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(54) Title: COMPOUNDS AND KITS FOR TREATING MUSCLE DISORDERS AND METHODS OF USE THEREOF

(57) Abstract: Described herein are compounds and kits for treating muscle disorders and methods of use thereof.
COMPOUNDS AND KITS FOR TREATING MUSCLE DISORDERS AND METHODS OF USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. provisional application Serial Nos. 60/578,914 and 60/633,274, filed on June 12, 2004 and December 3, 2004, respectively. These applications are hereby incorporated by this reference in their entireties for all of their teachings.

BACKGROUND

The treatment of muscle disorders is a growing field of technology. Muscle disorders caused by genetic defects, trauma, or ischemia can be very debilitating and can substantially reduce the quality of life of the patient.

It is believed that calpain is the primary protease that initiates the degradation of skeletal muscle. Calpains are a family of Ca$^{2+}$ activated intracellular proteases whose activity is accelerated when abnormal amounts of Ca$^{2+}$ enter the cell by virtue of increased membrane permeability as a result of some traumatic or ischemic event and/or a genetic defect. Calpain is one of a relatively small family of cysteine proteases, which are active in promoting programmed cell death, or apoptosis. It has been implicated in the initiation of both necrotic and apoptotic cell death.

The trigger which activates calpain is Ca$^{2+}$ ions leaking into cells, where the levels are generally very low. The dystrophin gene responsible for muscular dystrophy, for instance, is involved in maintaining membrane integrity, and when it is mutated, the membrane is more permeable to calcium ions. Calpain has been implicated in the neurotoxicity that follows spinal cord injury. Tissues weakened by ischemia/reperfusion injury, such as occurs following stroke or myocardial infarct, admit Ca$^{2+}$ ions. Over the past ten years, it has emerged that calpain enzymatic activity plays a key role in a very large number of cellular degenerative conditions.

Leupeptin, a tripeptide aldehyde, has been shown to be a potent inhibitor of thiol proteases of the calpain class of enzymes. Leupeptin is produced by streptomyces strains. One of the problems in using leupeptin, either by oral or parenteral administration, is that it distributes itself indiscriminately to all parts of the body, when only muscle tissue should be targeted. One approach is to administer
larger doses that are necessary to get the desired result; however, this can cause potential dose-limiting side effects in other parts of the body and, in the case of leupeptin, is expensive. Thus, there is a need for compounds and methods to target more directly muscle tissue with greater efficacy at lower dosages and higher safety margin.

**SUMMARY**

Described herein are compounds and kits for treating muscle disorders and methods of use thereof. The advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the aspects described below. The advantages described below will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive.

**BRIEF DESCRIPTION OF THE DRAWINGS**

The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several aspects described below. Like numbers represent the same elements throughout the figures.

Figure 1 depicts a reaction scheme for synthesizing aminocarnityl-succinyl-leucyl-argininal.

Figure 2 shows the absorption of leupeptin (18 mg/kg) into plasma after intramuscular injection in hind-limbs of rats, which shows a peak of 4.5 μg/ml at 1 hr and a return to 0 by 24 hr.

Figure 3 shows the quantification of myofiber diameters, which shows statistically significant increased diameters in the right opponens and abductor muscles of treated animals, at both 12 and 24 weeks, after delayed nerve repair. Normal myofiber diameter, obtained as a mean in the left, unoperated hand of control animals, is shown by open arrows on the left of graph.

Figure 4 shows the mean myofiber diameters in the left and right opponens pollicis muscles 3 months after right median nerve repair. On the right, all leupeptin treatment groups show a significant increase compared to the right, control group (*p
Figure 5 shows mean myofiber diameters in the left and right abductor pollicis muscles 6 months after right median nerve repair. On the left, the 6-mg, 12-mg, and 18-mg leupeptin treatment groups show a small but significant increase, compared to the left, control group (*p <0.01). On the right, the 6-mg, 12-mg, and 18-mg leupeptin-treatment groups show a significant increase, compared to the right, control group (*p <0.01).

Figure 6 shows the appearance of spectrin breakdown products in RD cells that have been incubated with maitotoxin in the presence of calcium.

**DETAILED DESCRIPTION**

Before the present compounds, compositions, articles, devices, and/or methods are disclosed and described, it is to be understood that the aspects described below are not limited to specific compounds, synthetic methods, or uses as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting.

In this specification and in the claims that follow, reference will be made to a number of terms that shall be defined to have the following meanings:

It must be noted that, as used in the specification and the appended claims, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a pharmaceutical carrier” includes mixtures of two or more such carriers, and the like.

“Optional” or “optionally” means that the subsequently described event or circumstance can or cannot occur, and that the description includes instances where the event or circumstance occurs and instances where it does not. For example, the phrase “optionally substituted lower alkyl” means that the lower alkyl group can or can not be substituted and that the description includes both unsubstituted lower alkyl and lower alkyl where there is substitution.

Ranges may be expressed herein as from “about” one particular value, and/or to “about” another particular value. When such a range is expressed, another aspect includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent “about,” it will
be understood that the particular value forms another aspect. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint.

References in the specification and concluding claims to parts by weight, of a particular element or component in a composition or article, denote the weight relationship between the element or component and any other elements or components in the composition or article for which a part by weight is expressed. Thus, in a compound containing 2 parts by weight of component X and 5 parts by weight component Y, X and Y are present at a weight ratio of 2:5, and are present in such ratio regardless of whether additional components are contained in the compound.

A weight percent of a component, unless specifically stated to the contrary, is based on the total weight of the formulation or composition in which the component is included.

Variables such as R₁-R₆, R₁₀, m, n, o, X, Y, and Z used throughout the application are the same variables as previously defined unless stated to the contrary.

The term “alkyl group” as used herein is a branched or unbranched saturated hydrocarbon group of 1 to 25 carbon atoms, such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, t-butyl, pentyl, hexyl, heptyl, octyl, decyl, tetradecyl, hexadecyl, eicosyl, tetracosyl and the like. A “lower alkyl” group is an alkyl group containing from one to six carbon atoms.

The term “polyalkylene group” as used herein is a group having two or more CH₂ groups linked to one another. The polyalkylene group can be represented by the formula −(CH₂)ᵦ−, where n is an integer of from 2 to 25.

The term “polyether group” as used herein is a group having the formula −[(CHR)ₙO]ₘ−, where R is hydrogen or a lower alkyl group, n is an integer of from 1 to 20, and m is an integer of from 1 to 100. Examples of polyether groups include, polyethylene oxide, polypropylene oxide, and polybutylene oxide.

The term “polythioether group” as used herein is a group having the formula −[(CHR)ₙS]ₘ−, where R is hydrogen or a lower alkyl group, n is an integer of from 1 to 20, and m is an integer of from 1 to 100.

The term “polyimino group” as used herein is a group having the formula
–[(CHR<sub>h</sub>NR<sub>m</sub>)<sub>n</sub>], where each R is, independently, hydrogen or a lower alkyl group, n is an integer of from 1 to 20, and m is an integer of from 1 to 100.

The term “polyester group” as used herein is a group that is produced by the reaction between a compound having at least two carboxylic acid groups with a compound having at least two hydroxyl groups.

The term “polyamide group” as used herein is a group that is produced by the reaction between a compound having at least two carboxylic acid groups with a compound having at least two unsubstituted or monosubstituted amino groups.

The term “aryl group” as used herein is any carbon-based aromatic group including, but not limited to, benzene, naphthalene, etc. The term “aromatic” also includes “heteroaryl group,” which is defined as an aromatic group that has at least one heteroatom incorporated within the ring of the aromatic group. Examples of heteroatoms include, but are not limited to, nitrogen, oxygen, sulfur, and phosphorus. The aryl group can be substituted or unsubstituted. The aryl group can be substituted with one or more groups including, but not limited to, alkyl, alkenyl, alkenyl, aryl, halide, nitro, amino, ester, ketone, aldehyde, hydroxy, carboxylic acid, or alkoxy.

By “subject” is meant an individual. Preferably, the subject is a mammal such as a primate, and, more preferably, a human. The term “subject” can include domesticated animals, such as cats, dogs, etc., livestock (e.g., cattle, horses, pigs, sheep, goats, etc.), and laboratory animals (e.g., mouse, rabbit, rat, guinea pig, etc.).

By “contacting” is meant an instance of exposure by close physical contact of at least one substance to another substance. For example, contacting can include contacting a substance, such as a pharmacologic agent, with a cell. A cell can be contacted with a test compound, for example, a context dependent inhibitor, or putative context dependent inhibitor by adding the agent to the culture medium (by continuous infusion, by bolus delivery, or by changing the medium to a medium that contains the agent) or by adding the agent to the extracellular fluid in vivo (by local delivery, systemic delivery, intravenous injection, bolus delivery, or continuous infusion). The duration of contact with a cell or group of cells is determined by the time the test compound is present at physiologically effective levels or at presumed physiologically effective levels in the medium or extracellular fluid bathing the cell.

“Induce” means initiating a desired response or result that was not present
prior to the induction step. The term “potentiate” means sustaining a desired response at the same level prior to the potentiating step or increasing the desired response over a period of time.

“Treatment” or “treating” means to administer a composition to a subject or a system with an undesired condition or at risk for the condition. The condition can include a disease or a predisposition to a disease. The effect of the administration of the composition to the subject can have the effect of but is not limited to reducing or preventing the symptoms of the condition, a reduction in the severity of the condition, or the complete ablation of the condition.

By “effective amount” is meant a therapeutic amount needed to achieve the desired result or results, e.g., inhibiting enzymatic activity.

Disclosed are compounds, compositions, and components that can be used for, can be used in conjunction with, can be used in preparation for, or are products of the disclosed methods and compositions. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a number of different carrier molecules and protease inhibitors are disclosed and discussed, each and every combination and permutation of the carrier molecule and the protease inhibitor are specifically contemplated unless specifically indicated to the contrary. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not individually recited, each is individually and collectively contemplated. Thus, in this example, each of the combinations A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are specifically contemplated and should be considered disclosed from disclosure of A, B, and C; D, E, and F; and the example combination A-D. Likewise, any subset or combination of these is also specifically contemplated and disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E are specifically contemplated and should be considered disclosed from disclosure of A, B, and C; D, E, and F; and the example combination A-D. This concept applies to all aspects of this disclosure including, but not limited to, steps in methods of making
and using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific embodiment or combination of embodiments of the disclosed methods, and that each such combination is specifically contemplated and should be considered disclosed.

I. Compounds

In one aspect, the compounds described herein that can be used to treat or prevent muscle disorders are composed of a carrier molecule that is directly or indirectly bonded to one or more protease inhibitors. The term “compound” as used herein also includes the corresponding prodrug or pharmaceutically-acceptable salt or ester thereof of the compound.

The term “carrier molecule” as defined herein is any compound that can facilitate the delivery of the protease inhibitor to a particular muscle tissue. In one aspect, the carrier molecule can be any endogenous molecule. In an alternative embodiment, the carrier molecule can be a derivative of an endogenous compound. Examples of carrier molecules useful herein include, but are not limited to, carnitine or aminocarnitine.

