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(54) Titre : COMPOSITIONS ET METHODES DE TRAITEMENT DE TROUBLES NEURODEGENERATIFS,
MYODEGENERATIFS ET DU STOCKAGE LYSOSOMAL
(54) Title: COMPOSITIONS AND METHODS FOR TREATING NEURODEGENERATIVE, MYODEGENERATIVE, AND
LYSOSOMAL STORAGE DISORDERS

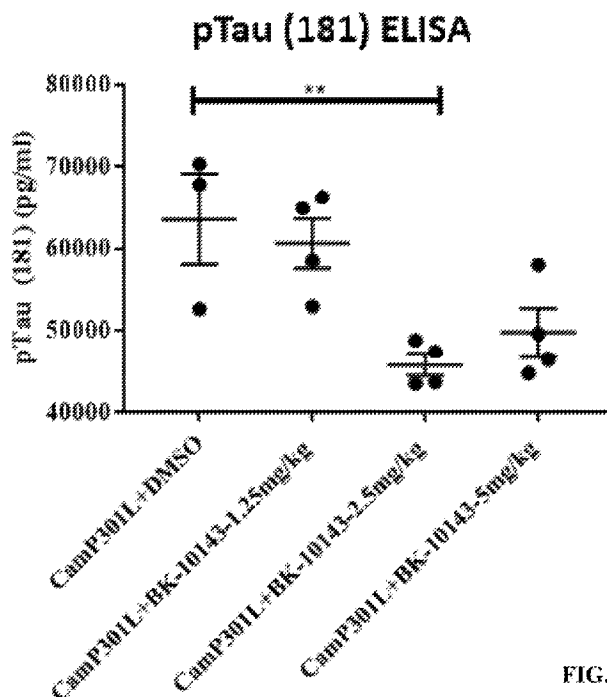


FIG. 6

(57) Abrégé/Abstract:

Provided herein are compositions and methods for treating or preventing a neurodegenerative disease, a myodegenerative disease, a prion disease or a lysosomal storage disease in a subject.

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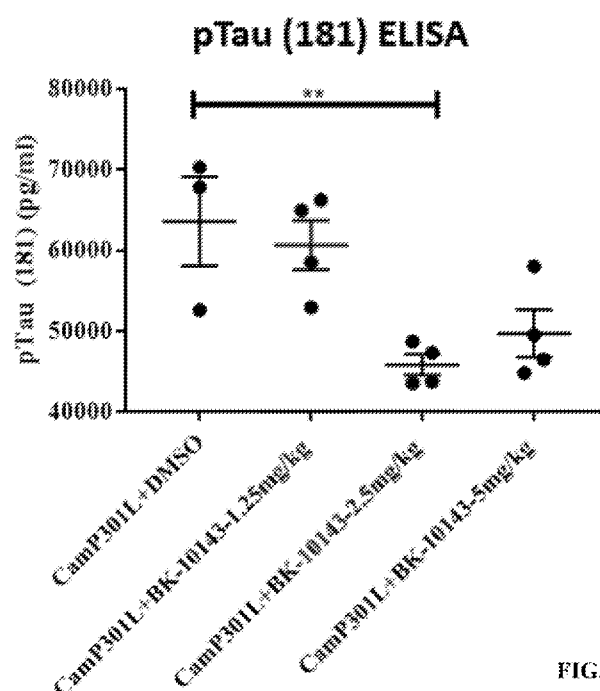


FIG. 6

(57) Abstract: Provided herein are compositions and methods for treating or preventing a neurodegenerative disease, a myodegenerative disease, a prion disease or a lysosomal storage disease in a subject.

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WO 2020/106825 A1

WO 2020/106825 A1

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- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
- *of inventorship (Rule 4.17(iv))*

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COMPOSITIONS AND METHODS FOR TREATING NEURODEGENERATIVE, MYODEGENERATIVE, AND LYSOSOMAL STORAGE DISORDERS

This application claims priority to U.S. Provisional Application No. 62/769,791, filed November 20, 2018, which is hereby incorporated in its entirety by this reference.

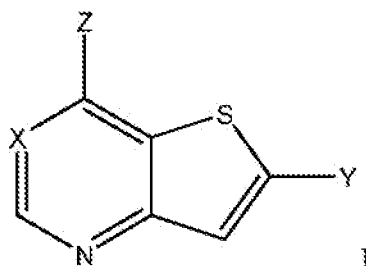
BACKGROUND

Neurodegenerative diseases include genetic and sporadic disorders associated with progressive nervous system dysfunction. These diseases are characterized by progressive deterioration of nerve cells or nerve cell function. It has been estimated that one of four Americans will develop a neurodegenerative condition in their lifetimes. Generally, however, the underlying mechanisms causing the conditions are not well understood and few effective treatment options are available for preventing or treating neurodegenerative diseases.

Lysosomal storage disorders represent some of the most devastating of genetic diseases, and the need to develop therapies for these disorders remains largely unmet. Many of these diseases cause damage to the central nervous system (CNS), but the mechanisms underlying such damage are largely unknown. Although the incidence of lysosomal storage disorders is rare (less than about 1:100,000 individuals is affected), lysosomal storage disorders affect mostly children who often die at a young age, many within a few months or years of birth. Many other children die following years of suffering from various symptoms of their particular lysosomal storage disorder.

SUMMARY

Provided herein are compositions and methods for treating or preventing a neurodegenerative disease, a myodegenerative disease, a prion disease or a lysosomal storage disease in a subject. Provided herein are compounds having Formula I



wherein

X is N or CH;

Y is C₆₋₁₀ aryl unsubstituted or substituted with R¹; or C₅₋₁₀ heteroaryl unsubstituted or substituted with R¹, or N-methylpiperazinyl;

R¹ is $-(CH_2)_n-R^2$, $-(CH_2)_n-C(O)-R^2$, or $-O(CH_2)_n-R^2$;

R² is -H, -CN, halogen, C₁₋₃ alkyl, C₁₋₃ alkoxy, phenyl, pyridinyl, amino, C₁₋₃ alkyl amino, di C₁₋₃ alkyl amino, hydroxyl C₁₋₃ alkyl amino, carboxy C₁₋₃ alkyl amino, C₃₋₆ cycloalkyl C₁₋₃ alkylamino, pyrrolidinyl, hydroxyl pyrrolidinyl, hydroxyl C₁₋₃ alkylpyrrolidinyl, carboxypyrrolidinyl, piperidinyl, C₁₋₃ alkylpiperidinyl, di C₁₋₃ alkyl piperidinyl, piperazinyl, C₁₋₃ alkylpiperazinyl, C₁₋₄ alkoxy carbonylpiperazinyl, or morpholinyl;

Z is heteroaryl, heterocyclyl, or NR³R⁴;

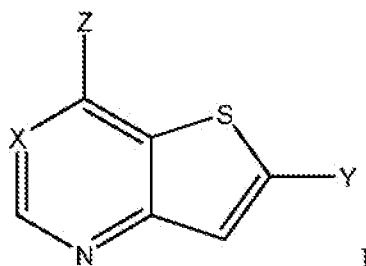
R³ and R⁴ are independently H, C₁₋₃ alkyl, C₁₋₃ alkoxy, or unsubstituted phenyl,

and

n is an integer selected from 0 to 3,

or an isomer or pharmaceutically acceptable salt thereof.

Also provided is a method of treating or preventing a neurodegenerative disease, a myodegenerative disease or a prion disease in a subject, comprising administering to the subject with the neurodegenerative disease, the myodegenerative disease, or the prion disease or at risk for developing the neurodegenerative disease, the myodegenerative disease or the prion disease an effective amount of a compound having Formula I



wherein

X is N or CH;

Y is C₆₋₁₀ aryl unsubstituted or substituted with R¹; or C₅₋₁₀ heteroaryl unsubstituted or substituted with R¹, or N-methylpiperazinyl;

R¹ is $-(CH_2)_n-R^2$, $-(CH_2)_n-C(O)-R^2$, or $-O(CH_2)_n-R^2$;

R² is -H, -CN, halogen, C₁₋₃ alkyl, C₁₋₃ alkoxy, phenyl, pyridinyl, amino, C₁₋₃ alkyl amino, di C₁₋₃ alkyl amino, hydroxyl C₁₋₃ alkyl amino, carboxy C₁₋₃ alkyl amino, C₃₋₆ cycloalkyl C₁₋₃ alkylamino, pyrrolidinyl, hydroxyl pyrrolidinyl, hydroxyl C₁₋₃ alkylpyrrolidinyl, carboxypyrrolidinyl, piperidinyl, C₁₋₃ alkylpiperidinyl, di C₁₋₃ alkyl piperidinyl, piperazinyl, C₁₋₃ alkylpiperazinyl, C₁₋₄ alkoxy carbonylpiperazinyl, or morpholinyl;

Z is heteroaryl, heterocyclyl, or NR³R⁴;

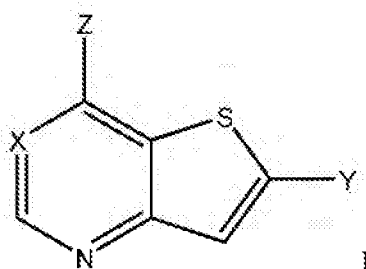
R³ and R⁴ are independently H, C₁₋₃ alkyl, C₁₋₃ alkoxy, or unsubstituted phenyl,

and

n is an integer selected from 0 to 3,

or an isomer or pharmaceutically acceptable salt thereof.

Also provided are methods of inhibiting or preventing toxic protein aggregation in a neuron. The methods comprise contacting the neuron with an effective amount of a compound having Formula I:



wherein,

X is N or CH;

Y is C₆₋₁₀ aryl unsubstituted or substituted with R¹; or C₅₋₁₀ heteroaryl unsubstituted or substituted with R¹, or N-methylpiperazinyl;

R¹ is -(CH₂)_n-R², -(CH₂)_n-C(O)-R², or -O(CH₂)_n-R²;

R² is -H, -CN, halogen, C₁₋₃ alkyl, C₁₋₃ alkoxy, phenyl, pyridinyl, amino, C₁₋₃ alkyl amino, di C₁₋₃ alkyl amino, hydroxyl C₁₋₃ alkyl amino, carboxy C₁₋₃ alkyl amino, C₃₋₆ cycloalkyl C₁₋₃ alkylamino, pyrrolidinyl, hydroxyl pyrrolidinyl, hydroxyl C₁₋₃ alkylpyrrolidinyl, carboxypyrrolidinyl, piperidinyl, C₁₋₃ alkylpiperidinyl, di C₁₋₃ alkyl piperidinyl, piperazinyl, C₁₋₃ alkylpiperazinyl, C₁₋₄ alkoxy carbonylpiperazinyl, or morpholinyl;

Z is heteroaryl, heterocyclyl, or NR³R⁴;

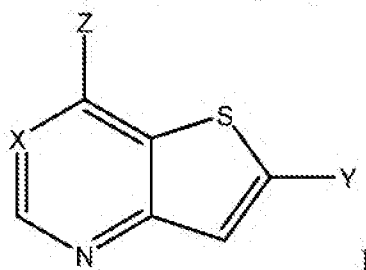
R³ and R⁴ are independently H, C₁₋₃ alkyl, C₁₋₃ alkoxy, or unsubstituted phenyl,

and

n is an integer selected from 0 to 3,

or an isomer or pharmaceutically acceptable salt thereof.

Also provided are methods of treating or preventing a lysosomal storage disorder (LSD) in a subject. The methods comprise administering to the subject having the LSD or at risk of developing the LSD an effective amount of a compound having Formula I:



wherein,

X is N or CH;

Y is C₆₋₁₀ aryl unsubstituted or substituted with R¹; or C₅₋₁₀ heteroaryl unsubstituted or substituted with R¹, or N-methylpiperazinyl;

R^1 is $-(CH_2)_n-R^2$, $-(CH_2)_n-C(O)-R^2$, or $-O(CH_2)_n-R^2$;

R^2 is $-H$, $-CN$, halogen, C_{1-3} alkyl, C_{1-3} alkoxy, phenyl, pyridinyl, amino, C_{1-3} alkyl amino, di C_{1-3} alkyl amino, hydroxyl C_{1-3} alkyl amino, carboxy C_{1-3} alkyl amino, C_{3-6} cycloalkyl C_{1-3} alkylamino, pyrrolidinyl, hydroxyl pyrrolidinyl, hydroxyl C_{1-3} alkylpyrrolidinyl, carboxypyrrolidinyl, piperidinyl, C_{1-3} alkylpiperidinyl, di C_{1-3} alkyl piperidinyl, piperazinyl, C_{1-3} alkylpiperazinyl, C_{1-4} alkoxy carbonylpiperazinyl, or morpholinyl;

Z is heteroaryl, heterocyclyl, or NR^3R^4 ;

R^3 and R^4 are independently H , C_{1-3} alkyl, C_{1-3} alkoxy, or unsubstituted phenyl, and

n is an integer selected from 0 to 3,

or an isomer or pharmaceutically acceptable salt thereof.

DESCRIPTION OF THE FIGURES

The present application includes the following figures. The figures are intended to illustrate certain embodiments and/or features of the compositions and methods, and to supplement any description(s) of the compositions and methods. The figures do not limit the scope of the compositions and methods, unless the written description expressly indicates that such is the case.

Fig. 1 (left panel and middle panel) shows that, after 16 hours of treatment, neuroprotective effects were observed in B35 cells treated with of 1 μM BK41043, as evidenced by the decrease in lactate dehydrogenase (LDH) and the increase in (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl- tetrazolium bromide (MTT), respectively, as compared to control. Fig. 1 (right panel) shows that, after five hours of treatment, there was a stepwise increase in cell viability via decrease in LDH with decreasing concentrations of BK40143.

Fig. 2 shows cell viability of B35 cells after 24 hour transfection with pTau (left panel) or α -synuclein (right panel) and five hours of treatment with BK41043. Dehydrogenase (LDH) measured in the media, reflecting cell death, is not different between control and cells expressing tau or α -synuclein, with or without BK40143, indicating that this compound does not result in toxicity or cell death.

Fig. 3 shows the level of pTau (181) after 24 hour transfection (left panel, students t-test: unpaired, two-tailed, $*p<0.05$, $***p<0.01$) and that BK41043 reduces pTau (181) levels in pTau transfected B35 cells (right panel, $n=6$, ANOVA: ordinary, one-way, Dunnett's multiple comparisons test, $p<0.05$, $****p<0.0001$).

Fig. 4 shows the level of α -synuclein after 24 hour transfection (left panel), and that BK41043 reduces α -synuclein in α -synuclein-transfected B35 cells (right panel). Students t-test: unpaired, two-tailed, $*p<0.05$, $**p<0.01$, $n=6$). Doses of BK40143 ranging from 10uM-1mM appear to reduce the level of alpha-synuclein in this cell culture model.

Fig. 5 shows cell viability of B35 cells after five hours of treatment with BK40197.

Fig. 6 shows that BK40143 reduces pTau (181) in Tau expressing transgenic mice, after seven days of treatment. The levels of pTau(181) were measured via ELISA. Students t-test: unpaired, two-tailed, Welch's correction, $*p<0.05$, $***p<0.01$.

Fig. 7 shows that treatment with BK-40143 (1.25 mg/kg, 2.5 mg/kg or 5.0 mg/kg) did not affect the levels of pTau (231) (AT180) in rTG4510 transgenic mice ($n=4$), using Western blot analysis.

Fig. 8 shows that Tau (HT7) was significantly reduced in Tau transgenic mice after seven days of treatment with BK-41043, at 1.25 mg/kg and 2.5 mg/kg. Students t-test: unpaired, two-tailed, Welch's correction, $*p<0.05$, $***p<0.01$, $n=4$.

Fig. 9 shows that treatment with BK-40143 (1.25 mg/kg or 2.5 mg/kg) results in *in vivo* inhibition of DDR1, as measured by detection of phosphorylated (active) DDR1 (pMCK10). This data indicate that BK40143 potently inhibits DDR *in vivo*.

Fig. 10 shows that pTau (191) was reduced in Camp301L mice after treatment with 2.5 mg/kg or 5 mg/kg BK-40143. Student's t-test: unpaired, Welch's correction, 1 tailed, $*p>0.05$, $p=0.02$, $n=4$.

Fig. 11A shows that 1mM and 100uM of BK40196 significantly reduced the level of alpha-synuclein in transfected B35 cells.

Fig. 11B shows that BK40197 does not significantly reduce alpha-synuclein levels in transfected B35 cells.

Figs. 12A-D shows that BK40143 significantly reduces alpha-synuclein in A53T mice. Male and female 12-month old A53T mice were treated i.p. with 2.5mg/kg of BK40143 for 21 consecutive days. Fig. 12A is an ELISA for human alpha-synuclein show a significant 39%

reduction in the level of alpha-synuclein in BK40143 treated animals compared to DMSO treated control A53T mice. C57BL/6J mice were used as controls and show no detectable (N.D.) human alpha-synuclein. Fig. 12B shows that BK40143 increases the overall level of dopamine (30%). BK40143 did increase the level of the dopamine metabolite, homovanillic acid (HVA), in A53T mice indicating more dopamine turnover which may result in better dopamine neurotransmission. Figs. 12C and 12D are immunoblots for alpha-synuclein which mirrored the 40% reduction in alpha-synuclein seen in the ELISA.

Fig. 13 shows that BK40143 improves velocity of locomotion in A53T mice. A53T mice were tested on the open field test for overall locomotor ability over a 60-minute trial. Although the mice did not show any difference in the total distance travelled or total time spent moving, the velocity of their movements significantly increased with BK40143 treatment.

Figs. 14A-E show that BK40143 selectively deactivates DDRs (about 50%) but not Src or Abl and reduces phosphorylated tau in the rTG4510 tauopathy mouse model. Male and female, 3-month-old rTG4530 mice were treated i.p. with 1.25mg/kg, 2.5mg/kg, or 5mg/kg of BK40143 or DMSO for 7 consecutive days. Fig. 14A, an immunoblot probing for activated (phosphorylated) DDR1 demonstrates that 1.25 and 2.5 but not 5 mg/kg of BK40143 deactivated DDR1. Fig. 14B, an immunoblot probing for activated Src, demonstrates that BK40143 did not engage this tyrosine kinase. Fig. 14C, an immunoblot probing for activated Abl, demonstrates that BK40143 did not engage this tyrosine kinase. Fig. 14D, an immunoblot probing for phosphorylated Tau (AT8), shows that all three doses of BK40143 reduced the levels of phosphorylated tau by 41-49% percent in the same mice, indicating that DDR inhibition is concurrent with pTau reduction. Fig. 14E is an ELISA for phosphorylated Tau (AT181) showing that 2.5mg/kg of BK40143 significantly reduced phosphorylated Tau.

Figs. 15A-F show that BK40143 significantly reduces amyloid, phosphorylated tau, and deactivates DDR1 in transgenic APP mice. Male and female, 7-month-old APP mice were treated i.p. with 1.25 and 2.5mg/kg of BK40143 or DMSO for 21 consecutive days. Fig. 15A is an immunoblot for aggregating extracellular amyloid-beta (6E10) which demonstrates that 1.25 and 2.5mg/kg of BK40143 significantly reduced amyloid-beta plaques. Fig. 15B is an immunoblot probing for phosphorylated DDR1 which demonstrates that 1.25 and 2.5mg/kg of BK40143 deactivated DDR1 by 40% and 31%, respectively. Fig. 15C, probing for activated (phosphorylated Abl (245), demonstrates that BK40143 did not engage Abl. Figs. 15D and 15E.

1.25 and 2.5mg/kg BK40143 respectively, significantly reduced soluble human amyloid-beta via ELISA but did not significantly reduce the insoluble amyloid-beta. Fig. 15F shows that 2.5mg/kg of BK40143 significantly reduced human phosphorylated tau (Ser396) by over 80%.

Fig. 16 shows that BK40143 could improve performance on Morris Water Maze test for cognition in APP mice. Measurements include the number of platform entries (left panel), the latency to the first entry (middle panel), and the distance travelled before their first entry (right panel) to the platform. Although there was no significant difference between groups, 1.25mg/kg of BK showed a trend for increased performance with higher numbers of platform entries, and a lower latency to first entry and lower distance travelled before the first entry.

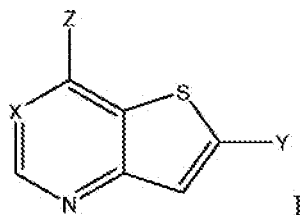
Fig. 17 shows that BK40143 does not cause cell death in the hippocampus of APP mice. Representative 20um hippocampal sections were stained for nissl substance (left panel). 4x and 20x images for DMSO, 1.25mg/kg, and 2.5mg/kg of BK40143 are shown (left panel). Mean staining intensity was quantified in imagej software as the total amount of nissl staining in all 4x images (right panel).

DETAILED DESCRIPTION

Provided herein are compositions and methods for treating or preventing a neurodegenerative disease, a myodegenerative disease, a prion disease or a lysosomal storage disease in a subject.

Compounds

In some examples, a class of compounds described herein includes compounds represented by Formula I:



or an isomer or pharmaceutically acceptable salt thereof.

In Formula I, X is N or CH.

Also, in Formula I, Y is C₆₋₁₀ aryl unsubstituted or substituted with R¹; or C₅₋₁₀ heteroaryl unsubstituted or substituted with R¹, or N-methylpiperazinyl;

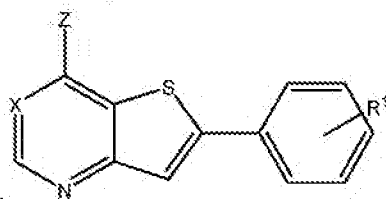
Also, in Formula I, R¹ is -(CH₂)_n-R², -(CH₂)_n-C(O)-R², or -O(CH₂)_n-R²;

Additionally, in Formula I, R² is -H, -CN, halogen, C₁₋₃ alkyl, C₁₋₃ alkoxy, phenyl, pyridinyl, amino, C₁₋₃ alkyl amino, di C₁₋₃ alkyl amino, hydroxyl C₁₋₃ alkyl amino, carboxy C₁₋₃ alkyl amino, C₃₋₆ cycloalkyl C₁₋₃ alkylamino, pyrrolidinyl, hydroxyl pyrrolidinyl, hydroxyl C₁₋₃ alkylpyrrolidinyl, carboxypyrrolidinyl, piperidinyl, C₁₋₃ alkylpiperidinyl, di C₁₋₃ alkyl piperidinyl, piperazinyl, C₁₋₃ alkylpiperazinyl, C₁₋₄ alkoxy carbonyl piperazinyl, or morpholinyl;

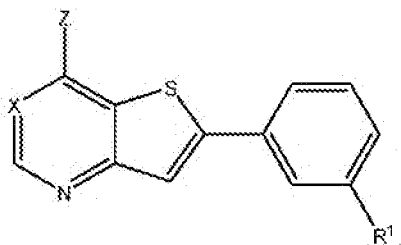
Z is heteroaryl, heterocyclyl, or NR³R⁴;

Also, in Formula I, R³ and R⁴ are independently selected from H, C₁₋₃ alkyl, C₁₋₃ alkoxy, or unsubstituted phenyl, and n is an integer selected from 0 to 3.

