Title: NITRIC OXIDE AMINO ACID ESTERS FOR ENHANCEMENT OF PHYSICAL OR MUSCULAR PERFORMANCE AND THE TREATMENT OF MUSCULAR OR NEUROMUSCULAR DISEASES

Abstract: Use of nitric oxide amino acid esters to treat muscular or neuromuscular diseases, increase muscle growth and/or muscle strength, or to stimulate vasodilation prior to physical training. Preferred muscular or neuromuscular diseases are muscular dystrophy and muscular atrophy. Preferably the nitric oxide acid esters are administered in a transdermal composition further comprising a penetration enhancer and a pharmaceutically acceptable carrier.
Title: NITRIC OXIDE AMINO ACID ESTERS FOR ENHANCEMENT OF PHYSICAL OR MUSCULAR PERFORMANCE AND THE TREATMENT OF MUSCULAR OR NEUROMUSCULAR DISEASES.

CROSS-REFERENCE TO RELATED APPLICATIONS
This application claims priority of US provisional patent applications 61/428,901, 61/428,903 and 61/428,906 filed on December 31, 2010 the specifications of which is hereby incorporated by reference.

BACKGROUND
(a) Field
[0001] The subject matter disclosed generally relates to transdermal compositions, and more specifically to transdermal compositions comprising nitric oxide amino acid ester compounds for the treatment of muscular and/or neuromuscular diseases, for the increase of muscle growth and/or an increase in muscular strength. The subject matter also relates to methods of use of compositions for physical training enhancement.

(b) Related Prior Art
[0002] Nitric oxide (NO) mediates activation of satellite precursor cells to enter the cell cycle. This provides new precursor cells for skeletal muscle growth and muscle repair from injury or disease. Targeting a drug that specifically delivers NO to muscle has the potential to promote normal function and treat muscular and/or neuromuscular disease, and would also help to avoid side effects of NO from other treatment modalities such as nitroglycerin nitrates or even large amounts of arginine. Currently, transdermal methods of stimulating the growth of specific muscle groups through the transdermal applications of a nitric oxide donor are lacking. Preliminary experimental work proving the theory has been done using a transdermal combination of isosorbide mononitrate and guanifeisen.
[0003] Physical exercise is any bodily activity that enhances or maintains physical fitness and overall health or wellness. It is performed for various reasons. These include strengthening muscles and the cardiovascular system, honing athletic skills, weight loss or maintenance and for enjoyment. Frequent and regular physical exercise boosts the immune system, and helps prevent the "diseases of affluence" such as heart disease, cardiovascular disease, Type 2 diabetes and obesity. It also improves mental health, helps prevent depression, helps to promote or maintain positive self-esteem, and can even augment an individual's sex appeal or body image, which again is also linked with higher levels of self-esteem.

[0004] Sufficient blood flow to muscles and other organs that are recruited during any types of physical exercise is necessary to meet an increased demand in nutrients by these muscles and organs. Nitric oxide (NO) is known as the "endothelium-derived relaxing factor", or "EDRF", is biosynthesized endogenously from L-arginine and oxygen by various nitric oxide synthase (NOS) enzymes and by reduction of inorganic nitrate. The endothelium (inner lining) of blood vessels uses nitric oxide to signal the surrounding smooth muscle to relax, thus resulting in vasodilation and increasing blood flow. Therefore, NO production is critical for increasing the vasodilation necessary to meet the increased demand in nutrients by muscles and organs during physical exercise. To improve this naturally occurring process, compound capable to generating NO in vivo have been used, and boost physical exercise performance. These compounds have not been shown to have any effect upon muscle growth or performance.

[0005] Nitric oxide donating compounds such as nitroglycerin and isosorbide nitrates are commonly used for their vasodilatory properties. However, there are several drawbacks to the manufacture, storage and use of nitroglycerin. Nitroglycerin is an explosive compound that is difficult to produce and stabilize. It
is inherently unstable over the long term resulting in a maximum shelf life of a product containing of about six months. These compounds

[0006] A major drawback to the long term usage of nitroglycerin for the treatment of diseases is that the metabolic pathway for the liberation of nitric oxide from nitroglycerin occurs in the mitochondria, utilizing the aldehyde dehydrogenase 2 enzyme. The liberation of large amounts of nitric oxide within the mitochondria from the use of nitroglycerin proves to be toxic to the mitochondria over time and eventually causes extensive metabolic disruption. Also, certain classes of patient suffering from nitric oxide deficiencies, mainly of Asian descent, have been shown to carry a recessive allele of the gene producing aldehyde dehydrogenase 2 which renders them non-responsive to the use of nitroglycerin.

[0007] Therefore there still exists a need for an efficient muscle enhancing nitric oxide donor that would not possess negative aspects, such as poor stability, short half life and inherent toxicity. The use of such replacement molecules for the treatment for conditions such as muscle atrophy in disuse and aging, and for the promotion of muscle tissue repair as required after injury or in neuromuscular diseases such as muscular dystrophy, is highly desirable.

[0008] Also there is still a need for molecules that deliver NO for promoting muscle growth and enhance muscular strength that would not possess the negative aspects of other nitrates such as the systemic effects caused by oral dosing or the long term inherent toxicity.

[0009] Also, there still exists a need for efficient molecules capable of transdermally donating NO. The use of such molecules for the stimulation of vasodilation prior to physical exercise.

[0010] It is thus desirable to provide a composition and method for the treatment of muscular and/or neuromuscular condition which contains an
alternative compound to nitroglycerin, and does not require any special operational procedures other than the application of a composition.

[0011] It is also desirable to provide a composition and method for the promotion of muscle growth and enhance muscular strength which contains an alternative compound to known nitrates, and does not require any special operational procedures other than the application of a composition.

[0012] It is also desirable to provide a composition and method for stimulation of vasodilation prior to physical exercise that does not require any special operational procedures other than the use of a composition.

SUMMARY

[0013] According to an embodiment, there is provided a transdermal composition comprising:

- a therapeutically effective amount of a compound of formula (I):

\[
\begin{align*}
\text{O} & \quad \text{(CH}_2\text{n)}) \quad \text{NO}_2 \\
\text{CH} & \quad \text{O} \\
\text{NH} & \\
\text{R}_1 & \\
\text{R}_2 &
\end{align*}
\]

wherein \( n \) may be 1 to 10;

wherein \( R_1 \) may be an amino acid side chain group (D or L configuration),

wherein \( R_2 \) may be a hydrogen atom, or an amino acid (D or L configuration) forming a peptide bond, or any pharmaceutically acceptable salts thereof; and

- a skin penetration enhancer,
in association with a pharmaceutically acceptable topical carrier.

[0014] The compound of formula (I) may be (2-nitrooxy)-2-ethylamino-3-methylbutanoate:

[0015]

[0016] or any pharmaceutically acceptable salts thereof.

[0017] The compound of formula (I) may be L-(2'-nitrooxyethyl)-2-amino-3-methylbutanoate:

[0018]

[0019] or any pharmaceutically acceptable salts thereof.

[0020] The compound of formula (I) may be D-(2'-nitrooxyethyl)-2-amino-3-methylbutanoate:

[0021]

[0022] or any pharmaceutically acceptable salts thereof.

[0023] The compound of formula (I) may be valine butylene glycol nitrate:

[0024]

[0025] or any pharmaceutically acceptable salts thereof.
The compound of formula (I) may be 2'-nitrooxy ethyl 2-amino-pentanoate:

or any pharmaceutically acceptable salts thereof.

The compound of formula (I) may be L-(2'-nitroxyethyl)-2-amino-pentanoate:

or any pharmaceutically acceptable salts thereof.

The compound of formula (I) may be 2'-nitroxy butyl 2-amino-pentanoate:

or any pharmaceutically acceptable salts thereof.

The $R_2$ may be a hydrogen atom.

The $R_1$ may be chosen from:
[0037] H, \( \text{CH}_3 \), \( \text{C}_2 \), \( \text{C}_2 \)-SH, \( \text{C}_2 \)-COOH, and \( \text{SeH} \),

[0038] wherein when \( R_1 \) is \( \text{H} \) or \( \text{OH} \), the \( R_1 \) may be also linked to an \( \text{NH}_2 \) of the Formula (I) to form a proline or hydroxyproline amino acid side chain.

[0039] The \( R_2 \) may be an amino acid of formula (II) (D or L configuration) and derivatives thereof, forming a peptide bond:
[0040] (II)

[0041] wherein Rₓ may be chosen from:

[0042] H, \text{CH₃,} \ldots
wherein when $R_1$ is \( \text{HO-} \), or \( \text{HO-} \), the $R_1$ may be also linked to an NH$_2$ of the Formula (I) to form a proline or hydroxyproline amino acid side chain.

The compound may be in a liposomal liquid.

The skin penetration enhancer may be chosen from a C$_8$-C$_{22}$ fatty acid, a C$_8$-C$_{22}$ fatty alcohol, a lower alkyl ester of a C$_8$-C$_{22}$ fatty acid, a di(lower)alkyl ester of C$_8$-C$_{22}$ diacid, a monoglyceride of C$_8$-C$_{22}$ fatty acid, tetrahydrofurfuryl alcohol polyethylene glycol ether, polyethylene glycol, propylene glycol, 2-(2-ethoxyethoxy)ethanol (transcutol), diethylene glycol monomethyl ether, diethylene glycol monoethyl ether, an alkylaryl ether of polyethylene oxide, a polyethylene oxide monomethyl ether, a polyethylene oxide dimethyl ether; dimethyl sulfoxide (DMSO), glycerol, ethyl acetate, acetoacetic ester, N-alkylpyrrolidone, a terpenes, dimethyl formamide (DMF), N,N-dimethylacetamide (DMA), methyl laurate, glycerol monolaurate, a fatty acid ester of a C$_2$ to C$_4$ alkanediol having a fatty acid portion of the ester from about 8 to 22 carbon atoms, a fatty alcohol ether of a C$_2$ to C$_4$ alkanediols having a fatty acid portion of the ether from about 8 to 22 carbon atoms, triglycerides of coconut oil, isopropyl palmitate, isopropyl myristate, laurocapram, and combinations thereof.

The skin penetration enhancer may be propylene glycol.

The skin penetration enhancer may be isopropyl myristate.

The C$_8$-C$_{22}$ fatty acid may be chosen from isostearic acid, octanoic acid, and oleic acid.

The C$_8$-C$_{22}$ fatty alcohol may be chosen from oleyl alcohol and lauryle alcohol.
The lower alkyl ester of a C₆-C₂₂ fatty acid may be chosen from ethyl oleate, isopropyl myristate (IPM), butyl stearate, and methyl laurate.

The di(lower)alkyl esters of a C₆-C₂₂ diacid may be diisopropyl adipate.

The monoglyceride of a C₆-C₂₂ fatty acid may be glycercylnonolaurate.

The pharmaceutically acceptable carrier may be chosen from a water base or an oil base carrier.

The composition may be further comprising a thickening agent.

The thickening agent may be chosen from CARBOPOL®, carboxypolymethylene, carboxymethylcellulose Carbopol® Ultrace 10, Carbopol® 940, Carbopol® 941, Carbopol® 954, Carbopol® 980, Carbopol® 981, Carbopol® ETD 2001, Carbopol® EZ-2 and Carbopol® EZ-3.

The composition may be further comprising a wetting agent.

The wetting agent may be chosen from benzalkonium chloride, benzethonium chloride, cetylpyridinium chloride; dioctyl sodium sulfosuccinate; a polyoxyethylene alkylphenyl ether, a poloxamers, a polyoxyethylene fatty acid glyceride, a polyoxyethylene alkyl ethers, a polyoxyethylene fatty acid ester, a polyoxyethylene sorbitan ester, a propylene glycol fatty acid ester, sodium lauryl sulfate, sodium laurate sulfate oleic acid, sodium oleate, triethanolamine oleate, a glycercylnafatty acid ester, a sorbitan ester, sorbitan monooleate, sorbitan monopalmitate, sorbitan monostearate, tyloxapol and mixtures thereof.

The polyoxyethylene sorbitan ester may be polysorbate 80.

The composition may be further comprising a lubricant.

The lubricant may be chosen from glyceryl behenate, stearic acid, magnesium stearate, calcium stearate, sodium stearate; a hydrogenated vegetable oil, colloidal silica, talc, a waxe, boric acid, sodium benzoate, sodium
acetate, sodium fumarate, sodium chloride, DL-leucine, sodium oleate, sodium lauryl sulfate, sodium laureth sulfate, magnesium lauryl sulfate, glycerol, sorbitol, a water soluble cellulose, a polysorbate, a carbomer, a polyethylene glycol (PEG), a polyethylene, and a thickening agent.

[0061] The lubricant may be sodium benzoate.

[0062] The water soluble cellulose may be chosen from modified starch, methylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methocel® MC, carboxymethyl cellulose, ethyl cellulose, hydroxyl ethyl cellulose, and any combination thereof.

[0063] The hydroxypropyl methylcellulose may be hydroxypropyl methylcellulose E5.

[0064] The polysorbate may be chosen from polyoxyethylene (20) sorbitan monolaurate (polysorbate 20), polyoxyethylene (20) sorbitan monopalmitate (polysorbate 40), polyoxyethylene (20) sorbitan monostearate (polysorban 60), polyoxyethylene (20) sorbitan tristearate (polysorban 65), and polyoxyethylene (20) sorbitan monooleate (polysorban 80), and any combination thereof.

[0065] The carbomer may be a carbopol® polymer chosen from carbopol® polymer 71G NF, carbopol® polymer 971P NF, carbopol® polymer 974P NF, carbopol® polymer 980 NF, carbopol® polymer 981 NF, carbopol® polymer 5984 EP and carbopol® polymer Ultrez 10 NF, and any combination thereof.

[0066] The polyethylene glycol (PEG) may be chosen from PEG 200, PEG 200E, PEG 300, PEG 300E, PEG 400, PEG 400E, PEG 600 and PEG 600E, and any combination thereof.

[0067] The polyethylene glycol (PEG) may be PEG 400.

[0068] The polyethylene glycol (PEG) may be PEG 3350.

[0069] The thickening agent may be chosen from alginic acid, sodium alginate, potassium alginate, ammonium alginate, calcium alginate, agar,
carrageenan, locust bean gum, xanthan gum, pectin, and gelatin, and any combination thereof.

[0070] The composition may be further comprising at least one antiseptic agent.

[0071] The antiseptic agent may be selected from chlorhexidine gluconate, glucono delta-lactone, a paraben compound, benzoic acid, imidazolidinyl urea, a quaternary ammonium compound, methyl hydroxybenzoate, propyl hydroxybenzoate, chlorocresol, benzalkonium chloride and Octenidine dihydrochloride.

[0072] The paraben may be methyl paraben.

[0073] The may be further comprising a preservative agent.

[0074] The preservative agent may be chosen from EDTA, EGTA, hydroxytoluene butoxide, hydroxyanisol butoxide, sodium hydroxide, calcium propionate, sodium nitrate, sodium nitrite, sulfur dioxide, sodium bisulfite, and potassium hydrogen sulfite.

[0075] The may be further comprising an emollient.

