Title: TREATING PROTEIN MISFOLDING DISEASES

Abstract: This document provides methods and materials related to treating a protein misfolding disease. For example, methods and materials relating to the use of a lobeline compound to treat a protein misfolding disease are provided.
TREATING PROTEIN MISFOLDING DISEASES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit of priority from U.S. Provisional Application Serial No. 60/985,897, filed on November 6, 2007.

Statement as to Federally Sponsored Research

This invention was made with government support under NS44230 awarded by the National Institute of Neurological Disorders and Stroke. The government has certain rights in the invention.

BACKGROUND

Technical Field

This document relates to methods and materials involved in treating protein misfolding diseases. For example, this document relates to methods and materials involved in using lobeline (e.g., α-lobeline) and analogs thereof to treat a protein misfolding disease such as Krabbe disease.

Background Information

Globoid cell leukodystrophy (GLD), or Krabbe disease, is a lysosomal storage disorder with major neurological manifestations. It is a devastating, autosomal recessive disease that mainly affects infants (about 90 percent of all cases). Krabbe disease is caused by mutations in the galactocerebrosidase (GALC) gene that severely impair its function. In general, less than five percent of normal enzymatic activity is found in patients with GLD. The resulting accumulation of a GALC substrate, psychosine, causes extensive demyelination in both the central and peripheral nervous system.

SUMMARY

This document provides methods and materials related to treating protein misfolding diseases. For example, this document relates to the use lobeline (e.g., α-lobeline) and analogs thereof and pharmaceutical composition comprising them, to treat protein misfolding diseases, such as Krabbe disease. Research evidence suggests that disease-related mutations in polypeptides, which can be account for different
human congenital conditions, can cause misfolding of newly-synthesized polypeptides in the endoplasmic reticulum (ER). As a result, misfolded polypeptides can be degraded or accumulated in the ER. Recent studies demonstrate that low-dose treatment with reversible inhibitors can act as chaperones for some misfolded polypeptides, allowing the polypeptides to "escape" from degradation and to traffic to appropriate organelles. This discovery has revealed new treatment options for diseases that involve protein misfolding.

The methods and materials provided herein can allow clinicians to treat a mammal having a protein misfolding disease, thereby providing the mammal with a longer and healthier quality of life.

In general, one aspect of this document features a method for treating a mammal having a protein misfolding disease. The method comprises, or consists essentially of, administering a composition comprising a lobeline compound to the mammal under conditions wherein a symptom of the protein misfolding disease is reduced in severity or eliminated. The protein misfolding disease can be Krabbe disease. The mammal can be a human. The lobeline compound can be α-lobeline. The composition can comprise two or more lobeline compounds. The method can comprise identifying the mammal as having the protein misfolding disease before the administering step. The method can comprise monitoring the mammal for the reduction in severity or elimination of the symptom after the administering step.

In another aspect, this document features a method for treating a protein misfolding disease in a mammal in need thereof. The method comprises, or consists essentially of, administering to the mammal a therapeutically effective amount of a compound of Formula II:

\[ \text{Formula II} \]

or a pharmaceutically acceptable salt thereof, wherein:

- \( R^1 \) is hydrogen, -OR, -OC(O)N(R\( \text{OR}^{10} \)), -OC(O)OR, -OC(O)R, -OSO\( 2(R^{10}) \), -OSO\( 2N(R^{10}XR^{11}) \), or -OSO\( 2(OR^{10}) \); or \( R^1 \) and \( R^5 \) taken together form a bond;
- \( R^2 \) is hydrogen, -OR, -OC(O)N(R\( \text{OR}^{10} \)), -OC(O)OR, -OC(O)R, -OSO\( 2(R^{10}) \), -OSO\( 2N(R^{10}XR^{11}) \), or -OSO\( 2(OR^{10}) \); or \( R^2 \) and \( R^6 \) taken together form a bond;
R³ is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl; or R¹ and R³ taken together with the carbon to which they are attached form -(C=O)-; R⁴ is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl; or R² and R⁴ taken together with the carbon to which they are attached form -(C=O)-; or each of R⁴ and R⁵ independently for each occurrence is hydrogen; R⁷ is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl; each of R¹⁰ and R¹¹ independently for each occurrence is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl; or -

[C(R¹²)₂]m-R¹³; or R¹⁰ and R¹¹ taken together with the nitrogen to which they are bonded represent a 3-10 member optionally substituted heterocyclic ring; R¹² independently for each occurrence is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl; R¹³ independently for each occurrence is hydrogen -OR¹⁴, -N(R¹⁴)COR¹⁵, -N(R¹⁴)C(O)OR¹⁵, -N(R¹⁴)SO₂(R¹⁵), -CON(R¹⁴)(R¹⁵), -OC(O)N(R¹⁴XR¹⁵), -OC(O)OR¹⁴, -CO₂R¹⁴, -OC(O)R¹⁴, -C(O)N(O(R¹⁴)R¹⁵), -SO₂N(R¹⁴XR¹⁵), -OSO₂N(R¹⁴)(R¹⁵), -SO₂(R¹⁴), -OSO₂(R¹⁴), or -N(R¹⁴)S(O)₂OR¹⁵; and each of R¹⁴ and R¹⁵ independently for each occurrence is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl; or R¹⁴ and R¹⁵ taken together represent a 5-8 member optionally substituted heterocyclic ring. Each of R³ and R⁴ can be hydrogen. Each of R¹ and R², independently for each occurrence, can be hydrogen, -OR¹⁰, or -OSO₂(R¹⁰). R³ can be H, or R¹ and R³ taken together with the carbon to which they are attached can form -(C=O)-, and R² can be hydrogen; or R² and R³ taken together with the carbon to which they are attached can form -(C=O)-.

R⁷ can be alkyl. R¹ can be hydrogen, -OR¹⁰, -OC(O)OR¹⁰, -OC(O)R¹⁰, or -OSO₂(R¹⁰); or R¹ and R⁵ taken together can form a bond; R² can be hydrogen, -OR¹⁰, or -OSO₂(R¹⁰); or R² and R⁶ taken together can form a bond; R³ can be hydrogen; or R¹ and R³ taken together with the carbon to which they are attached can form -(C=O)-; R⁴ can be hydrogen; or R² and R⁴ taken together with the carbon to which they are attached can form -(C=O)-; or each of R⁴ and R⁵ independently for each occurrence can be hydrogen; R⁷ can be alkyl; and R¹⁰ can be hydrogen, alkyl, aryl, heteroaryl, or heteroaralkyl. The compound of Formula II can have the relative stereochemistry depicted in Formula III:
In another aspect, this document features a method for treating a protein misfolding disease in a mammal in need thereof. The method comprises, or consists essentially of, administering to the mammal a therapeutically effective amount of a compound selected from the group consisting of:

pharmaceutically acceptable salt thereof.