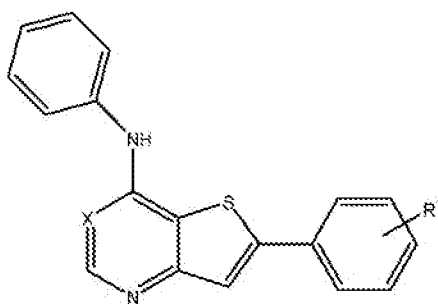
In some examples of Formula I, Y is benzyl substituted with R¹:



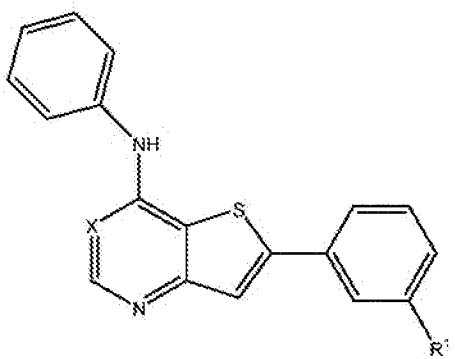
In some examples of Formula I, Y is benzyl substituted with R¹ in the meta position:



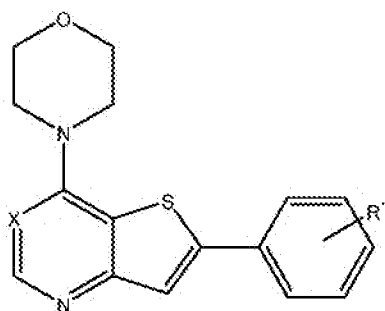
In some examples of Formula I, Z is NR³R⁴, R³ is benzyl or H, R⁴ is benzyl or H, and Y is benzyl substituted with R¹;



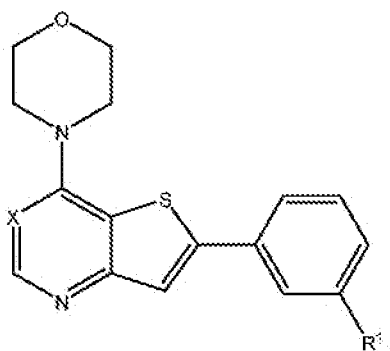
In some examples of Formula I, Z is NR^3R^4 , R^3 is benzyl or H, R^4 is benzyl or H, and Y is benzyl substituted with R^1 in the meta position:



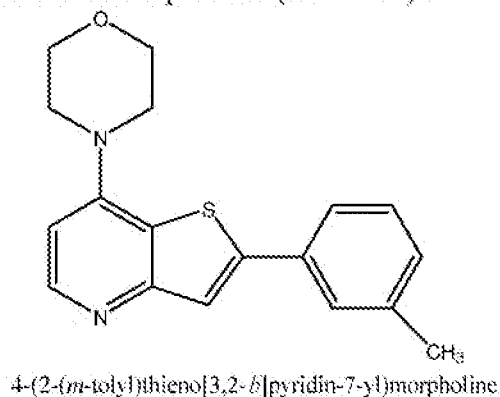
In some examples of Formula I, Z is morpholinyl and Y is benzyl substituted with R^1 :



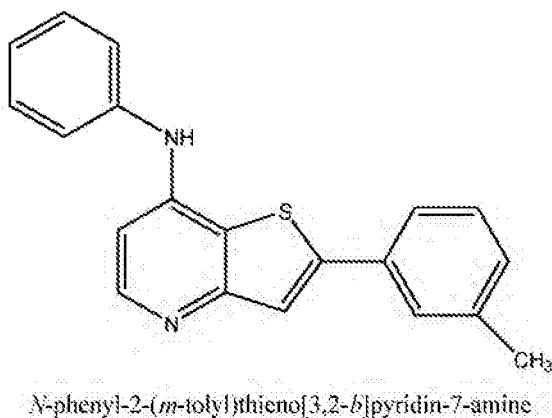
In some examples of Formula I, Z is morpholinyl and Y is benzyl substituted with R^1 in the meta position:



A compound of Formula I is Compound 1 (BK40197):



Another compound of Formula I is Compound 2 (BK40193):



In some examples of Formula I, the compound does not comprise one or more halogen atoms. In some examples of Formula I, Y is 2-*m*-toluyl. In some examples of Formula I, Z is heterocyclyl. In some examples of Formula I, Z is morpholin-1-yl. In some examples of Formula I, R⁵ is H and R⁴ is unsubstituted phenyl.

As used herein, the terms alkyl, alkenyl, and alkynyl include straight- and branched-chain monovalent substituents. Examples include methyl, ethyl, isobutyl, 3-butenyl, and the like. Ranges of these groups useful with the compounds and methods described herein include C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, and C₂-C₂₀ alkynyl. Additional ranges of these groups useful with the compounds and methods described herein include C₁-C₁₂ alkyl, C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₄ alkyl, C₂-C₄ alkenyl, and C₂-C₄ alkynyl.

The term alkoxy as used herein is an alkyl group bound through a single, terminal ether linkage. The term hydroxy as used herein is represented by the formula —OH.

The terms amine or amino as used herein are represented by the formula —NR³R⁴, where R³ and R⁴ can each be substitution group as described herein, such as hydrogen, an alkyl, a cycloalkyl, a halogenated alkyl, alkenyl, or alkynyl group described above.

The alkoxy, amino, alkyl, alkenyl, alkynyl, or carbonyl molecules used herein can be substituted or unsubstituted. As used herein, the term substituted includes the addition of an alkoxy, amino, alkyl, alkenyl, alkynyl, or carbonyl group to a position attached to the main chain of the alkoxy, amino, alkyl, alkenyl, alkynyl, or carbonyl, e.g., the replacement of a hydrogen by one of these molecules. Examples of substitution groups include, but are not limited to, hydroxy, halogen (e.g., F, Br, Cl, or I), and carboxyl groups. Conversely, as used herein, the term unsubstituted indicates the alkoxy, amino, alkyl, alkenyl, alkynyl, or carbonyl has a full complement of hydrogens, i.e., commensurate with its saturation level, with no substitutions, e.g., linear decane (—(CH₂)₉—CH₃).

Heteroalkyl, heteroalkenyl, and heteroalkynyl are defined similarly as alkyl, alkenyl, and alkynyl, but can contain O, S, or N heteroatoms or combinations thereof within the backbone. Ranges of these groups useful with the compounds and methods described herein include C₁-C₂₀ heteroalkyl, C₂-C₂₀ heteroalkenyl, and C₂-C₂₀ heteroalkynyl. Additional ranges of these groups useful with the compounds and methods described herein include C₁-C₁₂ heteroalkyl, C₂-C₁₂ heteroalkenyl, C₂-C₁₂ heteroalkynyl, C₁-C₆ heteroalkyl, C₂-C₆ heteroalkenyl, C₂-C₆ heteroalkynyl, C₁-C₄ heteroalkyl, C₂-C₄ heteroalkenyl, and C₂-C₄ heteroalkynyl.

The terms cycloalkyl, cycloalkenyl, and cycloalkynyl include cyclic alkyl groups having a single cyclic ring or multiple condensed rings. Examples include cyclohexyl, cyclopentylethyl, and adamantanyl. Ranges of these groups useful with the compounds and methods described herein include C₃-C₂₀ cycloalkyl, C₃-C₂₀ cycloalkenyl, and C₃-C₂₀ cycloalkynyl. Additional

ranges of these groups useful with the compounds and methods described herein include C₅-C₁₂ cycloalkyl, C₅-C₁₂ cycloalkenyl, C₅-C₁₂ cycloalkynyl, C₅-C₆ cycloalkyl, C₅-C₆ cycloalkenyl, and C₅-C₆ cycloalkynyl.

The terms heterocycloalkyl, heterocycloalkenyl, and heterocycloalkynyl are defined similarly as cycloalkyl, cycloalkenyl, and cycloalkynyl, but can contain O, S, or N heteroatoms or combinations thereof within the cyclic backbone. Ranges of these groups useful with the compounds and methods described herein include C₃-C₂₀ heterocycloalkyl, C₃-C₂₀ heterocycloalkenyl, and C₃-C₂₀ heterocycloalkynyl. Additional ranges of these groups useful with the compounds and methods described herein include C₅-C₁₂ heterocycloalkyl, C₅-C₁₂ heterocycloalkenyl, C₅-C₁₂ heterocycloalkynyl, C₅-C₆ heterocycloalkyl, C₅-C₆ heterocycloalkenyl, and C₅-C₆ heterocycloalkynyl.

Aryl molecules include, for example, cyclic hydrocarbons that incorporate one or more planar sets of, typically, six carbon atoms that are connected by delocalized electrons numbering the same as if they consisted of alternating single and double covalent bonds. An example of an aryl molecule is benzene. Heteroaryl molecules include substitutions along their main cyclic chain of atoms such as O, N, or S. When heteroatoms are introduced, a set of five atoms, e.g., four carbon and a heteroatom, can create an aromatic system. Examples of heteroaryl molecules include furan, pyrrole, thiophene, imadazole, oxazole, pyridine, and pyrazine. Aryl and heteroaryl molecules can also include additional fused rings, for example, benzofuran, indole, benzothiophene, naphthalene, anthracene, and quinoline. The aryl and heteroaryl molecules can be attached at any position on the ring, unless otherwise noted.

Optionally, the compounds having Formula I are tyrosine kinase inhibitors that inhibit one or more receptor tyrosine kinases selected from the group consisting of Abl, PDGFR α , PDGFR β , DDR 1, DDR2, cKIT, arginase II, Src, Fyn, VEGFR and Zac. In some examples, the compound having Formula I selectively inhibits Abl, PDGFR α , PDGFR β , DDR 1, DDR2, cKIT, arginase II, Src, Fyn or VEGFR or Zac. In some examples, the compound having Formula I inhibits DDR 1 and/or DDR2.

As used herein, the term pharmaceutically acceptable salt refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are

well known in the art. Pharmaceutically acceptable salts of the compounds provided herein, for example, pharmaceutically acceptable salts of nilotinib, bosutinib, pazopanib and a compound of Formula I, include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, trifluoroacetic acid, undecanoate, valerate salts, and the like.

The compounds described herein can be prepared in a variety of ways. The compounds can be synthesized using various synthetic methods, including those provided in the Examples. At least some of these methods are known in the art of synthetic organic chemistry. The compounds described herein can be prepared from readily available starting materials. Optimum reaction conditions can vary with the particular reactants or solvent used, but such conditions can be determined by one skilled in the art by routine optimization procedures.

Variations on Formula I include the addition, subtraction, or movement of the various constituents as described for each compound. Similarly, when one or more chiral centers are present in a molecule, all possible chiral variants are included. Additionally, compound synthesis can involve the protection and deprotection of various chemical groups. The use of protection and deprotection, and the selection of appropriate protecting groups can be determined by one skilled in the art. The chemistry of protecting groups can be found, for example, in Wuts, Greene's Protective Groups in Organic Synthesis, 5th. Ed., Wiley & Sons, 2014, which is incorporated herein by reference in its entirety.

Reactions to produce the compounds described herein can be carried out in solvents, which can be selected by one of skill in the art of organic synthesis. Solvents can be substantially nonreactive with the starting materials (reactants), the intermediates, or products under the conditions at which the reactions are carried out, i.e., temperature and pressure. Reactions can be carried out in one solvent or a mixture of more than one solvent. Product or intermediate formation can be monitored according to any suitable method known in the art. For example, product formation can be monitored by spectroscopic means, such as nuclear magnetic resonance spectroscopy (e.g., ^1H or ^{13}C) infrared spectroscopy, spectrophotometry (e.g., UV-visible), or mass spectrometry, or by chromatography such as high performance liquid chromatography (HPLC) or thin layer chromatography.

Any of the compounds described herein can be modified to enhance blood-brain barrier permeability. Optionally, one or more of the compounds described herein can be administered with an agent that enhances the blood brain barrier permeability of the compound(s).

Methods for Treating or Preventing Neurodegenerative Diseases, Myodegenerative Diseases or Prion Diseases

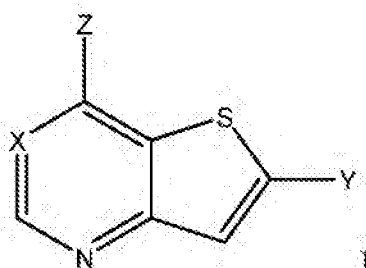
Provided herein are methods of treating or preventing a neurodegenerative disease, a myodegenerative disease or a prion disease. The neurodegenerative disease or disorder can be a neurodegenerative disease of the central nervous system. These include, but are not limited to, amyotrophic lateral sclerosis, Alzheimer's disease, frontotemporal dementia, TDP-43 pathologies, including frontotemporal dementia with TDP-43, frontotemporal dementia linked to chromosome-17, amyloidosis, Pick's disease, Huntington's disease, mild cognitive impairment, an α -synucleinopathy (e.g., Parkinson's disease, Lewy body disease), multiple sclerosis, Glial Cytoplasmic Inclusions, including multiple system atrophy, chronic traumatic encephalopathies, a Tauopathy, progressive supranuclear palsy, and cortico-basal degeneration. The neurodegenerative disease can also be a secondary neurodegenerative disease induced by a traumatic brain injury, stroke or an infection, for example, a bacterial or a viral infection (e.g., HIV, Herpes simplex virus (HSV)).

Myodegenerative diseases or disorders include but are not limited to a dystrophy (for example, muscular dystrophy), a myopathy (for example, nemaline myopathy, multiminicore

myopathy, centronuclear myopathy, mitochondrial myopathy, metabolic myopathy, etc.) or myotonia (for example, myotonia congenita, paramyotonia congenita or myotonic dystrophy).

Prion diseases or disorders include but are not limited to Creutzfeldt-Jakob Disease, Variant Creutzfeldt-Jakob Disease, Gerstmann-Straussler-Scheinker Syndrome, Fatal Familial Insomnia, Kuru, Bovine Spongiform Encephalopathy, Chronic Wasting Disease and Scrapie, to name a few.

The methods comprise administering to the subject with the neurodegenerative disease, myodegenerative disease or prion disease, or at risk of developing the neurodegenerative disease, the myodegenerative disease or the prion disease an effective amount of a compound having Formula I:



wherein,

X is N or CH;

Y is C₆₋₁₀ aryl unsubstituted or substituted with R¹; or C₃₋₁₀ heteroaryl unsubstituted or substituted with R¹, or N-methylpiperazinyl;

R¹ is -(CH₂)_n-R², -(CH₂)_n-C(O)-R², or -O(CH₂)_n-R²;

R² is -H, -CN, halogen, C₁₋₃ alkyl, C₁₋₃ alkoxy, phenyl, pyridinyl, amino, C₁₋₃ alkyl amino, di C₁₋₃ alkyl amino, hydroxyl C₁₋₃ alkyl amino, carboxy C₁₋₃ alkyl amino, C₃₋₆ cycloalkyl C₁₋₃ alkylamino, pyrrolidinyl, hydroxyl pyrrolidinyl, hydroxyl C₁₋₃ alkylpyrrolidinyl, carboxypyrrolidinyl, piperidinyl, C₁₋₃ alkylpiperidinyl, di C₁₋₃ alkyl piperidinyl, piperazinyl, C₁₋₃ alkylpiperazinyl, C₁₋₄ alkoxy carbonylpiperazinyl, or morpholinyl;

Z is heteroaryl, heterocyclyl, or NR³R⁴;

R³ and R⁴ are independently H, C₁₋₃ alkyl, C₁₋₃ alkoxy, or unsubstituted phenyl,

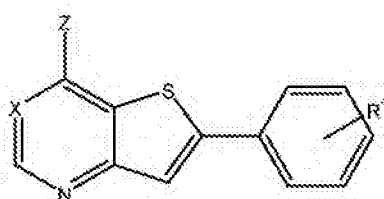
and

n is an integer selected from 0 to 3,

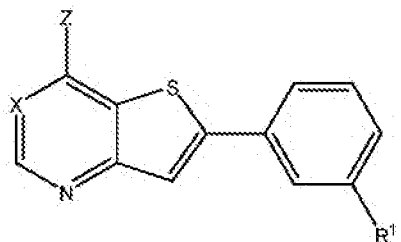
or an isomer or pharmaceutically acceptable salt thereof.

In some methods, the compound having Formula I does not comprise one or more halogen atoms. In some methods, Y is 2-*m*-toluyl in the compound having Formula I. In some methods, Z is heterocyclyl in the compound having Formula I. In some methods, Z is morpholin-1-yl in the compound having Formula I. In some methods, R³ is H and R⁴ is unsubstituted phenyl in the compound having Formula I.

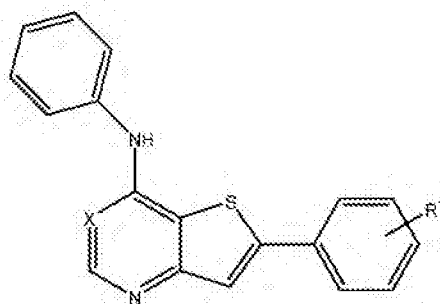
In some methods, a compound having Formula I, wherein Y is benzyl substituted with R¹ is administered to the subject:



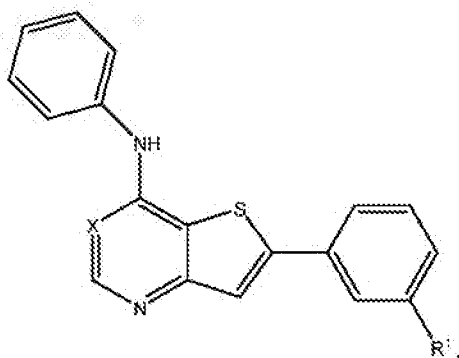
In some methods, a compound having Formula I, wherein Y is benzyl substituted with R¹ in the meta position, is administered to the subject:



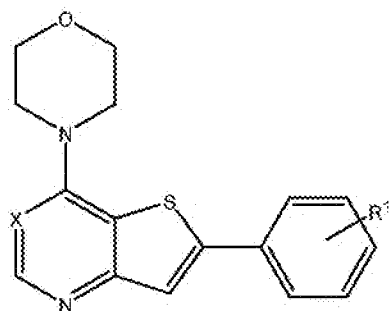
In some methods, a compound having Formula I, wherein Z is NR³R⁴, R³ is benzyl or H, R⁴ is benzyl or H, and Y is benzyl substituted with R¹ is administered to the subject:



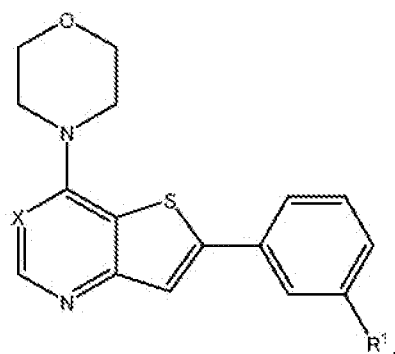
In some methods, a compound of Formula I, wherein Z is NR^3R^4 , R^3 is benzyl or H, R^4 is benzyl or H, and Y is benzyl substituted with R^1 in the meta position, is administered to the subject:



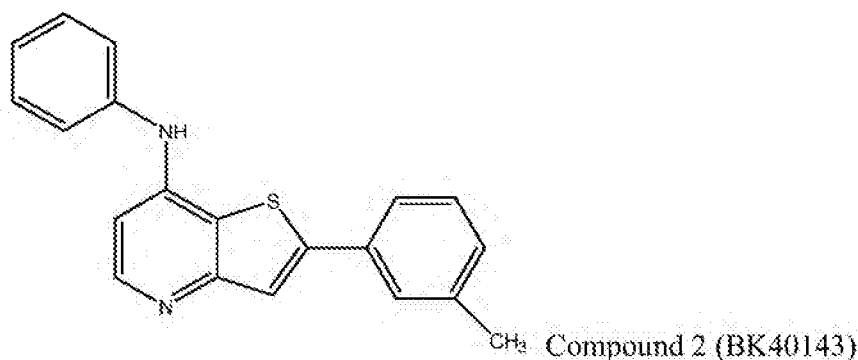
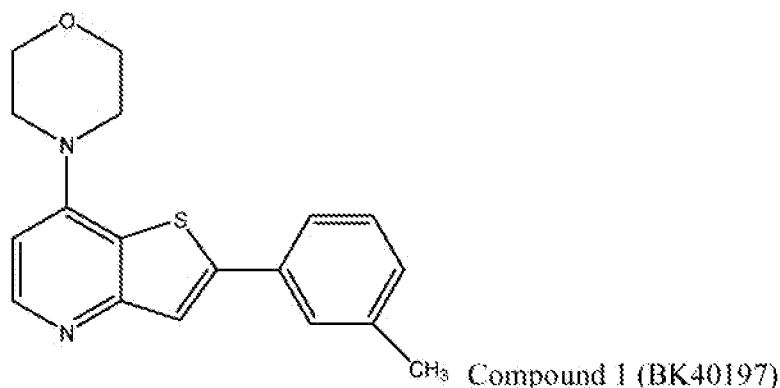
In some methods, a compound of Formula I, herein Z is morpholinyl and Y is benzyl substituted with R^1 is administered to the subject:



In some methods, a compound of Formula I, wherein Z is morpholinyl and Y is benzyl substituted with R^1 in the meta position is administered to the subject:



Examples of Formula I that can be used in any of the methods described herein include the following compounds:



The methods provided herein optionally include selecting a subject with a neurodegenerative disease, a myodegenerative disease or a prion disease or at risk for developing a neurodegenerative disease, a myodegenerative disease or a prion disease. One of skill in the art knows how to diagnose a subject with or at risk of developing a neurodegenerative disease, a myodegenerative disease or a prion disease. For example, one or more of the follow tests can be used: a genetic test (e.g., identification of a mutation in TDP-43 gene) or familial analysis (e.g., family history), central nervous system imaging (e.g., magnetic resonance imaging and positron emission tomography), electroencephalography, clinical or behavioral tests (e.g., assessments of muscle weakness, tremor, gait, or memory), or laboratory tests.

The method optionally further includes administering a second therapeutic agent to the subject. The second therapeutic agent is selected from the group consisting of levadopa, a dopamine agonist, an anticholinergic agent, a cholinergic agent (e.g., 5-hydroxytryptamine (5-

HT) inhibitors), a monoamine oxidase inhibitor, a COMT inhibitor, donepezil, memantine, risperidone, amantadine, rivastigmine, an NMDA antagonist, an acetylcholinesterase inhibitor, a cholinesterase inhibitor, riluzole, an anti-psychotic agent, an antidepressant, a glucocorticoid (for example, prednisone), a tyrosine kinase inhibitor (e.g., nilotinib, bosutinib, imatinib, pazopanib, etc.), and tetrabenazine. The second therapeutic agent or therapy can be administered to the subject prior to, simultaneously with, or subsequent to administration of the compound having Formula I.

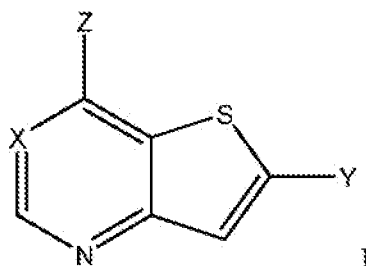
In the methods where a tyrosine kinase inhibitor is administered as a second therapeutic agent, the tyrosine kinase inhibitor can be a tyrosine kinase inhibitor that does not inhibit a tyrosine kinase receptor that is inhibited by the compound of Formula I or has decreased selectivity for a tyrosine kinase receptor, as compared to a compound of Formula I.

Also provided herein is a method of inhibiting or preventing toxic protein aggregation in a neuron and/or rescuing a neuron from degeneration. As used herein, references to inhibiting, decreasing or reducing include a change of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater as compared to a control level.

