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(54) Title: INHIBITORS OF CDC2-LIKE KINASES (CLKS) AND METHODS OF USE THEREOF

(57) Abstract: Provided herein are compounds which are CDC2-Like Kinase (CLK) inhibiting compounds and methods of use thereof. The CLK-inhibiting compounds may be used for increasing the lifespan of a cell, and treating and/or preventing a wide variety of diseases and disorders including, for example, diseases or disorders related to aging or stress, diabetes, obesity, neurodegenerative diseases, cardiovascular disease, blood clotting disorders, inflammation, ocular disorders, and/or flushing as well as diseases or disorders that would benefit from increased mitochondrial activity. Also provided are compositions comprising a Clk-inhibiting compound in combination with another therapeutic agent.



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INHIBITORS OF CDC2-LIKE KINASES (CLKS) AND METHODS OF USE THEREOF

RELATED APPLICATIONS

This application claims the benefit of priority from United States provisional application number 61/008,964, filed December 21, 2007. The specification of the
5 foregoing application is hereby incorporated by reference in its entirety.

BACKGROUND

A class of non-receptor protein kinases are implicated in regulating RNA splicing (Fu, 1995 RNA 1:663-680; Staknis and Reed, 1994, Mol. Cell. Biol. 14:7670-7682). These protein kinases phosphorylate polypeptides rich in serine and
10 arginine (SR proteins). SR proteins are characterized as containing at least one amino-terminal RNA recognition motif and a basic carboxyterminal domain rich in serine and arginine residues, often arranged in tandem repeats (Zahler et al., 1992, Genes Dev 6:837-847). Experimental evidence supports the idea that the SR domain is involved in protein-protein interactions (Kohtz et al., 1994, Nature 368:119-124)
15 as well as protein-RNA interactions (Harada et al., 1996, Nature 380:175-179), and may contribute to a localization signal directing proteins to nuclear speckles (Hedley et al., 1995, Proc. Natl. Acad. Sci. USA 92:11524-11528).

The selection of splice site can be altered by numerous extracellular stimuli, including growth factors, cytokines, hormones, depolarization, osmotic shock, and
20 UVC irradiation through synthesis, phosphorylation, and a change in localization of serine/arginine-rich (SR) proteins (Stamm (2002) Hum. Mol. Genet. 11: 2409).

SR proteins are a family of essential factors required for constitutive splicing of pre-mRNA (Krainer et al. (1991) Cell 66: 383) and play an important role in modulating alternative splicing (Blencowe (2000) Trends Biochem. Sci. 25: 106).
25 They are highly conserved in eukaryotes and are characterized by having one or two RNA-recognition motifs at the amino terminus and an RS domain at the carboxyl terminus (Zahler et al. (1992) Genes Dev. 6: 837; Caceres et al. (1993) EMBO J. 12: 4715). RS domains consist of multiple consecutive RS/SR dipeptide repeats and differ in length among different SR proteins. Extensive phosphorylation of serines in
30 the RS domain occurs in all SR proteins (Kohtz, et al (1994) Nature 368: 119; Gui et

al. (1994) *Nature* 369: 678). Although its precise physiological role is still unknown, phosphorylation of SR proteins affects their protein-protein and protein-RNA interactions (Xiao et al. (1997) *Genes Dev.* 11: 334), intracellular localization and trafficking (Caceres et al. (1998) *Genes Dev.* 12: 55; Misteli et al. (1998) *J. Cell Biol.* 143: 297), and alternative splicing of pre-mRNA (Duncan et al. (1997) *Mol. Cell. Biol.* 17: 5996). Spliceosome assembly may be promoted by phosphorylation of SR proteins that facilitate specific protein interactions, while preventing SR proteins from binding randomly to RNA (Xiao et al. (1997) *Genes Dev.* 11: 334). Once a functional spliceosome has formed, dephosphorylation of SR proteins appears to be necessary to allow the transesterification reactions to occur (Cao et al. (1997) *RNA (New York)* 3: 1456). Therefore, the sequential phosphorylation and dephosphorylation of SR proteins may mark the transition between stages in each round of the splicing reaction. To date, several kinases have been reported to phosphorylate SR proteins, including a family of kinases termed CLK (Cdc2-like kinase), or LAMMER kinases from the consensus motif, consisting of four members (CLK1/Sty and CLK2, CLK3 and CLK4) (Colwill et al. (1996) *EMBO J.* 15: 265; Nayler et al. (1997) *Biochem. J.* 326: 693).

Mammalian CLK family kinases contain an SR domain and are demonstrated to phosphorylate SR proteins *in vitro* and SF2/ASF *in vivo* (Nayler et al. (1997) *Biochem. J.* 326: 693). Clks are shown to be dual-specificity kinases that autophosphorylate on tyrosine, serine, and threonine residues in overexpression systems and *in vitro* (Nayler et al. (1997) *Biochem. J.* 326: 693; Ben-David et al. (1991) *EMBO J.* 10: 317; Howell et al. (1991) *Mol. Cell. Biol.* 11: 568). When overexpressed, the catalytically inactive mutant kinases localize to nuclear speckles where splicing factors are concentrated, whereas the wild-type enzymes distribute throughout the nucleus and cause speckles to dissolve (Colwill et al. (1996) *EMBO J.* 15: 265). The overexpression of CLKs also affects splicing site selection of pre-mRNA of both its own transcript and adenovirus E1A transcripts *in vivo* (Duncan et al. (1997) *Mol. Cell. Biol.* 17: 5996).

CLK's are well conserved in many organisms. mCLK1 is a dual specificity protein kinase originally isolated in mouse expression libraries (Ben-David et al., 1991, *EMBO J.* 10:317-325; Howell et al., 1991, *Mol. Cell. Biol.* 11:568-572) and

human (hCLK1, hCLK2, hCLK3, hCLK4), plant (AFC1, AFC2, AFC3) and fly (DOA) CLK protein kinases have since been identified (Johnson and Smith, 1991, J. Biol. Chem. 266:3402-3407; Hanes et al., 1994, J. Mol. Biol. 244:665-672; Bender and Fink, 1994, Proc. Natl. Acad. Sci. USA 91:12105-12109; Yun et al., 1994, Genes. Dev. 8:1160-1173). Three of the genes for human CLKs have been mapped to unique chromosomal locations; specifically hCLK1 – 2q33, hCLK2 – 1q21 and hCLK3 – 15q24 (Talmadge et al., Hum Genet. 1998 103(4):523-4). The amino terminal domain of these proteins is rich in serine and arginine, whereas the catalytic domain can be most similar to CDC2, a serine/threonine protein kinase (Ben-David et al., 1991, EMBO J. 10:317-325). CLKs are also known as STY or LAMMER kinases (the latter based on a signature motif 'EHLAMMERILG' conserved between the CLK family members).

To date, a number of diseases caused by mis-splicing have been reported; in some cases, mutation(s) found around splice sites appear to be responsible for changing the splicing pattern of a transcript by unusual exon inclusion or exclusion and/or alteration of 5' or 3' sites (reviewed in Stoss et al. (2000) Gene Ther. Mol. Biol. 5: 9; Philips et al. (2000) Cell. Mol. Life Sci. 57: 235; Faustino et al. (2003) Genes Dev. 17: 419). A typical example is beta-thalassemia, an autosomal recessive disease, which is often associated with mutations in intron 2 of the alpha-globin gene. The generation of aberrant 5' splice sites activates a common 3' cryptic site upstream of the mutations and induces inclusion of a fragment of the intron-containing stop codon. As a result, the amount of functional alpha-globin protein is reduced. For therapeutic modulation of alternative splicing, several trials with antisense oligonucleotide (Sazani et al. (2003) J. Clin. Investig. 112: 481), peptide nucleic acid oligonucleotide, and RNAi (Epstein (1998) Methods 14: 21; Celotto et al. (2002) RNA (New York) 8: 718) have been reported. These approaches could be useful for manipulating a specific splice site selection of a known target sequence like beta-globin (Sazani et al. (2003) J. Clin. Investig. 112: 481). However, the aberrant splicing, found in the patients of breast cancer, Wilm's tumor, and amyotrophic lateral sclerosis (ALS), are not always accompanied with mutations around splice sites. In sporadic ALS patients, EAAT2 (excitatory amino acid transporters 2) RNA processing is often aberrant in motor cortex and in spinal cord,

the regions specifically affected by the disease. As exon 9 is aberrantly skipped in some ALS patients without any mutation in the gene (Lin et al. (1998) Neuron 20: 589), the disorders could be attributed to abnormalities in regulatory factors of splicing. Actually the balance of alternative splicing products can be affected by changes in the ratio of heterogeneous nuclear ribonucleoprotein and SR proteins (Mayeda et al. (1992) Cell 68: 365; Caceres et al. (1994) Science 265: 1706) and in the phosphorylation state and localization of SR proteins (Duncan et al. (1997) Mol. Cell. Biol. 17: 5996).

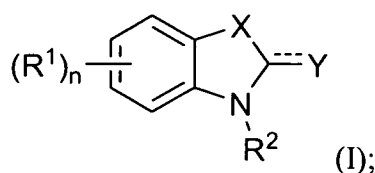
U.S. patent publication 2005/0171026 ("Therapeutic composition of treating abnormal splicing caused by the excessive kinase induction"), provides a composition for treating or preventing abnormal splicing caused by the excessive kinase induction, which comprises compounds and a method for using the compounds for treating or preventing abnormal splicing caused by the excessive kinase induction. The compositions and methods so described would be useful for treatment of diseases that have as a cause excessive kinase activity leading to abnormal splicing, including some forms of cancer and neurodegeneration as described within the application.

PCT Publication No. WO 2007/019417 discloses that in addition to the role CLKs play in splicing, CLKs directly phosphorylate proteins involved in, among other things, gene transcription; deacetylation of proteins that have been post-translationally modified by acetylation of specific lysine residues; and mitochondrial function, biogenesis, and/or activity. In particular, WO 2007/019417 discloses that CLKs have been shown to phosphorylate sirtuins and PGC-1 alpha thereby modulating pathways involved in gene transcription and mitochondrial function, biogenesis, and/or activity. Modulators of CLK activity are therefore useful for treating numerous diseases and disorders.

SUMMARY

Provided herein are novel CDC2-Like Kinase (CLK) inhibiting compounds and methods of use thereof.

In one aspect, the invention provides CLK-inhibiting compounds of Formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

X is selected from S or O;

Y is selected from -NH-C(O)-R⁵, =C(R³)-C(O)-N(R⁴)-R⁵,
 5 =C(R³)-C(O)-C(CH₃)₃, =C(R³)-C(O)-CH(R⁷)(R⁷), =C(R³)-C(=NOH)-R⁵, and
 =C(R³)-CH(OH)-R⁵, and when X is O, Y is additionally selected from =C(R³)-
 carbocyclic and =C(R³)-heterocyclic;

each R¹ is independently selected from -OH, halo, -R⁶, -OR⁶, -SR⁶, -NH₂,
 -NH(R⁶), -N(R⁶)(R⁶), -N(R³)C(O)R⁶, -N(R³)C(S)R⁶ and -SO₂N(R⁶)(R⁶);

10 R² is selected from -(C₁-C₅-alkyl), -(C₁-C₃-alkyl)-aryl,
 -(C₁-C₃-alkyl)-NH(R⁶), and -(C₁-C₃-alkyl)-N(R⁶)(R⁶);

each R³ is independently selected from hydrogen and optionally substituted
 -(C₁-C₄-alkyl);

R⁴ is selected from hydrogen and optionally substituted-(C₁-C₄-alkyl);

15 R⁵ is selected from hydrogen, -(C₁-C₄-alkyl), aryl, -(C₁-C₄-alkyl)-aryl,
 heteroaryl, and -(C₁-C₄-alkyl)-heteroaryl wherein the alkyl, aryl and heteroaryl are
 optionally substituted;

each R⁶ is independently selected from C₁-C₆ alkyl, C₂-C₆ alkenyl or alkynyl,
 carbocyclyl, and heterocyclyl, or wherein two R⁶ together with the nitrogen atom to
 20 which they are bound are taken together to form an optionally substituted
 heterocyclyl; and wherein each R⁶ is optionally and independently substituted;

each R⁷ is independently selected from hydrogen, -(C₁-C₄-alkyl),
 -(C₂-C₄-alkenyl), -(C₂-C₄-alkynyl), carbocyclyl, -(C₁-C₄-alkyl)-carbocyclyl,
 heterocyclyl, and -(C₁-C₄-alkyl)-heterocyclyl, wherein the alkyl, alkenyl, alkynyl,
 25 carbocyclyl and heterocyclyl are optionally substituted

n is an integer from 1 to 4; and

--- represents a single or a double bond.

Also provided are pharmaceutical compositions comprising compounds of
 Formula (I)-(V) or a salt, prodrug or metabolite thereof.

In another aspect, the invention provides methods for using CLK-inhibiting compounds, or compositions comprising CLK-inhibiting compounds. In certain embodiments, CLK-inhibiting compounds may be used for a variety of therapeutic applications including, for example, increasing the lifespan of a cell, and treating
5 and/or preventing a wide variety of diseases and disorders including, for example, diseases or disorders related to aging or stress, diabetes, obesity, neurodegenerative diseases, cardiovascular disease, blood clotting disorders, inflammation, and/or flushing, etc. CLK-inhibiting compounds may also be used for treating a disease or disorder in a subject that would benefit from increased mitochondrial activity, for
10 enhancing muscle performance, for increasing muscle ATP levels, or for treating or preventing muscle tissue damage associated with hypoxia or ischemia.

In certain aspects, the CLK-inhibiting compound may be administered alone or in combination with other compounds, including, for example, other CLK-inhibiting compounds, a sirtuin-activating compound, or other therapeutic agents.

15 DETAILED DESCRIPTION

1. Definitions

As used herein, the following terms and phrases shall have the meanings set forth below. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art.

20 The singular forms “a,” “an,” and “the” include plural reference unless the context clearly dictates otherwise.

The term “agent” is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule (such as a nucleic acid, an antibody, a protein or portion thereof, e.g., a peptide), or an extract made from
25 biological materials such as bacteria, plants, fungi, or animal (particularly mammalian) cells or tissues. The activity of such agents may render it suitable as a “therapeutic agent” which is a biologically, physiologically, or pharmacologically active substance (or substances) that acts locally or systemically in a subject.

The term “bioavailable” when referring to a compound is art-recognized and
30 refers to a form of a compound that allows for it, or a portion of the amount of compound administered, to be absorbed by, incorporated to, or otherwise physiologically available to a subject or patient to whom it is administered.

The terms “CLK protein” or “CLK” refer to a member of the Cdc2-like kinase protein family. Exemplary members of the Cdc2-like kinase family include, for example, CLK proteins from human (hCLK1, hCLK2, hCLK3, and hCLK4), mouse (mCLK1, mCLK2, mCLK3, and mCLK4), plant (AFC1, AFC2, and AFC3) and fly (DOA) CLK protein kinases, as well as homologs (e.g., orthologs and paralogs), variants, or fragments thereof. In an exemplary embodiment, a CLK protein refers to hCLK1 (GenBank Accession No. AAH31549), hCLK2 (GenBank Accession No. AAH53603), hCLK3 (GenBank Accession No. AAH19881), or hCLK4 (GenBank Accession No. NP_065717). In other embodiments, a CLK protein refers to a polypeptide comprising a sequence consisting of, or consisting essentially of, the amino acid sequence set forth in GenBank Accession Nos. AAH31549, AAH53603, AAH19881, or NP_065717; polypeptides comprising all or a portion of the amino acid sequence set forth in GenBank Accession Nos. AAH31549, AAH53603, AAH19881, or NP_065717; the amino acid sequence set forth in GenBank Accession Nos. AAH31549, AAH53603, AAH19881, or NP_065717 with 1 to about 2, 3, 5, 7, 10, 15, 20, 30, 50, 75 or more conservative amino acid substitutions; an amino acid sequence that is at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% identical to GenBank Accession Nos. AAH31549, AAH53603, AAH19881, or NP_065717; and functional fragments of any of the foregoing. CLK proteins preferably have protein kinase activity, including, for example, protein kinase activity toward a sirtuin protein or PGC-1 α . Fragments of the full length CLK proteins having kinase activity may be identified using techniques well known in the art, such as sequence comparisons and assays such as those described herein.

The term “companion animals” refers to cats and dogs. As used herein, the term “dog(s)” denotes any member of the species *Canis familiaris*, of which there are a large number of different breeds. The term “cat(s)” refers to a feline animal including domestic cats and other members of the family *Felidae*, genus *Felis*.

