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(54) Title: NAT10 MODULATORS FOR TREATING OR PREVENTING LAMINOPATHIES, AGING AND CANCER

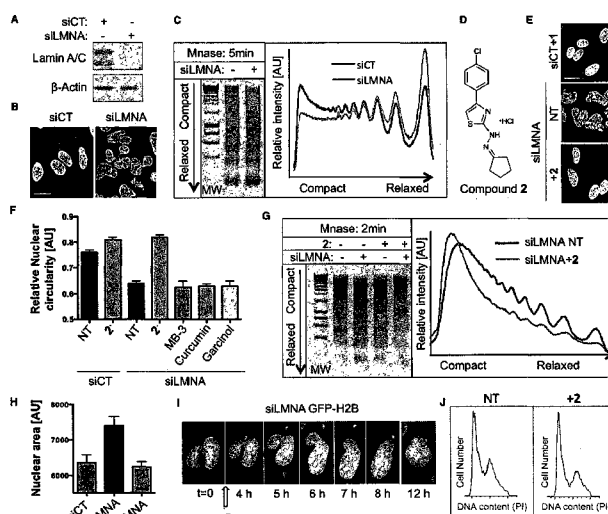


FIGURE 1

(57) Abstract: The invention relates to compounds in the treatment or prevention of disorders associated with Lamin A and/or Lamin C depletion or LMNA mutations, such as laminopathy, premature ageing disorders, normal ageing and cancer (such as a cancer characterised by low levels of LMNA expression).

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NAT10 MODULATORS FOR TREATING OR PREVENTING LAMINOPATHIES, AGING AND CANCER

**FIELD OF THE INVENTION**

The invention relates to compounds in the treatment or prevention of disorders associated with Lamin A and/or Lamin C depletion or *LMNA* mutations, such as laminopathy, premature ageing disorders, normal ageing and cancer.

**BACKGROUND OF THE INVENTION**

The nuclear lamina, composed of A and B-type lamins, maintains nuclear morphology and functions as an anchoring platform for tethering chromatin T. Dechat *et al.* (2008) *Genes Dev.* 22, 832). Lamins interact with numerous chromatin-bound proteins and are also indirectly linked to the cytoskeleton, via transmembrane proteins including Nesprins and SUN proteins (M. Crisp *et al.* (2006) *J. Cell Bio.* 172, 41). Mutations in *LMNA*, which encodes Lamin A and C, cause a wide spectrum of human diseases known as laminopathies (H. J. Worman, G. Bonne (2007) *Exp. Cell Res.* 313, 2121). These include Emery-Dreifuss muscular dystrophy (AD-EDMD) (G. Bonne *et al.* (1999) *Nat. Genet.* 21, 285) and the severe accelerated-ageing disease Hutchinson Gilford Progeria Syndrome (HGPS) (A. De Sandre-Giovannoli *et al.* (2003) *Science* 300, 2055; M. Eriksson *et al.* (2003) *Nature* 423, 293). Deregulation of A-type lamins has also been observed in various human cancers, where they are aberrantly expressed or localized (J. L. Broers *et al.* (1993) *Am. J. Pathol.* 143, 211; S. F. Moss *et al.* (1999) *Gut* 45, 723; R. S. Venables *et al.* (2001) *Br. J. Cancer* 84, 512).

Lamin A/C depletion or *LMNA* mutations cause enlarged, misshapen nuclei associated with loss of global chromatin organization (G. Galiova *et al.* (2008) *Eur. J. Cell Biol.* 87, 291). The exact molecular causes for the broad range of clinical phenotypes associated with laminopathies, however, remain to be defined. While some of these pathologies might reflect the primary molecular defect in lamin disorganization causing cellular fragility (the "structural hypothesis"), many seem likely to result from downstream effects on chromatin structure, gene expression or additional nuclear processes such as replication, transcription and DNA repair (K. L. Wilson *et al.* (2001) *Cell* 104, 647).

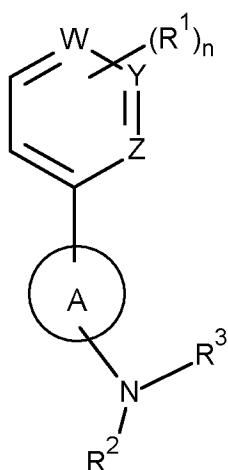
Interestingly, recent studies suggest that improving nuclear architecture of laminopathic cells can also ameliorate certain downstream defects in chromatin structure and other cellular processes, thus improving some disease-associated phenotypes (C. Y. Chen *et al.* (2012) *Cell* 149, 565; J. I. Toth *et al.* (2005) *PNAS* 102, 12873; M. Columbaro *et al.* (2005) *Cell Mol. Life Sci.* 62, 2669).

There is therefore a need to provide treatments which correct nuclear architecture defects and improve cellular fitness of laminopathic human cells, thereby treating laminopathies and premature ageing disorders.

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### SUMMARY OF THE INVENTION

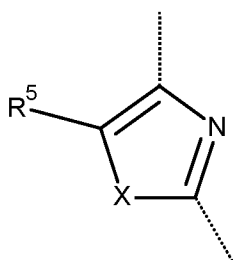
According to a first aspect of the invention, there is provided a compound of formula (I):



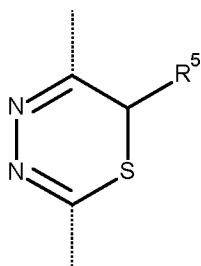
15 wherein:

each of W, Y and Z represent CH or one of W, Y and Z represents nitrogen and the other two groups represent CH;

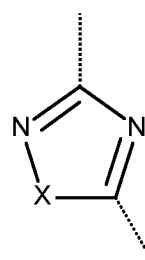
Ring A represents:



(i)



(ii)



(iii)

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X represents S, O or NR<sup>a</sup>;

R<sup>a</sup> represents hydrogen, hydroxyl, =O, C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, halogen, haloC<sub>1-6</sub>alkyl, C<sub>1-6</sub> alkoxy or haloC<sub>1-6</sub> alkoxy;

R<sup>5</sup> represents hydrogen, hydroxyl, C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, halogen, haloC<sub>1-6</sub>alkyl, C<sub>1-6</sub> alkoxy, haloC<sub>1-6</sub> alkoxy, COH, COOH, COOC<sub>1-6</sub>alkyl, cyano, NH<sub>2</sub> or NO<sub>2</sub>;

n represents an integer selected from 0 to 5;

each R<sup>1</sup> independently represents C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, halogen, haloC<sub>1-6</sub>alkyl, C<sub>1-6</sub> alkoxy, haloC<sub>1-6</sub> alkoxy, cyano, hydroxyl, COH, COOH, COOC<sub>1-6</sub>alkyl, NH<sub>2</sub> or NO<sub>2</sub>;

R<sup>2</sup> represents hydrogen, C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl or C<sub>2-6</sub> alkynyl;

R<sup>3</sup> is selected from either hydrogen or an -N=R<sup>4</sup> group, such that when Ring A represents formula (i) or (iii), R<sup>3</sup> represents -N=R<sup>4</sup>, and when Ring A represents formula (ii), R<sup>3</sup> represents hydrogen;

R<sup>4</sup> represents -C(R<sup>4a</sup>)(R<sup>4b</sup>), C<sub>3-8</sub> cycloalkyl, C<sub>3-8</sub> cycloalkenyl or benzyl wherein said cycloalkyl or benzyl is optionally substituted by C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, halogen, haloC<sub>1-6</sub>alkyl, C<sub>1-6</sub> alkoxy, haloC<sub>1-6</sub> alkoxy, cyano, hydroxyl, COH, COOH, COOC<sub>1-6</sub>alkyl, NH<sub>2</sub> or NO<sub>2</sub>;

R<sup>4a</sup> and R<sup>4b</sup> are independently selected from hydrogen, C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, halogen, haloC<sub>1-6</sub>alkyl, C<sub>1-6</sub> alkoxy, haloC<sub>1-6</sub> alkoxy, cyano, hydroxyl, COH, COOH, COOC<sub>1-6</sub>alkyl, NH<sub>2</sub>, NO<sub>2</sub>, C<sub>3-8</sub> cycloalkyl or benzyl wherein said cycloalkyl or benzyl is optionally substituted by C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, halogen, haloC<sub>1-6</sub>alkyl, C<sub>1-6</sub> alkoxy, haloC<sub>1-6</sub> alkoxy, cyano, hydroxyl, COH, COOH, COOC<sub>1-6</sub>alkyl, NH<sub>2</sub> or NO<sub>2</sub>;

or a pharmaceutically acceptable salt or solvate thereof, for use in the treatment or prevention of laminopathy, premature ageing disorders, normal ageing or cancer.

### BRIEF DESCRIPTION OF THE FIGURES

**Figure 1: Identification of a KAT inhibitor restoring nuclear shape and chromatin compaction in Lamin A/C depleted cells. A)** Analysis of Lamin A/C depletion (siLMNA) in U2OS cells compared to negative control (siCT). **B)** Nuclear shape observed by DAPI staining. **C)** Representative MNase digestion profile (left) from three independent experiments and corresponding

quantification (right). **D)** Molecular structure of cyclopentylidene-[4-(4'-chlorophenyl)thiazol-2-yl]hydrazone (**Compound 2**). **E)** Nuclear shape rescue observed by DAPI staining after treatment with **Compound 2**. **F)** Quantification of nuclear circularity in non-treated (NT) cells or cells treated with the indicated compounds (means of three independent experiments with  $n > 212 \pm \text{s.d.}$ ). **G)** Representative MNase digestion profile (left) from three independent experiments and corresponding quantification of siLMNA cells (right). **H)** Quantification of nuclear area (means of three independent experiments with  $n > 198 \pm \text{s.d.}$ ). **I)** Live imaging pictures of nuclear shape rescue in GFP-H2B expressing U2OS cells transfected with siLMNA and treated with **Compound 2**. **J)** Cell cycle profile analysed by flow cytometry. PI: propidium iodide. Scale bars: 20  $\mu\text{m}$ .

**Figure 2: Small molecule Compound 2 rescues nuclear shape defects in various Lamin A/C depleted cells.** **A)** and **B)** Nuclear shape visualization by DAPI staining of HeLa (**A**) or RPE-1 (**B**) cells depleted for Lamin A/C (siLMNA) and treated with various KATi. Scale bars: 50  $\mu\text{m}$ . **C)** and **D)** Cell Profiler quantification of nuclear circularity of HeLa (**C**) or RPE-1 (**D**) cells from DAPI staining as shown in **A)** and **B)** (means of three independent experiments with  $n > 252 \pm \text{s.d.}$ ). **E)** Cell cycle profile analysed by flow cytometry. PI: propidium iodide. **F)** Analysis of Lamin A/C expression levels in indicated cancer cell lines. Bottom panel: Image J quantification of Lamin A/C expression in cancer cells relative to normal RPE-1 cells. **G)** Representative pictures of DAPI staining showing nuclear shape improvement in low Lamin A/C expressing cancer cells after treatment with **Compound 2**. Scale bars: 20  $\mu\text{m}$ .

**Figure 3: Nuclear shape rescue of Lamin A/C depleted cells after treatment with Compound 2 is independent of mitosis.** Nuclear shape rescue observed by DAPI staining in cells synchronized in S phase by aphidicolin and then treated with **Compound 2** in the presence of aphidicolin to prevent mitotic entry. Scale bars: 20  $\mu\text{m}$ .

**Figure 4: The N-acetyltransferase NAT10 is a cellular target of small molecule Compound 2.** **A)** Molecular structure of clickable analogue

**Compound 3** and clickable inactive control molecule **Reference Compound A**. **B)** Quantification of U2OS nuclear circularity (means of three independent experiments with  $n > 224 \pm \text{s.d.}$ ) **C)** Principle of click-chemistry strategy for small molecule tagging. **D)** Molecular structure of biotinylated analogue of **Compound 3**. **E)** Silver staining of proteins after pull-down of small molecule biotinylated **Compound 3** and identification of specific bands (see arrows) decreasing in the presence of 5 equivalents of non-clickable molecule **Compound 2**. The band corresponding to NAT10 is magnified on the right (\*) together with other specific and non-specific bands. **F)** Pull-down of clickable molecules **Compound 3** and **Reference Compound A** pre-incubated in U2OS cells and analysis of bound proteins. **G)** High resolution representative microscopy pictures of NAT10 (red) and fluorescently labeled **Compound 3** (green) in control or NAT10 depleted cells (siNAT10) as observed by western blotting (bottom panel). Scale bars: 10  $\mu\text{m}$ . **H)** Analysis of purified (silver staining, left) NAT10 folding by circular dichroism spectroscopy (right), showing the incremental effects of increasing concentrations of **Compound 2** on NAT10 helical character. **I)** Molecular structure of Remodelin (**Compound 1**), a stable and potent analogue of **Compound 2**.

**Figure 5: Subcellular localization of the molecules and analysis of their effects on NAT10 localization.** **A)** Fluorescent labelling of clickable control molecule **Reference Compound A** showing no specific staining, contrary to molecule **Compound 3** (see Figure 2). Scale bar: 20  $\mu\text{m}$ . **B)** IF pictures of Lamin A/C and NAT10 staining within cells transfected or treated as indicated. Scale bar: 20  $\mu\text{m}$ .

**Figure 6: A screen of Compound 2 analogues to identify structural requirements for nuclear shape rescue.** **A)** Nuclear shape analysis by DAPI staining in siLMNA cells treated with the indicated analogues of **Compound 2**. **B)** Dose-dependent nuclear shape rescue observed by DAPI staining of siLMNA cells, showing an  $\sim 5$ -fold increase in the potency of Remodelin compared to **Compound 2**. Scale bar: 20  $\mu\text{m}$ .

**Figure 7: Inhibiting NAT10 activity by Remodelin mediates nuclear shape rescue of LMNA depleted cells. A)** Analysis of NAT10 and Lamin A/C depletion in U2OS cells. **B)** Nuclear shape visualised by DAPI staining (left) and quantification of nuclear circularity (right) (means of three independent experiments with  $n > 267 \pm \text{s.d.}$ ). Scale bar: 20  $\mu\text{m}$ . **C)** Representation of NAT10 with its known domains. The G641E mutation identified in **D)** is indicated by an asterisk. **D)** Modelled 3D structure of human NAT10 residues 10 to 917 showing the acetyl-CoA binding site (left) and disruption of Ac-CoA binding by NAT10 mutation (G641E, right) visualised with Swiss-Prot PDB Viewer. **E)** *In vitro* acetylation assay showing the acetyltransferase activity of NAT10 towards tubulin. **F)** Quantification of nuclear circularity (left) in cells stably expressing siRNA-resistant FLAG-NAT10 WT or FLAG-NAT10 G641E (FLAG-NAT10 MUT) (means of three independent experiments with  $n > 198 \pm \text{s.d.}$ ) and nuclear shape visualised by DAPI staining (right). Scale bar: 20  $\mu\text{m}$ .

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**Figure 8: NAT10 mutation G641E (MUT) does not affect its localization. A)** Alignment of NAT10 GNAT (Gcn5-related N-acetyltransferase) domain showing the conservation of the G641 residue. **B)** Silver staining of purified FLAG-NAT10 WT or G641E mutant (MUT) from Human HEK293 cells. These proteins were used for the acetyl-transferase activity assay. **C)** Characterization of U2OS cells stably expressing siRNA resistant constructs of FLAG-NAT10 WT and MUT. The expression of the constructs and the resistance to siRNA was observed by western blotting and **D)** the correct localization of both constructs was verified by IF staining using anti-NAT10 antibody or anti-FLAG antibody. Scale bars: 10  $\mu\text{m}$ .

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**Figure 9: Remodelin targets NAT10 to improve nuclear shape and fitness of HGPS cells. A)** Representative immunofluorescence (IF) pictures of Lamin A/C in HGPS cell lines compared to matched normal fibroblasts at the same population doubling. Scale bar: 10  $\mu\text{m}$ . **B)** Quantification of misshapen nuclei upon Remodelin treatment (means of three independent experiments with  $n > 213 \pm \text{s.e.m.}$ ). **C)** Lamin A/C staining in HGPS AG11498 cells (left) and quantification of misshapen nuclei (right; means of three independent experiments with  $n > 176 \pm \text{s.e.m.}$ ). Scale bar: 50  $\mu\text{m}$ . **D)** Western blotting

30



analysis of  $\gamma$ H2AX after Remodelin or FTI treatment. **E)** Immunofluorescence analysis of  $\gamma$ H2AX staining upon Remodelin or ATM/ATR inhibition (ATM/ATRi). **F)** Quantification of  $\gamma$ H2AX positive cells observed by IF (means of three independent experiments with  $n > 127 \pm$  s.e.m.). **G)** HGPS proliferation upon Remodelin or ATM/ATR-inhibitor treatment (means of nine replicates  $\pm$  s.e.m.). **H)** Quantification of senescence-associated  $\beta$ -galactosidase positive cells (means of three independent experiments with  $n > 257 \pm$  s.e.m.). **I)** Quantification of senescence-associated  $\beta$ -galactosidase positive cells in HGPS AG11498 after 8 days of Remodelin treatment at population doubling 12 (PDL 12) and after several weeks of Remodelin treatment and 12 cell divisions (PDL 24) (means of two independent experiments with  $n > 298 \pm$  s.d.).

**Figure 10: Remodelin rescues nuclear shape of aged MRC5 cells but not WS cells in a dose dependent manner. A)** Misshapen nuclei observed by DAPI staining in MRC5 aged cells in culture, at population doubling 44 (PD44) were rescued by Remodelin. **B)** Quantification of misshapen nuclei after increasing concentrations of Remodelin (mean of three independent experiments  $\pm$  s.e.m.). **C)** DAPI staining of non-laminopathic Werner Syndrome cells (WS) showing no nuclear shape improvement upon Remodelin treatment. **D)** Cell Profiler quantification of misshapen nuclei (mean of three independent experiments  $\pm$  s.d.  $n > 273$ ). Scale bars: 50  $\mu$ m.

**Figure 11: Remodelin prevents FTI induced nuclear shape defects in non-progeric cells. A)** Analysis of Lamin A/C processing after Remodelin or FTI treatment, showing that Remodelin is not a FTI. **B)** Representative pictures of Lamin A/C IF staining after the indicated treatments. Arrows indicate FTI induced misshapen nuclei in normal fibroblasts. Scale bar: 50  $\mu$ m. **C)** Quantification of misshapen nuclei with the indicated cell lines and inhibitor treatments (means of three independent experiments  $\pm$  s.e.m.  $n > 178$ ). **D)** Representative pictures of DAPI staining showing effects of FTI and Remodelin on nuclear shape of U2OS cells. Scale bars: 20  $\mu$ m.

**Figure 12: Remodelin improves global cellular fitness of HGPS cells. A)** NAT10 depletion in normal fibroblasts and HGPS AG11498 cells observed by

western blotting, and showing that siNAT10 has no effect on Lamin A/C expression and processing. **B)** IF images showing nuclear shape improvement and decreased numbers of  $\gamma$ H2AX foci in HGPS cells upon Remodelin treatment. Scale bars: 10  $\mu$ m. **C)** Western Blotting analysis showing that Remodelin decreases  $\gamma$ H2AX levels in both HGPS cell lines tested. **D)** IF images showing nuclear shape improvement and decreased intensity of  $\gamma$ H2AX staining in Emery-Dreifuss muscular dystrophy cells (EDMD) upon Remodelin treatment. Scale bars: 10  $\mu$ m. **E)** Western Blotting analysis showing that Remodelin decreases  $\gamma$ H2AX levels in EDMD. **F)** Western blotting analysis showing the effect of Remodelin on the p53 and DNA damage signalling pathways in HGPS AG11498 cells. **G)** Representative IF images of H3K9me3 or SUN1 patterns quantified in **H)**. Scale bars: 20  $\mu$ m. **I)** IF staining showing more intense and homogenous DAPI staining, as well as reorganization of nucleolus architecture as observed by NAT10 staining upon Remodelin treatment. Scale bars: 10  $\mu$ m. **J)** Flow cytometry analysis after 2 hours of EdU incorporation in HGPS cells upon Remodelin treatment showing enhancement of DNA replication rate. **K)** Quantification of senescence-associated  $\beta$ -galactosidase positive cells after treatment with p53 inhibitor (Pifithrin) or with the indicated KAT inhibitors, showing that none of these compounds decrease the senescence in HGPS AG11498.

**Figure 13: Inhibiting NAT10 acetyltransferase activity modifies microtubule organisation to rescue nuclear shape defects.** **A)** Microtubule network visualisation by inverted IF pictures of  $\alpha$ -Tubulin. **B)** Nuclear shape visualisation after treating cells with microtubule or actin cytoskeleton disrupting agents. **C)** Visualisation of nuclear shape (DAPI) and Golgi (anti-Giantin) integrity in siLMNA cells. **D)** Fractionation of polymerised (P) and soluble (S) tubulin upon Remodelin or nocodazole (Nocod.) treatment. **E)** Microtubule regrowth assay in cells transfected with siNAT10 and expressing the indicated siRNA resistant constructs.  $\alpha$ -tubulin IF staining (left) shows nucleation phase: t=5 minutes and microtubule anchorage (t=15min). Right: quantification of cells with the indicated patterns (means of three independent experiments with n>103  $\pm$  s.e.m.). **F)** Laminopathic cells have enlarged, misshapen nuclei associated with disorganized chromatin structure. Upon Remodelin treatment,

NAT10 acetyltransferase activity is inhibited, leading to disruption of microtubule anchorage at the centrosome and release of microtubule forces on the nuclear envelope. This release of mechanical stress on the nucleus contributes to nuclear shape rescue and global improvement of cellular fitness and chromatin organization. Scale bars: 20  $\mu\text{m}$ .

**Figure 14: Remodelin targets the microtubule network in HGPS cells to improve nuclear shape. A)** Nuclear shape visualisation and quantification after treating cells with microtubule-disrupting agents. **B)** Microtubule regrowth assay in HGPS AG11498 cells treated with Remodelin.  $\alpha$ -tubulin inverted IF staining shows normal microtubule depolymerisation (T=0) and nucleation phase (T=5 min) but defects in the microtubule anchorage (T=15 min) upon Remodelin treatment. **C)** Microtubule regrowth assay as in **B)** showing defects in the microtubule anchorage (T=15 min) upon NAT10 depletion (siNAT10) in the indicated HGPS cell lines and compared to cells transfected with control siRNA (siCT). Scale bars: 20  $\mu\text{m}$ .

**Figure 15: Compound 2 inhibits the proliferation, migration and invasion of cancer cells with low Lamin A/C expression. A)** Population doubling time of the indicated cancer cell lines treated or not with 50  $\mu\text{M}$  of **compound 2** for 1 week, showing that cells expressing low levels of Lamin A are more sensitive to the molecule. **B)** Representative pictures of matrigel invasion assay of prostate 22RV1 cancer cells, showing no invasion upon treatment with 50  $\mu\text{M}$  of **compound 2**. **C)** Quantification of transwell migration assays and matrigel invasion assays from the indicated low expressing cancer cell lines after treatment with 50  $\mu\text{M}$  of **compound 2**.

**Figure 16: Compound 2 decreases protein translation of Lamin A/C depleted cells. A)** U2OS cells transfected with siRNA control (siCT) or siRNA Lamin A/C (siLMNA) were plated at the same density, treated with cycloheximide (CHX) for 24 h to inhibit protein synthesis and stained with DAPI. Representative pictures of DAPI staining show that siLMNA cells are more resistant to CHX. **B)** Protein synthesis was measured by quantifying the incorporation of HPG, a

clickable analogue of methionine. FACS analysis shows quantification of HPG intensity in low Lamin A expressing U2OS cells **(B)** or in normal RPE cells **(C)** after increasing doses of **compound 2**.

5 **Figure 17: Pharmacokinetics assay of Remodelin in mice. A)** The pharmacokinetics of Remodelin was evaluated in ICR mice following IV at 1 mg/kg and PO at 5 mg/kg dose level. Following IV 1 mg/kg administration, the half-life (T<sub>1/2</sub>) was 0.915 h, and clearance was 139 mL/min/kg. Following PO 5 mg/kg administration, the half-life (T<sub>1/2</sub>) was 1.81 h, the maximum plasma  
10 concentration (C<sub>max</sub>, 409 ng/mL) was achieved at 0.25 h (T<sub>max</sub>) and Oral exposure was 259 ng/h/mL (AUC<sub>0-∞</sub>), bioavailability is 43.5%. **B)** Details of the results obtained in the curves shown in **A)**.

**Figures 18 and 19: Analysis of structure requirements for nuclear shape  
15 rescue.** The results of this analysis are described in Example 6.

**Figure 20: Compound 2 inhibits the growth of A431 melanoma cell line in a dose-dependent manner.** The results of this analysis are described in  
20 Example 7.

**Figure 21: Lamin A/C knock-out U2OS cells are more sensitive to Compound 2 and analogues than wild type cells.** The results of this analysis are described in Example 8.

25 **Figure 22: Compound 2 inhibits global protein translation specifically in LMNA KO cells.** The results of this analysis are described in Example 9.

**Figure 23: Depletion of NAT10 leads to similar inhibition of proliferation, migration and invasion than treatment with Compound 2.** The results of  
30 this analysis are described in Example 10.

**Figure 24: Assessment of Compound 2 *in vivo* toxicity and *in vitro* specificity.** The results of this analysis are described in Example 11.

**Figure 25: Effect of Compound 2 treatment on a mouse model of Hutchinson Gilford progeria syndrome.** The results of this analysis are described in Example 12.

5 **Figure 26: Molecular effects of Compound 2 *in vivo*.** The results of this analysis are described in Example 13.

**Figure 27: Assessment of acetyl tubulin as a potential biomarker for NAT10 inhibition.** The results of this analysis are described in Example 14.

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**Figure 28: Engineering a  $Lmna^{G609G/G609G}/NAT10^{+/-}$  mouse for genetic validation.** The results of this analysis are described in Example 15.

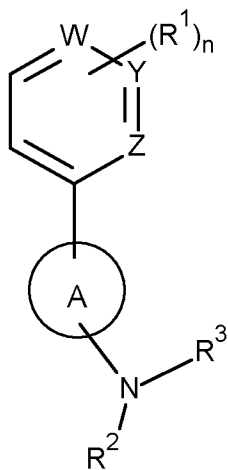
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**Figure 29: Microarray analysis shows specificity of Compound 2 towards gene expression regulation in HGPS cells.** The results of this analysis are described in Example 16.

## DETAILED DESCRIPTION OF THE INVENTION

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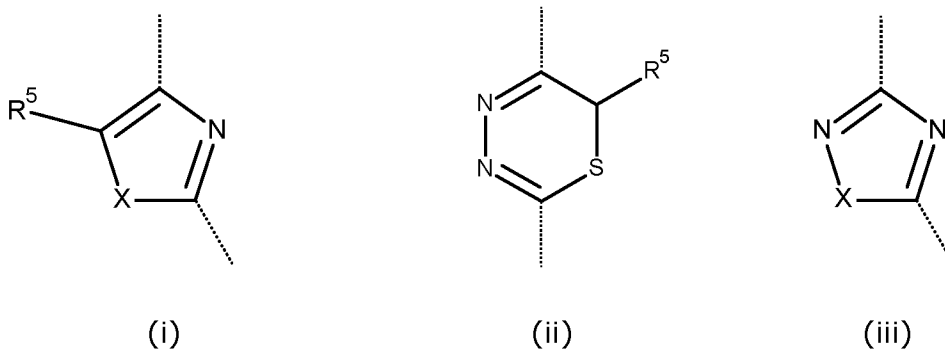
According to one particular aspect of the invention which may be mentioned, there is provided a compound of formula (I):



wherein:

each of W, Y and Z represent CH or one of W, Y and Z represents nitrogen and the other two groups represent CH;

25 Ring A represents:



X represents S, O or NR<sup>a</sup>;

R<sup>a</sup> represents hydrogen, hydroxyl, =O, C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, halogen, haloC<sub>1-6</sub>alkyl, C<sub>1-6</sub> alkoxy or haloC<sub>1-6</sub> alkoxy;

R<sup>5</sup> represents hydrogen, hydroxyl, C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, halogen, haloC<sub>1-6</sub>alkyl, C<sub>1-6</sub> alkoxy, haloC<sub>1-6</sub> alkoxy, COH, COOH, COOC<sub>1-6</sub>alkyl, cyano, NH<sub>2</sub> or NO<sub>2</sub>;

n represents an integer selected from 0 to 5;

each R<sup>1</sup> independently represents C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, halogen, haloC<sub>1-6</sub>alkyl, C<sub>1-6</sub> alkoxy, haloC<sub>1-6</sub> alkoxy, cyano, hydroxyl, COH, COOH, COOC<sub>1-6</sub>alkyl, NH<sub>2</sub> or NO<sub>2</sub>;

R<sup>2</sup> represents hydrogen, C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl or C<sub>2-6</sub> alkynyl;

R<sup>3</sup> is selected from either hydrogen or an -N=R<sup>4</sup> group, such that when Ring A represents formula (i) or (iii), R<sup>3</sup> represents -N=R<sup>4</sup>, and when Ring A represents formula (ii), R<sup>3</sup> represents hydrogen;

R<sup>4</sup> represents -C(R<sup>4a</sup>)(R<sup>4b</sup>), C<sub>3-8</sub> cycloalkyl or benzyl wherein said cycloalkyl or benzyl is optionally substituted by C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, halogen, haloC<sub>1-6</sub>alkyl, C<sub>1-6</sub> alkoxy, haloC<sub>1-6</sub> alkoxy, cyano, hydroxyl, COH, COOH, COOC<sub>1-6</sub>alkyl, NH<sub>2</sub> or NO<sub>2</sub>;

R<sup>4a</sup> and R<sup>4b</sup> are independently selected from hydrogen, C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, halogen, haloC<sub>1-6</sub>alkyl, C<sub>1-6</sub> alkoxy, haloC<sub>1-6</sub> alkoxy, cyano, hydroxyl, COH, COOH, COOC<sub>1-6</sub>alkyl, NH<sub>2</sub>, NO<sub>2</sub>, C<sub>3-8</sub> cycloalkyl or benzyl wherein said cycloalkyl or benzyl is optionally substituted by C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, halogen, haloC<sub>1-6</sub>alkyl, C<sub>1-6</sub> alkoxy, haloC<sub>1-6</sub> alkoxy, cyano, hydroxyl, COH, COOH, COOC<sub>1-6</sub>alkyl, NH<sub>2</sub> or NO<sub>2</sub>;

or a pharmaceutically acceptable salt or solvate thereof, for use in the treatment or prevention of laminopathy, premature ageing disorders, normal ageing or cancer.

The term 'halo' or 'halogen' as used herein refers to fluorine, chlorine, bromine or iodine.

- 5 The term 'cyano' as used herein refers to a group where a carbon atom is triple-bonded to a nitrogen atom (*i.e.*  $-\text{C}\equiv\text{N}$ ).

The term 'hydroxyl' as used herein refers to a group where an oxygen atom is bonded to a hydrogen atom.

10

The term 'C<sub>1-6</sub>alkyl' as used herein as a group or part of a group refers to a linear or branched saturated hydrocarbon group containing from 1 to 6 carbon atoms. Examples of such groups include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert butyl, n-pentyl, isopentyl, neopentyl or hexyl and  
15 the like.

The term 'C<sub>2-6</sub>alkenyl' as used herein as a group or part of a group refers to a linear or branched hydrocarbon group containing from 2 to 6 carbon atoms and containing a carbon carbon double bond.

20

The term 'C<sub>2-6</sub>alkynyl' as used herein as a group or part of a group refers to a linear or branched hydrocarbon group having from 2 to 6 carbon atoms and containing a carbon carbon triple bond.

- 25 The term 'C<sub>1-6</sub>alkoxy' as used herein as a group or part of a group refers to an -O-C<sub>1-6</sub>alkyl group wherein C<sub>1-6</sub>alkyl is as defined herein. Examples of such groups include methoxy, ethoxy, propoxy, butoxy, and the like.

The term 'haloC<sub>1-6</sub>alkyl' as used herein as a group or part of a group refers to a  
30 C<sub>1-6</sub>alkyl group as defined herein wherein one or more than one hydrogen atom is replaced with a halogen. The term 'haloC<sub>1-6</sub>alkyl' therefore includes monohaloC<sub>1-6</sub>alkyl and also polyhaloC<sub>1-6</sub>alkyl. There may be one, two, three or more hydrogen atoms replaced with a halogen, so the haloC<sub>1-6</sub>alkyl may have

one, two, three or more halogens. Examples of such groups include fluoroethyl, fluoromethyl, trifluoromethyl or trifluoroethyl and the like.

5 The term 'haloC<sub>1-6</sub>alkoxy' as used herein as a group or part of a group refers to a -O-C<sub>1-6</sub>alkyl group as defined herein wherein one or more than one hydrogen atom is replaced with a halogen. The term 'haloC<sub>1-6</sub>alkoxy' therefore includes monohaloC<sub>1-6</sub>alkoxy, and also polyhaloC<sub>1-6</sub>alkoxy. There may be one, two, three or more hydrogen atoms replaced with a halogen, so the haloC<sub>1-6</sub>alkoxy may have one, two, three or more halogens. Examples of such groups include  
10 fluoroethoxy, difluoromethoxy or trifluoromethoxy and the like.

The term 'C<sub>3-8</sub> cycloalkyl' as used herein as a group or part of a group refers to a saturated hydrocarbon ring containing from 3 to 8 carbon atoms. Examples of such groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl,  
15 cyclooctyl and the like.

The term 'benzyl' as used herein as a group or part of a group refers to a benzene ring attached to a CH<sub>2</sub> group.

20 For the avoidance of doubt, unless otherwise indicated, the term "substituted" means substituted by one or more defined groups. In the case where groups may be selected from a number of alternative groups, the selected groups may be the same or different.

25 For the avoidance of doubt, the term "independently" means that where more than one substituent is selected from a number of possible substituents, those substituents may be the same or different.

In one embodiment, each of W, Y and Z represent CH.

30

In one embodiment, Ring A represents formula (i) or (ii). In a further embodiment, Ring A represents formula (i). In an alternative embodiment, Ring A represents formula (ii). In a yet further alternative embodiment, Ring A represents formula (iii).



In one embodiment, X represents S or O. In a further embodiment, X represents S (*i.e.* a thiazole ring). In an alternative embodiment, X represents O (*i.e.* an oxazole ring).