[0076] The emollient may be chosen from mineral oil, a mixture of a mineral oil and a lanolin alcohol, cetyl alcohol, cetostearyl alcohol, petrolatum, petrolatum and a lanolin alcohol, cetyl esters wax, cholesterol, glycerin, glyceryl monostearate, isopropyl myristate (IPM), isopropyl palmitate, lecithin, allyl caproate, althea officinalis extract, arachidyl alcohol, argobase EUC, butylene glycol, dicaprylate/dicaprate, acacia, allantoin, carrageenan, cetyl dimethicone, cyclomethicone, diethyl succinate, dihydroabietyl behenate, dioctyl adipate, ethyl laurate, ethyl palmitate, ethyl stearate, isoamyl laurate, octanoate, PEG-75, lanolin, sorbitan laurate, walnut oil, wheat germ oil, super refined almond, super refined sesame, super refined soybean, octyl palmitate, caprylic/capric
triglyceride, butyrosperrnum parkii oil, olive oil, cetyl alcohol, behenyl alcohol, limnanthes alba seed oil, palmitic acid and glyceryl cocoate.

[0077] The emollient may be cetyl alcohol.

[0078] The emollient may be stearyl alcohol.

[0079] The emollient may be niacinamide.

[0080] The emollient may be lecithin.

[0081] The composition may be further comprising an essential oil.

[0082] The essential oil may be chosen from argan oil, cypress oil, chamomile oil, oil (bois de rose oil) lavender oil, tea tree oil, pine tree oil, eucalyptol oil, eucalyptus oil, birch oil, peppermint oil, ylang-ylang oil, cymbopogon martini oil (palmarosa oil), sweet almond oil and olive oil.

[0083] The essential oil may be tea tree oil.

[0084] The composition may be further comprising phosphocreatine.

[0085] The composition may be further comprising magnesium chloride.

[0086] The composition may be in a transdermal patch.

[0087] The composition may be further comprising a fragrance.

[0088] According to another embodiment, there is provided a method of treating a muscular or a neuromuscular disease in a patient which comprises:

[0089] (a) transdermally treating the patient with the composition of the present invention, to treat the muscular or neuromuscular disease.

[0090] The disease may be a muscular dystrophy.

[0091] The muscular dystrophy may be chosen from Becker's muscular dystrophy, congenital muscular dystrophy, Duchenne muscular dystrophy, distal muscular dystrophy, Emery-Dreifuss muscular dystrophy, facioscapulohumeral muscular dystrophy, limb-girdle muscular dystrophy, myotonic muscular
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dystrophy, oculopharyngeal muscular dystrophy, spinal muscular atrophy, Brown-Vialetto-Van Laere syndrome (BVVL), and Fazio-Londe (FL) syndrome.

[0092] The disease may be a muscular atrophy.

[0093] The muscular atrophy may be chosen from muscle atrophy associated with a cancer, muscle atrophy associated with AIDS, muscle atrophy associated with congestive heart failure, muscle atrophy associated with chronic obstructive pulmonary disease, muscle atrophy associated with renal failure, muscle atrophy associated with severe burns, and muscle atrophy associated with long bed rest.

[0094] The disease may be chosen from Charcot-Marie-Tooth disease, Dejerine-Sottas disease, and Kennedy’s disease.

[0095] According to another embodiment, there is provided a method to cause an increase muscle growth and/or an increase in muscular strength in a subject which comprises:

[0096] (a) transdermally treating the subject with the composition of the present invention, to increase muscular strength.

[0097] The increase in muscle growth and/or an increase in muscular strength may be through enhanced myosin and actin binding.

[0098] The increase in muscle growth and/or an increase in muscular strength may be a long term increase in muscular strength.

[0099] The long term increase in muscular strength may be through a recruitment of at least one satellite cell.

[0100] The long term increase in muscular strength may be through a lowering of a myostatin level.

[0101] The long term increase in muscular strength may be through an increase in follistatin level.
According to another embodiment, there is provided a use of a compound of formula (I) for the preparation of a medicament for the treatment of a muscular and/or neuromuscular disease:

\[
\begin{align*}
\text{O} & \quad \text{(I)} \\
\text{CH} & \quad (\text{CH}_2)_n \quad \text{NO}_2
\end{align*}
\]

wherein \( n \) may be 1 to 10;

wherein \( R_1 \) may be an amino acid side chain group (D or L configuration),

wherein \( R_2 \) may be a hydrogen atom, or an amino acid (D or L configuration) forming a peptide bond, or any pharmaceutically acceptable salts thereof.

According to another embodiment, there is provided a use of a compound of formula (I) for the treatment of a muscular and/or neuromuscular disease:

\[
\begin{align*}
\text{O} & \quad \text{(I)} \\
\text{CH} & \quad (\text{CH}_2)_n \quad \text{NO}_2
\end{align*}
\]

wherein \( n \) may be 1 to 10;

wherein \( R_1 \) may be an amino acid side chain group (D or L configuration),
wherein R₂ may be a hydrogen atom, or an amino acid (D or L configuration) forming a peptide bond, or any pharmaceutically acceptable salts thereof.

According to another embodiment, there is provided a use of a compound of formula (I) for the preparation of a medicament for causing an increase in muscle growth and/or an increase in muscular strength:

wherein n may be 1 to 10;

wherein R₁ may be an amino acid side chain group (D or L configuration),

wherein R₂ may be a hydrogen atom, or an amino acid (D or L configuration) forming a peptide bond, or any pharmaceutically acceptable salts thereof.

According to another embodiment, there is provided a use of a compound of formula (I) for causing an increase in muscle growth and/or an increase in muscular strength:
wherein n may be 1 to 10;

wherein $R_1$ may be an amino acid side chain group (D or L configuration),

wherein $R_2$ may be a hydrogen atom, or an amino acid (D or L configuration) forming a peptide bond, or any pharmaceutically acceptable salts thereof.

According to another embodiment, there is provided a use of a compound of formula (I) for the preparation of a medicament for stimulation of vasodilation prior to physical exercise:

$$\begin{align*}
\text{O} \\
\text{CH} \\
\text{NH} \\
\text{R_1} \quad \text{(I)} \\
\text{O} \quad \text{(CH_2)_n} \quad \text{ONO_2} \\
\text{R_2}
\end{align*}$$

wherein n may be 1 to 10;

wherein $R_1$ may be an amino acid side chain group (D or L configuration),

wherein $R_2$ may be a hydrogen atom, or an amino acid (D or L configuration) forming a peptide bond,

or any pharmaceutically acceptable salts thereof.

According to another embodiment, there is provided a use of a compound of formula (II) for stimulation of vasodilation prior to physical exercise:
wherein \( n \) may be 1 to 10;

wherein \( R_1 \) may be an amino acid side chain group (D or L configuration),

wherein \( R_2 \) may be a hydrogen atom, or an amino acid (D or L configuration) forming a peptide bond,

or any pharmaceutically acceptable salts thereof.

The compound of formula (I) may be (2-nitrooxy)-2-ethylamino-3-methylbutanoate:

or any pharmaceutically acceptable salts thereof.

The compound of formula (I) may be L-(2'-nitrooxyethyl)-2-amino-3-methylbutanoate:

or any pharmaceutically acceptable salts thereof.
The compound of formula (I) may be D-(2'-nitrooxyethyl)-2-amino-3-methylbutanoate:

or any pharmaceutically acceptable salts thereof.

The compound of formula (I) may be valine butylene glycol nitrate:

or any pharmaceutically acceptable salts thereof.

The compound of formula (I) may be 2'-nitrooxy ethyl 2-amino-pentanoate:

or any pharmaceutically acceptable salts thereof.

The compound of formula (I) may be L-(2'-nitrooxyethyl)-2-amino-pentanoate:

or any pharmaceutically acceptable salts thereof.
The compound of formula (I) may be 2'-nitrooxy butyl 2-amino-pentanoate:

or any pharmaceutically acceptable salts thereof.

The $R_2$ may be a hydrogen atom.

The $R_1$ may be chosen from:
[00159] wherein when $R_1$ is, or , the $R_1$ may be also linked to an $\text{NH}_2$ of the Formula (I) to form a proline or hydroxyproline amino acid side chain.

[00160] The $R_2$ may be an amino acid of formula (II) (D or L configuration) and derivatives thereof, forming a peptide bond:

\[
\begin{array}{c}
\text{H} \quad \text{H} \\
\text{O} \\
\text{H} - \text{N} - \text{C} - \text{C} - \text{O} - \text{H} \\
\text{R}_x
\end{array}
\]

[00161] (II)

[00162] wherein $R_x$ may be chosen from:

[00163] $\text{H}$, $\text{CH}_3$, $\text{CH}_3$, $\text{CH}_3$,

$\text{C} - \text{CH}_2 - \text{CH}_3$, $\text{C} - \text{CH}_2 - \text{CH}_3$, $\text{C} - \text{OH}$,

$\text{C} - \text{NH}_2$, $\text{C} - \text{NH}_2$, $\text{CH}_3 - \text{COOH}$, $\text{CH}_3 - \text{SH}$,
[00164] wherein when R1 is or , the R1 may be also linked to an NH2 of the Formula (I) to form a proline or hydroxyproline amino acid side chain.

[00165] The disease may be a muscular dystrophy.

[00166] The muscular dystrophy may be chosen from Becker's muscular dystrophy, congenital muscular dystrophy, Duchenne muscular dystrophy, distal muscular dystrophy, Emery-Dreifuss muscular dystrophy, facioscapulohumeral muscular dystrophy, limb-girdle muscular dystrophy, myotonic muscular dystrophy, oculopharyngeal muscular dystrophy, spinal muscular atrophy, Brown-Vialetto-Van Laere syndrome (BVVL), and Fazio-Londe (FL) syndrome.

[00167] The disease may be a muscular atrophy.

[00168] The muscular atrophy may be chosen from muscle atrophy associated with a cancer, muscle atrophy associated with AIDS, muscle atrophy associated with congestive heart failure, muscle atrophy associated with chronic obstructive pulmonary disease, muscle atrophy associated with renal failure,
muscle atrophy associated with severe burns, and muscle atrophy associated with long bed rest.

[00169] The disease may be chosen from Charcot-Marie-Tooth disease, Dejerine-Sottas disease, and Kennedy's disease.

[00170] The increase in muscle growth and/or an increase in muscular strength may be through enhanced myosin and actin binding.

[00171] The increase in muscle growth and/or an increase in muscular strength may be a long term increase in muscular strength.

[00172] The long term increase in muscular strength may be through a recruitment of at least one satellite cell.

[00173] The long term increase in muscular strength may be through a lowering of a myostatin level.

[00174] The long term increase in muscular strength may be through an increase in follistatin level.

[00175] According to another embodiment, there is provided a method for stimulation of vasodilation prior to physical exercise in a subject which comprises:

[00176] (a) administering prior to physical exercise to the subject a compound of formula (I)

\[
\begin{align*}
\text{O} &\quad \text{CH} \\
\text{CH} &\quad \text{O} \quad (\text{CH}_2)_n \quad \text{-ONO}_2 \\
\text{NH} &\quad \text{R}_1 \\
\text{R}_2 &\quad \text{(I)}
\end{align*}
\]

[00177] wherein n may be 1 to 10;
[00179] wherein R₁ may be an amino acid side chain group (D or L configuration),

[00180] wherein R₂ may be a hydrogen atom, or an amino acid (D or L configuration) forming a peptide bond,

[00181] or any pharmaceutically acceptable salts thereof.

[00182] The compound of formula (I) may be (2-nitrooxy)-2-ethylamino-3-methylbutanoate:

![Chemical structure 1]

[00183]

[00184] or any pharmaceutically acceptable salts thereof.

[00185] The compound of formula (I) may be L-(2'-nitrooxyethyl)-2-amino-3-methylbutanoate:

![Chemical structure 2]

[00186]

[00187] or any pharmaceutically acceptable salts thereof.

[00188] The compound of formula (I) may be D-(2'-nitrooxyethyl)-2-amino-3-methylbutanoate:

![Chemical structure 3]

[00189]

[00190] or any pharmaceutically acceptable salts thereof.

[00191] The compound of formula (I) may be valine butylene glycol nitrate:
or any pharmaceutically acceptable salts thereof.

The compound of formula (I) may be 2'-nitrooxy ethyl 2-amino-pentanoate:

or any pharmaceutically acceptable salts thereof.

The compound of formula (I) may be L-(2'-nitrooxyethyl)-2-amino-pentanoate:

or any pharmaceutically acceptable salts thereof.

The compound of formula (I) may be 2'-nitrooxy butyl 2-amino-pentanoate:

or any pharmaceutically acceptable salts thereof.

The R₂ may be a hydrogen atom.
The $R_1$ may be chosen from:

and

wherein when $R_1$ is $\text{HO}$, or $\text{NH}_2$, the $R_1$ may be also linked to an $\text{NH}_2$ of the Formula (I) to form a proline or hydroxyproline amino acid side chain.
The $R_2$ may be an amino acid of formula (II) (D or L configuration) and derivatives thereof, forming a peptide bond:

$$\text{H} - \text{N} - \text{C} - \text{C} - \text{O} - \text{H}$$

wherein $R_x$ may be chosen from:

- $\text{H}$
- $\text{CH}_3$
- $\text{H}_2\text{C} - \text{C} - \text{H}_2$
- $\text{C} - \text{H}_2 - \text{CH}_3$
- $\text{H}_2\text{C} - \text{C} - \text{NH}_2$
- $\text{C} - \text{H}_2 - \text{COOH}$
- $\text{H}_2\text{C} - \text{CH}_2 - \text{CH}_2$
- $\text{H}_2\text{NH} - \text{CH}_2$
- $\text{CH}_2\text{COOH}$
- $\text{H}_2\text{N} - \text{CH}_2 - \text{CH}_2$
- $\text{H}_2\text{N} - \text{NH}_2$
- $\text{H}_2\text{O}$
- $\text{H}_2\text{N} - \text{CH}_2 - \text{COOH}$
- $\text{H}_2\text{N} - \text{CH}_2 - \text{SH}$
wherein when $R_1$ is $\text{H}$, or $\text{H}_2$, the $R_1$ may be also linked to an $\text{NH}_2$ of the Formula (I) to form a proline or hydroxyproline amino acid side chain.

The following terms are defined below.

The term "Amino acid ester compound" is intended to mean the condensation product of an amino acid with mononitrated alkane or alkene diol. As will be evident to those familiar to the art, the condensation reaction could also involve, but not limited to, dipeptides or tripeptides, nitrated alcohols containing aliphatic, alkyl or aromatic moieties, as well as other nitric oxide groups attached to the alkane or alkene diols. Amino acid or dipeptide reactions are preferred as well as the condensation reaction with short chain mononitrated alkane diols such as 1,3 propanediol or 1,4 butanediol.

The expression "Therapeutically effective amount" is intended to mean the amount of the compound and/or composition that is effective to achieve its intended purpose.

The expression "transdermally absorbed" is intended to mean the delivery of a compound by passage through the unbroken skin and into the blood stream.

The terms "Carriers" or "vehicles" are intended to mean carrier materials suitable for compound administration and include any such material known in the art such as, for example, any liquid, lotion, gel, solvent, liquid
diluent, solubilizer, or the like, which is non-toxic and which does not interact with any components of the composition in a deleterious manner.

