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(54) Title: INHIBITION OF DCPS

(57) Abstract: The disclosure relates to methods and compositions (e.g., compounds and pharmaceutical compositions thereof) useful for increasing expression of SMN in a cell (*e.g., in vitro or in vivo*). As a deficiency in SMN can result in the development of an SMA condition in a subject, the methods and compositions described herein can also be used to, e.g., treat, or prevent, an SMA condition in a subject.

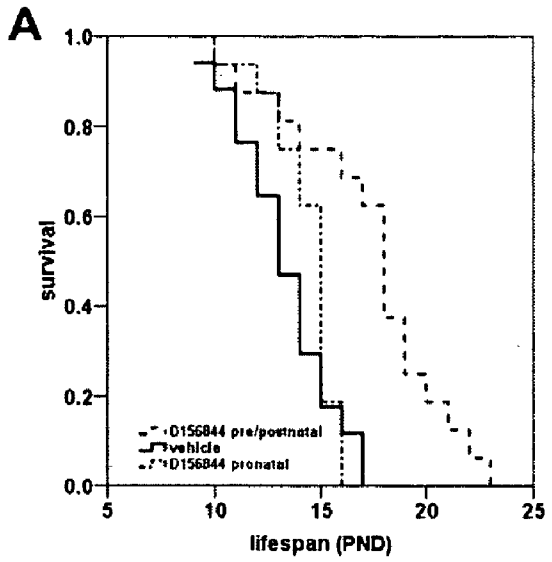

FIG. 10A

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SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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INHIBITION OF DCPS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application Serial No. 61/072,040, filed on March 26, 2008. The entire contents of the foregoing are incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] The research described in this application was supported by grant no. GM67005 awarded from the National Institute of General Medical Sciences, and grant no. NS038650 awarded from the National Institutes of Health. Thus, the government has certain rights in the invention.

TECHNICAL FIELD

[0003] The technology described herein generally relates to enhancing SMN expression by inhibiting DcpS. The technology more particularly relates to methods and compositions for increasing expression of SMN, and treating or preventing an SMA condition in a subject.

BACKGROUND

[0004] Spinal muscular atrophy (SMA) is a currently untreatable, autosomal recessive genetic disease caused by a deficiency of full-length survival motor neuron (SMN) protein. The symptoms are the result of progressive degeneration of motor neurons in the anterior horn of the spinal cord resulting in weakness and wasting of the voluntary muscles.

[0005] Type I (Acute) SMA is also called Werdnig-Hoffmann Disease. SMA type I is evident before birth or within the first few months of life. There may be a reduction in fetal movement in the final months of pregnancy. There is a general weakness in the intercostals and accessory respiratory muscles. The chest may appear concave. Symptoms include floppiness of the limbs and trunk, feeble movements of the arms and legs, swallowing and feeding difficulties, and impaired breathing. Affected children never sit or stand and usually die before the age of 2.

[0006] Type II (Chronic) SMA is usually diagnosed by 15 months. Children may have respiratory problems, floppy limbs, decreased or absent deep tendon reflexes, and twitching of arm, leg, or tongue muscles. These children may learn to sit but cannot stand or walk. Life expectancy varies. Feeding and swallowing problems are not usually characteristic of Type II, although in some patients a feeding tube may become necessary. Tongue fasciculations are less often found in children with Type II but a fine tremor in the outstretched fingers is common.

[0007] Type III (Mild) SMA, often referred to as Kugelberg-Welander or Juvenile Spinal Muscular Atrophy, is usually diagnosed between 2 and 17 years of age. Symptoms include abnormal manner of walking; difficulty running, climbing steps, or rising from a chair; and slight tremor of the fingers. The patient with Type III can stand alone and walk; tongue fasciculations are seldom seen. Types I, II and III progress over time, accompanied by deterioration of the patient's condition.

[0008] Type I, II and III SMA are caused by a mutation in a part of the DNA called the Survival of Motor Neuron (SMN1) gene, which normally produces a protein called SMN. Because of their gene mutation, people with SMA make less SMN protein, which results in the loss of motor neurons. SMA symptoms may be improved by increasing the levels of SMN protein. Normally the SMN1 gene provides instructions for making a protein called Survival of Motor Neuron 1. The SMN1 protein helps to assemble the cellular machinery needed to process pre-mRNA. More than 90 percent of individuals with spinal muscular atrophy lack part or all of both copies of the SMN1 gene. A small percentage of people with this condition lack one copy of the SMN1 gene and have a point mutation in the remaining copy. About 30 different mutations have been identified. The most frequent of these mutations replaces the amino acid tyrosine with cysteine at position 272 in the SMN1 protein. Other mutations replace amino acids at different positions or produce an abnormally short protein. As a result of these missing or altered genes, cells have a shortage of functional SMN1 protein. It remains unclear why motor neurons are particularly vulnerable to a shortage of this protein. Loss of the SMN1 protein from motor neurons results in the degeneration of these nerve cells, leading to the signs and symptoms of spinal muscular atrophy.

[0009] There is a second, almost identical copy of the SMN gene called SMN2. In all cases of spinal muscular atrophy, particularly the milder cases, there are multiple copies of SMN2. Typically, people who do not have spinal muscular atrophy have zero to three copies of the gene, but more copies have been observed. On a limited basis, increased SMN2 gene copy number can help replace the protein needed for the survival of motor neurons. In general, symptoms are less severe and begin later in life in affected individuals with more copies of the SMN2 gene. The SMN1 and SMN2 genes provide instructions for making a protein called Survival of Motor Neuron. However, the SMN2 gene makes full-length, functional SMN protein in much lower amounts than the SMN1 gene. This protein is made in four different versions by both the SMN1 and SMN2 genes. Only isoform d is full size and functional. Due to a single point mutation at the beginning of exon 7 in the SMN2 gene, the full length form is made at a much higher proportion in the SMN1 gene versus the SMN2 gene. The other isoforms (a, b, and c) are smaller and are not fully functional when made from either gene. The SMN2 gene primarily makes the form of the protein missing exon 7 due to the single point mutations. SMN protein missing exon 7 cannot oligomerize, which is required for function, and is very unstable. Among individuals with spinal muscular atrophy (who lack functional SMN1 genes), additional copies of the SMN2 gene can modify the course of the disorder. On a limited basis, the extra SMN2 genes can help replace the protein needed for the survival of motor neurons. Spinal muscular atrophy still occurs, however, because most of the proteins produced by SMN2 genes are isoforms a, b, and c, which are smaller than the SMN1 protein and cannot fully compensate for the loss of SMN1 genes.

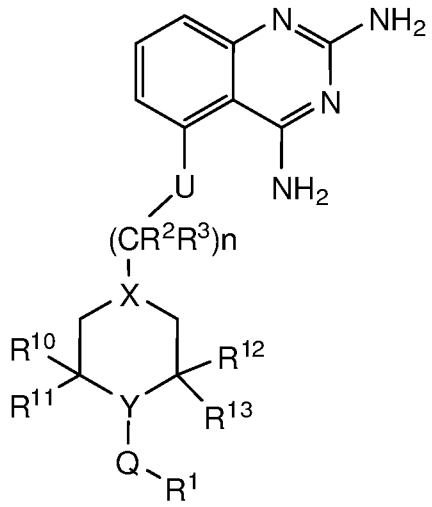
SUMMARY

[0010] The present disclosure is based, at least in part, on methods and compositions for inhibiting the mRNA decapping protein DcpS (a scavenger pyrophosphatase enzyme that hydrolyzes the residual cap structure following mRNA degradation in the 3' to 5' exoribonuclease pathway and the m^7GDP product of the Dcp2 decapping enzyme) and thereby enhancing the expression of SMN in a cell. Moreover, such compounds are able to enhance the lifespan of SMN-deficient mice, when administered either before and after birth, or only after birth. Accordingly, the disclosure provides methods and compositions (e.g., compounds, pharmaceutical compositions containing the compositions, and articles of manufacture) useful for increasing expression of SMN in a cell (e.g., *in vitro* or *in vivo*) as well as methods and

compositions for use in treating, or preventing, or ameliorating one or more symptoms of an SMA condition in a subject.

[0011] A method for increasing expression of SMN protein in a cell, the method comprising contacting a cell with a DcpS inhibitor in an amount effective to increase the expression of SMN protein in the cell.

[0012] The DcpS inhibitor can be a compound having the following structural formula:



wherein:

U is O or a bond directly from the quinazoline ring to X;

n = an integer from 0 – 3;

R^2 and R^3 are each independently H or lower alkyl;

n is 0, 1, 2, or 3;

X and Y are each independently CH or N, so that the ring that contains X and Y is cyclohexane, piperidine, or piperazine;

R^{10}, R^{11}, R^{12}, and R^{13} are independently H or alkyl;

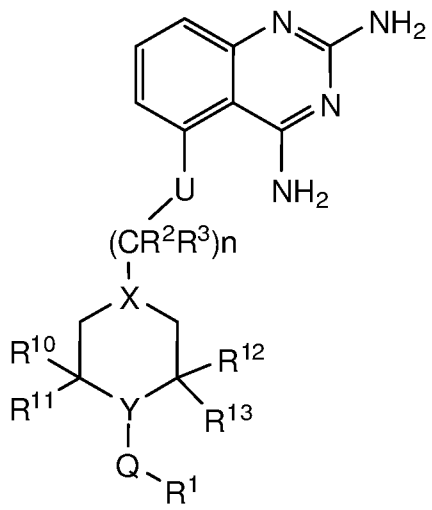
R^{10} and R^{11} together can be =O, and independently R^{12} and R^{13} together can be =O, so that the ring containing X and Y can be glutarimide, or piperidinone;

Q is selected from the group consisting of: $–CR^{14}R^{15}–$, –C(=O)–, $–SO_2–$, $–CH_2C(=O)–$, $–C(=O)CH_2S–$, and a direct bond from N to R^1, wherein R^{14} and R^{15} are each independently H, or lower alkyl;

R^1 can be H, alkyl, alkoxy, carbocycle, substituted carbocycle, heteroaryl or substituted heteroaryl, heterocyclyl or substituted heterocyclyl, fluoroalkyl, and alkoxyalkyl.

[0013] A method for treating an SMA condition in a subject, the method comprising administering to the subject a compound that inhibits DcpS.

[0014] The compound can have the following structural formula:



wherein:

U is O or a bond directly from the quinazoline ring to X;

n = an integer from 0 – 3;

R^2 and R^3 are each independently H or lower alkyl;

n is 0, 1, 2, or 3;

X and Y are each independently CH or N, so that the ring that contains X and Y is cyclohexane, piperidine, or piperazine;

R^{10}, R^{11}, R^{12}, and R^{13} are independently H or alkyl;

R^{10} and R^{11} together can be =O, and independently R^{12} and R^{13} together can be =O, so that the ring containing X and Y can be glutarimide, or piperidinone;

Q is selected from the group consisting of: $–CR^{14}R^{15}–$, $–C(=O)–$, $–SO_2–$, $–CH_2C(=O)–$, $–C(=O)CH_2S–$, and a direct bond from N to R^1, wherein R^{14} and R^{15} are each independently H, or lower alkyl;

R^1 can be H, alkyl, alkoxy, carbocycle, substituted carbocycle, heteroaryl or substituted heteroaryl, heterocyclyl or substituted heterocyclyl, fluoroalkyl, and alkoxyalkyl.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] **FIG. 1A** is a schematic diagram depicting the structure of an exemplary C5-substituted quinazoline compound (Cpd. 1) shown with a 2-fluoro-benzyl piperidine substituent. Atoms of the quinazoline numbered. The structure of Cpd. 1 is depicted in Table 1, herein.

[0016] **FIG. 1B** is a line graph depicting the ability of Cpd. 1 to induce β-lactamase expression from an SMN promoter-driven β-lactamase reporter construct in NSC-34 cells (mean ± SD, triplicate wells). The Y-axis represents the "fold" increase over control of β-lactamase activity and the X-axis represents the log concentration of Cpd.1 (μM). The EC_{50} was 9.1 nM.

[0017] **FIG. 1C** is a bar graph depicting the ability of Cpd. 1 to induce SMN mRNA expression from an endogenous SMN gene in NSC-34 cells. The Y-axis represents the "fold" increase over control of SMN mRNA expression and the X-axis represents the concentration of Cpd.1 (50 or 500 nM), trichostatin A (TSA; 100 nM), or a DMSO control. "Fold" increase values are presented mean ± SD, n=9.

[0018] **FIG. 1D** is a bar graph depicting diminution of DcpS levels, which led to an increase in SMN2 that was not further accentuated by Cpd. 1 in human 293T cells. SMN2 mRNA levels are presented relative to beta-actin mRNA levels.

[0019] **FIG. 2** is a photograph of an immunoblot depicting the effect of three exemplary compounds (Cpd. 1 and Cpd. 12), or DMSO control, on SMN protein expression in human fibroblasts obtained from a type I SMA patient (3813). 60 μg of cell lysate prepared from the treated cells (or from human fibroblasts obtained from an SMA carrier/SMN positive patient, 3814) were subjected to sodium dodecyl-polyacrylamide gel electrophoresis (SDS-PAGE) and SMN protein was detected using a mouse anti-SMN monoclonal antibody (MANSMA2 (8F7)). As a control for equal protein loading on the gel, β-tubulin was also detected using a mouse anti-β-tubulin monoclonal antibody. As used herein, Cpd. 12 refers to 5-[1-(3-chlorobenzyl)piperidin-4-ylmethoxy]quinazoline-2,4-diamine, and Cpd. 1 refers to 5-[1-(2-fluorobenzyl)piperidin-4-ylmethoxy]quinazoline-2,4-diamine. The structure for each of these compounds is also provided herein).

[0020] **FIG. 3** is a schematic diagram of a synthetic reaction to produce Cpd. 3. A tin derivative of Cpd. 1 was synthesized (Cpd. 2) and ^{125}I sodium iodide/chloramine T was used to displace the $SnBu_3$ of Cpd. 2, yielding the desired radiolabeled C5-quinazoline (Cpd. 3).

[0021] **FIG. 4 A** is a pair of photographs of array images identifying DcpS as a potential Cpd. 3 target protein. Arrays comprised of 5,000 immobilized human proteins were probed with ^{125}I-Cpd. 3 in the presence and absence of unlabeled small molecules. Specific displacement of ^{125}I-Cpd. 3 (at 100 nM concentration) by bioactive, but distinct C5-quinazolines (Cpd. 3 and Cpd. 1; unlabeled at 10 μM concentration) was observed. Specific displacement of ^{125}I-Cpd. 3 (at 100 nM concentration) by control compounds staurosporine and tertbutylquinone was not observed. Duplicate features boxed in white (upper right box within each of the enlarged boxes) correspond to the positional mapping reagent ^{125}I-streptavidin binding to a biotinylated control protein. The lower left box within each enlarge box corresponds to the binding between the ^{125}I-labeled compound and DcpS protein on the array (in the presence or absence of unlabeled annotated small molecules).

[0022] **FIG. 4 B** is a bar graph depicting the quantitation of the array images depicted in FIG. 4 A. The quantified competition binding data was normalized against signals arising from ^{125}I-streptavidin binding to the biotinylated control protein. Standard deviations for the replicate assays are indicated.

[0023] **FIG. 5A** is a pair of photographs of autoradiograms depicting the effect of C5-substituted quinazolines on DcpS decapping activity *in vitro*. Radiolabeled m7Gp*pppG was incubated with purified human DcpS (5 nM) in the presence of increasing concentrations of C5-quinazolines (Cpd. 1 or Cpd. 4; left photograph) or a positive control for DcpS inhibition (Cap structure) or negative vehicle (DMSO) control (right photograph). As used herein, "*" in the name of a radiolabeled compound denotes that the phosphate group ("p") preceding the "*" is detectably labeled (e.g., radioisotope labeled). The reaction products were separated by thin layer chromatography and visualized with a Phosphorimager. Calculated percentages of decapping activity relative to total substrate input are indicated below each photograph. The structure of Cpd. 4 is depicted in Table 1.

[0024] **FIG. 5B** is a line graph depicting the results of the experiment described in FIG. 5A (numerical data for the relative decapping rate vs. concentration of compounds (nM)). The Y-axis represents the % of DcpS decapping activity and the X-axis depicts the concentration of the compound used in the experiment (nM).

[0025] **FIG. 5C** is a line graph depicting the results of an experiment to determine the effect of Cpds. 1, 4, 5, 6, 7, and 8 on DcpS decapping activity (*in vitro*). The Y-axis represents the % of DcpS decapping activity and the X-axis depicts the concentration of the compound used in the experiment (nM). The structures of Cpds. 1, 4, 5, 6, 7, and 8 are depicted in Table 1.

[0026] **FIG. 5D** is a line graph depicting the linear correlation between SMN2 promoter activity and DcpS inhibition (e.g., as determined in FIGs. 1C and 5C). The Y-axis represents the DcpS Assay IC_{50} values (nM) and the X-axis represents the SMN2 promoter assay EC_{50} values (nM).

[0027] **FIG. 6A** is a picture of a representative immunoblot showing SMN and β-actin protein expression in spinal cord extracts from SMNΔ7 SMA mice treated with either Cpd. 1 or vehicle for 5 days.

[0028] **FIG. 6B** is a bar graph showing changes in SMN protein levels relative to β-actin protein in Cpd. 1- or vehicle-treated spinal cord extracts.

[0029] **FIG. 7A** is a Kaplan-Meier plot showing survival of SMNΔ7 SMA mice receiving either vehicle or Cpd. 1 (3 mg/kg/d) beginning at either PND04 or PND09.

[0030] **FIG. 7B** is a Kaplan-Meier plot showing onset of body mass loss for SMNΔ7 SMA mice receiving either vehicle or Cpd. 1 (3 mg/kg/d) beginning at either PND04 or PND09.

[0031] **FIG. 7C** is body mass curves for SMNΔ7 SMA mice receiving either vehicle or cpd. 1 (3 mg/kg/d) beginning at PND04.

[0032] **FIGS. 8A – 8E** are bar graphs showing that oral administration of Cpd. 1 improved the motor phenotype of SMNΔ7 SMA mice and reduced motor neuron loss in the lumbar spinal cord. A) Righting reflex latencies at PND07 and PND11 for SMNΔ7 SMA mice treated with (3

mg/kg/d) or vehicle as compared to carrier mice. B) Vectorial movement latencies at PND07, PND11 and PND14 for SMNΔ7 SMA mice treated with or vehicle. C) Spontaneous locomotor activity as measured as the number of grids crossed in 1 min at PND07, PND11 and PND14 for SMNΔ7 SMA mice treated with or vehicle. D) The number of pivots (90° turns) made in 1 min at PND07, PND11 and PND14 for SMNΔ7 SMA mice treated with or vehicle. E) The number of motor neurons in the lumbar (L4-5) spinal cord of SMNΔ7 SMA mice (PND11) treated with or vehicle. (*, $p \leq 0.05$)

[0033] **FIG. 9A** is a line graph showing Cpd. 1 levels in prenatal mouse brains whose dams were dosed with Cpd. 1 (0-60 mg/kg/d) beginning at ED11.5.

[0034] **FIG. 9B** is a bar graph showing β-galactosidase activity—a marker for mSmn promoter activity—in the brains of mice whose dams received Cpd. 1 (0-60 mg/kg/d) beginning at ED11.5.

[0035] **FIG. 10A** is a Kaplan-Meier plot showing survival of SMNΔ7 SMA mice receiving either vehicle or Cpd. 1 (3 mg/kg/d) beginning ED11.5 and continuing after birth or ending at birth.

[0036] **FIG. 10B** is a Kaplan-Meier plot showing onset of body mass loss for SMNΔ7 SMA mice receiving either vehicle or Cpd. 1 beginning ED11.5 and continuing after birth or ending at birth.

[0037] **FIG. 10C** is body mass curves for SMNΔ7 SMA mice receiving either vehicle or Cpd. 1 (3 mg/kg/d) beginning at ED11.5.

[0038] **FIG. 11** is a bar graph showing therapeutic window of opportunity for protective effects of SMN2 induction by Cpd.1.

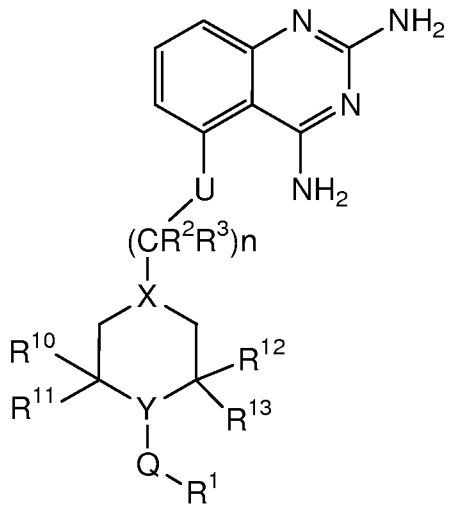
DETAILED DESCRIPTION

[0039] The disclosure relates to methods and compositions (e.g., compounds and pharmaceutical compositions thereof) useful for increasing expression of SMN in a cell (e.g., *in vitro* or *in vivo*). As a deficiency in SMN can result in the development of an SMA condition in

a subject, the methods and compositions described herein can also be used to treat, or prevent, an SMA condition in a subject. Exemplary methods and compositions are set forth below.

Exemplary Compounds and Pharmaceutical Compositions Thereof

[0040] Compounds that can be used to increase expression of SMN (e.g., SMN protein or an mRNA encoding an SMN protein) in a cell and/or treat (or prevent) an SMA condition in a subject include a compound having the formula (I),



(I)

wherein:

U is O or a bond directly from the quinazoline ring to X;

n = an integer from 0 – 3;

R^2 and R^3 are each independently H, or lower alkyl;

X and Y are each independently CH or N, so that the ring that contains X and Y is cyclohexane, piperidine, or piperazine;

R^{10}, R^{11}, R^{12}, and R^{13} are independently H or alkyl;

R^{10} and R^{11} together can be =O, and independently R^{12} and R^{13} together can be =O, so that the ring containing X and Y can be glutarimide, or piperidinone;

Q is selected from the group consisting of: $–CR^{14}R^{15}–$, –C(=O)–, $–SO_2–$, $–CH_2C(=O)–$, $–C(=O)CH_2S–$, and a direct bond from N to R^1, wherein R^{14} and R^{15} are each independently H, or lower alkyl; and

R^1 is H, alkyl, alkoxy, carbocycle, substituted carbocycle, heteroaryl or substituted heteroaryl, heterocyclyl or substituted heterocyclyl, fluoroalkyl, and alkoxyalkyl.

