AUTOPHAGY ENHANCERS

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0001] This invention was made with government support under DK096990 awarded by the National Institutes of Health (NIH). The government has certain rights in the invention.

FIELD OF INVENTION

[0002] The present disclosure provides tetracyclic analogs that induce autophagy in a subject suffering from alpha-1 antitrypsin deficiency (ATD) and possibly other autophagy associated diseases or disorders, such as Alzheimer's, Parkinson's, and Huntington's diseases and amyotrophic lateral sclerosis.

BACKGROUND OF INVENTION

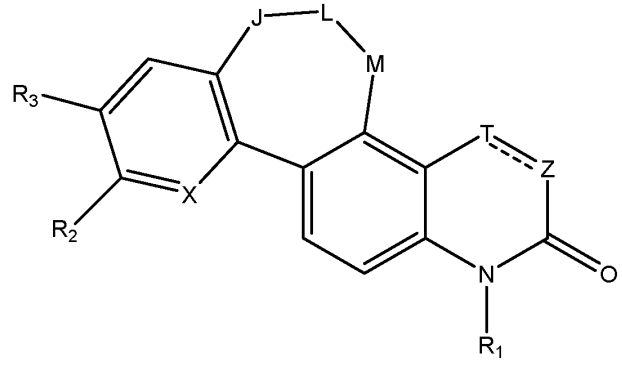
[0003] Autophagy refers to the process involving the decomposition of intracellular components via lysosomes and plays an important role in maintaining and regulating cell homeostasis by degrading intracellular components and providing degradation products to cells. Autophagy also typically declines with age and may contribute to damaged cells and protein accumulation. While autophagy may play an important role in preventing and responding to disease, problems with the autophagy process are believed to be associated with many diseases and disorders. There is great interest in the role of protein degradation via autophagy in such conditions where the protein is found in the cytoplasm. Autophagy is an intracellular process in which cytoplasmic materials are engulfed by double membrane structures, which form autophagosomes. The autophagosomes first fuse with endosomes to form hybrid organelles called amphisomes that later fuse with lysosomes, where the entrapped cytosolic contents are degraded. The process of autophagy has been proposed to be important in protein misfolding disorders, both as a contributing factor, through inhibition of the process, and a potential therapeutic strategy, through its upregulation.

[0004] A need exists for improved agents for enhancing autophagy in a subject to treat or prevent diseases, disorders, or conditions where autophagy is affected.

SUMMARY OF INVENTION

[0005] The present disclosure provides compounds, pharmaceutical compositions, and methods for treatment of autophagy-related diseases.

[0006] One aspect of the disclosure is a compound of Formula I



(Formula I)

wherein R_1 is hydrogen, substituted alkyl, or unsubstituted alkyl; R_2 is hydrogen, halo, substituted alkyl, unsubstituted alkyl, alkoxyl, cyano, amino, $-N(R_4)C(O)R_5$, $-C(O)N(R_4)(R_5)$, $-SO_2R_6$, $-N(R_4)SO_2R_6$, $-SO_2N(R_4)(R_5)$, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl; R_3 is hydrogen, halo, substituted alkyl, unsubstituted alkyl, alkoxyl, cyano, amino, $-N(R_4)C(O)R_5$, $-C(O)N(R_4)(R_5)$, $-SO_2R_6$, $-N(R_4)SO_2R_6$, $-SO_2N(R_4)(R_5)$, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl; J is $-CH(R_4)-$, $-C(R_4)-$, $-N(R_5)-$, or -O-; L is $-CH(R_4)-$, $-C(R_4)-$, or -C(O)-; M is $-CH(R_4)-$, $-C(R_4)-$, $-N(R_5)-$, or -O-; T is absent, $-CH_2CH_2-$, $-CH(R_4)-$, $-C(R_4)-$, -O-, or together with Z form a cycloalkyl group fused to the 6-membered ring; Z is $-CH(R_4)-$, $-C(R_4)-$, $-N(R_5)-$, -N-, -O-, or together with T form a cycloalkyl group fused to the 6-membered ring; T and Z can be joined by a single bond or a double bond depending on the valence of the groups; when T is absent the ring is a 5-membered ring; when T is $-CH_2CH_2-$ the ring is a 7-membered ring; R_4 and R_5 are independently hydrogen or substituted or unsubstituted alkyl; and R_6 is substituted or unsubstituted alkyl.

[0007] Another aspect of the disclosure is a pharmaceutical composition comprising a pharmaceutically acceptable excipient and a compound of Formula I.

[0008] Yet another aspect of the disclosure is a method for treating an autophagy-related disease, the method comprising administering the compound of Formula I described herein or the pharmaceutical compositions described herein to a subject in need thereof.

[0009] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

[0010] The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] Figure 1 depicts the effect of Compound 4 on ATZ levels in HTO/Z cell line model via western blot. ATZ and β-actin levels are shown for varying concentrations of Compound 4, and ATZ values normalized to β-actin levels are shown above.

[0012] Figure 1B depicts the effect of Compound 4 on ATZ levels in HTO/Z cellular models using MSD assay.

[0013] Figure 2A depicts mean plasma concentration of Compounds 4 and 13 after PO dosing of mice with both Compound 4 and Compound 13 at 5 mg/kg.

[0014] Figure 2B depicts mean brain concentration of Compounds 4 and 13 after PO dosing of mice with Compound 4 and Compound 13 at 5 mg/kg.

[0015] Figure 2C depicts mean liver concentration of Compounds 4 and 13 after PO dosing of mice with Compound 4 and Compound 13 at 5 mg/kg.

[0016] Figure 2D depicts plasma concentrations for different subjects of Compound 13 after PO dosing of mice at 1 mg/kg.

[0017] Figure 3 depicts the amount of collagen per mg of liver using the hydroxyproline assay in livers of PiZ mice at harvest treated with Compound 13 versus placebo treated mice.

[0018] Figure 4 depicts percent of SYTOX-positive cells in LOAD (late-onset Alzheimer's disease) cortical neurons with and without Compound 4 treatment. Young control (YC) and old control (OC) cells are also shown.

[0019] Figure 5 depicts caspase 3/7 signal per area in LOAD (late-onset Alzheimer's disease) cortical neurons treated with varying concentrations of Compound 4.

[0020] Figure 6 depicts annexin V signal per area in LOAD (late-onset Alzheimer's disease) cortical neurons treated with varying concentrations of Compound 4.

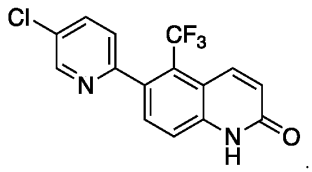
DETAILED DESCRIPTION OF INVENTION

[0021] The present disclosure provides for tetracyclic analogs that can modulate autophagy in a subject suffering from alpha-1 antitrypsin deficiency (ATD) and possibly other autophagy associated diseases or disorders, such as Alzheimer's Disease, Parkinson's disease, and Huntington's diseases and amyotrophic lateral sclerosis.

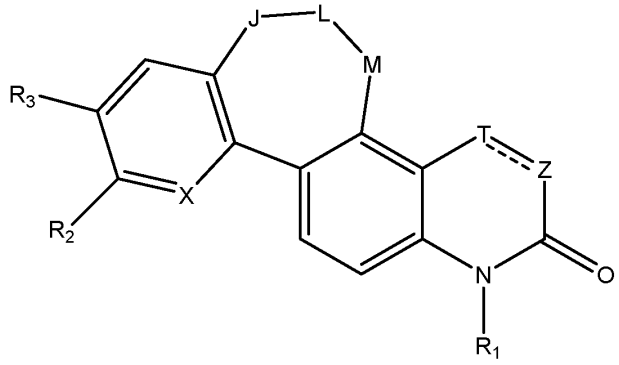
[0022] These tetracyclic analogs mediate reduced cellular accumulation of misfolded aggregated alpha-1 antitrypsin Z variant (ATZ) load in cell line models of ATD and fibrosis in a PiZ mouse model of ATD without affecting insulin secretion. The most common alpha-1 antitrypsin mutant variant is a missense mutation (E342K), commonly referred to as PiZ. Furthermore, these analogs improved the survival of human striatal neurons derived from patients with Huntington's disease and lowered huntingtin (HTT) inclusion body in HD-MSN in contrast to the inactive form.

[0023] The seven-membered ring analogs (e.g., Compound 4) yield an unexplored position for chemical modifications over first generation six-membered ring analogs (e.g., 2-quinolong A), possibly providing improved pharmacokinetics (PK) and the opportunity for placement of a photoaffinity tag. Similar to 2-quinolone A, Compound 4 is metabolically stable in human hepatocytes (100% parent), but more stable than 2-quinolone A in mouse hepatocytes (84.4% vs 37.5% parent) – with implications, potentially, of having superior pharmacokinetics (PK) to 2-quinolone A.

[0024] 2-Quinolone A has the following structure:



[0025] This disclosure describes compounds of Formula I



(Formula I)

wherein R_1 is hydrogen or substituted or unsubstituted alkyl; R_2 is hydrogen, halo, substituted alkyl, unsubstituted alkyl, alkoxyl, cyano,
amino, -N(R_4)C(O)R_5, -C(O)N(R_4)(R_5), -SO_2R_6, -N(R_4)SO_2R_6, -SO_2N(R_4)(R_5), cycloalkyl, heterocycloalkyl, aryl, or heteroaryl; R_3 is hydrogen, halo, substituted alkyl, unsubstituted alkyl, alkoxyl, cyano,
amino, -N(R_4)C(O)R_5, -C(O)N(R_4)(R_5), -SO_2R_6, -N(R_4)SO_2R_6, -SO_2N(R_4)(R_5), cycloalkyl, heterocycloalkyl, aryl, or heteroaryl; J is –CH(R_4)-, -C(R_4)-, -N(R_5)-, or -O-; L is –CH(R_4)-, -C(R_4)-, or -C(O)-; M is –CH(R_4)-, -C(R_4)-, -N(R_5)-, or -O-; T is absent, –CH_2CH_2–, –CH(R_4)-, -C(R_4)-, -O-, or together with Z form a cycloalkyl group fused to the 6-membered ring; Z is –CH(R_4)-, -C(R_4)-, -N(R_5)-, -N-, -O-, or together with T form a cycloalkyl group fused to the 6-membered ring; T and Z can be joined by a single bond or a double bond depending on the valence of the groups; when T is absent the ring is a 5-membered ring; when T is –CH_2CH_2– the ring is a 7-membered ring; R_4 and R_5 are independently hydrogen or substituted or unsubstituted alkyl; and R_6 is substituted or unsubstituted alkyl.

