl acetate. The resulting mixture was washed with 1x40 mL of brine. The resulting  
 solution was dried over sodium sulfate and concentrated under vacuum. This resulted in 0.65  
 g (78%) of 5-cyano-N-(4-cyanophenyl)-2-methylbenzamide as a light brown solid.

[0211] Into a 50-mL round-bottom flask, was placed 5-cyano-N-(4-cyanophenyl)-2-  
 methylbenzamide (300 mg, 1.15 mmol, 1.00 equiv), 2-methylpropan-2-ol (12 mL), water (12  
 25 mL). To this was added tetraoxo(potassio)manganese (908 mg, 5.75 mmol, 5.00 equiv) in  
 several batches at 100°C in 27 mins. The resulting solution was stirred for 6.5 h at 100°C in

an oil bath. The reaction mixture was cooled to 40 degree C with a water bath. The pH value of the solution was adjusted to 2 with aqueous hydrogen chloride (2.4 mol/L). The resulting solution was extracted with 4x50 ml of ethyl acetate and the organic layers combined and concentrated under vacuum. Aqueous sodium hydroxide (1 mol/L) was employed to adjust the pH to 9. The crude product (200 mg) was purified by Prep-HPLC with the following conditions (1#-Pre-HPLC-001(SHIMADZU)): Column, SunFire Prep C18, 19\*150mm 5um; mobile phase, Water with 50mmolNH<sub>4</sub>HCO<sub>3</sub> and CH<sub>3</sub>CN (25% CH<sub>3</sub>CN up to 30% in 15 min, up to 100% in 2 min ,down to 25% in 1 min); Detector, Waters2545 UvDector 254&220nm. 8.5 mg product was obtained. This resulted in 8.5 mg (2%) of sodium 4-cyano-2-[(4-cyanophenyl)carbamoyl]benzoate as a white solid. <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>OD): δ 7.99 (s, 1H), 7.88 (m, 4H), 7.70 (d, *J* = 6.3 Hz, 2H). MS (ESI): 289.90 [M-Na-1]; calcd for [C<sub>16</sub>H<sub>8</sub>N<sub>3</sub>NaO<sub>3</sub> - Na - 1] 289.06.

#### B10

[0212] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 13.49 (brs, 2H), 10.92 (1H), 8.47 (s, 1H), 8.20 (m, 1H), 7.85 (m, 4H), 7.71 (d, *J* = 7.8 Hz, 1H). MS (ESI): 281.90 [M + H<sup>+</sup>]; calcd for [C<sub>16</sub>H<sub>10</sub>N<sub>2</sub>O<sub>5</sub> + H<sup>+</sup>] 282.09. MS (ESI): 311.00 [M + H<sup>+</sup>]; calcd for [C<sub>16</sub>H<sub>11</sub>N<sub>3</sub>O<sub>3</sub> + H<sup>+</sup>] 311.07.

#### B12

[0213] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 8.04 (m, 1H), 7.67-7.98 (m, 4H), 7.32-7.62 (m, 2H). MS (ESI): 333.40 [M + Na<sup>+</sup>]; calcd for [C<sub>16</sub>H<sub>10</sub>N<sub>2</sub>O<sub>5</sub> + Na<sup>+</sup>] 333.05.

#### B19

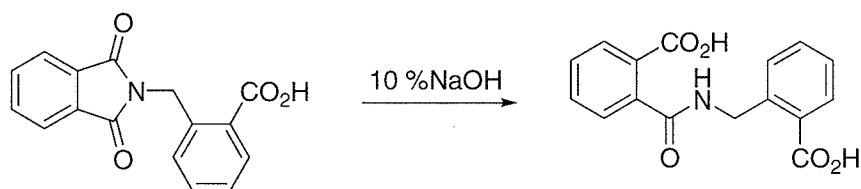
[0214] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 13.61 (brs, 1H), 10.94 (s, 1H), 9.10 (s, 1H), 8.89 (d, *J* = 4.8 Hz, 1H), 7.83 (s, 4H), 7.63 (d, *J* = 4.8 Hz, 1H). MS (ESI): 268.00 [M + H<sup>+</sup>]; calcd for [C<sub>14</sub>H<sub>9</sub>N<sub>3</sub>O<sub>3</sub> + H<sup>+</sup>] 268.07.

#### B20

[0215] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 13.48 (brs, 1H), 11.04 (s, 1H), 8.82 (m, 2H), 8.26 (dd, 7.8, 1.2 Hz, 1H), 7.97 (m, 2H), 7.85 (m, 2H), 7.73 (m, 1H). MS (ESI): 268.00 [M + H<sup>+</sup>]; calcd for [C<sub>14</sub>H<sub>9</sub>N<sub>3</sub>O<sub>3</sub> + H<sup>+</sup>] 268.07.

#### B32

[0216] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 11.83 (brs, 1H), 7.67 (m, 6H), 7.50 (m, 2H), 7.32-7.40 (m, 2H), 7.08-7.20 (m, 3H). MS (ESI): 365.05 [M + Na<sup>+</sup>]; calcd for [C<sub>21</sub>H<sub>14</sub>N<sub>2</sub>O<sub>3</sub> + H<sup>+</sup>] 365.09.

MS 01

[0217] 2-(N-Phthalimidomethyl)benzoic acid (as prepared by Bornstein, J. et al, Organic Syntheses, 38, No pp. given; 1958) was dissolved in 10% NaOH solution and stirred at room temperature overnight. The pH value of the solution was adjusted to 2 with aqueous hydrogen chloride (2.4 mol/L). The resulting solution was extracted with 4x50 ml of ethyl acetate and the organic layers combined and concentrated under vacuum. Aqueous sodium hydroxide (1 mol/L) was employed to adjust the pH to 9. The crude product was purified by Prep-HPLC to give the title compound. <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 13.00 (s, 1H), 8.79 (t, *J* = 6.0 Hz, 1H), 7.90 (d, *J* = 6.9 Hz, 1H), 7.54-7.82 (m, 6H), 7.38 (t, *J* = 7.5 Hz, 1H), 4.79 (d, *J* = 5.7 Hz, 2H). MS (ESI): 300.00 [M + H<sup>+</sup>]; calcd for [C<sub>16</sub>H<sub>13</sub>NO<sub>5</sub> + H<sup>+</sup>] 300.09.

MS02

[0218] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 12.95 (s, 2H) 8.93 (t, *J* = 6.0 Hz, 1H), 7.97 (s, 1H), 7.47-7.87 (m, 7H), 4.51 (d, *J* = 5.7 Hz, 2H). MS (ESI): 322.00 [M + Na<sup>+</sup>]; calcd for [C<sub>16</sub>H<sub>13</sub>NO<sub>5</sub> + Na<sup>+</sup>] 322.07.

MS04

[0219] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 13.01 (brs, 1H), 9.03 (t, *J* = 5.7 Hz, 1H), 7.83 (t, *J* = 8.1 Hz, 2H), 7.46-7.80 (m, 6H), 4.62 (d, *J* = 6.0 Hz, 2H). MS (ESI): 281.00 [M + H<sup>+</sup>]; calcd for [C<sub>16</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub> + H<sup>+</sup>] 281.09.

MS06

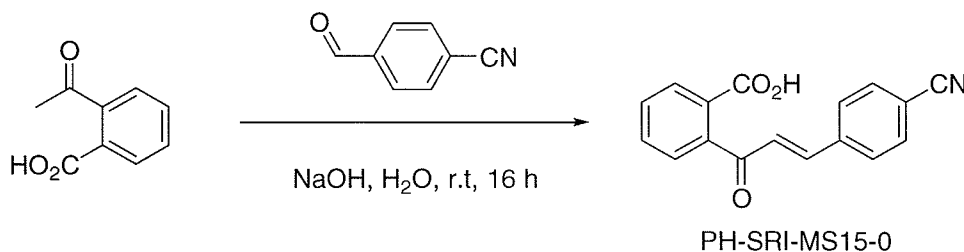
[0220] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 12.99 (brs, 1H), 8.95 (t, *J* = 6.0 Hz, 1H), 7.95 (m, 3H), 7.49-7.83 (m, 5H), 4.53 (d, *J* = 6.0 Hz, 2H). MS (ESI): 280.85 [M + H<sup>+</sup>]; calcd for [C<sub>16</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub> + H<sup>+</sup>] 281.09.

MS11

[0221] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>OD): δ 7.94-8.03 (m, 3H), 7.86 (m, 2H), 7.50 (m, 2H), 7.38 (m, 1H). MS (ESI): 281.25 [M + H<sup>+</sup>]; calcd for [C<sub>16</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub> + H<sup>+</sup>] 281.09.

MS14

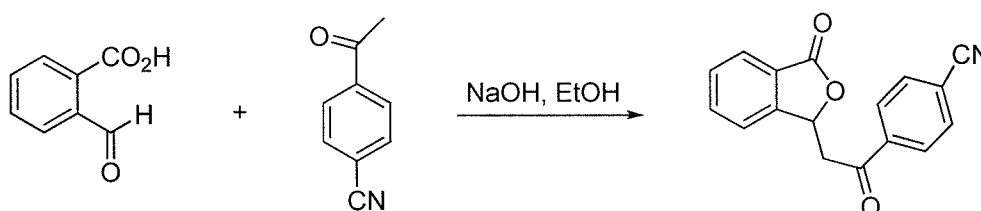
[0222] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 11.13 (s, 1H), 8.48 (d, *J* = 6.3 Hz, 1H) 7.94 (m, 3H), 7.58 (t, *J* = 5.4 Hz, 1H), 7.49 (d, *J* = 6.3 Hz, 2H), 7.15 (t, *J* = 5.7 Hz, 1H), 3.87 (s, 2H). MS (ESI): 321.90 [M + Na<sup>+</sup>]; calcd for [C<sub>16</sub>H<sub>13</sub>NO<sub>5</sub> + Na<sup>+</sup>] 322.07.

MS15

[0223] Into a 50-mL round-bottom flask, was placed 2-acetylbenzoic acid (1.64 g, 9.99 mmol, 1.00 equiv), ethanol (5 mL), 4-formylbenzonitrile (1.31 g, 9.99 mmol, 1.00 equiv). To this was added sodium hydroxide(aq.) (10 mL, 1.5N) with an ice/water bath. The resulting solution was stirred for 16 h at room temperature. The reaction was then quenched by the addition of 20 mL of water. The pH value of the solution was adjusted to 2-3 with hydrogen chloride (4 mol/L). The resulting solution was extracted with 3x20 mL of dichloromethane and the organic layers combined. The resulting mixture was washed with 1x30 mL of H<sub>2</sub>O. The mixture was dried over anhydrous sodium sulfate and concentrated under vacuum. The crude product (250 mg) was purified by Prep-HPLC with the following conditions (1#-Pre-HPLC-006(Waters)): Column, SunFire Prep C18, 19\*150mm 5um; mobile phase, WATER WITH 0.5% TFA and CH<sub>3</sub>CN (20% CH<sub>3</sub>CN up to 60% in 10 min, up to 100% in 2 min); Detector, uv 254/220nm. 16.2 mg product was obtained. This resulted in 16.2 mg (1%) of 2-[(2E)-3-(4-cyanophenyl)prop-2-enoyl]benzoic acid as a white solid. <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>OD): δ 8.07 (m, 1H), 7.50-7.77 (m, 8H), 7.24 (m, 2H). MS (ESI): 278.15 [M + H<sup>+</sup>]; calcd for [C<sub>17</sub>H<sub>11</sub>NO<sub>3</sub> + H<sup>+</sup>] 278.08.

MS16

[0224] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 13.10 (brs, 1H), 7.96 (m, 3H), 7.83 (m, 2H), 7.64-7.76 (m, 2H), 7.52 (m, 1H), 7.28 (m, 2H). MS (ESI): 297.25 [M + H<sup>+</sup>]; calcd for [C<sub>17</sub>H<sub>12</sub>O<sub>5</sub> + H<sup>+</sup>] 297.08.

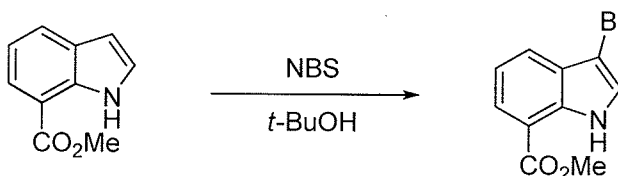
MS 17-1

[0225] To a solution of 2-carboxybenzaldehyde (150 mg, 1 mmol) in 2M NaOH in water /ethanol (1:1, 5 mL) was added 4'-cyanoacetophenone (145 mg, 1 mmol) dropwise at 0 °C. The resultant mixture was stirred for 48 h at room temperature. The reaction mixture was

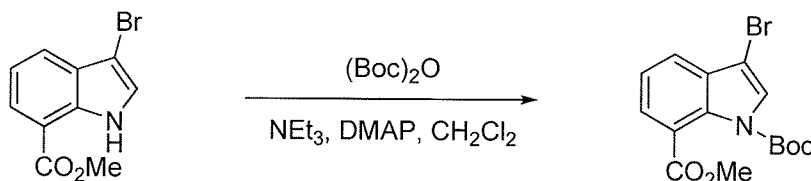
diluted with ethyl acetate and subsequently washed with brine. The organic extracts were dried over anhydrous sodium sulfate and concentrated under vacuum with subsequent purification by column chromatography to give the title compound. <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 8.15 (d, *J* = 6.0 Hz, 2H), 8.04 (d, *J* = 6.3 Hz, 2H), 7.87 (d, *J* = 5.7 Hz, 1H), 7.81 (t, *J* = 5.4 Hz, 1H), 7.74 (d, *J* = 5.7 Hz, 1H), 7.63 (t, *J* = 5.7 Hz, 1H), 6.12 (m, 1H), 3.77-3.98 (m, 2H). MS (ESI): 275.85 [M - 1]; calcd for [C<sub>17</sub>H<sub>11</sub>NO<sub>3</sub> - 1] 276.07.

MS18-1

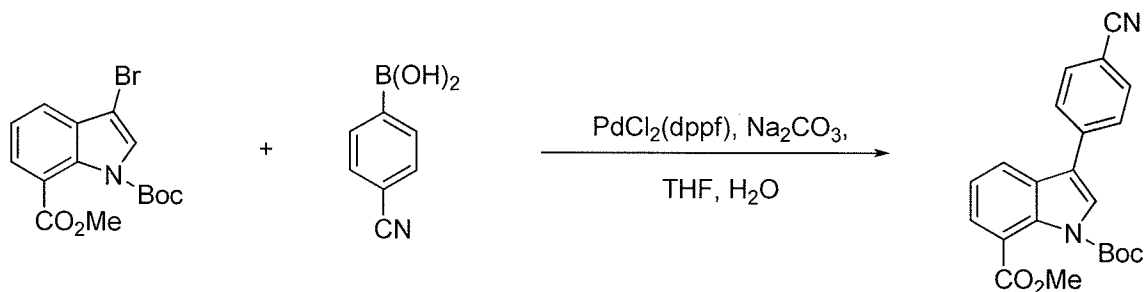
[0226] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 13.35 (brs, 1H), 8.08 (m, 4H), 7.75-7.88 (m, 3H), 7.62 (m, 1H), 6.13 (m, 1H), 3.93 (m, 2H). HRMS (ESI): 297.0766 [M + H<sup>+</sup>]; calcd for [C<sub>17</sub>H<sub>12</sub>O<sub>5</sub> + H<sup>+</sup>] 297.0757.

MS 21

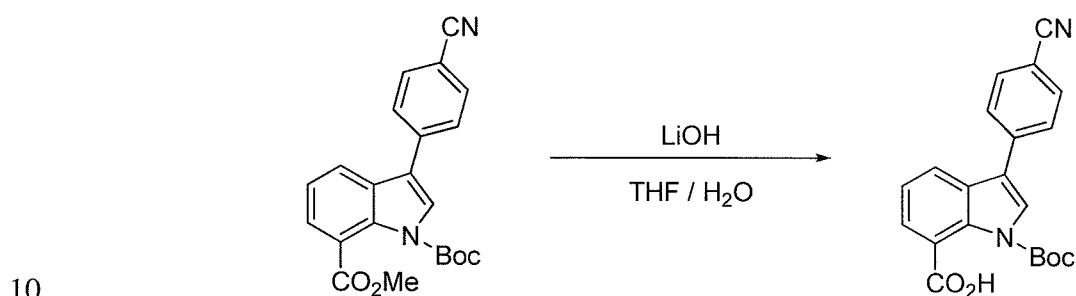
[0227] (The title compound was prepared according to the procedure of Nozawa, Eisuke et al from PCT Int. Appl., 2009005076, 08 Jan 2009.) To a mixture of methyl 1H-indole-7-carboxylate (6.00 g, 34.25 mmol) and tert-butanol (300 mL) was added *N*-bromosuccinimide (3.30 g, 18.5 mmol) portionwise at room temperature. This solution was stirred for 2h at room temperature. The reaction mixture was concentrated under reduced pressure. To the residue was added a saturated sodium bicarbonate solution followed by extraction with ethyl acetate. The organic layer was dried over sodium sulfate, filtered, and then concentrated under reduced pressure. The crude product was purified by flash column chromatography to give the title compound (4.2 g, 48%).



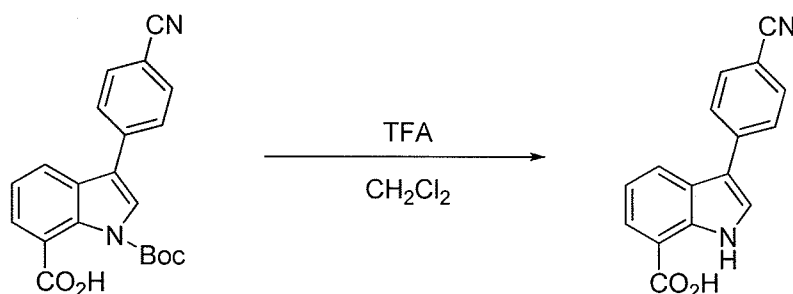
[0228] To a solution of methyl 3-bromo-1H-indole-7-carboxylate (4.0 g, 15.75 mmol) and DMAP (38.5 mg, 0.315 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (15 mL) was added di-*tert*-buty dicarbonate (3.78 g, 17.33 mmol). The reaction was stirred for 3h at room temperature. The reaction was quenched with 1*N* HCl. The organic layer was extracted with ethyl acetate, dried over sodium sulfate, filtered, and then concentrated under reduced pressure. The crude product was purified by flash column chromatography to give the title compound (5.03g, 90%).



[0229] To a solution of 1-tert-butyl 7-methyl 3-(4-cyanophenyl)-1H-indole-1,7-dicarboxylate (753.0 mg, 2 mmol,) 4-cyanophenylboronic acid (441.0 mg, 3.0 mmol), sodium carbonate (318.0 mg, 3.0 mmol) in THF and H<sub>2</sub>O (3:1, 80 mL) was added PdCl<sub>2</sub>(dppf) (0.2 mmol, 146.3 mg). The reaction mixture was heated at reflux for 10 h. The reaction was quenched by addition of ethyl acetate and sodium bicarbonate. The organic layer was separated, washed with brine, dried over sodium sulfate, filtered, and then concentrated under reduced pressure. The crude product was purified by flash column chromatography to give the title compound (587.2 mg, 78%).



[0230] 1-tert-butyl 7-methyl 3-(4-cyanophenyl)-1H-indole-1,7-dicarboxylate (376.4 mg, 1 mmol) was added to a solution of 1M LiOH·H<sub>2</sub>O in THF and H<sub>2</sub>O (1:1, 10 mL). The reaction mixture was stirred at room temperature for 12h. The aqueous layer was first washed with ethyl acetate and then acidified with 4N HCl. The aqueous layer was then extracted with ethyl acetate. The organic layer was dried over sodium sulfate, filtered, and then concentrated under reduced pressure. The crude product was used in the next step without further purification.



[0231] Crude 1-(tert-butoxycarbonyl)-3-(4-cyanophenyl)-1H-indole-7-carboxylic acid (300 mg) was added to a 10% solution of trifluoroacetic acid in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) at 0 °C. The

reaction mixture was warmed to room temperature and stirred for one hour. The solvent was removed under reduced pressure. The crude product was purified by Prep-HPLC to give the title compound. <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 13.16 (brs, 1H), 11.59 (s, 1H), 8.22 (d, *J* = 8.1 Hz, 1H), 7.86-7.97 (m, 6H), 7.29 (t, *J* = 7.5 Hz, 1H). MS (ESI): 261.00 [M - 1]; calcd for [C<sub>16</sub>H<sub>10</sub>N<sub>2</sub>O<sub>5</sub> - 1] 261.07.

MS26

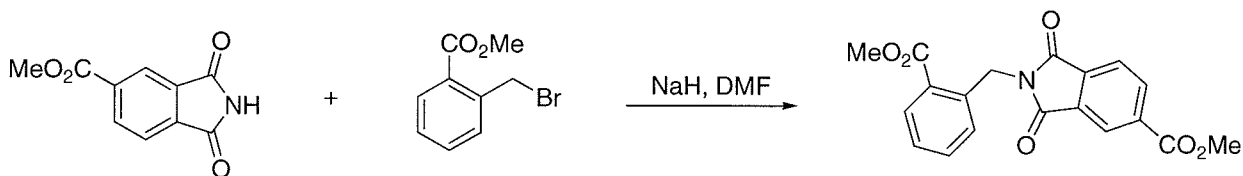
[0232] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 8.14 (d, *J* = 6.6 Hz, 2H), 7.92 (d, *J* = 6.6 Hz, 2H), 7.83 (d, *J* = 5.7 Hz, 1H), 7.71 (m, 2H), 7.57 (t, *J* = 5.1 Hz, 1H), 5.09 (s, 2H). MS (ESI): 235.00 [M + H+]; calcd for [C<sub>15</sub>H<sub>10</sub>N<sub>2</sub>O + H+] 235.09.

10 MS28

[0233] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 7.96-8.07 (m, 4H), 7.86 (s, 2H), 7.74 (d, *J* = 8.4 Hz, 2H).

MS29

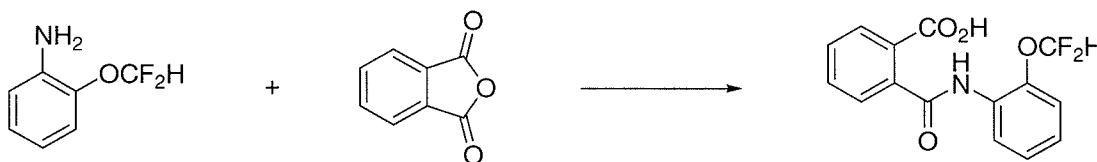
[0234] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 8.10 (d, *J* = 8.4 Hz, 2H), 7.93-8.03 (m, 4H), 7.60 (d, *J* = 8.4 Hz, 2H). MS (ESI): 266.00 [M - 1 calcd for [C<sub>15</sub>H<sub>19</sub>NO<sub>4</sub> - 1] 266.05.

MS 39

[0235] To a solution of NaH (60% dispersion in oil, 1.2 eq) in DMF (10 mL) was added methyl 1,3-dioxoisindoline-5-carboxylate (353 mg, 1.00 mmol) (as prepared by Mazzocchi, P. H. et al Journal of Organic Chemistry, 48(18), 2981-9; 1983) dropwise at 0°C. The solution was warmed to room temperature and stirred for one hour. Methyl 2-(bromomethyl)benzoate (275 mg, 1.2 mmol) was subsequently added dropwise and the reaction mixture stirred overnight. The mixture was diluted with ethyl acetate and washed with brine. The organic extracts were dried over anhydrous sodium sulfate and concentrated under vacuum to give an oil which was used in the next step without further purification. <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 8.37 (m, 2H), 7.92 (t, *J* = 7.8 Hz, 2H), 7.37 (t, *J* = 7.8 Hz, 1H), 7.27 (t, *J* = 7.8 Hz, 1H), 7.07 (d, *J* = 7.8 Hz, 1H), 5.22 (s, 2H). MS (ESI): 347.80 [M + Na+]; calcd for [C<sub>17</sub>H<sub>11</sub>NO<sub>6</sub> + Na+] 348.05.