5

In one embodiment, R<sup>5</sup> represents hydrogen or halogen (such as bromo). In a further embodiment, R<sup>5</sup> represents hydrogen.

10 In one embodiment, n represents an integer from 0 to 3. In one embodiment, n represents an integer from 1 to 2. In a further embodiment, n represents 1. In an alternative embodiment, n represents 2.

15 In one embodiment, R<sup>1</sup> represents cyano, hydroxyl, halogen (such as chloro or bromo), C<sub>1-6</sub>alkoxy (such as methoxy), COOH, NO<sub>2</sub>, haloC<sub>1-6</sub>alkyl (such as trifluoromethyl) or haloC<sub>1-6</sub>alkoxy (such as trifluoromethoxy). In a further embodiment, R<sup>1</sup> represents cyano, hydroxyl, halogen (such as chloro or bromo), C<sub>1-6</sub>alkoxy (such as methoxy), COOH or NO<sub>2</sub>. In a yet further embodiment, R<sup>1</sup> represents cyano, halogen (such as chloro or bromo) or NO<sub>2</sub>, such as cyano or halogen. In a yet further embodiment, R<sup>1</sup> represents cyano. In a yet further  
20 alternative embodiment, R<sup>1</sup> represents halogen, such as chloro. In a yet further alternative embodiment, R<sup>1</sup> represents haloC<sub>1-6</sub>alkyl, such as trifluoromethyl.

It will be understood by persons skilled in the art that the R<sup>1</sup> group may be attached in the *para*, *ortho* or *meta* positions on the phenyl ring of formula (I).  
25 In one embodiment, R<sup>1</sup> is in the 2, 3, or 4 position (*i.e.* *ortho*, *meta* or *para* position), in particular the *meta* (3) and/or *para* (4) position. In a further embodiment, R<sup>1</sup> is in the *meta* (3) position, for example when R<sup>1</sup> is halogen or cyano, in particular halogen. In an alternative embodiment, R<sup>1</sup> is in the *para* (4) position, for example when R<sup>1</sup> is cyano, chloro or trifluoromethyl.

30

In one embodiment, n represents 1 and said R<sup>1</sup> group is present on the 3 or 4 position on the phenyl ring of formula (I).

In one embodiment, n represents 1 and R<sup>1</sup> represents cyano, hydroxyl, halogen (such as chloro or bromo), C<sub>1-6</sub>alkoxy (such as methoxy), COOH, NO<sub>2</sub> or haloC<sub>1-6</sub>alkyl (such as trifluoromethyl). In a further embodiment, n represents 1 and R<sup>1</sup> represents cyano, hydroxyl, halogen (such as chloro or bromo), C<sub>1-6</sub>alkoxy (such as methoxy), COOH or NO<sub>2</sub>.

In one embodiment, n represents 2 and said R<sup>1</sup> group is present on the 2 and 4 or 3 and 4 or 2 and 3 positions on the phenyl ring of formula (I), such as the 2 and 4 or 3 and 4 positions.

In one embodiment, n represents 2 and both R<sup>1</sup> groups represent chloro (*i.e.* 3,4-dichloro) or R<sup>1</sup> represents C<sub>1-6</sub>alkoxy (e.g. methoxy) and hydroxyl (*i.e.* 2-hydroxy-4-methoxy).

In an alternative embodiment, n represents 2 and both R<sup>1</sup> groups represent hydroxy (*i.e.* 3,4-dihydroxy).

In one embodiment, R<sup>2</sup> represents hydrogen or C<sub>2-6</sub>alkynyl (such as ethynyl). In a further embodiment, R<sup>2</sup> represents hydrogen. In a further alternative embodiment, R<sup>2</sup> represents ethynyl.

In one embodiment, R<sup>3</sup> represents -N=R<sup>4</sup>.

In one embodiment, R<sup>4</sup> represents -C(R<sup>4a</sup>)(R<sup>4b</sup>) (such as -C(Me)<sub>2</sub>), unsubstituted C<sub>3-8</sub> cycloalkyl, unsubstituted C<sub>3-8</sub> cycloalkenyl or unsubstituted benzyl. In a further embodiment, R<sup>4</sup> represents unsubstituted C<sub>3-8</sub> cycloalkyl or unsubstituted benzyl.

In one embodiment, R<sup>4</sup> represents C<sub>3-8</sub> cycloalkyl, such as cyclopentyl or cyclohexyl (such as unsubstituted cyclopentyl or unsubstituted cyclohexyl). In a further embodiment, R<sup>4</sup> represents C<sub>3-8</sub> cycloalkyl, such as cyclopentyl (such as unsubstituted cyclopentyl).

In an alternative embodiment, R<sup>4</sup> represents C<sub>3-8</sub> cycloalkenyl, such as cyclopentenyl or cyclohexenyl (such as unsubstituted cyclopentenyl or unsubstituted cyclohexenyl).

- 5 In one embodiment, R<sup>4</sup> is optionally substituted by 1 or 2 groups, selected from C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, halogen, haloC<sub>1-6</sub>alkyl, C<sub>1-6</sub> alkoxy, haloC<sub>1-6</sub> alkoxy or hydroxyl.

In one embodiment, the compound of formula (I) is selected from Compounds 1  
10 to 21 or an alternative pharmaceutically acceptable salt, solvate, free acid preparation or free base preparation thereof. In a further embodiment, the compound of formula (I) is selected from Compounds 1 to 12 or an alternative pharmaceutically acceptable salt, solvate, free acid preparation or free base preparation thereof. In a yet further embodiment, the compound of formula (I)  
15 is selected from Compounds 1 to 11, such as Compounds 1 to 8, or an alternative pharmaceutically acceptable salt, solvate, free acid preparation or free base preparation thereof. In a yet further embodiment, the compound of formula (I) is selected from Compounds 1 to 3 or an alternative pharmaceutically acceptable salt, solvate, free acid preparation or free base  
20 preparation thereof.

In an alternative embodiment, the compound of formula (I) is a compound selected from: Compounds 1, 3, 4, 5, 6, 7 and 8, such as Compound 3 or 8 or  
25 an alternative pharmaceutically acceptable salt, solvate, free acid preparation or free base preparation thereof.

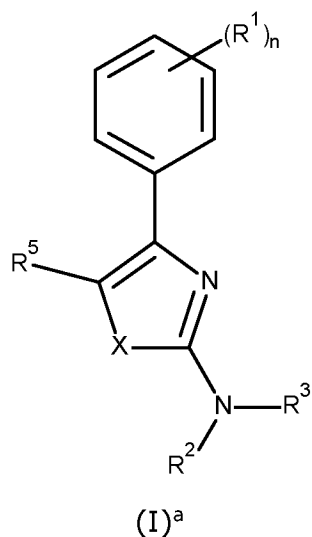
In one embodiment, the compound of formula (I) is 4-(4-cyanophenyl)-2-(2-cyclopentylidenehydrazinyl)thiazole (Compound 1) or a pharmaceutically acceptable salt, solvate, free acid preparation or free base preparation thereof.

30 In an alternative embodiment, the compound of formula (I) is 4-(4-chlorophenyl)-2-(2-cyclopentylidenehydrazinyl)thiazole or a pharmaceutically acceptable salt, solvate, free acid preparation or free base preparation thereof, such as the hydrochloride salt.

In an alternative embodiment, the compound of formula (I) is 4-(4-chlorophenyl)-2-(2-cyclopentylidene-1-(prop-2-yn-1-yl)hydrazinyl)thiazole (Compound 3) or a pharmaceutically acceptable salt, solvate, free acid preparation or free base preparation thereof.

5 In an alternative embodiment, the compound of formula (I) is 4-(4-trifluoromethylphenyl)-2-(2-cyclopentylidenehydrazinyl)thiazole (Compound 13) or a pharmaceutically acceptable salt, solvate, free acid preparation or free base preparation thereof, such as the hydrobromide salt.

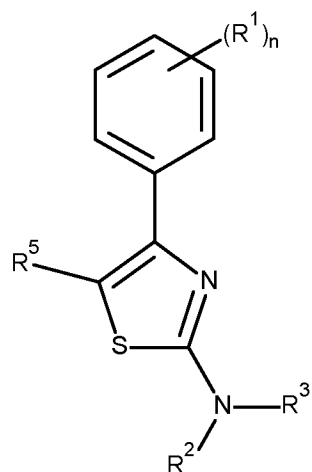
10 According to a further aspect of the invention, there is provided a compound of formula (I)<sup>a</sup>:



15 wherein R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>5</sup>, X and n are as defined hereinbefore, for use in the treatment or prevention of laminopathy, premature ageing disorders, normal ageing or cancer.

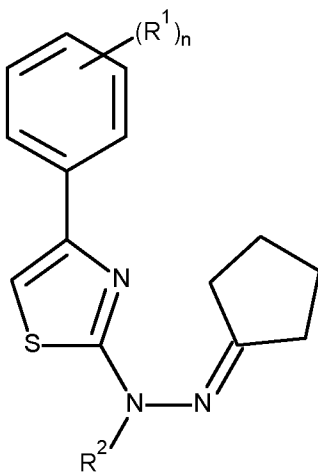
According to a further aspect of the invention, there is provided a compound of formula (I)<sup>b</sup>:

19

(I)<sup>b</sup>

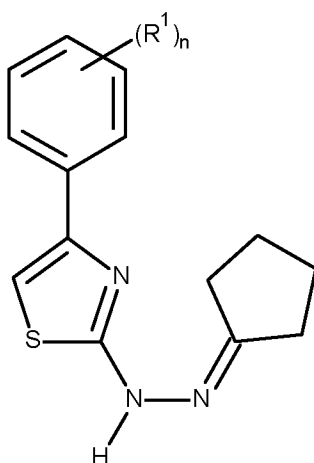
wherein R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>5</sup> and n are as defined hereinbefore, for use in the treatment or prevention of laminopathy, premature ageing disorders, normal ageing or cancer.

According to a further aspect of the invention, there is provided a compound of formula (I)<sup>c</sup>:

(I)<sup>c</sup>

wherein R<sup>1</sup>, R<sup>2</sup> and n are as defined hereinbefore, for use in the treatment or prevention of laminopathy, premature ageing disorders, normal ageing or cancer.

According to a further aspect of the invention, there is provided a compound of formula (I)<sup>d</sup>:

(I)<sup>d</sup>

wherein R<sup>1</sup> and n are as defined hereinbefore, for use in the treatment or prevention of laminopathy, premature ageing disorders, normal ageing or cancer.

Certain compounds of formula (I) are novel, thus according to a further aspect of the invention, there is provided a compound of formula (I) selected from: Compound 3, Compound 8, Compound 11, Compounds 13-17 and Compounds 19-21; and salts and solvates of any one thereof.

In one embodiment, the compound is selected from Compound 3 or 8.

### **NAT10 inhibition**

More generally, it will be understood that the inventors have discovered a novel link between NAT10 inhibition and improving nuclear architecture and chromatin organisation in Lamin A/C depleted cells or cells with *LMNA* mutations. Therefore, in a further aspect of the invention, there is provided a NAT10 inhibitor for use in the treatment or prevention of laminopathy, premature ageing disorders, normal ageing and cancer (such as a cancer characterised by low levels of LMNA expression), in particular, laminopathy and premature ageing disorders.

N-acetyltransferase 10, also referred to as 'NAT10' (or initially as hALP), is a N-acetyltransferase enzyme that is highly conserved from bacteria to human (see

Figure 8). Mammalian NAT10 has been proposed to be a lysine acetyltransferase (KAT), while its bacterial homolog, TmcA, is a known RNA cytosine acetyltransferase (Chimnaronk *et al.* (2009) *Embo. J.* 28, 1362). KAT enzymes have the ability to modulate the acetylation status of histones and other proteins, thus affecting global chromatin organization. Therefore, references herein to the term 'NAT10 inhibitor' refer to molecules which are able to inhibit the acetyl-transferase activity of NAT10. Known substrates of mammalian NAT10 are histones and tubulin (Shen *et al.* (2009) *Exp. Cell Res.* 315, 1653).

The inventors have identified small molecule inhibitors of NAT10 which result in nuclear morphology rescue via reorganization of the microtubule network. Thus, the identified small molecule inhibitors of NAT10 disclosed herein provide new opportunities to study laminopathy-associated processes with valuable spatial and temporal resolution, and can be used to alleviate laminopathies or diseases which cause premature ageing.

It will be apparent to persons skilled in the art that inhibition may occur either by binding to NAT10 directly or indirectly.

#### **Methods of screening**

According to a further aspect of the invention, a method of screening for substances capable of inhibiting NAT10 activity is provided, as are NAT10 inhibitors and their use in therapy.

In light of the fact that the inventors have described a link between NAT10 inhibition and the treatment or prevention of laminopathy, premature ageing disorders, normal ageing or cancer, there is also provided a method of screening for a candidate drug substance intended to prevent or treat or prevent laminopathy, premature ageing disorders, normal ageing or cancer in a subject which comprises identifying a test substance capable of inhibiting NAT10 activity by measuring the effects of said test substance on NAT10 activity.

In one embodiment, the method of screening comprises:

- a. contacting Lamin A and/or Lamin C depleted cells with a test substance;

- b. measuring the level of NAT10 activity after a set period; and
- c. comparing the level of NAT10 activity measured to that observed when no test substance is added.

5 In one embodiment, the method of screening is performed *in vitro*.

In one embodiment, the Lamin A and/or Lamin C depleted cells are obtained by using small interfering RNA (siRNA) to interfere with the expression of the *LMNA* gene. In a further embodiment, the Lamin A and/or Lamin C depleted cells are  
10 obtained by using siRNA duplexes of **SEQ ID NOs: 1** and **2**.

It will be appreciated by the person skilled in the art that the methods of screening provide concrete guidance for the identification of compounds capable of inhibiting NAT10 activity and rescuing nuclear shape defects.

15

### ***Preparation of compounds***

In the present context, the term "pharmaceutically acceptable salt" is intended to indicate salts which are not harmful to the patient. Such salts include pharmaceutically acceptable acid addition salts, pharmaceutically acceptable  
20 metal salts and pharmaceutically acceptable alkaline addition salts. Acid addition salts include salts of inorganic acids as well as organic acids.

A reference to a compound of formula (I) and sub-groups thereof also includes ionic forms, salts, solvates, isomers (including geometric and stereochemical  
25 isomers), tautomers, N-oxides, esters, prodrugs, isotopes and protected forms thereof, for example, as discussed below; preferably, the salts or tautomers or isomers or N-oxides or solvates thereof; and more preferably, the salts or tautomers or N-oxides or solvates thereof, even more preferably the salts or tautomers or solvates thereof. Hereinafter, compounds and their ionic forms,  
30 salts, solvates, isomers (including geometric and stereochemical isomers), tautomers, N-oxides, esters, prodrugs, isotopes and protected forms thereof as defined in any aspect of the invention (except intermediate compounds in chemical processes) are referred to as "compounds of the invention".



The compounds described herein include their use in the form of any and all stereoisomers (e.g. diastereoisomers and enantiomers as appropriate). For example, chiral compounds are claimed or claimed to be used as single enantiomers or mixtures of enantiomers (e.g. a racemic mixture).

5

Many compounds of formula (I) can exist in the form of salts, for example acid addition salts or, in certain cases salts of organic and inorganic bases such as carboxylate, sulfonate and phosphate salts. All such salts are within the scope of this invention, and references to compounds of formula (I) include the salt forms  
10 of the compounds.

The salts of the present invention can be synthesized from the parent compound that contains a basic or acidic moiety by conventional chemical methods such as methods described in *Pharmaceutical Salts: Properties, Selection, and Use*, P.  
15 Heinrich Stahl (Editor), Camille G. Wermuth (Editor), ISBN: 3-90639-026-8, Hardcover, 388 pages, August 2002. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media such as ether, ethyl acetate, ethanol, isopropanol,  
20 or acetonitrile are used.

Acid addition salts (mono- or di-salts) may be formed with a wide variety of acids, both inorganic and organic. Examples of acid addition salts include mono- or di-salts formed with an acid selected from the group consisting of acetic, 2,2-  
25 dichloroacetic, adipic, alginic, ascorbic (e.g. L-ascorbic), L-aspartic, benzenesulfonic, benzoic, 4-acetamidobenzoic, butanoic, (+) camphoric, camphor-sulfonic, (+)-(1S)-camphor-10-sulfonic, capric, caproic, caprylic, cinnamic, citric, cyclamic, dodecylsulfuric, ethane-1,2-disulfonic, ethanesulfonic, 2-hydroxyethanesulfonic, formic, fumaric, galactaric, gentisic, glucoheptonic, D-  
30 gluconic, glucuronic (e.g. D-glucuronic), glutamic (e.g. L-glutamic),  $\alpha$ -oxoglutaric, glycolic, hippuric, hydrohalic acids (e.g. hydrobromic, hydrochloric, hydriodic), isethionic, lactic (e.g. (+)-L-lactic, ( $\pm$ )-DL-lactic), lactobionic, maleic, malic, (-)-L-malic, malonic, ( $\pm$ )-DL-mandelic, methanesulfonic, naphthalene-2-sulfonic, naphthalene-1,5-disulfonic, 1-hydroxy-2-naphthoic, nicotinic, nitric,

oleic, orotic, oxalic, palmitic, pamoic, phosphoric, propionic, pyruvic, L-pyroglutamic, salicylic, 4-amino-salicylic, sebacic, stearic, succinic, sulfuric, tannic, (+)-L-tartaric, thiocyanic, *p*-toluenesulfonic, undecylenic and valeric acids, as well as acylated amino acids and cation exchange resins.

5

One particular group of salts consists of salts formed from acetic, hydrochloric, hydriodic, phosphoric, nitric, sulfuric, citric, lactic, succinic, maleic, malic, isethionic, fumaric, benzenesulfonic, toluenesulfonic, methanesulfonic (mesylate), ethanesulfonic, naphthalenesulfonic, valeric, acetic, propanoic, 10 butanoic, malonic, glucuronic and lactobionic acids. One particular salt is the hydrochloride salt. Another particular salt is the hydrogensulfate salt, also known as a hemisulfate salt

Where the compounds of formula (I) contain an amine function, these may form 15 quaternary ammonium salts, for example by reaction with an alkylating agent according to methods well known to the skilled person. Such quaternary ammonium compounds are within the scope of formula (I).

It will be understood that the compounds of the invention may exist as mono- or 20 di-salts.

The salt forms of the compounds of the invention are typically pharmaceutically acceptable salts, and examples of pharmaceutically acceptable salts are discussed in Berge *et al.*, 1977, "Pharmaceutically Acceptable Salts," *J. Pharm. Sci.*, Vol. 66, pp. 1-19. However, salts that are not pharmaceutically acceptable 25 may also be prepared as intermediate forms which may then be converted into pharmaceutically acceptable salts. Such non-pharmaceutically acceptable salts forms, which may be useful, for example, in the purification or separation of the compounds of the invention, also form part of the invention.

30

Those skilled in the art of organic chemistry will appreciate that many organic compounds can form complexes with solvents in which they are reacted or from which they are precipitated or crystallized. These complexes are known as "solvates". For example, a complex with water is known as a "hydrate".

Pharmaceutically acceptable solvates of the compound of the invention are within the scope of the invention. In one embodiment, the pharmaceutically acceptable solvates of the compounds of the invention include hydrates thereof.

5 It will be appreciated by those skilled in the art that certain protected derivatives of compounds of formula (I), which may be made prior to a final deprotection stage, may not possess pharmacological activity as such, but may, in certain instances, be administered orally or parenterally and thereafter metabolised in the body to form compounds of the invention which are pharmacologically active. Such  
10 derivatives may therefore be described as "prodrugs". All such prodrugs of compounds of the invention are included within the scope of the invention. Examples of pro-drug functionality suitable for the compounds of the present invention are described in *Drugs of Today*, Volume 19, Number 9, 1983, pp 499 – 538 and in *Topics in Chemistry*, Chapter 31, pp 306 – 316 and in "Design of  
15 Prodrugs" by H. Bundgaard, Elsevier, 1985, Chapter 1 (the disclosures in which documents are incorporated herein by reference). It will further be appreciated by those skilled in the art, that certain moieties, known to those skilled in the art as "pro-moieties", for example as described by H. Bundgaard in "Design of Prodrugs" (the disclosure in which document is incorporated herein by reference) may be  
20 placed on appropriate functionalities when such functionalities are present within compounds of the invention.

Also included within the scope of the compound and various salts of the invention are polymorphs thereof.

25 Compounds of formula (I) may exist in a number of different geometric isomeric, and tautomeric forms and references to compounds of formula (I) include all such forms.

30 The present invention includes all pharmaceutically acceptable isotopically-labelled compounds of the invention, *i.e.* compounds of formula (I), wherein one or more atoms are replaced by atoms having the same atomic number, but an atomic mass or mass number different from the atomic mass or mass number usually found in nature.

Examples of isotopes suitable for inclusion in the compounds of the invention comprise isotopes of hydrogen, such as  $^2\text{H}$  (D) and  $^3\text{H}$  (T), carbon, such as  $^{11}\text{C}$ ,  $^{13}\text{C}$  and  $^{14}\text{C}$ , chlorine, such as  $^{36}\text{Cl}$ , fluorine, such as  $^{18}\text{F}$ , iodine, such as  $^{123}\text{I}$ ,  $^{125}\text{I}$  and  $^{131}\text{I}$ , nitrogen, such as  $^{13}\text{N}$  and  $^{15}\text{N}$ , oxygen, such as  $^{15}\text{O}$ ,  $^{17}\text{O}$  and  $^{18}\text{O}$ , phosphorus, such as  $^{32}\text{P}$ , and sulfur, such as  $^{35}\text{S}$ .

Certain isotopically-labelled compounds of formula (I), for example, those incorporating a radioactive isotope, are useful in drug and/or substrate tissue distribution studies. The compounds of formula (I) can also have valuable diagnostic properties in that they can be used for detecting or identifying the formation of a complex between a labelled compound and other molecules, peptides, proteins, enzymes or receptors. The detecting or identifying methods can use compounds that are labelled with labelling agents such as radioisotopes, enzymes, fluorescent substances, luminous substances (for example, luminol, luminol derivatives, luciferin, aequorin and luciferase), etc. The radioactive isotopes tritium, *i.e.*  $^3\text{H}$  (T), and carbon-14, *i.e.*  $^{14}\text{C}$ , are particularly useful for this purpose in view of their ease of incorporation and ready means of detection.

Substitution with heavier isotopes such as deuterium, *i.e.*  $^2\text{H}$  (D), may afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased *in vivo* half-life or reduced dosage requirements, and hence may be preferred in some circumstances.

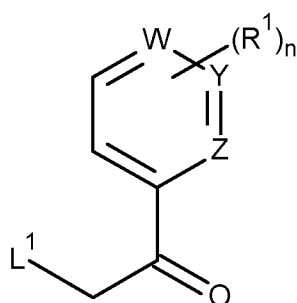
Substitution with positron emitting isotopes, such as  $^{11}\text{C}$ ,  $^{18}\text{F}$ ,  $^{15}\text{O}$  and  $^{13}\text{N}$ , can be useful in Positron Emission Topography (PET) studies for examining target occupancy.

Isotopically-labeled compounds of formula (I) can generally be prepared by conventional techniques known to those skilled in the art or by processes analogous to those described in the accompanying Examples and Preparations using appropriate isotopically-labeled reagents in place of the non-labeled reagent previously employed.

**Processes for preparing compounds**

According to a further aspect of the invention there is provided a process for preparing a compound of formula (I) as herein defined which comprises:

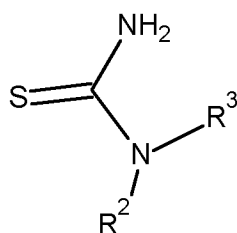
- 5 (a) when A represents (i), reacting a compound of formula (II)



(II)

wherein W, Y, Z, R<sup>1</sup> and n are as defined hereinbefore and L<sup>1</sup> represents a suitable leaving group, such as chlorine or bromine; with a compound of formula

- 10 (III):



(III)

wherein R<sup>2</sup> and R<sup>3</sup> are as defined hereinbefore;

- 15 (b) deprotection of a protected derivative of a compound of formula (I);  
and/or
- (c) interconversion of a compound of formula (I) or protected derivative thereof to a further compound of formula (I) or protected derivative thereof; and
- 20 (d) optional formation of a pharmaceutically acceptable salt of a compound of formula (I).

Process (a) typically comprises reacting a compound of formula (II) with a compound of formula (III) in the presence of a suitable solvent, such as isopropanol at a suitable temperature, such as room temperature for between 6 and 24 hours. It will be appreciated by the skilled person that preparation of  
5 compounds of formula (I) wherein A represents (ii) and (iii) may be made in an analogous manner to the procedure described in process (a).

It will be appreciated by those skilled in organic synthesis that two or more chemical steps in the schemes above may be run sequentially without isolation  
10 of intermediate materials.

Process (b) typically comprises any suitable deprotection reaction, the conditions of which will depend upon the nature of the protecting group. When the protecting group represents tBoc, such a deprotection reaction will typically  
15 comprise the use of a suitable acid in a suitable solvent. For example, the acid may suitably comprise trifluoroacetic acid or hydrogen chloride and the solvent may suitably comprise dichloromethane ethyl acetate, 1,4-dioxane, methanol or water. Optionally a mixture of solvents may be used, for example aqueous methanol or ethyl acetate / 1,4-dioxane.

It will be appreciated that, when the protecting group represents tBoc, deprotection using a suitable acid as described above may generate a compound of formula (I) as a pharmaceutically acceptable salt, which may be isolated directly. Alternatively, the compound of formula (I) may be isolated as the free  
20 base using methods well known in the art and thereafter optionally converted to a pharmaceutically acceptable salt according to process (d).

A hydroxy group may be protected, for example, as an ether (-OR) or an ester (-OC(=O)R), for example, as: a t-butyl ether; a tetrahydropyranyl (THP) ether; a  
30 benzyl, benzhydryl (diphenylmethyl), or trityl (triphenylmethyl) ether; a trimethylsilyl or t-butyldimethylsilyl ether; or an acetyl ester (-OC(=O)CH<sub>3</sub>).

An aldehyde or ketone group may be protected, for example, as an acetal (R-CH(OR)<sub>2</sub>) or ketal (R<sub>2</sub>C(OR)<sub>2</sub>), respectively, in which the carbonyl group (>C=O)

is treated with, for example, a primary alcohol. The aldehyde or ketone group is readily regenerated by hydrolysis using a large excess of water in the presence of acid.

- 5 An amine group may be protected, for example, as an amide (-NRCO-R) or a carbamate (-NRCO-OR), for example, as: a methyl amide (-NHCO-CH<sub>3</sub>); a benzyl carbamate (-NHCO-OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>, -NH-Cbz or NH-Z); as a t-butyl carbamate (-NHCO-OC(CH<sub>3</sub>)<sub>3</sub>, -NH-Boc); a 2-biphenyl-2-propyl carbamate (-NHCO-OC(CH<sub>3</sub>)<sub>2</sub>C<sub>6</sub>H<sub>4</sub>C<sub>6</sub>H<sub>5</sub>, -NH-Boc), as a 9-fluorenylmethyl carbamate (-NH-Fmoc), as  
10 a 6-nitroveratryl carbamate (-NH-Nvoc), as a 2-trimethylsilylethyl carbamate (-NH-Teoc), as a 2,2,2-trichloroethyl carbamate (-NH-Troc), as an allyl carbamate (-NH-Alloc), or as a 2(-phenylsulphonyl)ethyl carbamate (-NH-Psec).

Other protecting groups for amines, such as cyclic amines and heterocyclic N-H  
15 groups, include toluenesulphonyl (tosyl) and methanesulphonyl (mesyl) groups, benzyl groups such as a *para*-methoxybenzyl (PMB) group and tetrahydropyranyl (THP) groups.

Process (c) typically comprises interconversion procedures known by one skilled  
20 in the art. For example, in compounds of formula (I), a first substituent may be converted by methods known by one skilled in the art into a second, alternative substituent. A wide range of well known functional group interconversions are known by a person skilled in the art for converting a precursor compound to a compound of formula I and are described in *Advanced Organic Chemistry* by  
25 Jerry March, 4<sup>th</sup> Edition, John Wiley & Sons, 1992. For example possible metal catalysed functionalisations such as using organo-tin reagents (the Stille reaction), Grignard reagents and reactions with nitrogen nucleophiles are described in 'Palladium Reagents and Catalysts' [Jiro Tsuji, Wiley, ISBN 0-470-85032-9] and Handbook of Organopalladium Chemistry for Organic Synthesis  
30 [Volume 1, Edited by Ei-ichi Negishi, Wiley, ISBN 0-471-31506-0].

Process (d) may be carried out by treatment of a compound of formula (I) in the free base form, dissolved in a suitable solvent, with a stoichiometric amount or an excess of a pharmaceutically acceptable organic or inorganic acid, then

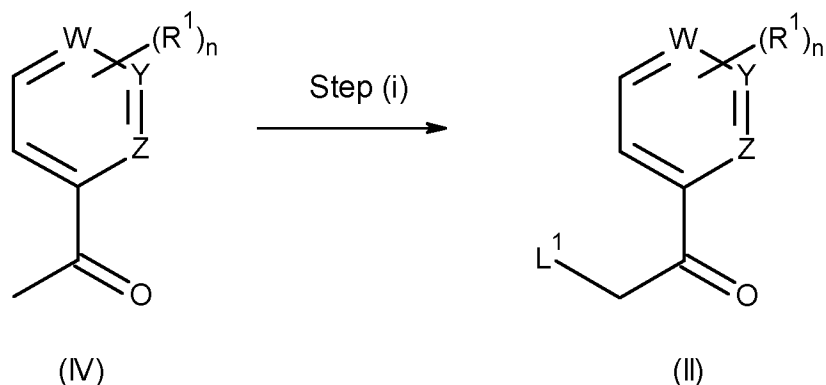
isolation of the resulting salt by methods well known in the art, e.g. evaporation of solvent or crystallisation.

If appropriate, the reactions previously described in processes (a), (b) and (c) are followed or preceded by one or more reactions known to the skilled of the art and are performed in an appropriate order to achieve the requisite substitutions on  $R^1$ ,  $R^2$ ,  $R^3$ ,  $R^4$  and  $R^5$  defined above to afford other compounds of formula (I). Non-limiting examples of such reactions whose conditions can be found in the literature include:

- 10 protection of reactive functions,
- deprotection of reactive functions,
- halogenation,
- dehalogenation,
- dealkylation,
- 15 alkylation and arylation of amine, aniline, alcohol and phenol,
- Mitsunobu reaction on hydroxyl groups,
- cycloaddition reactions on appropriate groups,
- reduction of nitro, esters, cyano, aldehydes,
- transition metal-catalyzed coupling reactions,
- 20 acylation,
- sulfonylation/introduction of sulfonyl groups,
- saponification/hydrolysis of ester groups,
- amidification or transesterification of ester groups,
- esterification or amidification of carboxylic groups,
- 25 halogen exchange,
- nucleophilic substitution with amine, thiol or alcohol,
- reductive amination,
- oxime formation on carbonyl and hydroxylamine groups,
- S-oxidation,
- 30 N-oxidation,
- salification.

Compounds of formula (II) may be prepared from compounds of formula (IV) in accordance with the following Scheme 1:



**Scheme 1**

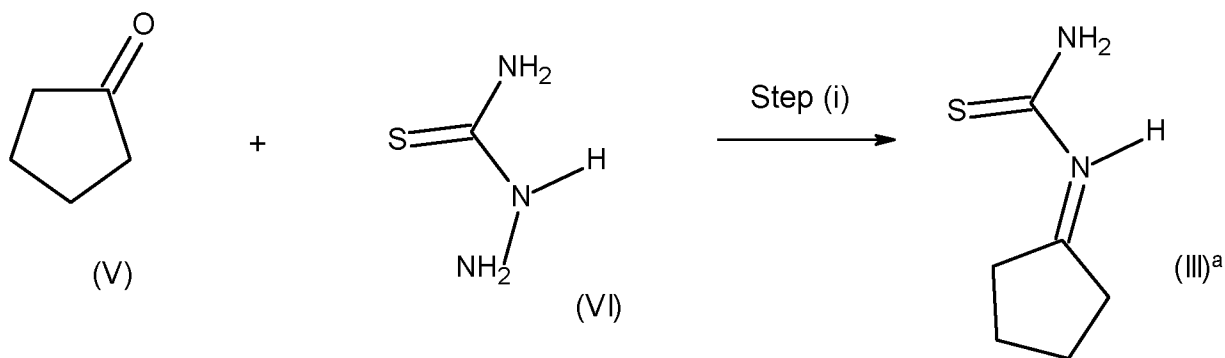
wherein W, Y, Z, R<sup>1</sup>, n and L<sup>1</sup> are as defined hereinbefore.

5

Step (i) typically comprises the use of suitable reagents, such as 1.1 eq NXS, 1.5 eq pTSA and MeCN followed by heating to reflux for 2 to 24 hours.

10 It will be appreciated by the skilled person that compounds of formula (III) are either known or may be prepared from known materials in accordance with known procedures. For example, compounds of formula (V) where R<sup>2</sup> represents hydrogen and R<sup>3</sup> represents -N=cyclopropyl may be prepared in accordance with the following Scheme 2:

15

**Scheme 2**

Step (i) typically comprises reacting compounds of formula (V) and (VI) in a suitable solvent such as isopropanol followed by heating to reflux for 16 hours.

20 The required intermediates, for example compounds of formula (IV), (V) and (VI) are either commercially available, known in the literature, prepared by

methods analogous to those in the literature or prepared by methods analogous to those described in the example experimental procedures below.

