

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
29 May 2008 (29.05.2008)

PCT

(10) International Publication Number
WO 2008/064296 A1

(51) International Patent Classification:

A61K 31/415 (2006.01) A61K 31/426 (2006.01)
A61K 31/421 (2006.01) A61P 25/18 (2006.01)

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(21) International Application Number:

PCT/US2007/085358

(22) International Filing Date:

21 November 2007 (21.11.2007)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

60/860,787 22 November 2006 (22.11.2006) US

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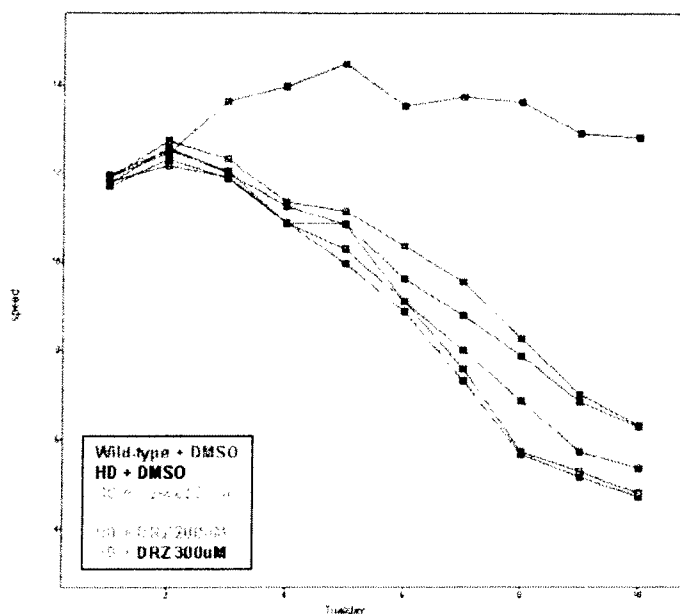
(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH,
CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG,
ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL,
IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK,
LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW,
MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL,
PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY,
TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA,
ZM, ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,
FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL,
PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM,
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

(54) Title: METHOD OF TREATING NEUROLOGICAL DISORDERS WITH CARBONIC ANHYDRASE INHIBITORS



(57) Abstract: Described herein are methods for treating a subject, such as an animal or human, having a neurological disorder or at risk for developing a neurological disorder (e.g., a patient that has a genetic defect associated with a neurological disorder, but does not appear to suffer from any symptoms commonly associated with the neurological disorder) by administering to the subject having the neurological disorder an effective amount of a carbonic anhydrase inhibitor.

METHOD OF TREATING NEUROLOGICAL DISORDERS WITH CARBONIC ANHYDRASE INHIBITORS

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BACKGROUND

[0001] Improved treatments for Huntington's disease (HD), Alzheimer's disease, Parkinson's disease, severe seizure disorders (e.g., epilepsy and familial dysautonomia), and injury or trauma to the nervous system are needed.

10 [0002] HD is a progressive hereditary disorder of the neurodegenerative type involving the basal ganglia (cerebral areas in charge of controlling involuntary movement), can cause highly debilitating motor and psychiatric symptoms. In most cases, onset of Huntington's disease occurs between ages 35 and 40, with an incidence of one case in 10,000 and a mean duration of the disease of about 17 years. The onset is insidious and is characterized by
15 abnormalities of coordination, movement, and behavior. Movement abnormalities include restlessness, mild postural abnormalities, and quick jerking movements of the fingers, limbs, and trunk. The movement abnormalities may be accompanied by substantial weight loss. Depression is common, and cognitive abnormalities and inappropriate behavior may develop. In contrast to the choreic movements typical of onset in adults, juvenile patients may exhibit
20 rigidity, tremor, and dystonia. In the course of eight to 15 years, the disorder progresses to complete incapacitation, with most patients dying of aspiration pneumonia or inanition.

[0003] Huntington's disease was the first major inherited disorder with an unidentified basic defect to be linked with a DNA marker. The genetic defect responsible for the disease consists of an expansion of the CAG triplet encoding glutamine (polyQ expansion) at the
25 amino-terminal end of the protein known as huntingtin. In healthy subjects there are 36 glutamine residues at the amino terminus. In those affected by HD there is an increase in these repetitions ranging from about 38 to about 120 units. Within the scope of this variability, it has been observed that the greater the number of repetitions, the earlier the onset of the disease occurs. One hundred per cent of subjects with the mutation are affected,
30 and the disease is transmitted with dominant autosomic characteristics; just one mutant allele is sufficient to evoke the pathology (Brinkman et al., Am J Hum Genet 60, 1202-1210 (1997)).

SUMMARY

[0004] Described herein are methods for treating a subject, such as an animal or human, having a neurological disorder or at risk for developing a neurological disorder (e.g., a patient that has a genetic defect associated with a neurological disorder, but does not appear to suffer from any symptoms commonly associated with the neurological disorder) by administering to the subject having the neurological disorder an effective amount of a carbonic anhydrase inhibitor. In other embodiments the method comprises administering a carbonic anhydrase inhibitor to the subject at risk for developing a neurological disorder.

[0005] Described herein is a method for treating a subject having a neurological disorder, the method comprising administering to the subject an effective amount of a carbonic anhydrase inhibitor. In some cases the neurological disorder is selected from the group consisting of Huntington's disease, Alzheimer's disease, and a neurological disorder arising from a polyglutamine expansion. In some cases the carbonic anhydrase inhibitor is selected from the group consisting of dichlorphenamide, dorzolamide, methazolamide, topiramate or acetazolamide. In some cases the carbonic anhydrase inhibitor is selected from the group consisting of benzthiazide, chlorothiazide, hydroflumethiazide, diazoxide, celecoxib, metolazone and althiazide. In some cases the carbonic anhydrase inhibitor is selected from the group consisting of bendroflumethiazide, hydrochlorthiazide, trichlormethiazide, benzolamide and furosemide.

[0006] The method comprises of administering a carbonic anhydrase inhibitor to a patient, either as the sole active agent or in combination with one or more additional active agents. The carbonic anhydrase inhibitor can be administered as a pharmaceutical composition that further contains a pharmaceutically acceptable excipient, carrier or diluent. The pharmaceutical composition can be administered in a variety of solid and liquid dosage forms, and through various routes of administration, including oral delivery or continuous injection via a shunt.

[0007] The neurological disorder can be a neurodegenerative disorder, a disorder of movement, an extrapyramidal disorder, a cerebellar disorder or a hyperkinetic movement disorder. Neurological disorders that can be treated include, but are not limited to, neurological diseases arising from a polyglutamine expansion, such as Huntington's disease.

[0008] Pharmaceutical compositions for treating neurological disorders containing an effective amount of carbonic anhydrase inhibitor or a derivative are described in the disclosure.

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FIGURES

[0009] Figure 1 depicts the results of a study analyzing the age-dependent decline in speed of locomotion between the wild type flies treated with vehicle (DMSO) and HD model flies treated with vehicle, HD model flies treated with the positive control compounds Trichostatin A (TSA) and different concentrations of dorzolamide (DRZ). From the top, the lines depict:
10 wt-DMSO; HD-TSA 250 micromolar; HD-DRZ 300 micromolar; HD-DRZ 200 micromolar; HD-DRZ 100 micromolar; and HD-DMSO.

[0010] Figure 2 depicts the results of a study analyzing the age-dependent decline in speed of locomotion between the wild type flies treated with vehicle (DMSO) and HD model flies
15 treated with vehicle, HD model flies treated with the positive control compounds Trichostatin A (TSA) and different concentrations of dichlorphenamide (DCP).

[0011] Figure 3 is a table that shows the effect sizes of compound-treated vs. vehicle-treated HD *Drosophila* model flies in locomotor assay.

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[0012] Figure 4 depicts the result of an experiment analyzing the effect of dichlorphenamide on survival in the HD fly model. Dichlorphenamide treatment results are shown in the upper line. The control (DMSO) results are shown in the lower line.

25 [0013] Figure 5 is a table that shows the effect sizes of compound-treated vs. vehicle treated AD *Drosophila* model flies in a locomotor assay.

DETAILED DESCRIPTION

30 [0014] Methods and pharmaceutical compositions for treating a subject, such as a human or an animal that has a neurological disorder by administering a carbonic anhydrase inhibitor or a derivative, analog, or a salt thereof are described as are pharmaceutical compositions and methods for treating a patient having a neurological disorder, comprising administering to the

patient an effective amount of a carbonic anhydrase inhibitor or a pharmaceutically acceptable salt thereof.

[0015] As used herein, the term “small molecule” refers to compounds having a molecular mass of less than 3000 Daltons.

- 5 [0016] As used herein, a “therapeutic agent” includes a carbonic anhydrase inhibitor that ameliorates one or more of the symptoms of a neurological disorder.

[0017] A therapeutic agent can reduce one or more symptoms of the disorder, delay onset of one or more symptoms, or prevent or cure the disease.

- 10 [0018] As used herein, an “additional active agent” refers to an agent that ameliorates one or more of the symptoms of a neurological disorder, including a neurodegenerative disorder. An “additional active agent” can reduce one or more symptoms of the disorder, delay onset of one or more symptoms, or prevent or cure the disease. An “additional active agent” can mean a second (different) therapeutic agent (i.e., a different carbonic anhydrase inhibitor), or a structurally distinct active agent for treating a neurological disorder.

- 15 [0019] An “effective amount” as referred to herein, relates to the amount of the carbonic anhydrase inhibitor that is capable of rendering a beneficial clinical outcome of the condition being treated with a derivative or analog thereof compared with the absence of such treatment. The effective amount of the therapeutic agent administered will depend on the degree, severity, and type of the disease or condition, the amount of therapy desired, and the
20 release characteristics of the pharmaceutical formulation. It will also depend on the subject’s health, size, weight, age, sex and tolerance to specific compounds, which are determinable pharmaceutical parameters to those skilled in the field. Generally, treatment is considered “effective” if one or more symptoms of the disease or disorder improves (e.g., at least 10% relative to pre-treatment) during the course of treatment. The compounds described herein
25 can also be given to prevent or delay the onset of symptoms in an individual predisposed to such disorder, e.g. Huntington’s disease. A delay or absence of the onset of one or more symptoms relative to the time one would expect such symptoms to arise in a similar individual not treated with the drug would indicate efficacy.

