A

	Con	JH4	JH13	JH18	JH59	SCL14	SCL43	SCL109	NC7	NC21	NC24	

LMNA- M

GST-progerin

PPT

LMNA-M

Sup

B

GFP-progerin

\- JH4 JH13FTI

GFP

GST

PPT

GFP

Actin

SUP

GST-LMNA Pull down

C

Con   JH4

GFP-LMNA | progerin | DAPI   GFP-LMNA | progerin | DAPI

293-GFP-LMNA+NT-progerin

A GFP-progerin transfected 293

B 293-GFP-progerin

C

D IP: progerin

E HGPS

F

G

A

GFP (p14) | GST | PPT
GFP (p14) | Actin | SUP
- JH1 JH4 JH13

GST-progerin pull down

B

p53 | GST | PPT
p53 | Actin | SUP
Con JH4 JH13

GST-p14 pull down

C

PPT SUP
JH4 - + - +
GFP-progerin — GFP-progerin + FLAG-BMI1
GFP-progerin — GFP-progerin + FLAG-MEL18

IP: FLAG

D

FLAG-MEL18 | FLAG-BMI1
PPT SUP | PPT SUP
GST-progerin-C - + - + | - + - +
GST-LMNA-C + - + - | + - + -
FLAG
GST
Actin

GST pull-down

E

FLAG-BMI1
PPT SUP
His-LMNA: N M N M
FLAG
His
Actin

IP: His

F

p53 p14 progerin LMNA MEL18/BMI1 LMNB
JH4 JH4

G

JH4

B33

07: JH4-Biotin
Linker-Biotin

Linker-Biotin 08: Biotin

H

GFP-LMNA | GFP-progerin
- JH4 B33 07 08 | - JH4 B33 07 08
GFP | GST | PPT
GFP | Actin | SUP

progerin-C GST pull down   LMNA-C GST pull down

I

Con | 07: JH4-biotin | 08: biotin
GFP-progerin
GFP-LMNA

J

07: JH4-biotin | 08: biotin
24hr 48hr | 24hr 48hr
N9
HGPS

A

LMNA

progerin

GAPDH

N9 N81 HGPS1 HGPS2

B

N81

LMNA/C

DAPI

C

N9   N81

LMNA/C   DAPI   LMNA/C   DAPI

Con

JH1

JH4

JH13

FTI

D

Con   JH4   Con   JH4

N9

N81

HGPS

X25   X40

Trypan Blue Staining

E

N9   HGPS   N81

DMSO

JH4

JH4 3 µM / 7days

F

LMNA/C   Ki-67   DAPI

Con

JH1

JH4

JH13

N81

G

Con   JH4

Con   0%   0%

IGF-1   29.5%   31.2%

WI-38/SA-β-Gal

H

Con   JH4

EV   1.5%   1.0%

H-ras   23.5%   21.5%

WI-38/SA-β-Gal

I

WI-38   HGPS

JH4 (5µM) -   -   -   +   +   +   -
            - IGF Ras - IGF Ras -

Progerin

p16

p-ERK

t-ERK

Actin

A

JH4- 72 h

Absorbance (OD=540)

■ DMSO ■ 1 µM ■ 3 µM
■ 5 µM ■ 10 µM

HCT116 p53+/-   A549

C

JH 4 -36 h

Absorbance (OD=540)

■ DMSO □ 1 µM ■ 3 µM
■ 5 µM □ 10 µM

N.S.   N.S.

C2   C2V

B

C2   Caki-2

LMNA/C   DAPI   LMNA/C   DAPI

Con

FTI (5 µM)

JH4 (5 µM)

JH13 (5 µM)

GGTI (5 µM)

A

Total 14.4K array

↓

1921 genes species; heat map (2 fold cut-off)

↓

Elimination of NS genes of HS/N-9
(selection HS related genes; 1396; Table S1)

↓ ↓

HS specific 873
genes (Table S2)

523 genes; HS and N81
altered genes (Table S3)

↓ ↓ ↓

JH4 responding
384 genes
(table S5)

JH4 non-
responding 384
genes (table S6)

298 genes; obviously
responding genes for JH4
(Table S4)

B

C

D

E

F

G

Related function	No. (%)	Representative genes
Cell cycle, chromosome seg regation	104 (34.9)	Cdc25C, BLM, CDK1, CENPs
DNA replication	28 (9.4)	ESCO2, MCM, Mis18a, ki-67
Immune system	16 (5.4)	IL6, IL8, IL33
Regulation of transcription	15 (5.0)	STAT4, TCF19, ZNF724P
Cell structure	15 (5.0)	LMNB1, TACC3, SDC1
DNA repair	13 (4.4)	BRCA1, BRCA2, Rad51, CHEK
Development	12 (4.0)	STIL, FOXM1
Signal transduction	11 (3.7)	ALS
Metabolic process	9 (3.0)	sulfatase2
Cell adhesion	9 (3.0)	protocadherin7
Homeostasis	6 (2.0)	senescence marker protein-30
Cell death	5 (1.7)	coagulation factor III
Cancer	4 (1.3)	TGFβ-R1
Cardiac disease	3 (0.5)	Integrin-α2
Proteolysis	3 (1.0)	Carboxypeptidase A4
Metabolic disease	2 (0.7)	single-minded homolog 1
Unknown & Etc.	43 (14.4)	MMP3
Total	298 (100)	-

A

N9

DMSO | JH4
LMNA/C | DAPI | LMNA/C | DAPI

Noc

Taxol

N81

DMSO | JH4
LMNA/C | DAPI | LMNA/C | DAPI

Noc

Taxol

Taxol

B 293 (GFP-LMNA+progerin)

Con | Noc | Taxol

DMSO

JH4

FTI

C

LMNA +progerin | DAPI

Taxol

Noc

JH4

A

	DMSO		JH4		FTI	
	GFP	DAPI	GFP	DAPI	GFP	DAPI

Con

Noc

Taxol

293-GFP-progerin

B

Con   JH4

GFP   ER   DAPI   GFP   ER   DAPI

Taxol   JH4+ Taxol

GFP   ER   DAPI   GFP   ER   DAPI

C

	Con			Taxol	
GFP-progerin	ER	DAPI	GFP-progerin	ER	DAPI

DMSO

JH4

D

293-GFP-LMNA+NT-progerin

	DMSO			JH4	
GFP-LMNA	progerin	DAPI	GFP-LMNA	progerin	DAPI

Taxol-treated condition

A 4 times injected *Lmna^G609G/G609G* mice

B 6 times injected *Lmna^G609G/G609G* mice

A

B

40 weeks old-*Lmna*^{wt/G609G}

C
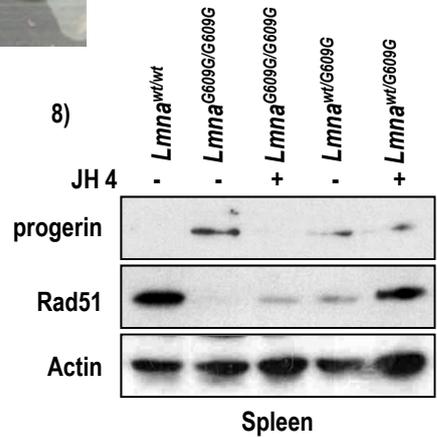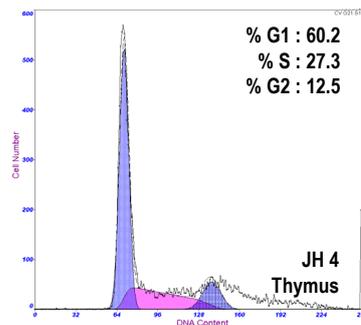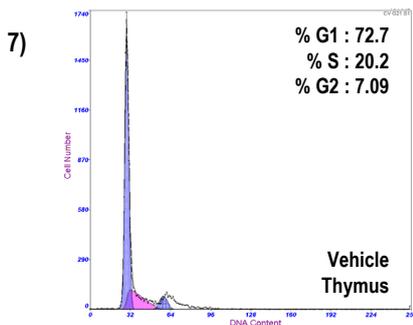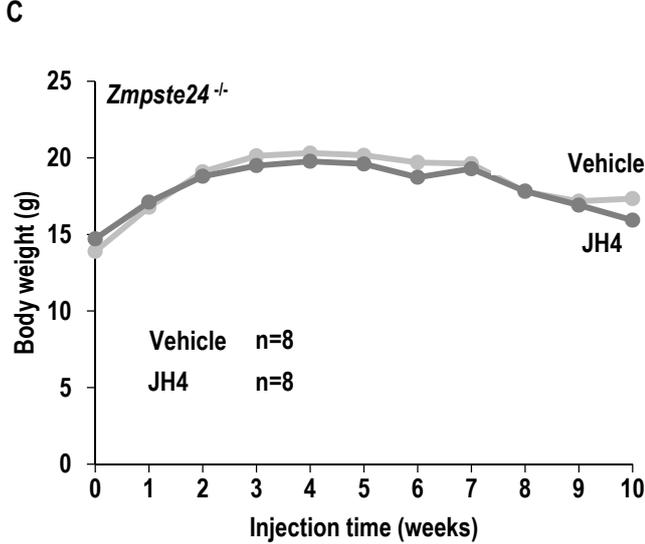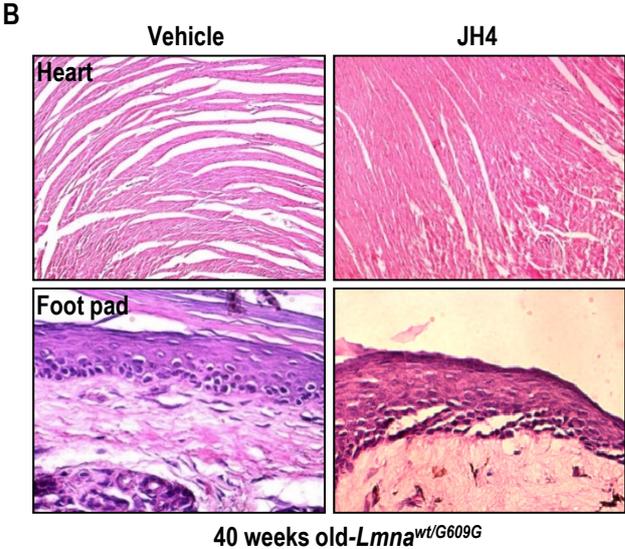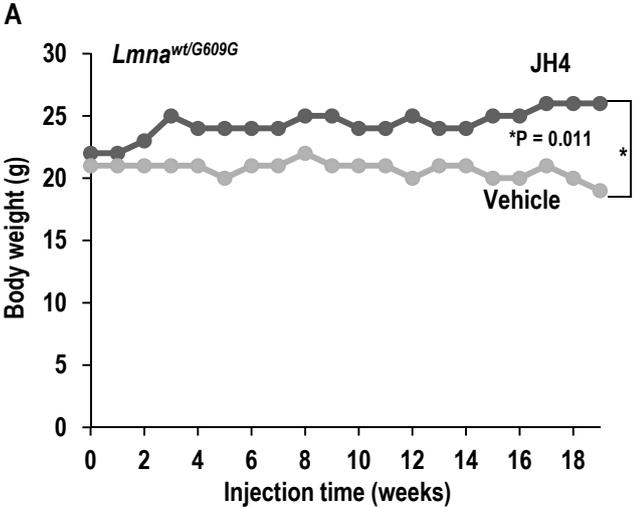
Supplementary Information to:

Novel therapeutic chemicals for Hutchinson-Gilford Progeria Syndrome

Lee *et al.*
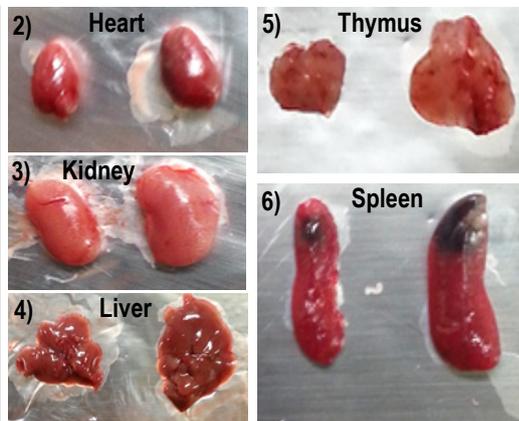
Materials and Methods

Animal experiments

The experiments were performed in the Association for Assessment and Accreditation of Laboratory Animal Care certified facility, in compliance with animal policies approved by Pusan National University. $Lmna^{G609G/G609G}$ mice were generated as previously described (37). Chemicals, mixed with sunflower oil, were intraperitoneally injected in mice (10 mg/kg twice per week from 4-week-old). $Zmpste24^{-/-}$ mice were generated as described (38) and used in the Animal Facility of the Universidad de Oviedo, in accordance with the guidelines of the Committee for Animal Experimentation of the University. We administrated JH4 at a concentration of 10 mg/kg in olive oil intraperitoneally to mice three times a week. Control mice were treated in the same conditions with olive oil alone. Mice were treated throughout life span, starting between 5 and 6 weeks of age. For histological analysis, tissues were embedded in paraffin after fixing with 10% formalin for 12 h, and sectioned at 5 μm thickness on microtome. Sectioned tissues were deparaffinized and stained with hematoxylin and eosin.

Chemical screening

For chemical screening, an ELISA assay was established by modifying the previous platform (Lee et al., 2010). Synthetic methods are provided in supplementary material. Chemical libraries were provided by GY Song (Chungnam National University, Deajeon, Korea), HY Moon (Pusan National University, Busan, Korea) and Korean Chemical Bank (Korea). About 12,000 chemical species were used in total in this screening.

Cell culture and reagents

Human fibroblast cells from HGPS patients (AG03198, 10-year-old female; AG03199; 10-year-old female; AG01972, 14-year-old female; AG03513, 15-year-old male) and controls (GM 00038, 9-year-old female; AG06103, 29-year-old male; AG09603, 81-year-old female) were obtained from the Coriell Cell Repositories (Camden, New Jersey, USA) and were maintained in EMEM, containing 15% FBS, 2 mM glutamine or HEMEM with 26 mM HEPES without antibiotics. Human cancer cell lines, obtained from ATCC (HCT116, A549HEK 293,), were maintained in liquid medium containing 10% FBS and 1% antibiotics at 37 °C. Non-human cell lines such as Vero (Monkey), HICIC7 (Mouse), MDCK (Canine) and Mv1Lu (Mink) were obtained from Korea cell line bank (KCLB, Seoul, Korea).

Antibodies and reagents

General chemical inhibitors included FTI-277 (5 µM; F9803; Sigma Aldrich), ALLN(; 208719; Calbiochem), cycloheximide (100 µg; C4857; Sigma Aldrich), nocodazol (; 487928; Calbiochem) and taxol (1 µM; T7402; Sigma Aldrich). Antibodies used for experiments included GFP (1:1000; sc-9996; Santa Cruz Biotechnology); GST (1:5000; sc-138; Santa Cruz Biotechnology); p53 (1:5000; sc-126; Santa Cruz Biotechnology); His (1:1000; sc-8036; Santa Cruz Biotechnology); Emerin (Clone 4G5; 1;5000; Novocastra); Actin (1:1000; sc-1616; Santa Cruz Biotechnology); Lamin B (1:1000; sc-6216; Santa Cruz Biotechnology); p16 (1:500; sc-759; Santa Cruz Biotechnology); Lamin A/C (1:1000; sc-6215; Santa Cruz Biotechnology); Progerin (1:200; sc-81611; Santa Cruz Biotechnology); BRCA1 (1:500; sc-6954; Santa Cruz Biotechnology); Cyclin B1 (1:1000; sc-594; Santa Cruz Biotechnology); Rad51 (1:1000; sc-53428; Santa Cruz Biotechnology); Cdc25C (1:1000; 4668; Cell Signaling Technology); BLM (1:200; sc-13584; Santa Cruz Biotechnology); phospho Cdc2 (1:1000; 9111; Cell Signaling Technology); DCR2 (1:200; sc-65310; Santa Cruz Biotechnology); Ki-67 (1:200; sc-15402; Santa Cruz Biotechnology); H3K9me3 (1:3000; Ab8898; Abcam); DNA-PKcs (1:5000; sc-9051; Santa Cruz Biotechnology); FLAG (1:2000; F3165; Sigma Aldrich).

Immunoprecipitation and Western-blot analysis

Whole-cell lysates were prepared in RIPA and centrifuged at 14,000 rpm for 30 min. 20 µg of cell extracts were separated by SDS-PAGE and transferred onto a PVDF membrane. The membrane was incubated (1 h to overnight) at 4 °C with primary antibody, followed by reaction with a secondary antibody at room temperature for 1 h. Peroxidase activity was detected by chemiluminescence with ECL kit (Intron, Seoul, Korea) as recommended by its manufacturer. To examine the interaction between progerin and lamin A, the protein extracts were added the antibody against GFP (2 µg/sample). After incubation for 2 h at 4 °C with agitation, protein A and protein G were added. After washing twice with PBS, the precipitates were dissolved in RIPA buffer and SDS sample buffer.

Far-western blot analysis

Recombinant His-LMNA-N and His-LMNA-M were subjected to SDS-polyacrylamide gel electrophoresis and transferred onto a PVDF membrane. The membrane was incubated 1 h ~ overnight at 4 °C with the GST-LMNA or progerin or RKIP, followed by reaction with an appropriate primary antibody and a secondary antibody at room temperature for 1 h. Peroxidase activity was detected by chemiluminescence with ECL kit (Intron, Seoul, Korea) as recommended by the manufacturer.