In another aspect, this document features a method for treating a protein misfolding disease in a mammal in need thereof. The method comprises, or consists essentially of, administering to the mammal a therapeutically effective amount of a compound of Formula IV:
or a pharmaceutically acceptable salt thereof.

In another aspect, this document features the use of a compound in the manufacture of a medicament for the treatment of a protein misfolding disease, wherein the compound has Formula II:

or a pharmaceutically acceptable salt thereof, wherein: \( R^1 \) is hydrogen, -OR\(^{10} \), -OC(O)N(R\(^{10}XR^{11}\)), -OC(O)OR\(^{10} \), -OC(O)R\(^{10} \), -OSO\(^2\)(R\(^{10} \)), -OSO\(^2\)N(R\(^{10}XR^{11}\)), or -OSO\(^2\)(OR\(^{10} \)); or \( R^1 \) and \( R^5 \) taken together form a bond; \( R^2 \) is hydrogen, -OR\(^{10} \), -OC(O)N(R\(^{10}XR^{11}\)), -OC(O)OR\(^{10} \), -OC(O)R\(^{10} \), -OSO\(^2\)(R\(^{10} \)), -OSO\(^2\)N(R\(^{10}XR^{11}\)), or -OSO\(^2\)(OR\(^{10} \)); or \( R^2 \) and \( R^6 \) taken together form a bond; \( R^3 \) is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl; or \( R^1 \) and \( R^3 \) taken together with the carbon to which they are attached form -(C=O)-; \( R^4 \) is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl; or \( R^2 \) and \( R^4 \) taken together with the carbon to which they are attached form -(C=O)-; or each of \( R^4 \) and \( R^5 \) independently for each occurrence is hydrogen; \( R^7 \) is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl; each of \( R^{10} \) and \( R^{11} \) independently for each occurrence is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or -[C(R\(^{12}\)]\(_m\)-R\(^{13}\); or \( R^{10} \) and \( R^{11} \) taken together with the nitrogen to which they are bonded represent a 3-10 member optionally substituted heterocyclic ring; \( R^{12} \) independently for each occurrence is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl; \( R^{13} \) independently for each occurrence is hydrogen -OR\(^{14} \), -N(R\(^{14}\))COR\(^{15} \), -N(R\(^{14}\))C(O)OR\(^{15} \), -N(R\(^{14}\))SO\(^2\)(R\(^{15}\)), -CON(R\(^{14}\))(R\(^{15}\)), -OC(O)N(R\(^{14}XR^{15}\)), -OC(O)OR\(^{14} \), -CO\(^2\)R\(^{14} \), -OC(O)R\(^{14} \), -C(O)N(R\(^{14}\))OR\(^{14} \)(R\(^{15}\)), -SO\(^2\)N(R\(^{14}XR^{15}\)), -OSO\(^2\)N(R\(^{14}XR^{15}\)), -SO\(^2\)(R\(^{14}\)), -OSO\(^2\)(R\(^{14}\)), -SO\(^2\)(OR\(^{14} \)), -}
OSO₂(OR¹⁴), or -N(R¹⁴)S(O)₂OR¹⁵; and each of R¹⁴ and R¹⁵ independently for each occurrence is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl; or R¹⁴ and R¹⁵ taken together represent a 5-8 member optionally substituted heterocyclic ring.

All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

**DESCRIPTION OF THE DRAWINGS**

Figure 1 is a diagram of a chemical reaction for detecting GALC polypeptide activity.

Figure 2 is a graph plotting recombinant GALC polypeptide activity in an *in vitro* assay containing the indicated amounts of αLB (µM) as a percentage of recombinant GALC polypeptide activity detected in a control reaction lacking αLB.

Figure 3 contains graphs plotting intracellular (lysate) and extracellular (medium) GALC activity in H4-hGALC cells expressing wild-type (WT) or mutant (I546T or D528N) GALC and treated with the indicated amount of αLB (µg/mL). The cells were treated at the indicated concentration of αLB in a humidified CO₂ incubator at 37°C for three days.

Figure 4 contains a graph plotting intracellular (lysate) GALC activity in H4-hGALC cells expressing mutant (I546T) GALC and treated with 20 µg/mL of αLB. Figure 4 also contains a Western blot analysis of GALC expression in cells treated with or without αLB.

Figure 5 contains a bar graph plotting GALC polypeptide activity detected in human skin fibroblasts obtained from Krabbe patients together with a Western blot analysis of GALC expression in the human skin fibroblasts. The GALC genotype and phenotype of each sample is set forth in Figure 6.

Figure 6 contains a table listing the GALC genotype and phenotype of the skin fibroblast samples used in Figure 5.

Figure 7 contains chemical structures for various lobeline compounds.
Figure 8 contains chemical structures for various lobeline compounds.

DETAILED DESCRIPTION

This document provides methods and materials related to treating a mammal having a protein misfolding disease. For example, this document provides methods and materials related to the use of lobeline (e.g., α-lobeline) and analogs thereof to treat a protein misfolding disease in a mammal. The methods and materials provided herein can be used to treat a protein misfolding disease in any type of mammal including, without limitation, mice, rats, dogs, cats, horses, cows, pigs, monkeys, and humans.

Any type of protein misfolding disease, such as Krabbe disease, other leukodystrophies, synucleinopathies, or tauopathies, can be treated using a lobeline compound. For example, symptomatic or asymptomatic Krabbe disease can be treated using a lobeline compound.

In general, a protein misfolding disease can be treated by administering a lobeline compound to a mammal having a protein misfolding disease. It will be appreciated that a single lobeline compound or a combination of lobeline compounds (e.g., two, three, four, five, or more lobeline compounds) can be used to treat a protein misfolding disease upon administration. For example, a mammal having a protein misfolding disease can be treated by administering a composition containing α-lobeline and lobelane to a mammal.

The compounds provided herein (e.g., compounds of Formula I-IV, and others as described herein) can be used to treat disease states or conditions related to protein misfolding, e.g., leukodystrophies, synucleinopathies, and tauopathies.

Examples of leukodystrophies include, but are not limited to metachromatic leukodystrophy, Krabbe disease, adrenoleukodystrophy, Pelizaeus-Merzbacher disease, Canavan disease, childhood ataxia with central nervous system hypomyelination (also known as vanishing white matter disease), Alexander disease, Refsum disease, and cerebrotendinous xanthomatosis.