[00217] The term "Nitric oxide adduct" or "NO adduct" is intended to mean compounds and functional groups which, under physiological conditions, can donate, release and/or directly or indirectly transfer any of the three redox forms of nitrogen monoxide (NO\(^+\), NO\(^-\), NO\(^\cdot\)), such that the biological activity of the nitrogen monoxide species is expressed at the intended site of action.

[00218] The term "Nitric oxide releasing" or "nitric oxide donating" is intended to mean methods of donating, releasing and/or directly or indirectly transferring any of the three redox forms of nitrogen monoxide (NO\(^+\), NO\(^-\), NO\(^\cdot\)), such that the biological activity of the nitrogen monoxide species is expressed at the intended site of action.

[00219] The term "Nitric oxide donor" or "NO donor" is intended to mean compounds that donate, release and/or directly or indirectly transfer a nitrogen monoxide species, and/or stimulate the endogenous production of nitric oxide or endothelium-derived relaxing factor (EDRF) in vivo and/or elevate endogenous levels of nitric oxide or EDRF in vivo and/or are oxidized to produce nitric oxide and/or are substrates for nitric oxide synthase and/or cytochrome P450. "NO donor" also includes compounds that are precursors of L-arginine, inhibitors of the enzyme arginase and nitric oxide mediators.

[00220] The term "pharmaceutical acceptable carrier" is intended to mean a preservative solution, a saline solution, an isotonic (about 0.9%) saline solution, or about a 5% albumin solution, suspension, sterile water, phosphate buffered saline, and the like. Other buffering agents, dispersing agents, and inert non-toxic substances suitable for delivery to a patient may be included in the compositions of the present invention. The compositions may be solutions, suspensions or any appropriate formulation suitable for administration, and are typically sterile and
free of undesirable particulate matter. The compositions may be sterilized by conventional sterilization techniques.

[00221] The term "long term" is intended to mean a period of time of about one week or more, and preferably one month or more.

[00222] The term "lubricant" is intended to mean a substance (often a liquid) introduced between two moving surfaces to reduce the friction between them, hydrate the surface as well as reducing wear of the body parts.

[00223] Features and advantages of the subject matter hereof will become more apparent in light of the following detailed description of selected embodiments, as illustrated in the accompanying figures. As will be realized, the subject matter disclosed and claimed is capable of modifications in various respects, all without departing from the scope of the claims. Accordingly, the drawings and the description are to be regarded as illustrative in nature, and not as restrictive and the full scope of the subject matter is set forth in the claims.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[00224] The composition of the present invention contains vasoactive amino acid ester compounds. The nitric oxide amino acid ester compounds of the present invention possess many of the required characteristics necessary to fulfill the role of a primary boosting of NO levels. The compounds easily dissociate in water into the amino acid derivative and associated ion forming the pharmaceutical salt. The compounds of the present invention are extremely stable in the form of the salts, and thus possess long shelf lives and stability.

[00225] The compounds may be D- or L-isomers. The D- or L- nitric oxide amino acid ester compounds of the present invention possess many of the required characteristics necessary to fulfill the role of a primary boosting of NO levels. The compounds easily dissociate in water into the amino acid derivative and associated ion forming the pharmaceutical salt. The compounds of the
present invention are extremely stable in the form of the salts, and thus possess long shelf lives and stability.

[00226] The nitric oxide releasing groups of the compounds of the present invention are preferably nitro groups (i.e. NO₂), and/or nitroso groups (i.e. NO) that are linked to the amino acid ester compounds through one or more sites such as oxygen (hydroxyl condensation), sulfur (sulffhydryl condensation) and/or nitrogen.

[00227] Amino acids molecules are actively transported across cell membranes by transporters such as the PEPT1 transporter that mediates their uptake. Without wishing to be bound by theory, it is believed that the fusion of a nitric oxide donating moiety to an amino acid molecule enhances the absorption of the compound through the active transport of the compound as an amino acid, and enhance the bioavailability of such compound. Branched chain amino acids such as leucine, isoleucine and valine are believed to have the most preferred absorption patterns.

[00228] The ester bonds of the compounds are susceptible to degradation by carboxylesterase enzymes and since a maximum achievable pulse of nitric oxide is desired the preferred embodiments would be the L-isomers. Since the enzymes are ubiquitous to all body fluids and are found in pulmonary fluids and in wound fluids the release of nitric oxide within these applications ex-vivo would be foersen within the enzymatic degradation profile of the nitric oxide releasing amino acid esters. Degradation of the compounds before their absorption may cause release of the active moiety before it reaches systemic circulation, and therefore decrease the amount of actual effective compound present to treat a condition. This represents a net disadvantage. The rate of hydrolysis (degradation) of compounds in vivo may sometime be changed by effecting some manipulation to the stereochemistry of compounds in order to increase or decrease their rate of degradation as may be desired. According to the present invention, using D- or L-isomers of the compounds of the present invention may
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affect their biological half-life and thus render them better suited for certain treatment, as may be desired.

[00229] As used herein a “slow acting” NO precursor may provide for the release of NO in the tissues over the course of a few hours to several hours after initial exposure to the “slow acting” NO precursor. In contrast, a “fast acting” NO precursor, generally provides almost instantaneous release of detectable levels of NO in the plasma or tissue (e.g., within about a few minutes of exposure to the “fast acting” NO precursor and lasting for several minutes). Such “fast acting” agents may be quickly depleted, however. Thus, a fast acting NO donor may be used to provide a burst of NO, while a slow acting NO donor may be used to provide a more sustained, protracted level of NO release. Since the antimicrobial action could be maintained over several hours to prevent re-infection or as a prophylactic measure, a slow acting D-isomer of the abovementioned compounds of the present invention may be used.

[00230] The preferred compounds of the present invention are the valine or norvaline derivatives of the nitric oxide amino acid ester of the present invention. They may be in the D- or L- configuration, depending on the desired release profile and treatment. The most preferred compounds are known as valine nitrooxy ethyl ester (or valine ethylene glycol nitrate), valine nitrooxy butyl ester (or valine butylene glycol nitrate), or any pharmaceutically acceptable salt thereof, which possess many of the required characteristics necessary to fulfill the role of boosting NO levels. The compound easily dissociates in water into the valine derivative valine ethylene or butylene glycol nitrate and the salt forming acid. The compounds are extremely stable in the form of the salt and thus possesses a long shelf life. It has been observed that the preferred compounds of the present invention do not cause hypotension in normotensive or hypotensive individuals. Therefore, upon administration of the preferred compounds of the present invention, an hypertensive individual will experience the vasodilatory effect caused by the preferred compounds, which will result in a
decrease in blood pressure. The decrease in blood pressure may be up to a normotensive blood pressure. Individuals with normal blood pressure will not experience the vasodilatory effect caused by the preferred compounds, and their blood pressure will remain stable (unchanged). Individuals with lower than normal blood pressure (hypotensive) will not experience a further drop in blood pressure and their blood pressure will remain stable (unchanged). Without wishing to be bound by theory, the compounds of the present invention are believed to be subject to a 2 phase metabolism. The preferred compounds of the present invention have an initial first phase half-life of approximately 1 to about 3 minutes, or from about 1 to about 2.5 minutes, or from about 1 to about 2 minutes, or from about 1 to about 1.5 minutes, or from about 1.5 to about 3 minutes, or from about 1.5 to about 2.5 minutes, or from about 1.5 to about 2 minutes, or from about 2 to about 3 minutes, or from about 2 to about 2.5 minutes, or from about 2.5 to about 3 minutes in the L-configuration, and approximately about 3 to about 6 hours, or from about 3 to about 5 hours, or from about 3 to about 4 hours, or from about 4 to about 6 hours, or from about 4 to about 5 hours, or from about 5 to about 6 in the D-configuration. The half-life of the compounds of the present invention, combined with the half-life of the nitric oxide donating moiety may result in a combined half-life of 9-12 hours or even longer.

[00231] Preferably, a therapeutically effective amount of the D- or L-compounds of the present invention are administered. Therapeutically effective amounts include but are not limited to 0.5 to 200 mg of the compound of the present invention. Preferably, therapeutically effective amounts include 1 to 15 mg, 0.5 to 5 mg, 1 to 5 mg, 5 to 10 mg, 10 to 15 mg, 1 to 15 mg, 1 to 30 mg, 5 to 20 mg, 5 to 15 mg, 5 to 30 mg, 10 to 20 mg, 10 to 30 mg and 15 to 30 mg. Other preferable therapeutically effective amounts also include from about 0.05 mg to about 200 mg, or from about 0.05 mg to about 150 mg, or from about 0.05 mg to about 100 mg, or from about 0.05 mg to about 50 mg, or from about 0.05 mg to
about 40 mg, or from about 0.05 to about 30 mg, or from about 0.05 mg to about 20 mg, or from about 0.05 mg to about 15 mg, or from about 0.05 mg to about 10 mg, or from about 0.05 mg to about 5 mg, or from about 0.05 mg to about 1 mg, or from about 0.5 mg to about 150 mg, or from about 0.5 mg to about 200 mg, or from about 0.5 mg to about 100 mg, or from about 0.5 mg to about 50 mg, or from about 0.5 mg to about 40 mg, or from about 0.5 to about 30 mg, or from about 0.5 mg to about 20 mg, or from about 0.5 mg to about 15 mg, or from about 0.5 mg to about 10 mg, or from about 0.5 mg to about 5 mg, or from about 0.5 mg to about 1 mg, about 1 mg to about 200 mg, or from about 1 mg to about 150 mg, or from about 1 mg to about 100 mg, or from about 1 mg to about 50 mg, or from about 1 mg to about 40 mg, or from about 1 to about 30 mg, or from about 1 mg to about 20 mg, or from about 1 mg to about 15 mg, or from about 1 mg to about 10 mg, or from about 1 mg to about 5 mg, about 5 mg to about 200 mg, or from about 5 mg to about 150 mg, or from about 5 mg to about 100 mg, or from about 5 mg to about 50 mg, or from about 5 mg to about 40 mg, or from about 5 to about 30 mg, or from about 5 mg to about 20 mg, or from about 5 mg to about 15 mg, or from about 5 mg to about 10 mg, about 10 mg to about 200 mg, or from about 10 mg to about 150 mg, or from about 10 mg to about 100 mg, or from about 10 mg to about 50 mg, or from about 10 mg to about 40 mg, or from about 10 to about 30 mg, or from about 10 mg to about 20 mg, or from about 10 mg to about 15 mg, or from about 10 mg to about 10 mg, or from about 15 mg to about 200 mg, or from about 15 mg to about 150 mg, or from about 15 mg to about 100 mg, or from about 15 mg to about 50 mg, or from about 15 mg to about 40 mg, or from about 15 to about 30 mg, or from about 15 mg to about 20 mg, about 20 mg to about 200 mg, or from about 20 mg to about 150 mg, or from about 20 mg to about 100 mg, or from about 20 mg to about 50 mg, or from about 20 mg to about 40 mg, or from about 20 to about 30 mg, about 30 mg to about 200 mg, or from about 30 mg to about 150 mg, or from about 30 mg to about 100 mg, or from about 30 mg to about 50 mg, or from about 30 mg to about 40 mg, about 40
mg to about 200 mg, or from about 40 mg to about 150 mg, or from about 40 mg to about 100 mg, or from about 40 mg to about 50 mg, 50 mg to about 200 mg, or from about 50 mg to about 150 mg, or from about 50 mg to about 100 mg, about 100 mg to about 200 mg, or from about 100 mg to about 150 mg, or 150 mg to about 200 mg.

[00232] The compounds and compositions of the invention are described in more detail herein.

[00233] In embodiments, the amino acid ester compounds comprising at least one nitric oxide releasing group of the present invention are D- or L-isomers. The choice of a D- or L-isomer for a given amino acid ester compounds of the present invention will depend on the desired properties of the compound. For example, when an increase in nitric oxide levels is required for a short, rather punctual period of time, a compound with a shorter biological half-life may be desired. On the other hand, when an increase in nitric oxide levels is desired for long periods of time, for example to match the therapeutic duration of an anti-inflammatory compound such as naproxen and modulate its gastric damaging effect, a compound with a longer biological half-life may be desired.

[00234] In embodiments the amino acid ester compounds comprising at least one nitric oxide releasing group, and pharmaceutically acceptable salts thereof, the compounds compound of formula (I) is:

\[
\begin{align*}
\text{O} & \\
\text{CH} & \\
\text{NH} & \\
\text{R}_1 & \\
\text{O} & -(\text{CH}_2)_n-\text{NO}_2 \\
\text{R}_2 &
\end{align*}
\]

wherein,

\[n = 1 \text{ to } 10;\]
R₁ is an amino acid side chain group (D or L configuration), which may be chosen from:

\[ \text{CH}_3, \quad \text{CH}_3, \quad \text{CH}_3, \quad \text{CH}_3, \quad \text{CH}_3, \quad \text{R}_2, \quad \text{C}_2, \quad \text{OH}, \quad \text{C}_2, \quad \text{OH}, \quad \text{C}_2, \quad \text{SH}, \quad \text{C}_2, \quad \text{NH}_2, \quad \text{C}_2, \quad \text{NH}_2, \quad \text{C}_2, \quad \text{NH}_2, \quad \text{C}_2, \quad \text{CH}_2, \quad \text{COOH}, \quad \text{C}_2, \quad \text{CH}_2, \quad \text{COOH}, \quad \text{C}_2, \quad \text{CH}_2, \quad \text{NH}, \quad \text{H}_2, \quad \text{OH}, \quad \text{C}_2, \quad \text{SH}, \quad \text{C}_2, \quad \text{SeH}, \]

[00235] wherein when R₁ is \[ \text{H}, \quad \text{NH}_2, \quad \text{CH}_2, \text{CH}_3 \], or \[ \text{R}_1 \text{H}, \quad \text{HO}, \quad \text{HO}, \quad \text{HO}, \quad \text{HO}, \quad \text{HO} \], R₁ is also linked to an NH₂ of the Formula (I) to form a proline or hydroxyproline amino acid side chain.
The R₂ is a hydrogen atom, or an amino acid (D or L configuration) forming a peptide bond, which may be chosen from:

and

wherein when R₂ is _______ or _______, the R₂ is also linked to an NH₂ of the Formula (I) to form a proline or hydroxyproline amino acid side chain.
Preferably, the R₁ may be chosen from valine, leucine or isoleucine. Preferably, the R₂ may a hydrogen atom.

[00237] The preferred compounds of formula (I) is:

[00238] L-(2'-nitrooxyethyl-2-amino-3-methylbutanoate:

[00239] or any pharmaceutically acceptable salts thereof.

[00240] Another preferred compound of formula (I) is:

[00241] D-(2'-nitrooxyethyl)-2-amino-3-methylbutanoate:

[00242] or any pharmaceutically acceptable salts thereof.