“Diabetes” refers to high blood sugar or ketoacidosis, as well as chronic, general metabolic abnormalities arising from a prolonged high blood sugar status or a decrease in glucose tolerance. “Diabetes” encompasses both the type I and type II (Non Insulin Dependent Diabetes Mellitus or NIDDM) forms of the disease. The

risk factors for diabetes include the following factors: waistline of more than 40 inches for men or 35 inches for women, blood pressure of 130/85 mmHg or higher, triglycerides above 150 mg/dl, fasting blood glucose greater than 100 mg/dl or high-density lipoprotein of less than 40 mg/dl in men or 50 mg/dl in women.

5 The term “ED50” is art-recognized. In certain embodiments, ED50 means the dose of a drug which produces 50% of its maximum response or effect, or alternatively, the dose which produces a pre-determined response in 50% of test subjects or preparations. The term “LD50” is art-recognized. In certain
10 embodiments, LD50 means the dose of a drug which is lethal in 50% of test subjects. The term “therapeutic index” is an art-recognized term which refers to the therapeutic index of a drug, defined as LD50/ED50.

 The term “hyperinsulinemia” refers to a state in an individual in which the level of insulin in the blood is higher than normal.

 The term “insulin resistance” refers to a state in which a normal amount of
15 insulin produces a subnormal biologic response relative to the biological response in a subject that does not have insulin resistance.

 An “insulin resistance disorder,” as discussed herein, refers to any disease or condition that is caused by or contributed to by insulin resistance. Examples
20 include: diabetes, obesity, metabolic syndrome, insulin-resistance syndromes, syndrome X, insulin resistance, high blood pressure, hypertension, high blood cholesterol, dyslipidemia, hyperlipidemia, dyslipidemia, atherosclerotic disease including stroke, coronary artery disease or myocardial infarction, hyperglycemia, hyperinsulinemia and/or hyperproinsulinemia, impaired glucose tolerance, delayed insulin release, diabetic complications, including coronary heart disease, angina
25 pectoris, congestive heart failure, stroke, cognitive functions in dementia, retinopathy, peripheral neuropathy, nephropathy, glomerulonephritis, glomerulosclerosis, nephrotic syndrome, hypertensive nephrosclerosis some types of cancer (such as endometrial, breast, prostate, and colon), complications of pregnancy, poor female reproductive health (such as menstrual irregularities,
30 infertility, irregular ovulation, polycystic ovarian syndrome (PCOS)), lipodystrophy, cholesterol related disorders, such as gallstones, cholecystitis and cholelithiasis,

gout, obstructive sleep apnea and respiratory problems, osteoarthritis, and prevention and treatment of bone loss, e.g. osteoporosis.

The term “livestock animals” refers to domesticated quadrupeds, which includes those being raised for meat and various byproducts, e.g., a bovine animal including cattle and other members of the genus *Bos*, a porcine animal including domestic swine and other members of the genus *Sus*, an ovine animal including sheep and other members of the genus *Ovis*, domestic goats and other members of the genus *Capra*; domesticated quadrupeds being raised for specialized tasks such as use as a beast of burden, e.g., an equine animal including domestic horses and other members of the family Equidae, genus *Equus*.

The term “mammal” is known in the art, and exemplary mammals include humans, primates, livestock animals (including bovines, porcines, etc.), companion animals (e.g., canines, felines, etc.) and rodents (e.g., mice and rats).

“Obese” individuals or individuals suffering from obesity are generally individuals having a body mass index (BMI) of at least 25 or greater. Obesity may or may not be associated with insulin resistance.

The terms “parenteral administration” and “administered parenterally” are art-recognized and refer to modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intra-articular, subcapsular, subarachnoid, intraspinal, and intrasternal injection and infusion.

A “patient”, “subject”, “individual” or “host” refers to either a human or a non-human animal.

The term “pharmaceutically acceptable carrier” is art-recognized and refers to a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting any subject composition or component thereof. Each carrier must be “acceptable” in the sense of being compatible with the subject composition and its components and not injurious to the patient. Some examples of materials which may serve as pharmaceutically acceptable carriers include: (1) sugars, such as

lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as
5 peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18)
10 Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

The term "prophylactic" or "therapeutic" treatment is art-recognized and refers to administration of a drug to a host. If it is administered prior to clinical manifestation of the unwanted condition (e.g., disease or other unwanted state of the
15 host animal) then the treatment is prophylactic, i.e., it protects the host against developing the unwanted condition, whereas if administered after manifestation of the unwanted condition, the treatment is therapeutic (i.e., it is intended to diminish, ameliorate or maintain the existing unwanted condition or side effects therefrom).

The term "pyrogen-free", with reference to a composition, refers to a
20 composition that does not contain a pyrogen in an amount that would lead to an adverse effect (e.g., irritation, fever, inflammation, diarrhea, respiratory distress, endotoxic shock, etc.) in a subject to which the composition has been administered. For example, the term is meant to encompass compositions that are free of, or substantially free of, an endotoxin such as, for example, a lipopolysaccharide (LPS).

25 "Replicative lifespan" of a cell refers to the number of daughter cells produced by an individual "mother cell." "Chronological aging" or "chronological lifespan," on the other hand, refers to the length of time a population of non-dividing cells remains viable when deprived of nutrients. "Increasing the lifespan of a cell" or "extending the lifespan of a cell," as applied to cells or organisms, refers to
30 increasing the number of daughter cells produced by one cell; increasing the ability of cells or organisms to cope with stresses and combat damage, e.g., to DNA, proteins; and/or increasing the ability of cells or organisms to survive and exist in a

living state for longer under a particular condition, e.g., stress (for example, heatshock, osmotic stress, high energy radiation, chemically-induced stress, DNA damage, inadequate salt level, inadequate nitrogen level, or inadequate nutrient level). Lifespan can be increased by at least about 20%, 30%, 40%, 50%, 60% or
5 between 20% and 70%, 30% and 60%, 40% and 60% or more using methods described herein.

“CLK-inhibiting compound” refers to a compound that decreases the level of a CLK protein and/or decreases at least one activity of a CLK protein. In an exemplary embodiment, a CLK-inhibiting compound may decrease at least one
10 biological activity of a CLK protein by at least about 10%, 25%, 50%, 75%, 100%, or more. Exemplary biological activities of CLK proteins include, for example, kinase activity, ability to phosphorylate a sirtuin protein, ability to phosphorylate a SIRT1 protein, ability to phosphorylate PGC-1alpha, ability to phosphorylate a SR protein, ability to autophosphorylate, ability to phosphorylate a splicing factor,
15 ability to regulate splicing, ability to bind to a sirtuin protein, ability to bind to a SIRT1 protein, or ability to bind to PGC-1alpha.

“Sirtuin protein” refers to a member of the Sirtuin deacetylase protein family, or preferably to the sir2 family, which include yeast Sir2 (GenBank Accession No. P53685), *C. elegans* Sir-2.1 (GenBank Accession No. NP_501912),
20 and human SIRT1 (GenBank Accession No. NM_012238 and NP_036370 (or AF083106)) and SIRT2 (GenBank Accession No. NM_012237, NM_030593, NP_036369, NP_085096, and AF083107) proteins. Other family members include the four additional yeast Sir2-like genes termed “HST genes” (homologues of Sir two) HST1, HST2, HST3 and HST4, and the five other human homologues hSIRT3,
25 hSIRT4, hSIRT5, hSIRT6 and hSIRT7 (Brachmann et al. (1995) *Genes Dev.* 9:2888 and Frye et al. (1999) *BBRC* 260:273). Preferred sirtuins are those that share more similarities with SIRT1, i.e., hSIRT1, and/or Sir2 than with SIRT2, such as those members having at least part of the N-terminal sequence present in SIRT1 and absent in SIRT2 such as SIRT3 has.

30 “SIRT1 protein” refers to a member of the sir2 family of sirtuin deacetylases. In one embodiment, a SIRT1 protein includes yeast Sir2 (GenBank Accession No. P53685), *C. elegans* Sir-2.1 (GenBank Accession No. NP_501912), human SIRT1

(GenBank Accession No. NM_012238 or NP_036370 (or AF083106)), and human SIRT2 (GenBank Accession No. NM_012237, NM_030593, NP_036369, NP_085096, or AF083107) proteins, and equivalents and fragments thereof. In another embodiment, a SIRT1 protein includes a polypeptide comprising a sequence
5 consisting of, or consisting essentially of, the amino acid sequence set forth in GenBank Accession Nos. NP_036370, NP_501912, NP_085096, NP_036369, or P53685. SIRT1 proteins include polypeptides comprising all or a portion of the amino acid sequence set forth in GenBank Accession Nos. NP_036370, NP_501912, NP_085096, NP_036369, or P53685; the amino acid sequence set forth in GenBank
10 Accession Nos. NP_036370, NP_501912, NP_085096, NP_036369, or P53685 with 1 to about 2, 3, 5, 7, 10, 15, 20, 30, 50, 75 or more conservative amino acid substitutions; an amino acid sequence that is at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% identical to GenBank Accession Nos. NP_036370, NP_501912, NP_085096, NP_036369, or P53685, and functional fragments thereof.
15 Polypeptides of the invention also include homologs (e.g., orthologs and paralogs), variants, or fragments, of GenBank Accession Nos. NP_036370, NP_501912, NP_085096, NP_036369, or P53685.

“Sirtuin-activating compound” refers to a compound that increases the level of a sirtuin protein and/or increases at least one activity of a sirtuin protein. In an
20 exemplary embodiment, a sirtuin-activating compound may increase at least one biological activity of a sirtuin protein by at least about 10%, 25%, 50%, 75%, 100%, or more. Exemplary biological activities of sirtuin proteins include deacetylation, e.g., of histones and p53; extending lifespan; increasing genomic stability; silencing transcription; and controlling the segregation of oxidized
25 proteins between mother and daughter cells. Examples of sirtuin-activating compounds include, for example, resveratrol, butein, fisetin, piceatannol, quercetin, nicotinamide riboside, and derivatives of the foregoing, as well as the sirtuin-activating compounds described in U.S. Patent Publication No. 2005/0136537. Additional sirtuin-activating compounds can be found in U.S. Patent Publication
30 Nos. 2006/0229265, 2007/0014833, 2007/0149466, 2007/0037810, 2007/0037809, 2006/0292099, 2007/0037827, 2007/0043050, 2007/0037865 and 2008/0249103 and PCT Publication Nos. WO 2006/078941, WO 2006/094237, WO 2006/094235,

WO 2006/094248, WO 2006/094210, WO 2006/094209, WO 2006/094246 WO 2006/094233, WO 2008/060400, WO 2008/073451 and WO 2008/100376. In an exemplary embodiment, a sirtuin-activating compound has no substantial modulating activity for a CLK protein.

5 The terms “systemic administration,” “administered systemically,” “peripheral administration” and “administered peripherally” are art-recognized and refer to the administration of a subject composition, therapeutic or other material other than directly into the central nervous system, such that it enters the patient’s system and, thus, is subject to metabolism and other like processes.

10 The term “therapeutic agent” is art-recognized and refers to any chemical moiety that is a biologically, physiologically, or pharmacologically active substance that acts locally or systemically in a subject. The term also means any substance intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease or in the enhancement of desirable physical or mental development and/or
15 conditions in an animal or human.

 The term “therapeutic effect” is art-recognized and refers to a local or systemic effect in animals, particularly mammals, and more particularly humans caused by a pharmacologically active substance. The phrase “therapeutically-effective amount” means that amount of such a substance that produces some
20 desired local or systemic effect at a reasonable benefit/risk ratio applicable to any treatment. The therapeutically effective amount of such substance will vary depending upon the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art. For
25 example, certain compositions described herein may be administered in a sufficient amount to produce a desired effect at a reasonable benefit/risk ratio applicable to such treatment.

 “Treating” a condition or disease refers to curing as well as ameliorating at least one symptom of the condition or disease.

30 The term “vision impairment” refers to diminished vision, which is often only partially reversible or irreversible upon treatment (e.g., surgery). Particularly severe vision impairment is termed “blindness” or “vision loss”, which refers to a

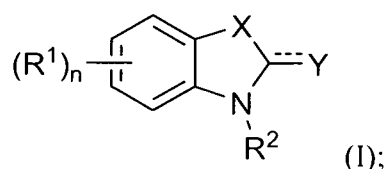
complete loss of vision, vision worse than 20/200 that cannot be improved with corrective lenses, or a visual field of less than 20 degrees diameter (10 degrees radius).

Other technical terms used herein have their ordinary meaning in the art that they are used, as exemplified by a variety of technical dictionaries, such as the McGraw-Hill Dictionary of Chemical Terms and the Stedman's Medical Dictionary.

2. CLK Inhibitors

In one aspect, the invention provides novel CLK-inhibiting compounds for treating and/or preventing a wide variety of diseases and disorders including, for example, diseases or disorders related to aging or stress, diabetes, obesity, neurodegenerative diseases, ocular diseases and disorders, cardiovascular disease, blood clotting disorders, inflammation, and/or flushing, etc. CLK-inhibiting compounds may also be used for treating a disease or disorder in a subject that would benefit from increased mitochondrial activity, for enhancing muscle performance, for increasing muscle ATP levels, or for treating or preventing muscle tissue damage associated with hypoxia or ischemia. Other compounds disclosed herein may be suitable for use in a pharmaceutical composition and/or one or more methods disclosed herein.

In one embodiment, CLK-inhibiting compounds of the invention are represented by Formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

X is selected from S or O;

Y is selected from $-N(R^3)-C(O)-R^5$ (e.g., $-NH-C(O)-R^5$), $=C(R^3)-C(O)-N(R^4)-R^5$, $=C(R^3)-C(O)-C(CH_3)_3$, $=C(R^3)-C(O)-CH(R^7)(R^7)$, $=C(R^3)-C(=NOH)-R^5$, and $=C(R^3)-CH(OH)-R^5$, and when X is O, Y is additionally selected from $=C(R^3)$ -carbocyclic and $=C(R^3)$ -heterocyclic;

each R^1 is independently selected from $-OH$, halo, $-R^6$, $-OR^6$, $-SR^6$, $-NH_2$, $-NH(R^6)$, $-N(R^6)(R^6)$, $-N(R^3)C(O)R^6$, $-N(R^3)C(S)R^6$ and $-SO_2N(R^6)(R^6)$;

R^2 is selected from $-(C_1-C_5\text{-alkyl})$, $-(C_1-C_3\text{-alkyl})\text{-aryl}$,
 $-(C_1-C_3\text{-alkyl})\text{-heteroaryl}$, $-(C_1-C_3\text{-alkyl})\text{-NH}(R^6)$, and $-(C_1-C_3\text{-alkyl})\text{-N}(R^6)(R^6)$;
 each R^3 is independently selected from hydrogen and optionally substituted
 $-(C_1-C_4\text{-alkyl})$;

5 R^4 is selected from hydrogen and optionally substituted $-(C_1-C_4\text{-alkyl})$;

R^5 is selected from hydrogen, $-(C_1-C_4\text{-alkyl})$, aryl, $-(C_1-C_4\text{-alkyl})\text{-aryl}$,
 heteroaryl, and $-(C_1-C_4\text{-alkyl})\text{-heteroaryl}$ wherein the alkyl, aryl and heteroaryl is
 optionally substituted;

each R^6 is independently selected from C_1-C_6 alkyl, C_2-C_6 alkenyl or alkynyl,
 10 carbocyclyl, and heterocyclyl, or wherein two R^6 together with the nitrogen atom to
 which they are bound are taken together to form an optionally substituted
 heterocyclyl; and wherein each R^6 is optionally and independently substituted;

each R^7 is independently selected from hydrogen, $-(C_1-C_4\text{-alkyl})$,
 $-(C_2-C_4\text{-alkenyl})$, $-(C_2-C_4\text{-alkynyl})$, carbocyclyl, $-(C_1-C_4\text{-alkyl})\text{-carbocyclyl}$,
 15 heterocyclyl, and $-(C_1-C_4\text{-alkyl})\text{-heterocyclyl}$, wherein the alkyl, alkenyl, alkynyl,
 carbocyclyl and heterocyclyl are optionally substituted

n is an integer from 1 to 4; and

--- represents a single or a double bond.

In certain embodiments, when X is O, R^2 is ethyl, Y is $=CH-C(O)-CH_2CH_3$,
 20 and n is 1, then R^1 is not methoxy.