The method includes contacting the neuron with an effective amount of a compound of Formula I. Optionally, the compound having Formula I is Compound 1 or Compound 2. The toxic protein aggregate optionally comprises one or more of an amyloidogenic protein, alpha-synuclein, tau, or TDP-43. By amyloidogenic protein is meant a peptide, polypeptide, or protein that has the ability to aggregate. An example of an amyloidogenic protein is β -amyloid. The contacting is performed *in vivo* or *in vitro*. The *in vivo* method is useful in treating a subject with or at risk of developing toxic protein aggregates and comprises administering the compound of Formula I to the subject as described below. The *in vitro* method is useful, for example, in treating neural cells prior to transplantation. In such case, the compound of Formula I is generally added to a culture medium. Optionally, the target neurons are contacted with a second therapeutic agent as described above.

Methods for Treating or Preventing Lysosomal Storage Disorders

Also provided are methods for treating or preventing a LSD in a subject. The methods comprise administering to the subject with the LSD or at risk of developing the LSD an effective amount of a compound having Formula I:



wherein,

X is N or CH;

Y is C₆₋₁₀ aryl unsubstituted or substituted with R¹; or C₅₋₁₀ heteroaryl unsubstituted or substituted with R¹, or N-methylpiperazinyl;

R¹ is $-(CH_2)_n-R^2$, $-(CH_2)_n-C(O)-R^2$, or $-O(CH_2)_n-R^2$;

R² is -H, -CN, halogen, C₁₋₃ alkyl, C₁₋₃ alkoxy, phenyl, pyridinyl, amino, C₁₋₃ alkyl amino, di C₁₋₃ alkyl amino, hydroxyl C₁₋₃ alkyl amino, carboxy C₁₋₃ alkyl amino, C₃₋₆ cycloalkyl C₁₋₃ alkylamino, pyrrolidinyl, hydroxyl pyrrolidinyl, hydroxyl C₁₋₃ alkylpyrrolidinyl, carboxypyrrolidinyl, piperidinyl, C₁₋₃ alkylpiperidinyl, di C₁₋₃ alkyl piperidinyl, piperazinyl, C₁₋₃ alkylpiperazinyl, C₁₋₄ alkoxy carbonylpiperazinyl, or morpholinyl;

Z is heteroaryl, heterocyclyl, or NR³R⁴;

R³ and R⁴ are independently H, C₁₋₃ alkyl, C₁₋₃ alkoxy, or unsubstituted phenyl,

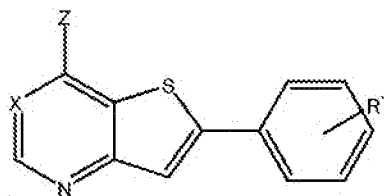
and

n is an integer selected from 0 to 3,

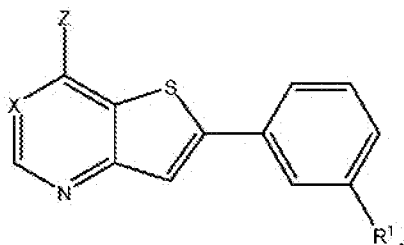
or an isomer or pharmaceutically acceptable salt thereof.

In some methods, the compound having Formula I does not comprise one or more halogen atoms. In some methods, Y is 2-*m*-toluyl in the compound having Formula I. In some methods Z is heterocyclyl in the compound having Formula I. In some methods, Z is morpholin-1-yl in the compound having Formula I. In some methods, R³ is H and R⁴ is unsubstituted phenyl in the compound having Formula I.

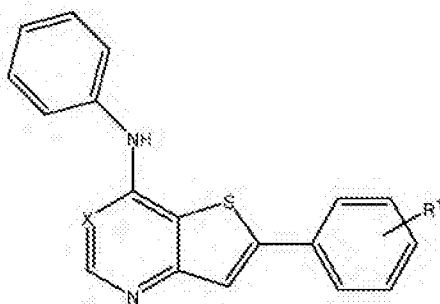
In some methods, a compound having Formula I, wherein Y is benzyl substituted with R¹ is administered to the subject:



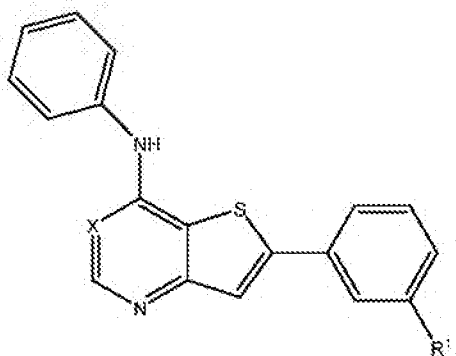
In some methods, a compound having Formula I, wherein Y is benzyl substituted with R¹ in the meta position, is administered to the subject:



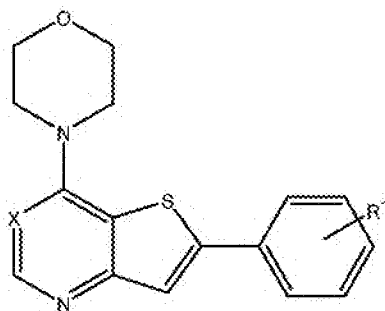
In some methods, a compound having Formula I, wherein Z is NR³R⁴, R³ is benzyl or H, R⁴ is benzyl or H, and Y is benzyl substituted with R¹ is administered to the subject:



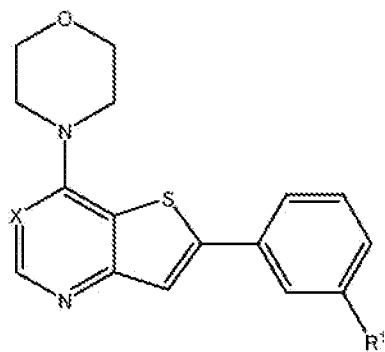
In some methods, a compound of Formula I, wherein Z is NR^3R^4 , R^3 is benzyl or H, R^4 is benzyl or H, and Y is benzyl substituted with R^1 in the meta position, is administered to the subject:



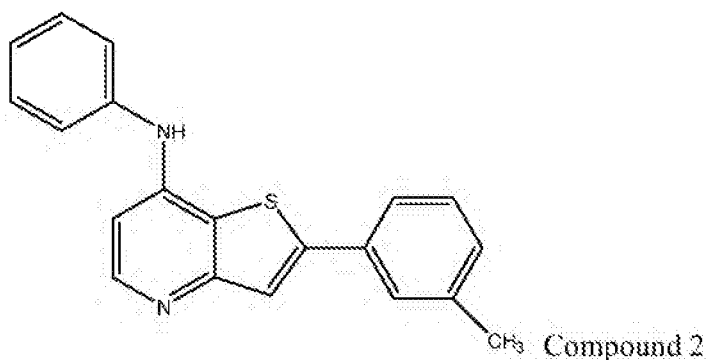
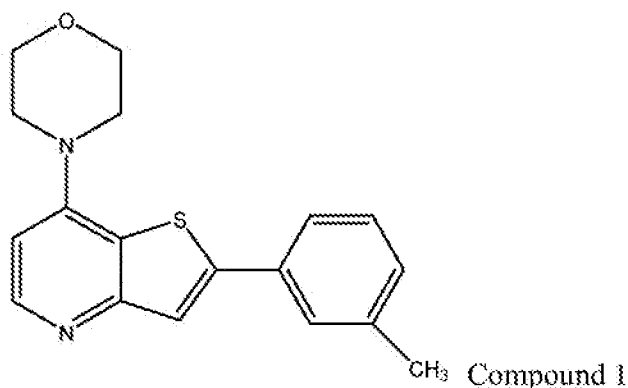
In some methods, a compound of Formula I, wherein Z is morpholinyl and Y is benzyl substituted with R^1 is administered to the subject:



In some methods, a compound of Formula I, wherein Z is morpholinyl and Y is benzyl substituted with R^1 in the meta position is administered to the subject:



Examples of Formula I that can be used to treat or prevent LSD include the following compounds:



Optionally, the compound of Formula I inhibits one or more receptor tyrosine kinases selected from the group consisting of Abl, PDGFR α , PDGFR β , DDR 1, DDR2, cKIT, arginase II, Src, Fyn, VEGFR and Zac. In some examples, the compound of Formula I selectively inhibits Abl, PDGFR α , PDGFR β , DDR 1, DDR2, cKIT, arginase II, Src, Fyn or VEGFR or Zac. In some examples, the compound having Formula I inhibits DDR 1 and/or DDR2. For example, and not to be limiting, Compound 1 or Compound 2 can be used to inhibit DDR1 and/or DDR2. In another example, the compound having Formula I, for example, Compound 1 or Compound 2 selectively inhibits DDR 1 or DDR2.

LSDs are inherited metabolic disorders that result from defects in lysosomal function. In the majority of cases, LSDs are caused by a deficiency of specific enzymes responsible for degradation of lipids and glycoproteins present in lysosomes. In some cases, defective non-enzymatic lysosomal proteins or non-lysosomal proteins involved in lysosomal biogenesis cause

LSDs. The progressive lysosomal accumulation of undegraded metabolites results in generalized cell and tissue dysfunction, and, therefore, multi-systemic pathology. LSDs that can be treated or prevented using the methods provided herein include, but are not limited to, Mucopolysaccharidosis Type I (for example, Hurler syndrome, Hurler-Scheie syndrome and Scheie syndrome), Mucopolysaccharidosis Type I (for example, Hunter syndrome), Mucopolysaccharidosis Type III (for example, Sanfillipo syndrome A, Sanfillipo syndrome B, Sanfillipo syndrome C and Sanfillipo syndrome D), Mucopolysaccharidosis Type IV (for example, Morquio syndrome A and Morquio syndrome B), Mucopolysaccharidosis Type VI (for example, Maroteaux-Lamy syndrome), Mucopolysaccharidosis Type VII (for example, Sly syndrome), Mucopolysaccharidosis Type IX (for example, Natowicz syndrome), Pseudo-Hurler polydystrophy, Tay-Sachs, Gaucher disease, Niemann-Pick disease, Fucosidosis, Galactosialidosis, Globoid-cell leukodystrophy, GM1 Gangliosidosis, GM2 Gangliosidosis, α -Mannosidosis, Metachromatic leukodystrophy and Pompe disease. The LSDs provided herein are examples of diseases or disorders associated with decreased lysosomal clearance.

Also provided are methods of promoting lysosomal clearance in one or more cells of a subject comprising administering to the subject that has a disorder associated with decreased lysosomal clearance an effective amount of a compound having Formula I. . Optionally, the compound having Formula I is Compound 1 or Compound 2. As used throughout, lysosomal clearance is a process by which accumulating lipids, proteins, glycoproteins or a combination thereof are metabolized or degraded in the lysosome of one or more cells in the subject. A decrease in lysosomal clearance means a decrease in degradation of lipids, proteins and/or glycoproteins in the lysosome of one or more cells of the subject as compared to a control, for example as compared to lysosomal clearance in one or more cells of a healthy subject. Any disorder associated with decreased lysosomal clearance can be treated using the methods provided herein, including, but limited to, any of the LSDs set forth throughout. As used herein, references to promoting or increasing include a change of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% 100%, 200%, 400% or greater as compared to a control level. Optionally, promoting lysosomal clearance decreases the amount of a lipid, a protein, a glycoprotein or a combination thereof in existing aggregates in the lysosome of one or more cells in a subject. Optionally, promoting lysosomal clearance inhibits or prevents formation of aggregates comprising a lipid, a protein, a glycoprotein or a combination thereof in the lysosome of one or

more cells in a subject. Optionally, promoting lysosomal clearance decreases the amount of time required to degrade or metabolize a lipid, a protein, a glycoprotein or a combination thereof in one or more cells of the subject as compared to a control.

Optionally, in the methods provided herein, the effective amount of a compound having Formula I inhibits or prevents toxic substance aggregation or accumulation in one or more cells of the subject as compared to a control. As used herein, references to decreasing, reducing, or inhibiting include a change of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater as compared to a control level. Such terms can include, but do not necessarily include, complete elimination of the toxic substance in one or more cells of the subject. Optionally, the one or more cells are brain cells, cells in one or more peripheral tissues of the subject, or a combination thereof. Optionally, the brain cells can be neurons and/or glial cells. In the methods provided herein, a toxic substance that can aggregate or accumulate in cells can be one or more of a lipid, a protein or a glycoprotein. The toxic substance(s) can increase cell damage and/or increase cell death in one or more cells of the subject. In the methods provided herein, the toxic substance(s) can be in the lysosome or elsewhere in one or more cells of the subject. For example, and not to be limiting, LSDs characterized by an accumulation of lipids in the cells of a subject include, but are not limited to, sphingolipidoses (including Gaucher's and Niemann-Pick diseases), gangliosidosis (including Tay-Sachs disease), leukodystrophies; mucopolysaccharidoses (including Hunter syndrome and Hurler disease), glycoprotein storage disorders, mucolipidoses, and glycogen storage disease type II (Pompe disease).

Lipids and glycoproteins that accumulate in sphingolipidoses include sphingomyelin in brain and red blood cells (Niemann Pick Disease); glycolipids, including ceramide trihexoside, in brain heart and kidney (Fabry disease); galactocerebroside in oligodendrocytes (Krabbe disease); glucocerebrosides in red blood cells, spleen and liver (Gaucher disease); GM2 gangliosides in neurons (Tay-Sachs disease) and Sandhoff disease; GM1 gangliosides; and sulfatide compounds in neural tissue (metachromatic leukodystrophy).

Lysosomal storage diseases also include mucopolysaccharidoses (MP) that have a deficiency in one or more lysosomal enzymes, for example, α -L-iduronidase (Hurler disease, Scheie syndrome and Hurler Scheie syndrome); iduronate sulphate (Hunter disease) heparan sulfate (Sanfilippo type A), N-acetyl- α -D-glucosamine (Sanfilippo type B), CoA- α -glucosaminide-N-acetyltransferase (Sanfilippo type C), N-Acetyl- α -D-glucosaminide-6-sulfate (Sanfilippo type D and

Morquio syndrome type A), B-galactose (Morquio syndrome type B) and N-acetylgalactosamine (Maroteaux-Lamy disease) but all of these MPs diseases are a result of lysosomal accumulation of heparan sulfate, dermatan sulfate or keratan sulfate. Glycogen storage diseases (i.e. Pompe disease) result from storage of sugars and phosphorylated sugars in the lysosomes.

The methods provided herein optionally include selecting a subject with a LSD. One of skill in the art knows how to diagnose a subject with a LSD. For example, one or more of the following tests can be used: a genetic test (e.g., identification of a mutation associated with a LSD) or familial analysis (e.g., family history, genetic testing of parents), central nervous system imaging (e.g., magnetic resonance imaging and positron emission tomography), clinical or behavioral tests (e.g., assessments to identify mood disorders, aggressiveness and/or cognitive abnormalities), or laboratory tests (e.g., blood and/or urine tests to identify abnormal levels of metabolites or enzymatic deficiencies).

The methods provided herein optionally further include administering an effective amount of a second therapeutic agent or therapy to the subject. The second therapeutic agent or therapy can be administered to the subject prior to, simultaneously with, or subsequent to administration of the compound of Formula I. The second therapeutic agent or therapy is selected from the group consisting of an enzyme, hematopoietic stem cells, a bone marrow transplant, gene therapy or a small molecule. For example, and not to be limiting, LSDs associated with an enzymatic deficiency can be treated with an enzyme to increase the amount of the deficient enzyme in the subject. For example, enzyme replacement therapy (ERT) with a recombinant enzyme, such as imiglucerase (Cerezyme®), velaglucerase alfa (VPRIV®) or taliglucerase alfa (Elelyso®), can be used as a second therapeutic agent to treat Type I Gaucher disease. Small molecules that inhibit glycosylceramide synthase, for example, miglustat and eliglustat, can also be used to treat Type I Gaucher disease. A small molecule that acts as a chaperone to stabilize a defective enzyme produced by the subject or a small molecule that reduces the amount of one or more substrates that would normally be processed by an enzyme in the subject can also be used.

One or more therapeutic agents that reduce the symptoms of a LSD can also be administered. For example, an anti-epileptic such as gabapentin or lamotrigine can be used to prevent seizures in a subject. Antibiotics can be used to treat bacterial infections such as pneumonia. Other agents include, but are not limited to, anti-inflammatory agents (e.g., NSAIDs

and anti-inflammatory steroids), and muscle relaxants. Dialysis, physical therapy and surgery are also contemplated herein as therapies to treat a LSD.

In some methods for treating or preventing a LSD, the second therapeutic agent can be a tyrosine kinase inhibitor (e.g., nilotinib, bosutinib, imatinib, pazopanib, etc.). Therefore, in some examples, a tyrosine kinase and a compound of Formula I are administered to the subject. In the methods where a tyrosine kinase is administered as a second therapeutic agent, the tyrosine kinase can be a tyrosine kinase inhibitor that differs in selectivity for one or more receptor tyrosine kinases as compared to the compound of Formula I.

Pharmaceutical Compositions

The term effective amount, as used throughout, is defined as any amount necessary to produce a desired physiologic response, for example, inhibiting or preventing toxic protein aggregation in a neuron or promoting lysosomal clearance.

Exemplary dosage amounts for administration of any compound described herein, for example, a compound of Formula I, include doses from about 0.5 to about 200 mg/kg of body weight of active compound per day, which may be administered in a single dose or in the form of individual divided doses, such as from 1 to 4 times per day. Alternatively, the dosage amount can be from about 0.5 to about 150 mg/kg of body weight of active compound per day, about 0.5 to 100mg/kg of body weight of active compound per day, about 0.5 to about 75 mg/kg of body weight of active compound per day, about 0.5 to about 50 mg/kg of body weight of active compound per day, about 0.5 to about 25 mg/kg of body weight of active compound per day, about 1 to about 50mg/kg of body weight of active compound per day, about 1 to about 40mg/kg of body weight of active compound per day, about 1 to about 30 mg/kg of body weight of active compound per day, about 1 to about 30 mg/kg of body weight of active compound per day, about 30 mg/kg of body weight of active compound per day about 20 mg/kg of body weight of active compound per day, about 10 mg/kg of body weight of active compound per day, or about 5 mg/kg of body weight of active compound per day.

Optionally, the dosage is less than about 10 mg/kg and can be less than about 9.5, 9, 8.5, 8, 7.5, 7, 6.5, 6, 5.5, 5, 4.5, 4, 3.5, 3, 2.5, 2, 1.5, 1.25, 1.0, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 mg/kg or any dosage in between these amounts. The dosage can range from about 0.1 mg/kg to about 10 mg/kg, from about 0.1 mg/kg to about 9 mg/kg, from about 0.1 mg/kg to about 8

mg/kg, from about 0.1 mg/kg to about 7 mg/kg, from about 0.1 mg/kg to about 6 mg/kg, from about 0.1 mg/kg to about 5 mg/kg, from about 0.1 mg/kg to about 4 mg/kg, from about 0.1 mg/kg to about 3 mg/kg, from about 0.1 mg/kg to about 2 mg/kg, from about 0.1 mg/kg to about 1 mg/kg, or from about 0.1 mg/kg to about 0.5 mg/kg. One of skill in the art would adjust the dosage as described below based on specific characteristics of the inhibitor and the subject receiving it.

The composition can comprise a single unit dose of a compound of Formula I, for example, a single unit dose of about 50 mg/kg or less, 40 mg/kg or less, 30 mg/kg or less, 20 mg/kg or less, 10 mg/kg or less, of about 5 mg/kg or less, of about 2.5 mg/kg or less or about 1.5 mg/kg or less of Compound 1 or Compound 2, or a pharmaceutically acceptable salt thereof. Packages including one or multiple, single unit doses of a compound having Formula I, for example, multiple, single unit doses of Compound 1 or Compound 2 are also provided. The package can further comprise single or multiple unit doses of one or more second therapeutic agents described herein.

Effective amounts and schedules for administering one or more of the compounds having Formula I described herein can be determined empirically and making such determinations is within the skill in the art. The dosage ranges for administration are those large enough to produce the desired effect in which one or more symptoms of the disease or disorder are affected (e.g., reduced or delayed). The dosage should not be so large as to cause substantial adverse side effects, such as unwanted cross-reactions, unwanted cell death, and the like. Generally, the dosage will vary with the type of inhibitor, the species, age, body weight, general health, sex and diet of the subject, the mode and time of administration, rate of excretion, drug combination, and severity of the particular condition and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any contraindications. Dosages can vary and can be administered in one or more dose administrations daily.

The compounds having Formula I and other agents described herein can be provided in a pharmaceutical composition. These include, for example, a pharmaceutical composition comprising a therapeutically effective amount of one or more compounds having Formula I and a pharmaceutical carrier. The term carrier means a compound, composition, substance, or structure that, when in combination with a compound or composition, aids or facilitates preparation, storage, administration, delivery, effectiveness, selectivity, or any other feature of

the compound or composition for its intended use or purpose. For example, a carrier can be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject. Such pharmaceutically acceptable carriers include sterile biocompatible pharmaceutical carriers, including, but not limited to, saline, buffered saline, artificial cerebral spinal fluid, dextrose, and water.

Depending on the intended mode of administration, the pharmaceutical composition can be in the form of solid, semi-solid or liquid dosage forms, such as, for example, tablets, suppositories, pills, capsules, powders, liquids, or suspensions, preferably in unit dosage form suitable for single administration of a precise dosage. The compositions will include a therapeutically effective amount of the agent described herein or derivatives thereof in combination with a pharmaceutically acceptable carrier and, in addition, may include other medicinal agents, pharmaceutical agents, carriers, or diluents. By pharmaceutically acceptable is meant a material that is not biologically or otherwise undesirable, which can be administered to an individual along with the selected agent without causing unacceptable biological effects or interacting in a deleterious manner with the other components of the pharmaceutical composition in which it is contained.

As used herein, the term carrier encompasses any excipient, diluent, filler, salt, buffer, stabilizer, solubilizer, lipid, stabilizer, or other material known in the art for use in pharmaceutical formulations. The choice of a carrier for use in a composition will depend upon the intended route of administration for the composition. The preparation of pharmaceutically acceptable carriers and formulations containing these materials is described in, *e.g.*, Remington: The Science and Practice of Pharmacy, 22nd edition, Loyd V. Allen et al, editors, Pharmaceutical Press (2012).

Examples of physiologically acceptable carriers include buffers such as phosphate buffers, citrate buffer, and buffers with other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as

sodium; and/or nonionic surfactants such as TWEEN[®] (ICI, Inc.; Bridgewater, New Jersey), polyethylene glycol (PEG), and PLURONICS[™] (BASF; Florham Park, NJ).

Compositions containing the agent(s) described herein suitable for parenteral injection may comprise physiologically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, and sterile powders for reconstitution into sterile injectable solutions or dispersions. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (propyleneglycol, polyethyleneglycol, glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants.

These compositions may also contain adjuvants such as preserving, wetting, emulsifying, and dispensing agents. Prevention of the action of microorganisms can be promoted by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. Isotonic agents, for example, sugars, sodium chloride, and the like may also be included. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin.

