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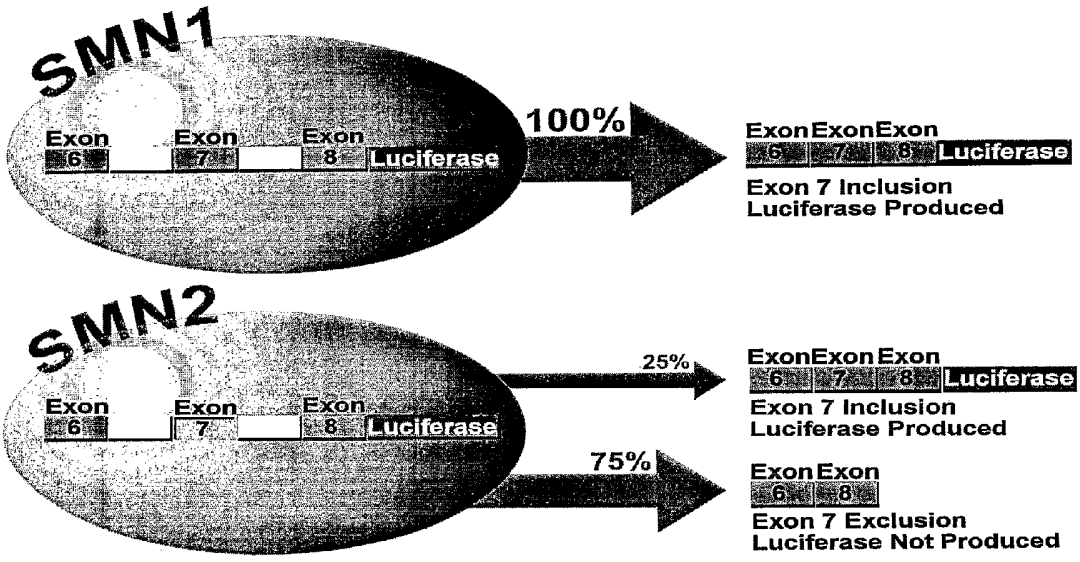
(54) **Title:** METHODS AND COMPOSITIONS TO TREAT MOTOR NEURON DISEASE



(57) **Abstract:** The invention features compositions and methods for treating motor neuron diseases, such as spinal muscular atrophy and amyotrophic lateral sclerosis. The compositions and methods include derivatives of indoprofen.

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METHODS AND COMPOSITIONS TO TREAT MOTOR NEURON DISEASE

TECHNICAL FIELD

This invention relates to compositions and methods for treating motor neuron diseases and related disorders.

BACKGROUND OF THE INVENTION

Motor neuron diseases (MNDs) are progressive, degenerative disorders that affect nerves in the upper or lower parts of the body. MNDs include spinal muscular atrophy (SMA), amyotrophic lateral sclerosis (ALS), progressive muscular atrophy, and postpolio syndrome. Generally, symptoms of MNDs may include difficulty swallowing, limb weakness, slurred speech, impaired gait, facial weakness, and muscle cramps. Respiration may be affected in the later stages of these diseases.

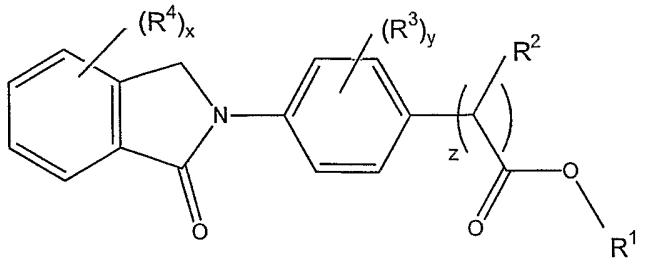
SMA is an autosomal recessive disorder characterized by the degeneration of α-motor neurons of the spinal cord anterior horn, which leads to progressive muscular atrophy, paralysis, respiratory failure and infant death. The most severe form of SMA, called Werdnig-Hoffman syndrome (SMA type I) and the two milder forms (types II and III) are caused by deletions or mutations of the Survival Motor Neurons 1 (SMN1) gene.

ALS, also called Lou Gehrig's disease, occurs when specific nerve cells in the brain and spinal cord that control voluntary movement gradually degenerate. The loss of these motor neurons causes the muscles under their control to weaken and waste away, leading to paralysis. Deletions of the SMN2 have been linked to the development and progression of some forms of ALS.

SUMMARY OF THE INVENTION

The invention features compositions and methods for treating MNDs, such as SMA and ALS. The compositions and methods include derivatives of indoprofen.

One aspect of the invention features compounds having the structure of formula I:



Formula I

In some embodiments, each of R^4 and R^3 of Formula I is independently an optionally substituted C_1-C_{12} alkyl, an optionally independently substituted C_1-C_{12} alkenyl, an optionally independently substituted C_1-C_{12} alknyl, an optionally independently substituted C_1-C_{12} aryl, an optionally independently substituted C_1-C_{12} cycloaryl, –OH or a halogen; x is 0, 1, 2, 3 or 4; y is 0, 1, 2, 3 or 4; z is 1, 2, 3, or 4 and each R^2 is independently an optionally independently substituted C_1-C_{12} alkyl, an optionally independently substituted C_1-C_{12} alkenyl, an optionally independently substituted C_1-C_{12} alknyl, an optionally independently substituted C_1-C_{12} aryl, or an optionally independently substituted C_1-C_{12} cycloaryl; and R^1 is an optionally independently substituted C_1-C_{12} alkyl, an optionally independently substituted C_1-C_{12} alkenyl, an optionally independently substituted C_1-C_{12} alknyl, an optionally independently substituted C_1-C_{12} aryl, or an optionally independently substituted C_1-C_{12} cycloaryl. The invention also features pharmaceutically acceptable salts of the compounds having these structures.

In some embodiments, R^4 and R^3 are not substituted, or R^4 and R^3 are independently an optionally independently substituted C_1-C_{12} alkyl, an optionally independently substituted C_1-C_6 alkenyl, an optionally independently substituted C_1-C_{12} alknyl, an optionally independently substituted C_1-C_6 aryl, an optionally independently substituted C_1-C_6 cycloaryl, –OH or a halogen. Each of R^4 and R^3 can independently be a halogen, such as chlorine or fluorine.

In some embodiments, both x and y of Formula I are 0.

In some embodiments, R^1 of Formula I is not substituted, or R^1 is substituted with -OH or a halogen.

In other embodiments, n of Formula I is 1 and R^2 is a C_1 or C_2 alkyl. Further, R^2 of Formula I may or may not be substituted.

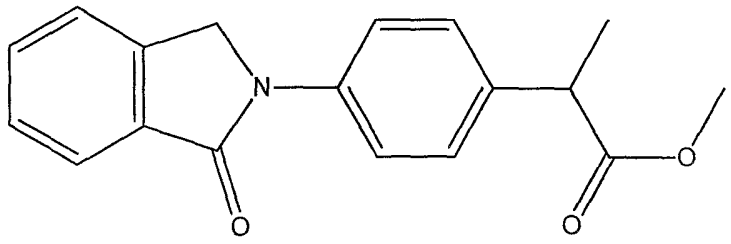
In some embodiments, R^1 of Formula I is a straight chain C_1 to C_6 alkyl.

In other embodiments, R^1 is a branched chain C_1 to C_6 alkyl.

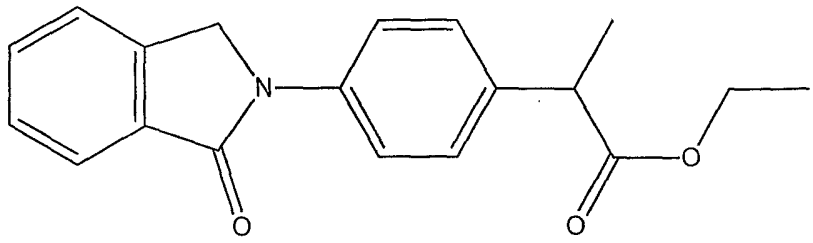
In yet other embodiments, R^1 is any of $-CH_3$, $-CH_2CH_3$, $-CH(CH_3)_2$ any $-C(CH_3)_3$, optionally independently singly or multiply substituted with one or more –OH or halogen.

In some embodiments, R^2 is $-CH_3$, n is 1 and R^1 is any of $-CH_3$, $-CH_2CH_3$, $-CH(CH_3)_2$ and $-C(CH3)_3$. In addition, in some embodiments, R^4 and R^3 are both H.

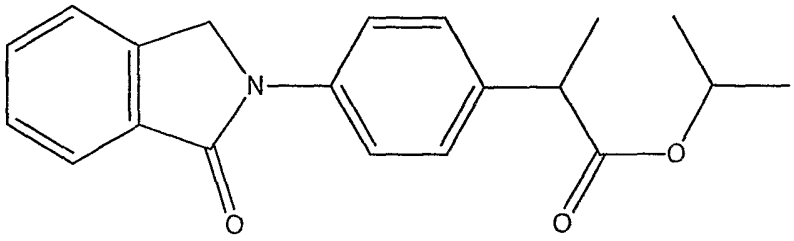
Some compounds featured in the invention and having the structure of formula I are methyl, ethyl or isopropyl ester derivatives of indoprofen and have the structure of Formula II, III, or IV, below, respectively.



Formula II



Formula III



Formula IV

Also featured in the invention are methods of synthesizing ester derivatives of indoprofen, including but not limited to methyl, ester, isopropyl, or tert-butyl esters of indoprofen. The methods generally include mixing an alcohol, such as methyl, ethyl, or isopropyl alcohol, with indoprofen in the presence of an organic solvent (e.g., chloroform, polypropylene glycol, or ethanolamine) in an acidic solution. The mixture is refluxed in solution at about 50°C for about 2 to 3 hours. The method can further include monitoring the synthesis reaction by performing an assay, such as thin layer chromatography, on a sample of the solution.

The synthesis methods featured in the invention can also include purifying the ester derivative of indoprofen by extracting the solution at least once with a mixture of an organic solvent (e.g., methylene chloride), and sodium bicarbonate, extracting the solution at least once with water, and evaporating the organic solvent.

Certain compounds described herein may exist in stereoisomeric forms such as enantiomers, diastereomers and mixtures thereof. Mixtures can be separated into stereoisomerically pure constituents. Certain compounds described herein may be tautomeric, and the invention encompasses the various tautomeric mixtures.

Synthesis reaction products including the ester derivatives can be evaluated, such as for purity. For example, the mass of ester derivatives synthesized by the methods feature in the invention can be verified by liquid chromatography-mass spectrometry analysis.

As used herein, the term "halo" or "halogen" refers to any radical of fluorine, chlorine, bromine or iodine.

The term "alkyl" refers to a hydrocarbon chain that may be a straight chain or branched chain, containing the indicated number of carbon atoms. For example, C_1-C_{12} alkyl indicates that the group may have from 1 to 12 (inclusive) carbon atoms in it (i.e., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12) and includes methyl, ethyl, n-propyl, n-butyl, i-propyl, i-butyl, t-butyl, s-butyl, n-pentyl, i-pentyl, t-pentyl, neo-pentyl, n-hexyl, and i-hexyl. The term "haloalkyl" refers to an alkyl in which one or more hydrogen atoms are replaced by halo, and includes alkyl moieties in which all hydrogens have been replaced by halo (e.g., perfluoroalkyl). The terms "arylalkyl" or "aralkyl" refer to an alkyl moiety in which an alkyl hydrogen atom is replaced by an aryl group. Examples of "arylalkyl" or "aralkyl" include benzyl and 9-fluorenyl groups.

The term "alkenyl" refers to a hydrocarbon chain that may be a straight chain or branched chain, containing the indicated number of carbon atoms and at least one double bond. For example, C_1-C_{12} alkenyl indicates that the group may have from 1 to 12 (inclusive) carbon atoms in it (i.e., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12), e.g., ethenyl, i-propenyl, n-butenyl, i-butenyl, allyl, 1,3-butadienyl. Where there are two double bonds or more than two double bonds they can be conjugated or non-conjugated.

The term "alknyl" refers to a hydrocarbon chain that may be a straight chain or branched chain, containing the indicated number of carbon atoms and at least one triple bond. For example, C_1-C_{12} alknyl indicates that the group may have from 1 to 12 (inclusive) carbon atoms in it (i.e., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12).