In certain embodiments, when X is S, Y is $=CH-C(O)-C(CH_3)_3$, or
 $=CH-C(O)-CH(R^7)(R^7)$; each R^7 is independently selected from hydrogen,
 unsubstituted $-(C_1-C_4\text{-alkyl})$, unsubstituted carbocyclyl, and unsubstituted
 $-(C_1-C_4\text{-alkyl})\text{-carbocyclyl}$; n is 1 or 2; each R^1 is independently selected from halo,
 25 $-R^6$ and $-OR^6$; each R^6 is independently selected from C_1-C_6 straight or branched
 alkyl, C_2-C_6 straight or branched alkenyl or alkynyl, a carbocyclic moiety, and a
 heterocyclic moiety consisting of carbon, hydrogen and oxygen atoms; and each R^6
 is optionally substituted with one or more substituents selected from alkyl, O-alkyl
 and $-OH$; then R^2 is not $-(C_2-C_5\text{-alkyl})$, or $-(C_1-C_3\text{-alkyl})\text{-aryl}$. In such certain
 30 embodiments, when X is S, Y is $=CH-C(O)-C(CH_3)_3$, or $=CH-C(O)-CH(R^7)(R^7)$;
 each R^7 is independently selected from hydrogen, unsubstituted $-(C_1-C_4\text{-alkyl})$,
 unsubstituted carbocyclyl, and unsubstituted $-(C_1-C_4\text{-alkyl})\text{-carbocyclyl}$; n is 1 or 2;

each R^1 is independently selected from halo, $-R^6$ and $-OR^6$; each R^6 is independently selected from C_1 - C_6 straight or branched alkyl, C_2 - C_6 straight or branched alkenyl or alkynyl, a carbocyclic moiety, and a heterocyclic moiety consisting of carbon, hydrogen and oxygen atoms; and each R^6 is optionally substituted with one or more
 5 substituents selected from alkyl, O-alkyl and $-OH$; then R^2 is not $-(C_1-C_5\text{-alkyl})$, or $-(C_1-C_3\text{-alkyl})\text{-aryl}$.

In certain embodiments, when X is S, Y is $=CH-C(O)-CH_3$, R^2 is $-ethyl$, n is 2, and one R^1 is methoxy; then the other R^1 is not NH_2 or when one R^1 is $-OH$, then the other R^1 is not $-CH_2-N(CH_3)_2$.

10 In certain embodiments, when X is S, Y is $=CH-C(O)-CH_3$, R^2 is $ethyl$, and n is 1; then R^1 is not $-OH$.

In certain embodiments, when X is S, Y is $=CH-C(O)-CH_2\text{-tetrahydrofuran-2-yl}$, R^2 is $ethyl$, and n is 1; then R^1 is not methyl or methoxy.

In certain embodiments, when X is S, Y is $=CH-C(O)-N(CH_3)-R^5$, and R^2 is
 15 methyl; then R^5 is not $-CH_2\text{-(1-methyl-1H-indol-2-yl)}$.

In certain embodiments, when X is S, Y is $=CH-C(O)-N(H)\text{-(4-dimethylaminophenyl)}$, R^2 is $ethyl$, and n is 1; then R^1 is not $-S\text{-methyl}$.

In certain embodiments, R^1 is selected from $-NH(R^6)$, $-N(R^6)(R^6)$ and a heterocyclic moiety comprising one or more heteroatoms selected from N and S.

20 Exemplary values of R^1 include $-N(R^6)(R^6)$, pyridyl, $-N(R^3)C(O)R^6$, $-SO_2N(R^6)(R^6)$ and $-N(R^3)C(S)R^6$.

In certain embodiments, R^2 is selected from $-(C_1-C_5\text{-alkyl})$, $-(C_1-C_3\text{-alkyl})\text{-aryl}$, $-(C_1-C_3\text{-alkyl})\text{-NH}(R^6)$, and $-(C_1-C_3\text{-alkyl})\text{-N}(R^6)(R^6)$. In certain such embodiments, $-(C_1-C_3\text{-alkyl})\text{-aryl}$ is $-(C_1-C_3\text{-alkyl})\text{-phenyl}$. In other such
 25 embodiments, R^2 is selected from $-(C_1-C_3\text{-alkyl})\text{-aryl}$, $-(C_1-C_3\text{-alkyl})\text{-NH}(R^6)$, and $-(C_1-C_3\text{-alkyl})\text{-N}(R^6)(R^6)$, such as $-(C_1-C_3\text{-alkyl})\text{-NH}(R^6)$ and $-(C_1-C_3\text{-alkyl})\text{-N}(R^6)(R^6)$.

Exemplary values of R^2 include aralkyl where the aryl has one or more substituents comprising a heteroatom or a halogen (optionally in addition to other
 30 substituents), $-(C_1-C_3\text{-alkyl})\text{-N}(R^6)(R^6)$, and heterocyclalkyl.

In certain embodiments, a compound of the invention includes one of the specific values of R^1 in combination with one of the specific values of R^2 . For

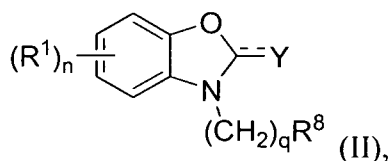
example, a compound may include an R^1 selected from $-NH(R^6)$, $-N(R^6)(R^6)$, a heterocyclic moiety comprising one or more heteroatoms selected from N and S, and $-N(R^3)C(O)R^6$, $-SO_2N(R^6)(R^6)$ and $-N(R^3)C(S)R^6$ and an R^2 selected from $-(C_1-C_5\text{-alkyl})$, $-(C_1-C_3\text{-alkyl})\text{-aryl}$, $-(C_1-C_3\text{-alkyl})\text{-NH}(R^6)$, and $-(C_1-C_3\text{-alkyl})\text{-N}(R^6)(R^6)$. Such combinations of R^1 and R^2 may be present in conjunction with the values of Y described both above and below.

In certain embodiments, Y is selected from $-NH-C(O)-R^5$, $=C(R^3)-C(=NOH)-R^5$ and $=C(R^3)-CH(OH)-R^5$, and when X is O, Y is additionally selected from $=C(R^3)\text{-carbocyclic}$ and $=C(R^3)\text{-heterocyclic}$.

In certain embodiments, Y is $=C(R^3)-C(O)-NR^4R^5$; R^5 is selected from hydrogen, $-(C_1-C_4\text{-alkyl})$, aryl, $-(C_1-C_4\text{-alkyl})\text{-aryl}$, heteroaryl, and $-(C_2-C_4\text{-alkyl})\text{-heteroaryl}$, wherein the alkyl, aryl and heteroaryl are optionally substituted; and each R^1 is independently selected from $-OH$, halo, $-R^6$, $-OR^6$, and $-NH_2$.

In embodiments where $-N(R^6)(R^6)$ moieties form an optionally substituted heterocyclyl, the heterocyclyl group optionally contains one or more additional heteroatoms (e.g., N, O).

In certain embodiments, CLK-inhibiting compounds of the invention are represented by Formula (II):



or a pharmaceutically acceptable salt thereof, wherein:

Y is selected from $-NH-C(O)-R^5$, $=C(R^3)-C(O)-N(R^4)-R^5$, $=C(R^3)-C(O)-C(CH_3)_3$, $=C(R^3)-C(O)-CH(R^7)(R^7)$, $=C(R^3)-C(=NOH)-R^5$, $=C(R^3)-CH(OH)-R^5$, $=C(R^3)\text{-carbocyclic}$ and $=C(R^3)\text{-heterocyclic}$;

each R^1 is independently selected from $-OH$, halo, $-R^6$, $-OR^6$, $-SR^6$, $-NH_2$, $-NH(R^6)$, $-N(R^6)(R^6)$, $-N(R^3)C(O)R^6$, $-N(R^3)C(S)R^6$ and $-SO_2N(R^6)(R^6)$;

each R^3 is independently selected from hydrogen and optionally substituted $-(C_1-C_4\text{-alkyl})$;

R^4 is selected from hydrogen and optionally substituted-(C₁-C₄-alkyl);

R^5 is selected from hydrogen, -(C₁-C₄-alkyl), aryl, -(C₁-C₄-alkyl)-aryl, heteroaryl, and -(C₁-C₄-alkyl)-heteroaryl wherein the alkyl, aryl and heteroaryl is optionally substituted;

each R^6 is independently selected from C₁-C₆ alkyl, C₂-C₆ alkenyl or alkynyl, carbocyclyl, and heterocyclyl, or wherein two R^6 together with the nitrogen atom to which they are bound are taken together to form an optionally substituted heterocyclyl; and wherein each R^6 is optionally and independently substituted;

each R^7 is independently selected from hydrogen, -(C₁-C₄-alkyl), -(C₂-C₄-alkenyl), -(C₂-C₄-alkynyl), carbocyclyl, -(C₁-C₄-alkyl)-carbocyclyl, heterocyclyl, and -(C₁-C₄-alkyl)-heterocyclyl, wherein the alkyl, alkenyl, alkynyl, carbocyclyl and heterocyclyl are optionally substituted

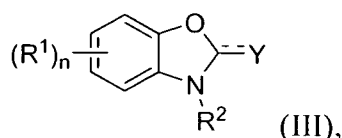
R^8 is selected from -N(R^6)(R^6), carbocycle, and heterocycle, wherein carbocycle and heterocycle are optionally substituted;

n is an integer from 1 to 4;

q is an integer from 1 to 3; and

--- represents a single or a double bond.

In certain embodiments, CLK-inhibiting compounds of the invention are represented by Formula (III):



or a pharmaceutically acceptable salt thereof, wherein:

Y is selected from -NH-C(O)- R^5 , =C(R^3)-C(O)-N(R^4)- R^5 , =C(R^3)-C(O)-C(CH₃)₃, =C(R^3)-C(O)-CH(R^7)(R^7), =C(R^3)-C(=NOH)- R^5 , =C(R^3)-CH(OH)- R^5 , =C(R^3)-carbocyclic and =C(R^3)-heterocyclic;

each R^1 is independently selected from -OH, halo, - R^6 , -OR⁶, -SR⁶, -NH₂, -NH(R^6), -N(R^6)(R^6), -N(R^3)C(O)R⁶, -N(R^3)C(S)R⁶ and -SO₂N(R^6)(R^6);

R^2 is selected from $-(C_1-C_5\text{-alkyl})$, $-(C_1-C_3\text{-alkyl})\text{-aryl}$, $-(C_1-C_3\text{-alkyl})\text{-NH}(R^6)$, and $-(C_1-C_3\text{-alkyl})\text{-N}(R^6)(R^6)$;

each R^3 is independently selected from hydrogen and optionally substituted $-(C_1-C_4\text{-alkyl})$;

5 R^4 is selected from hydrogen and optionally substituted $-(C_1-C_4\text{-alkyl})$;

R^5 is selected from hydrogen, $-(C_1-C_4\text{-alkyl})$, aryl, $-(C_1-C_4\text{-alkyl})\text{-aryl}$, heteroaryl, and $-(C_1-C_4\text{-alkyl})\text{-heteroaryl}$ wherein the alkyl, aryl and heteroaryl is optionally substituted;

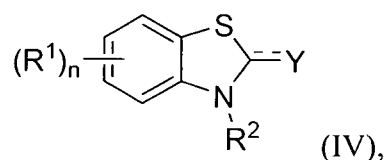
10 each R^6 is independently selected from C_1-C_6 alkyl, C_2-C_6 alkenyl or alkynyl, carbocyclyl, and heterocyclyl, or wherein two R^6 together with the nitrogen atom to which they are bound are taken together to form an optionally substituted heterocyclyl; and wherein each R^6 is optionally and independently substituted;

15 each R^7 is independently selected from hydrogen, $-(C_2-C_4\text{-alkyl})$, $-(C_2-C_4\text{-alkenyl})$, $-(C_3-C_4\text{-alkynyl})$, carbocyclyl, $-(C_1-C_4\text{-alkyl})\text{-carbocyclyl}$, heterocyclyl, and $-(C_1-C_4\text{-alkyl})\text{-heterocyclyl}$, wherein the alkyl, alkenyl, alkynyl, carbocyclyl and heterocyclyl are optionally substituted;

n is an integer from 1 to 4; and

20 --- represents a single or a double bond.

In certain embodiments, CLK-inhibiting compounds of the invention are represented by Formula (IV):



or a pharmaceutically acceptable salt thereof, wherein:

25 Y is selected from $-\text{NH}-\text{C}(\text{O})-\text{R}^5$, $-\text{C}(\text{R}^3)=\text{C}(\text{R}^7)(\text{R}^7)$, $=\text{C}(\text{R}^3)-\text{C}(\text{O})-\text{O}(\text{R}^3)$, $=\text{C}(\text{R}^3)-\text{C}(\text{O})-\text{N}(\text{R}^4)-\text{R}^5$, $=\text{C}(\text{R}^3)-\text{C}(=\text{NOH})-\text{R}^5$, and $=\text{C}(\text{R}^3)-\text{CH}(\text{OH})-\text{R}^5$;

each R^1 is independently selected from $-\text{OH}$, halo, $-\text{R}^6$, $-\text{OR}^6$, $-\text{NH}_2$, $-\text{NH}(\text{R}^6)$, $-\text{N}(\text{R}^6)(\text{R}^6)$, $-\text{N}(\text{R}^3)\text{C}(\text{O})\text{R}^6$, $-\text{N}(\text{R}^3)\text{C}(\text{S})\text{R}^6$ and $-\text{SO}_2\text{N}(\text{R}^6)(\text{R}^6)$;

R^2 is selected from $-(C_1-C_5\text{-alkyl})$, $-(C_1-C_3\text{-alkyl})\text{-aryl}$,
 $-(C_1-C_3\text{-alkyl})\text{-NH}(R^6)$, and $-(C_1-C_3\text{-alkyl})\text{-N}(R^6)(R^6)$;

each R^3 is independently selected from hydrogen and optionally
substituted $-(C_1-C_4\text{-alkyl})$;

5 R^4 is selected from hydrogen and optionally
substituted $-(C_1-C_4\text{-alkyl})$;

R^5 is selected from hydrogen, $-(C_1-C_4\text{-alkyl})$, aryl,
 $-(C_1-C_4\text{-alkyl})\text{-aryl}$, heteroaryl, and $-(C_2-C_4\text{-alkyl})\text{-heteroaryl}$ wherein the
aryl, aryl and heteroaryl is optionally substituted;

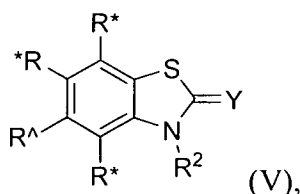
10 each R^6 is independently selected from C_1-C_6 alkyl, C_2-C_6 alkenyl or
alkynyl, carbocyclyl, and heterocyclyl, or wherein two R^6 together with the
nitrogen atom to which they are bound are taken together to form an
optionally substituted heterocyclyl; and wherein each R^6 is optionally and
independently substituted;

15 each R^7 is independently selected from hydrogen, $-(C_1-C_4\text{-alkyl})$,
 $-(C_2-C_4\text{-alkenyl})$, $-(C_2-C_4\text{-alkynyl})$, carbocyclyl, $-(C_1-C_4\text{-alkyl})\text{-carbocyclyl}$,
heterocyclyl, and $-(C_1-C_4\text{-alkyl})\text{-heterocyclyl}$, wherein the alkyl, alkenyl,
alkynyl, carbocyclyl and heterocyclyl are optionally substituted

n is an integer from 1 to 4; and

20 $---$ represents a single or a double bond.

In certain embodiments, CLK-inhibiting compounds of the invention are
represented by Formula (V):



or a pharmaceutically acceptable salt thereof, wherein:

25 Y is selected from $=C(R^3)\text{-C(O)-C(CH}_3)_3$ and
 $=C(R^3)\text{-C(O)-CH}(R^7)(R^7)$;

each R^* at each occurrence is independently selected from OH , $-$
 CH_3 , $-\text{CF}_3$, $-\text{OCH}_3$, $-\text{OCH}_2\text{CH}_3$, $-\text{O}(\text{C}=\text{O})\text{CH}_3$, pyridyl, morpholinyl,
pyrrolidinyl, piperidinyl, $-\text{NH}(R^6)$, $-\text{N}(R^6)(R^6)$, $-\text{N}(R^3)\text{C(O)}R^6$,

-N(R³)C(S)R⁶, and -SO₂N(R⁶)(R⁶); wherein when R* is selected from pyridyl, morpholinyl, pyrrolidinyl or piperidinyl, R* is optionally substituted; and

5 R[^] is selected from, pyridyl, morpholinyl, pyrrolidinyl, piperidinyl, -NH(R⁶), -N(R⁶)(R⁶), -N(R³)C(O)R⁶, -N(R³)C(S)R⁶, and -SO₂N(R⁶)(R⁶); wherein when R* is selected from pyridyl, morpholinyl, pyrrolidinyl or piperidinyl, R* is optionally substituted;

R² is selected from -(C₁-C₅-alkyl), -(C₁-C₃-alkyl)-aryl, -(C₁-C₃-alkyl)-NH(R⁶), and -(C₁-C₃-alkyl)-N(R⁶)(R⁶);
10 each R³ is independently selected from hydrogen and optionally substituted -(C₁-C₄-alkyl);

R⁴ is selected from hydrogen and optionally substituted-(C₁-C₄-alkyl);

R⁵ is selected from hydrogen, -(C₁-C₄-alkyl), aryl, 15 -(C₁-C₄-alkyl)-aryl, heteroaryl, and -(C₁-C₄-alkyl)-heteroaryl wherein the alkyl, aryl and heteroaryl is optionally substituted;

each R⁶ is independently selected from C₁-C₆ alkyl, C₂-C₆ alkenyl or alkynyl, carbocyclyl, and heterocyclyl, or wherein two R⁶ together with the nitrogen atom to which they are bound are taken together to form an 20 optionally substituted heterocyclyl; and wherein each R⁶ is optionally and independently substituted;

each R⁷ is independently selected from hydrogen, -(C₁-C₄-alkyl), -(C₂-C₄-alkenyl), -(C₂-C₄-alkynyl), carbocyclyl, -(C₁-C₄-alkyl)-carbocyclyl, heterocyclyl, and -(C₁-C₄-alkyl)-heterocyclyl, wherein the alkyl, alkenyl, 25 alkynyl, carbocyclyl and heterocyclyl are optionally substituted; and

n is an integer from 1 to 4.