[00243] According to one embodiment, the D-isomers of the compounds of the present invention, depending on the amino acid side chain attached, may exhibit a biological half-life of up to about 6 hours. According to one embodiment, the biological half-life may be from about 3 hours to about 6 hours. According to one embodiment, branched amino acid side chains (e.g. isoleucine, leucine, and valine) may exhibit enhanced uptake and biological half-life from about 3 hours to about 6 hours, or from about 4 hours to about 6 hours. The increase in biological half-life of the D-isomers is thought to contribute to a release of the nitric oxide donating moiety over a longer time frame, which is a very desirable property that may result in a more uniform release of nitric oxide over time, and thus a better prophylactic profile. Release over a longer time may also allow preparation of formulations containing the compound that may be only administered twice daily.
Longer half-lives are important in certain applications when conditions being treated are persistent ones, such as pulmonary hypertension, hypertension, vascular insufficiencies, and the likes.

[00244] According to some embodiments, the L-isomers of the compounds of the present invention, depending on the amino acid side chain attached, may exhibit a biological half-life of up to about 3 hours. According to one embodiment, the biological half-life may be from about 1 to about 3 hours, or from about 1 to about 2.5 hours, or from about 1 to about 2 hours, or from about 1 to about 1.5 hours, or from about 1.5 to about 3 hours, or from about 1.5 to about 2.5 hours, or from about 1.5 to about 2 hours, or from about 2 to about 3 hours, or from about 2 to about 2.5 hours, or from about 2.5 to about 3 hours. According to some embodiments, branched amino acid side chains (e.g. isoleucine, leucine, and valine) may exhibit enhanced uptake and biological half-life from about from about 1 hour to about 3 hours.

[00245] According to one embodiment, the branched amino acid side chains may exhibit good oral bioavailability.

[00246] According to an embodiment, the compounds of the present invention display very fast onset of nitric oxide release when hard physical activity is performed, and they are capable of long sustained release of nitric oxide for up to 24 hours, with a 40% increase in nitric oxide. Interestingly, the 24 hours sustained release highlights that the molecular mechanism involved in the release of nitric oxide from the D- or L- compounds of the present invention does not involve a mechanism that develops tolerance like other nitric oxide releasing molecules. According to some embodiments, the compounds of the present invention may be administered systemically, for example through oral administration or injection, or locally, for example through transdermal administration. Surprisingly, upon local application the effect of the compounds of the present invention is localized to the tissue where the transdermal formulation has been applied. Without wishing to be bound by theory, it is possible that the
mechanism of action of the compounds of the present invention is related to the laminar flow activation of receptors (e.g. GST isoform) in the endothelial cells that metabolize the nitrooxy alcohol to nitric oxide. Thus, the observed localized effect of the compounds suggest that the activation of the compounds of the present invention for release of nitric oxide upon hypertensive conditions may not involve the liver cytosol but rather a localized activation pathway. Furthermore, the sustained release over a period of 24 hours suggests that the nitrooxy alcohol molecules resulting from the breakdown of the compounds of the present invention are being sequestered in the cells of the target tissue(s). These observations also suggest that the physiological effects of the molecule may be related to the two step metabolism of the D- or L- compounds of the present invention. The first step comprises the carboxylesterase cleavage of the D- or L-amino acid moiety from the nitrooxy alcohol, and the second step the metabolism of nitrooxy alcohol in the cytosol of endothelial cells to nitric oxide, which may be glutathione transferase regulated.

[00247] The fast onset of nitric oxide release suggests that the (L-) bond between the amino acid moiety and the nitrooxy alcohol moiety is being quickly cleaved by carboxylesterases enzyme (or carboxylic-ester hydrolase) in the tissue, which have been shown to exist in many tissue types in humans. According to some embodiments of the present invention, the L-isomers of the compounds of the present invention are believed to be more susceptible to carboxylesterase enzymes and to be more rapidly cleaved by these enzymes for fast release of the nitrooxy alcohol moiety in the target tissue.

[00248] According to another embodiment, the D-isomers of the compounds of the present invention are believed to be less susceptible to the carboxylesterase enzymes, affording them greater stability and longer half-lives. The D-isomer compounds, because of their greater stability and longer half-lives may distribute throughout the body into the tissue, to be metabolized to nitrooxy
alcohol, which may either be sequestered in the cytosol of the cells for a period of time, or react and be liberated as nitric oxide.

[00249] Compounds of the invention that have one or more asymmetric carbon atoms may exist as the optically pure enantiomers, pure diastereomers, mixtures of enantiomers, mixtures of diastereomers, racemic mixtures of enantiomers, diastereomeric racemates or mixtures of diastereomeric racemates. It is to be understood that the invention anticipates and includes within its scope all such isomers and mixtures thereof.

[00250] Compounds of the invention that have one or more double bounds may exist as a single tautomers or a mixture of tautomers. It is to be understood that the invention anticipates and includes within its scope all such tautomers and mixtures thereof.

[00251] The compounds and compositions of the invention can be formulated as pharmaceutically acceptable salt forms. Pharmaceutically acceptable salts include, for example, alkali metal salts and addition salts of free acids or free bases. The nature of the salt is not critical, provided that it is pharmaceutically-acceptable. Suitable pharmaceutically-acceptable acid addition salts may be prepared from an inorganic acid or from an organic acid. Examples of such inorganic acids include, but are not limited to, hydrochloric, hydrobromic, hydroiodic, nitric, carbonic, sulfuric and phosphoric acid and the like. Appropriate organic acids include, but are not limited to, aliphatic, cycloaliphatic, aromatic, heterocyclic, carboxylic and sulfonic classes of organic acids, such as, for example, formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, fumaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, mesylic, salicylic, p-hydroxybenzoic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, benzenesulfonic, pantothenic, toluenesulfonic, 2-hydroxyethanesulfonic, sulfanilic, stearic, algenic, β-hydroxybutyric, cyclohexylaminosulfonic, galactaric and galacturonic acid and
the like. Suitable pharmaceutically-acceptable base addition salts include, but are not limited to, metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from primary, secondary and tertiary amines, cyclic amines, N, N'-dibenzylethlenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine and the like. All of these salts may be prepared by conventional means from the corresponding compound by reacting, for example, the appropriate acid or base with the compound. In one embodiment, the pharmaceutically acceptable salts of the compounds of the invention include the nitrate salts.

[00252] The compounds of the present invention may be incorporated into liposomal liquid formulation. Any suitable liposomal formulation known in the art for use for drug delivery or as carriers of drugs or dietary supplements may be used. The liposomes or liposomal formulations are vesicles made out of the same material as a cell membrane. Liposomes can be filled with drugs, and used to deliver drugs for treating diseases or conditions. They are usually made of phospholipids like phosphatidylethanolamine and phosphatidylcholine. The material making up the vesicles are usually amphiphilic with the hydrocarbon tail of the molecule being hydrophobic; and the polar head being hydrophilic. The head is attracted to water, and the tail, which is made of a long hydrocarbon chain, is repelled by water.

[00253] The amino acid ester compound of the present invention is dipped into a liposomal composition. The liposome preparation can be prepared by using a suitable blend of phospholipids and additives to maximize the entrapment of the amino acid ester compound into the liposomes. Typically, the process is carried out by mixing the right proportion of the amino acid ester compound and phospholipids and additives into a suitable solvent and removing the solvent to obtain liposomes. The ratio of phospholipids to amino acid ester compound may vary from 1-30, depending on the process of mixing and the blend of phospholipids.
[00254] The compounds of the present invention, because of the small size of the molecule, can be other choices of linkages and/or amino acids or their derivatives. For example, as alternatives to the above choices, propyl, butyl, or longer chains may be linked to any amino acid. Salts such as chloride or hydrochloride salts may be used. Other amino acid derivatives may also be chosen. Derivatives of the base amino acids whether they are in the L or D configuration of these amino acids can be chosen. Non standard amino acids, or synthetic derivative of standard and non-standard amino acids may be elected, such as those containing acetyl groups attached to the amide of the molecule or nor derivatives of the amino acids, when such derivatives can be achieved.

[00255] The amino acid esters compounds may be based on natural, non-standard or even modified amino acids, with the basic structure as depicted below, where the Rx represents the side chain of the amino acid (wherein Rx may be R1, R2 or R3, as applicable to the specific molecule described herein):

```
  H—N—C—C—O—H
    \   /   \     /
      Rx
```

Basic amino acid structure

[00256] Natural Amino Acids

<table>
<thead>
<tr>
<th>No.</th>
<th>Originating Amino Acid</th>
<th>Formula</th>
<th>Rx = R1 or R2 or R3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glycine</td>
<td>H</td>
<td>-H</td>
</tr>
<tr>
<td>2</td>
<td>Alanine</td>
<td>CH₃</td>
<td>(\text{V}\text{V} \text{V} \text{CH}_3)</td>
</tr>
<tr>
<td>3</td>
<td>Valine*</td>
<td>(CH₃)₂</td>
<td>CH₃ (\text{V} \text{V} \text{V} \text{CH} \text{CH}_3)</td>
</tr>
<tr>
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</tr>
<tr>
<td>4</td>
<td>Leucine*</td>
<td>CH₂CH(CH₃)₂</td>
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</tr>
<tr>
<td></td>
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<tr>
<td>5</td>
<td>Isoleucine*</td>
<td>CH(CH₃)CH₂CH₃</td>
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</tr>
<tr>
<td>6</td>
<td>Phenylalanine*</td>
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<td>Tyrosine</td>
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<td>8</td>
<td>Tryptophane*</td>
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<td>Serine</td>
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<td>Threonine*</td>
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<td>11</td>
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<tr>
<td>12</td>
<td>Methionine*</td>
<td>CH₂CH₂SCH₃</td>
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<tr>
<td>13</td>
<td>Proline</td>
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<td>14</td>
<td>Asparagine</td>
<td>CH₃COCH₂</td>
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<tr>
<td></td>
<td>Amino Acid</td>
<td>Chemical Structure</td>
<td>Molecular Structure</td>
</tr>
<tr>
<td>---</td>
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<td>--------------------</td>
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<td>15</td>
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<td><img src="image" alt="Glutamic acid Structure" /></td>
</tr>
<tr>
<td>18</td>
<td>Lysine*</td>
<td>CH₂CH₂CH₂CH₂NH₂</td>
<td><img src="image" alt="Lysine Structure" /></td>
</tr>
<tr>
<td>19</td>
<td>Histidine*</td>
<td>CH₃C₃N₂H₃</td>
<td><img src="image" alt="Histidine Structure" /></td>
</tr>
<tr>
<td>20</td>
<td>Arginine*</td>
<td>(CH₂)₃CN₃H₄</td>
<td><img src="image" alt="Arginine Structure" /></td>
</tr>
</tbody>
</table>

* essential amino acids
**Modified Amino Acids**

<table>
<thead>
<tr>
<th>No.</th>
<th>Originating Amino acid</th>
<th>Formula</th>
<th>( R_x = R_1 ) or ( R_2 ) or ( R_3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Cystine</td>
<td>( \text{CH}_2\text{S}_2\text{CH}_2\text{CHNH}_2\text{COOH} )</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Hydroxyproline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>( \varepsilon )-N-methyllysine</td>
<td>( \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NHCH}_3 )</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>diiodotyrosine</td>
<td>( \text{CH}_2\text{C}_6\text{H}_2\text{OH} )</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>homocysteine</td>
<td>( \text{CH}_2\text{CH}_2\text{SH} )</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>ornithine</td>
<td>( \text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2 )</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>Norvaline</td>
<td>( \text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_3 )</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>selenocysteine</td>
<td>( \text{CH}_2\cdot\text{SeH} )</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Hypusine</td>
<td>( \text{CH}_2\text{CH}_2\text{CH}_2\text{NHCH}_2\text{CH(OH)}\text{CH}_2\text{CH}_2\text{NH}_2 )</td>
<td></td>
</tr>
<tr>
<td>J</td>
<td>Dehydroalanine</td>
<td>( \text{CH}_2 )</td>
<td></td>
</tr>
</tbody>
</table>

The nitric oxide amino acid ester compounds of the present invention are not limited to a single amino acid molecule. The compounds of the present invention may be dipeptide or even tripeptide molecules, with the general
formula depicted below and where \( R_x \) and \( R_y \) independently are any of the amino acid side chains described herein.

![Chemical Structure](image)

[00259] The composition containing a compound as defined in the present invention may include a wide variety of additional components, including, for example, one or more of gases, gaseous precursors, liquids, oils, stabilizing materials, pharmaceutical acceptable carriers, and photoactive agents.

[00260] The compositions described herein include those which are suitable for administration orally, bucally, sublingually, parenterally, transdermally, rectally, by inhalation, intranasally, topically, intramuscularly, intravenously, or by injection, as known in the art. Compositions described herein include those which are suitable for oral administration, such as solid dosage forms. Peroral compound administration, which is known to one skilled in the art, involves the delivery of pharmaceutical compounds via the oral passage of the compound into the systemic circulation of the patient. Solid dosage forms for oral administration can include capsules, sustained-release capsules, tablets, sustained release tablets, chewable tablets, sublingual tablets, effervescent tablets, pills, powders, granules and gels. In such solid dosage forms, the active compounds can be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms can also comprise, as in normal practice, additional substances other than inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets, effervescent tablets, and pills, the dosage forms
can also comprise buffering agents. Soft gelatin capsules can be prepared to contain a mixture of the active compounds or compositions of the invention and vegetable oil. Hard gelatin capsules can contain granules of the active compound in combination with a solid, pulverulent carrier such as lactose, saccharose, sorbitol, mannitol, potato starch, corn starch, amylopectin, cellulose derivatives of gelatin. Tablets and pills can be prepared with enteric coatings.

[00261] The compounds and compositions of the invention can include an enteric coating to prevent release of the amino acid ester compound before it reaches the small intestine. Enteric coatings are often used to prevent stomach irritation caused by some medicine or to safeguard the medicine or compounds against the stomach acids or enzymes of the gastric environment. Therefore, an enteric coating will control the location in the digestive system where it is absorbed.

[00262] Enteric coatings are selectively insoluble substances that will not dissolve in the acidic juices (pH ~3) of the stomach, but upon reaching the relatively higher pH (pH >5.5) environment of the small intestine will readily dissolve. Materials used for enteric coatings include fatty acids, waxes, and shellac as well as plastics. Suitable materials used for enteric coatings include but are not limited to: methacrylic acid copolymers, cellulose acetate (including succinate and phthalate versions), styrol maleic acid co-polymers, polymethacrylic acid/ acrylic acid copolymer, hydroxypropyl methyl cellulose phthalate, polyvinyl acetate phthalate, hydroxyethyl ethyl cellulose phthalate, hydroxypropyl methyl cellulose acetate succinate, cellulose acetate tetrahydrophthalate, acrylic resin, timellitate, shellac, alginic acid, medium chain triglycerides, oleic acid, stearic acid.

[00263] Compositions described herein also include those which are suitable for transdermal administration of the compound as define in the present invention and optionally include a vehicle or carrier for the transdermal administration of the compounds described herein as well as further comprising
one or more of the following: pharmacologically active agents, solvents, thickening agents, skin penetration enhancers, wetting agents, lubricants, emollients, substances added to mask or counteract a disagreeable odor, fragrances, essential oils, preservative agents and antiseptic agents.

[00264] The compounds and compositions of the present invention can be administered transdermally in dosage unit formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles, as desired. In one embodiment of the invention the amino acid ester compound comprising at least one nitric oxide releasing group is administered transdermally.