The terms "alkylamino" and "dialkylamino" refer to –NH(alkyl) and –N(alkyl)$_2$ radicals respectively. The term "aralkylamino" refers to a –NH(aralkyl) radical. The term "alkoxy" refers to an -O-alkyl radical. The term "mercapto" refers to an SH radical. The term "thioalkoxy" refers to an -S-alkyl radical.

The term "aryl" refers to an aromatic monocyclic, bicyclic, or tricyclic hydrocarbon ring system, wherein any ring atom capable of substitution can be substituted by a substituent. Examples of aryl moieties include, but are not limited to, phenyl, naphthyl, and anthracenyl.

The term "cycloalkyl" as employed herein includes saturated monocyclic, bicyclic, tricyclic, or polycyclic hydrocarbon groups having 3 to 12 carbons, wherein any ring atom capable of substitution can be substituted by a substituent. Examples of cycloalkyl moieties include, but are not limited to, cyclopentyl, norbornyl, and adamantyl.

The term "acyl" refers to an alkylcarbonyl, cycloalkylcarbonyl, arylcarbonyl, heterocyclylcarbonyl, or heteroarylcarbonyl substituent, any of which may be further substituted by substituents.

The term "oxo" refers to an oxygen atom, which forms a carbonyl when attached to carbon, an N-oxide when attached to nitrogen, and a sulfoxide or sulfone when attached to sulfur.

The term "substituents" refers to a group "substituted" on an alkyl, cycloalkyl, alkenyl, alkynyl, heterocyclyl, heterocycloalkenyl, cycloalkenyl, aryl, or heteroaryl group at any atom of that group. Suitable substituents include, without limitation, alkyl, alkenyl, alkynyl, alkoxy, acyloxy, halo, hydroxy, cyano, nitro, amino, SO_3H, sulfate, phosphate, perfluoroalkyl, perfluoroalkoxy, methylenedioxy, ethylenedioxy, carboxyl, oxo, thioxo, imino (alkyl, aryl, aralkyl), $S(O)_n$alkyl (where n is 0-2), $S(O)_n$ aryl (where n is 0-2), $S(O)_n$ heteroaryl (where n is 0-2), $S(O)_n$ heterocyclyl (where n is 0-2), amine (mono-, di-, alkyl, cycloalkyl, aralkyl, heteroaralkyl, and combinations thereof), ester (alkyl, aralkyl, heteroaralkyl), amide (mono-, di-, alkyl, aralkyl, heteroaralkyl, and combinations thereof), sulfonamide (mono-, di-, alkyl, aralkyl, heteroaralkyl, and combinations thereof), unsubstituted aryl, unsubstituted heteroaryl, unsubstituted heterocyclyl, and unsubstituted cycloalkyl. In one aspect, the substituents on a group are independently any one single, or any subset of the aforementioned substituents.

The invention features methods for treating an individual having or at risk for developing an MND, such as SMA or ALS, by administering a compound having the structure of Formula I, II, III, or IV. Individuals suitable for the treatments methods may have a deletion of or a mutation in one or both alleles of the SMN1 or SMN2

genes. Administration of a compound featured in the invention may lead to in an increase in full-length SMN protein levels.

The treatment methods featured in the invention can include diagnosing the individual as having or at risk for developing a motor neuron disease. For example, the diagnosing step can include providing a biological sample from the individual, where the biological sample contains a nucleic acid, and performing an assay to determine whether the nucleic acid includes a mutation of the SMN1 gene of the individual. The assay may include Northern blot analysis, polymerase chain reaction (PCR), or a restriction fragment length polymorphism (RFLP), ARMS™ (Newton *et al., Nucleic Acids Res.* 17:2503-16, 1989), or Amplification Refractory Mutation System Linear Extension (ALEX™) (Haque *et al., Diagn Mol Pathol.* 7:248-52, 1998) assay. If the nucleic acid includes a mutation in the SMN1 gene, such as a point mutation in exon 6, 7, or 8, or in an intron flanking exon 6 or 7, or a complete or partial deletion of the SMN1 gene, the individual can be diagnosed as having or at risk for developing SMA.

Diagnosis of an individual may include testing the nucleic acid in the biological sample for a mutation in the SMN2 gene, such as a deletion of a fragment of or the entire SMN2 gene. An individual determined to have a mutation in this gene may be diagnosed as having or at risk for developing ALS.

The invention also features pharmaceutical compositions including a compound having the structure of Formula I, II, III, or IV, and a pharmaceutically acceptable carrier.

Certain indoprofen derivatives may have advantages over other MND therapies. For example, our laboratory observed that indoprofen does not traverse the blood-brain barrier in mice (see Examples below). The presence of the carboxylic acid group on indoprofen contributes to a negative charge on the compound, and it is hypothesized that negatively charged compounds are generally less likely to traverse the blood-brain barrier (reviewed in Clark, *Drug Disc. Today*, 8:927-933, 2003). This carboxylic acid is eliminated in ester derivatives of indoprofen, such as the methyl, ethyl and isopropyl derivatives featured in the invention. Thus the ester derivatives of indoprofen are more likely to traverse the blood-brain barrier, and may therefore prove to increase the efficacy of indoprofen-derived compounds in MND therapy.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, useful methods and materials are described below. The materials, methods, and exemplification are illustrative only and not intended to be limiting. Other features and advantages of the invention will be apparent from the accompanying drawings and description, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic diagram illustrating the SMN1-luciferase (SMN1-luc) and SMN2-luciferase (SMN2-luc) used in a screen for compounds that can increase SMN2-Luc protein production, but not SMN1-Luc protein production. In vivo, SMN1 transcripts are spliced to include exons 6, 7, and 8 in a processed RNA. SMN2 transcripts are alternatively spliced such that 25% of processed RNA includes exons 6, 7, and 8, and 75% of processed RNA excludes exon 7.

FIG. 2A is the structure of indoprofen [4-(1,3-dihydro-1-oxo-isoindol-2-yl)-benzeneacetic acid].

FIG. 2B is the structure of ibuprofen.

FIG. 2C is the structure of ketoprofen.

FIG. 2D is the structure of suprofen.

FIG. 2E is the structure of aspirin.

FIG. 2F is the structure of acetominophen.

FIG. 2G is the structure of isoindolinone.

FIG. 2H is the structure of an esterified indoprofen.

FIG. 3A is a graph showing the effect of increasing doses of indoprofen on SMN1-luc and SMN2-luc minigene expression in stably transfected c33a cells.

FIG. 3B is a graph showing the effect of increasing doses of indoprofen-related compounds on SMN1-luc and SMN2-luc minigene expression in stably transfected c33a cells.

FIG. 3C is a Western blot analysis comparing SMN protein levels in Type I SMA patient fibroblasts ("13"), which express no SMN1 protein; carrier fibroblasts

("14"), which have 1 copy of the SMN1 gene, and NSC34 mouse neuronal cells ("34"), which have two copies of the SMN1 gene.

FIG. 3D is a Western blot analysis comparing SMN protein levels in indoprofen-treated cells and in control cells, which were not treated with indoprofen ("Ctrl"). Initiation factor, eIF-4e, was used as a loading control.

FIG. 3E is a graph illustrating the number of gems observed in indoprofen-treated type I SMA patient fibroblasts (2806) and untreated cells. Circles represent samples within each treatment. Squares represent the mean number of gems per 100 nuclei within each treatment (0 μM: 1.3; 5 μM: 6.5; and 15 μM: 8.3).

DETAILED DESCRIPTION

The present invention features methods and compositions for treating MNDs, such as SMA and ALS. In particular, a method is presented for treating MNDs using the small molecule indoprofen (FIG. 2A) and structural analogs of this compound. Indoprofen can be obtained commercially from Sigma-Aldrich (St. Louis, MO).

Indoprofen derivatives and methods of making indoprofen derivatives

The invention features indoprofen ester derivatives having the general structure of formula I illustrated above. The ester derivatives can be, for example, methyl, ethyl, isopropyl, or tert-butyl ester derivatives.

The invention also features methods of making the indoprofen ester derivatives featured in the invention. For example, an alcohol including the desired carbon chain derivative (e.g., methanol, ethanol, isopropanol, tert-butanol, and the like) can be mixed with indoprofen in the presence of an appropriate organic solvent, such as chloroform, propylene glycol, or ethanolamine, to dissolve the indoprofen. The solution can be refluxed with heat at about 40°C to about 60°C (e.g., about 45°C, 50°C, or 55°C) in acidic conditions for about 1 to about 24 hours, preferably from about 1 to about 4 hours (e.g., about 2 to about 3 hours). Acidic conditions can be achieved by adding an acid, such as sulfuric acid, to the solution. The exact amount of acid added to the solution to achieve acidic conditions is not essential. For example, one drop, e.g., about 1 to 100 μL , of sulfuric acid can be sufficient for making ester derivatives of indoprofen.

The reaction can be checked periodically to monitor progress of the reaction, until the reaction is completed. Various methods can be used to monitor the progress, including chromatographic methods such as thin layer chromatography, or ultraviolet or visible spectroscopy.

To purify the ester derivatives, the impurities are extracted out of the organic solution, such as with an organic solvent (e.g., methylene chloride), and sodium bicarbonate. The solution is mixed, such as by vortexing, and the phases allowed to separate. Separation of the phases can be facilitated by centrifugation. The aqueous layer is removed, and the organic layer (which contains the ester) is extracted at least one more time (e.g., one, two, or three more times) with sodium bicarbonate. In a final extraction step, to remove excess salt, water is added to the organic layer, the solution mixed and the layers allowed to separate. Finally, the solvent is evaporated, leaving the purified esters behind. The structure and purity of the esters can be confirmed by a number of methods known in the art, including, but not limited to mass spectrometry (e.g., liquid chromatography-mass spectrometry (LC-MS)), nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy, and ultraviolet or visible spectroscopy.

The racemic mixture of purified esters can be further purified by methods known in the art such as by the method described in Haynes *et al.* (*J. Chromatogra. A*, 803:261-271, 1998), and methods referenced therein.

Motor Neuron Disease and SMN Protein Activity

The compounds and methods featured in the invention can be used to treat MNDs, such as SMA, a disease caused by a mutant SMN1 gene. Normally, humans have two copies of the survival motor neurons SMN gene (SMN1 and SMN2). The two SMN genes differ by a translationally silent C to T mutation at nucleotide position 6 in exon 7 (codon 280) of SMN2, which alters the splicing pattern of the transcript. The SMN1 gene is primarily transcribed to generate mRNA transcripts that are spliced to encode full-length SMN protein, while SMN2 transcripts are alternatively spliced such that exons 5 and/or 7 are removed. The alternatively spliced SMN2 transcript encodes a truncated protein. The full-length proteins produced by the two genes are identical. Almost all of the protein produced from the SMN1 transcript is full-length SMN protein, while only about 30% of the protein produced from the SMN2 transcript is full-length.

SMA is caused by a deletion of or mutation in the SMN1 gene. One consequence of this deletion is the loss of full-length SMN protein synthesis from the SMN gene, thus providing that any full-length SMN protein is synthesized from SMN2. The result is a reduction in the total amount of SMN protein production. Homozygous deletions of the SMN2 gene have been associated with some cases of ALS.

In SMA type I patients, the SMN1 gene is deleted entirely and SMN2 is spliced such that only ~30% of mRNAs are full-length, and ~70% of spliced transcripts exclude exon 7. The full-length SMN2 gene product is sufficient to maintain fetal development, but infected individuals manifest the symptoms of SMA early in life and typically die as infants.

Full-length SMN protein expressed from SMN1 and SMN2 is present in the cytoplasm and in small subnuclear bodies, including gems and Cajal bodies (also called coiled bodies) in most normal cells. Full-length SMN has also been reported to interact with transcription factors, and the gem-associated profilins. In addition, SMN binds to the zinc-finger protein ZPR1, the function of which is unknown, but cytoplasmic ZPR1 translocates with SMN1 to the subnuclear bodies upon treatment of mammalian cells with mitogens. Truncated proteins translated from an alternatively spliced SMN2 transcript, SMN2Δexon7, are found only in the cytoplasm. Further, SMN2Δexon7 does not interact with ZPR1, and in the absence of full-length SMN, ZPR fails to translocate to the nucleus of mammalian cells following treatment with mitogens. Other protein interactions interrupted by SMN2Δexon7 include SMN oligomerization and the interaction of SMN with sm proteins within snRNPs. Interactions with the antiapoptotic protein Bcl-2 are disrupted, and in addition, the SMN2Δexon7 allele has a dominant negative effect on full-length SMN2.