Unless otherwise specified, alkyl, alkenyl, alkynyl and all cyclic (including heterocyclic) groups are substituted. Suitable substituents for "optionally substituted" moieties are discussed below or directly in the claims or description of 30 the moiety.

An alkyl group is a straight chained or branched non-aromatic hydrocarbon which is completely saturated. Typically, a straight chained or branched alkyl group

has from 1 to about 20 carbon atoms, preferably from 1 to about 10. Examples of straight chained and branched alkyl groups include methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, tert-butyl, pentyl, hexyl, pentyl and octyl. A C₁-C₄ straight chained or branched alkyl group is also referred to as a "lower alkyl" group.

5 An alkenyl group is a straight chained or branched non-aromatic hydrocarbon which contains one or more double bonds. Typically, the double bonds are not located at the terminus of the alkenyl group, such that the double bond is not adjacent to another functional group.

10 An alkynyl group is a straight chained or branched non-aromatic hydrocarbon which contains one or more triple bonds. Typically, the triple bonds are not located at the terminus of the alkynyl group, such that the triple bond is not adjacent to another functional group.

A carbocyclic group is a monocyclic or polycyclic ring system that contains only carbon atoms.

15 Aromatic (aryl) groups are fully carbocyclic aromatic groups having one or more rings such as phenyl, naphthyl, and anthracyl. The terms "aryl" and "fully aromatic carbocyclic" are used interchangeably in this application.

20 Heteroaryl groups consist of one or more rings, where each ring is aromatic and at least one ring includes at least one heteroatom. Examples include imidazolyl, thienyl, furyl, pyridyl, pyrimidyl, pyranyl, pyrazolyl, pyrrolyl, pyrazinyl, thiazolyl, oxazolyl, and tetrazolyl.

25 Heteroaryl groups also include fused polycyclic aromatic ring systems in which a carbocyclic aromatic ring or heteroaryl ring is fused to one or more other heteroaryl rings. Examples include benzothienyl, benzofuryl, indolyl, quinolinyl, benzothiazole, benzoxazole, benzimidazole, quinolinyl, isoquinolinyl and isoindolyl. The terms "heteroaryl" and "fully aromatic heterocyclyl" are used interchangeably in this application.

30 Non-aromatic heterocyclic groups consist of one or more rings, where each ring is non-aromatic and at least rings contains one or more heteroatoms such as nitrogen, oxygen or sulfur. Non-aromatic heterocyclic group include fully saturated ring systems and ring systems having one or more degrees of unsaturation, provided that the ring system does not have aromatic character. Each ring in the ring system

can be five, six, seven or eight-membered. Examples include tetrahydrofuryl, tetrahydrothiophenyl, dihydropyridine, dihydropyran, thiopyran, hexahydrochromene, hexahydroquinoline, tetrahydroquinone, morpholino, thiomorpholino, pyrrolidinyl, piperazinyl, oxabicyclooctane, oxabicycloheptane, and thiabicyclooctane, azaspirodecane, piperidinyl, and thiazolidinyl, along with the cyclic form of sugars.

Non-aromatic carbocyclic groups consist of one or more rings, where each ring is non-aromatic and each ring atom is a carbon atom. Non-aromatic carbocyclic groups include fully saturated ring systems and ring systems having one or more degrees of unsaturation. Each ring in a non-aromatic heterocyclic group ring can be five, six, seven or eight-membered. Examples include cyclohexadiene, cyclohexene, cyclohexane, cyclopentane, cyclooctane, cyclopentadiene, hexahydronaphthalene, octahydroindene, bicycloheptane, bicyclooctane, spirodecane and spirononadiene.

Suitable substituents on an alkyl, alkenyl, alkynyl, aryl, non-aromatic heterocyclic or partially or fully aryl group (carbocyclic and heteroaryl) are those which do not substantially interfere with the ability of the disclosed compounds to have one or more of the properties disclosed herein. A substituent substantially interferes with the properties of a compound when the magnitude of the property is reduced by more than about 50% in a compound with the substituent compared with a compound without the substituent. Examples of suitable substituents include -OH, halogen (-Br, -Cl, -I and -F), -OR^a, -SH, -SR^a, -O-COR^a, -COR^a, -C(O)R^a, -COOH, -COOR^a, -OCO₂R^a, -C(O)NR^aR^b, -OC(O)NR^aR^b, -SO₃H, -NH₂, -NHR^a, -N(R^aR^b), -COOR^a, -CHO, -CONH₂, -CONHR^a, -CON(R^aR^b), -NHCOR^a, -NRCOR^a, -NHCONH₂, -NHCONR^aH, -NHCON(R^aR^b), -NR^cCONH₂, -NR^cCONR^aH, -NR^cCON(R^aR^b), -C(=NH)-NH₂, -C(=NH)-NHR^a, -C(=NH)-N(R^aR^b), -C(=NR^c)-NH₂, -C(=NR^c)-NHR^a, -C(=NR^c)-N(R^aR^b), -NH-C(=NH)-NH₂, -NH-C(=NH)-NHR^a, -NH-C(=NH)-N(R^aR^b), -NH-C(=NR^c)-NH₂, -NH-C(=NR^c)-NHR^a, -NH-C(=NR^c)-N(R^aR^b), -NR^dH-C(=NH)-NH₂, -NR^d-C(=NH)-NHR^a, -NR^d-C(=NH)-N(R^aR^b), -NR^d-C(=NR^c)-NH₂, -NR^d-C(=NR^c)-NHR^a, -NR^d-C(=NR^c)-N(R^aR^b), -NHNH₂, -NHNHR^a, -NHR^aR^b, -SO₂NH₂, -SO₂NHR^a, -SO₂NR^aR^b, -CH=CHR^a, -CH=CR^aR^b, -CR^c=CR^aR^b, CR^c=CHR^a, -CR^c=CR^aR^b, -CCR^a, -SO_kR^a (k is 0, 1 or 2), -S(O)_kOR^a (k is 0, 1 or 2) and -NH-C(=NH)-NH₂. R^a-R^d are each independently an aliphatic, substituted aliphatic, benzyl, substituted

benzyl, aromatic or substituted aromatic group, preferably an alkyl, benzylic or aryl group. In addition, $-NR^aR^b$, taken together, can also form a substituted or unsubstituted non-aromatic heterocyclic group. A cyclic group can also have a linear or branched alkyl, alkenyl or alkynyl group as a substituent. A substituted
5 group can have more than one substituent, unless otherwise specified.

Combinations of substituents and variables envisioned by this invention are only those that result in the formation of stable compounds. As used herein, the term "stable" refers to compounds that possess stability sufficient to allow manufacture and that maintain the integrity of the compound for a sufficient period
10 of time to be useful for the purposes detailed herein.

Also included in the present invention are salts, particularly pharmaceutically acceptable salts, of the compounds described herein. The compounds of the present invention that possess a sufficiently acidic, a sufficiently basic, or both functional groups, can react with any of a number of inorganic bases, and inorganic and
15 organic acids, to form a salt. Alternatively, compounds that are inherently charged, such as those with a quaternary nitrogen, can form a salt with an appropriate counterion (e.g., a halide such as bromide, chloride, or fluoride, particularly bromide).

Acids commonly employed to form acid addition salts are inorganic acids
20 such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, phosphoric acid, and the like, and organic acids such as p-toluenesulfonic acid, methanesulfonic acid, oxalic acid, p-bromophenyl-sulfonic acid, carbonic acid, succinic acid, citric acid, benzoic acid, acetic acid, and the like. Examples of such salts include the sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate,
25 monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, isobutyrate, caproate, heptanoate, propiolate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, butyne-1,4-dioate, hexyne-1,6-dioate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, hydroxybenzoate,
30 methoxybenzoate, phthalate, sulfonate, xylenesulfonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, gamma-hydroxybutyrate,

glycolate, tartrate, methanesulfonate, propanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, mandelate, and the like.

Base addition salts include those derived from inorganic bases, such as ammonium or alkali or alkaline earth metal hydroxides, carbonates, bicarbonates, and the like. Such bases useful in preparing the salts of this invention thus include sodium hydroxide, potassium hydroxide, ammonium hydroxide, potassium carbonate, and the like.

According to another embodiment, the present invention provides methods of producing the above-defined compounds. The compounds may be synthesized using conventional techniques. Advantageously, these compounds are conveniently synthesized from readily available starting materials.

3. Exemplary Uses

In certain embodiments, the invention provides methods for using CLK-inhibiting compounds. CLK-inhibiting compounds may be useful for a variety of therapeutic applications including, for example, increasing the lifespan of a cell, and treating and/or preventing a wide variety of diseases and disorders including, for example, diseases or disorders related to aging or stress, diabetes, obesity, neurodegenerative diseases, cardiovascular disease, blood clotting disorders, inflammation, cancer, and/or flushing, etc. CLK-inhibiting compounds may also be used for treating a disease or disorder in a subject that would benefit from increased mitochondrial activity, for enhancing muscle performance, for increasing muscle ATP levels, or for treating or preventing muscle tissue damage associated with hypoxia or ischemia. The methods comprise administering to a subject in need thereof a pharmaceutically effective amount of a CLK-inhibiting compound.

In certain embodiments, a CLK-inhibiting compound described herein may be taken alone or in combination with other compounds. In one embodiment, a mixture of two or more CLK-inhibiting compounds may be administered to a subject in need thereof. In another embodiment, a CLK-inhibiting compound may be administered with one or more sirtuin-activating compounds. Exemplary sirtuin-activating compounds include, for example, resveratrol, butein, fisetin, piceatannol, quercetin, nicotinamide riboside, and derivatives or analogs of the foregoing as well as the sirtuin-activating compounds described in U.S. Patent Publication No.

2005/0136537. In an exemplary embodiment, a CLK-inhibiting compound may be administered in combination with nicotinic acid. In yet another embodiment, one or more CLK-inhibiting compounds may be administered with one or more therapeutic agents for the treatment or prevention of various diseases, including, for example, diabetes, neurodegenerative diseases, cardiovascular disease, blood clotting, inflammation, flushing, obesity, ageing, stress, ocular disorders, etc. In another embodiment, a CLK-inhibiting compound may be administered with one or more agents that promote mitochondrial biogenesis, mitochondrial activity, and/or muscle performance. In various embodiments, combination therapies comprising a CLK-inhibiting compound may refer to (1) pharmaceutical compositions that comprise one or more CLK-inhibiting compounds in combination with one or more therapeutic agents; and (2) co-administration of one or more CLK-inhibiting compounds with one or more therapeutic agents wherein the CLK-inhibiting compound and therapeutic agent have not been formulated in the same compositions. When using separate formulations, the CLK-inhibiting compound may be administered at the same time, intermittent, staggered, prior to, subsequent to, or combinations thereof, with the administration of another therapeutic agent.

i. Aging/Stress

In one embodiment, the invention provides a method extending the lifespan of a cell, extending the proliferative capacity of a cell, slowing aging of a cell, promoting the survival of a cell, delaying cellular senescence in a cell, mimicking the effects of calorie restriction, increasing the resistance of a cell to stress, or preventing apoptosis of a cell, by contacting the cell with a CLK-inhibiting compound.

The methods described herein may be used to increase the amount of time that cells, particularly primary cells (i.e., cells obtained from an organism, e.g., a human), may be kept alive in a cell culture. Embryonic stem (ES) cells and pluripotent cells, and cells differentiated therefrom, may also be treated with a CLK-inhibiting compound to keep the cells, or progeny thereof, in culture for longer periods of time. Such cells can also be used for transplantation into a subject, e.g., after *ex vivo* modification.

In one embodiment, cells that are intended to be preserved for long periods of time may be treated with a CLK-inhibiting compound. The cells may be in suspension (e.g., blood cells, serum, biological growth media, etc.) or in tissues or organs. For example, blood collected from an individual for purposes of
5 transfusion may be treated with a CLK-inhibiting compound to preserve the blood cells for longer periods of time. Additionally, blood to be used for forensic purposes may also be preserved using a CLK-inhibiting compound. Other cells that may be treated to extend their lifespan or protect against apoptosis include cells for consumption, e.g., cells from non-human mammals (such as meat) or plant cells
10 (such as vegetables).

CLK-inhibiting compounds may also be applied during developmental and growth phases in mammals, plants, insects or microorganisms, in order to alter, retard or accelerate the developmental and/or growth process.

In another embodiment, a CLK-inhibiting compound may be used to treat
15 cells useful for transplantation or cell therapy, including, for example, solid tissue grafts, organ transplants, cell suspensions, stem cells, bone marrow cells, etc. The cells or tissue may be an autograft, an allograft, a syngraft or a xenograft. The cells or tissue may be treated with the CLK-inhibiting compound prior to administration/implantation, concurrently with administration/implantation, and/or
20 post administration/implantation into a subject. The cells or tissue may be treated prior to removal of the cells from the donor individual, *ex vivo* after removal of the cells or tissue from the donor individual, or post implantation into the recipient. For example, the donor or recipient individual may be treated systemically with a CLK-inhibiting compound or may have a subset of cells/tissue treated locally with
25 a CLK-inhibiting compound. In certain embodiments, the cells or tissue (or donor/recipient individuals) may additionally be treated with another therapeutic agent useful for prolonging graft survival, such as, for example, an immunosuppressive agent, a cytokine, an angiogenic factor, etc.

In yet other embodiments, cells may be treated with a CLK-inhibiting
30 compound *in vivo*, e.g., to increase their lifespan or prevent apoptosis. For example, skin can be protected from aging (e.g., developing wrinkles, loss of elasticity, etc.) by treating skin or epithelial cells with a CLK-inhibiting compound.

In an exemplary embodiment, skin is contacted with a pharmaceutical or cosmetic composition comprising a CLK-inhibiting compound. Exemplary skin afflictions or skin conditions that may be treated in accordance with the methods described herein include disorders or diseases associated with or caused by inflammation, sun
5 damage or natural aging. For example, compositions comprising a CLK-inhibiting compound find utility in the prevention or treatment of contact dermatitis (including irritant contact dermatitis and allergic contact dermatitis), atopic dermatitis (also known as allergic eczema), actinic keratosis, keratinization disorders (including eczema), epidermolysis bullosa diseases (including penfigus),
10 exfoliative dermatitis, seborrheic dermatitis, erythemas (including erythema multiforme and erythema nodosum), damage caused by the sun or other light sources, discoid lupus erythematosus, dermatomyositis, psoriasis, skin cancer and the effects of natural aging. In another embodiment, a CLK-inhibiting compound may be used for the treatment of wounds and/or burns to promote healing,
15 including, for example, first-, second- or third-degree burns and/or thermal, chemical or electrical burns. The formulations may be administered topically, to the skin or mucosal tissue, as an ointment, lotion, cream, microemulsion, gel, solution or the like, as further described herein, within the context of a dosing regimen effective to bring about the desired result.

20 Topical formulations comprising one or more CLK-inhibiting compounds may also be used as preventive, e.g., chemopreventive, compositions. When used in a chemopreventive method, susceptible skin is treated prior to any visible condition in a particular individual.

CLK-inhibiting compounds may be delivered locally or systemically to a
25 subject. In one embodiment, a CLK-inhibiting compound is delivered locally to a tissue or organ of a subject by injection, topical formulation, etc.

In another embodiment, a CLK-inhibiting compound may be used for treating or preventing a disease or condition induced or exacerbated by cellular senescence in a subject; methods for decreasing the rate of senescence of a subject,
30 e.g., after onset of senescence; methods for extending the lifespan of a subject; methods for treating or preventing a disease or condition relating to lifespan; methods for treating or preventing a disease or condition relating to the

proliferative capacity of cells; and methods for treating or preventing a disease or condition resulting from cell damage or death. In certain embodiments, the method does not act by decreasing the rate of occurrence of diseases that shorten the lifespan of a subject. In certain embodiments, a method does not act by reducing
5 the lethality caused by a disease, such as cancer.