[0041] Substituted carbocycles, such as monsubstituted or 2,6-dihalo substituted phenyls, are preferred for R_1.

[0042] In one embodiment of compounds of formula (I), when X = CH, Y = N, U = O, n = 1, Q = CH_2, and $R^2 = R^3 = R^{10}$–R^{13} = H, then R^1 is not 3,4-dichlorophenyl.

[0043] In another embodiment of compounds of formula (I), when X = CH, Y = N, U = O, n = 1, Q = C(=O), and $R^2 = R^3 = R^{10}$–R^{13} = H, then R^1 is not 2-, 3-, or 4-iodophenyl, and R^1 is not 2-, 3-, or 4-chlorophenyl.

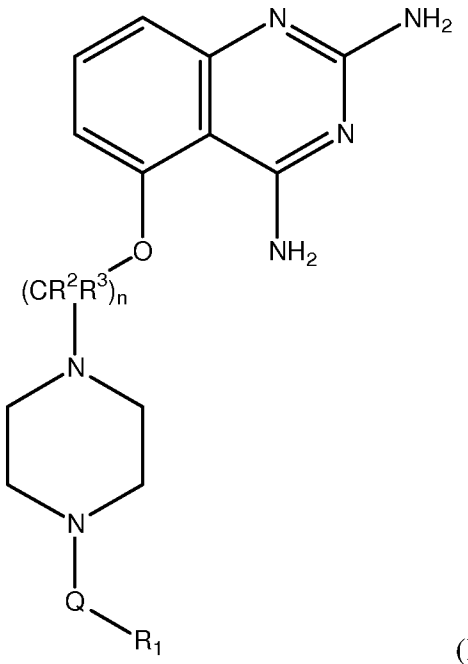
[0044] In yet another embodiment of compounds of formula (I), when X = CH, Y = N, U = O, n = 1, Q = C(=O), and $R^2 = R^3 = R^{10} - R^{13}$ = H, then R^1 is not t-butoxy (*i.e.*, Q and R^1 when taken together are not tBoc).

[0045] In still another embodiment of compounds of formula (I), when X = CH, Y = N, U = O, n = 1, Q = a bond, and $R^2 = R^3 = R^{10} - R^{13}$ = H, then R^1 is not H (*i.e.*, Q and R^1 when taken together are not H).

[0046] In a still further embodiment of compounds of formula (I), such compounds are excluded when U is O, n is 1, X and Y are each CH, R^{10}–R^{13} are each H, one of R^2 and R^3 is H, and the other is H or lower alkyl, Q is a bond, and R^1 is H. That is, when the ring containing X and Y is cyclohexyl and one of R^2 and R^3 is H other is H or lower alkyl, then Q-R^1 is other than H.

[0047] In some embodiments, R^1 is H or lower alkyl; in certain embodiments, R^1–R^3 and R^{10}–R^{13} are each independently H or lower alkyl.

[0048] A compound useful for increasing expression of SMN (e.g., SMN protein or an mRNA encoding an SMN protein) in a cell and/or treating (or preventing) an SMA condition in a subject can have the formula (II),



(II)

wherein:

U is O or a bond directly from the quinazoline ring to X;

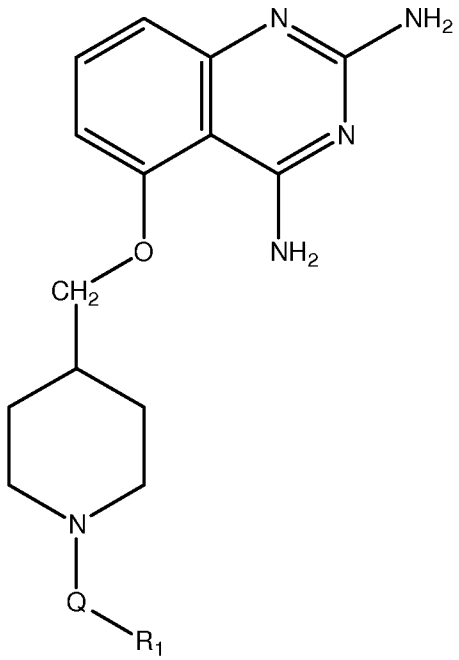
n = an integer from 0 – 3;

R^2 and R^3 are each independently H, or lower alkyl;

Q is selected from the group consisting of: $–CR^{14}R^{15}–$; –C(=O)–; $–SO_2–$; $–CH_2C(=O)–$; $–C(=O)CH_2S–$; and a direct bond from N to R^1, wherein R^{14} and R^{15} are each independently H, or lower alkyl; and

R^1 is selected from the group consisting of: H; alkyl; alkoxy; carbocycle; heterocyclyl; substituted heterocyclyl; substituted carbocycle; fluoroalkyl and alkoxyalkyl.

[0049] A compound useful for increasing expression of SMN (e.g., SMN protein or an mRNA encoding an SMN protein) in a cell and/or treating (or preventing) an SMA condition in a subject can have the formula (III),



(III)

wherein:

U is O or a bond directly from the quinazoline ring to X;

n = an integer from 0 – 3;

Q is selected from the group consisting of: $–CR^{14}R^{15}–$, –C(=O)–, $–SO_2–$, $–CH_2C(=O)–$, $–C(=O)CH_2S–$, and a direct bond from N to R^1, wherein R^{14} and R^{15} are each independently H, or lower alkyl; and

R^1 is selected from the group consisting of H, alkyl, alkoxy, carbocycle, substituted carbocycle, heteroaryl or substituted heteroaryl, heterocyclyl, and substituted heterocyclyl, fluoroalkyl, and alkoxyalkyl.

[0050] In one embodiment of formula (III), when Q = CH_2, and $R^2 = R^3 = R^{10}–R^{13}$ = H, then R^1 is not 3,4-dichlorophenyl.

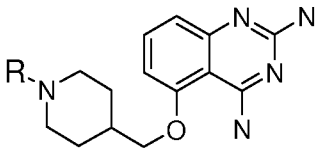
[0051] In yet another embodiment of formula (III), when Q = C(=O), and $R^2 = R^3 = R^{10} – R^{13}$ = H, then R^1 is not t-butoxy (*i.e.*, Q and R^1 when taken together are not tBoc).

[0052] In yet another embodiment of formula (III), when Q = C(=O), and $R^2 = R^3 = R^{10} – R^{13}$ = H, then R^1 is not chloro-phenyl, and is not iodo-phenyl.

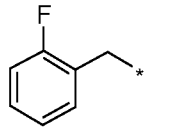
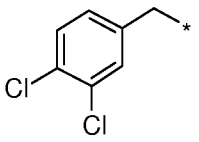
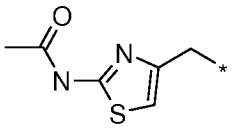
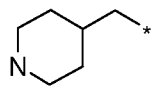
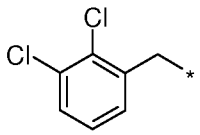
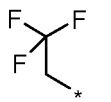
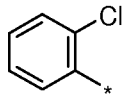
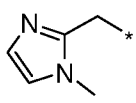
[0053] In still another embodiment of formula (III), when Q = a bond, and $R^2 = R^3 = R^{10} - R^{13}$ = H, then R^1 is not H (*i.e.*, Q and R^1 when taken together are not H).

[0054] A compound useful for increasing expression of SMN (*e.g.*, SMN protein or an mRNA encoding an SMN protein) in a cell and/or treating (or preventing) an SMA condition in a subject can have the formula (III) wherein Q is chosen from $-CH_2-$, $-C(=O)-$, $-SO_2-$, $-CH_2C(=O)-$ and $-SCH_2C(=O)-$; R^1 is selected from the group consisting of H, alkyl, alkoxy, carbocycle, heterocyclyl, substituted carbocycle and substituted heterocyclyl, with the proviso that when Q = CH_2, R^1 is not 3,4-dichlorophenyl.

[0055] Exemplary compounds can have the following formulae:



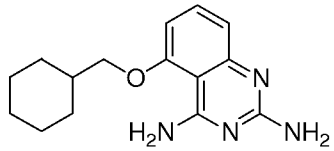
wherein R is selected from the group consisting of:



and


.





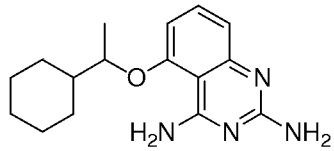
[0056] Various other embodiments of the compounds disclosed herein exclude compounds wherein Q is–CH_2– and R^1 is a phenyl ring having any two halo substituents in *meta*, or *meta* and *para*, positions relative to its benzylic position.

[0057] Various other embodiments of the compounds disclosed herein exclude compounds wherein Q is–C(=O)– and R^1 is a phenyl ring mono-substituted with any halogen in *ortho*, *meta*, or *para* positions relative to its benzylic position.

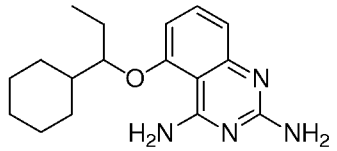
[0058] Accordingly, the compounds listed immediately below are exemplary compounds that are excluded from the various embodiments of the 2,4-diaminoquinazoline compounds disclosed herein:



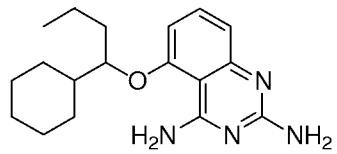
5-(Cyclohexylmethoxy)quinazoline-2,4-diamine



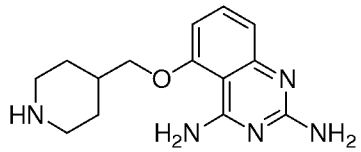
5-(1-Cyclohexyl-ethoxy)-quinazoline-2,4-diamine



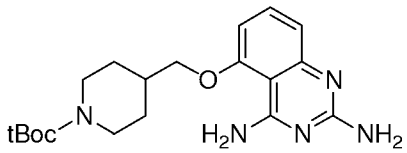
5-(1-Cyclohexylpropoxy)-quinazoline-2,4-diamine



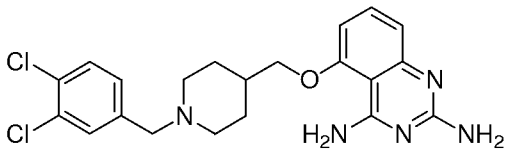
5-(1-Cyclohexyl-butoxy)-quinazoline-2,4-diamine



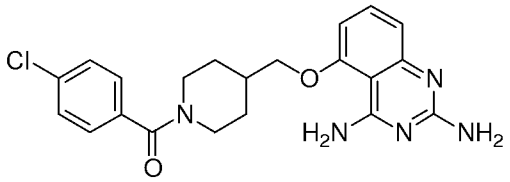
5-(Piperidin-4-ylmethoxy)-quinazoline-2,4-diamine



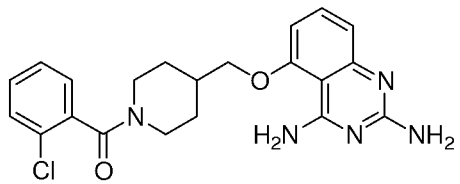
4-(2,4-diamino-quinazolin-5-yloxymethyl)-piperidin-1-carboxylic acid tert-butyl ester



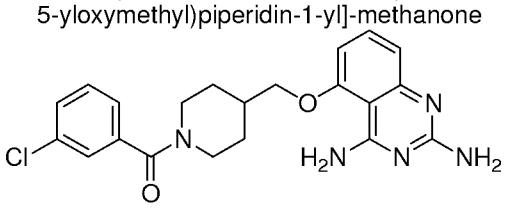
5-[1-(3,4-Dichlorobenzyl)-piperidin-4-ylmethoxy]-quinazoline-2,4-diamine



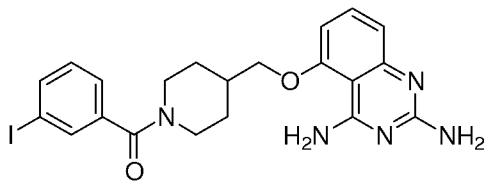
(4-Chlorophenyl)-[4-(2,4-diamino-quinazolin-5-yloxymethyl)piperidin-1-yl]-methanone



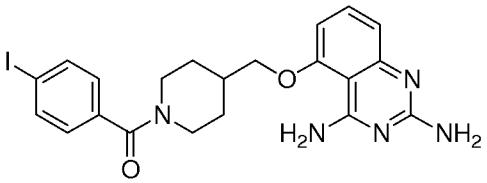
(2-Chlorophenyl)-[4-(2,4-diamino-quinazolin-5-yloxymethyl)piperidin-1-yl]-methanone



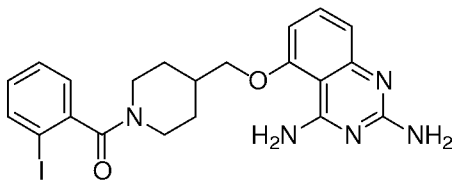
(3-Chlorophenyl)-[4-(2,4-diamino-quinazolin-5-yloxymethyl)piperidin-1-yl]-methanone



[4-(2,4-Diaminoquinazolin-5-yloxymethyl)piperidin-1-yl]-(3-iodophenyl)methanone



[4-(2,4-Diaminoquinazolin-5-yloxymethyl)piperidin-1-yl]-(4-iodophenyl)methanone



[4-(2,4-Diaminoquinazolin-5-yloxymethyl)piperidin-1-yl]-(2-iodophenyl)methanone

[0059] Suitable methods for synthesizing any of the compounds described herein are known in the art and described in detail in, e.g., Singh et al. (U.S. Application Serial No. 11/832,255, filed on August 1, 2007 and published as U.S. Patent Application Publication No. 2009-0042900), the disclosure of which is incorporated herein by reference in its entirety.

Definitions

[0060] A comprehensive list of abbreviations utilized by organic chemists (i.e., persons of ordinary skill in the art), which is typically presented in a table entitled "Standard List of Abbreviations," appears in the first issue of each volume of the *Journal of Organic Chemistry*. The list, as it appears in the most recent edition of that journal prior to the filing date of the instant application is incorporated herein by reference in its entirety.

[0061] Alkyl includes linear, branched, or cyclic saturated hydrocarbon structures and combinations thereof (*e.g.*, t-butyl-cyclohexyl). Lower alkyl refers to alkyl groups having from 1 to 6 carbon atoms. Examples of lower alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, s- and t-butyl. Preferred alkyl groups are those of having 20 carbon atoms or fewer.

[0062] Cycloalkyl is a subset of alkyl and includes cyclic hydrocarbon groups having single rings, as well as polycyclic hydrocarbons having two or more rings that share an edge or bridgeheads. (A polycyclic ring system is one in which two or more rings have two or more carbons in common.) Preferred cycloalkyl groups have single rings from 3 to 8 carbon atoms, and two rings with from 7 to 10 carbon atoms. Examples of cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, and the like. Examples of polycyclic hydrocarbons include ring systems such as norbornyl, adamantyl, and decalin.

[0063] "Alkenyl" or "alkenylene" includes linear, branched, or cyclic unsaturated hydrocarbon structures having the specified number of carbon atoms and one or more carbon-carbon double bonds at any point. Examples of alkenyl include, but are not limited to, ethenyl, 1-propenyl, 2-propenyl, 2-butenyl, 3-butenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, 5-hexenyl, 2-methyl-2-propenyl, 4-methyl-3-pentenyl, and the like.

[0064] Cycloalkenyl is a subset of alkenyl and includes non-aromatic cyclic hydrocarbon groups having one or more double bonds in single rings, as well as polycyclic hydrocarbons

having one or more double bonds and two or more rings that share an edge or bridgeheads. Preferred cycloalkenyl groups have single rings from 3 to 8 carbon atoms, and two rings with from 7 to 10 carbon atoms. Examples of cycloalkenyls include cyclohexenyl, nobornenyl, and the like.

[0065] "Alkynyl" or "alkynylene" includes linear, branched, or cyclic unsaturated hydrocarbon structures having one or more carbon-carbon triple bonds at any point. Examples include ethynyl, propynyl, butynyl, pentynyl, hexynyl and the like.

[0066] Alkoxy or alkoxyl refers to an alkyl group attached to a parent structure through an oxygen. Examples include methoxy, ethoxy, propoxy, isopropoxy, cyclopropyloxy, cyclohexyloxy and the like. Lower-alkoxy refers to alkoxy groups containing one to six carbons.

[0067] Oxaalkyl refers to alkyl groups in which one or more carbon atoms together with its bonded hydrogens has been replaced by oxygen. Examples include methoxypropoxy, 3,6,9-trioxadecyl and the like.

[0068] Acyl refers to hydrocarbon structures, attached to a parent structure through a carbonyl functionality. One or more carbons in the acyl group may be independently replaced by nitrogen, oxygen or sulfur as long as the point of attachment to the parent remains at the carbonyl. Examples include acetyl, benzoyl, propionyl, isobutyryl, t-butoxycarbonyl, benzyloxycarbonyl and the like. Lower-acyl refers to acyl groups containing one to six carbons.

[0069] Aryl means a 5- or 6-membered aromatic ring, a bicyclic 9- or 10-membered aromatic ring system, or a tricyclic 12-, 13- or 14-membered aromatic ring system. The aromatic 6- to 14-membered carbocyclic rings include, e.g., benzene, naphthalene, indane, tetralin, and fluorene.

[0070] Heteroaryl means a 5- or 6-membered heteroaromatic ring containing 0-3 heteroatoms independently selected from O, N, or S; a bicyclic 9- or 10-membered heteroaromatic ring system containing 0-3 heteroatoms selected from O, N, or S; or a tricyclic 12-, 13- or 14-membered heteroaromatic ring system containing 0-3 heteroatoms selected from O, N, or S. The 5- to 10-membered aromatic heterocyclic rings include, *e.g.*, pyrrole, imidazole, pyridine, indole, thiophene, benzopyranone, thiazole, furan, benzimidazole, quinoline, isoquinoline, quinoxaline, pyrimidine, pyrazine, tetrazole, and pyrazole. The nitrogen and sulfur

heteroatoms may optionally be oxidized, and the nitrogen heteroatom may optionally be quaternized.

[0071] Heterocycle means heteroaryl group, or a cycloalkyl group in which from one to three carbons is independently replaced by a heteroatom selected from the group consisting of N, O and S. The nitrogen and sulfur heteroatoms may optionally be oxidized, and the nitrogen heteroatom may optionally be quaternized. Examples of heterocycles that fall within the scope of the definitions include (without limitation) pyrrolidine, pyrazole, pyrrole, indole, quinoline, isoquinoline, tetrahydroisoquinoline, benzofuran, benzodioxan, benzodioxole (commonly referred to as methylenedioxyphenyl, when occurring as a substituent), tetrazole, morpholine, thiazole, pyridine, pyridazine, pyrimidine, thiophene, furan, oxazole, oxazoline, isoxazole, dioxane, tetrahydrofuran and the like. Examples of heterocyclyl residues additionally include piperazinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxo-pyrrolidinyl, 2-oxoazepinyl, azepinyl, 4-piperidinyl, pyrazolidinyl, imidazolyl, imidazolinyl, imidazolidinyl, pyrazinyl, oxazolidinyl, isoxazolidinyl, thiazolidinyl, isothiazolyl, quinuclidinyl, isothiazolidinyl, benzimidazolyl, thiadiazolyl, benzopyranyl, benzothiazolyl, tetrahydrofuryl, tetrahydropyranyl, thienyl, benzothienyl, thiamorpholinyl, thiamorpholinylsulfoxide, thiamorpholinylsulfone, oxadiazolyl, triazolyl and tetrahydroquinolinyl.

[0072] Carbocycle is the complement of heterocycle. Carbocycle as used herein means a cycloalkyl or aryl residue in which all of the ring atoms are carbon. It includes polycyclic rings. Examples include cyclohexane, benzene, cyclopentadiene, naphthalene, phenanthrene, fluorene, norbornane, bicycloheptadiene, indane, and bicyclooctane.

[0073] Substituted alkyl, substituted aryl, substituted cycloalkyl, substituted heterocyclyl, etc., refer respectively to alkyl, aryl, cycloalkyl, or heterocyclyl, etc. wherein one or more hydrogen atoms in each group are independently replaced with an atom or group selected from: halogen, haloalkyl, hydroxy, lower alkoxy, carboxylic acid, carboalkoxy (also referred to as alkoxycarbonyl), carboxamido (also referred to as alkylaminocarbonyl), cyano, nitro, amino, alkylamino, dialkylamino, mercapto, alkylthio, sulfoxide, sulfone, acylamino, amidino, phenyl, benzyl, halobenzyl, heteroaryl, phenoxy, benzyloxy, heteroaryloxy, benzoyl, halobenzoyl, or

loweralkylhydroxy. In preferred embodiments, in a substituted group, up to three hydrogen atoms are replaced by substituents.

[0074] The term hydrocarbon includes alkyl, cycloalkyl, cycloalkenyl, alkenyl, alkynyl, cycloalkynyl, aryl and combinations thereof. Examples include phenethyl, cyclohexylmethyl, camphoryl and naphthylethyl.

[0075] The term "halogen" means fluorine, chlorine, bromine or iodine.

[0076] It will be understood that "substitution", "substituted" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, automerization, dissociation, etc.

[0077] As used herein, and as would be understood by the person of skill in the art, the recitation of "a compound" is intended to include salts, solvates, co-crystals and inclusion complexes of that compound.