### ***Methods of treatment***

- 5 As discussed hereinabove, it is believed that compounds of the invention may be useful in the treatment or prevention of disorders associated with Lamin A and/or Lamin C depletion or *LMNA* mutations, such as laminopathy, premature ageing disorders, normal ageing and cancer, in particular, laminopathy and premature ageing disorders.
- 10 Therefore, according to a further aspect of the invention, there is provided compounds of the invention for use as a medicament, preferably a human medicament.
- 15 According to a further aspect the invention provides the use of compounds of the invention in the manufacture of a medicament for treating or preventing laminopathy, premature ageing disorders, normal ageing or cancer, in particular laminopathy or premature ageing.
- 20 According to a further aspect of the invention there is provided a method of treating or preventing laminopathy, premature ageing disorders, normal ageing or cancer in a human in need thereof comprising administering to said human a therapeutically effective amount of a compound as defined herein.
- 25 References herein to "laminopathy" refer to a group of genetic disorders which are caused by mutations in genes encoding proteins of the nuclear lamina, *e.g.* Lamin A/C depletion and/or defects in the *LMNA* gene. Laminopathies comprise dystrophic and progeric (*i.e.* premature ageing) diseases.
- 30 Examples of laminopathies include, but are not limited to: Atypical Werner syndrome; Barraquer-Simons syndrome; Buschke-Ollendorff syndrome; Cardiomyopathy; Charcot-Marie-Tooth disease; Emery-Dreifuss muscular dystrophy (X-linked (EDMD), autosomal dominant (EDMD2) or autosomal recessive (EDMD3)); Familial partial lipodystrophy of the Dunnigan type (FPLD);

Greenberg dysplasia; Hutchinson-Gilford progeria syndrome (HGPS); Leukodystrophy, demyelinating, adult-onset, autosomal dominant (ADLD); Limb-girdle muscular dystrophy type 1B (LGMD1B); Lipoatrophy with diabetes, hepatic steatosis, hypertrophic cardiomyopathy, and leukomelanodermic papules (LDHCP); Mandibuloacral dysplasia with type A lipodystrophy (MADA);  
5 Mandibuloacral dysplasia with type B lipodystrophy (MADB); Pelger-Huet anomaly (PHA); Pelizaeus-Merzbacher disease; or Restrictive Dermopathy (RD).

References herein to "premature ageing" refer to a disorder that causes  
10 accelerated ageing in an individual. Such disorders may be referred to as "progeric disorders" and include: Hutchinson-Gilford progeria syndrome (HGPS); Werner syndrome; Bloom syndrome; Rothmund-Thomson syndrome; Cockayne syndrome; or Xeroderma pigmentosum.

15 *LMNA* down-regulation has also been reported in several types of cancer (see Zink *et al.* (2004) *Nat. Rev.*; Wu *et al.* (2009) *J. Exp. Clin. Cancer Res.*). The complete loss of Lamin A expression that is observed in some cancers suggests that lamins may act as tumour suppressors (J.L. Broers, *et al.*, *Am J Pathol* 1993, S.F. Moss, *et al.*, *Gut* 1999, R.S. Venables, *et al.*, *Br J Cancer* 2001).  
20 Importantly, downregulation of Lamin A/C expression is correlated with cancer aggressiveness and poor prognosis (E.J. Belt, *et al.*, *European journal of cancer* 2011, N.D. Willis, *et al.*, *PLoS One* 2008, N.D. Willis, *et al.*, *Biochem Soc Trans* 2008). Loss of a functional lamina at the inner nuclear membrane seems to contribute to misshapen nuclei of cancer cells. Moreover, decreased Lamin A/C  
25 expression is associated with softer nuclei that can squeeze more easily into small blood vessels, that could contribute to increase the invasive potential of these cancer cells. Therefore, since the compounds of the invention are able to improve nuclear shape of Lamin A/C depleted cells, it will be understood that the compounds of the invention may also be used to treat these cancers. Indeed,  
30 higher doses of the compounds reduce cancer cells invasion and migration as well as cell proliferation, in particular in cancer cells expressing low levels of Lamin A/C.

Examples of types of cancer which may be treated by the compounds described herein include, but are not limited to, breast, bowel, bladder, bone, brain, cervical, colon, endometrial, oesophageal, kidney, liver, lung, ovarian, pancreatic, prostate, skin, stomach, testicular, thyroid or uterine cancer, 5 leukemia, lymphoma, myeloma or melanoma. Cancer stem cells might also be targeted by the compounds.

The term 'medicament' as used herein refers to a pharmaceutical formulation that is of use in treating, curing or improving a disease or in treating, 10 ameliorating or alleviating the symptoms of a disease. A pharmaceutical formulation comprises a pharmacologically active ingredient in a form not harmful to the subject it is being administered to and additional constituents designed to stabilise the active ingredient and affect its absorption into the circulation or target tissue. In one embodiment, the medicament described 15 herein is a human medicament.

It will be appreciated that references herein to "treatment" extend to prophylaxis, prevention of recurrence and suppression or amelioration of symptoms (whether mild, moderate or severe) as well as the treatment of 20 established conditions.

### ***Pharmaceutical compositions***

According to a further aspect, the invention provides a pharmaceutical composition comprising a compound of the invention, in association with one or 25 more pharmaceutically acceptable carrier(s), diluents(s) and/or excipient(s) for use in the treatment or prevention of laminopathy, premature ageing disorders, normal ageing or cancer. The carrier, diluent and/or excipient must be "acceptable" in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof.

30

The compounds of the invention may also be used in combination with one or more further active ingredients. The invention thus provides, in a further aspect, a combination comprising a compound of the invention or a

pharmaceutically acceptable derivative thereof together with one or more further active ingredients.

When a compound of the invention or a pharmaceutically acceptable derivative thereof is used in combination with one or more further active ingredients against the same disease state the dose of each compound may differ from that when the compound is used alone. Appropriate doses will be readily appreciated by those skilled in the art. It will be appreciated that the amount of a compound of the invention required for use in treatment will vary with the nature of the condition being treated and the age and the condition of the patient and will be ultimately at the discretion of the attendant physician or veterinarian.

The one or more further active ingredients can be active ingredients for the treatment or prevention of laminopathy, premature ageing disorders, normal ageing or cancer. Suitable non limiting examples of such active ingredients include: pravastatin, zoledronic acid, rapamycin, farnesyl-transferase inhibitors such as lonafarnib, etc.

The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation and thus pharmaceutical formulations comprising a combination as defined above together with a pharmaceutically acceptable carrier or excipient comprise a further aspect of the invention. The individual components of such combinations may be administered either sequentially or simultaneously in separate or combined pharmaceutical formulations by any convenient route.

When administration is sequential, either the compound of the invention or the one or more further active ingredients may be administered first. When administration is simultaneous, the combination may be administered either in the same or different pharmaceutical composition.

When combined in the same formulation it will be appreciated that the two compounds must be stable and compatible with each other and the other components of the formulation. When formulated separately they may be

provided in any convenient formulation, conveniently in such manner as are known for such compounds in the art.

### ***Administration***

- 5 The compound of the invention may be administered as the raw chemical but the active ingredient is preferably presented as a pharmaceutical formulation.

The compounds of the invention may be administered in conventional dosage forms prepared by combining a compound of the invention with standard  
10 pharmaceutical carriers or diluents according to conventional procedures well known in the art. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation.

- 15 The pharmaceutical compositions of the invention may be formulated for administration by any route, and include those in a form adapted for oral, topical or parenteral administration to mammals including humans.

The compositions may be in the form of tablets, capsules, powders, granules,  
20 lozenges, creams or liquid preparations, such as oral or sterile parenteral solutions or suspensions.

The topical formulations of the present invention may be presented as, for instance, ointments, creams or lotions, eye ointments and eye or ear drops,  
25 impregnated dressings and aerosols, and may contain appropriate conventional additives such as preservatives, solvents to assist drug penetration and emollients in ointments and creams.

The formulations may also contain compatible conventional carriers, such as  
30 cream or ointment bases and ethanol or oleyl alcohol for lotions. Such carriers may be present as from about 1% up to about 98% of the formulation. More usually they will form up to about 80% of the formulation.

Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatine, sorbitol, tragacanth, or polyvinylpyrrolidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tableting lubricants, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants, for example potato starch; or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives, such as suspending agents, for example sorbitol, methyl cellulose, glucose syrup, gelatine, hydroxyethyl cellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats, emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl *p*-hydroxybenzoate or sorbic acid, and, if desired, conventional flavouring or colouring agents.

20

Suppositories will contain conventional suppository bases, *e.g.* cocoa-butter or other glyceride.

For parenteral administration, fluid unit dosage forms are prepared utilising the compound and a sterile vehicle, water being preferred. The compound, depending on the vehicle and concentration used, can be either suspended or dissolved in the vehicle. In preparing solutions the compound can be dissolved in water for injection and filter-sterilised before filling into a suitable vial or ampoule and sealing.

30

Advantageously, agents such as a local anaesthetic, preservative and buffering agents can be dissolved in the vehicle. To enhance the stability, the composition can be frozen after filling into the vial and the water removed under vacuum. The dry lyophilised powder is then sealed in the vial and an accompanying vial of

water for injection may be supplied to reconstitute the liquid prior to use. Parenteral suspensions are prepared in substantially the same manner except that the compound is suspended in the vehicle instead of being dissolved and sterilisation cannot be accomplished by filtration. The compound can be sterilised  
5 by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the compound.

The compositions may contain from 0.1% by weight, for example from 10-60%  
10 by weight, of the active material, depending on the method of administration. Where the compositions comprise dosage units, each unit will for example contain from 5-1000 mg of the active ingredient. The dosage as employed for adult human treatment may range from 10 to 3000 mg per day depending on the route and frequency of administration. For oral administration a typical dose  
15 may be in the range of 50 to 1500 mg per day, for example 120 to 1000 mg per day.

It will be recognised by one of skill in the art that the optimal quantity and spacing of individual dosages of a compound of the invention will be determined  
20 by the nature and extent of the condition being treated, the form, route and site of administration, and the particular mammal being treated, and that such optimums can be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, *i.e.*, the number of doses of a compound of the invention given per day for a defined  
25 number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

All publications, including, but not limited to, patents and patent applications cited in this specification, are herein incorporated by reference as if each  
30 individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

## **EXAMPLES**

The invention is illustrated by the Examples described below.



In the procedures that follow, after each starting material, reference to a Description or Example by number is typically provided. This is provided merely for assistance to the skilled chemist. The starting material may not necessarily  
5 have been prepared from the batch referred to.

Where reference is made to the use of a "similar" procedure, as will be appreciated by those skilled in the art, such a procedure may involve minor variation, for example reaction temperature, reagent/solvent amount, reaction  
10 time, work-up conditions or chromatographic purification conditions.

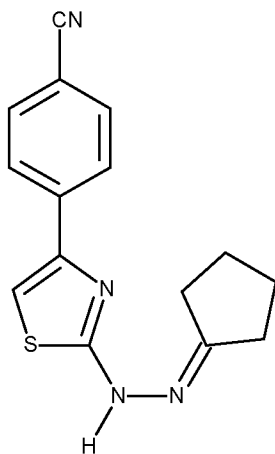
All solvents and reagents were purified using standard techniques or used as supplied from commercial sources (Sigma-Aldrich). NMR spectra were acquired on a Bruker 500 MHz instrument using deuterated solvents at 300 K. Notation  
15 for the  $^1\text{H}$  NMR spectral splitting patterns includes: singlet (s), doublet (d), triplet (t), broad (br) and multiplet/overlapping peaks (m). Signals are quoted as  $\delta$  values in ppm and coupling constants (J) are quoted in Hertz. Mass spectra were recorded on a Micromass<sup>®</sup> Q-ToF (ESI) spectrometer.

## 20 **General procedure**

The appropriate ketone or aldehyde was dissolved in isopropanol at a final concentration of 0.5 M and refluxed for 24 hours in the presence of an equimolar amount of thiosemicarbazide. The corresponding thiosemicarbazones were isolated by filtration and recrystallized from hot ethanol. Equimolar amounts of  
25 thiosemicarbazones and the desired haloketones were stirred at room temperature in isopropanol overnight at a final concentration of 0.2 M. The resulting products were recrystallized from hot ethanol several times to yield pure products and were used without further purification.

## 30 **Compound 1**

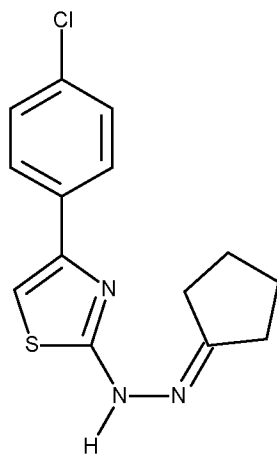
***4-(4-Cyanophenyl)-2-(2-cyclopentylidenehydrazinyl)thiazole  
(Remodelin)***



2-Cyclopentylidenehydrazine-1-carbothioamide (which may be prepared as described in Scheme 2 hereinbefore) (1 g, 4.46 mmol) and 2-bromo-4'-  
5 hereinbefore) (700 mg, 4.45 mmol) were stirred overnight in 12 ml of isopropanol at room temperature. The precipitate was filtered and recrystallized from hot ethanol to yield the hydrobromide salt of the desired compound (559 mg, 1.98 mmol, 45%) as light yellow needles. This was resuspended in DMSO at a concentration of 10mg/mL for use in cellular assays. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 12.11 (br s), 7.84 (d, J = 9.0 Hz, 2H), 7.81 (d, J = 9.0 Hz, 2H), 6.84 (s, 1H), 2.61 (t, J = 9.0 Hz, 2H), 2.51 (t, J = 9.0 Hz, 2H), 1.94–1.80 (m, 4H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): δ 173.8, 169.5, 138.8, 133.5, 131.3, 126.3, 118.0, 114.1, 103.8, 33.7, 31.2, 25.2, 25.0; HRMS (m/z): [M]<sup>+</sup> calcd. for C<sub>15</sub>H<sub>15</sub>N<sub>4</sub>S, 283.1009; found, 283.1017.

15

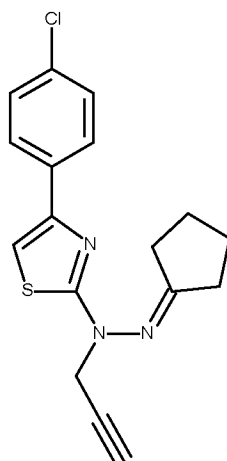
**Compound 2*****4-(4-Chlorophenyl)-2-(2-cyclopentylidenehydrazinyl)thiazole hydrochloride salt***



Thiosemicarbazide (20 g, 220 mmol) and cyclopentanone (19.43 ml, 220 mmol) were refluxed in 500 ml of isopropanol for 24 hours. The precipitate was filtered and recrystallized from hot ethanol to provide the corresponding thiosemicarbazone (2-cyclopentylidenehydrazine-1-carbothioamide) as pale yellow crystals. 2-cyclopentylidenehydrazine-1-carbothioamide (10 g, 63 mmol) and 2,4'-dichloroacetophenone (12 g, 63 mmol) were stirred overnight in 300 ml of isopropanol at room temperature. The precipitate was filtered and recrystallized from hot ethanol to yield the hydrochloride salt of the desired compound (16 g, 48 mmol, 77%) as light yellow needles. This was resuspended in DMSO at a concentration of 10mg/mL for use in cellular assays. Spectral data were in agreement with those previously described in the literature (F. Chimenti *et al.*, (2009) *J. Med. Chem.* 52, 530).

15 **Compound 3**

**4-(4-Chlorophenyl)-2-(2-cyclopentylidene-1-(prop-2-yn-1-yl)hydrazinyl)thiazole**



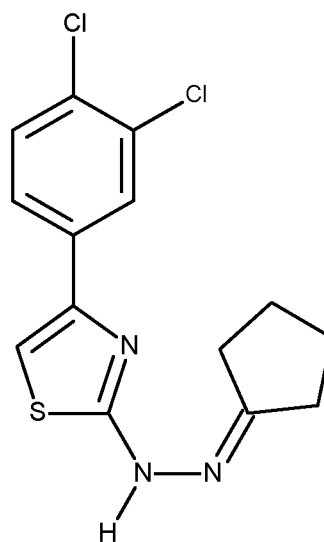
To a solution of **Compound 2** (1 g, 3 mmol) in freshly distilled DMF (75 ml) was added  $K_2CO_3$  (1.375 g, 10 mmol), triethylamine (1.4 ml, 10 mmol) and propargyl bromide (1.2 ml, 5 mmol, 80 wt. % in toluene). The solution turned purple after 12 hours at room temperature. Propargyl bromide (1.2 ml) was added and the reaction mixture was stirred for another 12 hours. The solvent was then evaporated under reduced pressure and the crude residue dissolved in  $CH_2Cl_2$ , washed several times with saturated solutions of  $NH_4Cl$  and  $NaCl$  and dried over  $MgSO_4$ . The solvent was evaporated under reduced pressure and the desired product (0.35 g, 1 mmol, 34%) was obtained as a brown oil after regular column chromatography. TLC (Hexane :  $CH_2Cl_2$ , 80 : 20):  $R_f = 0.25$ ;  $^1H$  NMR (500 MHz,  $CDCl_3$ ):  $\delta$  7.78 (d,  $J = 8.0$  Hz, 2H), 7.32 (d,  $J = 8.0$  Hz, 2H), 6.86 (s, 1H), 4.65 (s, 2H), 2.64–2.55 (m, 4H), 2.22 (s, 1H), 1.86–1.82 (m, 4H);  $^{13}C$  NMR (125 MHz,  $CDCl_3$ ):  $\delta$  184.9, 171.8, 150.6, 133.4, 133.3, 128.6 (2C), 127.3 (2C), 105.2, 78.3, 72.5, 42.7, 33.8, 31.5, 24.9, 24.4; HRMS (m/z):  $[M]^+$  calcd. for  $C_{17}H_{16}ClN_3S$ , 329.0758; found, 329.0747.

It will be understood that Compounds 4-12 may be made in an analogous manner to Compounds 1 to 3, as described above:

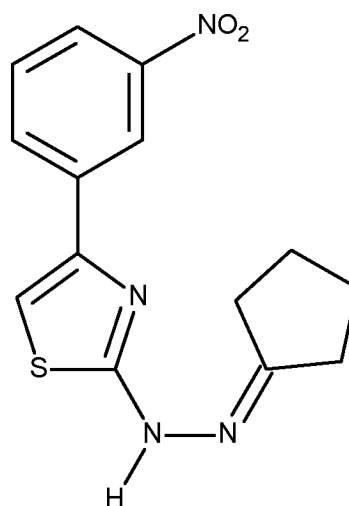
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Compound	Structure
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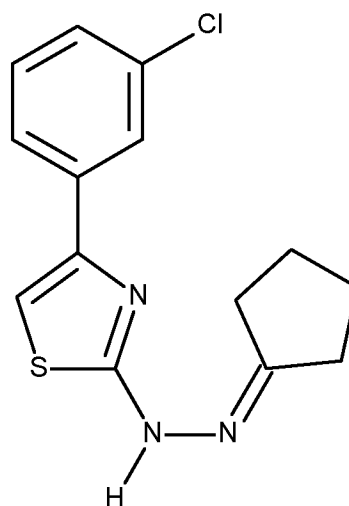
**2-(2-Cyclopentylidenehydrazinyl)-4-(3,4-dichlorophenyl)thiazole (Compound 4)**



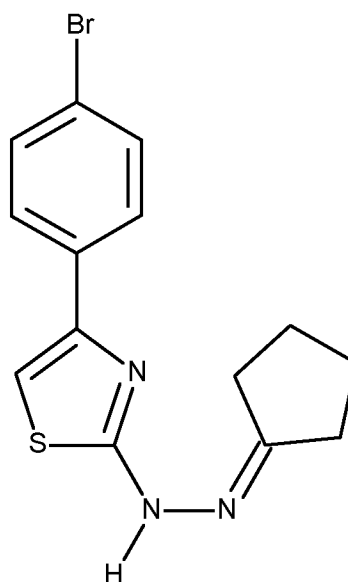
**2-(2-Cyclopentylidenehydrazinyl)-4-(3-nitrophenyl)thiazole (Compound 5)**



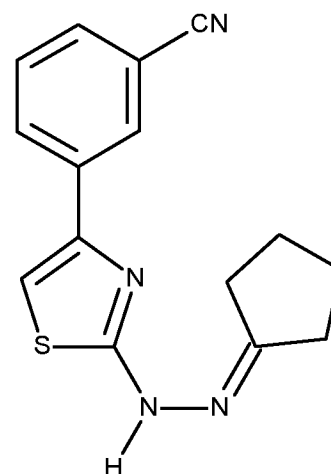
**4-(3-Chlorophenyl)-2-(2-cyclopentylidenehydrazinyl)thiazole (Compound 6)**



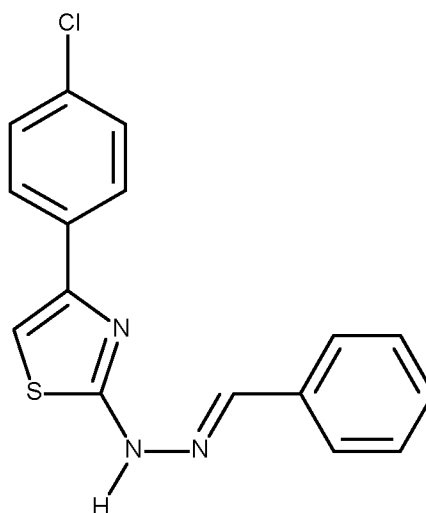
**4-(4-Bromophenyl)-2-(2-cyclopentylidenehydrazinyl)thiazole (Compound 7)**



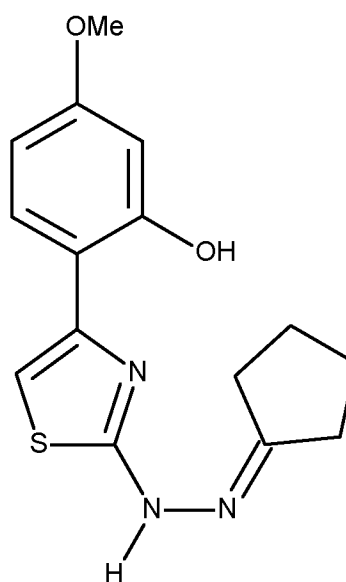
**3-(2-(2-Cyclopentylidenehydrazinyl)thiazol-4-yl)benzonitrile (Compound 8)**



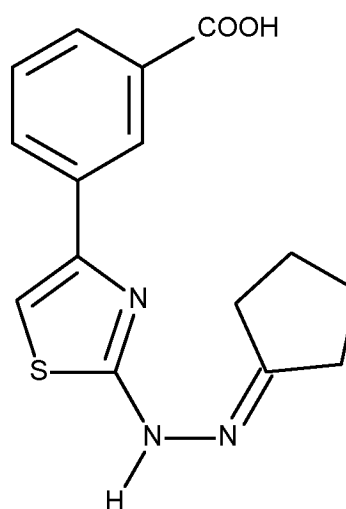
**(Z)-2-(2-Benzylidenehydrazinyl)-4-(4-chlorophenyl)thiazole (Compound 9)**

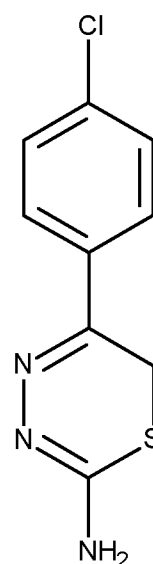
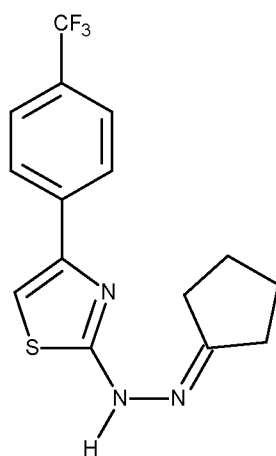


**2-(2-(2-Cyclopentylidenehydrazinyl)thiazol-4-yl)-5-methoxyphenol (Compound 10)**



**3-(2-(2-Cyclopentylidenehydrazinyl)thiazol-4-yl)benzoic acid (Compound 11)**



**5-(4-Chlorophenyl)-6H-1,3,4-thiadiazin-2-amine (Compound 12)****Compound 13****4-(4-Trifluoromethylphenyl)-2-(2-cyclopentylidenehydrazinyl)thiazole hydrobromide salt**

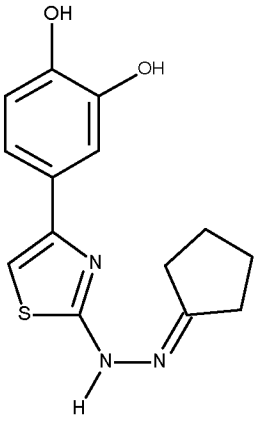
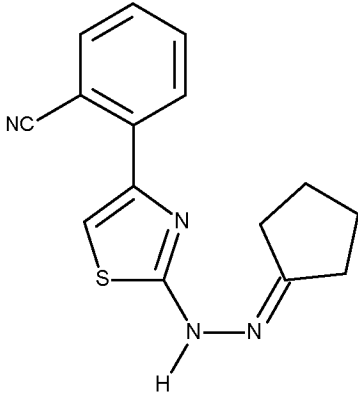
5

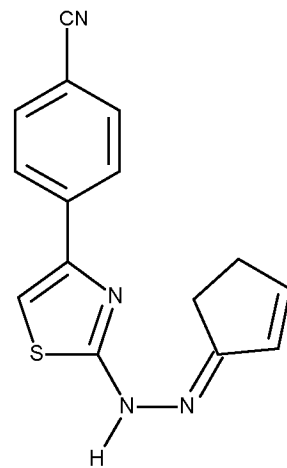
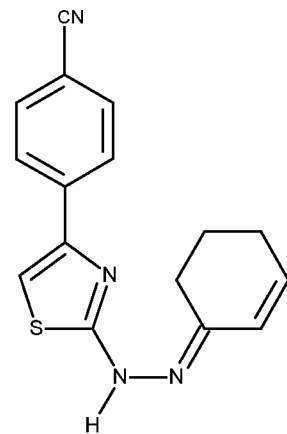
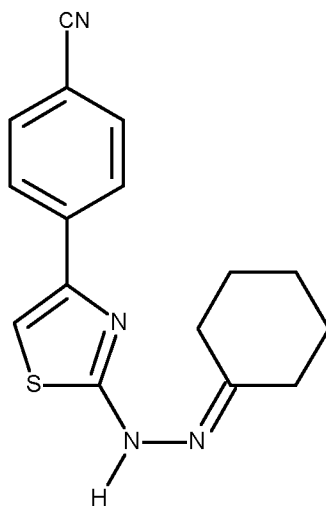
2-Cyclopentylidenehydrazine-1-carbothioamide (3.0 g, 18.7 mmol) and 2-bromo-4'-(trifluoromethyl)acetophenone (5.0g, 18.7 mmol) were stirred in isopropanol (150 mL) at r.t. for 24 h. The precipitate was filtered and recrystallized three times from hot ethanol to yield the hydrobromide salt of the title compound as bright yellow needles (1.5 g, 3.7mmol, 20%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 11.12 (br s, 2H), 7.80 (d, *J* = 8.5 Hz, 2H), 7.65 (d, *J* = 8.5 Hz, 2H), 6.87 (s, 1H), 2.53 (t, *J* = 7.0 Hz, 2H), 2.47 (t, *J* = 7.0 Hz, 2H), 1.93–1.75 (m, 4H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 171.9, 169.4, 140.3, 131.8 (q, *J* = 32.0 Hz), 131.6, 126.5 (br q, *J* = 3.5 Hz, 2C), 126.1 (2C), 123.7 (q, *J* = 274.0 Hz),

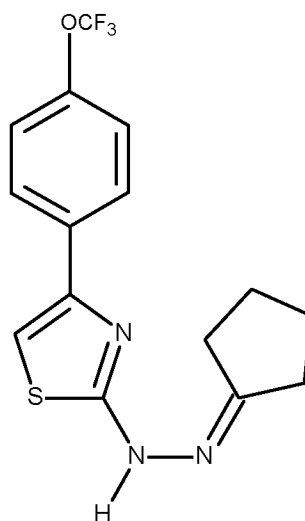
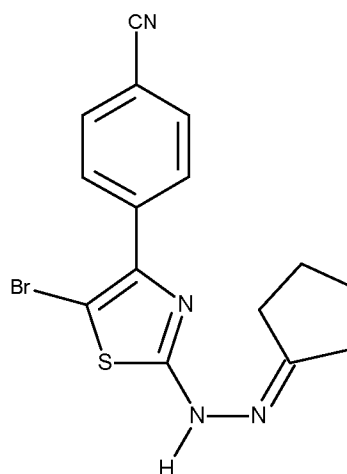
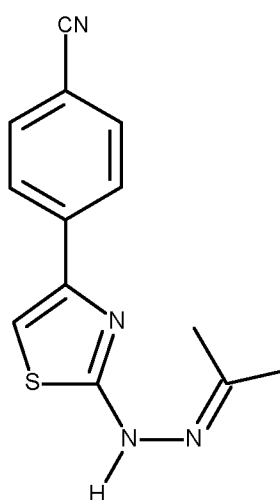


103.5, 33.5, 30.6, 25.1, 24.9. HRMS (m/z): [M+H]<sup>+</sup> calcd. for C<sub>15</sub>H<sub>15</sub>F<sub>3</sub>N<sub>3</sub>S, 326.0933; found, 326.0950.

5 It will be understood that Compounds 14-21 may be made in an analogous manner to Compounds 1 to 3 and 13, as described above:

Compound Number	Structure
<b>Compound 14</b>	
<b>Compound 15</b>	

**Compound 16****Compound 17****Compound 18**

**Compound 19****Compound 20****Compound 21****EXPERIMENTAL DATA**

## **Material and Methods**

### **Cell culture and transfections**

U2OS and SaOS-2 osteosarcoma cells, HeLa cervical cancer cells, A431 melanoma cells, NCI-SNU5 gastric cancer cells, MRC-5 lung fibroblast cells and Werner syndrome patient-derived SV40 fibroblast cells were grown in Dulbecco's modified Eagle medium (DMEM, Sigma-Aldrich) supplemented with 10% fetal bovine serum (BioSera), 2mM L-glutamine, 100U per ml penicillin, 100 µg ml<sup>-1</sup> streptomycin. Normal skin primary fibroblasts GM03440 and Hutchinson Gilford Progeria Syndrome (HGPS) skin primary fibroblasts AG11498 and AG06297 were purchased from Coriell Cell Repositories and used between passage number 9-12 and 20-23 respectively. Cells were grown in DMEM supplemented as above. RPE-1 retinal pigment epithelial cells were grown in DMEM and Ham F12 mix medium supplemented as above and buffered with sodium bicarbonate. HMV-II melanoma cell line, 22RV1 prostate carcinoma cells, NCI-H82 and NCI-H69 lung carcinoma cells, and NCI-N87 gastric cancer cell lines were grown in RPMI-1640 medium (Sigma-Aldrich) supplemented as above. Stably transfected U2OS cells were maintained in standard medium containing 1mg ml<sup>-1</sup> G418 (Invitrogen). The siRNA duplexes were obtained from Life Sciences: Lamin A/C stealth RNAi: CCAUGAAGGAGGAACUGGACUCCA (**SEQ ID NO: 1**) and GCGUGAGGAGUUUAAGGAGCUGAAA (**SEQ ID NO: 2**), NAT10 stealth RNAi: GAGCAUGGACCUCUCUGAAUACAUA (**SEQ ID NO: 3**), and as control siRNA, stealth RNAi negative control duplexes were used. Plasmid DNA and siRNA transfections were carried out using Lipofectamine 2000 and Lipofectamine RNAiMax (Life Sciences) respectively, following the manufacturer's instructions. Cells were analysed 48 to 72 hours after transfection.

### **Drug treatments**

The following KAT and KDAC inhibitors were used in the nuclear shape rescue screen, incubating cells with the molecules for 16 hours: trichostatin A (1 µM), sodium butyrate (5 mM), tubacin (10 µM), SAHA (5 µM), curcumin (10 µM), garcinol (50 µM), anacardic acid (1 µM), MB-3 (5 µM), 4-(4-chlorophenyl)-2-(2-cyclopentylidenehydrazinyl)thiazole (**Compound 2**) (50 µM), 4-(4-chlorophenyl)-2-(2-cyclopentylidene-1-(prop-2-yn-1-yl)hydrazinyl)thiazole

(**Compound 3**) (50  $\mu\text{M}$ ) and cyclopentylidene-[4-(4-cyanophenyl)thiazol-2-yl]hydrazine (**Compound 1**) (Remodelin) (10 to 50  $\mu\text{M}$ ). For HGPS cellular fitness assays, Remodelin was incubated at 1  $\mu\text{M}$  for 1 to 10 days, renewing the medium every 3 days. For long-term senescence assay, cells were kept and  
5 passaged in medium containing 1  $\mu\text{M}$  Remodelin or DMSO only for 12 population doublings. Senescence was assessed after 8 days of Remodelin treatment when cells were at Population Doubling 12, and then after several weeks of Remodelin treatment, when cells reached Population Doubling 24 using the Senescence  $\beta$ -Galactosidase Staining Kit #9860 from Cell Signaling. Nocodazole, colchicine and  
10 latrunculin A (Sigma-Aldrich) were used at 200ng/ml, 1  $\mu\text{g/ml}$  and 1  $\mu\text{M}$  respectively. Aphidicolin (Sigma-Aldrich) was used at 5  $\mu\text{g/ml}$  for 16 h. Farnesyltransferase inhibitor FTI-277 was purchased from Tocris Bioscience and used at 5  $\mu\text{M}$ . ATMi (KU55933) and ATRi (ATR-45) were obtained from Tocris Bioscience and from the Ohio State University respectively and used at 10  $\mu\text{M}$   
15 and 500 nM. Pifithrin alpha (Sigma-Aldrich) was used at 10  $\mu\text{M}$ .

### Live imaging of GFP-H2B cells

U2OS cells were transfected with siLMNA for 48 hours before addition of (**Compound 2**) at 50  $\mu\text{M}$ . Pictures were acquired every 15 minutes for 16 hours  
20 in z-stack of 0.2  $\mu\text{m}$  interval with a Deltavision PersonalDV (Applied Precision, 512x512 CoolSNAP HQ2 camera) equipped with a 100x UPlanSApo/1.40 oil objective (Olympus) and controlled with SoftWoRx software (Applied Precision). Movies were then assembled from pictures with ImageJ software.

### 25 Flow cytometry

10  $\mu\text{M}$  EdU was incubated for 2 hours when indicated. Cells were fixed in 4% paraformaldehyde (Sigma-Aldrich). EdU was fluorescently labeled using the Click-iT® EdU Flow Cytometry Assay Kit (Life Sciences). DNA was stained with 50  $\text{mg ml}^{-1}$  propidium iodide (Sigma-Aldrich) in phosphate buffer solution (PBS)  
30 containing 0.1% Triton-X-100 and 0.5 $\text{mg ml}^{-1}$  DNase free RNase A (Sigma-Aldrich). Samples were processed on a FACSCalibur flow cytometer equipped with CellQuest software (Becton Dickinson). Results were analysed using FlowJo software (TreeStar).