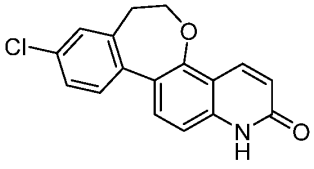
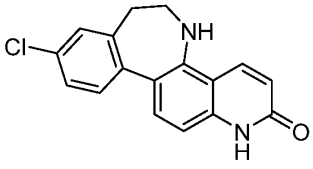
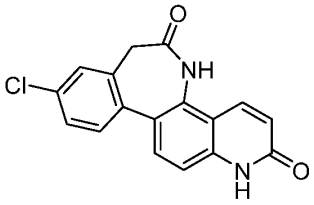
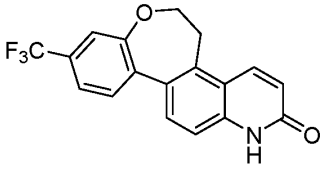
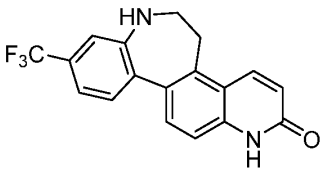
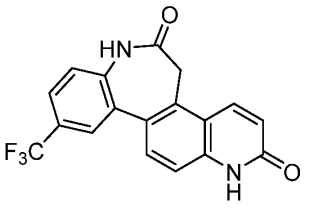
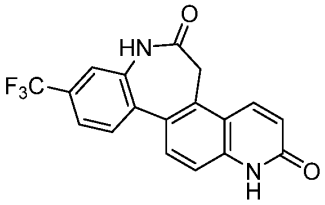
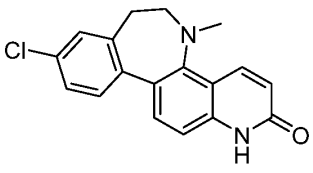
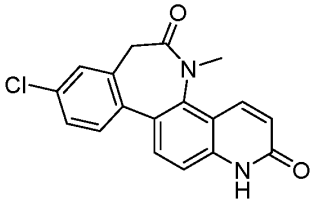
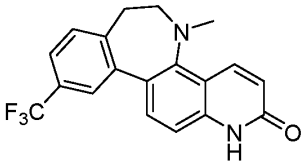
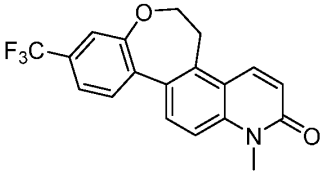
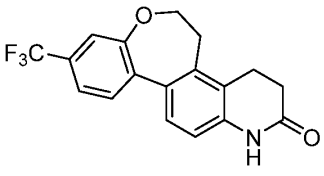
[0026] The compounds of Formula I disclosed herein, wherein R_1 is hydrogen or unsubstituted C_1 to C_3 alkyl; R_2 is hydrogen, halo, or halo-substituted C_1 to C_3 alkyl; R_3 is hydrogen, halo, or halo-substituted C_1 to C_3 alkyl; J is –CH(R_4)-, -C(R_4)-, or -O-; L is –CH(R_4)-, -C(R_4)-, or -C(O)-; M is –CH(R_4)- or -O-; T is –C(R_4)-; Z is –C(R_4)-; T and Z share a double bond; and R_4 is hydrogen or unsubstituted C_1 to C_3 alkyl.

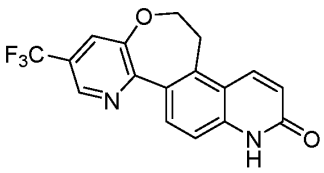
[0027] Also, the compounds of Formula I disclosed herein, wherein R_1 is hydrogen or methyl; R_2 is hydrogen, chloro, or chloro-substituted C_1 to C_3 alkyl; R_3 is hydrogen, chloro, or chloro-substituted C_1 to C_3 alkyl; J is -O-; L is –CH(R_4)-; M is –CH(R_4)-; T is –C(R_4)-; Z is –C(R_4)-; T and Z share a double bond; and R_4 is independently hydrogen or methyl.

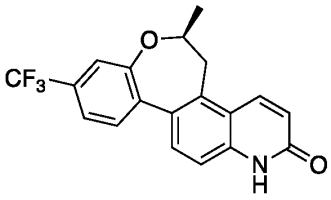
[0028] Additionally, the compounds of Formula I disclosed herein, wherein R_1 is

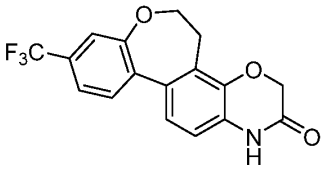
hydrogen; R_2 is hydrogen; R_3 is chloro or chloromethyl; J is -O- or –CH(R_4)-; L is –CH(R_4)-; M is –CH(R_4)- or -N(R_5)-; T is –C(R_4)-; Z is –C(R_4)-; T and Z share a double bond; and R_4 is hydrogen.

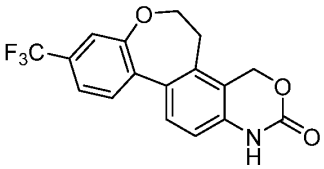
[0029] The compounds of Formula I, wherein the compound is:

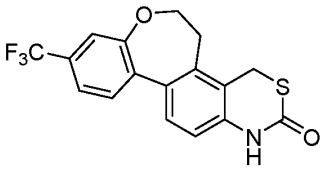

(Ex. 1),

(Ex. 2),

(Ex. 3),

(Ex. 4),

(Ex. 5),

(Ex. 6),

(Ex. 7),

(Ex. 8),

(Ex. 9),

(Ex. 10),

(Ex. 11),

(Ex. 12),

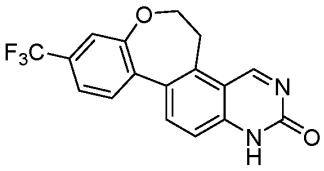

(Ex. 13),

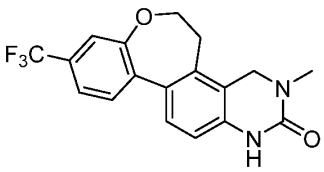

(Ex. 14),

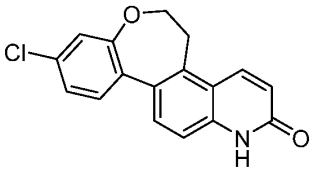

(Ex. 15),

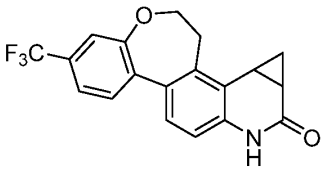

(Ex. 16),

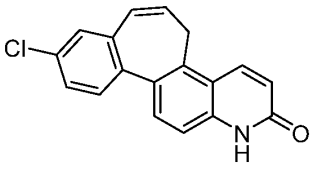

(Ex. 17),

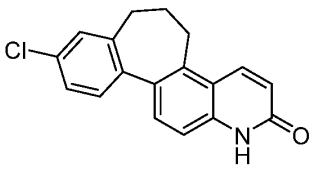

(Ex. 18),

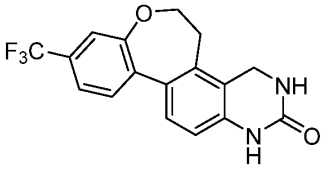

(Ex. 19),

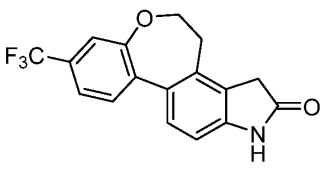

(Ex. 20),

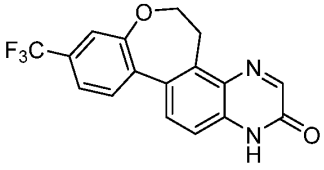

(Ex. 21),

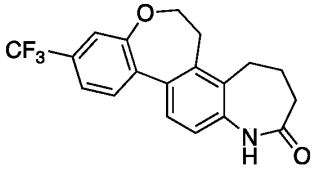

(Ex. 22),

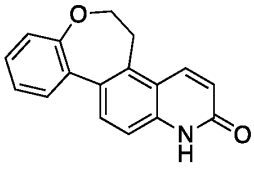

(Ex. 23),

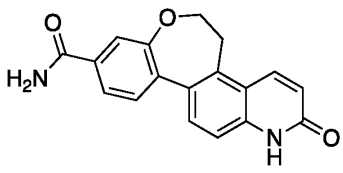
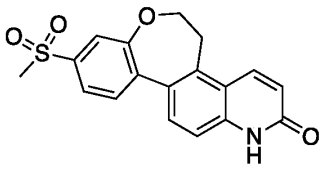

(Ex. 24),

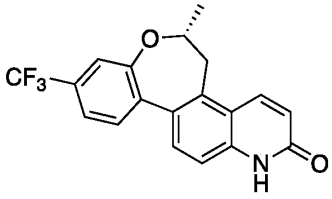

(Ex. 25),


(Ex. 26),

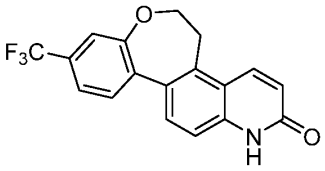
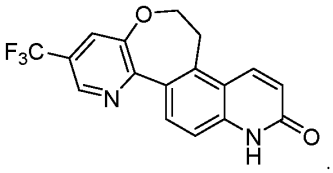

(Ex. 27),


(Ex. 28),

 (Ex. 29),  (Ex. 30), or

 (Ex. 31).

[0030] Preferably, the compound of Formula I is described in Ex. 4 or Ex. 13:

Pharmaceutical Compositions or Formulations

[0031] As noted, the present disclosure relates to pharmaceutical compositions comprising a therapeutically effective amount of at least one of the compounds as described herein (e.g., a compound of Formula (I) or salt or prodrug thereof).

[0032] Another aspect of the disclosure is a pharmaceutical composition comprising a pharmaceutically acceptable excipient and the compound of Formula I described herein.

[0033] Yet another aspect of the disclosure is a pharmaceutical compositions disclosed herein, wherein the compound of Formula I has a concentration from about 0.01 wt.% to about 1 wt.%, from about 0.05 wt.% to about 0.5 wt.%, from about 0.05 wt.%, about 0.1 wt.%, or about 0.5 wt.%, or of about 0.05 wt.%, about 0.1 wt.%, or about 0.5 wt.%, based on the total weight of the pharmaceutical composition.

[0034] A further aspect of the disclosure is a pharmaceutical composition as described herein, wherein the pharmaceutically acceptable excipient comprises a protic polar solvent, a non-protic polar solvent, a non-ionic surfactant, a cyclodextrin, or a combination thereof.

[0035] Preferably, the pharmaceutically acceptable excipient water, Solutol® HS-15 (polyethylene glycol 12-hydroxystearate), Labrasol® ALF (polyethylene glycol-8 mono- and diesters of caprilic (C_8) and capric (C_{10}) acids with a small fraction of mono-, di-, and

triglycerides), methylcellulose, Tween® 80 (polyoxyethylenesorbitan monooleate), N-methyl-2-pyrrolidone (NMP), polyethylene glycol with number average molecular weight of 400 (PEG 400), sulfobutylether-β-cyclodextrin (SBE-β-CD), Cremophor® EL (macrogolglycerol ricinoleate), dimethyl sulfoxide (DMSO), or a combination thereof.

[0036] The agents and compositions described herein can be formulated by any conventional manner using one or more pharmaceutically acceptable carriers or excipients as described in, for example, Remington's Pharmaceutical Sciences (A.R. Gennaro, Ed.), 21st edition, ISBN: 0781746736 (2005), incorporated herein by reference in its entirety. Such formulations will contain a therapeutically effective amount of a biologically active agent described herein, which can be in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the subject.

[0037] The term "formulation" refers to preparing a drug in a form suitable for administration to a subject, such as a human. Thus, a "formulation" can include pharmaceutically acceptable excipients, including diluents or carriers. Pharmaceutically acceptable excipients for use in the compositions of the present invention are selected based upon a number of factors including the particular compound used, and its concentration, stability and intended bioavailability; the subject, its age, size and general condition; and the route of administration.

[0038] The term "pharmaceutically acceptable" as used herein can describe substances or components that do not cause unacceptable losses of pharmacological activity or unacceptable adverse side effects. Examples of pharmaceutically acceptable ingredients can be those having monographs in United States Pharmacopeia (USP 29) and National Formulary (NF 24), United States Pharmacopeial Convention, Inc, Rockville, Maryland, 2005 ("USP/NF"), or a more recent edition, and the components listed in the continuously updated Inactive Ingredient Search online database of the FDA. Other useful components that are not described in the USP/NF, etc. may also be used.