Examples of tauopathies include, but are not limited to Alzheimer's disease, progressive supranuclear palsy, corticobasal degeneration, frontotemporal lobar degeneration (Pick's disease).

Examples of synucleinopathies include, but are not limited to Parkinson's disease, dementia with Lewy bodies, pure autonomic failure, and multiple system
atrophy.

Other protein misfolding diseases include, but are not limited to cystic fibrosis, marfan syndrome, Fabry disease, Gaucher's disease, retinitis pigmentosa 3, Alzheimer's disease, Type II diabetes, Parkinson's disease, spongiform encephalopathies such as Creutzfeldt-Jakob disease, primary systemic amyloidosis, secondary systemic amyloidosis, senile systemic amyloidosis, familial amyloid polyneuropathy I, hereditary cerebral amyloid angiopathy, hemodialysis-related amyloidosis, familial amyloid polyneuropathy III, Finnish hereditary systemic amyloidosis, medullary carcinoma of the thyroid, atrial amyloidosis, hereditary non-neuropathic systemic amyloidosis, injection-localized amyloidosis, and hereditary renal amyloidosis.

Any lobeline compound such as α-lobeline can be used to treat a protein misfolding disease. Examples of lobeline compounds that can be capable of treating a protein misfolding disease include, without limitation, α-lobeline, lobelane, lobelanide, those compounds set forth in Figure 7 or 8, and those compounds of Formula I-IV. In certain instances, the lobeline compound can be a compound of Formula I without regard to chirality:

\[
\text{I}
\]

where \( R^1 \) and \( R^2 \) each independently represents hydrogen, lower alkyl, lower alkenyl, lower alkylcarbonyl, arylcarbonyl (e.g., phenylcarbonyl), aralkylcarbonyl (e.g., alkylphenylcarbonyl), lower alkoxy carbonyl, lower alkyaminocarbonyl, higher alkylcarbonyl, poly(alkyleneoxide)carbonyl, or lower haloalkyl; \( R^3 \) and \( R^4 \) each independently represents hydrogen or is absent, (in which case, \( R^1 \) and/or \( R^2 \) is absent respectively) and a C=O is present; and X represents hydrogen or lower alkyl. Whenever a carbonyl-containing substituent is provided as \( R^1 \) or \( R^2 \), it is understood that the carbonyl group is covalently bonded to the respective O atom appearing in Formula I. Thus, in the instances where the substituent is an alkoxy carbonyl or alkyaminocarbonyl, a carbonate or carbamate linkage is present in the molecule.

In some cases, \( R^1 \) and \( R^2 \) can include methylcarbonyl (acetyl), phenylcarbonyl...
(benzoyl), natural fatty acid groups (e.g., palmitoyl, oleyl, linoleyl, stearyl, and lauryl), and polyethyleneglycol (PEG) covalently bonded to the molecule via a carbonate linkage.

The definition of each expression, e.g., alkyl, m, n, and the like, when it occurs more than once in any structure, is intended to be independent of its definition elsewhere in the same structure.

As used herein, the term "alkyl" includes saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups.

In certain embodiments, a straight chain or branched chain alkyl has about 30 or fewer carbon atoms in its backbone (e.g., C1-C30 for straight chain, C1-C30 for branched chain), and alternatively, about 20 or fewer. Likewise, cycloalkyls have from about 3 to about 10 carbon atoms in their ring structure, and alternatively about 5, 6 or 7 carbons in the ring structure. As used herein, the terms "lower alkyl," "lower alkenyl," "lower alkoxy," and the like, refer to normal, branched, and cyclic hydrocarbyl groups containing one to six carbon atoms. The term "higher alkyl" includes alkyl groups containing seven to about 20 carbon atoms.

The term "aryl" includes 5-, 6- and 7-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, naphthalene, anthracene, pyrene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles" or "heteroaromatics." The aromatic ring may be substituted at one or more ring positions with such substituents as described above, for example, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, aldehyde, ester, heterocyclyl, aromatic or heteroaromatic moieties, -CF3, -CN, or the like. The term "aryl" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings (the rings are "fused rings") wherein at least one of the rings is aromatic, e.g., the other cyclic rings may be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls. One example of an aryl is estradiol and derivatives thereof. The term "aralkyl" refers to an aryl group covalently bonded to an alkyl group.
The terms "heterocyclyl", "heteroaryl", or "heterocyclic group" include 3- to 10-membered ring structures, alternatively 3- to 7-membered rings, whose ring structures include one to four heteroatoms. Heterocycles may also be polycycles. Heterocyclyl groups include, for example, thiophene, thianthrene, furan, pyran, iso benzofuran, chromene, xanthene, phenoxanthenes, pyrrole, imidazole, pyrazole, isothiazole, isoxazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthyridine, quinoxaline, quinoxoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, pyrimidine, phenanthroline, phenazine, phenarsazine, phenothiazine, furazan, phenoxazine, pyrrolidinone, oxolane, thiolane, oxazole, piperidine, piperazine, morpholine, lactones, lactams such as azetidinones and pyrrolidinones, sultams, sultones, and the like. The heterocyclic ring may be substituted at one or more positions with such substituents as described above, as for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, -CF3, -CN, or the like.

The term "optionally substituted" refers to any chemical group, such as alkyl, cycloalkyl aryl, and the like, wherein one or more hydrogen may be replaced with a substituent as described herein, for example, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, aldehyde, ester, heterocyclyl, aromatic or heteroaromatic moieties, -CF3, -CN, or the like; or has the formula -[(CRSoRSi)n]RS₂, wherein each of RS₀ and RS₁ independently for each occurrence is hydrogen, alkyl, aralkyl, cycloalkyl, or aryl; RS₂ is hydrogen, amino, acylamino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, aldehyde, ester; and n is an integer selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In some cases, a lobeline compound provided herein can be converted into a different molecule upon metabolism in a mammal. For example, an acetyl group that is present at R¹ and/or R² of a lobeline compound administered to a mammal can be removed by metabolic processes such as those that occur in the gastrointestinal tract or the liver.
In some cases, a lobeline compound can have the structure of Formula I where (a) both $R^3$ and $R^4$ are hydrogen, (b) either $R^3$ or $R^4$ is hydrogen and the other is absent (as is either $R^1$ or $R^2$) in which case a C=O is present in the appropriate location, or (c) both $R^3$ and $R^4$ are absent as are both $R^1$ and $R^2$ in which case two C=O are present in those locations. In such cases, $X$ can be a methyl group. In some cases, the chirality at the 2 and 6 positions of the piperidyl ring of a lobeline compound can be the same as in naturally occurring $\alpha$-lobeline.