Immunofluorescence staining and senescence-specific acidic β-galactosidase activity staining

Cells were seeded on a cover glass and transfected with the indicated vectors. After fixing with 100% methanol for 10 min at 4 °C, cells were incubated with blocking buffer (PBS+anti-human-Ab; 1: 500) for 1 h. After washing with PBS twice, cells were incubated with anti-lamin A/C, H3K9Me3 or DNA-PKcs in blocking buffer (1: 200) for 2 h and sequentially with anti-goat Ab-FITC or anti-rabbit Ab-rhodamin in blocking buffer (1: 1000) for 2 h and mounted. For endoplasmic reticulum staining, cells were incubated with ER-tracker (1:10000; E34250?; Invitrogen) for 2 h at 37 °C with 5% CO_2 chamber. Nucleus was stained with DAPI. Immunofluorescence signal was detected by fluorescence

microscopy (Zeiss). For senescence specific acidic-β-galactosidase activity staining, cells were washed once with PBS (pH 7.2), fixed with PBS containing 0.5% glutaraldehyde. After washing with PBS, cells were stained in X-gal solution (9860; Cell Signaling Technology) overnight at 37 °C.

RNA isolation and RT-PCR

For RT-PCR, total cellular RNA was extracted using RNA extraction kit (Qiagen). After measurement of RNA concentration, 1 µg of total RNA was reverse transcribed to cDNA using MMLV RT (Invitrogen) and random hexamers. RT-PCR was performed with the following specific primers: Lamin A/C, 5'-AAGGAGATGACCTGCTCCATC-3' and 5'-TTTCTTTGGCTTCAAGCCCCC-3'; DcR2, 5'-ACTGGGAAAACCCCAGCAGCG-3' and 5'-TTCCAGCAGACGCTGTGGCTC-3'; p16, 5'-GCCGCGAGTGAGGGTTTTCG-3' and 5'- GTGCACGGGTCGGGTGAGAG-3'; Rad51, 5'-TCTTTGGTTTTGGAGGAGGGG-3'and 5'- GTAGGTTTGGCACAAGACTCC-3'; BRCA1, 5'-AGAGTGTCCCATCTGTCTGG-3' and 5'-CGCTGCTTTGTCCTCAGAG-3'; BLM, 5'-GGATTATGGCTGCTGTTCCTC-3' and 5'-CAA AGTGACTTTGGGGTGGTG-3'; TNFSF18, 5'-GAAGCTGTGGCTCTTTTGCTC-3' and 5'- GTAGTTTGCATTGGGAGCCAC-3'; IL6, 5'-AAATGCCAGCCTGCTGACGAAC-3' and 5'-AACAACAATCTGAGGTGCCCATGCTAC-3'; IL8, 5'-TGGCAGCCTTCCTGATTTCTG-3' and 5'-AACTTCTCCACAACCCTCTGC-3'; IL33, 5'-CCACCAAAAGGCCTTCACTG-3' and 5'-GTTGGCATGCAACCAGAAGT-3'; ESCO2, 5'-GGCATTTCGTGTCCTGTCTG-3' and 5'-AAC TTGCCATCTGGTGTTGG-3'; Mis 18α, 5'-CGGTCACTGAGGTGTAGCAG-3' and 5'- AAGATTGAGTGAGCACCCCG-3' and GAPDH 5'-ATCTTCCAGGAGCGAGATCCC-3' and 5'- AGTGAGCTTCCC GTTCAGCTC-3'.

MTT assay and cell proliferation analysis

To measure the cell viability, cells were treated with the indicated chemicals for 24 h. For MTT assay, cells were incubated with 0.5 mg/ml of 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution for 4 h at 37 °C. After removing excess solution, the precipitated materials were

dissolved in 200 µl DMSO and quantified by measuring the absorbance at 540 nm. For cell counting, cells were collected with medium and stained with trypan blue for 10 min at room temperature. Using a hemocytometer, the survival cells were determined.

FACS analysis

To analyze cell cycle progression, cells were treated with JH4 or vehicle for 24 h after transfection with the indicated vectors. For cytometry, cells were fixed with ethanol and then incubated with 25 µg/ml propidium iodide (Sigma-Aldrich) and 10 µM RNase A (Novagen). At least 10,000 stained cells were sorted by FACS (FC500; Beckman Coulter) and analyzed by CXP software 2.0. To check the cell cycle in splenocytes and thymocytes of JH4 injected animals, freshly isolated tissues were washed with autoclaved PBS and dissociated by chopping. Release cells were collected by centrifugation and fixed with 70% Et-OH.

ELISA

To isolate progerin-lamin A binding inhibitors, ELISA analysis was performed according to the previous literature (Lee et al., 2010). GST-progerin was fixed on a 96-well plate using 0.5% paraformaldehyde. After drying and washing, His-LMNA-M with 10 µM of chemicals (final concentration) was incubated for 1 h. 96-well plates were washed with TBST and incubated with anti-His-Ab (1: 10000, 45 min), and anti-mouse-IgG-HRP (1: 50000, 30 min). After washing twice, plates were incubated with TMB solution (CL07; Calbiochem) and Stop solution (1N H_2SO_4) and the absorbance at 650 nm was determined using an ELISA reader.

Inhibition assays and IC50 value determination

Reactions were performed under the conditions described for ELISA assay. Briefly, JH4 was diluted in DMSO in a range from 0.01 to 30 µM. His-LMNA-M at 10 µM was coated on 96-well plates. Followed by incubation with GST-progerin-C or GST-LMNA-C, and differently diluted JH4 for 1 h,

plate was reacted with anti-GST-ab (1:10000, 45 min), and anti-mouse-IgG-HRP (1: 50000, 30 min). After washing, plates were incubated with TMB solution (CL07; Calbiochem) and Stop solution (1N H_2SO_4).   The absorbance of the plate was read at 450 nm. The IC50 value was determined by fitting a dose response curve of the data using GraphPad Prism (GraphPad Software)

Microarray analysis

500 ng of total RNA extracted using the RNAeasy kit (Qiagen) were used. RNA labelling, hybridization on Human Gene 1.0 ST arrays (Affymetrix) and data analysis were performed by DNA Link (Seoul, Korea (rep. of)). Genes showing at least two-fold differences in either cell line were selected for further analysis.

Statistical analysis and protein network analysis

To test the statistical significance of the observed differences, we performed the student's *t*-test. For protein network analysis, we collected 100 genes from HARG/JR set and ran the network program in cBioportal package (http://www.cbioportal.org).

Supplementary Figure legends

Figure S1. **Direct interaction between lamin A and progerin**

(A) Selective interaction between progerin and lamin A (LMNA). GFP-LMNA or GFP-lamin B (LMNB)-transfected lysates were mixed and incubated with GST-LMNA or GST-progerin. Both recombinant proteins were selectively associated with GFP-LMNA but not GFP-LMNB.

(B) Interaction between lamin C (LMNC) and progerin. The lysates of GFP-LMNC-transfected 293 cells were incubated with GST-LMNA or GST-progerin for 0.5 h at RT. GST-associated GFP-LMNC was determined by WB analysis.

(C) Binding affinity analysis of progerin-LMNA middle region by far western blot analysis. His-tagged N-terminal (LMNA-N; 1-300) and middle region (LMNA-M) of lamin A were subjected to SDS-PAGE and transferred to a PVDF membrane. The membrane was incubated with the indicated recombinant proteins, and the associated proteins were determined with an anti-GST antibody.

(D) Progerin induces nuclear deformation. 293 cells were co-transfected with GFP-LMNA and non-tagged progerin (NT-progerin) for 12 h. After fixation, the cells were stained with a progerin-specific Ab (red). Co-localization of lamin A and progerin is indicated with white arrowheads. Scale bar indicates 10 μm.

(E) Co-localization of lamin A (green) and progerin (red) in nuclear membrane knobs (arrows).

(F) Progerin did not induce nuclear deformation in neuron or embryonic neuron cell. Transfection of GFP-progerin or laminA alone into Neuroblastoma cell line, SH-SY-5Y (F) and mouse neuro-embryonic cell line, N2A, did not induce nuclear deformation. However, co-transfection could induce it. Cells were transfected with indicated vectors for 24 hr. For visualizing the nuclear membrane, cells were stained with emerin (red) and DAPI for DNA (blue). Scale bar indicates 10 μm.

(G) Western blot analysis for expression level of transfected genes. Despite low expression of progerin in N2A, co-transfection induced nuclear deformation.

Figure S2. Chemical screening

(**A**) Experimental scheme for the chemical screening

7

(B) Representative result of ELISA-based chemical screening. BL indicates the basal level (negative control), which is determined by the value of reaction without His-LMNA-M, and PL is positive level, which is determined by the reaction without chemical. We performed the ELISA assay twice (gray and black bar).

(C) Chemical structures of selected compounds: JH1, JH4, JH13, and LG-02. M.W., molecular weight of each chemical.

(D) MTT assay. The cell viability of RCC cells (C2, C2V, and Caki2), HCT116 and A549 cells, after incubation with 10 µM of the indicated chemicals for 48 h was measured using the MTT assay.

(E) Dose-response relationship of the inhibition of the binding between lamin A and progerin by JH4. His-tagged LMNA-M was fixed in a 96-well plate and incubated with GST-LMNA or GST-progerin, and differently diluted JH4. After reaction with GST-ab and mouse-HRP, the value was determined by ELISA reader and the data was analyzed by prism software.