### **Nuclear circularity and nuclear area quantification**

CellProfiler software was used to quantify nuclear circularity and nuclear area from DAPI staining pictures, using the "object size shape" measurement. The AreaShape measurement allowed the calculation of the Form Factor index ( $4 \times \pi \times \text{Area}/\text{Perimeter}^2$ ) corresponding to circularity (a Form Factor of 1 reflecting a perfect circle), as well as the calculation of nuclear Area.

### **Proliferation assay**

HGPS cells were plated at the same number in 24 well dishes. Next day, Remodelin (1  $\mu\text{M}$ ) or small molecule **Compound 2** (1 to 10  $\mu\text{M}$ ), were added to the wells and plates were transferred into an IncuCyte microscope (Essen BioScience). Phase contrast pictures were acquired every 2 hours over several days. Percentage of cell confluence was calculated by the Cell Player integrated software (Essen BioScience) and analysed with GraphPad Prism® software.

### **Protein purification from human cells**

HEK293 cells were transiently transfected with NAT10 constructs and harvested after 48 hours in PBS. Cells were lysed for 5 minutes at room temperature in IP lysis buffer (20 mM Tris pH 7.5, 40 mM NaCl, 2 mM  $\text{MgCl}_2$ , 0.5% NP-40) freshly supplemented with 50 U/ml benzonase and EDTA-free protease inhibitor cocktail (Roche). After this initial lysis step, NaCl concentration was adjusted to 450 mM and samples were incubated at 4°C with rotation. Lysates were clarified by centrifugation (13,200 rpm, 20 minutes at 4°C), and after recovery NaCl concentration was equilibrated to 150 mM. Lysates were used for immunoprecipitation reaction in IP buffer (25 mM Tris pH 7.5, 150 mM NaCl, 1.5 mM DTT, 10% glycerol, 0.5% NP-40) supplemented with protease inhibitors. Target proteins were captured with anti-FLAG antibody (M2, Sigma-Aldrich) coupled to protein A/G-Dynabeads (Life Sciences). Complexes were washed with IP buffer containing incrementally increasing amounts of NaCl (250mM, 500mM, and 1M). Following this, the immuno-complexes were washed in TBS containing gradually decreasing amounts on NaCl (1M, 500mM, 250mM, and 150mM). At this point, elution was carried out using excess triple-Flag peptide (Sigma Aldrich). Eluted proteins were loaded on a gel and the purification was verified by Silver Staining (SilverQuest kit, Life Sciences).

**Analysis of soluble or polymerised tubulin**

U2OS cells pre-treated with 5  $\mu$ M Remodelin for 16 hours or with 10  $\mu$ M nocodazole for 8 hours, were lysed in MT Stabilization Buffer (MSB) (85 mM PIPES [pH 6.9], 1 mM EGTA, 1 mM MgCl<sub>2</sub>, 2M glycerol, 0.5% Triton with 4  $\mu$ g/ml Taxol). Lysates were kept at 4°C for 2 minutes and then centrifuged for 10 minutes at 13,000 rpm. Supernatants, representing the soluble fraction of proteins, were transferred to new tubes, and Laemmli buffer was added. Pellets, representing the polymerized fraction of proteins, were washed once in MSB without Triton, and then resuspended in Laemmli buffer. Equal amounts of lysate were loaded on a gradient gel.

**Microtubules regrowth assay**

48 hours after siRNA transfections, microtubules were depolymerized by cold treatment at 4°C for 1 hour in cells pre-treated or not with 5 to 10  $\mu$ M Remodelin for 16 hours. Cold medium was replaced by pre-warmed medium and cells were incubated at 37°C for 5 or 15 minutes to allow microtubules nucleation and anchorage respectively. Cells were fixed in PFA and stained with anti  $\alpha$ -Tubulin antibody as described in the immunofluorescence procedure.

**Immunoblotting**

Total cell extracts were prepared by scraping cells in SDS lysis buffer (4% SDS, 20% glycerol, and 120 mM Tris-HCl, pH 6.8), boiling for 5 minutes at 95°C, followed by 10 strokes through a 25-gauge needle. Before loading, lysates were diluted with a solution of 0.01% bromophenol blue and 200 mM DTT and boiled for 5 minutes at 95°C. Proteins were resolved by SDS-PAGE on 4-12% gradient gels (NUPAGE, Life Sciences) and transferred onto nitrocellulose membrane (Protran; Whatman). Secondary antibodies coupled to IRDye fluorophores were from LI-COR Biosciences. Detection and quantification was performed with an imager (Odyssey; LI-COR Biosciences).

**Immunofluorescence**

Cells were washed with PBS and fixed for 20 minutes with 2% PFA in PBS. Cells were permeabilised for 5 minutes with PBS/0.2% Triton X-100, and blocked with

PBS/0.2% Tween 20 (PBS-T) containing 5% BSA. Coverslips were incubated for 1 hour with primary antibodies and for 30 minutes with appropriate secondary antibodies coupled to Alexa Fluor 488 or 594 fluorophores (Life Technologies), before being incubated with 2µg/ml DAPI. Pictures were acquired with a  
5 FluoView 1000 confocal microscope (Olympus). For high resolution imaging, z-stacks were acquired with a Deltavision PersonalDV (Applied Precision, 1024x1024 CoolSNAP HQ2 camera, z-stack of 0.2 µm interval) or with a Deltavision OMX V3 in conventional mode (Applied Precision, 512x512 Cascade II cameras (Photometrics), z-stack of 0.125 µm interval) both equipped with a  
10 100x UPlanSApo/1.40 oil objective (Olympus) and controlled with SoftWoRx software (Applied Precision). Deconvolutions were then performed with SoftWoRx (Applied Precision) in conservative mode. The different channels were acquired sequentially.

### 15 **Fluorescent labelling of clickable molecules**

Cells were pre-incubated with clickable molecules **Compound 3** or **Reference Compound A** (1-Chloro-4-ethynilbenzene, purchased from Sigma-Aldrich) for 2 hours before being fixed and permeabilised as described above. Click reaction was prepared using Invitrogen Click-iT reagents with Alexa fluor 488 Azide and  
20 incubated with fixed cells for 1 hour in the dark. In case of co-labelling with another protein, the click reaction was performed before the antibody incubations.

### **Antibodies**

25 The antibodies used in this study are:

#### Abcam antibodies:

Lamin A/C ab40567

HMG1 ab18256

H2AX total ab11175

30 Histone H3 ab1791

β-actin ab8226

ATM (phospho S1981) ab81292

SUN1 ab124770

anti-Giantin ab24586

#### Santa Cruz antibodies:

Lamin A/C sc-6215

p53 (DO-1) sc-126

p21 sc-397

#### Cell Signaling antibodies:

γH2AX 2577

p-Rb (Ser 807/811) 9308

Rb 9309



$\alpha$ -tubulin T9026

p-p53 (Ser15) 9284

H3K9me3 ab8898

Sigma-Aldrich antibodies:

Others:

$\alpha$ -tubulin FITC F2168

$\gamma$ H2AX 05-636 Millipore

5 FLAG F3165

NAT10 13365-1-AP ProteinTech

### Micrococcal nuclease digestion sensitivity assay

1x10<sup>6</sup> cells were trypsinized, harvested, and washed once with 1ml of 1 x RSB buffer (10 mM Tris, pH 7.6, 15 mM NaCl, and 1.5 mM MgCl<sub>2</sub>). After centrifugation (3,00 x g), the cell pellet was resuspended in 1 ml of 1 x RSB buffer with 1% Triton-X 100 and homogenized by five strokes with a loose-fitting glass pestle to release nuclei. Nuclei were collected by centrifugation (13,000 x g) and washed twice with 1 ml of buffer A (15 mM Tris, pH 7.5, 15 mM NaCl, 60 mM KCl, 0.34 M sucrose, 0.5 mM spermidine, 0.15 mM spermine, 0.25 mM PMSF, and 0.1%  $\beta$ -mercaptoethanol). Nuclei were resuspended in Buffer A and aliquoted into 100  $\mu$ l aliquots. 1.2  $\mu$ l of 0.1 M CaCl<sub>2</sub> was added to each aliquot and nuclei were digested by addition of 0.25  $\mu$ l of 200 U/ml MNase (Sigma-Aldrich) and incubated at 30°C. Each aliquot was put back on ice at different time points and digestion was immediately stopped by addition of 3  $\mu$ l EDTA. DNA was purified using the Qiagen PCR purification kit and 1500 ng of DNA was analysed on a 1.5% agarose gel. Digestion profiles were analysed using ImageJ and values were adjusted relative to the global intensity of each lane to compensate for DNA loading variations.

### 25 Lysine acetyltransferase (KAT) assay

The KAT assay was performed using the Fluorescent HAT Assay kit (Active Motif) using NAT10 purified from HEK293 cells and 5  $\mu$ g of purified MAP enriched porcine tubulin (Tebu-bio) as a substrate. Remodelin and clickable molecule **2** were used at 50  $\mu$ M.

30

### Circular Dichroism (CD) Spectroscopy

CD experiments were performed using a Chirascan Circular Dichroism Spectrophotometer (Applied Photophysics, UK). 200  $\mu$ l of purified FLAG-NAT10 at a final concentration of 10  $\mu$ M in TBS 0.1% NP-40 (Sigma-Aldrich) was placed

in a quartz cuvette with an optical path length of 1 mm, transferred to the spectrophotometer. CD scans were recorded at 25°C over the wavelength range of 180 to 350 nm with a 1 second response time, 1 nm pitch and 1.5-nm bandwidth. **Compound 2** solubilized in DMSO was added and the solution was incubated for 5 minutes before recording scans. CD spectra were buffer subtracted, zero corrected at 300 nm and normalized (Molar ellipticity  $\theta$  is quoted in  $10^5 \text{ deg cm}^2 \text{ dmol}^{-1}$ ).

### DNA manipulations

All DNA constructs were validated to be mutation-free by DNA sequencing. A list of DNA oligonucleotides used in this study is provided below. pICE-FLAG was generated by cloning annealed primers FLAG-S and FLAG-AS into HindIII- and BamHI-digested pICE, a new synthetic plasmid conferring puromycin-resistance to mammalian cells (Britton S, Coates J, Jackson SP. J Cell Biol. 2013). To generate NAT10 cDNA resistant to NAT10 siRNA, NAT10 cDNA was amplified from IMAGE clone 5166101 (Source Bioscience) using primer pairs NAT10-F and NAT10-siR-R, or NAT10-siR-F and NAT10-R.. The resulting PCR products were fused together by PCR using primers NAT10-F and NAT10-R. The resulting PCR product was digested with BamHI and MluI and cloned into pICE-FLAG digested with the same restriction enzymes. To generate pICE-FLAG-NAT10-G641E, the G641E mutation was introduced into pICE-FLAG-NAT10 using QuickChange Site-Directed Mutagenesis kit (Agilent Technologies), according to the manufacturer's instructions and using primers NAT10-G641E-F and NAT10-G641E-R.

**TABLE 1: List of DNA oligonucleotides**

Name	Sequence 5' to 3'	SEQ ID NO.
FLAG-S	AGCTTGCGGCCGCGCCACCATGGATTACAAGG ATGACGACGATAAGG	4
FLAG-AS	GATCCCTTATCGTCGTCATCCTTGTAATCCATGGT GGCGGCCGCGCA	5
NAT10-F	GCCGGATCCATGCATCGGAAAAAGGTGGATAAC CG	6
NAT10-R	CGGACGCGTCTATTTCTTCCGCTTCAGTTTCATAT	7

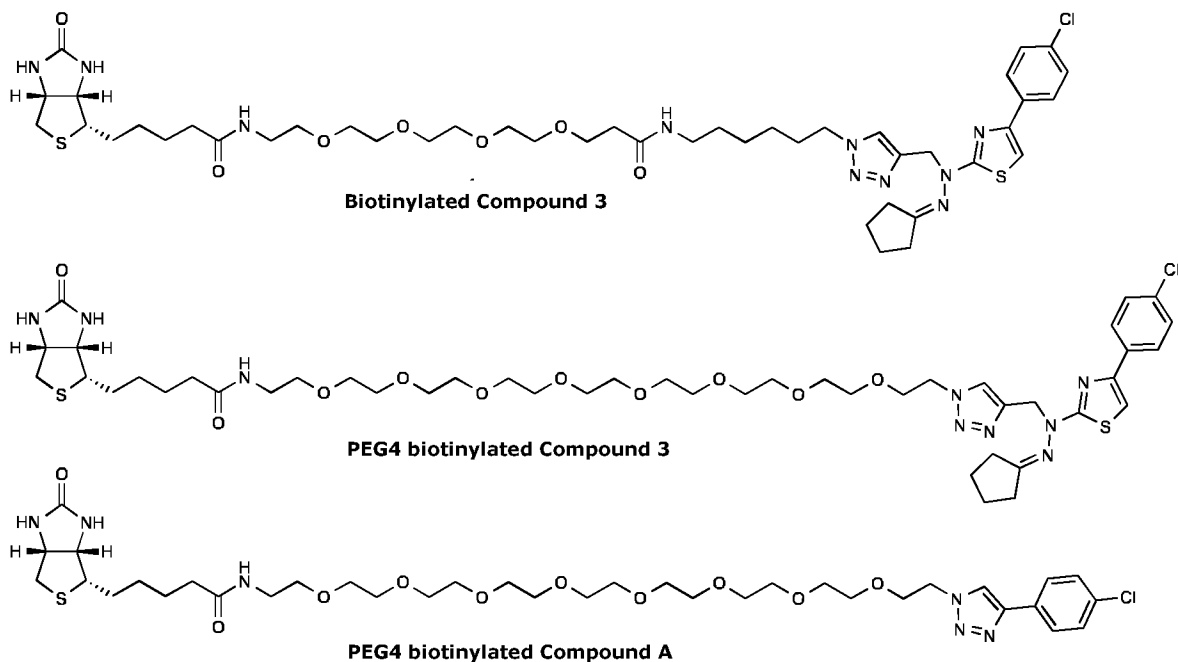
	C	
NAT10-siR-F	CTGAAATCAATGGATTTGAGTGAATATATTATCC GTGGGGACGATGAAGAGTGG	8
NAT10-siR-R	GGATAATATATTTCACTCAAATCCATTGATTTTCAGC TTCCCTACTTCCTTCTTG TG	9
NAT10-G641E-F	CAAGGGATGGGCTATGAGAGCCGTGCTCTGCAG	10
NAT10-G641E-R	CTGCAGAGCACGGCTCTCATAGCCCATCCCTTG	11

### Small molecules for protein pull-down assays

Biotinylated derivative was synthesized from **Compound 3** ("biotinylated **Compound 3**") using commercially available O-(2-aminoethyl)-O'-(2-azidoethyl)heptaethylene glycol and (+)-biotin N-hydroxysuccinimide. Further biotinylated derivatives of **Compound 3** ("PEG4 biotinylated **Compound 3**") and control **Reference Compound A** ("PEG4 biotinylated **Compound A**") were generated *in situ* by addition of PEG4 carboxamide-6-azido hexanyl biotin (Life Sciences) and click reagents in cell extracts.

### Biotinylated small molecule pull-downs

Cells were harvested in PBS and lysed in RIPA buffer supplemented with 1 mM PMSF and protease inhibitors (Roche) for 30 minutes at 4°C with rotation. The supernatants were collected by centrifugation at 16,000 x g for 10 minutes. Supernatants were then incubated with 40 µM of biotinylated small molecule (biotinylated **Compound 3**) or for the competition experiment with 200 µM of (**Compound 2**) and 40 µM of (biotinylated **Compound 3**) for 2 hours. Streptavidin coated magnetic beads (Dynabeads M-280 Streptavidin, Life Sciences) were washed 3 times with binding buffer (25 mM Tris.HCl, 150 mM NaCl, 0.1% Triton) and incubated with supernatants for 1 hour at 4°C with rotation. The magnetic beads were washed 3 times with the binding buffer followed by boiling in Laemmli buffer for 5 minutes to elute the proteins. Samples were then loaded on 4-12% gradient gels (NUPAGE, Life Sciences), analyzed by silver staining (SilverQuest staining kit, Life Sciences) and specific bands were cut and analysed by LC-MS/MS.



### Clickable small molecule pull-downs

5 PEG4 biotinylated **Compound 3** was synthesized from Compounds **2**, commercially available O-(2-aminoethyl)-O'-(2-azidoethyl)heptaethylene glycol and (+)-biotin N-hydroxysuccinimide, prior to being incubated with cell extracts (used in pull-down and mass spectrometry analyses).

10 Biotinylated Compound **3** and PEG4 biotinylated Compound **A** were generated *in situ* from Compounds **2** and **3**, pre-incubated in live cells for 2h prior to adding commercially available PEG4 carboxamide-6-azidohexanyl biotin and click reagents in cell extracts (used in click-pull-down and western blotting validation).

15 Click reaction reagents were added to the cell lysates as follows: 10  $\mu$ M biotin azide (PEG4 carboxamide-6-Azidohexanyl Biotin, Life Sciences), 10 mM sodium ascorbate (Sigma-Aldrich), 2 mM CuSO<sub>4</sub> (Life Sciences). Click reactions were incubated in the dark for 1 hour at 4°C with rotation. Streptavidin beads were then incubated for 1 hour and samples were eluted and loaded on a gel as described above, before immunoblotting.

20

## RESULTS

### Example 1: Nuclear shape rescue by a KAT inhibitor

Because lamin proteins act as a scaffold to maintain nuclear architecture and to anchor chromatin at the nuclear periphery (J. Gotzmann, R. Foisner (1999) *Crit. Rev. Eukaryot. Gene Expr.* 9, 257), small-interfering RNA (siRNA) mediated depletion of Lamin A/C (siLMNA) causes nuclear shape defects in various human cell lines (**Figure 1A, B** and **Figure 2A, B**). Notably, it was found that Lamin A/C depletion was also associated with global chromatin relaxation as observed by increased micrococcal nuclease (MNase) sensitivity and increased nuclear area (**Figure 1B, C**). Thus, it was reasoned that re-compacting chromatin in Lamin A/C depleted cells might improve some of their nuclear architecture defects. Lysine acetyltransferase (KAT) and lysine deacetylase (KDAC) enzymes have the ability to modulate the acetylation status of histones and other proteins, thus affecting global chromatin organization. Therefore, the effects of various natural and synthetic small molecules (see Material and Methods) known to inhibit the activity of individual KAT or KDAC enzymes, were evaluated on chromatin compaction and nuclear shape of siLMNA cells. This identified the KAT inhibitor 4-(4-chlorophenyl)-2-(2-cyclopentylidenehydrazinyl)thiazole (**Compound 2**) (F. Chimenti *et al.*, (2009) *J. Med. Chem.* 52, 530) (**Figure 1D**) as completely restoring nuclear shape and circularity in various cell lines depleted of Lamin A/C (**Figure 1E, F** and **Figure 2A-D**). Despite small molecule **Compound 2** having been initially identified as a GCN5 network inhibitor in *Saccharomyces cerevisiae* (F. Chimenti *et al.*, (2009) *J. Med. Chem.* 52, 530), the classical GCN5 inhibitor MB-3 (M. Biel *et al.* (2004) *Angew. Chem. Int. Ed. Engl.* 43, 3974), did not improve nuclear circularity of siLMNA cells (**Figure 1F**), suggesting that the small molecule-dependent nuclear shape rescue was independent of GCN5 inhibition. Molecule **Compound 2** also improved global chromatin compaction in siLMNA cells as observed by decreased MNase sensitivity (**Figure 1G**), and reduced nuclear area (**Figure 1H**). By using cells expressing GFP-tagged histone H2B (GFP-H2B), it was observed by live imaging that complete nuclear shape rescue in siLMNA cells occurred within 12 hours upon treatment with **Compound 2**, independently of mitosis (**Figure 1I** and **Figure 3**) and without markedly affecting the cell cycle profile (**Figure 1J** and **Figure 2E**). Moreover, **Compound 2** improved the nuclear morphology of several cancer cell lines displaying reduced Lamin A/C expression (**Figure 2F, G**), indicating that the observed effects were not specific to siRNA-mediated

Lamin A/C depletion.

**Example 2: Protein target identification by chemical labelling of Compound 2**

5 To identify putative biological targets of molecule **Compound 2** and elucidate the mechanisms by which it improves nuclear morphology, a molecular-based strategy was established involving cellular functionalization of the molecule by means of "click chemistry" to retrieve and validate drug-associated protein complexes. To this end, an alkyne group was strategically introduced onto the  
10 hydrazone moiety of **Compound 2**, thereby producing "clickable" analogue **Compound 3 (Figure 4A)** that exhibited equivalent cellular activity as **Compound 2 (Figure 4B)**, and used the inactive clickable molecule **Reference Compound A** as a negative control (**Figure 4A, B**). While the alkyne moiety is biologically inert, it can selectively react with any molecule carrying an azide  
15 group upon copper exposure (V. V. Rostovtsev *et al.* (2002) *Angew. Chem. Int. Ed. Engl.* 41, 2596), thus allowing tagging of the small molecule in cells (**Figure 4C**). As a first step, a biotinylated derivative of **Compound 3** was generated (biotinylated **Compound 3**) (**Figure 4D**) and incubated with cell extracts. Proteins associated with biotinylated **Compound 3** were then retrieved with  
20 streptavidin beads and resolved by gel electrophoresis (**Figure 4E**). Through carrying out competition experiments, several protein species were identified whose staining intensities were reduced in the presence of an excess of competitor **Compound 2**. Proteins selectively interacting with biotinylated **Compound 3** by such competition criteria were then excised from gels and  
25 identified by mass spectrometry (LC-MS/MS).

Strikingly, N-acetyltransferase 10 (NAT10) was the only KAT protein identified by the above procedures (see **Table 2**), thus being the only likely relevant target of **Compound 2**.

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**TABLE 2: Biotin analogue of Compound 2 pulls-down the acetyltransferase protein NAT10.** Specific protein hits retrieved by small molecule biotinylated **Compound 3** and identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

<b><i>Cytoskeleton</i></b>	<b><i>Peptide Hits</i></b>	<b><i>Protein Name</i></b>
A2BDB0	21	Gamma-actin
P15924	2	Desmoplakin
P21333	8	Filamin-A
Q14315	7	Filamin-C
P35527	6	Keratin, type I
Q5R844	3	Myosin light polypeptide 6
P48681	2	Nestin
A6QQJ3	2	Peripherin
Q15149	46	Plectin
O94915	4	Protein furry homolog- like
Q54HF0	4	Putative actin-25
Q9NYL9	3	Tropomodulin-3
P23729	2	Type III intermediate filament
O43795	15	Unconventional myosin- Ib
O00159	21	Unconventional myosin- Ic
O94832	3	Unconventional myosin- Id
P08670	52	Vimentin
<b><i>RNA/Nucleolus</i></b>	<b><i>Peptide Hits</i></b>	<b><i>Protein Name</i></b>
Q3SZ63	2	Nucleolar protein 56
A4FUD3	3	116 kDa U5 small nuclear ribonucleoprotein component
Q1HR24	3	40S ribosomal protein S14
Q76I82	3	40S ribosomal protein

		S15a
Q3T0X6	3	40S ribosomal protein S16
Q6Q311	3	40S ribosomal protein S25
Q4R5I3	2	60S ribosomal protein L22
Q3T057	3	60S ribosomal protein L23
Q3T0D5	2	60S ribosomal protein L30
Q3T171	3	60S ribosomal protein L36
Q02878	5	60S ribosomal protein L6
Q99020	2	Heterogeneous nuclear ribonucleoprotein A/B
A5A6H4	3	Heterogeneous nuclear ribonucleoprotein A1
Q6URK4	5	Heterogeneous nuclear ribonucleoprotein A3
Q5RA82	2	Heterogeneous nuclear ribonucleoprotein C
Q14103	3	Heterogeneous nuclear ribonucleoprotein D0
Q4R4M6	6	Heterogeneous nuclear ribonucleoprotein K
Q8VEK3	8	Heterogeneous nuclear ribonucleoprotein U
Q2HJ60	8	Heterogeneous nuclear ribonucleoproteins A2/B1
<u>Q9H0A0</u>	<u>8</u>	<u>N-acetyltransferase 10</u>
Q9NR30	5	Nucleolar RNA helicase 2
Q3T160	2	Nucleophosmin



Q08E38	2	Pre-mRNA-processing factor 19
A1A5S1	3	Pre-mRNA-processing factor 6
Q5RAS1	3	pre-rRNA processing protein FTSJ3
Q4R6M5	16	Probable ATP-dependent RNA helicase DDX5
Q9D903	2	Probable rRNA-processing protein EBP2
Q28009	5	RNA-binding protein FUS
Q9UKM9	8	RNA-binding protein Raly
O75691	41	Small subunit processome component 20 homolog
<b>Chromatin</b>	<b>Peptide Hits</b>	<b>Protein Name</b>
Q91ZW3	2	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 5
Q9QZQ8	3	Core histone macro-H2A.1
Q8CCK0	2	Core histone macro-H2A.2
Q6URC2	3	High mobility group protein HMG-I/HMG-Y
P0C0S8	3	Histone H2A type 1
A9UMV8	3	Histone H2A.J
P02272	3	Histone H2A.V
Q2M2T1	4	Histone H2B type 1-K
P18437	2	Non-histone chromosomal protein

		HMG-17
<b><i>Nuclear Envelope</i></b>	<b><i>Peptide Hits</i></b>	<b><i>Protein Name</i></b>
Q5SRE5	4	Nucleoporin NUP188 homolog
P20700	5	Lamin-B1
Q03252	22	Lamin-B2
<b><i>Trafficking</i></b>	<b><i>Peptide Hits</i></b>	<b><i>Protein Name</i></b>
Q6P5F9	4	Exportin-1
Q9EPL8	2	Importin-7
<b><i>Other</i></b>	<b><i>Peptide Hits</i></b>	<b><i>Protein Name</i></b>
P46940	2	Ras GTPase-activating-like protein IQGAP1
Q86VI3	4	Ras GTPase-activating-like protein IQGAP3

Further highlighting its potential as a biologically relevant target of **Compound 2**, it was noted that NAT10 had previously been linked with the SUN1 nuclear envelope protein (Y. H. Chi *et al.* (2007) *J. Bio. Chem.* 282, 27447), whose

5 depletion was recently shown to rescue nuclear shape in LMNA depleted cells (C. Y. Chen *et al.* (2012) *Cell* 149, 565), and that the KAT activity of NAT10 has been demonstrated towards microtubule and histone substrates (Shen *et al.* (2009) *Exp. Cell Res.* 315, 1653). To validate the interaction between NAT10 and **Compound 2**, cells were pre-incubated with the clickable bioactive

10 analogue **Compound 3** or non-bioactive control **Reference Compound A** to allow their binding to protein targets in live cells. Next, the molecules were functionalized by addition of a biotin-containing linker, then streptavidin beads were used to retrieve bound proteins. This analysis revealed that **Compound 3** but not **Reference Compound A** specifically retrieved NAT10 (**Figure 4F**),

15 thereby establishing that NAT10 is a target of **Compound 2** in the context of living cells and indicating that this protocol selected for specific protein partners without the use of photo-crosslinking agents (S. E. Ong *et al.* (2009) *PNAS* 106, 4617; X. Li, T. M. Kapoor (2010) *J. Am. Chem. Soc.* 132, 2504). In parallel studies, cells were treated with **Compound 3** or **Reference Compound A**,

20 fixed and then **Compound 3** was functionalized with a fluorophore (R. Rodriguez

*et al.* (2009) *Nat. Chem. Biol.* 8, 301) to allow visualization of their sub-cellular distributions by high-resolution microscopy (**Figure 4G**). Importantly, while little or no cellular staining was observed for inactive control **Reference Compound A** (**Figure 5A**), there was a predominant accumulation of **Compound 3** in nucleoli as well as some staining at the nuclear periphery and in the cytoplasm (**Figure 4G** and **Figure 5A**). Moreover, a striking overlap of labelled **Compound 3** and NAT10 staining was observed, further corroborating the affinity pull-down studies. In line with these observations, siRNA-mediated depletion of NAT10 (**Figure 4G**, bottom panel) led to a marked reduction of molecule **Compound 3** accumulation in the nucleolus (**Figure 4G**), without changes in nucleolar architecture. Collectively, these data established that NAT10 is a specific target of **Compound 2** in cells.

To assess whether the interaction between **Compound 2** and NAT10 was direct, NAT10 purified from human HEK293 cells (**Figure 4H**, left panel) was titrated with **Compound 2** and NAT10 conformation was monitored by circular dichroism spectroscopy (**Figure 4H**, right panel). In the absence of compound, the NAT10 spectra displayed a characteristic negative signal at 210 nm, reflecting the presence of  $\alpha$ -helices. Upon addition of **Compound 2**, a clear increase in the absolute molar ellipticity was observed in a concentration-dependent manner, consistent with stabilization of the protein by supramolecular stapling, a process reminiscent of covalent intramolecular stapling previously described (R. E. Moellering *et al.* (2009) *Nature* 462, 182). These data demonstrated a direct physical interaction between **Compound 2** and NAT10, and suggested that stabilization of protein folding by the molecule might inhibit NAT10 activity. During our analyses, it was found that **Compound 2** was rapidly degraded upon exposure to light or air. Because of this and to try to identify more potent molecules, the ability of structural variants of **Compound 2** were explored to rescue nuclear shape of LMNA depleted cells at varying doses. This revealed that while the central thiazolehydrazone core was required for nuclear shape rescue, the cyclopentane ring could be drastically altered, whereas only subtle aromatic modifications were tolerated (**Figure 6A**). These studies thus identified **Compound 1**, containing a cyano function in the para position (**Figure 4I**) as the most potent and stable analogue of the series, with a five-fold potency

improvement as compared to **Compound 2 (Figure 6B)**. This analogue was named "Remodelin" based on its ability to remodel nuclear architecture of siLMNA cells and was used in the following experiments.

### 5 **Example 3: NAT10 inhibition mediates nuclear shape rescue**

The above studies suggested that Remodelin and related compounds might reverse nuclear shape of Lamin A/C depleted cells through NAT10 targeting. To explore this idea, NAT10 and Lamin A/C was co-depleted in human U2OS cells (**Figure 7A**). Strikingly, this revealed that, comparable to the effects observed  
10 with Remodelin, NAT10 depletion completely rescued nuclear morphology defects caused by Lamin A/C depletion (**Figure 7B**; similar effects were observed in RPE-1 and HeLa cells). Because molecule **Compound 2**, the analogue of Remodelin, was discovered as a KAT inhibitor, it was assessed whether NAT10 acetyltransferase activity was altered by Remodelin and involved  
15 in nuclear shape rescue in Lamin A/C depleted cells. To this end, a 3D-model for region 10 to 917 of human NAT10 was generated from the structure of its highly conserved bacterial homolog TmcA (Chimnaronk *et al.* (2009) *Embo. J.* 28, 1362), using the Phyre2 online server (L. A. Kelley, M. J. Sternberg (2009) *Nat. Protoc.* 4, 363) (**Figure 7C, D**). The site of acetyl-CoA binding was then  
20 predicted by aligning this model with the original TmcA structure in complex with Ac-CoA (**Figure 7D**). The conserved Gly 641 was then mutated to Glu (G641E) (**Figure 8A**) which was predicted to block NAT10 acetyltransferase activity (**Figure 7D**, right). Indeed, biochemical assays with wild-type (WT) and G641E mutant (MUT) NAT10 proteins expressed and purified from human HEK293 cells  
25 (**Figure 8B**) showed that the G641E mutation abolished NAT10 KAT activity (**Figure 7E**). Moreover, the inclusion of Remodelin or clickable bioactive **Compound 3** in these reactions abolished NAT10 WT KAT activity, revealing that Remodelin is a NAT10 inhibitor.

30 To explore the function of NAT10 KAT activity in controlling nuclear shape, stable cell lines expressing siRNA-resistant NAT10 WT or G641E constructs were generated. Given the correct expression and subcellular localization of both proteins (**Figure 8C, D**), Lamin A/C and the endogenous NAT10 protein were co-depleted and the nuclear shape morphology was studied. Strikingly, while

Lamin A/C depletion impaired nuclear circularity in cells expressing NAT10 WT, this was not the case in cells expressing catalytically inactive NAT10 MUT (**Figure 7F**). Collectively, these data thereby established that inactivating NAT10 KAT activity by mutation or by Remodelin action restores normal nuclear morphology of siLMNA cells.

#### **Example 4: Remodelin targets NAT10 to improve fitness of HGPS cells**

Having found that Remodelin rescues nuclear shape defects of Lamin A/C depleted cells, its effects on nuclear shape of *LMNA* mutated cells was investigated by using two primary fibroblast cell lines derived from HGPS patients (AG11498 and AG06267) carrying the heterozygous dominant point mutation *LMNA* c.1824C>T, p.G608G. On account of this mutation, HGPS cells express a permanently farnesylated, truncated form of Lamin A called Progerin, which accumulates with cell passage number at the nuclear rim. This leads to nuclear membrane folding and nuclear blebbing (**Figure 9A**), and is at least in part responsible for the premature ageing phenotypes of HGPS patients (K. Cao *et al.* (2011) *J. Clin. Invest.* 121, 2833). Notably, Remodelin significantly reduced the number of HGPS cells with misshapen nuclei (**Figure 9A, B**) and also had this effect on primary MRC5 fibroblasts aged in culture (**Figure 10A, B**) that also become misshapen and accumulate Progerin upon extended passaging (P. Scaffidi, T. Misteli (2006) *Science* 312, 1059). By contrast, Remodelin had no detectable effect on misshapen nuclei of non-laminopathic Werner syndrome cells, indicating that nuclear shape rescue is specific to lamin-associated defects (**Figure 10C, D**).