[0078] The term "solvate" refers to a compound of Formulae (I), (II), or (III) in the solid state, wherein molecules of a suitable solvent are incorporated in the crystal lattice. A suitable solvent for therapeutic administration is physiologically tolerable at the dosage administered. Examples of suitable solvents for therapeutic administration are ethanol and water. When water is the solvent, the solvate is referred to as a hydrate. In general, solvates are formed by dissolving the compound in an appropriate solvent and isolating the solvate by cooling or using an antisolvent. The solvate is typically dried or azeotroped under ambient conditions.

[0079] Co-crystals are combinations of two or more distinct molecules arranged to create a unique crystal form whose physical properties are different from those of its pure constituents. Pharmaceutical co-crystals have recently become of considerable interest for improving the solubility, formulation and bioavailability of such drugs as itraconazole (see, e.g., Remenar et al. (2003) J. Am. Chem. Soc., 125:8456-8457) and fluoxetine. Inclusion complexes are described in Remington: The Science and Practice of Pharmacy 19th Ed. (1995) volume 1, page 176-177, which is incorporated herein by reference in its entirety. The most commonly employed

inclusion complexes are those with cyclodextrins, and all cyclodextrin complexes, natural and synthetic, are specifically encompassed within the claims.

[0080] The term "pharmaceutically acceptable salt" refers to salts prepared from pharmaceutically acceptable non-toxic acids or bases including inorganic acids and bases and organic acids and bases. When the compounds of the present invention are basic, salts may be prepared from pharmaceutically acceptable non-toxic acids including inorganic and organic acids. Suitable pharmaceutically acceptable acid addition salts for the compounds of the present invention include acetic, benzenesulfonic (besylate), benzoic, camphorsulfonic, citric, ethenesulfonic, fumaric, gluconic, glutamic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phosphoric, succinic, sulfuric, tartaric acid, p-toluenesulfonic, and the like. When the compounds contain an acidic side chain, suitable pharmaceutically acceptable base addition salts for the compounds of the present invention include metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from lysine, N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine), and procaine.

[0081] As used herein, a DcpS inhibitor can be any compound capable of inhibiting the activity or the expression of a DcpS protein. For example, a DcpS inhibitor can be a compound that binds to an active site of a DcpS protein and inhibit the activity of the DcpS protein. Alternatively, a DcpS inhibitor can be a compound that: (i) destabilizes a DcpS protein; (ii) destabilizes an mRNA encoding a DcpS protein; (iii) inhibits the transcription of an mRNA encoding a DcpS protein; or (iv) inhibits the translation of an mRNA encoding a DcpS protein. The DcpS protein, or mRNA encoding the DcpS protein, can be, e.g., a mammalian (e.g., a rodent, a non-human primate, or a human) form of the DcpS protein or mRNA. An exemplary amino acid sequence for a human form of a DcpS protein is as follows:
MADAAPQLGKRKRELDVEEAHAASTEEKEAGVGNGTCAPVRLPFSGFRLQKVLRESAR
DKIIFLHGKVNEASGDGDGEDAVVILEKTPFQVEQVAQLLTGSPELQLQFSNDIYSTYHL
FPPRQLNDVKTTVVYPATEKHLQKYLRQDLRLIRETGDDYRNITLPHLESQSLSIQWVYN
ILDKKAEADRIVFENPDPSDGFVLIPDLKWNQQQLDDLYLIAICHRRGIRSLRDLTPEHLP
LLRNILHQGQEAILQRYRMKGDHLRVYLHYLPSYYHLHVHFTALGFEAPGSGVERAHL

LAEVIENLECDPRHYQQRTLTFALRADDPLLKLLQEAQQS (SEQ ID NO:1; NCBI Accession No. Q96C86).

[0082] As used herein, a subject can be, e.g., an animal such as an insect, a fish, a bird, a reptile, or a mammal (e.g., a rodent (e.g., a mouse, rat, hamster, or rabbit), cat, dog, goat, cow, goat, whale, non-human primate (e.g., a chimpanzee or macaque) or a human).

Pharmaceutical Compositions

[0083] The pharmaceutical compositions provided herein contain therapeutically effective amounts of one or more of the compounds described herein that are useful in increasing the expression of SMN in a cell and/or in the prevention, treatment, or amelioration of one or more of the symptoms of Spinal Muscular Atrophy (SMA) condition. SMA conditions include, without limitation, e.g., infantile SMA (Werdnig-Hoffmann disease); severe infantile SMA; intermediate SMA; juvenile SMA (Kugelberg-Welander disease); and adult SMA.

[0084] Pharmaceutical carriers suitable for administration of the compounds described above include any such carriers known to those skilled in the art to be suitable for the particular mode of administration. In addition, the compounds may be formulated as the sole pharmaceutically active ingredient in the composition or may be combined with other active ingredients. The compositions contain one or more compounds described above. The compounds are, in one embodiment, formulated into suitable pharmaceutical preparations such as solutions, suspensions, tablets, dispersible tablets, pills, capsules, powders, sustained release formulations or elixirs, for oral administration or in sterile solutions or suspensions for parenteral administration, as well as transdermal patch preparation and dry powder inhalers. In one embodiment, the compounds described above are formulated into pharmaceutical compositions using techniques and procedures well known in the art (see, e.g., Ansel, Introduction to Pharmaceutical Dosage Forms, Fourth Edition, (1985), 126).

[0085] In the compositions, effective concentrations of one or more compounds or pharmaceutically acceptable derivatives thereof is (are) mixed with a suitable pharmaceutical carrier. The compounds may be derivatized as the corresponding salts, esters, enol ethers or esters, acetals, ketals, orthoesters, hemiacetals, hemiketals, acids, bases, solvates, hydrates or prodrugs prior to formulation, as described above. The concentrations of the compounds in the

compositions are effective for delivery of an amount, upon administration, that treats, prevents, or ameliorates one or more of the symptoms of an SMA condition.

[0086] In one embodiment, the compositions are formulated for single dosage administration. To formulate a composition, the weight fraction of a compound is dissolved, suspended, dispersed, or otherwise mixed in a selected carrier at an effective concentration such that the treated condition is relieved, prevented, or one or more symptoms are ameliorated. The active compound is included in the pharmaceutically acceptable carrier in an amount sufficient to exert a therapeutically useful effect in the absence of undesirable side effects on the patient treated. The therapeutically effective concentration may be determined empirically by testing the compounds in *in vitro* and *in vivo* systems such as those described herein and then extrapolated therefrom for dosages for humans.

[0087] The concentration of active compound in the pharmaceutical composition will depend on absorption, inactivation and excretion rates of the active compound, the physicochemical characteristics of the compound, the dosage schedule, and amount administered as well as other factors known to those of skill in the art. For example, the amount that is delivered is sufficient to ameliorate one or more of the symptoms of any SMA condition that is known in the art or described herein.

[0088] Therapeutically effective doses are further described elsewhere herein.

[0089] The active ingredient can be administered once, or can be divided into a number of smaller doses to be administered at intervals of time. It is understood that the precise dosage and duration of treatment is a function of the disease being treated and may be determined empirically using known testing protocols or by extrapolation from *in vivo* or *in vitro* test data (for example, test data collected from the murine models described in the accompanying Examples). It is to be noted that concentrations and dosage values may also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed compositions.

[0090] In instances in which the compounds exhibit insufficient solubility, methods for solubilizing compounds may be used. Such methods are known to those of skill in this art, and include, but are not limited to, using cosolvents such as dimethylsulfoxide (DMSO), using surfactants such as TWEEN®, or dissolution in aqueous sodium bicarbonate. Derivatives of the compounds, such as prodrugs of the compounds may also be used in formulating effective pharmaceutical compositions. Upon mixing or addition of the compound(s), the resulting mixture may be a solution, suspension, emulsion, or the like. The form of the resulting mixture depends upon a number of factors, including the intended mode of administration and the solubility of the compound in the selected carrier or vehicle. The effective concentration is sufficient for ameliorating the symptoms of the disease, disorder or condition treated and may be empirically determined.

[0091] The pharmaceutical compositions are provided for administration to humans and animals in unit dosage forms, such as tablets, capsules, pills, powders, granules, sterile parenteral solutions or suspensions, and oral solutions or suspensions, and oil-water emulsions containing suitable quantities of the compounds or pharmaceutically acceptable derivatives thereof.

[0092] The pharmaceutically therapeutically active compounds and derivatives thereof are, in one embodiment, formulated and administered in unit-dosage forms or multiple-dosage forms. Unit-dose forms as used herein refers to physically discrete units suitable for human and animal subjects and packaged individually as is known in the art. Each unit-dose contains a predetermined quantity of the therapeutically active compound sufficient to produce the desired therapeutic effect, in association with the required pharmaceutical carrier, vehicle or diluent. Examples of unit-dose forms include ampoules and syringes and individually packaged tablets or capsules. Unit-dose forms may be administered in fractions or multiples thereof. A multiple-dose form is a plurality of identical unit-dosage forms packaged in a single container to be administered in segregated unit-dose form. Examples of multiple-dose forms include vials, bottles of tablets or capsules or bottles of pints or gallons. Hence, multiple dose form is a multiple of unit-doses which are not segregated in packaging.

[0093] Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, or otherwise mixing an active compound as defined above and optional

pharmaceutical adjuvants in a carrier, such as, for example, water, saline, aqueous dextrose, glycerol, glycols, ethanol, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered can also contain minor amounts of nontoxic auxiliary substances such as wetting agents, emulsifying agents, solubilizing agents, pH buffering agents and the like, for example, acetate, sodium citrate, cyclodextrine derivatives, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, and other such agents. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see Remingtons' Pharmaceutical Sciences, Mack Publishing Company, Easton, PA, 15th Edition, 1975.

[0094] Dosage forms or compositions containing active ingredient in the range of 0.005% to 100% with the balance made up from non-toxic carrier may be prepared. Methods for preparation of these compositions are known to those skilled in the art. The contemplated compositions may contain 0.001%-100% active ingredient, in one embodiment 0.1-95%, in another embodiment 75-85%.

Compositions for oral administration

[0095] Oral pharmaceutical dosage forms are either solid, gel or liquid. The solid dosage forms are tablets, capsules, granules, and bulk powders. Types of oral tablets include compressed, chewable lozenges and tablets which may be enteric-coated, sugar-coated or film-coated. Capsules may be hard or soft gelatin capsules, while granules and powders may be provided in non-effervescent or effervescent form with the combination of other ingredients known to those skilled in the art.

[0096] In certain embodiments, the formulations are solid dosage forms, in one embodiment, capsules or tablets. The tablets, pills, capsules, troches and the like can contain one or more of the following ingredients, or compounds of a similar nature: a binder; a lubricant; a diluent; a glidant; a disintegrating agent; a coloring agent; a sweetening agent; a flavoring agent; a wetting agent; an emetic coating; and a film coating. Examples of binders include microcrystalline cellulose, gum tragacanth, glucose solution, acacia mucilage, gelatin solution, molasses, polvinylpyrrolidine, povidone, crospovidones, sucrose and starch paste. Lubricants include talc, starch, magnesium or calcium stearate, lycopodium and stearic acid. Diluents include, for

example, lactose, sucrose, starch, kaolin, salt, mannitol and dicalcium phosphate. Glidants include, but are not limited to, colloidal silicon dioxide. Disintegrating agents include crosscarmellose sodium, sodium starch glycolate, alginic acid, corn starch, potato starch, bentonite, methylcellulose, agar and carboxymethylcellulose. Coloring agents include, for example, any of the approved certified water soluble FD and C dyes, mixtures thereof; and water insoluble FD and C dyes suspended on alumina hydrate. Sweetening agents include sucrose, lactose, mannitol and artificial sweetening agents such as saccharin, and any number of spray dried flavors. Flavoring agents include natural flavors extracted from plants such as fruits and synthetic blends of compounds which produce a pleasant sensation, such as, but not limited to peppermint and methyl salicylate. Wetting agents include propylene glycol monostearate, sorbitan monooleate, diethylene glycol monolaurate and polyoxyethylene laural ether. Emetic-coatings include fatty acids, fats, waxes, shellac, ammoniated shellac and cellulose acetate phthalates. Film coatings include hydroxyethylcellulose, sodium carboxymethylcellulose, polyethylene glycol 4000 and cellulose acetate phthalate.

[0097] The compound, or pharmaceutically acceptable derivative thereof, could be provided in a composition that protects it from the acidic environment of the stomach. For example, the composition can be formulated in an enteric coating that maintains its integrity in the stomach and releases the active compound in the intestine. The composition may also be formulated in combination with an antacid or other such ingredient.

[0098] When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar and other enteric agents. The compounds can also be administered as a component of an elixir, suspension, syrup, wafer, sprinkle, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

[0099] The active materials can also be mixed with other active materials which do not impair the desired action, or with materials that supplement the desired action, such as antacids, H2 blockers, and diuretics. The active ingredient is a compound or pharmaceutically acceptable

derivative thereof as described herein. Higher concentrations, up to about 98% by weight of the active ingredient may be included.

[00100] In all embodiments, tablets and capsules formulations can be coated as known by those of skill in the art in order to modify or sustain dissolution of the active ingredient. Thus, for example, they may be coated with a conventional enterically digestible coating, such as phenylsalicylate, waxes and cellulose acetate phthalate.

[00101] Liquid oral dosage forms include aqueous solutions, emulsions, suspensions, solutions and/or suspensions reconstituted from non-effervescent granules and effervescent preparations reconstituted from effervescent granules. Aqueous solutions include, for example, elixirs and syrups. Emulsions are either oil-in-water or water-in-oil.

[00102] Elixirs are clear, sweetened, hydroalcoholic preparations. Pharmaceutically acceptable carriers used in elixirs include solvents. Syrups are concentrated aqueous solutions of a sugar, for example, sucrose, and may contain a preservative. An emulsion is a two-phase system in which one liquid is dispersed in the form of small globules throughout another liquid. Pharmaceutically acceptable carriers used in emulsions are non-aqueous liquids, emulsifying agents and preservatives. Suspensions use pharmaceutically acceptable suspending agents and preservatives. Pharmaceutically acceptable substances used in non-effervescent granules, to be reconstituted into a liquid oral dosage form, include diluents, sweeteners and wetting agents. Pharmaceutically acceptable substances used in effervescent granules, to be reconstituted into a liquid oral dosage form, include organic acids and a source of carbon dioxide. Coloring and flavoring agents are used in all of the above dosage forms.

[00103] Solvents include glycerin, sorbitol, ethyl alcohol and syrup. Examples of preservatives include glycerin, methyl and propylparaben, benzoic acid, sodium benzoate and alcohol. Examples of non-aqueous liquids utilized in emulsions include mineral oil and cottonseed oil. Examples of emulsifying agents include gelatin, acacia, tragacanth, bentonite, and surfactants such as polyoxyethylene sorbitan monooleate. Suspending agents include sodium carboxymethylcellulose, pectin, tragacanth, Veegum and acacia. Sweetening agents include sucrose, syrups, glycerin and artificial sweetening agents such as saccharin. Wetting agents include propylene glycol monostearate, sorbitan monooleate, diethylene glycol monolaurate and

polyoxyethylene lauryl ether. Organic acids include citric and tartaric acid. Sources of carbon dioxide include sodium bicarbonate and sodium carbonate. Coloring agents include any of the approved certified water soluble FD and C dyes, and mixtures thereof. Flavoring agents include natural flavors extracted from plants such fruits, and synthetic blends of compounds which produce a pleasant taste sensation.

[00104] For a solid dosage form, the solution or suspension, in for example propylene carbonate, vegetable oils or triglycerides, is in one embodiment encapsulated in a gelatin capsule. Such solutions, and the preparation and encapsulation thereof, are disclosed in U.S. Patent Nos. 4,328,245; 4,409,239; and 4,410,545. For a liquid dosage form, the solution, e.g., for example, in a polyethylene glycol, may be diluted with a sufficient quantity of a pharmaceutically acceptable liquid carrier, e.g., water, to be easily measured for administration.

[00105] Alternatively, liquid or semi-solid oral formulations may be prepared by dissolving or dispersing the active compound or salt in vegetable oils, glycols, triglycerides, propylene glycol esters (e.g., propylene carbonate) and other such carriers, and encapsulating these solutions or suspensions in hard or soft gelatin capsule shells. Other useful formulations include those set forth in U.S. Patent Nos. RE28,819 and 4,358,603. Briefly, such formulations include, but are not limited to, those containing a compound provided herein, a dialkylated mono- or poly-alkylene glycol, including, but not limited to, 1,2-dimethoxymethane, diglyme, triglyme, tetraglyme, polyethylene glycol-350-dimethyl ether, polyethylene glycol-550-dimethyl ether, polyethylene glycol-750-dimethyl ether wherein 350, 550 and 750 refer to the approximate average molecular weight of the polyethylene glycol, and one or more antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate, vitamin E, hydroquinone, hydroxycoumarins, ethanolamine, lecithin, cephalin, ascorbic acid, malic acid, sorbitol, phosphoric acid, thiodipropionic acid and its esters, and dithiocarbamates.

[00106] Other formulations include, but are not limited to, aqueous alcoholic solutions including a pharmaceutically acceptable acetal. Alcohols used in these formulations are any pharmaceutically acceptable water-miscible solvents having one or more hydroxyl groups, including, but not limited to, propylene glycol and ethanol. Acetals include, but are not limited to, di(lower alkyl) acetals of lower alkyl aldehydes such as acetaldehyde diethyl acetal.

Injectables, solutions, and emulsions

[00107] Parenteral administration, in one embodiment characterized by injection, either subcutaneously, intramuscularly or intravenously is also contemplated herein. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. The injectables, solutions and emulsions also contain one or more excipients. Suitable excipients are, for example, water, saline, dextrose, glycerol or ethanol. In addition, if desired, the pharmaceutical compositions to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents, stabilizers, solubility enhancers, and other such agents, such as for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate and cyclodextrins.

[00108] Implantation of a slow-release or sustained-release system, such that a constant level of dosage is maintained (see, e.g., U.S. Patent No. 3,710,795) is also contemplated herein. Briefly, a compound provided herein is dispersed in a solid inner matrix, e.g., polymethylmethacrylate, polybutylmethacrylate, plasticized or unplasticized polyvinylchloride, plasticized nylon, plasticized polyethyleneterephthalate, natural rubber, polyisoprene, polyisobutylene, polybutadiene, polyethylene, ethylene-vinylacetate copolymers, silicone rubbers, polydimethylsiloxanes, silicone carbonate copolymers, hydrophilic polymers such as hydrogels of esters of acrylic and methacrylic acid, collagen, cross-linked polyvinylalcohol and cross-linked partially hydrolyzed polyvinyl acetate, that is surrounded by an outer polymeric membrane, e.g., polyethylene, polypropylene, ethylene/propylene copolymers, ethylene/ethyl acrylate copolymers, ethylene/vinylacetate copolymers, silicone rubbers, polydimethyl siloxanes, neoprene rubber, chlorinated polyethylene, polyvinylchloride, vinylchloride copolymers with vinyl acetate, vinylidene chloride, ethylene and propylene, ionomer polyethylene terephthalate, butyl rubber epichlorohydrin rubbers, ethylene/vinyl alcohol copolymer, ethylene/vinyl acetate/vinyl alcohol terpolymer, and ethylene/vinyloxyethanol copolymer, that is insoluble in body fluids. The compound diffuses through the outer polymeric membrane in a release rate controlling step. The percentage of active compound contained in such parenteral compositions is highly dependent on the specific nature thereof, as well as the activity of the compound and the needs of the subject.

[00109] Parenteral administration of the compositions includes intravenous, subcutaneous and intramuscular administrations. Preparations for parenteral administration include sterile solutions ready for injection, sterile dry soluble products, such as lyophilized powders, ready to be combined with a solvent just prior to use, including hypodermic tablets, sterile suspensions ready for injection, sterile dry insoluble products ready to be combined with a vehicle just prior to use and sterile emulsions. The solutions may be either aqueous or nonaqueous.

[00110] If administered intravenously, suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, and polypropylene glycol and mixtures thereof.

[0100] Pharmaceutically acceptable carriers used in parenteral preparations include aqueous vehicles, nonaqueous vehicles, antimicrobial agents, isotonic agents, buffers, antioxidants, local anesthetics, suspending and dispersing agents, emulsifying agents, sequestering or chelating agents and other pharmaceutically acceptable substances.

[0101] Examples of aqueous vehicles include sodium chloride injection, Ringers injection, isotonic dextrose injection, sterile water injection, dextrose and lactated Ringers injection. Nonaqueous parenteral vehicles include fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil and peanut oil. Antimicrobial agents in bacteriostatic or fungistatic concentrations must be added to parenteral preparations packaged in multiple-dose containers which include phenols or cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoic acid esters, thimerosal, benzalkonium chloride and benzethonium chloride. Isotonic agents include sodium chloride and dextrose. Buffers include phosphate and citrate. Antioxidants include sodium bisulfate. Local anesthetics include procaine hydrochloride. Suspending and dispersing agents include sodium carboxymethylcelluose, hydroxypropyl methylcellulose and polyvinylpyrrolidone. Emulsifying agents include Polysorbate 80 (TWEEN 80). A sequestering or chelating agent of metal ions include EDTA. Pharmaceutical carriers also include ethyl alcohol, polyethylene glycol and propylene glycol for water miscible vehicles; and sodium hydroxide, hydrochloric acid, citric acid or lactic acid for pH adjustment.

[0102] The concentration of the pharmaceutically active compound is adjusted so that an injection provides an effective amount to produce the desired pharmacological effect. The exact dose depends on the age, weight and condition of the patient or animal as is known in the art.

[0103] The unit-dose parenteral preparations are packaged in an ampoule, a vial or a syringe with a needle. All preparations for parenteral administration must be sterile, as is known and practiced in the art.

[0104] Illustratively, intravenous or intraarterial infusion of a sterile aqueous solution containing an active compound is an effective mode of administration. Another embodiment is a sterile aqueous or oily solution or suspension containing an active material injected as necessary to produce the desired pharmacological effect.

[0105] Injectables are designed for local and systemic administration. In one embodiment, a therapeutically effective dosage is formulated to contain a concentration of at least about 0.1% w/w up to about 90% w/w or more, in certain embodiments more than 1% w/w of the active compound to the treated tissue(s).