In some cases, $R^1$ and $R^2$ can include methylcarbonyl (acyetyl), phenylcarbonyl (benzoyl), natural fatty acid groups (e.g., palmitoyl, oleyl, linoleyl, stearyl, and lauryl), and polyethyleneglycol (PEG).

In certain instances the lobeline compound can be a compound of Formula II:

![Formula II](image)

or a pharmaceutically acceptable salt thereof, where

- $R^1$ can be hydrogen, -OR$^{10}$, -OC(O)N(R$^{10}$X$^{11}$), -OC(O)OR$^{10}$, -OC(O)R$^{10}$, -OSO$_2$(R$^{10}$), -OSO$_2$N(R$^{10}$X$^{11}$), or -OSO$_2$(OR$^{10}$); or $R^1$ and $R^5$ taken together can form a bond;
- $R^2$ can be hydrogen, -OR$^{10}$, -OC(O)N(R$^{10}$)(R$^{10}$)$^{R^2}$, -OC(O)OR$^{10}$, -OC(O)R$^{10}$, -OSO$_2$(R$^{10}$), -OSO$_2$N(R$^{10}$X$^{11}$), or -OSO$_2$(OR$^{10}$); or $R^2$ and $R^6$ taken together can form a bond;
- $R^3$ can be hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl; or $R^1$ and $R^3$ taken together with the carbon to which they are attached can form -(C=O)-;
- $R^4$ can be hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl; or $R^2$ and $R^4$ taken together with the carbon to which they are attached can form -(C=O)-; or each of $R^4$ and $R^5$ independently for each occurrence can be hydrogen or as described above;
- $R^7$ can be hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl;
- each of $R^{10}$ and $R^{11}$ independently for each occurrence can be hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl,
or \([C(R^{15})_2]m-R^{13}\); or \(R^{10}\) and \(R^{11}\) taken together with the nitrogen to which they are bonded represent a 3-10 member optionally substituted heterocyclic ring;

\(R^{15}\) independently for each occurrence can be hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl;

\(R^{10}\) independently for each occurrence can be hydrogen \(-\text{OR}^{14}\), \(-\text{N}(\text{R}^{14})\text{COR}^{15}\), \(-\text{N}(\text{R}^{14})\text{C}(\text{O})\text{OR}^{15}\), \(-\text{N}(\text{R}^{14})\text{SO}_2(\text{R}^{15})\), \(-\text{CON}(\text{R}^{14})(\text{R}^{15})\), \(-\text{OC}(\text{O})\text{N}(\text{R}^{14})(\text{R}^{15})\), \(-\text{OC}(\text{O})\text{OR}^{14}\), \(-\text{CO}_2(\text{R}^{14})\), \(-\text{OC}(\text{O})\text{R}^{14}\), \(-\text{C}(\text{O})\text{N}(\text{OR}^{14})(\text{R}^{15})\), \(-\text{SO}_2\text{N}(\text{R}^{14})(\text{R}^{15})\), \(-\text{OSO}_2\text{N}(\text{R}^{14}\text{XR}^{15})\), \(-\text{SO}_2(\text{R}^{14})\), \(-\text{OSO}_2(\text{R}^{14})\), \(-\text{SO}_2(\text{OR}^{14})\), \(-\text{OSO}_2(\text{OR}^{14})\), or \(-\text{N}(\text{R}^{14})\text{S}(\text{O})_2\text{OR}^{15}\); and

5 each of \(\text{R}^{14}\) and \(\text{R}^{15}\) independently for each occurrence can be hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl; or \(\text{R}^{14}\) and \(\text{R}^{15}\) taken together represent a 5-8 member optionally substituted heterocyclic ring.

In certain instances, each of \(\text{R}^{3}\) and \(\text{R}^{4}\) can be hydrogen; and/or each of \(\text{R}^{1}\) and \(\text{R}^{2}\) independently for each occurrence can be hydrogen, \(-\text{OR}^{10}\), or \(-\text{OSO}_2\text{OR}^{10}\).

In certain instances, \(\text{R}^{3}\) can be \(\text{H}\), or \(\text{R}^{1}\) and \(\text{R}^{3}\) taken together with the carbon to which they are attached can form \(-\text{(C=O)}\)-, and \(\text{R}^{2}\) can be hydrogen; or \(\text{R}^{2}\) and \(\text{R}^{3}\) taken together with the carbon to which they are attached can form \(-\text{(C=O)}\)-.

In certain instances, \(\text{R}^{7}\) can be alkyl.

In certain instances, \(\text{R}^{1}\) can be hydrogen, \(-\text{OR}^{10}\), \(-\text{OC}(\text{O})\text{OR}^{10}\), \(-\text{OC}(\text{O})\text{R}^{10}\), or \(-\text{OSO}_2(\text{R}^{10})\); or \(\text{R}^{1}\) and \(\text{R}^{5}\) taken together can form a bond; \(\text{R}^{2}\) can be hydrogen, \(-\text{OR}^{10}\), or \(-\text{OSO}_2(\text{R}^{10})\); or \(\text{R}^{2}\) and \(\text{R}^{6}\) taken together can form a bond; \(\text{R}^{3}\) can be hydrogen; or \(\text{R}^{1}\) and \(\text{R}^{3}\) taken together with the carbon to which they are attached can form \(-\text{(C=O)}\)-; \(\text{R}^{4}\) can be hydrogen; or \(\text{R}^{2}\) and \(\text{R}^{4}\) taken together with the carbon to which they are attached can form \(-\text{(C=O)}\)-; or each of \(\text{R}^{3}\) and \(\text{R}^{5}\) independently for each occurrence can be hydrogen; \(\text{R}^{7}\) is alkyl; and \(\text{R}^{10}\) is hydrogen, alkyl, aryl, heteroaryl, or heteroaralkyl.

In certain instances, the lobeline compound can have the relative stereochemistry depicted in Formula III:

![Formula III](image)

Also provided, is a method for treating a protein misfolding disease in a
mammal in need thereof, comprising administering to said mammal a therapeutically effective amount of a compound selected from the group consisting of:

![Chemical structures](image)

or a pharmaceutically acceptable salt thereof.

Also provided, is a method for treating a protein misfolding disease in a mammal in need thereof, comprising administering to said mammal a therapeutically effective amount of a compound of Formula IV:

![Chemical structure of Formula IV](image)

or a pharmaceutically acceptable thereof.