[00265] Transdermal compound administration, which is known to one skilled in the art, involves the delivery of pharmaceutical compounds via percutaneous passage of the compound into the systemic circulation of the patient. Topical administration can also involve the use of transdermal administration such as transdermal patches or iontophoresis devices. Other components can be incorporated into the transdermal patches as well. For example, compositions and/or transdermal patches can be formulated with one or more preservatives or bacteriostatic agents including, but not limited to, methyl hydroxybenzoate, propyl hydroxybenzoate, chlorocresol, benzalkonium chloride, and the like. Dosage forms for topical administration of the compounds and compositions can include creams, sprays, lotions, gels, ointments, eye drops, nose drops, ear drops, and the like. In such dosage forms, the compositions of the invention can be mixed to form white, smooth, homogeneous, opaque cream or lotion with, for example, benzyl alcohol 1% or 2% (wt/wt) as a preservative, emulsifying wax, glycerin, isopropyl palmitat, lactic acid, purified water and sorbitol solution. In addition, the compositions can contain polyethylene glycol 400. They can be mixed to form ointments with, for example, benzyl alcohol 2% (wt/wt) as preservative, white petrolatum, emulsifying wax, and tenox II (butylated hydroxyanisole, propyl gallate, citric acid, propylene glycol). Woven
pads or rolls of bandaging material, e.g., gauze, can be impregnated with the compositions in solution, lotion, cream, ointment or other such form can also be used for topical application.

[00266] The compositions can also be applied topically using a transdermal system, such as one of an acrylic-based polymer adhesive with a resinous crosslinking agent impregnated with the composition and laminated to an impermeable backing. In a particular embodiment, the compositions of the invention are administered as a transdermal patch, more particularly as a sustained-release transdermal patch. The transdermal patches of the invention can include any conventional form such as, for example, adhesive matrix, polymeric matrix, reservoir patch, matrix or monolithic-type laminated structure, and are generally comprised of one or more backing layers, adhesives, skin penetration enhancers, an optional rate controlling membrane and a release liner which is removed to expose the adhesives prior to application. Polymeric matrix patches also comprise a polymeric-matrix forming material. Suitable transdermal patches are described in more detail in, for example, U.S. Patents Nos. 5,262,165; 5,948,433; 6,010,715 and 6,071,531.

Skin penetration enhancers

[00267] As used herein, "enhancement," "skin penetration enhancement," or "skin permeation enhancement," refer to an increase in the permeability of the skin to a drug, so as to increase the rate at which the drug permeates through the skin. Thus, "skin permeation enhancer," "skin penetration enhancer," or simply "enhancer" refers to an agent, or mixture of agents that achieves such permeation enhancement. Several compounds have been investigated for use as skin penetration enhancers. See, for example, U.S. Pat. Nos. 5,601,839; 5,006,342; 4,973,468; 4,820,720; 4,006,218; 3,551,154; and 3,472,931.

[00268] A skin penetration enhancer as used herein means any compound that augments movement of active compound through the dermis, for instance,
that allows a colloidal dispersion of lipid with a non-lipid so it can penetrate body tissues which are composed of lipids and water along with other dermis components. In one embodiment, the skin penetration enhancer is DMSO, however, any skin penetration enhancer suitable in and known in the art for transdermal formulations may be used, such a those that allow a colloidal dispersion of a lipid with a non lipid so it can penetrate body tissues which are composed of lipids and water along with other dermis components. In one embodiment, DMSO has been shown to be a preferred skin penetration enhancer and the invention provides a transdermal formulation or composition comprising a therapeutic compound, or pharmaceutically acceptable salts thereof and DMSO with or without nano colloidal silica.

[00269] In one embodiment, the composition may comprise one or more skin penetration enhancing agents for transdermal drug delivery. Non-limiting examples of skin penetration enhancing agents include C₆-C₂₂ fatty acids such as isostearic acid, octanoic acid, and oleic acid; C₆-C₂₂ fatty alcohols such as oleyl alcohol and lauryl alcohol; lower alkyl esters of C₆-C₂₂ fatty acids such as ethyl oleate, isopropyl myristate (IPM), butyl stearate, and methyl laurate; di(lower)alkyl esters of C₆-C₂₂ diacids such as diisopropyl adipate; monoglycerides of C₆-C₂₂ fatty acids such as glyceryl monolaurate; tetrahydrofurfuryl alcohol polyethylene glycol ether; polyethylene glycol, propylene glycol; 2-(2-ethoxyethoxy)ethanol (transcutol); diethylene glycol monomethyl ether; alkylaryl ethers of polyethylene oxide; polyethylene oxide monomethyl ethers; polyethylene oxide dimethyl ethers; dimethyl sulfoxide; glycerol; ethyl acetate; acetoacetic ester; N-alkylpyrrolidone; terpenes, dimethyl formamide (DMF), N,N-dimethylacetamide (DMA), diethylene glycol monoethyl or monomethyl ether with propylene glycol monolaurate and methyl laurate; glycerol monolaurate and ethanol, fatty acid esters or fatty alcohol ethers of C₂ to C₄ alkanediols, where each fatty acid/alcohol portion of the ester/ether is of about 8 to 22 carbon atoms.
The skin penetration enhancing agent is present in an amount sufficient to provide the desired physical properties and skin penetration profile for the composition. Illustratively, one or more pharmaceutically acceptable skin penetration enhancer can be present in a total amount by weight of the composition of about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 1.0%, about 1.5%, about 2.0%, about 2.5%, about 3.0%, about 3.5%, about 4.0%, about 4.5%, about 5.0%, about 5.5%, about 6.0%, about 6.5%, about 7.0%, about 7.5%, about 8.0%, about 8.5%, about 9.0%, about 9.5%, about 10.0%, about 10.5%, about 11.0%, about 11.5%, about 12.0%, about 12.5%, about 13.0%, about 13.5%, about 14.0%, about 14.5%, and or 15.0%. As a further illustration, one or more pharmaceutically acceptable skin penetration enhancer is present in a total amount by weight between about 0.1% and about 15%; between about 0.1% and about 10%; between about 0.5% and about 10%; or between about 3% and about 8%.

As a further illustration, one or more pharmaceutically acceptable skin penetration enhancer is present in a total amount by weight between about 0.5% and about 10%, between about 1% and about 10%, between about 2% and about 10%, between about 3% and about 10%, between about 4% and about 10%, between about 5% and about 10%, between about 6% and about 10%, between about 7% and about 10%, between about 8% and about 10%, between about 9% and about 10%, between about 0.5% and about 9%, between about 1% and about 9%, between about 2% and about 9%, between about 3% and about 9%, between about 4% and about 9%, between about 5% and about 9%, between about 6% and about 9%, between about 7% and about 9%, between about 8% and about 9%, between about 0.5% and about 8%, between about 1% and about 8%, between about 2% and about 8%, between about 3% and about 8%, between about 4% and about 8%, between about 5% and about 8%, between about 6% and about 8%, between about 7% and about 8%, between about 1% and about 7%, between about 2% and about 7%, between about 3%
and about 7%, between about 4% and about 7%, between about 5% and about 7%, between about 6% and about 7%, between about 0.5% and about 6%, between about 1% and about 6%, between about 2% and about 6%, between about 3% and about 6%, between about 4% and about 6%, between about 5% and about 6%, between about 0.5% and about 5%, between about 1% and about 5%, between about 2% and about 5%, between about 3% and about 5%, between about 4% and about 5%, between about 0.5% and about 4%, between about 1% and about 4%, between about 2% and about 4%, between about 3% and about 4%, between about 0.5% and about 3%, between about 1% and about 3%, between about 2% and about 3% and between about 0.5% and about 2%, between about 1% and about 2%, between about 0.5% and about 1%.

Thickening agents

[00272] In one embodiment, the composition may comprise a thickening or gelling agent to increase the viscosity of the composition. Non-limiting examples of thickening agents (aka gelling agents) which may be used herein include neutralized anionic polymers such as polyacrylic acid (CARBOPOL® by Noveon, Inc., Cleveland, Ohio), carboxypolymethylene, carboxymethylcellulose and the like, including derivatives of Carbopol® polymers, such as Carbopol® Ultrace 10, Carbopol® 940, Carbopol® 941, Carbopol® 954, Carbopol® 980, Carbopol® 981, Carbopol® ETD 2001, Carbopol® EZ-2 and Carbopol® EZ-3. Also suitable are other known polymeric thinking agents, such as Pemulen® polymeric emulsifiers, and Noveon® polycarbophils and Klucel®. Additional thickening agents, enhancers and adjuvants may generally be found in Remington's The Science and Practice of Pharmacy as well as the Handbook of Pharmaceutical Excipients, Arthur H. Kibbe ed. 2000. Thickening agents or gelling agents are present in an amount sufficient to provide the desired rheological properties of the composition. Illustratively, one or more pharmaceutically acceptable thickening agent or gelling agent are present in a total amount by weight of about 0.1%, about 0.25%, about 0.5%, about 0.75%, about 1%, about 1.25%, about
1.5%, about 1.75%, about 2.0%, about 2.25%, about 2.5%, about 2.75%, about 3.0%, about 3.25%, about 3.5%, about 3.75%, about 4.0%, about 4.25%, about 4.5%, about 4.75%, about 5.0%, about 5.25%, about 5.5%, about 5.75%, about 6.0%, about 6.25%, about 6.5%, about 6.75%, about 7.0%, about 7.25%, about 7.5%, about 7.75%, about 8.0%, about 8.25%, about 8.5%, about 8.75%, about 9.0%, about 9.25%, about 9.5%, about 9.75%, about 10%, about 11%, about 11.5%, about 12%, about 12.5%, about 13%, about 13.5%, about 14%, about 14.5% or about 15%. As a further illustration, one or more pharmaceutically acceptable thickening or gelling agent are present in a total amount by weight between about 0.1% and about 15%; about 0.5% and about 5%; or about 1% and about 3%.

Wetting agents

[00273] Compositions described herein optionally comprise one or more pharmaceutically acceptable wetting agents as excipients. Non-limiting examples of surfactants that can be used as wetting agents in compositions of the disclosure include quaternary ammonium compounds, for example benzalkonium chloride, benzethonium chloride and cetlypyridinium chloride; dioctyl sodium sulfo succinate; polyoxyethylene alkylphenyl ethers, for example nonoxynol 9, nonoxynol 10, and octoxynol 9; poloxamers (polyoxyethylene and polyoxypolypropylene block copolymers); polyoxyethylene fatty acid glycerides and oils, for example polyoxyethylene (8) caprylic/capric mono- and diglycerides (e.g., Labrasol™ of Gattefossé), polyoxyethylene (35) castor oil and polyoxyethylene (40) hydrogenated castor oil; polyoxyethylene alkyl ethers, for example polyoxyethylene (20) cetostearyl ether; polyoxyethylene fatty acid esters, for example polyoxyethylene (40) stearate; polyoxyethylene sorbitan esters, for example polysorbate 20 and polysorbate 80 (e.g., Tween™ 80 of ICI); propylene glycol fatty acid esters, for example propylene glycol laurate (e.g., Lauroglycol™ of Gattefossé); sodium lauryl sulfate, sodium laureth sulfate, laureth 23, fatty acids and salts thereof, for example oleic acid, sodium oleate
and triethanolamine oleate; glyceryl fatty acid esters, for example glyceryl monostearate; sorbitan esters, for example sorbitan monolaurate, sorbitan monooleate, sorbitan monopalmitate and sorbitan monostearate; tyloxapol; and mixtures thereof. Such wetting agents, if present, constitute in total about 0.25% to about 15%, about 0.25% to about 5%, about 0.25% to about 2%, about 0.25% to about 1%, about 0.4% to about 10%, or about 0.5% to about 5%, of the total weight of the composition. Illustratively, one or more pharmaceutically acceptable wetting agents are present in a total amount by weight of about 0.25%, about 0.5%, about 0.75%, about 1%, about 1.25%, about 1.5%, about 1.75%, about 2.0%, about 2.25%, about 2.5%, about 2.75%, about 3.0%, about 3.25%, about 3.5%, about 3.75%, about 4.0%, about 4.25%, about 4.5%, about 4.75%, about 5.0%, about 5.25%, about 5.5%, about 5.75%, about 6.0%, about 6.25%, about 6.5%, about 6.75%, about 7.0%, about 7.25%, about 7.5%, about 7.75%, about 8.0%, about 8.25%, about 8.5%, about 8.75%, about 9.0%, about 9.25%, about 9.5%, about 9.75% or about 10%.

Lubricants

[00274] Compositions described herein optionally comprise one or more pharmaceutically acceptable lubricants (including anti-adherents and/or glidants) as excipients. Suitable lubricants include, either individually or in combination, glycercyl behapate (e.g., Compritol™ 888); stearic acid and salts thereof, including magnesium (magnesium stearate), calcium and sodium stearates; hydrogenated vegetable oils (e.g., Sterotex™); colloidal silica; talc; waxes; boric acid; sodium benzoate; sodium acetate; sodium fumarate; sodium chloride; DL-leucine; PEG (e.g., Carbowax™ 4000 and Carbowax™ 6000); sodium oleate; sodium lauryl sulfate; sodium laureth sulfate and magnesium lauryl sulfate. Such lubricants, if present, constitute in total about 0.1% to about 10%, about 0.2% to about 8%, or about 0.25% to about 5%, of the total weight of the composition. Illustratively, one or more pharmaceutically acceptable lubricants are present in a total amount by weight of about 0.1%, about 0.2%, about 0.3%, about 0.4%,
about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1.0%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2.0%, about 2.1%, about 2.2%, about 2.3%, about 2.4%, about 2.5%, about 2.6%, about 2.7%, about 2.8%, about 2.9%, about 3.0%, about 3.1%, about 3.2%, about 3.3%, about 3.4%, about 3.5%, about 3.6%, about 3.7%, about 3.8%, about 3.9%, about 4.0%, about 4.1%, about 4.2%, about 4.3%, about 4.4%, about 4.5%, about 4.6%, about 4.7%, about 4.8%, about 4.9%, about 5.0%, about 5.1%, about 5.2%, about 5.3%, about 5.4%, about 5.5%, about 5.6%, about 5.7%, about 5.8%, about 5.9%, about 6.0%, about 6.1%, about 6.2%, about 6.3%, about 6.4%, about 6.5%, about 6.6%, about 6.7%, about 6.8%, about 6.9%, about 7.0%, about 7.1%, about 7.2%, about 7.3%, about 7.4%, about 7.5%, about 7.6%, about 7.7%, about 7.8%, about 7.9%, about 8.0%, about 8.1%, about 8.2%, about 8.3%, about 8.4%, about 8.5%, about 8.6%, about 8.7%, about 8.8%, about 8.9%, about 9.0%, about 9.1%, about 9.2%, about 9.3%, about 9.4%, about 9.5%, about 9.6%, about 9.7%, about 9.8%, about 9.9% or about 10.0%.