[0106] The compound may be suspended in micronized or other suitable form or may be derivatized to produce a more soluble active product or to produce a prodrug. The form of the resulting mixture depends upon a number of factors, including the intended mode of administration and the solubility of the compound in the selected carrier or vehicle. The effective concentration is sufficient for ameliorating the symptoms of the condition and may be empirically determined.

Lyophilized powders

[0107] Of interest herein are also lyophilized powders, which can be reconstituted for administration as solutions, emulsions and other mixtures. They may also be reconstituted and formulated as solids or gels.

[0108] The sterile, lyophilized powder is prepared by dissolving a compound provided herein, or a pharmaceutically acceptable derivative thereof, in a suitable solvent. The solvent may contain an excipient which improves the stability or other pharmacological component of the powder or reconstituted solution, prepared from the powder. Excipients that may be used

include, but are not limited to, dextrose, sorbital, fructose, corn syrup, xylitol, glycerin, glucose, sucrose or other suitable agents. The solvent may also contain a buffer, such as citrate, sodium or potassium phosphate or other such buffer known to those skilled in the art at, in one embodiment, about neutral pH. Subsequent sterile filtration of the solution followed by lyophilization under standard conditions known to those skilled in the art provides the desired formulation. In one embodiment, the resulting solution will be apportioned into vials for lyophilization. Each vial will contain a single dosage or multiple dosages of the compound. The lyophilized powder can be stored under appropriate conditions, such as at about 4 °C to room temperature.

[0109] Reconstitution of a lyophilized powder with water for injection provides a formulation for use in parenteral administration. For reconstitution, the lyophilized powder is added to sterile water or other suitable carrier. The precise amount depends upon the selected compound. Such amount can be empirically determined.

Topical administration

[0110] Topical mixtures are prepared as described for the local and systemic administration. The resulting mixture may be a solution, suspension, emulsions or the like and are formulated as creams, gels, ointments, emulsions, solutions, elixirs, lotions, suspensions, tinctures, pastes, foams, aerosols, irrigations, sprays, suppositories, bandages, dermal patches or any other formulations suitable for topical administration.

[0111] The compounds or pharmaceutically acceptable derivatives thereof may be formulated as aerosols for topical application, such as by inhalation (see, e.g., U.S. Patent Nos. 4,044,126, 4,414,209, and 4,364,923, which describe aerosols for delivery of a steroid useful for treatment of inflammatory diseases, particularly asthma). These formulations for administration to the respiratory tract can be in the form of an aerosol or solution for a nebulizer, or as a microfine powder for insufflation, alone or in combination with an inert carrier such as lactose. In such a case, the particles of the formulation will, in one embodiment, have diameters of less than 50 microns, in one embodiment less than 10 microns.

[0112] The compounds may be formulated for local or topical application, such as for topical application to the skin and mucous membranes, such as in the eye, in the form of gels, creams,

and lotions and for application to the eye or for intracisternal or intraspinal application. Topical administration is contemplated for transdermal delivery and also for administration to the eyes or mucosa, or for inhalation therapies. Nasal solutions of the active compound alone or in combination with other pharmaceutically acceptable excipients can also be administered.

[0113] These solutions, particularly those intended for ophthalmic use, may be formulated as 0.01% - 10% isotonic solutions, pH about 5-7, with appropriate salts.

Compositions for other routes of administration

[0114] Other routes of administration, such as transdermal patches, including iontophoretic and electrophoretic devices, and buccal and rectal administration, are also contemplated herein. Transdermal patches, including iontophoretic and electrophoretic devices, are well known to those of skill in the art. For example, such patches are disclosed in U.S. Patent Nos. 6,267,983, 6,261,595, 6,256,533, 6,167,301, 6,024,975, 6,010715, 5,985,317, 5,983,134, 5,948,433, and 5,860,957.

[0115] For example, pharmaceutical dosage forms for rectal administration are rectal suppositories, capsules and tablets for systemic effect. Rectal suppositories are used herein mean solid bodies for insertion into the rectum which melt or soften at body temperature releasing one or more pharmacologically or therapeutically active ingredients. Pharmaceutically acceptable substances utilized in rectal suppositories are bases or vehicles and agents to raise the melting point. Examples of bases include cocoa butter (theobroma oil), glycerin-gelatin, carbowax (polyoxyethylene glycol) and appropriate mixtures of mono-, di- and triglycerides of fatty acids. Combinations of the various bases may be used. Agents to raise the melting point of suppositories include spermaceti and wax. Rectal suppositories may be prepared either by the compressed method or by molding. The weight of a rectal suppository, in one embodiment, is about 2 to 3 g.

[0116] Tablets and capsules for rectal administration are manufactured using the same pharmaceutically acceptable substance and by the same methods as for formulations for oral administration.

Targeted Formulations

[0117] The compounds provided herein, or pharmaceutically acceptable derivatives thereof, may also be formulated to be targeted to a particular tissue (e.g., a motor neuron), receptor, or other area of the body of the subject to be treated. Many such targeting methods are well known to those skilled in the art. All such targeting methods are contemplated herein for use in the instant compositions. For non-limiting examples of targeting methods, see, e.g., U.S. Patent Nos. 6,316,652, 6,274,552, 6,271,359, 6,253,872, 6,139,865, 6,131,570, 6,120,751, 6,071,495, 6,060,082, 6,048,736, 6,039,975, 6,004,534, 5,985,307, 5,972,366, 5,900,252, 5,840,674, 5,759,542 and 5,709,874.

[0118] In one embodiment, liposomal suspensions, including tissue-targeted liposomes, such as tumor-targeted liposomes, may also be suitable as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art. For example, liposome formulations may be prepared as described in U.S. Patent No. 4,522,811. Briefly, liposomes such as multilamellar vesicles (MLV's) may be formed by drying down egg phosphatidyl choline and brain phosphatidyl serine (7:3 molar ratio) on the inside of a flask. A solution of a compound provided herein in phosphate buffered saline lacking divalent cations (PBS) is added and the flask shaken until the lipid film is dispersed. The resulting vesicles are washed to remove unencapsulated compound, pelleted by centrifugation, and then resuspended in PBS.

Methods for Increasing Expression of SMN in a Cell

[0119] Methods for increasing expression of SMN in a cell can include the step of contacting a cell with (or introducing into a cell) a DcpS inhibitor (such as a compound described herein) in an amount effective to increase the expression of SMN in the cell. Such contacting can occur *in vitro* (e.g., in cell culture) or *in vivo*. Suitable *in vivo* methods for contacting a cell with a DcpS inhibitor include, e.g., any of the methods of treatment described below under "Methods for Treating an SMA Condition."

[0120] Suitable *in vitro* methods for contacting a cell with a DcpS inhibitor include, e.g., plating a cell (or a population of cells) on solid support matrix (e.g., a plastic tissue culture plate, or a multiwell (96 or 386-well) tissue culture plate) and culturing the cell at a physiological

temperature (e.g., 37°C) in an aqueous buffered medium (e.g., a tissue culture medium such as Dulbecco's Modified Eagle Medium (DMEM) or Roswell Park Memorial Institute (RPMI) medium) containing the DcpS inhibitor.

[0121] In embodiments where the DcpS inhibitor is a protein, an siRNA, or an antisense RNA, the cell can be transfected with a nucleic acid encoding the protein, siRNA, or antisense RNA and the cell is then cultured under conditions that permit the expression of the protein, siRNA, or antisense RNA. The transfection step can be accomplished by any standard means used for such nucleic acid delivery including, e.g., calcium phosphate, lipofection, electroporation, viral infection, and biolistic gene transfer. (See, e.g., Sambrook et al. (2001) Molecular Cloning, a Lab Manual, 3rd Edition). Alternatively, liposomes or polymeric microparticles can be used. It is understood that the delivery method will depend, at least in part, on the type of cell being transfected.

[0122] The cell can be any cell that expresses, or is capable of expressing, SMN protein or an mRNA encoding an SMN protein. The cell can endogenously express (or be capable of endogenously expressing) SMN, or the cell can exogenously express (or be capable of exogenously expressing) SMN. For example, the cell can contain an endogenous nucleic acid (or gene) that encodes an SMN protein (that is, the cell can naturally express SMN) or the cell can contain a transgene (e.g., an expression vector) encoding a recombinant SMN.

[0123] The cell can be a prokaryotic or eukaryotic cell. For example, the cell can be a bacterial cell, a fungal cell (e.g., a yeast cell), a plant cell, or an animal cell (e.g., a cell from a fish, a reptile, or a mammal (e.g., a mouse, rat, guinea pig, dog, cat, pig, horse, goat, cow, a non-human primate (e.g., a chimpanzee or a macaque) or a human)). The cell can be of any of a variety of histological types including, without limitation, an epithelial cell, a lymphoid cell, a macrophage, a monocyte, a dendritic cell, a motor neuron, a keratinocyte, or a muscle cell. The cell can also be obtained from any of a diverse group of tissues such as, but not limited to, lung, breast, colon, pancreas, kidney, stomach, liver, bone, blood, brain, skin, thyroid, ovary, testes, cervix, vagina, or bladder.

[0124] As used herein, an increased expression of SMN is an increase in the amount or stability of SMN protein or an increase in the amount or stability of an mRNA encoding an SMN

protein. It is understood that an increase in the amount or stability of an mRNA encoding a SMN protein can result in an increase in the amount of SMN protein. For example, while not bound by any particular theory or mechanism of action, a DcpS inhibitor can increase the expression of SMN protein by increasing the stability of a capped mRNA encoding the SMN protein.

[0125] In some embodiments, the methods can include the steps of detecting the presence or amount of SMN protein or mRNA in the cell and/or determining whether an increase in SMN expression has occurred in the cell. Numerous methods can be used for detecting SMN expression in a cell. Such methods depend on, e.g., whether mRNA or protein expression is being detected. For example, one suitable method for detecting SMN protein expression in a cell is a solid-phase immunoassay (e.g., a "sandwich" type immunoassay), wherein a first anti-SMN antibody is adhered to a solid-phase matrix (e.g., sepharose, agarose, magnetic beads, or a multi-well assay plate). A SMN protein-containing sample (e.g., a cell lysate containing or suspected of containing SMN protein) is then added to the antibody-coupled matrix and incubated for a time sufficient to allow binding of the SMN protein (if present) to the immobilized anti-SMN antibody. Unbound proteins, if any, are removed in subsequent wash steps. SMN proteins, if bound by the immobilized anti-SMN antibody, can then be detected using a second anti-SMN antibody. The second antibody can optionally have a different epitope specificity than the first antibody. For the purposes of detection, the second anti-SMN antibody can be directly coupled to a detection moiety. Detection moieties include, for example, fluorescent labels (e.g., cy5, cy3, green fluorescent protein, or fluorescein). Detection moieties can also be radioisotope labels, such as ^{35}S, ^{32}P, ^{33}P, ^{3}H, or ^{125}I. Detection labels can also be enzymes, e.g., alkaline phosphatase (AP), horseradish peroxidase (HRP), luciferase, or chloramphenicol acetyl transferase (CAT). Alternatively, it is often useful that the detection moiety be coupled to a secondary antibody that specifically recognizes the first, detection antibody (for example, to amplify the assay signal strength). In another embodiment, the detection antibody or secondary antibody can be conjugated to a first member of a binding pair (e.g., biotin or streptavidin) and the detection moiety can be linked to a second member of a binding pair (e.g., streptavidin or biotin).

[0126] SMN protein can be detected by western blotting using anti-SMN antibodies described herein. Western blotting methods are described in, for example, Sambrook et al. (*supra*). A sample containing SMN protein can be suspended in a denaturing buffer (e.g., Laemmli's buffer) containing both detergent (e.g., sodium dodecyl sulfate) and a reducing agent (e.g., DTT or beta-mercaptoethanol). The sample can then be subjected to SDS-polyacrylamide gel electrophoresis (PAGE). PAGE resolved proteins, separated by size, can then be transferred to a filter membrane (e.g., nitrocellulose) and subjected to western blot techniques using antibodies specific to SMN. The level of SMN in a sample can be determined by comparison to a control or reference sample containing a known amount of SMN. In an alternative embodiment of the western technique, sometimes called a dot-blot method, the protein sample can be directly adhered to a filter membrane without prior SDS-PAGE resolution.

[0127] Methods for detecting the expression of an mRNA encoding SMN protein include, e.g., northern blot analysis and reverse transcription polymerase chain reaction (RT-PCR). Such methods are well known in the art and described in detail in Sambrook et al. (*supra*).

[0128] Methods of assessing the level of a SMN protein or SMN mRNA in a sample can be quantitative, semi-quantitative, or qualitative. Thus, for example, the level of SMN in a sample can be determined as a discrete value. For example, where quantitative immunoassays are necessary, the level of SMN can be measured as a numerical value by correlating the detection signal derived from the quantitative assay to the detection signal of a known concentration of SMN protein or the signal presence of SMN protein in a reference sample provided from a second cell. Alternatively, the level of SMN protein can be assessed using any of a variety of semi-quantitative/qualitative systems. Thus, the level of expression of SMN protein useful in the immunoassay in a sample can be expressed as, for example, (a) one or more of "very high," "high," "average," "low," and /or "very low" or (b) one or more of "++++," "+++," "++," "+," "+/–," and/or "–." In this aspect, where it is also desired, the level of SMN protein in a cell can be expressed relative to the SMN protein levels in a reference sample (e.g., from a reference cell).

[0129] The DcpS inhibitor can be a compound selected from a variety of chemical classes, so long as the compound possesses the appropriate activity. Compounds can be biomolecules

including, but not limited to, peptides, polypeptides, peptidomimetics (e.g., peptoids), amino acids, amino acid analogs (e.g., non-naturally-occurring amino acids), saccharides, fatty acids, steroids, purines, pyrimidines, derivatives or structural analogues thereof, polynucleotides, and polynucleotide analogs (e.g., non-naturally-occurring polynucleotides). Compounds can be both small or large molecule compounds.

[0130] Typically small molecule compounds are relatively small organic molecules having a molecular weight in the range of about 50 to 2,500 Daltons. These compounds can comprise functional groups necessary for structural interaction with proteins (e.g., hydrogen bonding), and can include at least an amine, carbonyl, hydroxyl, or carboxyl group, and preferably at least two of the functional chemical groups. These compounds can often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures (e.g., purine core) substituted with one or more of the above functional groups.

[0131] Exemplary DcpS inhibitors include compounds containing, or having, the structure of any one of Formulas (I), (II), or (III). Exemplary DcpS inhibitors also include compounds that contain, or have, the structure of one of any of the compounds depicted in Table 1. Compounds can also be any of those described under the section herein titled "Exemplary Compounds and Pharmaceutical Compositions Thereof."

[0132] Also of interest as compounds in the methods described herein are nucleic acid aptamers, which are relatively short nucleic acid (DNA, RNA or a combination of both) sequences that bind with high avidity to a variety of proteins and inhibit the binding to such proteins of ligands, receptors, and other molecules. Aptamers are generally about 25 – 40 nucleotides in length and have molecular weights in the range of about 6 – 18 kDa. Aptamers with high specificity and affinity for targets can be obtained by an *in vitro* evolutionary process termed SELEX (systemic evolution of ligands by exponential enrichment) (see, for example, Zhang et al., *Arch. Immunol. Ther. Exp.*, 52:307-315, (2004), the disclosure of which is incorporated herein by reference in its entirety). For methods of enhancing the stability (by using nucleotide analogs, for example) and enhancing *in vivo* bioavailability (e.g., *in vivo* persistence in a subject's circulatory system) of nucleic acid aptamers see Zhang, et al. (2004)

and Brody et al. (*Reviews in Molecular Biotechnology*, (2000) 74:5-13, the disclosure of which is incorporated herein by reference in its entirety).

[0133] Molecules that are targeted to an mRNA encoding the DcpS protein are useful for the methods described herein, e.g., enhancing the expression of a DcpS protein, e.g., for treating an SMA condition. Examples of nucleic acids include siRNAs. Other such molecules that function using the mechanisms associated with RNAi can also be used, including chemically modified siRNAs and vector driven expression of hairpin RNA that are then cleaved to siRNA. The nucleic acid molecules or constructs that are useful as described herein include dsRNA (e.g., siRNA) molecules comprising 16-30, e.g., 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in each strand, wherein one of the strands is substantially identical, e.g., at least 80% (or more, e.g., 85%, 90%, 95%, or 100%) identical, e.g., having 3, 2, 1, or 0 mismatched nucleotide(s), to a target region in the mRNA, and the other strand is complementary to the first strand. The dsRNA molecules can be chemically synthesized, can transcribed be *in vitro* from a DNA template, or can be transcribed *in vivo* from, e.g., shRNA. The dsRNA molecules can be designed using methods known in the art, e.g., Dharmacon.com (see, siDESIGN CENTER) or "The siRNA User Guide," available on the Internet at mpibpc.gwdg.de/abteilunge-n/100/105/sirna.html.

[0134] Negative control siRNAs ("scrambled") generally have the same nucleotide composition as the selected siRNA, but without significant sequence complementarity to the appropriate genome. Such negative controls can be designed by randomly scrambling the nucleotide sequence of the selected siRNA; a homology search can be performed to ensure that the negative control lacks homology to any other gene in the appropriate genome. Controls can also be designed by introducing an appropriate number of base mismatches into the selected siRNA sequence.

[0135] In some cases, a pool of siRNA's is used to modulate the expression of a target gene. The pool is composed of at least 2 (e.g., 3, 4, 5, 8, or 10) different sequences targeted to the target gene.

[0136] Antisense nucleic acids are also useful for inhibiting the expression of a DcpS protein by decreasing the stability of an mRNA encoding the DcpS protein. Such antisense nucleic acid

molecules, i.e., nucleic acid molecules whose nucleotide sequence is complementary to all or part of an mRNA encoding a DcpS protein. An antisense nucleic acid molecule can be antisense to all or part of a non-coding region of the coding strand of a nucleotide sequence encoding an inhibitor protein. The non-coding regions ("5' and 3' untranslated regions") are the 5' and 3' sequences that flank the coding region and are not translated into amino acids.

[0137] Based upon the nucleotide sequences disclosed herein, one skilled in the art can easily choose and synthesize any of a number of appropriate antisense molecules to target a gene described herein. For example, a "gene walk" comprising a series of oligonucleotides of 15-30 nucleotides spanning the length of a nucleic acid (e.g., a target nucleic acid) can be prepared, followed by testing for inhibition of expression of the gene. Optionally, gaps of 5-10 nucleotides can be left between the oligonucleotides to reduce the number of oligonucleotides synthesized and tested.

[0138] An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 nucleotides or more in length. An antisense nucleic acid described herein can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic

acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation.

[0139] An antisense nucleic acid molecule can be an alpha-anomeric nucleic acid molecule. An alpha-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual beta-units, the strands run parallel to each other (Gaultier et al., *Nucleic Acids Res.*, 15:6625-6641, (1987)). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue et al., *Nucleic Acids Res.*, 15:6131-6148, 1987) or a chimeric RNA-DNA analog (Inoue et al., *FEBS Lett.*, 215:327-330, 1987).

[0140] Large molecule compounds can include large proteins (e.g., a dominant negative form of a DcpS protein) or macromolecular complexes comprising two or more proteins. Large molecule compounds, particularly those that are composed of more than one polypeptide, can be covalently joined or non-covalently joined, e.g., by hydrogen bonding, Van der Waals forces, or hydrophobic interactions.

[0141] DcpS inhibitors can be identified from a number of potential sources, including: chemical libraries, natural product libraries, and combinatorial libraries comprised of systematically randomized peptides, oligonucleotides, or organic molecules. Chemical libraries can consist of random chemical structures, some of which are analogs of known compounds or analogs or compounds that have been identified as "hits" or "leads" in other drug discovery screens, while others are derived from natural products, and still others arise from non-directed synthetic organic chemistry. Natural product libraries are collections of compounds from microorganisms, animals, plants, or marine organisms, which can be obtained by, e.g.: (1) fermentation and extraction of broths from soil, plant, or marine microorganisms, or (2) extraction of plants or marine organisms. Natural product libraries include polypeptides, non-ribosomal peptides, and variants (non-naturally occurring) thereof. For a review, see Science 282:63-68 (1998). Combinatorial libraries are composed of large numbers of peptides, oligonucleotides, or organic compounds as a mixture. These libraries are relatively easy to

prepare by traditional automated synthesis methods, PCR, cloning, or proprietary synthetic methods. Of particular interest are non-peptide combinatorial libraries.

[0142] Still other libraries of interest include peptide, protein, peptidomimetic, multiparallel synthetic collection, recombinatorial, and polypeptide libraries. For a review of combinatorial chemistry and libraries created therefrom, see Myers, Curr. Opin. Biotechnol. 8:701-707 (1997).

[0143] Suitable methods for determining whether a compound is a DcpS inhibitor include in vitro DcpS decapping assays described in the accompanying Examples. A variety of compounds such as Cpd. 1 (herein) can be used as positive controls in such assays.

Methods of Treatment

[0144] Disclosed herein are methods for treating a condition (e.g., an SMA condition) in a subject, which methods can include the step of administering to a subject a compound that inhibits DcpS (e.g., any of the compounds or compositions described herein). As evidenced by the foregoing Examples, cells contacted with DcpS inhibitors exhibited an increased expression of SMN mRNA and SMN protein. Thus, the methods described herein can be used for treating any condition (e.g., a genetic disorder) that results from, or whose symptoms or progression are exacerbated by, an insufficient expression of a gene product, the steady-state mRNA level of the gene product being regulated by DcpS. The gene product can be, e.g., the SMN protein. In some embodiments, the condition can be one that is characterized (and/or whose symptoms or progression is exacerbated) by the absence, or low levels, of expression of SMN protein in a cell (or in many cells in a multicellular organism) such as an SMA condition (e.g., Proximal SMA; see further herein), Amyotrophic Lateral Sclerosis (ALS or Lou Gehrig's disease, see, *e.g.*, *Biochem. Biophys. Res. Commun.*, (2007) Dec. 28; 364(4):850-5.; *Neurology* 2006 Oct 10;67(7):1147-50, *Neurology* 2005 Sep 27;65(6):820-5), or other motor neuron diseases. In practicing the methods, effective amounts of any of the compounds or compositions described herein having the appropriate activity are administered. Such amounts are sufficient to achieve a therapeutically effective concentration of the compound or active component of the composition *in vivo*.