Certain compounds provided herein may exist in particular geometric or stereoisomeric forms. All such compounds, including cis- and trans-isomers, R- and S-enantiomers, diastereomers, (D)-isomers, (L)-isomers, the racemic mixtures thereof, and other mixtures thereof, fall within the scope of this document. Additional
asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are included in this document.

In some instances, the lobeline compound described herein can have basic functionality. Such basic functionality can exist in free base form or as salt. In some instances, salts of the lobeline compounds described herein can include hydrochloride, hydrobromide, nitrate, sulfate, tartrate, fumarate, citrate, maleate, ascorbate, lactate, aspartate, mesylate, benzene sulfonate, propionate, or succinate salts. In some cases, an anionic moiety such as a fatty acid salt (e.g., palmitate salt) can be used.

The term "pharmaceutically-acceptable salts" in this respect, refers to the relatively non-toxic, inorganic and organic acid addition salts of the compounds provided herein. These salts can be prepared in situ in the administration vehicle or the dosage form manufacturing process, or by separately reacting a purified compound are provided herein in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed during subsequent purification. Representative pharmaceutically acceptable salts include the hydrobromide, hydrochloride, sulfate, bisulfate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like. (See, for example, Berge et al. (1977) "Pharmaceutical Salts", J. Pharm. Sci. 66:1-19)

The pharmaceutically acceptable salts of the compounds described herein include the conventional nontoxic salts or quaternary ammonium salts of the compounds, e.g., from non-toxic organic or inorganic acids. For example, such conventional nontoxic salts include those derived from inorganic acids such as hydrochloride, hydrobromic, sulfuric, sulfamic, phosphoric, nitric, and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, palmitic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isothionic, and the like.

In some cases, the compounds provided herein can contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically-acceptable salts with pharmaceutically-acceptable bases. These salts can likewise be prepared in situ in the administration vehicle or the dosage form manufacturing process, or by separately reacting the purified compound in its free acid form with a suitable base,
such as the hydroxide, carbonate or bicarbonate of a pharmaceutically-acceptable metal cation, with ammonia, or with a pharmaceutically-acceptable organic primary, secondary or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like.

Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like. (See, for example, Berge et al., supra)

Any appropriate method can be used to obtain a lobeline compound. For example, α-lobeline can be chemically synthesized or isolated from a natural source (e.g., Lobelia inflate).

The lobeline compounds described herein can be synthesized using numerous methodologies well known in the chemical arts. The secondary hydroxyl of lobeline (Figure 7, Compound 1) can be modified, e.g., by alkylation or acylation, to yield compounds of Formula II where R₁ is -OR₁⁰, -OC(O)(R₁⁰)(R₂⁰), -OC(O)OR₁⁰, or -OC(O)R₁⁰. The hydroxyl group can also be oxidized, e.g., by swern oxidation, TEMPO/NMO, etc, to yield compounds of Formula II where R₁ and R₃ taken together can form -(C=O)- (see, e.g., Figure 7, compound 5). The hydroxyl group may also be eliminated, e.g., by acid catalyzed elimination or by first forming a leaving group (e.g., tosylate, bromide, chloride, etc) and reacting the resulting analog with a base, to yield compounds where R₁ and R⁵ form a bond (see, e.g., Figure 7, step a). The resulting double bond can be reduced, e.g., using hydrogen/palladium, to yield, for example, lobeline compounds where R₁, R₃, and R⁵ are hydrogen (see, e.g., Figure 7, step c).

Lobeline analogs wherein groups R₂, R₄, and R⁶ are modified can be accessed via a number of synthetic routes. In certain instance, the free hydroxyl group of lobeline is suitably protected and the ketone is then reduced (see, e.g., Figure 7, step b). If a particular diastereomer is desired, it can either be separated by purification or can be produced using an asymmetric reduction. The resulting alcohol can then be modified as described above to yield the desired analog of lobeline.

In addition to the reactions described above, synthetic chemists can use a broad array of chemical transformations to access other lobeline analogs of interest. Representative examples include palladium coupling reactions to alkenylhalides or aryl halides, oxidations, reductions, reactions with nucleophiles, reactions with
electrophiles, pericyclic reactions, installation of protecting groups, removal of protecting groups, and the like.

If, for instance, a particular enantiomer of a compound provided herein is desired, it may be prepared by asymmetric synthesis, or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. In instances where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts are formed with an appropriate optically-active acid or base, followed by resolution of the diastereomers thus formed by fractional crystallization or chromatographic means well known in the art, and subsequent recovery of the pure enantiomers.

Also provided are pharmaceutically acceptable compositions which comprise a therapeutically effective amount of one or more of the compounds described herein, formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents. The pharmaceutical compositions of the present disclosure may be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; and (2) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, e.g., those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue.

Pharmaceutically acceptable carriers, adjuvants and vehicles that may be used in the pharmaceutical compositions described herein include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

If required, the solubility and bioavailability of the compounds described herein in pharmaceutical compositions may be enhanced by methods well-known in

Another known method of enhancing bioavailability is the use of an amorphous form of a compound described herein optionally formulated with a poloxamer, such as LUTROL™ and PLURONIC™ (BASF Corporation), or block copolymers of ethylene oxide and propylene oxide. See United States patent 7,014,866; and United States patent publications 20060094744 and 20060079502.

The pharmaceutical compositions described herein include those suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. In certain embodiments, the compound of the formulae herein is administered transdermally (e.g., using a transdermal patch or iontophoretic techniques). Other formulations may conveniently be presented in unit dosage form, e.g., tablets, sustained release capsules, and in liposomes, and may be prepared by any methods well known in the art of pharmacy. See, for example, Remington's Pharmaceutical Sciences, Mack Publishing Company, Philadelphia, PA (17th ed. 1985).

Such preparative methods include the step of bringing into association with the molecule to be administered ingredients such as the carrier that constitutes one or more accessory ingredients. In certain instances, the compositions are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers, liposomes or finely divided solid carriers, or both, and then, if necessary, shaping the product.

In certain embodiments, the compounds are administered orally. The compositions described herein suitable for oral administration may be presented as discrete units such as capsules, sachets, or tablets each containing a predetermined amount of the active ingredient; a powder or granules; a solution or a suspension in an aqueous liquid or a non-aqueous liquid; an oil-in-water liquid emulsion; a water-in-oil liquid emulsion; packed in liposomes; or as a bolus, etc. Soft gelatin capsules can be useful for containing such suspensions, which may beneficially increase the rate of compound absorption.
In the case of tablets for oral use, carriers that are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried cornstarch. When aqueous suspensions are administered orally, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

Compositions suitable for oral administration include lozenges comprising the ingredients in a flavored basis, usually sucrose and acacia or tragacanth; and pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia.