N01

[0236]  $^1\text{H-NMR}$  (300 MHz,  $(\text{CD}_3)_2\text{SO}$ ):  $\delta$  13.03 (brs, 1H), 11.00 (brs, 1H), 8.47 (d,  $J = 6.0$  Hz, 2H), 7.92 (d,  $J = 7.2$  Hz, 1H), 7.64 (m, 5H). **MS** (ESI): 242.90 [M + H<sup>+</sup>]; calcd for  $[\text{C}_{13}\text{H}_{10}\text{N}_2\text{O}_3 + \text{H}^+]$  243.08.

5 N04

[0237] Prepared from 2-(Difluoromethoxy)aniline and phthalic anhydride under standard conditions.  $^1\text{H-NMR}$  (300 MHz,  $(\text{CD}_3)_2\text{SO}$ ):  $\delta$  9.95 (s, 1H), 7.88 (m, 2H), 7.52-7.71 (m, 3H), 7.30 (m, 3H) 7.09 (s, 1H). **MS** (ESI): 307.95 [M + H<sup>+</sup>]; calcd for  $[\text{C}_{15}\text{H}_{11}\text{F}_2\text{NO}_4 + \text{H}^+]$

10 308.07.

N05

[0238]  $^1\text{H-NMR}$  (300 MHz,  $(\text{CD}_3)_2\text{SO}$ ):  $\delta$  13.09 (brs, 1H), 10.54 (s, 1H), 7.91 (d,  $J = 6.9$  Hz, 1H), 7.48-7.90 (m, 5H), 7.39 (t,  $J = 8.1$  Hz, 1H), 7.23 (s, 1H), 6.91 (d,  $J = 6.6$  Hz, 1H). **MS** (ESI): 307.85 [M + H<sup>+</sup>]; calcd for  $[\text{C}_{15}\text{H}_{11}\text{F}_2\text{NO}_4 + \text{H}^+]$  308.07.

15 N08

[0239]  $^1\text{H-NMR}$  (300 MHz,  $(\text{CD}_3)_2\text{SO}$ ):  $\delta$  13.18 (brs, 1H), 10.66 (brs, 1H), 8.42 (brs, 1H), 7.69-8.02 (m, 7H), 2.62 (s, 3H). **MS** (ESI): 306.05 [M + Na<sup>+</sup>]; calcd for  $[\text{C}_{16}\text{H}_{13}\text{NO}_4 + \text{Na}^+]$  306.07.

N09

20 [0240]  $^1\text{H-NMR}$  (300 MHz,  $(\text{CD}_3)_2\text{SO}$ ):  $\delta$  13.11 (brs, 1H), 10.70 (s, 1H), 7.83-7.99 (m, 5H), 7.58-7.72 (m, 3H), 2.52 (m, 3H). **MS** (ESI): 284.05 [M + H<sup>+</sup>]; calcd for  $[\text{C}_{16}\text{H}_{13}\text{NO}_4 + \text{H}^+]$  284.09.

N10

25 [0241]  $^1\text{H-NMR}$  (300 MHz,  $(\text{CD}_3)_2\text{SO}$ ):  $\delta$  13.12 (brs, 1H), 12.23 (s, 1H), 8.62 (d,  $J = 8.1$  Hz, 1H), 8.34 (s, 1H), 7.22-7.89 (m, 7H), 7.17 (t,  $J = 6.9$  Hz, 1H). **MS** (ESI): 285.00 [M + H<sup>+</sup>]; calcd for  $[\text{C}_{15}\text{H}_{12}\text{N}_2\text{O}_4 + \text{H}^+]$  285.09.

N11

30 [0242]  $^1\text{H-NMR}$  (300 MHz,  $(\text{CD}_3)_2\text{SO}$ ):  $\delta$  13.02 (brs, 1H), 10.46 (s, 1H), 8.20 (s, 1H), 7.82-7.94 (m, 3H), 7.57-7.71 (m, 4H), 7.35-7.44 (m, 2H). **MS** (ESI): 307.25 [M + Na<sup>+</sup>]; calcd for  $[\text{C}_{15}\text{H}_{12}\text{N}_2\text{O}_4 + \text{Na}^+]$  307.07.



N13

[0243] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 13.40 (brs, 1H), 11.55 (s, 1H), 8.61 (d, *J* = 6.3 Hz, 1H), 8.02 (m, 2H), 7.23-7.88 (m, 4H), 7.20 (t, *J* = 5.4 Hz, 1H). MS (ESI): 308.20 [M + Na<sup>+</sup>]; calcd for [C<sub>15</sub>H<sub>11</sub>NO<sub>5</sub> + Na<sup>+</sup>] 308.05.

5 N14

[0244] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 13.02 (brs, 2H), 10.53 (s, 1H), 8.38 (s, 1H), 7.89 (t, *J* = 6.0 Hz, 2H), 7.56-7.69 (m, 4H), 7.46 (t, *J* = 5.7 Hz, 1H). MS (ESI): 286.20 [M + H<sup>+</sup>]; calcd for [C<sub>15</sub>H<sub>11</sub>NO<sub>5</sub> + H<sup>+</sup>] 286.07

N17

10 [0245] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 13.00 (brs, 1H), 10.65 (s, 1H), 8.34 (s, 1H), 7.90 (d, *J* = 5.7 Hz, 1H), 7.77 (m, 1H) 7.39-7.69 (m, 5H), 7.32 (s, 2H). MS (ESI): 320.85 [M + H<sup>+</sup>]; calcd for [C<sub>14</sub>H<sub>12</sub>N<sub>2</sub>O<sub>5</sub>S + H<sup>+</sup>] 321.05.

N19

15 [0246] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 13.13 (brs, 1H), 10.71 (s, 1H), 8.18 (s, 1H), 7.90-7.94 (m, 2H), 7.55-7.73 (m, 5H). MS (ESI): 267.00 [M + H<sup>+</sup>]; calcd for [C<sub>15</sub>H<sub>10</sub>N<sub>2</sub>O<sub>3</sub> + H<sup>+</sup>] 267.08.

N20

20 [0247] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 13.10 (brs, 1H), 9.76 (s, 1H), 7.88 (d, *J* = 7.8 Hz, 1H), 7.57-7.71 (m, 4H), 7.45 (d, *J* = 7.2 Hz, 1H), 7.19-7.32 (m, 2H), 5.29 (brs, 1H), 4.61 (s, 2H). MS (ESI): 293.90 [M + Na<sup>+</sup>]; calcd for [C<sub>15</sub>H<sub>13</sub>NO<sub>4</sub> + Na<sup>+</sup>] 294.07.

N24

[0248] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 8.05 (m, 2H), 7.45-7.74 (m, 5H), 7.28 (m, 1H), 4.23 (s, 2H), 2.77 (s, 3H). MS (ESI): 284.90 [M + H<sup>+</sup>]; calcd for [C<sub>16</sub>H<sub>16</sub>N<sub>2</sub>O<sub>3</sub> + H<sup>+</sup>] 285.12.

N26

25 [0249] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 12.98 (brs, 1H), 10.24 (s, 1H), 7.88 (d, *J* = 6.6 Hz, 1H), 7.53-7.87 (m, 5H), 7.17 (d, *J* = 8.4 Hz, 2H), 3.59 (t, *J* = 6.9 Hz, 2H), 2.70 (t, *J* = 7.2 Hz, 2H). MS (ESI): 308.25 [M + Na<sup>+</sup>]; calcd for [C<sub>16</sub>H<sub>15</sub>NO<sub>4</sub> + Na<sup>+</sup>] 308.09.

N28

30 [0250] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 12.84 (brs, 1H), 10.13 (s, 1H), 7.86 (d, *J* = 7.2 Hz, 1H), 7.52-7.68 (m, 5H), 6.93 (d, *J* = 9.0 Hz, 2H), 3.76 (m, 4H), 3.07 (m, 4H). MS (ESI): 326.90 [M + H<sup>+</sup>]; calcd for [C<sub>18</sub>H<sub>18</sub>N<sub>2</sub>O<sub>4</sub> + H<sup>+</sup>] 327.13.

N30

[0251] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 9.41 (brs, 1H), 7.60 (m, 1H), 7.51 (m, 1H), 7.40 (m, 2H), 3.74 (m, 1H), 2.73 (m, 1H), 2.03 (m, 2H), 1.87 (m, 2H), 1.60 (m, 2H), 1.36 (m, 2H). MS (ESI): 295.10 [M + Na<sup>+</sup>]; calcd for [C<sub>15</sub>H<sub>16</sub>N<sub>2</sub>O<sub>3</sub> + Na<sup>+</sup>] 295.11.

5 N31

[0252] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 7.67 (d, *J* = 5.7 Hz, 1H), 7.27-7.36 (m, 3H), 1.99 (m, 1H), 1.47-1.64 (m, 8H). MS (ESI): 314.30 [M + Na<sup>+</sup>]; calcd for [C<sub>15</sub>H<sub>17</sub>NO<sub>5</sub> + Na<sup>+</sup>] 314.10.

N33

10 [0253] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 7.54 (m, 1H), 7.26-7.52 (m, 3H), 3.07 (d, *J* = 7.2 Hz, 2H), 2.28 (m, 1H), 1.80 (m, 2H), 1.38-1.58 (m, 5H), 1.23 (m, 2H). MS (ESI): 306.10 [M + H<sup>+</sup>]; calcd for [C<sub>16</sub>H<sub>19</sub>NO<sub>5</sub> + H<sup>+</sup>] 306.13.

N34

15 [0254] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 7.81 (m, 1H), 7.50-7.80 (m, 3H), 6.74 (s, 2H). MS (ESI): 232.10 [M + H<sup>+</sup>]; calcd for [C<sub>11</sub>H<sub>9</sub>N<sub>3</sub>O<sub>3</sub> + H<sup>+</sup>] 232.07.

N35

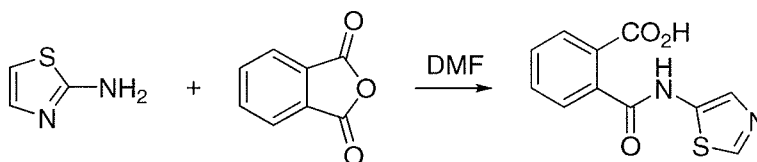
[0255] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 7.95 (d, *J* = 7.2 Hz, 1H), 7.82 (m, 2H), 7.42-7.52 (m, 3H), 7.09 (s, 1H). MS (ESI): 232.90 [M + H<sup>+</sup>]; calcd for [C<sub>11</sub>H<sub>8</sub>N<sub>2</sub>O<sub>4</sub> + H<sup>+</sup>] 233.06.

N38

20 [0256] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 13.16 (brs, 1H), 11.81 (s, 1H), 8.41 (s, 1H), 7.93 (d, *J* = 7.8 Hz, 1H), 7.57-7.73 (m, 5H), 7.45 (m, 2H), 7.35 (m, 1H). MS (ESI): 309.00 [M + H<sup>+</sup>]; calcd for [C<sub>17</sub>H<sub>12</sub>N<sub>2</sub>O<sub>4</sub> + H<sup>+</sup>] 309.09.

N39

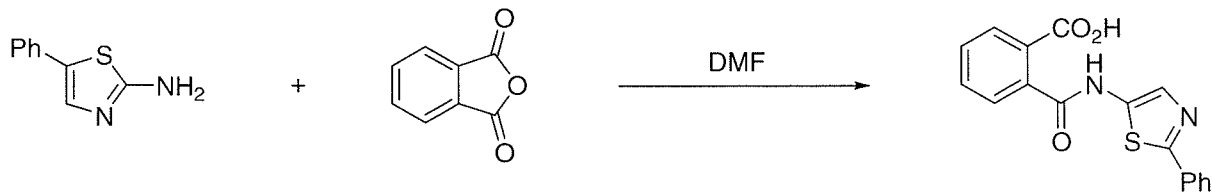
25 [0257] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 7.87-8.05 (m, 4H), 7.64 (s, 1H), 7.43-7.58 (m, 4H), 7.34 (t, *J* = 7.5 Hz, 1H). MS (ESI): 325.00 [M + H<sup>+</sup>]; calcd for [C<sub>17</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>S + H<sup>+</sup>] 325.06.

N42

[0258] Prepared from 2-Aminothiazole + phthalic anhydride under standard conditions.

<sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 13.07 (brs, 1H), 11.78 (s, 1H), 8.63 (s, 1H), 7.93 (m, 1H), 7.58-7.91 (m, 4H). MS (ESI): 248.85 [M + H<sup>+</sup>]; calcd for [C<sub>11</sub>H<sub>8</sub>N<sub>2</sub>O<sub>3</sub>S + H<sup>+</sup>] 249.03.

N45



[0259] 2-Amino-4-phenylthiazole + phthalic anhydride under standard conditions. <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 13.16 (brs, 1H), 11.90 (s, 1H), 7.93 (m, 3H), 7.62-7.92 (m, 4H), 7.42-7.53 (m, 3H). MS (ESI): 325.00 [M + H<sup>+</sup>]; calcd for [C<sub>17</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>S + H<sup>+</sup>] 325.06.

10 N50

[0260] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 8.61 (d, *J* = 4.8 Hz, 2H), 7.84 (d, *J* = 7.5 Hz, 1H), 7.50-7.62 (m, 3H), 7.17 (t, *J* = 4.8 Hz, 1H). MS (ESI): 243.85 [M + H<sup>+</sup>]; calcd for [C<sub>12</sub>H<sub>9</sub>N<sub>3</sub>O<sub>3</sub> + H<sup>+</sup>] 244.07.

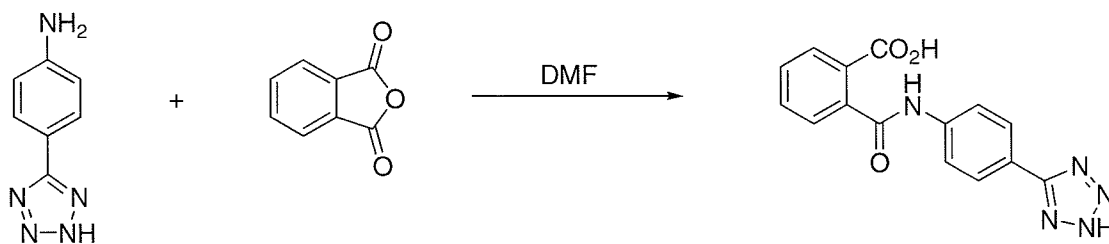
N52

15 [0261] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 13.12 (brs, 1H), 11.22 (s, 1H), 9.43 (s, 1H), 8.42 (m, 2H), 7.91 (m, 1H), 7.57-7.69 (m, 3H). MS (ESI): 243.85 [M + H<sup>+</sup>]; calcd for [C<sub>12</sub>H<sub>9</sub>N<sub>3</sub>O<sub>3</sub> + H<sup>+</sup>] 244.07.

PA02

20 [0262] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 13.07 (brs, 1H), 11.14 (s, 1H), 10.56 (s, 1H), 8.97 (brs, 1H), 7.92 (d, *J* = 7.2 Hz, 1H), 7.57-7.77 (m, 7H). MS (ESI): 300.95 [M + H<sup>+</sup>]; calcd for [C<sub>15</sub>H<sub>12</sub>N<sub>2</sub>O<sub>5</sub> + H<sup>+</sup>] 301.08.

PA06



25 [0263] Prepared from 5-(4-Aminophenyl)tetrazole + phthalic anhydride under standard conditions. <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 13.11 (brs, 1H), 10.68 (s, 1H), 8.05 (d, *J* = 8.7

Hz, 2H), 7.92 (d,  $J = 8.1$  Hz, 3H), 7.59-7.70 (m, 3H). **MS** (ESI): 310.25 [M + H<sup>+</sup>]; calcd for [C<sub>15</sub>H<sub>11</sub>N<sub>5</sub>O<sub>3</sub> + H<sup>+</sup>] 310.09.

PA07

5 [0264] **<sup>1</sup>H-NMR** (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO):  $\delta$  10.39 (s, 1H), 8.92 (m, 2H), 8.57 (m, 1H), 8.05 (m, 2H), 7.87 (m, 1H), 7.54-7.76 (m, 8H) (1:1 complex with pyridine). **MS** (ESI): 320.05 [M - 1]; calcd for [C<sub>14</sub>H<sub>11</sub>NO<sub>6</sub>S - 1] 320.03.

PA08

[0265] **<sup>1</sup>H-NMR** (300 MHz, (CD<sub>3</sub>OD):  $\delta$  8.06 (d,  $J = 7.2$  Hz, 1H), 7.78 (m, 4H), 7.56-7.70 (m, 3H). **MS** (ESI): 321.95 [M + H<sup>+</sup>]; calcd for [C<sub>14</sub>H<sub>12</sub>NO<sub>6</sub>P + H<sup>+</sup>] 322.05.

10 PM02

[0266] **<sup>1</sup>H-NMR** (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO):  $\delta$  13.03 (brs, 1H), 10.37 (s, 1H), 7.87 (d,  $J = 6.9$  Hz, 1H), 7.55-7.71 (m, 5H), 7.40 (t,  $J = 8.1$  Hz, 2H) 7.29 (t,  $J = 7.8$  Hz, 2H), 6.91-7.02 (m, 3H), 5.05 (s, 2H). **MS** (ESI): 370.30 [M + Na<sup>+</sup>]; calcd for [C<sub>21</sub>H<sub>17</sub>NO<sub>4</sub> + Na<sup>+</sup>] 370.11.

PM05

15 [0267] **<sup>1</sup>H-NMR** (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO):  $\delta$  13.08 (brs, 1H), 10.52 (s, 1H), 8.66 (m, 1H), 8.10 (d,  $J = 8.7$  Hz, 2H), 7.83-7.97 (m, 5H), 7.59-7.72 (m, 3H), 7.32 (m, 1H). **MS** (ESI): 318.85 [M + H<sup>+</sup>]; calcd for [C<sub>19</sub>H<sub>14</sub>N<sub>2</sub>O<sub>3</sub> + H<sup>+</sup>] 319.11.

PM10

20 [0268] **<sup>1</sup>H-NMR** (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO):  $\delta$  12.50 (brs, 1H), 7.63-7.76 (m, 4H), 7.47 (m, 2H), 7.21 (d,  $J = 8.4$  Hz, 2H), 6.40 (t,  $J = 6.0$  Hz, 1H), 5.52 (s, 2H), 4.15 (d,  $J = 5.7$  Hz, 2H). **MS** (ESI): 314.00 [M + H<sup>+</sup>]; calcd for [C<sub>16</sub>H<sub>15</sub>N<sub>3</sub>O<sub>4</sub> + H<sup>+</sup>] 314.11.

PM11

25 [0269] **<sup>1</sup>H-NMR** (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO):  $\delta$  13.00 (brs, 1H), 10.31 (s, 1H), 7.89 (d,  $J = 6.9$  Hz, 1H), 7.54-7.70 (m, 6H), 7.22 (d,  $J = 8.4$  Hz, 2H), 4.16 (d,  $J = 5.7$  Hz, 2H), 3.57 (s, 3H). **MS** (ESI): 329.00 [M + H<sup>+</sup>]; calcd for [C<sub>17</sub>H<sub>16</sub>N<sub>2</sub>O<sub>5</sub> + H<sup>+</sup>] 329.11.

PM18

[0270] **<sup>1</sup>H-NMR** (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO):  $\delta$  12.73 (brs, 1H), 11.37 (brs, 1H), 7.97 (m, 2H), 7.38-7.87 (m, 6H), 7.05 (m, 2H). **MS** (ESI): 307.95 [M + H<sup>+</sup>]; calcd for [C<sub>17</sub>H<sub>13</sub>N<sub>3</sub>O<sub>3</sub> + H<sup>+</sup>] 308.10.

R01

[0271] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 8.36 (m, 1H), 7.93 (d, *J* = 6.0 Hz, 1H), 7.78 (m, 1H), 7.65 (d, *J* = 5.7 Hz, 1H), 7.48 (m, 2H), 7.33 (t, *J* = 6.0 Hz, 1H). MS (ESI): 264.00 [M + H<sup>+</sup>]; calcd for [C<sub>15</sub>H<sub>9</sub>N<sub>3</sub>O<sub>2</sub> + H<sup>+</sup>] 264.08.

5 R06-1

[0272] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 8.64 (m, 1H), 8.14 (d, *J* = 8.4 Hz, 1H), 7.75-7.88 (m, 2H), 7.34-7.51 (m, 3H). MS (ESI): 280.90 [M + H<sup>+</sup>]; calcd for [C<sub>15</sub>H<sub>8</sub>N<sub>2</sub>O<sub>2</sub>S + H<sup>+</sup>] 281.04.

R07

10 [0273] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO + D<sub>2</sub>O): δ 7.79-7.89 (m, 5H), 7.33 (m, 2H), 7.00 (m, 1H). MS (ESI): 300.95 [M - 1]; calcd for [C<sub>14</sub>H<sub>10</sub>N<sub>2</sub>O<sub>4</sub>S - 1] 301.04.

R10

[0274] <sup>1</sup>H-NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 11.50 (brs, 1H), 7.87-7.99 (m, 4H), 7.64 (m, 1H), 7.36 (m, 3H), 7.16 (m, 2H). MS (ESI): 281.90 [M + H<sup>+</sup>]; calcd for [C<sub>15</sub>H<sub>11</sub>N<sub>3</sub>O<sub>3</sub> + H<sup>+</sup>]  
15 282.09.

**Example 2. High throughput screen for inducers of chondrogenesis**

[0275] This example describes the assay used to identify compounds that induce chondrogenesis. The assay system targeted the resident mesenchymal stem cells (MSCs) in cartilage and identified mediators that stimulated the natural repair potential and enhance  
20 integrated cartilage regeneration. This unbiased cell-based screening approach with chemical or genomic libraries has proven a powerful approach for identifying molecules that control stem cell self-renewal and fate.

[0276] The primary screening assay was based upon the development of chondrogenic nodules. Chondrocytes can grow in monolayer *in vitro*, but to more closely mimic their native  
25 environment in the joint, the cells are routinely cultured in non-adherent conditions such as in pellet cultures or alginate bead suspensions.

[0277] During the design of a high throughput screen of hMSCs in 384 well plates we discovered that cells, even when plated under monolayer conditions initially, if exposed to the proper stimulation and environment, develop a nodule containing the characteristics of  
30 chondrocytes grown in pellet cultures. In direct comparison with the pellet cultures of MSCs or chondrocytes, all populations of the chondrogenic nodules had high levels of cartilage specific matrix production (both proteoglycans and type II collagen expression) suggesting

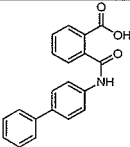
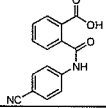
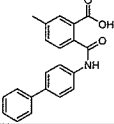
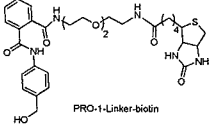
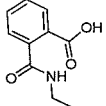
that chondrogenic differentiation has occurred. To initially image nodules, the wells were fixed and stained with 1µg/ml Rhodamine B (Figure 1) where the nodules were easily detected by eye and images captured by light microscopy. In the actual screen of a library of 22,000 structurally diverse heterocycles, 10<sup>4</sup> primary hMSCs were plated in 384 well plates in serum free DMEM. The cells were treated with 5µM of each compound and incubated for 4 days. During this period the chondrogenic nodule formed in wells that were considered a positive hit. To facilitate high throughput imaged-based detection, the chondrogenic nodules were stained with Nile red which binds non-specifically to collagens. The Nile Red stained nodules were quantified on an Acumen eX3 (high content imaging device) by excitation with a 488 laser for rapid detection of the nodules. PRO1 was thus identified as having EC<sub>50</sub>=100nM (see Table 1).