In one aspect, the carrier molecule has the formula I or IX

\[
\text{HOOC} \quad \begin{array}{c}
\text{m} \\
\text{X}
\end{array} \quad \begin{array}{c}
\text{n} \\
\text{NR}^1 R^2 R^3^+ \quad \text{Y}
\end{array} \quad \begin{array}{c}
\text{I}
\end{array}
\]

\[
\text{HOOC} \quad \begin{array}{c}
\text{m} \\
\text{X}
\end{array} \quad \begin{array}{c}
\text{n} \\
\text{NR}^1 R^2
\end{array} \quad \begin{array}{c}
\text{IX}
\end{array}
\]

wherein each \( R^1, R^2, \) and \( R^3 \) comprises, independently, hydrogen or a branched- or straight chain alkyl group, 
\( X \) comprises \( \text{OH} \) or \( \text{NHR}^6 \), wherein \( R^6 \) comprises hydrogen or a branched- or straight-chain alkyl group;
\( Y \) comprises a pharmaceutically-acceptable anion; and
\( m \) and \( n \) can be an integer from 1 to 10,
or the pharmaceutically-acceptable salt or ester thereof.
The carrier molecules having the formula I or IX can be the L- or D-isomer, or a mixture thereof. In one aspect, the carrier molecule having the formula I is the L-isomer. In one aspect, m and n can be an integer from 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, or 1 to 3. In another aspect, m and n are 1. In a further aspect, R\(^1\), R\(^2\), and R\(^3\) in formula I can be a C\(_1\)-C\(_3\) alkyl group. In another aspect, R\(^1\), R\(^2\), and R\(^3\) in formula I can each be a methyl group. In another aspect, X is an OH group, R\(^1\), R\(^2\), and R\(^3\) in formula I are each a methyl group, and m and n are 1. In this aspect, the carrier molecule is referred to as carnitine. In another aspect, X is NH\(_2\), R\(^1\), R\(^2\), and R\(^3\) in formula I are each a methyl group, and m and n are 1. In this aspect, the carrier molecule is referred to as aminocarnitine.

The carrier molecules useful herein can be the substantially pure L- or D-isomer, or a mixture thereof. The term “substantially pure” with respect to the L- or D-isomer refers to greater than 95%, greater than 97%, greater than 98%, greater than 99%, greater than 99.5%, or 100% of one isomer with respect to the other enantiomer.

The term “protease inhibitor” is defined herein as any molecule that interacts with a protease and reversibly or irreversibly inhibits proteolytic activity. The term “protease inhibitor” also includes precursor molecules that can be converted to a protease inhibitor upon administration to a subject. In one aspect, the protease inhibitor is a calpain inhibitor. In one aspect, the protease inhibitor is a peptide aldehyde, a peptide boronate, or a vinyl sulfone. In another aspect, the protease inhibitor is a peptide having at least one aldehyde group or oxirane group. Examples of protease inhibitors include, but are not limited to, pepstatin, bestatin, Bowman-Birk inhibitor, chymostatin, bacitracin, lactacystin, clasto-lactacystin-\(\beta\)-lactone, ritonavir, saquinavir, indinavir, nelfinavir, amprenavir, calpastatin or a peptide fragment thereof, or the like. In another aspect, the protease inhibitor is a peptide-aldehyde protease inhibitor. The protease inhibitors disclosed in Lee D.H. and Goldberg A.L. “Proteasome inhibitors: valuable new tools for cell biologist,” Cell Biology, 8, 397-399, 1998; Goll DE, Thompson VF, Li H, Wei W, Cong J. “The calpain system,” Physiol Rev. 2003 Jul;83(3):731-801; Hernandez AA, Roush WR “Recent advances in the synthesis, design and selection of cysteine protease inhibitors,” Curr Opin Chem Biol. 2002 Aug;6(4):459-65; Perrin BJ, Huttenlocher A. “Calpain,” Int J Biochem Cell Biol. 2002 Jul;34(7):722-5; Laval SH, Bushby KM “Limb-girdle

In one aspect, the protease inhibitor is a calpain inhibitor. Calpain inhibitors of the present invention are known and have been described in numerous scientific publications and patent literature. For example, U.S. Pat. No. 5,081,204 (Higuchi), U.S. Pat. No. 5,486,623 (Zimmerman), U.S. Pat. No. 5,498,616 (Mallamo), U.S. Pat. No. 5,506,243 (Ando), and U.S. Pat. No. 5,514,694 (Powers) describe a variety of different chemical entities for the inhibition of calpain including: N-substituted peptidyl compounds, peptidyl ketone heterocyclic ethers, heterocyclic-N-heteroatom methyl ketones, sulfonamide pyrrolidines, and peptidyl ketoamides, respectively. Additional examples of calpain inhibitors in the patent literature include WIPO Publication Nos. WO 92/11850 (Cortex Pharmaceutical), WO 94/00095 (Cortex) and WO 95/00535 (Alkermes Inc.) which disclose peptide keto compounds, peptide aldehydes and alpha-ketoamides, respectively. Other examples of calpain inhibitors have been published in European Patent Application Publications. Still other calpain inhibitors in the scientific literature include alpha-mercaptoacrylic acids, disclosed in Proc. Natl. Acad. Sci. USA, volume 93, pages 6687-6692 (1996). Examples of calpain inhibitors include, but are not limited to, leucyl-argininal, benzamidine derivatives, leupeptin, PhCH₂OCO-leucine-norvaline-CONH-CH₂-2-pyridyl, Ph₂CHCO-leucine-alpha-aminobutyric acid-CONH-CH₂-2-pyridyl, Ph₂CHCO-
leucine-alpha-aminobutyric acid-CONH-(CH₂)₃-4-morpholinyl, PhCH₂OCO-leucine-alpha-aminobutyric acid-CONH-CH₂-2-pyridyl, and PhCH₂OCO-leucine-alpha-aminobutyric acid-CONH-CH₂-CH(OH)Ph.

The carrier molecule and protease inhibitor can be directly or indirectly bonded to one another. When the carrier molecule and protease inhibitor are directly bonded to one another, a new covalent bond is formed between the carrier molecule and the protease inhibitor. For example, when X in formula I or IX is a hydroxyl group, the hydroxyl group can react with a COOH group or ester thereof present on the protease inhibitor to form a new ester linkage. In another embodiment, functional groups present on the carrier molecule and/or protease inhibitor can be chemically modified prior to direct or indirect bonding.

Alternatively, the carrier molecule and protease inhibitor can be indirectly bonded to one another with the use of a linker molecule. A “linker molecule” is any compound that has at least one group that can form a covalent bond with the carrier molecule and at least one group that can form a covalent bond with the protease inhibitor. In one aspect, the linker group can have up to 25 carbon atoms. In one aspect, the linker can be a polyether having two or more groups capable of reacting with the carrier molecule and protease inhibitor. For example, the polyether can have two or more hydroxyl groups, amino groups, carboxyl groups, or combinations thereof. It is contemplated that the polyether can be of varying molecular weight. Examples of linker molecules include, but are not limited to, ε-aminocaproic acid, polyethylene glycol, glutaraldehyde, and the like. It is contemplated that the linker molecule can be covalently attached to the carrier molecule, the protease inhibitor, or both prior to linking the carrier molecule to the protease inhibitor. In one aspect, the linker has the formula -C₁^(O)(CH₂)ₙ(O)C²⁻, wherein n is an integer of from 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, or 1 to 5, 1 to 4, 1 to 3, or 2, wherein C₁ is covalently bonded to the carrier molecule and C² is covalently bonded to the protease inhibitor.

The techniques disclosed in U.S. Patent Nos. 4,742,081; 4,866,040; 5,008,288; and 5,876,747 for directly or indirectly bonding a carrier molecule to a protease inhibitor, which are incorporated by reference in their entireties, can be used herein.

Any of the compounds described herein can exist as a prodrug. The term “prodrug” is defined herein as an inactive form of a parent drug that has been created
to overcome one or more barriers to their effective use. For example, a prodrug can be a compound that has a protecting group that is cleaved upon administration to a subject to produce the active form of the drug. In certain aspects, if the active compound is unstable, it can be prepared as its salt form in order to increase stability in dry form (e.g., powder). Methods for converting prodrugs of the compounds described herein will be discussed in greater detail below.

The term “protecting group” as used herein is a group that can be chemically bound to an atom, and subsequently removed (either chemically, in-vitro, or in-vivo) from the atom by predictable methods. Examples of many of the possible protective groups can be found in *Protective Groups in Organic Synthesis* by T.W. Green, John Wiley and Sons, 1981, which is incorporated herein by reference in its entirety. In one embodiment, if the active compound has an aldehyde group, the aldehyde group can be converted to an acetal or hemiacetal using techniques known in the art, which can be cleaved under acidic conditions to produce the active form of the compound (i.e., reproduce the aldehyde group). Prodrug forms of the compounds described herein can also be more stable when compared to the active form, which also prolongs the shelf-life of the compound. It is contemplated that the carrier molecule, the protease inhibitor, or both can have groups that can be protected to convert the compound to the prodrug.

Any of the compounds described herein can be the pharmaceutically-acceptable salt or ester thereof. In one aspect, pharmaceutically-acceptable salts are prepared by treating the free acid with an appropriate amount of a pharmaceutically-acceptable base. Representative pharmaceutically-acceptable bases are ammonium hydroxide, sodium hydroxide, potassium hydroxide, lithium hydroxide, calcium hydroxide, magnesium hydroxide, ferrous hydroxide, zinc hydroxide, copper hydroxide, aluminum hydroxide, ferric hydroxide, isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, lysine, arginine, histidine, and the like. In one aspect, the reaction is conducted in water, alone or in combination with an inert, water-miscible organic solvent, at a temperature of from about 0 °C to about 100 °C such as at room temperature. In certain aspects where applicable, the molar ratio of the compounds described herein to base used are chosen to provide the ratio desired for any particular
salts. For preparing, for example, the ammonium salts of the free acid starting
material, the starting material can be treated with approximately one equivalent of
pharmaceutically-acceptable base to yield a neutral salt.

In another aspect, if the compound possesses a basic group, it can be
protonated with an acid such as, for example, HCl, HBr, or H₂SO₄, to produce the
cationic salt. For example, the techniques disclosed in U.S. Patent No. 5,436,229 for
producing the sulfate salts of argininal aldehydes, which is incorporated by reference
in its entirety, can be used herein. In the case of H₂SO₄, the corresponding sulfate
\((SO₄^{2-})\) or hydrogen sulfate \((HSO₄^-)\) salt can be produced depending upon the amount
of acid that is employed. In one aspect, the reaction of the compound with the acid or
base is conducted in water, alone or in combination with an inert, water-miscible
organic solvent, at a temperature of from about 0 °C to about 100 °C such as at room
temperature. In certain aspects where applicable, the molar ratio of the compounds
described herein to base used are chosen to provide the ratio desired for any particular
salts. For preparing, for example, the ammonium salts of the free acid starting
material, the starting material can be treated with approximately one equivalent of
pharmaceutically-acceptable base to yield a neutral salt.

Ester derivatives are typically prepared as precursors to the acid form of the
compounds--as illustrated in the examples below--and accordingly can serve as
prodrugs. Generally, these derivatives will be lower alkyl esters such as methyl,
ethyl, and the like. Amide derivatives -(CO)NH₂, -(CO)NHR and -(CO)NR₂, where R
is an alkyl group defined above, can be prepared by reaction of the carboxylic acid-
containing compound with ammonia or a substituted amine.

In one aspect the compound is produced from a carrier molecule having the
formula I that is indirectly linked to a protease inhibitor by a linker molecule. In one
aspect, the compound is produced from carnitine or aminocarnitine, leucine or a
derivative thereof, and arginine or a derivative thereof. In one aspect, the compound
can be (1) carnitine or aminocarnitine covalently bonded to leucine or a derivative
thereof through a linker such as, for example, glutaric acid or succinic acid, and (2)
the leucine residue is covalently bonded to arginine or a derivative thereof. In another
aspect, the compound has the formulae II or III
wherein $R^1$-$R^5$, $X$, $Y$, $m$ and $n$ are defined as above, $o$ is an integer from 1 to 10, and $Z$ is a pharmaceutically-acceptable anion, and $R^4$ and $R^5$ can be, independently, a branched- or straight-chain alkyl group having from 1 to 20 carbon atoms or protecting group, or $C(O)R^{10}$, where $R^{10}$ can be independently a branched- or straight-chain alkyl group having from 1 to 20 carbon atoms. Alternatively, $R^4$-$R^5$ can be part of a ring. In one aspect, the compound has the formula II, wherein $R^1$, $R^2$, and $R^3$ are methyl, $m$ and $n$ are 1, $X$ is NH, and $o$ is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10. In another aspect, the compound has the formula III, wherein $R^1$, $R^2$, and $R^3$ are methyl, $R^4$ and $R^5$ are butyl, $m$ and $n$ are 1, $X$ is NH, and $o$ is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10. In another aspect, the compound has the formula III, wherein $m$ and $n$ are 1, $R^1$-$R^3$ are methyl, $R^4$ and $R^5$ are each $C(O)R^{10}$, $X$ is NH, and $o$ is 2. In a further aspect, the compound has the formula III, wherein the compound can be hydrolyzed at a pH of 1 to 3 at a temperature of 37 °C.