Farnesyltransferase inhibitors (FTIs) have been used in HGPS cells to prevent the toxic accumulation of farnesylated Progerin at the nuclear membrane. Indeed, non-farnesylated Progerin is relocalized away from the nuclear envelope and, as a result, nuclear blebbing is reduced (B. C. Capell *et al.* (2005) *PNAS* 102, 12879). To determine whether Remodelin leads to farnesyltransferase inhibition, Lamin A processing was examined by western blotting (**Figure 11A**). Importantly, this demonstrated that Remodelin did not block pre-Lamin A cleavage and maturation, unlike FTI that caused accumulation of the pre-Lamin A intermediate. While this indicated that Remodelin does not inhibit

farnesyltransferase activity, it was found that Remodelin and FTI did not act synergistically on nuclear shape improvement, suggesting that they impact on a common pathway (**Figure 11B, C**). Significantly, unlike what was had observed for Remodelin, FTI does not improve nuclear morphology in LMNA-depleted cells, in accordance with its proposed mode of action. Furthermore, although FTI improves the nuclear shape of HGPS cells, it has the opposite effect on normal cells that do not express Progerin, (**Figure 11B, C**) probably through FTI treatment leading to the accumulation and inappropriate localization of unprocessed Lamin A and B together with centrosome separation defects (V. L. Verstraeten *et al.* (2011) *PNAS* 108, 4997; M. W. Glynn, T. W. Glover (2005) *Hum. Mol. Genet.* 14, 2959; Y. Wang *et al.* (2012) *Nucleus* 3, 452). By contrast, Remodelin did not induce nuclear shape defects in normal human fibroblasts; and indeed, it prevented FTI-induced nuclear shape defects in such fibroblasts (**Figure 11B-D**). Crucially, a comparable decrease in the number of misshapen nuclei was observed in HGPS cells upon Remodelin treatment or NAT10 depletion (**Figure 9C and Figure 12A**), leading us to conclude that – as was the case for siLMNA cells – NAT10 inhibition by Remodelin was responsible for nuclear shape improvement in HGPS cells.

While nuclear shape improvement is not always associated with overall amelioration of HGPS cell phenotypes (M. X. Ibrahim *et al.* (2013) *Science* 340, 1330), the inventors found that Remodelin improved global HGPS-cell fitness as observed by decreased steady-state levels of  $\gamma$ H2AX and autophosphorylated ATM (markers of unrepaired DNA double-strand breaks) and decreased DNA damage signaling (**Figure 9D and Figure 12B, C, F**), improved chromatin and nucleolar organization as assessed by H3K9me3 and NAT10 staining, and decreased SUN1 accumulation at the nuclear envelope (**Figure 12G-I**). That, Remodelin does not detectably affect the expression and localization of SUN1 in siLMNA cells, implies that the mechanism of nuclear shape rescue by Remodelin is distinct from that observed upon SUN1 depletion (C. Y. Chen *et al.* (2012) *Cell* 149, 565). Interestingly, cells derived from patients with Emery-Dreifuss muscular dystrophy (EDMD), also displayed decreased levels of  $\gamma$ H2AX upon Remodelin treatment (**Figure 12D, E**), suggesting that Remodelin might have broader applications to other types of laminopathies. Blocking signaling by the

apical DNA-damage response kinases ATM and ATR also decreased  $\gamma$ H2AX (**Figure 9E, F**). However, unlike Remodelin – which also improved DNA replication (**Figure 12J**), enhanced cell proliferation capacity (**Figure 9G**) and decreased senescence (**Figure 9H, I**; it should be noted from **Figure 12K** that other KAT inhibitors do not decrease senescence) – inhibiting ATM and ATR decreased proliferation and induced senescence (**Figure 9G, H**). As shown in **Figure 12K** similar effects were observed upon p53-pathway inhibition. These data thus supported the notion that Remodelin reduces the amount of DNA damage in HGPS cells, while damage is still present but no longer signaled properly upon ATM/ATR inhibition, leading to cell growth arrest and senescence. In addition, Remodelin improved HGPS cell chromatin compaction as observed by DAPI staining intensity (**Figure 12G**) and nucleolar organization, without altering NAT10 localization (**Figure 12I**). Importantly, these effects of Remodelin were associated with globally increased fitness of HGPS cells, as observed by increased DNA replication (**Figure 12J**), enhanced cell proliferation capacity (**Figure 9G**) and decreased proportion of senescent cells (**Figure 9H**). Highlighting its potential for long-term benefits, it was found that Remodelin prevented HGPS cell senescence even after several weeks of treatment (**Figure 9I**).

20

### **Example 5: NAT10 inhibition reorganizes microtubules to restore nuclear shape**

NAT10 localizes mainly in the nucleolus, a nuclear compartment whose role in maintaining global nuclear organization and nuclear shape has been demonstrated by studies showing that depleting the nucleolar proteins nucleophosmin (NPM1), fibrillarin or SENP5 leads to changes in nuclear shape (M. A. Amin *et al.* (2007) *Biochem. Biophys. Res. Commun.* 360, 320; A. Di Bacco *et al.* (2006) *Mol. Cell Biol.* 26, 4489; M. A. Amin *et al.* (2008) *Biochem. J.* 415, 345). In the case of NPM1, this has been established to occur through changes in microtubule stability (G. Wang *et al.* (2010) *J. Biol. Chem.* 285, 19060), which then impact on the nucleus via connections between the cytoskeleton and the nuclear envelope. Because of this and since tubulin is a known NAT10 substrate, the microtubule network of cells was examined by immunofluorescence and observed a striking reorganization upon Remodelin

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treatment. Moreover, by using the cell complementation system, it was established that equivalent microtubule reorganization was triggered by NAT10 depletion or by mutational inactivation of the NAT10 catalytic domain (**Figure 13A**). In accordance with there being a functional link between microtubule reorganization and nuclear shape rescue by Remodelin treatment or NAT10 inhibition, the microtubule destabilizing drugs nocodazole and colchicine also rescued the nuclear shape defects of siLMNA cells (**Figure 13B**) and HGPS cells (**Figure 14A**), whereas latrunculin A, an inhibitor of actin polymerization, increased nuclear distortion (**Figure 13B**). These results thereby indicated that microtubule but not actin cytoskeleton reorganization can rescue nuclear shape in the context of Lamin A/C depletion. Importantly, it was established that the effects of Remodelin were not linked to Golgi apparatus fragmentation or tubulin depolymerization, because in contrast to nocodazole or colchicine, Remodelin did not affect Golgi apparatus integrity (**Figure 13C**) or tubulin assembly into polymers (**Figure 13D**). These differences help explain why, unlike the situation in response to nocodazole or colchicine exposure, cells did not accumulate in mitosis upon Remodelin treatment.

To gain insight into how NAT10 modifies microtubule organization, microtubule dynamics were analysed by performing regrowth assays after cold-induced depolymerization and release into warm medium. While initial microtubule regrowth (the nucleation phase) appeared normal after NAT10 inhibition in siLMNA cells (**Figure 13E**, t = 5 minutes) and HGPS cells (**Figure 14B**), anchorage of microtubules to centrosomes was clearly affected by Remodelin treatment in both siLMNA cells (**Figure 13E**) and HGPS cells (**Figure 14B**) or by the NAT10 G641E mutation (**Figure 13E**, t = 15 minutes), indicating that NAT10 KAT activity promotes microtubule anchorage. In accord with this, nuclear shape rescue was observed in siLMNA cells upon depletion of the PCM-1 protein that is involved in microtubule anchorage (A. Dammermann, A. Merdes (2002) *J. Cell Biol.* 159, 255). Since microtubules exert an external force on the nucleus that contributes to nuclear envelope deformation (M. C. King *et al.* (2008) *Cell* 134, 427), the results therefore support a model in which inhibiting NAT10 KAT activity in laminopathic cells reduces microtubule anchorage, thereby releasing an external force on the nuclear envelope and contributing to nuclear shape



rescue and global enhancement of cellular fitness (**Figure 13F**). This model is in accordance with previous work suggesting that releasing microtubule forces on the nucleus by modifying substrate stiffness normalizes the nuclear shape of laminopathic cells (C. Tamiello *et al.* (2013) *Nucleus* 4, 61) and helps explain why Remodelin, like other microtubule reorganizing agents (N. Suzuki *et al.* (1998) *PNAS* 95, 10499), corrects FTI-induced nuclear shape defects in non progeric cells (**Figure 11**).

#### **Example 6: Analysis of structure requirements for nuclear shape rescue**

Lamin A/C was depleted by siRNA in U2OS cells (siLMNA) to induce misshapen nuclei. Cells were then treated for 16h with various analogues of **Compound 2** and nuclear shape rescue was analysed by DAPI staining. The results are shown in **Figure 18** and **Figure 19** which display the structures of the tested molecules and their ability to rescue nuclear shape.

#### **Example 7: Compound 2 inhibits the growth of A431 melanoma cell line in a dose-dependent manner**

A431 cells expressing a low level of Lamin A protein (see **Figure 2**) were plated at low density and treated with the indicated concentration of **Compound 2**. Cell growth was monitored automatically over time using an IncuCyte ZOOM ®. The results are shown in **Figure 20**. The proliferation assay shows that A431 cell growth is inhibited in a dose-dependent manner. A concentration of 10µM Remodelin is enough to reduce the proliferation of these cells by about 50% whereas cells expressing high levels of lamin A/C were not affected by up to 50µM Remodelin (see Figure 15).

#### **Example 8: Lamin A/C knock-out U2OS cells are more sensitive to Compound 2 and analogues than wild type cells**

LMNA knock out (KO) U2OS cell line was engineered using CripR/Cas9 technology. The sensitivity of LMNA KO cells to **Compound 2** was compared to cells expressing wild type LMNA (LMNA WT). Cell growth was monitored automatically over time using an IncuCyte ZOOM ®. The results are shown in **Figure 21** which has been split into two graphs for easier reading of the curves. The upper panel shows the specificity of **Compound 2** towards inhibiting cell

growth of LMNA KO cells. The lower panel shows an increased potency of **Compound 13**, compared to **Compound 2** on LMNA KO cell growth inhibition.

**Example 9: Compound 2 inhibits global protein translation specifically in LMNA KO cells**

U2OS cells expressing wild type or KO LMNA were treated with **Compound 2** for 24h and incubated for 1h with the clickable methionine analogue HPG to measure global protein translation. HPG was then clicked with an Alexa fluor 488 and global fluorescence intensity was quantified by FACS on 10 000 cells. The graphs in **Figure 22** show a strong reduction of global protein translation upon **Compound 2** addition in LMNA KO cells but not in LMNA WT cells, which might account for the difference of cell growth inhibition observed in the previous figure.

**Example 10: Depletion of NAT10 leads to similar inhibition of proliferation, migration and invasion than treatment with Compound 2**

NAT10 was depleted by siRNA in U2OS cells (siNAT10) and cells were then treated with **Compound 2** for 16h before being seeded on matrigel for migration/invasion assays. In parallel, cells from the same plates were seeded separately to assess cell proliferation. The results are shown in **Figure 23** and are presented as percentage of cell number compared to untreated control cells (siCT). The graphs show that inhibiting NAT10 with **Compound 2** or depleting it with siRNA leads to similar reduction of cell proliferation, migration and invasion.

**Example 11: Assessment of Compound 2 *in vivo* toxicity and *in vitro* specificity**

**Compound 2** was administered to mice orally with the indicated daily dose, for 14 days and the results are shown in **Figure 24**. Left panel of **Figure 24**: body weight change shows that the compound is well tolerated orally, up to high doses of 400mg/kg/day, without significant decrease of body weight. Right panel of **Figure 24**: measurement of **Compound 2** concentration in heart and muscles of mice treated with the indicated dose, at the end of the 14 days treatment. The graph shows a very high concentration of the compound in both heart and muscles, suggesting compound accumulation over time.

Lower panel of **Figure 24** shows that **Compound 2** does not inhibit the activity of the main lysine acetyltransferase proteins (KAT) *in vitro* towards their histone substrates, suggesting high specificity towards NAT10 inhibition, and thus potentially low toxicity *in vivo*.

5

### **Example 12: Effect of Compound 2 treatment on a mouse model of Hutchinson Gilford progeria syndrome**

Left panel of **Figure 25**: An HGPS mouse model engineered to carry the G609G mutation on both alleles displayed smaller size and back curvature compared to wild type mice. Middle panel of **Figure 25**: HGPS mice were treated with a daily oral dose of 100mg/kg/day of **Compound 2** from 3 weeks of age and until culled due to >20% body weight loss. After several days of treatment with **Compound 2**, mice displayed hair graying that could reflect target engagement. Right panel of **Figure 25**: Global survival of HGPS mice (Lmna<sup>G609G/G609G</sup>) treated with vector only (Lmna<sup>G609G/G609G</sup> V) or with **Compound 2** (Lmna<sup>G609G/G609G</sup> **2**) showing a significant increase of the median survival of mice upon **Compound 2** treatment (+25%). Number of mice/group n=10.

### **Example 13: Molecular effects of Compound 2 *in vivo***

Tissues from wild type mice or Lmna<sup>G609G/G609G</sup> mice treated with **Compound 2** were harvested and proteins were extracted and loaded on a western blot which is shown in **Figure 26**. Lmna<sup>G609G/G609G</sup> mice indeed show progerin expression (readily detectable mainly in the Lung tissues), confirming their genotype. DNA damage was assessed by the DNA double strand break marker  $\gamma$ H2AX that was quantified using a Licor Odyssey Imaging system. The relative amount of  $\gamma$ H2AX compared to total H2AX is represented below the western blot and shows a clear decrease of DNA damage in tissues from Lmna<sup>G609G/G609G</sup> mice that were treated with **Compound 2**, compared to the untreated mice, in both heart and lung. No effect was observed in liver extracts, suggesting that **Compound 2** might not accumulate in the liver.

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### **Example 14: Assessment of acetyl tubulin as a potential biomarker for NAT10 inhibition**

As Tubulin is a direct substrate of NAT10, Tubulin acetylation levels in cells derived from HGPS patients (left and bottom panel of **Figure 27**) were assessed as well as in  $Lmna^{G609G/G609G}$  mice (right panel of **Figure 27**). Tubulin acetylation appears higher in both patient cells and  $Lmna^{G609G/G609G}$  mice, suggesting that NAT10 might be hyper-activated. Treated of  $Lmna^{G609G/G609G}$  mice with **Compound 2** led to a decrease of tubulin acetylation levels (right panel of **Figure 27**), suggesting that this acetylation mark could be a good biomarker for NAT10 inhibition *in vivo*.

10 **Example 15: Engineering a  $Lmna^{G609G/G609G}/NAT10^{+/-}$  mouse for genetic validation**

Top left panel of **Figure 28**: Assessment of NAT10 expression level in Lung protein extracts from shows about 50% reduction of NAT10 expression in  $NAT10^{+/-}$  mice, compared to WT or  $Lmna^{G609G/G609G}$  (top right panel of **Figure 28**). Lower panel of **Figure 28**: Body weight of the indicated mouse genotypes, showing a strong increase of body weight in  $Lmna^{G609G/G609G}/NAT10^{+/-}$  mouse, compared to  $Lmna^{G609G/G609G}$  mouse and suggesting that 50% reduction of NAT10 is sufficient to rescue, at least partially, the phenotype of  $Lmna^{G609G/G609G}$  HGPS mice.

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**Example 16: Microarray analysis shows specificity of Compound 2 towards gene expression regulation in HGPS cells**

Normal fibroblasts or HGPS fibroblasts were kept in culture for 12 population doublings with chronic treatment of 1 $\mu$ M **Compound 2** (top panels of **Figure 29**) or transfected with siRNA against NAT10 for 3 days (bottom panels of **Figure 29**). RNA was extracted and global gene expression was analysed by microarray. Volcano plots show a very high specificity of **Compound 2** towards gene expression regulation of HGPS cells, with about 1000 genes affected more than 2 fold, whereas only 24 genes were affected in normal fibroblasts. Venn diagrams (panel 2 of **Figure 29**) show that about half of the genes that are misregulated in HGPS compared to normal fibroblasts are rescued by **Compound 2** treatment. Bottom panels of **Figure 29**: Volcano plots and Venn diagrams show that NAT10 depletion (see WB below for efficiency of NAT10 depletion) leads to change in the expression of about twice more genes in HGPS

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cells as compared to normal fibroblasts. About 30% of the genes affected by NAT10 depletion in HGPS cells were also affected by long-term **Compound 2** treatment, suggesting that these are the genes regulated by NAT10 acetyltransferase activity.

5

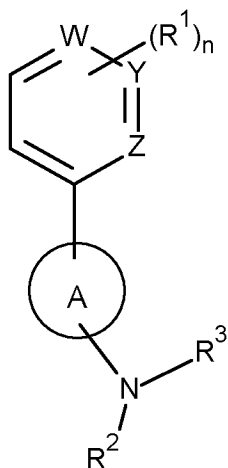
In conclusion, the present inventors have identified and characterized the small molecule Remodelin as a new agent that improves nuclear shape and fitness of both progeric and Lamin A/C depleted cells. To their knowledge, Remodelin is the first molecule that also reduces the steady state level of  $\gamma$ H2AX in HGPS  
10 cells, which is believed to contribute to their premature ageing phenotype. Importantly, by using an unbiased *in vivo* and *in vitro* click-chemistry based approach, NAT10 was identified as the cellular target of Remodelin responsible for nuclear morphology rescue, via reorganization of the microtubule network. Thus, "small molecule inhibitors" of NAT10 provide new opportunities to study  
15 laminopathy-associated processes with valuable spatial and temporal resolution (T. U. Mayer *et al.* (1999) *Science* 286, 971), and might in due course yield new classes of drugs for alleviating dystrophic and premature ageing diseases.

Throughout the specification and the claims which follow, unless the context  
20 requires otherwise, the word 'comprise', and variations such as 'comprises' and 'comprising', will be understood to imply the inclusion of a stated integer, step, group of integers or group of steps but not to the exclusion of any other integer, step, group of integers or group of steps.

25 The application of which this description and claims forms part may be used as a basis for priority in respect of any subsequent application. The claims of such subsequent application may be directed to any feature or combination of features described herein. They may take the form of product, composition, process, or use claims and may include, by way of example and without  
30 limitation, the following claims:

**CLAIMS**

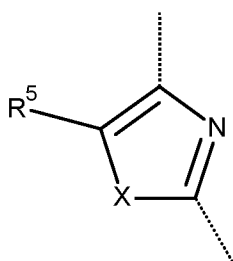
1. A compound of formula (I):



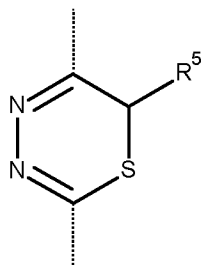
5 wherein:

each of W, Y and Z represent CH or one of W, Y and Z represents nitrogen and the other two groups represent CH;

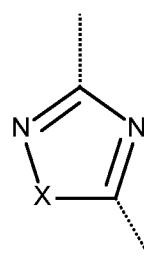
Ring A represents:



(i)



(ii)



(iii)

10

X represents S, O or  $NR^a$ ;

$R^a$  represents hydrogen, hydroxyl, =O,  $C_{1-6}$  alkyl,  $C_{2-6}$  alkenyl,  $C_{2-6}$  alkynyl, halogen, halo $C_{1-6}$ alkyl,  $C_{1-6}$  alkoxy or halo $C_{1-6}$  alkoxy;

15  $R^5$  represents hydrogen, hydroxyl,  $C_{1-6}$  alkyl,  $C_{2-6}$  alkenyl,  $C_{2-6}$  alkynyl, halogen, halo $C_{1-6}$ alkyl,  $C_{1-6}$  alkoxy, halo $C_{1-6}$  alkoxy, COH, COOH, COOC $_{1-6}$ alkyl, cyano,  $NH_2$  or  $NO_2$ ;

n represents an integer selected from 0 to 5;

each  $R^1$  independently represents  $C_{1-6}$  alkyl,  $C_{2-6}$  alkenyl,  $C_{2-6}$  alkynyl, halogen, halo $C_{1-6}$ alkyl,  $C_{1-6}$  alkoxy, halo $C_{1-6}$  alkoxy, cyano, hydroxyl, COH, COOH,

20 COOC $_{1-6}$ alkyl,  $NH_2$  or  $NO_2$ ;

R<sup>2</sup> represents hydrogen, C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl or C<sub>2-6</sub> alkynyl;

R<sup>3</sup> is selected from either hydrogen or an -N=R<sup>4</sup> group, such that when Ring A represents formula (i) or (iii), R<sup>3</sup> represents -N=R<sup>4</sup>, and when Ring A represents formula (ii), R<sup>3</sup> represents hydrogen;

- 5 R<sup>4</sup> represents -C(R<sup>4a</sup>)(R<sup>4b</sup>), C<sub>3-8</sub> cycloalkyl, C<sub>3-8</sub> cycloalkenyl or benzyl wherein said cycloalkyl or benzyl is optionally substituted by C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, halogen, haloC<sub>1-6</sub>alkyl, C<sub>1-6</sub> alkoxy, haloC<sub>1-6</sub> alkoxy, cyano, hydroxyl, COH, COOH, COOC<sub>1-6</sub>alkyl, NH<sub>2</sub> or NO<sub>2</sub>;

- 10 R<sup>4a</sup> and R<sup>4b</sup> are independently selected from hydrogen, C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, halogen, haloC<sub>1-6</sub>alkyl, C<sub>1-6</sub> alkoxy, haloC<sub>1-6</sub> alkoxy, cyano, hydroxyl, COH, COOH, COOC<sub>1-6</sub>alkyl, NH<sub>2</sub>, NO<sub>2</sub>, C<sub>3-8</sub> cycloalkyl or benzyl wherein said cycloalkyl or benzyl is optionally substituted by C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, halogen, haloC<sub>1-6</sub>alkyl, C<sub>1-6</sub> alkoxy, haloC<sub>1-6</sub> alkoxy, cyano, hydroxyl, COH, COOH, COOC<sub>1-6</sub>alkyl, NH<sub>2</sub> or NO<sub>2</sub>;

- 15 or a pharmaceutically acceptable salt or solvate thereof, for use in the treatment or prevention of laminopathy, premature ageing disorders, normal ageing or cancer (such as a cancer characterised by low levels of LMNA expression).

2. The compound for use of claim 1, wherein Ring A represents formula (i).

20

3. The compound for use of claim 1 or claim 2, wherein X represents S or O, such as S.

4. The compound for use of any one of claims 1 to 3, wherein R<sup>5</sup> represents  
25 hydrogen or halogen (i.e. bromo), such as hydrogen.

5. The compound for use of any one of claims 1 to 4, wherein n represents an integer from 1 to 2, such as 1.

30 6. The compound for use of any one of claims 1 to 5, wherein R<sup>1</sup> represents halogen (such as chloro or bromo), C<sub>1-6</sub>alkoxy (such as methoxy), cyano, hydroxyl, COOH, NO<sub>2</sub>, haloC<sub>1-6</sub>alkyl (such as trifluoromethyl) or haloC<sub>1-6</sub>alkoxy (such as trifluoromethoxy).

7. The compound for use of claim 6, wherein R<sup>1</sup> represents halogen (such as chloro or bromo), cyano, NO<sub>2</sub> or haloC<sub>1-6</sub>alkyl (such as trifluoromethyl), such as cyano or trifluoromethyl.
- 5 8. The compound for use of any one of claims 1 to 7, wherein R<sup>1</sup> is in the *meta* (3) and/or *para* (4) position, such as the *para* (4) position.
9. The compound for use of any one of claims 1 to 8, wherein R<sup>2</sup> represents hydrogen or C<sub>2-6</sub>alkynyl, such as hydrogen.
- 10 10. The compound for use of any one of claims 1 to 9, wherein R<sup>3</sup> represents -N=R<sup>4</sup>.
11. The compound for use of any one of claims 1 to 10, wherein R<sup>4</sup> represents  
15 C<sub>3-8</sub> cycloalkyl, such as cyclopentyl, in particular unsubstituted cyclopentyl.
12. The compound for use of any one of claims 1 to 10, wherein each of W, Y and Z represent CH.
- 20 13. The compound for use of any one of claims 1 to 12, wherein the compound is selected from Compounds 1 to 21 or an alternative pharmaceutically acceptable salt, solvate, free acid preparation or free base preparation thereof.
- 25 14. The compound for use of any one of claims 1 to 13, for use in the treatment or prevention of laminopathy, premature ageing disorders, normal ageing or cancer (such as a cancer characterised by low levels of LMNA expression).
- 30 15. The compound for use of any one of claims 1 to 14, wherein the laminopathy is selected from: Hutchinson-Gilford progeria syndrome (HGPS); Atypical Werner syndrome; Barraquer-Simons syndrome; Buschke-Ollendorff syndrome; Cardiomyopathy; Charcot-Marie-Tooth disease; Emery-Dreifuss muscular dystrophy (X-linked, autosomal dominant or autosomal recessive);



Familial partial lipodystrophy of the Dunnigan type (FPLD); Greenberg dysplasia; Leukodystrophy, demyelinating, adult-onset, autosomal dominant (ADLD); Limb-girdle muscular dystrophy type 1B (LGMD1B); Lipoatrophy with diabetes, hepatic steatosis, hypertrophic cardiomyopathy, and leukomelanodermic papules (LDHCP); Mandibuloacral dysplasia with type A lipodystrophy (MADA);  
5 Mandibuloacral dysplasia with type B lipodystrophy (MADB); Pelger-Huet anomaly (PHA); Pelizaeus-Merzbacher disease or Restrictive Dermopathy (RD).

16. The compound for use of claim 15, wherein the laminopathy is selected  
10 from: Hutchinson-Gilford progeria syndrome (HGPS), Emery-Dreifuss muscular dystrophy or Atypical Werner syndrome.

17. The compound for use of any one of claims 1 to 16, in combination with  
one or more further active ingredients.

15

18. A pharmaceutical composition comprising: (a) a compound, or a  
pharmaceutically acceptable salt thereof, according to any one of claims 1 to 16;  
and (b) a pharmaceutically acceptable excipient, for use in the treatment or  
prevention of laminopathy, premature ageing disorders, normal ageing or cancer  
20 (such as a cancer characterised by low levels of LMNA expression).

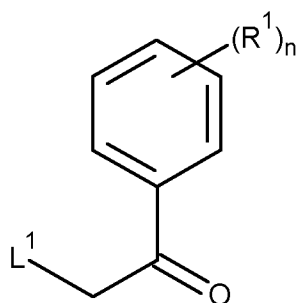
19. A compound of formula (I) selected from: Compound 3, Compound 8,  
Compound 11, Compounds 13-17 and Compounds 19-21; and salts or solvates  
of any one thereof.

25

20. A process for preparing a compound of formula (I) of any one of claims 1  
to 19 which comprises:

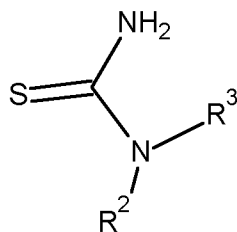
(a) when A represents (i), reacting a compound of formula (II)

80



(II)

wherein  $R^1$  and  $n$  are as defined in claim 1 and  $L^1$  represents a suitable leaving group, such as chlorine or bromine; with a compound of formula (III):



(III)

wherein  $R^2$  and  $R^3$  are as defined in claim 1;

- (b) deprotection of a protected derivative of a compound of formula (I);  
 10 and/or
- (c) interconversion of a compound of formula (I) or protected derivative thereof to a further compound of formula (I) or protected derivative thereof; and
- 15 (d) optional formation of a pharmaceutically acceptable salt of a compound of formula (I).

21. Use of a compound of any one of claims 1 to 19 in the manufacture of a medicament for treating or preventing laminopathy, premature ageing disorders,  
 20 normal ageing or cancer (such as a cancer characterised by low levels of LMNA expression).

22. A method of treating or preventing laminopathy, premature ageing disorders, normal ageing or cancer (such as a cancer characterised by low levels

of LMNA expression) in a human in need thereof comprising administering to said human a therapeutically effective amount of a compound of any one of claims 1 to 19.

5 23. A method of treating cancer (such as a cancer characterised by low levels of LMNA expression) in a human in need thereof comprising administering to said human a therapeutically effective amount of a compound of any one of claims 1 to 19.

10 24. A NAT10 inhibitor for use in the treatment or prevention of laminopathy, premature ageing disorders, normal ageing or cancer (such as a cancer characterised by low levels of LMNA expression).

15 25. A NAT10 inhibitor for use in the treatment of cancer (such as a cancer characterised by low levels of LMNA expression).