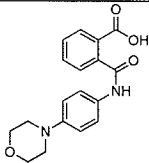
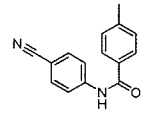
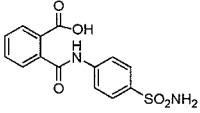
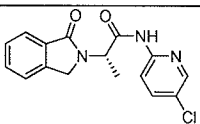
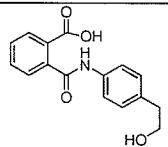
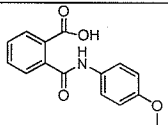
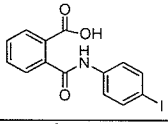
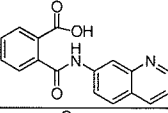
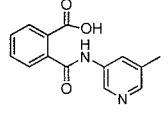
[0278] Following the initial screen, the hits were subsequently characterized in multiple secondary assays. No toxicity was found after treatment with 100µM PRO1 in hMSCs, synovial fibroblasts, chondrocytes or HEK 293 cells (data not shown). Furthermore, a small proliferative advantage was demonstrated in human chondrocytes (<1.5 fold), but was not significant when re-evaluated in cartilage organ cultures (data not shown). The specificity of chondrocyte differentiation was confirmed by immunocytochemical staining for type II collagen (Figure 2A), Sox9 and aggrecan in cultures of hMSCs after 18 days. In addition mRNA from pellet cultures after 7 and 21 days demonstrated expression of lubricin, aggrecan and type II collagen, but not osteocalcin or type X collagen. Similar data was generated using a mouse mesenchymal cell line, ATCD5, suggesting that PRO1 has mouse and human cross-reactivity. These data prompted an initial structure-activity relationship (SAR) be completed on the core scaffold of PRO1.

[0279] The chondrogenic differentiation event was initiated quickly even though a fully differentiated chondrocyte phenotype cannot be assessed prior to 14 days in culture. In an 18 day assay, compound treatment was only required for the first 48 hrs and followed by removal and replacement of the media (in the absence of stimuli, Figure 2B). The promotion of chondrogenic differentiation was not altered or enhanced upon longer treatment with the stimuli (data not shown). In addition, treatment of hMSCs with PRO1 or an analogue, PRO1-CN (Table 1), was more potent and efficient than treatment with known inducers of chondrogenic differentiation (TGFβ3, BMP7 or BMP2). The chondrocyte proliferative biologic in Phase II clinical trials, FGF18, did not effectively induce hMSC differentiation (Figure 2B).

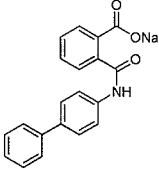
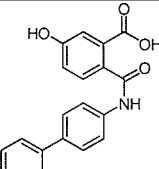
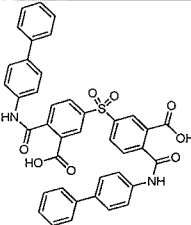
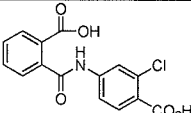
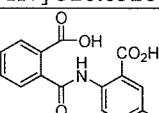
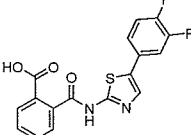
[0280] In the screen, hMSCs were used as a surrogate to future targeting of the resident MSCs with the cartilage joint. It was in that setting upon which we identified PRO1. In a joint injury, the primary cell type a therapeutic would have contact with is the chondrocyte. Although cartilage or chondrocyte-targeted screening has not lead to the development of a successful drug modifying osteoarthritis drug (DMOAD) to date, it is believed that a stem cell-based therapeutic would protect and even repair the existing chondrocytes. Therefore, protection of the articular chondrocytes and cartilage from a mature bovine knee was assessed. PRO1 and its analogues were incubated with primary bovine chondrocytes or cartilage explants cultures grown in the presence of TNF $\alpha$  and oncostatin M to mimic the cytokine-induced damage that can occur during OA. In the presence of the cytokine cocktail, *in vitro* cultured primary chondrocytes released 4-5 fold more nitric oxide (NO) than the untreated chondrocytes (50 $\mu$ M vs 10 $\mu$ M) as measured by the Greiss reaction. In cells treated with the cytokine cocktail and PRO1, the small molecule could effectively block the release of NO by up to 70% (Figure 2C). Similarly, in *ex vivo* treated cartilage explants, cytokine-induced release of glycosaminoglycans (GAG) was reduced by up to 60% by PRO1 (Figure 2D). Finally, hMSC were grown in pellet culture for 21 days. Upon immunohistochemical staining of the pellets, it was demonstrated that PRO1 could increase type II collagen and aggrecan expression in a three dimensional culture environment (Figure 2E).

**Table 1. Table of compounds synthesized and selected by SAR**

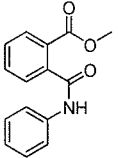
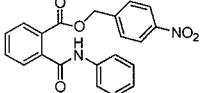
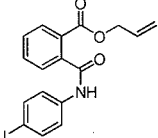
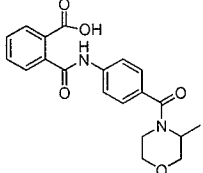
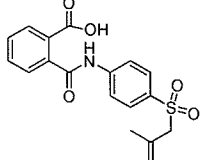
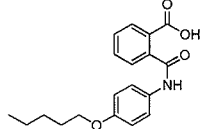
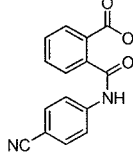
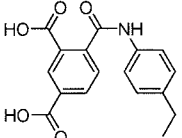
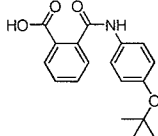
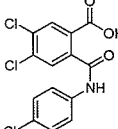
PRODUCT	STRUCTURE	CHONDROGENIC $\mu$ M EC <sub>50</sub> / NO INHIBITION $\mu$ M IC <sub>50</sub>	COLLAGEN TYPE II $\mu$ M EC <sub>50</sub>
AKT, AKT093, PRO1		0.225/0.45	0.08
AKT-CN, LK3, LK81, PRO1-CN		0.137/0.03	0.108
AKT-Me L3		>10/>10	>10
AKT-biotine LK14		0.04/0.001	>10
AKT-NHEt LK19b		2.01>10	>10

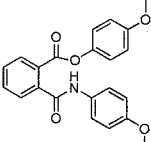
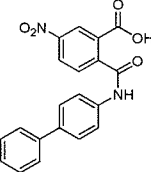
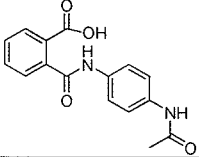
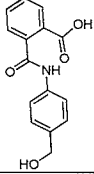

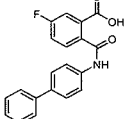
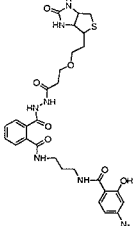
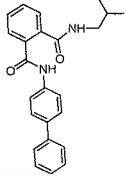
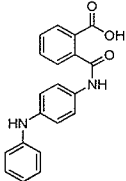
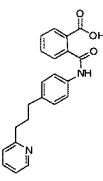
PRODUCT	STRUCTURE	CHONDROGENIC $\mu\text{M EC}_{50}$ / NO INHIBITION $\mu\text{M IC}_{50}$	COLLAGEN TYPE II $\mu\text{M EC}_{50}$
LK6		9.1/>10	>10
LK20b		0.014/>10	>10
Resynthesized LK8	 HRMS (ESI): 320.0425 [M + H <sup>+</sup> ]; calcd for [C <sub>14</sub> H <sub>12</sub> N <sub>2</sub> O <sub>5</sub> S + H <sup>+</sup> ] 320.0427.	9.1/>10	>10
SMA-1* LK17b		>10/0.04	0.37
L11c		0.125/>10	>10
Resynthesized L2		>10/ND	>10
Resynthesized L11a		>10/>10	>10
L9		>10/>10	>10
L5	 HRMS (ESI): 256.0852 [M + H <sup>+</sup> ]; calcd for [C <sub>14</sub> H <sub>12</sub> N <sub>2</sub> O <sub>3</sub> + H <sup>+</sup> ] 256.0848.	0.6/2.4	>10

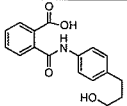
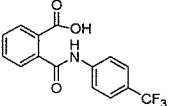
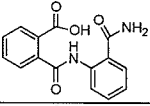
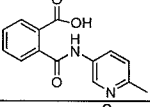
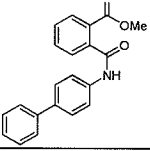
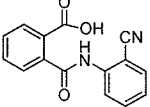
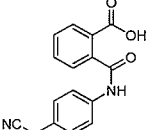
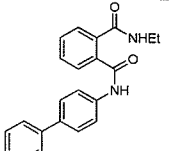
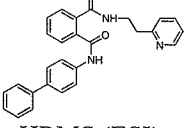
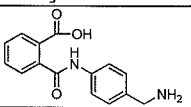


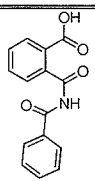
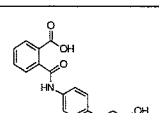
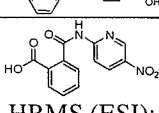
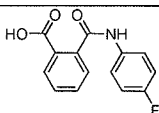
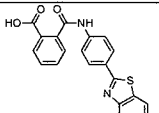
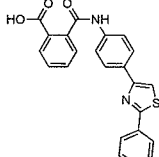
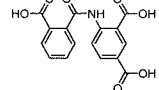
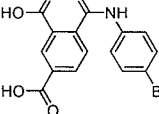
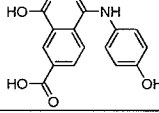
PRODUCT	STRUCTURE	CHONDROGENIC $\mu\text{M EC}_{50}$ / NO INHIBITION $\mu\text{M IC}_{50}$	COLLAGEN TYPE II $\mu\text{M EC}_{50}$
LK18b	 <p><b>HRMS (ESI):</b> 339.0866 [M + H<sup>+</sup>]; calcd for [C<sub>20</sub>H<sub>14</sub>NNaO<sub>3</sub> + H<sup>+</sup>] 339.0871.</p>	0.0015/2.7	0.05
LK22b		0.0046/>10	>10
LK4	 <p><b>HRMS (ESI):</b> 697.1634 [M + H<sup>+</sup>]; calcd for [C<sub>40</sub>H<sub>28</sub>N<sub>2</sub>O<sub>8</sub>S + H<sup>+</sup>] 697.1645.</p>	3.8/>10	33.34
LK63	 <p><b>HRMS (ESI):</b> 320.0332 [M + H<sup>+</sup>]; calcd for [C<sub>15</sub>H<sub>10</sub>ClNO<sub>5</sub> + H<sup>+</sup>] 320.0326</p>	0.126/.0298	ND
LK60		0.014/1.245	ND
LK35	 <p><b>HRMS (ESI):</b> 361.0452 [M + H<sup>+</sup>]; calcd for [C<sub>17</sub>H<sub>10</sub>F<sub>2</sub>N<sub>2</sub>O<sub>3</sub>S + H<sup>+</sup>] 361.0458.</p>	>10/0.03	>10

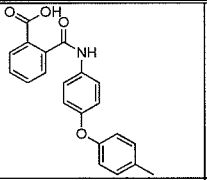
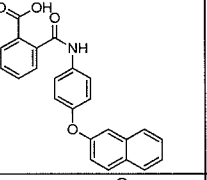
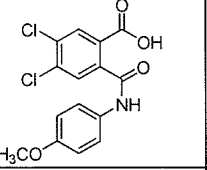
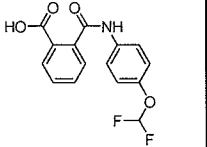
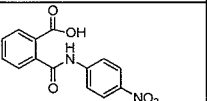
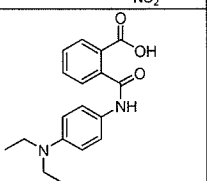
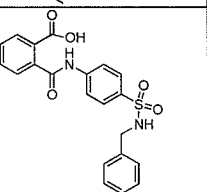
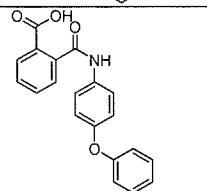
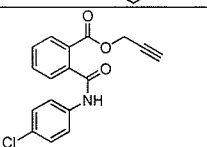
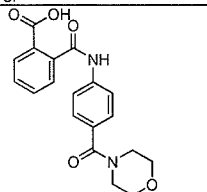
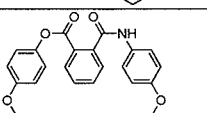
PRODUCT	STRUCTURE	CHONDROGENIC $\mu\text{M EC}_{50}$ / NO INHIBITION $\mu\text{M IC}_{50}$	COLLAGEN TYPE II $\mu\text{M EC}_{50}$
LK62		3.36/0.145	ND
Resynthesized LK57		>10/ND	ND
LK61		0.052/1.69	ND
Resynthesized LK49		5/>10	ND
		>10/2.5	ND
LK53		0.005/0.146	ND
		>10/0.624	ND
		ND/>10	ND
		ND/2.7	ND
		ND/>10	ND
		ND/>10	ND
		ND/>10	ND

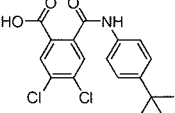
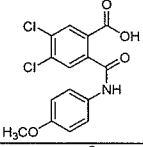
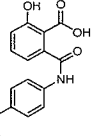
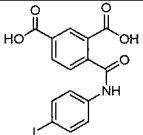
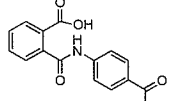
PRODUCT	STRUCTURE	CHONDROGENIC $\mu\text{M EC}_{50}$ / NO INHIBITION $\mu\text{M IC}_{50}$	COLLAGEN TYPE II $\mu\text{M EC}_{50}$
	 <p><b>HRMS (ESI):</b> 255.0895 [M + H<sup>+</sup>]; calcd for [C<sub>15</sub>H<sub>13</sub>NO<sub>3</sub> + H<sup>+</sup>] 255.0895.</p>	ND/>10	ND
		ND/>10	ND
		ND/>10	ND
		ND/2.64	ND
		ND/0.925	ND
		ND/>10	ND
		ND/>10	ND
		ND/0.925	ND
		ND/>10	ND
		ND/>10	ND

PRODUCT	STRUCTURE	CHONDROGENIC $\mu\text{M EC}_{50}$ / NO INHIBITION $\mu\text{M IC}_{50}$	COLLAGEN TYPE II $\mu\text{M EC}_{50}$
		ND/>10	ND
		ND/>10	ND
		ND/>10	ND
<b>AKT-CH2OH L1</b>		0.04/0.27	<b>8.46</b>
<b>AKT-NHMe LK23</b>		2.7/0.0046	<b>0.24</b>
<b>AKT-F LK5</b>		>10/>10	>10
<b>AKT-azide LK80 LK80b</b>		ND/0.12	<b>0.654</b>
<b>AKT-iPr LK14b</b>		0.0015/>10	<b>6.978</b>
<b>Resynthesized LK7</b>		>10/>10	>10
<b>LK15</b>		0.12/>10	>10

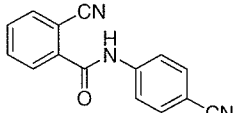
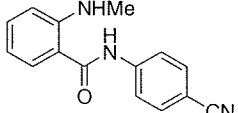
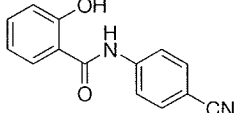
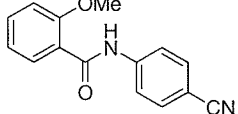
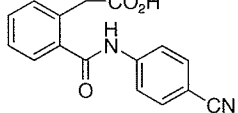
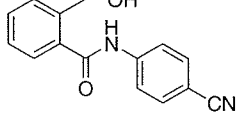
PRODUCT	STRUCTURE	CHONDROGENIC $\mu\text{M EC}_{50}$ / NO INHIBITION $\mu\text{M IC}_{50}$	COLLAGEN TYPE II $\mu\text{M EC}_{50}$
L12		ND/ND	ND
L4		9.5/22.1	11.1
Resynthesized L7		>10/>10	>10
L6		>10/>10	>10
LK55b		0.124/.234	ND
LK13b	 HRMS (ESI): 266.2519 [M + H <sup>+</sup> ]; calcd for [C <sub>15</sub> H <sub>10</sub> N <sub>2</sub> O <sub>3</sub> + H <sup>+</sup> ] 266.2515.	>10/ND	ND
L13	 HRMS (ESI): 280.0853 [M + H <sup>+</sup> ]; calcd for [C <sub>16</sub> H <sub>12</sub> N <sub>2</sub> O <sub>3</sub> + H <sup>+</sup> ] 280.0848.	>10/>10	>10
LK51		ND/ND	ND
LK52	 HRMS (ESI): 421.1796 [M + H <sup>+</sup> ]; calcd for [C <sub>27</sub> H <sub>23</sub> N <sub>3</sub> O <sub>2</sub> + H <sup>+</sup> ] 421.1790.	ND/ND	ND
LK55		0.126/0.216	ND

PRODUCT	STRUCTURE	CHONDROGENIC $\mu\text{M EC}_{50}$ / NO INHIBITION $\mu\text{M IC}_{50}$	COLLAGEN TYPE II $\mu\text{M EC}_{50}$
LK58	 <p>HRMS (ESI): 269.0692 [M + H+]; calcd for [C<sub>15</sub>H<sub>11</sub>NO<sub>4</sub> + H+] 269.0688.</p>	ND/ND	ND
Resynthesized LK64		0.398/0.981	ND
LK59	 <p>HRMS (ESI): 287.2277 [M + H+]; calcd for [C<sub>13</sub>H<sub>9</sub>NO<sub>5</sub> + H+] 287.2277.</p>	0.371/0.491	ND
Resynthesized	 <p>HRMS (ESI): 259.0639 [M + H+]; calcd for [C<sub>14</sub>H<sub>10</sub>FNO<sub>3</sub> + H+] 259.0645.</p>	>10uM/0.0125	ND
		ND/0.987	ND
		ND/>10	ND
		ND/>10	ND
		ND/>10	ND
		ND/>10	ND

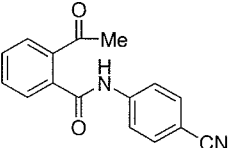
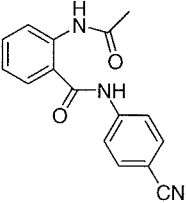
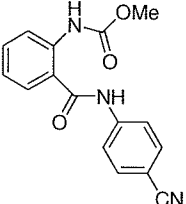
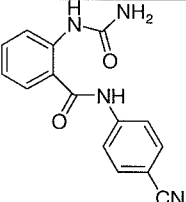
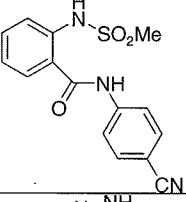
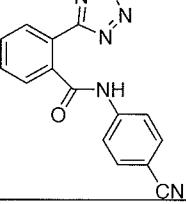
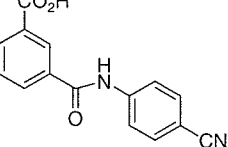
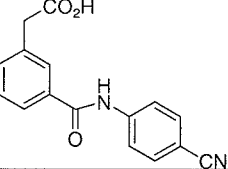
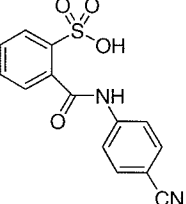
PRODUCT	STRUCTURE	CHONDROGENIC $\mu\text{M EC}_{50}$ / NO INHIBITION $\mu\text{M IC}_{50}$	COLLAGEN TYPE II $\mu\text{M EC}_{50}$
		ND/>10	ND
		ND/>10	ND
		ND/>10	ND
		ND/>10	ND
		ND/>10	ND
		ND/2.98	ND
		ND/>10	ND
		ND/>10	ND
		ND/>10	ND
		ND/>10	ND
		ND/>10	ND

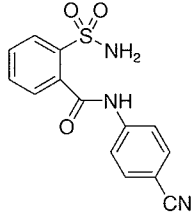
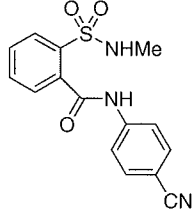
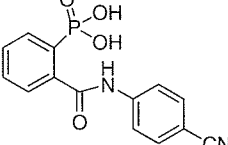
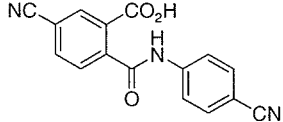
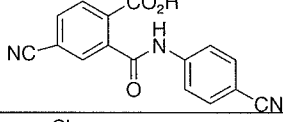
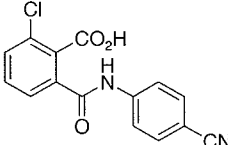
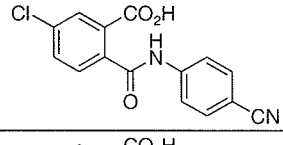
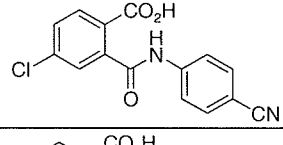
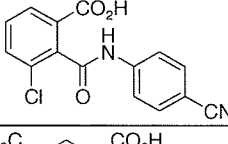
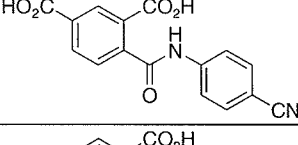
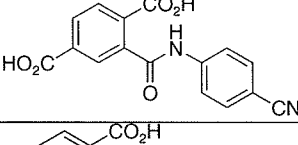
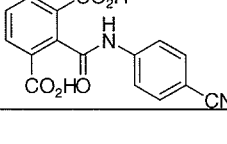
PRODUCT	STRUCTURE	CHONDROGENIC $\mu\text{M EC}_{50}$ / NO INHIBITION $\mu\text{M IC}_{50}$	COLLAGEN TYPE II $\mu\text{M EC}_{50}$
		ND/>10	ND
		ND/>10	ND
		ND/>10	ND
		ND/>10	ND
		ND/>10	ND

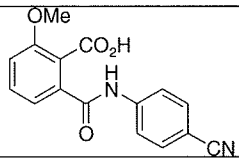
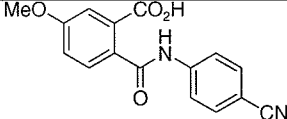
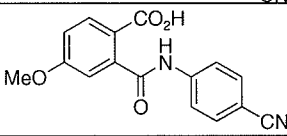
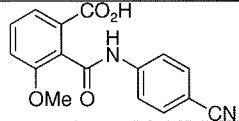
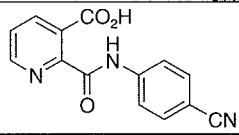
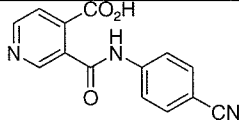
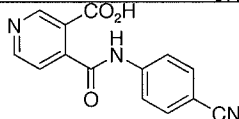
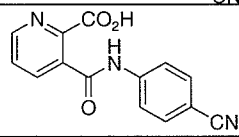
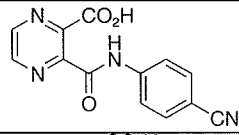
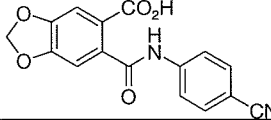
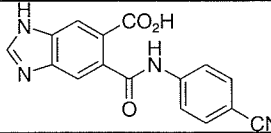
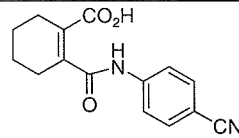
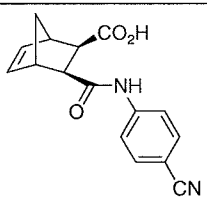
**Table 2. Additional Compounds**