In another aspect, the compound has the formulae IV or V.
where compound IV has the chemical name aminocarnityl-succinyl-leucyl-argininal, which is also referred to herein as CLA. In one aspect, a synthetic scheme for producing compound IV is depicted in Figure 1. Prodrugs of compounds IV and V are also contemplated. In one aspect, the prodrug can have the formulae VI and VII
wherein R^4 and R^5 can be, independently, a branched- or straight-chain alkyl group having from 1 to 20 carbon atoms or protecting group, or C(O)R^{10}, where R^{10} can be independently a branched- or straight-chain alkyl group having from 1 to 20 carbon atoms. In this aspect, formulae VI and VII are the diacetals and diesters of formulae IV and V, respectively. Alternatively, R^4-R^5 can be part of a ring. In this aspect, formulae VI and VII are cyclic acetals of formulae IV and V. In one aspect, R^4 and R^5 of formulae VI and VII can be, independently, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, isopentyl, or hexyl. In another aspect, R^4 and R^5 in formulae VI and VII are ethyl. In formulae II-VII, any pharmaceutically-acceptable salt or ester thereof is contemplated. In the case of formulae VI and VII, in one aspect, the acetal can be readily converted to the aldehyde by exposing the aldehyde to an acid. The synthetic techniques disclosed in U.S. Patent Nos. 4,742,081; 4,866,040; 5,008,288; and 5,876,747, which are incorporated by reference for their teachings, can be used to produce the compounds and prodrugs in formulae II-VII, respectively.

In another aspect, the compound has the formula VIII:
which is the sulfate salt of aminocarnityl-succinyl-leucyl-argininal. The compound having the formula VIII can also exist as the hydrogen sulfate salt (HSO₄⁻).

In one aspect, when the protease inhibitor is leucyl-argininal in any of the formulae described above, the stereochemistry about the two stereocenters in leucyl-argininal is L and L. In other aspects, the stereochemistry about the two stereocenters is L, D; D, L; or D, D.

II. Kits

Described herein are kits useful for treating or preventing a muscle disorder. In one aspect, described herein is a kit for treating or preventing a muscle disorder in a subject comprising:

a. an effective amount of a prodrug of a compound, wherein the compound comprises a carrier molecule and a protease inhibitor, wherein the carrier molecule is directly or indirectly bonded to one or more protease inhibitors, and

b. an activator, wherein the activator converts the prodrug to the active form of the compound.

It is contemplated that prodrugs of any of the compounds described can be used in the kit. In one aspect, a neutralizing base can be added to the mixture of prodrug and activator, to reduce the acidity of the activated drug prior to administration. In another aspect, any of the compounds depicted in formulae III, VI, and VII can be
used as the prodrug in the kit.

The activator used in the kit is a compound or mixture of compounds that converts the prodrug to the active form of the compound. The term “active compound” as defined herein is the compound that induces or potentiates a desired response. For example, the compounds depicted in formula III are acetals. Treatment of the acetal III with an acid produces compounds having the formula II, which is an aldehyde. Not wishing to be bound by theory, it is believed that the aldehyde group in formula II makes compounds in formula II the active compound that can treat or prevent a muscle disorder in a subject. The selection of the activator will vary depending upon the prodrug (i.e., the nature of the protecting group). Examples of activators include, but are not limited to, inorganic acids such as, for example, hydrochloric acid, sulfuric acid, nitric acid, phosphoric acid, organic acids such as, for example, maleic, oxalic, citric, tartaric, and acetic acids. In addition, activators may include substances that act as catalysts for activation such as, for example, enzymes, metals, salts, polymers, detergents, and zeolites. Examples of enzymes useful herein include hydrolases, esterases, glycosidases, glycanases, proteases, lipid-metabolizing enzymes, oxidases, and cytochrome P450 enzymes.

Depending upon the activator that is used, a neutralizing agent can be an optional component of the kit. The neutralizing agent is any compound or mixture of compounds that renders the activating agent safe for consumption or administration to a subject after the prodrug has been converted to the active compound. For example, the neutralizing agent can render the activator inactive after the prodrug has been converted to the active compound or, in the alternative, convert the activator to a compound that is not harmful to a subject. The neutralizing agent will vary depending upon the selection of the activator. For example, when the activator is an acid, the neutralizing agent can be a strong inorganic base such as sodium hydroxide or a weak base such as dibasic sodium phosphate. In another aspect, once the activator has converted the prodrug to the active compound, the mixture can be diluted in a beverage to neutralize any remaining activator.

Depending upon the end-use of the kit, the prodrug, activator, and neutralizing agent can exist as solids or solutions. For example, it is contemplated that the prodrug be in a solid or aqueous solution and the activator be in aqueous solution.
Alternatively, it is possible that the prodrug and the activator are both in solid form together or separately. In this aspect, water alone or in combination with other components can be added to the solid mixture. For example, sterile water, saline, and buffered solutions at physiological pH can be used to store the prodrug or activate the prodrug with the activator.

Depending upon the selection of the activator and the prodrug, it may be desirable to heat the prodrug and the activator upon mixing in order to expedite the conversion of the prodrug to the active compound. In one aspect, the kit further comprises a heating device. In one aspect, the heating device can be a Peltier block modified to hold a vial containing a mixture of the prodrug and activator. It is also contemplated that the kit can comprise a cooling device to cool the solution of the active compound if the solution was heated by the heating device.

In one aspect, the prodrug has the formula XI

```
CO₂H
CH₂
HC-NH-C-CH₂CH₂-C-NH-C-NH-CH-CH₂
     O   O   O   O
N(CH₃)₃⁺ Y⁻   OEt   OEt
CH₂
H₃C  CH  CH₃
H₂C
CH₂CH₂NHCNH₂
H₂⁺ Z⁻
```

and the activator is HCl.

The prodrug and activator can be admixed in any order. The duration of the admixing can vary depending upon the prodrug and activator selected as well as the relative amount of activator to prodrug. The duration of admixing is sufficient to convert the prodrug substantially (i.e., greater than 80%, greater than 85%, greater than 90%, greater than 95%, or greater than 99%) or completely to the active compound. Once the activator and the prodrug are admixed to produce the active compound, the active compound can be administered to the subject by a variety of techniques. Methods for delivering the compounds described herein including the active compound produced from the kits will be described below.
III. Methods of Use

*Delivery*

As used throughout, administration of any of the compounds, which include the prodrugs and the active compounds produce by the kits described herein, can occur in conjunction with other therapeutic agents. Thus, the compound can be administered alone or in combination with one or more therapeutic agents. For example, a subject can be treated with a compound alone, or in combination with chemotherapeutic agents, antibodies, antivirals, steroidal and non-steroidal anti-inflammatory agents, corticosteroids, conventional immunotherapeutic agents, cytokines, chemokines, and/or growth factors. Combinations may be administered either concomitantly (e.g., as an admixture), separately but simultaneously (e.g., via separate intravenous lines into the same subject), or sequentially (e.g., one of the compounds or agents is given first followed by the second). Thus, the term “combination” or “combined” is used to refer to either concomitant, simultaneous, or sequential administration of two or more agents.

The compounds, which include the prodrugs and the active compounds produce by the kits described herein, can be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. Administration may be topically (including ophthalmically, vaginally, rectally, intranasally), orally, by inhalation, or parenterally, for example by intravenous drip, subcutaneous, intraperitoneal or intramuscular injection. The disclosed compounds can be administered intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity, transdermally, intratracheally, extracorporeally, sublingually, rectally, or topically (e.g., topical intranasal administration or administration by inhalant). As used herein, “topical intranasal administration” means delivery of the compositions into the nose and nasal passages through one or both of the nares and can comprise delivery by a spraying mechanism. The latter can be effective when a large number of subjects are to be treated simultaneously. Administration of the compositions by inhalant can be through the nose or mouth via delivery by a spraying mechanism or droplet mechanism. Delivery can also be directly to any area of the respiratory system (e.g., lungs) via intubation.

In one aspect, when the compound is a prodrug, the compound can be
administered orally, so that after digestion, the acidic conditions present in the stomach can convert the prodrug to the active form. For example, a compound having the formula VI, which is an acetal, can be administered orally and converted to the corresponding aldehyde IV by cleavage of the acetal group in vivo.

Parenteral administration of the compound, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution of suspension in liquid prior to injection, or as emulsions. Alternatively, parenteral administration can involve the use of a slow release or sustained release system such that a constant dosage is maintained.

The exact amount of the compounds described herein required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the severity of the disorder being treated, the particular muscle tissue to be targeted, its mode of administration and the like. Thus, it is not possible to specify an exact amount for every compound. However, an appropriate amount can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein. The time at which the compounds can be administered will also vary depending upon the subject, the disorder, mode of administration, etc. The compound can be administered to the subject prior to the onset of the muscle disorder or during a time when the subject is experiencing symptoms of the muscle disorder. The compound can be administered over several weeks or months at varying intervals depending upon the subject and disorder to be treated. It is also contemplated that the compounds described herein can be administered daily in single or multiple doses.

The present compounds are generally administered in a therapeutically effective amount. That is, an amount that is sufficient to achieve the desired therapeutic result or to have an effect on undesired symptoms, but is generally insufficient to cause adverse side affects. For example, the compounds described herein can be administered in a therapeutically effective amount at a dosage from 0.01 mg/kg to 1,000 mg/kg. In another aspect, the lower endpoint of the dosage is 0.01, 0.05, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80, 0.90, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0, 45.0, 50.0, 60.0, 70.0, 80.0, 90.0,
100.0, 150.0, 200.0, 250.0, 300.0, 350.0, 400.0, 450.0, 500.0, 600.0, 700.0, 800.0, or 900.0 mg/kg, and the upper endpoint of the dosage is 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 15.0, 20.0, 25.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0, 100.0, 150.0, 200.0, 250.0, 300.0, 350.0, 400.0, 450.0, 500.0, 550.0, 600.0, 650.0, 700.0, 750.0, 800.0, 850.0, 900.0, 950.0, or 1,000.0 mg/kg, where any lower endpoint can be used with any upper endpoint, where the lower endpoint is less than the upper endpoint. In another aspect, the dosage is from 1 to 10 mg/kg or 1 to 5 mg/kg.

**Pharmaceutically-Acceptable Carriers**

In one aspect, any of the compounds described above, including prodrugs and active compounds produced by the kit, can be combined with at least one pharmaceutically-acceptable carrier to produce a pharmaceutical composition. The pharmaceutical compositions can be prepared using techniques known in the art. In one aspect, the composition is prepared by admixing the compound with a pharmaceutically-acceptable carrier. The term "admixing" is defined as mixing the two components together. Depending upon the components to be admixed, there may or may not be a chemical or physical interaction between two or more components.

Pharmaceutically-acceptable carriers are known to those skilled in the art. These most typically would be standard carriers for administration to humans, including solutions such as sterile water, saline, and buffered solutions at physiological pH.

Molecules intended for pharmaceutical delivery may be formulated in a pharmaceutical composition. Pharmaceutical compositions may include carriers, sweeteners, thickeners, diluents, buffers, preservatives, surface active agents and the like in addition to the molecule of choice. Pharmaceutical compositions may also include one or more active ingredients such as antimicrobial agents, antiinflammatory agents, anesthetics, and the like.

The pharmaceutical composition may be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. Administration may be topically (including ophthalmically, vaginally, rectally, intranasally).