In yet another embodiment, a CLK-inhibiting compound may be administered to a subject in order to generally increase the lifespan of its cells and to protect its cells against stress and/or against apoptosis. It is believed that treating a subject with a compound described herein is similar to subjecting the subject to
10 hormesis, i.e., mild stress that is beneficial to organisms and may extend their lifespan.

CLK-inhibiting compounds may be administered to a subject to prevent aging and aging-related consequences or diseases, such as stroke, heart disease, heart failure, arthritis, high blood pressure, and Alzheimer's disease. Other
15 conditions that can be treated include ocular disorders, e.g., associated with the aging of the eye, such as cataracts, glaucoma, and macular degeneration. CLK-inhibiting compounds can also be administered to subjects for treatment of diseases, e.g., chronic diseases, associated with cell death, in order to protect the cells from cell death. Exemplary diseases include those associated with neural cell
20 death, neuronal dysfunction, or muscular cell death or dysfunction, such as Parkinson's disease, Alzheimer's disease, multiple sclerosis, amyotrophic lateral sclerosis, and muscular dystrophy; AIDS; fulminant hepatitis; diseases linked to degeneration of the brain, such as Creutzfeldt-Jakob disease, retinitis pigmentosa and cerebellar degeneration; myelodysplasia such as aplastic anemia; ischemic
25 diseases such as myocardial infarction and stroke; hepatic diseases such as alcoholic hepatitis, hepatitis B and hepatitis C; joint-diseases such as osteoarthritis; atherosclerosis; alopecia; damage to the skin due to UV light; lichen planus; atrophy of the skin; cataract; and graft rejections. Cell death can also be caused by surgery, drug therapy, chemical exposure or radiation exposure.

30 CLK-inhibiting compounds can also be administered to a subject suffering from an acute disease, e.g., damage to an organ or tissue, e.g., a subject suffering

from stroke or myocardial infarction or a subject suffering from a spinal cord injury. CLK-inhibiting compound may also be used to repair an alcoholic's liver.

ii. Cardiovascular Disease

In another embodiment, the invention provides a method for treating and/or
5 preventing a cardiovascular disease by administering to a subject in need thereof a CLK-inhibiting compound.

Cardiovascular diseases that can be treated or prevented using a CLK-inhibiting compound include cardiomyopathy or myocarditis; such as idiopathic cardiomyopathy, metabolic cardiomyopathy, alcoholic cardiomyopathy, drug-
10 induced cardiomyopathy, ischemic cardiomyopathy, and hypertensive cardiomyopathy. Also treatable or preventable using the CLK-inhibiting compounds and methods described herein are atheromatous disorders of the major blood vessels (macrovascular disease) such as the aorta, the coronary arteries, the carotid arteries, the cerebrovascular arteries, the renal arteries, the iliac arteries, the
15 femoral arteries, and the popliteal arteries. Other vascular diseases that can be treated or prevented include those related to platelet aggregation, the retinal arterioles, the glomerular arterioles, the vasa nervorum, cardiac arterioles, and associated capillary beds of the eye, the kidney, the heart, and the central and peripheral nervous systems. The CLK-inhibiting compounds may also be used for
20 increasing HDL levels in plasma of an individual.

Yet other disorders that may be treated with CLK-inhibiting compounds include restenosis, e.g., following coronary intervention, and disorders relating to an abnormal level of high density and low density cholesterol.

In one embodiment, a CLK-inhibiting compound may be administered as
25 part of a combination therapeutic with another cardiovascular agent including, for example, an anti-arrhythmic agent, an antihypertensive agent, a calcium channel blocker, a cardioplegic solution, a cardiotonic agent, a fibrinolytic agent, a sclerosing solution, a vasoconstrictor agent, a vasodilator agent, a nitric oxide donor, a potassium channel blocker, a sodium channel blocker, statins, or a natriuretic
30 agent.

In one embodiment, a CLK-inhibiting compound may be administered as part of a combination therapeutic with an anti-arrhythmia agent. Anti-arrhythmia

agents are often organized into four main groups according to their mechanism of action: type I, sodium channel blockade; type II, beta-adrenergic blockade; type III, repolarization prolongation; and type IV, calcium channel blockade. Type I anti-arrhythmic agents include lidocaine, moricizine, mexiletine, tocainide, 5 procainamide, encainide, flecainide, tocainide, phenytoin, propafenone, quinidine, disopyramide, and flecainide. Type II anti-arrhythmic agents include propranolol and esmolol. Type III includes agents that act by prolonging the duration of the action potential, such as amiodarone, artileide, bretylium, clofilium, isobutilide, sotalol, azimilide, dofetilide, dronedarone, ersentilide, ibutilide, tedisamil, and 10 trecetilide. Type IV anti-arrhythmic agents include verapamil, diltiazem, digitalis, adenosine, nickel chloride, and magnesium ions.

In another embodiment, a CLK-inhibiting compound may be administered as part of a combination therapeutic with another cardiovascular agent. Examples of cardiovascular agents include vasodilators, for example, hydralazine; angiotensin 15 converting enzyme inhibitors, for example, captopril; anti-anginal agents, for example, isosorbide nitrate, glyceryl trinitrate and pentaerythritol tetranitrate; anti-arrhythmic agents, for example, quinidine, procainamide and lignocaine; cardioglycosides, for example, digoxin and digitoxin; calcium antagonists, for example, verapamil and nifedipine; diuretics, such as thiazides and related 20 compounds, for example, bendrofluazide, chlorothiazide, chlorothalidone, hydrochlorothiazide and other diuretics, for example, furosemide and triamterene, and sedatives, for example, nitrazepam, flurazepam and diazepam.

Other exemplary cardiovascular agents include, for example, a cyclooxygenase inhibitor such as aspirin or indomethacin, a platelet aggregation 25 inhibitor such as clopidogrel, ticlopidine or aspirin, fibrinogen antagonists or a diuretic such as chlorothiazide, hydrochlorothiazide, flumethiazide, hydroflumethiazide, bendroflumethiazide, methylchlorthiazide, trichloromethiazide, polythiazide or benzthiazide as well as ethacrynic acid, triacrynafene, chlorthalidone, furosemide, bumetanide, triamterene, amiloride and spironolactone and 30 salts of such compounds, angiotensin converting enzyme inhibitors such as captopril, zofenopril, fosinopril, enalapril, ceranopril, cilazapril, delapril, pentopril, quinapril, ramipril, lisinopril, and salts of such compounds, angiotensin II

antagonists such as losartan, irbesartan or valsartan, thrombolytic agents such as tissue plasminogen activator (tPA), recombinant tPA, streptokinase, urokinase, prourokinase, and anisoylated plasminogen streptokinase activator complex (APSAC, Eminase, Beecham Laboratories), or animal salivary gland plasminogen
5 activators, calcium channel blocking agents such as verapamil, nifedipine or diltiazem, thromboxane receptor antagonists such as ifetroban, prostacyclin mimetics, or phosphodiesterase inhibitors. Such combination products if formulated as a fixed dose employ the compounds of this invention within the dose range described above and the other pharmaceutically active agent within its approved
10 dose range.

Yet other exemplary cardiovascular agents include, for example, vasodilators, e.g., bencyclane, cinnarizine, citicoline, cyclandelate, cyclonicate, ebumamonine, phenoxezyl, flunarizine, ibudilast, ifenprodil, lomerizine, naphlole, nikamate, nosergoline, nimodipine, papaverine, pentifylline, nifedipine, vincamin,
15 vinpocetine, vichizyl, pentoxifylline, prostacyclin derivatives (such as prostaglandin E1 and prostaglandin I2), an endothelin receptor blocking drug (such as bosentan), diltiazem, nicorandil, and nitroglycerin. Examples of the cerebral protecting drug include radical scavengers (such as edaravone, vitamin E, and vitamin C), glutamate antagonists, AMPA antagonists, kainate antagonists, NMDA antagonists, GABA
20 agonists, growth factors, opioid antagonists, phosphatidylcholine precursors, serotonin agonists, $\text{Na}^+/\text{Ca}^{2+}$ channel inhibitory drugs, and K^+ channel opening drugs. Examples of the brain metabolic stimulants include amantadine, tiapride, and .gamma.-aminobutyric acid. Examples of the anticoagulant include heparins (such as heparin sodium, heparin potassium, dalteparin sodium, dalteparin calcium, heparin
25 calcium, parnaparin sodium, reviparin sodium, and danaparoid sodium), warfarin, enoxaparin, argatroban, batroxobin, and sodium citrate. Examples of the antiplatelet drug include ticlopidine hydrochloride, dipyridamole, cilostazol, ethyl icosapentate, sarpogrelate hydrochloride, dilazep hydrochloride, trapidil, a nonsteroidal antiinflammatory agent (such as aspirin), beraprost sodium, iloprost, and indobufene.
30 Examples of the thrombolytic drug include urokinase, tissue-type plasminogen activators (such as alteplase, tisokinase, nateplase, pamiteplase, monteplase, and rateplase), and nasaruplase. Examples of the antihypertensive drug include

angiotensin converting enzyme inhibitors (such as captopril, alacepril, lisinopril, imidapril, quinapril, temocapril, delapril, benazepril, cilazapril, trandolapril, enalapril, ceronapril, fosinopril, imadapril, mobertpril, perindopril, ramipril, spirapril, and randolapril), angiotensin II antagonists (such as losartan, candesartan, valsartan, eprosartan, and irbesartan), calcium channel blocking drugs (such as aranidipine, efonidipine, nicardipine, bamidipine, benidipine, manidipine, cilnidipine, nisoldipine, nitrendipine, nifedipine, nilvadipine, felodipine, amlodipine, diltiazem, bepridil, clentiazem, phendilin, galopamil, mibefradil, prenylamine, semotiadil, terodiline, verapamil, cilnidipine, elgodipine, isradipine, lacidipine, lercanidipine, nimodipine, cinnarizine, flunarizine, lidoflazine, lomerizine, bencyclane, etafenone, and perhexiline), β -adrenaline receptor blocking drugs (propranolol, pindolol, indenolol, carteolol, bunitrolol, atenolol, acebutolol, metoprolol, timolol, nipradilol, penbutolol, nadolol, tilisolol, carvedilol, bisoprolol, betaxolol, celiprolol, bopindolol, bevantolol, labetalol, alprenolol, amosulalol, arotinolol, befunolol, bucumolol, bufetolol, buferalol, buprandolol, butylidine, butofilolol, carazolol, cetamolol, cloranolol, dilevalol, epanolol, levobunolol, mepindolol, metipranolol, moprolol, nadoxolol, nevibolol, oxprenolol, practol, pronetalol, sotalol, sufinalol, talindolol, tertalol, toliprolol, xybenolol, and esmolol), α -receptor blocking drugs (such as amosulalol, prazosin, terazosin, doxazosin, bunazosin, urapidil, phentolamine, arotinolol, dapiprazole, fenspiride, indoramin, labetalol, naftopidil, nicergoline, tamsulosin, tolazoline, trimazosin, and yohimbine), sympathetic nerve inhibitors (such as clonidine, guanfacine, guanabenz, methyldopa, and reserpine), hydralazine, todralazine, budralazine, and cadralazine. Examples of the antianginal drug include nitrate drugs (such as amyl nitrite, nitroglycerin, and isosorbide), β -adrenaline receptor blocking drugs (such as propranolol, pindolol, indenolol, carteolol, bunitrolol, atenolol, acebutolol, metoprolol, timolol, nipradilol, penbutolol, nadolol, tilisolol, carvedilol, bisoprolol, betaxolol, celiprolol, bopindolol, bevantolol, labetalol, alprenolol, amosulalol, arotinolol, befunolol, bucumolol, bufetolol, buferalol, buprandolol, butylidine, butofilolol, carazolol, cetamolol, cloranolol, dilevalol, epanolol, levobunolol, mepindolol, metipranolol, moprolol, nadoxolol, nevibolol, oxprenolol, practol, pronetalol, sotalol, sufinalol, talindolol, tertalol, toliprolol, andxybenolol), calcium channel blocking drugs (such

as aranidipine, efonidipine, nicardipine, bamidipine, benidipine, manidipine, cilnidipine, nisoldipine, nitrendipine, nifedipine, nilvadipine, felodipine, amlodipine, diltiazem, bepridil, clentiazem, phendiline, galopamil, mibefradil, prenylamine, semotiadil, terodiline, verapamil, cilnidipine, elgodipine, isradipine, lacidipine, lercanidipine, nimodipine, cinnarizine, flunarizine, lidoflazine, lomerizine, bencyclane, etafenone, and perhexiline) trimetazidine, dipyridamole, etafenone, dilazep, trapidil, nicorandil, enoxaparin, and aspirin. Examples of the diuretic include thiazide diuretics (such as hydrochlorothiazide, methyclothiazide, trichlormethiazide, benzylhydrochlorothiazide, and penflutizide), loop diuretics (such as furosemide, etacrynic acid, bumetanide, piretanide, azosemide, and torasemide), K⁺ sparing diuretics (spironolactone, triamterene, and potassium canrenoate), osmotic diuretics (such as isosorbide, D-mannitol, and glycerin), nonthiazide diuretics (such as meticrane, tripamide, chlorthalidone, and mefruside), and acetazolamide. Examples of the cardiotonic include digitalis formulations (such as digitoxin, digoxin, methyl digoxin, deslanoside, vesnarinone, lanatoside C, and proscillaridin), xanthine formulations (such as aminophylline, choline theophylline, diprophylline, and proxiphylline), catecholamine formulations (such as dopamine, dobutamine, and docarpamine), PDE III inhibitors (such as amrinone, olprinone, and milrinone), denopamine, ubidecarenone, pimobendan, levosimendan, aminoethylsulfonic acid, vesnarinone, carperitide, and colforsin daropate. Examples of the antiarrhythmic drug include ajmaline, pirmenol, procainamide, cibenzoline, disopyramide, quinidine, aprindine, mexiletine, lidocaine, phenytoin, pilsicainide, propafenone, flecainide, atenolol, acebutolol, sotalol, propranolol, metoprolol, pindolol, amiodarone, nifekalant, diltiazem, bepridil, and verapamil. Examples of the antihyperlipidemic drug include atorvastatin, simvastatin, pravastatin sodium, fluvastatin sodium, clofibrate, clofibrate, simfibrate, fenofibrate, bezafibrate, colestimide, and colestyramine. Examples of the immunosuppressant include azathioprine, mizoribine, cyclosporine, tacrolimus, gusperimus, and methotrexate.

30 *iii. Cell Death*

CLK-inhibiting compounds may be administered to subjects who have recently received or are likely to receive a dose of radiation or toxin. In one

embodiment, the dose of radiation or toxin is received as part of a work-related or medical procedure, e.g., working in a nuclear power plant, flying an airplane, an X-ray, CAT scan, or the administration of a radioactive dye for medical imaging; in such an embodiment, the compound is administered as a prophylactic measure. In
5 another embodiment, the radiation or toxin exposure is received unintentionally, e.g., as a result of an industrial accident, habitation in a location of natural radiation, terrorist act, or act of war involving radioactive or toxic material. In such a case, the compound is preferably administered as soon as possible after the exposure to inhibit apoptosis and the subsequent development of acute radiation
10 syndrome.

iv. Neuronal Diseases/Disorders

In certain aspects, CLK-inhibiting compounds can be used to treat patients suffering from neurodegenerative diseases, and traumatic or mechanical injury to the central nervous system (CNS), spinal cord or peripheral nervous system (PNS).
15 Neurodegenerative disease typically involves reductions in the mass and volume of the human brain, which may be due to the atrophy and/or death of brain cells, which are far more profound than those in a healthy person that are attributable to aging. Neurodegenerative diseases can evolve gradually, after a long period of normal brain function, due to progressive degeneration (e.g., nerve cell dysfunction and death) of specific brain regions. Alternatively, neurodegenerative diseases can
20 have a quick onset, such as those associated with trauma or toxins. The actual onset of brain degeneration may precede clinical expression by many years. Examples of neurodegenerative diseases include, but are not limited to, Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic
25 lateral sclerosis (ALS; Lou Gehrig's disease), diffuse Lewy body disease, chorea-acanthocytosis, primary lateral sclerosis, ocular diseases (ocular neuritis), chemotherapy-induced neuropathies (e.g., from vincristine, paclitaxel, bortezomib), diabetes-induced neuropathies and Friedreich's ataxia. CLK-inhibiting compounds can be used to treat these disorders and others as described below.
30 AD is a chronic, incurable, and unstoppable CNS disorder that occurs gradually, resulting in memory loss, unusual behavior, personality changes, and a decline in thinking abilities. These losses are related to the death of specific types of

brain cells and the breakdown of connections and their supporting network (e.g. glial cells) between them. The earliest symptoms include loss of recent memory, faulty judgment, and changes in personality.