Solid dosage forms for oral administration of the compounds described herein or derivatives thereof include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the compounds described herein or derivatives thereof are admixed with at least one inert customary excipient (or carrier) such as sodium citrate or dicalcium phosphate or (a) fillers or extenders, as for example, starches, lactose, sucrose, glucose, mannitol, and silicic acid, (b) binders, as for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and acacia, (c) humectants, as for example, glycerol, (d) disintegrating agents, as for example, agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain complex silicates, and sodium carbonate, (e) solution retarders, as for example, paraffin, (f) absorption accelerators, as for example, quaternary ammonium compounds, (g) wetting agents, as for example, cetyl alcohol, and glycerol monostearate, (h) adsorbents, as for example, kaolin and bentonite, and (i) lubricants, as for example, talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, or mixtures thereof. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethyleneglycols, and the like.

Solid dosage forms such as tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells, such as enteric coatings and others known in the art. They may contain opacifying agents and can also be of such composition that they release the active compound or compounds in a certain part of the intestinal tract in a delayed manner. Examples of embedding compositions that can be used are polymeric substances and waxes. The active compounds can also be in micro-encapsulated form, if appropriate, with one or more of the above-mentioned excipients.

The compounds described herein can be incorporated into pharmaceutical compositions which allow for immediate release or delivery of those compounds to a mammal. The compounds described herein can also be incorporated into pharmaceutical compositions which allow for modified release, for example, delayed release or extended release (for example, sustained release or controlled release) of those compounds to a mammal for a period of several days, several weeks, or a month or more. Such formulations are described, for example, in U.S. Patent Nos. 5,968,895 and 6,180,608 and are otherwise known in the art. Any pharmaceutically-acceptable, delayed release or sustained-release formulation known in the art is contemplated.

Liquid dosage forms for oral administration of the compounds described herein or derivatives thereof include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art, such as water or other solvents, solubilizing agents, and emulsifiers, such as for example, ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propyleneglycol, 1,3-butyleneglycol, dimethylformamide, oils, in particular, cottonseed oil, groundnut oil, corn germ oil, olive oil, castor oil, sesame oil, glycerol, tetrahydrofurfuryl alcohol, polyethyleneglycols, and fatty acid esters of sorbitan, or mixtures of these substances, and the like.

Besides such inert diluents, the composition can also include additional agents, such as wetting, emulsifying, suspending, sweetening, flavoring, or perfuming agents.

The compositions are administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. The compositions are administered

via any of several routes of administration, including orally, parenterally, intravenously, intraperitoneally, intracranially, intraspinally, intrathecally, intraventricularly, intramuscularly, subcutaneously, intracavity or transdermally. Pharmaceutical compositions can also be delivered locally to the area in need of treatment, for example by topical application or local injection. Effective doses for any of the administration methods described herein can be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

Throughout, treat, treating, and treatment refer to a method of reducing or delaying one or more effects or symptoms of a neurodegenerative disease, a myodegenerative disease, a prion disease or a lysosomal storage disease. The subject can be diagnosed with a disease or disorder. Treatment can also refer to a method of reducing the underlying pathology rather than just the symptoms. The effect of the administration to the subject can have the effect of, but is not limited to, reducing one or more symptoms of the disease, a reduction in the severity of the disease, the complete ablation of the disease, or a delay in the onset or worsening of one or more symptoms. For example, a disclosed method is considered to be a treatment if there is about a 10% reduction in one or more symptoms of the disease in a subject when compared to the subject prior to treatment or when compared to a control subject or control value. Thus, the reduction can be about a 10, 20, 30, 40, 50, 60, 70, 80, 90, 100%, or any amount of reduction in between.

As used throughout, by subject is meant an individual. The subject can be an adult subject or a pediatric subject. Pediatric subjects include subjects ranging in age from birth to eighteen years of age. Thus, pediatric subjects of less than about 10 years of age, five years of age, two years of age, one year of age, six months of age, three months of age, one month of age, one week of age or one day of age are also included as subjects. Preferably, the subject is a mammal such as a primate, and, more preferably, a human. Non-human primates are subjects as well. The term subject includes domesticated animals, such as cats, dogs, etc., livestock (for example, cattle, horses, pigs, sheep, goats, etc.) and laboratory animals (for example, ferret, chinchilla, mouse, rabbit, rat, gerbil, guinea pig, etc.). Thus, veterinary uses and medical formulations are contemplated herein.

Disclosed are materials, compositions, and components that can be used for, can be used in conjunction with, can be used in preparation for, or are products of the disclosed methods and compositions. These and other materials are disclosed herein, and it is understood that when

combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutations of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a method is disclosed and discussed and a number of modifications that can be made to a number of molecules including in the method are discussed, each and every combination and permutation of the method, and the modifications that are possible are specifically contemplated unless specifically indicated to the contrary. Likewise, any subset or combination of these is also specifically contemplated and disclosed. This concept applies to all aspects of this disclosure including, but not limited to, steps in methods using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed, it is understood that each of these additional steps can be performed with any specific method steps or combination of method steps of the disclosed methods, and that each such combination or subset of combinations is specifically contemplated and should be considered disclosed.

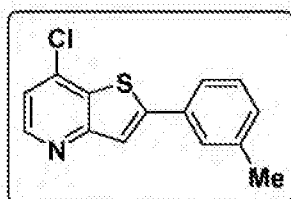
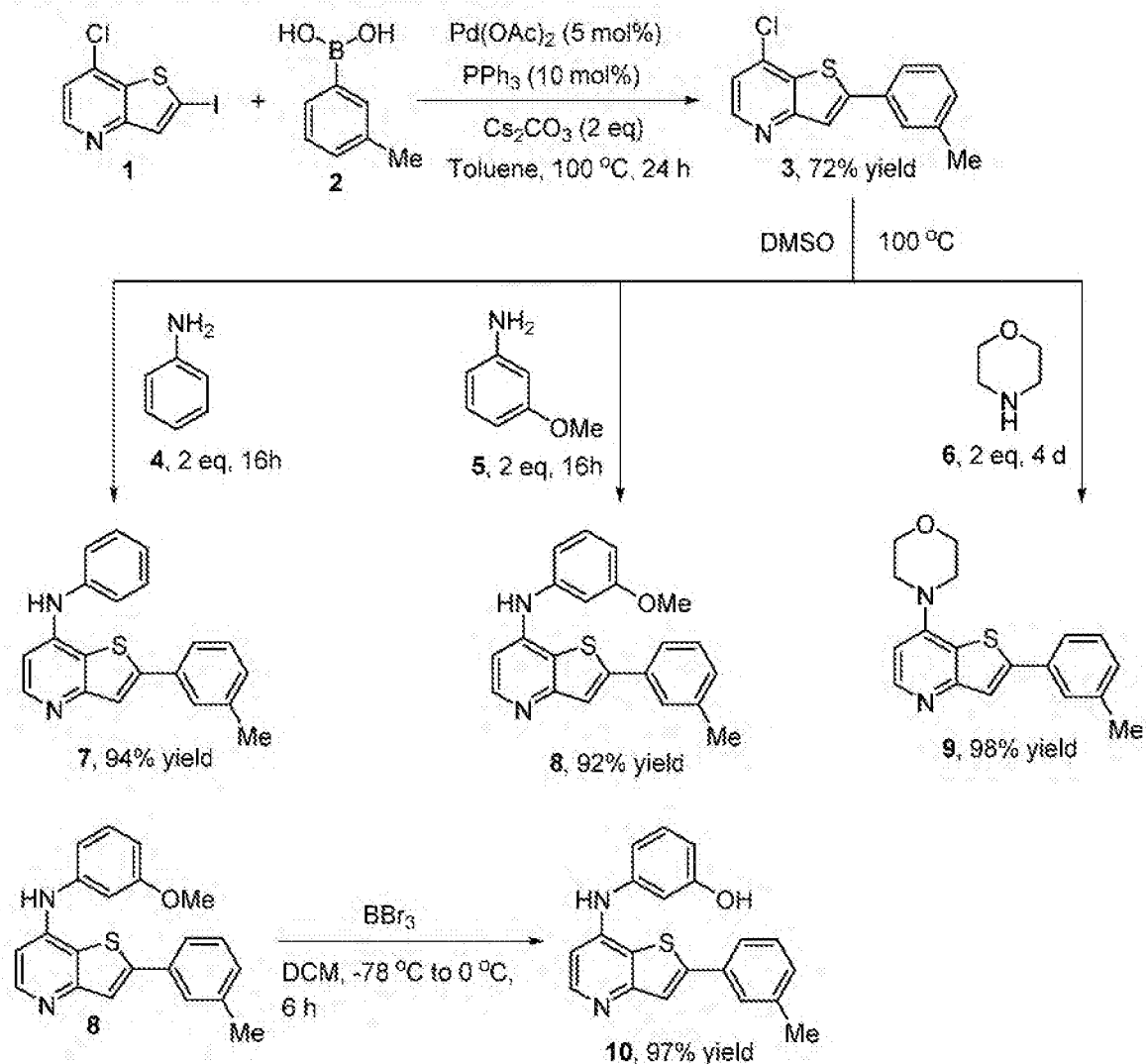
Publications cited herein and the material for which they are cited are hereby specifically incorporated by reference in their entireties.

EXAMPLES

Synthesis and characterization of thieno[3,2-*b*]pyridine derivatives

General Information

Commercially available 7-chloro-2-iodothieno[3,2-*b*]pyridine (**1**), *m*-tolylboronic acid (**2**), aniline (**4**), *m*-anisidine (**5**), morpholine (**6**), reagents, catalysts and solvents were used as purchased without further purification. NMR spectra were obtained at 400 MHz (¹H NMR) and 100 MHz (¹³C NMR) in deuterated solvents. Reaction products were purified by column chromatography on silica gel (particle size 40-63 μm) as described below.

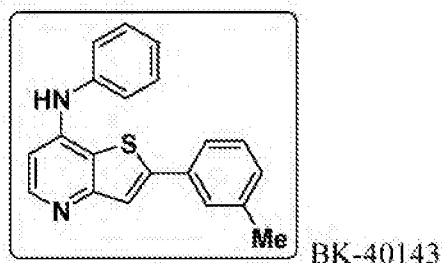
Synthetic methods and compound characterization**Synthesis of thieno[3,2-*b*]pyridine compounds 7-10**

7-Chloro-2-(*m*-tolyl)thieno[3,2-*b*]pyridine (3**).** A mixture of 7-chloro-2-iodothieno[3,2-*b*]pyridine (**1**) (500 mg, 1.69 mmol), 3-methylphenylboronic acid (**2**) (230 mg, 1.69 mmol), palladium(II) acetate (19 mg, 0.084 mmol), triphenylphosphine (44 mg, 0.169 mmol) and cesium carbonate (1.101 g, 3.38 mmol) in 15 mL of toluene was heated at reflux for 24 h. The reaction

mixture was cooled to room temperature and partitioned between water and dichloromethane. The organic layer was washed with saturated aqueous sodium chloride, dried over sodium sulfate, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel using hexanes-ethyl acetate (8:2) as mobile phase. Compound 3 was obtained as a colorless solid in 72% yield (315 mg, 1.21 mmol), $R_f = 0.2$ (hexanes/EtOAc, 1:1); ^1H NMR (400 MHz, Chloroform-*d*) $\delta = 8.54$ (d, $J = 5.1$ Hz, 1H), 7.73 (s, 1H), 7.55 – 7.52 (m, 2H), 7.34 (dd, $J = 7.8, 7.8$ Hz, 1H), 7.23 (m, 1H), 7.21 (d, $J = 5.1$ Hz, 1H), 2.42 (s, 3H); ^{13}C NMR (100 MHz, Chloroform-*d*) $\delta = 158.2, 149.6, 148.2, 139.0, 137.6, 133.2, 133.0, 130.4, 129.2, 127.3, 123.8, 120.8, 118.6, 21.5$; Anal. Calcd. for $\text{C}_{14}\text{H}_{10}\text{ClNS}$: C, 64.74; H, 3.88; N, 5.39. Found: C, 64.76; H, 4.05; N, 5.28.

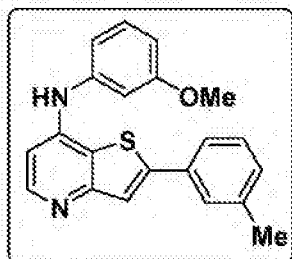
General procedure for the nucleophilic aromatic substitution reactions

A 5 mL pressure vessel was charged with 7-chloro-2-(*m*-tolyl)thieno[3,2-*b*]pyridine (3) (0.3 mmol), the amine (0.6 mmol) and DMSO (1.0 mL). The pressure vessel was then placed in a 100 °C oil bath and stirred for 16 h to 4 days. After full conversion was achieved based on ^1H NMR analysis, the reaction mixture was extracted with EtOAc and washed with water. The combined organic layers were dried over sodium sulfate and the solvent was removed *in vacuo*. The crude product was purified by flash chromatography on silica gel using with hexanes-ethyl acetate as mobile phase as described below.



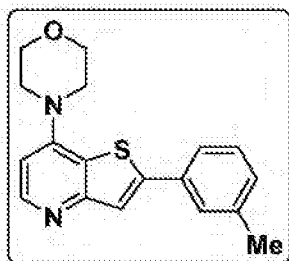
***N*-Phenyl-2-(*m*-tolyl)thieno[3,2-*b*]pyridin-7-amine (7).** Compound 7 was obtained as a colorless solid in 94% yield (89 mg, 0.282 mmol) from 7-chloro-2-(*m*-tolyl)thieno[3,2-*b*]pyridine (78 mg, 0.3 mmol) and aniline (56 mg, 0.6 mmol) in 1 mL of DMSO after 16 hours at 100 °C by following the general procedure described above. $R_f = 0.2$ (hexanes/EtOAc, 1:1); ^1H NMR (400 MHz, Chloroform-*d*) $\delta = 8.38$ (m, 1H), 7.70 (s, 1H), 7.57 – 7.50 (m, 2H), 7.43 – 7.38 (m, 2H), 7.37 – 7.23 (m, 3H), 7.21 – 7.17 (m, 2H), 6.90 (m, 1H), 6.15 (s, 1H), 2.43 (s, 3H); ^{13}C

NMR (100 MHz, Chloroform-*d*) δ = 158.5, 148.9, 146.5, 145.9, 139.4, 139.0, 133.7, 129.9, 129.7, 129.6, 129.1, 127.3, 124.8, 123.8, 122.7, 122.5, 121.6, 120.7, 102.6, 21.6; Anal. Calcd. for $C_{20}H_{16}N_2S$: C, 75.92; H, 5.10; N, 8.85. Found: C, 75.71; H, 5.32; N, 9.11.



BK40195

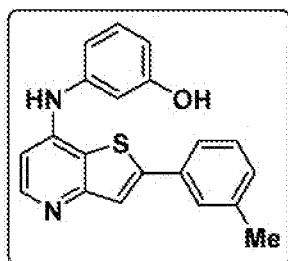
***N*-(3-Methoxyphenyl)-2-(*m*-tolyl)thieno[3,2-*b*]pyridin-7-amine (8).** Compound **8** was obtained as a colorless solid in 92% yield (95 mg, 0.276 mmol) from 7-chloro-2-(*m*-tolyl)thieno[3,2-*b*]pyridine (78 mg, 0.3 mmol) and *m*-anisidine (74 mg, 0.6 mmol) in 1 mL of DMSO after 16 hours at 100 °C by following the general procedure described above. R_f = 0.2 (hexanes/EtOAc, 2:1); ^1H NMR (400 MHz, Chloroform-*d*) δ = 8.41 (d, J = 5.6 Hz, 1H), 7.70 (s, 1H), 7.58 – 7.51 (m, 2H), 7.36 – 7.29 (m, 2H), 7.21 (d, J = 7.9 Hz, 1H), 6.96 (d, J = 5.6 Hz, 1H), 6.87 (dd, J = 7.9, 2.4 Hz, 1H), 6.83 (dd, J = 7.8, 7.7 Hz, 1H), 6.73 (dd, J = 7.9, 2.5 Hz, 1H), 6.07 (s, 1H), 3.83 (s, 3H), 2.44 (s, 3H); ^{13}C NMR (100 MHz, Chloroform-*d*) δ = 160.8, 158.6, 148.9, 146.5, 145.6, 140.7, 139.0, 133.7, 130.5, 130.0, 129.1, 127.3, 123.8, 121.7, 120.9, 114.5, 110.1, 108.1, 103.0, 55.5, 21.6; Anal. Calcd. for $C_{21}H_{18}N_2OS$: C, 72.80; H, 5.24; N, 8.09. Found: C, 72.53; H, 5.61; N, 8.19.



BK40197

4-(2-(*m*-Tolyl)thieno[3,2-*b*]pyridin-7-yl)morpholine (9). Compound **9** was obtained as a colorless solid in 98% yield (91 mg, 0.294 mmol) from 7-chloro-2-(*m*-tolyl)thieno[3,2-*b*]pyridine (78 mg, 0.3 mmol) and morpholine (52 mg, 0.6 mmol) in 1 mL of DMSO after 4 days at 100 °C by following the general procedure described above. R_f = 0.2 (hexanes/EtOAc, 1:1); ^1H

NMR (400 MHz, Chloroform-*d*) δ = 8.48 (d, J = 5.4 Hz, 1H), 7.69 (s, 1H), 7.59 – 7.52 (m, 2H), 7.34 (dd, J = 7.9, 7.8 Hz, 1H), 7.20 (dd, J = 7.9, 2.1 Hz, 1H), 6.64 (d, J = 5.4 Hz, 1H), 4.03 – 3.85 (m, 4H), 3.54 – 3.39 (m, 4H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, Chloroform-*d*) δ = 158.8, 153.0, 149.0, 146.6, 139.0, 133.6, 129.9, 129.1, 127.2, 123.7, 123.4, 121.4, 105.9, 66.9, 49.7, 21.6; Anal. Calcd. for $\text{C}_{18}\text{H}_{18}\text{N}_2\text{O}_2\text{S}$: C, 69.65; H, 5.85; N, 9.02. Found: C, 69.89; H, 5.72; N, 9.38.



BK40196

3-((2-(*m*-Tolyl)thieno[3,2-*b*]pyridin-7-yl)amino)phenol (10). To a solution of *N*-(3-methoxyphenyl)-2-(*m*-tolyl)thieno[3,2-*b*]pyridin-7-amine (**8**) (69 mg, 0.2 mmol) in dry dichloromethane (3 mL) was added boron tribromide (4 equiv) at -78 °C under inert atmosphere. The mixture was stirred for 4 h and the reaction temperature was allowed to reach 0 °C. After quenching with 1M HCl, the crude reaction mixture was extracted with EtOAc and washed with water. The combined organic layers were dried over sodium sulfate and the solvent was removed *in vacuo*. The crude product was purified by flash chromatography on silica gel using DCM-MeOH (19:1) as mobile phase. Compound **10** was obtained as a colorless solid in 97% yield (64 mg, 0.194 mmol). R_f = 0.4 (DCM/MeOH, 9:1); ^1H NMR (399 MHz, Methanol-*d*₄) δ = 8.22 (d, J = 6.7 Hz, 1H), 7.70 (s, 1H), 7.65 (s, 1H), 7.61 (dd, J = 7.5, 2.1 Hz, 1H), 7.40 (dd, J = 7.6, 7.6 Hz, 1H), 7.34 – 7.31 (m, 2H), 6.93 (d, J = 6.7 Hz, 1H), 6.88 (m, 1H), 6.85 – 6.79 (m, 2H), 2.44 (s, 3H); ^{13}C NMR (100 MHz, Methanol-*d*₄) δ = 160.1, 154.7, 153.5, 149.5, 141.1, 140.7, 139.5, 133.3, 132.4, 131.9, 131.7, 130.5, 128.2, 125.0, 117.4, 115.7, 114.9, 113.5, 102.7, 21.3; Anal. Calcd. for $\text{C}_{20}\text{H}_{16}\text{N}_2\text{O}_2\text{S}$: C, 72.26; H, 4.85; N, 8.43. Found: C, 72.29; H, 4.97; N, 8.61.

Cell Culture

Rat neuroblastoma B35 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) with 10% Fetal Bovine Serum (FBS) and 1% penicillin/streptomycin and incubated at

37°C with 5% CO₂. For the experiments, cells were transferred to 12-well plates (Cat. # 150628, ThermoFisher, Waltham, MA) and grown to at least 70% confluence. Transient transfection was performed with 3 µg P301L tau (Cat. #30145, Addgene) cDNA or 3 µg human α-synuclein cDNA using Fugene HD transfection reagent (Cat. #E2311, Promega, Madison WI) for 24 hours. Cells were treated with 1 mM, 100 µM, 10 µM, 1 µM, 0.1 µM, 0.01 µM, and 0.001 µM dissolved in DMSO or an equivalent 5 µL of DMSO for 5 hours. Cell culture media was collected and cells were harvested using sodium-tris, EDTA, NP-40 (STEN) buffer and centrifuged at 10,000 x g for 20 minutes at 4°C and supernatant was collected. Cell viability was determined via lactate dehydrogenase assay (Cat. # 88954, Thermofisher) and MTT assay (Cat. #V13154, Thermofisher). Protein was extracted by removing culture medium and adding 0.2 ml 1x STEN buffer (50 mM Tris (pH 7.6), 150 mM NaCl, 2 mM EDTA, 0.2 % NP-40, 0.2 % BSA, 20 mM PMSF and protease cocktail inhibitor) to cell layer and incubated on ice for 10 minutes. The bottom of the well was scraped and allowed to incubate on ice for an additional 10 minutes. Cell lysates were collected, stored at -80°C, and used for additional analyses..

Drug Preparation

Compound 1 (BK40197) and Compound 2 (BK40143), with molecular weights of 310.1 and 316.4 g/mol, respectively, were diluted in Dimethyl Sulfoxide (DMSO) to final concentrations of 100 µM, 10 µM, 1 µM, 0.1 µM, 0.01 µM, and 0.001 µM. Drugs were stored at -80 °C.

MTT assay

To measure cell viability, cells were incubated with 500 µL of Dulbecco's Modified Eagle's Medium (DMEM) containing 50 µL of (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT)) for 4 hours at 37°C and 5% CO₂. The media was aspirated so that 125 µL of media remained. The formazan salts were dissolved in 250 µL of DMSO. Absorbance was read against a blank containing 125 µL of media and MTT, and 250 µL of DMSO at 570 nm.

Lactate Dehydrogenase (LDH) Assay

Cellular cytotoxicity was quantitatively measured by assessment of LDH, a cytosolic enzyme that is released from damaged cells into the cellular media, after exposure to the drug 5

hours after initial dosage. The cell culture media was collected, and an aliquot was coupled with a lactate and NAD⁺. LDH catalyzes the reaction that converts lactate into pyruvate to produce NADH. NADH, in turn, reduces a tetrazolium salt (INT) into a red formazan product. The amount of LDH in the media is proportional to the amount of formazan, which was measured at 490 nm. The absorbance at 680 nm, to measure the background signal from the instrument, was subtracted from the absorbance at 490 nm to calculate the LDH activity.

Cell Culture Transfection and Treatment

To perform transient transfection of α -synuclein into the rat neuroblastoma B35 cells FuGene® HD Transfection Reagent (Promega Corporation, Madison, WI) was used. Cells were grown in 12-well dishes. A mixture containing 12 μ g of cDNA, 540 μ g of DMEM containing 2% FBS, and 60 μ l of FuGene® HD Transfection Reagent was incubated for 10 minutes. The cells were treated with 50 μ l of the FuGene® HD Transfection Reagent/DNA mixture for 24 hours. Cells were harvested after transfection, media was aspirated, the cells were treated with 200 μ l of Sodium Tris EDTA NP40 (STEN) lysis buffer then scraped off the plate and collected into a 1.5 ml centrifuge tube.