Preparations for administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of aqueous or non-aqueous carriers
include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles, if needed for collateral use of the disclosed compositions and methods, include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles, if needed for collateral use of the disclosed compositions and methods, include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

Formulations for topical administration may include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

It will be appreciated that the actual preferred amounts of compound in a specified case will vary according to the specific compound being utilized, the particular compositions formulated, the mode of application, and the particular situs and mammal being treated. Dosages for a given host can be determined using conventional considerations, e.g. by customary comparison of the differential activities of the subject compounds and of a known agent, e.g., by means of an appropriate conventional pharmacological protocol. Physicians and formulators, skilled in the art of determining doses of pharmaceutical compounds, will have no problems determining dose according to standard recommendations (Physicians Desk Reference, Barnhart Publishing (1999)).

In another aspect, any of the compounds described herein can be administered to a subject with the use of a liposome. In one aspect, the carrier molecule and/or protease inhibitor of the compound can be covalently attached to the liposome by reacting the carrier molecule and/or protease inhibitor with the fatty acid used to produce the liposome. In another aspect, the compounds described herein can be enclosed within the inner volume of the liposome and not covalently attached to the liposome. Examples of liposomes useful herein include, but are not limited to, conventional liposomes, long-circulating liposomes, immunoliposomes, and cationic liposomes. The methods disclosed in U.S. Patent Nos. 4,866,040; 5,008,288; and
5,876,747, which are incorporated by reference, can be used to produce liposome formulations with the compounds described herein. In another aspect, the compounds described herein can be delivered to a subject via micelles, nanoparticles, microspheres, and lipoproteins.

5 Therapeutic Uses

In one aspect, disclosed are methods for preventing or treating a muscle disorder in a subject, which involves administering an effective amount of any of the compounds described herein, including prodrugs and active compounds produced by the kits, to a subject in need of such treatment or prevention. Examples of muscle disorders that can be treated or prevented by the methods described herein include, but are not limited to, muscular dystrophies (e.g., DMD, BMD, LGMD, FSHMD); muscle wasting caused from, for example, cancer, denervation atrophy, AIDS, diabetes, malnutrition, or disuse; and cardiovascular disorders such as, for example, cardiomyopathies and cardiac ischemia.

15 In one aspect, the compounds described herein can treat muscular dystrophy in a subject. Muscular dystrophy (MD) refers to a group of genetic diseases characterized by progressive weakness and degeneration of the skeletal or voluntary muscles which control movement. The muscles of the heart and some other involuntary muscles are also affected in some forms of MD, and a few forms involve other organs as well. The major forms of MD include myotonic, Duchenne (DMD), Becker (BMD), limb-girdle, and other forms. DMD is the most common form of MD affecting children. MD can affect people of all ages. Although some forms first become apparent in infancy or childhood, others may not appear until middle age or later. There is no definitive therapy for this horrible disease. In another aspect, when a prodrug is administered to a subject, the prodrug diffuses into muscle cells or is taken up into muscle cells by an active or passive transport system and converted to the active form of the compound. In this aspect, this is particularly useful because the prodrug does not get converted to the active compound until it reaches the desired target, which is muscle cells and tissue. In another aspect, the active compounds produced by the kits described herein can be used to treat muscular dystrophy.
In one aspect, any of the compounds described herein can inhibit Ca-activated proteases when an effective amount of compound is administered to a subject. Calpains are a family of Ca$^{+2}$ activated intracellular proteases, whose activity is accelerated when abnormal amounts of Ca$^{+2}$ enter the cell by virtue of increased membrane permeability as a result of some traumatic or ischemic event and/or a genetic defect. In one aspect, the prodrugs and active compounds produced by the kits described herein can be used to inhibit Ca-activated proteases.

In another aspect, any of the compounds described herein can increase the myofiber diameter of muscle tissue when the muscle tissue is contacted with an effective amount of the compound. In another aspect, the compounds described herein can prevent the further reduction of myofiber diameter or breakdown of myofibers when the muscle tissue is contacted with an effective amount of the compound. Alternatively, in another aspect, the compounds described herein can increase the weight of existing muscle tissue when the muscle tissue is contacted with an effective amount of compound. In one aspect, the compounds described herein can increase myoblast formation. In these aspects, the muscle tissue can be contacted in vitro, in vivo, or ex vivo. In one aspect, the prodrugs and active compounds produced by the kits described herein can be used to increase the myofiber diameter of muscle tissue.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the compounds, compositions, and methods described and claimed herein are made and evaluated, and are intended to be purely exemplary and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.) but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric. There are numerous variations and combinations of reaction conditions, e.g., component concentrations, desired solvents, solvent mixtures, temperatures, pressures and other reaction ranges and conditions that can be used to optimize the
product purity and yield obtained from the described process. Only reasonable and routine experimentation will be required to optimize such process conditions.

**Example 1: MDX Mouse Studies**

It is well documented that as, observed in DMD and BMD, dystrophin is dysfunctional/absent in the murine MDX model of muscular dystrophy. In this mouse model for DMD/BMD, significantly elevated levels of intracellular calcium mediate an elevated rate of protein degradation in muscle cells. A series of studies to test the ability of the calpain inhibitor, leupeptin, to delay and/or prevent features of calcium-induced damage to dystrophic myofibers was initiated. The results are presented in Tables 1 and 2. Control C57BL/10SNJ mice were treated with intramuscular injections of normal saline. The MDX mice were treated intramuscularly with either normal saline or 12 mg/kg leupeptin or 18 mg/kg leupeptin. All injections were delivered in the hind-limbs in various locations to avoid muscle damage due to repeated injections at the same location. The gastrocnemius, soleus, anterior tibialis, and diaphragm muscles were examined in all animals. Table 1 shows the results of this analysis.
TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Gastrocnemius</th>
<th>Soleus</th>
<th>Anterior tibialis</th>
<th>Diaphragm</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/10 SNJ</td>
<td>37.9 ± 3.8</td>
<td>20.3 ± 2.2</td>
<td>28.7 ± 1.8</td>
<td>22.1 ± 2.0</td>
</tr>
<tr>
<td>mdx</td>
<td>20.9 ± 0.9</td>
<td>19.2 ± 2.8</td>
<td>24.4 ± 1.5</td>
<td>16.4 ± 1.7</td>
</tr>
<tr>
<td>mdx 12 mg/kg leupeptin</td>
<td>32.9 ± 2.6</td>
<td>24.7 ± 1.8</td>
<td>37.5 ± 2.8</td>
<td>21.8 ± 0.9</td>
</tr>
<tr>
<td>mdx 18 mg/kg leupeptin</td>
<td>34.0 ± 2.7</td>
<td>25.5 ± 2.3</td>
<td>38.3 ± 1.3</td>
<td>21.7 ± 1.8</td>
</tr>
</tbody>
</table>

Comparison of mdx (untreated) muscles to 12 mg/kg and 18 mg/kg leupeptin treatment was significant, p<0.01, for the gastrocnemius, anterior tibialis, and diaphragm muscles. Significance was at p<0.05 in the soleus muscle (Student's two-tail t-test).

As shown above in Table 1, both doses of leupeptin, increased myofiber diameters in MDX leupeptin-treated muscles when compared with saline-treated control MDX muscles. Not wishing to be bound by theory, it is believed that leupeptin prevents a decrease in myofiber size rather than increasing an already degenerated myofiber. Further, in comparison to untreated MDX muscle in the gastrocnemius, treatment with 12 mg/kg leupeptin induced a 37% increase in myofiber diameter and, at 18 mg/kg, a 39% increase in myofiber diameter. In the soleus muscle, 12 mg/kg induced a 22% and 18 mg/kg a 25% increase in diameter. In the anterior tibialis, 12 mg/kg induced a 35% increase and, at 18 mg/kg, a 36% increase, demonstrating large increases in diameter. In the diaphragm, treatment at both 12mg/kg and 18 mg/kg induced a 25% increase in myofiber diameter.

C57BL/10SNJ control mice showed normal histology. The histologic appearance of untreated MDX myofibers was characterized by evidence of myofiber degeneration consistent with other reports. Electron microscopy confirmed light-microscopic findings of larger myofibers with features of normal ultra structure after leupeptin treatment at both doses compared with saline-treated MDX muscle.

To further examine whether the beneficial/protective action of leupeptin was associated with a change in calpain activity, the activity of the enzyme was assessed in the muscle various muscles taken from the control, and MDX rats ± treatment with leupeptin. As shown in Table 2, in the muscle taken from leupeptin-treated MDX mice, calpain activities decreased substantially in response to leupeptin treatment.
TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Gastrocnemius</th>
<th>Soleus</th>
<th>Anterior tibialis</th>
<th>Diaphragm</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/10 SNJ</td>
<td>56 ± 5</td>
<td>123 ± 9</td>
<td>75 ± 7</td>
<td>22 ± 5</td>
</tr>
<tr>
<td>mdx</td>
<td>168 ± 11</td>
<td>335 ± 21</td>
<td>311 ± 17</td>
<td>38 ± 4</td>
</tr>
<tr>
<td>12 mg/kg</td>
<td>27 ± 7</td>
<td>187 ± 16</td>
<td>155 ± 16</td>
<td>26 ± 4</td>
</tr>
<tr>
<td>18 mg/kg</td>
<td>67 ± 8</td>
<td>102 ± 9</td>
<td>71 ± 11</td>
<td>17 ± 5</td>
</tr>
</tbody>
</table>

aActivities based on cpn/260—μg protein; average of 3 determinations per sample; calpain assay described in Methods.

Comparison of mdx (untreated) muscles to 12-mg/kg and 18-mg/kg leupeptin treatment was significant, p<0.01 for the gastrocnemius; p<0.05 for the soleus (12 mg); p<0.01 (18 mg); p<0.05 for the anterior tibialis; and p<0.05 for the diaphragm (Student’s two-tail t-test).

In comparing the results of leupeptin-treated to untreated MDX muscles, in the gastrocnemius, at 12 mg/kg, there was an 84% decrease in calpain activity and, at 18 mg/kg, a 60% decrease. In the soleus muscle at 12 mg/kg leupeptin, there was a 44% decrease and, at 18 mg/kg leupeptin a 70% decrease in calpain activity. In the anterior tibialis at 12 mg/kg leupeptin, there was a 50% decrease, and a 77% decrease in calpain activity at 18 mg/kg of treatment. In the diaphragm muscle at 12 mg/kg, there was a 51% decrease, and a striking 71% decrease in activity at 18 mg/kg of leupeptin treatment.

This study demonstrates that leupeptin can inhibit muscle degeneration in this dystrophin deficient murine MDX model. In fact, the increase in myofiber diameter actually exceeded the normal size in treatments at both doses in both soleus and tibialis.

**Example 2: Monkey Studies**

**First set of monkey studies:** *Intramuscular injection of Leupeptin.*

In order to test the hypothesis that in vivo inhibition of calpains by leupeptin can facilitate neuromuscular recovery by inhibiting denervation atrophy of the skeletal-muscle target after complete peripheral nerve transection and delayed nerve repair (7), a monkey study (Cebus apella) was undertaken. This median-nerve model was chosen to approximate human anatomic and regenerative aspects.

**Toxicology:** There were no significant differences in all mean values between treated and control animals. Complete blood counts and clotting times after intramuscular leupeptin administration at 18 mg/kg, twice daily for 24 weeks, were
not affected in treated animals, when compared to controls. In controls, mean values were as follows: WBC = 4.83 ± 1.04 \times 10^3; RBC = 5.34 ± 0.34 \times 10^6;
 hematocrit = 37.97% ± 2.09; platelets = 3.30 ± 0.05 \times 10^5; prothrombin time = 9.68 ± 0.36 sec; partial thromboplastin time = 28.78 ± 1.32 sec. In treated animals, mean values were: WBC = 6.67 ± 2.26 \times 10^3; RBC = 5.55 ± 0.26 \times 10^6; hematocrit = 38.18% ± 1.69; platelets 4.33 ± 0.18 \times 10^5; prothrombin time = 10.88 ± 0.49 sec; partial thromboplastin time = 33.06 ± 0.76 sec. Further, blood chemistry analyses also showed no significant differences between treated and control animals, which included: uric acid, triglycerides, cholesterol, creatine kinase, lactate dehydrogenase, SGOT, SGPT, SGGT, alkaline phosphatase, bilirubin, total protein, albumin, phosphate, calcium, and amylase.