[0039] The term "pharmaceutically acceptable excipient," as used herein, can include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic, or absorption delaying agents. The use of such media and agents for pharmaceutical active substances is well known in the art (see generally Remington's Pharmaceutical Sciences (A.R. Gennaro, Ed.), 21st edition, ISBN: 0781746736 (2005)). Except insofar as any conventional media or agent is incompatible with an active ingredient, its use in the

therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

[0040] A "stable" formulation or composition can refer to a composition having sufficient stability to allow storage at a convenient temperature, such as between about 0 ºC and about 60 ºC, for a commercially reasonable period of time, such as at least about one day, at least about one week, at least about one month, at least about three months, at least about six months, at least about one year, or at least about two years.

[0041] The formulation should suit the mode of administration. Routes of administration include, but are not limited to, oral, parenteral (e.g., intravenous, intra-arterial, subcutaneous, rectal, subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intraperitoneal, or intrasternal), topical (nasal, transdermal, intraocular), intravesical, intrathecal, enteral, pulmonary, intralymphatic, intracavital, vaginal, transurethral, intradermal, aural, intramammary, buccal, orthotopic, intratracheal, intralesional, percutaneous, endoscopical, transmucosal, sublingual and intestinal administration. For example, the agents of use with the current disclosure can be formulated by known methods for administration to a subject using several routes including: parenteral, pulmonary, oral, topical, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, ophthalmic, buccal, and rectal. The individual agents may also be administered in combination with one or more additional agents or together with other biologically active or biologically inert agents. Such biologically active or inert agents may be in fluid or mechanical communication with the agent(s) or attached to the agent(s) by ionic, covalent, Van der Waals, hydrophobic, hydrophilic or other physical forces.

[0042] The pharmaceutical compositions can be formulated, for example, for oral administration. The pharmaceutical compositions can be formulated as tablets, dispersible powders, pills, capsules, gel-caps, granules, solutions, suspensions, emulsions, syrups, elixirs, troches, lozenges, or any other dosage form that can be administered orally. Pharmaceutical compositions can include one or more pharmaceutically acceptable excipients. Suitable excipients for solid dosage forms include sugars, starches, and other conventional substances including lactose, talc, sucrose, gelatin, carboxymethylcellulose, agar, mannitol, sorbitol, calcium phosphate, calcium carbonate, sodium carbonate, kaolin, alginic acid, acacia, corn starch, potato starch, sodium saccharin, magnesium carbonate, microcrystalline cellulose, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, and stearic acid.

Further, such solid dosage forms can be uncoated or can be coated to delay disintegration and absorption.

[0043] The pharmaceutical compositions can also be formulated for parenteral administration, e.g., formulated for injection via intravenous, intra-arterial, subcutaneous, rectal, subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intraperitoneal, or intrasternal routes. Dosage forms suitable for parenteral administration include solutions, suspensions, dispersions, emulsions or any other dosage form that can be administered parenterally.

[0044] Pharmaceutically acceptable excipients are identified, for example, in The Handbook of Pharmaceutical Excipients, (American Pharmaceutical Association, Washington, D.C., and The Pharmaceutical Society of Great Britain, London, England, 1968). Additional excipients can be included in the pharmaceutical compositions of the invention for a variety of purposes. These excipients can impart properties which enhance retention of the compound at the site of administration, protect the stability of the composition, control the pH, facilitate processing of the compound into pharmaceutical compositions, and so on. Other excipients include, for example, fillers or diluents, surface active, wetting or emulsifying agents, preservatives, agents for adjusting pH or buffering agents, thickeners, colorants, dyes, flow aids, non-volatile silicones, adhesives, bulking agents, flavorings, sweeteners, adsorbents, binders, disintegrating agents, lubricants, coating agents, and antioxidants.

[0045] Compound described herein can be prepared as a salt. "Salt" as used herein refers to pharmaceutically acceptable salts of the compounds described herein which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge, et al. describes pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 66: 1-19 (1977). Examples of pharmaceutically acceptable salts include, but are not limited to, nontoxic acid addition salts which are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include, but are not limited to, adipate, alginate, ascorbate, aspartate, benzenesulfonate,

benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, alkyl having from 1 to 6 carbon atoms, sulfonate and aryl sulfonate

[0046] Controlled-release (or sustained-release) preparations may be formulated to extend the activity of the agent(s) and reduce dosage frequency. Controlled-release preparations can also be used to effect the time of onset of action or other characteristics, such as blood levels of the agent, and consequently affect the occurrence of side effects. Controlled-release preparations may be designed to initially release an amount of an agent(s) that produces the desired therapeutic effect, and gradually and continually release other amounts of the agent to maintain the level of therapeutic effect over an extended period of time. In order to maintain a near-constant level of an agent in the body, the agent can be released from the dosage form at a rate that will replace the amount of agent being metabolized or excreted from the body. The controlled-release of an agent may be stimulated by various inducers, *e.g.*, change in pH, change in temperature, enzymes, water, or other physiological conditions or molecules.

[0047] In other embodiments, the compounds may be prepared as "prodrugs" in a pharmaceutically acceptable composition/formulation. As used herein, the term "prodrug" refers to a derivative of a compound that can hydrolyze, oxidize, or otherwise react under biological conditions (*in vitro* or *in vivo*) to provide a compound as described herein. Prodrugs may only become active upon some reaction under biological conditions, but they may have activity in their unreacted forms. Examples of prodrug moieties include substituted and unsubstituted, branch or unbranched lower alkyl ester moieties, (e.g., propionoic acid esters), lower alkenyl esters, di-lower alkyl-amino lower-alkyl esters (e.g.,

dimethylaminoethyl ester), acylamino lower alkyl esters (e.g., acetyloxymethyl ester), acyloxy lower alkyl esters (e.g., pivaloyloxymethyl ester), aryl esters (phenyl ester), aryl-lower alkyl esters (e.g., benzyl ester), substituted (e.g., with methyl, halo, or methoxy substituents) aryl and aryl-lower alkyl esters, amides, lower-alkyl amides, di-lower alkyl amides, and hydroxy amides. Prodrugs and their uses are well known in the art (see, e.g., Berge, et al. 1977 J. Pharm. Sci. 66:1-19). Prodrugs can typically be prepared using well-known methods, such as those described in Burger's Medicinal Chemistry and Drug Discovery (1995, Manfred E. Wolff ed., 5thed. 172-178, 931-932).

[0048] Agents or compositions described herein can also be used in combination with other therapeutic modalities, as described further below. Thus, in addition to the therapies described herein, one may also provide to the subject other therapies known to be efficacious for treatment of the disease, disorder, or condition.

Methods of Use

[0049] Another aspect of the present disclosure provides for treating an autophagy-related disease by modulation of autophagy in a subject in need thereof.

[0050] A further aspect of the invention is a method for treating an autophagy-related disease, the method comprising administering the compound of Formula I disclosed herein or the pharmaceutical compositions disclosed herein to a subject in need thereof.

[0051] Additionally, the methods of treatment disclosed herein can have the autophagy-related disease is alpha-1 antitrypsin deficiency (ATD), Alzheimer's disease, Parkinson's disease, Huntington's disease, or amyotrophic lateral sclerosis.

[0052] Further, the methods of treatment described herein can have the subject be a mammal; preferably the subject is a human.

[0053] Autophagy can be described as a pathway or process in a cell or organism that involves degradation of cellular components via delivery to a lysosome. For example, the pathway can be chaperone-mediated autophagy, microautophagy, macroautophagy, or variants of macroautophagy, such as LC3-associated phagocytosis. An autophagy-associated disease, disorder, or condition may also be one that involves membrane trafficking pathways that are dependent on autophagy genes, such as unconventional protein secretion, exocytosis of secretory granules/lysosomes, exosome secretion, or retromer- dependent trafficking. These pathways are capable of removing misfolded proteins, aggregated proteins, or parts of organelles by delivery to the plasma membrane for exocytosis without involving degradation

and/or delivery to the lysosome. As such, an autophagy modulating agent can be capable of modulating such autophagic pathways.

[0054] As described herein, an autophagy-associated disease, disorder, or condition can be a disease resulting from defects in, or abnormal function of, autophagic processes or autophagic pathways in a cell or organism; diseases and disorders that are caused by misfolded and/or aggregated proteins, which can include age-dependent degenerative diseases; or diseases in which autophagy function has been implicated. Defects in, or abnormal function of, an autophagic pathway or process may involve a defect in, or abnormal function of the action of various cellular components, such as an organelle or protein. For example, the organelle can be a lysosome, a vesicle, an autophagosome, a vacuole, a phagophore, or a plasma membrane.

[0055] For example, an autophagy modulating agent described herein and analogs thereof can be used as a therapeutic for ATD, Huntington's disease, Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, and other misfolded protein and age-dependent degenerative diseases in which enhancing autophagy will be beneficial.

[0056] For example, an autophagy-associated disease, disorder, or condition can be adult polyglucosan body disease, Afibrinogenemia, alpha-1 antitrypsin deficiency (ATD), Alzheimer's disease (AD), amyotrophic lateral sclerosis, an age- dependent degenerative disease, autism spectrum disorders, Becker muscular dystrophy, beta-propellar protein-associated neurodegeneration, Birt-Hogg-Dube syndrome, Blau syndrome, cancer, Centronuclear myopathy, Chanarin-Dorfman syndrome, Charcot-Marie-Tooth (CMT) disease, childhood ataxia, Chorea- acanthocytosis, Chronic progressive external ophthalmoplegia, Congenital disorders of glycosylation, Congenital dyserythropoietic anemia, Congenital myasthenic syndrome, Congenital myotonic dystrophy, Corneal dystrophy Avellino type, cortical atrophy, Crohn's disease, Danon disease, Danon's cardiomyopathy, diabetes, distal myopathy, Dysferlinopathy, Emery-Dreifuss muscular dystrophy, epilepsy, Familial encephalopathy with neuroserpin inclusion bodies, familial Mediterranean fever, Familial partial lipodystrophy, Fanconi anemia congenital syndrome, frontotemporal dementia, Galloway-Mowat syndrome, Gaucher's disease, Gerstmann-Straussler-Scheinker disease, Glycogen storage disease type 2, Griscelli syndrome, Groenouw type I corneal dystrophy, Hermansky-Pudlak syndrome, Huntington's disease (HD), Idiopathic pulmonary fibrosis, inflammatory bowel disease, juvenile arthritis, Kearns-Sayre syndrome, Keshan disease, LEOPARD syndrome, Li-Fraumeni syndrome, Limb- girdle muscular dystrophy type 1D, 2B, LRBA

deficiency, Macrophagic myofasciitis, Marek disease, Martsolf syndrome, Miyoshi myopathy, Mulibrey Nanism, multiple sclerosis (MS), multisystem disorder, cystinosis, Myofibrillar myopathy, Myostatin-related muscle hypertrophy, Myotonic dystrophy, Nemaline myopathy, neuronal ceroid lipofuscinosis, Neuronal ceroid lipofuscinosis, non- alcoholic fatty liver disease, NORSE, osteoarthritis, osteopetrosis, Paget's disease of the bone, Papillon Lefevre syndrome, Parkinson's disease, Pelger- Huet anomaly, Perry syndrome, Peters anomaly, Phosphoglycerate kinase deficiency, primary microcephaly, primary open angle glaucoma, Progeria, Proteus syndrome, Reducing body myopathy, Retinitis pigmentosa, Rett syndrome, Salla disease, SAPHO syndrome, Schaaf-Yang syndrome, Sengers syndrome, sensory and autonomic neuropathy type II, SHORT syndrome, Simpson-Golabi-Behmel syndrome, Sitosterolemia, Smith-Magenis syndrome, Snyder-Robinson syndrome, spastic paraplegia, spinocerebellar ataxia, Stargardt disease, systematic lupus erythematosus, systemic sclerosis, Tangier disease, tuberculosis, ulcerative colitis, Vici syndrome, Wiskott Aldrich syndrome, X-linked myopathy with excessive autophagy, X-linked myotubular myopathy, Yunis-Varon syndrome, or Zellweger syndrome spectrum disorders.