[0020] **I. Neurological Disorders**

[0021] The neurological disorder treated in the disclosure can be, but is not limited to, a disorder of movement, an extrapyramidal disorder, a cerebellar disorder excluding episodic ataxia-2, or a hyperkinetic movement disorder. The neurological disorder also can be, but is not limited to, Huntington's disease, age-related memory impairment, amyotrophic lateral sclerosis, ataxia-telangiectasia, Biswanger's disease, cerebral amyloid angiopathies, Creutzfeldt-Jacob disease including variant form, corticobasal degeneration, multi infarct dementia, subcortical dementia, dementia with Lewy Bodies, dementia due to human immunodeficiency virus (HIV), Friedreich ataxia, fronto-temporal dementia linked to chromosome 17 (FTDP-17), frontotemporal lobar degeneration, frontal lobe dementia, Kennedy disease, Korsakoff's syndrome, mild cognitive impairment, neurological manifestations of HIV, neurological conditions arising from polyglutamine expansions excluding polyglutamine expansion in the CACNA1A gene affected in SCA-6, Pick's disease, prion diseases, Kuru disease, fatal familial insomnia, Gerstmann-Straussler-Scheinker disease, prion protein cerebral amyloid angiopathy, postencephalitic Parkinsonism, progressive supranuclear palsy, spinal muscular atrophy, transmissible spongiform encephalopathies or vascular dementia. The disclosure also can provide therapeutic benefit to diseases or conditions including, but is not limited to, argyrophilic grain dementia, Parkinsonism-dementia complex of Guam, auto-immune conditions such as Guillain-Barre syndrome or Lupus, brain and spinal tumors (including neurofibromatosis), cerebral amyloid angiopathies, cerebral palsy, chronic fatigue syndrome, corticobasal degeneration, conditions due to developmental dysfunction of the CNS parenchyma, conditions due to developmental dysfunction of the cerebrovasculature, dementia lacking distinct histology, Dementia Pugilistica, diffuse neurofibrillary tangles with calcification, diseases of the eye, ear and vestibular systems involving neurodegeneration (including macular degeneration and glaucoma), Down's syndrome, dyskinesias (Paroxysmal), dystonias, essential tremor, Fahr's syndrome, hepatic encephalopathy, hereditary spastic paraplegia, hydrocephalus, pseudotumor cerebri and other conditions involving CSF dysfunction, Gaucher's disease, Hallervorden-Spatz disease, Korsakoff's syndrome, mild cognitive impairment, monomelic amyotrophy, motor neuron diseases, multiple system atrophy, multiple sclerosis and other demyelinating conditions (e.g., leukodystrophies), myalgic encephalomyelitis, myoclonus, neurodegeneration induced by chemicals, drugs and toxins, neurological/cognitive manifestations and consequences of bacterial and/or viral infections, including but not restricted to enteroviruses, Niemann-Pick disease, non-Guamanian motor neuron disease with neurofibrillary tangles, non-ketotic hyperglycinemia, olivo-ponto cerebellar atrophy,

oculopharyngeal muscular dystrophy, neurological manifestations of Polio myelitis including non-paralytic polio and post-polio-syndrome, primary lateral sclerosis, restless leg syndrome, Sandhoff disease, spasticity, sporadic fronto-temporal dementias, striatonigral degeneration, subacute sclerosing panencephalitis, sulphite oxidase deficiency, Sydenham's chorea, tangle
5 only dementia, Tay-Sach's disease, Tourette's syndrome, vascular dementia, and Wilson disease.

[0022] In one embodiment, the neurological disease is a neurological condition arising from a polyglutamine expansion, excluding polyglutamine expansion in the CACNA1A gene. In some cases, the polyglutamine expansion can be of at least about 10, at least about 20, at least
10 about 30, at least about 40, at least about 50, at least about 60, at least about 70, at least about 80, or at least about 100 or more residues. In Huntington's disease, for example, the polyglutamine expansion is typically between 21 and 33 residues in length.

[0023] The subject that the method of treatment is administered to can be an animal, such as *Drosophila* or a mammal. The mammal can be, but is not limited to, a mouse, a rat, a cat, a
15 dog, a primate, or a human.

[0024] II. Animal Models

[0025] The methods and pharmaceutical compositions for treating neurological disorders, such as neurodegenerative diseases, comprising a carbonic anhydrase inhibitor are disclosed herein. The suitability of a compound for treatment of a neurodegenerative disease can be
20 assessed in any of a number of animal models for neurodegenerative disease. For example, mice transgenic for Huntington's disease (see, e.g., Mangiarini et al., 1996, Cell 87: 493-506, Lin et al., 2001, Hum. Mol. Genet. 10: 137-144) and can be used to determine the efficacy of a given compound in the treatment or prevention of neurodegenerative disease. Alzheimer's disease (Hsiao, 1998, Exp. Gerontol. 33: 883-889; Hsiao et al., 1996, Science 274: 99-102),
25 Parkinson's disease (Kim et al., 2002, Nature 418: 50-56), amyotrophic lateral sclerosis (Zhu et al., 2002, Nature 417: 74-78), Pick's disease (Lee & Trojanowski, 2001, Neurology 56 (Suppl. 4): S26-S30, and spongiform encephalopathies (He et al., 2003, Science 299: 710-712) can be used to evaluate the efficacy of the carbonic anhydrase inhibitor in a similar manner.

[0026] Animal models are not limited to mammalian models. For example, *Drosophila* strains provide accepted models for a number of neurodegenerative disorders (reviewed in
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Fortini & Bonini, 2000, Trends Genet. 16: 161-167; Zoghbi & Botas, 2002, Trends Genet. 18: 463-471). These models include not only flies bearing mutated fly genes, but also flies bearing human transgenes, optionally with targeted mutations. Among the *Drosophila* models available are, for example, Huntington's disease (Kazemi-Esfarjani & Benzer, 2000, Science 287: 1837-1840), Parkinson's disease (Feany et al., 2000, Nature 404: 394-398; Auluck et al., 2002, Science 295: 809-810), age-dependent neurodegeneration (Palladino et al., 2002, Genetics 161: 1197-1208), Alzheimer's disease (Selkoe et al., 1998, Trends Cell Biol. 8: 447-453; Ye et al., 1999, J. Cell Biol. 146: 1351-1364), amyotrophic lateral sclerosis (Parkes et al., 1998, Nature Genet. 19: 171-174), and adrenoleukodystrophy.

[0027] The use of *Drosophila* as a model organism has proven to be an important tool in the elucidation of human neurodegenerative pathways, as the *Drosophila* genome contains many relevant human orthologs that are extremely well conserved in function (Rubin, G.M., et al., Science 287: 2204-2215 (2000)). For example, *Drosophila melanogaster* carries a gene that is homologous to human APP which is involved in nervous system function. The gene, APP-like (APPL), is approximately 40% identical to APP695, the neuronal isoform (Rosen et al., Proc. Natl. Acad. Sci. U.S.A. 86:2478-2482 (1988)), and like human APP695 is exclusively expressed in the nervous system. Flies deficient for the APPL gene show behavioral defects which can be rescued by the human APP gene, suggesting that the two genes have similar functions in the two organisms (Luo et al., Neuron 9:595-605 (1992)). In addition, *Drosophila* models of polyglutamine repeat diseases (Jackson, G.R., et al., Neuron 21:633-642 (1998); Kazemi-Esfarjani, P. and Benzer, S., Science 287:1837-1840 (2000); Fernandez-Funez et al., Nature 408:101-6 (2000)), Parkinson's disease (Feany, M.B. and Bender, W.W., Nature 404:394-398 (2000)) and other diseases have been established which closely mimic the disease state in humans at the cellular and physiological levels, and have been successfully employed in identifying other genes that may be involved in these diseases. Thus, the power of *Drosophila* as a model system has been demonstrated in the ability to represent the disease state and to perform large scale genetic screens to identify critical components of disease.

[0028] The transgenic flies exhibit progressive neurodegeneration which can lead to a variety of altered phenotypes including locomotor phenotypes, behavioral phenotypes (e.g., appetite, mating behavior, and/or life span), and morphological phenotypes (e.g., shape, size, or location of a cell, organ, or appendage; or size, shape, or growth rate of the fly).

[0029] Test animals, such as transgenic flies, are administered carbonic anhydrase inhibitor and evaluated for symptoms relative to animals not administered the compound. A change in the severity of symptoms (e.g., a 1%, 2%, 5%, 10%, or greater improvement in one or more symptoms), or a delay in the onset of symptoms, in treated versus untreated animals can be indicative of therapeutic efficacy.

[0030] For example dichlorphenamide has shown reproducible positive effects in a *Drosophila* model of HD. The flies performed better than carrier controls using two metrics: early speed (days 1-7) and late speed (days 8-10).

10 [0031] **III. Dosage and Administration**

[0032] The pharmaceutical composition for treating a subject having or at risk of developing a neurological disorder can comprise a therapeutically effective amount of a carbonic anhydrase inhibitor, a derivative or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient, carrier or diluent. The pharmaceutical composition can comprise carbonic anhydrase inhibitor, or a derivative, analog, or a pharmaceutically acceptable salt thereof.

[0033] The pharmaceutical composition of the disclosure can be administered in a variety of dosage forms including, but not limited to, a solid dosage form or in a liquid dosage form, an oral dosage form, a parenteral dosage form, an intranasal dosage form, a suppository, a lozenge, a troche, buccal, a controlled release dosage form, a pulsed release dosage form, an immediate release dosage form, an intravenous solution, a suspension or combinations thereof. The dosage can be an oral dosage form that is a controlled release dosage form. The oral dosage form can be a tablet or a caplet. The compounds employed can be administered, for example, by oral or parenteral routes, including intravenous, intramuscular, intraperitoneal, subcutaneous, transdermal, airway (aerosol), rectal, vaginal and topical (including buccal and sublingual) administration. In one embodiment, the compounds or pharmaceutical compositions comprising the compounds are delivered to a desired site, such as the brain, by continuous injection via a shunt.

[0034] In another embodiment, the carbonic anhydrase inhibitor can be administered parenterally, such as intravenous (IV) administration. The formulations for administration will commonly comprise a solution of the carbonic anhydrase inhibitor (e.g.,

Dichlorphenamide) dissolved in a pharmaceutically acceptable carrier. Among the acceptable vehicles and solvents that can be employed are water and Ringer's solution, an isotonic sodium chloride. In addition, sterile fixed oils can conventionally be employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid can likewise be used in the preparation of injectables. These solutions are sterile and generally free of undesirable matter. These formulations may be sterilized by conventional, well known sterilization techniques. The formulations may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents, e.g., sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of compound of the carbonic anhydrase inhibitor in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight, and the like, in accordance with the particular mode of administration selected and the patient's needs. For IV administration, the formulation can be a sterile injectable preparation, such as a sterile injectable aqueous or oleaginous suspension. This suspension can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation can also be a sterile injectable solution or suspension in a nontoxic parenterally-acceptable diluent or solvent, such as a solution of 1,3-butanediol.