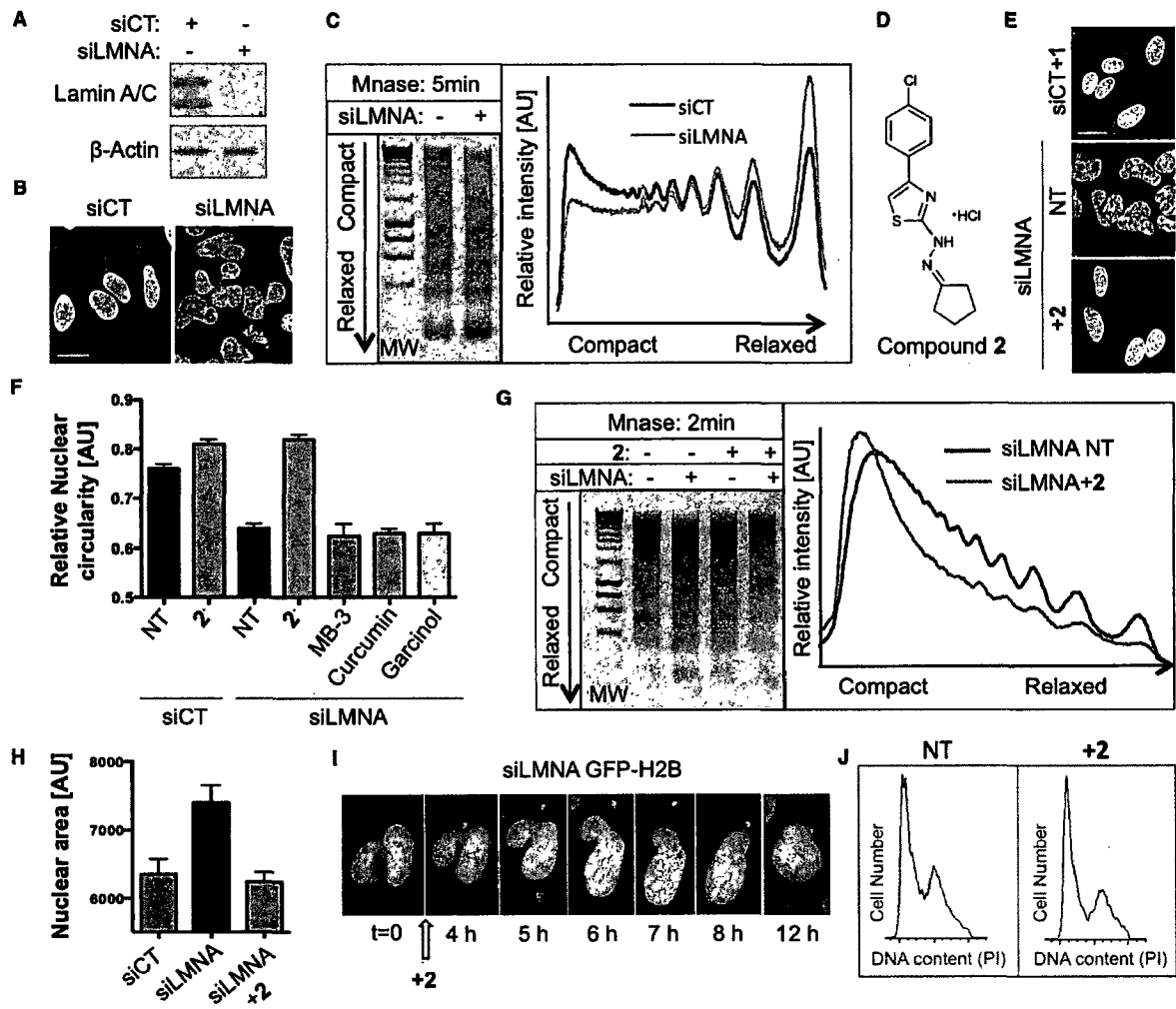


FIGURE 1

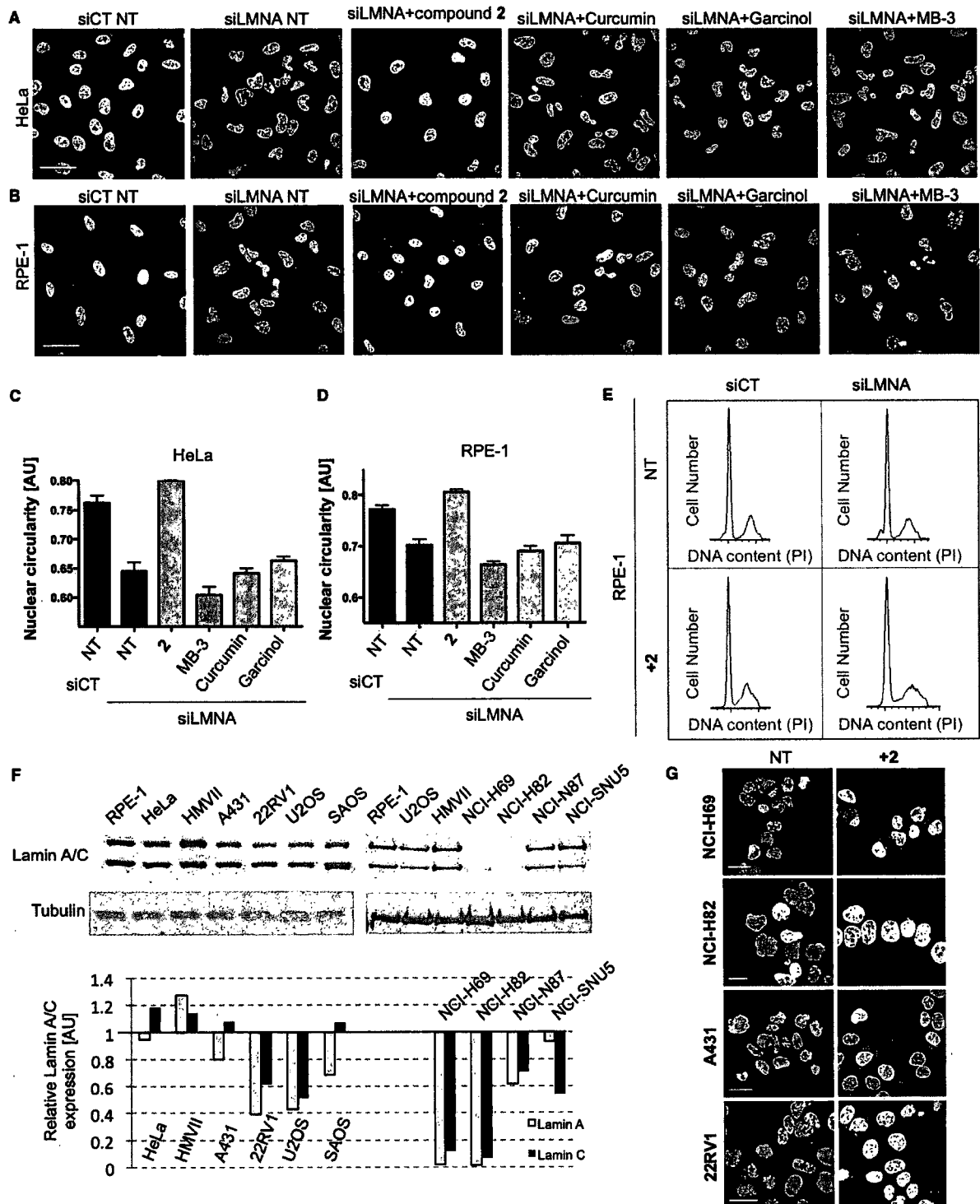


FIGURE 2

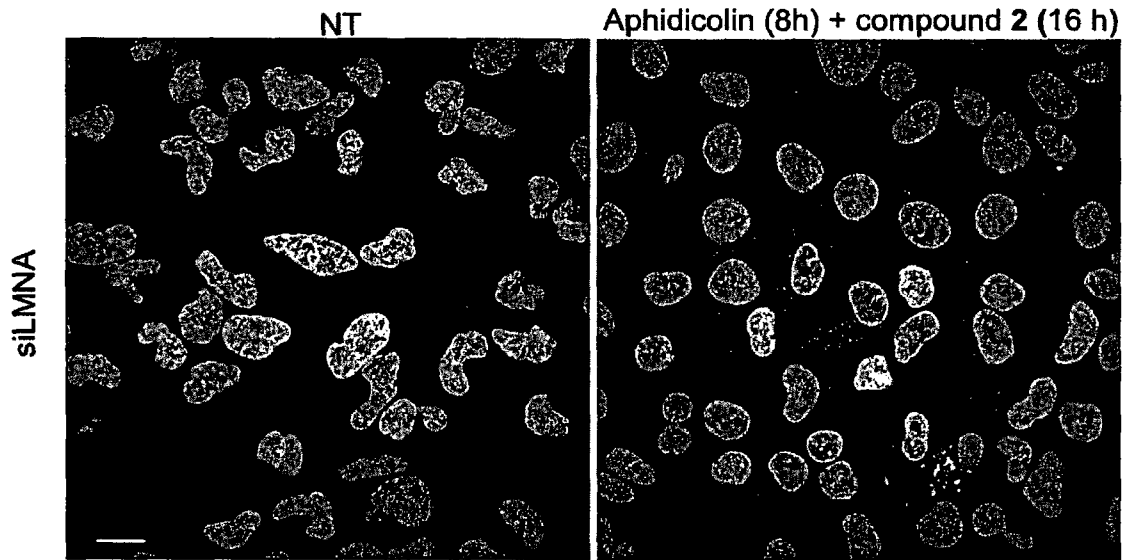


FIGURE 3

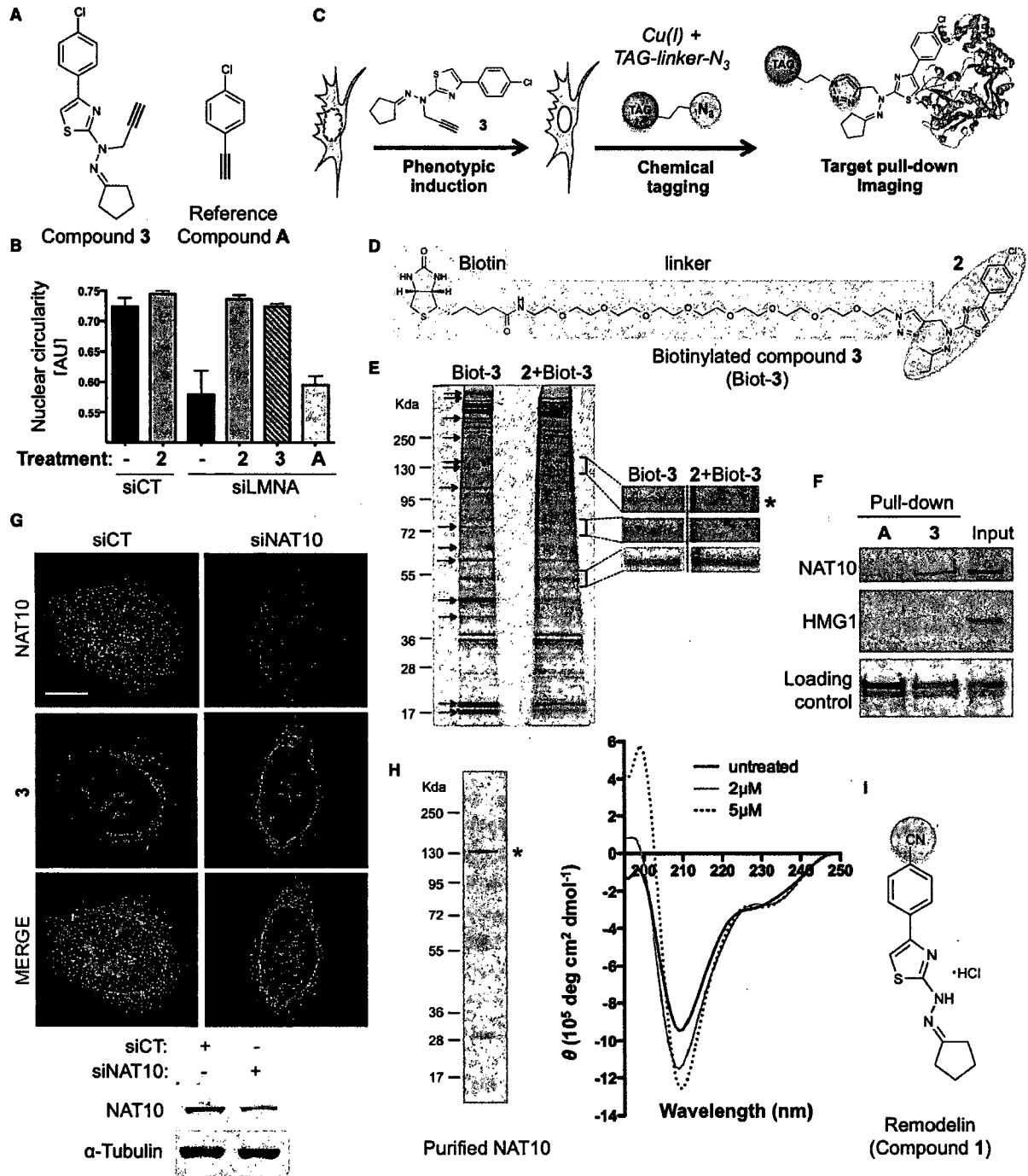


FIGURE 4

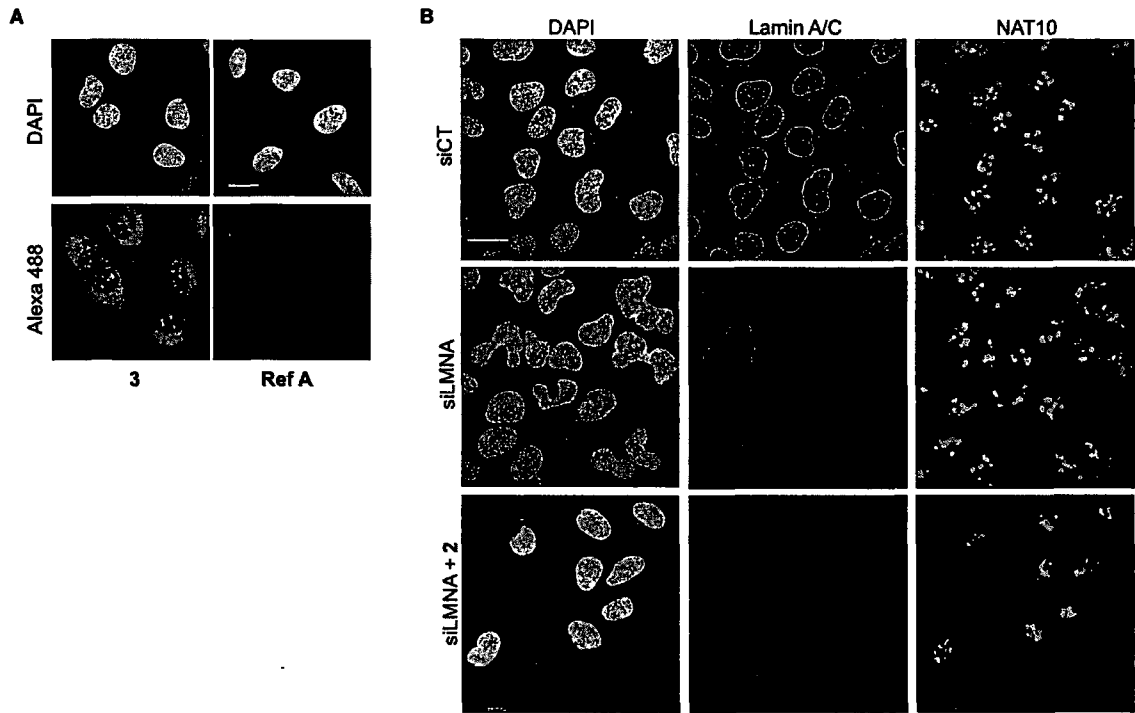


FIGURE 5



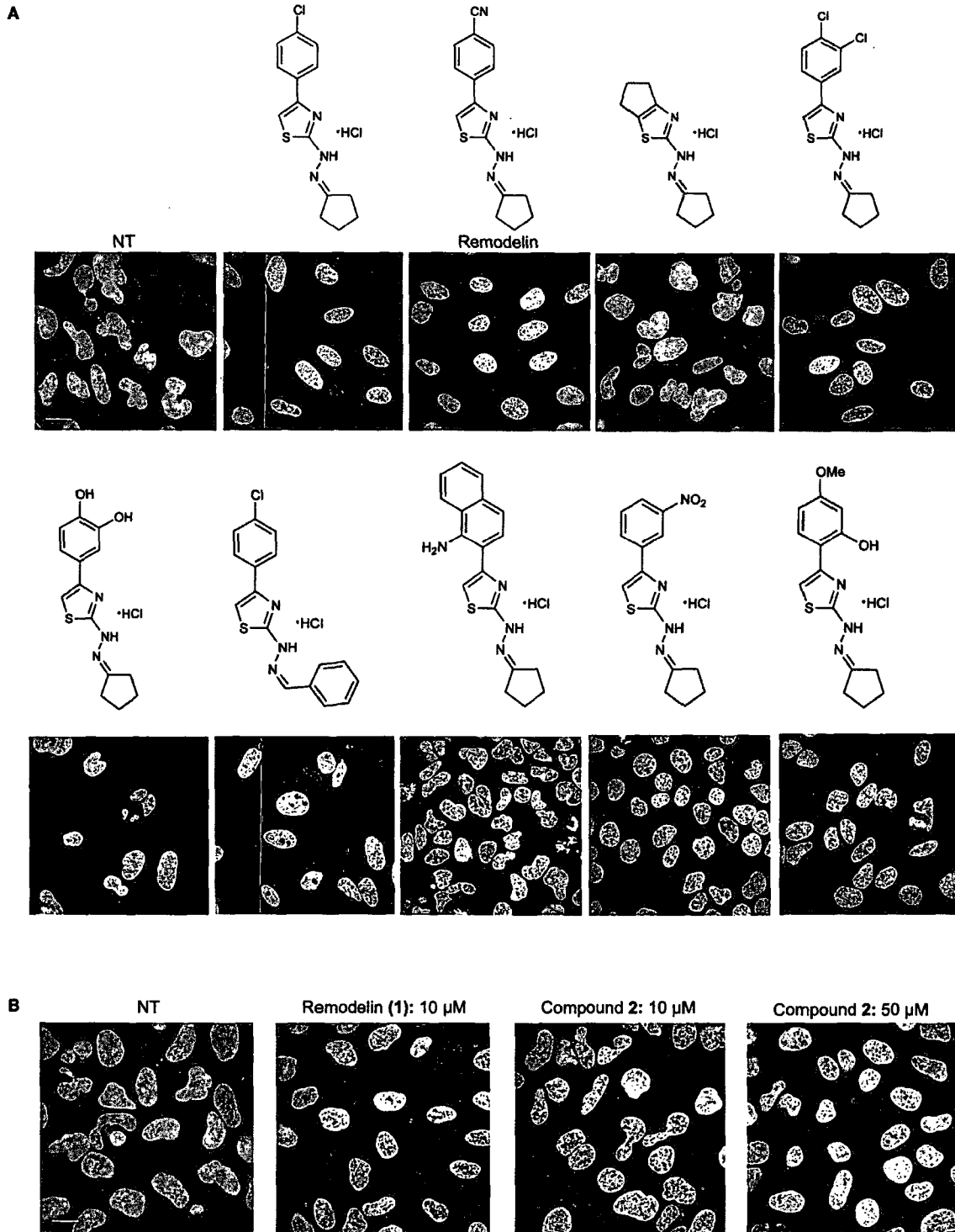


FIGURE 6

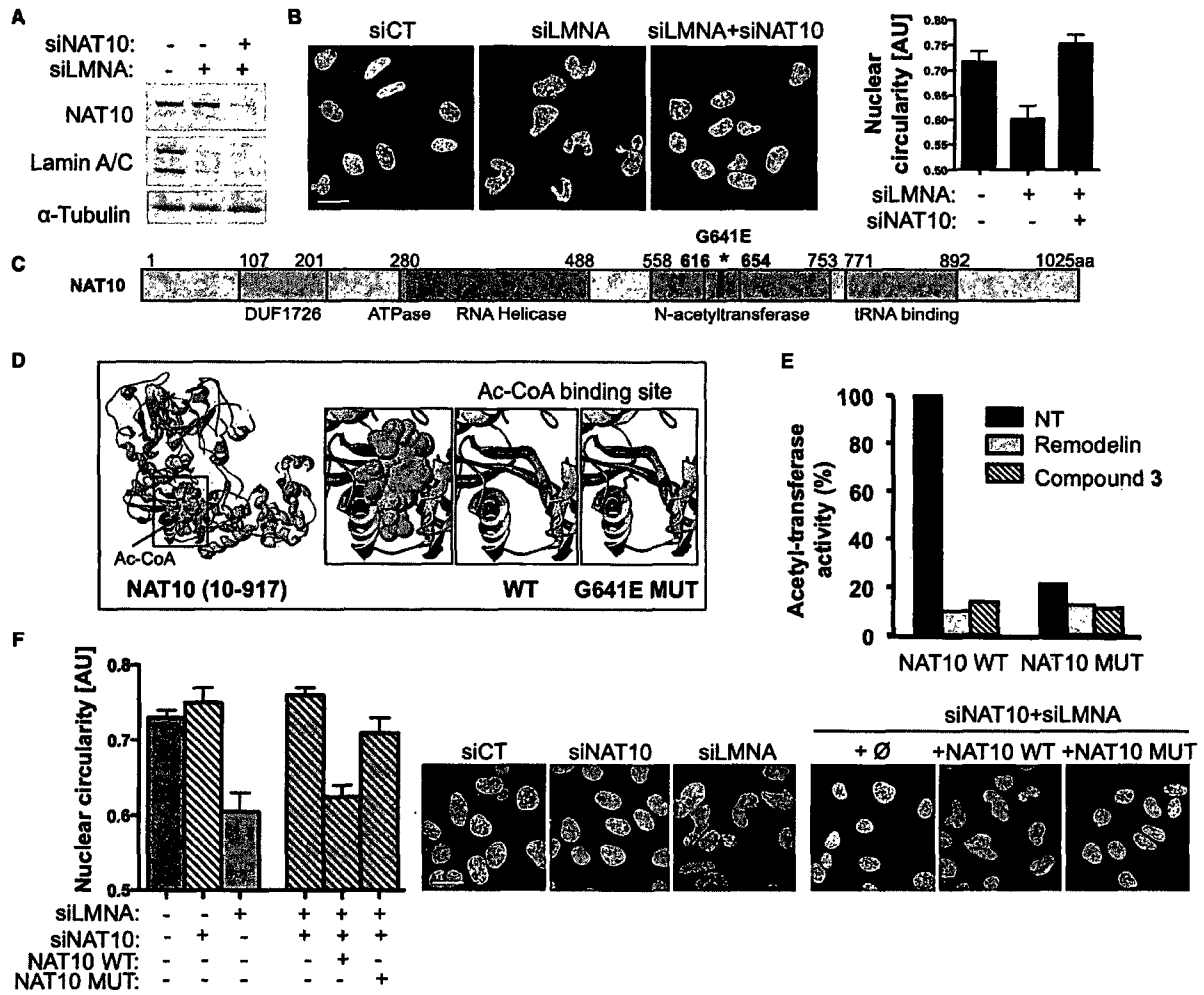
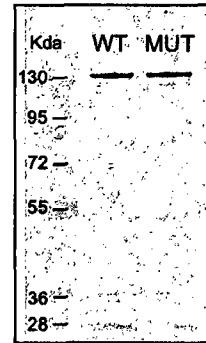


FIGURE 7

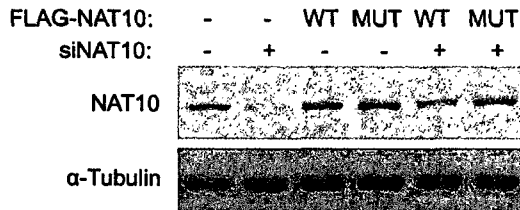
A

<i>Vibrio cholerae</i>	460	-CNRIVRIAVHPCPCROSLSSQLTHF	485
<i>Nitrosococcus halophilus</i>	511	-CARIVRIAVHPPVGGSLGHLVDTH	536
<i>Erwinia carotovora</i>	469	-ARRVSRIVAVSSRRQSLGRALADQ	494
<i>Shigella flexneri</i>	417	-GRVSRIVAVHPPARESLGROLIAGA	442
<i>Escherichia coli</i>	434	-GRVSRIVAVHPPARESLGROLIAGA	459
<i>Salmonella typhimurium</i>	454	-GRVSRIVAVHPPARESLGROLIAGA	479
<i>Citrobacter koseri</i>	455	-GRVSRIVAVHPPARESLGROLIAGA	480
<i>Haemophilus influenzae</i>	468	-SLRISRIAVQPNWQKSLGNLQAN	493
<i>Haemophilus ducreyi</i>	405	-SIRISRIAVQPLQNGSLGSLVDF	430
<i>Actinobacillus pleuropneumoniae</i>	400	-SVRISRIAVQPLQKSLGKRLISDF	425
<i>Sulfolobus islandicus</i>	525	-GRVSRIVAVHPPARESLGROLIAGA	550
<i>Thermophilum pendens</i>	556	-GRVSRIVAVHPPARESLGROLIAGA	581
<i>Methanopyrus kandleri</i>	584	-GRVSRIVAVHPPARESLGROLIAGA	609
<i>Pyrococcus abyssi</i>	583	-GRVSRIVAVHPPARESLGROLIAGA	608
<i>Arabidopsis thaliana</i>	611	-GARIVRIAVHPPNRMVGYGSRALQIL	636
<i>Drosophila melanogaster</i>	595	-GVRIVRIAVHPPNRMVGYGSRALQIL	620
<i>Caenorhabditis elegans</i>	616	-GRVSRIVAVHPPARESLGROLIAGA	641
<i>Dictyostelium discoideum</i>	615	-GVRIVRIAVHPPNRMVGYGSRALQIL	640
<i>Saccharomyces cerevisiae</i>	632	-GARIVRIAVHPPNRMVGYGSRALQIL	657
<i>Schizosaccharomyces pombe</i>	620	-GARIVRIAVHPPNRMVGYGSRALQIL	645
<i>Xenopus tropicalis</i>	623	-GRVSRIVAVHPPARESLGROLIAGA	648
<i>Gallus gallus</i>	623	-GRVSRIVAVHPPARESLGROLIAGA	648
<i>Oryctolagus cuniculus</i>	623	-GRVSRIVAVHPPARESLGROLIAGA	648
<i>Mus musculus</i>	623	-GRVSRIVAVHPPARESLGROLIAGA	648
<i>Sus scrofa</i>	623	-GRVSRIVAVHPPARESLGROLIAGA	648
<i>Homo sapiens</i>	623	-GRVSRIVAVHPPARESLGROLIAGA	648
Consensus		.....*	