The effect of a drug on SMN2 gene expression can be assayed by the criteria discussed herein, including assays to test for the modulation of SMN2 protein interactions, such as with transcription factors (*e.g.*, the E2 protein of papilloma virus); profilin; ZPR1; sm proteins; Bcl-2; modulation of SMN2 oligomerization; and localization to the nucleus and/or nuclear bodies. The assays are alternatives to, or can be complementary to assays to test directly for an effect on SMN2 expression levels. The drug may modulate (e.g., increase) SMN2 expression levels by altering the splicing pattern of the SMN2 transcript such that more full-length protein is produced. Alternatively, the drug may increase translation of full-length SMN protein from the

SMN2 transcript. For example, drugs that increase translation of the SMN2 transcript are indoprofen, and ester derivatives of indoprofen, including the methyl, ethyl, and isopropyl ester derivatives of indoprofen (see FIGs. 2A and 2H).

Assays for monitoring total SMN2 mRNA levels and mRNA isoform levels include reverse-transcription coupled with polymerase chain reaction (RT-PCR), Northern blot analysis, and *in situ* analysis. Assays for monitoring SMN2 protein levels, including full-length and truncated SMN2 levels include Western blot analysis, immunohistochemistry analysis, and reporter gene assays. The assays are also alternatives to, or can be complementary to, the cell-based and transgenic animal-based experiments described herein, which utilize SMN2 minigene constructs, including a reporter gene, for *in vivo* analyses.

Minigene constructs that include the differential exons of SMN1 and SMN2 can be used to identify or characterize compounds that modulate expression differentially between the SMN1 and SMN2 transcripts. For example, compounds that increase expression from one gene and not the other may have more specific effects, and thus fewer general effects, therefore potentially causing fewer unwanted side effects *in vivo*. The minigene constructs can include the differentially spliced exons (e.g., at least exons 6, 7, and 8 (or fragments thereof), and all or fragments of the intervening introns, and where the exon 8 or a fragment thereof is fused to a reporter gene, such as luciferase or GFP). The invention features compounds that modulate expression differentially between SMN1 and SMN2 transcripts. In particular, the invention features compounds that increase expression of SMN2 *in vivo*. The increased expression can be attributed to a modification of the splicing pattern such that more exon 7 is more frequently included in the processed mRNA transcript, or the increased expression can be attributed to an increase in translation levels.

Treatment methods

The compositions featured in the invention, including pharmaceutical compositions containing indoprofen and ester derivatives of indoprofen, can be administered to subjects (e.g., humans) determined to carry a deletion or mutation in the SMN1 or SMN2 gene, or diagnosed as having an MND. Patients diagnosed as having different types of SMA (e.g., SMA I, SMA II, SMA III and SMA IV) or ALS are suitable for treatment with the pharmaceutical compositions featured in the

invention. Patients suitable for treatment can carry, for example, a deletion of the entire SMN1 gene, a point mutation (e.g., Tyr272Cys mutation), short deletions including all or part of the consensus splice sites of introns 6 and 7, a deletion of exon 7, a deletion of exon 8, a gene conversion event (e.g., an event that replaces the telomeric SMN1 gene with SMN2), or a deletion of all or part of the SMN2 gene. The patient can be homozygous or heterozygous for one or more of these genetic abnormalities. In addition to deletions or mutations in the SMN1 gene, patients may carry a homozygous or heterozygous deletion of the neighboring gene, (neuronal apoptosis inhibitory protein gene (NAIP)), or a mutation in NAIP, e.g., a deletion of NAIP exon 5.

The treatment methods featured in the invention include the diagnosis of a human having or at risk for developing SMA or ALS. For example, diagnosis of SMA can include determining that the human carries a genetic mutation in the SMN1 gene. The diagnostic methods include providing a biological sample containing nucleic acid from the human and testing the nucleic acid for the presence, on at least one allele, of a mutation in the SMN1 gene, particularly in exon 6, 7, or 8, and/or in the introns flanking exons 6 or 7. The mutation can be any mutation described herein, including a point mutation or a deletion mutation. If a mutation is discovered in at least one allele, the human can be diagnosed as being at risk of having or developing SMA. An assessment of the phenotype of the human (e.g., the neurological phenotype) can be performed to verify that a patient has SMA. Identification of the genetic mutation can include methods known in the art including, but not limited to, Restriction Fragment Length Polymorphism (RFLP) assays, polymerase chain reaction (PCR) (e.g., restriction site based PCR), Amplification Refractory Mutation System (ARMS™), Competitive Oligonucleotide Priming System (COPS), Taqman™, Molecular Beacons, Amplification Refractory Mutation System Linear Extension (ALEX™). The biological sample from the human can be, for example, a tissue or fluid sample (e.g., a blood, urine, or saliva sample).

Diagnosis of ALS can include determining that the human carries a deletion of the SMN2 gene.

A patient who exhibits the symptoms or a phenotype of an MND can be administered a pharmaceutical composition featured in the invention. For example, SMA is generally characterized by the degeneration of the anterior horn cells leading to

symmetrical muscle weakness and wasting of voluntary muscles. SMA types I-IV differ primarily by the age of onset and severity. Type I SMA typically presents soon after birth, and death usually comes during infancy. Type II SMA is characterized by onset between about 3 and 15 months and survival beyond 4 years and usually until adolescence or later. Proximal muscle weakness is the cardinal feature, as in other forms of spinal muscular atrophy. Type III SMA is characterized by onset usually between 2 and 17 years of age. Symptoms include atrophy and weakness of proximal limb muscles, primarily in the legs, followed by distal phenotypes. Patients demonstrate fasciculations, and pulmonary dysfunction is often a cause of morbidity in these patients. Type IV SMA is characterized by onset over the age of about 20 years. Symptoms can include tongue fasciculations, hand tremor, symmetrical weakness of the proximal muscles, muscle atrophy, and bilateral hypertrophy of muscles.

A common first symptom of ALS is a painless weakness in a hand, foot, arm or leg, and other early symptoms include speech swallowing or walking difficulty. As nerve cells degenerate, the voluntary muscles weaken and become immobile. Onset of ALS generally occurs between the ages of 40 and 70. Patients diagnosed with MND can be administered a pharmaceutical composition featured in the invention.

Suitable administration can be selected, for example, based on the patient and the severity of disease. For example, administration to infants and children may be by intravenous injection or by liquid formulations. In addition, a mother carrying a fetus determined to have a deletion or mutation in the SMN1 gene (and therefore at high risk for SMA) may be administered a drug featured in the invention (e.g., indoprofen or an ester derivative of indoprofen) as a means of treating the fetus with the drug. Administration to the mother can be, e.g., by oral, intravenous, or intraperitoneal injection.

Effective Dose

Toxicity and therapeutic efficacy of a drug disclosed herein (e.g., indoprofen or an indoprofen ester derivative) can be determined by standard pharmaceutical procedures, using either cells in culture or experimental animals to determine 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, which can be expressed as the ratio LD_{50}/ED_{50}. Drugs

that exhibit large therapeutic indices are preferred. While drugs that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such drugs to the site of affected tissue to minimize potential damage to uninfected cells and, thereby, reduce side effects.

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 preferably 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 any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (that is, 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 may be measured, for example, by high performance liquid chromatography or by LC-MS.

Indoprofen and ester derivatives of indoprofen, for example, can be administered at a dosage of 1 mg-2000 mg per day, preferably 50-1500 mg per day, more preferably 80-1200 mg per day. For example, indoprofen and ester derivatives of indoprofen can be administered at 10 mg, 50 mg, 80 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 500 mg, or 600 mg per day or more per day. Administration can be once or more per day (e.g., once, twice, or three times per day), and the drug can be administered at regular intervals, such as daily or weekly.

Formulations and use

Pharmaceutical compositions for use in accordance with the present invention can be formulated in a conventional manner using one or more physiologically acceptable carriers or excipients.

Thus, the drugs (*e.g.*, compounds or molecules, such as indoprofen and ester derivatives of indoprofen) and their physiologically acceptable salts and solvates may be formulated for administration by oral or parentarel administration. Other suitable compositions are formulated for inhalation or insufflation (either through the mouth or the nose), or buccal administration.

For oral administration, the pharmaceutical compositions may take the form of, for example liquid preparations, such as solutions, syrups or suspensions, or they can be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (for example, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (for example, lecithin or acacia); non-aqueous vehicles (for example, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (for example, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations can also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. The pharmaceutical compositions may also take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (for example, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (for example, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (for example, magnesium stearate, talc or silica); disintegrants (for example, potato starch or sodium starch glycolate); or wetting agents (for example, sodium lauryl sulphate). The tablets can be coated by methods well known in the art. Preparations for oral administration can be suitably formulated to give controlled release of the active compound.

The drugs can be formulated for parenteral administration by injection, for example, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, for example, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, for example, sterile pyrogen-free water, before use. The formulations can be injected, for example, intravenously or by stereotactic injection.

For buccal administration the compositions can take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the drugs for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, for example,

dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, for example, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

In addition to the formulations described previously, the drugs can also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the drugs can be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The compositions can be presented in a pack or dispenser device that may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device can be accompanied by instructions for administration.

The therapeutic compositions of the invention can also contain a carrier or excipient, many of which are known to skilled artisans. Excipients that can be used include buffers (for example, citrate buffer, phosphate buffer, acetate buffer, and bicarbonate buffer), amino acids, urea, alcohols, ascorbic acid, phospholipids, proteins (for example, serum albumin), EDTA, sodium chloride, liposomes, mannitol, sorbitol, and glycerol. The drugs of the invention can be administered by any standard route of administration. For example, administration can be parenteral, intravenous, subcutaneous, intramuscular, intracranial, intraorbital, opthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, transmucosal, or oral. A modulatory compound can be formulated in various ways, according to the corresponding route of administration. For example, liquid solutions can be made for ingestion or injection; gels or powders can be made for ingestion, inhalation, or topical application. Methods for making such formulations are well known and can be found in, for example, "Remington's Pharmaceutical Sciences." It is expected that the preferred route of administration will be intravenous.

It is recognized that the pharmaceutical compositions and methods described herein can be used independently or in combination with one another. That is, subjects can be administered one or more of the pharmaceutical compositions, *e.g.*, pharmaceutical compositions comprising a drug of the invention, such as indoprofen or an ester derivative of indoprofen, or combinations of these, in temporally overlapping or non-overlapping regimens. When therapies overlap temporally, the therapies may generally occur in any order and can be simultaneous (*e.g.*, administered simultaneously together in a composite composition or simultaneously but as separate compositions) or interspersed. By way of example, a subject afflicted with spinal muscular atrophy can be simultaneously or sequentially administered both a drug that increases expression of full-length SMN2 and an antibody which can be conjugated or linked with a therapeutic agent, a cytotoxic agent, an imaging agent, or the like. Those skilled in the art will recognize that appropriate dosing and administering regimens can be applied to the other therapeutics listed above.

This invention is further illustrated by the following example, which should not be construed as limiting. The teachings of all references, patents and published patent applications cited throughout this application are incorporated herein by reference.

EXAMPLES

Example 1. Indoprofen was identified in an assay for increased SMN production from SMN2.

In a screen to identify compounds capable of enhancing the inclusion of exon 7 in SMN2 mRNA, cells of the human cervical carcinoma cell line c33A (ATCC#HTB-31) were stably transformed with an SMN fragment/luciferase reporter gene construct that included exons 6-8 of SMN1 or SMN2, a neomycin resistance gene and a luciferase reporter gene (Zhang *et al.*, *Gene Ther.* 8:1532-1538, 2001) (see FIG. 1). If the minigenes were transcribed and properly expressed, luciferase protein would be produced. An increase in luciferase expression would indicate that the minigenes were transcribed and properly spliced to include exon 7. An increase in luciferase expression from the SMN1 minigene construct would likely indicate increased transcription levels. An increase in luciferase expression from the SMN2 minigene construct could be an indication of splicing modification such that more transcripts including exon 7 were

produced, or an indication of increased transcription levels. A compound that causes either effect (an increase in transcription or a modification of splicing of SMN2) is identified as a compound capable of increasing full-length SMN protein levels *in vivo*.