[0035] In one embodiment, the carbonic anhydrase inhibitor of the disclosure can be administered by introduction into the central nervous system of the subject, e.g., into the cerebrospinal fluid of the subject. The formulations for administration will commonly comprise a solution of the compound of the carbonic anhydrase inhibitor (e.g. Dichlorphenamide) dissolved in a pharmaceutically acceptable carrier. In certain aspects,, the carbonic anhydrase inhibitor is introduced intrathecally, e.g., into a cerebral ventricle, the lumbar area, or the cisterna magna. In another aspect, the carbonic anhydrase inhibitor is introduced intraocularly, to thereby contact retinal ganglion cells.

[0036] The pharmaceutically acceptable formulations can easily be suspended in aqueous vehicles and introduced through conventional hypodermic needles or using infusion pumps. Prior to introduction, the formulations can be sterilized with , gamma radiation or electron

beam sterilization, described in U.S. Pat. No. 436,742 the contents of which are incorporated herein by reference.

[0037] In one embodiment, the pharmaceutical composition comprising a compound of carbonic anhydrase inhibitor is administered into a subject intrathecally. As used herein, the term "intrathecal administration" is intended to include delivering a pharmaceutical composition comprising carbonic anhydrase inhibitor directly into the cerebrospinal fluid of a subject, by techniques including lateral cerebroventricular injection through a burrhole or cisternal or lumbar puncture or the like (described in Lazorthes et al. *Advances in Drug Delivery Systems and Applications in Neurosurgery*, 143-192 and Omayya et al., *Cancer Drug Delivery*, 1: 169-179, the contents of which are incorporated herein by reference). The term "lumbar region" is intended to include the area between the third and fourth lumbar (lower back) vertebrae. The term "cisterna magna" is intended to include the area where the skull ends and the spinal cord begins at the back of the head. The term "cerebral ventricle" is intended to include the cavities in the brain that are continuous with the central canal of the spinal cord. Administration of a carbonic anhydrase inhibitor to any of the above mentioned sites can be achieved by direct injection of the pharmaceutical composition comprising the carbonic anhydrase inhibitor or by the use of infusion pumps. For injection, the pharmaceutical compositions can be formulated in liquid solutions, for example physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the pharmaceutical compositions may be formulated in solid form and re-dissolved or suspended immediately prior to use. Lyophilized forms are also included. The injection can be, for example, in the form of a bolus injection or continuous infusion (e.g., using infusion pumps) of pharmaceutical composition.

[0038] In one embodiment, the pharmaceutical composition comprising a carbonic anhydrase inhibitor is administered by lateral cerebro ventricular injection into the brain of a subject. The injection can be made, for example, through a burr hole made in the subject's skull. In another embodiment, said encapsulated therapeutic agent is administered through a surgically inserted shunt into the cerebral ventricle of a subject. For example, the injection can be made into the lateral ventricles, which are larger, even though injection into the third and fourth smaller ventricles can also be made.

[0039] In yet another embodiment, the pharmaceutical composition is administered by injection into the cisterna magna, or lumbar area of a subject.

[0040] For oral administration, the compounds that are useful will generally be provided in unit dosage forms of a tablet, pill, dragee, lozenge or capsule; as a powder or granules; or as an aqueous solution, suspension, liquid, gels, syrup, slurry, etc. suitable for ingestion by the patient. Tablets for oral use may include the active ingredients mixed with pharmaceutically acceptable excipients such as inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavoring agents, coloring agents and preservatives. Suitable inert diluents include sodium and calcium carbonate, sodium and calcium phosphate, and lactose, while corn starch and alginic acid are suitable disintegrating agents. Binding agents may include starch and gelatin, while the lubricating agent, if present, will generally be magnesium stearate, stearic acid or talc. If desired, the tablets may be coated with a material such as glyceryl monostearate or glyceryl distearate, to delay absorption in the gastrointestinal tract.

[0041] Pharmaceutical preparations for oral use can be obtained through combination of a carbonic anhydrase inhibitor with a solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable additional compounds, if desired, to obtain tablets or dragee cores. Suitable solid excipients in addition to those previously mentioned are carbohydrate or protein fillers that include, but are not limited to, sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose such as methyl cellulose, hydroxypropylmethyl-cellulose or sodium carboxymethylcellulose; and gums including arabic and tragacanth; as well as proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

[0042] Capsules for oral use include hard gelatin capsules in which the active ingredient is mixed with a solid diluent, and soft gelatin capsules wherein the active ingredients is mixed with water or an oil such as peanut oil, liquid paraffin or olive oil.

[0043] Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable

organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

[0044] For transmucosal administration (e.g., buccal, rectal, nasal, ocular, etc.), penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

[0045] Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or a salicylate. Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate. For intramuscular, intraperitoneal, subcutaneous and intravenous use, the compounds will generally be provided in sterile aqueous solutions or suspensions, buffered to an appropriate pH and isotonicity. Suitable aqueous vehicles include Ringer's solution and isotonic sodium chloride. Aqueous suspensions may include suspending agents such as cellulose derivatives, sodium alginate, polyvinyl-pyrrolidone and gum tragacanth, and a wetting agent such as lecithin. Suitable preservatives for aqueous suspensions include ethyl and n-propyl p-hydroxybenzoate.

[0046] The suppositories for rectal administration of the drug can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperatures and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

[0047] The compounds of the disclosure can be delivered transdermally, by a topical route, formulated as applicator sticks, solutions, suspensions, emulsions, gels, creams, ointments, pastes, jellies, paints, powders, or aerosols.

[0048] The compounds useful may also be presented as aqueous or liposome formulations. Aqueous suspensions contain a carbonic anhydrase inhibitor in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients include a suspending agent, such as sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents such as a naturally occurring phosphatide (e.g., lecithin), a condensation product of an alkylene oxide with a fatty acid (e.g., polyoxyethylene

stearate), a condensation product of ethylene oxide with a long chain aliphatic alcohol (e.g., heptadecaethylene oxycetanol), a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol (e.g., polyoxyethylene sorbitol mono-oleate), or a condensation product of ethylene oxide with a partial ester derived from fatty acid and a hexitol anhydride (e.g., polyoxyethylene sorbitan monooleate). The aqueous suspension can also contain one or more preservatives such as ethyl or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents and one or more sweetening agents, such as sucrose, aspartame or saccharin. Formulations can be adjusted for osmolarity.

[0049] Oil suspensions can be formulated by suspending a carbonic anhydrase inhibitor in a vegetable oil, such as arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin; or a mixture of these. The oil suspensions can contain a thickening agent, such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents can be added to provide a palatable oral preparation, such as glycerol, sorbitol or sucrose. These formulations can be preserved by the addition of an antioxidant such as ascorbic acid. As an example of an injectable oil vehicle, see Minto, J. Pharmacol. Exp. Ther. 281:93-102, 1997. The pharmaceutical formulations can also be in the form of oil-in-water emulsions. The oily phase can be a vegetable oil or a mineral oil, described above, or a mixture of these. Suitable emulsifying agents include naturally-occurring gums, such as gum acacia and gum tragacanth, naturally occurring phosphatides, such as soybean lecithin, esters or partial esters derived from fatty acids and hexitol anhydrides, such as sorbitan mono-oleate, and condensation products of these partial esters with ethylene oxide, such as polyoxyethylene sorbitan mono-oleate. The emulsion can also contain sweetening agents and flavoring agents, as in the formulation of syrups and elixirs. Such formulations can also contain a demulcent, a preservative, or a coloring agent.

[0050] In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation or transcutaneous delivery (e.g., subcutaneously or intramuscularly), intramuscular injection or a transdermal patch. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (e.g., as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[0051] The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

- 5 [0052] For administration by inhalation, the compounds for use are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount.
- 10 Capsules and cartridges of e.g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

- [0053] In general a suitable dose will be in the range of 0.01 to 1000 mg per kilogram body weight of the recipient per day. The desired dose is can be presented once daily, but may be
- 15 dosed as two, three, four, five, six or more sub-doses administered at appropriate intervals throughout the day.

- [0054] The compounds can be administered as the sole active agent, or in combination with other known therapeutics to be beneficial in the treatment of neurological disorders. In any event, the administering physician can provide a method of treatment that is prophylactic or
- 20 therapeutic by adjusting the amount and timing of drug administration on the basis of observations of one or more symptoms (e.g., motor or cognitive function as measured by standard clinical scales or assessments) of the disorder being treated.

- [0055] Details on techniques for formulation and administration are well described in the scientific and patent literature, see, e.g., the latest edition of Remington's Pharmaceutical
- 25 Sciences, Maack Publishing Co, Easton Pa. ("Remington's"). Therapeutically effective amounts of a carbonic anhydrase inhibitor may range from about 0.01 to about 1000 milligrams per kilogram (mg/kg). A person of ordinary skill in the art will be able without undue experimentation, having regard to that skill and this disclosure, to determine a therapeutically effective amount of a particular carbonic anhydrase inhibitor.

- 30 [0056] After a pharmaceutical composition of the disclosure has been formulated in an acceptable carrier, it can be placed in an appropriate container and labeled for treatment of an

indicated condition. For administration of the carbonic anhydrase inhibitor, such labeling would include, e.g., instructions concerning the amount, frequency and method of administration. In one embodiment, a kit for inhibiting or reversing AP-induced weight gain in a human which includes a carbonic anhydrase inhibitor and instructional material teaching the indications, dosage and schedule of administration of the carbonic anhydrase inhibitor is provided.

[0057] IV. **Compound Toxicity**

[0058] The ratio between toxicity and therapeutic effect for a particular compound is its therapeutic index and can be expressed as the ratio between LD₅₀ (the amount of compound lethal in 50% of the population) and ED₅₀ (the amount of compound effective in 50% of the population). Compounds that exhibit high therapeutic indices are desirable. Therapeutic index data obtained from cell culture assays and/or animal studies can be used in formulating a range of dosages for use in humans. The dosage of such compounds can lie within a range of plasma concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. See, e.g. Fingl et al., In. The Pharmacological Basis of Therapeutics, Ch.1, p.1, 1975. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition and the particular method in which the compound is used.

[0059] V. **Experiment Details**

[0060] A library of compounds was screened to identify compounds that reduce the polyglutamine-induced neurodegeneration and behavior in a *Drosophila* HD model. The *Drosophila* HD model used to screen compounds can be generated using standard genetic engineering technology, such as those described below.