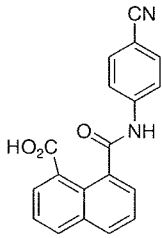
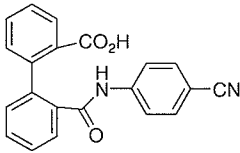
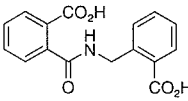
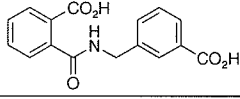
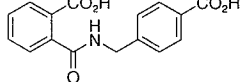
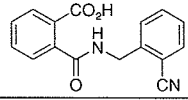
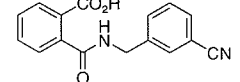
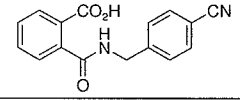
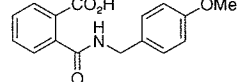
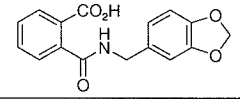
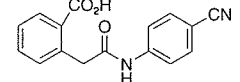
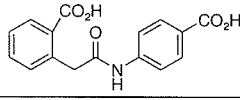
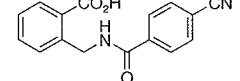
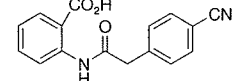
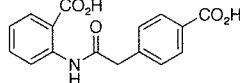
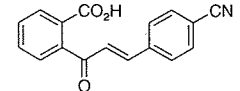
Compound	Structure	Activity <sup>1</sup>
A01		**
A02		
A04		
A05		
A06		***
A08		

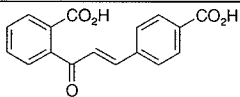
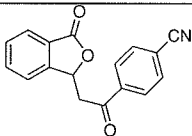
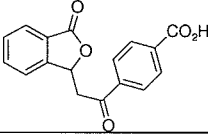
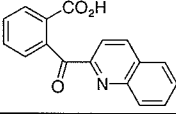
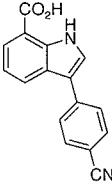
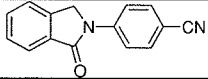
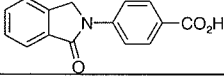
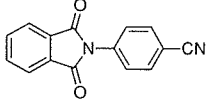
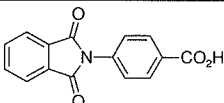
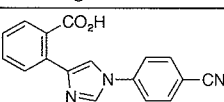
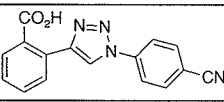
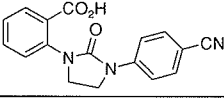
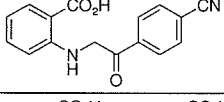
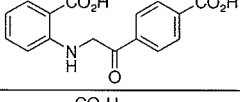
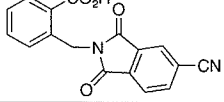
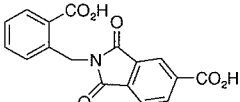


Compound	Structure	Activity <sup>1</sup>
A09		**
A10		
A11		**
A12		**
A13		**
A14		
A17		
A18		
A22		**

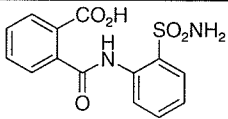
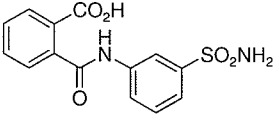
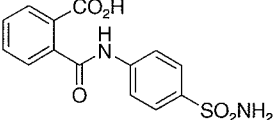
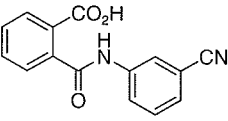
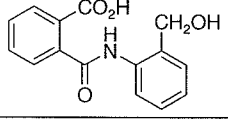
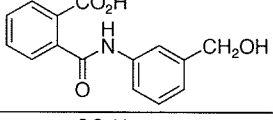
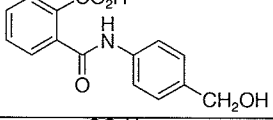
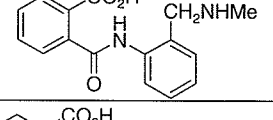
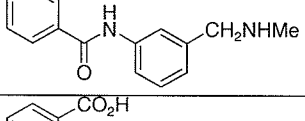
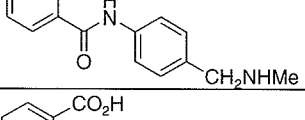
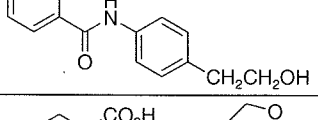
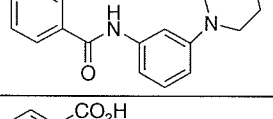
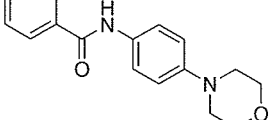
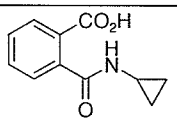
Compound	Structure	Activity <sup>1</sup>
A23		**
A24		**
A25		
B02		**
B03		***
B05		
B06		
B07		
B08		
B10		**
B11		
B12		**

Compound	Structure	Activity <sup>1</sup>
B13		
B14		
B15		
B16		
B17		
B18		
B19		***
B20		**
B21		
B27		
B28		
B29		
B30		

Compound	Structure	Activity <sup>1</sup>
B31		
B32		**
MS01		***
MS02		**
MS03		
MS04		**
MS05		
MS06		**
MS07		
MS08		
MS09		
MS10		
MS11		**
MS13		
MS14		**
MS15		***

Compound	Structure	Activity <sup>1</sup>
MS16		**
MS17-1		***
MS18-1		**
MS19		
MS21		***
MS26		**
MS27		
MS28		**
MS29		***
MS30		
MS31		
MS32		
MS35		
MS36		
MS38		
MS39		***

Compound	Structure	Activity <sup>1</sup>
N01		***
N02		
N04		***
N05		**
N06		
N07		
N08		****
N09		**
N10		**
N11		**
N12		
N13		**
N14		**
N15		

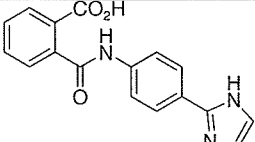
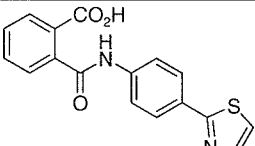
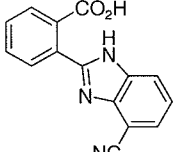
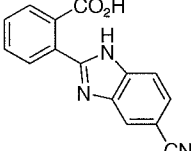
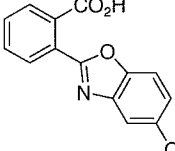
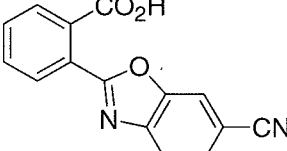
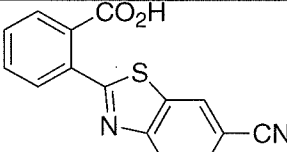
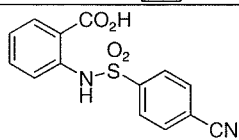
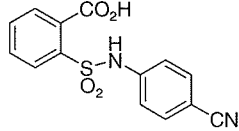
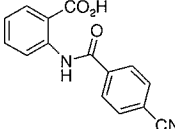
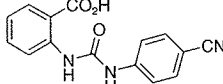
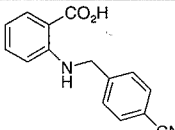
Compound	Structure	Activity <sup>1</sup>
N16		
N17		**
N18		
N19		***
N20		**
N21		
N22		
N23		
N24		***
N25		
N26		***
N27		
N28		**
N29		

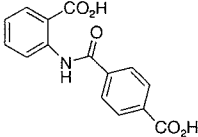
Compound	Structure	Activity <sup>1</sup>
N30		**
N31		**
N32		
N33		**
N34		**
N35		**
N36		
N37		
N38		**
N39		**
N42		***
N45		***
N46		
N47		



Compound	Structure	Activity <sup>1</sup>
N48		
N49		
N50		**
N51		
N52		**
N53		
PA02		**
PA03		
PA04		
PA05		
PA06		***
PA07		***
PA08		**

Compound	Structure	Activity <sup>1</sup>
PM01		
PM02		**
PM04		
PM05		**
PM06		
PM08		
PM09		
PM10		**
PM11		**
PM12		
PM15		
PM16		
PM17		

Compound	Structure	Activity <sup>1</sup>
PM18		**
PM19		
R01		**
R02		
R04		
R04-1		
R06-1		**
R07		**
R08		
R09		
R10		**
R11		

Compound	Structure	Activity <sup>1</sup>
R14		

<sup>1</sup> \*\*, chondrogenic nodules formed in at least 25% but less than 50% of cells. \*\*\*, chondrogenic nodules formed in at least 50% but less than 75% of cells. \*\*\*\*, chondrogenic nodules formed in at least 75% of cells.

5

### **Example 3. Rapid phenotypic cartilage nodule formation by hMSC cultures after incubation with 100nM PRO1.**

[0281] Primary hMSCs grown in a 96 well Greiner plates (50,000 / well) were incubated for 4 days in the presence of the 5uM of each DMSO or 100nM of PRO1. On day 4, the cells were fixed with 4% formalin for 10 minutes, washed and stained with 1µg/ml of Rhodamine B for 2 hours at room temperature. The wells were imaged to display the formation of a cartilage nodule and phenotypic change associated with stimulation of MSCs by PRO1.

### **Example 4. Confirmation of cartilage-specific protein expression and chondro-protective properties of PRO1**

[0282] Primary hMSCs were plated (10,000/well) in 384-well plates and cultured for 18 days. The cells were fixed with 4% formalin, permeablized with triton (0.5%), digested with 0.5mg/ml collagenase II to unwind the collagen fibrils, and then immunostained with an antibody recognizing type II collagen. DAPI (2µg/ml) was added to visualize the nuclei. The chondrogenic nodules stained positive for type II collagen but lacked expression of type X collagen and osteocalcin, markers for chondrocyte hypertrophy and osteoblasts, respectively (not shown). 10,000 hMSCs (passages 3-5) were plated in Greiner 384-well low-bottom plates on day 1. The cells were allowed to adhere and stimulated for 48 hrs (days 2-3) with the indicated stimuli in MSC growth media (Millipore). The media was then removed and replaced with serum-free DMEM for an additional 16 days. This media was replenished on days 10 and 15 of the assay. Primary bovine chondrocytes were isolated from the articular surfaces from mature knees. After one week of culture, 8,500 cells were trypsinized and plated in Greiner 384-well white clear-bottom plates in growth media. Following 24 hrs of culture, the media was removed and replaced with DMEM containing 20 ng/ml TNFα and 10 ng/ml oncostatin M. The cells were treated for 48 hours with and without the small molecules to assess the inhibition of the cytokine induced-NO release. 20 µl of the supernatant was

mixed with 20  $\mu$ l of the Greiss reagent and quantitated at 540 nm as described by the Promega kit instructions. Bovine cartilage explants (cut in circular explants in a 96 well plate) were cultured in the presence of 20 ng/ml TNF $\alpha$  and 10 ng/ml oncostatin M to induce GAG release for 72 hours (Figure 2D). PRO1 was added at the indicated concentrations during the entire 72 hrs. Incubation with 1 $\mu$ M TGF $\beta$ 3 (or less) and multiple doses of 1X ITS / 100nM dexamethasone did not have the same degree of chondro-protective activities. Parafin sections were hydrated, incubated in low pH Antigen unmasking solution (Vector) at 90 C for 10 minutes, permeabilized with 0.1% collagenase II (only for the sections stained with type II collagen) for 10 minutes, blocked with CAS block for 10 minutes and then incubated with the primary antibody (type II collagen, aggrecan or Timp1) antibody (Abcam) overnight at 4 C. The antigens were visualized through standard ABC detection methods and developed with DAB. Representative of 3 sections stained / treatment group (independent experiments). See Figure 2A-E.

**Example 5. PRO1 reduced cartilage damage and circulating COMP levels after induction of knee joint trauma by collagenase VII**

[0283] IA injections of C57BL/10 mice with collagenase VII (12U/knee) induced joint damage through digestion of the ligament of the knee. The mice were dosed intra-articularly on days 7 and 21 with 10 $\mu$ M PRO1 or PBS. On day 56 the mice were euthanized for histopathological analyses. Parafin blocks were sectioned at 5 $\mu$ m and stained with Safranin O, and representative data was collected for the PBS- and PRO1-treated mice (Figure 3A). The total medial tibial plateau joint score was determined by two blinded observers and averaged to represent the data shown here using the OARSI scoring system (Figure 3B). Peripheral blood was collected from each mouse by retro-orbital bleeding on day 1, day 13 and day 56. The circulating COMP was determined by the Animal COMP ELISA kit according to the manufacturer's instructions (MDBioproducts, St Paul, MN). Pooled data, 10 mice / group (Figure 3C).

**Example 6. Surgical induction of Cartilage Injury: Inhibition of CTX-II peptide in plasma and protection of cartilage damage by PRO1**

[0284] Surgical transection of the ACL, MCL, and MMTL was performed to induce cartilage injury in female 129SVE mice. Mice were dosed by IA injection on days 7, 14 and 21. The medial tibial plateaus from representative mice were assessed on day 28 (Figure 4A). Histomorphometric analyses and grading by two blinded observers using a modified OARSI scoring system determined the joint severity scores of the lateral tibial plateau on day 28 after

surgical induction (Figure 4B). At 28 days, peripheral blood was collected by retro-orbital bleeding and the serum was used to determine the cleaved type II collagen fragments (CTX-II levels) by ELISA (Serum Pre-Clinical Cartilaps, Immunodiagnostic Systems, Fountain Hills, AZ). n=15 / treatment group (Figure 4C).

#### 5 **Example 7. Surgical induction of OA: alleviation of OA-induced pain**

[0285] Surgical transection of the ACL, MCL, and MMTL was performed to induce cartilage injury in female 129SVE mice. Mice were dosed by IA injection on days 7, 14, 21 and 28 once / week. On day 42, incapacitance measurements were used to determine the percentage of weight the mouse distributed to each hind leg. 10 measurements were taken per  
10 mouse and averaged for each point in Figure 5A. (n=10 mice / group except n=4 for sham and surgical groups). Histomorphometric analyses and grading by two blinded observers using a modified OARSI scoring system determined the joint severity scores of the lateral tibial plateau on day 56 after surgical induction (Figure 5B). Parafin sections were hydrated, incubated in low-pH antigen unmasking solution (Vector) at 90 °C for 10 minutes,  
15 permeabilized with 0.1% collagenase II for 10 minutes, blocked with CAS block for 10 minutes, and then incubated with 1:200 dilution of type II collagen antibody (Abcam) overnight at 4 °C. The antigen was visualized through standard ABC detection methods and developed with DAB (Figure 5C).

#### 20 **Example 8. PRO1 interacts with FLNA and regulates the PEBP2 $\beta$ / Runx1 pathway to induce chondrogenesis**

[0286] To identify the interaction of PRO1 and FLNA, 5  $\mu$ M biotin-PRO1-azide was added to hMSCs in the differentiation media in the absence or presence of a 50-fold molar excess of PRO1 and incubated for 30 min. 254-nm light was applied to the cells for another 30 min. Cells were lysed in lysis buffer (20 mM Tris-HCl, pH = 7.4, 150 mM NaCl, 1 mM EDTA, 1  
25 mM DTT, 0.5% v/v Triton X-100, protease inhibitor cocktail), passed through 26 1/2 gauge needles 20 times, and centrifuged at 12,000 rpm for 30 min. The supernatant was subjected to ammonium sulfate precipitation fractionation. Fractions were analyzed by Western blotting using an anti-biotin antibody (Figure 6A). Fractions containing 20-40% ammonium sulfate were precipitated using trichloroacetic acid (TCA) and resuspended in isoelectric focusing  
30 sample buffer and subjected to 2D SDS-PAGE and Western blotting against biotin. Proteins specifically labeled by biotin-PRO1-azide were recognized, and the corresponding spots on a parallel gel visualized by silver staining were excised. These proteins were identified by mass spectrometry proteomics analysis. hMSCs were treated in monolayer or pellet culture (as

indicated in Figure 6B-6C) for 24-72 hrs. 15µg of protein was separated by SDS-PAGE and hMSCs were fractionated (as indicated in Figure 6B-6C) into cytosolic and nuclear fractions or monomeric and filamentous components as previously described.

**Example 9. PRO1 inhibits the protein interactions between FLNA PEBP2β**

5 [0287] hMSCs were treated with PRO1 for 12 hr and lysed as above. 2 µg of anti-PEBP2β antibody was added to the cell lysate and incubated overnight at 4 °C prior to addition of agarose beads bearing immobilized protein A/G. After incubation of the lysate with the protein A/G beads for 1 hour, the beads were collected by centrifugation at 2,000 rpm and washed 3 times with lysis buffer. 30 µL of 2X SDS sample buffer was added to the beads and  
10 boiled for 5 min. Proteins were analyzed by Western blotting using antibodies as indicated in Figure 7.

Compound	Route	Dose mg/kg	Day	Animal ID	T <sub>1/2</sub> h	T <sub>max</sub> h	C <sub>max</sub> nM	AUC <sub>0-∞</sub> h*nM	Plasma Conc. (nM)			
									1.00	3.00	7.00	24.00
AKT093	intra- articular (into the joint)	1.6 x 10 <sup>-4</sup> mmol/kg	1	1	1.9	1.0	133.6	342.3	134	28	13	BLQ
		(0.05 mg/kg)		2	1.6	1.0	77.8	213.4	78	24	6	BLQ
		(QD)		3	2.4	1.0	95.5	376.9	95	49	16	BLQ

LOQ = 1 ng/mL

(Accuracy: 99.4%)

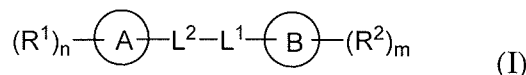
Mean 2.0 1.0 102.3 310.9 102.3 33.6 11.8 BLQ

Comments: 1) Compound was dosed at 0.05 mg/kg by intra-articular injection.  
2) After IA dosing, the compound was rapidly absorbed and the C<sub>max</sub> was ~ 100 nM.  
3) The bioavailability was ~22%  
4) Short T<sub>1/2</sub> (~2h).

15 [0288] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, one of skill in the art will appreciate that certain changes and modifications may be practiced within the scope of the appended claims. In addition, each reference provided herein is incorporated by reference in its entirety to the same extent as if each reference was individually incorporated by reference. Where a conflict exists between the instant application and a reference provided herein, the instant application shall dominate.

WHAT IS CLAIMED IS:

1           1.       A method of ameliorating arthritis or joint injury in a mammal, the  
2 method comprising administering to a joint of the mammal a composition comprising a  
3 therapeutically effective amount of a compound of formula I:



4           wherein

5           each of ring A and ring B are independently selected from the group consisting of  
6           cycloalkyl, aryl and heteroaryl;

7           each R<sup>1</sup> and R<sup>2</sup> is independently selected from the group consisting of H, C<sub>1-6</sub> alkyl,

8           C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, C<sub>1-6</sub> alkoxy, C<sub>1-6</sub> heteroalkyl, halogen,

9           C<sub>1-6</sub> haloalkyl, C<sub>1-6</sub> haloalkoxy, C<sub>1-6</sub> alkyl-CN, C<sub>1-6</sub> alkylhydroxy, -OR<sup>2a</sup>,

10           -NR<sup>2b</sup>R<sup>2d</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>R<sup>2d</sup>, -C(O)R<sup>1a</sup>, -C(O)R<sup>2d</sup>, -C(O)OR<sup>2a</sup>,

11           C<sub>1-6</sub> alkyl-C(O)OR<sup>2b</sup>, -OC(O)R<sup>2b</sup>, -OC(O)OR<sup>2b</sup>, -C(O)NR<sup>2a</sup>R<sup>2b</sup>,

12           -C(O)N(OH)R<sup>2b</sup>, -NR<sup>2b</sup>C(O)R<sup>2c</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)R<sup>2c</sup>, -NR<sup>2b</sup>C(O)OR<sup>2c</sup>,

13           C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)OR<sup>2c</sup>, -OC(O)NR<sup>2b</sup>R<sup>2c</sup>, -NR<sup>2b</sup>C(O)NR<sup>2b</sup>R<sup>2c</sup>,

14           -NR<sup>2b</sup>C(NR<sup>2b</sup>)NR<sup>2b</sup>R<sup>2c</sup>, -C(O)NR<sup>2b</sup>C(O)R<sup>2b</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)NR<sup>2b</sup>R<sup>2c</sup>,

15           -SR<sup>2a</sup>, -SO<sub>2</sub>R<sup>2b</sup>, -SO<sub>2</sub>OR<sup>2b</sup>, -SO<sub>2</sub>NR<sup>2b</sup>R<sup>2d</sup>, -NR<sup>2b</sup>SO<sub>2</sub>R<sup>2b</sup>, -P(O)(OR<sup>2b</sup>)<sub>2</sub>,

16           -B(OR<sup>2b</sup>), -CN, -NO<sub>2</sub>, -N<sub>3</sub>, heterocycloalkyl, aryl, heteroaryl,

17           C<sub>1-6</sub> alkyl-heterocycloalkyl, C<sub>1-6</sub> alkyl-aryl, C<sub>1-6</sub> alkyl-O-aryl,

18           C<sub>1-6</sub> alkyl-heteroaryl, and heteroaryl-aryl, and wherein the heterocycloalkyl,

19           aryl and heteroaryl groups are optionally substituted with 1 to 2 R<sup>2a</sup> groups;

20           R<sup>1a</sup> is selected from the group consisting of -OR<sup>1b</sup> and -NR<sup>1b</sup>R<sup>1c</sup>;

21           R<sup>1b</sup> and R<sup>1c</sup> are each independently selected from the group consisting of H,

22           C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, cycloalkyl, heterocycloalkyl, aryl,

23           heteroaryl, C<sub>1-6</sub> alkyl-aryl, and C<sub>1-6</sub> alkyl-heteroaryl, wherein the cycloalkyl,

24           heterocycloalkyl, aryl and heteroaryl groups are optionally substituted with

25           from 1 to 4 R<sup>1d</sup> groups;

26           each R<sup>1d</sup> is independently selected from the group consisting of H, C<sub>1-6</sub> alkyl,

27           C<sub>1-6</sub> alkoxy, and -NO<sub>2</sub>;

28           each R<sup>2a</sup> is independently selected from the group consisting of H, C<sub>2-6</sub> alkenyl,

29           C<sub>2-6</sub> alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl,

30           C<sub>1-6</sub> alkyl-cycloalkyl, C<sub>1-6</sub> alkyl-heterocycloalkyl, C<sub>1-6</sub> alkyl-aryl and

31           C<sub>1-6</sub> alkyl-heteroaryl, optionally substituted with 1 to 2 R<sup>2b</sup> groups;

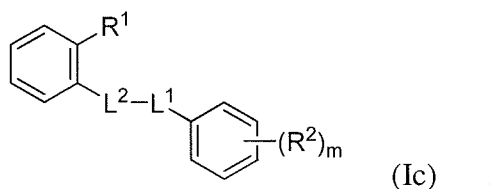
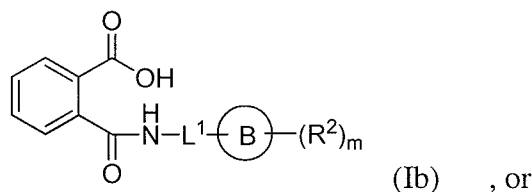
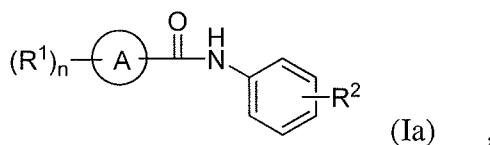