Cells transfected with the minigene constructs were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 10% (v/v) Fetal Bovine Serum (Sigma F 2442), 50 units/mL penicillin, 50 μg/mL streptomycin sulfate and 400 mg/L G418 (*i.e.* Geneticin, Gibco, cat. No. 11811-031). Cells were allowed to grow at 37°C with 5% carbon dioxide in Corning 175 cm^2 vented tissue culture flasks (VWR Scientific/#29560-970).

The medium used to perform the luciferase assay was identical to the growth medium except the G418 selection agent was omitted. To perform the luciferase assay, a single reagent (25 mM glycylglycine, 15 mM magnesium sulfate, 4 mM EGTA, adjusted to pH 7.85, to which is added 10 μM D-luciferin (Sigma L9504), 10 mM dithiothreitol (DTT, Roche Molecular Biochemicals 100034), 2 mM adenosine 5'-triphosphate (ATP, Sigma A7699), 50 μM Coenzyme A, sodium salt (Sigma C 3144) and 1.5% Triton X-100) was used to lyse the cells and to initiate the luciferase reaction.

Compound libraries for use in the screen were either obtained at a concentration of 4 mg/mL in dimethyl sulfoxide (DMSO) or were solubilized and plated at this concentration in 384-well stock "mother" plates. These compounds were then diluted into an aqueous medium to create "daughter" plates as follows: 147 μL of Dulbecco's Modified Eagle Medium (DMEM) were dispensed, using a Zymark SciClone ALH, into each well of a Greiner 384-well, clear, polypropylene, 22 mm deep daughter plate (E&K Scientific / #EK-30202). Three (3) μL from the mother plate were transferred to the daughter plates using a Zymark SciClone ALH with 384-well fixed tip pipetting head. This resulted in a compound concentration of 80 μg/mL in each well of the daughter plates. Compounds tested in the screen included 20,000 compounds from a combinatorial library, 1,040 compounds from a National Institute for Neurological Disorders and Stroke (NINDS) library, 2,337 compounds from our Annotated Compound Library (Root *et al., Chem. Biol.* 10:881-892, 2003), and 23,685 compounds from our TIC library, which is a composite of compounds purchased from TimTec, IBS, and ChemBridge that were selected for specific properties, including stereochemical complexity.

To perform the screens, c33a cells were detached from the flasks using trypsin-EDTA (0.25% trypsin, 1 mM EDTA · 4 Na) (Life Technologies / #15050065). The cells were rinsed with 3 mL trypsin-EDTA, which was immediately aspirated. The cells were then incubated for 5-10 minutes at 37°C with an additional 3 mL of trypsin-EDTA. The trypsin enzyme was neutralized with 7 mL of media (normal C33a culture media lacking G418). Two to three 10 mL aliquots were combined and centrifuged at 228 g (1000 rpm) for 5 minutes. The supernatant was aspirated, and the cells were resuspended at a concentration of 200,000 cells/mL in G418-free media. The cell suspension was kept at 14°C, and constant stirring at 300 rpm ensured that the suspension was maintained. 57 µL of cell suspension was dispensed, using a Zymark SciClone ALH, into each well of a Nunc 384-well, opaque, white, tissue culture treated, 13 mm deep, assay plate (VWR Scientific / #62409-072) for a concentration of 11,400 cells/well. Three (3) µL from the daughter plate were transferred to each assay plate using a Zymark SciClone ALH with 384-well fixed tip pipetting head; this resulted in a compound concentration of 4 µg/mL. The plate was covered with a lid and incubated at 37°C and 5% carbon dioxide for 48 hours.

The white plastic assay plates were observed to be weakly luminescent. To avoid interference with the luminescence reading of the luciferase assay, care was taken to keep plate exposure to light at a minimum. Using a CCS Packard MiniTrak with 96/384 wash head and multi-position dispenser, the media was aspirated out of each plate, and the plate was washed 5 times with phosphate buffered saline (PBS) (pH ~7.2). Fifty-three (53) µL of assay reagent [2 mM adenosine 5'-triphosphate (Sigma / #A7699), 10 mM dithiothreitol (Roche Molecular Biochemicals / #100034), 20 mM coenzyme A (Sigma / #C3144), 10 µM D-Luciferin (Sigma / #L9504), and 65 mM Triton X-100 (Sigma / #T9284) in a buffer of 15 mM magnesium sulfate (anhydrous) (Mallinckrodt / #6070), 25 mM glycylglycine (American Bioanalytical / #AB680), 4 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EDTA) (Sigma / #E4378) at pH 7.85] was added to each well. The plate was transferred from the MiniTrak to a CCS Packard Fusion Analyzer via a CCS Packard SideTrak. Luminescence in each well was read using the Fusion Analyzer with a 0.8 second integration time. We discovered that indoprofen significantly increased reporter activity from SMN2-luc cells relative to SMN1-luc cells (Table 1 and FIG. 3A). The

most effective concentration, 2.8 μg/mL (~10 μM, MW = 281.3), resulted in a three-fold increase in luminescence.

Table 1. Effect of indoprofen and indoprofen analogs on minigene expression.

Compound ID No.	Compound	Luminescence Enhancement SMN1-luc	% Luminescence Enhancement SMN2-luc	Source
1	Indoprofen	44%	184%	Sigma / #I3132
2	Ibuprofen	5.6%	-0.8%	Sigma / #I4883
3	Ketoprofen	-30%	-21%	Sigma / #K1751
4	Suprofen	-4.0%	-5.0%	Sigma / #S9894
5	Acetylsalicylic Acid	-5.1%	-3.0%	Sigma / #A5376
6	Acetominophen	-17%	-9.5%	Sigma / #A7085
7	Isoindolinone	18%	21%	Interbioscreen / #STOCK1N-30308
8	Indoprofen, methyl ester derivative	38%	72%	Stockwell laboratory
9	Indoprofen, ethyl ester derivative	18%	98%	Stockwell laboratory
10	Indoprofen, isopropyl ester derivative	18%	70%	Stockwell laboratory

To find related active compounds, we tested those with structures similar to indoprofen, including non-steroidal anti-inflammatory drugs (NSAIDs), and found none that increased luminescence (Table 1, Compounds 2-7; see also FIGs. 2B-2G). Compounds with only the 2-phenylpropionic acid group or the isoindolinone group did not enhance luminescence (FIG. 3B). Methyl, ethyl and isopropyl esters of indoprofen retained some degree of activity and selectivity (Table 1, Compounds 8-10). This activity may be attributed to hydrolysis in cells and generation of indoprofen.

Example 2. Indoprofen increases SMN protein levels in human type I SMA patient fibroblasts.

Despite the noteworthy increase in SMN2-minigene-reporter activity, real time RT-PCR using Type I SMA-affected human primary fibroblasts (cell line 3813 from Coriell Cell Repositories) failed to show an increase in the ratio of full-length to truncated transcripts, or in the absolute level of transcripts following indoprofen treatment. Considering indoprofen's selective activity for SMN2-luc, it is unlikely that indoprofen is acting post-translationally, as both SMN1-luc and SMN2-luc encode the same protein. We propose that indoprofen has a pre- or co-translational effect on protein production from SMN2, through a cyclooxygenase-independent mechanism. For example, it is possible that indoprofen, resembling a nucleotide, binds to the SMN2 pre-mRNA and displaces proteins that reduce the rate of translation. We sought evidence as to whether indoprofen also affects the level of endogenous SMN protein in human cells.

Type I SMA fibroblasts (cell line 3813) were used to assay endogenous SMN protein levels. These cells carry a homozygous deletion of the SMN1 gene, and therefore generate no SMN1 protein. Carrier fibroblasts are heterozygous for the SMN1 gene deletion, and the mouse neuronal cell line NSC34 carries two wildtype copies of SMN1. Western blot analysis indicates that Type I SMA patient fibroblasts produce less SMN protein than Type I SMA and carrier fibroblasts (FIG. 3C).

We treated 3813 cells with 5 μM and 20 μM indoprofen for three days with daily media and compound changes (even though LC-MS analysis revealed that there was no significant degradation of indoprofen over four days in cell culture medium). Western blot analysis (described below) revealed that both concentrations of indoprofen treatments increased SMN protein levels to a similar extent, although occasionally, higher levels were observed from either treatment concentration (FIG. 3D). Combining data from both treatments, indoprofen-treated cells resulted in a mean 13% increase in SMN protein production versus untreated cells [independent, one-tailed t-test; n=17 (9 treated samples, 8 control samples), $\nu=15$, $p<0.014$].

For protein analysis experiments, Type I SMA fibroblast cells (cell line 3813) were cultured in Minimum Essential Medium (MEM) with Earle's salts and non-essential amino acids (Gibco / #10370) that was supplemented with 15% (v/v) FBS (Sigma / #F2442), 2 mM L-glutamine (US Biologicals / #G7120), and antibiotics (50,000 units penicillin/L MEM, 50 mg streptomycin/L MEM) (Sigma / #P4333).

Cells were allowed to grow at 37°C with 5% carbon dioxide in Corning 175 cm^2 vented tissue culture flasks (VWR Scientific / #29560-970).

To assess whether indoprofen treatment affected SMN protein production, cells were seeded in Corning six-well plates (VWR International / #29442-036) at a density of ~35,000 cells per well in 2 mL of normal cell culture media (see above). Five (5) µL or 20 µL of 2 mM indoprofen (Sigma / #I3132) solution (in PBS) was added to each well to create a treatment concentration of 5 µM or 20 µM, respectively. The cell culture media was changed daily. Retreatment with indoprofen occurred immediately after the media change. After the three-day treatment, the samples were prepared for western blotting.

To prepare the samples, the media was aspirated, and each well was washed with 1 mL of PBS. One hundred (100) µL of RIPA buffer [50 mM HEPES (Calbiochem / #391338), 40 mM sodium chloride (American Bioanalytical / #AB01915), 2 nM EDTA (American Bioanalytical / #AB00500), 0.5% Triton X-100 (Sigma / #X100), 1.5 mM sodium vanadate (Sigma / #S6383), 50 mM sodium fluoride (Sigma / #S7920), 10 mM sodium pyrophosphate (Sigma / #S9515), 1 Complete Mini, EDTA-free protease inhibitor cocktail tablet (Roche / #1836170) per 10 mL of buffer, adjusted to pH 7.4 with potassium hydroxide] was added to induce cell lysis. Each well was scraped for one minute, and the solution was transferred to a 1.5 mL microcentrifuge tube. Cell lysates were centrifuged at 16,000 g for 15 minutes at 4°C. Supernatants were transferred to a new, clean 1.5 mL microcentrifuge tube, while the pellet was discarded.

To prepare for polyacrylamide gel electrophoresis (PAGE), 33 µL of 4X loading buffer [10 mL aliquot: 4.8 mL 10% sodium dodecyl sulfate (SDS) (BioRad / #161-0301), 2.8 mL glycerol (Sigma / #G5516), 2.4 mL 0.5 M Tris at pH 6.8 (American Bioanalytical / #AB02000), 100 µL of 1% bromphenol blue (Sigma / #B5525), 0.5 mL β-mercaptoethanol (Sigma / #M3148), and double distilled water (ddH2O) to bring up to 10 mL]. Samples were heated to ~95°C for 5 minutes and recentrifuged at 11,000 g for ~5 minutes at 4°C. An equal volume, usually ~15 µL, was loaded on a Novex 12% Tris-Glycine 12-well (Invitrogen / #EC600052) gel. The gel was placed in the Invitrogen XCell SureLock gel electrophoresis apparatus (#EI0001) in electrophoresis running buffer [1 L water, 3 g Tris (American Bioanalytical / #AB02000), 14.40 g glycine, 1 g SDS (BioRad / #161-0301)] and was run for 1-1.5 hours at 120 volts.

The gel's contents were transferred to a PVDF (BioRad / #162-0177) membrane using standard blotting techniques in a BioRad PROTEAN 3 Cell with Mini Trans-Blot Module (#165-3323) with transfer buffer [4L aliquot: 3.6 L ddH20, 400 mL methanol (EM Science / #MX0488-1), 8.85 g (3-cyclohexylamino)-1-propanesulfonic acid (CAPS) (American Bioanalytical / #AB00287), 1.4 g sodium hydroxide (American Bioanalytical / #AB01916)]. The transfer apparatus was run for 1 hour at a constant voltage of 100 volts.