[0061] A “tissue-specific” expression control sequence, as used herein, refers to expression control sequences that drive expression in one tissue or a subset of tissues, while being essentially inactive in at least one other tissue. “Essentially inactive” means that the expression of a sequence operatively linked to a tissue-specific expression control sequence is less than 5% of the level of expression of that sequence in that tissue where the expression control sequence is most active. “Tissue-specific expression control sequences” include those that are specific for organs such as the eye, wing, notum, brain, as well as tissues of the

central and peripheral nervous systems. Tissue-specific expression control sequences can either be used as a “driver” in the UAS-GAL4 system, or alternatively can be linked to a transgene to control its expression in a *cis* acting manner.

[0062] Examples of tissue specific control sequences include but are not limited to:

- 5 promoters/enhancers important in eye development, such as *sevenless* (Bowtell et al., Genes Dev. 2:620-34 (1988)), *eyeless* (Bowtell et al., Proc. Natl. Acad. Sci. U.S.A. 88:6853-7 (1991)), and *GMR/glass* (Quiring et al., Science 265:785-9 (1994)); promoters/enhancers derived from any of the rhodopsin genes, that are useful for expression in the eye; enhancers/promoters derived from the *dpp* or *vestigial* genes useful for expression in the
- 10 wing (Staehling-Hampton et al., Cell Growth Differ. 5:585-93 (1994), Kim et al., Nature 382:133-8 (1996)); promoters/enhancers specific for nerve, e.g., *elav* (Yao and White, J. Neurochem. 63:41-51 (1994)) which is specific for pan-neuronal expression in post-mitotic neurons, *scabrous* (*sca*) (Song et al., Genetics 162:1703-24 (2002) which is specific for pan-neuronal expression in neuroblasts to neurons, *APPL* (Martin-Morris and White,
- 15 Development 110: 185-95 (1990)), *Nervana 2* (*Nrv2*) (Sun et al., Proc. Nat'l. Acad. Sci. U.S.A. 96:10438-43 (1999)) which is specific for expression in the central nervous system, *Cha* (Barber et al., J. Comp. Neurol. 22:533-43 (1989)) which is specific for cholinergic neurons, *TH* (Friggi-Grelín et al., J. Neurobiol. 54:618-27 (2003)) which is specific for dopaminergic neurons, *CaMKII* (Takmatsu et al., Cell Tissue Res. 310:237-52 (2002)) which
- 20 is specific for central nervous system of embryos and larvae as well as brain, thoracic ganglion and gut of adult, *P* (Gendre et al., Development 131:83-92 (2004)) which is specific for pharyngeal sensory neurons, *Dmef2* (Mao et al., Proc. Natl. Acad. Sci. USA 101:198-203 (2004), Gal4 line named “P247”) which is specific for adult mushroom bodies of the brain, and promoters/enhancers derived from other neural-specific genes; and *gcm* (Dumstreit et al.,
- 25 J. Neurosci. 23:3325-35 (2003)) which is specific for glial cells; all of which references are incorporated by reference herein. Other examples of expression control sequences include, but are not limited to the heat shock promoters/enhancers from the *hsp70* and *hsp83* genes, useful for temperature induced expression; and promoters/enhancers derived from ubiquitously expressed genes, such as tubulin, actin, or ubiquitin.

- 30 [0063] Transgenic flies that have incorporated into their genome a DNA sequence that encodes exons 1-4 of huntingtin can be utilized.

[0064] The transgenic flies for screening compounds for treating neurological diseases can be generated by any means known to those skilled in the art. Methods for production and analysis of transgenic *Drosophila* strains are well established and described in Brand et al., Methods in Cell Biology 44:635-654 (1994); Hay et al., Proc. Natl. Acad. Sci. USA 94(10):5195-200 (1997); and in *Drosophila: A Practical Approach* (ed. D. B. Roberts), pp175-197, IRL Press, Oxford, UK (1986), herein incorporated by reference in their entirety.

[0065] In general, to generate a transgenic fly, a transgene of interest is stably incorporated into a fly genome. Any fly can be used, such as a member of the Drosophilidae family. An exemplary fly is *Drosophila melanogaster*.

[0066] A variety of transformation vectors are useful for the generation of transgenic flies, and include, but are not limited to, vectors that contain transposon sequences, which mediate random integration of a transgene into the genome, as well as vectors that use homologous recombination (Rong and Golic, Science 288:2013-2018 (2000)). One vector is pUAST (Brand and Perrimon, Development 118:401-415 (1993)) which contains sequences from the transposable P-element which mediate insertion of a transgene of interest into the fly genome. Another vector is PdL, which is able to yield doxycycline-dependent overexpression (Nandis, Bhole and Tower, Genome Biology 4 (R8):1-14, (2003)). Yet another vector is pExp-UAS because of its ease of cloning and mapping genomic location.

[0067] P-element transposon mediated transformation is a commonly used technology for the generation of transgenic flies and is described in detail in Spradling, P element mediated transformation, in *Drosophila: A Practical Approach* (ed. D. B. Roberts), pp175-197, IRL Press, Oxford, UK (1986), herein incorporated by reference. Other transformation vectors based on transposable elements include, for example, the hobo element (Blackman et al., Embo J. 8:211-7 (1989)), the mariner element (Lidholm et al., Genetics 134:859-68 (1993)), the hermes element (O'Brochta et al., Genetics 142:907-14 (1996)), the Minos element (Loukeris et al., Proc. Natl. Acad. Sci. USA 92:9485-9 (1995)), or the piggyBac element (Handler et al., Proc. Natl. Acad. Sci. USA 95:7520-5 (1998)). In general, the terminal repeat sequences of the transposon that are required for transposition are incorporated into a transformation vector and arranged such that the terminal repeat sequences flank the transgene of interest. It is desirable that the transformation vector contains a marker gene

used to identify transgenic animals. Commonly used marker genes affect the eye color of *Drosophila*, such as derivatives of the *Drosophila white* gene (Pirrotta V., & C. Brockl, EMBO J. 3(3):563-8 (1984)) or the *Drosophila rosy* gene (Doyle W. et al., Eur. J Biochem. 239(3):782-95 (1996)) genes. Any gene that results in a reliable and easily measured

5 phenotypic change in transgenic animals can be used as a marker. Examples of other marker genes used for transformation include the *yellow* gene (Wittkopp P. et al., Curr Biol. 12(18):1547-56 (2002)) that alters bristle and cuticle pigmentation; the *forked* gene (McLachlan A., Mol Cell Biol. 6(1):1-6 (1986)) that alters bristle morphology; the *Adh*⁺ gene used as a selectable marker for the transformation of *Adh*⁻ strains (McNabb S. et al.,

10 Genetics 143(2):897-911 (1996)); the *Ddc*⁺ gene used to transform *Ddc*^{ts2} mutant strains (Scholnick S. et al., Cell 34(1):37-45(1983)); the *lacZ* gene of *E. coli*; the *neomycin*^R gene from the *E. coli* transposon Tn5; and the green fluorescent protein (GFP; Handler and Harrell, Insect Molecular Biology 8:449-457 (1999)), which can be under the control of different promoter/enhancer elements, e.g. eyes, antenna, wing and leg specific promoter/enhancers, or

15 the poly-ubiquitin promoter/enhancer elements.

[0068] Plasmid constructs for introduction of the desired transgene are coinjected into *Drosophila* embryos having an appropriate genetic background, along with a helper plasmid that expresses the specific transposase needed to incorporate the transgene into the genomic

20 DNA. Animals arising from the injected embryos (G0 adults) are selected, or screened manually, for transgenic mosaic animals based on expression of the marker gene phenotype and are subsequently crossed to generate fully transgenic animals (G1 and subsequent generations) that will stably carry one or more copies of the transgene of interest.

25 [0069] Binary systems are commonly used for the generation of transgenic flies, such as the UAS/GAL4 system. This is a well established system which employs the UAS upstream regulatory sequence for control of promoters by the yeast GAL4 transcriptional activator protein, as described in Brand and Perrimon, Development 118:401-15 (1993)) and Rorth et al, Development 125:1049-1057 (1998), herein incorporated by reference in their entireties.

30 In this approach, transgenic *Drosophila*, termed "target" lines, are generated where the gene of interest (e.g., huntingtin) is operatively linked to an appropriate promoter (e.g., hsp70 TATA box, see Brand and Perrimon, Development 118:401-15 (1993)) controlled by UAS. Other transgenic *Drosophila* strains, termed "driver" lines, are generated where the GAL4 coding region is operatively linked to promoters/enhancers that direct the expression of the

GAL4 activator protein in specific tissues, such as the eye, antenna, wing, or nervous system or by induction, such as by heat shock. The gene of interest is not expressed in the "target" lines for lack of a transcriptional activator to "drive" transcription from the promoter joined to the gene of interest. However, when the UAS- target line is crossed with a GAL4 driver line, the gene of interest is induced. The resultant progeny display a specific pattern of expression that is characteristic for the GAL4 line.

[0070] The technical simplicity of this approach makes it possible to sample the effects of directed expression of the gene of interest in a wide variety of tissues by generating one transgenic target line with the gene of interest, and crossing that target line with a panel of pre-existing driver lines. Numerous GAL4 driver *Drosophila* strains with specific drivers have been described in the literature and others can readily be prepared using established techniques (Brand and Perrimon, Development 118:401-15 (1993)). Driver strains for use include, for example, *apterous*-Gal4 for expression in wings, brain, and interneurons; *elav*-Gal4 for pan-neuronal expression in post-mitotic neurons; *scabrous*-GAL4 for pan-neuronal expression in the developing nervous system from neuroblasts to neurons; *sevenless*-Gal4, *eyeless*-Gal4, and *GMR*-Gal4 for expression in eyes; *Nervana 2*-Gal4 for expression in the central nervous system; *Cha*- (choline acetyltransferase) Gal4 for expression in cholinergic neurons, *TH*- (tyrosine hydroxylase) for expression in dopaminergic neurons; *CaMKII*- (calmodulin dependent kinase II) for expression in the central nervous system of embryos and larvae as well as the brain, thoracic ganglion, and gut of adults; *P*-Gal4 for expression in pharangeal sensory neurons; and *gcm*-Gal4 for expression in glial cells.