[0145] One genus of conditions that can be treated, or whose symptoms can be ameliorated, by the compounds or compositions (and/or methods) described herein are Proximal SMA

conditions. A "Proximal SMA condition" is a term applied to a number of different disorders, all having mutations in the SMN1 gene (resulting in a reduction in SMN protein expression) and the manifestation of muscle weakness and atrophy due to loss of the motor neurons of the spinal cord and brainstem. Proximal SMA conditions can include, without limitation, any of the SMA conditions known in the art such as, but not limited to infantile SMA (Werdnig-Hoffmann disease); severe infantile SMA; intermediate SMA; juvenile SMA (Kugelberg-Welander disease); and adult SMA. In some embodiments, the Proximal SMA condition is not adult SMA.

[0146] In some embodiments, the compound can be administered to a pregnant mother carrying a child having, suspected of having, or at risk of developing, a condition that is characterized by the absence, or low levels, of expression of SMN in a cell (or in many cells in a multicellular organism) such an any of the SMA conditions described herein.

[0147] As used herein, a subject "suspected of having an SMA condition" is one who exhibits one or more symptoms of an SMA condition. Symptoms of an SMA condition can include one or more of muscle weakness; poor muscle tone; weak cry; limpness or a tendency to flop; difficulty sucking or swallowing; accumulation of secretions in the lungs or throat; tongue fasciculation; legs that tend to be weaker than the arms; hypotonia; areflexia; multiple congenital contractures (arthrogryposis) associated with loss of anterior horn cells; feeding difficulties; increased susceptibility to respiratory tract infections; or failure to reach developmental milestones, such as lifting the head or sitting up. Symptoms of an SMA condition can appear at any stage of development; however, In general, the earlier the symptoms appear, the shorter the life span for the subject. The onset is often sudden and dramatic.

[0148] A subject "at risk of developing" an SMA condition is one with a genetic predisposition for developing an SMA condition. For example, mutations in the SMN gene (e.g., a complete loss of function mutation or deletion in both copies of the SMN gene). Thus, a subject having a mutation in the SMN gene can be at risk of developing an SMA condition.

[0149] In some embodiments, the methods can include the step of determining whether a subject has an SMA condition. Methods for determining whether a subject has an SMA condition include any of a number of qualitative and quantitative tests. For example, a medical practitioner (e.g., a doctor or nurse) can review the medical history of a subject and/or determine

the number or type of symptoms that a particular subject presents. In some instances, a medical practitioner can include genetic tests to determine whether one or both copies of the SMN gene in a subject are present or mutated. In some instances (e.g., when the SMN gene test is not possible or does not show any abnormality), tests such as an electromyography (EMG) test or muscle biopsy can be performed as a diagnostic test.

[0150] A compound useful for treating, preventing, or ameliorating one or more symptoms of an SMA condition can be administered to a subject, *e.g.*, a human subject, by a variety of methods. For many applications, the route of administration is one of: intravenous injection or infusion (IV), subcutaneous injection (SC), intraperitoneally (IP), or intramuscular injection. In some cases, administration can be directly into the CNS, *e.g.*, intrathecal, intracerebroventricular (ICV), intracerebral, or intracranial. The compound can be administered as a fixed dose, or in a mg/kg dose. In other instances, administration can be oral (e.g., administered by inhalation), transdermal (topical), transmucosal, or rectal. (See, e.g., the section herein entitled "Pharmaceutical Compositions").

[0151] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

[0152] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[0153] Where the compound is a polypeptide or otherwise particularly antigenic, the dose can also be chosen to reduce or avoid production of antibodies against the compound. The route and/or mode of administration of the agent can also be tailored for the individual case.

[0154] Dosage regimens are adjusted to provide the desired response, *e.g.*, a therapeutic response or, when administered in combination with another agent, a combinatorial therapeutic effect. The dosage regimen can, for example, be capable of treating, preventing, or ameliorating one or more symptoms of an SMA condition. The dose of a compound can be, optionally, formulated separately or together with an appropriate dose of a second therapeutic agent can be used to provide a subject with the agent. Suitable dosages and/or dose ranges for the compound include an amount sufficient to treat, prevent, or ameliorate one or more symptoms of an SMA condition. Such dosages can include, e.g., about 0.001 μg/kg to 10,000 μg/kg body weight of the subject, per dose. In another example, the dosage can be about 1 μg/kg to 100 μg/kg body weight of the subject, per dose. In another example, the dosage can be about 1 μg/kg to 30 μg/kg body weight of the subject, per dose, e.g., from 3 μg/kg to 10 μg/kg body weight of the subject, per dose.

[0155] A dose of a compound required to treat, prevent, or ameliorate one or more symptoms of an SMA condition can depend on a variety of factors including, for example, the age, sex, and weight of a subject to be treated. Other factors affecting the dose administered to the subject include, e.g., the type or severity of the subject's SMA condition. For example, a patient with advanced form of an SMA condition can require a administration of a different dosage of a compound than a patient with a milder form of an SMA condition. Other factors can include, e.g., other disorders concurrently or previously affecting the subject, the general health of the subject, the genetic disposition of the subject, diet, time of administration, rate of excretion, drug combination, and any other additional therapeutics that are administered to the subject. It should also be understood that a specific dosage and treatment regimen for any particular subject will depend upon the judgment of the treating medical practitioner. The amount of any active ingredients will also depend upon the particular described compound and the presence or absence and the nature of the additional therapeutic agents in a composition comprising the compound.

[0156] The dose of a compound that inhibits DcpS can be determined by the efficacy of the particular compound employed and the condition of the subject, as well as the gender or body weight of the subject to be treated. The size of the dose also will be determined by the existence, nature, and extent of any adverse side effects that accompany the administration of a particular

compound in a particular subject. In determining the effective amount of the compound to be administered in the treatment or prophylaxis of the disease being treated, the physician can evaluate factors such as the circulating plasma levels of the compound, compound toxicities, and/or the progression of the disease, etc.

[0157] Toxicity and therapeutic efficacy of such compounds can be determined by known pharmaceutical procedures in cell cultures or experimental animal models (e.g., animal models of an SMA condition; see, e.g., Monani et al. (2000) *Human Molecular Genetics* 9:2451-2457 or the working Examples). These procedures can be used, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compounds that exhibit high therapeutic indices are preferred. While compounds that exhibit toxic side effects can be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[0158] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies generally within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For a compound used as described herein (e.g., for treating an SMA condition in a subject), the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography. Methods for determining an IC_{50} for a DcpS inhibitor in cell culture are detailed in the accompanying Examples.

[0159] Dosage unit form or "fixed dose" as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect (e.g., treating,

preventing, or ameliorating one or more symptoms of an SMA condition) in association with the required pharmaceutical carrier and optionally in association with the other agent. Suitable administration frequencies are described elsewhere herein.

[0160] Following administration of one or more of the compounds described herein (or pharmaceutical compositions thereof) to a subject (e.g., a human patient), the efficacy of the treatment in ameliorating one or more symptoms of an SMA condition can be assessed by comparing the number and/or severity of one or more symptoms presented by a patient before and after treatment. Alternatively, where administration of the compounds is used to prevent the occurrence of an SMA condition, treatment efficacy can be assessed as a delay in presentation of, or a failure to present, one or more symptoms of an SMA condition. The efficacy of a treatment (e.g., a compound or composition described herein) over time (e.g., a progressive improvement) in ameliorating one or more symptoms of an SMA condition can be determined by assessing, e.g., the number or severity of one or more symptoms at multiple time points following treatment. For example, a subject (e.g., a patient) can have an initial assessment of the severity of his or her disorder (e.g., the number or severity of one or more symptoms of an SMA condition), administered treatment, and then assessed subsequently to the treatment two or more times (e.g., at one week and one month; at one month at two months; at two weeks, one month, and six months; or six weeks, six months, and a year). Where one or more compounds or compositions are administered to a subject for a limited period of time (e.g., a predetermined duration) or number of administrations, the effect of treatment on ameliorating one or more symptoms of an SMA condition can be assessed at various time points after the final treatment. For example, following the last administration of a dose of one or more compounds, the number or severity of a patient's symptoms can be assessed at 1 month (e.g., at 2 months, at 6 months, at one year, at two years, at 5 years or more) subsequent to the final treatment.

[0161] The efficacy of a treatment with one or more compounds (or compositions) described herein on one or more symptoms of an SMA condition can be assessed as a monotherapy or as part of a multi-therapeutic regimen. For example, the compound(s) can be administered in conjunction with other clinically relevant treatments for an SMA condition including, but not limited to, physical or respiratory therapy, a decongestant, butyrates, valproic acid, hydroxyurea (HU), histone deacetylase (HDAC) inhibitors, methylase inhibitors, and/or Rilutek® (riluzole).

Exemplary HDAC inhibitors include, but are not limited to, valproic acid, hydroxybutyrate, phenylbutyrate, phenylbutyrate derivatives, butyrate prodrugs, trichostatin A (TSA), suberoylanilide hydroxamic acid (SAHA), AR-42 (OSU-HDAC42; Arno Therapeutics, Fairfield, NJ), and those described in, e.g., Herman et al., *Nat Chem Biol.*, (2006 Oct.); 2(10):551-8), the disclosure of which is incorporated herein by reference. An exemplary methylase inhibitor is 5-azacytidine.

[0162] Additional clinically relevant agents for an SMA condition that may be used in conjunction with any of compounds (or compositions) described herein include, without limitation: polyphenol botanical compounds (see, e.g., *Hum Genet.* (2008) 122(6):635-43); PP1 inhibitors (*Hum Mol Genet.* (2008) 17(1):52-70); tetracycline derivatives (e.g., alcarubicin; see *Hum Mol Genet.* (2001) 10(24):2841); aminoglycosides (see, e.g., *Hum Genet.* (2006) 120(4):589-601); 5-(N-ethyl-N-isopropyl)-amiloride (see, e.g., *Ann Neurol.* (2008) 63(1):26-34); indoprofen (see, e.g., *Chem Biol.* (2004) 11(11):1489-93); salbutamol (see, e.g., *J Med Genet.* (2008) 45(1):29-31); PTC124 (PTC Therapeutics, Inc., South Plainfield, NJ); and albuterol (see, e.g., *Neurology* (2002) 59(4):609-10).

[0163] As defined herein, a "therapeutically effective amount" of a DcpS inhibitor is an amount of the DcpS inhibitor that is capable of producing a medically desirable result (e.g., amelioration of one or more symptoms of an SMA condition and/or increasing the expression of SMN in a cell) in a treated subject. A therapeutically effective amount of a DcpS inhibitor (i.e., an effective dosage) includes milligram, microgram, nanogram, or picogram amounts of the DcpS inhibitor per kilogram of subject or sample weight (e.g., as described herein).

[0164] A compound or pharmaceutical composition thereof described herein can be administered to a subject as a combination therapy with another treatment (another active ingredients), e.g., a treatment for an SMA condition. For example, the combination therapy can include administering to the subject (e.g., a human patient) one or more additional agents that provide a therapeutic benefit to the subject who has, or is at risk of developing, (or suspected of having) an SMA condition. Thus, the compound or pharmaceutical composition and the one or more additional agents are administered at the same time. Alternatively, the compound can be administered first in time and the one or more additional agents administered second in time.

The one or more additional agents can be administered first in time and the compound administered second in time. The compound can replace or augment a previously or currently administered therapy. For example, upon treating with a compound of the invention, administration of the one or more additional agents can cease or diminish, *e.g.*, be administered at lower levels. Administration of the previous therapy can also be maintained. In some instances, a previous therapy can be maintained until the level of the compound (e.g., the dosage or schedule) reaches a level sufficient to provide a therapeutic effect. The two therapies can be administered in combination.

[0165] It will be appreciated that in instances where a previous therapy is particularly toxic (e.g., a treatment for an SMA condition that carries significant side-effects) or poorly tolerated by a subject (e.g., a patient), administration of the compound can be used to offset and/or lessen the amount of the previous therapy to a level sufficient to give the same or improved therapeutic benefit, but without the toxicity.

[0166] In some instances, when the subject is administered a compound or pharmaceutical composition described herein, the first therapy is halted. The subject can be monitored for a first pre-selected result, *e.g.*, an improvement in one or more symptoms of an SMA condition such as any of those described herein (e.g., see above). In some cases, where the first pre-selected result is observed, treatment with the compound is decreased or halted. The subject can then be monitored for a second pre-selected result after treatment with the compound is halted, *e.g.*, a worsening of a symptom of an SMA condition. When the second pre-selected result is observed, administration of the compound to the subject can be reinstated or increased, or administration of the first therapy is reinstated, or the subject is administered both a compound and first therapy, or an increased amount of the compound and the first therapeutic regimen.

[0167] In addition, while the invention is not limited by any particular theory or mechanism of action, because the compounds identified herein can function at the molecular level to correct the SMA condition, assessing the effect of a therapy on subject having an SMA condition can be done by assessing, e.g., whether an increase in the expression of SMN in one or more affected cells in a subject having (or suspected of having) an SMA condition occurred.

[0168] The DcpS inhibitor can also be administered with a treatment for one or more symptoms of an SMA condition. For example, the DcpS inhibitor can be co-administered (e.g., at the same time or by any combination regimen described above) with, e.g., a pain medication, an antibiotic, or a decongestant.

[0169] In some embodiments, the methods can be used to prevent an SMA condition, or one or more symptoms of an SMA condition, from occurring in a subject. When the terms "prevent," "preventing," "prevention," or "prophylaxis" are used herein in connection with a given treatment for a given SMA condition, they mean that the treated subject either does not develop a clinically observable level of an SMA condition at all (e.g., the subject does not exhibit one or more symptoms of the an SMA condition), or the condition develops more slowly and/or to a lesser degree (e.g., as a result of increased expression of SMN) in the subject than it would have absent the treatment. These terms are not limited solely to a situation in which the subject experiences no aspect of an SMA condition whatsoever. For example, a treatment will be said to have "prevented" an SMA condition if it is given prior to the onset of any symptoms of the SMA condition in a subject at risk of developing an SMA condition and results in the subject's experiencing fewer and/or milder symptoms of the SMA condition than otherwise expected. A treatment can "prevent" an SMA condition when the subject displays only mild overt symptoms of an SMA condition. "Prevention" does not imply that there must have been no symptoms of an SMA condition nor that those symptoms are entirely absent in a treated subject.

Articles of Manufacture

[0170] Also included are articles of manufacture that include: a container; and a composition contained within the container, wherein the composition comprises an active ingredient for increasing the expression of SMN in a cell, wherein the active ingredient comprises one or more (e.g., two, three, four, five, six, seven, eight, nine, or 10 or more) of any of the compounds described herein, and wherein the container has a label indicating that the composition is for use in increasing the expression of SMN in a cell and/or treating (or preventing) an SMA condition in a subject (e.g., a human). The label can further indicate that the composition is to be administered to a subject having, suspected of having, or at risk of developing, an SMA condition such as any of those described herein. In some embodiments, the label can further

indicate that the composition is to be given to a pregnant woman carrying a child having, suspected of having, or at risk of developing, an SMA condition. The composition of the article of manufacture can be dried or lyophilized and can include, e.g., one or more solutions (and/or instructions) for solubilizing a dried or lyophilized composition.

[0171] The articles of manufacture can also include instructions for administering the composition to a subject (e.g., any of the methods described above under "Methods for Treating an SMA Condition").

[0172] Such articles of manufacture or the various components thereof can also be included in kits.

EXAMPLES

Example 1. Effect of C5-Quinazolines on Endogenous SMN mRNA and Protein Expression in Cells

[0173] The C5-substituted quinazolines described in the following experiments were synthesized according to published methods as described in Thurmond et al. (2008) J. Med. Chem., 51:449-469. To synthesize ^{125}I-Cpd. 3, 30 μL of 2% acetic acid in methanol was added to 21 μL (10.1 mCi, 5.05 nmol) of aqueous NaI^{125}. Next, 15 μL (30 μg, 45 nmol) of the stannane Cpd. 2 in methanol/DMF (1:1) followed by a 20 μL solution of chloramine T (200 μg, 712 nmol) of 10 μg/μL solution in methanol were added. The reaction mixture was incubated for 3 minutes at room temperature and then 20 μL (200μg, 1.05 μmoles) of aqueous sodium metabisulfite was added. Next, the reaction mixture was injected on to a preparative HPLC column (MacMod: ACE 300SB, C18, 5 μm, 300A, 4.6 mm x 250 mm) using 0.4 mL of initial HPLC eluent, 0.1% TFA in water. The samples were eluted using 20-70% eluent B over 50min, (eluent A 0.1% TFA in water, eluent B 1% TFA in ACN). Four 0.85 mL column fractions were collected and to the fractions was added 0.2 mL water. The samples were then re-injected into the HPLC. Two fractions (having a total activity of about ~6 mCi) were collected. A sample of the product co-eluted with authentic cold standard, Cpd. 3. A solution made with 400 mg sucrose, 7.6 mL water and 0.4 mL ethanol was used as a medium for freeze drying the isolated compounds. A quality control check was performed by analyzing one of the samples on a Jupiter 7.5 cm C4 column, and indicated that the purified compounds had < 0.1% free iodine and > 98.1% radiochemical

purity (specific radioactivity 2000 Ci/mmol). Five 230 μCi samples were shipped on cold pack for biological evaluations. Radioiodination was performed at GE Healthcare, Bio-Science Division, Woburn, MA.

[0174] Cpd. 1 and TSA (Trichostatin A, Sigma, T-8552; Sigma Aldrich, St. Louis, MO) were formulated in DMSO and added to a culture of NSC-34 cells (which cells resulted from a fusion of motor neuron enriched, embryonic mouse spinal cord cells with mouse neuroblastoma) at a final concentration of 500 nM/50 nM and 100 nM, respectively. The final concentration of DMSO was 0.5%. The cells were incubated for 18 hours and then harvested for RNA isolation using RNeasy Mini Kit (Qiagen) according to manufactures protocol. RNA quality and quantity was measured on an Agilent 2100 Bioanalyzer (Agilent Technologies). cDNA synthesis was performed using High Capacity cDNA Archive kit (Applied Biosystems), according to manufactures protocol. Gene expression analysis was performed using TaqMan Assays-on-Demand Gene Expression Products (Applied Biosystems) according to manufactures protocol. Each assay was run in triplicate and the data was normalized against an internal housekeeping gene, B-actin. The relative difference in expression was calculated using the equation $2e^{(-\Delta CT)}$. Statistical comparisons between groups in the gene expression studies, was done by using Students t-test, an associated probability of <5% was considered significant.

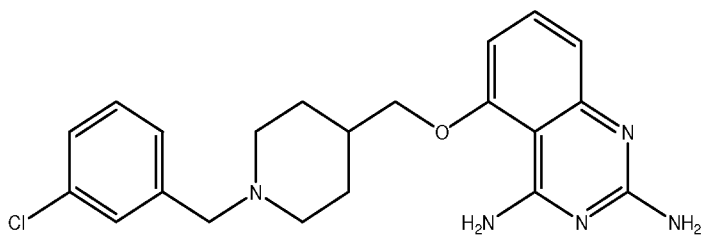
[0175] Optimization of the C5-quinazoline series for human SMN2 promoter inducing activity utilized an NSC-34 derived cell line expressing an intronless β-lactamase gene under control of the human SMN2 promoter (Jarecki, et al. (2005) *Human Mol. Genet.* 14(14):2003-18). NSC-34 cells expressing the β-lactamase gene as above were treated with increasing concentrations of Cpd. 1 and lysates prepared from the cells were analyzed for β-lactamase activity. Increasing concentrations of Cpd. 1 increased the total β-lactamase activity in the cells, suggesting that Cpd. 1 affected the amount of β-lactamase in the cell.

[0176] Since NSC-34 cells are a hybrid cell line resulting from the fusion of mouse neuroblastoma N18TG2 cells with motor neuron-enriched embryonic day 12-14 cells harvested from mouse spinal cord (Cashman et al. (1992) Dev. Dyn. 194:209-21), the cells contain the mouse SMN gene in its normal chromosomal context. Therefore, an effort was made to ascertain if the C5-quinazolines would act on the NSC-34 cells to increase endogenous mouse

SMN mRNA. To that end, real-time PCR studies were carried out to measure the mRNA levels in cells treated with Cpd. 1 (see FIG. 1A and Table 1), a potent C5-quinzaoline, and compared that to the induction of β-lactamase activity driven by the human SMN2 promoter (FIGS. 1B and 1C). Potent induction of mouse SMN mRNA was also observed in this cellular context suggesting that compounds such as Cpd. 1 affect processes related to SMN mRNA synthesis, processing, or degradation.

[0177] Diminution of DcpS levels led to an increase in SMN2 that was not further accentuated by Cpd. 1. A human 293T cell line expressing 90% reduced levels of DcpS due to a stably integrated DcpS specific shRNA (Shen et al. (2008) RNA 14:1132-42) or a control knockdown cell line were treated either with Cpd. 1 or DMSO for 24 hours. SMN2 mRNA levels were determined with qPCR as described previously (Britcha et al. (2008) Hum. Genet. 123:141-53). SMN2 levels in the DcpS knockdown cell increased approximately 3 fold and the addition of the Cpd. 1 did not further increase the levels strongly (FIG. 1D), indicating that Cpd. 1 functions through DcpS.

[0178] C5-quinazolines were also tested for their ability to enhance SMN protein expression in human fibroblasts obtained from a type I SMA patient (hereinafter referred to as "3813" cells). 3813 cells were treated with Cpd. 1 or Cpd. 12, each at 10, 100, or 1000 nM for 5 days. (As used herein, Cpd. 12 refers to 5-[1-(3-chlorobenzyl)piperidin-4-ylmethoxy]quinazoline-2,4-diamine), and Cpd 1. refers to 5-[1-(2-fluorobenzyl)piperidin-4-ylmethoxy]quinazoline-2,4-diamine. The structure of Cpd. 12 is as follows:



Cpd. 12.