PD is a chronic, incurable, and unstoppable CNS disorder that occurs gradually and results in uncontrolled body movements, rigidity, tremor, and dyskinesia. These motor system problems are related to the death of brain cells in an area of the brain that produces dopamine, a chemical that helps control muscle activity. In most people with PD, symptoms appear after age 50. The initial symptoms of PD are a pronounced tremor affecting the extremities, notably in the hands or lips. Subsequent characteristic symptoms of PD are stiffness or slowness of movement, a shuffling walk, stooped posture, and impaired balance. There are wide ranging secondary symptoms such as memory loss, dementia, depression, emotional changes, swallowing difficulties, abnormal speech, sexual dysfunction, and bladder and bowel problems.

ALS (motor neuron disease) is a chronic, incurable, and unstoppable CNS disorder that attacks the motor neurons, components of the CNS that connect the brain to the skeletal muscles. In ALS, the motor neurons deteriorate and eventually die, and though a person's brain normally remains fully functioning and alert, the command to move never reaches the muscles. Most people who get ALS are between 40 and 70 years old. The first motor neurons that weaken are those controlling the arms or legs. Those with ALS may have trouble walking, they may drop things, fall, slur their speech, and laugh or cry uncontrollably. Eventually the muscles in the limbs begin to atrophy from disuse.

HD is another neurodegenerative disease resulting from genetically programmed degeneration of neurons in certain areas of the brain. This degeneration causes uncontrolled movements, loss of intellectual faculties, and emotional disturbance. HD is a familial disease, passed from parent to child through a dominant mutation in the wild-type gene. Some early symptoms of HD are mood swings, depression, irritability or trouble driving, learning new things, remembering a fact, or making a decision. As the disease progresses, concentration on intellectual tasks becomes increasingly difficult and the patient may have difficulty feeding himself or herself and swallowing.

Tay-Sachs disease and Sandhoff disease are glycolipid storage diseases caused by the lack of lysosomal β -hexosaminidase (Gravel et al., in *The Metabolic Basis of Inherited Disease*, eds. Scriver et al., McGraw-Hill, New York, pp. 2839-2879, 1995). In both disorders, GM2 ganglioside and related glycolipidssubstrates
5 for β -hexosaminidase accumulate in the nervous system and trigger acute neurodegeneration. In the most severe forms, the onset of symptoms begins in early infancy. A precipitous neurodegenerative course then ensues, with affected infants exhibiting motor dysfunction, seizure, visual loss, and deafness. Death usually occurs by 2-5 years of age. Neuronal loss through an apoptotic mechanism has been
10 demonstrated (Huang et al., *Hum. Mol. Genet.* 6: 1879-1885, 1997).

It is well-known that apoptosis plays a role in AIDS pathogenesis in the immune system. However, HIV-1 also induces neurological disease. Shi et al. (*J. Clin. Invest.* 98: 1979-1990, 1996) examined apoptosis induced by HIV-1 infection of the CNS in an in vitro model and in brain tissue from AIDS patients, and found
15 that HIV-1 infection of primary brain cultures induced apoptosis in neurons and astrocytes in vitro. Apoptosis of neurons and astrocytes was also detected in brain tissue from 10/11 AIDS patients, including 5/5 patients with HIV-1 dementia and 4/5 nondemented patients.

There are four main peripheral neuropathies associated with HIV, namely
20 sensory neuropathy, AIDP/CIPD, drug-induced neuropathy and CMV-related.

The most common type of neuropathy associated with AIDS is distal symmetrical polyneuropathy (DSPN). This syndrome is a result of nerve degeneration and is characterized by numbness and a sensation of pins and needles. DSPN causes few serious abnormalities and mostly results in numbness or tingling
25 of the feet and slowed reflexes at the ankles. It generally occurs with more severe immunosuppression and is steadily progressive. Treatment with tricyclic antidepressants relieves symptoms but does not affect the underlying nerve damage.

A less frequent, but more severe type of neuropathy is known as acute or chronic inflammatory demyelinating polyneuropathy (AIDP/CIDP). In AIDP/CIDP
30 there is damage to the fatty membrane covering the nerve impulses. This kind of neuropathy involves inflammation and resembles the muscle deterioration often identified with long-term use of AZT. It can be the first manifestation of HIV

infection, where the patient may not complain of pain, but fails to respond to standard reflex tests. This kind of neuropathy may be associated with seroconversion, in which case it can sometimes resolve spontaneously. It can serve as a sign of HIV infection and indicate that it might be time to consider antiviral therapy. AIDP/CIDP may be auto-immune in origin.

Drug-induced, or toxic, neuropathies can be very painful. Antiviral drugs commonly cause peripheral neuropathy, as do other drugs e.g. vincristine, dilantin (an anti-seizure medication), high-dose vitamins, isoniazid, and folic acid antagonists. Peripheral neuropathy is often used in clinical trials for antivirals as a dose-limiting side effect, which means that more drugs should not be administered. Additionally, the use of such drugs can exacerbate otherwise minor neuropathies. Usually, these drug-induced neuropathies are reversible with the discontinuation of the drug.

CMV causes several neurological syndromes in AIDS, including encephalitis, myelitis, and polyradiculopathy.

Neuronal loss is also a salient feature of prion diseases, such as Creutzfeldt-Jakob disease in human, BSE in cattle (mad cow disease), Scrapie Disease in sheep and goats, and feline spongiform encephalopathy (FSE) in cats. CLK-inhibiting compounds may be useful for treating or preventing neuronal loss due to these prior diseases.

In another embodiment, a CLK-inhibiting compound may be used to treat or prevent any disease or disorder involving axonopathy. Distal axonopathy is a type of peripheral neuropathy that results from some metabolic or toxic derangement of peripheral nervous system (PNS) neurons. It is the most common response of nerves to metabolic or toxic disturbances, and as such may be caused by metabolic diseases such as diabetes, renal failure, deficiency syndromes such as malnutrition and alcoholism, or the effects of toxins or drugs. The most common cause of distal axonopathy is diabetes, and the most common distal axonopathy is diabetic neuropathy. The most distal portions of axons are usually the first to degenerate, and axonal atrophy advances slowly towards the nerve's cell body. If the noxious stimulus is removed, regeneration is possible, though prognosis decreases depending on the duration and severity of the stimulus. Those with distal axonopathies usually

present with symmetrical glove-stocking sensori-motor disturbances. Deep tendon reflexes and autonomic nervous system (ANS) functions are also lost or diminished in affected areas.

Diabetic neuropathies are neuropathic disorders that are associated with diabetes mellitus. These conditions usually result from diabetic microvascular injury involving small blood vessels that supply nerves (*vasa nervorum*). Relatively common conditions which may be associated with diabetic neuropathy include third nerve palsy; mononeuropathy; mononeuritis multiplex; diabetic amyotrophy; a painful polyneuropathy; autonomic neuropathy; and thoracoabdominal neuropathy. Clinical manifestations of diabetic neuropathy include, for example, sensorimotor polyneuropathy such as numbness, sensory loss, dysesthesia and nighttime pain; autonomic neuropathy such as delayed gastric emptying or gastroparesis; and cranial neuropathy such as oculomotor (3rd) neuropathies or Mononeuropathies of the thoracic or lumbar spinal nerves.

Peripheral neuropathy is the medical term for damage to nerves of the peripheral nervous system, which may be caused either by diseases of the nerve or from the side-effects of systemic illness. Peripheral neuropathies vary in their presentation and origin, and may affect the nerve or the neuromuscular junction. Major causes of peripheral neuropathy include seizures, nutritional deficiencies, and HIV, though diabetes is the most likely cause. Mechanical pressure from staying in one position for too long, a tumor, intraneural hemorrhage, exposing the body to extreme conditions such as radiation, cold temperatures, or toxic substances can also cause peripheral neuropathy.

In an exemplary embodiment, a CLK-inhibiting compound may be used to treat or prevent multiple sclerosis (MS), including relapsing MS and monosymptomatic MS, and other demyelinating conditions, such as, for example, chronic inflammatory demyelinating polyneuropathy (CIDP), or symptoms associated therewith.

MS is a chronic, often disabling disease of the central nervous system. Various and converging lines of evidence point to the possibility that the disease is caused by a disturbance in the immune function, although the cause of this disturbance has not been established. This disturbance permits cells of the immune

system to "attack" myelin, the fat containing insulating sheath that surrounds the nerve axons located in the central nervous system ("CNS"). When myelin is damaged, electrical pulses cannot travel quickly or normally along nerve fiber pathways in the brain and spinal cord. This results in disruption of normal electrical conductivity within the axons, fatigue and disturbances of vision, strength,
5 coordination, balance, sensation, and bladder and bowel function.

As such, MS is now a common and well-known neurological disorder that is characterized by episodic patches of inflammation and demyelination which can occur anywhere in the CNS, however, almost always without any involvement of the peripheral nerves associated therewith. Demyelination produces a situation
10 analogous to that resulting from cracks or tears in an insulator surrounding an electrical cord. That is, when the insulating sheath is disrupted, the circuit is "short circuited" and the electrical apparatus associated therewith will function intermittently or not at all. Such loss of myelin surrounding nerve fibers results in
15 short circuits in nerves traversing the brain and the spinal cord that thereby result in symptoms of MS. It is further found that such demyelination occurs in patches, as opposed to along the entire CNS. In addition, such demyelination may be intermittent. Therefore, such plaques are disseminated in both time and space.

It is believed that the pathogenesis involves a local disruption of the blood
20 brain barrier which causes a localized immune and inflammatory response, with consequent damage to myelin and hence to neurons.

Clinically, MS exists in both sexes and can occur at any age. However, its most common presentation is in the relatively young adult, often with a single focal lesion such as a damage of the optic nerve, an area of anesthesia (loss of sensation),
25 or paraesthesia (localized loss of feeling), or muscular weakness. In addition, vertigo, double vision, localized pain, incontinence, and pain in the arms and legs may occur upon flexing of the neck, as well as a large variety of less common symptoms.

An initial attack of MS is often transient, and it may be weeks, months, or years before a further attack occurs. Some individuals may enjoy a stable, relatively
30 event free condition for a great number of years, while other less fortunate ones may experience a continual downhill course ending in complete paralysis. There is, most commonly, a series of remission and relapses, in which each relapse leaves a patient

somewhat worse than before. Relapses may be triggered by stressful events, viral infections or toxins. Therein, elevated body temperature, i.e., a fever, will make the condition worse, or as a reduction of temperature by, for example, a cold bath, may make the condition better.

5 In yet another embodiment, a CLK-inhibiting compound may be used to treat trauma to the nerves, including, trauma due to disease, injury (including surgical intervention), or environmental trauma (e.g., neurotoxins, alcoholism, etc.).

 CLK-inhibiting compounds may also be useful to prevent, treat, and alleviate symptoms of various PNS disorders, such as the ones described below. The PNS is
10 composed of the nerves that lead to or branch off from the spinal cord and CNS. The peripheral nerves handle a diverse array of functions in the body, including sensory, motor, and autonomic functions. When an individual has a peripheral neuropathy, nerves of the PNS have been damaged. Nerve damage can arise from a number of causes, such as disease, physical injury, poisoning, or malnutrition.

15 These agents may affect either afferent or efferent nerves. Depending on the cause of damage, the nerve cell axon, its protective myelin sheath, or both may be injured or destroyed.

 The term “peripheral neuropathy” encompasses a wide range of disorders in which the nerves outside of the brain and spinal cord—peripheral nerves—have
20 been damaged. Peripheral neuropathy may also be referred to as peripheral neuritis, or if many nerves are involved, the terms polyneuropathy or polyneuritis may be used.

 Peripheral neuropathy is a widespread disorder, and there are many underlying causes. Some of these causes are common, such as diabetes, and others
25 are extremely rare, such as acrylamide poisoning and certain inherited disorders. The most common worldwide cause of peripheral neuropathy is leprosy. Leprosy is caused by the bacterium *Mycobacterium leprae*, which attacks the peripheral nerves of affected people.

 Leprosy is extremely rare in the United States, where diabetes is the most
30 commonly known cause of peripheral neuropathy. It has been estimated that more than 17 million people in the United States and Europe have diabetes-related polyneuropathy. Many neuropathies are idiopathic; no known cause can be found.

The most common of the inherited peripheral neuropathies in the United States is Charcot-Marie-Tooth disease, which affects approximately 125,000 persons.

Another of the better known peripheral neuropathies is Guillain-Barré syndrome, which arises from complications associated with viral illnesses, such as
5 cytomegalovirus, Epstein-Barr virus, and human immunodeficiency virus (HIV), or bacterial infection, including *Campylobacter jejuni* and Lyme disease. The worldwide incidence rate is approximately 1.7 cases per 100,000 people annually. Other well-known causes of peripheral neuropathies include chronic alcoholism, infection of the varicella-zoster virus, botulism, and poliomyelitis. Peripheral
10 neuropathy may develop as a primary symptom, or it may be due to another disease. For example, peripheral neuropathy is only one symptom of diseases such as amyloid neuropathy, certain cancers, or inherited neurologic disorders. Such diseases may affect the PNS and the CNS, as well as other body tissues.

Other PNS diseases treatable with CLK-inhibiting compound include:

15 Brachial Plexus Neuropathies (diseases of the cervical and first thoracic roots, nerve trunks, cords, and peripheral nerve components of the brachial plexus. Clinical manifestations include regional pain, paresthesia; muscle weakness, and decreased sensation in the upper extremity. These disorders may be associated with trauma, including birth injuries; thoracic outlet syndrome; neoplasms, neuritis, radiotherapy;
20 and other conditions. See Adams et al., Principles of Neurology, 6th ed, pp1351-2); Diabetic Neuropathies (peripheral, autonomic, and cranial nerve disorders that are associated with diabetes mellitus). These conditions usually result from diabetic microvascular injury involving small blood vessels that supply nerves (*vasa nervorum*). Relatively common conditions which may be associated with diabetic
25 neuropathy include third nerve palsy; mononeuropathy; mononeuritis multiplex; diabetic amyotrophy; a painful polyneuropathy; autonomic neuropathy; and thoracoabdominal neuropathy (see Adams et al., Principles of Neurology, 6th ed, p1325); mononeuropathies (disease or trauma involving a single peripheral nerve in isolation, or out of proportion to evidence of diffuse peripheral nerve dysfunction).
30 Mononeuritis multiplex refers to a condition characterized by multiple isolated nerve injuries. Mononeuropathies may result from a wide variety of causes, including ischemia; traumatic injury; compression; connective tissue diseases; cumulative

trauma disorders; and other conditions; Neuralgia (intense or aching pain that occurs along the course or distribution of a peripheral or cranial nerve); and Peripheral Nervous System Neoplasms (neoplasms which arise from peripheral nerve tissue). This includes neurofibromas; Schwannomas; granular cell tumors; and malignant
5 peripheral nerve sheath tumors. See DeVita Jr et al., *Cancer: Principles and Practice of Oncology*, 5th ed, pp1750-1); and Nerve Compression Syndromes (mechanical compression of nerves or nerve roots from internal or external causes). These may result in a conduction block to nerve impulses, due to, for example, myelin sheath dysfunction, or axonal loss. The nerve and nerve sheath injuries may be caused by
10 ischemia; inflammation; or a direct mechanical effect; Neuritis (a general term indicating inflammation of a peripheral or cranial nerve). Clinical manifestation may include pain; paresthesias; paresis; or hyperesthesia; Polyneuropathies (diseases of multiple peripheral nerves). The various forms are categorized by the type of nerve affected (e.g., sensory, motor, or autonomic), by the distribution of nerve
15 injury (e.g., distal vs. proximal), by nerve component primarily affected (e.g., demyelinating vs. axonal), by etiology, or by pattern of inheritance.

In another embodiment, a CLK-inhibiting compound may be used to treat or prevent chemotherapeutic induced neuropathy. The CLK-inhibiting compounds may be administered prior to administration of the chemotherapeutic agent,
20 concurrently with administration of the chemotherapeutic drug, and/or after initiation of administration of the chemotherapeutic drug. If the CLK-inhibiting compound is administered after the initiation of administration of the chemotherapeutic drug, it is desirable that the CLK-inhibiting compound be administered prior to, or at the first signs, of chemotherapeutic induced neuropathy.