Emollients

[00275] In another embodiment, the compositions described herein optionally comprise an emollient. Illustrative emollients include mineral oil, mixtures of mineral oil and lanolin alcohols, cetyl alcohol, cetostearyl alcohol, stearyl alcohol, petrolatum, petrolatum and lanolin alcohols, cetyl esters wax, cholesterol, glycerin, glyceryl monostearate, isopropyl myristate (IPM), isopropyl palmitate, lecithin, allyl caproate, althea officinalis extract, arachidyl alcohol, argobase EUC, butylene glycol, dicaprylyl/dicaprate, acacia, allantoin, carrageenan, cetyl dimethicone, cyclomethicone, diethyl succinate, dihydroabietyl behenate, dioctyl adipate, ethyl laurate, ethyl palmitate, ethyl stearate, isoamyl laurate, octanoate, PEG-75, niacinamide, lanolin, sorbitan laurate, walnut oil, wheat germ oil, super refined almond, super refined sesame,
super refined soybean, octyl palmitate, caprylic/capric triglyceride and glyceryl cocoate.

[00276] An emollient, if present, is present in the compositions described herein in an amount of about 0.25% to about 30%, 0.25% to about 1%, 0.25% to about 2%, 0.25% to about 3%, about 0.25% to about 5%, about 0.25% to about 10%, about 0.5% to about 30%, 0.5% to about 1%, 0.5% to about 2%, 0.5% to about 3%, about 0.5% to about 5%, about 0.5% to about 10%, about 1% to about 30%, about 3% to about 25%, or about 5% to about 15%, by weight. Illustratively, one or more emollients are present in a total amount by weight of about 0.5%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, or about 30%.

Water soluble celluloses

[00277] Celluloses are organic compounds with the general formula \((C_6H_{10}O_5)_n\), a polysaccharide consisting of a linear chain of several hundred to over ten thousands \(\beta(1\rightarrow4)\) linked D-glucose units. Preferred celluloses include water-soluble celluloses, and modified water-soluble celluloses such as those known in the art and have properties similar to cellulose. Examples are methylcellulose of different viscosity, ethylcellulose, hydroxypropyl cellulose, hydroxymethylcellulose, and hydroxyethylcellulose, hydroxypropyl methylcellulose, such as HMPC E5 (e.g. methocel® E5), methocel® MC, and carboxymethylcellulose. These cellulose compounds, like cellulose itself, are not digestible by humans, and they are not toxic, and not allergenic.

Polysorbates

[00278] Polysorbates are a class of emulsifiers used in some pharmaceuticals and food preparation. Polysorbates are oily liquids derived from
PEG-ylated sorbitan (a derivative of sorbitol) esterified with fatty acids. Polysorbates include but are not limited to polyoxyethylene (20) sorbitan monolaurate (polysorbate 20), polyoxyethylene (20) sorbitan monooleate (polysorbate 40), polyoxyethylene (20) sorbitan monooleate (polysorbate 60), polyoxyethylene (20) sorbitan tristearate (polysorbate 65), and polyoxyethylene (20) sorbitan monooleate (polysorbate 80).

**Carbomers**

[00279] Carbomer is a generic name for synthetic polymers of acrylic acid used as emulsion stabilizers or thickening agents in pharmaceuticals and cosmetic products. They may be homopolymers of acrylic acid, crosslinked with an allyl ether pentaerythritol, allyl ether of sucrose, or allyl ether of propylene. Carbomers include but are not limited to carbopol® polymer 71G NF, carbopol® polymer 971P NF, carbopol® polymer 974P NF, carbopol® polymer 980 NF, carbopol® polymer 981 NF, carbopol® polymer 5984 EP and carbopol® polymer Ultrez 10 NF.

[00280] A carbomer, if present, is present in the compositions described herein in an amount of about 0.25% to about 30%, 0.25% to about 1%, 0.25% to about 2%, 0.25% to about 3%, about 0.25% to about 5%, about 0.25% to about 10%, about 0.5% to about 30%, 0.5% to about 1%, 0.5% to about 2%, 0.5% to about 3%, about 0.5% to about 5%, about 0.5% to about 10%, about 1% to about 30%, about 3% to about 25%, or about 5% to about 15%, by weight. Illustratively, one or more emollients are present in a total amount by weight of about 0.25, about 0.5%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, or about 30%.
Polyethylene glycol (PEG)

[00281] PEG refers to an oligomer or polymer of ethylene oxide and are prepared by polymerization of ethylene oxide and are commercially available over a wide range of molecular weights from 300 g/mol to 10,000,000 g/mol. The preferred PEG to be used in the present invention are liquid PEGs including but not limited to PEG 200, PEG 200E, PEG 300, PEG 300E, PEG 400, PEG 400E, PEG 600 and PEG 600E. Also included among the preferred PEG is PEG 3350, which may be used as a humectants as well as a solvent and carrier. The present invention will be more readily understood by referring to the following examples which are given to illustrate the invention rather than to limit its scope.

[00282] A PEG, if present, is present in the compositions described herein in an amount of about about 0.5% to about 70%, 1% to about 70%, 0.5% to about 65%, 0.5% to about 60%, about 0.5% to about 50%, about 0.5% to about 40%, about 0.5% to about 30%, about 0.5% to about 10%, about 0.5% to about 5%, about 1% to about 70%, 1% to about 65%, about 1% to about 60%, about 1% to about 50%, about 1% to about 40%, about 1% to about 30%, about 1% to about 20%, about 1% to about 10%, about 1% to about 5%, about 3% to about 25%, about 5% to about 10%, about 60% to about 65%, or about 5% to about 15%, by weight. Illustratively, one or more emollients are present in a total amount by weight of about 0.25, about 0.5%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57%, about 58%, about 59%, about 60%, about 61%, about 62%,
about 63%, about 64%, about 65%, about 66%, about 67%, about 68%, about 69%, or about 70%.

Essential oils

The composition of the present invention may include essential oils as active agents to help penetration of the compounds of the present invention. Oil are "essential" in the sense that they carry distinctive scent, or essence, of a plant. Preferred essential oils include but are not limited to argan oil, cypress oil, chamomile oil, oil (bois de rose oil) lavender oil, tea tree oil (melaleuque oil), pine tree oil, eucalyptol oil, eucalyptus oil, birch oil, peppermint oil, ylang-ylang oil, cymbopogon martinii oil (palmarosa oil), sweet almond oil and olive oil. The most preferred essential oil is tea tree oil (melaleuque oil) since it has beneficial medical properties when applied topically, including antiseptic, antibacterial, antifungal, and antiviral action, and is also believed to have beneficial cosmetic properties.

An essential oil, if present, is present in the compositions described herein in an amount of about 0.25% to about 30%, 0.25% to about 1%, 0.25% to about 2%, 0.25% to about 3%, about 0.25% to about 5%, about 0.25% to about 10%, about 0.5% to about 30%, 0.5% to about 1%, 0.5% to about 2%, 0.5% to about 3%, about 0.5% to about 5%, about 0.5% to about 10%, about 1% to about 30%, about 3% to about 25%, or about 5% to about 15%, by weight. Illustratively, one or more emollients are present in a total amount by weight of about 0.5%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, or about 30%.
Antiseptic agents

[00285] The composition of the present invention may also be prepared by the addition of an antiseptic agent in order to keep the composition sterile and disinfect the surfaces onto which it is applied during use. The preferred antiseptic agents include but are not limited to chlorhexidine gluconate, glucono deltla-lactone, a paraben compound, such as methyl-paraben, Isopropyl-paraben, Isobutyl-paraben, Butyl-paraben, benzoic acid, imidazolidinyl urea, a quaternary ammonium compound, methyl hydroxybenzoate, propyl hydroxybenzoate, chlorocresol, benzalkonium chloride and Octenidine dihydrochloride.

Preservative agent

[00286] Furthermore, in order to stabilize and keep the composition for extended periods of time, preservative agents may be added to the composition. The preferred preservative agents include but are not limited to EDTA, EGTA, hydroxytoluene butoxide, hydroxyanisol butoxide, sodium hydroxide, calcium propionate, sodium nitrate, sodium nitrite, sulfur dioxide, sodium bisulfite, benzoic acid, caprylyl glycol, Diazolidinyl urea, Phenoxyethanol, Dehydroacetic acid, Iodopropynylbutylcarbamate, Sorbic acid, methyl-paraben, Isopropyl-paraben, Isobutyl-paraben, Butyl-paraben, and potassium hydrogen sulfite. The present invention will be more readily understood by referring to the following examples which are given to illustrate the invention rather than to limit its scope.

Other ingredients

[00287] According to another embodiment of the present invention, the composition may further include phosphocreatine. Phosphocreatine is a phosphorylated creatine molecule that serves as a rapidly mobilizable reserve of high-energy phosphates in skeletal muscle and brain. Studies have shown that phosphocreatine supplementation may improve performance during intense exercise, such as sprint.
According to another embodiment, the composition may also further include magnesium chloride. Magnesium is a cofactor to the enzyme creatine kinase which converts creatine into phosphocreatine. Since creatine monohydrate supplements are extremely popular and proven to be effective, magnesium may be an important mineral in helping to optimize creatine function. In active muscle, creatine kinase also helps phosphocreatine combine with ADP to resynthesize ATP in contractile activity. This process, which involves magnesium, basically increases anaerobic endurance. Phosphocreatine possesses a higher phosphate group transfer potential than ATP so it may be able to form ATP quickly and provide energy for muscular activity.

**Use of the composition**

In use, according to an embodiment, the compositions of the present invention may be used for favoring muscle growth and enhance muscular strength by supplementing the muscle nitric oxide level through transdermal administration. The composition of the present invention is applied to a desired area of the skin where the target muscle that are to be enhanced, and the compound of the present invention is capable of causing vasodilation and providing an increase in NO in the target muscle. Without wishing to be bound by theory, it is believe that the increase in NO enhances muscle growth and muscular strength through an enhancement of the myosin and actin binding. The long term enhancement of muscular strength is thought to be caused by an increase in the number and/or an increase recruitment of satellite cells to the target muscle, so that they differentiate and grow the muscle fiber. This may be caused by a lowering of the myostatin levels and/or an increase in the follistatin levels.

According to another embodiment, the composition of the present invention may be used for stimulation of vasodilation prior to physical exercise. The composition may be used, for example, prior to a workout at the gym, or before a training or game of any sports.
Use of the composition of the present invention is suitable to enhance most types of physical exercise, including but not limited to aerobic exercises, such as cycling, swimming, walking, skipping rope, rowing, running, hiking or playing tennis, and focus on increasing cardiovascular endurance. Also including but not limited to anaerobic exercises, such as weight training, functional training or sprinting, increase short-term muscle strength.

The increases blood flow generated by the compositions of the present invention is of interest to bodybuilders, as increased blood flow will serve to deliver more nutrients to muscles, thus helping muscles become larger when subject to training. The fact that NO acts to reduce inflammation should also make it of interest to bodybuilders as it has the potential to reduce the pain associated with subjecting muscles to training.

The composition of the present invention may thus be administered orally, transdermally, or in any suitable manner prior to physical exercise to maintain or enhance NO production and thereby stimulate vasodilation. Enhanced vasodilation may be found to be beneficiary for the enhancement of physical exercise performance.

According to another embodiment, the composition of the present invention may be used for treating muscular or neuromuscular diseases in a patient, by transdermally treating the patient with the composition according to an embodiment of the present invention to treat and/or alleviate the muscular or neuromuscular disease. Diseases such as muscular dystrophy may be treated. Non limiting example of muscular dystrophies include Becker's muscular dystrophy, congenital muscular dystrophy, Duchenne muscular dystrophy, distal muscular dystrophy, Emery-Dreifuss muscular dystrophy, facioscapulohumeral muscular dystrophy, limb-girdle muscular dystrophy, myotonic muscular dystrophy, oculopharyngeal muscular dystrophy, spinal muscular atrophy, Brown-Vialetto-Van Laere syndrome (BVVL), and Fazio-Londe (FL) syndrome.
[00295] Other types of muscular or neuromuscular diseases include but are not limited to muscular atrophy, which includes muscle atrophy associated with a cancer, AIDS, congestive heart failure, chronic obstructive pulmonary disease, renal failure, severe burns, and muscle atrophy associated with long (prolonged) bed rest. Also included in the muscular or neuromuscular diseases are Charcot-Marie-Tooth disease, Dejerine-Sottas disease, and Kennedy's disease.

[00296] The present invention will be more readily understood by referring to the following examples which are given to illustrate the invention rather than to limit its scope.

**EXAMPLE 1**

Transdermal gel with an emulsion base

[00297] The ingredient are mixed according to the provided order, and the compound of the present invention [(2-nitrooxy)-2-ethylamino-3-methylbutanoate] (also referred to as veen or valine ethyl ester nitrate) is provided as part of a liposomal formulation in liquid form, which provides a final concentration of the [(2-nitrooxy)-2-ethylamino-3-methylbutanoate (or valine ethyl ester nitrate) compound that is 20.2% by weight.
<table>
<thead>
<tr>
<th>%</th>
<th>Ingredient</th>
<th>mg/g</th>
<th>mg/1.5g</th>
<th>Order of mixing</th>
</tr>
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<tbody>
<tr>
<td>63.59</td>
<td>PEG 400</td>
<td>635.9</td>
<td>953.85</td>
<td>1</td>
</tr>
<tr>
<td>7.27</td>
<td>PEG 3350</td>
<td>72.7</td>
<td>109.05</td>
<td>2</td>
</tr>
<tr>
<td>0.98</td>
<td>Cetyl Alcohol</td>
<td>9.8</td>
<td>14.70</td>
<td>3</td>
</tr>
<tr>
<td>0.98</td>
<td>Stearyl Alcohol</td>
<td>9.8</td>
<td>14.70</td>
<td>4</td>
</tr>
<tr>
<td>0.98</td>
<td>Propylene Glycol</td>
<td>9.8</td>
<td>14.70</td>
<td>5</td>
</tr>
<tr>
<td>9.78</td>
<td>Isopropyl Myristate</td>
<td>97.8</td>
<td>146.70</td>
<td>6</td>
</tr>
<tr>
<td>2.64</td>
<td>Veen Liposomal Liquid*</td>
<td>26.4</td>
<td>39.60</td>
<td>7</td>
</tr>
<tr>
<td>0.98</td>
<td>HPMC E5</td>
<td>9.8</td>
<td>14.70</td>
<td>8</td>
</tr>
<tr>
<td>0.98</td>
<td>Laureth 23</td>
<td>9.8</td>
<td>14.70</td>
<td>9</td>
</tr>
<tr>
<td>0.66</td>
<td>Niacinamide</td>
<td>6.6</td>
<td>9.90</td>
<td>10</td>
</tr>
<tr>
<td>0.49</td>
<td>Lecithin</td>
<td>4.9</td>
<td>7.35</td>
<td>11</td>
</tr>
<tr>
<td>0.49</td>
<td>Polysorbate 80</td>
<td>4.9</td>
<td>7.35</td>
<td>12</td>
</tr>
<tr>
<td>0.20</td>
<td>Methyl Paraben</td>
<td>2</td>
<td>3.00</td>
<td>13</td>
</tr>
<tr>
<td>0.20</td>
<td>Sodium Benzoate</td>
<td>2</td>
<td>3.00</td>
<td>14</td>
</tr>
<tr>
<td>9.78</td>
<td>Tea Tree Oil</td>
<td>97.8</td>
<td>146.70</td>
<td>15</td>
</tr>
<tr>
<td>100.00</td>
<td></td>
<td>1000.0</td>
<td>1500.00</td>
<td></td>
</tr>
</tbody>
</table>

* 20.2% VEEN

[00299] While preferred embodiments have been described above and illustrated in the accompanying drawings, it will be evident to those skilled in the art that modifications may be made without departing from this disclosure. Such modifications are considered as possible variants comprised in the scope of the disclosure.
CLAIMS:

1. A transdermal composition comprising:
   - a therapeutically effective amount of a compound of formula (I):
     \[
     \begin{align*}
     &\text{O} \\
     &\text{CH} \\
     &\text{NH} \\
     &\text{R}_2
     \end{align*}
     \begin{align*}
     &\text{O} \\
     &\text{(CH}_2)_n\text{O} \text{NO}_2
     \end{align*}
     \]
     wherein \( n = 1 \) to 10;
     wherein \( R_1 \) is an amino acid side chain group (D or L configuration),
     wherein \( R_2 \) is a hydrogen atom, or an amino acid (D or L configuration) forming a peptide bond, or any pharmaceutically acceptable salts thereof; and
   - a skin penetration enhancer,

in association with a pharmaceutically acceptable topical carrier.