Following blotting, the membrane was blocked for 1 hour at room temperature on a shaker in PBST [PBS with 0.5 mL Tween 20/L PBS] with 5% (w/v) nonfat milk. The membrane was subsequently washed 3 times in PBST for 5 minutes/wash. The membrane was incubated with 1.5 μg of the primary antibody for SMN (mouse polyclonal) (BD Biosciences / #610646) in PBST with 2.5% (w/v) nonfat milk for 1 hour at room temperature. Washing of the membrane was performed as previously described. A goat anti-mouse antibody (Santa Cruz Biotechnology / #sc-2005) conjugated to horseradish peroxidase (HRP) was used as the secondary antibody. The membrane was incubated with 1.5 μg of this secondary antibody in PBST with 5% (w/v) nonfat milk for 1 hour at room temperature. Washing of the membrane was performed as previously described, and enhanced chemiluminescence (ECL) was performed using Western Lightning™ (PerkinElmer Life Sciences / #NEL101) with 2 mL of each reagent per membrane.

Example 3. Indoprofen increased the number of nuclear gems in human type I SMA patient fibroblasts.

The increase in SMN protein production led us to inquire about the effect of indoprofen on the overall number of gems – punctate structures in the nucleus. The number of gems (short for "Gemini of Coiled (Cajal) Bodies") directly correlates with SMN protein production (Coovert *et al., Hum. Mol. Genet.* 6:1205-1214, 1997), and they are found in many adult cell types (especially neurons) and in all fetal tissues. Fibroblasts from normal patients, SMA carriers, and type I SMA patients have ~80 gems, ~40 gems, and ~1-2 gems per 100 nuclei, respectively (Coovert *et al., Supra*).

To perform the gem count assay, Type I SMA fibroblasts were seeded into dishes containing three 1 cm^2 cover slips at a density of 5,000 cells/cm^2. After 24 hours, the media (DMEM supplemented with 10% FBS, 90 U/mL penicillin, 90 μg/mL

streptomycin, and 1.8 mM L-glutamine) was changed to media containing indoprofen. The media was changed every 24 hours with media containing fresh drug for a total treatment period of 5 days. After treatment, the cover slips were removed and washed briefly with PBS, fixed in ice cold solution of 50% acetone/50% methanol for 7 minutes, and air dried. After fixation, the cover slips were rehydrated with PBS for 5 minutes and blocked for 30 minutes with 1x BLOCK (1% FBS, 1% horse serum, 0.1% BSA in PBS). The cells were probed for one hour with MANSMA1 anti-SMN monoclonal antibody, which was diluted 1:1,000 in 1x BLOCK containing 0.1% Tween 20. Cover slips were then washed three times with 0.1% Tween 20 in PBS for 10 minutes each. The cells were then incubated with goat anti-mouse TRITC-conjugated secondary antibody (Sigma) diluted 1:2,000 in 1x BLOCK, 0.1% Tween 20. After 1 hour, the cover slips were washed as above and mounted on Superfrost slides (Fisher Scientific / #12-550-15) using 1:3 dilution of Vectashield containing DAPI I in Vectashield without DAPI (Vector Laboratories / #H-1200 and #H-1000, respectively). Gem counting was performed on a Nikon microscope equipped with a TRITC/DAPI dual band pass filter and a Magnafire digital camera (Optronics).

The Type I SMA fibroblasts (cell line 2806) were treated with indoprofen (5 μM and 15 μM) and we recorded the resulting changes in gem count. Both indoprofen treatments yielded a significant increase in gem count [5 μM: independent, one-tailed t-test; n=17 (8 treated, 9 untreated), ν=15, p=6.9x10-4 and 15 μM: independent, one-tailed t-test; n=16 (7 treated, 9 untreated), ν=14, p=1.7x10-4] (FIG. 3E). Pooling the two treatments also generated a significant result [independent, one-tailed t-test; n=24 (15 treated, 9 untreated), ν=22, p=3.2x10-7]. Additionally, we tested one other type I SMA fibroblast cell line (3813) and one mouse fibroblast cell line (Pellizzoni *et al.*, *Cell* 95:615-624, 1998).

In 3813 cells, only the 15 μM indoprofen treatment yielded a significant increase in gem count [independent, one-tailed t-test; n=12 (6 treated, 6 untreated), ν=10, p=2.0x10-3]. Five (5) μM indoprofen treatment resulted in a p-value of 0.10 [independent one-tailed t-test, n=12 (6 treated, 6 untreated), ν=10]. Combining both concentrations, treatment significantly increased gem count over untreated cells [independent, one-tailed t-test, n=18 (12 treated, 6 untreated), ν=16, p=2.2x10-3].

Both indoprofen concentrations (5 μM and 15 μM) resulted in significant gem count increases in mouse fibroblasts (Pellizzoni *et al.*, *Cell* 95:615-624, 1998) [5 μM:

independent, one-tailed t-test; n=12 (6 treated, 6 untreated), v=10, p=0.025 and 15 μM: independent, one-tailed t-test; n=12 (6 treated, 6 untreated), v=10, $p=2.1x10^{-3}$]. As expected, pooling samples from both treatments revealed a p-value of $3.3x10^{-4}$ [independent, one-tailed t-test, n=18 (12 treated, 6 untreated), v=10].

Example 4. Indoprofen does not reach the brain of treated mice, and is detected in embryos of treated pregnant mice.

In an attempt to find a maximum effective dose, the pharmacokinetics of indoprofen in mice were studied. Pregnant mice were treated with a single dose of 20 mg/kg of indoprofen, using either intraperitoneal (IP) injection or oral gavage, to examine indoprofen accumulation in the brain, blood, and embryo.

A 20 mM indoprofen solution was prepared in PBS immediately before dosing. In order to assess efficacy, 2 administration methods were evaluated. Oral dosing (PO) was performed using a 1 cm^3 syringe and a 23 gauge, 1.5 inch gavage needle. Intraperitoneal injection (IP) was performed using a 1 cm^3 syringe with a 27 gauge, 0.5 inch needle.

Mice were euthanized by CO_2 asphyxiation 1 hour after dosing. Seven hundred (700) mL of blood was collected by cardiac puncture, was transferred to a Microtainer Serum Separator Tube (BD Biosciences / #365956), and was allowed to clot at room temperature for 30 minutes. The tube was centrifuged at 20°C for ~18,000 g for 5 minutes. Serum was collected and acidified to pH 3.0 with 1 M hydrochloric acid (Sigma / #H9892). One (1) mL of methylene chloride (Sigma / #D65100) was added to the serum, and the mixture was vortexed for 1 minute. The tube was subsequently centrifuged at 20°C at ~1000 g for 10 minutes. The organic phase was transferred to a glass scintillation vial and placed in a fume hood for evaporation. The dried sample was reconstituted in 1 mL of 5% acetonitrile (EM Science / #AX0142) and spun at room temperature at ~11,000 g for 10 minutes. One hundred and fifty (150) mL of the sample was removed for LC-MS analysis.

Both routes of administration resulted in ~5.3 μM indoprofen plasma concentration. Further analysis showed that no indoprofen was present in brain tissue 4 hours after treatment.

To analyze embryos and placenta, the two aforementioned treatment methods (PO and IP) were performed on pregnant mice at embryonic day 13 (E13). One hour

later, the uterus was dissected and placed in a 100 mm tissue culture dish (Corning / #430167) containing sterile PBS. The uterus was opened, and each intact embryo sac was removed and placed in a sterile 100 mm dish. The sacs were opened using sterile forceps and scissors. Placentas were separated from each embryo and were pooled in a 15 mL polystyrene centrifuge tube (Corning / #430055). Similarly, all embryos were pooled in a 15 mL centrifuge tube.

To begin the extraction, ice-cold methanol (EM Science / #MX0488) (4 mL/g tissue) was added and each tube was vortexed for 2 minutes and sonicated for 1 minute to break up the tissue completely. Water (4 mL/g tissue) was added, and samples were vortexed for another minute. Both tubes were centrifuged at room temperature at ~1000 g for 5 minutes. The liquid portions in each tube were transferred to new 15 mL tubes, and the solid portions were discarded. The samples were acidified to pH 3.0, and methylene chloride was added to bring the liquid volume up to 15 mL. The tubes were again vortexed for 1 minute. Each tube was spun at ~1000 g for 10 minutes. The organic phases were transferred to clean 20 mL glass scintillation vials and placed in a fume hood for evaporation. The dried samples were reconstituted in 1 mL of 5% acetonitrile and subsequently spun at room temperature at ~11,000 g for 10 minutes. One hundred and fifty (150) mL of each sample was removed for LC-MS analysis.

LC-MS analysis was performed using a ThermoFinnigan LCQ DECA XP instrument. Separation was performed using a reverse phase C18 HPLC column (Grace-VYDAC / #218TP51) with a linear gradient and solvents, H_2O/CH_3CN, 0.1% TFA. The LC-MS gradient was: 0 min = 5% CH_3CN; 30 min = 100% CH_3CN with a flow rate of 100 µL/min. Indoprofen was found to peak at 16.7 min (52.9% CH_3CN/47.1% H_2O), and our standard was found to peak at 22.3 min (70.6% CH_3CN/29.4% H_2O). The chromatograms were established as a combined absorbance of 280 nm and 220 nm. The samples were analyzed as positive ions under ESI conditions (mass range = 200-2000 m/z). A full 40 minute scan was performed to detect the compounds in each sample.

Indoprofen was found in the plasma of both the pregnant mice and their embryos one hour after treatment. Both routes of administration resulted in ~5.3 µM indoprofen plasma concentration, while the IP method was shown to be more effective than gavage at delivering indoprofen to embryonic tissue. An average concentration of

~3.0 μM indoprofen was found in embryos of IP-treated mice at embryonic day 13 (E13).

Example 5. Indoprofen increased mouse viability.

We tested the effect of indoprofen on the viability of SMA model mice, which lack murine SMN but contain a human SMN2 transgene (i.e., $Smn^{-/-}$; $TgSMN2^{+/-}$) (Monani *et al., Hum. Mol. Genet.* 9:333-339, 2000). To generate litters that contained 25% $Smn^{-/-}$; $TgSMN2^{+/-}$ mice, we mated $Smn^{+/-}$; $TgSMN2^{+/+}$ mice with $Smn^{+/-}$; $TgSMN2^{-/-}$ mice. Our SMA model mice were derived from the published transgenic model created in C57BL/6 and crossed with FVB (Taconic Labs, Germantown, New York) (Monani et al., *supra*). These mice were backcrossed once with a wild-type FVB mouse to generate our C57BL/6/FVB mice. Our laboratory backcrossed the male C57BL/6/FVB mouse with a female wild-type FVB (The Jackson Laboratory / #001800) mouse. The offspring were bred with each other, and the resulting offspring were used for this study. We found that in this strain of $Smn^{-/-}$; $TgSMN2^{+/-}$, embryos die at approximately embryonic day 11 (E11).

Because our mice came from two different strains, phenotype modifying genes could be present on the background. This may result in an embryonic death that varies (i.e., that is earlier) from the previously published data. Additionally, genetic drift may have resulted over time or may have occurred by using FVB mice from different vendors. In the Ohio State University colony, $Smn^{-/-}$; $TgSMN2^{+/-}$ mice are viable until birth (Monani et al., *supra*). These mice have been backcrossed over six times to the Taconic FVB strain.

We generated $Smn^{-/-}$; $TgSMN2^{+/+}$ mice by crossing our $Smn^{+/-}$; $TgSMN2^{+/?}$ mice with wild-type FVB mice (The Jackson Laboratory) and genotyping the offspring. $Smn^{+/-}$; $TgSMN2^{+/?}$ mice generating ~9 or more embryos all containing TgSMN2 were known to be $TgSMN2^{+/+}$. These homozygous mice were mated with each other and the offspring were, again, genotyped to ensure the presence of SMN2 in all of them. To avoid potential maternal imprinting, approximately 50% of the males possessed the transgene (i.e., $TgSMN2^{+/+}$) in these crosses.

To evaluate the therapeutic effect of orally administered indoprofen on developing SMA carrier embryos, we treated pregnant $Smn^{+/-}$; $TgSMN2^{+/+}$ (after mating with $Smn^{+/-}$; $TgSMN2^{-/-}$ male) SMA mice with indoprofen. Pregnant mice were

identified by the presence of a vaginal copulatory plug. Treatment with indoprofen (Sigma / #I3132) began on day 1 of the pregnancy. A 5 mM indoprofen solution in sterile PBS was created, and the pH was controlled to 7.0 using sodium hydroxide. Mice were weighed before dosing to ensure an accurate 5 mg/kg indoprofen dose. Restraining the mouse with its head and body extended, the 27 gauge, 0.5 inch needle was introduced into the abdomen. Mice were dosed twice daily (Monday through Friday) through embryonic day 14 (E14). On E14, pregnant mice were dissected and the embryos removed. A Roche High Pure PCR prep kit (Roche / #1732668) was used to purify the embryos for genotyping.