- 33 each  $R^{2b}$  and  $R^{2c}$  is independently selected from the group consisting of H, and  
 34  $C_{1-6}$  alkyl;
- 35 each  $R^{2d}$  is independently selected from the group consisting of H,  $C_{1-6}$  alkyl,  
 36 cycloalkyl, heterocycloalkyl, aryl, heteroaryl,  $C_{1-6}$  alkyl-cycloalkyl,  
 37  $C_{1-6}$  alkyl-heterocycloalkyl,  $C_{1-6}$  alkyl-aryl and  $C_{1-6}$  alkyl-heteroaryl, each  
 38 optionally substituted with 1 to 2  $R^{2b}$  groups;
- 39 each of  $L^1$  and  $L^2$  are independently selected from the group consisting of a bond,  
 40  $C_{1-6}$  alkylene,  $C_{2-6}$  alkenylene,  $C_{1-6}$  alkylene-O-, -O- $C_{1-6}$  alkylene,  
 41  $C_{1-6}$  alkylene-NR<sup>3a</sup>-, -NR<sup>3a</sup>- $C_{1-6}$  alkylene, -C(O)-,  $C_{1-6}$  alkylene-C(O)-,  
 42 -C(O)- $C_{1-6}$  alkylene-NH-, -NH- $C_{1-6}$  alkylene-C(O)-, -C(O)NH-, -NHC(O)-,  
 43  $C_{1-6}$  alkylene-NHC(O)-, -SO<sub>2</sub>NH-, -NHSO<sub>2</sub>-, -NHC(O)NH-, cycloalkylene,  
 44 -N=N-, and -C(R<sup>3a</sup>)=N(R<sup>3c</sup>)-, wherein the alkylene group is optionally  
 45 substituted with from 1-4  $R^{3b}$  groups;  $R^{3a}$  is selected from the group consisting  
 46 of H, and  $C_{1-6}$  alkyl;
- 47 each  $R^{3b}$  is independently selected from the group consisting of H,  $C_{1-6}$  alkyl, halogen,  
 48 -OR<sup>3a</sup> and -NR<sup>3a</sup>R<sup>3a</sup>;
- 49  $R^{3c}$  is absent or -OH;
- 50 alternatively,  $L^2$  is combined with  $R^1$ ,  $L^1$  is combined with  $L^2$ ,  $L^1$  is combined with  
 51  $R^2$ , two  $R^1$  groups on adjacent ring atoms, or two  $R^2$  groups on adjacent ring  
 52 atoms are combined to form a 5-6 membered heterocycloalkyl with from 1 to  
 53 3 heteroatoms selected from N, O and S, or a 5-6 membered heteroaryl with  
 54 from 1 to 3 heteroatoms selected from N, O and S, and optionally substituted  
 55 with from 1 to 3 groups selected from the group consisting of H,  $C_{1-6}$  alkyl and  
 56 oxo;
- 57 subscripts m and n are each an integer from 1 to 3;
- 58 wherein:
- 59 (a)  $L^1$  is a bond,  $L^2$  is -C(O)NH-, ring B is phenyl, and at least one  $R^2$  is  
 60 -CN or phenyl, or
- 61 (b) at least one  $R^1$  is -C(O)OH, ring A is phenyl,  $L^2$  is -C(O)NH-, and  $L^1$  is  
 62 a bond or  $C_{1-6}$  alkylene, or
- 63 (c) each of ring A and ring B is phenyl, at least one  $R^1$  is -C(O)OH or  
 64 combined with  $L^2$ , and at least one  $R^2$  is selected from the group  
 65 consisting of H, -CN and -C(O)OH;

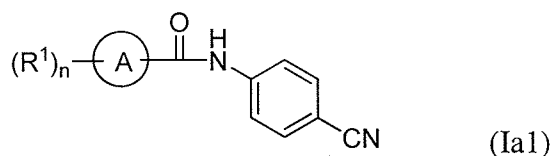
66 wherein when R<sup>1</sup> is -CO<sub>2</sub>H, subscript n is 1, ring A is phenyl, L<sup>2</sup> is -C(O)NH-, L<sup>1</sup> is a  
 67 bond, ring B is phenyl, subscript m is 1, and R<sup>2</sup> is phenyl, then the phenyl of  
 68 R<sup>2</sup> is substituted with C<sub>1-6</sub> alkyl,  
 69 or salts and isomers thereof, thereby ameliorating arthritis or joint injury in the  
 70 mammal.

1 2. The method of claim 1, wherein  
 2 (a) L<sup>1</sup> is a bond, L<sup>2</sup> is -C(O)NH-, ring B is phenyl, R<sup>2</sup> is -CN or phenyl, and  
 3 subscript m is 1, or  
 4 (b) R<sup>1</sup> is -C(O)OH, subscript n is 1, ring A is phenyl, L<sup>2</sup> is -C(O)NH-, and L<sup>1</sup> is a  
 5 bond or -CH<sub>2</sub>-, or  
 6 (c) each of ring A and ring B is phenyl, R<sup>1</sup> is -C(O)OH or combined with L<sup>2</sup>,  
 7 subscript n is 1, and at least one R<sup>2</sup> is selected from the group consisting of H,  
 8 -CN and -CO<sub>2</sub>H.

1 3. The method of any of claims 1 or 2, wherein the compound has the  
 2 structure:



1 4. The method of any of claims 1-3, wherein the compound has the  
 2 structure:



4 wherein

5 each R<sup>1</sup> is independently selected from the group consisting of C<sub>2-6</sub> alkenyl,  
6 C<sub>2-6</sub> alkynyl, C<sub>1-6</sub> alkoxy, C<sub>1-6</sub> heteroalkyl, halogen, C<sub>1-6</sub> haloalkyl,  
7 C<sub>1-6</sub> haloalkoxy, C<sub>1-6</sub> alkyl-CN, C<sub>1-6</sub> alkylhydroxy, -OR<sup>2a</sup>, -NR<sup>2b</sup>R<sup>2d</sup>,  
8 C<sub>1-6</sub> alkyl-NR<sup>2b</sup>R<sup>2d</sup>, -C(O)R<sup>1a</sup>, -C(O)R<sup>2d</sup>, -C(O)OR<sup>2a</sup>, C<sub>1-6</sub> alkyl-C(O)OR<sup>2b</sup>,  
9 -OC(O)R<sup>2b</sup>, -OC(O)OR<sup>2b</sup>, -C(O)NR<sup>2a</sup>R<sup>2b</sup>, -C(O)N(OH)R<sup>2b</sup>, -NR<sup>2b</sup>C(O)R<sup>2c</sup>,  
10 C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)R<sup>2c</sup>, -NR<sup>2b</sup>C(O)OR<sup>2c</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)OR<sup>2c</sup>,  
11 -OC(O)NR<sup>2b</sup>R<sup>2c</sup>, -NR<sup>2b</sup>C(O)NR<sup>2b</sup>R<sup>2c</sup>, -NR<sup>2b</sup>C(NR<sup>2b</sup>)NR<sup>2b</sup>R<sup>2c</sup>,  
12 -C(O)NR<sup>2b</sup>C(O)R<sup>2b</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)NR<sup>2b</sup>R<sup>2c</sup>, -SR<sup>2a</sup>, -SO<sub>2</sub>R<sup>2b</sup>, -SO<sub>2</sub>OR<sup>2b</sup>,  
13 -SO<sub>2</sub>NR<sup>2b</sup>R<sup>2d</sup>, -NR<sup>2b</sup>SO<sub>2</sub>R<sup>2b</sup>, -P(O)(OR<sup>2b</sup>)<sub>2</sub>, -B(OR<sup>2b</sup>)<sub>2</sub>, -CN, -NO<sub>2</sub>, -N<sub>3</sub>,  
14 heterocycloalkyl, aryl, heteroaryl, C<sub>1-6</sub> alkyl-heterocycloalkyl, C<sub>1-6</sub> alkyl-aryl,  
15 C<sub>1-6</sub> alkyl-O-aryl, C<sub>1-6</sub> alkyl-heteroaryl, and heteroaryl-aryl, and wherein the  
16 heterocycloalkyl, aryl and heteroaryl groups are optionally substituted with 1  
17 to 2 R<sup>2a</sup> groups;  
18 each of R<sup>2a</sup>, R<sup>2b</sup>, R<sup>2c</sup> and R<sup>2d</sup> are independently selected from the group consisting of  
19 H and C<sub>1-6</sub> alkyl; and  
20 ring A is selected from the group consisting of phenyl, biphenyl and pyridyl,  
21 wherein when ring A is phenyl and at least one R<sup>1</sup> is -C(O)OH, then subscript n is 2  
22 or 3.

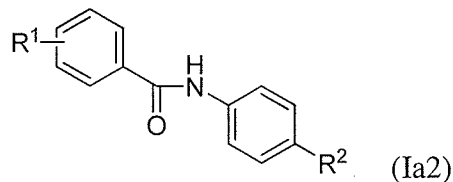
1 5. The method of claim 4, wherein each R<sup>1</sup> is independently selected  
2 from the group consisting of -C(O)R<sup>2d</sup>, -C(O)OR<sup>2b</sup>, C<sub>1-6</sub> alkyl-C(O)OR<sup>2b</sup>, -NR<sup>2b</sup>C(O)OR<sup>2c</sup>,  
3 -NR<sup>2b</sup>C(O)NR<sup>2b</sup>R<sup>2c</sup>, -SO<sub>2</sub>OR<sup>2b</sup>, -SO<sub>2</sub>NR<sup>2b</sup>R<sup>2d</sup>, -NR<sup>2b</sup>SO<sub>2</sub>R<sup>2b</sup>, and -CN.

1 6. The method of claim 4, wherein each R<sup>1</sup> is independently selected  
2 from the group consisting of -CH<sub>2</sub>C(O)OH, -C(O)Me, -NHC(O)NH<sub>2</sub>, -NHC(O)OMe,  
3 -NHSO<sub>2</sub>Me, -SO<sub>2</sub>NH<sub>2</sub>, -SO<sub>2</sub>NHMe, -SO<sub>3</sub>H, -C(O)OH, and -CN.

1 7. The method of any of claims 4-7, wherein  
2 ring A is phenyl; and  
3 subscript n is 1.

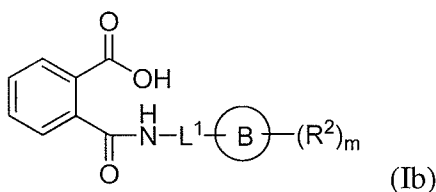
1 8. The method of any of claims 4-7, wherein  
2 ring A is selected from the group consisting of biphenyl and pyridyl, or  
3 subscript n is 2.

1                    9.        The method of any of claims 1-3, wherein the compound has the  
2 structure:



3  
4 wherein  
5  $R^1$  is selected from the group consisting of  $C_{1-6}$  alkyl and  $-C(O)OR^{2b}$ ; and  
6  $R^2$  is selected from the group consisting of  $-CN$  and Ph.

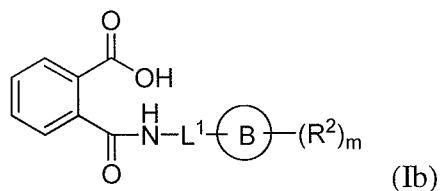
1                    10.        The method of any of claims 1-3, wherein the compound has the  
2 structure:



3  
4 wherein  
5 each  $R^2$  is independently selected from the group consisting of H,  $C_{1-6}$  haloalkoxy,  
6  $C_{1-6}$  alkyl- $NR^{2b}R^{2d}$ ,  $-C(O)OR^{2b}$ ,  $-C(O)N(OH)R^{2b}$ ,  $C_{1-6}$  alkyl- $NR^{2b}C(O)OR^{2c}$ ,  
7  $C_{1-6}$  alkyl- $NR^{2b}C(O)NR^{2b}R^{2c}$ ,  $-SO_2OR^{2b}$ ,  $-PO_3H$ ,  $-CN$ , aryl, heteroaryl, and  
8  $C_{1-6}$  alkyl-O-aryl;  
9 ring B is selected from the group consisting of cyclohexyl, phenyl, imidazole,  
10 oxazole, thiazole, pyrimidine, and pyrazine; and  
11  $L^1$  is selected from the group consisting of a bond and  $-CH_2-$ ;  
12 wherein  
13 when  $R^2$  is  $C_{1-6}$  alkyl- $NR^{2b}R^{2d}$ , then one of  $R^{2b}$  and  $R^{2d}$  is  $C_{1-6}$  alkyl,  
14 when  $R^2$  is  $-C(O)OH$ , then  $L^1$  is  $-CH_2-$  or ring B is cyclohexyl, or both,  
15 when  $R^2$  is  $-CN$ , then  $L^1$  is  $-CH_2-$  or ring B is cyclohexyl, or both, and  
16 when ring B is 2-thiazole,  $R^2$  is unsubstituted phenyl.

1                    11.        The method of claim 10, wherein each  $R^2$  is independently selected  
2 from the group consisting of H,  $-CH_2NHCONH_2$ ,  $-CH_2NHCOOMe$ ,  $-CH_2NHMe$ ,  $-CH_2OPh$ ,  
3 2-CN, 4-CN,  $-C(O)OH$ ,  $-CONHOH$ ,  $-OCF_2H$ ,  $-PO_3H$ ,  $-SO_3H$ , phenyl, pyridyl, imidazole and  
4 tetrazole.

1 12. The method of any of claims 1-3, wherein the compound has the  
2 structure:



3  
4 wherein

5 each  $R^2$  is independently selected from the group consisting of  $C_{1-6}$  alkyl, halogen,  
6  $C_{1-6}$  alkylhydroxy,  $C_{1-6}$  alkyl- $NR^{2b}R^{2d}$ ,  $-C(O)R^{2d}$ ,  $-C(O)OR^{2b}$ ,  $-C(O)NR^{2b}R^{2c}$ ,  
7  $-SO_2NR^{2b}R^{2d}$ ,  $-CN$ , - heterocycloalkyl, and aryl, wherein the aryl groups are  
8 optionally substituted with halogen;

9 alternatively, two  $R^2$  groups on adjacent ring atoms can be combined to form a 5-  
10 membered heterocycloalkyl;

11 ring B is selected from the group consisting of phenyl, thiazole and pyridyl; and

12  $L^1$  is selected from the group consisting of a bond and  $-CH_2-$ ;

13 wherein

14 when  $R^2$  is  $C_{1-6}$  alkyl- $NR^{2b}R^{2d}$ , then both of  $R^{2b}$  and  $R^{2d}$  are H,

15 when  $R^2$  is  $-C(O)OH$ , then  $L^1$  is a bond and ring B is phenyl,

16 when  $R^2$  is  $-CN$ , then  $L^1$  is a bond and ring B is phenyl, and

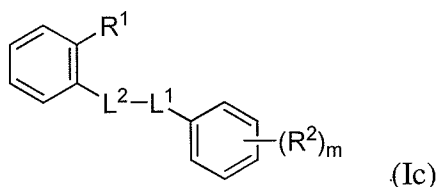
17 when ring B is 2-thiazole, then  $R^2$  is substituted phenyl.

1 13. The method of claim 12, wherein

2 each  $R^2$  is independently selected from the group consisting of H, Me,  $-Cl$ ,  $-CH_2OH$ ,  
3  $-CH_2CH_2OH$ ,  $-CH_2NH_2$ ,  $-C(O)Me$ ,  $-C(O)OH$ ,  $-C(O)NH_2$ ,  $-CN$ , morpholine,  
4 3,4-difluorophenyl and  $-SO_2NH_2$ ; and

5 alternatively, two  $R^2$  groups on adjacent ring atoms are combined to form a 1,3-  
6 dioxole or 1-methylpyrrolidine-2,5-dione.

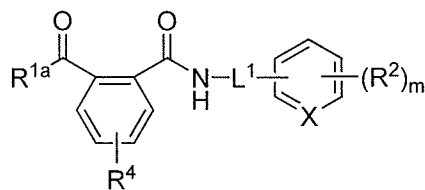
1 14. The method of any of claims 1-3, wherein the compound has the  
2 structure:



3  
4 wherein

5 R<sup>1</sup> is -C(O)OH;  
 6 each R<sup>2</sup> is independently selected from the group consisting of -CN and -C(O)OH;  
 7 each of L<sup>1</sup> and L<sup>2</sup> are independently selected from the group consisting of a bond,  
 8 C<sub>1-6</sub> alkylene, C<sub>2-6</sub> alkenylene, -C(O)-, C<sub>1-6</sub> alkylene-C(O)-,  
 9 -C(O)-C<sub>1-6</sub> alkylene-NH-, -NH-C<sub>1-6</sub> alkylene-C(O)-, -NHC(O)-, -SO<sub>2</sub>NH-,  
 10 -NHSO<sub>2</sub>-, and -NHC(O)NH-;  
 11 wherein at least one of L<sup>1</sup> and L<sup>2</sup> is selected from the group consisting of -C(O)-,  
 12 C<sub>1-6</sub> alkylene-C(O)-, -C(O)-C<sub>1-6</sub> alkylene-NH-, -NH-C<sub>1-6</sub> alkylene-C(O)-,  
 13 -NHC(O)-, -SO<sub>2</sub>NH-, -NHSO<sub>2</sub>-, and -NHC(O)NH-;  
 14 alternatively, L<sup>2</sup> is combined with R<sup>1</sup>, or L<sup>1</sup> is combined with R<sup>2</sup>, to form a 5-6  
 15 membered heterocycloalkyl with from 1 to 3 heteroatoms selected from N, O  
 16 and S, or a 5-6 membered heteroaryl with from 1 to 3 heteroatoms selected  
 17 from N, O and S.

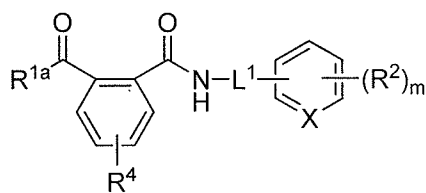
1 15. The method of claim 1, wherein the compound has the structure:



2 R<sup>1a</sup> is selected from the group consisting of -OR<sup>1b</sup> and -NR<sup>1b</sup>R<sup>1c</sup>;  
 3 R<sup>1b</sup> and R<sup>1c</sup> are each independently selected from the group consisting of H,  
 4 C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, cycloalkyl, heterocycloalkyl, aryl,  
 5 heteroaryl, C<sub>1-6</sub> alkyl-aryl, and C<sub>1-6</sub> alkyl-heteroaryl, wherein the cycloalkyl,  
 6 heterocycloalkyl, aryl and heteroaryl groups are optionally substituted with  
 7 from 1 to 4 R<sup>1d</sup> groups;  
 8 each R<sup>1d</sup> is independently selected from the group consisting of H, C<sub>1-6</sub> alkyl,  
 9 C<sub>1-6</sub> alkoxy, and -NO<sub>2</sub>;  
 10 each R<sup>2</sup> is independently selected from the group consisting of C<sub>1-6</sub> alkyl, C<sub>1-6</sub> alkoxy,  
 11 halogen, C<sub>1-6</sub> haloalkyl, C<sub>1-6</sub> haloalkoxy, C<sub>1-6</sub> alkylamine, C<sub>1-6</sub> alkyl-CN,  
 12 C<sub>1-6</sub> alkyl-OH, heterocycloalkyl, aryl, heteroaryl, C<sub>1-6</sub> alkyl-aryl,  
 13 C<sub>1-6</sub> alkyl-heteroaryl, heteroaryl-aryl, -OR<sup>2a</sup>, -NR<sup>2b</sup>R<sup>2d</sup>, -C(O)R<sup>2d</sup>, -C(O)OR<sup>2b</sup>,  
 14 -OC(O)R<sup>2b</sup>, -C(O)NR<sup>2b</sup>R<sup>2c</sup>, -NR<sup>2b</sup>C(O)R<sup>2c</sup>, -NR<sup>2b</sup>C(O)OR<sup>2c</sup>, -OC(O)NR<sup>2b</sup>R<sup>2c</sup>,  
 15 -SO<sub>2</sub>R<sup>2a</sup>, -SO<sub>2</sub>NR<sup>2b</sup>R<sup>2d</sup>, -CN, -NO<sub>2</sub>, and -N<sub>3</sub>, wherein the heterocycloalkyl,  
 16 aryl and heteroaryl groups are optionally substituted with 1 to 2 R<sup>2b</sup> groups;  
 17

18 alternatively, two  $R^2$  groups on adjacent ring atoms are combined to form a 5 to 6  
 19 membered heterocyclic ring having from 1 to 3 heteroatoms each  
 20 independently selected from the group consisting of N, O and S, and  
 21 optionally substituted with from 1 to 3 groups selected from the group  
 22 consisting of H,  $C_{1-6}$  alkyl and oxo;  
 23 each  $R^{2a}$  is independently selected from the group consisting of H,  $C_{2-6}$  alkenyl,  
 24  $C_{2-6}$  alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl,  
 25  $C_{1-6}$  alkyl-cycloalkyl,  $C_{1-6}$  alkyl-heterocycloalkyl,  $C_{1-6}$  alkyl-aryl and  
 26  $C_{1-6}$  alkyl-heteroaryl, optionally substituted with 1 to 2  $R^{2b}$  groups;  
 27 each  $R^{2b}$  and  $R^{2c}$  is independently selected from the group consisting of H, and  
 28  $C_{1-6}$  alkyl;  
 29 each  $R^{2d}$  is independently selected from the group consisting of H,  $C_{1-6}$  alkyl,  
 30 cycloalkyl, heterocycloalkyl, aryl, heteroaryl,  $C_{1-6}$  alkyl-cycloalkyl,  
 31  $C_{1-6}$  alkyl-heterocycloalkyl,  $C_{1-6}$  alkyl-aryl and  $C_{1-6}$  alkyl-heteroaryl, each  
 32 optionally substituted with 1 to 2  $R^{2b}$  groups;  
 33  $R^4$  is independently selected from the group consisting of  $C_{1-6}$  alkyl, halogen,  
 34  $C_{1-6}$  haloalkyl,  $C_{1-6}$  alkoxy,  $C_{1-6}$  haloalkoxy, OH,  $-CO_2H$ , and  $-NO_2$ ;  
 35  $L^1$  is selected from the group consisting of a bond,  $C_{1-6}$  alkylene,  $-C(O)-$ ,  
 36  $-C_{1-6}$  alkylene-NH-,  $-C_{1-6}$  alkylene-NHC(O)-, and heteroarylene; and  
 37 X is selected from the group consisting of  $-CH-$  and  $-N-$ .