[0057] As another example, an autophagy-associated disease, disorder, or condition can be diseases and disorders that are caused by misfolded and/or aggregated proteins, which can include age-dependent degenerative diseases, Amyotonia congenita, Benign hereditary chorea, Bethlem myopathy, Bourneville syndrome, Brown syndrome, Central diabetes insipidus, Charcot-Marie-Toothdisease, Cholesteryl ester storage disease, Chorea minor, Cramp-fasciculation syndrome, Dentatorubral-pallidoluysian atrophy, Doxorubicin-induced cardiomyopathy, Episodic ataxia with nystagmus, Fabry disease, Familial Mediterranean fever, Froster-Huch syndrome, Hypergonadotropic ovarian failure, familial or sporadic, Idiopathic inflammatory myopathy, Inclusion body myositis, Kennedy disease, Lafora disease, Leber congenital amaurosis 11, Leber congenital amaurosis 3, Limb-girdle muscular dystrophy, Marinesco-Sjogren syndrome, Oculopharyngeal muscular dystrophy, Pancreatitis, pediatric, Pelizaeus-Merzbacher disease, Phenylketonuria, Pigment-dispersion syndrome, Refsum disease, infantile form, Spinal muscular atrophy, Spinocerebellar ataxia, or Tubular aggregate myopathy.

[0058] As another example, an autophagy-associated disease, disorder, or condition can be a disease resulting from defects in, or abnormal function of, autophagic processes or autophagic pathways in a cell or organism or diseases in which autophagy function has been implicated such as adult polyglucosan body disease, Afibrinogenemia, Centronuclear myopathy, Congenital dyserythropoietic anemia, Congenital myotonic dystrophy, Danon disease, Familial

encephalopathy with neuroserpin inclusion bodies, Hermansky-Pudlak syndrome, Idiopathic pulmonary fibrosis, Miyoshi myopathy, Myofibrillar myopathy, Myotonic dystrophy, Progeria, Retinitis pigmentosa, Stargardt disease, X-linked myopathy with excessive autophagy, or X-linked myotubular myopathy.

Alpha-1 Antitrypsin Deficiency (ATD)

[0059] An aspect of the present disclosure provides for treatment of autophagy-related diseases in terms of modulation of autophagy in a subject suffering from alpha-1 antitrypsin deficiency (ATD). ATD is an inherited disorder that can result in liver disease, due to accumulation of misfolded mutant alpha-1 antitrypsin protein (ATZ). ATD is a well-known genetic cause of severe liver disease including cirrhosis and hepatocellular carcinoma in adults. The classical form of ATD is characterized by a point mutation that substitutes lysine for glutamate 342 in the mutant variant called ATZ.

[0060] The compounds of Formula I disclosed herein have activity in modulating the autophagy process. Analogs show reduced hepatic ATZ load and fibrosis in a PiZ mouse model of ATD without affecting insulin secretion. These analogs also decreased cellular ATZ load in the C. *elegans* model of ATD in a dose-dependent fashion.

ATD Disease Models

[0061] Transgenic C.*elegans* ('Z worm') expressing the human Z mutant form of alpha-1 antitrypsin (ATZ) fused to green fluorescent protein (GFP) can be used as a model of ATD for screening and testing of autophagy modulating agents to treat ATD. The C.*elegans* model of ATD exhibits ATZ aggregation within the endoplasmic reticulum, slow growth, reduced fertility, and shortened lifespan. These phenotypes are also exhibited in humans with ATD, proving that C.*elegans* is a representative model of the disease.

[0062] A transgenic mouse model expressing the human mutant variant of alpha-1 antitrypsin, referred to as PiZ mouse, can also be used as a model of ATD. The PiZ mouse exhibits accumulation of mutant alpha-1 antitrypsin aggregates, liver fibrosis, and development of malignant liver tumors.

Methods of Administration

[0063] Agents and compositions described herein can be administered according to methods described herein in a variety of means known to the art. The agents and composition can be used therapeutically either as exogenous materials or as endogenous

materials. Exogenous agents are those produced or manufactured outside of the body and administered to the body. Endogenous agents are those produced or manufactured inside the body by some type of device (biologic or other) for delivery within or to other organs in the body.

[0064] As discussed above, administration can be parenteral, pulmonary, oral, topical, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, ophthalmic, buccal, or rectal administration.

[0065] Agents and compositions described herein can be administered in a variety of methods well known in the arts. Administration can include, for example, methods involving oral ingestion, direct injection (e.g., systemic or stereotactic), implantation of cells engineered to secrete the factor of interest, drug-releasing biomaterials, polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, implantable matrix devices, mini-osmotic pumps, implantable pumps, injectable gels and hydrogels, liposomes, micelles (e.g., up to 30 μm), nanospheres (e.g., less than 1 μm), microspheres (e.g., 1-100 μm), reservoir devices, a combination of any of the above, or other suitable delivery vehicles to provide the desired release profile in varying proportions. Other methods of controlled-release delivery of agents or compositions will be known to the skilled artisan and are within the scope of the present disclosure.

[0066] Delivery systems may include, for example, an infusion pump which may be used to administer the agent or composition in a manner similar to that used for delivering insulin or chemotherapy to specific organs or tumors. Typically, using such a system, an agent or composition can be administered in combination with a biodegradable, biocompatible polymeric implant that releases the agent over a controlled period of time at a selected site. Examples of polymeric materials include polyanhydrides, polyorthoesters, polyglycolic acid, polylactic acid, polyethylene vinyl acetate, and copolymers and combinations thereof. In addition, a controlled release system can be placed in proximity of a therapeutic target, thus requiring only a fraction of a systemic dosage.

[0067] Agents can be encapsulated and administered in a variety of carrier delivery systems. Examples of carrier delivery systems include microspheres, hydrogels, polymeric implants, smart polymeric carriers, and liposomes (see generally, Uchegbu and Schatzlein, eds. (2006) Polymers in Drug Delivery, CRC, ISBN-10: 0849325331). Carrier-based systems for molecular or biomolecular agent delivery can: provide for intracellular delivery; tailor biomolecule/agent release rates; increase the proportion of biomolecule that reaches its site of

action; improve the transport of the drug to its site of action; allow colocalized deposition with other agents or excipients; improve the stability of the agent *in vivo*; prolong the residence time of the agent at its site of action by reducing clearance; decrease the nonspecific delivery of the agent to nontarget tissues; decrease irritation caused by the agent; decrease toxicity due to high initial doses of the agent; alter the immunogenicity of the agent; decrease dosage frequency, improve taste of the product; or improve shelf life of the product.

[0068] Compositions and methods described herein utilizing molecular biology protocols can be according to a variety of standard techniques known to the art (see, e.g., Sambrook and Russel (2006) Condensed Protocols from Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, ISBN-10: 0879697717; Ausubel et al. (2002) Short Protocols in Molecular Biology, 5th ed., Current Protocols, ISBN-10: 0471250929; Sambrook and Russel (2001) Molecular Cloning: A Laboratory Manual, 3d ed., Cold Spring Harbor Laboratory Press, ISBN-10: 0879695773; Elhai, J. and Wolk, C. P. 1988. Methods in Enzymology 167, 747-754; Studier (2005) Protein Expr Purif. 41(1), 207–234; Gellissen, ed. (2005) Production of Recombinant Proteins: Novel Microbial and Eukaryotic Expression Systems, Wiley-VCH, ISBN-10: 3527310363; Baneyx (2004) Protein Expression Technologies, Taylor & Francis, ISBN-10: 0954523253).

[0069] Definitions and methods described herein are provided to better define the present disclosure and to guide those of ordinary skill in the art in the practice of the present disclosure. Unless otherwise noted, terms are to be understood according to conventional usage by those of ordinary skill in the relevant art.

[0070] In some embodiments, numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth, used to describe and claim certain embodiments of the present disclosure are to be understood as being modified in some instances by the term “about.” In some embodiments, the term “about” is used to indicate that a value includes the standard deviation of the mean for the device or method being employed to determine the value. In some embodiments, the numerical parameters set forth in the written description and attached claims are approximations that can vary depending upon the desired properties sought to be obtained by a particular embodiment. In some embodiments, the numerical parameters should be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of some embodiments of the present disclosure are approximations, the numerical values set forth in the specific

examples are reported as precisely as practicable. The numerical values presented in some embodiments of the present disclosure may contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements. The recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein.

Definitions

[0071] The term "imine" or "imino", as used herein, unless otherwise indicated, can include a functional group or chemical compound containing a carbon-nitrogen double bond. The expression "imino compound", as used herein, unless otherwise indicated, refers to a compound that includes an "imine" or an "imino" group as defined herein. The "imine" or "imino" group can be optionally substituted.

[0072] The term "hydroxyl", as used herein, unless otherwise indicated, can include -OH. The "hydroxyl" can be optionally substituted.

[0073] The terms "halogen" and "halo", as used herein, unless otherwise indicated, include a chlorine, chloro, Cl; fluorine, fluoro, F; bromine, bromo, Br; or iodine, iodo, or I.

[0074] The term "acetamide", as used herein, is an organic compound with the formula CH_3CONH_2. The "acetamide" can be optionally substituted.

[0075] The term "aryl", as used herein, unless otherwise indicated, include a carbocyclic aromatic group. Examples of aryl groups include, but are not limited to, phenyl, benzyl, naphthyl, or anthracenyl. The "aryl" can be optionally substituted.

[0076] The terms "amine" and "amino", as used herein, unless otherwise indicated, include a functional group that contains a nitrogen atom with a lone pair of electrons and wherein one or more hydrogen atoms have been replaced by a substituent such as, but not limited to, an alkyl group or an aryl group. The "amine" or "amino" group can be optionally substituted. The amino group can preferably be –NR'R", wherein R' and R" are independently hydrogen or an alkyl, substituted alkyl, aryl, substituted aryl, alkaryl, heterocyclo, or heteroaryl as defined herein.

[0077] The term "alkyl", as used herein, unless otherwise indicated, can include saturated monovalent hydrocarbon radicals having straight or branched moieties, such as but not limited to, methyl, ethyl, propyl, butyl, pentyl, hexyl, octyl groups, etc. Representative

straight-chain lower alkyl groups include, but are not limited to, -methyl, -ethyl, -n-propyl, -n-butyl, -n-pentyl, -n-hexyl, -n-heptyl and -n-octyl; while branched lower alkyl groups include, but are not limited to, -isopropyl, -sec-butyl, -isobutyl, -tert-butyl, -isopentyl, 2-methylbutyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl, 2,2-dimethylpentyl, 2,3-dimethylpentyl, 3,3-dimethylpentyl, 2,3,4-trimethylpentyl, 3-methylhexyl, 2,2-dimethylhexyl, 2,4-dimethylhexyl, 2,5-dimethylhexyl, 3,5-dimethylhexyl, 2,4-dimethylpentyl, 2-methylheptyl, 3-methylheptyl, unsaturated C1-10 alkyls include, but are not limited to, -vinyl, -allyl, -1-butenyl, -2-butenyl, -isobutylenyl, -1-pentenyl, -2-pentenyl, -3-methyl-1-butenyl, -2-methyl-2-butenyl, -2,3-dimethyl-2-butenyl, 1-hexyl, 2-hexyl, 3-hexyl, -acetylenyl, -propynyl, -1-butynyl, -2-butynyl, -1-pentynyl, -2-pentynyl, or -3-methyl-1 butynyl. An alkyl can be saturated, partially saturated, or unsaturated. The "alkyl" can be optionally substituted.