Figure S3. JH chemicals disrupt the binding between lamin A and progerin

(A) *In vitro* binding assay using His-LMNA-M after incubation with GST-progerin upon treatment with indicated chemicals (10 µM).

(B) JH4 blocks the interaction of progerin and GST-LMNA. GST pull-down assay using lysates from 293 cells transfected with GFP-progerin after incubation with GST-LMNA under treatment with the indicated chemicals.

(C) JH4 blocks the co-localization of lamin A and progerin. For this, 293 cells were co-transfected with GFP-LMNA and non-tagged progerin (NT-progerin) for 24 h and incubated with JH4 for additional 24 h. JH4 can abolish the co-localization of lamin A (green) and progerin (red) in the nuclear membrane or nuclear speckles. DAPI was used for DNA staining. Scale bar indicates 10 µm.

Figure S4. JH chemicals ameliorate the progerin-induced nuclear deformation

(A) Quantification of nuclear abnormalities in GFP-progerin-transfected 293 cells after incubation

with the indicated chemicals (5 µM) for 24 h. At least 200 cells/sample/experiment were counted by two independent researchers. The statistical significance was analyzed by Student's t-test. For all data sets, $P< 0.05$ was considered to be statistical significant.

(B) The JH chemicals suppress progerin-induced nuclear deformation. GFP-progerin-transfected 293 cells were incubated with the indicated concentration of the JH chemicals for 24 h. Scale bar indicates 10 µm.

(C) Effect of the JH chemicals on *LMNA* mutants. 293 cells were transfected with several types of GFP-LMNA mutants, which were isolated from laminopathies, and GFP-progerin for 24 h and then incubated with JH4 (5 µM) for 24 h. Scale bar indicates 10 µm.

(D) JH chemicals disrupt the interaction between lamin A and progerin in HGPS cells. Immunoprecipitation was performed using a progerin-specific antibody, and the co-precipitated lamin A/C proteins were analyzed by WB analysis. Whole cell lysates (WCL) were obtained from HGPS cells treated for 24 h with the chemicals (5 µM).

(E) Transcripts of progerin are not affected by the JH chemicals or the FTI. HGPS cells were incubated with the indicated chemicals for 48 h. After the cells were washed, total RNA was extracted and RT-PCR was performed.

(F) The effect of JH chemicals on nuclear deformation. Scale bar indicates 10 µm.

(G) The effect of JH4 on other HGPS cells. Similarly with HGPS4, JH4 could block the nuclear deformation. Cells were incubated with JH4 for 48 hr and counted on the basis of nuclear morphology.

Figure S5. JH chemicals specifically disrupt the interactions via C-terminal region of progerin

(A) The effect of chemicals on p14^{ARF} and progerin binding. p14^{ARF}-transfected 293 cell lysates were incubated with GST-progerin and the JH chemicals for 2 h. The associated p14^{ARF} proteins were analyzed by WB.

(B) JH chemicals do not affect p53/p14^{ARF} binding. 293 cell lysates were incubated with GST-p14 and the JH chemicals for 2 h. The associated p53 proteins were analyzed by WB.

(C) JH4 does not disrupt the interaction of progerin and BMI1 or MEL18. 293 cell lysates, co-transfected with GFP-progerin and BMI1 or GFP-progerin and MEL18, were immunoprecipitated using FLAG antibody and co-precipitated GFP-progerin were analyzed by WB analysis.

(D) C-terminal regions of lamin A and progerin do not interact with MEL18 and BMI1. MEL18 or BMI1-transfected 293 cell lysates were incubated with GST-progerin or GST-LMNA. The associated MEL18 or BMI1 proteins were analyzed by WB.

(E) The middle region of lamin A (LMNA-M) is responsible for the binding to BMI1. After incubation with LMNA-N or LMNA-M, BMI1-transfected 293 cell lysates were immunoprecipitated using His antibody and co-precipitated BMI1 was analyzed by WB analysis.

(F) Scheme of the interaction between lamin A / progerin and associated proteins. JH4 blocks the progerin-C-terminal mediated interaction.

(G) Chemical structures of biotinylated JH4. B33 is a precursor for biotinylation and 07 is biotin conjugated JH4. 08 is covered from modified linker and biotin that is used for biotin control.

(H) The effect of biotinylated JH4 on the interaction of lamin A and progerin. GST pull-down assay using lysates from HEK293 cells transfected with GFP-progerin or GFP-LMNA after incubation with GST-LMNA or GST-progerin under treatment with the indicated chemicals.

(I) The biotinylated JH4 (07: JH4-biotin) suppresses progerin -induced nuclear deformation. GFP-progerin-transfected HEK293 cells were incubated with the indicated concentration of the JH chemicals for 24 hr.

(J) Nuclear membrane location of JH4-biotin in HGPS cells. By contrast, JH4-biotin was not detected in normal cells.

Figure S6. The effect of the JH chemicals on cellular senescence

(A) SA-β-gal staining of HGPS cells incubated with or without chemicals for 48 h. After fixation, the cells were stained using a SA-β-gal staining kit.

(B) JH chemicals suppress SA-β-gal expression in HGPS cells. Based on SA-β-gal assay of HGPS

10

cells (see Figures 4D and S6A), senescent cells were counted. JH chemicals (5 µM, for 48 h) suppressed SA-β-gal activity. The statistical significance was analyzed by Student's *t*-test. For all data sets, *P*< 0.05 was considered to be statistical significant.

(C) JH4 suppresses *DcR2* expression, but does not alter *LMNA* transcript levels. RT-PCR analysis was performed in two different HGPS cell lines, derived from female and male patients, after treatment with JH4 for 48 h. *GAPDH* was used as loading control.

(D) Reduction of DcR2 and progerin in HGPS cells in response to JH chemicals. Cells were incubated with the indicated concentration of JH1, JH4 or JH13 for 48 h and harvested for WB assay.

(E) Increase of H3K9Me3 in JH4-treated HGPS cells. 3 kinds of HGPS cells were incubated with JH4 for 48 hr and stained with H3K9Me3 Ab.

(F) Increase of H3K9Me3 expression in HGPS cells by JH chemical treatment. HGPS-1 cells were incubated with of JH chemicals (5 µM) or FTI (FTI-277) for 24 h. After being fixed with 100% MeOH, the cells were stained with lamin A/C Ab (green) and H3K9Me3 Ab (red).

(G) JH chemicals induce DNA-PK in HGPS cells. IF was performed in chemical-treated HGPS cells with anti-progerin (green) and DNA-PK (red) antibodies. Scale bar indicates 10 µm.

(H) DNA-PK is induced by JH4 at the protein level. WB of DNA PK was performed in HGPS and normal cells after treatment with the indicated chemicals for 48 h.

Figure S7. Effect of the JH chemicals on aged cells

(A) Transcripts of progerin accumulate in aged cells. Lower molecular weighted products indicate progerin. Total RNA was extracted from normal (N9; derived from 9 years old person, N81; derived from 81 years old person) and HGPS (HGPS1, HGPS2) cells and RT-PCR was performed.

(B) Nuclear deformation in aged cells. Cells obtained from an elderly healthy individual (N81) were stained with lamin A/C Ab (green) and DAPI (blue).

(C) Amelioration of nuclear irregularity in aged cells by JH chemicals and FTI. Normal cells (N9 and N81) were incubated with JH chemicals (5 µM) or FTI (FTI-277) for 24 h. After fixation with 100%

11

MeOH, cells were stained with lamin A/C Ab (green) and DAPI (blue).

(D) JH4 increases cell growth in normal (N9 and N81) and HGPS cells for long term incubation. Despite 10 days incubation, cell growth was promoted without cell death.

(E) Colony forming assay of N9, N81 and HGPS cells treated with 3 μM JH4 for 10 days. After fixation, cells were stained with trypan blue.

(F) JH4 induces Ki-67 expression in aged fibroblasts. Immunostaining for lamin A/C (green) and Ki-67 (red) was performed in N81 treated 48 h with JH4 (5 μM). Scale bar indicates 10 μm.

(G and H) JH4 does not block the growth factor or oncogene-induced senescence (OIS) in normal WI-38 cell. To check the engagement of JH4 in OIS, normal WI-38 cells was incubated with IGF-1 (10 ng/ml) in serum free condition (G) or tranfected with oncogenic H-Ras (H; H-Ras^{12V}). Senescence, induced by oncogenic signal, did not suppressed by JH4. Cells were incubated in indicated conditions for 48 hr and stained with SA-β-gal. Numbers in figures indicated % of SA-β-gal positive cells.

(I) Western blot analysis. Induced p16INK4A by IGF-1 (IGF) treatment in serum free condition or H-Ras (Ras) transfected WI-38 was not inhibited by JH4. However, progerin is not expressed under p16-induced condition. HGPS cell lysate was used for positive control of progerin.

Figure S8. Effect of JH4 on cancer cell lines

(A) MTT assay. The cell viability of human cancer cell lines is not altered by JH4. HCT116 and A549 cells were incubated with the indicated concentrations of JH4 (1-10 μM) for 72 h and determined their viability by MTT assay.