Compositions suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in a freeze dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets.

Such injection solutions may be in the form, for example, of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents (such as, for example, Tween 80) and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are mannitol, water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium.

For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant.
The pharmaceutical compositions described herein may be administered in the form of suppositories for rectal administration. These compositions can be prepared by mixing a compound described herein with a suitable non-irritating excipient which is solid at room temperature but liquid at the rectal temperature and therefore will melt in the rectum to release the active components. Such materials include, but are not limited to, cocoa butter, beeswax and polyethylene glycols.

The pharmaceutical compositions described herein can be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art. See, e.g.: Rabinowitz JD and Zaffaroni AC, US Patent 6,803,031, assigned to Alexza Molecular Delivery Corporation.

Topical administration of the pharmaceutical compositions described herein are useful when the desired treatment involves areas or organs readily accessible by topical application. For topical application topically to the skin, the pharmaceutical composition can be formulated with a suitable ointment containing the active components suspended or dissolved in a carrier. Carriers for topical administration of the compounds described herein include, but are not limited to, mineral oil, liquid petroleum, white petroleum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax, and water. In certain instances, the pharmaceutical composition can be formulated with a suitable lotion or cream containing the active compound suspended or dissolved in a carrier. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldecanol, benzyl alcohol, and water. The pharmaceutical compositions described herein may also be topically applied to the lower intestinal tract by rectal suppository formulation or in a suitable enema formulation.

Application of the subject therapeutics may be local, so as to be administered at the site of interest. Various techniques can be used for providing the subject compositions at the site of interest, such as injection, use of catheters, trocars, projectiles, pluronic gel, stents, sustained drug release polymers or other device which provides for internal access.

A composition containing a lobeline compound as described herein can be in any appropriate form. For example, a composition described herein can be in the
form of a solution or powder with or without a diluent to make an injectable
suspension. A composition also can contain additional ingredients including, without
limitation, pharmaceutically acceptable vehicles, methyl cellulose, ethanol, various
oils such as peanut oil, and dimethyl sulfoxide. A pharmaceutically acceptable
vehicle can be, for example, saline, water, lactic acid, and mannitol.

The compounds described herein can be administered to a mammalian subject
either alone or in combination with pharmaceutically acceptable carriers or diluents in
a pharmaceutical composition according to standard pharmaceutical practice. In
certain instances, the compounds or pharmaceutical compositions can be administered
topically, orally, or parenterally. Parenteral administration includes intravenous,
imtramuscular, intraperitoneal, intrathecal, subcutaneous and transdermal.

In some cases, a combination of lobeline compounds can be administered by
different routes. For example, one lobeline compound can be administered orally and
a second lobeline compound can be administered via injection.

In some cases, provided is a method for treating a protein misfolding disease,
comprising contacting a cell with a lobeline compound as provided herein. In certain
instances, the contacting occurs in vivo or in vitro.

In some cases, provided is a method for treating a protein misfolding disease,
comprising contacting a misfolded protein with a lobeline compound. In certain
instances, the contacting occurs in vivo or in vitro, e.g., in a mammal. In certain
instances, the misfolded protein is a GALT protein.

Before administering a lobeline compound to a mammal, the mammal can be
assessed to determine whether or not the mammal has a protein misfolding disease.
Any appropriate method can be used to determine whether or not a mammal has a
protein misfolding disease. For example, a mammal (e.g., human) can be identified as
having a protein misfolding disease using standard diagnostic techniques such as
enzymatic assays. In some cases, a diagnostic GALT enzyme assay can be used to
determine whether or not a mammal has a protein misfolding disease.

After identifying a mammal as having a protein misfolding disease, the
mammal can be administered a composition containing a lobeline compound. A
composition containing a lobeline compound can be administered to a mammal in any
amount, at any frequency, and for any duration effective to achieve a desired outcome
(e.g., to reduce a symptom of a protein misfolding disease). In some cases, a
composition containing a lobeline compound can be administered to a mammal.
having a protein misfolding disease to reduce or eliminate a symptom of a protein misfolding disease 5, 10, 25, 50, 75, 80, 85, 90, 95, or 100 percent.

Effective doses will also vary, as recognized by those skilled in the art, depending on the diseases treated, the severity of the disease, the route of administration, the sex, age and general health condition of the subject, excipient usage, the possibility of co-usage with other therapeutic treatments such as use of other agents and the judgment of the treating physician.

An effective amount of a composition containing a lobeline compound can be any amount that reduces the severity of a symptom of a protein misfolding disease without producing significant toxicity to the mammal. For example, an effective amount of a lobeline compound can be from about 0.05 mg/kg to about 100 mg/kg (e.g., from about 0.1 mg/kg to about 50 mg/kg, from about 0.2 mg/kg to about 25 mg/kg, or from about 0.5 mg/kg to about 10 mg/kg). Typically, an effective amount of a lobeline compound such as α-lobeline can be from about 0.01 mg/kg to about 10 mg/kg. If a particular mammal fails to respond to a particular amount, then the amount of lobeline compound can be increased by, for example, two fold. After receiving this higher concentration, the mammal can be monitored for both responsiveness to the treatment and toxicity symptoms, and adjustments made accordingly. The effective amount can remain constant or can be adjusted as a sliding scale or variable dose depending on the mammal’s response to treatment. Various factors can influence the actual effective amount used for a particular application. For example, the frequency of administration, duration of treatment, use of multiple treatment agents, route of administration, and severity of the protein misfolding disease may require an increase or decrease in the actual effective amount administered.

The frequency of administration can be any frequency that reduces the severity of a symptom of a protein misfolding disease without producing significant toxicity to the mammal. For example, the frequency of administration can be from about once a week to about three times a day, or from about twice a month to about six times a day, or from about twice a week to about once a day. The frequency of administration can remain constant or can be variable during the duration of treatment. A course of treatment with a composition containing a lobeline compound can include rest periods. For example, a composition containing a lobeline compound can be administered daily over a two week period followed by a two week rest period,
and such a regimen can be repeated multiple times. As with the effective amount, various factors can influence the actual frequency of administration used for a particular application. For example, the effective amount, duration of treatment, use of multiple treatment agents, route of administration, and severity of the protein misfolding disease may require an increase or decrease in administration frequency.

An effective duration for administering a composition containing a lobeline compound can be any duration that reduces the severity of a symptom of a protein misfolding disease without producing significant toxicity to the mammal. Thus, the effective duration can vary from several days to several weeks, months, or years. In general, the effective duration for the treatment of a protein misfolding disease can range in duration from several weeks to several months. In some cases, an effective duration can be for as long as an individual mammal is alive. Multiple factors can influence the actual effective duration used for a particular treatment. For example, an effective duration can vary with the frequency of administration, effective amount, use of multiple treatment agents, route of administration, and severity of the protein misfolding disease.