[0179] A set of cells was also treated with DMSO, the vehicle, as a control. Following treatment with the compounds, cell lysates were prepared from the cells. Sixty μg of each cell lysate was subjected to SDS-PAGE and SMN protein was detected by western blotting using mouse anti-SMN monoclonal antibody (MANSMA2) (FIG. 2). As a protein loading control, β-tubulin was also detected using a mouse anti-β-tubulin antibody. An increase in the amount of SMN protein in 3813 cells was observed in cells treated with each of Cpds. 1 and 12, but not with DMSO. These results demonstrated that the C5-quinazolines are able to enhance SMN protein expression in human fibroblasts.

Example 2. Probing Immobilized Protein Target Arrays for Potential C5-Quinazoline Binders

[0180] All clones used to generate the human protein collection were fully sequenced and subcloned into the expression vector, pDEST™20 (Invitrogen). These clones were then used to express proteins in Sf9 insect cells as N-terminal GST-fusions using the Bac-to-Bac® Baculovirus Expression System (Invitrogen). Insect cell lysates were loaded directly into 96-well plates containing glutathione resin (GE Healthcare). After washing, purified proteins were eluted under conditions designed to obtain native proteins, then stored at –80 °C. Protein arrays were printed on 1x3 inch modified glass slides using a 48 pin contact arrayer (OmniGrid, Genomics Solutions) at 4 °C. Protein arrays were stored at –20 °C until use.

[0181] Protein microarrays were blocked for 1 hour in Tris buffer (50 mM Tris-HCl pH 7.5, 5 mM $MgSO_4$, 0.1% v/v Tween20) with low-speed orbital shaking. A solution of 100 nM ^{125}I-Cpd. 3 tracer with 10 nM ^{125}I-Streptavidin (GE Healthcare, Amersham) in 50 mM Tris-HCl pH 7.5, 5 mM $MgSO_4$, 0.1% v/v Tween20 was applied to the surface of the microarrays and allowed to incubate under a Hyperslip™ coverslip at 4°C for 90 minutes. Following incubation, arrays were washed three times with the aforementioned Tris buffer using 28 ml per wash. After washing, arrays were placed into a slide holder and spun for 2 minutes at 2000 rpm in a plate centrifuge. Dried arrays were exposed to a phosphorimager screen, images were acquired using a PerkinElmer Phosphorimager, and data were extracted with microarray data acquisition software (GenePix Pro, Molecular Devices).

[0182] Protein microarrays were assayed as described above with probing solutions comprised of 100 nM ^{125}I-Cpd. 3 mixed with 10 nM ^{125}I-streptavidin for positional mapping, in

the presence or absence of 10 μM unlabeled competitor compound. Unlabeled competitor molecules included Cpd. 3, Cpd. 1, staurosporine, and tertbutylquinone. All assays were carried out in duplicate.

[0183] In order to identify candidate cellular targets of the C5-quinazolines, radiolabeled analogs that retained potent SMN2 promoter activity were produced. A meta-iodo derivative of Cpd. 3 was found to be an active piperidine amide-based analog with an EC_{50} of 35.6 nM (n=2) (Thurmond et al. (2008) J Med Chem 51:449-469) (see Table 1 herein).

[0184] Having identified an appropriate iodinated C5-quinazoline for binding studies (Cpd. 3), a tin derivative of Cpd. 3 was first prepared (Cpd. 2), which supported radioiodination to the corresponding ^{125}I labeled analog using the iododestannylation reaction (FIG. 3). This reaction with 100 mole percent incorporation of iodine under carrier-free conditions provided a theoretical specific activity of 2200 Ci/mmol. Additional quinazolines with potent SMN2 promoter activity to use as unlabeled competitors of ^{125}I-Cpd. 3 in binding experiments were selected and tested as follows.

[0185] High density human protein arrays were used as the test bed for probing with ^{125}I-Cpd. 3 tracer compound. Recombinant human proteins were expressed as fusion proteins with N-terminal glutathione-S-transferase (GST) purified from Baculovirus-infected insect cells. Represented on the protein microarrays were soluble proteins of potential therapeutic interest including kinases, phosphatases, nuclear receptors, and enzymes of intermediary metabolism. Proteins were purified under non-denaturing conditions and printed as adjacent duplicate spots on chemically modified glass slides. Additionally, the arrays contain control elements suitable for positional mapping of the data acquisition grid as well as positive and negative assay controls. The Human ProtoArray® Protein Microarray v3.0 mg containing more than 5,000 human proteins was probed with 100 nM of the ^{125}I-Cpd. 3 tracer in the presence of ^{125}I-streptavidin to facilitate accurate positional mapping. High resolution images were generated through phosphoimaging. Pixel intensity data was extracted from these images and the resultant data normalized against the signals obtained for the ^{125}I-streptavidin positional mapping reagent binding to a biotinylated control protein.

[0186] Evaluation of the resultant data set identified an interaction that was consistently above background, corresponding to the human mRNA scavenger decapping enzyme DcpS, a member of the histidine triad (HIT) superfamily of hydrolases (Liu et al. (2002) EMBO J 21:4699-708) (FIG. 4A). DcpS functions in the 3'-5' exonucleolytic pathway for mRNA decay to hydrolyse the residual cap structure m^7GpppN to m^7GMP + pN (Wang et al. (1999) Mol Cell Biol 19:4552-60); Liu et al. (2002) EMBO J 21:4699-708) and in the 5'-3' pathway to dephosphorylate the m^7GDP decapping product to m^7GMP (van Dijk et al. (2003) Proc. Natl. Acad. Sci. USA 100:12081-6). To further evaluate the specificity of DcpS for binding to the C5-quinazoline, the ProtoArrays were subsequently probed with ^{125}I-Cpd. 3 in the absence or presence of 10 μM unlabeled competitor molecules. Analysis of the signal intensity data revealed that C5-quinazoline competitors including Cpd. 3 and Cpd. 1 effectively competed with ^{125}I-Cpd. 3 for binding to DcpS, while other compounds such as staurosporine and tertbutylquinone did not compete (FIG. 4B).

Example 3. C5-Quinazolines Inhibited DcpS

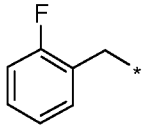
[0187] RNA corresponding to the pcDNA3 (Invitrogen) polylinker (pcP) was transcribed *in vitro* by SP6 polymerase according to the manufacturer's directions (Promega) from a PCR-generated template using T7 and SP6 promoter primers. Cap labeling of the RNA was carried out with the vaccinia virus capping enzyme in the presence of [α-^{32}P] GTP and S-adenosyl-methionine (SAM) as previously described (Wang et al. (1999) *Mol. Cell Biol.*, 19:4552-60) to label the α-phosphate (relative to the terminal guanosine; m^7Gp*ppN-Labeled cap structure was generated by treating the cap-labeled RNA with 1 unit nuclease P1 (Sigma) to degrade the RNA leaving the cap structure containing the pyrophosphate linkage, intact as previously described (Liu et al. (2002) EMBO J. 21:4699-708). Decapping assays were carried out with 5 ng Flag-tagged recombinant DcpS protein and 20 nM unlabeled cap structure (purchased from New England Biolab) spiked with radioactive cap structure in decapping buffer (10 mM Tris-HCl pH 7.5, 100 mM KOAc, 2 mM $Mg(OAc)_2$, 2 mM DTT) for 30 seconds at room temperature. Decapping reactions were terminated with the addition of 1.7 N formic acid. An aliquot of each reaction was spotted onto polyethylenimine (PEI) cellulose thin-layer chromatography (TLC) plates (Sigma) that were pre-run in water, air dried, and then developed with 0.45 M $(NH_4)_2SO_4$ at room temperature (Liu et al. (2002) EMBO J, 21:4699-708). The TLC plates were air dried

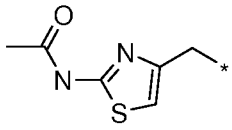
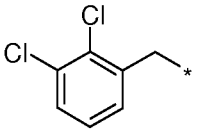
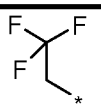
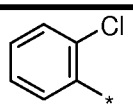
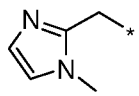
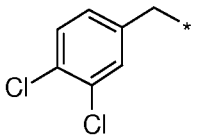
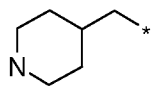
and exposed to PhosphorImager for quantification of radioactive spots, performed using the Molecular Dynamics Phosphorimager (Storm860) using ImageQuant-5 software.

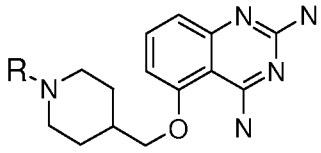
[0188] The ability of the C5-quinazolines to modulate DcpS decapping activity in purified enzyme assays was determined by measuring the hydrolysis of m7GpppG to m7G in the presence of various compounds. Decapping reactions were carried out with ^{32}P-labeled m7Gp*ppG cap structure where the label was at the first phosphate following the methylated guanosine. Decapping reactions were carried out with a fixed amount of DcpS in the presence of increasing titration of Cpd. 1 and reaction products and substrate resolved by polyethylenimine (PEI) cellulose thin-layer chromatography (TLC). The decapping assays revealed that Cpd.1, but not a related negative control Cpd. 4, was highly effective at inhibiting recombinant DcpS decapping activity (FIGS. 5A and 5B). The inhibition observed with Cpd. 1 (IC_{50} =~15 nM) was more efficient than the natural cap analog m7GpppG (IC_{50} = ~30 nM). Specificity of inhibition was underscored by the lack of inhibition observed with the Cpd. 4 compound, that had reduced potency in cell based assays detecting stimulation of SMN2 expression (EC_{50} = 2540 nM; Table 1). These data demonstrate that Cpd. 1 (and compounds of similar structure) can inhibit DcpS decapping activity and suggest that the observed increase in SMN2 expression by this compound could be directed through its modulation of DcpS.

[0189] To determine the extent of correlation between SMN2 promoter activation and DcpS decapping inhibition, a panel of compounds was profiled in the DcpS decapping assay with EC_{50} in the SMN2 promoter assay varying nearly 1,000 fold from 4 nM to 2.5 μM. A linear correlation between SMN2 promoter activation and DcpS decapping inhibition (r^2 = 0.9857) was observed (FIGS. 5C and 5D; Table 1). This suggests that DcpS is a molecular target of the C5-quinazolines.

Table 1

Cpd. No.	R group[a]	SMN2 NSC-34 Cellular Assay EC_{50} nM Ave. (Low-Hi)	SMN2 NSC-34 Cellular Assay Max Fold Induction Ave. (Low-Hi)	*In Vitro* DcpS Assay IC_{50} nM
1		4.0 (1.4-6.7)	2.29 (2.06-2.50)	7.62

Cpd. No.	R group[a]	SMN2 NSC-34 Cellular Assay EC_{50} nM Ave. (Low-Hi)	SMN2 NSC-34 Cellular Assay Max Fold Induction Ave. (Low-Hi)	*In Vitro* DcpS Assay IC_{50} nM
4		2540	1.95	339.45
5		67 (17-109)	2.20 (2.10-2.40)	43.94
6		223 (81-364)	1.7 (1.67-1.74)	23.35
7		795 (791-800)	1.78 (1.66-1.91)	101.34
8		108	1.5	9.38
9		104 (51-142)	1.9 (1.7-2.0)	n.d.
10		921 (370-2239)	2.19 (1.90-2.40)	n.d.

[a]: Each of the compounds depicted in Table 1 contain the following structure in which the R group varies:  .

Example 4. C5-Substitued Quinazoline Crossed the Blood-Brain Barriers of Neonatal Mice and Increased Smn Expression in the Central Nervous System

[0190] For survival and phenotype analyses, SMNΔ7 SMA mice (*SMN2*$^{+/+}$; *SmnΔ7*$^{+/+}$; *mSmn*$^{-/-}$) were used (Butchbach et al. (2007) Neurobiol. Dis. 27:207-219). These mice were generated from males and females of the genotype *SMN2*$^{+/+}$; *SmnΔ7*$^{+/+}$; *mSmn*$^{+/-}$ (line 4299;

FVB.Cg-Tg(SMN2*delta7)4299Ahmb Tg(SMN2)89Ahmb *Smn1*^{tm1Msd}). These mice originated from our colony but can be obtained from Jackson (#005025). Neonatal offspring were genotyped using a PCR-based assay on genomic DNA from tail biopsies as described previously (Butchbach et al. (2007) Neurobiol Dis 27:207-219, incorporated herein by reference). Only pups of the genotypes *SMN2*$^{+/+}$;*SmnΔ7*$^{+/+}$;*mSmn*$^{+/-}$ (carrier) and *SMN2*$^{+/+}$;*SmnΔ7*$^{+/+}$;*mSmn*$^{-/-}$ (SMA) were used in these experiments.

[0191] Carrier and SMA littermate mice were treated with Cpd. 1 (3 mg/kg/d) or the appropriate vehicle via oral administration beginning at PND04—with birth being defined as PND01— or PND09 as described previously (Butchbach et al. (2007) J. Neurosci. Methods 161:282-290). Mice were monitored daily for changes in body mass and for death. SMNΔ7 carrier mice received differing doses of Cpd. 1 (0.1-30 mg/kg/d) or the appropriate vehicle beginning at PND04. Cpd. 1 or vehicle were delivered via oral administration as described previously (Butchbach et al. (2007) J. Neurosci. Methods 161:282-290). Treatment lasted for 5 days for the maximum tolerable dose (MTD) analysis at which time the pups were euthanized 1 hour after final dosing. Serum samples were taken and the brains were dissected and rapidly frozen in liquid nitrogen. Tail snips were also taken for genotyping.

[0192] Serum and forebrain samples were obtained from neonatal mice treated with differing doses and processed as described elsewhere (Thurmond et al. (2008) J Med Chem 51:449-469, incorporated herein by reference). Drug levels were measured using LC/MS/MS. Protein concentrations were measured for each sample after LC-MS-MS analysis; serum and forebrain drug levels were expressed as ng/mg protein.

[0193] β-Galactosidase activity was measured using the Galacto-Light chemiluminescent reporter gene assay (Applied Biosystems, Bedford, MA) according to manufacturer's directions except that the lysis buffer contained 0.1% Triton X-100. Luminescence was monitored using a Luminoskan Ascent luminometer.

[0194] The maximum tolerable dose (MTD) for Cpd. 1 was first determined in SMNΔ7 carrier (*SMN2*$^{+/+}$;*SMNΔ7*$^{+/+}$;*mSmn*$^{+/-}$) mice. These mice received different oral doses of Cpd. 1

(0.1–30 mg/kg/d) or the appropriate vehicle (ddH_2O)—for 5 days beginning at postnatal day (PND) 04. No adverse effects were observed for Cpd. 1 at any of the doses tested.

[0195] Cpd. 1 was able to cross the blood-brain barrier. Brain Cpd. 1 levels increased with dose in a linear manner at lower doses while serum Cpd. 1 levels were low and fairly constant.

[0196] The effect of Cpd. 1 on Smn expression *in vivo* using neonatal mice was also examined. In the mSmn knockout cassette, a LacZ:NeoR cassette was inserted into *mSmn* exon 2A to nullify the expression of mSmn protein (Schrank et al. (1997) Proc. Natl. Acad. Sci. USA 94:9920-9925), but also placed the LacZ reporter gene under the control of the *mSmn* promoter. β-Galactosidase activity was used to monitor SMN promoter activity since the promoter region of *mSmn* is very similar to those of human *SMN1* and *SMN2* (Monani et al. (1999) 1445:330-336; Echaniz-Laguna et al. (1999) Am. J. Hum. Genet. 64:1365-1370; Boda at al. (2004) Eur. J. Hum. Genet. 12:729-737; Rouget et al. (2005) Biochem. J. 385:433-443, all of which are incorporated herein by reference). Treatment of mice with Cpd. 1 increased *mSmn* promoter activity in forebrain and spinal cord extracts in a dose-dependent manner to a maximum at 3 mg/kg/d; *mSmn* promoter activity then decreases with increasing dose of Cpd. 1. Based on these observations and the MTD analysis, the optimal dose for Cpd. 1 is 3 mg/kg/d.

Example 5. C5-Substitued Quinazoline Increased SMN Protein Levels in the Spinal Cords of SMNΔ7 SMA Mice

[0197] Tissue protein extract (100 μg) was mixed with 0.16 volume of 6×loading dye (10.28% SDS, 36% glycerol and 0.012% bromophenol blue in 350 mM TrisHCl, pH 6.8) containing freshly added 100 mM DTT and resolved through a 12% polyacrylamide gel containing 0.1% SDS. Samples were then transferred onto a PVDF membrane via electroblotting. The resultant blots were incubated in 1×blocking buffer (5% nonfat milk, 1% BSA in PBST (0.2% Tween-20 in PBS)) overnight at 4 °C and then with a primary antibody diluted in 0.2×blocking buffer for 1 hour at room temperature. For detection of SMN protein, a mixture containing equivalent amount of the following mAbs was used: 8F7 (MANSMA2), 1F1(MANSMA21), 5E3 (MANSMA13) and 7Q12 (MANSMA19) (*e.g.*, Young et al. (2000), Exp. Cell Res., 256:365-374, incorporated herein by reference). This SMN cocktail was used at a titer of 1:500. After extensive washing with PBST (3×10 minutes), the blots were incubated with a horseradish peroxidase-conjugated goat anti-mouse secondary antibody (1:1000) diluted

in 0.2×blocking buffer for 1 hour at room temperature, washed extensive as above and the bound antibodies were detected by chemiluminescence (ECL Western Blotting Detection Reagents, Amersham Biosciences). To confirm equal loading of protein in each lane, the blots were stripped and reprobed using a mouse anti-β-actin mAb (1:20 000; clone AC-15, Sigma-Aldrich).

[0198] The effect of Cpd. 1 treatment on SMN protein levels was examined in the spinal cord of SMNΔ7 SMA mice. As shown in FIG. 6A, treatment of SMNΔ7 SMA mice with Cpd. 1 for 5 days (n=3/group) resulted in an increase in SMN protein levels in spinal cord extracts. In fact, oral administration of Cpd. 1 resulted in a ~70% increase in SMN protein levels in the spinal cord (FIG. 6B). Cpd. 1-treated SMNΔ7 SMA mice had spinal cord SMN proteins that were approximately 25% of the levels observed in SMNΔ7 carrier mice. Therefore, Cpd. 1 can increase SMN protein levels in the CNS of SMA mice *in vivo* but not to those levels observed in carrier mice.

Example 6. C5-Substitued Quinazoline Improved Survival and Motor Phenotype of SMNΔ7 SMA Mice

[0199] The effects of Cpd. 1 on the survival and phenotype of SMA mouse models were examined. The SMNΔ7 SMA mouse (*SMN2*$^{+/+}$;*SMNΔ7*$^{+/+}$;*mSmn*$^{-/-}$) model was used in these experiments because their phenotype and pathology strongly resemble what is observed in SMA and these mice provide a reasonably rapid measure of therapeutic benefit since they only live on average for 13.6±0.2 d (*e.g.*, Le et al. (2005) Hum Mol Genet 14:845-857; Butchbach et al. (2007) Neurobiol Dis 27:207-219, both incorporated herein by reference).

[0200] A cohort of drug-treated SMNΔ7 SMA mice were assessed for changes in righting reflex success and latency, spontaneous locomotor activity and pivoting activity as described elsewhere (Butchbach et al. (2007) Neurobiol. Dis. 27:207-219, incorporated herein by reference). For monitoring righting reflexes, each pup was turned onto its back and the time it takes to stably place all four paws on the ground was recorded (cutoff time of 60 s). Righting reflexes were assessed on PND07 and PND11. For spontaneous locomotor activity, each pup was placed in the center of a gridded (with 28 2.5-cm^2 grids) arena and the number of grids crossed in 1 min was counted as well as the latency for walking a distance greater than its body length (vectorial movement latency). For pivoting, each pup was placed in the center of a

gridded arena and the number of times the pup turned 90°C (pivots) during a 1-min time frame was counted. Spontaneous locomotor activity and pivoting were monitored on PND07, PND11 and PND14. To minimize the stress on the pup, spontaneous locomotor activity and pivoting tests was conducted simultaneously.

[0201] SMNΔ7 SMA mice received oral Cpd. 1 (3 mg/kg/d) beginning at PND04 and continued to receive drug for the duration of their lives. SMNΔ7 SMA mice treated with Cpd. 1 (n=14) lived, on average, 21% longer than vehicle-treated (n=15) controls (FIG. 7A, 17.0±0.5 d vs. 14.0±0.4 d; χ^2=16.783, p<0.001). Treatment of SMNΔ7 SMA mice with Cpd. 1 after the onset of motor neuron loss (PND09), however, did not improve survival of these mice (FIG. 7A, 14.9±0.4 d vs. 14.0±0.4 d; χ^2=2.829, p=0.093). Cpd. 1 also delayed the onset of body mass loss—an indicator for the end-stage of neurodegeneration in SMNΔ7 SMA mice (Butchbach et al. (2007) Neurobiol Dis 27:207-219)—slightly when the treatment started at PND04 (FIG. 7B, 12.0±0.4 d vs. 11.1±0.3 d; χ^2=3.247, p=0.072). No effect on the onset of body mass loss was observed in SMNΔ7 SMA mice treated with Cpd. 1 starting at PND09 (FIG. 7B). Even though the onset of body mass loss was slightly delayed in Cpd. 1-treated SMNΔ7 SMA mice, their body mass curve was not significantly different from the body mass curve of vehicle-treated SMNΔ7 SMA mice (FIG. 7C).