(B) Ameliorating effect of JH chemicals on the nuclear irregularity of RCC. C2 and Caki-2 cells were incubated with JH chemicals or FTI for 24 h. The cells were fixed and stained with lamin A/C Ab (green) and DAPI (blue). Scale bar indicates 10 μm.

(C) MTT assay. The cell viability of RCC cells (C2 and C2V) after incubation with the indicated concentrations of JH4 (1-10 μM) for 36 h were measured with the MTT assay. Despite the ameliorating effect of JH4 on nuclear deformation in C2, it did not promote cell viability. Viability

differences between control and JH4-treated cells were not statistically significant (N.S). The statistical significance between two groups was analyzed by Student's *t*-test. For all data sets, *P*< 0.05 was considered to be statistical significant.

Figure S9. Gene expression profile upon treatment with JH4

(A) Scheme outlining the identification of candidate target genes of JH4 in HGPS (HS) cells based on microarray results. From 14.4K microarray data, we removed non-significant (NS) genes (less than 2-fold change). For comparative analysis, we removed the NS genes and collected HGPS/aging related genes (Table S1). We also divided this gene set into two groups: genes altered both in HGPS and aged fibroblasts (N81), and genes altered only in HGPS (Table S3: HARG and Table S2: HUG, respectively). Genes in table S2 were divided by their response to JH4 treatment (JH4-responding genes; Table S5 and JH4-non-responding genes; Table S6). We collected JH4 responding genes from Table S3 and grouped as Table S4 (HGPS/Aging related gene/JH4 responding: HARG/JR). In this group, cell cycle regulators including chromosome segregation (CENP proteins) and moving (KIF family), DNA repair (Rad51, BRCA1) and check point, and histone protein, were found.

(B) Heat map of HARG/JR gene set. Based on (A), the heat map is classified in several categories.

(C) RT-PCR analysis of HARG/JR genes. Downregulated *IL33, BLM, Esco2, BRCA1, Rad51, CENP1, Mis18α* (upper panel) and upregulated *IL6, IL8, TNFSF18* (lower panel) in HGPS and N81 cells were restored by JH4 treatment but not by FTI in HGPS cells.

(D) RT-PCR analysis in aged fibroblasts and HGPS-2. Consistently with microarray results, in aged cells (N72, N81, and N94) and another HGPS cells, *IL-33, Rad51*, and *BRCA1* were reduced. In addition, increase of *IL-6* and *IL-8* were detected in these cells.

(E and **F)** Enlarged interaction network map of chromosome separation and cell cycle clusters (E), and DNA repair and DNA replication clusters (F). Bold circles indicate individual HARG/JH genes. Density of red color represents mutation frequency in human cancer.

(G) Representative genes in HARG/JR gene set (see table S4 in supplementary information). A large

13

portion of genes is related to cell cycle, DNA repair and chromosome segregation.

Figure S10. JH4 eliminates progerin-induced lamin A aggregation in G2/M phase

(A) JH4 allows lamin A/C dissemination during mitosis in aged cells. Normal cells were treated with JH4 or untreated for 48 h and then incubated with Nocodazol or Taxol (1 μM) for an additional 48 h. After fixation, the cells were stained with anti-lamin A/C Ab (green) and DAPI (blue). Mislocalization of lamin A (white arrows) in Taxol-treated N81 cells disappeared upon JH4-treatment (yellow arrows).

(B) JH4 eliminates progerin-induced lamin A aggregates. 293 cells transfected with GFP-LMNA or GFP-LMNA and NT-progerin were treated with JH4 or FTI for 24 h followed by treatment with nocodazol or taxol for an additional 24 h.

(C) JH4 can induce lamin A/C dissemination during mitosis when progerin is overexpressed. 293 cells transfected with GFP-LMNA and NT-progerin were treated with JH4 for 24 h followed by treatment with nocodazol or taxol for an additional 24 h. Scale bar indicates 10 μm.

Figure S11. JH4 abolishes progerin-induced mitosis defects

(A) JH4 abolishes progerin-induced lamin A aggregates during mitosis. 293 cells transfected with GFP-LMNA or GFP-LMNA and NT-progerin were treated with JH4 or FTI for 24 h followed by treatment with nocodazol or taxol for an additional 24 h.

(B and **C)** Immunostaining for ER (red) in 293 cells transfected with GFP-LMNA and NT-progerin or GFP-progerin and treated with JH4 for 24 h followed by treatment with taxol for an additional 24 h.

(D) Immunostaining for progerin (red) in 293 cells transfected with GFP-LMNA and NT-progerin treated with JH4 for 24 h followed by treatment with Taxol for an additional 24 h. Scale bar indicates 10 μm.

Figure S12. JH4 ameliorates progeria phenotypes in *Lmna*$^{G609G/G609G}$ mice

(A) Cumulative plot of body weight versus duration of JH4 treatment. *Lmna*$^{wt/wt}$ and *Lmna* $^{G609G/G609G}$ mice, untreated or treated with JH4, were observed. Dots represent mean values.

14

(B) Representative photograph of 15-week-old *Lmna*$^{G609G/G609G}$ untreated or JH4-treated mice. Hair-loss was recovered by JH4 injection.

(C) JH4 promotes cell cycle progression. Obvious increase of S phase in splenocytes (1.17%→2.49%) and thymocytes (0.89%→ 12.5%) from JH4-injected mice was observed.

(D) Increase of skin thickness and collagen density in JH4 treated mouse. Mouse foot pad was obtained from 18-week-old *Lmna*$^{G609G/G609G}$ mice.

(E) JH4 increases cell density (left) and suppresses nuclear deformation in liver (middle). Hematoxylin and eosin stained liver section of *Lmna* $^{G609G/G609G}$ mice treated with vehicle or JH4 (right).

(F) JH4 increases cell density (left) and suppresses nuclear deformation in lung (middle). Hematoxylin and eosin stained lung section of *Lmna* $^{G609G/G609G}$ mice treated with vehicle or JH4 (right). Arrows show deformed nuclei.

(G) JH4 ameliorates nuclear deformation in kidney tubular cells. Graph in upper panel was calculated based on nuclear morphology of H&E staining (lower panel). Arrows indicate deformed nuclei. The statistical significance between two groups was analyzed by Student's *t*-test. For all data sets, *P*< 0.05 was considered to be statistical significant. Scale bar indicates 10 μm.

(I) JH4 ameliorates early cysts and increases cell density in kidney *Lmna* $^{G609G/G609G}$ mice. Left panel showed the lower magnitude of kidneys and right upper graph showed number of cells at the same region. Right lower graph indicated cells per kidney tubes. Samples were taken from 18-week-old mice. The statistical significance between two groups was analyzed by Student's *t*-test. For all data sets, *P*< 0.05 was considered to be statistical significant. Scale bar indicates 20 μm.

Figure S13. Dose and time dependent effect of JH4 on progeria phenotypes in *Lmna*$^{G609G/G609G}$ mice

(A) Gross features of mice injected 4 times. Although increase of body size (1) was detected, organs (2-6) were not affected profoundly. Indeed, heart or kidney did not show significant difference

15

between vehicle and JH4-injected mice. In addition, increase of S-phase was less than 3% (7). WB analysis of spleen extract, obtained from mice injected 4 times, showed a partial reduction of progerin (8).

(B) Gross features of mice injected 6 times. In these mice, increase of body size (1) as well as organ volumes (2-6) was evident. For example, thymus of JH4-treated mice was enlarged about 2-fold in relation to vehicle treated mice. Cell cycle was also promoted more rapidly (7). WB analysis showed a reduction of progerin (8), more evident than in samples from animals injected 4 times. Scale bar indicates 20 μm.

Figure S14. JH4 shows anti-aging effect in *Lmna^wt/G609G* mice, but has no effect in a different laminopathy mouse model

(A) Cumulative plot of body weight versus JH4 injection period. *Lmna^wt/G609G* untreated or treated JH4 mice were followed and dots represent mean values.

(B) Hematoxylin and eosin stained heart and skin section of *Lmna^wt/G609G* mice treated with or JH4 or vehicle alone. Tissues were obtained from 32-week-old mice.

(C) Cumulative plot of body weight versus injection period in *Zmpste24^-/-* mice treated with JH4 or vehicle.

Chemical Synthesis
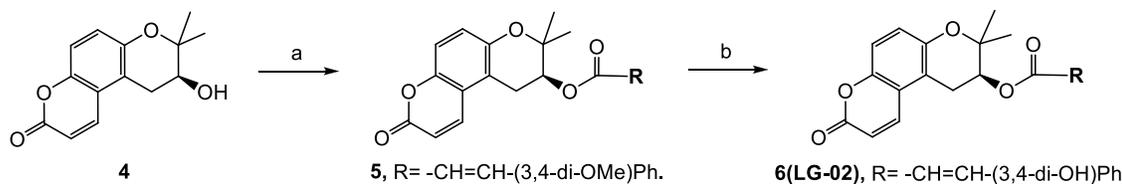
Scheme 1. Reagents and conditions: (a) Carboxylic acids, EDC, 4-DMAP, dry dichloromethane, 5-12 h, rt; (b) BBr₃ 1M solution in dichloromethane, dry dichloromethane, 0 °C → rt, 5 h.