B



C



D

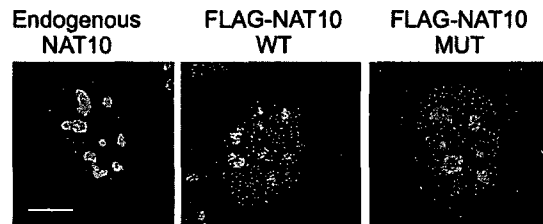


FIGURE 8

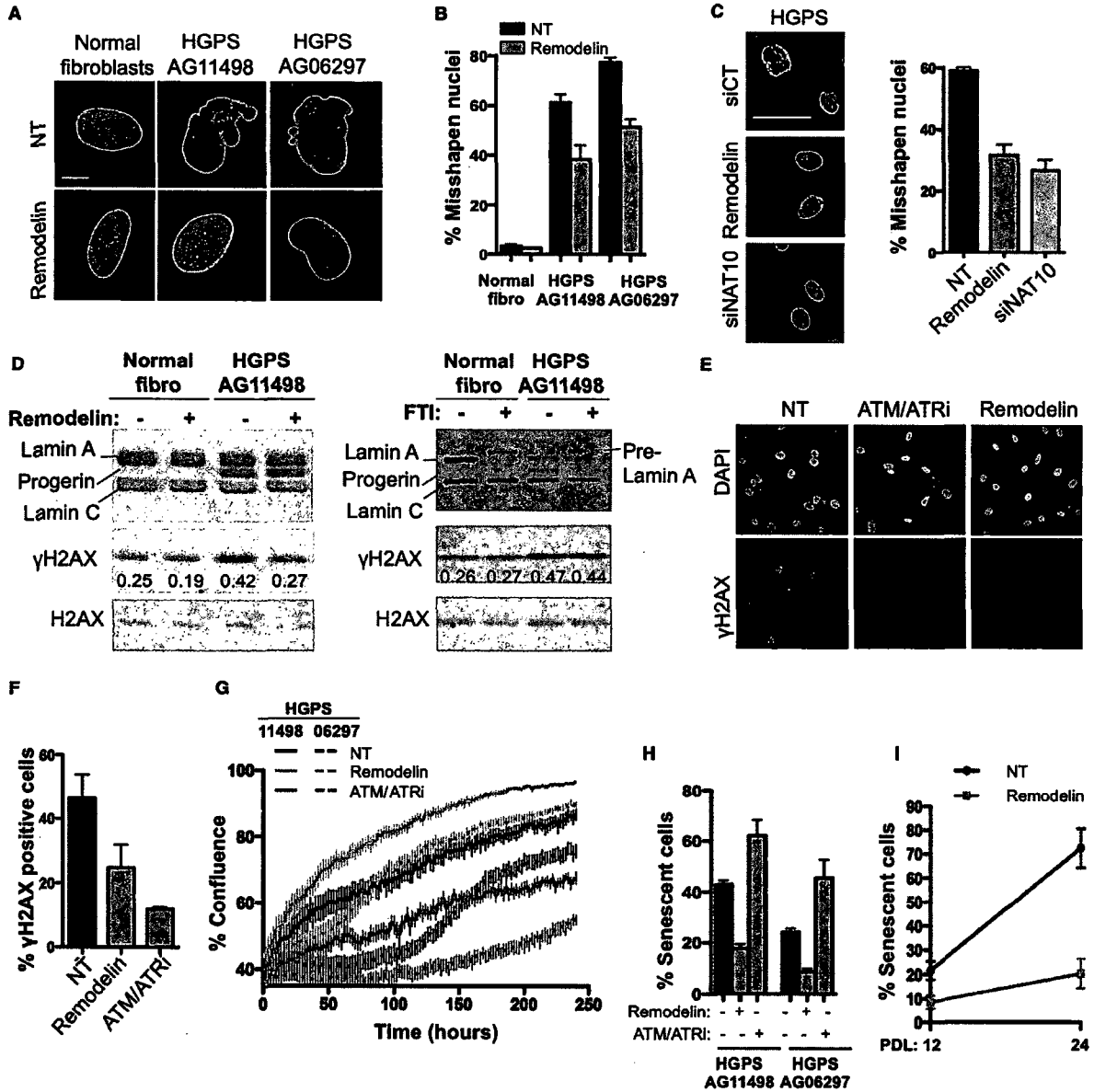


FIGURE 9

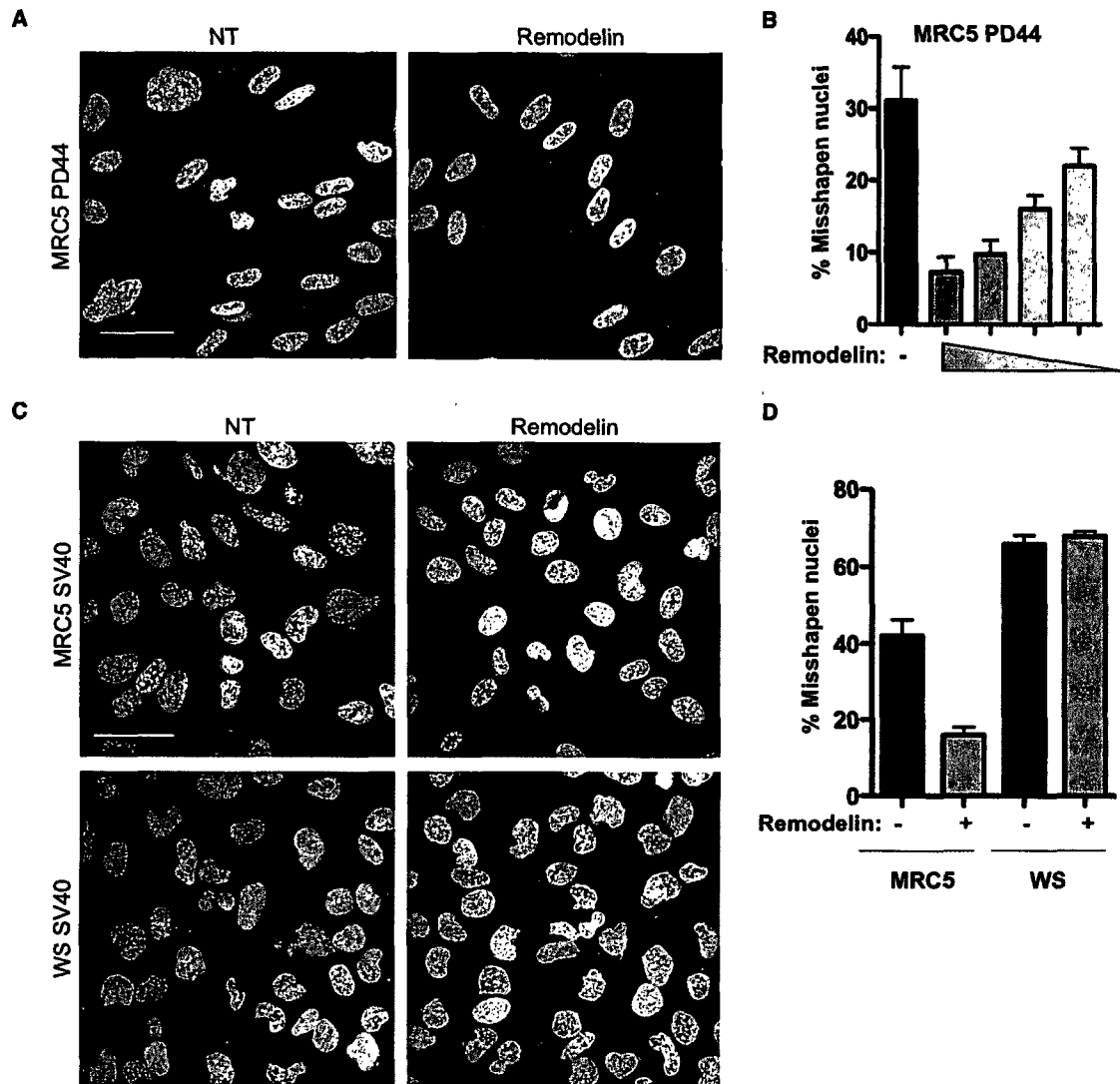


FIGURE 10

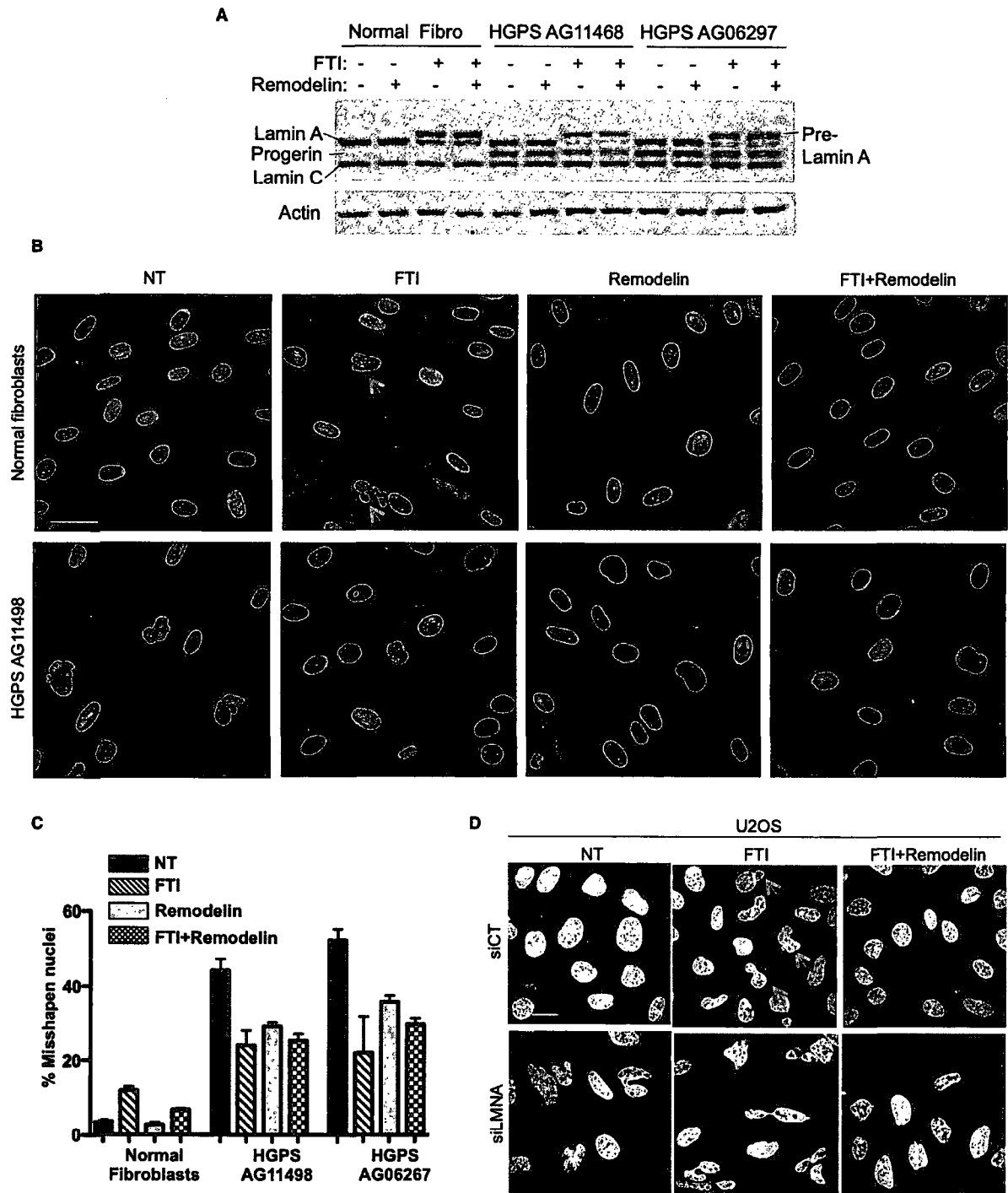


FIGURE 11

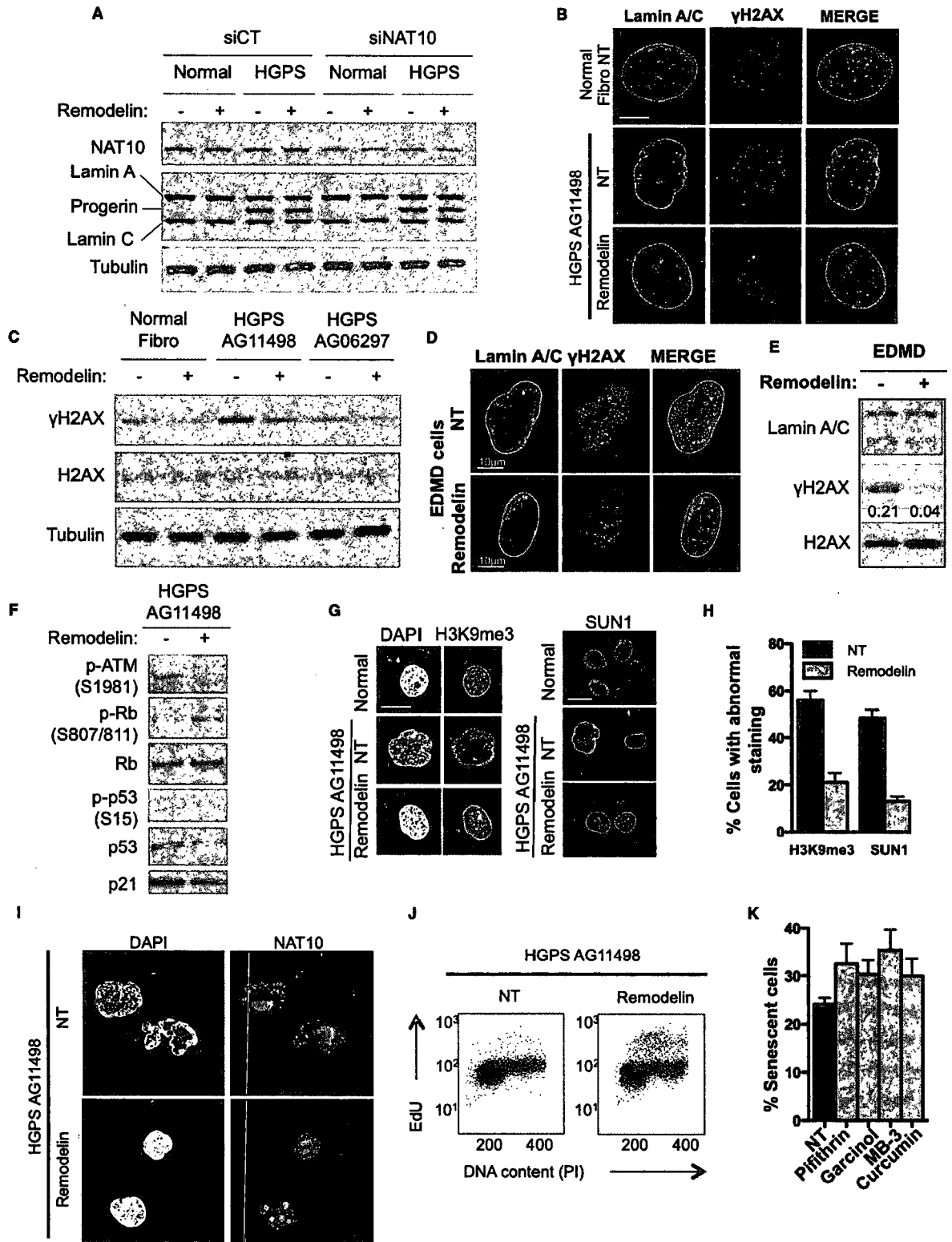


FIGURE 12

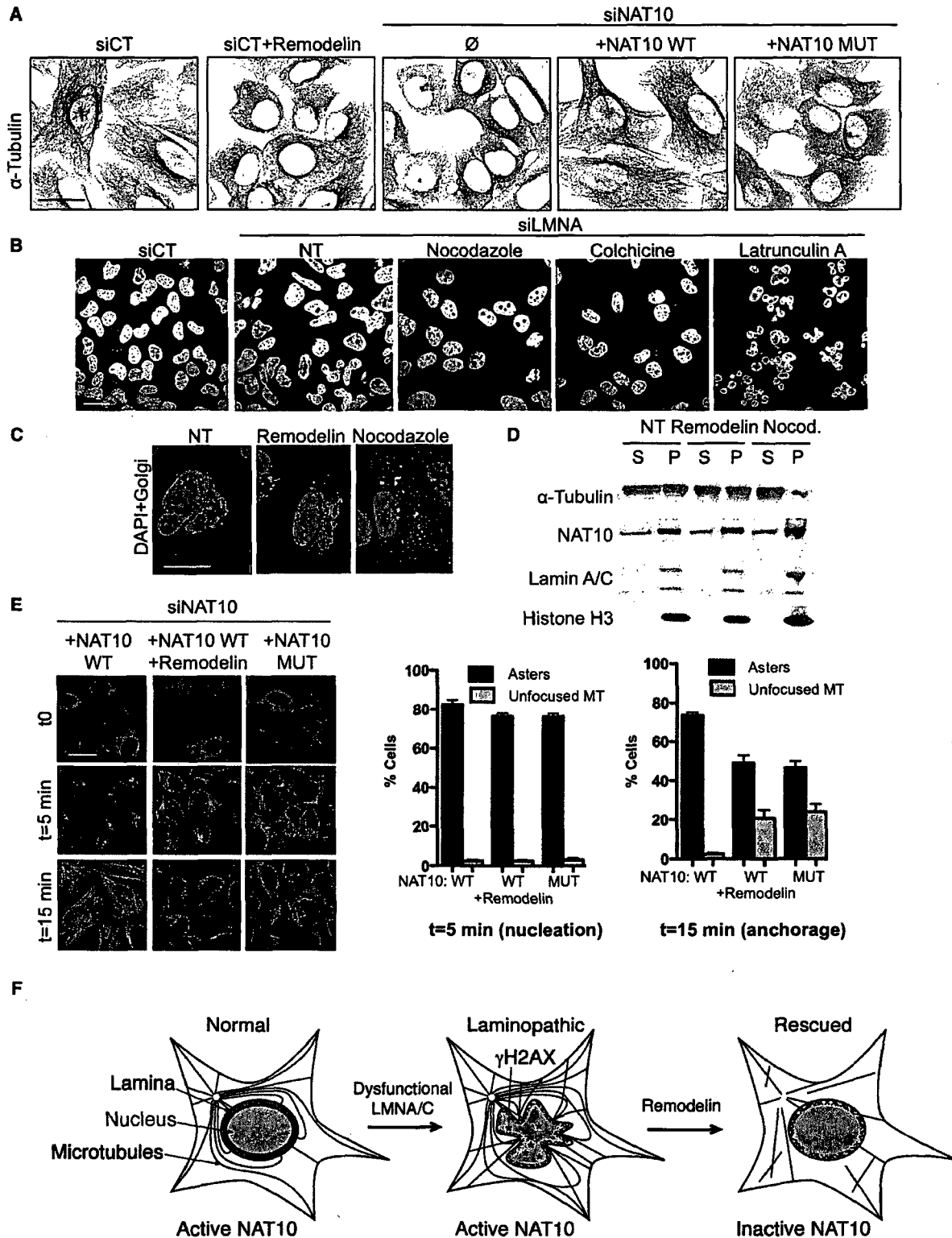


FIGURE 13



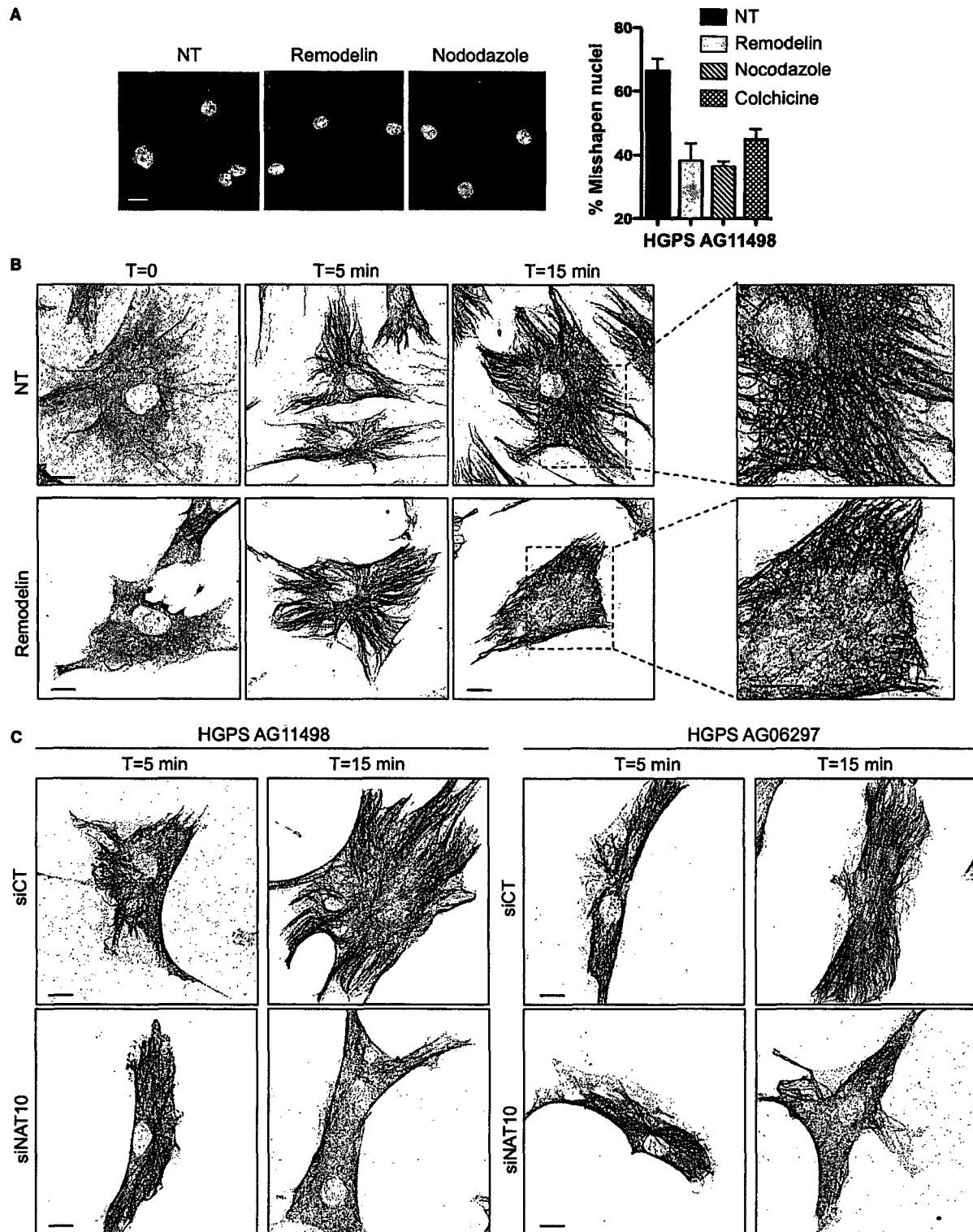


FIGURE 14

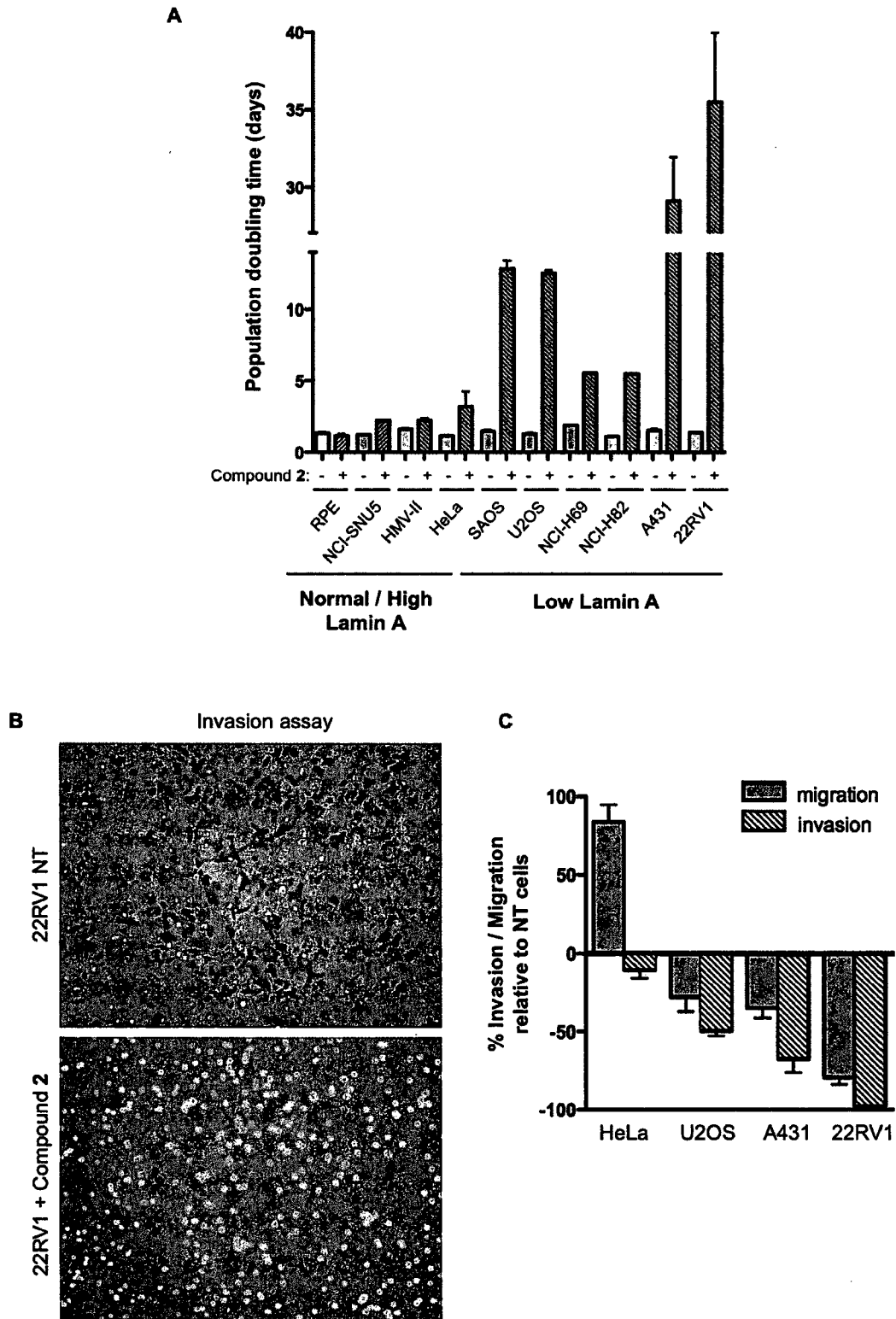


FIGURE 15

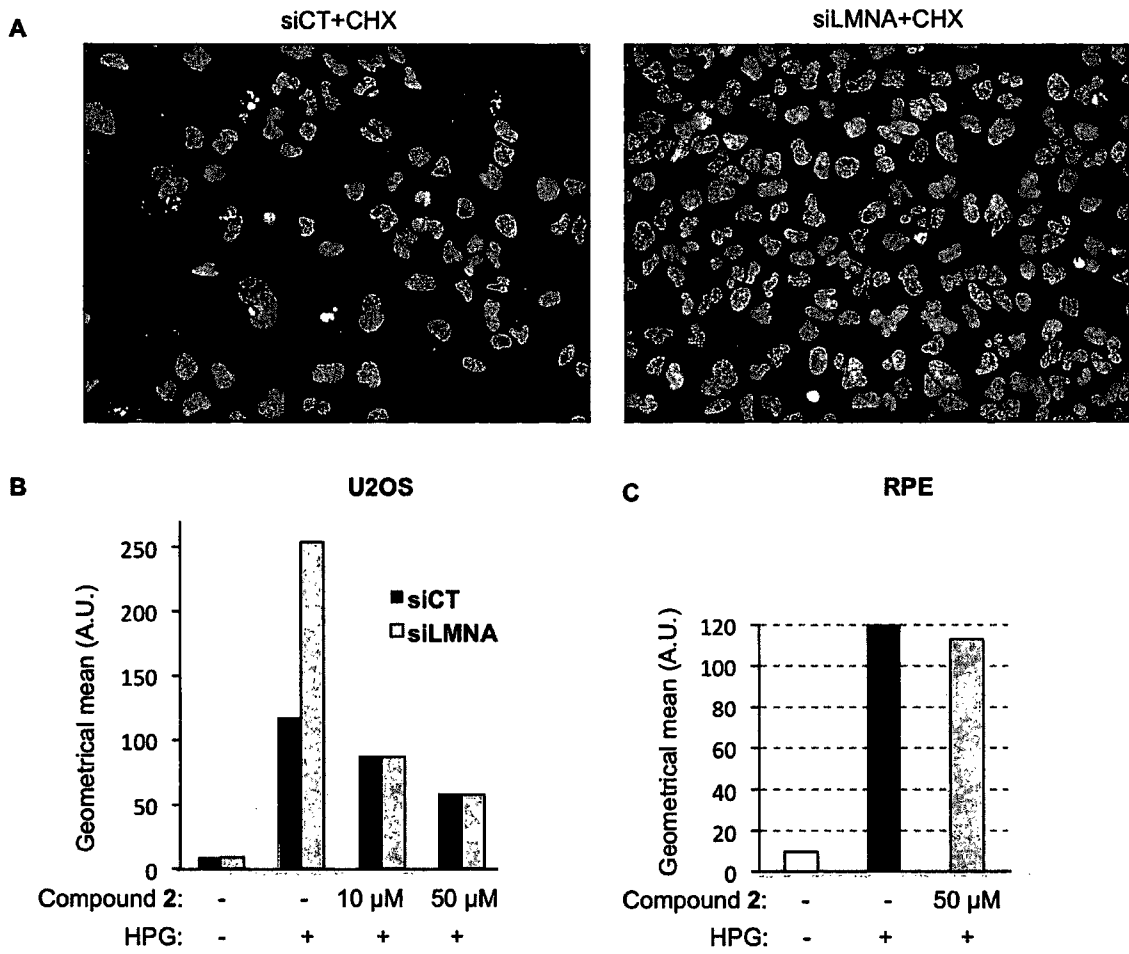
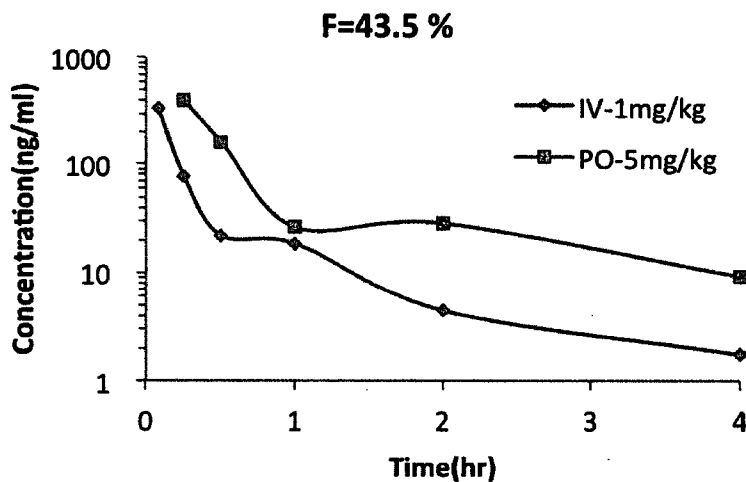


FIGURE 16

A



B

**PK Parameters and Plasma Concentration (ng/mL) for Remodelin**

$T_{1/2}$ (hr)	1.81
$T_{max}$ (hr)	0.250
$C_{max}$ (ng/mL)	409
$AUC_{0-t}$ (ng·hr/mL)	235
$AUC_{0-\infty}$ (ng·hr/mL)	259
MRT <sub>last</sub> (hr)	0.844
Bioavailability(%)	43.5%

**Study Details**

<b>Compound ID</b>	Remodelin
<b>Strain/Species/Sex</b>	ICR mouse/ male
<b>Nominal Dose</b>	1 mg/kg IV 5 mg/kg PO
<b>Formulation</b>	10% DMSO+ 90% (40% HP-β-CD)
<b>Matrix</b>	Plasma (EDTA-K <sub>2</sub> )

FIGURE 17

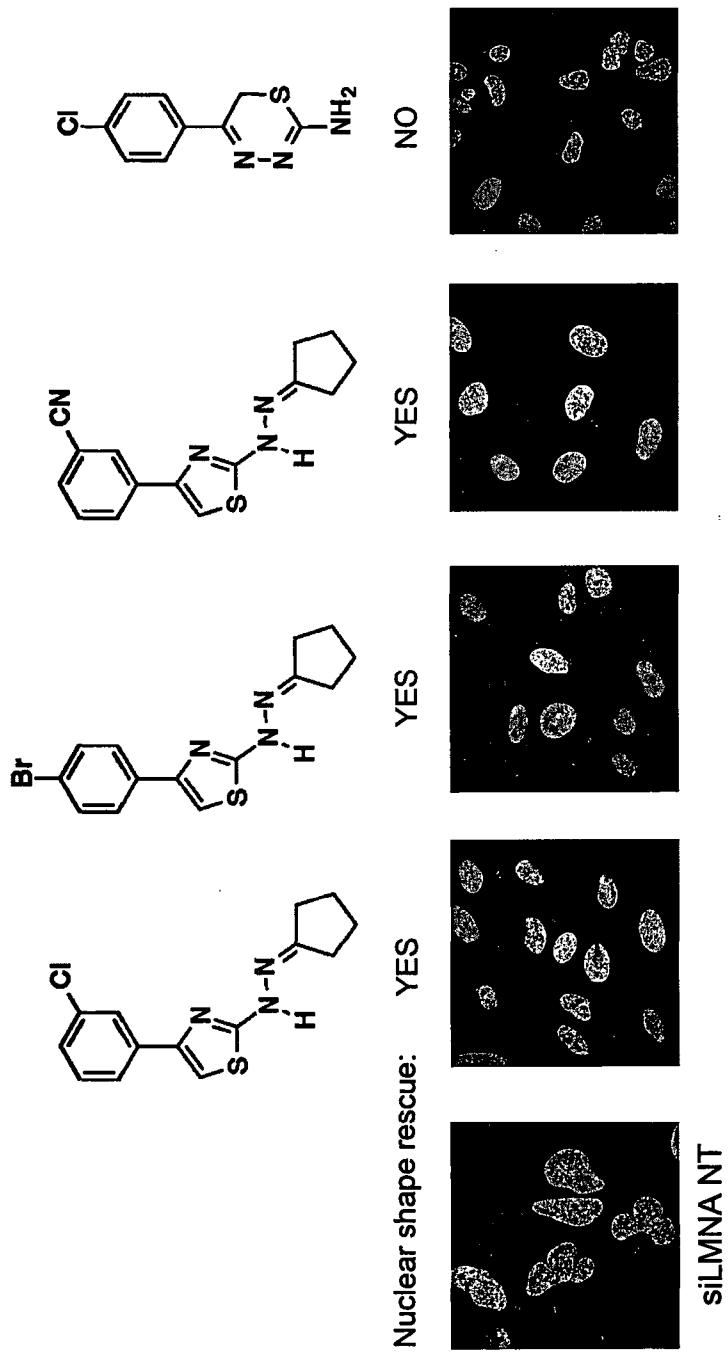


FIGURE 18

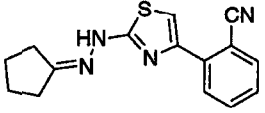
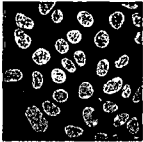
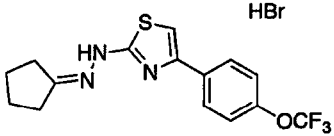
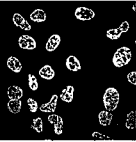
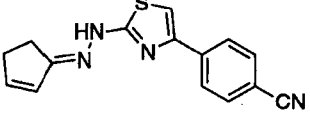
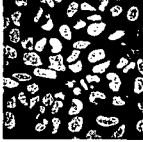
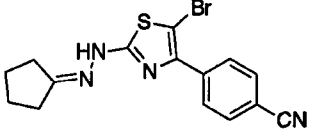
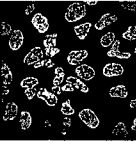
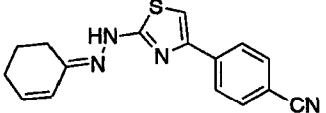
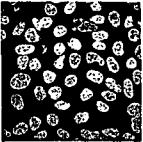
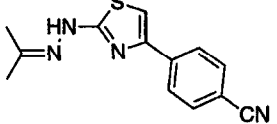
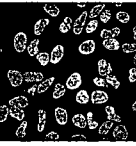
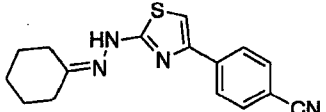
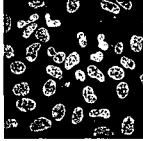
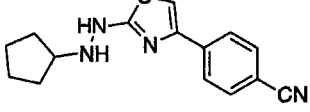
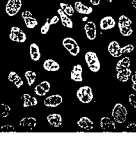
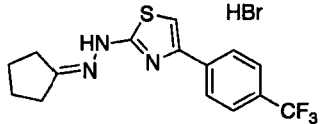
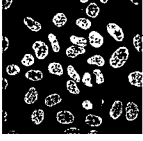
Molecular structure	Nuclear shape rescue	Molecular structure	Nuclear shape rescue
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 <p>Exact Mass: 280,0783 Molecular Weight: 280,3476</p>	<p>NO</p> 	 <p>Exact Mass: 360,0044 Molecular Weight: 361,2595</p>	<p>NO</p> 
 <p>Exact Mass: 294,0939 Molecular Weight: 294,3742</p>	<p>YES</p> 	 <p>Exact Mass: 256,0783 Molecular Weight: 256,3262</p>	<p>NO</p> 
 <p>Exact Mass: 296,1096 Molecular Weight: 296,3900</p>	<p>YES</p> 	 <p>Exact Mass: 284,1096 Molecular Weight: 284,3793</p>	<p>NO</p> 
 <p>Exact Mass: 405,01 Molecular Weight: 406,26</p>	<p>YES</p> 		

FIGURE 19

A431

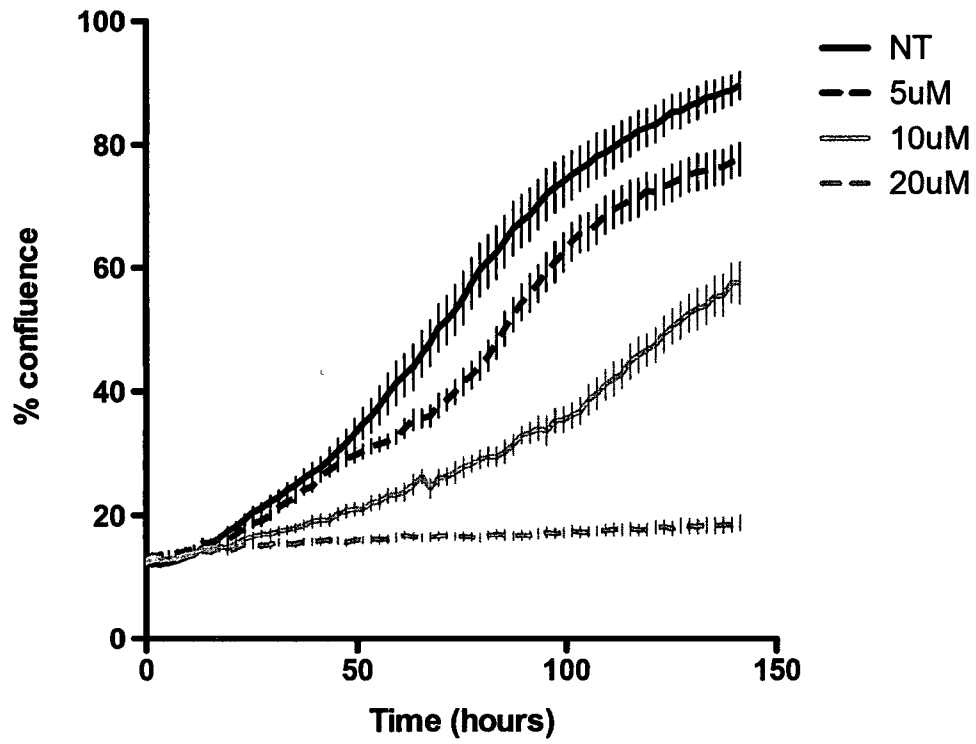


FIGURE 20

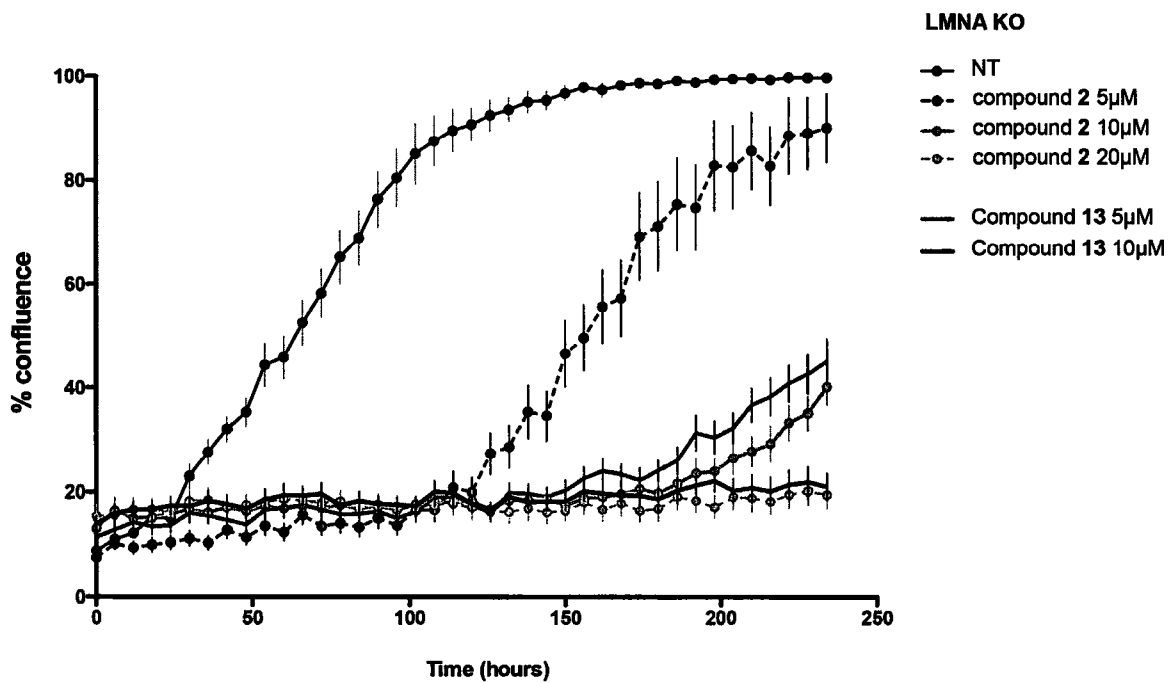
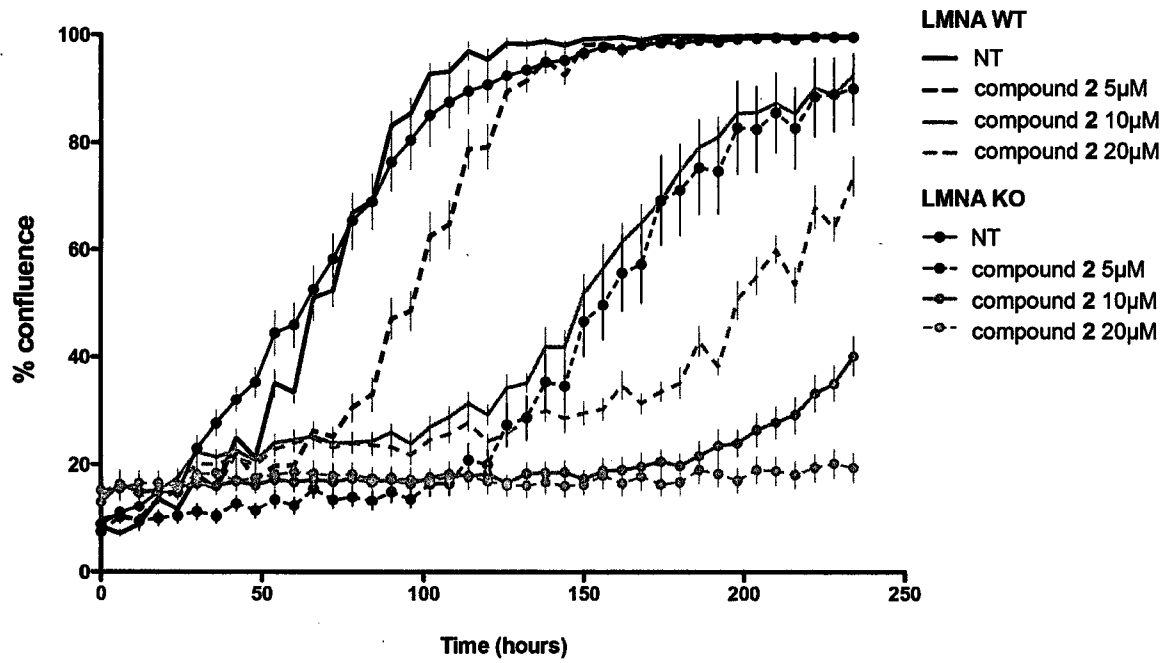