To determine that leupeptin was effectively being absorbed into plasma following intra muscular ("IM") administration in hind-limbs of 18 mg/kg, blood was withdrawn at 60, 75, 90, 120, 180 minutes and 24 hours after the IM injection (Figure 2).

Muscle morphology: Fibers biopsied at 24 weeks in the left, unoperated hands of control animals, showed typical histologic structure and a mean diameter of 34.83 ± 1.4 \mu m and 33.51 ± 2.2 \mu m, for opponens and abductor myofibers, respectively. Interestingly, opponens and abductor myofibers in the left, unoperated hand of leupeptin treated animals showed normal structure, and were significantly (p < 0.01) larger having a mean diameter of 43.25 ± 4.26 \mu m and 40.43 ± 2.23 \mu m, respectively.

Opponens pollicis myofibers and abductor pollicis myofibers in the right, nerve-repaired hands of control animals showed histologic and ultrastructural features consistent with denervation atrophy, including small, angulated myofibers and increased fibrosis. However, those muscles in the nerve-repaired hand of treated animals showed histologic and ultra-structural features consistent with control. As shown in Figure 3, muscles in leupeptin-treated animals showed a statistically significant increase in myofibers diameter, in comparison to control animals.

Opponens and abductor muscles from the left, unoperated hands of all animals showed a mean fiber-type type II [fast twitch] to type I [slow twitch]) ratio of 2:1. At 12 and 24 weeks after delayed nerve repair, right opponens and abductor muscles in
control animals had type II to type I fiber-type ratios of 1.5:1 and 1:1, respectively. Treated animals did not have a similar atrophy of type II myofibers. Treated-animal type II:type I fiber ratios 12 and 24 weeks after delayed nerve repair, were 2.3:1 and 2.5:1 in the opponens and abductor, respectively. Mean differences between treated and control animals were statistically significant, p<0.01.

Muscle biochemistry: Calpain activities were significantly (p < 0.01) increased by a mean 50 percent at both 12 and 24 weeks in the right, nerve-repaired opponens and abductor muscles of control animals, when compared to the right opponens and abductor muscles of leupeptin treated animals, indicating direct inhibition of enzyme activity by leupeptin.

Echocardiograms: To assure that leupeptin was causing no pathological effects on heart muscle, the cardiac lateral wall and septum were measured in millimeters in all animals prior to leupeptin treatment and 25 weeks after delayed nerve repair. Prior to treatment, in animals designated as controls, the lateral wall was 2.88 ± 0.33 mm and the septum was 2.84 ± 0.2 mm. Twenty-five weeks later in control animals, the lateral wall was 3.0 ± 0.5 mm and the septum was 2.9 ± 0.2 mm. Prior to treatment, in animals designated to receive leupeptin, the lateral wall was 3.5 ± 0.2 mm and the septum was 2.9 ± 0.4 mm. Twenty-five weeks later, in leupeptin-treated animals, the lateral wall was 3.2 ± 0.1 mm and the septum was 2.8 ± 0.3 mm. Importantly, there were no significant statistical differences within either the control or treatment groups or between the control and treatment groups (data not shown).

The results of this study indicated that leupeptin partially inhibits muscle denervation atrophy after immediate epineural nerve repair in a primate median-nerve model. After a complete median-nerve transection injury in the mid-forearm of primates, with a delay in nerve repair of 3-weeks duration followed by leupeptin treatment, the result was a significant increase in myofiber diameter, with retention of normal structure and less fibrosis. The mechanism of leupeptin action in denervated muscle appears to be the direct inhibition of the calpains, located at the Z-band, sarcolemma, and myofiber basal lamina. After denervation, nerve repair, and leupeptin treatment, the disassembly of the myofiber is prevented by inhibition of the calpains at these sites (data not shown).
Second set of Monkey studies: Oral administration of Leupeptin

Subsequent to the above studies another series of experiments were performed on the monkeys to assess another route of leupeptin administration in these animals (8). In this regard, oral delivery of leupeptin, after median nerve transection and epineural nerve repair, was studied for its potential benefits to neuromuscular recovery. The results of the controlled, dose-response study indicated that leupeptin was effectively absorbed into plasma by the oral route of administration. When plasma leupeptin concentrations were 3 pg/ml or greater, morphologic and functional motor recovery were facilitated after nerve repair. Serial testing of hematology, blood clotting, and serum biochemistry showed that there were no adverse effects, when leupeptin was administered twice daily for 6 months following nerve repair. These data suggest that leupeptin may be an effective and safe pharmaceutic adjunct to nerve repair, and may have clinical benefits in humans, where the oral route is a much preferred method of delivery.

Hematology/blood chemistry. Blood was drawn from the femoral vein at 1, 3, 4, 5, and 7 months postoperatively. Complete blood counts, clotting parameters, and serum chemistries in control animals and in animals after oral leupeptin administration at 3, 6, 12, and 18 mg/kg were quantified. Values were determined for hemoglobin, hematocrit, WBC, RBC, MCV, MCH, MCHC, differential (polymorphonuclear leukocytes, lymphocytes, monocytes, eosinophils, basophils), platelets, prothrombin time, partial thromboplastin time, glucose, urea nitrogen, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, SGPT, SGOT, lactic dehydrogenase, cholesterol, calcium, phosphorus, sodium, potassium, chloride, A/G, BUN/creatinine, globulin, amylase. There were no significant differences between controls vs. leupeptin treatment animals. Also, there were no significant differences between leupeptin-treatment groups (data not shown).

Muscle: Opponens pollicis myofibers biopsied at 3 months in the left, unoperated hands of all animals showed typical histologic structure. There were no significant statistical differences between the left control values in comparison to each, left, leupeptin-treatment group.

Opponens pollicis myofibers, biopsied from the right hand of all animals at 3 months
post nerve repair showed, in the controls, histologic features consistent with denervation atrophy, including small, angulated myofibers and areas of increased fibrosis. Animals in the 3-mg leupeptin treatment group also showed histologic features consistent with denervation atrophy. However, animals in the 6-mg, 12-mg, and 18-mg treatment groups showed histologic features consistent with normal myofibers (data not shown). Myofibers in the 12- and 18-mg/kg groups showed a more typically normal structure than myofibers in the 6-mg group. As shown in Figure 4, opponens pollicis myofibers in the right hands of leupeptin-treated animals showed increased myofiber diameter, in comparison with the right hands of the control group. The most significant increases occurred at the 6-mg, 12-mg, and 18-mg doses. When expressed as a percentage atrophy, in comparison to the left, unoperated control group, the right control group lost 63 percent of its myofiber diameter; the 3-mg group lost 53% of its myofiber diameter; the 6-mg group 23%; the 12-mg group 11%; and the 18-mg group only 8%.

Histologic examination of the left, unoperated abductor pollicis muscle in all animals revealed typically normal myofibers (Figure 5). Histologic examination of the right, nerve-repaired hands of controls abductor pollicis muscle biopsied 6 months after nerve repair, still showed evidence of denervation atrophy (Figure 5).

In comparison to the control group, all leupeptin-treatment abductor pollicis muscle groups demonstrated increased myofiber diameters (Figure 5). In the 3-mg and 6-mg leupeptin-treatment groups, there were foci of denervated myofibers, more so in the 3-mg than in the 6-mg treatment group (data not shown).

When expressed as a percent atrophy in comparison to the left, unoperated abductor pollicis muscle, the right abductor control group showed a 40 percent loss of myofiber diameter; the 3-mg group, a 30% loss; the 6-mg group, a 5% loss; the 12-mg group, no atrophy but an in crease of 1.2% in myofiber diameter; and the 18-mg group, no atrophy but an increase of 1.3% in myofiber diameter. Electron microscopic analysis of muscle samples was reflective of the findings by light microscopic analysis (data not shown).

Muscle calpain activities: Calpain activities in the left and right opponens pollicis and abductor pollicis, 3 and 6 months after right median nerve repair, showed some variability due to the pooling of small sample numbers. However, the trend in
activities, as was expected, when comparing control, left muscle to right, denervated, leupeptin-treated muscle, was an overall decrease in calpain activity after leupeptin treatment. The mean decrease in calpain activity in the right opponens pollicis muscles of leupeptin treated animals was 30%. The mean decrease in calpain activity in the right abductor pollicis muscles of leupeptin-treated animals was 24%.

The results of this study indicate that leupeptin, when delivered by the oral route of administration after median nerve transection and microsurgical repair in primates, inhibits muscle denervation atrophy. Calpain inhibition by leupeptin induced morphologic recovery in treated primates, as demonstrated by increased myofiber diameter, with retention of normal myofiber morphology distal to the nerve repair site. The results support the hypothesis that calpain inhibition by leupeptin prevents disassembly of myofibers, with retention of the end-target, skeletal muscle as a neurotrophic target after nerve repair.

An important finding of this study is that leupeptin treatment did not adversely affect hematology, clotting, or serum biochemistry parameters in primates. Finally, the oral method of peptide delivery is much preferred for human application, as any pharmacuetic adjunct to nerve repair will most likely require chronic (6 to 12 months) administration.

**Example 3: Clinical Studies**

Leupeptin’s low toxicity, non-immunogenicity and stability profile have made it a potential drug for eventual human use. In this regard, a preliminary safety trial of leupeptin in seven Italian DMD children with ages varying from 7-10 was performed. The trial was approved by the Italian Ministry of Health and the leupeptin supplied by the Nippon Kayaku Co., Japan. As seen in Table 3, leupeptin was administered orally at a total dose of 50 mg/twice daily in six children and 100 mg/twice daily in one child, both doses well below the efficacious dose [15-18 mg/kg/day] established in monkeys. During the process of muscle degeneration, muscle cells break open and their contents find their way into the bloodstream. Because most of the creatine kinase (CK) in the body normally exists in muscle, a rise in the amount of CK in the blood indicates that muscle damage has occurred, or is occurring. CK levels can be slightly elevated (500 U/L) in some nerve disorders, or grossly elevated (5,000 to 15,000 U/L)
in DMD or inflammatory myopathies. Importantly the significant decline in CK levels after treatment with leupeptin indicates a significant improvement (lessening of muscle breakdown) in these individuals, especially in the child given 100 mg twice daily.

5 TABLE 3

TREATMENT OF DUCHENNE PATIENTS WITH LEUPEPTIN

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>AGE (in yrs.)</th>
<th>DOSE (mg/day)</th>
<th>CREATINE KINASE (Units/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 Time</td>
</tr>
<tr>
<td>R.P</td>
<td>10</td>
<td>50</td>
<td>7,055</td>
</tr>
<tr>
<td>A.D</td>
<td>10</td>
<td>50</td>
<td>11,055</td>
</tr>
<tr>
<td>S.M</td>
<td>9.5</td>
<td>50</td>
<td>8,520</td>
</tr>
<tr>
<td>O.Z.</td>
<td>7.0</td>
<td>50</td>
<td>6,360</td>
</tr>
<tr>
<td>P.R.</td>
<td>7.0</td>
<td>50</td>
<td>9,910</td>
</tr>
<tr>
<td>I.M.</td>
<td>8.0</td>
<td>100</td>
<td>6,625</td>
</tr>
<tr>
<td>M.S.</td>
<td>10</td>
<td>50</td>
<td>6,270</td>
</tr>
</tbody>
</table>

Even at this lower dosage after one year of treatment, the measurement of creatine kinase (a routine measure of muscle integrity) showed values that were significantly decreased in 6 children at the 100mg/day and a very substantial decrease at the 200mg/day level (twice daily) in one child (these creatine kinase levels approached the upper end of the normal range). Importantly, as was observed in the monkey studies, the drug was well tolerated for one year and no adverse effects seen in any child. Blood chemistries measured over the year showed no abnormalities, and importantly, muscle function tests measured at 6 months and one year showed little decline in muscle strength when compared to the start of the trial.