Scheme 2. Reagents and conditions: (a) carboxylic acid, EDC, 4-DMAP, dry dichloromethane, 12 h, rt; (b) BBr₃ 1M solution in dichloromethane, dry dichloromethane, 0 °C → rt, 5 h.

General method for the synthesis of compounds (3a,b)

To a solution of (S)-(+)-decursinol (**1**, 0.406 mmol, 1eq) in anhydrous dichloromethane was added adequate carboxylic acid (0.609 mmol, 1.5eq), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC, 0.812 mmol, 2eq) and 4-(dimethylamino) pyridine (4-DMAP, 0.162 mmol, 0.4eq). The mixture was allowed to stir at room temperature for 5-12 h. Then the reaction mixture was concentrated under reduced pressure, and the residue was purified by flash silica gel column chromatography to obtain decursin derivatives (**2a-b**).

To a solution of decursin derivative with 3,4-dimethoxyphenyl group (**2a** or **2b**, 0.49 mmol, 1eq) in anhydrous dichloromethane (5 ml) was added 1M boron tribromide solution in dichloromethane (1.47 mmol, 3eq) in ice bath. The mixture was allowed to stir at room temperature for 5 h. The reaction solution was purified by silica gel short-column chromatography to obtain decursin derivatives with 3,4-dihydroxyphenyl group (**3a;JH13, 3b;JH1**).

(7S)-(+)-3-(3,4-Dihydroxy-phenyl)-acrylic acid, 8,8-dimethyl-2-oxo-6,7-dihydro-*2H,8H*-pyrano[3,2-*g*]chromen-7-yl-ester (3a, JH13)

Yield 93.2%, White Solid, mp: 115℃, R_f = 0.36 (1:2 *n*-hexane-ethyl acetate); $[\alpha]_D^{25}$ +19.3 (c=3, CHCl$_3$); ^{1}H NMR(400MHz, DMSO-d$_6$): δ_H 9.63(1H, s, OH-7'), 9.10(1H, s, OH-6'), 7.90(1H, d, *J*=9.6Hz, H-4), 7.46(1H, s, H-5), 7.45(1H, d, *J*=15.2Hz, H-3'), 7.00(1H, s, H-5'), 6.99(1H, d, *J*=8.4Hz, H-9'), 6.81(1H, s, H-10), 6.71(1H, d, *J*=8.4Hz, H-8'), 6.25(1H, d, *J*=9.6Hz, H-3), 6.22(1H, d, *J*=15.6Hz, H-2'), 5.14(1H, t, *J*=4.0Hz, H-7), 3.24(1H, dd, *J*=4.0, 17.6Hz, H-6a), 2.88(1H, dd, *J*=4.0, 17.6Hz, H-6b), 1.35(3H, s, CH$_3$-8), 1.31(3H, s, CH$_3$-8); ^{13}C NMR (100MHz, CDCl$_3$): δ_C 166.9(C-1'), 162.3(C-2), 156.6(C-9a), 154.0(C-10a), 147.0(C-7'), 146.2(C-6'), 144.2(C-3'), 143.9(C-4), 128.8(C-5), 126.8(C-4'), 122.5(C-9'), 116.0(C-5a), 115.3(C-8'), 114.3(C-2'), 114.1(C-3), 112.8(C-4a), 112.7(C-5'), 104.7(C-10), 76.8(C-8), 70.0(C-7), 27.8(C-6), 24.8(CH$_3$-8), 23.3(CH$_3$-8); IT-TOF/MS*: m/z*=409.1357 [M+H]$^+$, 431.1134 [M+Na]$^+$.

(7S)-(+)-3-(3,4-Dihydroxyphenyl)propionic acid, 8,8-dimethyl-2-oxo-6,7-dihydro-*2H,8H*-pyrano[3,2-*g*]chromen-7-yl-ester (3b, JH1)

Yield 86.8 %, Brown Solid, mp: 87℃, R_f = 0.21 (1:1 *n*-hexane-ethyl acetate); $[\alpha]_D^{25}$ +56.6 (c=1, MeOH); ^{1}H NMR(400MHz, DMSO-d$_6$): δ_H 8.69(1H, s, OH), 8.63(1H, s, OH), 7.89(1H, d, *J*=9.6Hz, H-4), 7.39(1H, s, H-5), 6.76(1H, s, H-10), 6.54(1H, d, *J*=8.0Hz, H-9'), 6.509(1H, s, H-5'), 6.34(1H, d, *J*=8.4Hz, H-8'), 6.24(1H, d, *J*=9.6Hz, H-3), 5.00(1H, t, *J*=4.2Hz, H-7), 3.14(1H, dd, *J*=4.4, 17.6Hz, H-6), 2.70(1H, dd, *J*=4.8, 17.6Hz, H-6), 2.61(2H, t, *J*=7.2Hz, H-3'), 2.490(2H, t, *J*=7.2Hz, H-2'), 1.23(3H, s, CH$_3$-8), 1.22(3H, s, CH$_3$-8); ^{13}C NMR (100MHz, DMSO-d$_6$): δ_C 171.8(C-1'), 160.2(C-2), 155.7(C-9a), 153.5(C-10a), 144.9(C-7'), 144.1(C-6'), 143.8(C-4), 130.9(C-5), 129.5(C-4'), 118.7(C-5a), 115.7(C-9'), 115.5(C-5'), 115.3(C-8'), 112.7(C-4a), 112.5(C-3), 103.4(C-10), 76.5(C-8), 69.3(C-7), 35.4(C-2'), 29.7(C-3'), 27.0(C-6), 24.3(CH$_3$-8), 23.2(CH$_3$-8); IT-TOF/MS: *m/z*=411.1393 [M+H]$^+$, 433.1283 [M+Na]$^+$.

Method for the synthesis of compounds (2c)

To a solution of (S)-(+)-decursinol (**1**, 0.406 mmol, 1eq) in anhydrous dichloromethane was added *trans*-3-(3-pyridyl)acrylic acid (0.609 mmol, 1.5eq), EDC(0.812 mmol, 2eq) and 4-DMAP(0.162 mmol, 0.4eq). The mixture was allowed to stir at room temperature for 12 h. Then the mixture was concentrated under reduced pressure, and the residue was purified by flash silica gel column chromatography to obtain decursin derivative (**2c, JH4**).

(7S)-(+)-3-(3-Pyridyl)-acrylic acid, 8,8-dimethyl-2-oxo-6,7-dihydro-*2H,8H*-pyrano[3,2-g]chromen-7-yl-ester (2c, JH4)

Yield 96.7%, White solid, mp: 105℃, R_f = 0.24 (1:1 *n*-hexane-ethyl acetate) ; $[\alpha]_D^{20}$ +48.5 (c=3, CHCl$_3$); ^{1}H NMR(400MHz, CDCl$_3$): δ_H 8.72(1H, d, *J*= 2.2Hz, H-5'), 8.60(1H, dd, *J*= 1.5, 4.9Hz, H-7'), 7.81(1H, d, *J*=8.4Hz, H-9'), 7.67(1H, d, *J*= 16.0Hz, H-3'), 7.58(1H, d, *J*= 9.6Hz, H-4), 7.32(1H, dd, *J*= 4.8, 8.0Hz, H-8'), 7.18(1H, s, H-5), 6.83(1H, s, H-10), 6.49(1H, d, *J*= 16.4Hz, H-2'), 6.24(1H, d, *J*= 9.6Hz, H-3), 5.21(1H, t, *J*= 4.8Hz, H-7), 3.26(1H, dd, *J*= 4.8, 17.2Hz, H-6a), 2.95(1H, dd, *J*= 4.8, 17.2Hz, H-6b), 1.44(3H, s, CH$_3$-8), 1.39(3H, s, CH$_3$-8); ^{13}C NMR (100 MHz, CDCl$_3$) δ_C 165.6(C-1'), 161.2(C-2), 156.3(C-9a), 154.2(C-10a), 151.2(C-5'), 149.8(C-7'), 143.1(C-4), 142.1(C-3'), 134.2(C-9'), 129.8(C-4'), 128.7(C-5), 123.7(C-8'), 119.5(C-2'), 115.5(C-5a), 113.4(C-3), 112.9(C-4a), 104.8(C-10), 76.5(C-8), 70.5(C-7), 27.8(C-6), 24.9(CH$_3$-8), 23.4(CH$_3$-8 IT-TOF/MS: *m/z*=378.1325 [M+H]$^+$, 400.1129 [M+Na]$^+$.

Method for the synthesis of compound (6)

To a solution of (S)-(+)-coumarin (**4**, 0.406 mmol, 1eq) in anhydrous dichloromethane was added 3,4-dimethoxycinnamic acid (0.609 mmol, 1.5eq), EDC (0.812 mmol, 2eq) and 4-DMAP (0.162 mmol, 0.4eq). The mixture was allowed to stir at room temperature for 12 h. Then the mixture was concentrated under reduced pressure, and the residue was purified by flash silica gel column

chromatography to obtain coumarin derivative (**5**).