[0078] The term "carboxyl", as used herein, unless otherwise indicated, can include a functional group consisting of a carbon atom double bonded to an oxygen atom and single bonded to a hydroxyl group (-COOH). The "carboxyl" can be optionally substituted.

[0079] The term "carbonyl", as used herein, unless otherwise indicated, can include a functional group consisting of a carbon atom double-bonded to an oxygen atom (C=O). The "carbonyl" can be optionally substituted.

[0080] The term "alkenyl", as used herein, unless otherwise indicated, can include alkyl moieties having at least one carbon-carbon double bond wherein alkyl is as defined above and including E and Z isomers of said alkenyl moiety. An alkenyl can be partially saturated or unsaturated. The "alkenyl" can be optionally substituted.

[0081] The term "alkynyl", as used herein, unless otherwise indicated, can include alkyl moieties having at least one carbon-carbon triple bond wherein alkyl is as defined above. An alkynyl can be partially saturated or unsaturated. The "alkynyl" can be optionally substituted.

[0082] The term "acyl", as used herein, unless otherwise indicated, can include a functional group derived from an aliphatic carboxylic acid, by removal of the hydroxyl (–OH) group. The "acyl" can be optionally substituted.

[0083] The term "alkoxyl", as used herein, unless otherwise indicated, can include O-alkyl groups wherein alkyl is as defined above and O represents oxygen. Representative alkoxyl groups include, but are not limited to, -O-methyl, -O-ethyl, -O-n-propyl, -O-n-butyl, -O-n-pentyl, -O-n-hexyl, -O-n-heptyl, -O-n-octyl, -O-isopropyl, -O-sec-butyl, -O-isobutyl, -O-

tert-butyl, -O-isopentyl, -O-2-methylbutyl, -O-2-methylpentyl, -O-3-methylpentyl, -O-2,2-dimethylbutyl, -O-2,3-dimethylbutyl, -O-2,2-dimethylpentyl, -O-2,3-dimethylpentyl, -O-3,3-dimethylpentyl, -O-2,3,4-trimethylpentyl, -O-3-methylhexyl, -O-2,2-dimethylhexyl, -O-2,4-dimethylhexyl, -O-2,5-dimethylhexyl, -O-3,5-dimethylhexyl, -O-2,4dimethylpentyl, -O-2-methylheptyl, -O-3-methylheptyl, -O-vinyl, -O-allyl, -O-1-butenyl, -O-2-butenyl, -O-isobutylenyl, -O-1-pentenyl, -O-2-pentenyl, -O-3-methyl-1-butenyl, -O-2-methyl-2-butenyl, -O-2,3-dimethyl-2-butenyl, -O-1-hexyl, -O-2-hexyl, -O-3-hexyl, -O-acetylenyl, -O-propynyl, -O-1-butynyl, -O-2-butynyl, -O-1-pentynyl, -O-2-pentynyl and -O-3-methyl-1-butynyl, -O-cyclopropyl, -O-cyclobutyl, -O-cyclopentyl, -O-cyclohexyl, -O-cycloheptyl, -O-cyclooctyl, -O-cyclononyl and -O-cyclodecyl, -O-CH2-cyclopropyl, -O-CH2-cyclobutyl, -O-CH2-cyclopentyl, -O-CH2-cyclohexyl, -O-CH2-cycloheptyl, -O-CH2-cyclooctyl, -O- CH2-cyclononyl, -O-CH2-cyclodecyl, -O-(CH2)2-cyclopropyl, -O-(CH2)2-cyclobutyl, -O-(CH2)2-cyclopentyl, -O-(CH2)2-cyclohexyl, -O-(CH2)2-cycloheptyl, -O-(CH2)2-cyclooctyl, -O-(CH2)2-cyclononyl, or -O-(CH2)2-cyclodecyl. An alkoxyl can be saturated, partially saturated, or unsaturated. The "alkoxyl" can be optionally substituted.

[0084] The term "cycloalkyl", as used herein, unless otherwise indicated, can include an aromatic, a non-aromatic, saturated, partially saturated, or unsaturated, monocyclic or fused, spiro or unfused bicyclic or tricyclic hydrocarbon referred to herein containing a total of from 1 to 10 carbon atoms (e.g., 1 or 2 carbon atoms if there are other heteroatoms in the ring), preferably 3 to 8 ring carbon atoms. Examples of cycloalkyls include, but are not limited to, C3-10 cycloalkyl groups include, but are not limited to, -cyclopropyl, -cyclobutyl, -cyclopentyl, -cyclopentadienyl, -cyclohexyl, -cyclohexenyl, -1,3-cyclohexadienyl, -1,4-cyclohexadienyl, -cycloheptyl, -1,3-cycloheptadienyl, -1,3,5-cycloheptatrienyl, -cyclooctyl, and -cyclooctadienyl. The term "cycloalkyl" also can include -lower alkyl-cycloalkyl, wherein lower alkyl and cycloalkyl are as defined herein. Examples of -lower alkyl-cycloalkyl groups include, but are not limited to, -CH2-cyclopropyl, -CH2-cyclobutyl, -CH2-cyclopentyl, -CH2-cyclopentadienyl, -CH2-cyclohexyl, -CH2-cycloheptyl, or -CH2-cyclooctyl. The "cycloalkyl" can be optionally substituted. A "cycloheteroalkyl", as used herein, unless otherwise indicated, can include any of the above with a carbon substituted with a heteroatom (e.g., O, S, N).

[0085] The term "heterocyclic" or "heteroaryl", as used herein, unless otherwise indicated, can include an aromatic or non-aromatic cycloalkyl in which one to four of the ring carbon atoms are independently replaced with a heteroatom from the group consisting of O,

S, and N. Representative examples of a heterocycle include, but are not limited to, benzofuranyl, benzothiophene, indolyl, benzopyrazolyl, coumarinyl, isoquinolinyl, pyrrolyl, pyrrolidinyl, thiophenyl, furanyl, thiazolyl, imidazolyl, pyrazolyl, triazolyl, quinolinyl, pyrimidinyl, pyridinyl, pyridonyl, pyrazinyl, pyridazinyl, isothiazolyl, isoxazolyl, (1,4)-dioxane, (1,3)-dioxolane, 4,5-dihydro-1H-imidazolyl, or tetrazolyl. Heterocycles can be substituted or unsubstituted. Heterocycles can also be bonded at any ring atom (i.e., at any carbon atom or heteroatom of the heterocyclic ring). A heterocyclic can be saturated, partially saturated, or unsaturated. The "hetreocyclic" can be optionally substituted.

[0086] The term "indole", as used herein, is an aromatic heterocyclic organic compound with formula C_8H_7N. It has a bicyclic structure, consisting of a six-membered benzene ring fused to a five-membered nitrogen-containing pyrrole ring. The "indole" can be optionally substituted.

[0087] The term "cyano", as used herein, unless otherwise indicated, can include a -CN group. The "cyano" can be optionally substituted.

[0088] The term "alcohol", as used herein, unless otherwise indicated, can include a compound in which the hydroxyl functional group (-OH) is bound to a carbon atom. In particular, this carbon center should be saturated, having single bonds to three other atoms. The "alcohol" can be optionally substituted.

[0089] The term "solvate" is intended to mean a solvate form of a specified compound that retains the effectiveness of such compound. Examples of solvates include compounds of the disclosure in combination with, for example, water, isopropanol, ethanol, methanol, dimethylsulfoxide (DMSO), ethyl acetate, acetic acid, or ethanolamine.

[0090] The term "mmol", as used herein, is intended to mean millimole. The term "equiv", as used herein, is intended to mean equivalent. The term "mL", as used herein, is intended to mean milliliter. The term "g", as used herein, is intended to mean gram. The term "kg", as used herein, is intended to mean kilogram. The term "μg", as used herein, is intended to mean micrograms. The term "h", as used herein, is intended to mean hour. The term "min", as used herein, is intended to mean minute. The term "M", as used herein, is intended to mean molar. The term "μL", as used herein, is intended to mean microliter. The term "μM", as used herein, is intended to mean micromolar. The term "nM", as used herein, is intended to mean nanomolar. The term "N", as used herein, is intended to mean normal. The term "amu", as used herein, is intended to mean atomic mass unit. The term "°C", as used herein, is intended to mean degree Celsius. The term "wt/wt", as used herein, is intended to

mean weight/weight. The term “v/v”, as used herein, is intended to mean volume/volume. The term “MS”, as used herein, is intended to mean mass spectroscopy. The term “HPLC”, as used herein, is intended to mean high performance liquid chromatograph. The term “RT”, as used herein, is intended to mean room temperature. The term "e.g.", as used herein, is intended to mean example. The term “N/A”, as used herein, is intended to mean not tested.

[0091] As used herein, the expression “pharmaceutically acceptable salt” refers to pharmaceutically acceptable organic or inorganic salts of a compound of the disclosure. Preferred salts include, but are not limited, to sulfate, citrate, acetate, oxalate, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucaronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, or pamoate (i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts. A pharmaceutically acceptable salt may involve the inclusion of another molecule such as an acetate ion, a succinate ion, or another counterion. The counterion may be any organic or inorganic moiety that stabilizes the charge on the parent compound. Furthermore, a pharmaceutically acceptable salt may have more than one charged atom in its structure. In instances where multiple charged atoms are part of the pharmaceutically acceptable salt, the pharmaceutically acceptable salt can have multiple counterions. Hence, a pharmaceutically acceptable salt can have one or more charged atoms and/or one or more counterion. As used herein, the expression “pharmaceutically acceptable solvate” refers to an association of one or more solvent molecules and a compound of the disclosure. Examples of solvents that form pharmaceutically acceptable solvates include, but are not limited to, water, isopropanol, ethanol, methanol, DMSO, ethyl acetate, acetic acid, and ethanolamine. As used herein, the expression “pharmaceutically acceptable hydrate” refers to a compound of the disclosure, or a salt thereof, that further can include a stoichiometric or non-stoichiometric amount of water bound by non-covalent intermolecular forces.

[0092] In some embodiments, the terms “a” and “an” and “the” and similar references used in the context of describing a particular embodiment (especially in the context of certain of the following claims) can be construed to cover both the singular and the plural, unless specifically noted otherwise. In some embodiments, the term “or” as used herein, including the claims, is used to mean “and/or” unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive.

[0093] The terms "comprise," "have" and "include" are open-ended linking verbs. Any forms or tenses of one or more of these verbs, such as "comprises," "comprising," "has," "having," "includes" and "including," are also open-ended. For example, any method that "comprises," "has" or "includes" one or more steps is not limited to possessing only those one or more steps and can also cover other unlisted steps. Similarly, any composition or device that "comprises," "has" or "includes" one or more features is not limited to possessing only those one or more features and can cover other unlisted features.

[0094] All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g. "such as") provided with respect to certain embodiments herein is intended merely to better illuminate the present disclosure and does not pose a limitation on the scope of the present disclosure otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the present disclosure.

[0095] Groupings of alternative elements or embodiments of the present disclosure disclosed herein are not to be construed as limitations. Each group member can be referred to and claimed individually or in any combination with other members of the group or other elements found herein. One or more members of a group can be included in, or deleted from, a group for reasons of convenience or patentability. When any such inclusion or deletion occurs, the specification is herein deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

[0096] All publications, patents, patent applications, and other references cited in this application are incorporated herein by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent application or other reference was specifically and individually indicated to be incorporated by reference in its entirety for all purposes. Citation of a reference herein shall not be construed as an admission that such is prior art to the present disclosure.