[0071] In an embodiment, transgenic *Drosophila* are produced using the UAS/GAL4 control system. Briefly, to generate a transgenic fly that expresses a mutant form of huntingtin containing a 128Q repeat in exon 1, a DNA sequence encoding *htt128Q* is cloned into a vector such that the sequence is operatively linked to the GAL4 responsive element UAS. Vectors containing UAS elements are readily available in the art, such as the pUAST vector (Brand and Perrimon, Development 118:401-415 (1993)), which places the UAS sequence element upstream of the transcribed region. The DNA is cloned using standard methods (Sambrook et al., *Molecular Biology: A laboratory Approach*, Cold Spring Harbor, N.Y. (1989); Ausubel, et al., *Current protocols in Molecular Biology*, Greene Publishing, Y, (1995)) and is described in more detail under the Molecular Techniques section of the present disclosure. After cloning the DNA into appropriate vector, such as pUAST, the vector is

injected into *Drosophila* embryos (e.g. *yw* embryos) by standard procedures (Brand et al., Methods in Cell Biology 44:635-654 (1994); Hay et al., Proc. Natl. Acad. Sci. USA 94:5195-200 (1997)) to generate transgenic *Drosophila*.

5 [0072] When the binary UAS/GAL4 system is used, the transgenic progeny can be crossed with *Drosophila* driver strains to assess the presence of an altered phenotype caused by expression of the transgene. A desirable *Drosophila* comprises the eye specific driver strain *GMR-GAL4*, which enables identification and classification of transgenic flies based on the severity of a eye phenotype.

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[0073] To evaluate locomotor and behavioral phenotypes (e.g., climbing assay), an *elav-GAL4* driver strain is used in the cross. Ectopic overexpression of *htt128Q* in *Drosophila* central nervous system (CNS) results in locomotor deficits, such as impaired movement, climbing and flying.

15

[0074] Expression of proteins such as huntingtin in transgenic flies is confirmed by standard techniques, such as Western blot analysis or by immunostaining of fly tissue cross-sections, both of which are described below.

20 [0075] *Locomotor Phenotypes*

[0076] Locomotor phenotypes can be assessed, for example, as described in U.S. Application Nos. 2004/0076583, 2004/0076318, and 2004/0076999, each of which is hereby incorporated by reference in its entirety. For example, locomotor ability can be assessed in a climbing
25 assay by placing flies in a vial, knocking them to the bottom of the vial, then counting the number of flies that climb past a given mark on the vial during a defined period of time. In this example, 100% locomotor activity of control flies is represented by the number of flies that climb past the given mark, while flies with an altered locomotor activity can have 80%, 70%, 60%, 50%, less than 50%, or less than 30% of the activity observed in a control fly
30 population.

[0077] In one aspect, the traits are measured by detecting and serially analyzing the movement of a population of flies in containers, e.g., vials. Movement of the flies can be monitored by a recording instrument, such as a CCD-video camera, the resultant images can

be digitized, analyzed using processor-assisted algorithms as described herein, and the analysis data stored in a computer-accessible manner. For example, in measuring traits related to fly movement, the trajectory of each animal may be monitored by calculation of one or more variables (e.g., speed, vertical only speed, vertical distance, turning frequency, frequency of small movements, etc.) for the animal. Values of such a variable are then averaged for population of animals in the vial and a global value is obtained describing the trait for each population (e.g., parental stock flies and transgenic flies).

[0078] "Movement trait data" as used herein refers to the measurements made of one or more movement traits. Examples of "movement trait data" measurements include, but are not limited to X-pos, X-speed, speed, turning, stumbling, size, T-count, P-count, T-length, Cross150, Cross250, and F-count. Descriptions of these particular measurements are provided below.

Examples of such "movement traits" include, but are not limited to:

- a) total distance (average total distance traveled over a defined period of time);
- b) X only distance (average distance traveled in X direction over a defined period of time);
- c) Y only distance (average distance traveled in Y direction over a defined period of time);
- d) average speed (average total distance moved per time unit);
- e) average X-only speed (distance moved in X direction per time unit);
- f) average Y-only speed (distance moved in Y direction per time unit);
- g) acceleration (the rate of change of velocity with respect to time);
- h) turning;
- i) stumbling;
- j) spatial position of one fly to a particular defined area or point (examples of spatial position traits include (1) average time spent within a zone of interest (e.g., time spent in bottom, center, or top of a container; number of visits to a defined zone within container); (2) average distance between a fly and a point of interest (e.g., the center of a zone); (3) average length of the vector connecting two sample points (e.g., the line distance between two flies or between a fly and a defined point or object); (4) average time the length of the vector connecting the two sample points is less than, greater than, or equal to a user define parameter; and the like);

k) path shape of the moving fly, *i.e.*, a geometrical shape of the path traveled by the fly (examples of path shape traits include the following: (1) angular velocity (average speed of change in direction of movement); (2) turning (angle between the movement vectors of two consecutive sample intervals); (3) frequency of turning (average amount of turning per unit of time); (4) stumbling or meandering (change in direction of movement relative to the distance); and the like. This is different from stumbling as defined above. Turning parameters may include smooth movements in turning (as defined by small degrees rotated) and/or rough movements in turning (as defined by large degrees rotated).

10 [0079] Movement traits can be quantified, for example, using the following parameters:

X-Pos: The X-Pos score is calculated by concatenating the lists of x-positions for all trajectories and then computing the average of all values in the concatenated list

X-Speed: The X-Speed score is calculated by first computing the lengths of the x-components of the speed vectors by taking the absolute difference in x-positions for subsequent frames. The resulting lists of x-speeds for all trajectories are then concatenated and the average x-speed for the concatenated list is computed.

Speed: The Speed score is calculated in the same way as the X-Speed score, but instead of only using the length of the x-component of the speed vector, the length of the whole vector is used. That is, $[\text{length}] = \text{square root of } ([\text{x-length}]^2 + [\text{y-length}]^2)$.

20 *Turning*: The Turning score is calculated in the same way as the Speed score, but instead of using the length of the speed vector, the absolute angle between the current speed vector and the previous one is used, giving a value between 0 and 90 degrees.

Stumbling: The Stumbling score is calculated in the same way as the Speed score, but instead of using the length of the speed vector, the absolute angle between the current speed vector and the direction of body orientation is used, giving a value between 0 and 90 degrees.

Size: The Size score is calculated in the same way as the Speed score, but instead of using the length of the speed vector, the size of the detected fly is used.

T-Count: The T-Count score is the number of trajectories detected in the movie.

P-Count: The P-Count score is the total number of points in the movie (*i.e.*, the number of points in each trajectory, summed over all trajectories in the movie).

T-Length: The T-Length score is the sum of the lengths of all speed vectors in the movie, giving the total length all flies in the movie have walked.

Cross150: The Cross150 score is the number of trajectories that either crossed the line at $x = 150$ in the negative x-direction (from bottom to top of the vial) during the movie,

or that were already above that line at the start of the movie. The latter criterion was included to compensate for the fact that flies sometimes don't fall to the bottom of the tube. In other words this score measures the number of detected flies that either managed to hold on to the tube or that managed to climb above the $x = 150$ line within the length of the movie.

5 *Cross250*: The *Cross250* score is equivalent to the *Cross150* score, but uses a line at $x = 250$ instead.

F-Count: The *F-Count* score counts the number of detected flies in each individual frame, and then takes the maximum of these values over all frames. It thereby measures the maximum number of flies that were simultaneously visible in any single frame during the
10 movie.

[0080] The assignment of directions in the X-Y coordinate system is arbitrary. For purposes of this disclosure, "X" refers to the vertical direction (typically along the long axis of the container in which the flies are kept) and "Y" refers to movement in the horizontal direction
15 (e.g., along the surface of the vial).

[0081] *Behavioral Phenotypes*

[0082] Neuronal degeneration in the central nervous system will give rise to alterations in
20 behavioral phenotype, including but not limited to locomotor deficits, that can be assayed and quantified in both larvae and adult *Drosophila*. For example, failure of *Drosophila* adult animals to climb in a standard climbing assay (see, e.g. Ganetzky and Flannagan, J. Exp. Gerontology 13:189-196 (1978); LeBourg and Lints, J. Gerontology 28:59-64 (1992)) is quantifiable, and indicative of the degree to which the animals have a motor deficit and
25 neurodegeneration. Neurodegenerative phenotypes include, but are not limited to, progressive loss of neuromuscular control, e.g. of the wings; progressive degeneration of general coordination; progressive degeneration of locomotion, and progressive loss of appetite. Other aspects of fly behavior that can be assayed include but are not limited to circadian behavioral rhythms, feeding behaviors, courtship behaviors, inhabitation to
30 external stimuli, and odorant conditioning. All of these phenotypes are measured by one skilled in the art by standard visual observation of the fly.

Another neural degeneration phenotype, is a reduced life span, for example, the *Drosophila* life span can be reduced by 10-80%, e.g., approximately, 30%, 40%, 50%, 60%, or 70%.

[0083] *Memory and Learning Phenotypes*

[0084] In *Drosophila*, the best characterized assay for associative learning and memory is an odor-avoidance behavioral task (T. Tully, et al. J. Comp. Physiol. A157, 263-277 (1985),
 5 incorporated herein by reference). This classical (Pavlovian) conditioning involves exposing the flies to two odors (the conditioned stimuli, or CS), one at a time, in succession. During one of these odor exposures (the CS+), the flies are simultaneously subjected to electric shock (the unconditioned stimulus, or US), whereas exposure to the other odor (the CS-) lacks this negative reinforcement. Following training, the flies are then placed at a “choice
 10 point,” where the odors come from opposite directions, and expected to decide which odor to avoid. By convention, learning is defined as the fly's performance when testing occurs immediately after training. A single training trial produces strong learning: a typical response is that >90% of the flies avoid the CS+. Performance of wild-type flies from this single-cycle training decays over a roughly 24-hour period until flies once again distribute evenly between
 15 the two odors. Flies can also form long-lasting associative olfactory memories, but normally this requires repetitive training regimens.

[0085] A common feature of many neurodegenerative diseases is the presence of protein aggregation in the brain. Examples of neurodegenerative diseases characterized by protein
 20 aggregation include Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Pick's disease, prion diseases, and other spongiform encephalopathies.

[0086] **Example 1: Production of Transgenic *Drosophila* Model of Huntington's Disease**

[0087] Female “activator” or “driver” flies were engineered to contain the neuronal specific
 25 drivers *elav-GAL4* or *nervana-GAL4* in a heat shock-*hid* Y (*phs-hid*Y or HS-*hid*+ on Y chromosome) background (Starz-Gaiano et al., 2001). The *hid* gene is a pro-apoptotic gene, which is expressed in this line by a heat inducible promoter. These flies were heat shocked in early development to kill male larvae and embryos and thereby produce massive numbers of *elav-GAL4* or *nervana-GAL4* virgin females.