Despite the study's limitations (subject age, drug dose, lack of power), it nonetheless demonstrated that: 1) leupeptin appeared to be well tolerated by all of the children
participating in the study; and 2) they all had a decrease in creatine kinase even at low doses.

**Example 4: Studies with aminocarnityl-succinyl-leucyl-argininal**

Aminocarnityl-succinyl-leucyl-argininal, which is Formula IV in the application and referred to below as CLA, is an aminocarnitine formulation of the tripeptide aldehyde leupeptin in which aminocarnitine is used to acylate a portion of the leupeptin molecule following removal of the acetyl-leucyl N-terminal end of the leupeptin molecule by enzymatic cleavage with subsequent covalent linkage of carnitine to the remaining leucyl argininal moiety.

An initial series of toxicology experiments was performed in rats to assess the effect of CLA at various doses, on animal viability. No toxic effect of CLA was observed at any of the doses given. A series of experiments to examine the ability of CLA vs. leupeptin to decrease activated calpain levels and inhibit muscle wasting was conducted. In the experiment shown in Table 4, rats were utilized to assess the effect of IP injection of either leupeptin or CLA into the hind limb muscle on their ability to inhibit calpain activity. To this end, four rats were injected IP with either leupeptin or CLA. After 2 hours, the muscle was isolated, homogenized, and assayed for remaining calpain activity by the standard assay.
TABLE 4

<table>
<thead>
<tr>
<th></th>
<th>% inhibition</th>
<th>% inhibition/μmole</th>
<th>Fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Leupeptin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(75 μmole)</td>
<td>20%</td>
<td>0.26%</td>
<td>1</td>
</tr>
<tr>
<td>CLA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(14 μmole)</td>
<td>48%</td>
<td>3.5%</td>
<td>13.5</td>
</tr>
</tbody>
</table>

The results of the experiment shown in Table 4, indicate that CLA is significantly (13.5-fold) more effective at penetrating the cell membrane to inhibit calpain intracellularly per μmole than leupeptin.

In another rat study (data shown in Table 5), the effectiveness of CLA in delaying muscle atrophy as compared to the parent compound leupeptin, was assessed. Denervation was carried out on one of the hind limb sciatic nerves of the rat. Denervation was allowed to proceed for 7 days. Either leupeptin or CLA was injected 2x/day in forelimb muscle for seven days. The non-denervated hind limb was used as the control. Leupeptin and/or CLA were injected into alternating forelimb muscles for a period of seven days after which, animals were sacrificed, muscles excised and weighed.
TABLE 5

<table>
<thead>
<tr>
<th></th>
<th>% loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leupeptin</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>32.9 ± 5</td>
</tr>
<tr>
<td>1.5 mg/kg</td>
<td>30.9 ± 4</td>
</tr>
<tr>
<td>15.0 mg/kg</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>CLA</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>28.0 ± 3</td>
</tr>
<tr>
<td>1.0 mg/kg</td>
<td>5.2 ± 1</td>
</tr>
<tr>
<td>3.0 mg/kg</td>
<td>&lt; 1.0</td>
</tr>
<tr>
<td>6.0 mg/kg</td>
<td>&lt; 1.0</td>
</tr>
</tbody>
</table>

As shown in Table 5, CLA was able to achieve similar inhibition of muscle wasting at 1 mg/kg as did leupeptin at 15 mg/kg. These results were in agreement with the data presented in Table 4, which showed a 13.5 fold improvement on the in vivo inhibition of calpain by CLA. These studies demonstrate that CLA can effectively target the attached leupeptin to the muscle so as to allow for a very effective inhibition of calpain activity, and amelioration of muscle atrophy due to denervation. All rats remained healthy without any apparent negative toxic effect of either CLA or leupeptin.

CLA was injected daily at the indicated concentrations into mdx mice from one to two months. Mdx controls were injected with saline alone. At two months, the animals were sacrificed and indicated muscles removed, fixed, and frozen for myofiber diameter analysis. It can be seen that CLA (formula IV) at both concentrations in both the diaphragm (Table 6) and gastrocnemins muscle (Table 7) significantly improved the retention of muscle size even at 5 mg/kg. This is in contrast to leupeptin alone, which showed efficacy only at 12-18 mg/kg. Thus, CLA is at least three to five times more effective than leupeptin alone.


**TABLE 6**

<table>
<thead>
<tr>
<th>Mouse type</th>
<th>Amount of compound III</th>
<th>Myofiber diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal (control)</td>
<td>0</td>
<td>33.5 ± 5.6</td>
</tr>
<tr>
<td>mdx</td>
<td>0</td>
<td>18.6 ± 6.1</td>
</tr>
<tr>
<td>mdx</td>
<td>5 mg</td>
<td>25.1 ± 7.3</td>
</tr>
<tr>
<td>mdx</td>
<td>10 mg</td>
<td>25.4 ± 7.3</td>
</tr>
</tbody>
</table>

**TABLE 7**

<table>
<thead>
<tr>
<th>Mouse type</th>
<th>Amount of compound III</th>
<th>Myofiber diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal (control)</td>
<td>0</td>
<td>34.3 ± 6</td>
</tr>
<tr>
<td>mdx</td>
<td>0</td>
<td>18.7 ± 6</td>
</tr>
<tr>
<td>mdx</td>
<td>5 mg</td>
<td>28.7 ± 7</td>
</tr>
<tr>
<td>mdx</td>
<td>10 mg</td>
<td>30.6 ± 11</td>
</tr>
</tbody>
</table>

**Example 5: Conversion of aminocarnityl-succinyl-leucyl-argininal diethyl acetal (CLA acetal) to aminocarnityl-succinyl-leucyl-argininal (CLA) using acid as an activating component**

To illustrate how an acid component could be used as part of a kit to generate the active aldehyde compound, experiments were performed to measure conversion of the diethyl acetal to aldehyde by incubating the acetal in solutions of variable acidity for variable lengths of time.

Solutions of CLA diethyl acetal in water were prepared at a concentration of 5 mg/mL and the pH was adjusted to values shown in Table 8 using concentrated hydrochloric acid. Each solution was incubated at different times at room temperature. Samples were removed at each time point and quenched using basic phosphate buffer, yielding a final pH of 9. The quenched samples were analyzed...
using an Agilent 1100 HPLC system. The aldehyde was chromatographically separated from the acetal on a C18 reverse-phase HPLC column, using a linear gradient where the starting mobile phase was 0.1% trifluoroacetic acid in a 99:1 water:acetonitrile mixture and the final mobile phase was 0.1% trifluoroacetic acid in a 50:50 water:acetonitrile mixture. The absorbance at 220 nm was monitored, and the area of the peak corresponding to elution of the CLA diethyl acetal was computed. The results are shown in Table 8. These results show that CLA acetal can be quantitatively and rapidly converted to an active aldehyde form using acid.
TABLE 8: Conversion of CLA acetal to aldehyde at pH 0 and pH 1

<table>
<thead>
<tr>
<th>pH Condition</th>
<th>Relative Peak Area</th>
<th>Activation Time (min)</th>
<th>Percent acetal remaining</th>
<th>Approximate t₁/₂ for activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH = 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>225</td>
<td>0</td>
<td>100.0</td>
<td></td>
<td>10 minutes</td>
</tr>
<tr>
<td>180</td>
<td>5</td>
<td>80.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>64.2</td>
<td>15</td>
<td>28.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.3</td>
<td>30</td>
<td>11.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.4</td>
<td>45</td>
<td>11.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.5</td>
<td>60</td>
<td>11.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.2</td>
<td>90</td>
<td>9.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24.5</td>
<td>120</td>
<td>10.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.9</td>
<td>240</td>
<td>9.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.5</td>
<td>1440</td>
<td>9.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH = 1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>315</td>
<td>0</td>
<td>100.0</td>
<td></td>
<td>2.5 hours</td>
</tr>
<tr>
<td>317</td>
<td>5</td>
<td>100.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>306.6</td>
<td>15</td>
<td>97.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>290.4</td>
<td>30</td>
<td>92.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>273.7</td>
<td>45</td>
<td>86.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>257.4</td>
<td>60</td>
<td>81.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>229.1</td>
<td>90</td>
<td>72.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>204</td>
<td>120</td>
<td>64.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>132.9</td>
<td>240</td>
<td>42.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.2</td>
<td>1440</td>
<td>6.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Example 6: Qualitative measurement of the oral bioavailability of the CLA acetal in the rat

This experiment was designed to measure in a qualitative manner the oral bioavailability of CLA acetal in rats. Four Lewis strain normal male rats, 9 weeks of age were administered a single dose of 50 mg/kg of the acetate salt of the CLA acetal compound, using a single oral gavage. Blood draws were taken at either 45 minutes or 1 hour after administration of drug. The blood samples were immediately centrifuged at 3000 rpm for 3-5 minutes to separate plasma from cells and the plasma and cells were frozen separately by immersion in liquid nitrogen and storage in a -20 °C freezer.

Selectively deuterated forms of the CLA acetal and aldehyde were added to each plasma sample at a final concentration of 150 ng/mL prior to extraction, to serve as internal standards in the analysis of drug levels. The aldehyde and acetal forms of
CLA were then extracted from the plasma using a Bligh Dyer-type liquid phase extraction followed by solid-phase extraction using a reverse phase column. The relative amounts of CLA acetal and aldehyde were then measured using HPLC separation with mass spectrometric detection, using an electrospray ionization source and a Micromass QToF detector. The peak areas were integrated and the quantity of compound found in plasma was estimated by comparison to the deuterated internal standards. Although this experiment is qualitative and does not yield a pharmacokinetic profile for the drug, the high levels of acetal found at a single time point in the blood of all of the rats indicates that the CLA acetal is absorbed intact when administered orally. The relatively high levels observed also indicate that, if conversion of prodrug to drug occurs after the CLA acetal enters the bloodstream, its oral bioavailability is likely to be high enough to provide a therapeutic benefit to human patients.
TABLE 9: CLA acetal and aldehyde concentrations of acetal and aldehyde in rat plasma.

<table>
<thead>
<tr>
<th>Rat</th>
<th>Time (minutes)</th>
<th>Estimated Plasma Concentration of CLA acetal (ng/mL)</th>
<th>Estimated Plasma Concentration of CLA aldehyde (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45</td>
<td>5,840</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>970</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>8240</td>
<td>72</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>51200</td>
<td>&lt; 20</td>
</tr>
</tbody>
</table>

Example 7: Study of toxicity of CLA acetal when administered orally to rats as a single dose

Sprague Dawley rats were administered single doses of CLA acetal by oral gavage at 7-8 weeks of age. The rats were divided into 4 groups, consisting of equal numbers of male and female rats, as shown in Table 10. Each group received a single oral dose level of CLA acetal, ranging in amount from 0 to 2000 mg/kg. The vehicle consisted of Dulbecco’s phosphate-buffered saline. No evidence of toxicity was observed at any of the dose levels at any time up to 24 hours after dosing.

TABLE 10: Single-dose oral rat toxicology study.