[0097] Having described the present disclosure in detail, it will be apparent that modifications, variations, and equivalent embodiments are possible without departing the scope of the present disclosure defined in the appended claims. Furthermore, it should be appreciated that all examples in the present disclosure are provided as non-limiting examples.

EXAMPLES

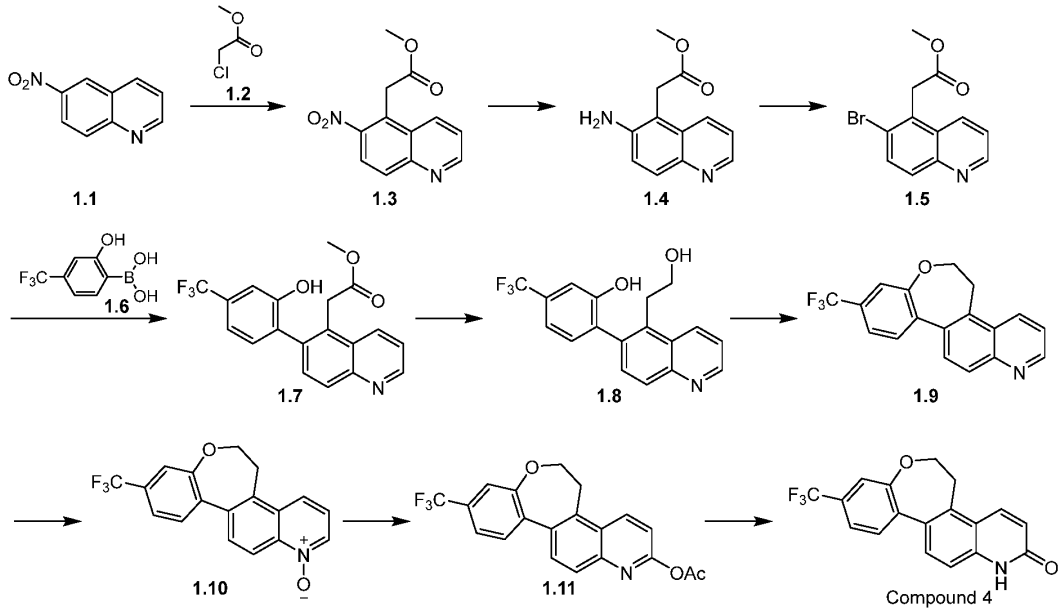
[0098] Compound 4 and Compound 13 are potent and more metabolically stable autophagy enhancer drugs. The following demonstrate evidence for *in vivo* systemic efficacy in the PiZ mouse model of alpha-1-antitrypsin deficiency and in human cellular models of tauopathy and Parkinsonism in addition to Huntington's disease.

[0099] It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the preceding description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

EXAMPLE 1: COMPOUND 4 SYNTHESIS

[00100] A series of analogs that were developed for further analysis are shown in the application above.

[00101] The scheme for the synthesis of Compound 4 (9-(trifluoromethyl)-5,6-dihydrobenzo[6,7]oxepino[4,5-f]quinolin-2(1H)-one) is shown below.



[00102] To a solution of 6-nitroquinoline **1.1** (90.00 g, 516.78 mmol, 1.00 *eq*) and methyl 2-chloroacetate **1.2** (168.24 g, 1550.34 mmol, 136.78 mL, 3.00 *eq*) in THF (3.0 L) was added NaH (62.02 g, 1550.34 mmol, 60% purity, 3.00 *eq*) at 0°C. The reaction mixture was stirred at 25°C for 12 h. The reaction mixture was quenched by saturated aqueous NH_4Cl 1.5 L at 0°C and extracted with ethyl acetate (1 L x 3), dried with anhydrous

Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography. Methyl 2-(6-nitroquinolin-5-yl)acetate **1.3** (123.50 g, 501.59 mmol, 97.06% yield) was obtained as a orange oil. MS: *m/z* 247.06 $(M+H)^+$. ^1H NMR (400 MHz): DMSO δ 9.13 (d, J = 4.2 Hz, 1H), 8.86 (d, J = 8.7 Hz, 1H), 8.31 - 8.24 (m, 1H), 8.23 - 8.16 (m, 1H), 7.78 (dd, J = 4.2, 8.7 Hz, 1H), 4.48 (s, 2H), 3.67 (s, 3H).

[00103] To a solution of methyl 2-(6-nitroquinolin-5-yl)acetate **1.3** (112.00 g, 454.88 mmol, 1.00 *eq*) in EtOAc (1.2 L) was added Pd/C (24.00 g, 10% purity). The reaction mixture was stirred at 25°C for 12 h under H_2(20 Psi) atmosphere. The mixture was filtered to obtain filtrate. The residue was purified by column chromatography. Methyl 2-(6-aminoquinolin-5-yl)acetate **1.4** (63.00 g, 291.35 mmol, 64.05% yield) was obtained as a brown oil. MS: *m/z* 217.09 $(M+H)^+$. ^1H NMR (400 MHz): DMSO δ 8.52 (dd, J = 1.4, 4.1 Hz, 1H), 8.06 (d, J = 8.3 Hz, 1H), 7.70 (d, J = 9.0 Hz, 1H), 7.36 (dd, J = 4.2, 8.6 Hz, 1H), 7.27 (d, J = 9.0 Hz, 1H), 5.58 (s, 2H), 3.97 (s, 2H), 3.61 (s, 3H).

[00104] To a solution of methyl 2-(6-aminoquinolin-5-yl)acetate **1.4** (5.00 g, 23.12 mmol, 1.00 *eq*) in ACN (100.0 mL) was added HBr (4.09 g, 24.28 mmol, 2.75 mL, 48% purity in H_2O, 1.05 *eq*), $NaNO_2$ (15.60 g, 226.24 mmol, 3.00 *eq*) at 0°C, the mixture was stirred at 25°C for 1 hr. Then the mixture was added $CuBr_2$ (6.20 g, 27.75 mmol, 1.20 *eq*). The reaction mixture was stirred at 80°C for 11 h. The reaction mixture was washed with THF (500 mL), then the mixture was filtered to obtain filtrate. The filtrate was quenched by saturated aqueous Na_2CO_3 100 mL and extracted with ethyl acetate (200 mL x 3), dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography. Methyl 2-(6-bromoquinolin-5-yl)acetate **1.5** (1.10 g, 3.93 mmol, 16.98% yield) was obtained as a yellow solid. MS: *m/z* 279.99 $(M+H)^+$. ^1H NMR (400 MHz): DMSO δ 8.94 (br d, J = 3.3 Hz, 1H), 8.31 (d, J = 8.6 Hz, 1H), 7.96 - 7.92 (m, 1H), 7.91 - 7.87 (m, 1H), 7.48 (dd, J = 4.1, 8.6 Hz, 1H), 4.33 (s, 2H), 3.71 (s, 3H).

[00105] To a solution of methyl 2-(6-bromoquinolin-5-yl)acetate **1.5** (1.00 g, 3.57 mmol, 1.00 *eq*) and (2-hydroxy-4-(trifluoromethyl)phenyl)boronic acid **1.6** (882.17 mg, 4.28 mmol, 1.20 *eq*) in dioxane (25.0 mL) and H_2O (5.0 mL) was added Amphos Pd Cl_2 (252.78 mg, 356.99 μmol, 252.78 μL, 0.10 *eq*) and K_3PO_4 (1.52 g, 7.14 mmol, 2.00 *eq*). The reaction mixture was stirred at 80°C for 12 h under N_2. The reaction mixture was washed with H_2O (50 mL) and extracted with ethyl acetate (50 ml x 3), dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column

chromatography. Methyl 2-(6-(2-hydroxy-4-(trifluoromethyl)phenyl)quinolin-5-yl)acetate **1.7** (1.21 g, 3.35 mmol, 93.81% yield) was obtained as a yellow solid. MS: *m/z* 362.09 $(M+H)^+$.

[00106] To a solution of methyl 2-(6-(2-hydroxy-4-(trifluoromethyl)phenyl)quinolin-5-yl)acetate **1.7** (1.10 g, 3.04 mmol, 1.00 *eq*) in THF (20.0 mL) was added LAH (231.10 mg, 6.09 mmol, 2.00 *eq*). The reaction mixture was stirred at 25°C for 1 h under N_2. The reaction mixture was quenched by $Na_2SO_4.H_2O$ (500 mg) at 0°C, then the mixture was filtered to obtain filtrate. The residue was purified by column chromatography. 2-(5-(2-hydroxyethyl)quinolin-6-yl)-5-(trifluoromethyl)phenol **1.8** (850.00 mg, 2.55 mmol, 83.77% yield) was obtained as a yellow solid. MS: *m/z* 334.10 $(M+H)^+$.

[00107] To a solution of 2-(5-(2-hydroxyethyl)quinolin-6-yl)-5-(trifluoromethyl)phenol **1.8** (750.00 mg, 2.25 mmol, 1.00 *eq*) in Tol. (30.0 mL) was added CMBP (1.09 g, 4.50 mmol, 2.00 *eq*) at 0 °C. The reaction mixture was stirred at 80°C for 11 h under N_2. The residue was purified by column chromatography. 9-(trifluoromethyl)-5,6-dihydrobenzo[6,7]oxepino[4,5-f]quinoline **1.9** (500.00 mg, 1.59 mmol, 70.48% yield) was obtained as a white solid. MS: *m/z* 316.09 $(M+H)^+$. ^1H NMR (400 MHz): DMSO δ 8.98 (dd, J = 1.4, 4.1 Hz, 1H), 8.80 (d, J = 8.5 Hz, 1H), 8.11 (d, J = 8.8 Hz, 1H), 7.91 (d, J = 8.6 Hz, 1H), 7.85 (d, J = 8.0 Hz, 1H), 7.71 (dd, J = 1.1, 8.1 Hz, 1H), 7.65 (dd, J = 4.1, 8.6 Hz, 1H), 7.54 (d, J = 1.3 Hz, 1H), 4.73 (t, J = 6.2 Hz, 2H), 3.30 (t, J = 6.2 Hz, 2H).

[00108] To a solution of 9-(trifluoromethyl)-5,6-dihydrobenzo[6,7]oxepino[4,5-f]quinoline **1.9** (200.00 mg, 634.34 μmol, 1.00 *eq*) in $CHCl_3$ (5.0 mL) was added m-CPBA (386.36 mg, 1.90 mmol, 85% purity, 3.00 *eq*) at 0°C. The reaction mixture was stirred at 50°C for 1 h. The reaction mixture was quenched by saturated aqueous Na_2SO_3 200 mL at 0°C and extracted with dichloromethane (100 mL x 3). The combined organic phase were washed with saturated aqueous Na_2CO_3 (100 mL x 3), dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was used in the next step without any purification. 9-(trifluoromethyl)-5,6-dihydrobenzo[6,7]oxepino[4,5-f]quinoline 1-oxide **1.10** (200.00 mg, crude) was obtained as a white solid. MS: *m/z* 332.08 $(M+H)^+$.

[00109] To a solution of 9-(trifluoromethyl)-5,6-dihydrobenzo[6,7]oxepino[4,5-f]quinoline 1-oxide **1.10** (200.00 mg, 603.70 μmol, 1.00 *eq*) in Ac_2O (5.0 mL) was stirred at 140°C for 3 h. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was used in the next step without any

purification. 9-(trifluoromethyl)-5,6-dihydrobenzo[6,7]oxepino[4,5-f]quinolin-2-yl acetate **1.11** (200.00 mg, crude) was obtained as brown oil. MS: *m/z* 374.09 $(M+H)^+$.