2. The composition as claimed in claim 1, wherein said compound of formula (I) is (2-nitrooxy)-2-ethylamino-3-methylbutanoate:

or any pharmaceutically acceptable salts thereof.
3. The composition as claimed in claim 1, wherein said compound of formula (I) is L-(2'-nitroxyethyl)-2-amino-3-methylbutanoate:

![Chemical Structure]

or any pharmaceutically acceptable salts thereof.

4. The composition as claimed in claim 1, wherein said compound of formula (I) is D-(2'-nitroxyethyl)-2-amino-3-methylbutanoate:

![Chemical Structure]

or any pharmaceutically acceptable salts thereof.

5. The composition as claimed in claim 1, wherein said compound of formula (I) is valine butylene glycol nitrate:

![Chemical Structure]

or any pharmaceutically acceptable salts thereof.

6. The composition as claimed in claim 1, wherein said compound of formula (I) is 2'-nitroxy ethyl 2-amino-pentanoate:

![Chemical Structure]

or any pharmaceutically acceptable salts thereof.

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7. The composition as claimed in claim 1, wherein said compound of formula (I) is L-(2'-nitrooxyethyl)-2-amino-pentanoate:

![Chemical Structure]

or any pharmaceutically acceptable salts thereof.

8. The composition as claimed in claim 1, wherein said compound of formula (I) is 2'-nitrooxy butyl 2-amino-pentanoate:

![Chemical Structure]

or any pharmaceutically acceptable salts thereof.

9. The composition as claimed in claim 1, wherein said R₂ is a hydrogen atom.

10. The composition as claimed in claim 1, wherein R₁ is chosen from:

![Chemical Structures]
wherein when $R_1$ is $\text{NH}_2$, or $\text{CH}_2\text{SeH}$, said $R_1$ is also linked to an $\text{NH}_2$ of said Formula (I) to form a proline or hydroxyproline amino acid side chain.

11. The composition as claimed in claim 1, wherein said $R_2$ is an amino acid of formula (II) (D or L configuration) and derivatives thereof, forming a peptide bond:

$$\text{H} - \text{N} - \text{C} - \text{C} - \text{O} - \text{H}$$

wherein $R_x$ is chosen from:
wherein when R₁ is \( \text{NH}_2 \), or \( \text{NH} \), said R₁ is also linked to an \( \text{NH}_2 \) of said Formula (I) to form a proline or hydroxyproline amino acid side chain.

12. The composition as claimed in any one of claims 1 - 11, wherein said compound is in a liposomal liquid.

13. The composition as claimed in claim 1, wherein said skin penetration enhancer is chosen from a C₈-C₂₂ fatty acid, a C₈-C₂₂ fatty alcohol, a
lower alkyl ester of a C₈-C₂₂ fatty acid, a di(lower)alkyl ester of C₆-C₂₂ diacid, a monoglyceride of C₈-C₂₂ fatty acid, tetrahydrofurfuryl alcohol polyethylene glycol ether, polyethylene glycol, propylene glycol, 2-(2-ethoxyethoxy)ethanol (transcutol), diethylene glycol monomethyl ether, diethylene glycol monoethyl ether, an alkylaryl ether of polyethylene oxide, a polyethylene oxide monomethyl ether, a polyethylene oxide dimethyl ether; dimethyl sulfoxide (DMSO), glycerol, ethyl acetate, acetoacetic ester, N-alkylpyrrolidone, a terpenes, dimethyl formamide (DMF), N,N-dimethylacetamide (DMA), methyl laurate, glycerol monolaurate, a fatty acid ester of a C₂ to C₄ alkanediol having a fatty acid portion of said ester from about 8 to 22 carbon atoms, a fatty alcohol ether of a C₂ to C₄ alkanediols having a fatty acid portion of said ether from about 8 to 22 carbon atoms, triglycerides of coconut oil, isopropyl palmitate, isopropyl myristate, laurocapram, and combinations thereof.

14. The composition of claim 13, wherein said skin penetration enhancer is propylene glycol.

15. The composition of claim 13, wherein said skin penetration enhancer is isopropyl myristate.

16. The composition as claimed in claim 13, wherein said C₆-C₂₂ fatty acid is chosen from isostearic acid, octanoic acid, and oleic acid.

17. The composition as claimed in claim 13, wherein said C₆-C₂₂ fatty alcohol is chosen from oleyl alcohol and lauryl alcohol.

18. The composition as claimed in claim 13, wherein said lower alkyl ester of a C₈-C₂₂ fatty acid is chosen from ethyl oleate, isopropyl myristate (IPM), butyl stearate, and methyl laurate.

19. The composition as claimed in claim 13, wherein said di(lower)alkyl esters of a C₆-C₂₂ diacid is diisopropyl adipate.
20. The composition as claimed in claim 13, wherein said monoglyceride of a C₈-C₂₂ fatty acid is glyceryl monolaurate.

21. The composition as claimed in any one of claim 1 - 20, wherein said pharmaceutically acceptable carrier is chosen from a water base or an oil base carrier.

22. The composition as claimed in any one of claims 1 - 21, further comprising a thickening agent.

23. The composition as claimed in claim 22, wherein said thickening agent is chosen from CARBOPOL®, carboxypolymethylene, carboxymethylcellulose Carbopol® Ultrace 10, Carbopol® 940, Carbopol® 941, Carbopol® 954, Carbopol® 980, Carbopol® 981, Carbopol® ETD 2001, Carbopol® EZ-2 and Carbopol® EZ-3.

24. The composition as claimed in any one of claims 1 - 23, further comprising a wetting agent.

25. The composition as claimed in claim 24, wherein said wetting agent is chosen from benzalkonium chloride, benzethonium chloride, cetlypyridinium chloride; dioctyl sodium sulfosuccinate; a polyoxyethylene alkylphenyl ether, a poloxamers, a polyoxyethylene fatty acid glyceride, a polyoxyethylene alkyl ethers, a polyoxyethylene fatty acid ester, a polyoxyethylene sorbitan ester, a propylene glycol fatty acid ester, sodium lauryl sulfate, sodium laureth sulfate oleic acid, sodium oleate, triethanolamine oleate, a glyceryl fatty acid ester, a sorbitan ester, sorbitan monooleate, sorbitan monopalmitate, sorbitan monostearate, tyloxapol and mixtures thereof.

26. The composition as claimed in claim 25, wherein said polyoxyethylene sorbitan ester is polysorbate 80.

27. The composition as claimed in any one of claims 1 - 25, further comprising a lubricant.
28. The composition as claimed in claim 27, wherein said lubricant is chosen from glyceryl behapate, stearic acid, magnesium stearate, calcium stearate, sodium stearate; a hydrogenated vegetable oil, colloidal silica, talc, a waxe, boric acid, sodium benzoate, sodium acetate, sodium fumarate, sodium chloride, DL-leucine, sodium oleate, sodium lauryl sulfate, sodium laureth sulfate, magnesium lauryl sulfate, glycerol, sorbitol, a water soluble cellulose, a polysorbate, a carbomer, a polyethylene glycol (PEG), a polyethylene, and a thickening agent.

29. The composition as claimed in claim 28, wherein said lubricant is sodium benzoate.

30. The composition as claimed in claim 28, wherein said water soluble cellulose is chosen from modified starch, methylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methocel® MC, carboxymethyl cellulose, ethyl cellulose, hydroxyl ethyl cellulose, and any combination thereof.

31. The composition of claim 30, wherein said hydroxypropyl methylcellulose is hydroxypropyl methylcellulose E5.

32. The composition as claimed in claim 28, wherein said polysorbate is chosen from polyoxyethylene (20) sorbitan monolaurate (polysorbate 20), polyoxyethylene (20) sorbitan monopalmitate (polysorbate 40), polyoxyethylene (20) sorbitan monostearate (polysorban 60), polyoxyethylene (20) sorbitan tristearate (polysorban 65), and polyoxyethylene (20) sorbitan monooleate (polysorban 80), and any combination thereof.

33. The composition as claimed in claim 28, wherein said carbomer is a carbopol® polymer chosen from carbopol® polymer 71G NF, carbopol® polymer 971P NF, carbopol® polymer 974P NF, carbopol® polymer 980 NF, carbopol® polymer 981 NF, carbopol® polymer 5984 EP and carbopol® polymer Ubtrez 10 NF, and any combination thereof.
34. The composition as claimed in claim 28, wherein said polyethylene glycol (PEG) is chosen from PEG 200, PEG 200E, PEG 300, PEG 300E, PEG 400, PEG 400E, PEG 600 and PEG 600E, and any combination thereof.

35. The composition as claimed in claim 34, wherein said polyethylene glycol (PEG) is PEG 400.

36. The composition as claimed in claim 34, wherein said polyethylene glycol (PEG) is PEG 3350.

37. The composition as claimed in claim 28, wherein said thickening agent is chosen from alginic acid, sodium alginate, potassium alginate, ammonium alginate, calcium alginate, agar, carrageenan, locust bean gum, xanthan gum, pectin, and gelatin, and any combination thereof.

38. The composition as claimed in any one of claims 1 - 37, further comprising at least one antiseptic agent.

39. The composition as claimed in claim 38, wherein said antiseptic agent is selected from chlorhexidine gluconate, glucono delta-lactone, a paraben compound, benzoic acid, imidazolidinyl urea, a quaternary ammonium compound, methyl hydroxybenzoate, propyl hydroxybenzoate, chlorocresol, benzalkonium chloride and Octenidine dihydrochloride.

40. The composition as claimed in claim 39, wherein said paraben is methyl paraben.

41. The composition as claimed in any one of claim 1 - 39, further comprising a preservative agent.

42. The composition as claimed in claim 41, wherein said preservative agent is chosen from EDTA, EGTA, hydroxytoluene butoxide, hydroxyanisol butoxide, sodium hydroxide, calcium propionate, sodium nitrate, sodium nitrite, sulfur dioxide, sodium bisulfite, and potassium hydrogen sulfite.
43. The composition as claimed in any one of claims 1 - 42, further comprising an emollient.

44. The composition as claimed in claim 43, wherein said emollient is chosen from mineral oil, a mixture of a mineral oil and a lanolin alcohol, cetyl alcohol, cetostearyl alcohol, petrolatum, petrolatum and a lanolin alcohol, cetyl esters wax, cholesterol, glycerin, glyceryl monostearate, isopropyl myristate (IPM), isopropyl palmitate, lecithin, allyl caproate, althea officinalis extract, arachidyl alcohol, argobase EUC, butylene glycol, dicaprylate/dicaprate, acacia, allantoin, carrageenan, cetyl dimethicone, cyclomethicone, diethyl succinate, dihydroabietyl behenate, dioctyl adipate, ethyl laurate, ethyl palmitate, ethyl stearate, isoamyl laurate, octanoate, PEG-75, lanolin, sorbitan laurate, walnut oil, wheat germ oil, super refined almond, super refined sesame, super refined soybean, octyl palmitate, caprylic/capric triglyceride, butyropermum parkii oil, olive oil, cetyl alcohol, behenyl alcohol, limnanthes alba seed oil, palmitic acid and glyceryl cocoate.

45. The composition as claimed in claim 44, wherein said emollient is cetyl alcohol.

46. The composition as claimed in claim 44, wherein said emollient is stearyl alcohol.

47. The composition as claimed in claim 44, wherein said emollient is niacinamide.

48. The composition as claimed in claim 44, wherein said emollient is lecithin.

49. The composition as claimed in any one of claims 1 - 48, further comprising an essential oil.

50. The composition as claimed in claim 49, wherein said essential oil is chosen from argan oil, cypress oil, chamomile oil, oil (bois de rose oil) lavender oil, tea tree oil, pine tree oil, eucalyptol oil, eucalyptus oil, birch oil, peppermint
oil, ylang-ylang oil, cymbopogon martinii oil (palmarosa oil), sweet almond oil and olive oil.

51. The composition as claimed in claim 49, wherein said essential oil is tea tree oil.

52. The composition as claimed in claim 1, further comprising phosphocreatine.

53. The composition as claimed in claim 1, further comprising magnesium chloride.

54. The composition as claimed in any one of claims 1 - 53, wherein said composition is in a transdermal patch.

55. The composition as claimed in any one of claims 1 - 54, further comprising a fragrance.

56. A method of treating a muscular or a neuromuscular disease in a patient which comprises:

   (a) transdermally treating said patient with the composition as claimed in any one of claims 1 - 34, to treat said muscular or neuromuscular disease.

57. The method as claimed in claim 56, wherein said disease is a muscular dystrophy.

58. The method as claimed in claim 57, wherein said muscular dystrophy is chosen from Becker's muscular dystrophy, congenital muscular dystrophy, Duchenne muscular dystrophy, distal muscular dystrophy, Emery-Dreifuss muscular dystrophy, facioscapulohumeral muscular dystrophy, limb-girdle muscular dystrophy, myotonic muscular dystrophy, oculopharyngeal muscular dystrophy, spinal muscular atrophy, Brown-Vialetto-Van Laere syndrome (BVVL), and Fazio-Londe (FL) syndrome.
59. The method as claimed in claim 56, wherein said disease is a muscular atrophy.

60. The method as claimed in claim 59, wherein said muscular atrophy is chosen from muscle atrophy associated with a cancer, muscle atrophy associated with AIDS, muscle atrophy associated with congestive heart failure, muscle atrophy associated with chronic obstructive pulmonary disease, muscle atrophy associated with renal failure, muscle atrophy associated with severe burns, and muscle atrophy associated with long bed rest.

61. The method as claimed in claim 56, wherein said disease is chosen from Charcot-Marie-Tooth disease, Dejerine-Sottas disease, and Kennedy's disease.

62. A method to cause an increase muscle growth and/or an increase in muscular strength in a subject which comprises:

(a) transdermally treating said subject with the composition as claimed in any one of claims 1 - 55, to increase said muscular strength.

63. The method according to claim 62, wherein said increase in muscle growth and/or an increase in muscular strength is through enhanced myosin and actin binding.