While we expected all of our crosses to yield 100% of the offspring containing SMN2, we occasionally obtained litters with only 50% SMN2-containing embryos. Embryos lacking SMN2 were not included in the embryo statistics. Of the 57 untreated embryos studied, none possessed the SMA genotype. The maximum expected number of SMA genotype embryos from untreated litters was 14 (25% of 57). Of the 39 treated embryos, the maximum expected number of SMA genotype embryos was 10 (25% of 39). Three (3) treated embryos were, in fact, found to have the SMA genotype.

To determine whether indoprofen could increase the viability of these SMA model embryos, we treated pregnant mice twice daily for the first 14 days of pregnancy by IP injection with 5 mg/kg indoprofen in phosphate buffered saline, the maximum dose that exhibited no toxicity within the 14 days. On embryonic day 14 (E14), we genotyped the embryos and ascertained the number of $Smn^{-/-}$; $TgSMN2^{+/-}$ (SMA genotype) embryos (Table 2B). At E14, none of the 7 untreated litters harbored any SMA genotype embryos, whereas 3 of 7 indoprofen-treated litters harbored SMA genotype embryos (Fisher Exact test, $v=1$, $p=0.096$). Thus, there was a trend in which indoprofen increased the viability of SMA model mice. As expected, indoprofen-treatment significantly increased the mean litter size from 5.7 embryos to 6.9 embryos [independent, one-tailed t-test; $n=14$, $v=12$, $p=0.040$]. There was also a trend in which indoprofen increased the number of SMA embryos (Fisher Exact test, $v=1$, $p=0.073$).

Table 2A. Number of non-SMA and SMA litters from untreated and indoprofen-treated mother mice.

	Non-SMA	SMA
Untreated	7	0
Indoprofen-treated	4	3

Table 2B. Number of non-SMA and SMA embryos from untreated and indoprofen-treated mother mice.

	Non-SMA	SMA
Untreated	57	0
Indoprofen-treated	39	3

Example 6. Ester derivatives of Indoprofen were synthesized.

To make methyl, ethyl, isopropyl, and t-butyl esters, 50 mg indoprofen was placed in a 2 mL vial with a stir bar. 250 microliters of methanol (EM Science, #MX0488-1), ethanol (AAPER, 200 proof), isopropanol (EM Science, #PX1835-2),or tert-butanol (JTBaker, #9056-01) were added to the indoprofen while stirring. 450 microliters chloroform was added to dissolve the indoprofen. One drop (about 20 µL) of sulfuric acid was added to the stirring solution. The solution was then heated to 50°C for 2-3 hours. Thin layer chromatography was performed to determine that the reaction was complete.

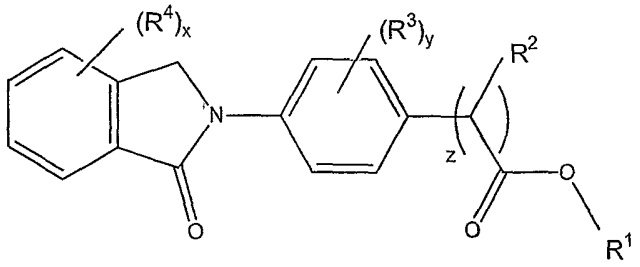
To purify the esters, the entire solution was transferred to a 20 mL vial (washed with methylene chloride) and 10 mL methylene chloride was added followed by the addition of 2 mL sodium bicarbonate (for compound 19, the 2 mL sodium bicarbonate was added before the 10 mL methylene chloride). The solution was vortexed, and then the phases allowed to separate. The aqueous (top) layer was removed, 2 mL sodium bicarbonate was added to the remaining organic layer and the mixture was vortexed. The phases were allowed to separate, and then the aqueous (top) layer was removed. 2 mL water was added to the organic layer, and the solution was swirled. The phases were allowed to separate and then the aqueous (top) layer was removed. The organic layer was transferred to a new vial and the solvent evaporated. The structure of the esters was confirmed by LC-MS. Mass spectrometry analysis indicated that the purified methyl derivative had the expected molecular weight of 296.2, the molecular weight of the ethyl ester derivative had the expected molecular weight of 310.2, the molecular weight of the isopropyl ester had the expected molecular weight of 324.1.

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from

the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A compound having the formula:



wherein each of R^4 and R^3 is independently an optionally substituted C_1-C_{12} alkyl, an optionally independently substituted C_1-C_{12} alkenyl, an optionally independently substituted C_1-C_{12} alknyl, an optionally independently substituted C_1-C_{12} aryl, an optionally independently substituted C_1-C_{12} cycloaryl, –OH or a halogen; x is 0, 1, 2, 3 or 4; y is 0, 1, 2, 3 or 4; z is 1, 2, 3, or 4 and each R^2 is independently an optionally independently substituted C_1-C_{12} alkyl, an optionally independently substituted C_1-C_{12} alkenyl, an optionally independently substituted C_1-C_{12} alknyl, an optionally independently substituted C_1-C_{12} aryl, or an optionally independently substituted C_1-C_{12} cycloaryl; and R^1 is an optionally independently substituted C_1-C_{12} alkyl, an optionally independently substituted C_1-C_{12} alkenyl, an optionally independently substituted C_1-C_{12} alknyl, an optionally independently substituted C_1-C_{12} aryl, or an optionally independently substituted C_1-C_{12} cycloaryl and pharmaceutically acceptable salts thereof.

2. The compound of claim 1 wherein R^4 and R^3 are not substituted.

3. The compound of claim 1 wherein each of R^4 and R^3 is independently an optionally independently substituted C_1-C_{12} alkyl, an optionally independently substituted C_1-C_6 alkenyl, an optionally independently substituted C_1-C_{12} alknyl, an optionally independently substituted C_1-C_6 aryl, an optionally independently substituted C_1-C_6 cycloaryl, –OH or a halogen.

4. The compound of claim 1 wherein each of R^4 and R^3 is independently a halogen.

5. The compound of claim 4 wherein the halogen is selected from Cl and F.

6. The compound of claim 1 wherein both x and y are 0.

7. The compound of claim 1 wherein R^1 is not substituted.

8. The compound of claim 1 wherein R^1 is substituted with –OH or a halogen.

9. The compound of claim 1 wherein n is 1 and R^2 is a C_1 or C_2 alkyl.

10. The compound of claim 9 wherein R^2 is not substituted.

11. The compound of claim 1 wherein R^1 is a straight chain C_1 to C_6 alkyl.

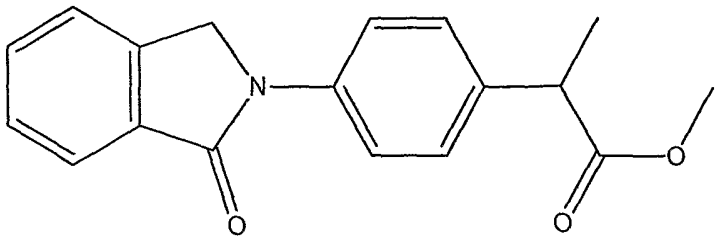
12. The compound of claim 1 wherein R^1 is a branched chain C_1 to C_6 alkyl.

13. The compound of claim 1 or claim 2 wherein R^1 is any of $-CH_3$, $-CH_2CH_3$, $-CH(CH_3)_2$ any $-C(CH_3)_3$, optionally independently singly or multiply substituted with one or more –OH or halogen.

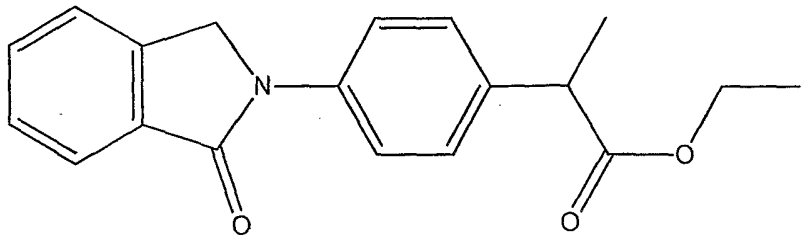
14. The compound of claim 1 wherein R^2 is $-CH_3$, n is 1 and R^1 is any of $-CH_3$, $-CH_2CH_3$, $-CH(CH_3)_2$ and $-C(CH3)_3$.

15. The compound of claim 14 wherein R^4 and R^3 are both H.

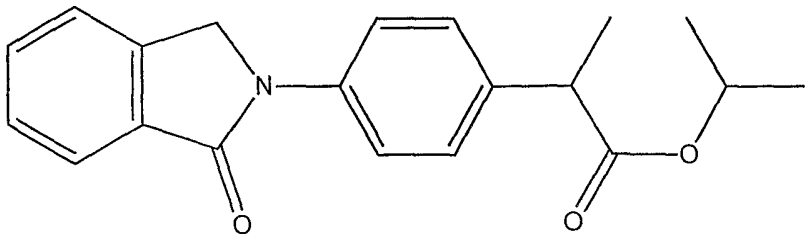
16. The compound of claim 1, wherein the compound has the formula:



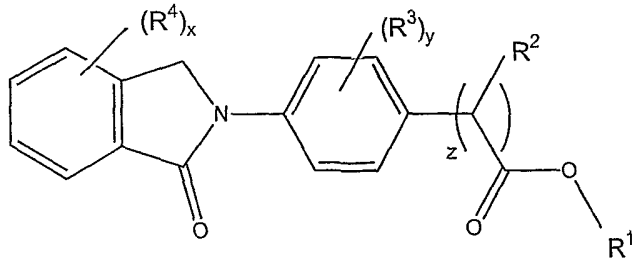
17. The compound of claim 1, wherein the compound has the formula:



18. The compound of claim 1, wherein the compound has the formula:



19. A method for treating an individual having or at risk for developing a motor neuron disease by administering a compound having the formula:



wherein each of R^4 and R^3 is independently an optionally substituted C_1-C_{12} alkyl, an optionally independently substituted C_1-C_{12} alkenyl, an optionally independently substituted C_1-C_{12} alknyl, an optionally independently substituted C_1-C_{12} aryl, an optionally independently substituted C_1-C_{12} cycloaryl, –OH or a halogen; x is 0, 1, 2, 3 or 4; y is 0, 1, 2, 3 or 4; z is 1, 2, 3, or 4 and each R^2 is independently an optionally independently substituted C_1-C_{12} alkyl, an optionally independently substituted C_1-C_{12} alkenyl, an optionally independently substituted C_1-C_{12} alknyl, an optionally independently substituted C_1-C_{12} aryl, or an optionally independently substituted C_1-C_{12} cycloaryl; and R^1 is an optionally independently substituted C_1-C_{12} alkyl, an optionally independently substituted C_1-C_{12} alkenyl, an optionally independently substituted C_1-C_{12} alknyl, an optionally independently substituted C_1-C_{12} aryl, or an optionally independently substituted C_1-C_{12} cycloaryl and pharmaceutically acceptable salts thereof.

20. The method of claim 19 wherein n is 1 and R^2 is a C_1 or C_2 alkyl.

21. The method of claim 20 wherein R^2 is not substituted.

22. The method of claim 19 wherein R^1 is an straight chain C_1 to C_6 alkyl.

23. The method of claim 19 wherein R^1 is an branched chain C_1 to C_6 alkyl.

24. The method of claim 19 or claim 10 wherein R^1 is any of $-CH_3$, $-CH_2CH_3$, $-CH(CH_3)_2$ any $-C(CH3)_3$, optionally independently singly or multiply substituted with one or more –OH or halogen.

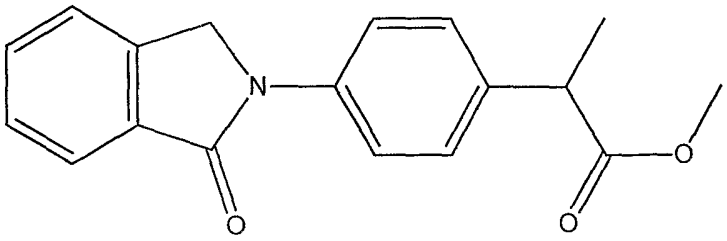
25. The method of claim 19 wherein R^2 is $-CH_3$, n is 1 and R^1 is any of $-CH_3$, $-CH_2CH_3$, $-CH(CH_3)_2$ any $-C(CH3)_3$.