30 [0088] For the mass production of males (*UAS-htt128Q[F27B]/UAS-htt128Q[F27B]*), the disease transgenes were moved into lines that contain a compound X (attached-X) chromosome with the same *phs-hid* element ($X^{X_{phs-hidY}}$) used above. In this scheme the *hid*

gene is present only in female flies and therefore was used to produce large numbers of male flies by heat shock. The massive collection of virgin female *elav-GAL4* flies were crossed with the massive collection of males that contain the silent disease-transgene. Their progeny, the F1 generation, were the “assay flies” (e.g., *elav-GAL4 / UAS-htt128Q[F27B]*). These

5 assay flies were robotically distributed into drugged assay vials.

[0089] The HD *Drosophila* model expresses exons 1 to 4 of the human huntingtin gene with 128Q repeats in exon 1. The construct was cloned into the *Drosophila* transformation vector, pUAST. Flies from a resulting transgenic line were then crossed to GAL4 driver lines that direct expression either in the eye (*GMR-GAL4* promoter) or in neurons (*elav-GAL4*

10 promoter). The model shows degeneration of photoreceptors when the transgene was expressed in the eye. Progressive degeneration of the photoreceptors was observed after several days of adult life. The properties of the HD128Q flies were consistent with those reported by others for two other strains in which exon-1-containing expanded repeats was expressed in the eye (Jackson et al., 1998; Steffan et al., 2001 –US20040142859). To test the

15 effects on motor function of the HD mutation, HD128Q lines were examined in which the transgene was expressed in all neurons. Motor function was assayed by tapping flies in a vial to the bottom and measuring the number of flies that are able to climb to a specified height within a specific length of time. When tapped to the bottom of a vial, wild-type flies rapidly climb to the top, where most of them remain. When assayed by this manual procedure, the

20 HD128Q transgenic lines showed normal behavior early in life. Starting at 10-12 days of life, however, the transgenic flies were observed to make short abortive climbs and fall back to the bottom. The decline in motor performance was progressive, leading to early death.

Examination of the HD128Q flies under the microscope revealed that their motor dysfunction was the result of spontaneous and uncoordinated movement of the limbs, which inhibited

25 normal locomotion. Examination of the neurons in these flies showed progressive degeneration and loss of particular neurons, accompanied by the formation of nuclear inclusions that stain positively for molecular chaperones, ubiquitin, and components of the proteasome. The HD128Q model thus has a functional deficit that in its phenotypic and neuropathological characteristics appears to be related to that seen in the human disease.

30 Moreover, as the deficit is quantifiable and reproducible, it is suitable for automated high-throughput screening.

[0090] Example 2: Locomotor Activity Assay Using the Transgenic *Drosophila* HD Model

[0091] Standard environmental conditions were followed to establish that different metrics detect differences between disease and control flies. A comparison of compound-treated HD flies and untreated HD flies was also included. The positive control compound tested was the histone deacetylase (HDAC) inhibitor Trichostatin-A (TSA).

[0092] In an assay run (typically corresponding to one day of an entire multi-day assay) the following process was followed. Each tube was dropped onto a surface, causing the flies to fall to the bottom of the tube and be subjected to impact shock stimulation. Their immediate negative geotaxis response to that event was thereafter captured in a 7.5 second movie. For each tube, this step was repeated four times, giving a total of four repeat videos per tube and run. The raw data for each video were analyzed and trajectories following the movements of individual flies in each tube were generated. The method for analyzing fly trajectories is described in published U.S. Patent Application 2004/0076318A1, which is hereby incorporated by reference in its entirety. The trajectories consisted of a list of x- and y-coordinates mapping out the positions of all the flies in the video, as well as measurements of size, orientation, length and width of the flies, with one list entry for every frame from when the fly started moving in one frame until it stopped in another. These data were then used to provide different scoring metrics for the test flies' behavior. The definitions of 11 metrics developed are summarized above.

[0093] The movies were first scored individually to give one value per metric and movie. Average values for all metrics were then calculated from the movie score values over all four repeat videos for a vial, resulting in one value per tube and metric. In a typical assay run, there were several tubes subjected to the same treatment. The data values from replicate tubes were subsequently used to calculate treatment means and standard errors for each specific treatment.

[0094] Average speed of the *Drosophila* flies were measured by a robot at both early and late speed, and early and late speed summary metrics were created. Early speed was the average speed of eight replicate vials, ten flies/vial, four repeat videos per trial day, averaged over trial days 1-7. Late speed was determined in the same manner but measured and averaged over days 8-10. Effect sizes were the differences between average mean speeds of the

summary metrics (i.e., differences between DMSO-carrier control and test drug) divided by the pooled standard deviation in the whole assay.

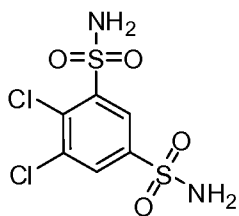
[0095] Carbonic anhydrase(s) are a collection of at least 14 Zinc containing metalloenzymes that are separated in three groups designated α , β , and γ . The enzymes catalyze the reversible dehydration of bicarbonate (HCO_3^-) which is characterized by Equation 1. The enzymes are also known to catalyze other chemical and biochemical reactions such as the hydrolysis of esters and lipids.

Equation 1

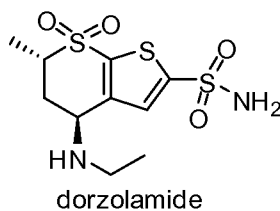


[0096] A carbonic anhydrase inhibitor is a compound that reduces the ability of a carbonic anhydrase to catalyze the conversion depicted in Equation 1 and/or one or more additional chemical or biochemical reactions catalyzed by a carbonic anhydrase. The reaction may be inhibited from progressing in any one or both directions.

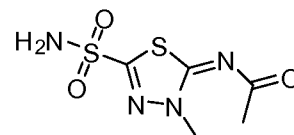
- 15 [0097] Examples of carbonic anhydrase inhibitors can be found in the following patents and applications US 5,095,026, US 5,157,044, US 6,214,381, US 6,683,066, US 6,242,442, US 2006/0088886A1, US 6,620,819, hereby incorporated by reference. The examples found in these documents are not meant to be limiting. Further examples of carbonic anhydrase inhibitors are depicted below. Other examples of carbonic anhydrase inhibitors include:
- 20 brinzolamide and ethoxbenzamide. Compounds that inhibit carbonic anhydrase, but have additional activities include: Ocufen (sodium 2-(3-fluoro-4-phenyl-phenyl)propanoate dehydrate; Furbiprofen – ((2R)-2-(3-fluoro-4-phenyl-phenyl)propanoic acid); homosulfamine (4-(aminomethyl)benzenesulfonamide hydrochloride; probenecid (4-(dipropylsulfamoyl)benzoic acid) and mafenide (4-(aminomethyl)benzenesulfonamide).
- 25 Other carbonic anhydrase inhibitors are known to those of ordinary skill in the art. One of ordinary skill in the art will recognize that certain carbonic anhydrase inhibitors can exist in multiple tautomeric forms.



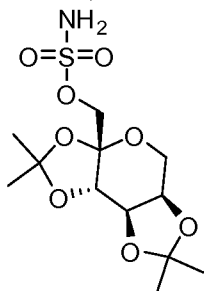
Dichlorphenamide
4,5-dichlorobenzene-1,3-disulfonamide



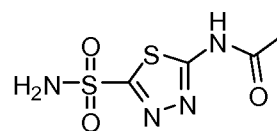
dorzolamide



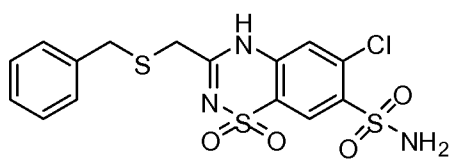
N-(3-methyl-5-sulfamoyl-1,3,4-thiadiazol-2(3*H*)-ylidene)acetamide
Methazolamide



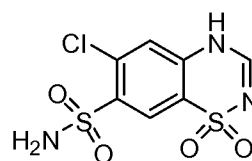
Topiramate
((3*aS*,5*aR*,8*aR*,8*bS*)-2,2,7,7-tetramethyltetrahydro-3*aH*-bis[1,3]dioxolo[4,5-*b*:4',5'-*d*]pyran-3*a*-yl)methyl sulfamate



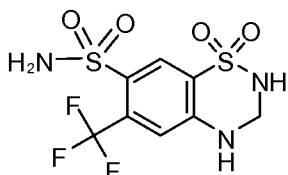
Acetazolamide
Acetazolamide
N-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)acetamide



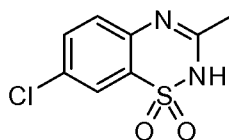
Benzthiazide



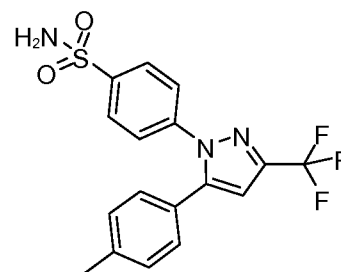
Chlorothiazide



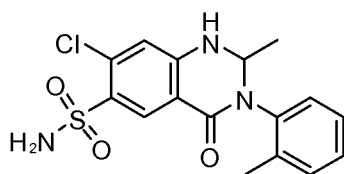
Hydroflumethiazide



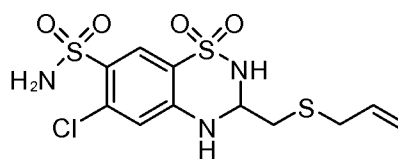
Diazoxide



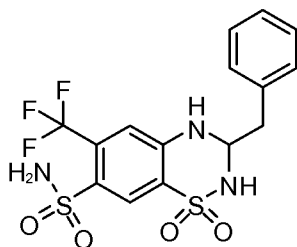
Celecoxib



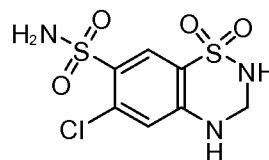
Metolazone



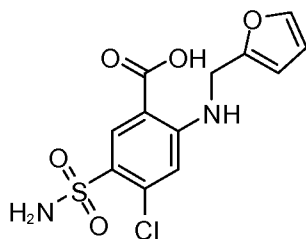
Althiazide



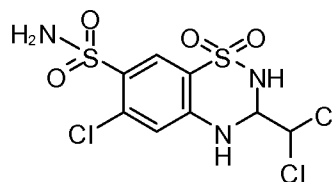
Bendroflumethiazide



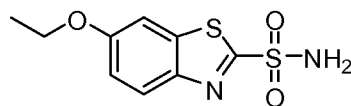
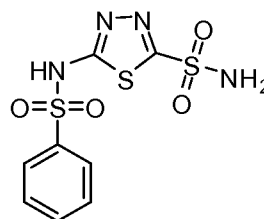
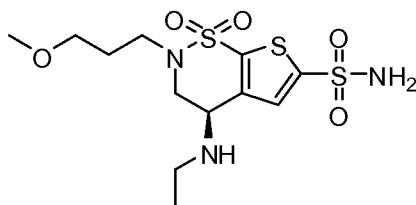
Hydrochlorthiazide



Furosemide



Trichlormethiazide

Ethoxyzolamide
6-ethoxybenzo[d]thiazole-2-sulfonamideBenzamide
5-(phenylsulfonamido)-1,3,4-thiadiazole-2-sulfonamide

brinzolamide

[0098] Figure 1 shows a comparison of the age-dependent decline in speed of locomotion
 5 between the wild type flies treated with vehicle (DMSO) and HD model flies treated with vehicle, HD model flies treated with the positive control compounds Trichostatin A (TSA) and different concentrations of dorzolamide (DRZ).