To a solution of coumarin derivative with 3,4-dimethoxycinnamoyl group (**5**, 0.49 mmol, 1eq) in anhydrous dichloromethane (5 ml) was added 1M boron tribromide solution in dichloromethane (1.47 mmol, 3eq) in ice bath. The mixture was allowed to stir at room temperature for 5 h. The reaction solution was purified by silica gel short-column chromatography to obtain coumarin derivatives with 3,4-dihydroxycinnamoyl group (**6, LG-02**).

(6S)-(-)-3-(3,4-Dihydroxy-phenyl)-acrylic acid, 7,7-dimethyl-2-oxo-5,6-dihydro-*7H*-pyrano[3,2-*f*]chromen-6-yl-ester (6, LG-02)

Yield 90.86%, White Solid, mp: 117℃, R_f = 0.15 (1:1 *n*-hexane-ethyl acetate); $[\alpha]_D^{20}$ -140.82 (c=2, CHCl$_3$); ^{1}H NMR(400MHz, DMSO-d$_6$): δ_H 9.63(1H, s, OH-7'), 9.09(1H, s, OH-6'), 8.09(1H, d, *J*=9.6Hz, H-4), 7.46(1H, d, *J*=16.0Hz, H-3'), 7.21(1H, d, *J*=9.2Hz, H-10)7.09(1H, d, *J*=9.2Hz, H-9), 7.01(1H, s, H-5'), 7.00(1H, d, *J*=8.0Hz, H-9'), 6.72(1H, d, *J*=8.0Hz, H-8'), 6.45(1H, d, *J*=10.0Hz, H-2'), 6.24(1H, d, *J*=15.6Hz, H-3), 5.20(1H, t, *J*=4.6Hz, H-6), 3.32(1H, dd, *J*=4.8, 17.6Hz, H-5), 3.06(1H, dd, *J*=4.0, 18.0Hz, H-5), 1.32(3H, s, CH$_3$-7), 1.27(3H, s, CH$_3$-7); ^{13}C NMR (100MHz, CDCl$_3$): δ_C 166.9(C-1'), 161.5(C-2), 149.1(C-8a), 148.7(C-10a), 146.8(C-7'), 146.1(C-6'), 144.0(C-3'), 139.6(C-4), 126.9(C-4'), 122.7(C-5'), 122.2(C-9'), 117.3(C-4a), 116.4(C-4b), 116.1(C-10), 115.3(C-8'), 114.4(C-2'), 114.0(C-3), 104.0(C-9), 75.3(C-7), 69.8(C-6), 25.4(C-5), 24.2(CH$_3$-7), 22.8(CH$_3$-7); IT-TOF/MS: *m/z*=409.1275 [M+H]$^+$, 431.1090 [M+Na]$^+$.

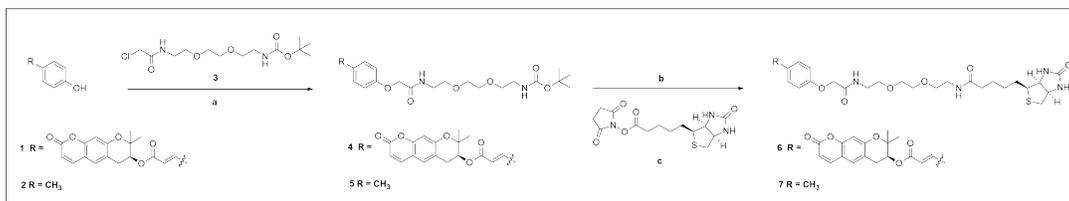**Biotin-JH4 synthesis**

<u>Chemistry</u>

  To confirm the binding of (+)-decursinol analogue **1** to which protein, biotin affinity probes were synthesized. The synthesis of the chemical probe **6** and its corresponding negative control **7** were accomplished according to the synthetic protocols as outlined in Scheme. The starting material **1** was synthesized from the (+)-decursinol, according to the procedures in the literature and shown in ref. 1. Another starting material *p*-cresol (**2**) was commercially available. As shown in Scheme, **1** and **2** were subjected to alkylation with Boc-protected PEG (polyethylene glycol) linker (**3**) in the presence of potassium carbonate, cesium carbonate and potassium iodide in anhydrous acetone and DMF to give **4** and **5** in good yield. The linker **3** was synthesized according to procedure as show in ref. 2. After deprotection of compounds, **4** or **5,** by using TFA, the residues were directly biotinylated with Biotin-OSu in the presence of triethylamine to provide the probe **6** or **7**, respectively.
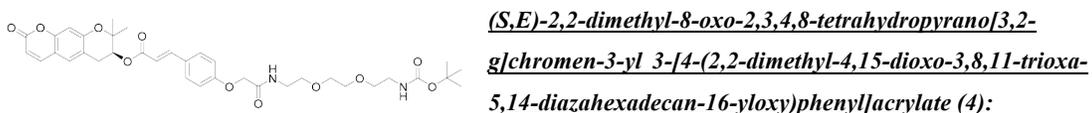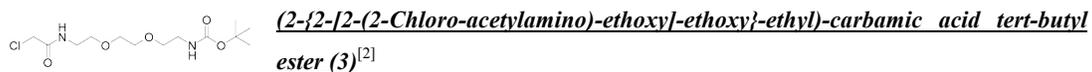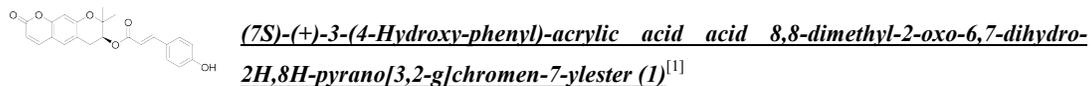
Synthesis

All of the commercial chemicals were of reagent grade and were used without further purification. All reactions were performed in oven-dried glassware, under an atmosphere of argon. Solvents were dried with standard procedures. The proton nuclear magnetic resonance ^{1}H-NMR were recorded on a Varian (400 MHz) spectrometer and ^{13}C NMR spectra were determined on a Varian (100 MHz) spectrometer. The chemical shifts are provided in parts per million (ppm) downfield with coupling constants in hertz (Hz). The mass spectra were recorded with high-resolution mass spectrometry (HR-MS) obtained on a G2 QTOF mass spectrometer. The products from all of the reactions were purified by flash column chromatography using silica gel 60 (230-400 mesh Kieselgel 60). Additionally, thin-layer chromatography on 0.25 mm silica plates (E. Merck, silica gel 60 Å F254) was used to monitor reactions. The chromatograms were visualized by UV irradiation or exposed to phosphomolybdic acid (PMA) staining. The purity of the final products was checked by reversed phase high-pressure liquid chromatography (RP-HPLC), which was performed on a Waters Corp. HPLC system equipped with a UV detector set at 254 nm. The mobile phases used were (A) H_2O containing 0.05 % TFA, and (B) CH_3CN. The HPLC employed an YMC Hydrosphere C18 (HS-302) column (5 μ particle size, 12 nm pore size) that was 4.6 mm dia. x 150 mm in size with a flow rate of 1.0 mL/min. The compound purity was assessed using gradient 25 % B to 100 % B in 35 min.

**Scheme .** Synthesis of biotinylated chemical probes 6-7.

Reagent and Conditions: a) K_2CO_3, Cs_2CO_3, KI, Acetone/DMF; b) TFA, CH_2Cl_2; c) TEA, DMF.

(7S)-(+)-3-(4-Hydroxy-phenyl)-acrylic acid acid 8,8-dimethyl-2-oxo-6,7-dihydro-2H,8H-pyrano[3,2-g]chromen-7-ylester (1)[1]

(2-{2-[2-(2-Chloro-acetylamino)-ethoxy]-ethoxy}-ethyl)-carbamic acid tert-butyl ester (3)[2]

(S,E)-2,2-dimethyl-8-oxo-2,3,4,8-tetrahydropyrano[3,2-g]chromen-3-yl 3-[4-(2,2-dimethyl-4,15-dioxo-3,8,11-trioxa-5,14-diazahexadecan-16-yloxy)phenyl]acrylate (4):

Compound **1** (0.39 g, 0.99 mmol), K_2CO_3 (0.21 g, 1.49 mmol), Cs_2CO_3 (0.16 g, 0.5 mmol), and KI (0.008 g, 0.05 mmol) were added to the solution of compound **3** (0.39 g, 1.19 mmol) synthesized according to procedure in the literature[2] in anhydrous acetone (5 mL) and DMF (5 mL). The reaction mixture was heated at 60°C for

36h and cooled to room temperature. The mixture was evaporated at reduced pressure, and the residue was washed with water. The solution was extracted with ethyl acetate, and the combined organic layers were dried over anhydrous MgSO$_4$, filtered, and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (*n*-hexane:EtOAc = 6:4 → *n*-hexane:EtOAc:MeOH = 5:4:1) to give compound **4** as a yellow oil (0.43 g, yield 63%).