26. The method of claim 25 wherein R^4 and R^3 are both H.

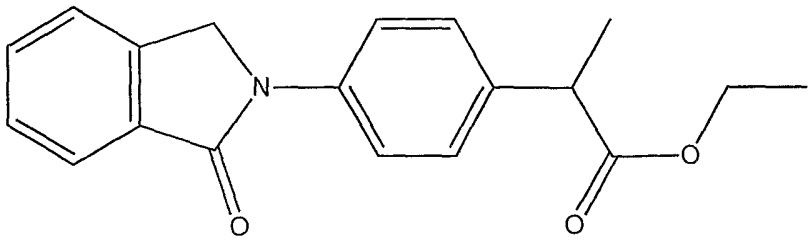
27. The method of claim 19, wherein the individual has a deletion of or a mutation in the SMN1 gene.

28. The method of claim 19, wherein the administration results in an increase in full-length SMN protein levels.

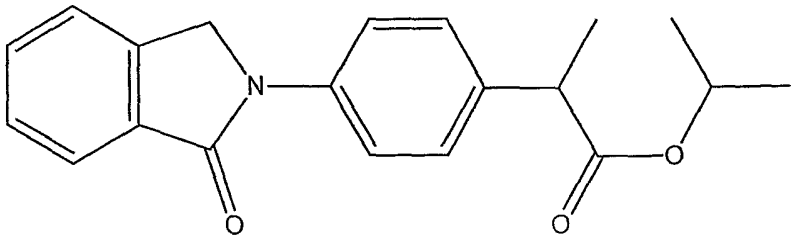
29. The method of claim 19, wherein the compound has the formula:



30. The method of claim 19, wherein the compound has the formula:

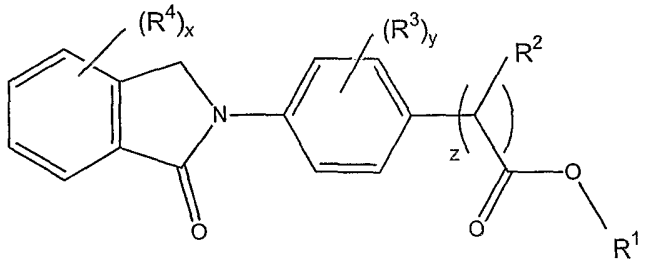


31. The method of claim 19, wherein the compound has the formula:



32. The method of claim 19, wherein the individual has or is at risk for developing spinal muscular atrophy or amyotrophic lateral sclerosis.

33. A pharmaceutical composition comprising a compound having the formula:



wherein each of R^4 and R^3 is independently an optionally substituted C_1-C_{12} alkyl, an optionally independently substituted C_1-C_{12} alkenyl, an optionally independently substituted C_1-C_{12} alknyl, an optionally independently substituted C_1-C_{12} aryl, an optionally independently substituted C_1-C_{12} cycloaryl, –OH or a halogen; x is 0, 1, 2, 3 or 4; y is 0, 1, 2, 3 or 4; z is 1, 2, 3, or 4 and each R^2 is independently an optionally independently substituted C_1-C_{12} alkyl, an optionally independently substituted C_1-C_{12} alkenyl, an optionally independently substituted C_1-C_{12} alknyl, an optionally independently substituted C_1-C_{12} aryl, or an optionally independently substituted C_1-C_{12} cycloaryl; and R^1 is an optionally independently substituted C_1-C_{12} alkyl, an optionally independently substituted C_1-C_{12} alkenyl, an optionally independently substituted C_1-C_{12} alknyl, an optionally independently substituted C_1-C_{12} aryl, or an optionally independently substituted C_1-C_{12} cycloaryl and pharmaceutically acceptable salts thereof, and

a pharmaceutically acceptable carrier.

34. The pharmaceutical composition of claim 33, wherein n is 1 and R^2 is a C_1 or C_2 alkyl.

35. The pharmaceutical composition of claim 34, wherein R^2 is not substituted.

36. The pharmaceutical composition of claim 33 wherein R^1 is an straight chain C_1 to C_6 alkyl.

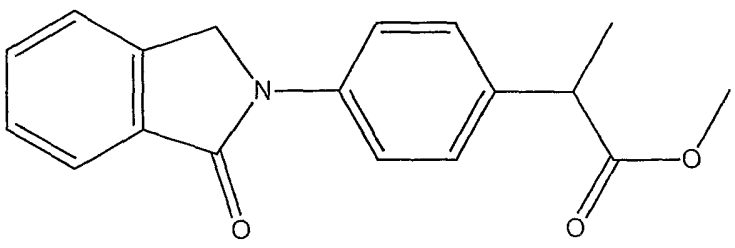
37. The pharmaceutical composition of claim 33 wherein R^1 is an branched chain C_1 to C_6 alkyl.

38. The pharmaceutical composition of claim 33 or claim 20 wherein R^1 is any of $-CH_3$, $-CH_2CH_3$, $-CH(CH_3)_2$ any $-C(CH_3)_3$, optionally independently singly or multiply substituted with one or more –OH or halogen.

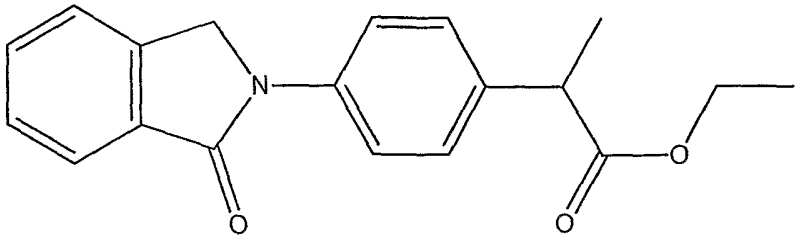
39. The pharmaceutical composition of claim 33 wherein R^2 is $-CH_3$, n is 1 and R^1 is any of $-CH_3$, $-CH_2CH_3$, $-CH(CH_3)_2$ any $-C(CH3)_3$.

40. The pharmaceutical composition of claim 39 wherein R^4 and R^3 are both H.

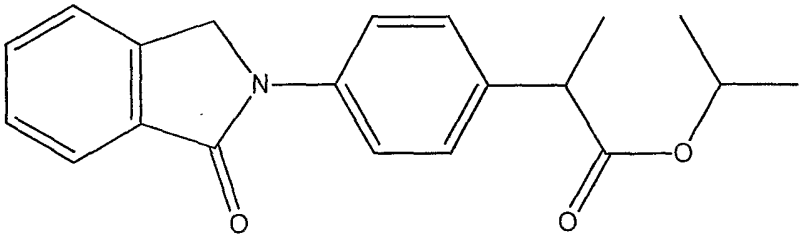
41. The pharmaceutical composition of claim 33, wherein the compound has the formula:



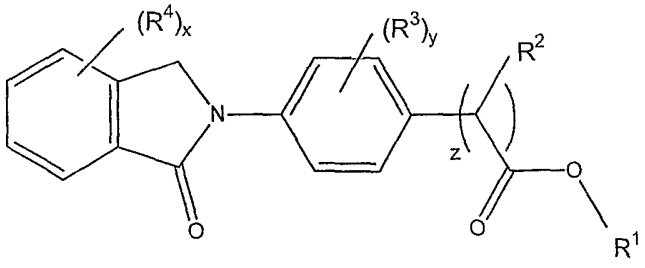
42. The pharmaceutical composition of claim 33, wherein the compound has the formula:



43. The pharmaceutical composition of claim 33, wherein the compound has the formula:



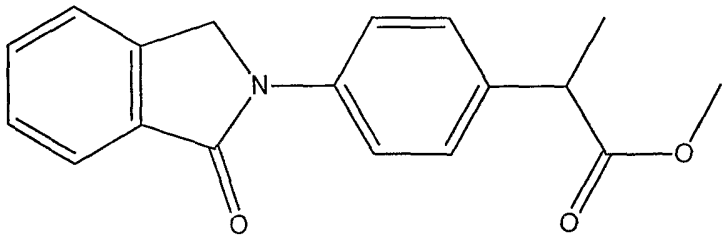
44. A method for treating an individual comprising diagnosing the individual as having or at risk for developing a motor neuron disease, and administering a compound having the formula:



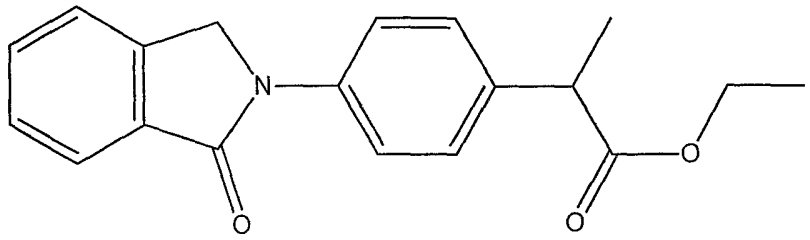
wherein each of R^4 and R^3 is independently an optionally substituted C_1-C_{12} alkyl, an optionally independently substituted C_1-C_{12} alkenyl, an optionally independently substituted C_1-C_{12} alknyl, an optionally independently substituted C_1-C_{12} aryl, an optionally independently substituted C_1-C_{12} cycloaryl, –OH or a halogen; x is 0, 1, 2, 3 or 4; y is 0, 1, 2, 3 or 4; z is 1, 2, 3, or 4 and each R^2 is independently an

optionally independently substituted C_1-C_{12} alkyl, an optionally independently substituted C_1-C_{12} alkenyl, an optionally independently substituted C_1-C_{12} alknyl, an optionally independently substituted C_1-C_{12} aryl, or an optionally independently substituted C_1-C_{12} cycloaryl; and R^1 is an optionally independently substituted C_1-C_{12} alkyl, an optionally independently substituted C_1-C_{12} alkenyl, an optionally independently substituted C_1-C_{12} alknyl, an optionally independently substituted C_1-C_{12} aryl, or an optionally independently substituted C_1-C_{12} cycloaryl and pharmaceutically acceptable salts thereof.

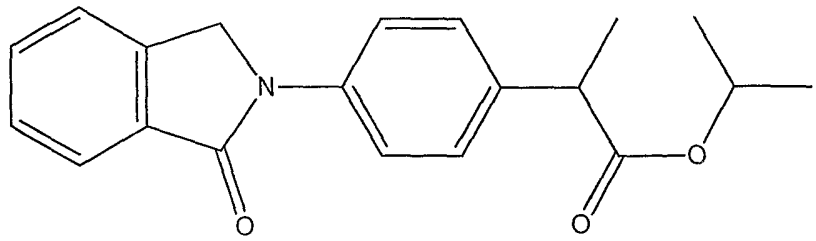
45. The method of claim 44, wherein the compound has the formula:



46. The method of claim 44, wherein the compound has the formula:



47. The method of claim 44, wherein the compound has the formula:



48. The method of claim 44, wherein the diagnosing step comprises

providing a biological sample from the individual, wherein the biological sample comprises nucleic acid, and

performing an assay to determine whether the nucleic acid comprises a mutation of the SMN1 gene of the individual,

wherein if the nucleic acid comprises a mutation of the SMN1 gene, the individual is diagnosed as having or at risk for developing the motor neuron disease spinal muscular atrophy (SMA).

49. The method of claim 48, wherein the assay to determine whether the nucleic acid comprises a mutation of the SMN1 gene of the individual is a Northern blot, polymerase chain reaction, restriction fragment length polymorphism, ARMS™ or ALEX™ assay.

50. The method of claim 48, wherein the mutation of the SMN1 gene is a point mutation in exon 6, 7, or 8, or in an intron flanking exon 6 or 7, or a complete or partial deletion of the SMN1 gene.

51. The method of claim 44, wherein the diagnosing step comprises

providing a biological sample from the individual, wherein the biological sample comprises nucleic acid,

performing an assay to determine whether the nucleic acid comprises a mutation of the SMN2 gene of the individual,

wherein if the nucleic acid comprises a mutation of the SMN2 gene, the individual is diagnosed as having or at risk for developing the motor neuron disease amyotrophic lateral sclerosis (ALS).

52. The method of claim 51, wherein the assay to determine whether the nucleic acid comprises a mutation of the SMN2 gene of the individual is a Northern blot, polymerase chain reaction, restriction fragment length polymorphism, ARMS™ or ALEX™ assay.