[0202] SMNΔ7 SMA mice develop a progressive impairment of neonatal motor behaviors including loss of surface righting and reduced spontaneous locomotion (Butchbach et al. (2007) Neurobiol Dis 27:207-219). Since Cpd. 1 improved survival of SMNΔ7 SMA mice when administered prior to motor neuron loss, the effect of this quinazoline on the amelioration of the SMA motor phenotype was examined in these mice. Gross observation of Cpd. 1-treated SMNΔ7 SMA mice showed an improvement in motor activity when compared to vehicle-treated SMNΔ7 SMA mice. Surface righting reflex responses are impaired in SMNΔ7 SMA mice; treatment with Cpd. 1 partially reduced the righting reflex latency (FIG. 8A; n=5/group) but this reduction was not statistically significant. When comparing the righting reflex latencies between SMA and non-SMA mice, however, the difference between SMA and non-SMA mice was no longer significant (p=0.906) in Cpd. 1-treated mice at PND11 but was significant in vehicle-treated mice (p=0.005). This observation suggests that Cpd. 1 does partially ameliorate surface

righting reflex response impairments in SMNΔ7 SMA mice. Vectorial movement latency is the amount the time it takes a neonatal mouse to move in one direction a distance that is greater than its body length (Butchbach et al. (2007) Neurobiol Dis 27:207-219). Vectorial movement latency was significantly reduced in Cpd. 1-treated SMNΔ7 SMA mice relative to vehicle-treated SMNΔ7 SMA mice at PND11 and PND14 (FIG. 8B). Spontaneous locomotor activity—measured by counting the number of grids crossed in an area within 1 min—was significantly improved in Cpd. 1-treated SMNΔ7 SMA mice at PND11 (FIG. 8C). Spontaneous locomotor activity was also greater in Cpd. 1-treated SMA mice than in vehicle-treated SMA mice but the difference is not statistically significant. Likewise, pivoting responses, another measure for spontaneous activity, were also ameliorated in Cpd. 1-treated SMNΔ7 SMA mice (FIG. 8D). Taken together, these behavioral observations showed that treatment of SMNΔ7 SMA mice with Cpd. 1 prior to motor neuron loss partially ameliorated the SMA motor phenotype.

[0203] SMNΔ7 SMA mice have a progressive loss of motor neurons in the lumbar spinal cord starting at ~PND09 (*e.g.*, Le et al. (2005) Hum Mol Genet 14:845-857). The effect of Cpd. 1 administration on the number of motor neurons in the lumbar spinal cord was examined. Dosing of SMNΔ7 SMA mice began at PND04 and continued daily until PND11. At PND11, vehicle-treated SMNΔ7 SMA mice had ~41% fewer motor neurons in the lumbar spinal cord than carrier mice (FIG. 8E); Cpd. 1-treated SMNΔ7 SMA mice, however, had motor neuron counts similar to those observed in carrier mice. Treatment of SMNΔ7 SMA mice with Cpd. 1 starting at PND04 delayed the loss of motor neurons at PND11.

Example 7. Prenatal Administration of C5-Substitued Quinazoline Further Improved Survival of SMNΔ7 SMA Mice

[0204] For prenatal drug administration, carrier dams received either Cpd. 1 (0.3-60 mg/kg/d for MTD and 3 mg/kg/d for survival analysis) or vehicle via oral administration (*e.g.*, Butchbach et al. (2007) J. Neurosci. Methods 161:285-290) beginning at embryonic day 11.5 (ED11.5). Treatment lasted for 5 days for the MTD analysis at which time the dams were euthanized 1 hour after final dosing and the brains of the feti were dissected and rapidly frozen in liquid nitrogen. Tail snips from the feti were also taken for genotyping. Additionally, the forebrains of the dams were dissected and rapidly frozen in liquid nitrogen. For survival analysis, dosing continued

through birth and the resultant pups were treated with or vehicle via oral administration beginning at PND02 until death; dosing of the dam ceased after PND01.

[0205] Transgenic mouse studies have shown that increasing SMN levels in prenatal SMA mouse neurons (beginning at embryonic day 13) ameliorated the motor phenotype of SMA mice (*e.g.*, Gavrilina et al. (2008) Hum Mol Genet 17:1063-1075). In order to assess whether prenatal administration of Cpd. 1 would improve the survival and motor phenotype of SMNΔ7 SMA mice, whether this compound can cross the placental barriers to reach the CNS of the fetal mice was first determined. Timed-pregnant SMNΔ7 carrier dams were treated orally with differing doses of Cpd. 1 (0-60 mg/kg/d) beginning at embryonic day 11 (ED11). After 5 days of treatment, the prenatal pups were dissected and analyzed for Cpd. 1 drug levels. As shown in FIG. 9A, there was a dose-dependent increase in Cpd. 1 levels in fetal brain extracts suggesting that this quinazoline can traverse the placental barriers to reach the fetal CNS. mSmn promoter activity, i.e. β-galactosidase expression, was also examined in these fetal brain samples. In fetal brain extracts, β-galactosidase activity was greater in Cpd. 1-treated mice at all doses tested but there was not a dose-dependent response during this dose range (FIG. 9B).

[0206] As Cpd. 1 was able to penetrate the blood-brain barrier, the effect of Cpd. 1 on survival of SMNΔ7 SMA mice when the drug was administered during prenatal development was determined. SMNΔ7 SMA mice received either Cpd. 1 (3 mg/kg/d) or vehicle beginning at ED11. Treatment continued through birth and was orally administered once the pups were born. Prenatal administration of Cpd. 1 (n=16) increased the average lifespan of SMNΔ7 SMA mice by 30% when compared to vehicle-treated (n=17) SMA mice (FIG. 10A, 17.3±0.9 days vs. 13.3±0.6 days; χ^2=14.973, p<0.001). If, however, Cpd. 1 was discontinued after birth, there was no significant difference in average lifespan between prenatally Cpd. 1-treated (n=16) SMNΔ7 SMA mice and vehicle-treated mice (FIG. 10A, 14.1±0.6 days vs. 13.3±0.6 days; χ^2=0.26, p=0.61). Although prenatal and postnatal administration of Cpd. 1 increased survival of SMNΔ7 SMA mice to a greater extent than only postnatal Cpd. 1 administration, the difference was not statistically significant (χ^2=2.577; p=0.108). Prenatal and postnatal administration of Cpd. 1 to SMNΔ7 SMA mice delayed the onset of body mass loss by 15% (FIG. 10B, 12.1±0.4 d vs. 10.5±0.3 d; χ^2=9.016, p=0.003). Treating SMNΔ7 SMA mice with Cpd. 1 only during the

prenatal period resulted in a small delay (10%) in the onset of body mass loss (FIG. 10B, 11.6±0.2 d vs. 10.5±0.3 d; χ^2=5.056, p=0.025). SMNΔ7 SMA mice treated prenatally and postnatally with Cpd. 1 tended to have larger body masses than those of vehicle-treated SMNΔ7 SMA mice, especially after PND11 (FIG. 10C). The effect of treatment of SMNΔ7 SMA mice with Cpd. 1 beginning at ED11 on motor phenotype was similar to that observed for SMNΔ7 SMA mice treated with Cpd. 1 beginning at PND04 (data not shown). Collectively, these observations showed that prenatal administration of Cpd. 1 to SMNΔ7 SMA mice improved their survival and motor phenotype.

Example 8. Therapeutic Window of Opportunity for Protective Effects of SMN2 Induction by C5-Substitued Quinazoline

[0207] Increasing SMN expression in the spinal cord improved the phenotype of mouse models of SMA. Even a low increase in SMN protein impacted the SMA phenotype in mice. The protective effect of Cpd. 1-mediated SMN induction was similar in magnitude to that observed with lentivirus-driven SMN gene replacement in SMNΔ7 SMA mice (*e.g.*, Azzouz et al. (2004) J. Clin. Invest. 114:1726-1731). Treatment of SMA mice beginning at PND04 had a marked effect on phenotype, and prenatal administration of this compound had a larger effect on survival of SMNΔ7 SMA mice. If treatment with Cpd. 1 began after the onset of motor neuron loss in SMNΔ7 SMA mice, there was no improvement in motor phenotype or survival. Prenatal treatment alone did not result in improved survival. Severe SMA mice that also harbor a SMN transgene driven by the prion promoter—which activates expression in neuron during early prenatal development (ED13)—showed no signs of neurodegeneration (Gavrilina et al. (2008) Hum. Mol. Genet. 17:1063-1075). Treatment of SMNΔ7 SMA mice with trichostatin A (TSA) improved survival of these mice if treatment began prior to motor neuron loss (Avila et al. (2007) J. Clin. Invest. 117:659-671; Narver, et al. (2008) Ann. Neurol. 64:465-470). These observations suggest that there is an optimal window of therapeutic opportunity for SMN induction in SMA (FIG. 11).

OTHER EMBODIMENTS

[0208] While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention,

which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method for increasing expression of SMN protein in a cell, the method comprising contacting a cell with a DcpS inhibitor in an amount effective to increase the expression of SMN protein in the cell.

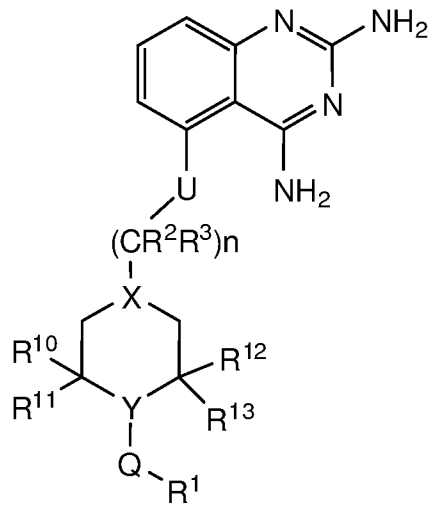
2. The method of claim 1, further comprising, prior to the contacting, determining whether the cell expresses DcpS.

3. The method of claim 1, wherein the compound inhibits the activity of a DcpS protein.

4. The method of claim 1, further comprising, after the contacting, detecting whether an increase in SMN expression has occurred.

5. The method of claim 1, wherein the SMN is a human SMN.

6. The method of claim 1, wherein the DcpS inhibitor is a compound having the following structural formula:



wherein:

U is O or a bond directly from the quinazoline ring to X;

n = an integer from 0 – 3;

R^2 and R^3 are each independently H or lower alkyl;

n is 0, 1, 2, or 3;

X and Y are each independently CH or N, so that the ring that contains X and Y is cyclohexane, piperidine, or piperazine;

R^{10}, R^{11}, R^{12}, and R^{13} are independently H or alkyl;

R^{10} and R^{11} together can be =O, and independently R^{12} and R^{13} together can be =O, so that the ring containing X and Y can be glutarimide, or piperidinone;

Q is selected from the group consisting of: $-CR^{14}R^{15}-$, $-C(=O)-$, $-SO_2-$, $-CH_2C(=O)-$, $-C(=O)CH_2S-$, and a direct bond from N to R^1, wherein R^{14} and R^{15} are each independently H, or lower alkyl;

R^1 can be H, alkyl, alkoxy, carbocycle, substituted carbocycle, heteroaryl or substituted heteroaryl, heterocyclyl or substituted heterocyclyl, fluoroalkyl, and alkoxyalkyl.

7. The method of claim 1, wherein the DcpS inhibitor comprises the structure of any one of the compounds depicted in Table 1.

8. The method of claim 1, wherein the DcpS inhibitor comprising the structure of Cpd. 1.

9. The method of claim 1, wherein the cell is an animal cell.

10. The method of claim 1, wherein the cell is a mammalian cell.

11. The method of claim 1, wherein the cell is a human cell.

12. The method of claim 1, wherein the contacting occurs *in vitro*.

13. The method of claim 1, wherein the contacting occurs *in vivo*.

14. A method for treating an SMA condition in a subject, the method comprising administering to the subject a compound that inhibits DcpS.

15. The method of claim 14, wherein the SMA condition is selected from the group consisting of infantile SMA (Werdnig-Hoffmann disease); severe infantile SMA; intermediate SMA; juvenile SMA (Kugelberg-Welander disease); and adult SMA.

16. The method of claim 14, wherein the subject is a human.

17. The method of claim 14, wherein the compound inhibits the expression of the DcpS.

18. The method of claim 14, wherein the compound inhibits the activity of the DcpS.

19. The method of claim 14, further comprising determining whether the subject has an SMA condition.

20. The method of claim 19, wherein the determining comprises detecting whether the subject has one or more copies of the SMN gene.

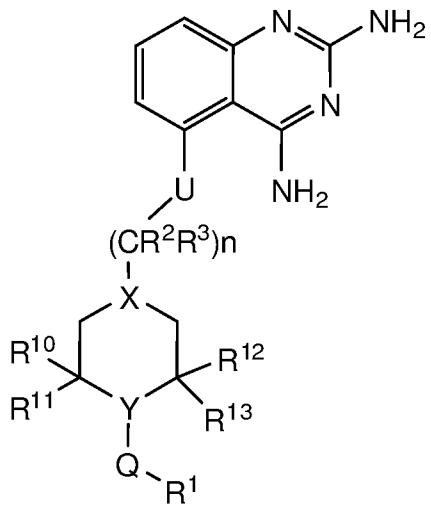
21. The method of claim 14, further comprising administering one or more additional therapeutic agents to the subject.

22. The method of claim 14, wherein the one or more additional agents are selected from the group consisting of butyrates, valproic acid, hydroxyurea, and riluzole.

23. The method of claim 14, further comprising, after administering the compound to the subject, detecting whether inhibition of the DcpS by the compound occurred.

24. The method of claim 14, wherein the DcpS is a human form of DcpS.

25. The method of claim 14, wherein the compound has the following structural formula:



wherein:

U is O or a bond directly from the quinazoline ring to X;

n = an integer from 0 – 3;

R^2 and R^3 are each independently H or lower alkyl;

n is 0, 1, 2, or 3;

X and Y are each independently CH or N, so that the ring that contains X and Y is cyclohexane, piperidine, or piperazine;

R^{10}, R^{11}, R^{12}, and R^{13} are independently H or alkyl;

R^{10} and R^{11} together can be =O, and independently R^{12} and R^{13} together can be =O, so that the ring containing X and Y can be glutarimide, or piperidinone;

Q is selected from the group consisting of: $-CR^{14}R^{15}-$, $-C(=O)-$, $-SO_2-$, $-CH_2C(=O)-$, $-C(=O)CH_2S-$, and a direct bond from N to R^1, wherein R^{14} and R^{15} are each independently H, or lower alkyl;

R^1 can be H, alkyl, alkoxy, carbocycle, substituted carbocycle, heteroaryl or substituted heteroaryl, heterocyclyl or substituted heterocyclyl, fluoroalkyl, and alkoxyalkyl.

26. The method of claim 14, wherein the compound comprises the structure of any one of the compounds depicted in Table 1.

27. The method of claim 14, wherein the compound comprises the structure of Cpd. 1.

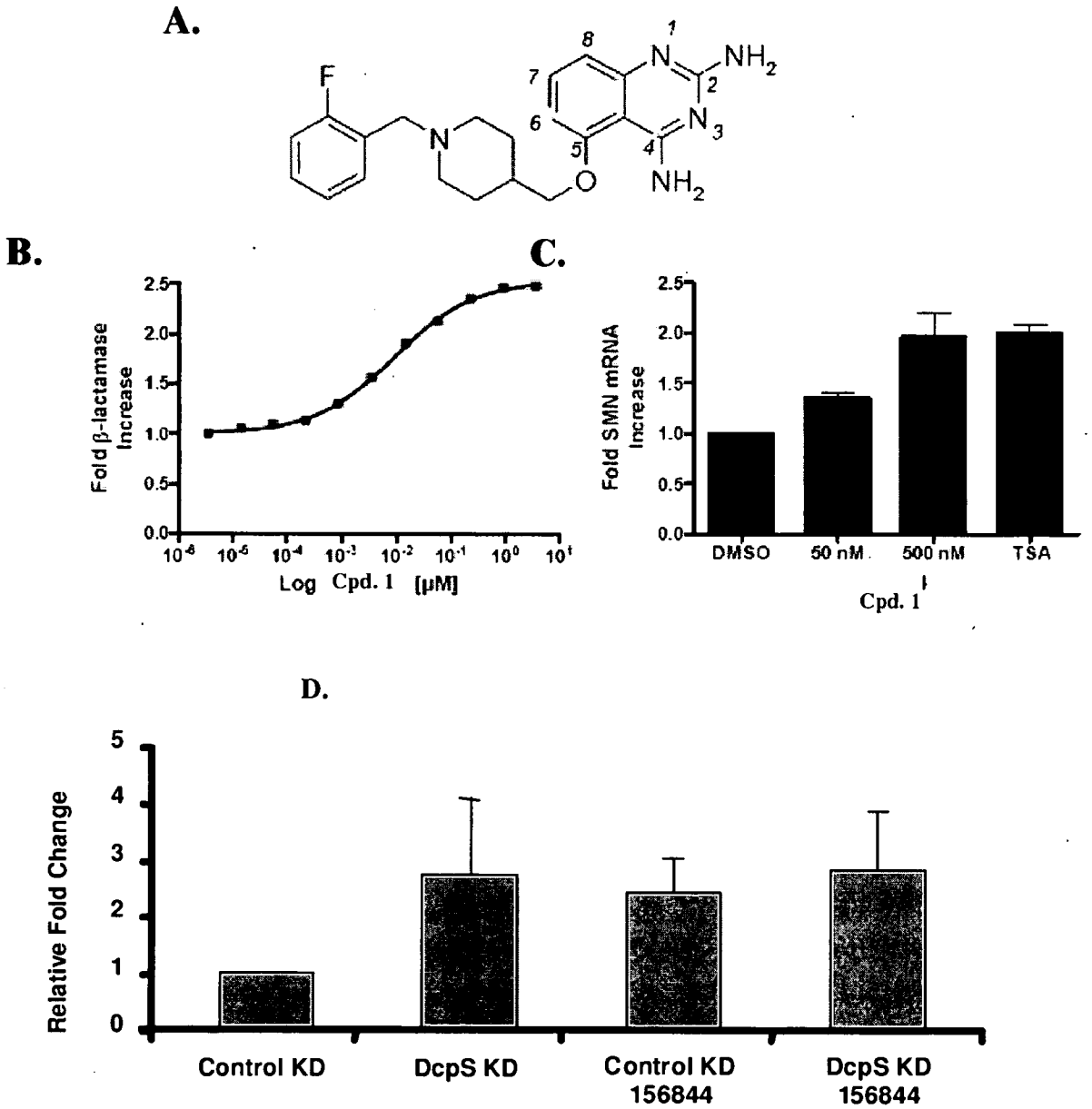

A.
F
N
O
NH2
NH2
N3
B.
Fold β-lactamase Increase
Log Cpd. 1 [μM]
C.
Fold SMN mRNA Increase
DMSO
50 nM
500 nM
TSA
Cpd. 1
D.
Relative Fold Change
Control KD
DcpS KD
Control KD 156844
DcpS KD 156844

FIG. 1A – 1D

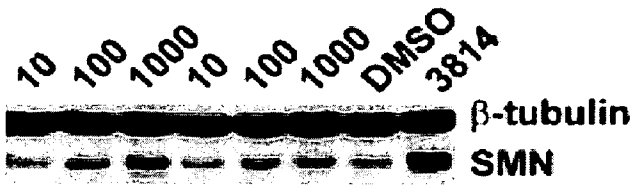

Cpd. 1
Cpd. 12
10
100
1000
10
100
1000
DMSO
3814
β-tubulin
SMN

FIG. 2

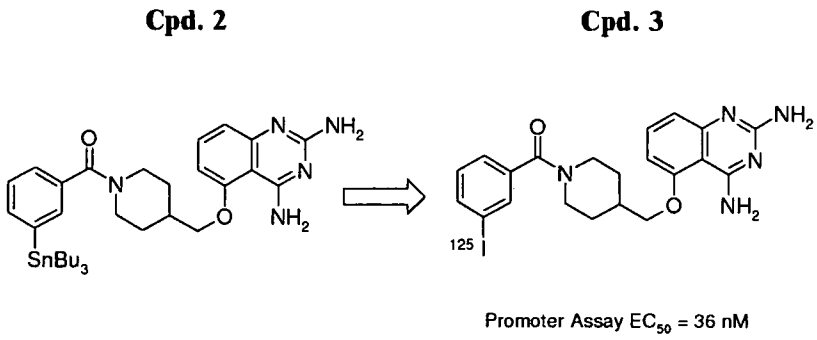

Cpd. 2
Cpd. 3
SnBu3
125
Promoter Assay EC50 = 36 nM

FIG. 3

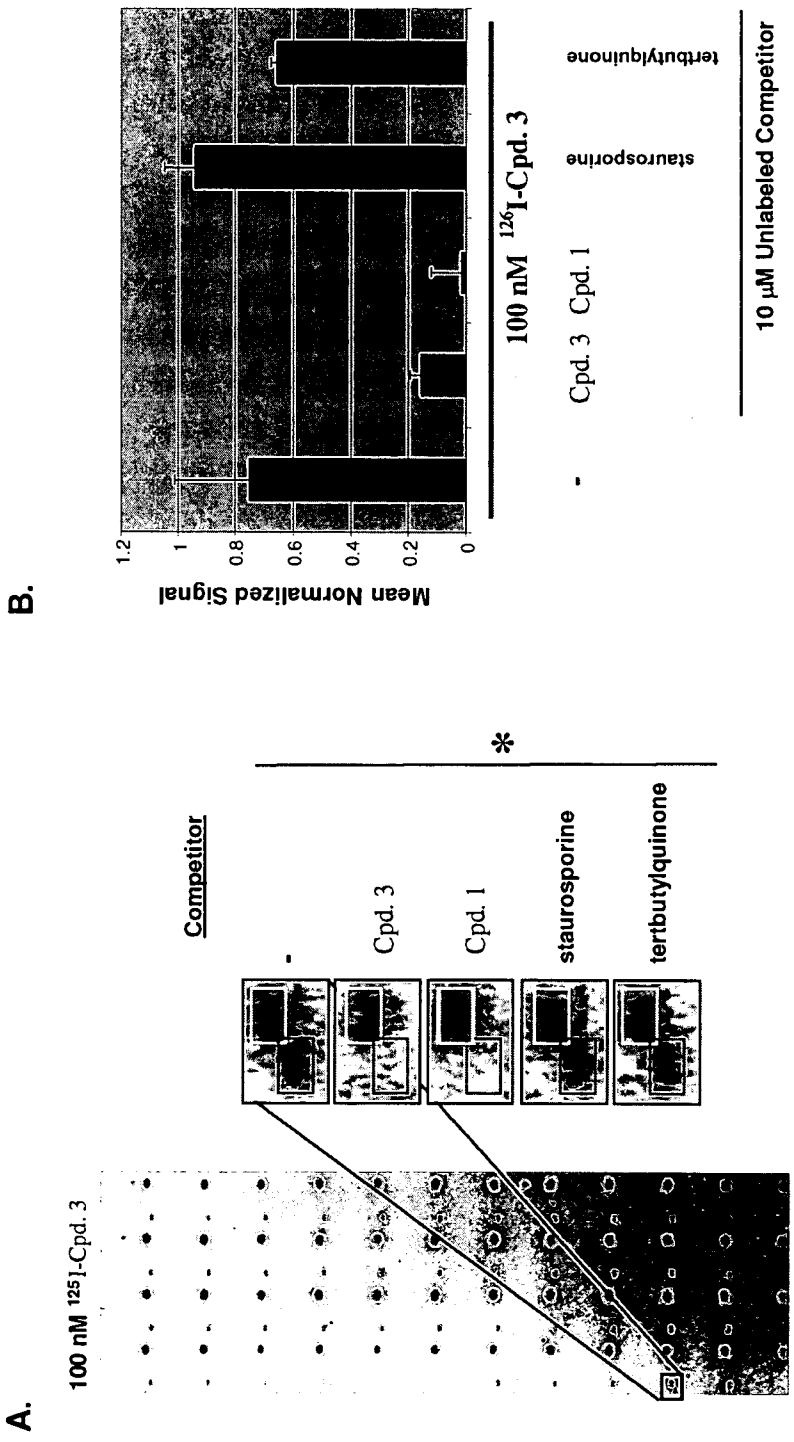

A.
100 nM 125I-Cpd. 3
Competitor
-
Cpd. 3
Cpd. 1
staurosporine
tertbutylquinone
*
* = 100 nM 126I-Cpd. 3
B.
Mean Normalized Signal
1.2
1
0.8
0.6
0.4
0.2
0
100 nM 126I-Cpd. 3
- Cpd. 3 Cpd. 1 staurosporine tertbutylquinone
10 µM Unlabeled Competitor