25 Chemotherapy drugs can damage any part of the nervous system. Encephalopathy and myelopathy are fortunately very rare. Damage to peripheral nerves is much more common and can be a side effect of treatment experienced by people with cancers, such as lymphoma. Most of the neuropathy affects sensory rather than motor nerves. Thus, the common symptoms are tingling, numbness or a
30 loss of balance. The longest nerves in the body seem to be most sensitive hence the fact that most patients will report numbness or pins and needles in their hands and feet.

The chemotherapy drugs which are most commonly associated with neuropathy, are the Vinca alkaloids (anti-cancer drugs originally derived from a member of the periwinkle - the Vinca plant genus) and a platinum-containing drug called Cisplatin. The Vinca alkaloids include the drugs vinblastine, vincristine and vindesine. Many combination chemotherapy treatments for lymphoma for example CHOP and CVP contain vincristine, which is the drug known to cause this problem most frequently. Indeed, it is the risk of neuropathy that limits the dose of vincristine that can be administered.

Studies that have been performed have shown that most patients will lose some reflexes in their legs as a result of treatment with vincristine and many will experience some degree of tingling (paresthesia) in their fingers and toes. The neuropathy does not usually manifest itself right at the start of the treatment but generally comes on over a period of a few weeks. It is not essential to stop the drug at the first onset of symptoms, but if the neuropathy progresses this may be necessary. It is very important that patients should report such symptoms to their doctors, as the nerve damage is largely reversible if the drug is discontinued. Most doctors will often reduce the dose of vincristine or switch to another form of Vinca alkaloid such as vinblastine or vindesine if the symptoms are mild. Occasionally, the nerves supplying the bowel are affected causing abdominal pain and constipation.

In another embodiment, a CLK-inhibiting compound may be used to treat or prevent a polyglutamine disease. Huntington's Disease (HD) and Spinocerebellar ataxia type 1 (SCA1) are just two examples of a class of genetic diseases caused by dynamic mutations involving the expansion of triplet sequence repeats. In reference to this common mechanism, these disorders are called trinucleotide repeat diseases. At least 14 such diseases are known to affect human beings. Nine of them, including SCA1 and Huntington's disease, have CAG as the repeated sequence (see Table 1, below). Since CAG codes for an amino acid called glutamine, these nine trinucleotide repeat disorders are collectively known as polyglutamine diseases.

Each polyglutamine disease is characterized by a progressive degeneration of a distinct group of nerve cells. The major symptoms of these diseases are similar, although not identical, and usually affect people in midlife. Given the similarities in symptoms, the polyglutamine diseases are hypothesized to progress via common

cellular mechanisms. Above a certain threshold, the greater the number of glutamine repeats in a protein, the earlier the onset of disease and the more severe the symptoms. This suggests that abnormally long glutamine tracts render their host protein toxic to nerve cells.

- 5 It is thought that the symptoms of SCA1 are not directly caused by the loss of normal ataxin-1 function but rather by the interaction between ataxin-1 and another protein called LANP. LANP is needed for nerve cells to communicate with one another and thus for their survival. When the mutant ataxin-1 protein accumulates inside nerve cells, it “traps” the LANP protein, interfering with its
- 10 normal function. After a while, the absence of LANP function appears to cause nerve cells to malfunction.

Table 1. Summary of Polyglutamine Diseases.

Disease	Gene name	Chromosomal location	Pattern of inheritance	Protein	Normal repeat length	Disease repeat length
Spinobulbar muscular atrophy (Kennedy disease)	<i>AR</i>	Xq13–21	X-linked recessive	androgen receptor (AR)	9–36	38–62
Huntington's disease	<i>HD</i>	4p16.3	autosomal dominant	huntingtin	6–35	36–121
Dentatorubral-pallidoluysian atrophy (Haw River syndrome)	<i>DRPLA</i>	12p13.31	autosomal dominant	atrophin-1	6–35	49–88
Spinocerebellar ataxia type 1	<i>SCA1</i>	6p23	autosomal dominant	ataxin-1	6–44	39–82
Spinocerebellar ataxia type 2	<i>SCA2</i>	12q24.1	autosomal dominant	ataxin-2	15–31	36–63
Spinocerebellar ataxia type 3 (Machado-Joseph disease)	<i>SCA3</i>	14q32.1	autosomal dominant	ataxin-3	12–40	55–84
Spinocerebellar ataxia type 6	<i>SCA6</i>	19p13	autosomal dominant	$\alpha 1_A$ -voltage-dependent calcium channel subunit	4–18	21–33
Spinocerebellar ataxia type 7	<i>SCA7</i>	3p12–13	autosomal dominant	ataxin-7	4–35	37–306
Spinocerebellar ataxia type 17	<i>SCA17</i>	6q27	autosomal dominant	TATA binding protein	25–42	45–63

Many transcription factors have also been found in neuronal inclusions in different diseases. It is possible that these transcription factors interact with the polyglutamine-containing proteins and then become trapped in the neuronal inclusions. This in turn might keep the transcription factors from turning genes on
5 and off as needed by the cell. Another observation is hypoacetylation of histones in affected cells. This has led to the hypothesis that Class I/II Histone Deacetylase (HDAC I/II) inhibitors, which are known to increase histone acetylation, may be a novel therapy for polyglutamine diseases (US Patent Publication No. 2004/0142859; “Method of treating neurodegenerative, psychiatric, and other disorders with
10 deacetylase inhibitors”).

In yet another embodiment, the invention provides a method for treating or preventing neuropathy related to ischemic injuries or diseases, such as, for example, coronary heart disease (including congestive heart failure and myocardial infarctions), stroke, emphysema, hemorrhagic shock, peripheral vascular disease
15 (upper and lower extremities) and transplant related injuries.

In certain embodiments, the invention provides a method to treat a central nervous system cell to prevent damage in response to a decrease in blood flow to the cell. Typically the severity of damage that may be prevented will depend in large part on the degree of reduction in blood flow to the cell and the duration of the
20 reduction. By way of example, the normal amount of perfusion to brain gray matter in humans is about 60 to 70 mL/100 g of brain tissue/min. Death of central nervous system cells typically occurs when the flow of blood falls below approximately 8-10 mL/100 g of brain tissue/min, while at slightly higher levels (i.e. 20-35 mL/100 g of brain tissue/min) the tissue remains alive but not able to function. In one
25 embodiment, apoptotic or necrotic cell death may be prevented. In still a further embodiment, ischemic-mediated damage, such as cytotoxic edema or central nervous system tissue anoxemia, may be prevented. In each embodiment, the central nervous system cell may be a spinal cell or a brain cell.

Another aspect encompasses administering a CLK-inhibiting compound to a
30 subject to treat a central nervous system ischemic condition. A number of central nervous system ischemic conditions may be treated by the CLK-inhibiting compounds described herein. In one embodiment, the ischemic condition is a stroke

that results in any type of ischemic central nervous system damage, such as apoptotic or necrotic cell death, cytotoxic edema or central nervous system tissue anoxia. The stroke may impact any area of the brain or be caused by any etiology commonly known to result in the occurrence of a stroke. In one alternative of this embodiment, the stroke is a brain stem stroke. Generally speaking, brain stem strokes strike the brain stem, which control involuntary life-support functions such as breathing, blood pressure, and heartbeat. In another alternative of this embodiment, the stroke is a cerebellar stroke. Typically, cerebellar strokes impact the cerebellum area of the brain, which controls balance and coordination. In still another embodiment, the stroke is an embolic stroke. In general terms, embolic strokes may impact any region of the brain and typically result from the blockage of an artery by a vaso-occlusion. In yet another alternative, the stroke may be a hemorrhagic stroke. Like ischemic strokes, hemorrhagic stroke may impact any region of the brain, and typically result from a ruptured blood vessel characterized by a hemorrhage (bleeding) within or surrounding the brain. In a further embodiment, the stroke is a thrombotic stroke. Typically, thrombotic strokes result from the blockage of a blood vessel by accumulated deposits.

In another embodiment, the ischemic condition may result from a disorder that occurs in a part of the subject's body outside of the central nervous system, but yet still causes a reduction in blood flow to the central nervous system. These disorders may include, but are not limited to a peripheral vascular disorder, a venous thrombosis, a pulmonary embolus, arrhythmia (e.g. atrial fibrillation), a myocardial infarction, a transient ischemic attack, unstable angina, or sickle cell anemia. Moreover, the central nervous system ischemic condition may occur as result of the subject undergoing a surgical procedure. By way of example, the subject may be undergoing heart surgery, lung surgery, spinal surgery, brain surgery, vascular surgery, abdominal surgery, or organ transplantation surgery. The organ transplantation surgery may include heart, lung, pancreas, kidney or liver transplantation surgery. Moreover, the central nervous system ischemic condition may occur as a result of a trauma or injury to a part of the subject's body outside the central nervous system. By way of example, the trauma or injury may cause a degree of bleeding that significantly reduces the total volume of blood in the subject's body.

Because of this reduced total volume, the amount of blood flow to the central nervous system is concomitantly reduced. By way of further example, the trauma or injury may also result in the formation of a vaso-occlusion that restricts blood flow to the central nervous system.

5 Of course it is contemplated that the CLK-inhibiting compounds may be employed to treat the central nervous system ischemic condition irrespective of the cause of the condition. In one embodiment, the ischemic condition results from a vaso-occlusion. The vaso-occlusion may be any type of occlusion, but is typically a cerebral thrombosis or an embolism. In a further embodiment, the ischemic
10 condition may result from a hemorrhage. The hemorrhage may be any type of hemorrhage, but is generally a cerebral hemorrhage or a subararachnoid hemorrhage. In still another embodiment, the ischemic condition may result from the narrowing of a vessel. Generally speaking, the vessel may narrow as a result of a vasoconstriction such as occurs during vasospasms, or due to arteriosclerosis. In yet
15 another embodiment, the ischemic condition results from an injury to the brain or spinal cord.

 In yet another aspect, a CLK-inhibiting compound may be administered to reduce infarct size of the ischemic core following a central nervous system ischemic condition. Moreover, a CLK-inhibiting compound may also be beneficially
20 administered to reduce the size of the ischemic penumbra or transitional zone following a central nervous system ischemic condition.

 In one embodiment, a combination drug regimen may include drugs or compounds for the treatment or prevention of neurodegenerative disorders or secondary conditions associated with these conditions. Thus, a combination drug
25 regimen may include one or more CLK-inhibiting compounds and one or more anti-neurodegeneration agents. For example, one or more CLK-inhibiting compounds can be combined with an effective amount of one or more of: L-DOPA; a dopamine agonist; an adenosine A_{2A} receptor antagonist; a COMT inhibitor; a MAO inhibitor; an N-NOS inhibitor; a sodium channel antagonist; a selective N-methyl D-aspartate
30 (NMDA) receptor antagonist; an AMPA/kainate receptor antagonist; a calcium channel antagonist; a GABA-A receptor agonist; an acetyl-choline esterase inhibitor; a matrix metalloprotease inhibitor; a PARP inhibitor; an inhibitor of p38 MAP

kinase or c-jun-N-terminal kinases; TPA; NDA antagonists; beta-interferons; growth factors; glutamate inhibitors; and/or as part of a cell therapy.

Exemplary N-NOS inhibitors include 4-(6-amino-pyridin-2-yl)-3-methoxyphenol 6-[4-(2-dimethylamino-ethoxy)-2-methoxy-phenyl]-pyridin-2-yl-amine, 6-[4-(2-dimethylamino-ethoxy)-2,3-dimethyl-phenyl]-pyridin-2-yl-amine, 6-[4-(2-pyrrolidinyl-ethoxy)-2,3-dimethyl-p-phenyl]-pyridin-2-yl-amine, 6-[4-(4-(n-methyl)piperidinyloxy)-2,3-dimethyl-p-phenyl]-pyridin-2-yl-amine, 6-[4-(2-dimethylamino-ethoxy)-3-methoxy-phenyl]-pyridin-2-yl-amine, 6-[4-(2-pyrrolidinyl-ethoxy)-3-methoxy-phenyl]-pyridin-2-yl-amine, 6-[4-(2,7-dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-ethoxy]-3-methoxy-phenyl]-pyridin-2-yl-amine, 6-[3-methoxy-4-[2-(4-phenethyl-piperazin-1-yl)-ethoxy]-phenyl]-pyridin-2-yl-amine, 6-[3-methoxy-4-[2-(4-methyl-piperazin-1-yl)-ethoxy]-phenyl]-pyridin-2-yl-amine, 6-[4-[2-(4-dimethylamino-piperidin-1-yl)-ethoxy]-3-methoxy-phenyl]-pyridin-2-yl-amine, 6-[4-(2-dimethylamino-ethoxy)-3-ethoxy-phenyl]-pyridin-2-yl-amine, 6-[4-(2-pyrrolidinyl-ethoxy)-3-ethoxy-phenyl]-pyridin-2-yl-amine, 6-[4-(2-dimethylamino-ethoxy)-2-isopropyl-phenyl]-pyridin-2-yl-amine, 4-(6-amino-pyridin-2-yl)-3-cyclopropyl-phenol 6-[2-cyclopropyl-4-(2-dimethylamino-ethoxy)-phenyl]-pyridin-2-yl-amine, 6-[2-cyclopropyl-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pyridin-2-yl-amine, 3-[3-(6-amino-pyridin-2-yl)-4-cyclopropyl-phenoxy]-pyrrolidine-1-carboxylic acid tert-butyl ester 6-[2-cyclopropyl-4-(1-methyl-pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 4-(6-amino-pyridin-2-yl)-3-cyclobutyl-phenol 6-[2-cyclobutyl-4-(2-dimethylamino-ethoxy)-phenyl]-pyridin-2-yl-amine, 6-[2-cyclobutyl-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pyridin-2-yl-amine, 6-[2-cyclobutyl-4-(1-methyl-pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 4-(6-amino-pyridin-2-yl)-3-cyclopentyl-phenol 6-[2-cyclopentyl-4-(2-dimethylamino-ethoxy)-phenyl]-pyridin-2-yl-amine, 6-[2-cyclopentyl-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pyridin-2-yl-amine, 3-[4-(6-amino-pyridin-2-yl)-3-methoxy-phenoxy]-pyrrolidine-1-carboxylic acid tert butyl ester 6-[4-(1-methyl-pyrrolidin-3-yl-oxy)-2-methoxy-phenyl]-pyridin-2-yl-amine, 4-[4-(6-amino-pyridin-2-yl)-3-methoxy-phenoxy]-piperidine-1-carboxylic acid tert butyl ester 6-[2-methoxy-4-(1-methyl-piperidin-4-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[4-(allyloxy)-2-methoxy-phenyl]-pyridin-2-yl-amine, 4-(6-amino-pyridin-2-yl)-3-methoxy-6-allyl-phenol 12 and 4-(6-

amino-pyridin-2-yl)-3-methoxy-2-allyl-phenol 13 4-(6-amino-pyridin-2-yl)-3-
 methoxy-6-propyl-phenol 6-[4-(2-dimethylamino-ethoxy)-2-methoxy-5-propyl-
 phenyl]-pyridin-yl-amine, 6-[2-isopropyl-4-(pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-
 yl-amine, 6-[2-isopropyl-4-(piperidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-
 5 isopropyl-4-(1-methyl-azetidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-
 isopropyl-4-(1-methyl-piperidin-4-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-
 isopropyl-4-(1-methyl-pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine 6-[2-
 isopropyl-4-(1-methyl-pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-
 isopropyl-4-(2-methyl-2-aza-bicyclo[2.2.1]hept-5-yl-oxy)-phenyl]-pyridin-2-yl-
 10 amine, 6-[4-(2-dimethylamino-ethoxy)-2-methoxy-phenyl]-pyridin-2-yl-amine, 6-
 {4-[2-(benzyl-methyl-amino)-ethoxy]-2-methoxy-phenyl}-pyridin-2-yl-amine, 6-[2-
 methoxy-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pyridin-2-yl-amine, 2-(6-amino-
 pyridin-2-yl)-5-(2-dimethylamino-ethoxy)-phenol 2-[4-(6-amino-pyridin-2-yl)-3-
 methoxy-phenoxy]-acetamide 6-[4-(2-amino-ethoxy)-2-methoxy-phenyl]-pyridin-2-
 15 yl-amine, 6-{4-[2-(3,4-dihydro-1h-isoquinolin-2-yl)-ethoxy]-2-methoxy-phenyl}-
 pyridin-2-yl-amine, 2-[4-(6-amino-pyridin-2-yl)-3-methoxy-phenoxy]-ethanol 6-
 {2-methoxy-4-[2-(2,2,6,6-tetramethyl-piperidin-1-yl)-ethoxy]-phenyl}-pyridin-2-
 yl-amine, 6-{4-[2-(2,5-dimethyl-pyrrolidin-1-yl)-ethoxy]-2-methoxy-phenyl}-
 pyridin-2-yl-amine, 6-{4-[2-(2,5-dimethyl-pyrrolidin-1-yl)-ethoxy]-2-methoxy-
 20 phenyl}-pyridin-2-yl-amine, 2-[4-(6-amino-pyridin-2-yl)-3-methoxy-phenoxy]-1-
 (2,2,6,6-tetramethyl-piperidin-1-yl)-ethanone 6-[2-methoxy-4-(1-methyl-pyrrolidin-
 2-yl-methoxy)-phenyl]-pyridin-2-yl-amine, 6-[4-(2-dimethylamino-ethoxy)-2-
 propoxy-phenyl]-pyridin-2-yl-amine, 6-{4-[2-(benzyl-methyl-amino)-ethoxy]-2-
 propoxy-phenyl}-pyridin-2-yl-amine 6-[4-(2-ethoxy-ethoxy)-2-methoxy-phenyl]-
 25 pyridin-2-yl-amine, 6-[4-(2-dimethylamino-ethoxy)-2-isopropoxy-phenyl]-pyridin-
 2-yl-amine, 6-[4-(2-ethoxy-ethoxy)-2-isopropoxy-phenyl]-pyridin-2-yl-amine, 6-[2-
 methoxy-4-(3-methyl-butoxy)-phenyl]-pyridin-2-yl-amine, 6-[4-(2-dimethylamino-
 ethoxy)-2-ethoxy-phenyl]-pyridin-2-yl-amine, 6-{4-[2-(benzyl-methyl-amino)-
 ethoxy]-2-ethoxy-phenyl}-pyridin-2-yl-amine, 6-[2-ethoxy-4-(3-methyl-butoxy)-
 30 phenyl]-pyridin-2-yl-amine, 1-(6-amino-3-aza-bicyclo[3.1.0]hex-3-yl)-2-[4-(6-
 amino-pyridin-2-yl)-3-ethoxy-phenoxy]-ethanone 6-[2-ethoxy-4-(2-pyrrolidin-1-yl-
 ethoxy)-phenyl]-pyridin-2-yl-amine, 3-{2-[4-(6-amino-pyridin-2-yl)-3-ethoxy-

phenoxy]-ethyl}-3-aza-bicyclo[3.1.0]hex-6-yl-amine, 1-(6-amino-3-aza-bicyclo[3.1.0]hex-3-yl)-2-[4-(6-amino-pyridin-2-yl)-3-methoxy-phenoxy]-ethanone