Mouse Treatment

Experiments were conducted on (a) TgAPP mice which express neuronally derived human APP gene, 770 isoform, containing the Swedish (K670N/M671L, Dutch E693Q, and Iowa D694N mutations under the control of the mouse thymus cell antigen 1, theta, Thy1, promoter (Davis et al. "Early-onset and robust cerebral microvascular accumulation of amyloid beta-protein in transgenic mice expressing low levels of a vasculotropic Dutch/Iowa mutant form of amyloid beta-protein precursor," *The Journal of biological chemistry*. 279(19):20296-20306 (2004); (b) rTg4510 mice which express human P301L tau and have the tet-responsive element (TRE or tetO) and mouse prion protein promoter sequences (PrP or Prnp) directing expression of the P301L mutant variant of human four-repeat microtubule-associated protein tau (4R0NTau P301L) (Santacruz et al. "Tau suppression in a neurodegenerative mouse model improves memory function," *Science* 309(5733):476-48 (2005); or (c) TgA53T mice which express mutant arginine to threonine (A53T) human α -synuclein under the control of the prion promoter (Giasson et al. "Neuronal alpha-synucleinopathy with severe movement disorder in mice

expressing A53T human alpha-synuclein," *Neuron* 34(4):521-533 (2002). Mice received treatments of daily intraperitoneal (i.p.) injections of BK40143 (BK) (Medicinal Chemistry Program, Georgetown University), Nilotinib (Nilo) (Cat. #S1033, Selleckchem Inc., Houston, TX), Bosutinib (bos) (Cat. #S1014, Selleckchem Inc.), or combined solutions of BK + Nilo, BK + Bos, or Nilo + Bos at 1.25mg/kg, 2.5mg/kg, or 5.0mg/kg dissolved in dimethyl sulfoxide (DMSO) (Cat. #D128-500, Fisher Scientific, Hampton, NH) or an equivalent dose of DMSO only. Treatment periods were either 7 consecutive days or 21 consecutive days as designated in the figure legends.

Xmap

Xmap technology uses magnetic microspheres that are internally coded with two fluorescent dyes. Through precise combinations of these two dyes, multiple proteins were measured within a sample. Each of these spheres is coated with a specific capture antibody. The capture antibody binds to the detection antibody and a reporter molecule, completing the reaction on the surface of the bead. 25 μ l of soluble brain tissue lysates, from transgenic Tg4510 mice treated with 5 mg/kg, 2.5 mg/kg and 1.25 mg/kg of BK40143 or DMSO were incubated overnight (16-20 hours) at room temperature with 25 μ l of detection antibody solution, and 25 μ l of a mixed bead solution, containing the following analyte: human phospho-tau (181). Following extensive washing of the plate, samples were incubated with 25 μ l of Streptavidin-Phycoerythrin was added to each well and incubated for 30 minutes at room temperature. Samples were then washed and suspended in 100ul of sheath fluid. After resuspension, samples were run on MAGPIX with Xponent software. The Median Fluorescent Intensity (MFI) data was analyzed using a 5- parameter logistic or spline curve-fitting method for calculating analyte concentrations in samples.

Protein Extraction

Brains from treated mice compared with DMSO treated mice were homogenized in STEN lysis buffer [50 mM sodium Tris (pH 7.6), 150 mM NaCl, 2 mM EDTA, 0.2% NP-40, 0.2% BSA, 20 mM PMSF and protease cocktail inhibitor], centrifuged at 10 000g for 20 min at 48 ° C and the supernatant containing the soluble protein fraction was collected. The supernatant was analyzed by Western Blot (WB) on SDS-NuPAGE Bis-Tris gel (Invitrogen, Carlsbad, CA) and ELISA.

Pharmacokinetics studies

C57BL/6J mice were injected one with i.p. injection of BK. Brain and serum were collected at 2, 4, 6, or 12h ($n=18$ per drug, $n=3$ per dose and time point). Animals injected with vehicle (DMSO) were used for background subtraction. Stock solutions of drug (approximately 1 mg/mL each) were prepared in methanol/dichloromethane (50:50). The serial dilutions for each of the standards were produced for the study separately in methanol/HPLC grade water (50:50). Preparation of the calibration curve standards and quality samples (QC) was performed by mixing the stock solutions in blank samples. Serum and brain samples were stored at -80°C then thawed to room temperature prior to preparation. The thawed serum samples (20 μL) were transfused to a tube containing 100 μL of water. The 500 μL extraction solvent, acetonitrile/methanol (50:50) was added to the sample. The mixture was vortexed and incubated on ice for 20min to accelerate protein precipitation. After incubation, the samples were vortexed again and centrifuged at 13,000 rpm for 20 min at 4°C . The supernatant was then collected and transferred to a new tube, dried using speed vac, and reconstituted in 200 μL of methanol/water (50:50). The mixture was spun again at 13,000 rpm for 20 min at 4°C . The supernatant was then collected into a mass spec sample tube cap and run in the mass spectrometer. For the brain, a small section of the thawed brain sample from each animal was transferred to a flat bottom tube. 200 μL of methanol/water (90:10) was added, and the tissue was homogenized. Acetonitrile was then added to the mixture facilitating protein precipitation. The mixture was then incubated on ice for 10 min. After incubation, the samples were vortexed and centrifuged at 13,000 rpm for 20 min at 4°C . The supernatant was then collected and transferred to a new tube, dried using speed vac, and reconstituted in 200 μL of methanol/water (50:50). The mixture was centrifuged at 13,000 rpm for 20 min at 4°C . The supernatant was collected into a mass spec sample tube cap and run in the mass spectrometer. The samples were resolved on an Acquity UPLC BEH C18 1.7 μm , 2.1 $\times$ 50 mm column online with a triple quadrupole mass spectrometer (Xevo-TQ-S, Waters Corporation) operating in the multiple reaction monitoring (MRM) mode. The sample cone voltage and collision energies were optimized for both analytes to obtain maximum ion intensity for parent and daughter ions using "IntelliStart" feature of MassLynx software (Waters Corporation). The instrument parameters were optimized to gain maximum specificity and sensitivity of ionization for the parent [$m/z = 438.25$] and daughter ions [$m/z = 357.33$]. Signal

intensities from all MRM Q1/Q3 ion pairs for analytes were ranked to ensure selection of the most intense precursor and fragment ion pair for MRM-based quantitation. This approach resulted in the selection of cone voltages and collision energies that maximized the generation of each fragment ion species. An analysis was performed with a six to eight-point calibration curve, the sample queue was randomized and solvent blanks were injected to assess sample carryover. MRM data were processed using TargetLynx 4.1. The relative quantification values of analytes were determined by calculating the ratio of peak areas of transitions of samples normalized to the peak area of the internal standard.

Tissue collection and protein extraction.

Animals were deeply anesthetized with a mixture of Xylazine and Ketamine (1:8), and 500µl of whole blood was collected via cardiac puncture, centrifuged at 2000×g to precipitate blood cells and the serum was collected. To wash out the remaining blood from vessels and reduce contamination, animals were perfused with 25ml of 1X phosphate buffered saline (PBS) for 5 mins. Brains were collected and homogenized in 1.0 ml 1x STEN buffer. Homogenized samples were centrifuged at 12,000 x g for 20 min at 4°C and supernatant (soluble protein fraction) was collected and stored at -80°C. Insoluble protein was extracted after removing the supernatant. The tissue pellet was washed with 1X STEN buffer. The pellet was re-suspended in 750ul of 70% formic acid and incubated for 30 min at room temperature followed by a centrifugation at 28,000 g at 4°C for 1hr. The supernatant was collected as the "insoluble fraction." Samples from the 70% formic acid fraction were stored at -80°C and neutralized with 1M Tris-base (1:20) immediately before use. Protein levels were quantified using Pierce BCA protein assay (ThermoFisher, 23225) via manufacturer's instructions.

Immunoblot analysis

Soluble and insoluble proteins extracted from mouse brain lysates were run on SDS NuPAGE Bis-Tris gel (Cat. #NP0301BOX, Invitrogen) and probed for phosphorylated tau with (1:1000) mouse monoclonal AT180 (Cat. #MN1040, ThermoFisher) and (1:1000) mouse monoclonal AT8 (Cat. #MN1020, ThermoFisher), total tau with (1:3000) mouse monoclonal Tau-5 antibody, phosphorylated DDR1 with (1:250) rabbit polyclonal MCK10 (Cat. #PA5-64780, ThermoFisher), ubiquitin with (1:5000) rabbit polyclonal (Cat. #PA3-16717,

ThermoFisher), Atg5 with (1:1000) rabbit monoclonal (Cat. #mAb 12994, Cell Signaling, Danvers, MA), Beclin-1 with (1:1000) rabbit monoclonal (Cat. #mAb 3495, Cell Signaling), and Actin with (1:8000) rabbit polyclonal (Cat. #MAB1501R, EMD Millipore, Burlington, MA). Blots were visualized using Super Signal™ West Dura Extended Duration Substrate (Cat. #37071, ThermoFisher) on the Amersham™ Imager 600 (GE Healthcare Life Sciences, Pittsburgh, PA). Western blots were quantified by densitometry using Image J software.

Enzyme Linked Immunosorbent Assay (ELISA)

Human α -synuclein and p-tau ELISA were performed using 50 μ l (1 μ g/ μ l) of cell lysates detected with 50 μ l primary antibody (3 h) and 100 μ l anti-rabbit secondary antibody (30 min) at RT. α -Synuclein levels were measured using human specific ELISA (Invitrogen Inc., Carlsbad, CA) according to manufacturer's protocols. Tau was measured using specific tau at serine 396 according to manufacturer's protocol. Each sample was duplicated.

ELISAs for total Tau, AB₄₀, and AB₄₂ (Millipore Cat# HNABTMAG 60K) were conducted using Milliplexed ELISA. As set forth above, Xmap technology uses magnetic microspheres that are internally coded with two fluorescent dyes. Through precise combinations of these two dyes, multiple proteins are simultaneously measured within a sample. Each of these spheres is coated with a specific capture antibody. The capture antibody binds to the detection antibody and a reporter molecule, completing the reaction on the surface of the bead. All samples including placebo and resveratrol at baseline and 52 weeks were analyzed in parallel using the same reagents. A total of 25 μ l soluble protein was incubated overnight at 4 °C with 25 μ l of a mixed bead solution containing total Tau, AB₄₀, and AB₄₂. After washing, samples were incubated with 25 μ l detection antibody solution for 1.5 h at room temperature. Streptavidin-phycoerythrin (25 μ l) was added to each well containing the 25 μ l of detection antibody solution. Samples were then washed and suspended in 100 μ l of sheath fluid. Samples were then run on MAGPIX with Xponent software. The median fluorescent intensity (MFI) data were analyzed using a 5-parameter logistic or spline curve-fitting method for calculating analyte concentrations in samples. Specific p-Tau ser396 (Invitrogen, KHB7031), human Tau thr181 (Invitrogen, KHO0631) and A β 1-42 (Invitrogen, KHB3442) were performed according to manufacturer's protocol on tissue soluble extracts from midbrain lysates in IXSTEN buffer (see above).

Behavior

Rotarod: mice were placed on an accelerating rod (Cat. #76-0770, Panlab, Harvard Apparatus) equipped with individual timers for each mouse. Mice were tested over 4 trials, 3 training and 1 testing. Mice were trained to stay on the rod at a constant 4 rotations per minute (rpm) for at least 5 minutes, and then the speed will gradually increase to 40 rpm over 300 seconds and the latency to fall was measured.

Open-field: Mice were placed in the open field arena apparatus (25 cm x 25 cm) for 60 minutes. Animals were tracked by photocell beams along the arena floor. Data were collected and analyzed for total distance traveled (cm), total time spent moving (sec), and velocity (distance/time) during the 60-minute trial. A center zone was digitally defined in the software as (25 cm x 25 cm) in the center of the apparatus and center zone entries, center zone distance travelled (cm), and time spent in center zone (sec) during the 60-minute trial were recorded.

Morris water maze: The water maze apparatus consisted of a 4-foot-diameter pool (San Diego Instruments) filled with water maintained at 25°C and made opaque with white paint and digitally divided into 4 quadrant zones (ANYMaze software, San Diego Instruments). Extra-maze visual cues were hung on the walls surrounding the pool and a hidden platform (4 inches in diameter) was submerged 1 cm below the surface of the water in the center of the 'platform zone'. Training consisted of three trials per day for four days leading up to a probe trial on the fifth day. The mice were introduced into the pool at one of three entry points, one per non-platform quadrant zone, with every entry point used over the course of the day. The location of the platform remained constant throughout the entire training period. The mice were given 60 sec to locate the platform, and remained on the platform for 10 sec before being removed. Mice that did not locate the platform within 60 sec were placed on the platform for 10 sec before removal from the maze. During the probe trial on the fifth day, the platform is removed and the tracking software (ANYMaze) was used to record latency to find the platform, platform quadrant zone, swim speed, and swim path. This training and probe trial paradigm was conducted pre-treatment and post-treatment.

Marble burial test: Marble burying test was performed as previously described [35] with modifications. Briefly, 20 marbles of 15 mm in diameter were spaced by 4 cm in five rows of four marbles each on a surface of gently packed 5-cm-deep corncob bedding in a double-sized rat cage. A mouse was left alone in the cage for 30 minutes. An observer blinded to the treatment

counted the number of marbles buried. Any marble buried more than two-thirds of its size was counted. Each mouse was assessed once at pre- and post- treatment and data were reported as average $\pm$ SEM of the percentage of buried marbles per animal. Kuskal-Wallis tests followed by Wilcoxon post hoc test was used to determine statistical significance of marble burying in mice treated with drug or DMSO.

Statistical analysis

All statistical analysis was performed using GraphPad Prism, version 8.0 (GraphPad software Inc.). For experiments involving mice, sample size (n) and female:male ratios used in each experiment are indicated in the figure legends. For experiments using cell lines the number of independent biological replicates is reported (N). Data are presented as mean $\pm$ SEM. When comparing averages in two groups, two-tailed Students *t* test or Welch's *t* test was performed. When comparing the averages on multiple groups, one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test was performed. Asterisks or the pound symbol denote actual p-value significances (* <0.05 , ** <0.01 , *** <0.001 , **** <0.0001) between groups or within groups and is noted in the individual figure legends.

Results

As shown in Fig. 1 (left panel and middle panel), after 16 hours of treatment, neuroprotective effects were observed in B35 cells treated with 1 μ M BK41043, as evidenced by the decrease in LDH and the increase in MTT, respectively, as compared to control. Fig. 1 (right panel) shows that, after five hours of treatment, there was a stepwise increase in cell viability, via decrease in LDH, with decreasing concentrations of BK40143.

Fig. 2 shows cell viability of B35 cells after 24 hour transfection with pTau (left panel) or α -synuclein (right panel) and five hours of treatment with BK41043.

B35 cells were grown in complete media and transfected with cDNA for human mutant Tau and alpha-synuclein using FuGene HD transfection reagents according to manufacturer's instructions. At 24 hours the transfection had produced significantly higher amounts of phospho-Tau and alpha-synuclein compared to non-transfected cells via ELISA. Fig. 3 shows the level of pTau (181) after 24 hour transfection (left panel) and that BK41043 reduces pTau (181) levels in pTau transfected B35 cells (right panel) back to control levels. This occurred without any

change to LDH levels indicating no increased toxicity during the experiment. Fig. 4 shows the level of α -synuclein after 24 hour transfection (left panel) and that BK41043 did not reduce α -synuclein in α -synuclein-transfected B35 cells (right panel).

Fig. 5 shows cell viability of B35 cells after five hours of treatment with BK40197.

Fig. 6 shows that BK40143 reduces pTau (181) in Tau expressing transgenic mice, after seven days of treatment. The levels of pTau(181) were measured via ELISA.

Fig. 7 shows that treatment with BK-40143 (1.25 mg/kg, 2.5 mg/kg or 5.0 mg/kg) did not affect the levels of pTau (231) (AT180) in transgenic mice.

Fig. 8 shows that Tau (HT7) was significantly reduced in Tau transgenic mice after seven days of treatment with BK-41043, at 1.25 mg/kg and 2.5 mg/kg.

Fig. 9 shows that treatment with BK-40143 (1.25 mg/kg or 2.5 mg/kg) results in *in vivo* inhibition of DDR1, as measured by detection of phosphorylated DDR1 (pMCK10).

Fig. 10 shows that pTau was reduced in Camp301L mice after treatment with 2.5 mg/kg or 5 mg/kg BK-40143.

As shown in Fig. 11, BK40196 reduced alpha-synuclein at high concentrations and BK40197 does not reduce alpha-synuclein. 1mM and 100uM of BK40196 significantly reduced the level of alpha-synuclein in transfected B35 cells (Fig. 11A). BK40197 does not display any ability to reduce alpha-synuclein levels in transfected B35 cells (Fig. 11B).

As shown in Fig. 12A, BK-40143 significantly decreased alpha-synuclein in A53T mice, compared to DMSO treated control A53T mice. C57BL/6J mice were used as controls and show no detectable (N.D.) human alpha-synuclein. Fig. 12B shows that BK-40143 insignificantly increases (about 30%) the overall level of dopamine, however BK-40143 did increase the level of the dopamine metabolite, homovanillic acid (HVA), in A53T mice indicating more dopamine turnover which could result in better dopamine neurotransmission. The immunoblots shown in Figs. 12C and 12D for alpha-synuclein (ThermoFisher, MA1-12874) mirrored the 40% reduction in alpha-synuclein seen in the ELISA.

Animal studies also showed that BK-40143 improved velocity of locomotion in A53T mice. A53T mice were tested on the open field test for overall locomotor ability over a 60-minute trial. Although the mice did not show any difference in the total distance travelled or total time spent moving, the velocity of their movements significantly increased with BK-40143

treatment (Fig. 13). This could be a reflection of better dopamine neurotransmission as indicated by the increased levels of HVA after BK (Fig. 12B).

It was also shown that BK40143 selectively deactivates DDRs but not Src or Abl and reduces phosphorylated tau in the rTG4510 tauopathy mouse model. Male and female, 3-month-old rTG4530 mice were treated i.p. with 1.25mg/kg, 2.5mg/kg, or 5mg/kg of BK40143 or DMSO for 7 consecutive days. Fig. 14A, an immunoblot probing for activated (phosphorylated) DDR1, demonstrates that 1.25 and 2.5 but not 5 mg/kg of BK40143 deactivated DDR1. Fig. 14B, an immunoblot probing for activated Src, demonstrates that BK-40143 does not engage this tyrosine kinase. Fig. 14C, an immunoblot probing for activated Abl, demonstrates that BK-40143 does not engage this tyrosine kinase, i.e., DDR1. Upon probing for phosphorylated Tau (AT8), Fig. 14D shows that all three doses of BK-40143 reduced the levels of phosphorylated tau by 41-49% percent. As shown in Fig. 14E, an ELISA for phosphorylated Tau (AT181) revealed that that 2.5mg/kg of BK40143 significantly reduces phosphorylated Tau.

BK40143 also significantly reduced amyloid, phosphorylated tau, and deactivated DDR1. Male and female, 7-month-old APP mice were treated i.p. with 1.25 and 2.5mg/kg of BK40143 or DMSO for 21 consecutive days. Immunoblotting for aggregating extracellular amyloid-beta (6E10) demonstrated that 1.25 and 2.5mg/kg of BK40143 significantly reduced amyloid-beta plaques (Fig. 15A). Probing for phosphorylated DDR1 demonstrated that 1.25 and 2.5mg/kg of BK40143 deactivated DDR1 by 40% and 31%, respectively (Fig. 15B). Probing for activated (phosphorylated Abl (245) demonstrated that BK40143 does not engage Abl (Fig. 15C). Figs 15D and 15E show that 1.25 and 2.5mg/kg BK-40143 significantly reduced soluble human amyloid-beta via ELISA, but did not significantly reduce the insoluble amyloid-beta. Also shown is that 2.5mg/kg of BK40143 significantly reduces human phosphorylated tau (Ser396) by over 80% (Fig. 15F).

Also shown is that BK40143 could improve performance on Morris Water Maze test for cognition in APP mice. APP mice were tested on the morris water maze post-treatment for their ability to find a target platform. Measurements include the number of platform entries (Fig. 16, left panel), the latency to the first entry (Fig. 16, middle panel), and the distance travelled before their first entry to the platform (Fig. 16, right panel). Although there was no significant difference between groups, 1.25mg/kg of BK-40143 shows a trend for increased performance with higher

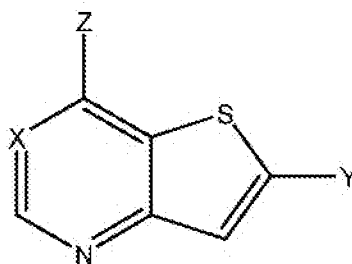
numbers of platform entries, and a lower latency to first entry and lower distance travelled before the first entry (Fig. 16).

Studies also showed that BK40143 does not cause cell death in the hippocampus of APP mice. Representative 20um hippocampal sections were stained for Nissl substance. 4x and 20x images for DMSO, 1.25mg/kg, and 2.5mg/kg of BK40143 are shown in Fig. 17 (left panel). Mean staining intensity was quantified in imagej software as the total amount of Nissl staining in all 4x images (Fig. 17, right panel).

These data suggest that a compound of Formula I, for example, BK41043 or BK40197 can be used to treat or prevent a neurodegenerative disorder, a myodegenerative disorder or a lysosomal storage disorder.

What is claimed is:

1. A compound having the following formula:



wherein

X is N or CH;

Y is C₆₋₁₀ aryl unsubstituted or substituted with R¹; or C₃₋₁₀ heteroaryl unsubstituted or substituted with R¹, or N-methylpiperazinyl;

R¹ is -(CH₂)_n-R², -(CH₂)_n-C(O)-R², or -O(CH₂)_n-R²;

R² is -H, -CN, halogen, C₁₋₃ alkyl, C₁₋₃ alkoxy, phenyl, pyridinyl, amino, C₁₋₃ alkyl amino, di C₁₋₃ alkyl amino, hydroxyl C₁₋₃ alkyl amino, carboxy C₁₋₃ alkyl amino, C₃₋₆ cycloalkyl C₁₋₃ alkylamino, pyrrolidinyl, hydroxyl pyrrolidinyl, hydroxyl C₁₋₃ alkylpyrrolidinyl, carboxypyrrolidinyl, piperidinyl, C₁₋₃ alkylpiperidinyl, di C₁₋₃ alkyl piperidinyl, piperazinyl, C₁₋₃ alkylpiperazinyl, C₁₋₄ alkoxy carbonylpiperazinyl, or morpholinyl;

Z is heteroaryl, heterocyclyl, or NR³R⁴;

R³ and R⁴ are independently H, C₁₋₃ alkyl, C₁₋₃ alkoxy, or unsubstituted phenyl,

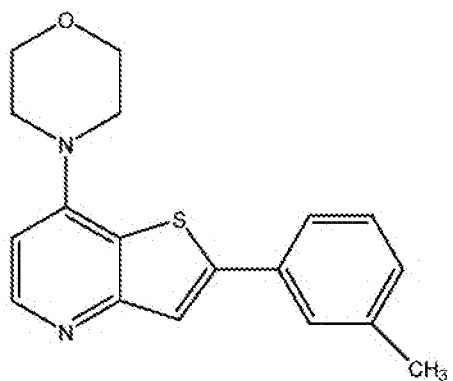
and

n is an integer selected from 0 to 3,

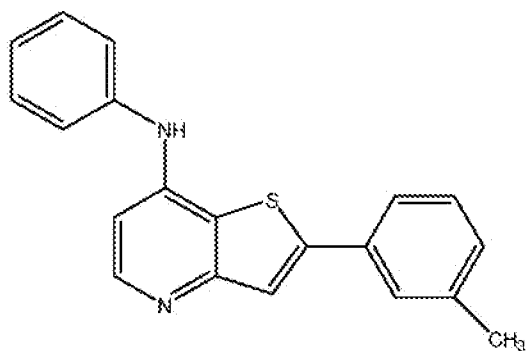
or an isomer or pharmaceutically acceptable salt thereof.