<table>
<thead>
<tr>
<th>Group Number</th>
<th>Treatment</th>
<th>Dose Level (mg/kg/day)</th>
<th>Concentration (mg/mL)</th>
<th>Dose Volume (mL/kg)</th>
<th>Number of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>Males 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Females 5</td>
</tr>
<tr>
<td>2</td>
<td>Low Dose</td>
<td>250</td>
<td>25</td>
<td>10</td>
<td>Males 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Females 5</td>
</tr>
<tr>
<td>3</td>
<td>Mid Dose</td>
<td>1000</td>
<td>100</td>
<td>10</td>
<td>Males 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Females 5</td>
</tr>
<tr>
<td>4</td>
<td>High Dose</td>
<td>2000</td>
<td>200</td>
<td>10</td>
<td>Males 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Females 5</td>
</tr>
</tbody>
</table>
Example 8: Study of toxicity of CLA when administered intravenously to rats as a single dose

In a pilot toxicology study, two groups of 2 male and 2 female Sprague-Dawley rats each were administered intravenous bolus doses of 250 and 500 mg/kg CLA. CLA aldehyde was found to be lethal at the 500 mg/kg dose level, with death occurring in 1 to 5 minutes. In a larger, follow-up study (Table 11), Sprague-Dawley rats were administered several lower doses of CLA intravenously at 7-8 weeks of age. The rats were divided into 4 groups consisting of equal numbers of male and female rats. Each group received a different dose level of CLA, as shown in Table 11, ranging from 0 to 200 mg/kg. The vehicle consisted of Dulbecco’s phosphate-buffered saline. The no adverse effect limit (NOAEL) was found to be 100 mg/kg in this study, and the maximum tolerated dose was 200 mg/kg. No toxicity was observed at 50 and 100 mg/kg.

**TABLE 11:** Single-dose intravenous toxicology study

<table>
<thead>
<tr>
<th>Group Number</th>
<th>Treatment</th>
<th>Dose Level (mg/kg/day)</th>
<th>Concentration (mg/mL)</th>
<th>Dose Volume (mL/kg)</th>
<th>Number of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>Low Dose</td>
<td>50</td>
<td>25</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>Mid Dose</td>
<td>100</td>
<td>50</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>High Dose</td>
<td>200</td>
<td>100</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>
**Example 9: Measurement of the inhibition of calpain activity by CLA in human muscle cells**

This study was designed to measure the effect of CLA, a calpain inhibitor, upon calpain activity inside living muscle cells. Alpha-II spectrin, a large cytoskeletal protein, is known to be a substrate of calpain, which is activated following calcium influx triggered by cellular damage or necrosis. Calcium-dependent cell death also occurs in many debilitating muscle diseases. Full length alpha-II spectrin has a molecular weight of approximately 250 kilodaltons; upon cleavage by calpain, calpain-specific spectrin breakdown products with molecular weights of approximately 150 and 145 kilodaltons, respectively, are generated sequentially. Human RD muscle cells are a rhabdomyosarcoma muscle cell line that expresses OCTN2, a transporter for carnitine. By monitoring the reduction in spectrin breakdown products caused by calpain inhibition in RD muscle cells, it is possible to measure the potency of inhibitors according to the extent to which they reduce the appearance of calpain-specific spectrin breakdown products. Figure 6 shows the appearance of spectrin breakdown products in RD cells that have been incubated with maitotoxin in the presence of calcium.

In the experiment, RD muscle cells were incubated with either CLA or leupeptin for 1 hour. 1 nM maitotoxin was added to the cells to stimulate calcium influx and calpain activation. The cells were incubated for another 60 minutes. The cells were then lysed in phosphate buffer containing 1% Triton X-100 and a protease inhibitor cocktail. The lysed samples were centrifuged and the supernatants were collected. The protein concentration in each supernatant was measured using a standard assay. 20 micrograms of each sample was electrophoresed on a 4% Tris-glycine polyacrylamide gel. Western blotting was performed using an antibody to alpha II spectrin that detects the full-length spectrin as well as the 150 and 145 kilodalton fragments.

Table 12 shows the results of the experiment comparing leupeptin (a peptide aldehyde with the sequence leu-leu-argininal) to CLA (aminocarnitine-leu-argininal). The IC$_{50}$ of CLA is shown to be approximately 200-fold lower than that of leupeptin. This result is attributable to improved transport of CLA into muscle cells due to the aminocarnitine moiety.
TABLE 12: IC₅₀ values for inhibition of spectrin breakdown product formation.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀, 150 kDa fragment (µM)</th>
<th>IC₅₀, 145 kDa fragment (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leupeptin</td>
<td>80,800</td>
<td>5750</td>
</tr>
<tr>
<td>CLA aldehyde</td>
<td>356</td>
<td>37</td>
</tr>
<tr>
<td>Ratio of IC₅₀s (leupeptin:CLA)</td>
<td>227:1</td>
<td>155:1</td>
</tr>
</tbody>
</table>

5 Example 10: Uptake and conversion of CLA in rat muscle

This experiment was designed to measure the uptake of CLA acetal in rat gastrocnemius muscle and to detect conversion of CLA acetal to CLA as it occurs in rat muscle. Four Lewis strain normal male rats, 9 weeks of age were administered a single dose of 50 mg/kg of the acetate salt of the CLA acetal compound, using a single oral gavage. At different times following administration of drug, each rat was sacrificed, the left and right gastrocnemius muscles were dissected from the rat, and the muscles were frozen by immersion in liquid nitrogen and storage in a -80 °C freezer.

The muscle tissues were thawed and homogenized using a blender.

Selectively deuterated forms of the CLA acetal and CLA were added to each muscle homogenate at a final concentration of 150 ng/mL prior to extraction, to serve as internal standards in the analysis of drug levels. The aldehyde and acetal forms of CLA were then extracted from the muscle homogenates using a Bligh Dyer-type liquid phase extraction followed by solid-phase extraction using a reverse phase column. The relative amounts of CLA acetal and CLA were then measured using HPLC separation with mass spectrometric detection, using an electrospray ionization source and micromass Qtof detector. The peak areas were integrated and the quantity of compound found in the homogenates was estimated by comparison to the deuterated internal standards.

The concentrations of CLA acetal and CLA shown in Table 13 indicate that most of the CLA taken up by muscle tissue is detected as the active aldehyde drug within 2 hours of oral administration of the inactive CLA acetal. These data show that CLA acetal is likely to be a prodrug that targets muscle tissue, reducing the risk
of potential toxicity due to exposure of other tissues to the active drug form.

TABLE 13

<table>
<thead>
<tr>
<th>Rat</th>
<th>Muscle</th>
<th>Time</th>
<th>Muscle concentration of prodrug, ng per gram</th>
<th>Muscle concentration of active drug, ng per gram</th>
<th>Percent of drug present as the active form</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Left gastrocnemius</td>
<td>2 hours</td>
<td>37.4</td>
<td>124.6</td>
<td>76.9</td>
</tr>
<tr>
<td>1.3</td>
<td>Right gastrocnemius</td>
<td>6 hours</td>
<td>9.7</td>
<td>683.6</td>
<td>98.6</td>
</tr>
<tr>
<td>2.1</td>
<td>Right gastrocnemius</td>
<td>8 hours</td>
<td>9.2</td>
<td>330.8</td>
<td>97.3</td>
</tr>
<tr>
<td>2.3</td>
<td>Right gastrocnemius</td>
<td>8 hours</td>
<td>10.3</td>
<td>758.4</td>
<td>98.7</td>
</tr>
</tbody>
</table>

Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the compounds, compositions and methods described herein.

Various modifications and variations can be made to the compounds, compositions and methods described herein. Other aspects of the compounds, compositions and methods described herein will be apparent from consideration of the specification and practice of the compounds, compositions and methods disclosed herein. It is intended that the specification and examples be considered as exemplary.
What is claimed:

1. A kit for treating or preventing a muscle disorder in a subject comprising:
   a. an effective amount of a prodrug of a compound, wherein the compound comprises a carrier molecule and a protease inhibitor, wherein the carrier molecule is directly or indirectly bonded to one or more protease inhibitors, and
   b. an activator, wherein the activator converts the prodrug to the active form of the compound.

2. The kit of claim 1, wherein the carrier molecule comprises the formula I or IX

\[ \text{HOOC} \quad \text{NR}^1 \text{R}^2 \text{R}^3 \quad \text{Y}^- \quad \text{I} \]

\[ \text{HOOC} \quad \text{NR}^1 \text{R}^2 \quad \text{IX} \]

wherein each \( R^1, R^2, \) and \( R^3 \) comprises, independently, hydrogen or a branched- or straight chain alkyl group,
\( X \) comprises OH or NHR\(^6\), wherein \( R^6 \) comprises hydrogen or a branched- or straight-chain alkyl group;
\( Y \) comprises a pharmaceutically-acceptable anion; and
\( m \) and \( n \) can be an integer from 1 to 10,
or the pharmaceutically-acceptable salt or ester thereof.

3. The kit of claim 2, wherein \( X \) is NH\(_2\).

4. The kit of claim 3, wherein \( R^1-R^3 \) in formula I and \( R^1 \) and \( R^2 \) in formula IX are methyl.

5. The kit of claim 1, wherein the carrier molecule is carnitine or aminocarnitine.

6. The kit in any of claims 1 to 5, wherein the protease inhibitor comprises
pepsatin, bestatin, Bowman-Birk inhibitor, chymostatin, bacitracin, lactacystin, clasto-lactacystin-β-lactone, ritonavir, saquinavir, indinavir, nelfinavir, amprenavir, calpastatin or a peptide fragment thereof, a derivative thereof, or a combination thereof.

7. The kit in any of claims 1 to 5, wherein the protease inhibitor comprises a peptide-aldehyde protease inhibitor.

8. The kit in any of claims 1 to 5, wherein the protease inhibitor comprises a calpain inhibitor.

9. The kit of claim 8, wherein the calpain inhibitor comprises a benzamidine derivative, leupeptin, PhCH₂OCO-leucine-norvaline-CONH-CH₂-2-pyridyl, Ph₂CHCO-leucine-alpha-aminobutyric acid-CONH-CH₂-2-pyridyl, Ph₂CHCO-leucine-alpha-aminobutyric acid-CONH-(CH₂)₄-4-morpholiny1, PhCH₂OCO-leucine-alpha-aminobutyric acid-CONH-CH₂-2-pyridyl, or PhCH₂OCO-leucine-alpha-aminobutyric acid-CONH-CH₂-CH(OH)Ph.

10. The kit of claim 8, wherein the calpain inhibitor is leucyl-argininal.

11. The kit in any of claims 1 to 10, wherein the carrier molecule is directly bonded to the protease inhibitor.

12. The kit in any of claims 1 to 10, wherein the carrier molecule is indirectly bonded to the protease inhibitor by a linker.

13. The kit of claim 12, wherein the linker comprises a polyalkylene group, a polyether group, a polyamide group, a polyimino group, a polyester, an aryl group, or a polythioether group.

14. The kit of claim 12, wherein the linker has the formula -C₁⁴(O)(CH₂)ₙ(O)C₂⁻, wherein n is an integer of from 1 to 10, wherein C₁ is covalently bonded to the carrier molecule and C₂ is covalently bonded to the protease inhibitor.

15. The kit in claim 14, wherein n is from 1 to 5.

16. The kit of claim 1, wherein the prodrug has the formula III
wherein each $R^1$-$R^3$ comprises, independently, hydrogen or a branched-or straight chain alkyl group,

$R^4$ and $R^5$ comprises, independently, hydrogen or a branched-or straight chain alkyl group, or $R^4$ and $R^5$ can be part of a ring, or $C(O)R^{10}$, where $R^{10}$ can be independently a branched-or straight-chain alkyl group having from 1 to 20 carbon atoms,

$X$ comprises $O$ or $NR^6$, wherein $R^6$ comprises hydrogen or a branched-or straight-chain alkyl group;

$Y$ and $Z$ comprises a pharmaceutically-acceptable anion; and $m, n, o$ can be an integer from 1 to 10, or the pharmaceutically-acceptable salt or ester thereof.

17. The kit of claim 16, wherein $m$ and $n$ are 1, $R^1$-$R^3$ are methyl, and $X$ is NH.

18. The kit of claim 16, wherein the compound has the formula III, wherein $m$ and $n$ are 1, $R^1$-$R^3$ are methyl, $R^4$ and $R^5$ are ethyl, $X$ is NH, and $o$ is 2.