^{1}H-NMR (CDCl$_3$, 400 MHz) δ 8.02 (s, 2H), 7.59-7.69 (m, 2H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.17 (s, 1H), 6.91 (d, *J* = 8.8 Hz, 2H), 6.83 (s, 1H), 6.31 (d, *J* = 15.6 Hz, 1H), 6.24 (d, *J* = 9.6 Hz, 1H), 5.19 (t, *J* = 4.8 Hz, 1H), 4.97 (brs, 1H), 4.52 (s, 2H), 3.55-3.62 (m, 6H), 3.52 (t, *J* = 5.2 Hz, 1H), 3.27-3.31 (m, 2H), 2.96 (s, 6H), 2.88 (s, 6H), 1.57 (s, 3H), 1.43 (s, 9H), 1.39 (s, 3H). HRMS (ESI) *m/z* calcd for C$_{36}$H$_{45}$N$_2$O$_{11}$ [(M+H)$^+$] 681.3023, found: 681.3048.`
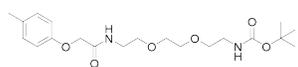
tert-butyl 2-(2-{2-[2-(p-tolyloxy)acetamido]ethoxy}ethoxy)ethylcarbamate (5):

p-Cresol (0.1 mL, 0.91 mmol), K$_2$CO$_3$ (0.19 g, 1.37 mmol), Cs$_2$CO$_3$ (0.15 g, 0.5 mmol), and KI (0.008 g, 0.05 mmol) were added to the solution of compound **3** (0.35 g, 1.09 mmol) synthesized according to procedure in the literature[2] in anhydrous acetone (3 mL) and DMF (3 mL). The reaction mixture was heated at 60°C for 36h and cooled to room temperature. The mixture was evaporated at reduced pressure, and the residue was washed with water. The solution was extracted with ethyl acetate, and the combined organic layers were dried over anhydrous MgSO$_4$, filtered, and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (*n*-hexane:EtOAc = 7:3 → *n*-hexane:EtOAc:MeOH = 6:3:1) to give compound **5** as a colorless oil (0.15 g, yield 41%).

^{1}H-NMR (CDCl$_3$, 400 MHz) δ 7.10 (d, *J* = 8.8 Hz, 2H), 6.81 (d, *J* = 8.4 Hz, 2H), 4.47 (s, 2H), 3.51-3.60 (m, 10H), 3.30 (d, *J* = 5.2 Hz, 2H), 2.30 (s, 3H), 1.44 (s, 9H). HRMS (ESI) *m/z* calcd for C$_{20}$H$_{33}$N$_2$O$_6$ [(M+H)$^+$] 397.2339, found: 397.2419.
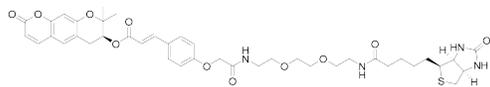

(E)-[(S)-2,2-dimethyl-8-oxo-2,3,4,8-tetrahydropyrano[3,2-g]chromen-3-yl] 3-(4-{2,13-dioxo-17-[(3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]-6,9-dioxa-3,12-diazaheptadecyloxy}phenyl)acrylate (6):

Compound **4** (0.18 g, 0.26 mmol) was dissolved in a mixture of TFA (0.8 mL) and CH$_2$Cl$_2$ (5 mL), and the solution was stirred at room temperature for 3 h. The solvents were removed and evaporated and concentrated *in vacuo*. The crude free amine product was not further purified but was dissolved in DMF (3 mL), to which was TEA (0.18 mL, 1.3 mmol) was added. Biotin-OSu (0.11 g, 0.31 mmol) was added to the solution, which was stirred at room temperature overnight. The reaction was quenched by water and extracted with ethyl acetate. The organic layers were dried over anhydrous MgSO$_4$, filtered, and concentrated *in vacuo*. the crude product was purified by silica gel column chromatography (CH$_2$Cl$_2$:MeOH = 10:1→9:1) to give compound **6** as a white solid (0.1 g, yield 48%).

^{1}H-NMR (CD$_3$OD, 400 MHz) δ 7.83 (d, *J* = 10.0 Hz, 1H), 7.63 (d, *J* = 16.0 Hz, 1H), 7.56 (d, *J* = 9.2 Hz, 2H), 7.38 (s, 1H), 7.00 (d, *J* = 9.2 Hz, 2H), 6.77 (s, 1H), 6.40 (d, *J* = 16.0 Hz, 1H), 6.22 (d, *J* = 9.6 Hz, 1H), 5.49 (s, 1H), 5.22 (t, *J* =4.8 Hz, 1H), 4.56 (s, 2H), 4.46 (q, *J* = 7.6 Hz, 1H), 4.27 (q, *J* = 8.0 Hz, 1H), 3.54-3.60 (m, 6H),

3.51 (t, J = 6.0 Hz, 2H), 3.46 (t, J = 5.6 Hz, 2H), 3.14-3.19 (m, 1H), 2.96 (dd, J = 4.8, 17.6 Hz, 1H), 2.90 (dd, J = 5.2, 12.8 Hz, 1H), 2.68 (d, J = 12.4 Hz, 1H), 2.17 (t, J = 7.2 Hz, 2H), 2.03 (d, J = 13.6 Hz, 1H), 1.54-1.71 (m, 5H), 1.42 (s, 3H), 1.39 (s, 3H), 1.28 (s, 1H), 1.17-1.24 (m, 1H). ^{13}C-NMR (CD$_3$OD, 100 MHz) δ 174.7, 169.3, 166.5, 164.7, 161.9, 159.7, 156.6, 154.0, 145.1, 144.2, 129.8, 129.2, 127.9, 116.4, 115.1, 114.9, 113.0, 112.2, 103.6, 76.7, 70.1, 69.9, 69.2, 69.0, 66.8, 61.9, 60.2, 55.6, 39.6, 38.9, 38.6, 35.3, 28.3, 28.1, 27.3, 25.4, 23.8, 22.3. HRMS (ESI) m/z calcd for C$_{41}$H$_{49}$N$_4$O$_{11}$S [(M-H)$^-$] 805.3119, found: 805.3130; Purity > 99% (as determined by RP-HPLC, t_R = 12.6 min).
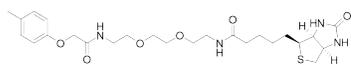
5-[(3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]-N-[2-(2-{2-[2-(p-tolyloxy)acetamido]ethoxy}ethoxy)ethyl]pentanamide (7):

Compound **5** (0.15 g, 0.37 mmol) was dissolved in a mixture of TFA (0.8 mL) and CH$_2$Cl$_2$ (4 mL), and the solution was stirred at room temperature for 1.5 h. The solvents were removed and evaporated and concentrated *in vacuo*. The crude free amine product was not further purified but was dissolved in DMF (3 mL), to which was TEA (0.26 mL, 1.85 mmol) was added. Biotin-OSu (0.15 g, 0.45 mmol) was added to the solution, which was stirred at room temperature overnight. The reaction was quenched by water and extracted with ethyl acetate. The organic layers were dried over anhydrous MgSO$_4$, filtered, and concentrated *in vacuo*. the crude product was purified by silica gel column chromatography (CH2Cl2:MeOH = 10:1→9:1) to give compound 7 as a white solid (0.085 g, yield 44%).

^{1}H-NMR (CD$_3$OD, 400 MHz) δ 7.10 (d, J = 9.2 Hz, 2H), 6.87 (d, J = 8.8 Hz, 2H), 4.48 (s, 2H), 4.28 (q, J = 7.6 Hz, 1H), 3.57-3.61 (m, 6H), 3.53 (t, J = 5.6 Hz, 2H), 3.47 (t, J = 5.6 Hz, 2H), 3.34 (t, J = 5.6 Hz, 2H), 3.16-3.21 (m, 1H), 2.91 (dd, J = 5.2, 12.4 Hz, 1H), 2.69 (d, J = 12.8 Hz, 1H), 2.27 (s, 3H), 2.20 (t, J = 7.6 Hz, 2H), 1.56-1.75 (m, 5H), 1.43 (q, J = 15.2 Hz, 2H). ^{13}C-NMR (CD$_3$OD, 100 MHz) δ 176.2, 171.5, 166.1, 157.1, 132.4, 131.1, 115.8, 71.3, 70.7, 70.5, 68.5, 63.4, 61.7, 57.0, 41.1, 40.4, 39.9, 36.8, 29.8, 29.5, 26.9, 20.6, 9.3. HRMS (ESI) m/z calcd for C$_{25}$H$_{37}$N$_4$O$_6$S [(M-H)$^-$] 521.2434, found: 521.2439; Purity > 95% (as determined by RP-HPLC, t_R = 7.7 min).

References of biotin probe synthesis

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[2] K. Lee, H.S. Ban, R. Naik, Y.S. Hong, S. Son, B.K. Kim, Y. Xia, K.B. Song, H.S. Lee, M. Won, *Angew. Chem. Int. Ed.* **2013**, *52*, 10286-10289.