2. The compound of claim 1, wherein the compound does not comprise one or more halogen atoms.
3. The compound of claim 1, wherein Y is 2-*m*-toluyl.
4. The compound of claim 1, wherein Z is heterocyclyl.

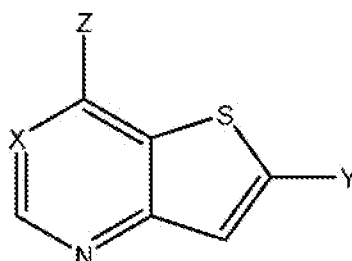
5. The compound of claim 4, wherein Z is morpholin-1-yl.
6. The compound of claim 1, wherein the compound has the following formula:



7. The compound of claim 1, wherein R³ is H and R⁴ is unsubstituted phenyl.
8. The compound of claim 1, wherein the compound has the following formula:



9. A method of treating or preventing a neurodegenerative disease, a myodegenerative disease or prion disease in a subject, comprising administering to the subject with the neurodegenerative disease of the central nervous system, the myodegenerative disease, or the prion disease or at risk for developing the neurodegenerative disease of the central nervous system, the myodegenerative disease or the prion disease an effective amount of a compound having the following formula:



wherein,

X is N or CH;

Y is C₆₋₁₀ aryl unsubstituted or substituted with R¹; or C₃₋₁₀ heteroaryl unsubstituted or substituted with R¹, or N-methylpiperazinyl;

R¹ is $-(CH_2)_n-R^2$, $-(CH_2)_n-C(O)-R^2$, or $-O(CH_2)_n-R^2$;

R² is -H, -CN, halogen, C₁₋₃ alkyl, C₁₋₃ alkoxy, phenyl, pyridinyl, amino, C₁₋₃ alkyl amino, di C₁₋₃ alkyl amino, hydroxyl C₁₋₃ alkyl amino, carboxy C₁₋₃ alkyl amino, C₃₋₆ cycloalkyl C₁₋₃ alkylamino, pyrrolidinyl, hydroxyl pyrrolidinyl, hydroxyl C₁₋₃ alkylpyrrolidinyl, carboxypyrrolidinyl, piperidinyl, C₁₋₃ alkylpiperidinyl, di C₁₋₃ alkyl piperidinyl, piperazinyl, C₁₋₃ alkylpiperazinyl, C₁₋₄ alkoxy carbonylpiperazinyl, or morpholinyl;

Z is heteroaryl, heterocyclyl, or NR³R⁴;

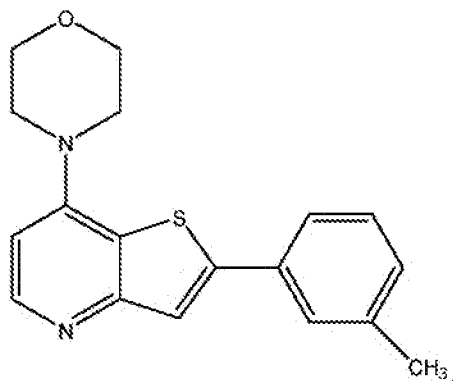
R³ and R⁴ are independently H, C₁₋₃ alkyl, C₁₋₃ alkoxy, or unsubstituted phenyl,

and

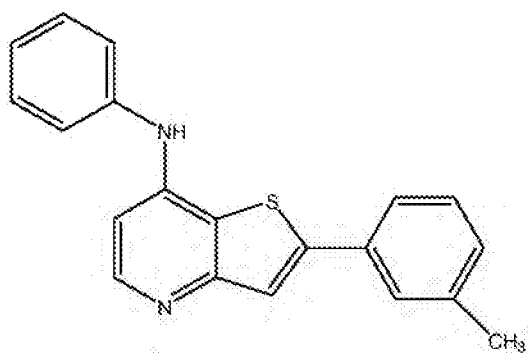
n is an integer selected from 0 to 3,

or an isomer or pharmaceutically acceptable salt thereof.

10. The method of claim 9, wherein the compound has the following formula:

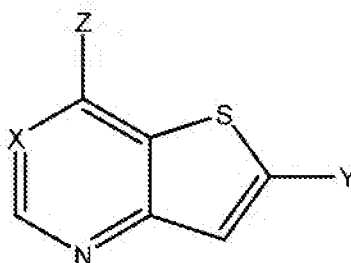


11. The method of claim 9, wherein the compound has the following formula:



12. The method of any one of claims 9-11, wherein the compound crosses the blood brain barrier.
13. The method of any one of claims 9-12, wherein the central nervous system neurodegenerative disease is selected from the group consisting of Amyotrophic Lateral Sclerosis, Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Mild Cognitive Impairment, an α -Synucleinopathy and a Tauopathy.
14. The method of any one of claims 9-13, wherein the compound is administered systemically.
15. The method of claim 13, wherein the compound is administered orally.
16. The method of any one of claims 9-15, wherein the compound is administered at a dosage of 10 mg/kg or less.

17. The method of claim any of claims 9-16, wherein the compound is administered at a dosage of 5 mg/kg or less.
18. The method of any one of claims 9-17, wherein the compound is administered at a dosage of 2.5 mg/kg or less.
19. The method of any one of claims 9-18, wherein the compound is administered daily.
20. The method of any one of claims 9-19, wherein the compound is in a pharmaceutical composition.
21. The method of any one of claims 9-20, further comprising administering a second therapeutic agent to the subject.
22. The method of claim 21, wherein the second therapeutic agent is selected from the group consisting of levadopa, a dopamine agonist, an anticholinergic agent, a monoamine oxidase inhibitor, a COMT inhibitor, amantadine, donepezil, memantine, risperidone, rivastigmine, an NMDA antagonist, an acetylcholinesterase inhibitor, a cholinesterase inhibitor, riluzole, an anti-psychotic agent, an antidepressant, and tetrabenazine.
23. A method of inhibiting or preventing toxic protein aggregation in a neuron comprising contacting the neuron with an effective amount of a composition comprising a compound having the following formula:



wherein,

X is N or CH;

Y is C₆₋₁₀ aryl unsubstituted or substituted with R¹; or C₅₋₁₀ heteroaryl unsubstituted or substituted with R¹, or N-methylpiperazinyl;

R^1 is $-(CH_2)_n-R^2$, $-(CH_2)_n-C(O)-R^2$, or $-O(CH_2)_n-R^2$;

R^2 is $-H$, $-CN$, halogen, C_{1-3} alkyl, C_{1-3} alkoxy, phenyl, pyridinyl, amino, C_{1-3} alkyl amino, di C_{1-3} alkyl amino, hydroxyl C_{1-3} alkyl amino, carboxy C_{1-3} alkyl amino, C_{3-6} cycloalkyl C_{1-3} alkylamino, pyrrolidinyl, hydroxyl pyrrolidinyl, hydroxyl C_{1-3} alkylpyrrolidinyl, carboxypyrrolidinyl, piperidinyl, C_{1-3} alkylpiperidinyl, di C_{1-3} alkyl piperidinyl, piperazinyl, C_{1-3} alkylpiperazinyl, C_{1-4} alkoxy carbonylpiperazinyl, or morpholinyl;

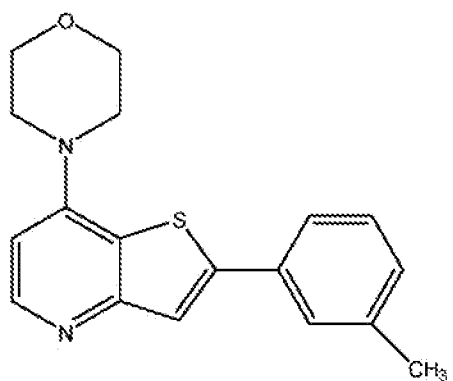
Z is heteroaryl, heterocyclyl, or NR^3R^4 ;

R^3 and R^4 are independently H , C_{1-3} alkyl, C_{1-3} alkoxy, or unsubstituted phenyl, and

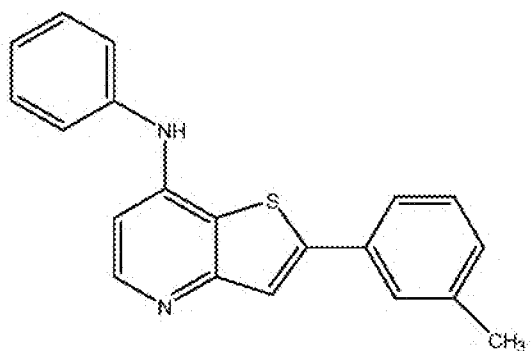
n is an integer selected from 0 to 3,

or an isomer or pharmaceutically acceptable salt thereof.

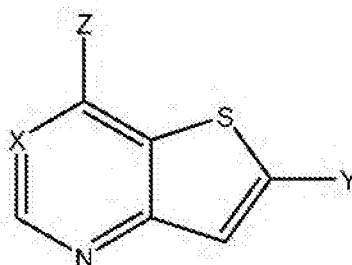
24. The method of claim 23, wherein the compound has the following formula:



25. The method of claim 23, wherein the compound has the following formula:



26. The method of any one of claims 23-25, wherein the protein is selected from the group consisting of an amyloidogenic protein, alpha-synuclein, tau and TDP-43.
27. The method of any of claims 23-26, wherein the contacting is performed *in vitro*.
28. The method of any of claims 23-26, wherein the contacting is performed *in vivo*.
29. A method of treating or preventing a lysosomal storage disease (LSD) in a subject, comprising administering to the subject having the LSD an effective amount of a compound having the following formula:



wherein,

X is N or CH;

Y is C₆₋₁₀ aryl unsubstituted or substituted with R¹; or C₅₋₁₀ heteroaryl unsubstituted or substituted with R¹, or N-methylpiperazinyl;

R¹ is -(CH₂)_n-R², -(CH₂)_n-C(O)-R², or -O(CH₂)_n-R²;

R² is -H, -CN, halogen, C₁₋₃ alkyl, C₁₋₃ alkoxy, phenyl, pyridinyl, amino, C₁₋₃ alkyl amino, di C₁₋₃ alkyl amino, hydroxyl C₁₋₃ alkyl amino, carboxy C₁₋₃ alkyl amino, C₃₋₆ cycloalkyl C₁₋₃ alkylamino, pyrrolidinyl, hydroxyl pyrrolidinyl, hydroxyl C₁₋₃ alkylpyrrolidinyl, carboxypyrrolidinyl, piperidinyl, C₁₋₃ alkylpiperidinyl, di C₁₋₃ alkyl piperidinyl, piperazinyl, C₁₋₃ alkylpiperazinyl, C₁₋₄ alkoxy carbonylpiperazinyl, or morpholinyl;

Z is heteroaryl, heterocyclyl, or NR³R⁴;

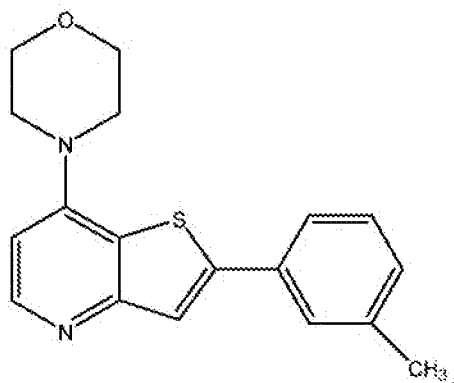
R³ and R⁴ are independently H, C₁₋₃ alkyl, C₁₋₃ alkoxy, or unsubstituted phenyl,

and

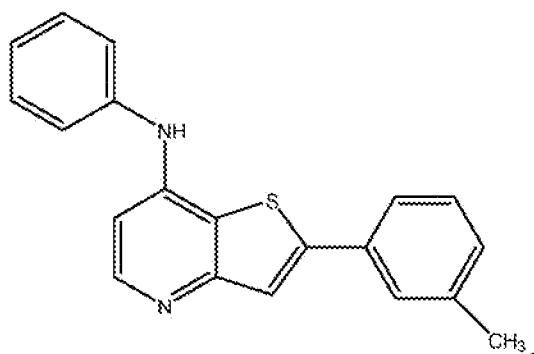
n is an integer selected from 0 to 3,

or an isomer or pharmaceutically acceptable salt thereof.

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



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



32. The method of any one of claims 29-31 wherein the lysosomal storage disease is selected from the group consisting of Hurler syndrome, Hurler-Scheie syndrome, Scheie syndrome, Hunter syndrome, Sanfillipo syndrome A, Sanfillipo syndrome B, Sanfillipo syndrome C, Sanfillipo syndrome D, Morquio syndrome A, Morquio syndrome B, Maroteaux-Lamy syndrome, Sly syndrome, Natowicz syndrome, Pseudo-Hurler polydystrophy, Tay-Sachs, Gaucher disease, Niemann-Pick disease, Fucosidosis, Galactosialidosis, Globoid-cell leukodystrophy, G_{M1} Gangliosidosis, G_{M2} Gangliosidosis, α -Mannosidosis, Metachromatic leukodystrophy, Niemann-Pick AB disease and Pompe disease.

33. The method of any one of claims 29-32, wherein the subject is a pediatric subject.
34. The method of any one of claims 29-33, wherein the effective amount of compound inhibits or prevents toxic substance aggregation in one or more cells of the subject.
35. The method of claim 34, wherein the one or more cells are brain cells, cells in one or more peripheral tissues of the subject, or a combination thereof.
36. The method of claim 35, wherein the brain cells are neurons and/or glial cells.
37. The method of any one of claims 29-36, wherein the effective amount is less than about 10 mg/kg.
38. The method of any of claims 29-37, further comprising administering a second therapeutic agent or therapy to the subject.
39. The method of claim 38, wherein the second therapeutic agent or therapy is selected from the group consisting of: enzyme replacement therapy, gene therapy, a hematopoietic stem cell transplant and a small molecule.

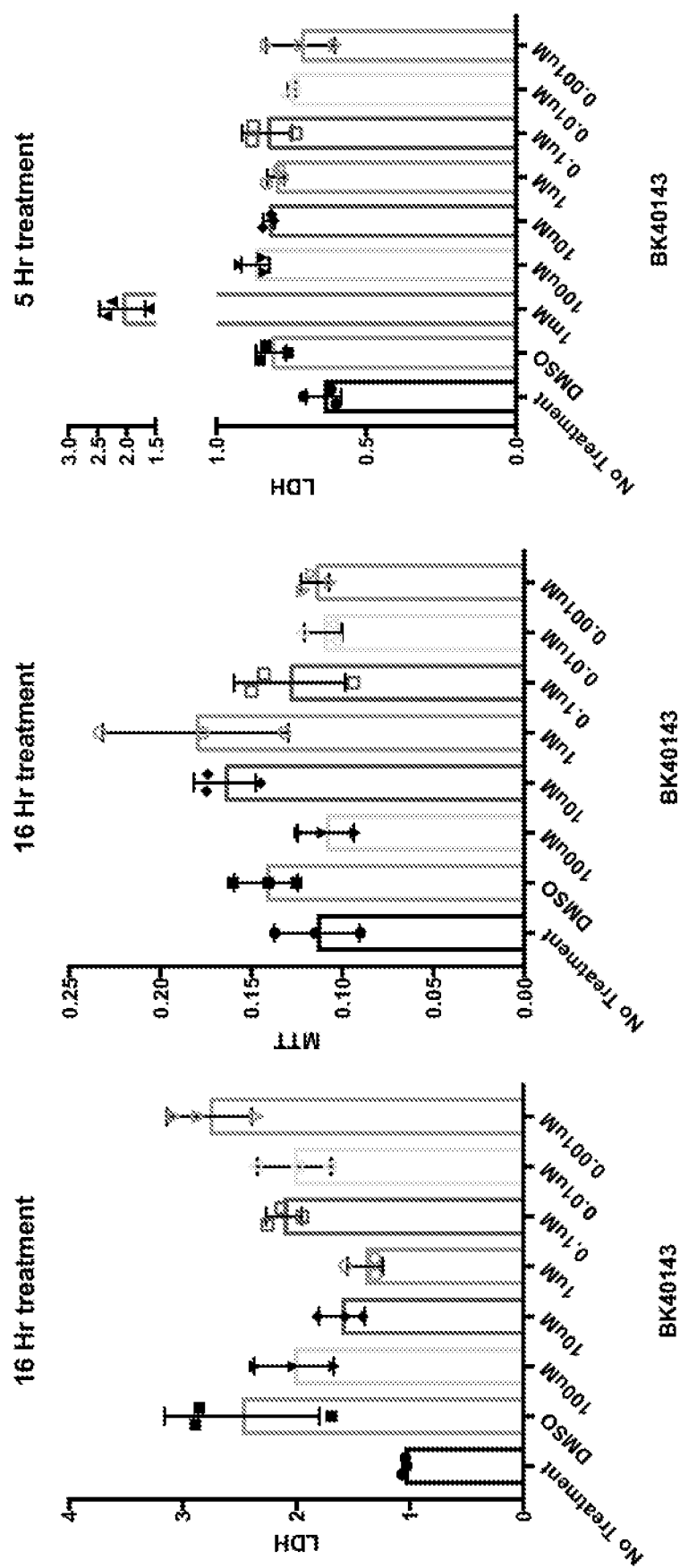
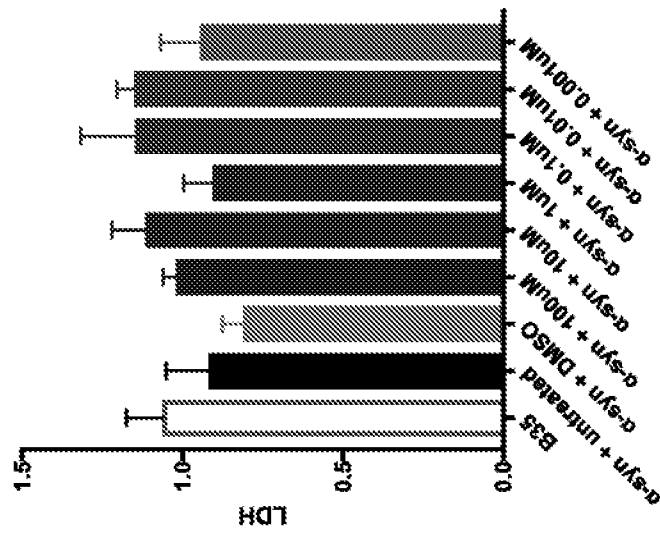


FIG. 1

α -synuclein transfected B35 cells
5 hour treatment BK40143



pTau (181) transfected B35 cells
5 hour treatment BK40143

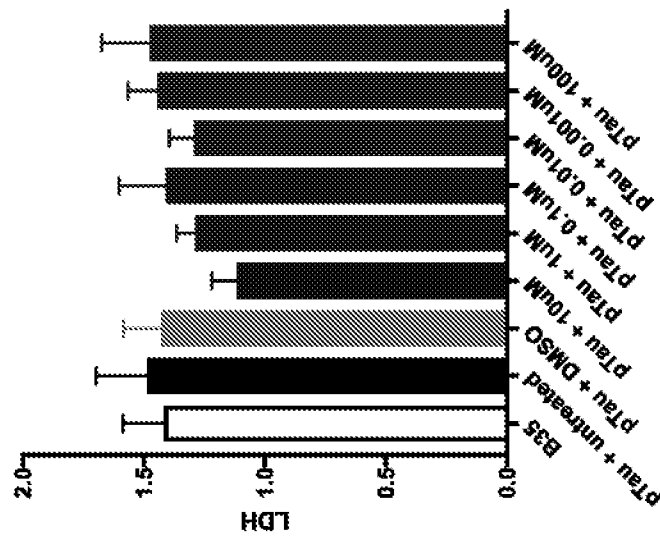


FIG. 2

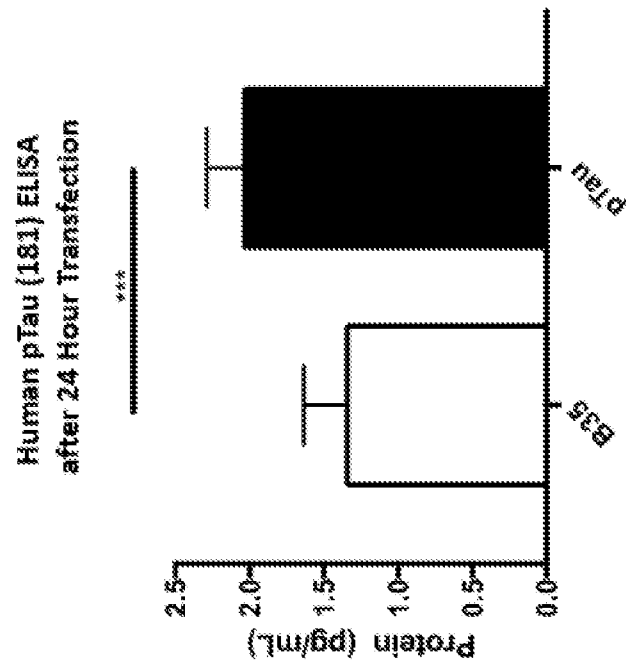
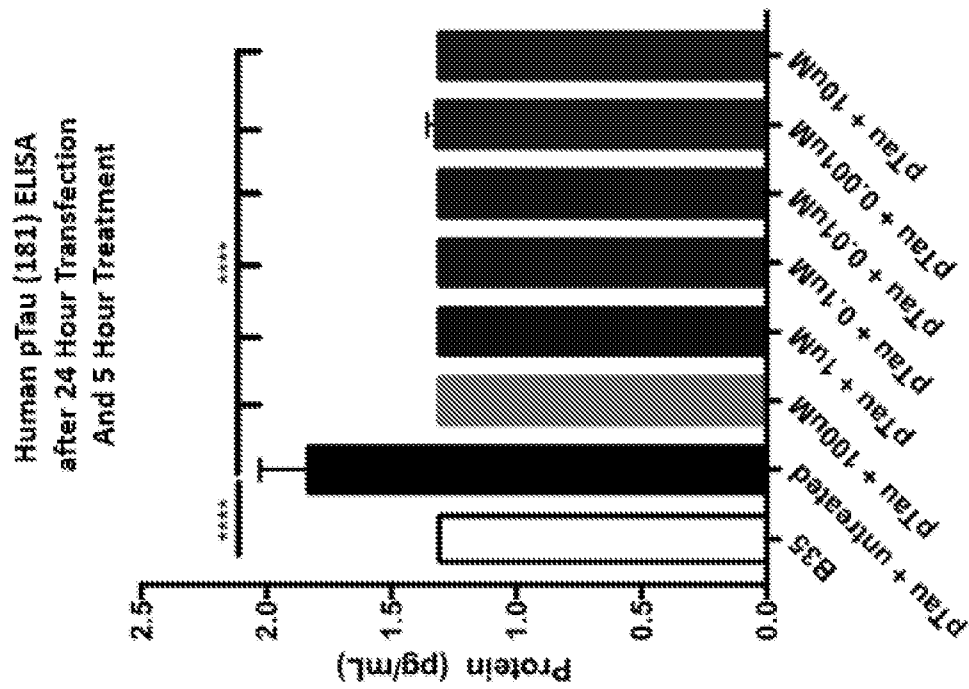