1 16. The method of claim 15, wherein the compound has the structure:

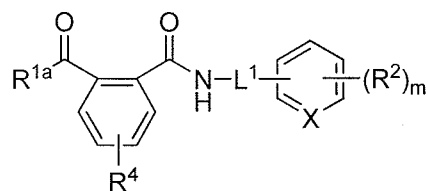


(Id)

2 wherein

3 each  $R^2$  is independently selected from the group consisting of  $C_{1-6}$  alkyl,  $C_{1-6}$  alkoxy,  
 4 halogen,  $C_{1-6}$  haloalkyl,  $C_{1-6}$  alkyl-CN,  $C_{1-6}$  alkyl-OH, heterocycloalkyl, aryl,  
 5 heteroaryl,  $C_{1-6}$  alkyl-aryl,  $C_{1-6}$  alkyl-heteroaryl, heteroaryl-aryl,  $-OR^{2a}$ ,  
 6  $-NR^{2b}R^{2d}$ ,  $-C(O)R^{2d}$ ,  $-OC(O)R^{2b}$ ,  $-C(O)NR^{2b}R^{2c}$ ,  $-NR^{2b}C(O)R^{2c}$ ,  
 7  $-NR^{2b}C(O)OR^{2c}$ ,  $-OC(O)NR^{2b}R^{2c}$ ,  $-SO_2R^{2a}$ ,  $-SO_2NR^{2b}R^{2d}$ ,  $-CN$ ,  $-NO_2$ , and  
 8  $-N_3$ , wherein the heterocycloalkyl, and aryl groups are optionally substituted  
 9 with 1 to 2  $R^{2b}$  groups;  
 10 wherein when  $R^2$  is  $-CN$ , then subscript m is 1,  $R^{1a}$  is OH,  $R^4$  is H, and  $L^1$  is a bond.  
 11

1 17. The method of claim 15, wherein the compound has the structure:



(Id)

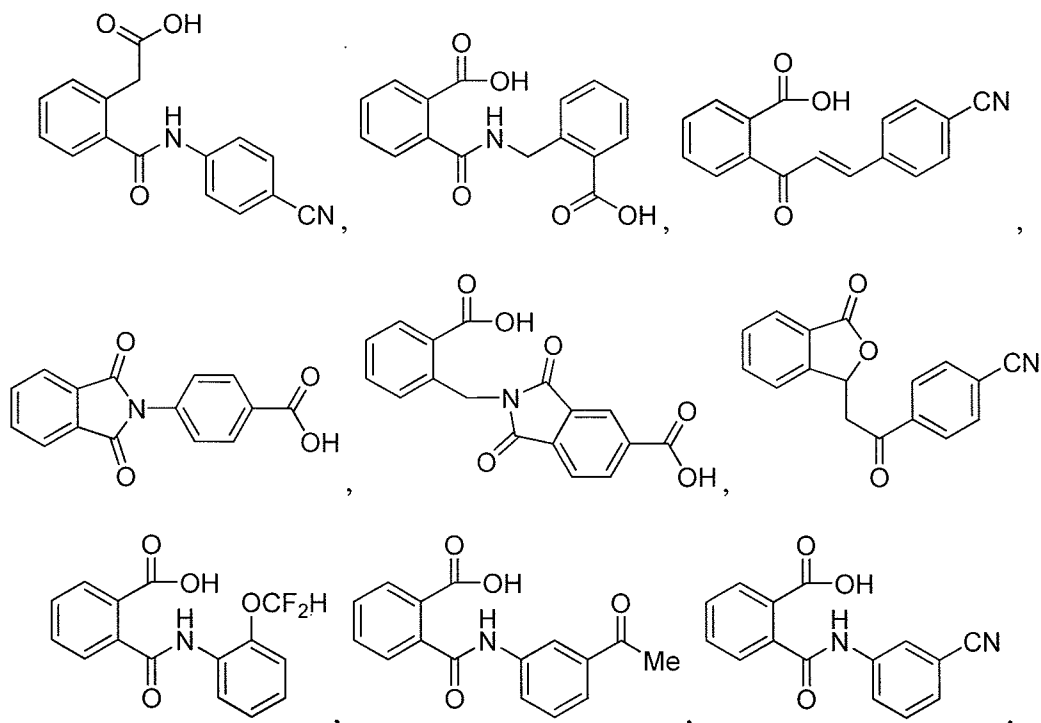
2 wherein

3 each  $R^2$  is independently selected from the group consisting of  $C_{1-6}$  alkyl, halogen,  
 4  $C_{1-6}$  haloalkyl,  $C_{1-6}$  alkyl-CN,  $C_{1-6}$  alkyl-OH, heterocycloalkyl, aryl,  
 5 heteroaryl,  $C_{1-6}$  alkyl-aryl,  $C_{1-6}$  alkyl-heteroaryl, heteroaryl-aryl,  $-OR^{2a}$ ,  
 6  $-NR^{2b}R^{2d}$ ,  $-C(O)R^{2d}$ ,  $-OC(O)R^{2b}$ ,  $-C(O)NR^{2b}R^{2c}$ ,  $-NR^{2b}C(O)R^{2c}$ ,  
 7  $-NR^{2b}C(O)OR^{2c}$ ,  $-OC(O)NR^{2b}R^{2c}$ ,  $-SO_2R^{2a}$ ,  $-SO_2NR^{2b}R^{2d}$ ,  $-CN$ ,  $-NO_2$ , and  
 8  $-N_3$ , wherein the heterocycloalkyl, and aryl groups are optionally substituted  
 9 with 1 to 2  $R^{2b}$  groups; and  
 10

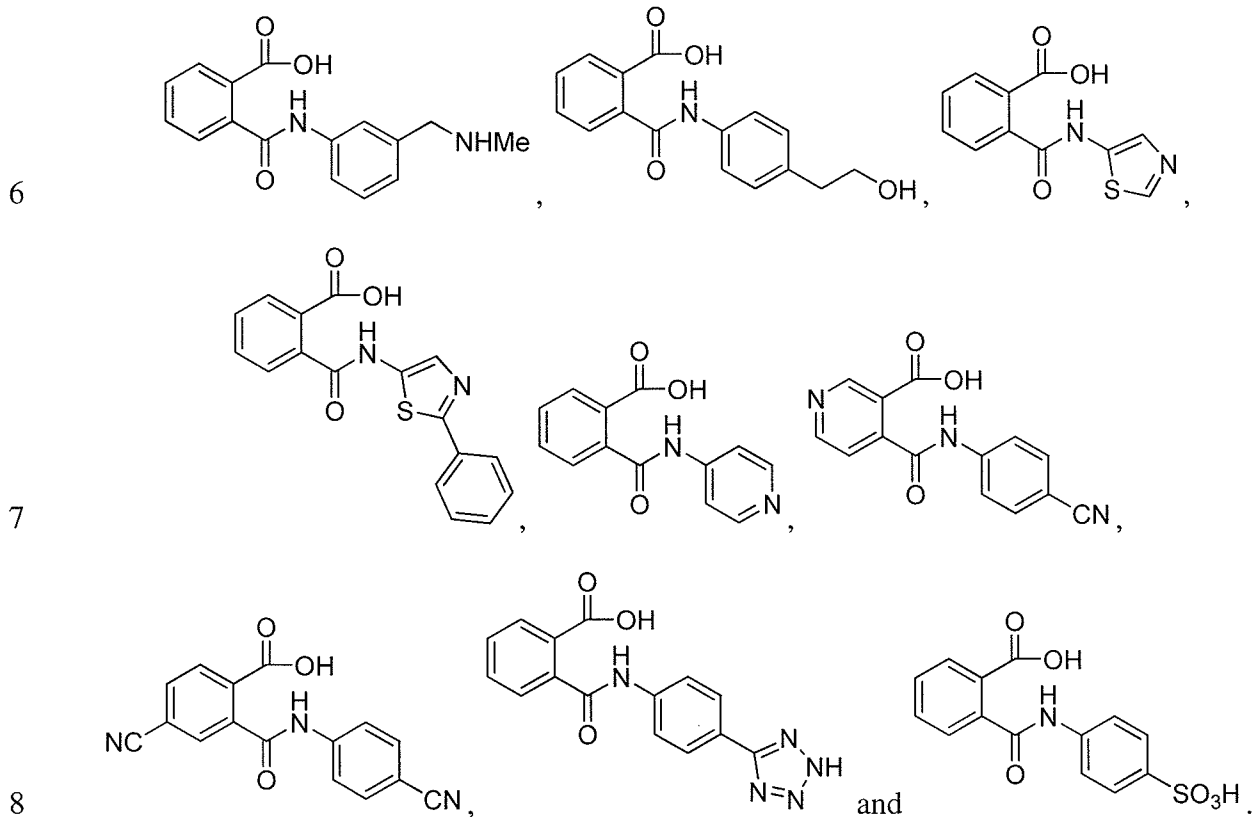
11  $L^1$  is a bond,

12 wherein when  $R^2$  is  $-CN$ , then subscript  $m$  is 1,  $R^{1a}$  is  $OH$ ,  $R^4$  is  $H$ , and  $L^1$  is a bond.

1 18. The method of claim 1, wherein the compound is selected from the  
 2 group consisting of:







1 19. The method of any one of claims 1-18, wherein the mammal has  
2 arthritis or joint injury.

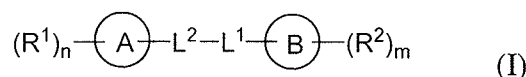
1 20. The method of any one of claims 1-18, wherein the mammal does not  
2 have, but is at increased risk for, arthritis or joint injury.

1 21. The method of any one of claims 1-18, wherein the arthritis is selected  
2 from the group consisting of osteoarthritis, trauma arthritis, and autoimmune arthritis.

1 22. The method of any one of claims 1-18, wherein the mammal is  
2 selected from the group consisting of a human, a dog, a horse and a cat.

1 23. The method of any one of claims 1-18, wherein the composition  
2 further comprises a member selected from the group consisting of angiotensin-like 3 protein  
3 (ANGPTL3), oral salmon calcitonin, SD-6010 (iNOS inhibitor), vitamin D3  
4 (cholecalciferol), collagen hydrolyzate, FGF18, BMP7, avocado soy unsaponifiables (ASU)  
5 and hyaluronic acid.

1                   24.     A method of inducing differentiation of mesenchymal stem cells into  
2 chondrocytes, the method comprising contacting mesenchymal stem cells with a sufficient  
3 amount of a compound having the structure:



4                   wherein

5                   each of ring A and ring B are independently selected from the group consisting of  
6 each of ring A and ring B are independently selected from the group consisting of  
7 cycloalkyl, aryl and heteroaryl;

8                   each R<sup>1</sup> and R<sup>2</sup> is independently selected from the group consisting of H, C<sub>1-6</sub> alkyl,

9 C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, C<sub>1-6</sub> alkoxy, C<sub>1-6</sub> heteroalkyl, halogen,

10 C<sub>1-6</sub> haloalkyl, C<sub>1-6</sub> haloalkoxy, C<sub>1-6</sub> alkyl-CN, C<sub>1-6</sub> alkylhydroxy, -OR<sup>2a</sup>,

11 -NR<sup>2b</sup>R<sup>2d</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>R<sup>2d</sup>, -C(O)R<sup>1a</sup>, -C(O)R<sup>2d</sup>, -C(O)OR<sup>2a</sup>,

12 C<sub>1-6</sub> alkyl-C(O)OR<sup>2b</sup>, -OC(O)R<sup>2b</sup>, -OC(O)OR<sup>2b</sup>, -C(O)NR<sup>2a</sup>R<sup>2b</sup>,

13 -C(O)N(OH)R<sup>2b</sup>, -NR<sup>2b</sup>C(O)R<sup>2c</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)R<sup>2c</sup>, -NR<sup>2b</sup>C(O)OR<sup>2c</sup>,

14 C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)OR<sup>2c</sup>, -OC(O)NR<sup>2b</sup>R<sup>2c</sup>, -NR<sup>2b</sup>C(O)NR<sup>2b</sup>R<sup>2c</sup>,

15 -NR<sup>2b</sup>C(NR<sup>2b</sup>)NR<sup>2b</sup>R<sup>2c</sup>, -C(O)NR<sup>2b</sup>C(O)R<sup>2b</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)NR<sup>2b</sup>R<sup>2c</sup>,

16 -SR<sup>2a</sup>, -SO<sub>2</sub>R<sup>2b</sup>, -SO<sub>2</sub>OR<sup>2b</sup>, -SO<sub>2</sub>NR<sup>2b</sup>R<sup>2d</sup>, -NR<sup>2b</sup>SO<sub>2</sub>R<sup>2b</sup>, -P(O)(OR<sup>2b</sup>)<sub>2</sub>,

17 -B(OR<sup>2b</sup>), -CN, -NO<sub>2</sub>, -N<sub>3</sub>, heterocycloalkyl, aryl, heteroaryl,

18 C<sub>1-6</sub> alkyl-heterocycloalkyl, C<sub>1-6</sub> alkyl-aryl, C<sub>1-6</sub> alkyl-O-aryl,

19 C<sub>1-6</sub> alkyl-heteroaryl, and heteroaryl-aryl, and wherein the heterocycloalkyl,  
20 aryl and heteroaryl groups are optionally substituted with 1 to 2 R<sup>2a</sup> groups;

21 R<sup>1a</sup> is selected from the group consisting of -OR<sup>1b</sup> and -NR<sup>1b</sup>R<sup>1c</sup>;

22 R<sup>1b</sup> and R<sup>1c</sup> are each independently selected from the group consisting of H,

23 C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, cycloalkyl, heterocycloalkyl, aryl,

24 heteroaryl, C<sub>1-6</sub> alkyl-aryl, and C<sub>1-6</sub> alkyl-heteroaryl, wherein the cycloalkyl,

25 heterocycloalkyl, aryl and heteroaryl groups are optionally substituted with

26 from 1 to 4 R<sup>1d</sup> groups;

27 each R<sup>1d</sup> is independently selected from the group consisting of H, C<sub>1-6</sub> alkyl,

28 C<sub>1-6</sub> alkoxy, and -NO<sub>2</sub>;

29 each R<sup>2a</sup> is independently selected from the group consisting of H, C<sub>2-6</sub> alkenyl,

30 C<sub>2-6</sub> alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl,

31 C<sub>1-6</sub> alkyl-cycloalkyl, C<sub>1-6</sub> alkyl-heterocycloalkyl, C<sub>1-6</sub> alkyl-aryl and

32 C<sub>1-6</sub> alkyl-heteroaryl, optionally substituted with 1 to 2 R<sup>2b</sup> groups;

- 33 each  $R^{2b}$  and  $R^{2c}$  is independently selected from the group consisting of H, and  
 34  $C_{1-6}$  alkyl;
- 35 each  $R^{2d}$  is independently selected from the group consisting of H,  $C_{1-6}$  alkyl,  
 36 cycloalkyl, heterocycloalkyl, aryl, heteroaryl,  $C_{1-6}$  alkyl-cycloalkyl,  
 37  $C_{1-6}$  alkyl-heterocycloalkyl,  $C_{1-6}$  alkyl-aryl and  $C_{1-6}$  alkyl-heteroaryl, each  
 38 optionally substituted with 1 to 2  $R^{2b}$  groups;
- 39 each of  $L^1$  and  $L^2$  are independently selected from the group consisting of a bond,  
 40  $C_{1-6}$  alkylene,  $C_{2-6}$  alkenylene,  $C_{1-6}$  alkylene-O-, -O- $C_{1-6}$  alkylene,  
 41  $C_{1-6}$  alkylene-NR<sup>3a</sup>-, -NR<sup>3a</sup>- $C_{1-6}$  alkylene, -C(O)-,  $C_{1-6}$  alkylene-C(O)-,  
 42 -C(O)- $C_{1-6}$  alkylene-NH-, -NH- $C_{1-6}$  alkylene-C(O)-, -C(O)NH-, -NHC(O)-,  
 43  $C_{1-6}$  alkylene-NHC(O)-, -SO<sub>2</sub>NH-, -NH-SO<sub>2</sub>-, -NHC(O)NH-, cycloalkylene,  
 44 -N=N-, and -C(R<sup>3a</sup>)=N(R<sup>3c</sup>)-, wherein the alkylene group is optionally  
 45 substituted with from 1-4  $R^{3b}$  groups;  $R^{3a}$  is selected from the group consisting  
 46 of H, and  $C_{1-6}$  alkyl;
- 47 each  $R^{3b}$  is independently selected from the group consisting of H,  $C_{1-6}$  alkyl, halogen,  
 48 -OR<sup>3a</sup> and -NR<sup>3a</sup>R<sup>3a</sup>;
- 49  $R^{3c}$  is absent or -OH;
- 50 alternatively,  $L^2$  is combined with  $R^1$ ,  $L^1$  is combined with  $L^2$ ,  $L^1$  is combined with  
 51  $R^2$ , two  $R^1$  groups on adjacent ring atoms, or two  $R^2$  groups on adjacent ring  
 52 atoms are combined to form a 5-6 membered heterocycloalkyl with from 1 to  
 53 3 heteroatoms selected from N, O and S, or a 5-6 membered heteroaryl with  
 54 from 1 to 3 heteroatoms selected from N, O and S, and optionally substituted  
 55 with from 1 to 3 groups selected from the group consisting of H,  $C_{1-6}$  alkyl and  
 56 oxo;
- 57 subscripts m and n are each an integer from 1 to 3;
- 58 wherein:
- 59 (a)  $L^1$  is a bond,  $L^2$  is -C(O)NH-, ring B is phenyl, and at least one  $R^2$  is  
 60 -CN or phenyl, or
- 61 (b) at least one  $R^1$  is -C(O)OH, ring A is phenyl,  $L^2$  is -C(O)NH-, and  $L^1$  is  
 62 a bond or  $C_{1-6}$  alkylene, or
- 63 (c) each of ring A and ring B is phenyl, at least one  $R^1$  is -C(O)OH or  
 64 combined with  $L^2$ , and at least one  $R^2$  is selected from the group  
 65 consisting of H, -CN and -C(O)OH;

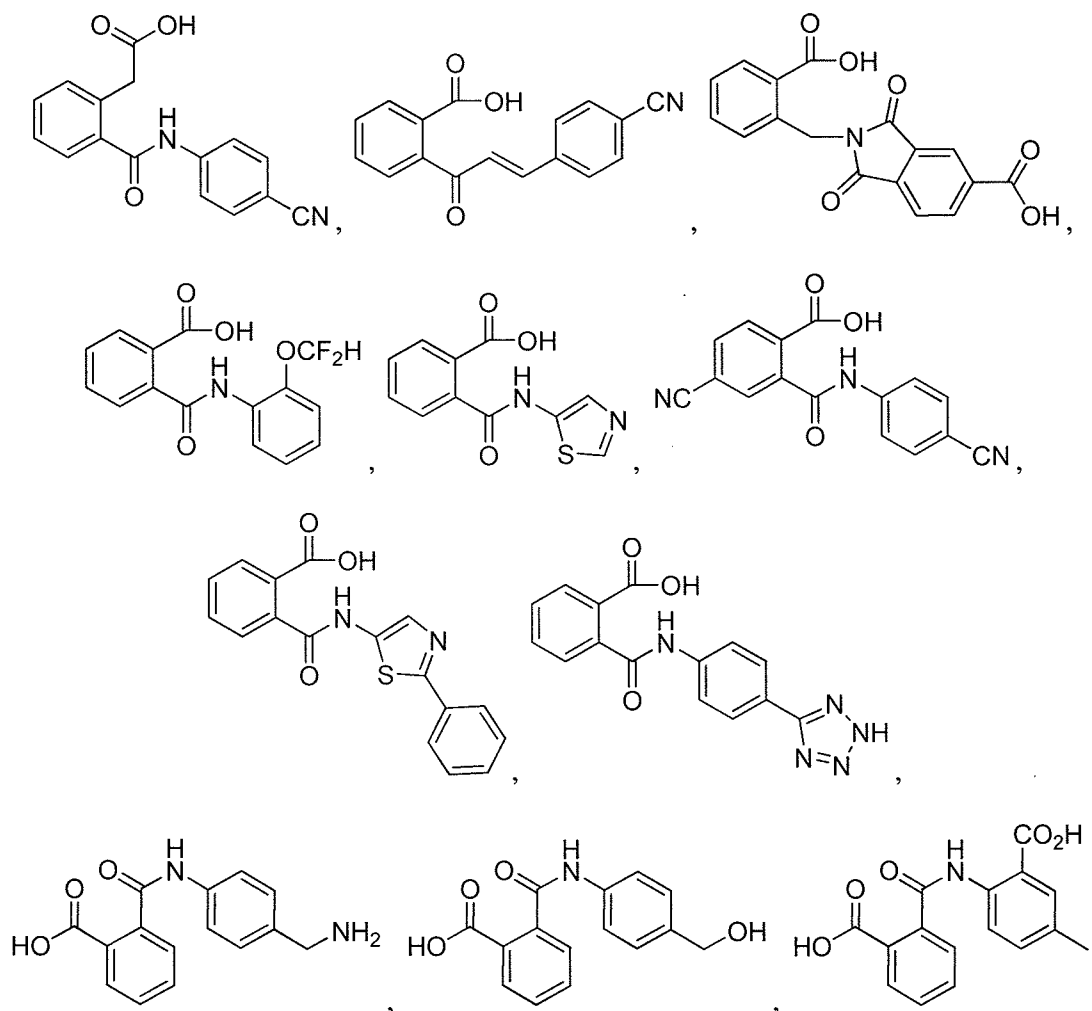
66 wherein when R<sup>1</sup> is -CO<sub>2</sub>H, subscript n is 1, ring A is phenyl, L<sup>2</sup> is -C(O)NH-, L<sup>1</sup> is a  
 67 bond, ring B is phenyl, subscript m is 1, and R<sup>2</sup> is phenyl, then the phenyl of  
 68 R<sup>2</sup> is substituted with C<sub>1-6</sub> alkyl,  
 69 or salts and isomers thereof, thereby inducing differentiation of the stem cells into  
 70 chondrocytes.

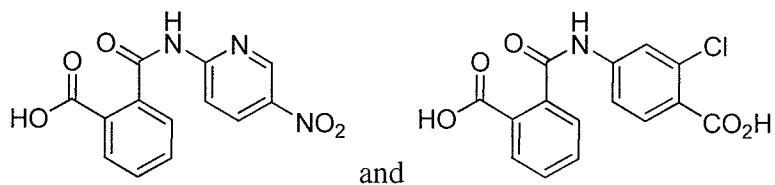
1 25. The method of claim 24, wherein the method is performed *in vitro*.

1 26. The method of claim 24, wherein the method is performed *in vivo* in a  
 2 mammal and the stem cells are present in the mammal.

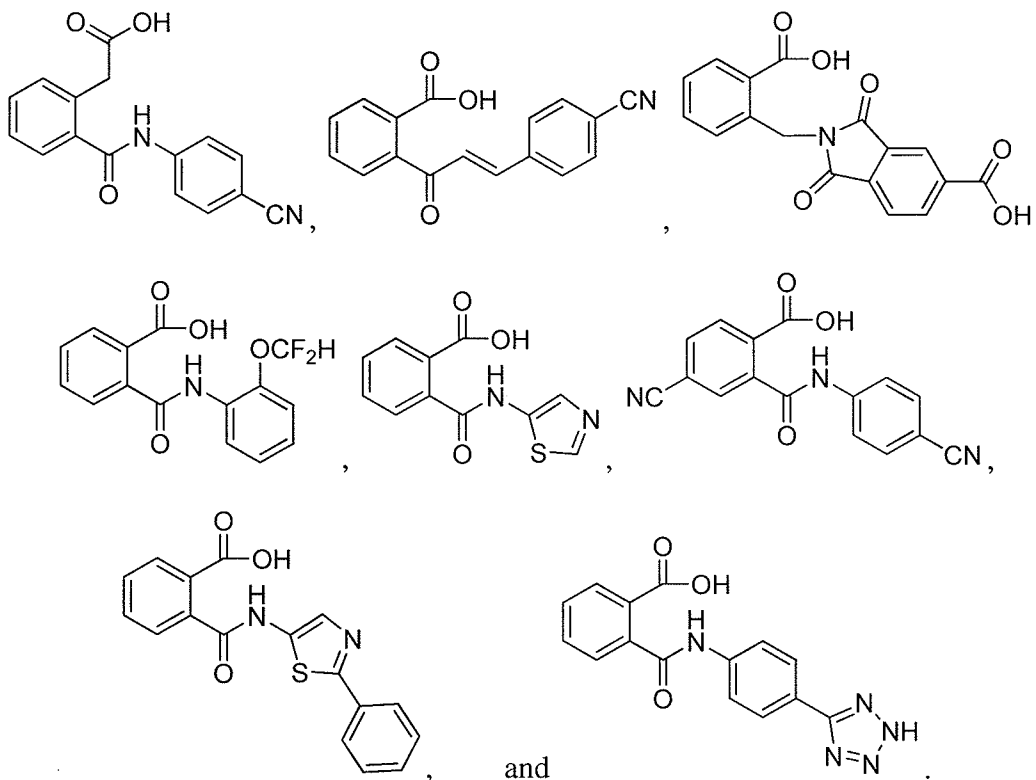
1 27. The method of claim 24, wherein the mammal is a human, a dog, a  
 2 horse or a cat.