64. The method according to any one of claims 62 - 63, wherein said increase in muscle growth and/or an increase in muscular strength is a long term increase in muscular strength.

65. The method according to claim 64, wherein said long term increase in muscular strength is through a recruitment of at least one satellite cell.

66. The method according to any one of claims 64 - 65, wherein said long term increase in muscular strength is through a lowering of a myostatin level.

67. The method according to any one of claims 64 - 66, wherein said long term increase in muscular strength is through an increase in follistatin level.
68. Use of a compound of formula (I) for the preparation of a medicament for the treatment of a muscular and/or neuromuscular disease:

\[
\begin{array}{c}
\text{O} \\
\text{R}_1 \text{CH} \\
\text{NH} \\
\text{R}_2 \\
\end{array}
\quad \text{(CH}_2\text{)}_n \quad \text{ONO}_2
\]

wherein \( n = 1 \) to 10;

wherein \( R_1 \) is an amino acid side chain group (D or L configuration),

wherein \( R_2 \) is a hydrogen atom, or an amino acid (D or L configuration) forming a peptide bond, or any pharmaceutically acceptable salts thereof.

69. Use of a compound of formula (I) for the treatment of a muscular and/or neuromuscular disease:

\[
\begin{array}{c}
\text{O} \\
\text{R}_1 \text{CH} \\
\text{NH} \\
\text{R}_2 \\
\end{array}
\quad \text{(CH}_2\text{)}_n \quad \text{ONO}_2
\]

wherein \( n = 1 \) to 10;

wherein \( R_1 \) is an amino acid side chain group (D or L configuration),

wherein \( R_2 \) is a hydrogen atom, or an amino acid (D or L configuration) forming a peptide bond, or any pharmaceutically acceptable salts thereof.
70. Use of a compound of formula (I) for the preparation of a medicament for causing an increase in muscle growth and/or an increase in muscular strength:

\[
\text{R}_1 \quad \text{C} \quad \text{O} \quad \text{N} \quad \text{H} \quad \text{R}_2 \quad \text{(CH}_2\text{n)NO}_2
\]

(1)

wherein \( n = 1 \) to 10;

wherein \( R_1 \) is an amino acid side chain group (D or L configuration),

wherein \( R_2 \) is a hydrogen atom, or an amino acid (D or L configuration) forming a peptide bond, or any pharmaceutically acceptable salts thereof.

71. Use of a compound of formula (I) for causing an increase in muscle growth and/or an increase in muscular strength:

\[
\text{R}_1 \quad \text{C} \quad \text{O} \quad \text{N} \quad \text{H} \quad \text{R}_2 \quad \text{(CH}_2\text{n)NO}_2
\]

(1)

wherein \( n = 1 \) to 10;

wherein \( R_1 \) is an amino acid side chain group (D or L configuration),

wherein \( R_2 \) is a hydrogen atom, or an amino acid (D or L configuration) forming a peptide bond, or any pharmaceutically acceptable salts thereof.
72. Use of a compound of formula (I) for the preparation of a medicament for stimulation of vasodilation prior to physical exercise:

\[
\begin{align*}
\text{CH} & \text{O} \\
\text{NH} & \text{R}_1 \\
\text{R}_2 & \text{(I)}
\end{align*}
\]

wherein \( n = 1 \) to 10;
wherein \( R_1 \) is an amino acid side chain group (D or L configuration),
wherein \( R_2 \) is a hydrogen atom, or an amino acid (D or L configuration) forming a peptide bond,
or any pharmaceutically acceptable salts thereof.

73. Use of a compound of formula (II) for stimulation of vasodilation prior to physical exercise:

\[
\begin{align*}
\text{CH} & \text{O} \\
\text{NH} & \text{R}_1 \\
\text{R}_2 & \text{(II)}
\end{align*}
\]

wherein \( n = 1 \) to 10;
wherein \( R_1 \) is an amino acid side chain group (D or L configuration),
wherein \( R_2 \) is a hydrogen atom, or an amino acid (D or L configuration) forming a peptide bond,
or any pharmaceutically acceptable salts thereof.
74. The use as claimed in any one of claims 68 - 73, wherein said compound of formula (I) is (2-nitroxy)-2-ethylamino-3-methylbutanoate:

or any pharmaceutically acceptable salts thereof.

75. The use as claimed in any one of claims 68 - 73, wherein said compound of formula (I) is L-(2'-nitrooxyethyl)-2-amino-3-methylbutanoate:

or any pharmaceutically acceptable salts thereof.

76. The use as claimed in any one of claims 68 - 73, wherein said compound of formula (I) is D-(2'-nitrooxyethyl)-2-amino-3-methylbutanoate:

or any pharmaceutically acceptable salts thereof.

77. The use as claimed in any one of claims 68 - 73, wherein said compound of formula (I) is valine butylene glycol nitrate:

or any pharmaceutically acceptable salts thereof.
78. The use as claimed in any one of claims 68 - 73, wherein said compound of formula (I) is 2'-nitrooxy ethyl 2-amino-pentanoate:

![Chemical Structure Image]

or any pharmaceutically acceptable salts thereof.

79. The use as claimed in any one of claims 68 - 73, wherein said compound of formula (I) is L-(2'-nitrooxyethyl)-2-amino-pentanoate:

![Chemical Structure Image]

or any pharmaceutically acceptable salts thereof.

80. The use as claimed in any one of claims 68 - 73, wherein said compound of formula (I) is 2'-nitrooxy butyl 2-amino-pentanoate:

![Chemical Structure Image]

or any pharmaceutically acceptable salts thereof.

81. The use as claimed in any one of claims 68 - 73, wherein said $R_2$ is a hydrogen atom.

82. The use as claimed in any one of claims 68 - 73, wherein $R_1$ is chosen from:
wherein when $R_1$ is $\text{--CH}_2\text{--CH}_2\text{--CH}_2\text{--CH}_2\text{--}$, or $\text{--CH}_2\text{--CH}_2\text{--CH}_2\text{--}$, said $R_1$ is also linked to an $\text{NH}_2$ of said Formula (I) to form a proline or hydroxyproline amino acid side chain.

83. The use as claimed in any one of claims 68 - 73, wherein said $R_2$ is an amino acid of formula (II) (D or L configuration) and derivatives thereof, forming a peptide bond:
wherein $R_x$ is chosen from:

- $H$, $\text{CH}_3$
- $\text{CH}_2\text{CH}_3$
- $\text{CH}_3\text{CH}_3$
- $\text{CH}_3\text{CH}_2\text{CH}_3$
- $\text{CH}_2\text{CH}_2\text{CH}_3$
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- $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$
- $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$
- $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$
wherein when \( R_1 \) is \( \text{HO} \), or \( \text{NH}_2 \), said \( R_1 \) is also linked to an \( \text{NH}_2 \) of said Formula (I) to form a proline or hydroxyproline amino acid side chain.

84. The use as claimed in any one of claims 68 - 69, wherein said disease is a muscular dystrophy.

85. The use as claimed in any one of claims 68 - 69, wherein said muscular dystrophy is chosen from Becker's muscular dystrophy, congenital muscular dystrophy, Duchenne muscular dystrophy, distal muscular dystrophy, Emery-Dreifuss muscular dystrophy, facioscapulohumeral muscular dystrophy, limb-girdle muscular dystrophy, myotonic muscular dystrophy, oculopharyngeal muscular dystrophy, spinal muscular atrophy, Brown-Vialette-Van Laere syndrome (BVVL), and Fazio-Londe (FL) syndrome.

86. The use as claimed in any one of claims 68 - 69, wherein said disease is a muscular atrophy.

87. The use as claimed in any one of claims 68 - 69, wherein said muscular atrophy is chosen from muscle atrophy associated with a cancer, muscle atrophy associated with AIDS, muscle atrophy associated with congestive heart failure, muscle atrophy associated with chronic obstructive pulmonary disease, muscle atrophy associated with renal failure, muscle atrophy associated with severe burns, and muscle atrophy associated with long bed rest.

88. The use as claimed in any one of claims 68 - 69, wherein said disease is chosen from Charcot-Marie-Tooth disease, Dejerine-Sottas disease, and Kennedy's disease.

89. The use as claimed in any one of claims 70 - 71, wherein said increase in muscle growth and/or an increase in muscular strength is through enhanced myosin and actin binding.
90. The use as claimed in any one of claims 70 - 71, wherein increase in muscle growth and/or an increase in muscular strength is a long term increase in muscular strength.

91. The use as claimed in claim 90, wherein said long term increase in muscular strength is through a recruitment of at least one satellite cell.

92. The use as claimed in any one of claims 90 - 91, wherein said long term increase in muscular strength is through a lowering of a myostatin level.

93. The method according to any one of claims 90 - 92, wherein said long term increase in muscular strength is through an increase in follistatin level.

94. A method for stimulation of vasodilation prior to physical exercise in a subject which comprises:

(a) administering prior to physical exercise to said subject a compound of formula (I)

\[
R_1 \quad \text{CH} \quad \text{O} \quad \text{O} \quad (\text{CH}_2)_n \quad \text{ONOO}_2
\]

wherein \( n = 1 \) to 10;

wherein \( R_1 \) is an amino acid side chain group (D or L configuration),

wherein \( R_2 \) is a hydrogen atom, or an amino acid (D or L configuration) forming a peptide bond,

or any pharmaceutically acceptable salts thereof.
The method as claimed in claim 94, wherein said compound of formula (I) is (2-nitrooxy)-2-ethylamino-3-methylbutanoate:

![Chemical structure](image)
or any pharmaceutically acceptable salts thereof.

The method as claimed in claim 94, wherein said compound of formula (I) is L-(2'-nitrooxyethyl)-2-amino-3-methylbutanoate:

![Chemical structure](image)
or any pharmaceutically acceptable salts thereof.

The method as claimed in claim 94, wherein said compound of formula (I) is D-(2'-nitrooxyethyl)-2-amino-3-methylbutanoate:

![Chemical structure](image)
or any pharmaceutically acceptable salts thereof.

The method as claimed in claim 94, wherein said compound of formula (I) is valine butylene glycol nitrate:

![Chemical structure](image)
or any pharmaceutically acceptable salts thereof.
99. The method as claimed in claim 94, wherein said compound of formula (I) is 2'-nitrooxy ethyl 2-amino-pentanoate:

or any pharmaceutically acceptable salts thereof.

100. The method as claimed in claim 94, wherein said compound of formula (I) is L-(2'-nitrooxyethyl)-2-amino-pentanoate:

or any pharmaceutically acceptable salts thereof.

101. The method as claimed in claim 94, wherein said compound of formula (I) is 2'-nitrooxy butyl 2-amino-pentanoate:

or any pharmaceutically acceptable salts thereof.

102. The method as claimed in claim 94, wherein said $R_2$ is a hydrogen atom.

103. The method as claimed in any one of claims 94 - 102, wherein $R_1$ is chosen from:
wherein when R₁ is or , said R₁ is also linked to an NH₂ of said Formula (I) to form a proline or hydroxyproline amino acid side chain.

104. The method as claimed in claim 94, wherein said R₂ is an amino acid of formula (II) (D or L configuration) and derivatives thereof, forming a peptide bond:
wherein $R_x$ is chosen from:

- $H$
- $\text{CH}_3$
- $\text{CH}_3\text{CH}_3$
- $\text{CH}_3\text{CH}_2\text{CH}_3$
- $\text{C}_3\text{H}_2$
- $\text{C}_3\text{H}_2\text{OH}$
- $\text{C}_3\text{H}_2\text{SH}$
- $\text{C}_3\text{H}_2\text{COOH}$
- $\text{C}_2\text{C}_2\text{SH}$
- $\text{C}_2\text{C}_2\text{COOH}$
- $\text{H}_2\text{NCH}_2\text{NH}_2$
- $\text{H}_2\text{NCH}_2\text{OH}$
wherein when $R_1$ is $\text{HO-}$ and $\text{amine group}$, said $R_1$ is also linked to an $\text{NH}_2$ of said Formula (I) to form a proline or hydroxyproline amino acid side chain.
### INTERNATIONAL SEARCH REPORT

**International application No.**

PCT/CA2011/001419

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**A. CLASSIFICATION OF SUBJECT MATTER**

IPC: A61K 31/223 (2006.01) , A61P 21/00 (2006.01) , A61P 9/08 (2006.01)

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

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**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

* A61K 31/223 *

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

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Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

STN, EPOQUE (Epocod, Medline), Canadian Patent Database (amino acid ester, valine butylene glycol, methyl butanolate, amipopentanoate, muscular disorder/disease, neuromuscular disorder/disease, muscular dystrophy, muscular/muscle atrophy, nitrile oxide donor, NO donor, oxidative stress, muscle strength, muscle mass, lean body mass, vasodilation, Charcot-Marie-Tooth disease, Dejermine-Stottas disease Kennedy’s disease and related terms)

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**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<td>WO 2010/034118 A (FARBER, Michael) 1 April 2010 (01-04-2010) see entire document</td>
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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex

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**Date of the actual completion of the international search**

1 March 2012 (01-03-2012)

**Date of mailing of the international search report**

25 April 2012 (25-04-2012)

**Name and mailing address of the ISA/CA**

Canadian Intellectual Property Office

Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9

Facsimile No.: 001-819-953-2476

**Authorized officer**

Tania Nish (819) 934-3592

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Form PCT/ISA/210 (second sheet) (July 2009)
INTERNATIONAL SEARCH REPORT

Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claim Nos.: 56-67 and 94-104 because they relate to subject matter not required to be searched by this Authority, namely:

   Claims 56-67 and 94-104 is directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search (Rule 39.1, PCT). However, this Authority has carried out a search based on the use of the compounds of formula (I) as defined in claims 56-67 and 94-104.

2. [ ] Claim Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box.

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [X] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

   Remark on Protest [ ] The additional search fees were accompanied by the applicant’s protest and, where applicable, the payment of a protest fee.

   [ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

   [ ] No protest accompanied the payment of additional search fees.
Continued from Box III

The claims are directed to a plurality of inventive concepts as follows:

**Group A** - Claims 1-55 (in part), 56-71, 74-83 (in part) and 84-93 are directed to method or use of compounds of formula (I), preferably in transdermal compositions, for increasing muscular growth or strength and the treatment of muscular and neuromuscular diseases.

**Group B** - Claims 1-55 (in part), 72-75, 74-83 (in part) and 94-104 are directed to method or use of compounds of formula (I), preferably in transdermal compositions, for stimulation of vasodilation prior to physical exercise.

The claims must be limited to one inventive concept as set out in Rule 13 of the PCT.

An *a posteriori* analysis has concluded that the nitric oxide amino acid ester compounds of formula (I) are known and their role as NO donors (specifically WO 2010/034118). Therefore, there lacks unity of invention within the subject matter of the claims because the linking feature, the nitric oxide amino acid ester compounds of formula (I), is not inventive.
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<td>Y</td>
<td>SCIORATI, C. et al.: “Co-administration of ibuprofen and nitric oxide is an effective experimental therapy for muscular dystrophy, with immediate applicability to humans” British Journal of Pharmacology (2010), 160: 1550-1560 see entire document</td>
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