In certain instances, a course of treatment and the severity of one or more symptoms related to the protein misfolding disease can be monitored. Any method can be used to determine whether or not the severity of a symptom of a protein misfolding disease is reduced. For example, the severity of a symptom of a protein misfolding disease can be assessed by determining GALC activity levels at different time points. The levels of GALC activity determined within tissue at different times can be compared to determine the level of increase of GALC activity following treatment.

The invention will be further described in the following examples, which do not limit the scope of the invention described herein.

EXEMPLARY

Example 1 - Identifying α-lobeline as a GALC inhibitor

Some human mutations cause protein misfolding of GALC. Expression of the D528N or the L629R GALC mutant in different mammalian cells lines, including COS-I, HEK 293, and H4, results in rapid turnover of the GALC precursor, protein misprocessing, and reduction in GALC activity. GALC activity from skin fibroblasts of Krabbe disease patients was equal to baseline levels when compared to control
cells (Figure 5, upper panel and Figure 6). In addition, Western blot analysis using anti-GALC antibodies revealed lower levels of GALC polypeptide in human skin fibroblasts from Krabbe patients as compared to the levels detected in control cells (Figure 5, lower panel and Figure 6). Expression of the 28 kDa GALC fragment in all of the mutant fibroblasts isolated from Krabbe disease patients was reduced, suggesting that the lack of GALC polypeptide processing is a characteristic molecular phenotype of the disease.

To identify potential chemical chaperones for stabilizing GALC carrying different disease mutations, an in vitro colorimetric GALC substrate turnover assay in 96-well plate format was developed. The GALC substrate turnover assay was adapted from an assay described elsewhere (Gal et al., Clinica Chimica Acta, 77:53-59 (1977)). Briefly, 150 ng of recombinant GALC polypeptide, 50 µg of total protein from cell lysate, or 17.5 µL of cell medium were mixed with citrate reaction buffer, taurocholate-oleic acid solution, and substrate, 2-hexadecanoylamino-4-nitrophenyl-b-D-galactopyranoside (FING), for 1 to 4 hours at 37°C (Figure 1). The reaction was stopped, developed with glycine/sodium hydroxide solution and ethanol, and the absorbance (410 nm) measured. Upon screening over 2500 candidates from several commercially available chemical libraries, α-lobelene was identified as an inhibitor of GALC. α-lobelene (αLB) is a main alkaloid found in the plant Lobelia inflate L. αLB down-regulated the activity of recombinant GALC by approximately 20 percent at 24 µM and reached about 32 percent reduction in activity when the concentration was increased to 300 µM (Figure 2).

Two GLD model cell lines carrying disease-related mutations, H4-hGALC (I546T) and H4-hGALC (D528N), exhibited GALC activity at a level that is only about 10 percent of the GALC activity exhibited from control cells (H4-hGALC WT). Treating H4 glioma cells engineered to over-express wild-type or mutant GALC polypeptides with αLB resulted in an increase in GALC activity (Figure 3). The rate of induction of GALC activity for the WT, I546T, and D528N cells were about 10, 20, and 27 percent, respectively, when the cells were treated with αLB at 20 µg/mL or 60 µM in culture media for three days (Figure 3, left panels and Figure 4). In addition, secretion of GALC polypeptide from the cells to culture media was also increased dose-dependently in all three cell lines (Figure 3; right panels). A corresponding increase in the 28 kDa processed fragment of GALC was detected in cell lysates treated with αLB (Figure 4).
The results described herein demonstrate that αLB is an in vitro GALC inhibitor that can be used to increase both intracellular and secretory GALC activity in cells. The detected αLB-induced increases in GALC activity in cells is similar to increases in polypeptide activity observed with other chemical chaperones. For example, the αLB-mediated induction of GALC activity in the I546T mutant corresponds with an increase in processing of the precursor into the 28 kDa GALC fragment as detected using a monoclonal GALC antibody. The results described herein also demonstrate that αLB can be used to treat protein misfolding disease such as Krabbe disease.

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.
WHAT IS CLAIMED IS:

1. A method for treating a mammal having a protein misfolding disease, wherein said method comprises administering a composition comprising a lobeline compound to said mammal under conditions wherein a symptom of said protein misfolding disease is reduced in severity or eliminated.

2. The method of claim 1, wherein said protein misfolding disease is Krabbe disease.

3. The method of claim 1, wherein said mammal is a human.

4. The method of claim 1, wherein said lobeline compound is α-lobeline.

5. The method of claim 1, wherein said composition comprises two or more lobeline compounds.

6. The method of claim 1, wherein said method comprises identifying said mammal as having said protein misfolding disease before said administering step.

7. The method of claim 1, wherein said method comprises monitoring said mammal for said reduction in severity or elimination of said symptom after said administering step.

8. A method for treating a protein misfolding disease in a mammal in need thereof, comprising administering to said mammal a therapeutically effective amount of a compound of Formula II:

   ![Chemical Structure](image)

   II

   or a pharmaceutically acceptable salt thereof, wherein:

   R^1 is hydrogen, -OR^{10}, -OC(O)N(R^{10}XR^{11}), -OC(O)OR^{10}, -OC(O)R^{10}, -OSO_2(R^{10}), -OSO_2N(R^{10}XR^{11}), or -OSO_2(OR^{10}); or R^1 and R^5 taken together form a bond;
R² is hydrogen, -OR¹₀, -OC(O)N(R¹⁰X²¹), -OC(O)OR¹⁰, -OC(O)R¹⁰, -OSO₂(R¹⁰), -OSO₂N(R¹⁰X²¹), or -OSO₂(OR¹⁰); or R² and R⁶ taken together form a bond; R³ is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl; or R¹ and R³ taken together with the carbon to which they are attached form -(C=O)-; R⁴ is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl; or R² and R⁴ taken together with the carbon to which they are attached form -(C=O)-; each of R⁴ and R⁵ independently for each occurrence is hydrogen; R⁷ is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl; each of R¹⁰ and R¹¹ independently for each occurrence is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or -[C(R¹²)]m-R¹³; or R¹⁰ and R¹¹ taken together with the nitrogen to which they are bonded represent a 3-10 member optionally substituted heterocyclic ring; R¹² independently for each occurrence is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl; R¹³ independently for each occurrence is hydrogen -OR¹⁴, -N(R¹⁴)COR¹⁵, -N(R¹⁴)C(O)OR¹⁵, -N(R¹⁴)SO₂(R¹⁵), -CON(R¹⁴)(R¹⁵), -OC(O)N(R¹⁴)(R¹⁵), -OC(O)OR¹⁴, -CO₂R¹⁴, -OC(O)R¹⁴, -C(O)N(OR¹⁴)(R¹⁵), -SO₂N(R¹⁴)(R¹⁵), -OSO₂N(R¹⁴X²¹), -SO₂(R¹⁴), -SO₂(OR¹⁴), -SO₂(OR¹⁴), or -N(R¹⁴)S(O)₂OR¹⁵; and each of R¹⁴ and R¹⁵ independently for each occurrence is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl; or R¹⁴ and R¹⁵ taken together represent a 5-8 member optionally substituted heterocyclic ring.