1 28. A compound selected from the group consisting of:

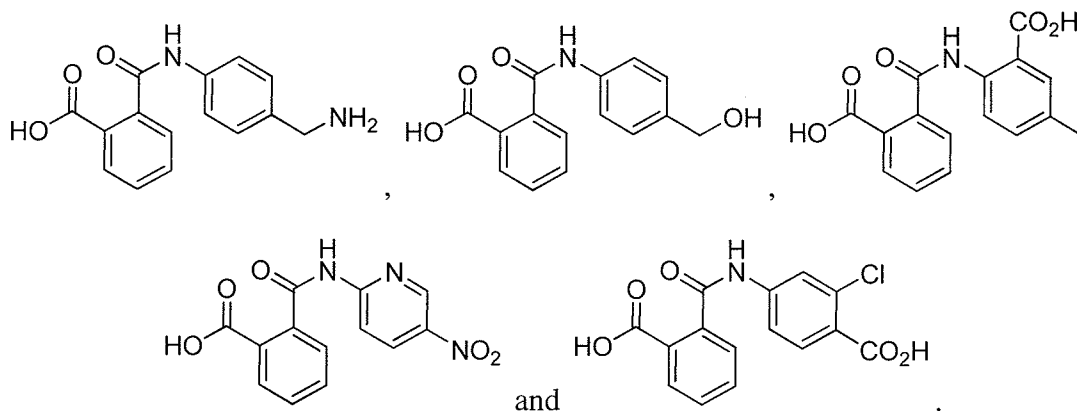




1 29. The compound of claim 28, selected from the group consisting of:

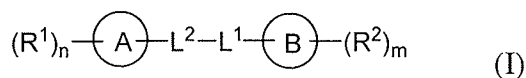


1 30. The compound of claim 28, selected from the group consisting of:



1 31. A pharmaceutical composition comprising a compound of any of  
2 claims 28-30 and a pharmaceutically acceptable excipient.

1 32. A pharmaceutical composition comprising a pharmaceutically  
2 acceptable excipient and a pharmaceutically effective amount of a compound of formula I:



wherein

each of ring A and ring B are independently selected from the group consisting of cycloalkyl, aryl and heteroaryl;

each R<sup>1</sup> and R<sup>2</sup> is independently selected from the group consisting of H, C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, C<sub>1-6</sub> alkoxy, C<sub>1-6</sub> heteroalkyl, halogen, C<sub>1-6</sub> haloalkyl, C<sub>1-6</sub> haloalkoxy, C<sub>1-6</sub> alkyl-CN, C<sub>1-6</sub> alkylhydroxy, -OR<sup>2a</sup>, -NR<sup>2b</sup>R<sup>2d</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>R<sup>2d</sup>, -C(O)R<sup>1a</sup>, -C(O)R<sup>2d</sup>, -C(O)OR<sup>2a</sup>, C<sub>1-6</sub> alkyl-C(O)OR<sup>2b</sup>, -OC(O)R<sup>2b</sup>, -OC(O)OR<sup>2b</sup>, -C(O)NR<sup>2a</sup>R<sup>2b</sup>, -C(O)N(OH)R<sup>2b</sup>, -NR<sup>2b</sup>C(O)R<sup>2c</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)R<sup>2c</sup>, -NR<sup>2b</sup>C(O)OR<sup>2c</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)OR<sup>2c</sup>, -OC(O)NR<sup>2b</sup>R<sup>2c</sup>, -NR<sup>2b</sup>C(O)NR<sup>2b</sup>R<sup>2c</sup>, -NR<sup>2b</sup>C(NR<sup>2b</sup>)NR<sup>2b</sup>R<sup>2c</sup>, -C(O)NR<sup>2b</sup>C(O)R<sup>2b</sup>, C<sub>1-6</sub> alkyl-NR<sup>2b</sup>C(O)NR<sup>2b</sup>R<sup>2c</sup>, -SR<sup>2a</sup>, -SO<sub>2</sub>R<sup>2b</sup>, -SO<sub>2</sub>OR<sup>2b</sup>, -SO<sub>2</sub>NR<sup>2b</sup>R<sup>2d</sup>, -NR<sup>2b</sup>SO<sub>2</sub>R<sup>2b</sup>, -P(O)(OR<sup>2b</sup>)<sub>2</sub>, -B(OR<sup>2b</sup>), -CN, -NO<sub>2</sub>, -N<sub>3</sub>, heterocycloalkyl, aryl, heteroaryl, C<sub>1-6</sub> alkyl-heterocycloalkyl, C<sub>1-6</sub> alkyl-aryl, C<sub>1-6</sub> alkyl-O-aryl, C<sub>1-6</sub> alkyl-heteroaryl, and heteroaryl-aryl, and wherein the heterocycloalkyl, aryl and heteroaryl groups are optionally substituted with 1 to 2 R<sup>2a</sup> groups;

R<sup>1a</sup> is selected from the group consisting of -OR<sup>1b</sup> and -NR<sup>1b</sup>R<sup>1c</sup>;

R<sup>1b</sup> and R<sup>1c</sup> are each independently selected from the group consisting of H, C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, C<sub>1-6</sub> alkyl-aryl, and C<sub>1-6</sub> alkyl-heteroaryl, wherein the cycloalkyl, heterocycloalkyl, aryl and heteroaryl groups are optionally substituted with from 1 to 4 R<sup>1d</sup> groups;

each R<sup>1d</sup> is independently selected from the group consisting of H, C<sub>1-6</sub> alkyl, C<sub>1-6</sub> alkoxy, and -NO<sub>2</sub>;

each R<sup>2a</sup> is independently selected from the group consisting of H, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, C<sub>1-6</sub> alkyl-cycloalkyl, C<sub>1-6</sub> alkyl-heterocycloalkyl, C<sub>1-6</sub> alkyl-aryl and C<sub>1-6</sub> alkyl-heteroaryl, optionally substituted with 1 to 2 R<sup>2b</sup> groups;

each R<sup>2b</sup> and R<sup>2c</sup> is independently selected from the group consisting of H, and C<sub>1-6</sub> alkyl;

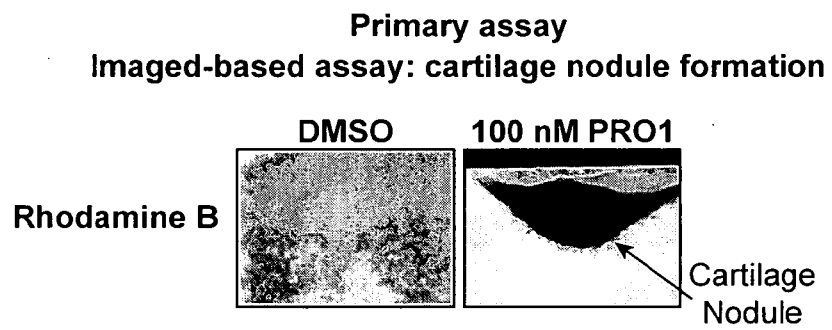
each R<sup>2d</sup> is independently selected from the group consisting of H, C<sub>1-6</sub> alkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, C<sub>1-6</sub> alkyl-cycloalkyl,

36 C<sub>1-6</sub> alkyl-heterocycloalkyl, C<sub>1-6</sub> alkyl-aryl and C<sub>1-6</sub> alkyl-heteroaryl, each  
37 optionally substituted with 1 to 2 R<sup>2b</sup> groups;  
38 each of L<sup>1</sup> and L<sup>2</sup> are independently selected from the group consisting of a bond,  
39 C<sub>1-6</sub> alkylene, C<sub>2-6</sub> alkenylene, C<sub>1-6</sub> alkylene-O-, -O-C<sub>1-6</sub> alkylene,  
40 C<sub>1-6</sub> alkylene-NR<sup>3a</sup>-, -NR<sup>3a</sup>-C<sub>1-6</sub> alkylene, -C(O)-, C<sub>1-6</sub> alkylene-C(O)-,  
41 -C(O)-C<sub>1-6</sub> alkylene-NH-, -NH-C<sub>1-6</sub> alkylene-C(O)-, -C(O)NH-, -NHC(O)-,  
42 C<sub>1-6</sub> alkylene-NHC(O)-, -SO<sub>2</sub>NH-, -NHSO<sub>2</sub>-, -NHC(O)NH-, cycloalkylene,  
43 -N=N-, and -C(R<sup>3a</sup>)=N(R<sup>3c</sup>)-, wherein the alkylene group is optionally  
44 substituted with from 1-4 R<sup>3b</sup> groups; R<sup>3a</sup> is selected from the group consisting  
45 of H, and C<sub>1-6</sub> alkyl;  
46 each R<sup>3b</sup> is independently selected from the group consisting of H, C<sub>1-6</sub> alkyl, halogen,  
47 -OR<sup>3a</sup> and -NR<sup>3a</sup>R<sup>3a</sup>;  
48 R<sup>3c</sup> is absent or -OH;  
49 alternatively, L<sup>2</sup> is combined with R<sup>1</sup>, L<sup>1</sup> is combined with L<sup>2</sup>, L<sup>1</sup> is combined with  
50 R<sup>2</sup>, two R<sup>1</sup> groups on adjacent ring atoms, or two R<sup>2</sup> groups on adjacent ring  
51 atoms are combined to form a 5-6 membered heterocycloalkyl with from 1 to  
52 3 heteroatoms selected from N, O and S, or a 5-6 membered heteroaryl with  
53 from 1 to 3 heteroatoms selected from N, O and S, and optionally substituted  
54 with from 1 to 3 groups selected from the group consisting of H, C<sub>1-6</sub> alkyl and  
55 oxo;  
56 subscripts m and n are each an integer from 1 to 3;  
57 wherein:  
58 (a) L<sup>1</sup> is a bond, L<sup>2</sup> is -C(O)NH-, ring B is phenyl, and at least one R<sup>2</sup> is  
59 -CN or phenyl, or  
60 (b) at least one R<sup>1</sup> is -C(O)OH, ring A is phenyl, L<sup>2</sup> is -C(O)NH-, and L<sup>1</sup> is  
61 a bond or C<sub>1-6</sub> alkylene, or  
62 (c) each of ring A and ring B is phenyl, at least one R<sup>1</sup> is -C(O)OH or  
63 combined with L<sup>2</sup>, and at least one R<sup>2</sup> is selected from the group  
64 consisting of H, -CN and -C(O)OH;  
65 wherein when R<sup>1</sup> is -CO<sub>2</sub>H, subscript n is 1, ring A is phenyl, L<sup>2</sup> is -C(O)NH-, L<sup>1</sup> is a  
66 bond, ring B is phenyl, subscript m is 1, and R<sup>2</sup> is phenyl, then the phenyl of  
67 R<sup>2</sup> is substituted with C<sub>1-6</sub> alkyl,  
68 or salts and isomers thereof.

1                   33.    The pharmaceutical composition of claim 32, wherein the composition  
2 is formulated for intra-articular delivery.

1                   34.    The composition of any of claims 32 or 33, further comprising a  
2 member selected from the group consisting of angiotensin-like 3 protein (ANGPTL3), oral  
3 salmon calcitonin, SD-6010 (iNOS inhibitor), vitamin D3 (cholecalciferol), collagen  
4 hydrolyzate, FGF18, BMP7, avocado soy unsaponifiables (ASU) and hyaluronic acid.

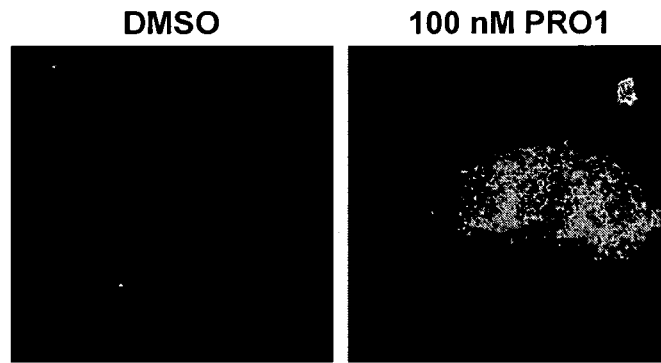




**FIG. 1**

A.

Type II collagen expression



B.

Quantitation of Type II collagen expression

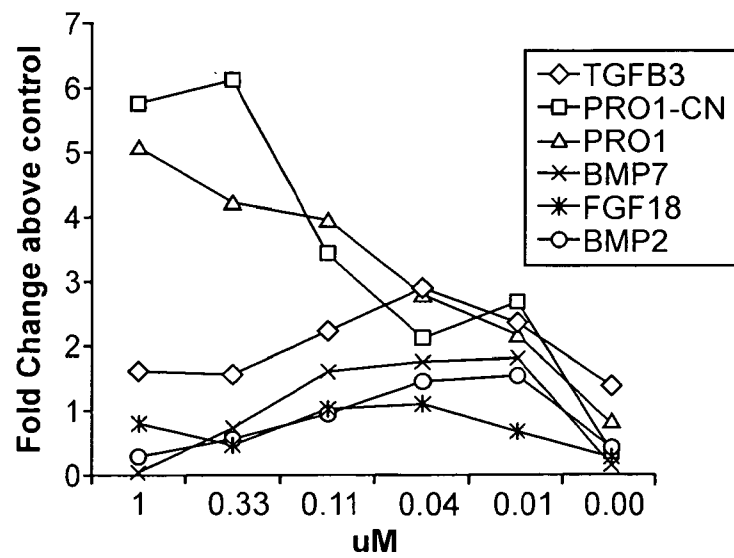
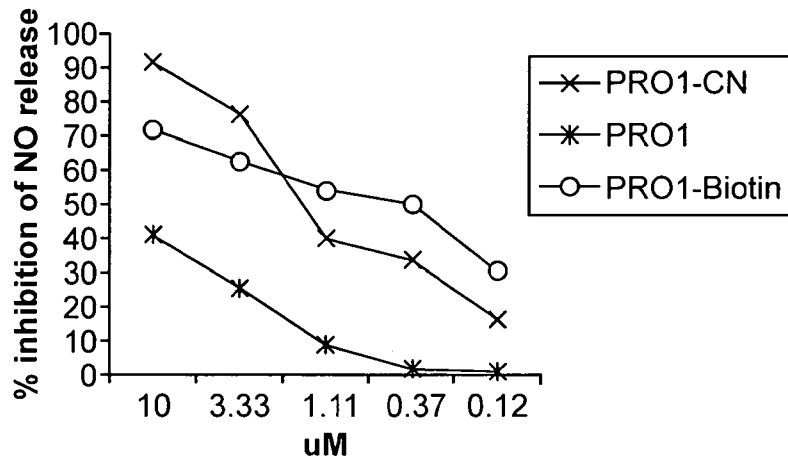


FIG. 2

C.

**Protection of chondrocytes following cytokine-induced nitric oxide release**



D.

**Protection of matrix loss following cytokine-induced GAG release**

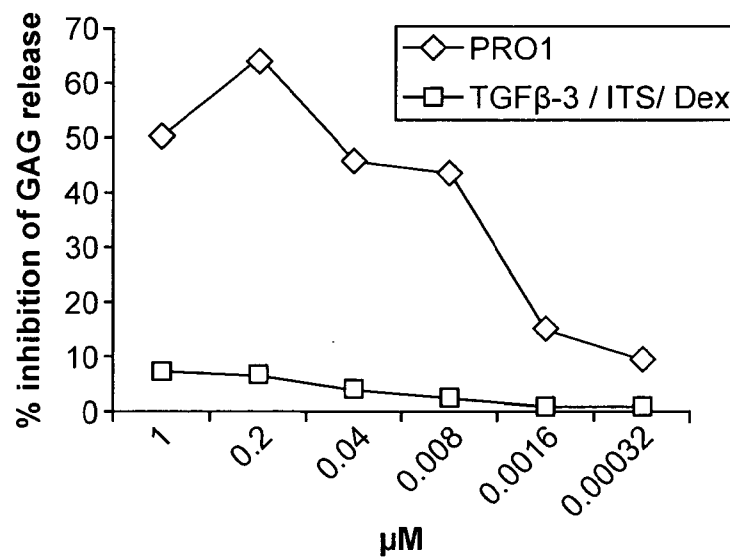


FIG. 2 (Cont. 1)

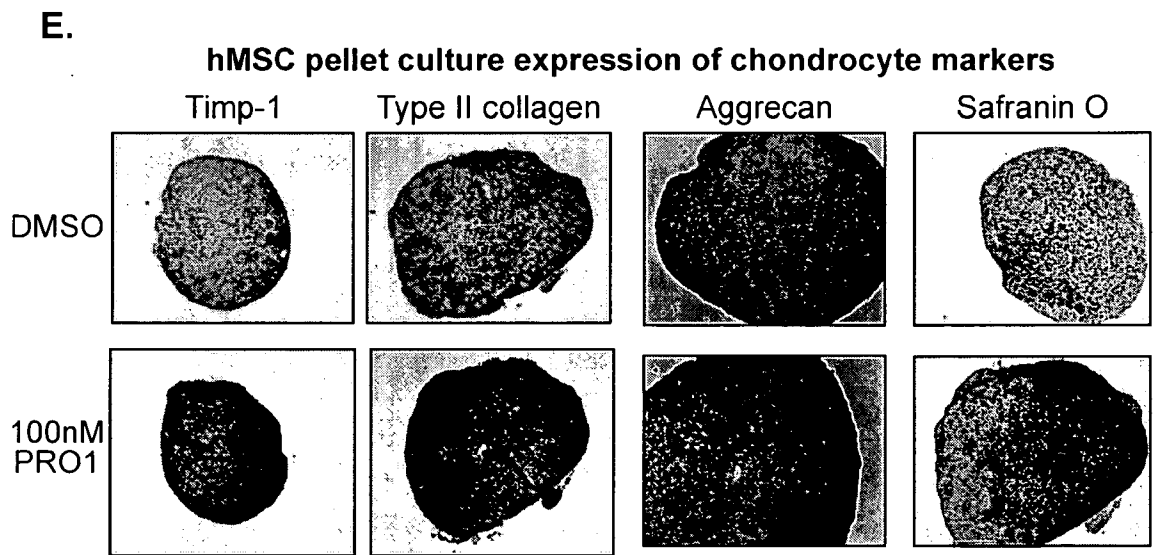
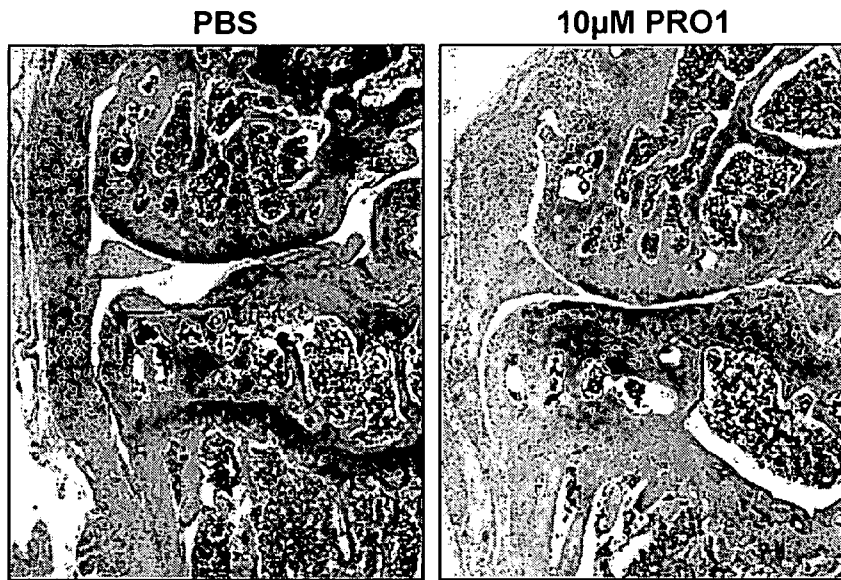


FIG. 2 (Cont. 2)

A.

Histological Representations of Cartilage Damage



B.

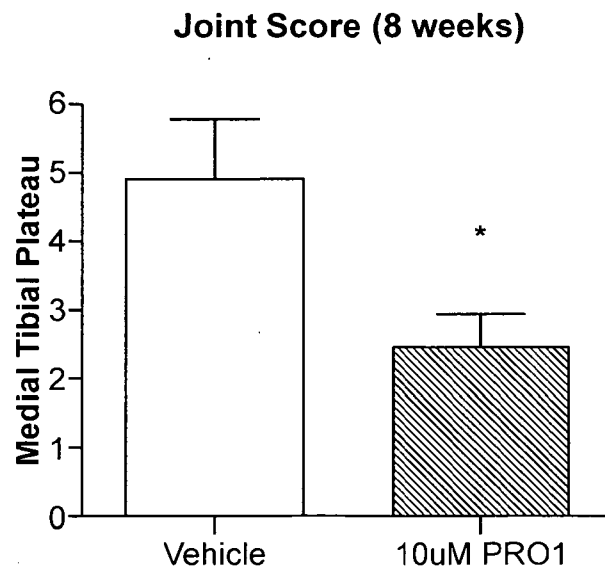
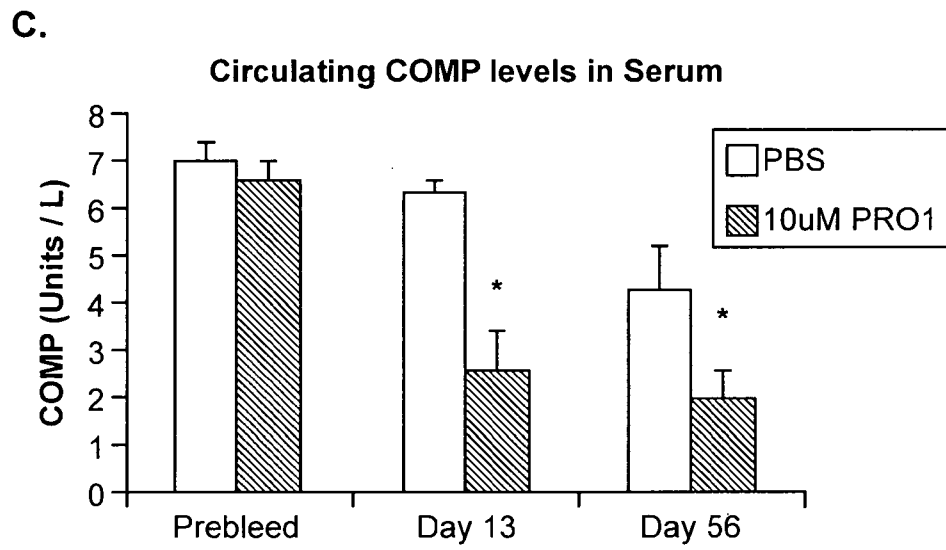


FIG. 3



**FIG. 3 (Cont.)**



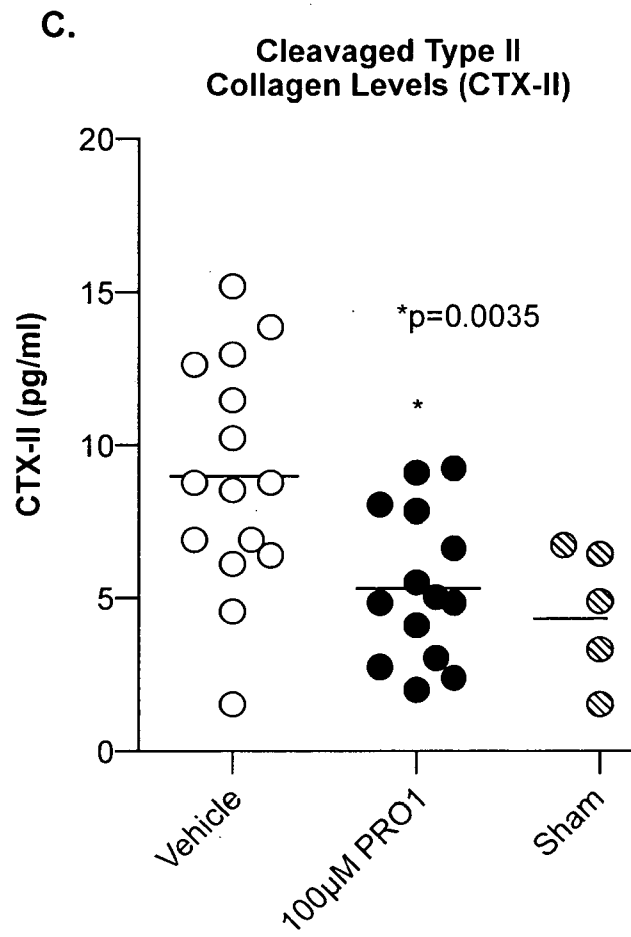


FIG. 4 (Cont.)