[00110] To a solution of 9-(trifluoromethyl)-5,6-dihydrobenzo[6,7]oxepino[4,5-f]quinolin-2-yl acetate **1.11** (200.00 mg, 535.73 µmol, 1.00 *eq*) in MeOH (10.0 mL) was added K_2CO_3 (222.12 mg, 1.61 mmol, 3.00 *eq*). The mixture was stirred at 25 °C for 1 h. The reaction mixture concentrated under reduced pressure to give a residue. The residue was washed with H_2O (50 mL), the mixture was filtered to obtain filter cake. The filter cake was washed with TBME (3 mL), and the filter cake concentrated under reduced pressure to give a residue. 9-(trifluoromethyl)-5,6-dihydrobenzo[6,7]oxepino[4,5-f]quinolin-2(1H)-one Compound 4 (74.68 mg, 223.78 µmol, 41.77% yield, 99.27% purity) was obtained as a yellow solid. MS: *m/z* 332.08 $(M+H)^+$. ^1H NMR (400 MHz): DMSO δ 11.95 (s, 1H), 8.35 (d, J = 10.0 Hz, 1H), 7.77 - 7.70 (m, 1H), 7.66 (d, J = 8.4 Hz, 2H), 7.49 (s, 1H), 7.40 (d, J = 8.6 Hz, 1H), 6.61 (d, J = 9.8 Hz, 1H), 4.64 (br t, J = 6.1 Hz, 2H), 3.13 (br t, J = 6.1 Hz, 2H).

EXAMPLE 2: COMPOUND 4 AND COMPOUND 13 WERE IDENTIFIED AS CANDIDATE ANALOGS

[00111] Example compounds have been tested for activity in HTO/Z and MSD assays.

[00112] The HTO/Z assay is carried out in the HTO/Z cell line, which is a HeLa line genetically engineered for expression of ATZ. The cells are incubated for 18 hours in the absence or presence of the example compound and then analyzed for steady state levels of ATZ by immunoblot.

[00113] For the MSD assay, HTO/Z cells were used after they had been passaged three times out of doxycycline. On day 1, HTO/Z cells (in 100 uL media) were plated at 10,000 cells/well in a 96-well plate. Compounds (11.1 uL of 10x inhibitor in media containing 1% DMSO) were added on day 2. On day 4, media was removed, and cells were immediately fixed by adding 150 µL of 4% PFA. After a 20 min incubation at room temperature without shaking, PFA was removed. A permeabilization solution of DPBS-0.1% Triton X-100 (200 uL) was added to plates with gentle shaking. The permeabilization step was repeated a total of five times. Licor Intercept blocking buffer (150 uL) was added and wells were blocked for 1.5 hours at room temperature with gentle shaking. The blocking buffer was removed and alpha-1-antitrypsin primary antibody in 50 uL blocking buffer (1:400 dilution) was added to each well.

Primary antibody was incubated overnight at 4°C without shaking. Primary antibody was removed, and wells were washed five times with 1X DPBS-0.1% Tween-20 (200 uL) for 5 minutes at room temperature with gentle shaking. IRDye 800CW goat anti-mouse IgG1 secondary antibody (1:600 dilution) and CellTag 700 (1:500 dilution) in 50 uL blocking buffer was added to each well followed by a 2-hour incubation at room temperature with gentle shaking. Plates were washed five times with 1X DPBS-0.1% Tween-20 (200 uL). Plates were centrifuged upside down at 1000 rpm for 1 min to remove excess liquid from wells. Plates were imaged on a Licor Odyssey CLx instrument. Mutant α1-antitrypsin Z protein was quantitated in the 800 nm channel; total cell counts from CellTag 700 was quantitated in the 700 nm channel.

[00114] Reagents used: Alpha-1-antitrypsin, human, mAb 2C1 (Hycult Biotech #HM2289), IRDye 800CW goat anti-mouse IgG1 secondary antibody (Licor # 926-32350), CellTag 700 stain (Licor # 926-41090), formaldehyde 16% in aqueous solution (VWR # 100503-020), Intercept (PBS) blocking buffer (Licor # 927-70001), DPBS (Corning # 21-031-CV), Triton X-100 (MilliporeSigma # T8787), Tween-20 (BioRad # 1610781), DMSO (MilliporeSigma #D2438), DMEM, high glucose (ThermoFisher # 11965-084), Tet System Approved FBS (Takara # 631101), penicillin-streptomycin (ThermoFisher # 15140-122), L-glutamine (ThermoFisher # 25030-081), G418 sulfate (ThermoFisher # 11811-031), hygromycin (ThermoFisher # 10687-010), doxycycline (MilliporeSigma #D9891)

[00115] Raw data was processed as follows: the ratio of 800 nm to 700 nm channels was determined for each well and then each well was normalized to the average DMSO value. After normalization, 0% inhibition has a value of 1.0 while maximum inhibition is typically in the 0.3-0.5 range.

[00116] IC50s were calculated by fitting data to a 4-parameter model using GraphPad Prism. Compounds were serially diluted 3-fold in DMSO and then diluted 100-fold into complete DMEM media. IC50 curves consisted of 7 inhibitor concentrations and 1 no inhibitor DMSO control all done in duplicate. Final DMSO concentration in the assay was 0.1%.

[00117] The results of the HTO/Z and MSD assays are summarized in the following table. For the HTO/Z assay, + indicates an effect at about 1.0-10.0 μM, ++ indicates an effect at about 0.10-1.0 μM, and +++ indicates an effect at about 0.01-0.1 μM or less. For the MSD assay, ND indicates not determined.

Compound	HTO/Z assay	MSD IC50 (nM)
Ex. 1	++	2.7
Ex. 2	++	ND
Ex. 3	++	ND
Ex. 4	+++	<1.0
Ex. 5	++	ND
Ex. 6	+	ND
Ex. 7	+	ND
Ex. 8	++	20
Ex. 9	++	ND
Ex. 10	++	ND
Ex. 11	++	ND
Ex. 12	++	ND
Ex. 13	++	<1.0
Ex. 14	++	ND
Ex. 15	++	16
Ex. 16	++	25
Ex. 17	++	1.2
Ex. 18	++	16
Ex. 19	++	6.3
Ex. 20	++	<1.0
Ex. 21	++	<1.0
Ex. 22	++	ND
Ex. 23	++	<1.0
Ex. 24	++	ND
Ex. 25	++	79
Ex. 26	++	18
Ex. 27	++	ND
Ex. 28	++	ND
Ex. 29	++	ND
Ex. 30	++	ND
Ex. 31	+	ND

[00118] Of particular interest are potent autophagy inducers Compound 4 and Compound 13. Compound 13 is the pyridine analog of Compound 4.

[00119] A western blot of ATZ levels normalized to β-actin levels indicates ATZ levels generally decrease as Compound 4 levels increase (Figure 1A). An MSD assay of Compound 4 resulted in an IC50 for reduction in cellular ATZ levels/accumulation of 0.00035 μM (Figure 1B).

EXAMPLE 3: COMPOUND 4 AND COMPOUND 13 *IN VIVO* METABOLISM

Pharmacology of Compound 4 and Compound 13

[00120] Oral administration of Compound 4 and Compound 13 resulted in high and stable drug levels in blood with rapid uptake and accumulation in brain and liver.

[00121] Plasma levels of Compound 4 and Compound 13 remain stable for up to 24 hours (Figures 2A-2D).

Compound 4 liver collagen

[00122] Male PIZ [h/h]GFP-LC3[h/h] mice were given either Compound 13 or placebo by gavage feeding 5 out of 7 days for 4 weeks.

[00123] Measuring the amount of collagen per mg of liver liver using the hydroxyproline assay in livers of PiZ mice at harvest treated with Compound 13 versus placebo treated mice found a decrease in Compound 13 treated mice compared to placebo treated mice ($P = 0.0447$) (Figure 3).

EXAMPLE 4: COMPOUND 4 CHARACTERIZATION IN NEURONS

[00124] Compound 4 was further characterized in young, healthy cortical neurons; age-matched (old), healthy cortical neurons; and late-onset Alzheimer's disease (LOAD) cortical neurons.

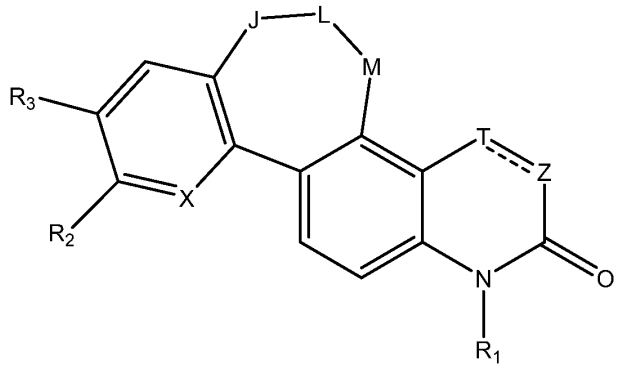
[00125] The first experiment tested the effect of Compound 4 on cell death in an AD human neuronal model system using a SYTOX™ assay. Compound 4 (5nM) treated LOAD (late-onset Alzheimer's disease) cortical neurons showed a decrease in cell death rate; young and old controls showed no significant difference (Figure 4).

[00126] The next experiment tested the effect of Compound 4 on cell death using a caspase assay in a model cell line of cortical cells from a patient with late-onset Alzheimer's disease created by re-programming (Figure 5). The results show that Compound 4 mediated a decrease in caspase 3/7 signal consistent with a reduction in cell death.

[00127] A further experiment tested the effect of Compound 4 on cell death in the re-programmed human cortical neuronal models from a patient with late-onset AD. Results from this line are shown in Figure 6. The results show that Compound 4 mediated a decrease in Annexin V, indicating a decrease in cell death, at doses of 2.5, 5, and 10 μM.

WHAT IS CLAIMED IS:

1. A compound of Formula I:



(Formula I)

wherein:

R_1 is hydrogen or substituted or unsubstituted alkyl;

R_2 is hydrogen, halo, substituted alkyl, unsubstituted alkyl, alkoxyl, cyano, amino, $-N(R_4)C(O)R_5$, $-C(O)N(R_4)(R_5)$, $-SO_2R_6$, $-N(R_4)SO_2R_6$, $-SO_2N(R_4)(R_5)$, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl;

R_3 is hydrogen, halo, substituted alkyl, unsubstituted alkyl, alkoxyl, cyano, amino, $-N(R_4)C(O)R_5$, $-C(O)N(R_4)(R_5)$, $-SO_2R_6$, $-N(R_4)SO_2R_6$, $-SO_2N(R_4)(R_5)$, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl;

J is $-CH(R_4)-$, $-C(R_4)-$, $-N(R_5)-$, or -O-;

L is $-CH(R_4)-$, $-C(R_4)-$, or -C(O)-;

M is $-CH(R_4)-$, $-C(R_4)-$, $-N(R_5)-$, or -O-;

T is absent, $-CH_2CH_2-$, $-CH(R_4)-$, $-C(R_4)-$, -O-, or together with Z form a cycloalkyl group fused to the 6-membered ring;

Z is $-CH(R_4)-$, $-C(R_4)-$, $-N(R_5)-$, -N-, -O-, or together with T form a cycloalkyl group fused to the 6-membered ring;

T and Z can be joined by a single bond or a double bond depending on the valence of the groups; when T is absent the ring is a 5-membered ring; when T is $-CH_2CH_2-$ the ring is a 7-membered ring;

R_4 and R_5 are independently hydrogen, substituted alkyl, or unsubstituted alkyl; and

R_6 is substituted alkyl or unsubstituted alkyl.