9. The method of claim 8, wherein each of R³ and R⁴ is hydrogen.

10. The method of claim 8, wherein each of R¹ and R², independently for each occurrence, is hydrogen, -OR¹⁰, or -OSO₂(OR¹⁰).

11. The method of claim 8, wherein R³ is H, or R¹ and R³ taken together with the carbon to which they are attached form -(C=O)-, and R² is hydrogen; or R² and R³
taken together with the carbon to which they are attached form -(C=O)-.

12. The method of any of claims 8-11, wherein R^7 is alkyl.

13. The method of claim 8, wherein R^1 is hydrogen, -OR, -OC(O)OR, -OC(O)R, or -OSO_2(R); or R^1 and R^5 taken together form a bond; R^2 is hydrogen, -OR, or -OSO_2(R); or R^2 and R^6 taken together form a bond; R^3 is hydrogen; or R^1 and R^3 taken together with the carbon to which they are attached form -(C=O)-; R^4 is hydrogen; or R^2 and R^4 taken together with the carbon to which they are attached form -(C=O)-; or each of R^4 and R^5 independently for each occurrence is hydrogen; R^7 is alkyl; and R^10 is hydrogen, alkyl, aryl, heteroaryl, or heteroaralkyl.

14. The method of claim 13, wherein said compound of Formula II has the relative stereochemistry depicted in Formula III:

III.

15. A method for treating a protein misfolding disease in a mammal in need thereof, comprising administering to said mammal a therapeutically effective amount of a compound selected from the group consisting of:
16. A method for treating a protein misfolding disease in a mammal in need thereof, comprising administering to said mammal a therapeutically effective amount of a compound of Formula IV:

![Chemical Structure](image)

or a pharmaceutically acceptable salt thereof.

17. Use of a compound in the manufacture of a medicament for the treatment of a protein misfolding disease, wherein said compound has Formula II:

![Chemical Structure](image)

or a pharmaceutically acceptable salt thereof, wherein:

- $R^1$ is hydrogen, -OR$^{10}$, -OC(O)N(R$^{10}$X$^{11}$), -OC(O)OR$^{10}$, -OC(O)R$^{10}$, -OSO$_2$(R$^{10}$), -OSO$_2$N(R$^{10}$X$^{11}$), or -OSO$_2$(OR$^{10}$); or $R^1$ and $R^5$ taken together form a bond;

- $R^2$ is hydrogen, -OR$^{10}$, -OC(O)N(R$^{10}$)(R$^{\pi}$), -OC(O)OR$^{10}$, -OC(O)R$^{10}$, -OSO$_2$(R$^{10}$), -OSO$_2$N(R$^{10}$)(R$^{11}$), or -OSO$_2$(OR$^{10}$); or $R^2$ and $R^6$ taken together form a bond;
R³ is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl; or R¹ and R³ taken together with the carbon to which they are attached form -(C=O)-;

R⁴ is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl; or R² and R⁴ taken together with the carbon to which they are attached form -(C=O)-; or each of R⁴ and R⁵ independently for each occurrence is hydrogen;

R⁷ is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl;

each of R¹⁰ and R¹¹ independently for each occurrence is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl; or C(R₁²)₂m-R¹³; or R¹⁰ and R¹¹ taken together with the nitrogen to which they are bonded represent a 3-10 member optionally substituted heterocyclic ring;

R¹² independently for each occurrence is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl;

R¹³ independently for each occurrence is hydrogen -OR¹⁴, -N(R¹⁴)COR¹⁵, -N(R¹⁴)C(O)OR¹⁵, -N(R¹⁴)SO₂(R¹⁵), -CON(R¹⁴)(R¹⁵), -OC(O)N(R¹⁴)(R¹⁵), -OC(O)OR¹⁴, -CO₂R¹⁴, -OC(O)R¹⁴, -C(O)N(OR¹⁴)(R¹⁵), -SO₂N(R¹⁴)(R¹⁵), -OSO₂N(R¹⁴XR¹⁵), -SO₂(R¹⁴), -OSO₂(R¹⁴), -SO₂(OR¹⁴), -OSO₂(OR¹⁴), or -N(R¹⁴)S(O)₂OR¹⁵; and each of R¹⁴ and R¹⁵ independently for each occurrence is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, aralkyl, heteroaryl, or heteroaralkyl; or R¹⁴ and R¹⁵ taken together represent a 5-8 member optionally substituted heterocyclic ring.
**GALC Substrate**

\[ ^* \text{HNG (2-hexadecanoylamino-4-nitrophenyl-\(\beta\)-D-galactopyranoside)} \]

\[ \text{GALC} \rightarrow \text{Product (yellow)} \]

\[ \text{galactose} \]

*Figure 1*
Figure 2

- Bar graph showing the percentage of control (% Control) against concentration (µM) from 0 to 600 µM.

- The graph indicates a decrease in % Control with increasing concentration, suggesting a concentration-dependent effect.
Figure 3
The H4-hGALC (I546T) mutant was treated with αLB (20 µg/ml) for 3 days.

A 20% increase in GALC activity was detected in the cell lysates.

A corresponding increase in the 28 kDa processed fragment of GALC was also noted in the cell lysates treated with αLB.

Figure 4
GALC activity from Krabbe skin fibroblasts are equal to baseline detection levels, when compared with control cells (# 14)

Western blot analysis with anti-GALC monospecific antibody

A reduction in the amount of the 28 kDa processed fragment of GALC was observed, when compared with control cells (# 14)

Figure 5
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<th>Label</th>
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<td>Galactosialidosis</td>
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</table>

Figure 6
Figure 7
Lobeline

Ketocalkene

10R-MESP

10S/10R-MEPP

Lobelanidine

Lobelanine

MTD

(-)-TTD

Lobelane

Lobeline Tosylate

Figure 8