[0099] Figure 2 shows a comparison of the age-dependent decline in speed of locomotion
 10 between the wild type flies treated with vehicle (DMSO) and HD model flies treated with vehicle, HD model flies treated with the positive control compounds Trichostatin A (TSA) and different concentrations of dichlorphenamide (DCP).

[0100] The table shown in Figure 3 shows the effect sizes compared to vehicle-treated HD flies of HD flies treated with carbonic anhydrase inhibitors at various concentrations. The effect sizes were calculated as described above for early speed as the average mean speed over trial days 1-7 and late speed as the average mean speed over days 8-10. Concentration is the final concentration of the drug or control compound in the food medium. The phenotype measured is the progressive decline in motor coordination and reduction in average walking speed following stimulation (tapping of the vial). The HD model flies also had a reduced lifespan (data not shown). The calculated effect sizes were weighted averages across experiments, using sample size as weight. Not all campaigns had the same number of replicates. The more replicates then a greater weight is attributed. The effect sizes shown in green are the calculated effect size averaged over at least three different assays with a total $n < 21$. Data shown in the white boxes are effect sizes in at least one assay with n greater than or equal to 8.

[0101] The effect of dichlorophenamide on survival in the HD fly model was analyzed. As shown in Figure 4, which is a Kaplan-Meier survival curve, dichlorophenamide (upper lines) significantly increases survival time compared to DMSO ($p \leq 0.05$).

[0102] **Example 3: Production of Transgenic Drosophila Model of Alzheimer's Disease**

[0103] Female "activator" or "driver" flies were engineered to contain the neuronal specific drivers *elav-GAL4* or *Cha-GAL4* in a heat shock-*hid* Y (*phs-hid*Y or HS-*hid*+ on Y chromosome) background (Starz-Gaiano et al., 2001). The *hid* gene is a pro-apoptotic gene, which is expressed in this line by a heat inducible promoter. These flies were heat shocked in early development to kill male larvae and embryos and thereby produce massive numbers of *elav-GAL4* or *Cha-GAL4* virgin females.

[0104] For the mass production of males (*UAS-APP-CT/UAS-APP-CT*, *UAS-tau/UAS-tau*), the human disease transgenes were moved into lines that contain a compound X (attached-X) chromosome with the same *phs-hid* element ($X^{Xphs-hidY}$) used above. In this scheme the *hid* gene is present only in female flies and therefore was used to produce large numbers of male flies by heat shock. The massive collection of virgin female *Cha-GAL4* flies were crossed with the massive collection of males that contain the silent disease-transgenes. Their

progeny, the F1 generation, were the “assay flies” (e.g., *Cha-GAL4/UAS-APP-CT*, *UAS-tau*). These assay flies were robotically distributed into drugged assay vials.

[0105] The AD *Drosophila* model contains the coding sequence of the carboxy-terminal portion of the human amyloid precursor protein gene (APP-CT), often referred to as the “C99” or “C100” fragment linked to a human APP signal sequence. The construct was cloned into the *Drosophila* transformation vector, pExP-UAS. Similarly, the human wild-type tau coding sequence was cloned into the pUAST *Drosophila* transformation vector. Both constructs were then incorporated into the fly to generate the AD disease model transgenic line. Flies from a resulting transgenic line were then crossed to GAL4 driver lines that direct expression either in the eye (*GMR-GAL4* promoter) or in cholinergic neurons (*Cha-GAL4* promoter). The model shows degeneration of photoreceptors when the transgenes were expressed in the eye. To test the effects on motor function of the expression of the human AD transgenes, APP-CT, tau lines were examined in which the transgenes were expressed in cholinergic neurons. Motor function was assayed by tapping flies in a vial to the bottom and measuring the number of flies that are able to climb to a specified height within a specific length of time. When tapped to the bottom of a vial, wild-type flies rapidly climb to the top, where most of them remain. When assayed by this manual procedure, the APP-CT, tau transgenic lines showed normal behavior early in adult life. Starting at 2-3 days of adult life, however, the transgenic flies were observed to make frequent falls before reaching the top of the vial. The flies also exhibited some erratic movement. The decline in motor performance was progressive, leading to early death. Examination of the AD assay flies under the microscope revealed that their motor dysfunction was the result of miscoordination of the limbs of the flies, which inhibited normal locomotion. Examination of the proteins in the brains of the AD model flies show production of beta-amyloid resulting from the cleavage of the APP-CT fragment, recapitulating the processing of the APP-CT fragment in humans. Furthermore, examination of aged brains of flies expressing the APP-CT transgene showed an age-dependent increase in insoluble beta-amyloid and an increase a plaque-like deposits, recapitulating the human disease. Moreover, as the locomotor deficit is quantifiable and reproducible, it is suitable for automated high-throughput screening.

[0106] Example 4: Locomotor Activity Assay Using the Transgenic Drosophila AD Model

[0107] Standard environmental conditions were followed to establish that different metrics detect differences between disease and control flies. A comparison of compound-treated AD flies and untreated AD flies was also included. The positive control compounds tested were the histone deacetylase (HDAC) inhibitor Trichostatin-A (TSA) and the cholinesterase inhibitor tacrine.

[0108] In an assay run (typically corresponding to one day of an entire multi-day assay) the following process was followed. Each tube was dropped onto a surface, causing the flies to fall to the bottom of the tube and be subjected to impact shock stimulation. Their immediate negative geotaxis response to that event was thereafter captured in a 7.5 second movie. For each tube, this step was repeated four times, giving a total of four repeat videos per tube and run. The raw data for each video were analyzed and trajectories following the movements of individual flies in each tube were generated. The method for analyzing fly trajectories is described in published U.S. Patent Application 2004/0076318A1, which is hereby incorporated by reference in its entirety. The trajectories consisted of a list of x- and y-coordinates mapping out the positions of all the flies in the video, as well as measurements of size, orientation, length and width of the flies, with one list entry for every frame from when the fly started moving in one frame until it stopped in another. These data were then used to provide different scoring metrics for the test flies' behavior. The definitions of 11 metrics developed are summarized above.

[0109] The movies were first scored individually to give one value per metric and movie. Average values for all metrics were then calculated from the movie score values over all four repeat videos for a vial, resulting in one value per tube and metric. In a typical assay run, there were several tubes subjected to the same treatment. The data values from replicate tubes were subsequently used to calculate treatment means and standard errors for each specific treatment.

[0110] The average number of trajectories (a single continuous path of an individual fly) above a 5.75 cm height in the vial for the first four seconds of the movie was measured by a robot over the 12 days of an assay, and the crosshigh summary metrics were created. Days 1-

9 was the average crosshigh 0-4 seconds averaged over eight replicate vials, ten flies/vial, four repeat videos/trial day for trial days 1-9. Similarly, crosshigh days 4-12 was calculated.

[0111] The percent of trajectories in the top of the vial for last 4.5 seconds of the movie was measured by dividing the number of trajectories in the top three centimeters of the vial for 4-

5 7.5 seconds in each movie. The summary metric percoftraj days 1-7 was calculated similarly as the metrics above by averaging over eight replicate vials, ten flies/vial, four repeat videos/trial day for trial days 1-7.

[0112] The mean of all detected fly speeds was calculated as the distance moved per frame for all pairs of detected points in all fly trajectories in the movie or movie segment.

10 WeightedSec4Speed was the mean of the detected fly speeds weighted by the time the detected speed occurred. The summary metric for days 1-10 was calculated similarly to the metrics above.

[0113] The results this analysis are shown in Figure 5 which is a table presenting the effect sizes of AD model flies treated with carbonic anhydrase inhibitors at various concentrations.

15 The category, as determined by effect size over several independent experiments, is listed under the effect size for each metric and concentration.

[0114] All patents, patent applications, and published references cited herein are hereby incorporated by reference in their entirety. While this disclosure has been particularly

20 illustrated and described with reference to particular examples, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope and spirit of the disclosure encompassed by the appended claims.

CLAIMS

1. A method of treating a subject having a neurological disorder, comprising administering
5 to the subject an effective amount of a carbonic anhydrase inhibitor.
2. The method of claim 1 wherein the neurological disorder is neurodegenerative disease.
3. The method of claim 1 wherein the neurological disorder is selected from the group
10 consisting of Huntington's disease, Alzheimer's disease, and a neurological disorder
arising from a polyglutamine expansion.
4. The method of claim 1 wherein the neurological disorder is Huntington's disease.
- 15 5. The method of any of claims 1-4 wherein the carbonic anhydrase inhibitor is selected
from the group consisting of dichlorphenamide, dorzolamide, methazolamide, topiramate
or acetazolamide.
6. The method of any of claims 1-4 wherein the carbonic anhydrase inhibitor is selected
20 from the group consisting of benzthiazide, chlorothiazide, hydroflumethiazide, diazoxide,
celecoxib, metolazone and althiazide.
7. The method of any of claims 1-4 wherein the carbonic anhydrase inhibitor is selected
from the group consisting of bendroflumethiazide, hydrochlorthiazide, trichlomethiazide,
25 benzolamide and furosemide.
8. The method of any of claims 1-4, wherein the carbonic anhydrase inhibitor is
administered as a pharmaceutical composition further comprising at least one excipient,
carrier or diluent.
- 30 9. The method of claim 8, wherein the pharmaceutical composition is administered in a solid
dosage form or in a liquid dosage form.