19. The kit of claim 16, wherein the compound has the formula III, wherein $m$ and $n$ are 1, $R^1$-$R^3$ are methyl, $R^4$ and $R^5$ are each $C(O)R^{10}$, $X$ is NH, and $o$ is 2.

20. The kit in any of claims 16 to 19, wherein the compound has the formula III, wherein the compound can be hydrolyzed at a pH of 1 to 3 at a temperature of 37 °C.

21. The kit in any of claims 1 to 20, wherein when the protease inhibitor is
leucyl-argininal having two stereocenters, wherein the stereochemistry about the two stereocenters is L and L.

22. The kit in any of claims 1 to 21, wherein the carrier molecule is the substantially pure L-isomer.

23. The kit in any of claims 1 to 21, wherein the carrier molecule is the substantially pure D-isomer.

24. The kit of claim 1, wherein the activator comprises an acid, an enzyme, a metal, a salt, a polymer, a detergent, or a zeolite.

25. The kit of claim 1, wherein the activator comprises an inorganic acid.

26. The kit of claim 1, wherein the activator comprises an organic acid.

27. The kit of claim 1, wherein the activator comprises hydrochloric acid or phosphoric acid.

28. The kit in any of claims 1 to 27, wherein the prodrug is an aqueous solution.

29. The kit in any of claims 1 to 27, wherein the prodrug is a solid.

30. The kit of claim 1, wherein the activator comprises HCl or phosphoric acid and the prodrug has the formula XI

\[
\begin{align*}
\text{CO}_2\text{H} & \quad \text{H}_3\text{C} \quad \text{CH} \quad \text{CH}_3 \\
\text{CH}_2 & \quad \text{CH} \quad \text{H}_2\text{C} \quad \text{CH}_2\text{CH}_2\text{NHCNH}_2 \\
\text{HC-NH-C-CH}_2\text{CH}_2\text{C-NH-CH-C-NH-CH-CH} & \quad \text{OEt} \quad \text{NH}_2^+ \quad \text{Z}^- \\
\text{CH}_2 & \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{OEt} \\
\text{N(CH}_3)_3^+ \quad \text{Y}^- 
\end{align*}
\]

wherein Y and Z comprises a pharmaceutically-acceptable anion.

31. The kit of claim 1, wherein the kit further comprises a neutralizing agent.

32. The kit of claim 31, wherein the neutralizing agent comprises a base.
33. The kit of claim 1, wherein the kit further comprises a heating device.

34. The kit of claim 33, wherein the heating device comprises a Peltier block.

35. A method for treating or preventing a muscle disorder in a subject comprising the kit in any of claims 1-34, comprising (a) admixing the prodrug of the compound and the activator to produce the activated form of the compound and (b) administering to the subject the activated form of the compound.

36. The method of claim 35, wherein the activated form of the compound is administered to the subject orally, intravenously, or parenterally.

37. The method of claim 35, wherein the muscle disorder comprises a muscular dystrophy, muscle wasting disease, cancer cachexia, cardiomyopathy, cardiac ischemia, denervation atrophy, or AIDS-related muscle wasting.

38. The method of claim 35, wherein the muscle disorder comprises muscular dystrophy, wherein the muscular dystrophy comprises myotonic, Duchenne (DMD), Becker (BMD), or limb-girdle.

39. A method for treating or preventing a muscle disorder in a subject, comprising administering to the subject an effective amount of a prodrug of a compound, wherein the compound comprises a carrier molecule and a protease inhibitor, wherein the carrier molecule is directly or indirectly bonded to one or more protease inhibitors.

40. The method of claim 39, wherein the carrier molecule comprises the formula I or IX

\[
\begin{align*}
\text{HOOC} & \quad \text{m} \\
\text{X} & \\
\text{NR}^1 \text{R}^2 \text{R}^3 & \\
\text{Y} & \\
\end{align*}
\]

\[
\begin{align*}
\text{HOOC} & \quad \text{m} \\
\text{X} & \\
\text{NR}^1 \text{R}^2 & \\
\end{align*}
\]
wherein each R\(^1\), R\(^2\), and R\(^3\) comprises, independently, hydrogen or a branched- or straight chain alkyl group,
X comprises OH or NHR\(^6\), wherein R\(^6\) comprises hydrogen or a branched- or straight-chain alkyl group;
Y comprises a pharmaceutically-acceptable anion; and
m and n can be an integer from 1 to 10,
or the pharmaceutically-acceptable salt or ester thereof.

41. The method of claim 40, wherein X is NH\(_2\).

42. The method of claim 41, wherein R\(^1\)-R\(^3\) in formula I and R\(^1\) and R\(^2\) in formula IX are methyl.

43. The method of claim 39, wherein the carrier molecule is carnitine or aminocarnitine.

44. The method in any of claims 39 to 43, wherein the protease inhibitor comprises pepstatin, bestatin, Bowman-Birk inhibitor, chymostatin, bacitracin, lactacystin, clasto-lactacystin-β-lactone, ritonavir, saquinavir, indinavir, nelfinavir, amprenavir, calpastatin or a peptide fragment thereof, a derivative thereof, or a combination thereof.

45. The method in any of claims 39 to 43, wherein the protease inhibitor comprises a peptide-aldehyde protease inhibitor.

46. The method in any of claims 39 to 43, wherein the protease inhibitor comprises a calpain inhibitor.

47. The method of claim 46, wherein the calpain inhibitor comprises a benzamidine derivative, leupeptin, PhCH\(_2\)OCO-leucine-norvaline-CONH-CH\(_2\)-2-pyridyl, Ph\(_2\)CHCO-leucine-alpha-aminobutyric acid-CONH-CH\(_2\)-2-pyridyl, Ph\(_2\)CHCO-leucine-alpha-aminobutyric acid-CONH-(CH\(_2\))\(_3\)-4-morpholiny1, PhCH\(_2\)OCO-leucine-alpha-aminobutyric acid-CONH-CH\(_2\)-2-pyridyl, or PhCH\(_2\)OCO-leucine-alpha-aminobutyric acid-CONH-CH\(_2\)-CH(OH)Ph.

48. The method of claim 46, wherein the calpain inhibitor is leucyl-argininal.
49. The method in any of claims 39 to 48, wherein the carrier molecule is directly bonded to the protease inhibitor.

50. The method in any of claims 39 to 48, wherein the carrier molecule is indirectly bonded to the protease inhibitor by a linker.

51. The method of claim 50, wherein the linker comprises a polyalkylene group, a polyether group, a polyamide group, a polyimino group, a polyester, an aryl group, or a polythioether group.

52. The method of claim 50, wherein the linker has the formula 

\[-C^1(O)(CH_2)_o(O)C^2-,\]

wherein \(o\) is an integer of from 1 to 10, wherein \(C^1\) is covalently bonded to the carrier molecule and \(C^2\) is covalently bonded to the protease inhibitor.

53. The method of claim 52, wherein \(o\) is from 1 to 5.

54. The method of claim 39, wherein the prodrug has the formula III

\[
\begin{align*}
\text{CO}_2\text{H} & \\
(\text{CH}_2)_m & \\
\text{H}_3\text{C} & \\
\text{CH} & \\
\text{CH}_3 & \\
\text{HC-X-C-(CH}_2)_o-C-\text{NH-CH-C-NH-CH-CH} & \\
\text{CH}_2\text{CH}_2\text{NHCHNH}_2 & \\
\text{OR}^4 & \\
\text{NH}_2^+ & \\
\text{Z}^- & \\
\text{OR}^5 & \\
\text{NR}^1\text{R}^2\text{R}^3^+ & \\
\text{Y}^- & 
\end{align*}
\]

wherein each \(R^1-R^3\) comprises, independently, hydrogen or a branched- or straight chain alkyl group,

\(R^4\) and \(R^5\) comprises, independently, hydrogen or a branched- or straight chain alkyl group, or \(R^4\) and \(R^5\) can be part of a ring, or \(C(O)R^{10}\), where \(R^{10}\) can be independently a branched- or straight-chain alkyl group having from 1 to 20 carbon atoms,

\(X\) comprises \(O\) or \(\text{NR}^6\), wherein \(\text{R}^6\) comprises hydrogen or a branched- or straight-chain alkyl group;
Y and Z comprises a pharmaceutically-acceptable anion; and
m, n, and o can be an integer from 1 to 10,
or the pharmaceutically-acceptable salt or ester thereof.

55. The method of claim 54, wherein m and n are 1, R^1-R^3 are methyl, and X is NH.

56. The method of claim 54, wherein the compound has the formula III,
wherein m and n are 1, R^1-R^3 are methyl, R^4 and R^5 are ethyl, X is NH, and
o is 2.

57. The method of claim 54, wherein the compound has the formula III,
wherein m and n are 1, R^1-R^3 are methyl, R^4 and R^5 are each C(O)R^{10}, X is
NH, and o is 2.

58. The method in any of claims 54 to 57, wherein the compound has the
formula III, wherein the compound can be hydrolyzed at a pH of 1 to 3 at a
temperature of 37 °C.

59. The method in any of claims 39 to 58, wherein when the protease inhibitor
is leucyl-argininal having two stereocenters, wherein the stereochemistry
about the two stereocenters is L and L.

60. The method in any of claims 39 to 59, wherein the carrier molecule is the
substantially pure L-isomer.

61. The method in any of claims 39 to 59, wherein the carrier molecule is the
substantially pure D-isomer.

62. The method of claim 39, wherein the muscle disorder comprises a muscular
dystrophy or muscle wasting, cancer cachexia, cardiomyopathy, cardiac
ischemia, denervation atrophy, or AIDS-related muscle wasting.

63. The method of claim 39, wherein the muscle disorder comprises muscular
dystrophy, wherein the muscular dystrophy comprises myotonic, Duchenne
(DMD), Becker (BMD), or limb-girdle.

64. The method of claim 39, wherein after administration of the prodrug of the
compound to the subject, the prodrug diffuses into muscle cells or is taken
up into muscle cells by an active or passive transport system and converted to the active form of the compound.

65. The method of claim 39, wherein the prodrug is in a pharmaceutical formulation.

66. A method for inhibiting Ca-activated proteases in a subject, comprising administering to the subject an effective amount of a prodrug of a compound, wherein the compound comprises a carrier molecule and a protease inhibitor, wherein the carrier molecule is directly or indirectly bonded to one or more protease inhibitors.

67. A method for increasing the myofiber diameter of muscle tissue, comprising contacting the muscle tissue with an effective amount of a prodrug of a compound, wherein the compound comprises a carrier molecule and a protease inhibitor, wherein the carrier molecule is directly or indirectly bonded to one or more protease inhibitors.

68. A compound having the formulae X or XI
wherein Y and Z comprises a pharmaceutically-acceptable anion.

69. A method for treating or preventing a muscle disorder in a subject, comprising administering to the subject an effective amount of the compound of claim 68.

70. A pharmaceutical formulation comprising the compound of claim 68 and a pharmaceutically acceptable carrier.
FIGURE 2

FIGURE 3
FIGURE 6

<table>
<thead>
<tr>
<th>Sample</th>
<th>Condition</th>
<th>MTX Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RD</td>
<td>1.8 mM</td>
</tr>
<tr>
<td>2</td>
<td>RD</td>
<td>5 mM</td>
</tr>
<tr>
<td>3</td>
<td>RD</td>
<td>25 mM</td>
</tr>
<tr>
<td>4</td>
<td>RD</td>
<td>50 mM</td>
</tr>
<tr>
<td>5</td>
<td>RD</td>
<td>1.8 mM 1 nM</td>
</tr>
<tr>
<td>6</td>
<td>RD</td>
<td>5 mM 1 nM</td>
</tr>
<tr>
<td>7</td>
<td>RD</td>
<td>25 mM 1 nM</td>
</tr>
<tr>
<td>8</td>
<td>RD</td>
<td>50 mM 1 nM</td>
</tr>
</tbody>
</table>

M  1  2  3  4  5  6  7  8

250kDa
150/145kDa
120kDa