FIG. 3

α -synuclein transfected B35 cells
5 hour treatment BK40143

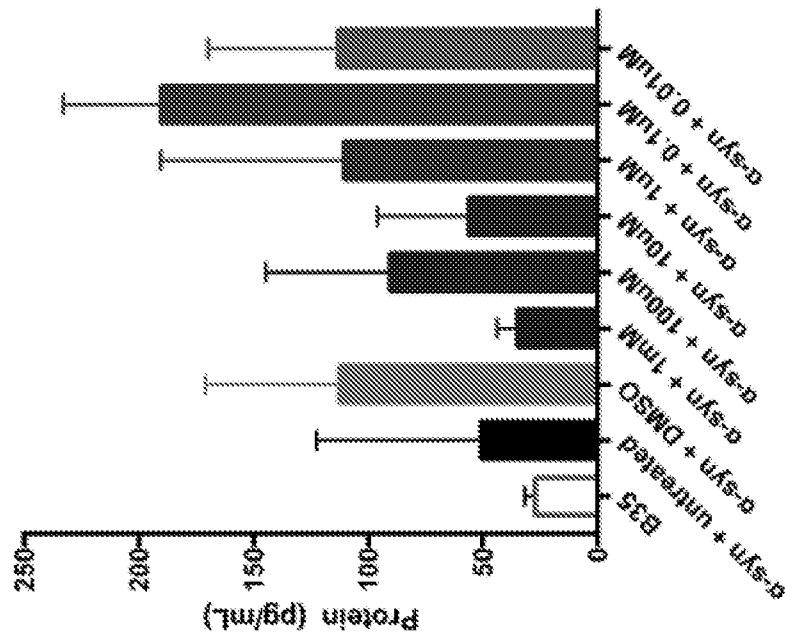
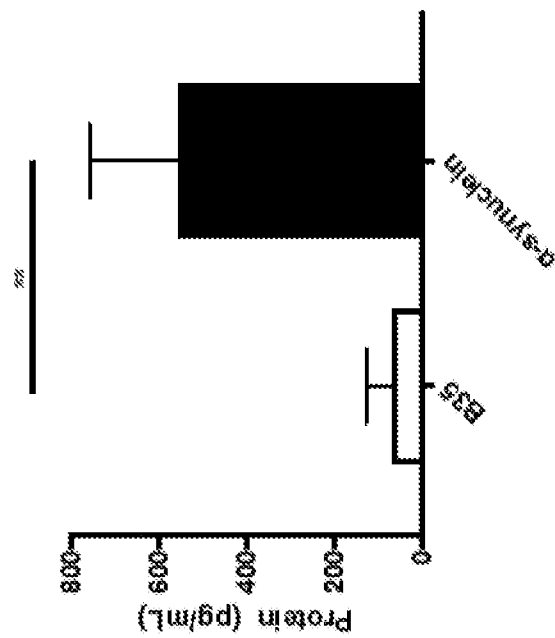


FIG. 4

α -Synuclein ELISA
after 24 Hour Transfection



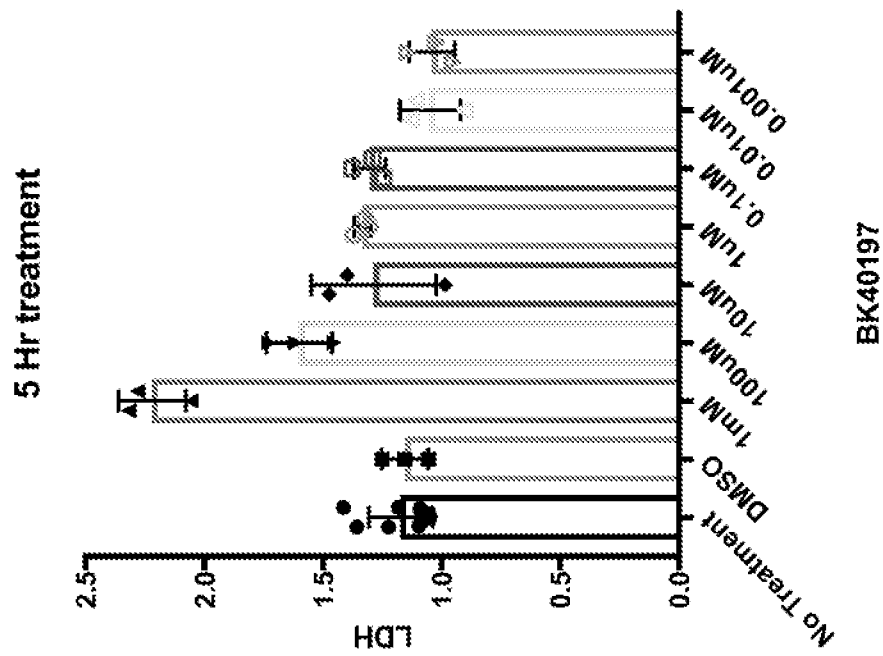


FIG. 5

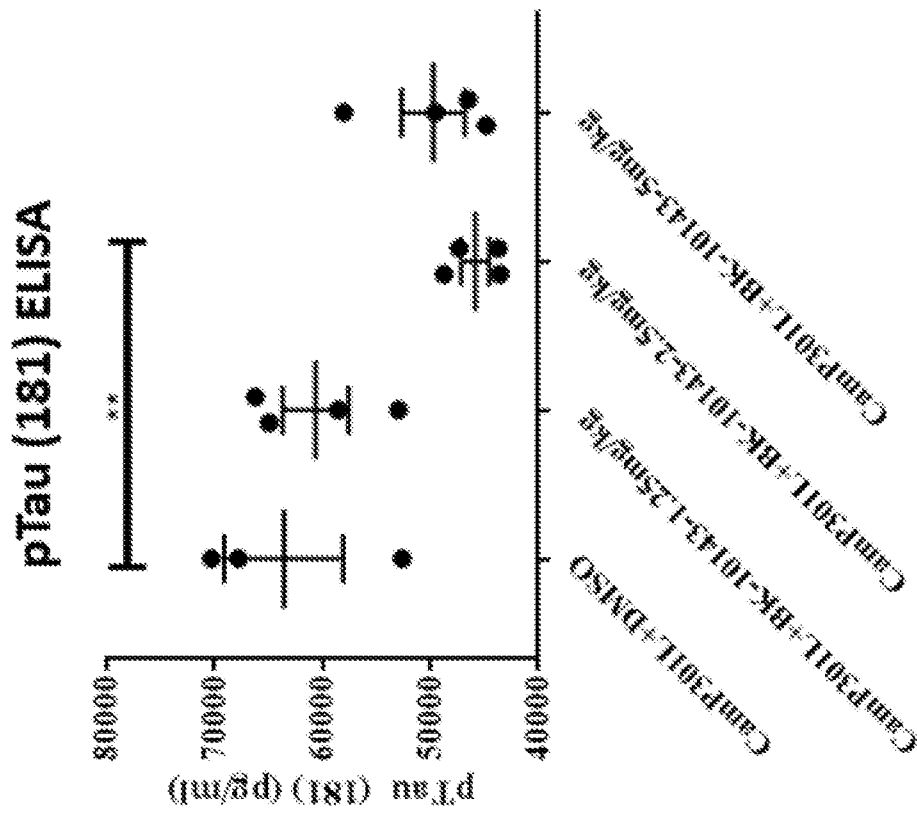


FIG. 6

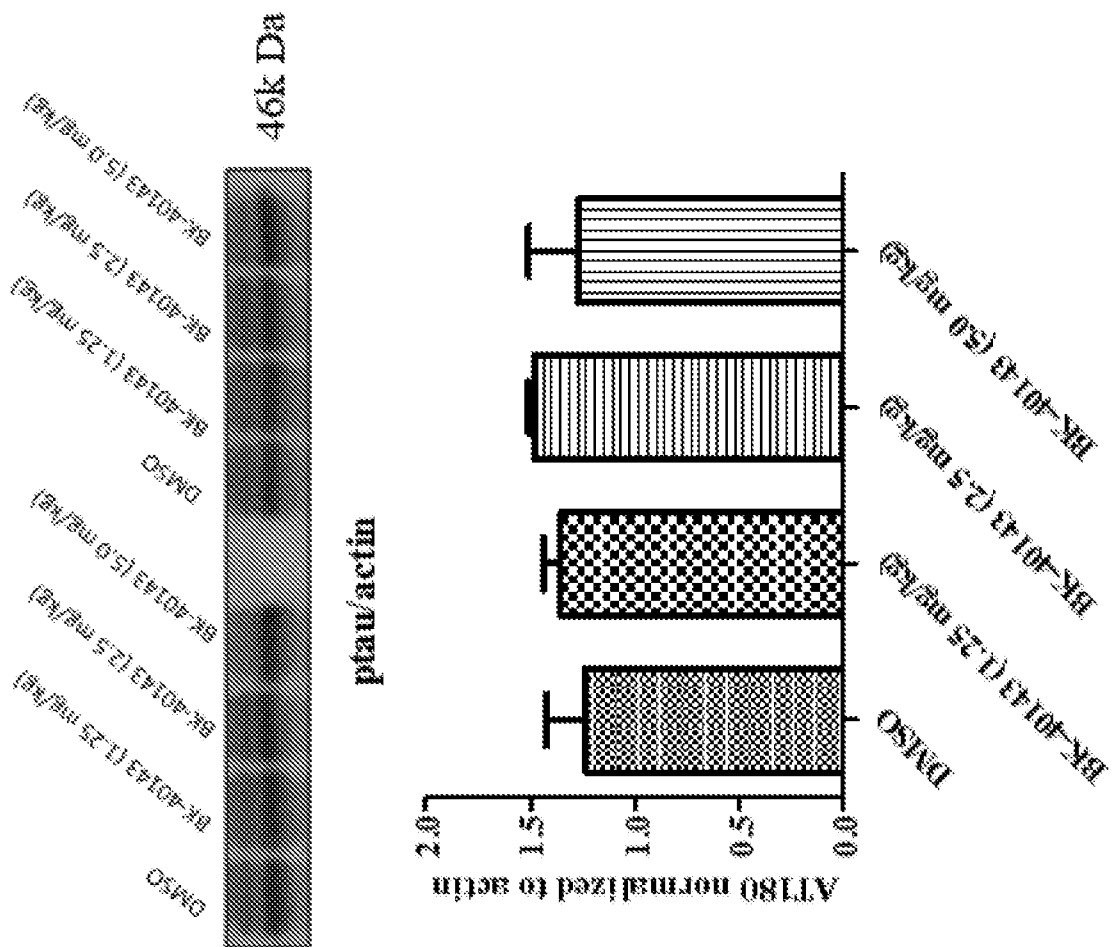


FIG. 7

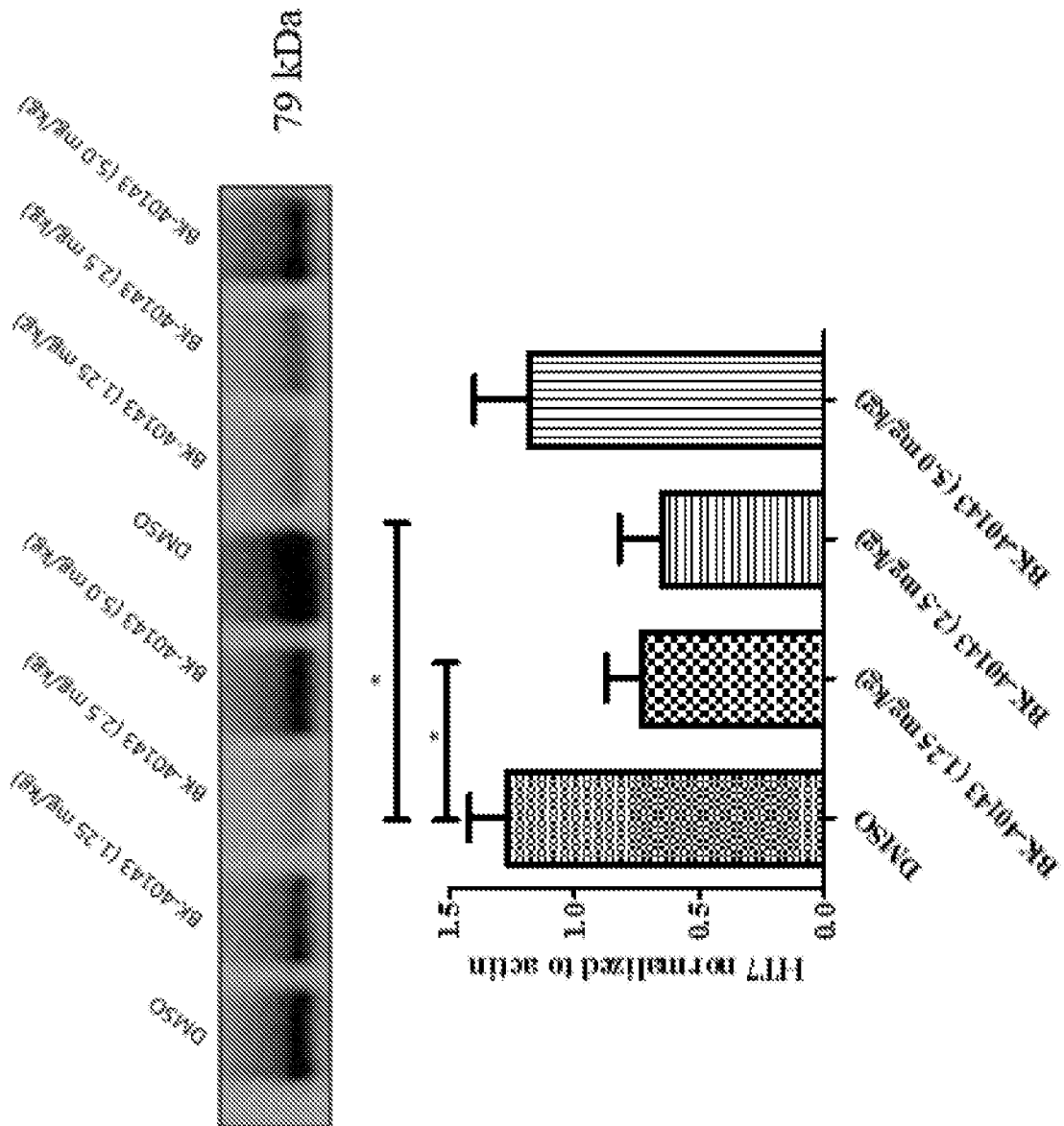


FIG. 8

9/17

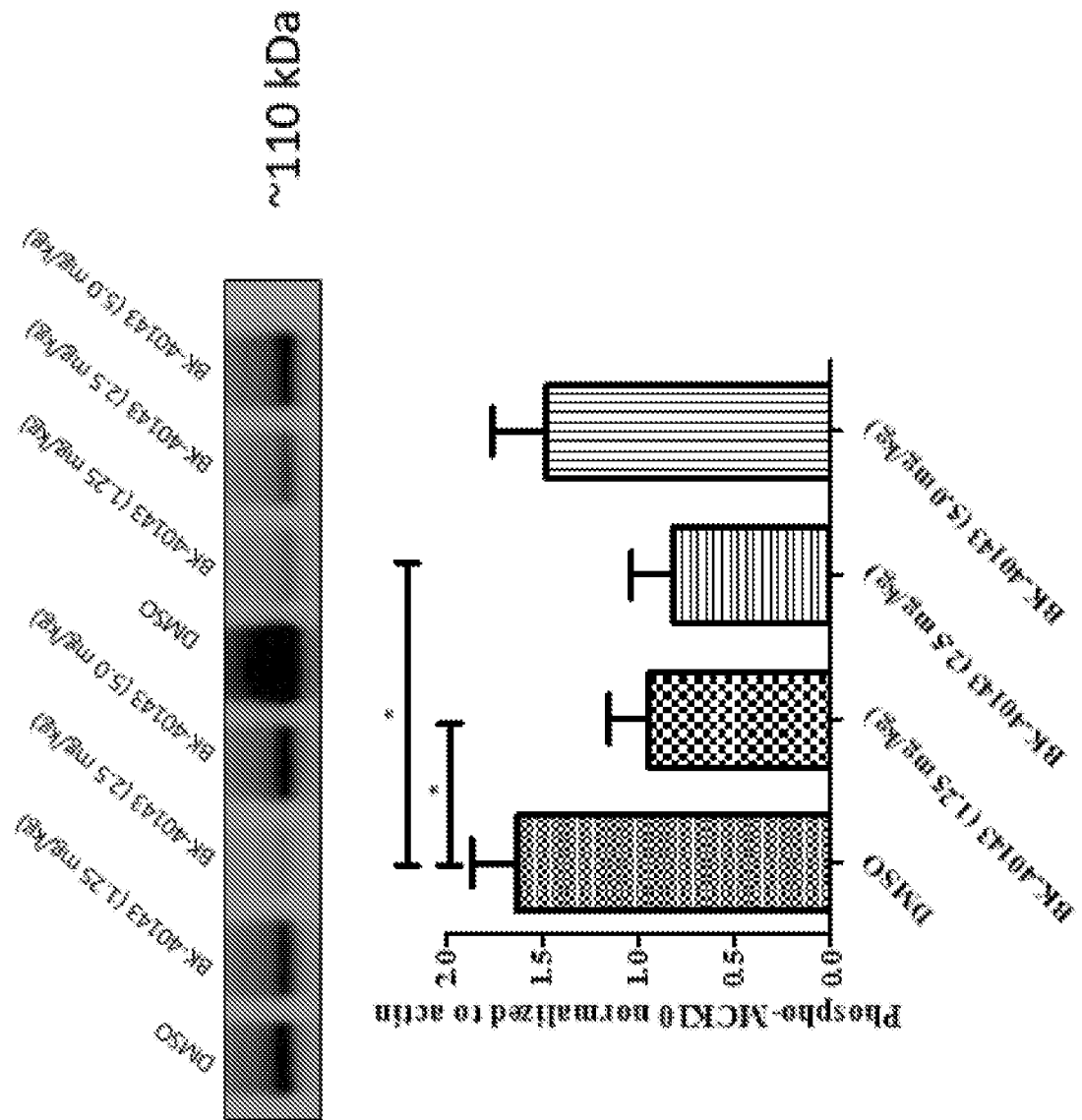


FIG. 9

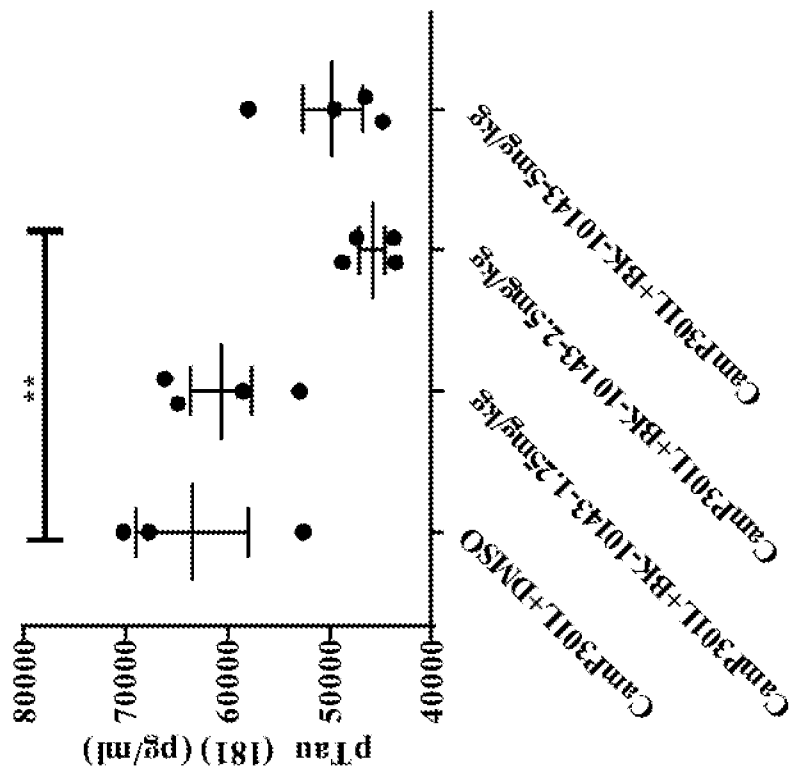
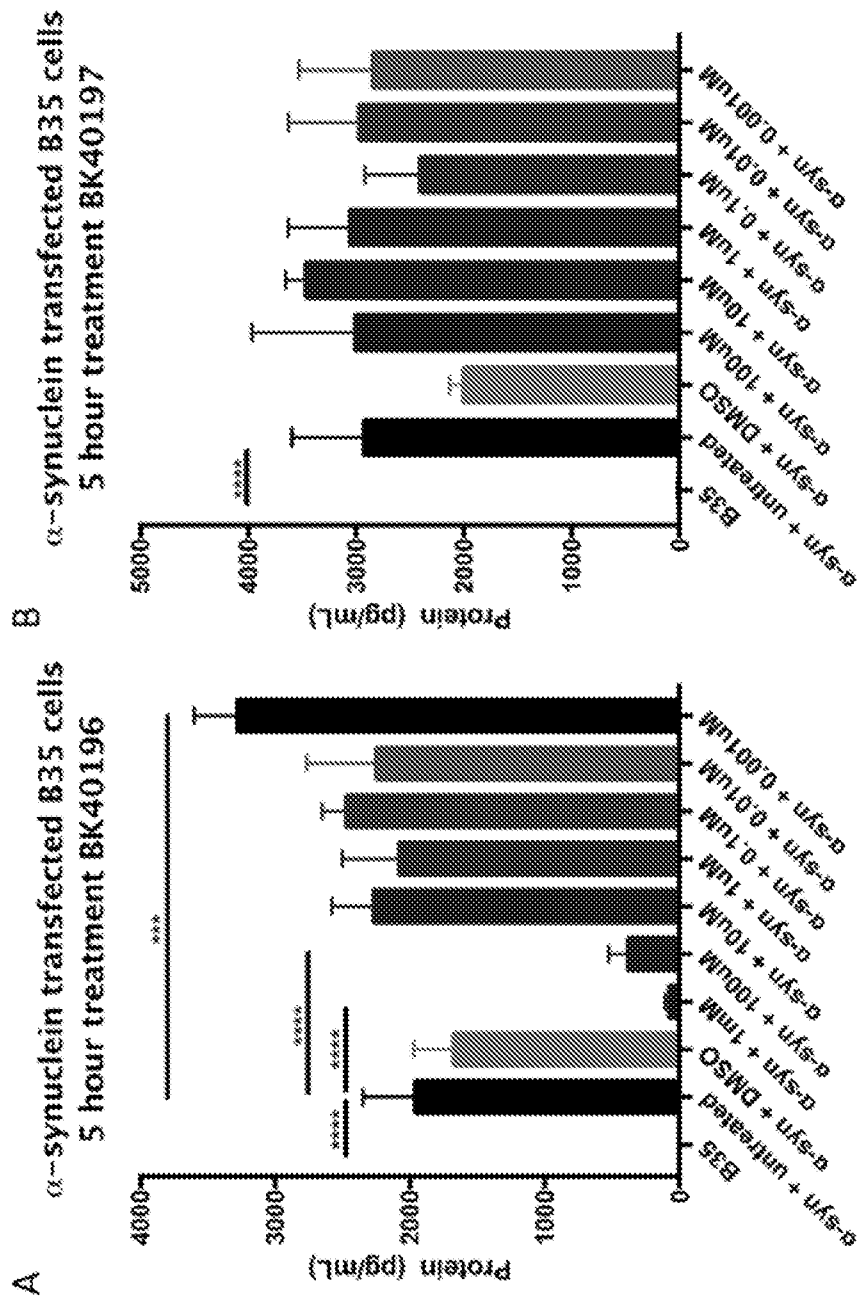