FIGURE 21



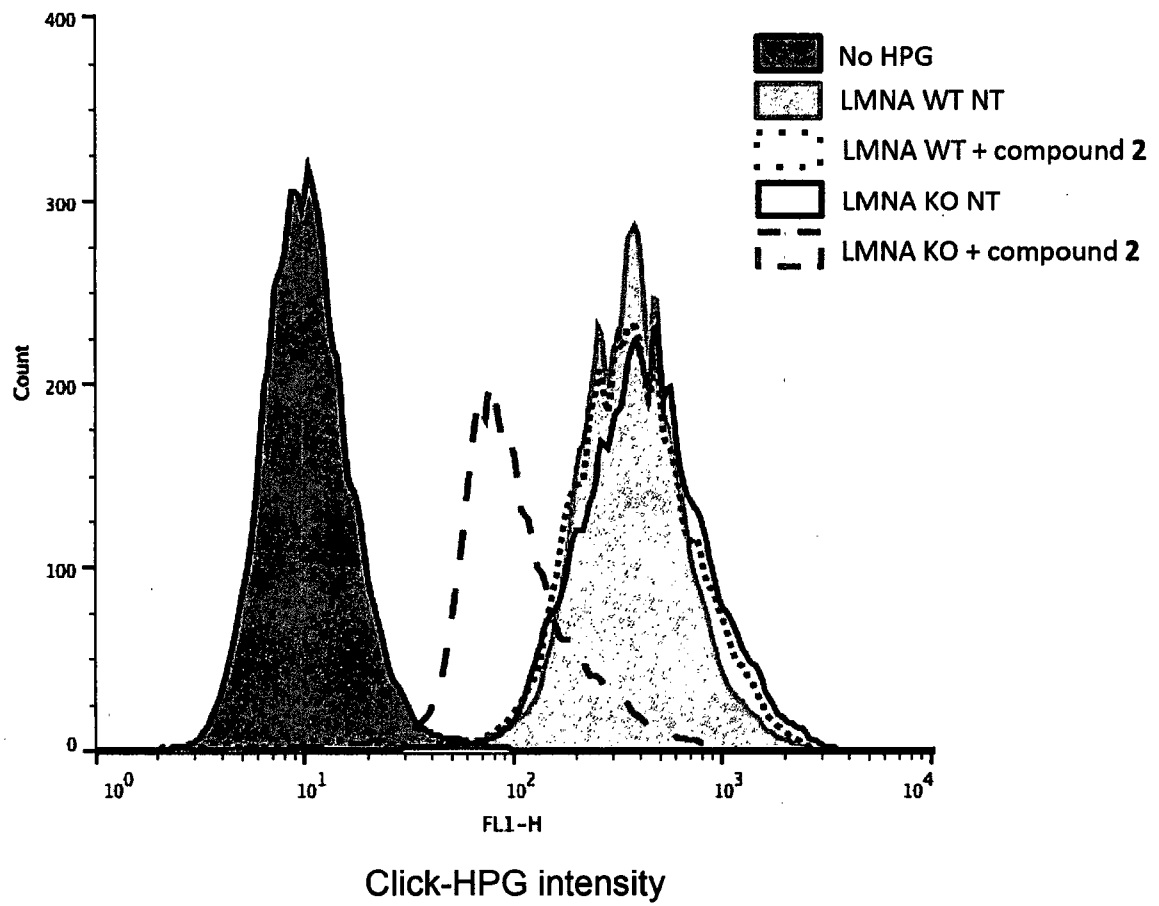


FIGURE 22

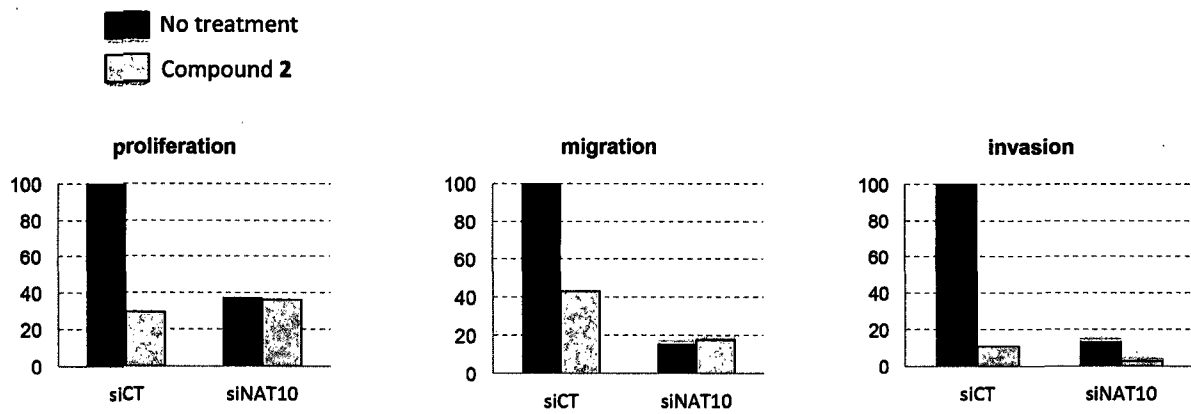
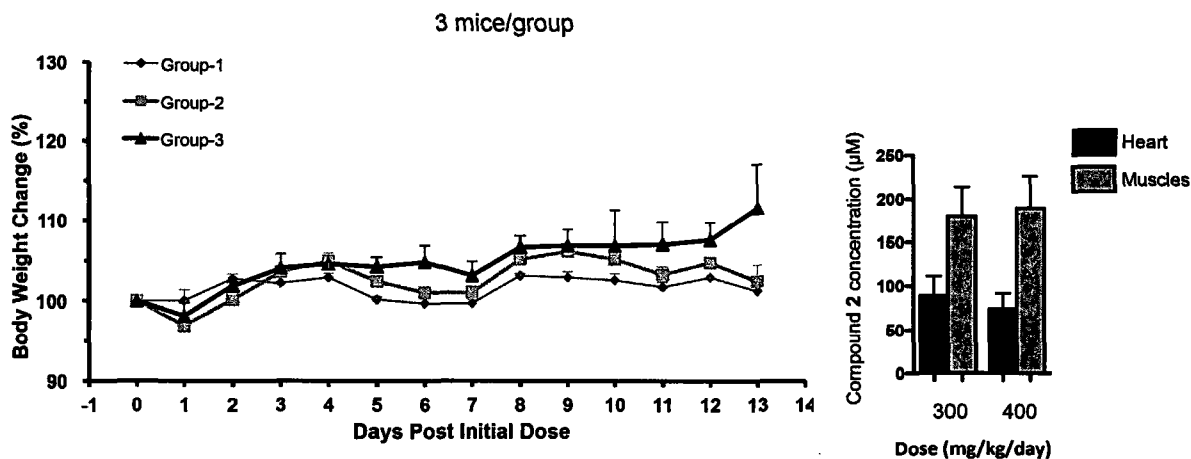


FIGURE 23

Group 1: compound 2 50mg/kg/day for 7 days + 300mg/kg/day for 7 days  
 Group 2: compound 2 200mg/kg/day for 7 days + 400mg/kg/day for 7 days  
 Group 3: compound 2 50mg/kg/day for 7 days + 100mg/kg/day for 7 days



Acetyltransferase:	Substrate:	Compound IC50* (M)		
		Compound 2	Anacardic Acid	C646
CBP	Histone H3		ND	3.66E-07
GCN5	Histone H3		4.60E-05	ND
KAT5	Histone H2A		ND	2.16E-05
MYST2/KAT7	Histone H3		ND	6.50E-06
MYST4/KAT6B/MORF	Histone H4		ND	3.26E-05
p300	Histone H3		ND	4.41E-07
pCAF	Histone H3		5.46E-05	ND

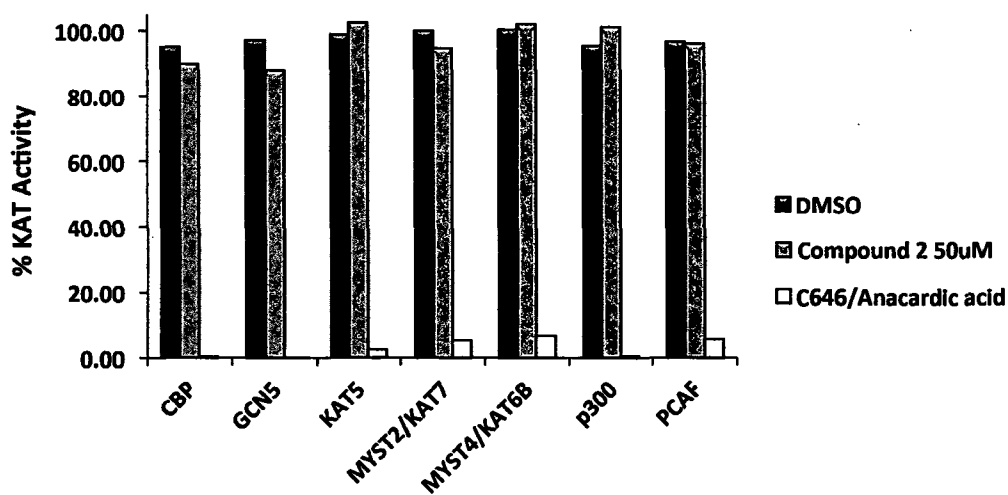


FIGURE 24

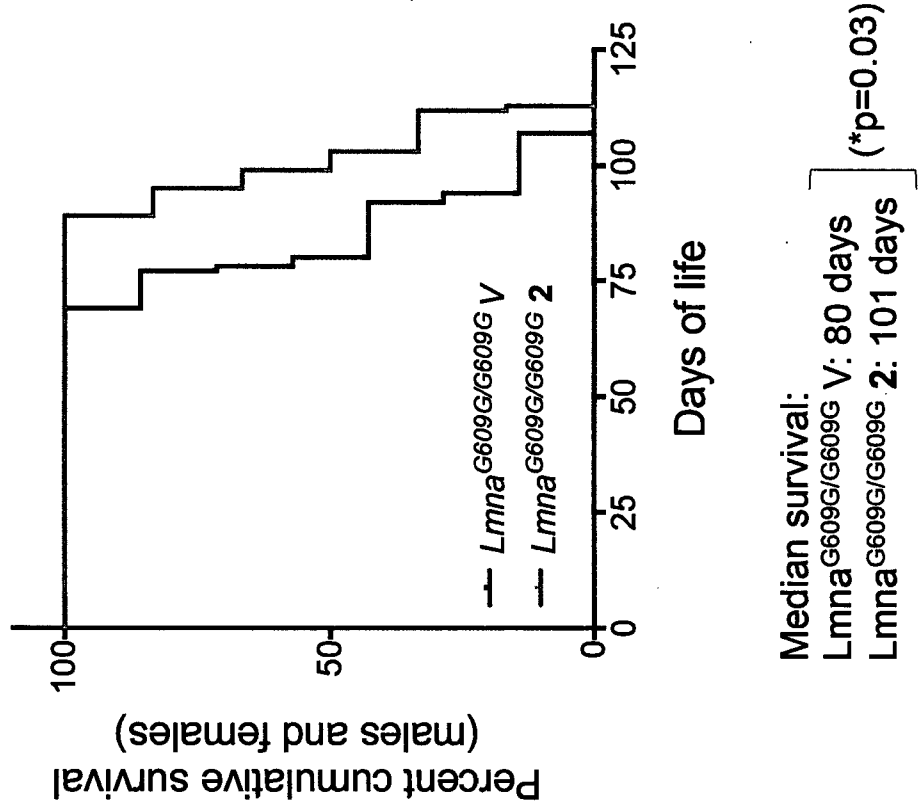
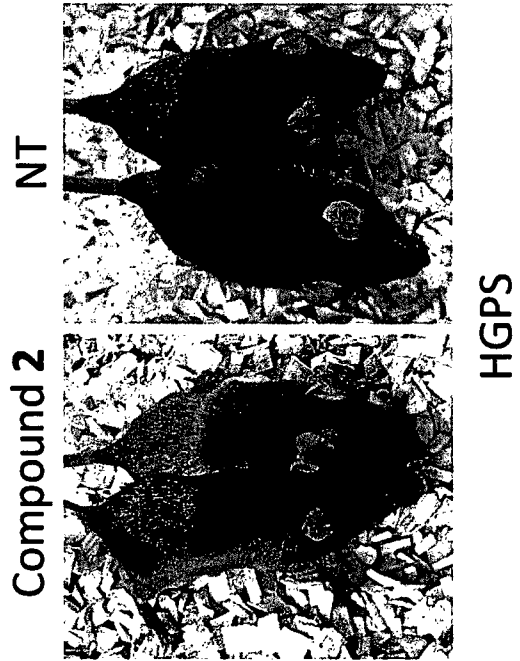
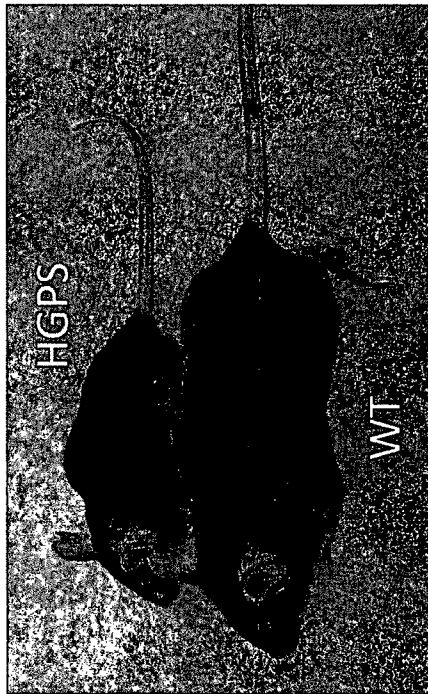


FIGURE 25

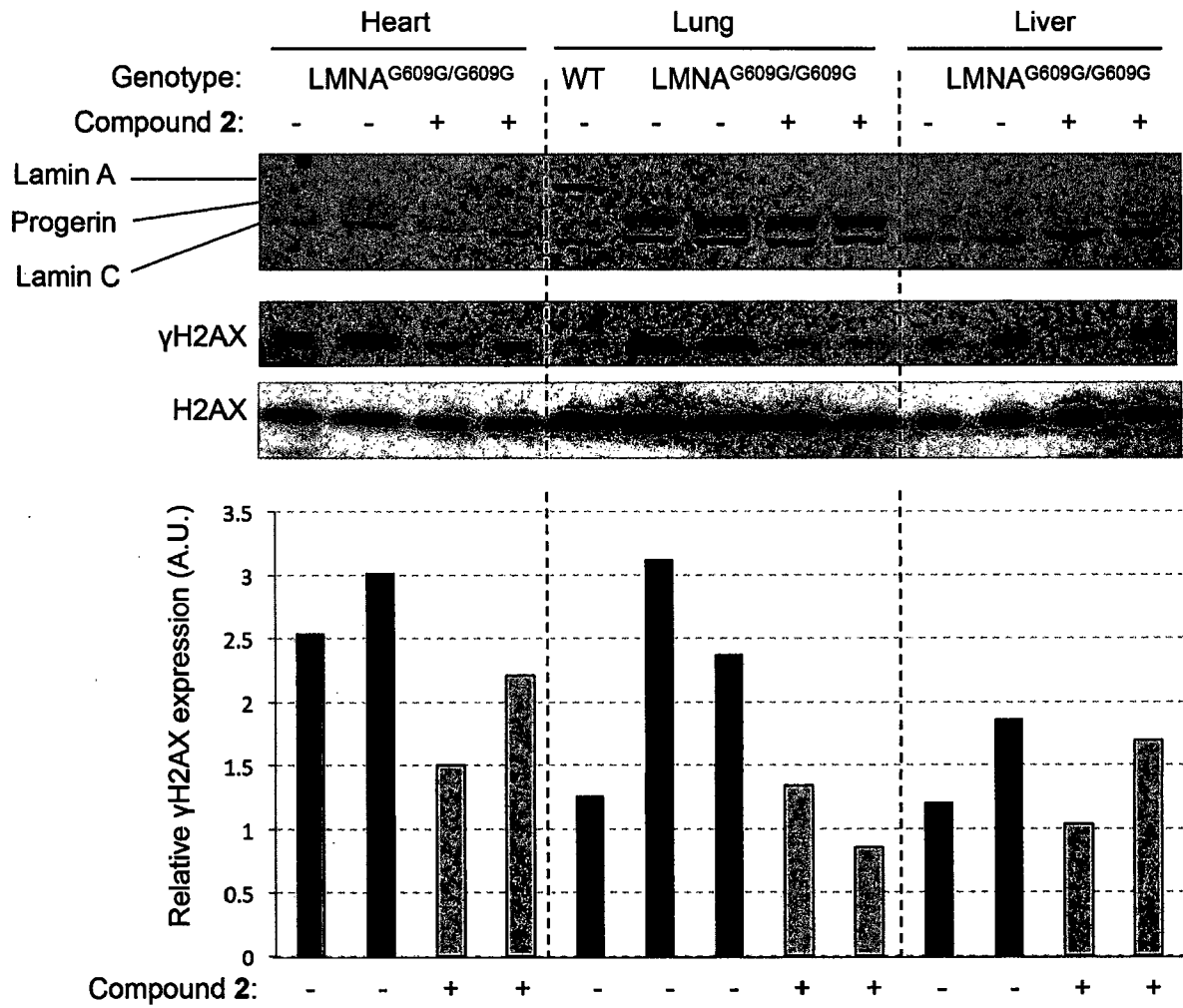


FIGURE 26

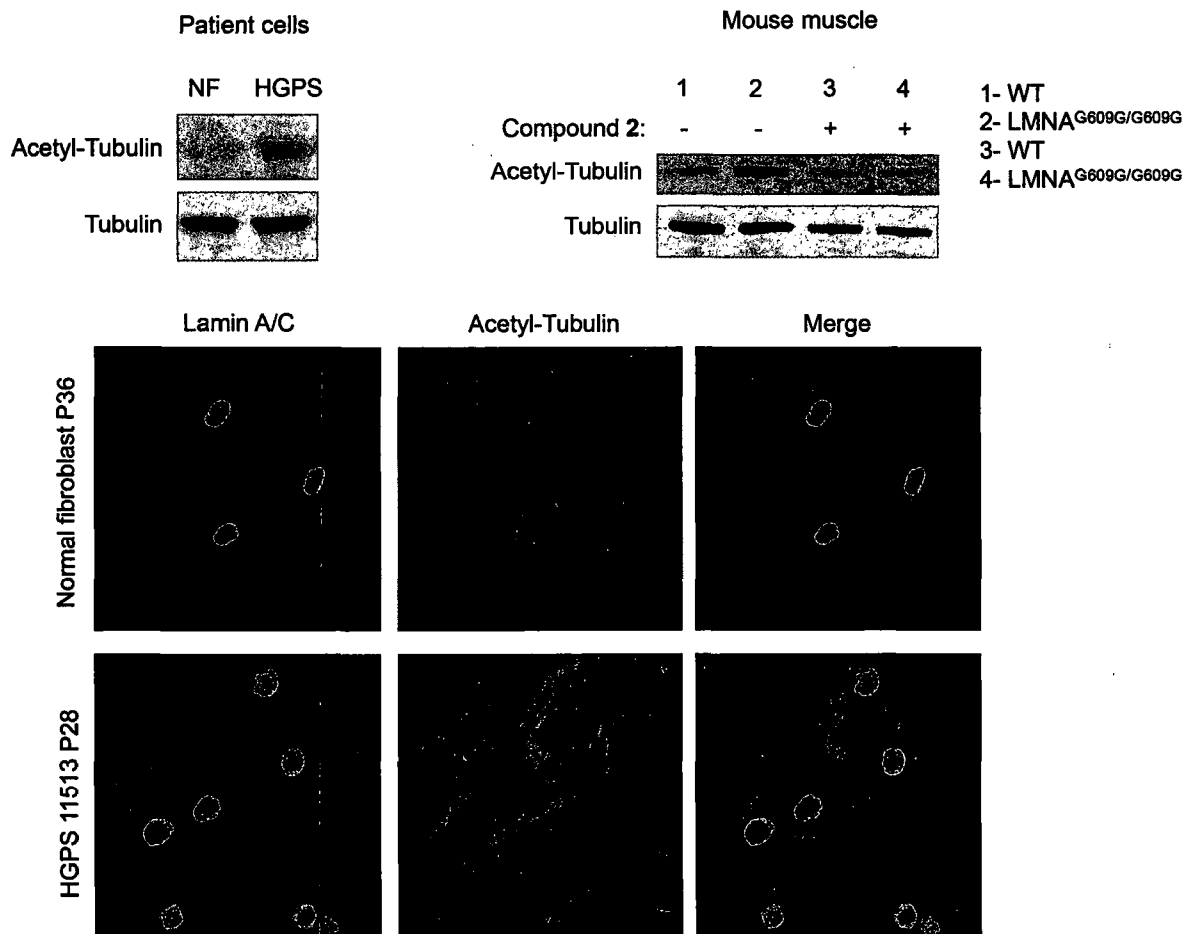


FIGURE 27

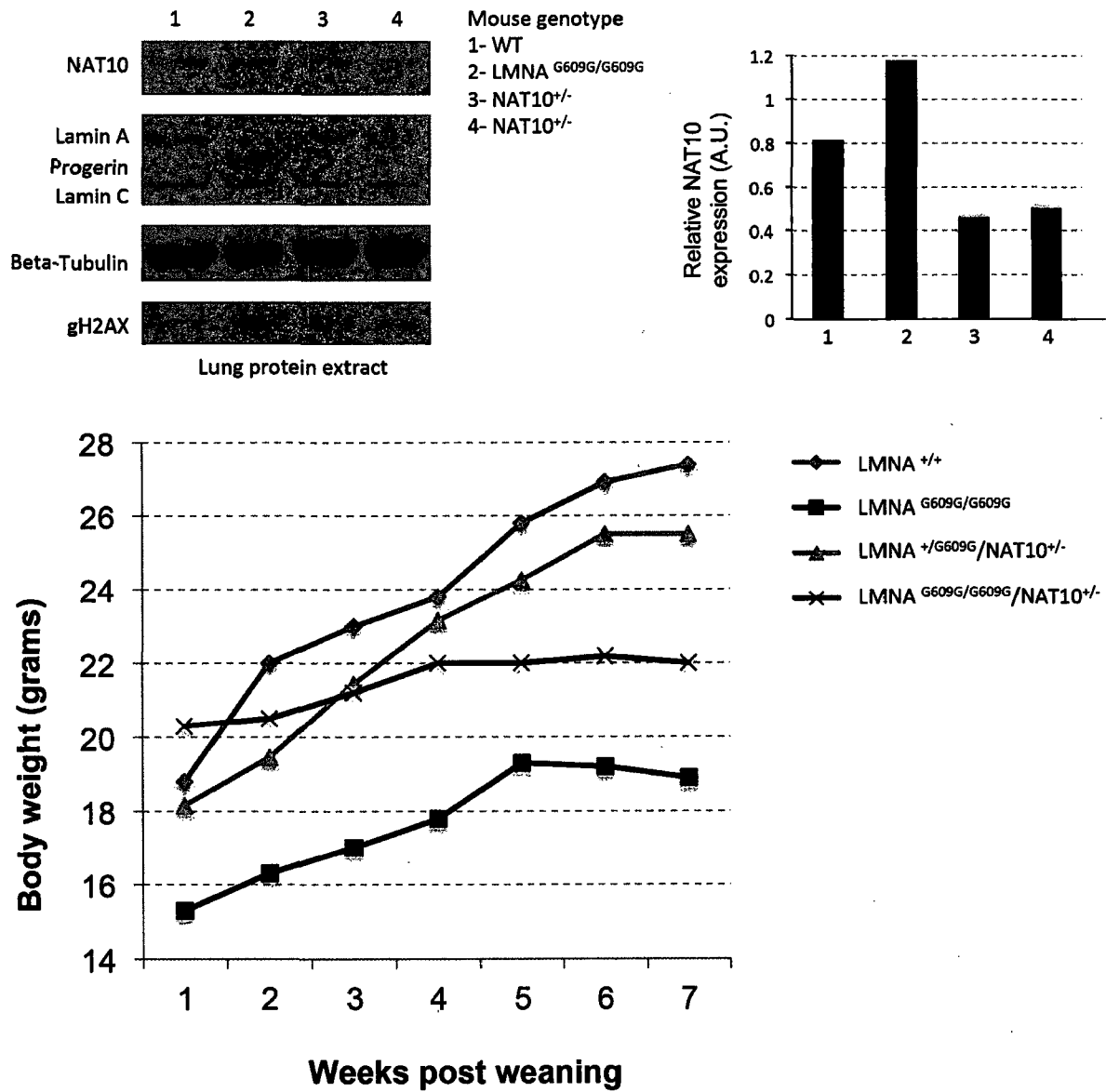


FIGURE 28

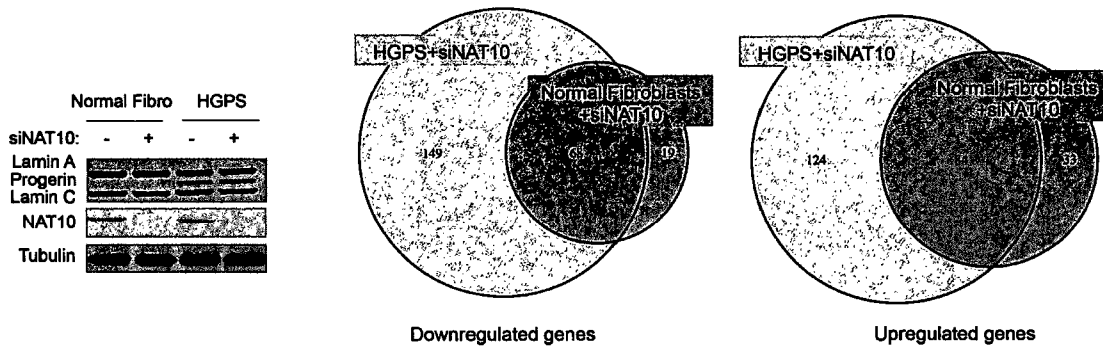
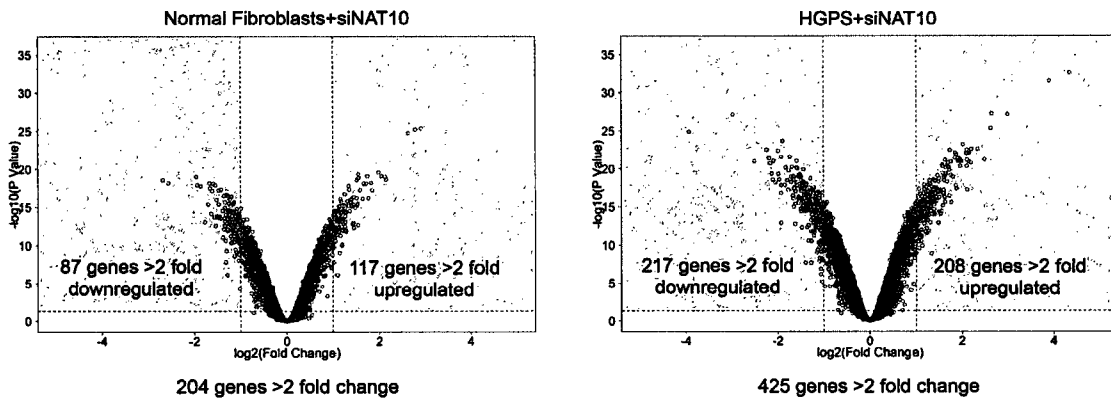
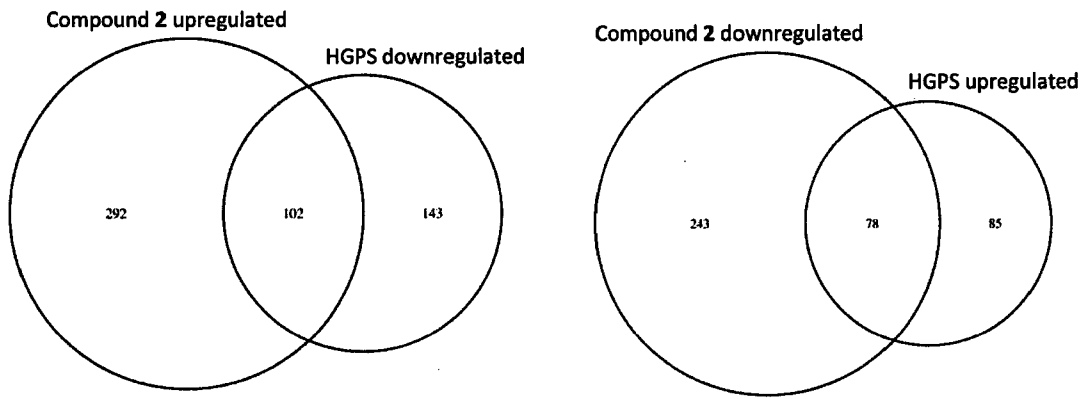
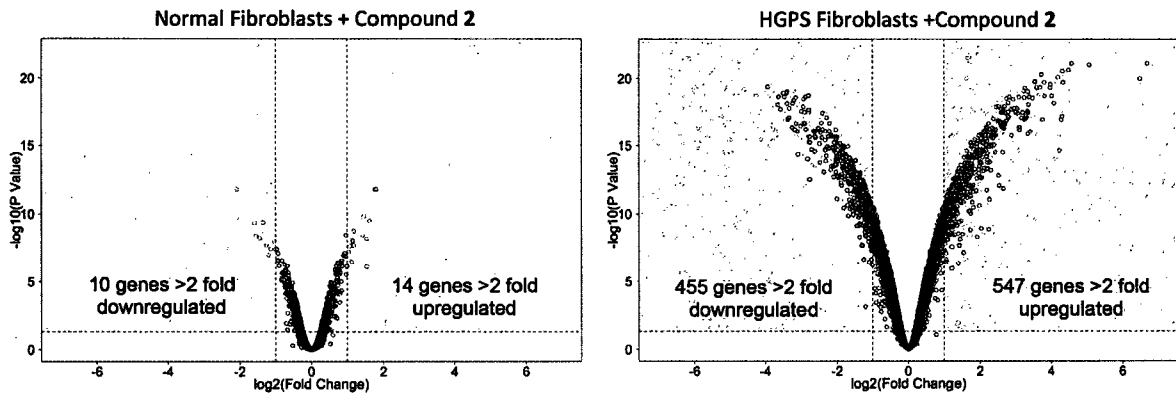


FIGURE 29



**INTERNATIONAL SEARCH REPORT**

International application No  
PCT/GB2015/051040

**A. CLASSIFICATION OF SUBJECT MATTER**  
 INV. A61K31/426 A61Q19/08 A61K45/06 A61K31/549 A61P35/00  
 A61P43/00 A61P9/00 A61P17/00 A61P39/00 C07D277/50  
 C07D285/18

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

**B. FIELDS SEARCHED**  
 Minimum documentation searched (classification system followed by classification symbols)  
 A61K A61Q C07D

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
 EPO-Internal, WPI Data, EMBASE, BIOSIS, CHEM ABS Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/144805 A1 (WAGNER GERHARD [US] ET AL) 10 June 2010 (2010-06-10)  claims 10,11 claim 46 abstract	1-7,9, 10,12, 14,17, 18,20-23
X	US 2013/178505 A1 (CHOREV MICHAEL [US] ET AL) 11 July 2013 (2013-07-11)  compound KH-152f; page 42; table 4 page 71 - page 71; example XIII; compounds KH-102BII compounds KH-102BIIs	1-6, 8-10,12, 14,17, 18,20-23

Further documents are listed in the continuation of Box C.       See patent family annex.

\* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search  19 June 2015	Date of mailing of the international search report  01/07/2015
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer  Langer, Oliver
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## INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2015/051040

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>EP 1 191 024 A1 (TSCHESCHE HARALD [DE]; SCHROEDER JOERG [DE]) 27 March 2002 (2002-03-27)</p> <p>abstract entry for compound 31 in Table 1 claims 3,7,8</p> <p style="text-align: center;">-----</p>	1,4-9, 12,14, 17,18, 21-23
X	<p>CN 103 006 653 A (UNIV EAST CHINA SCIENCE &amp; TECH) 3 April 2013 (2013-04-03)</p> <p>paragraph [0081] - paragraph [0085] first compound; paragraph [0039] paragraph [0051] - paragraph [0052]</p> <p style="text-align: center;">-----</p>	1-5, 7-10,12, 14,17, 18,21-23
X	<p>H. XU ET AL: "N- -Acetyltransferase 10 protein inhibits apoptosis through RelA/p65-regulated MCL1 expression", CARCINOGENESIS, vol. 33, no. 6, 1 June 2012 (2012-06-01), pages 1193-1202, XP055196270, ISSN: 0143-3334, DOI: 10.1093/carcin/bgs144 abstract page 1194, right-hand column, last paragraph</p> <p style="text-align: center;">-----</p>	24
X	<p>CHUNG-FAN LEE ET AL: "hNaa10p contributes to tumorigenesis by facilitating DNMT1-mediated tumor suppressor gene silencing", JOURNAL OF CLINICAL INVESTIGATION, vol. 120, no. 8, 2 August 2010 (2010-08-02), pages 2920-2930, XP055196230, ISSN: 0021-9738, DOI: 10.1172/JCI42275 abstract figure 2 page 2920 - page 2921</p> <p style="text-align: center;">-----</p>	24,25
X,P	<p>D. LARRIEU ET AL: "Chemical Inhibition of NAT10 Corrects Defects of Laminopathic Cells", SCIENCE, vol. 344, no. 6183, 2 May 2014 (2014-05-02), pages 527-532, XP055196219, ISSN: 0036-8075, DOI: 10.1126/science.1252651 the whole document</p> <p style="text-align: center;">-----</p> <p style="text-align: center;">-/--</p>	1-25

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/GB2015/051040

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2009/203747 A1 (CHAIMBAULT CORINNE [FR] ET AL) 13 August 2009 (2009-08-13) claims 1,18 paragraph [0008] -----	1-25
A	WO 2013/158046 A1 (AGENCY SCIENCE TECH & RES [SG]) 24 October 2013 (2013-10-24) claims 1,15 -----	1-23
A	US 2013/203799 A1 (GORDON LESLIE B [US] ET AL) 8 August 2013 (2013-08-08) claim 1 -----	1-23
A	WO 2013/123403 A1 (SANFORD BURNHAM MED RES INST [US]) 22 August 2013 (2013-08-22) claims 24,36-38 -----	1-23
A	US 2009/280111 A1 (ZHENG YIXIAN [US] ET AL) 12 November 2009 (2009-11-12) abstract claims 1,2 paragraph [0063] -----	1-23
A	M. SINGH ET AL: "Lamin A/C Depletion Enhances DNA Damage-Induced Stalled Replication Fork Arrest", MOLECULAR AND CELLULAR BIOLOGY, vol. 33, no. 6, 15 March 2013 (2013-03-15) , pages 1210-1222, XP055196209, ISSN: 0270-7306, DOI: 10.1128/MCB.01676-12 the whole document -----	1-23

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2015/051040

Patent document cited in search report	Publication date	Patent family member(s)	Publication date	
US 2010144805	A1	10-06-2010	AU 2006206286 A1	27-07-2006
			CA 2619153 A1	27-07-2006
			EP 1868604 A2	26-12-2007
			JP 5225691 B2	03-07-2013
			JP 2008528502 A	31-07-2008
			US 2010144805 A1	10-06-2010
			WO 2006078942 A2	27-07-2006
-----				
US 2013178505	A1	11-07-2013	AU 2011276539 A1	07-02-2013
			CA 2803880 A1	12-01-2012
			EP 2585453 A2	01-05-2013
			JP 2013533258 A	22-08-2013
			US 2013178505 A1	11-07-2013
			WO 2012006068 A2	12-01-2012
-----				
EP 1191024	A1	27-03-2002	NONE	
-----				
CN 103006653	A	03-04-2013	NONE	
-----				
US 2009203747	A1	13-08-2009	AT 503479 T	15-04-2011
			AU 2006264803 A1	11-01-2007
			CA 2613752 A1	11-01-2007
			EP 1898908 A2	19-03-2008
			ES 2363995 T3	22-08-2011
			FR 2887879 A1	05-01-2007
			JP 5635232 B2	03-12-2014
			JP 2009500312 A	08-01-2009
			US 2009203747 A1	13-08-2009
WO 2007003767 A2	11-01-2007			
-----				
WO 2013158046	A1	24-10-2013	EP 2838541 A1	25-02-2015
			WO 2013158046 A1	24-10-2013
-----				
US 2013203799	A1	08-08-2013	US 2008131375 A1	05-06-2008
			US 2011027806 A1	03-02-2011
			US 2012329066 A1	27-12-2012
			US 2013203799 A1	08-08-2013
			US 2015018381 A1	15-01-2015
-----				
WO 2013123403	A1	22-08-2013	US 2015018240 A1	15-01-2015
			WO 2013123403 A1	22-08-2013
-----				
US 2009280111	A1	12-11-2009	US 2007141652 A1	21-06-2007
			US 2009280111 A1	12-11-2009
-----				