2. The compound of claim 1, wherein:

R_1 is hydrogen or unsubstituted C_1 to C_3 alkyl;

R_2 is hydrogen, halo, or halo-substituted C_1 to C_3 alkyl;

R_3 is hydrogen, halo, or halo-substituted C_1 to C_3 alkyl;

J is $–CH(R_4)$-, $-C(R_4)$-, or -O-;

L is $–CH(R_4)$-, $-C(R_4)$-, or -C(O)-;

M is $–CH(R_4)$- or -O-;

T is $–C(R_4)$-;

Z is $–C(R_4)$-;

T and Z share a double bond; and

R_4 is hydrogen or unsubstituted C_1 to C_3 alkyl.

3. The compound of claim 1, wherein

R_1 is hydrogen or methyl;

R_2 is hydrogen, chloro, or chloro-substituted C_1 to C_3 alkyl;

R_3 is hydrogen, chloro, or chloro-substituted C_1 to C_3 alkyl;

J is -O-;

L is $–CH(R_4)$-;

M is $–CH(R_4)$-;

T is $–C(R_4)$-;

Z is $–C(R_4)$-;

T and Z share a double bond; and

R_4 is independently hydrogen or methyl.

4. The compound of claim 1, wherein

R_1 is hydrogen;

R_2 is hydrogen;

R_3 is chloro or chloromethyl;

J is -O- or $–CH(R_4)$-;

L is $–CH(R_4)$-;

M is $–CH(R_4)$- or $-N(R_5)$-;

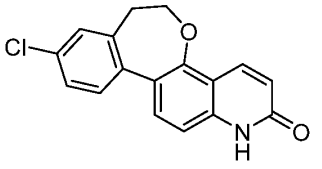
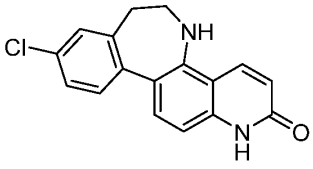
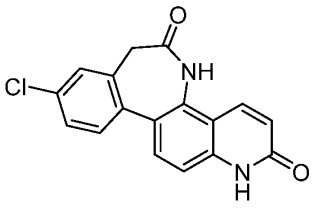
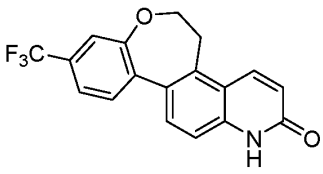
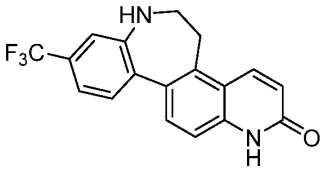
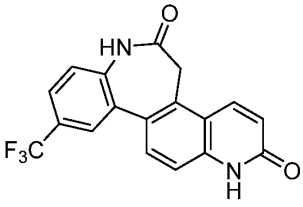
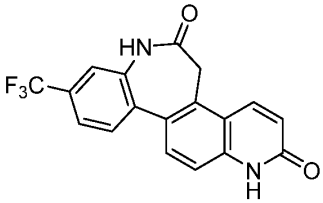
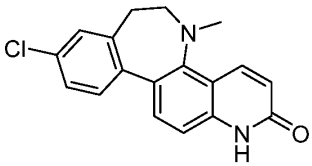
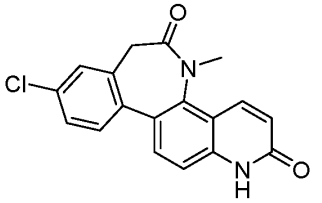
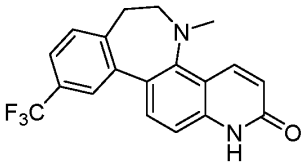
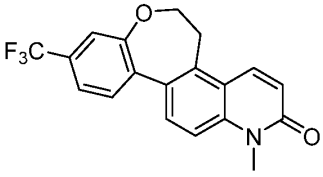
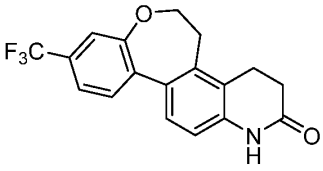
T is $–C(R_4)$-;

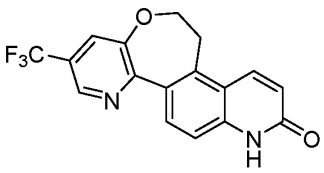
Z is $–C(R_4)$-;

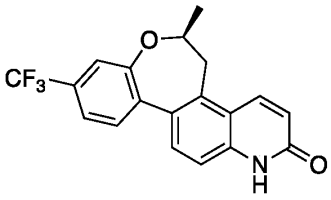
T and Z share a double bond; and

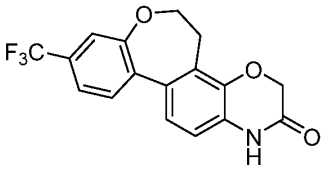
R_4 is hydrogen.

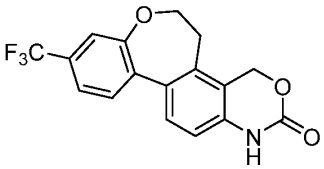
5. The compound of claim 1, wherein the compound is:

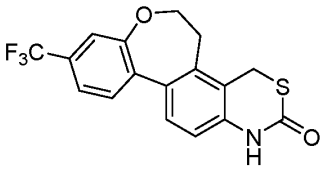

(Ex. 1),

(Ex. 2),

(Ex. 3),

(Ex. 4),

(Ex. 5),

(Ex. 6),

(Ex. 7),

(Ex. 8),

(Ex. 9),

(Ex. 10),

(Ex. 11),

(Ex. 12),

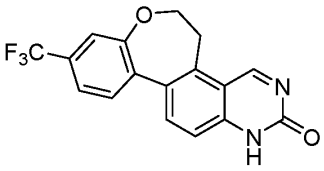

(Ex. 13),

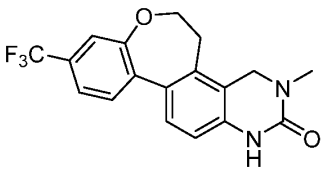

(Ex. 14),

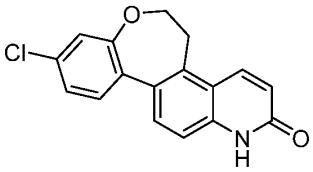

(Ex. 15),

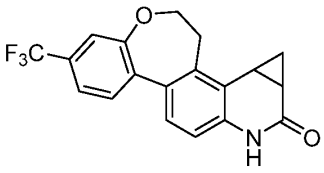

(Ex. 16),

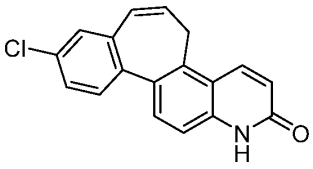

(Ex. 17),

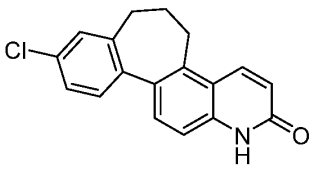

(Ex. 18),

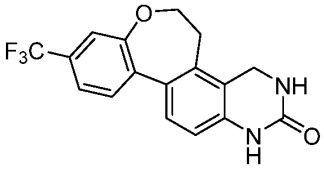

(Ex. 19),

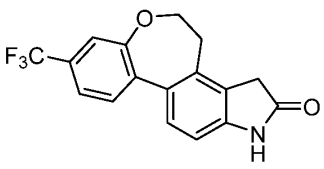

(Ex. 20),

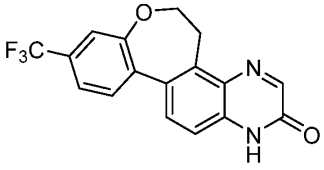

(Ex. 21),

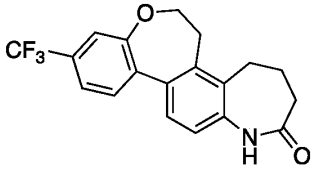

(Ex. 22),

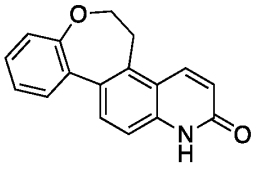

(Ex. 23),

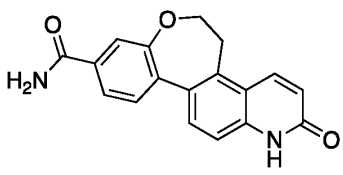
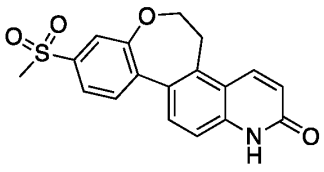

(Ex. 24),

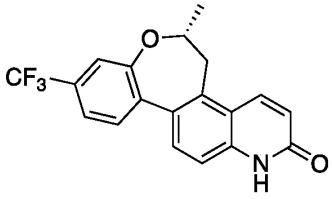

(Ex. 25),

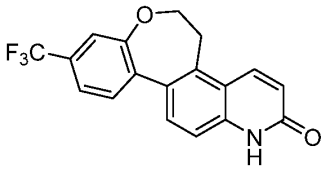

(Ex. 26),

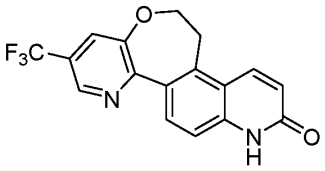

(Ex. 27),


(Ex. 28),

 (Ex. 29),  (Ex. 30), or

 (Ex. 31).

6. The compound of claim 1, wherein the compound is  or

.

7. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and the compound of Formula I of any one of claims 1 to 6.

8. The pharmaceutical composition of claim 7, wherein the compound of Formula I has a concentration of about 0.01 wt.% to about 1 wt.% based on the total weight of the pharmaceutical composition.

9. The pharmaceutical composition of claim 7 or 8, wherein the compound of Formula I has a concentration of about 0.05 wt.% to about 0.5 wt.%.

10. The pharmaceutical composition of any one of claims 7 to 9, wherein the compound of Formula I has a concentration of about 0.05 wt.%, about 0.1 wt.%, or about 0.5 wt.%.

11. The pharmaceutical composition of any one of claims 7 to 10, wherein the pharmaceutically acceptable excipient comprises a protic polar solvent, a non-protic polar solvent, a non-ionic surfactant, a cyclodextrin, or a combination thereof.

12. The pharmaceutical composition of any one of claims 7 to 10, wherein the pharmaceutically acceptable excipient comprises water, polyethylene glycol 12-hydroxystearate, polyethylene glycol-8 mono- and diesters of caprilic (C8) and capric (C10) acids with a small fraction of mono-, di-, and triglycerides, methylcellulose, polyoxyethylenesorbitan monooleate, N-methyl-2-pyrrolidone (NMP), polyethylene glycol with number average molecular weight of 400 (PEG 400), sulfobutylether-β-cyclodextrin (SBE-β-CD), macrogolglycerol ricinoleate, dimethyl sulfoxide (DMSO), or a combination thereof.

13. A method for treating an autophagy-related disease, the method comprising administering the compound of Formula I of any one of claims 1 to 6 or the pharmaceutical composition of any one of claims 7 to 12 to a subject in need thereof.

14. The method of claim 13, wherein the autophagy-related disease is alpha-1 antitrypsin deficiency (ATD), Alzheimer's disease, Parkinson's disease, Huntington's disease, or amyotrophic lateral sclerosis.

15. The method of claim 14, wherein the autophagy-related disease is alpha-1 antitrypsin deficiency (ATD).

16. The method of claim 14, wherein the autophagy-related disease is Alzheimer's disease.

17. The method of claim 14, wherein the autophagy-related disease is Parkinson's disease.

18. The method of claim 14, wherein the autophagy-related disease is Huntington's disease.

19. The method of claim 14, wherein the autophagy-related disease is amyotrophic lateral sclerosis.

20. The method of any one of claims 13 to 19, wherein the subject is a mammal.

21. The method of any one of claims 13 to 20, wherein the subject is a human.