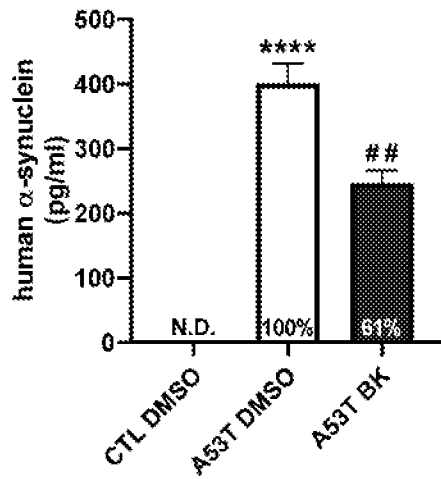
FIG. 10



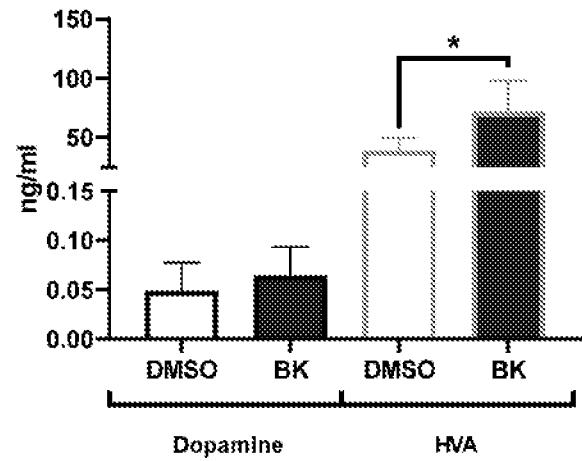
FIGS. 11A-B

12/17

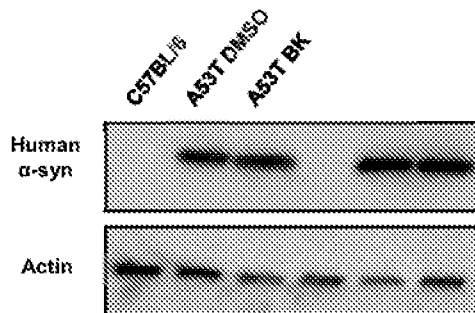
A



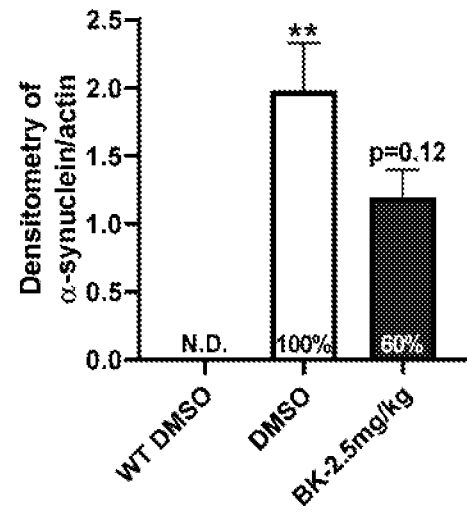
B



C



D



FIGS. 12A-D

REPLACEMENT SHEET

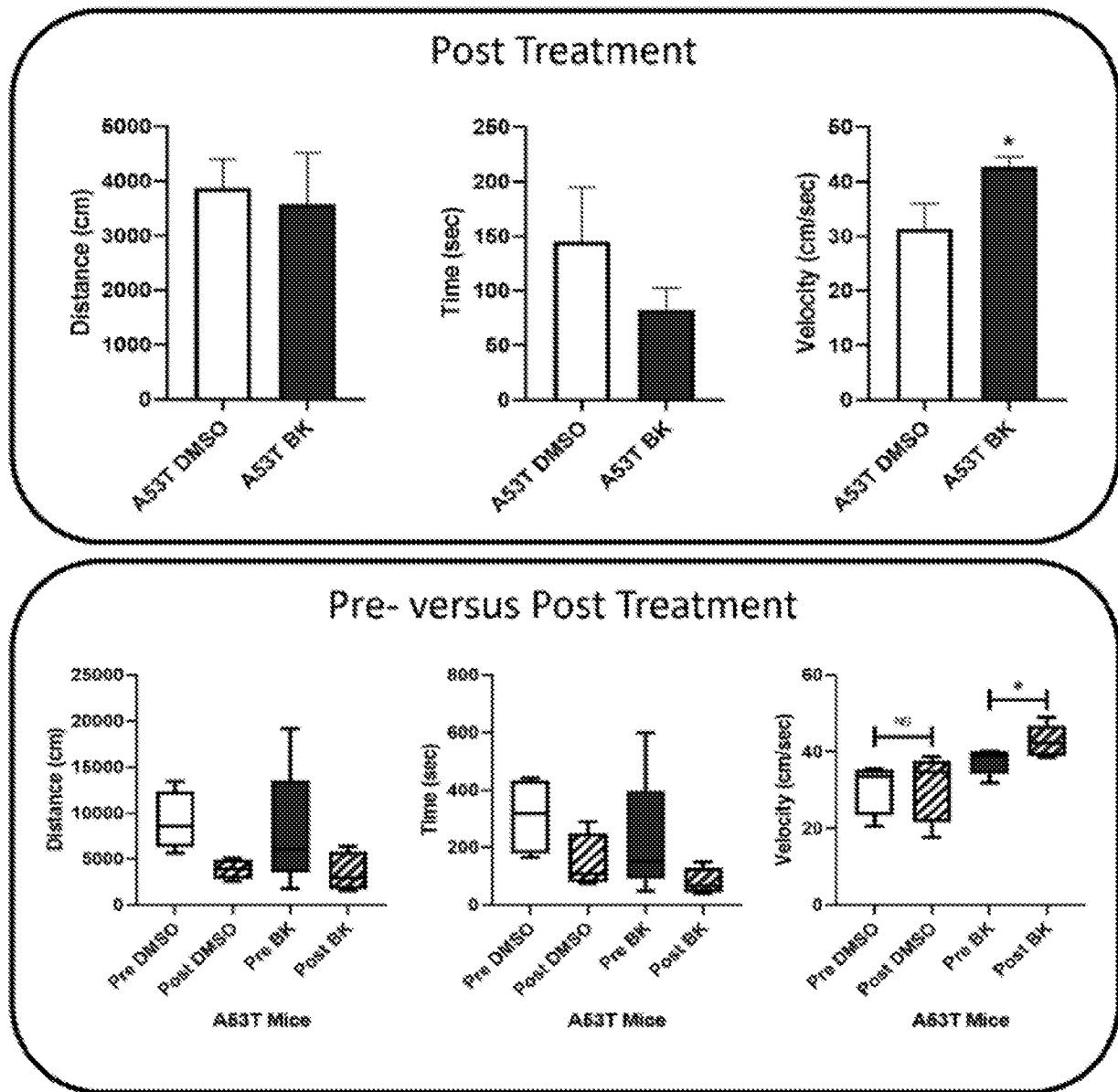
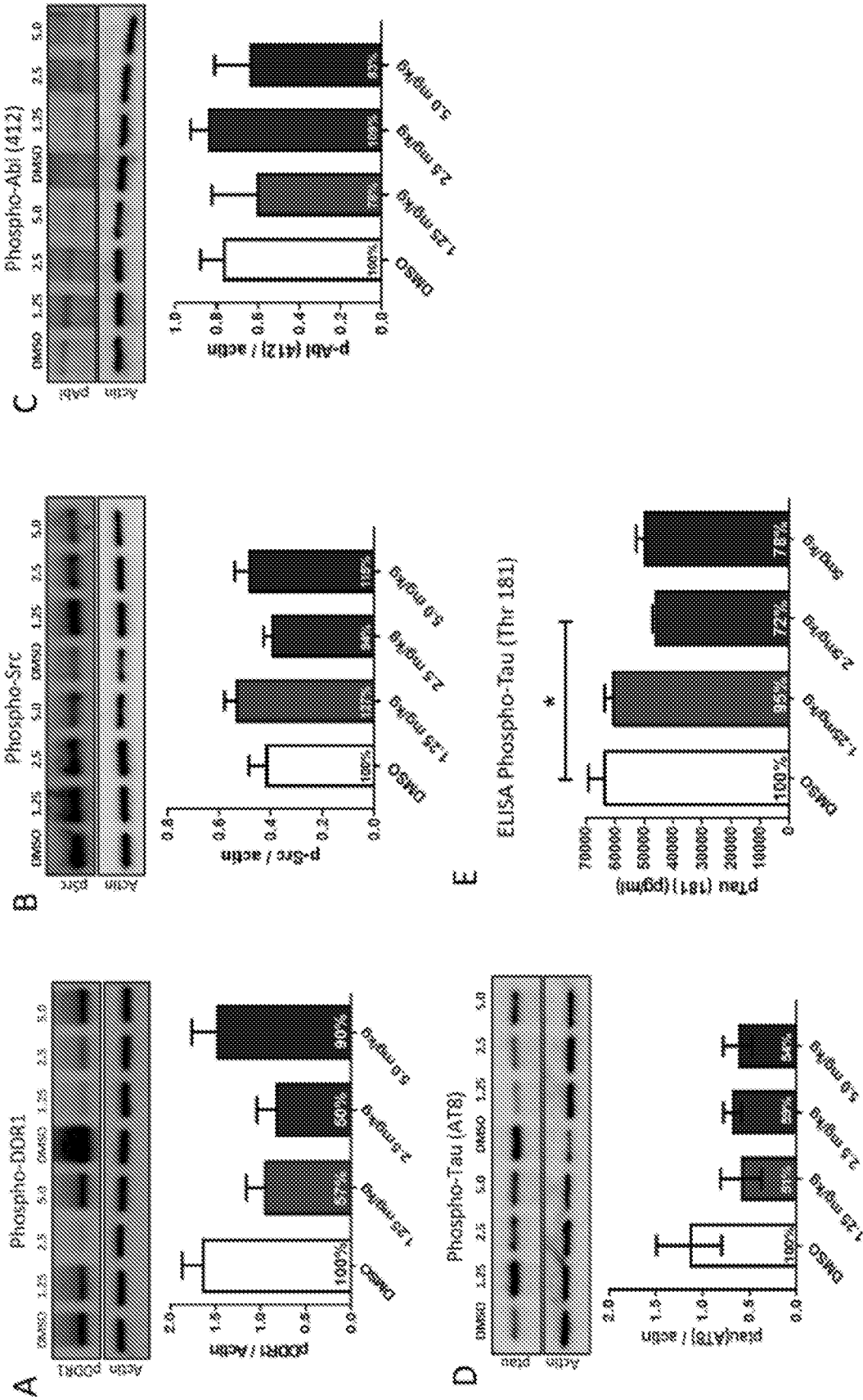
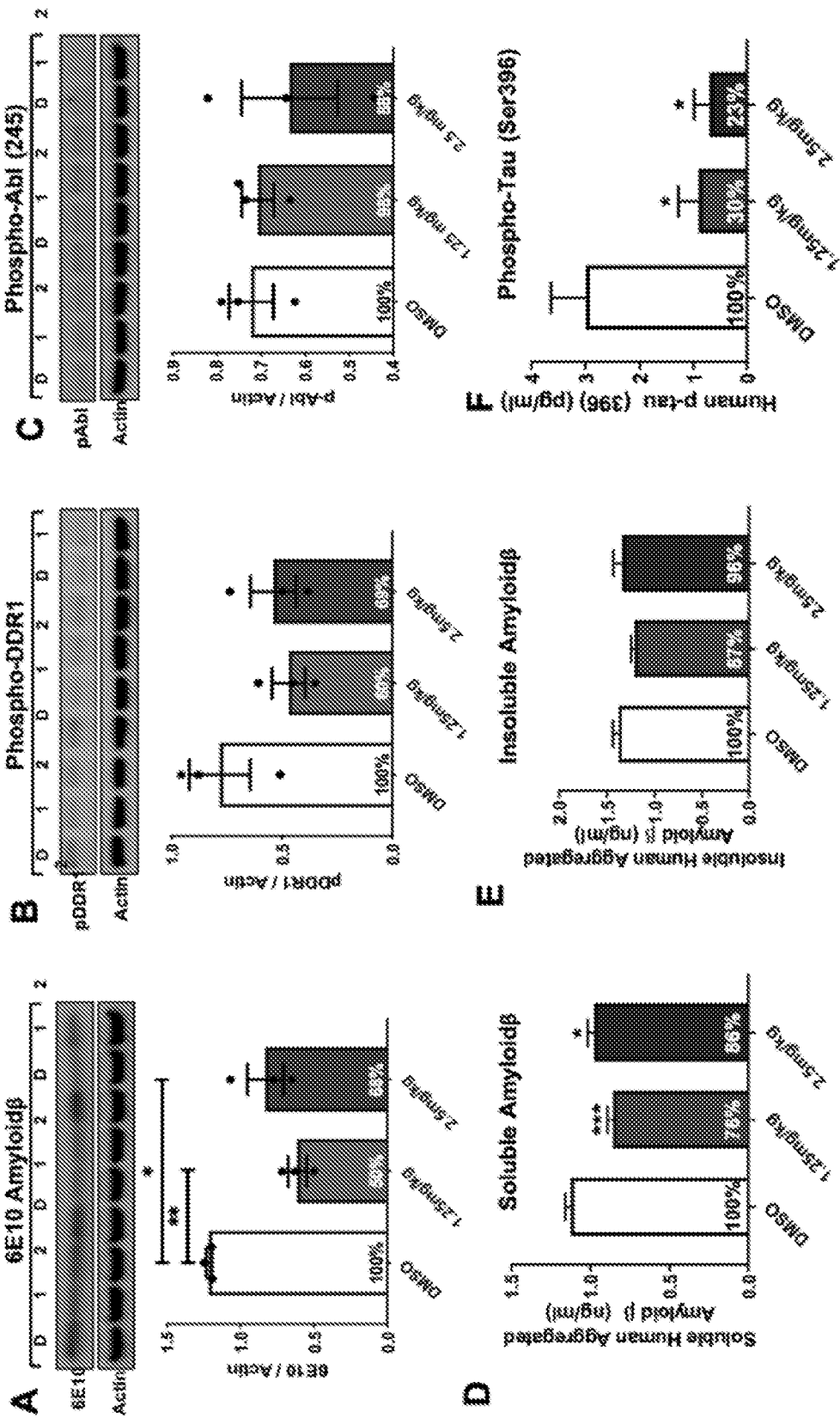


FIG. 13



FIGS. 14A-E



FIGS. 15A-F

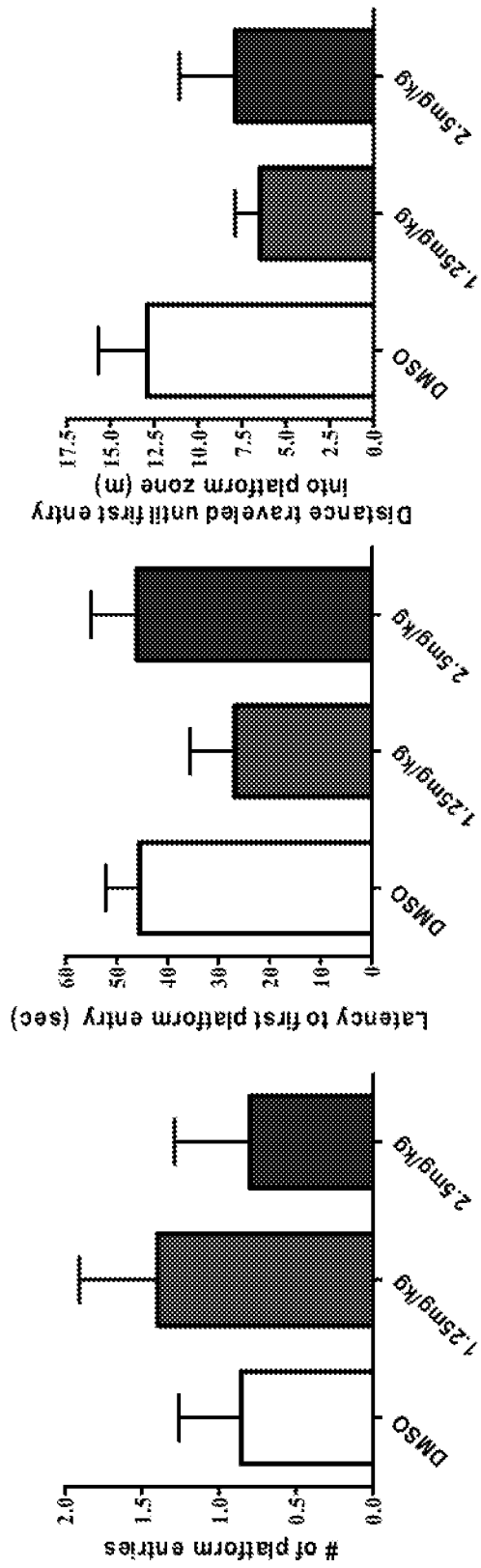


FIG. 16

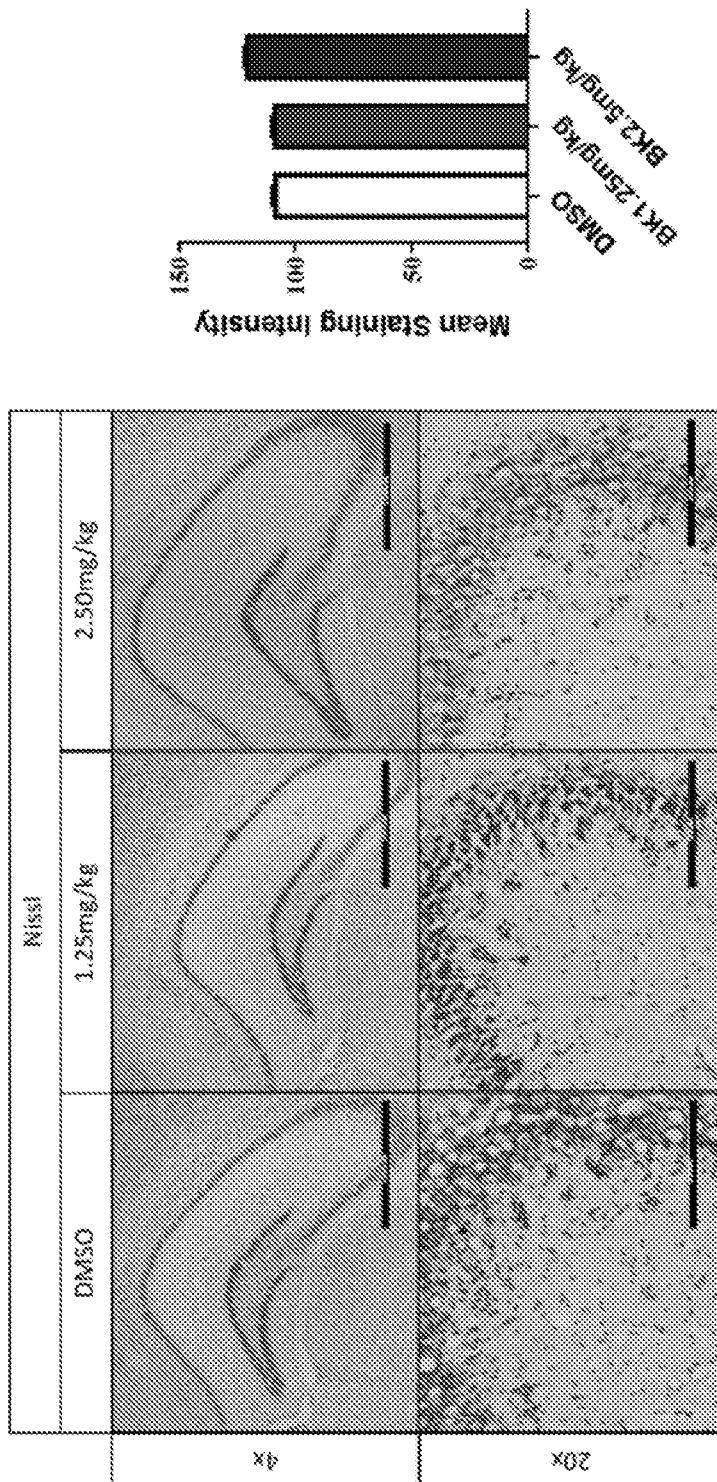


FIG. 17

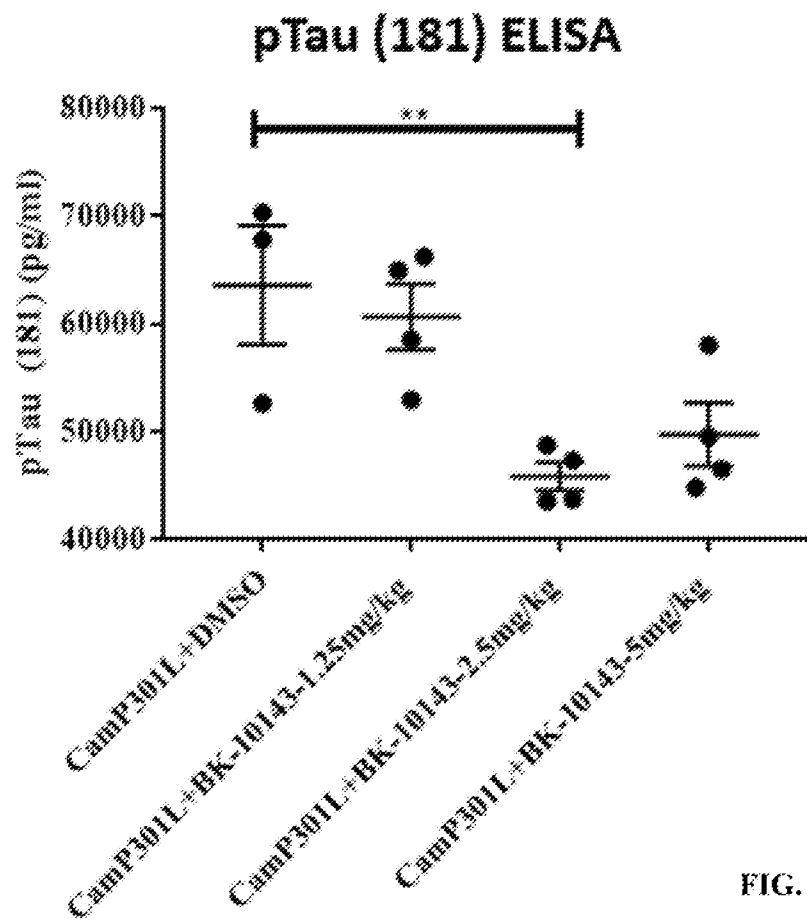


FIG. 6