FIG. 4A, 4B

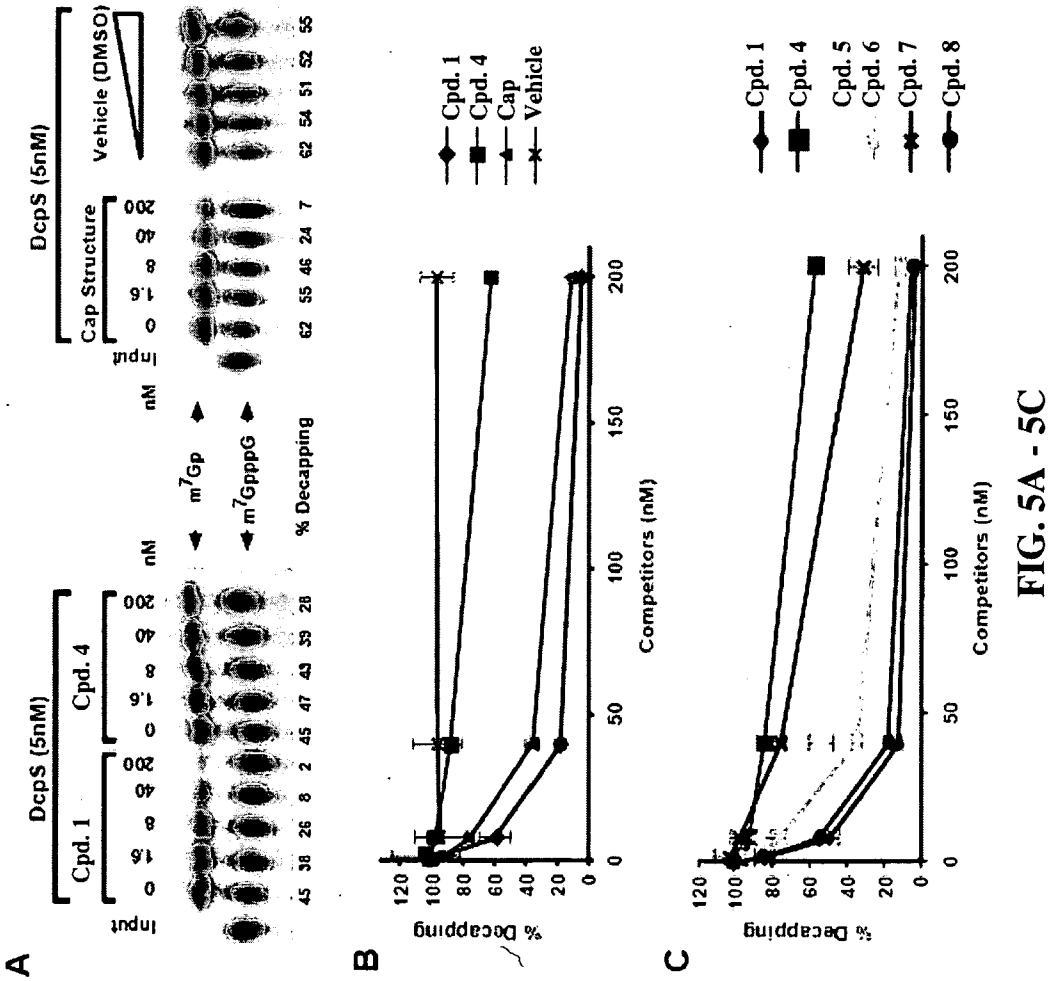

A
DcpS (5nM)
Cpd. 1
Cpd. 4
Input
nM
0
1.6
8
40
200
m7Gp
m7GpppG
% Decapping
45 36 26 8 2 45 47 43 39 28
DcpS (5nM)
Cap Structure
Vehicle (DMSO)
62 55 46 24 7 62 54 51 52 55
B
% Decapping
Competitors (nM)
Cpd. 1
Cpd. 4
Cap
Vehicle
C
% Decapping
Competitors (nM)
Cpd. 1
Cpd. 4
Cpd. 5
Cpd. 6
Cpd. 7
Cpd. 8

FIG. 5A - 5C

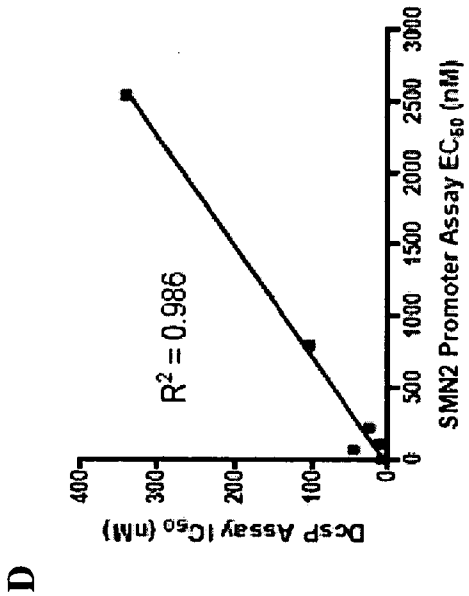

D
DcsP Assay IC50 (nM)
400
300
200
100
0
0
500
1000
1500
2000
2500
3000
SMN2 Promoter Assay EC50 (nM)
R2 = 0.986

FIG. 5D

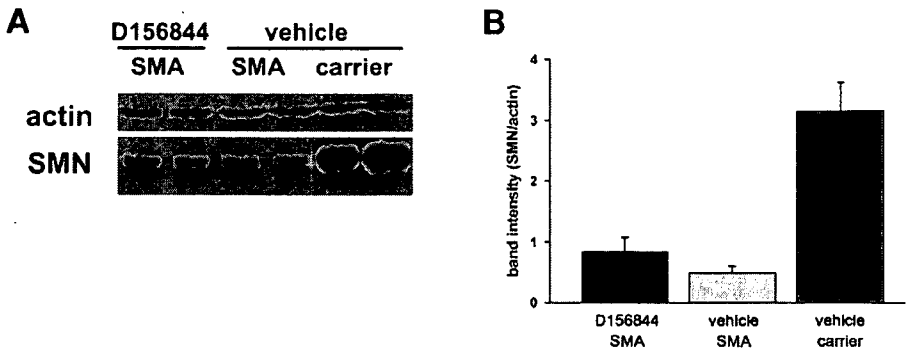

A
D156844
vehicle
SMA
SMA
carrier
actin
SMN
B
band intensity (SMN/actin)
0
1
2
3
4
D156844 SMA
vehicle SMA
vehicle carrier

FIG. 6A, 6B

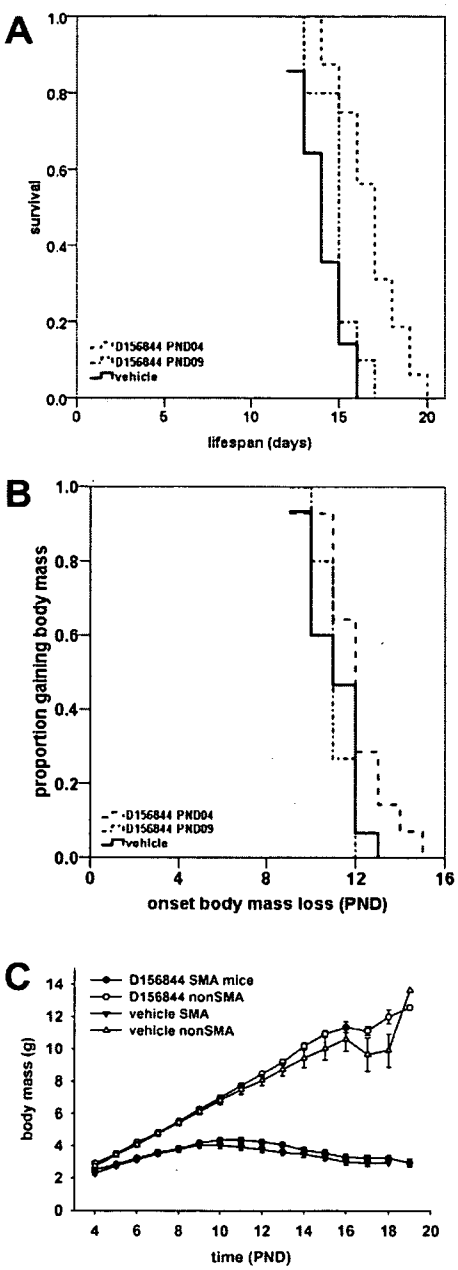

A
survival
lifespan (days)
D156844 PND04
D156844 PND09
vehicle
B
proportion gaining body mass
onset body mass loss (PND)
D156844 PND04
D156844 PND09
vehicle
C
body mass (g)
time (PND)
D156844 SMA mice
D156844 nonSMA
vehicle SMA
vehicle nonSMA

FIG. 7A - 7C

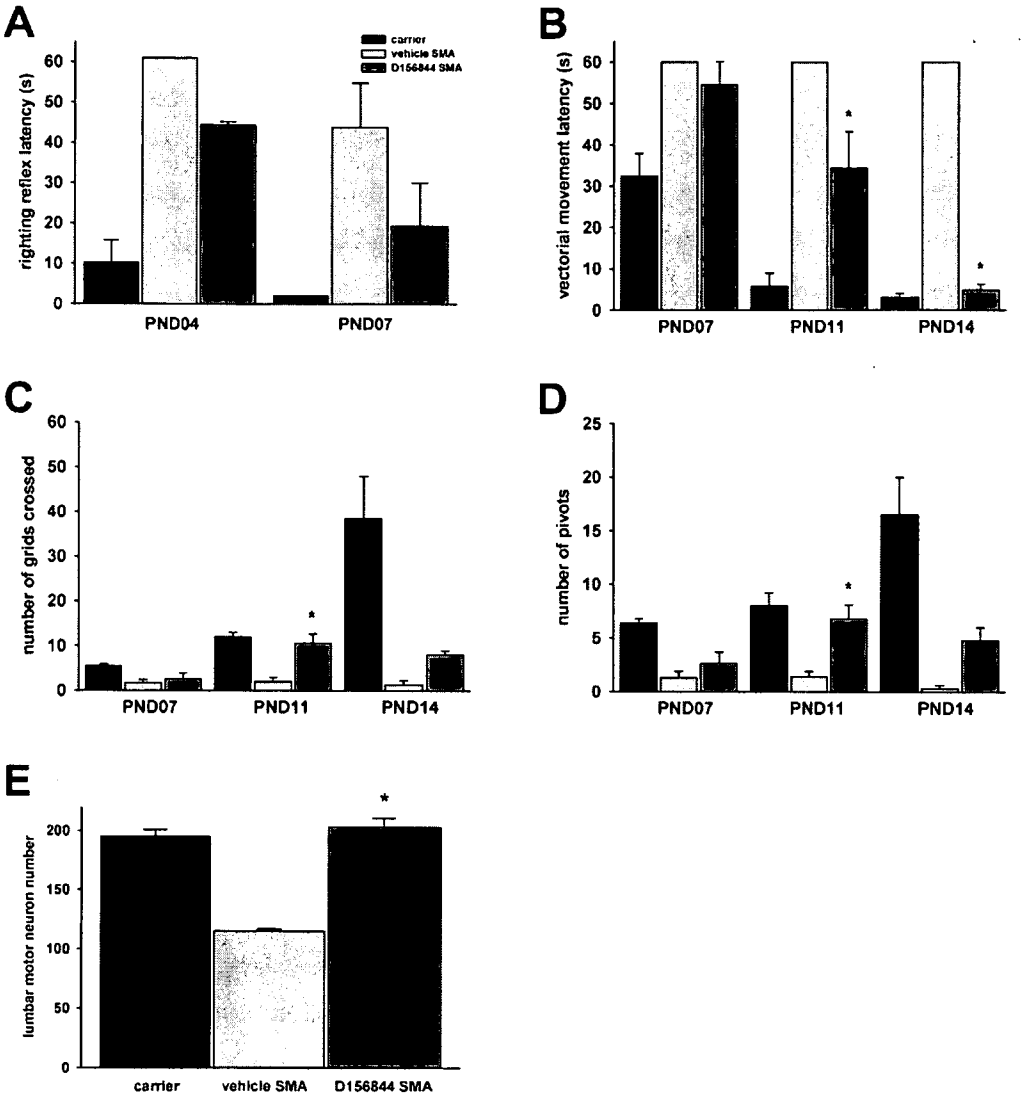

A
carrier
vehicle SMA
D156844 SMA
righting reflex latency (s)
0
10
20
30
40
50
60
PND04
PND07
B
vectorial movement latency (s)
0
10
20
30
40
50
60
*
*
PND07
PND11
PND14
C
number of grids crossed
0
10
20
30
40
50
60
*
PND07
PND11
PND14
D
number of pivots
0
5
10
15
20
25
*
PND07
PND11
PND14
E
lumbar motor neuron number
0
50
100
150
200
*
carrier
vehicle SMA
D156844 SMA

FIG. 8A – 8E

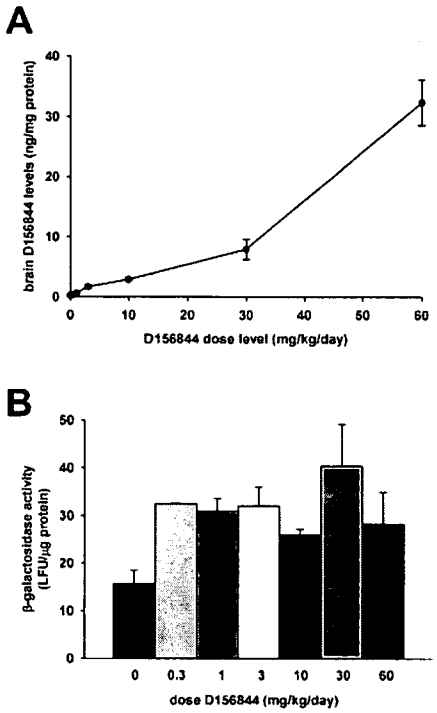

A
brain D156844 levels (ng/mg protein)
0
10
20
30
40
0
10
20
30
40
50
60
D156844 dose level (mg/kg/day)
B
β-galactosidase activity (LFU/μg protein)
0
10
20
30
40
50
0
0.3
1
3
10
30
60
dose D156844 (mg/kg/day)

FIG. 9A, 9B

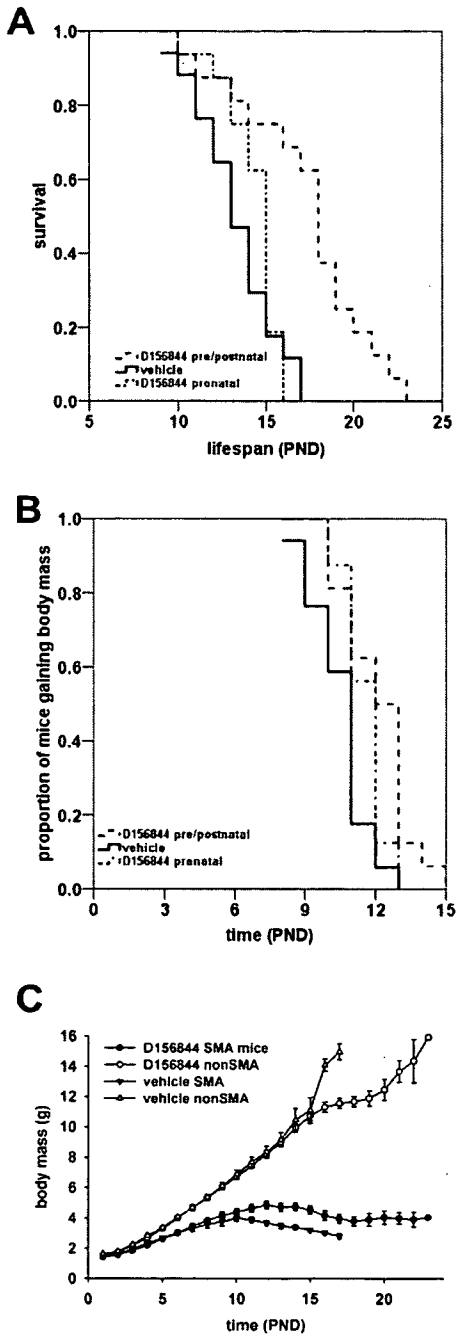

A
survival
lifespan (PND)
D156844 pre/postnatal
vehicle
D156844 prenatal
B
proportion of mice gaining body mass
time (PND)
D156844 pre/postnatal
vehicle
D156844 prenatal
C
body mass (g)
time (PND)
D156844 SMA mice
D156844 nonSMA
vehicle SMA
vehicle nonSMA

FIG. 10A - 10C

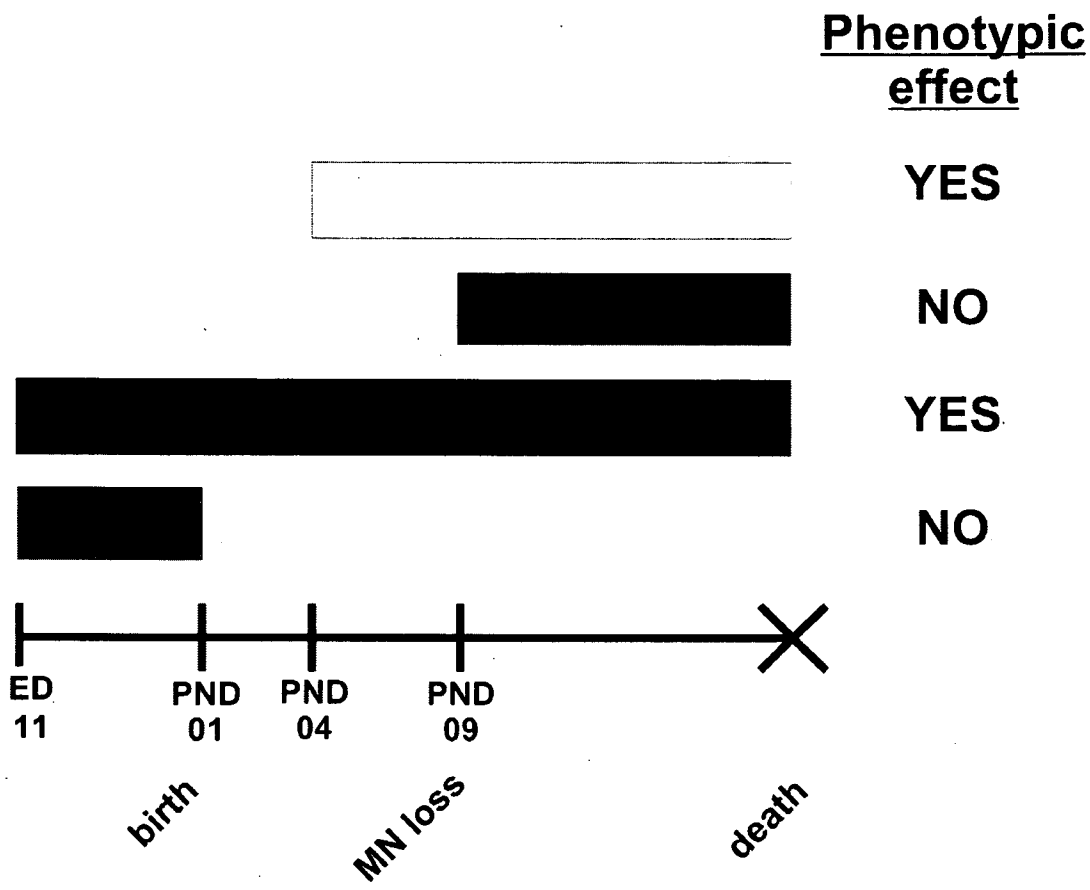

Phenotypic effect
YES
NO
YES
NO
ED 11
PND 01
PND 04
PND 09
birth
MN loss
death

FIG. 11