53. The method of claim 51, wherein the mutation of the SMN2 gene is a deletion of the SMN2 gene.

54. The method of claim 44, wherein the individual is diagnosed as having or at risk for developing SMA or ALS.

55. A method of synthesizing an ester derivative of indoprofen, comprising mixing an alcohol with indoprofen in the presence of an organic solvent in an acidic solution, and refluxing the solution at about 50°C for about 2 to 3 hours.

56. The method of claim 55, wherein the alcohol is methanol, ethanol, isopropanol, or tert-butanol.

57. The method of claim 55, wherein the organic solvent is chloroform, polypropylene glycol, or ethanolamine.

58. The method of claim 55, further comprising monitoring the synthesis by performing thin layer chromatography on a sample of the solution.

59. The method of claim 55, further comprising purifying the ester derivative of indoprofen by

extracting the solution at least once with a mixture of methylene salt and sodium bicarbonate,

extracting the solution at least once with water, and

evaporating the organic solvent.

60. The method of claim 59, further comprising separating enantiomers of the ester derivative.

61. The method of claim 59, further comprising verifying the mass of the ester derivative of indoprofen by performing liquid chromatography-mass spectrometry on the ester derivative.

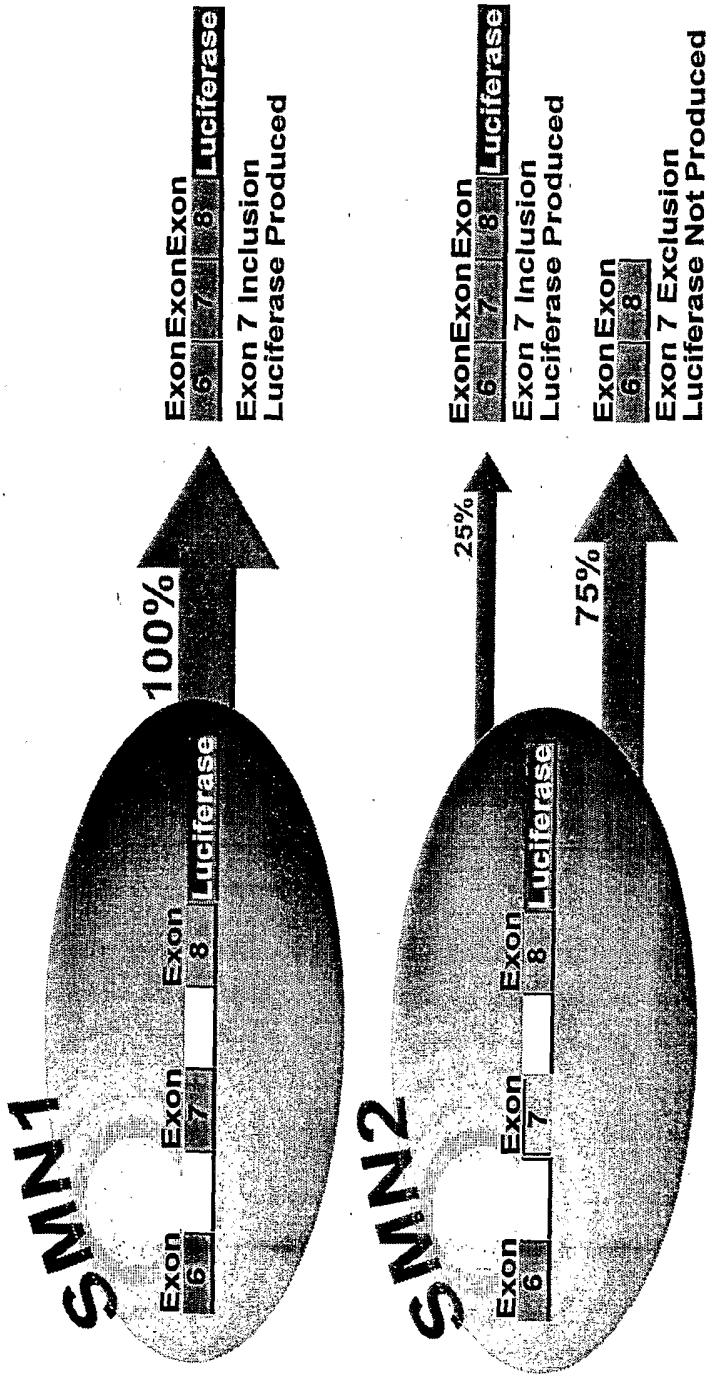

SMN1
Exon 6
Exon 7
Exon 8
Luciferase
100%
Exon Exon Exon
6 7 8 Luciferase
Exon 7 Inclusion
Luciferase Produced
SMN2
Exon 6
Exon 7
Exon 8
Luciferase
25%
Exon Exon Exon
6 7 8 Luciferase
Exon 7 Inclusion
Luciferase Produced
75%
Exon Exon
6 8
Exon 7 Exclusion
Luciferase Not Produced

Figure 1

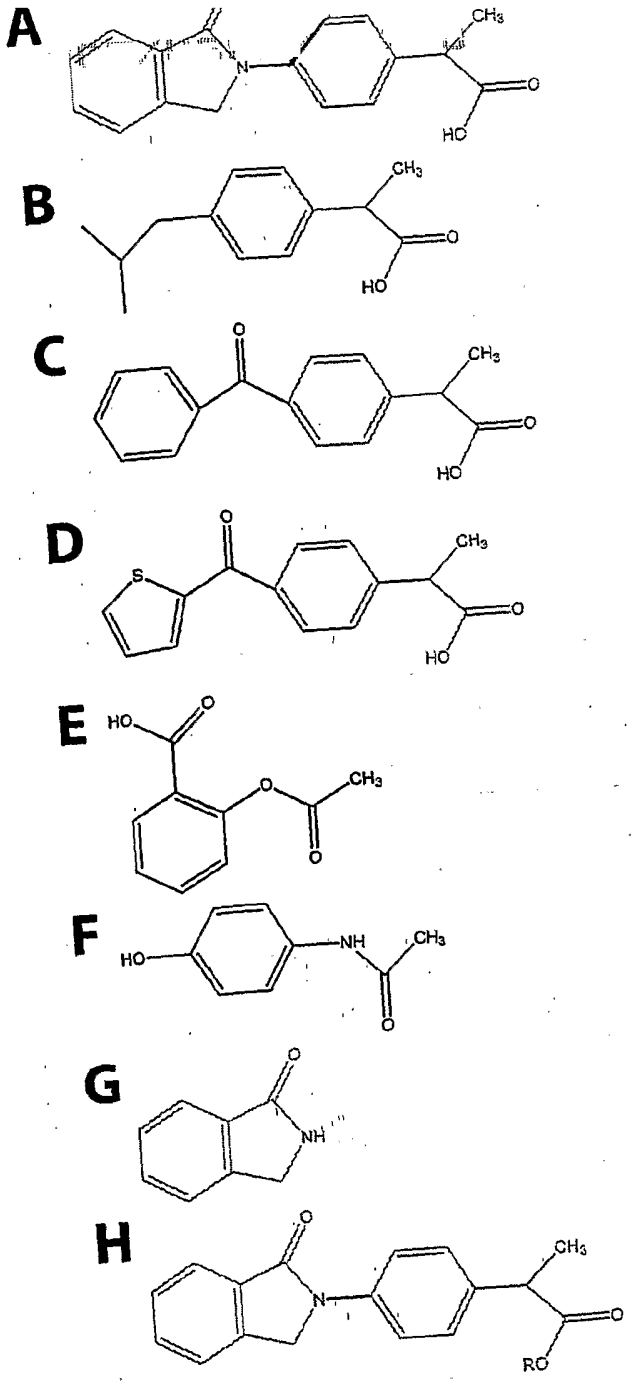

A
CH3
N
O
HO
B
CH3
O
HO
C
O
CH3
O
HO
D
O
S
CH3
O
HO
E
HO
O
O
CH3
O
F
HO
NH
CH3
O
G
O
NH
H
O
N
CH3
O
RO

FIGURE 2

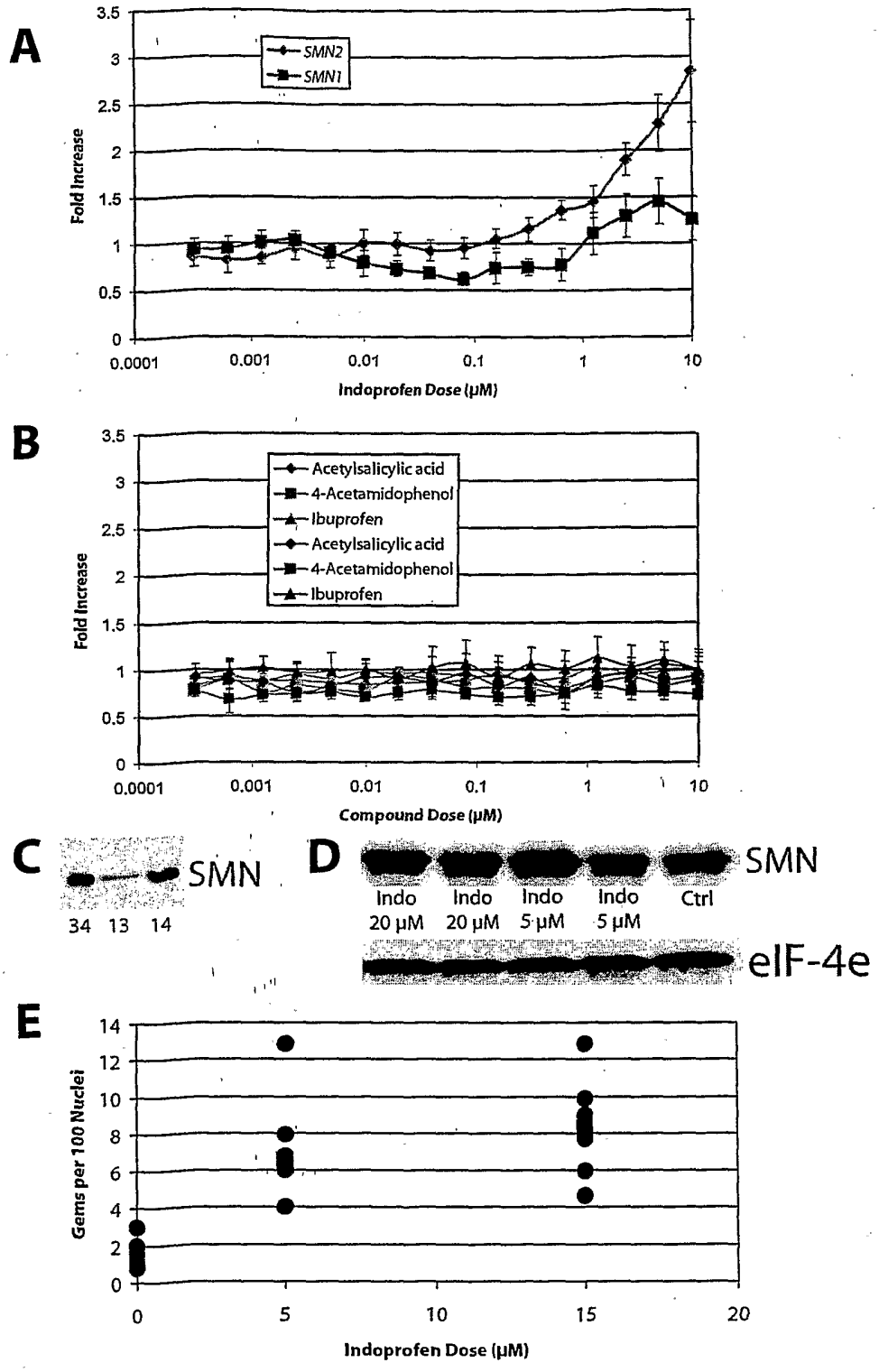

A
SMN2
SMN1
Fold Increase
Indoprofen Dose (μM)
B
Acetylsalicylic acid
4-Acetamidophenol
Ibuprofen
Acetylsalicylic acid
4-Acetamidophenol
Ibuprofen
Fold Increase
Compound Dose (μM)
C
SMN
34 13 14
D
SMN
Indo 20 μM
Indo 20 μM
Indo 5 μM
Indo 5 μM
Ctrl
eIF-4e
E
Gems per 100 Nuclei
Indoprofen Dose (μM)

FIGURE 3