10. The method of claim 9, wherein the dosage form is selected from the group consisting of an oral dosage form, a parenteral dosage form, an intranasal dosage form, a suppository, a lozenge, a troche, buccal, a controlled release dosage form, a pulsed release dosage form, an immediate release dosage form, an intravenous solution, a suspension and combinations thereof.
- 5
11. A method comprising administering a carbonic anhydrase inhibitor to a patient whose Huntingtin gene has a polyglutamine expansion.
12. The method of claim 11 wherein the carbonic anhydrase is selected from the group
- 10 dichlorphenamide, dorzolamide, methazolamide, topiramate or acetazolamide.
13. The method of claim 11 wherein the carbonic anhydrase is selected from the group consisting of benzthiazide, chlorothiazide, hydroflumethiazide, diazoxide, celecoxib, metolazone and althiazide
- 15
14. The method of claim 11 wherein the carbonic anhydrase is selected from the group consisting of bendroflumethiazide, hydrochlorthiazide, trichlomethiazide and furosemide.
15. A method comprising administering a carbonic anhydrase inhibitor to a patient having a
- 20 polyglutamine expansion in a gene wherein the polyglutamine expansion is associated with increased for developing a neurodegenerative disorder.

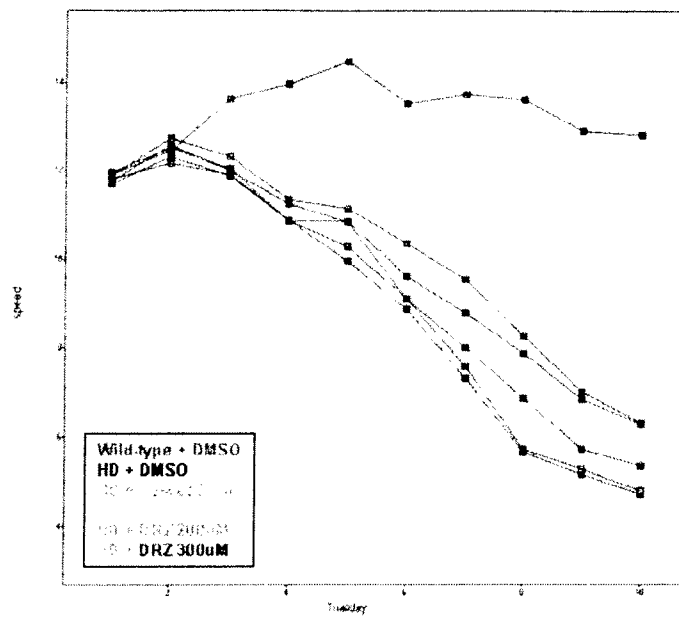


FIGURE 1

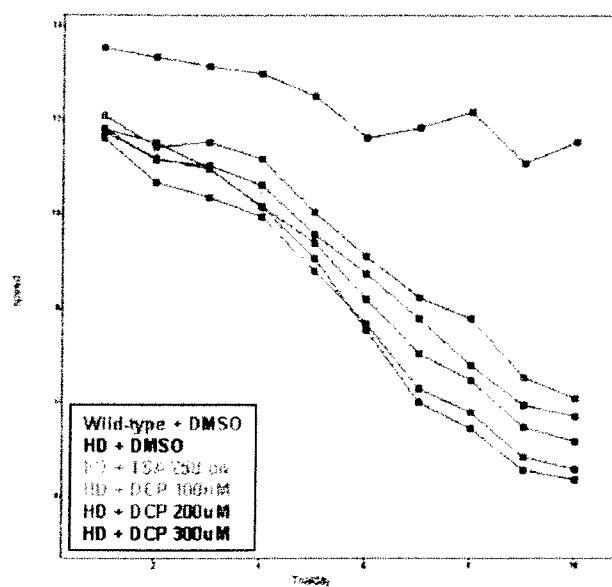
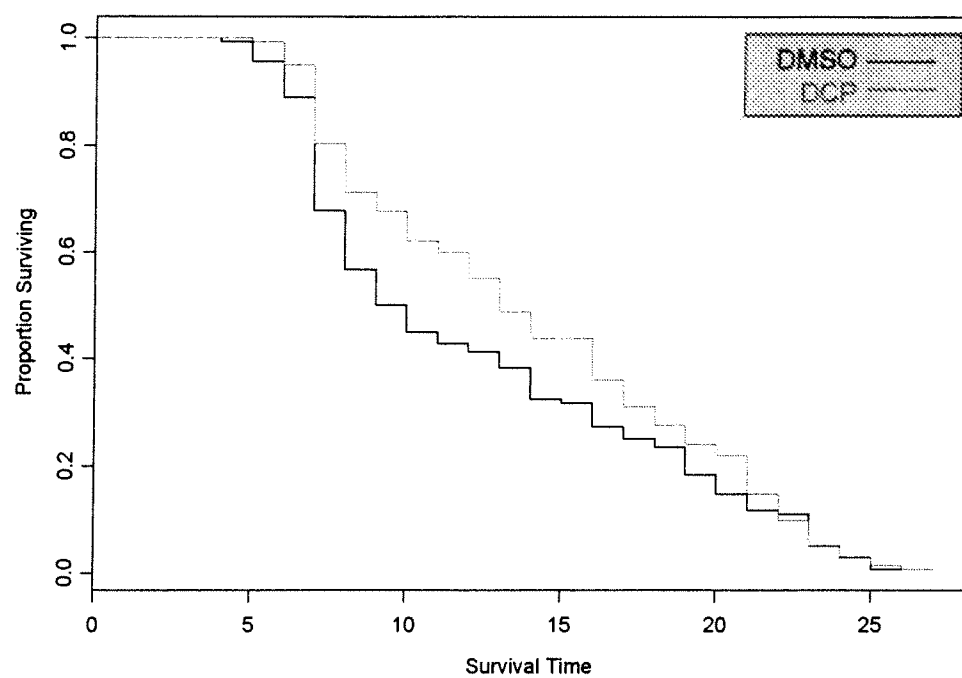


FIGURE 2

compound name	Early Speed (Days 1-7)						Late Speed (Days 8-10)					
	Effect Size						Effect Size					
	12.5 uM	25 uM	50 uM	100 uM	200 uM	300 uM	12.5 uM	25 uM	50 uM	100 uM	200 uM	300 uM
Dichlorphenamide			0.214	0.237	0.824	0.797			0.248	0.566	0.659	0.827
Dorzolamide HCl				0.167	0.814	0.639				0.245	0.802	0.868
Methazolamide		0.39	0.209	0.561	0.772			0.594	0.885	0.836	1.327	
Topiramate				0.182	-0.019	0.418				0.523	0.322	0.815
Acetazolamide		0.193	0.277	0.276	-0.157			0.32	0.322	0.252	0.836	
Benzthiazide		0.299	0.369	-0.72				0.542	0.502	-0.244		
Chlorothiazide		-0.281	0.511	-0.993				-0.086	0.544	-0.718		
Hydroflumethiazide		0.476	-0.155	-0.44				0.383	0.052	-0.359		
Diazoxide				0.009	0.373	-0.232				-0.318	0.697	-0.284
Celcoxib				-0.311	-0.007	-0.262				-0.245	0.028	-0.075
Metolazone		0.094	0.565	0.488				-0.173	-0.081	0.902		
Althiazide		0.659	0.899	-1.348				0.314	0.998	-0.474		
Bendroflumethiazide				-0.293	-0.261	-0.284				-0.263	-0.325	0.039
hydrochlorthiazide			0.163	-0.207	-0.088	-0.315			0.106	0.026	0.074	0.155
Trichlormethiazide			-0.206	0.117					-0.511	-0.436		
Furosemide	0.077	-0.18	-0.165	-1.477			1.047	0.117	-0.327	-1.086		

FIGURE 3

**FIGURE 4**

	crosshigh0-4s days 1-9				crosshigh0-4 days 4-12				percoffrajs day 1-7				weightedSec4Speed days 1-10			
	30 uM	100 uM	200 uM	300 uM	400 uM	30 uM	100 uM	200 uM	300 uM	400 uM	30 uM	100 uM	200 uM	300 uM	400 uM	400 uM
acetazolamide																
Effect Size	-0.19	-0.12		0.1		-0.19	-0.03		-0.11		0.66	0.21		-0.06		
Category	Indet.	Indet.		Indet.		Indet.	Indet.		Indet.		Indet.	Indet.		Indet.		-0.19
methazolamide																
Effect Size			0.49	0.94				0.58	0.92				0.5	0.76		
Category			Indet.	Active				Active	Active				Active	Active		0.7
dichlorphenamide																
Effect Size		0.53	-0.24		0.53		0.28	-0.55		0.5		0.43	-0.36		0.81	0.39
Category		Active	Inactive		Active		Indet.	Inactive		Active		Indet.	Inactive		Active	Indet.
dorzolamide																
Effect Size		-0.03	-0.15		-0.09		0.16	-0.18				0.28	0		0.22	-0.06
Category		Indet.	Indet.		Inactive		Indet.	Indet.				Indet.	Indet.		Indet.	Indet.
topiramate																
Effect Size		-0.05			0.74		-0.21			0.51		0.26			0.7	0.76
Category		Indet.			Indet.		Indet.			Indet.		Indet.			Indet.	Indet.

FIGURE 5

A. CLASSIFICATION OF SUBJECT MATTER*A61K 31/415(2006.01)i, A61K 31/421(2006.01)i, A61K 31/426(2006.01)i, A61P 25/18(2006.01)i*

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 8 as above

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

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

eKIPASS, PUBMED

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2003-0013692 A1 (STEVEN R. GULLANS, ET AL) 16 Jan. 2003	1-10
Y	See pages 1-7.	11-15
Y	ZHANG X. et al. 'A potent small molecule inhibits polyglutamine aggregation in Huntington's disease neurons and suppresses neurodegeneration in vivo.' Proc Natl Acad Sci U S A. 2005 Jan 18; 102(3): 892-7., Epub 2005 Jan 10. See the abstract.	11-15
A	US 6746678 B1 (HOWARD K. SHAPIRO) 8 Jun. 2004 See the abstract.	1-15



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

26 FEBRUARY 2008 (26.02.2008)

Date of mailing of the international search report

26 FEBRUARY 2008 (26.02.2008)

Name and mailing address of the ISA/KR

Korean Intellectual Property Office
Government Complex-Daejeon, 139 Seonsa-ro, Seo-
gu, Daejeon 302-701, Republic of Korea

Facsimile No. 82-42-472-7140

Authorized officer

YANG, In Soo

Telephone No. 82-42-481-5049



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2007/085358**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

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

1. ☒ Claims Nos.: 1-15
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 1-15 relate to a method of treatment of the human by therapy practiced on the human (e.g. a method of treating a subject having a neurological disorder). Nevertheless, a search has been done based on the alleged effects of the compounds/compositions.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2007/085358Patent document
cited in search reportPublication
datePatent family
member(s)Publication
date

US20030013692A1

16.01.2003

W02002056892A2

25.07.2002

W02002056892A3

31.10.2002

US06746678

08.06.2004

None