A.

Incapacitance Measurements

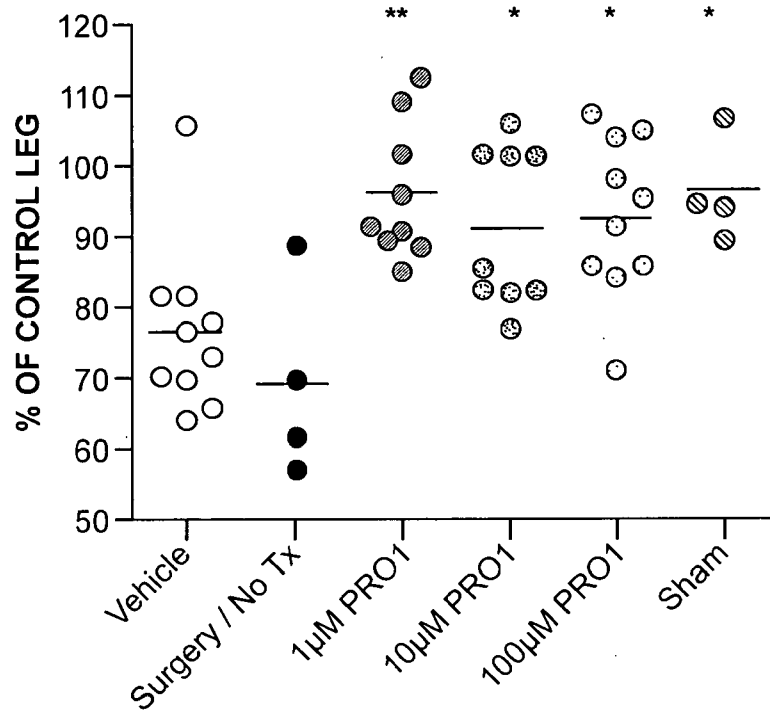
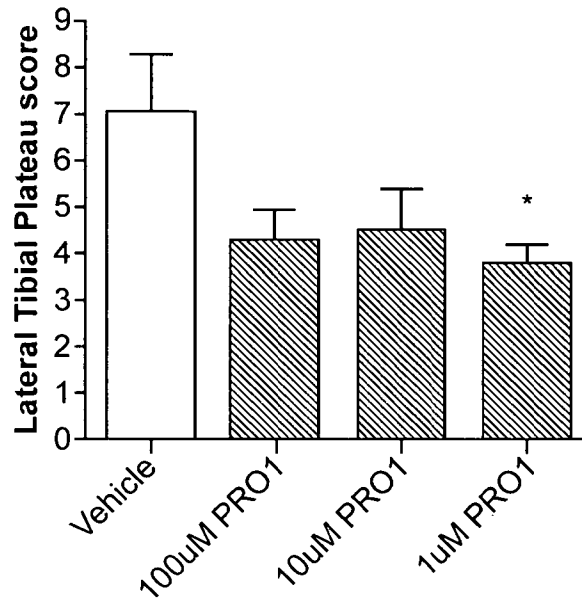


FIG. 5

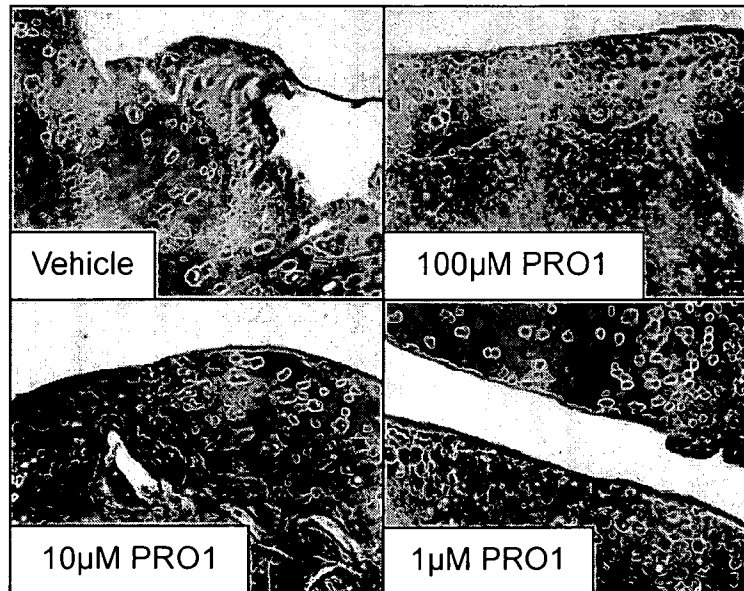
**B.**

**Joint Score**



**C.**

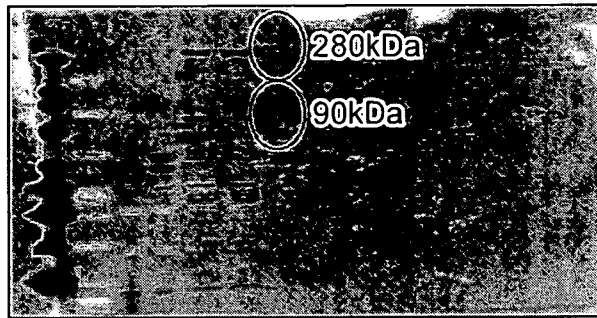
**Assesement of Type II collagen**



**FIG. 5 (Cont.)**

A.

Anti-Biotin identification of FLNA



B.

Expression of FLNA in hMSC

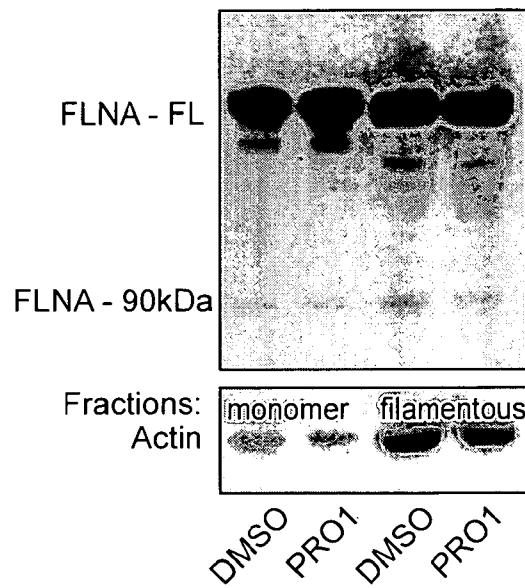


FIG. 6

C.

Protein expression in hMSC

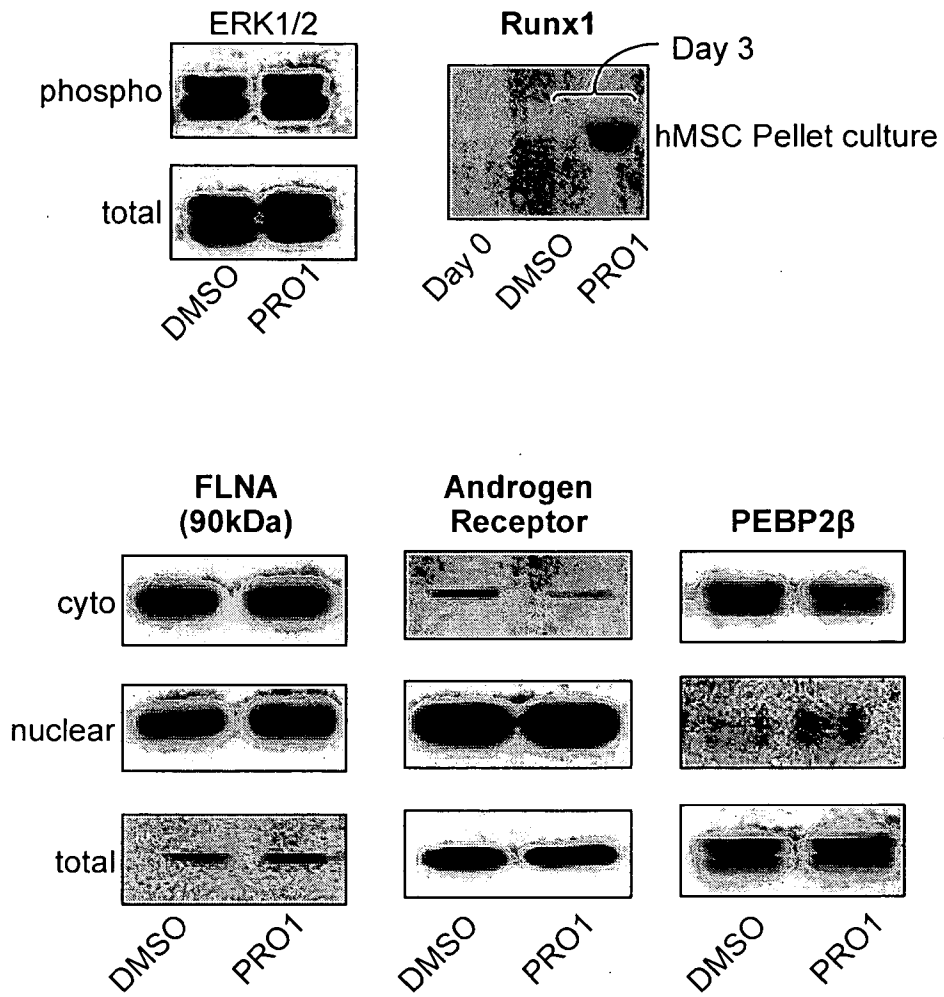
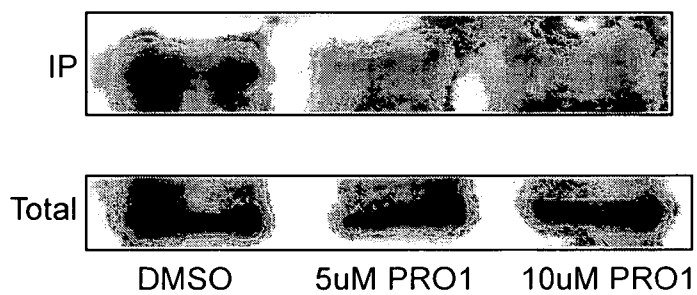


FIG. 6 (Cont.)

**Inhibition of PEPB2 $\beta$  / FLNA interaction**

**IP: PEPB2 $\beta$ , Western: FLNA (90 kDa fragment)**



**FIG. 7**

14 / 18

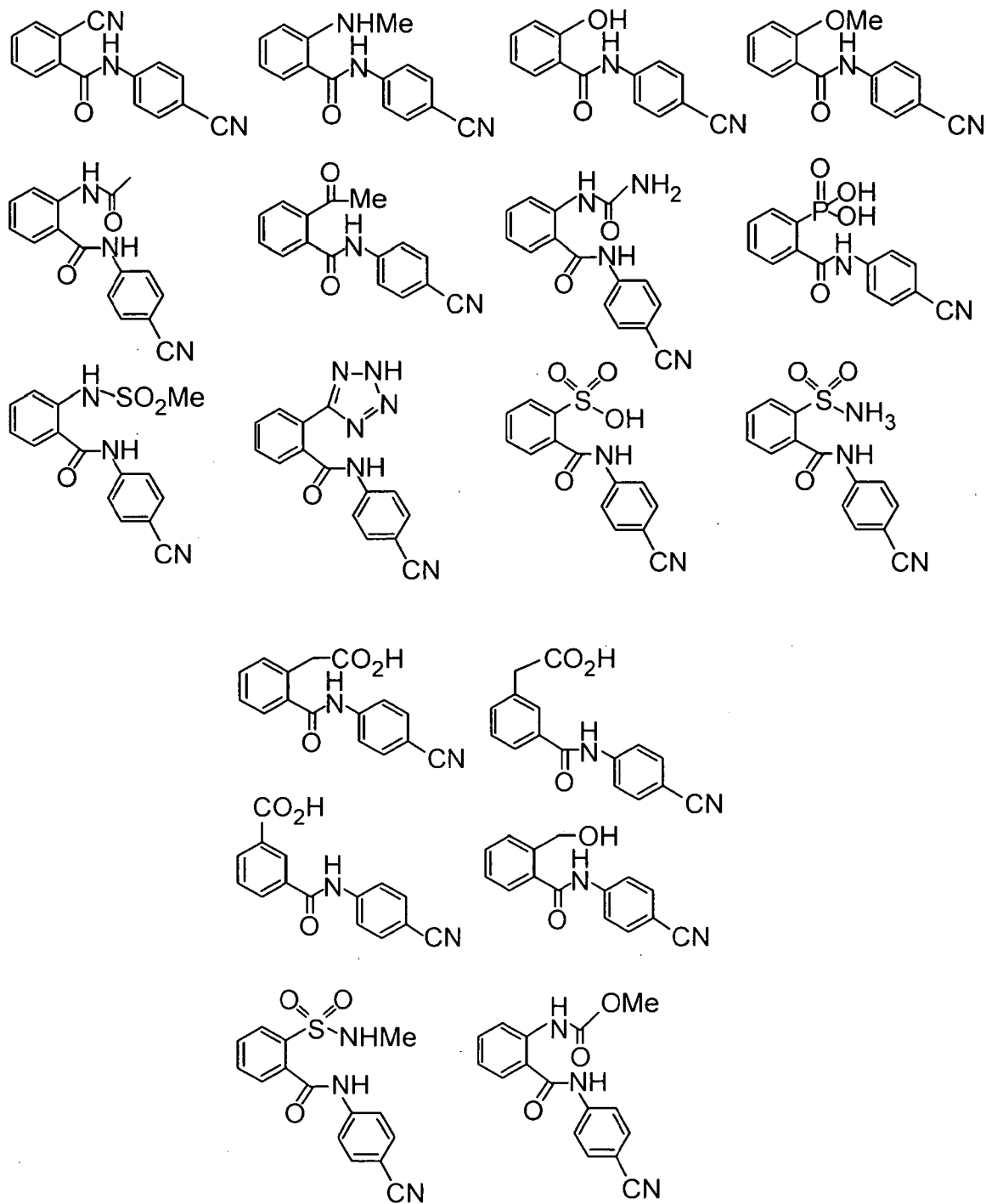


FIG. 8

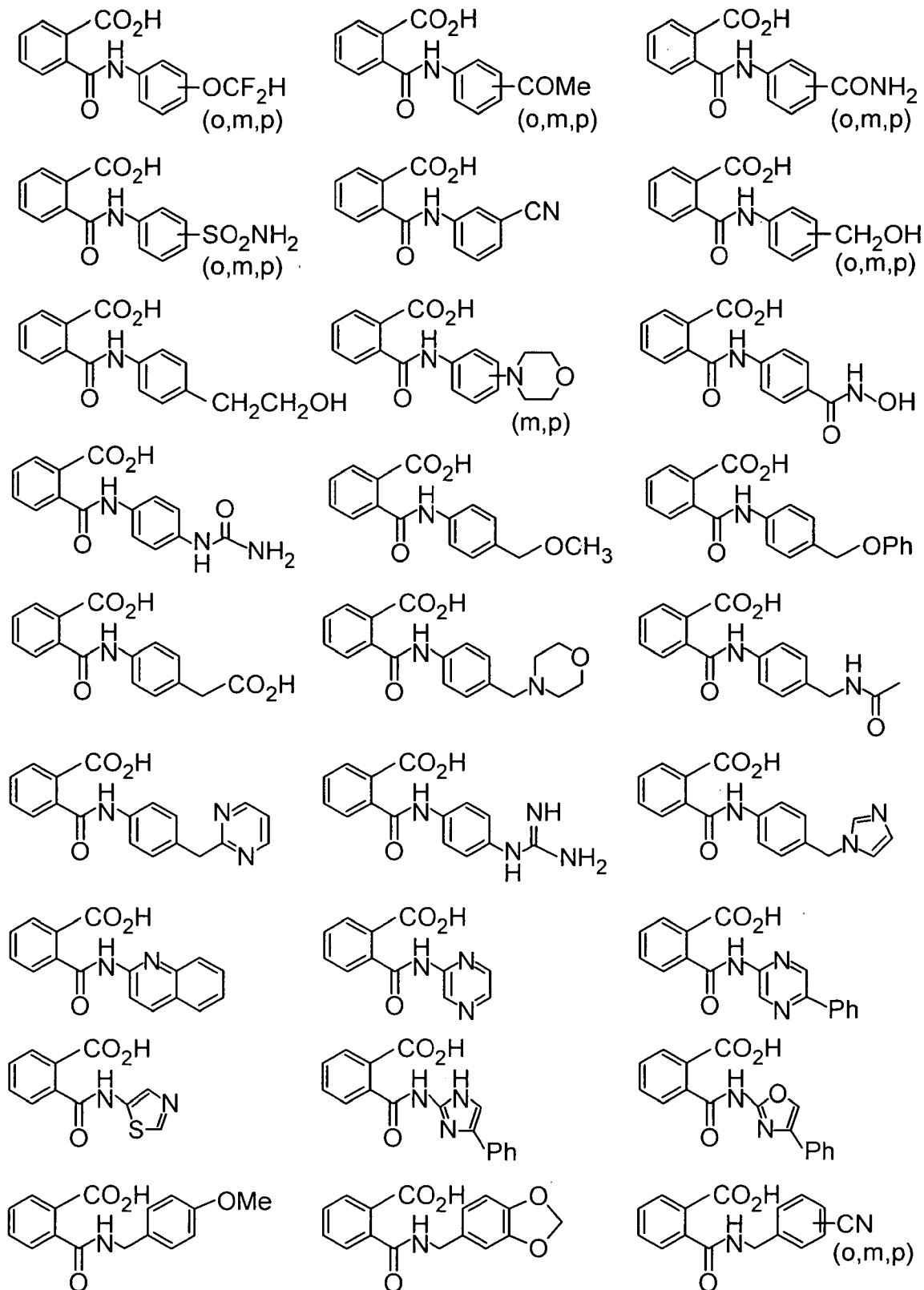


FIG. 8 (Cont. 1)

16 / 18

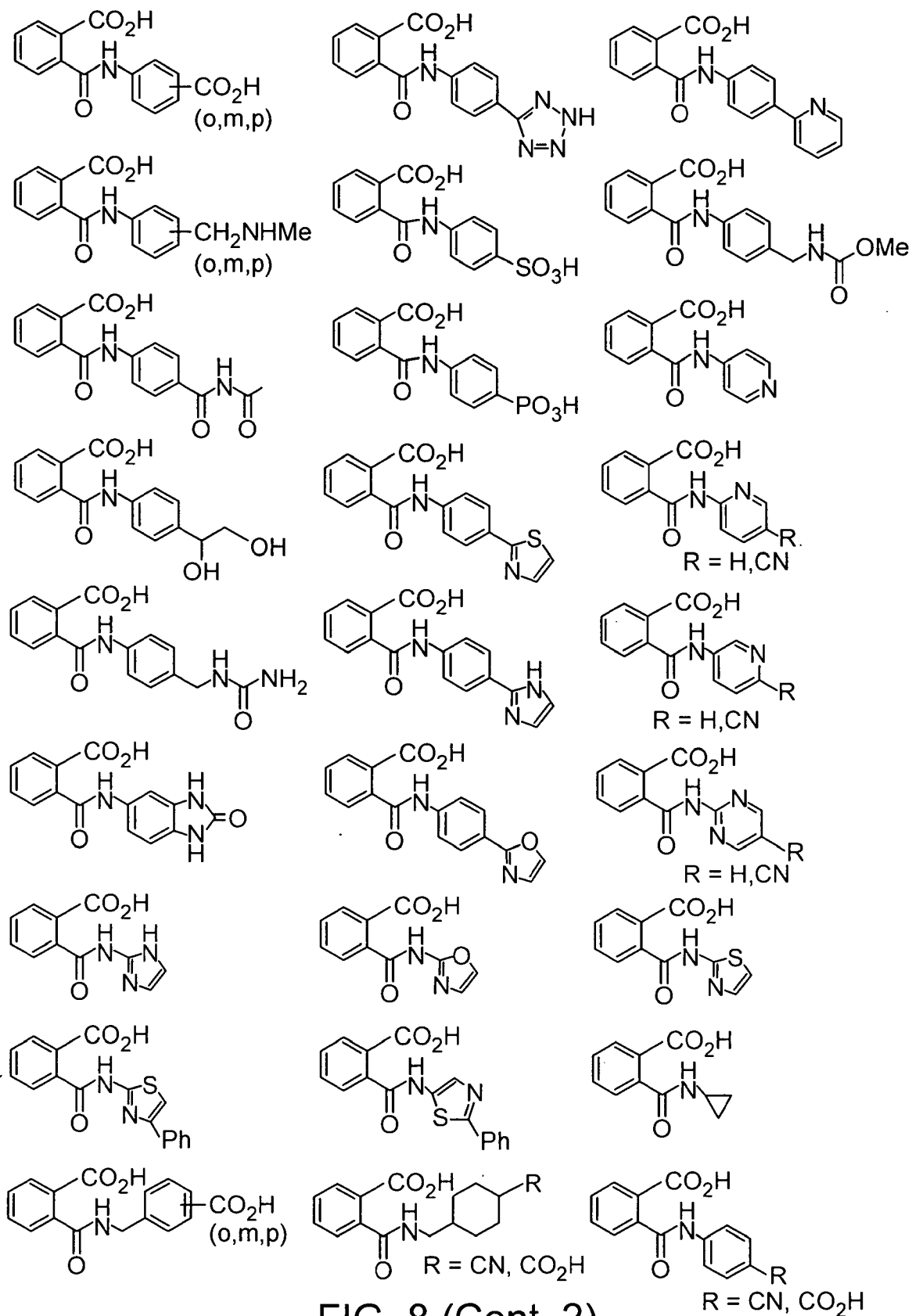


FIG. 8 (Cont. 2)





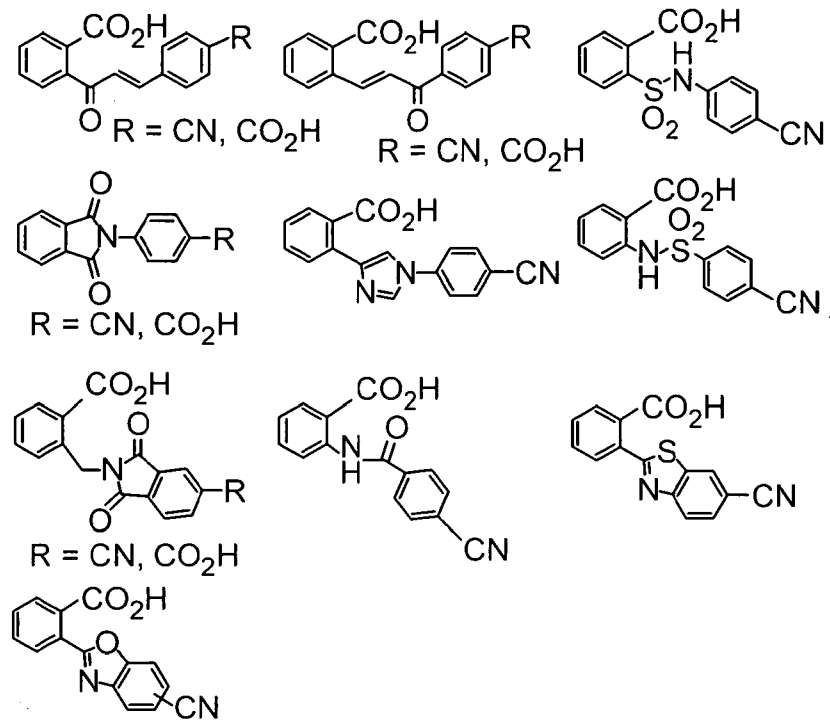


FIG. 8 (Cont. 4)