3-{2-[4-(6-amino-pyridin-2-yl)-3-methoxy-phenoxy]-ethyl}-3-aza-bicyclo[3.1.0]hex-6-yl-amine, 6-[2-isopropoxy-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pyridin-2-yl-amine, 6-{4-[2-(benzyl-methyl-amino)-ethoxy]-2-isopropoxy-phenyl}-pyridin-2-yl-amine, 6-[4-(2-dimethylamino-ethoxy)-2-methoxy-5-propyl-phenyl]-pyridin-2-yl-amine, 6-[5-allyl-4-(2-dimethylamino-ethoxy)-2-methoxy-phenyl]-pyridin-2-yl-amine, 6-[5-allyl-2-methoxy-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pyridin-2-yl-amine, 6-[3-allyl-4-(2-dimethylamino-ethoxy)-2-methoxy-phenyl]-pyridin-2-yl-amine, 6-[2-methoxy-4-(pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-methoxy-4-(1-methyl-pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-ethoxy-4-(pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-isopropoxy-4-(pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-methoxy-4-(piperidin-4-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-methoxy-4-(2,2,6,6-tetramethyl-piperidin-4-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-isopropoxy-4-(pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 3-[4-(6-amino-pyridin-2-yl)-3-methoxy-phenoxy]-azetidine-1-carboxylic acid tert-butyl ester 6-[4-(azetidin-3-yl-oxy)-2-methoxy-phenyl]-pyridin-2-yl-amine, 6-[2-methoxy-4-(1-methyl-azetidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-isopropoxy-4-(pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-isopropoxy-4-(pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-methoxy-4-(pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-methoxy-4-(1-methyl-pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-methoxy-4-(1-methyl-pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-methoxy-4-(2-methyl-2-azabicyclo[2.2.1]hept-5-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2-methoxy-4-(1-methyl-piperidin-4-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[4-(1-ethyl-piperidin-4-yl-oxy)-2-methoxy-phenyl]-pyridin-2-yl-amine, 6-[5-allyl-2-methoxy-4-(1-methyl-pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[4-(2-dimethylamino-ethoxy)-2,6-dimethyl-phenyl]-pyridin-2-yl-amine, 6-[2,6-dimethyl-4-(3-piperidin-1-yl-propoxy)-phenyl]-pyridin-2-yl-amine, 6-[2,6-dimethyl-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pyridin-2-yl-amine, 6-{2,6-dimethyl-4-[3-(4-methyl-piperazin-1-yl)-propoxy]-phenyl}-pyridin-2-yl-amine, 6-[2,6-dimethyl-4-(2-morpholin-4-yl-ethoxy)-phenyl]-pyridin-2-yl-amine, 6-{4-[2-(benzyl-methyl-amino)-ethoxy]-2,6-

- dimethyl-phenyl}-p-yrudin-2-yl-amine, 2-[4-(6-amino-pyridin-2-yl)-3,5-dimethyl-phenoxy]-acetam-ide 6-[4-(2-amino-ethoxy)-2,6-dimethyl-phenyl]-pyridin-2-yl-amine, 6-[2-isopropyl-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pyridin-2-yl-amine, 2-(2,5-dimethyl-pyrrolidin-1-yl)-6-[2-isopropyl-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pyridine 6-{4-[2-(3,5-dimethyl-piperidin-1-yl)-ethoxy]-2-isopropyl-phenyl}-pyridin-2-yl-amine, 6-[4-(2-dimethylamino-ethoxy)-2-isopropyl-phenyl]-pyridin-2-yl-amine, 6-[2-tert-butyl-4-(2-dimethylamino-ethoxy)-phenyl]-pyridin-2-yl-amine, 6-[2-tert-butyl-4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-pyridin-2-yl-amine, 6-[4-(2-pyrrolidinyl-ethoxy)-2,5-dimethyl-phenyl]-pyridin-2-yl-amine, 6-[4-(2-dimethylamino-ethoxy)-2,5-dimethyl-phenyl]-pyridin-2-yl-amine, 6-[4-(2-(4-phenethylpiperazin-1-yl)-ethoxy)-2,5-dimethyl-phenyl]-pyridin-2-yl-amine, 6-[2-cyclopropyl-4-(2-dimethylamino-1-methyl-ethoxy)-phenyl]-pyridin-2-yl-amine, 6-[cyclobutyl-4-(2-dimethylamino-1-methyl-ethoxy)-phenyl]-pyridin-2-yl-amine, 6-[4-(allyloxy)-2-cyclobutyl-phenyl]-pyridin-2-yl-amine, 2-allyl-4-(6-amino-pyridin-2-yl)-3-cyclobutyl-phenol and 2-allyl-4-(6-amino-pyridin-2-yl)-5-cyclobutyl-phenol 4-(6-amino-pyridin-2-yl)-5-cyclobutyl-2-propyl-phenol 4-(6-amino-pyridin-2-yl)-3-cyclobutyl-2-propyl-phenol 6-[2-cyclobutyl-4-(2-dimethylamino-1-methyl-ethoxy)-5-propyl-phenyl]-pyridin-2-yl-amine, 6-[2-cyclobutyl-4-(2-dimethylamino-1-methyl-ethoxy)-3-propyl-phenyl]-pyridin-2-yl-amine, 6-[2-cyclobutyl-4-(2-dimethylamino-ethoxy)-5-propyl-phenyl]-pyridin-2-yl-amine, 6-[2-cyclobutyl-4-(2-dimethylamino-ethoxy)-3-propyl-phenyl]-pyridin-2-yl-amine, 6-[2-cyclobutyl-4-(1-methyl-pyrrolidin-3-yl-oxy)-5-propyl-phenyl]-pyridin-2-yl-amine, 6-[cyclobutyl-4-(1-methyl-pyrrolidin-3-yl-oxy)-3-propyl-phenyl]-pyridin-2-yl-amine, 2-(4-benzyloxy-5-hydroxy-2-methoxy-phenyl)-6-(2,5-dimethyl-pyrrol-1-yl)-p-yridine 6-[4-(2-dimethylamino-ethoxy)-5-ethoxy-2-methoxy-phenyl]-pyridin-2-yl-amine, 6-[5-ethyl-2-methoxy-4-(1-methyl-piperidin-4-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[5-ethyl-2-methoxy-4-(piperidin-4-yl-oxy)-phenyl]-pyridin-2-yl-amine, 6-[2,5-dimethoxy-4-(1-methyl-pyrrolidin-3-yl-oxy)-phenyl]-pyridin-2-yl-amine, and 6-[4-(2-dimethylamino-ethoxy)-5-ethyl-2-methoxy-phenyl]-pyridin-2-yl-amine.
- Exemplary NMDA receptor antagonists include (+)-(1S, 2S)-1-(4-hydroxy-phenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol, (1S, 2S)-1-(4-hydroxy-3-methoxyphenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol, (3R, 4S)-3-(4-(4-

fluorophenyl)-4-hydroxypiperidin-1-yl)-chroman-4,7-diol, and (1R*, 2R*)-1-(4-hydroxy-3-methylphenyl)-2-(4-(4-fluoro-phenyl)-4-hydroxypiperidin-1-yl)-propan-1-ol-mesylate or a pharmaceutically acceptable acid addition salt thereof.

Exemplary dopamine agonists include ropininole; L-dopa decarboxylase
 5 inhibitors such as carbidopa or benserazide, bromocriptine, dihydroergocryptine, etisulergine, AF-14, alaptide, pergolide, piribedil; dopamine D1 receptor agonists such as A-68939, A-77636, dihydrexine, and SKF-38393; dopamine D2 receptor agonists such as carbergoline, lisuride, N-0434, naxagolide, PD-118440, pramipexole, quinpirole and ropinirole; dopamine/ β -adrenergic receptor agonists
 10 such as DPDMS and dopexamine; dopamine/5-HT uptake inhibitor/5-HT-1A agonists such as roxindole; dopamine/opiate receptor agonists such as NIH-10494; α 2-adrenergic antagonist/dopamine agonists such as terguride; α 2-adrenergic antagonist/dopamine D2 agonists such as ergolines and talipexole; dopamine uptake inhibitors such as GBR-12909, GBR-13069, GYKI-52895, and NS-2141;
 15 monoamine oxidase-B inhibitors such as selegiline, N-(2-butyl)-N-methylpropargylamine, N-methyl-N-(2-pentyl)propargylamine, AGN-1133, ergot derivatives, lazabemide, LU-53439, MD-280040 and mofegiline; and COMT inhibitors such as CGP-28014.

Exemplary acetyl cholinesterase inhibitors include donepezil, 1-(2-methyl-
 20 1H-benzimida-zol-5-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(2-phenyl-1H-benzimidazol-5-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(1-ethyl-2-methyl-1H-benzimidazol-5-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(2-methyl-6-benzothiazolyl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(2-methyl-6-benzothiazolyl)-3-[1-[(2-methyl-4-thiazolyl)methyl]-4-
 25 piperidinyl]-1-propanone; 1-(5-methyl-benzo[b]thien-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(6-methyl-benzo[b]thien-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(3,5-dimethyl-benzo[b]thien-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(benzo[b]thien-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(benzofuran-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(1-phenylsulfonyl-6-methyl-indol-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(6-methyl-indol-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(1-phenylsulfonyl-5-amino-indol-2-

- yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(5-amino-indol-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(5-acetylamino-indol-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(6-quinolyl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(5-indolyl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(5-benzthienyl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(6-quinazolyl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(6-benzoxazolyl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(5-benzofuranyl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(5-methyl-benzimidazol-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(6-methyl-benzimidazol-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(5-chloro-benzo[b]thien-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(5-azaindol-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(6-azabenzothien-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(1H-2-oxo-pyrrolo[2',3',5,6]benzo[b]thien-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(6-methyl-benzothiazol-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(6-methoxy-indol-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(6-methoxy-benzo[b]thien-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(6-acetylamino-benzo[b]thien-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 1-(5-acetylamino-benzo[b]thien-2-yl)-3-[1-(phenylmethyl)-4-piperidinyl]-1-propanone; 6-hydroxy-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-1,2-benzisoxazole; 5-methyl-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-1,2-benzisoxazole; 6-methoxy-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-1,2-benzisoxazole; 6-acetamide-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-1,2-benzisoxazole; 6-amino-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-1,2-benzisoxazole; 6-(4-morpholinyl)-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-1,2-benzisoxazole; 5,7-dihydro-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-6H-pyrrolo[4,5-f]-1,2-benzisoxazol-6-one; 3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-1,2-benzisothiazole; 3-[2-[1-(phenylmethyl)-4-piperidinyl]ethenyl]-1,2-benzisoxazole; 6-phenylamino-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-1,2-benzisoxazole; 6-(2-thiazolyl)-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-1,2-benzisoxazole; 6-(2-oxazolyl)-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-1,2-benzisoxazole; 6-pyrrolidinyl-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-1,2-

benzisoxazole; 5,7-dihydro-5,5-dimethyl-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-6H-pyrrolo[4,5-f]-1,2-benzisoxazole-6-one; 6,8-dihydro-3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-7H-pyrrolo[5,4-g]-1,2-benzisoxazole-7-one; 3-[2-[1-(phenylmethyl)-4-piperidinyl]ethyl]-5,6,8-trihydro-7H-isoxazolo[4,5-g]-quinolin-7-one; 1-benzyl-4-((5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine, 1-benzyl-4-((5,6-dimethoxy-1-indanon)-2-ylidenyl)methylpiperidine, 1-benzyl-4-((5-methoxy-1-indanon)-2-yl)methylpiperidine, 1-benzyl-4-((5,6-diethoxy-1-indanon)-2-yl)methylpiperidine, 1-benzyl-4-((5,6-methylenedioxy-1-indanon)-2-yl)methylpiperidine, 1-(m-nitrobenzyl)-4-((5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine, 1-cyclohexymethyl-4-((5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine, 1-(m-florobenzyl)-4-((5,6-dimethoxy-1-indanon)-2-yl)methylpiperidine, 1-benzyl-4-((5,6-dimethoxy-1-indanon)-2-yl)propylpiperidine, and 1-benzyl-4-((5-isopropoxy-6-methoxy-1-indanon)-2-yl)methylpiperidine.

Exemplary calcium channel antagonists include diltiazem, omega-conotoxin GVIA, methoxyverapamil, amlodipine, felodipine, lacidipine, and mibefradil.

Exemplary GABA-A receptor modulators include clomethiazole; IDDB; gaboxadol (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol); ganaxolone (3.alpha.-hydroxy-3.beta.-methyl-5.alpha.-pregnan-20-one); fengabine (2-[(butylimino)-(2-chlorophenyl)methyl]-4-chlorophenol); 2-(4-methoxyphenyl)-2,5,6,7,8,9-hexahydro-pyrazolo[4,3-c]cinnolin-3-one; 7-cyclobutyl-6-(2-methyl-2H-1,2,4-triazol-3-ylmethoxy)-3-phenyl-1,2,4-triazolo[4,3-b]pyridazine; (3-fluoro-4-methylphenyl)-N-({-1-[(2-methylphenyl)methyl]-benzimidazol-2-yl}methyl)-N-pentylcarboxamide; and 3-(aminomethyl)-5-methylhexanoic acid.

Exemplary potassium channel openers include diazoxide, flupirtine, pinacidil, levcromakalim, rilimakalim, chromakalim, PCO-400 and SKP-450 (2-[2''(1'', 3''-dioxolone)-2-methyl]-4-(2'-oxo-1'-pyrrolidinyl)-6-nitro-2H-1-benzopyran).

Exemplary AMPA/kainate receptor antagonists include 6-cyano-7-nitroquinoxalin-2,3-di-one (CNQX); 6-nitro-7-sulphamoylbenzo[f]quinoxaline-2,3-dione (NBQX); 6,7-dinitroquinoxaline-2,3-dione (DNQX); 1-(4-aminophenyl)-4-methyl-7,8-m-ethylenedioxy-5H-2,3-benzodiazepine hydrochloride; and 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo-[f]quinoxaline.

Exemplary sodium channel antagonists include ajmaline, procainamide, flecainide and riluzole.

Exemplary matrix-metalloprotease inhibitors include 4-[4-(4-fluorophenoxy)benzenesulfon-ylamino]tetrahydropyran-4-carboxylic acid
5 hydroxyamide; 5-Methyl-5-(4-(4'-fluorophenoxy)-phenoxy)-pyrimidine-2,4,6-trione; 5-n-Butyl-5-(4-(4'-fluorophenoxy)-phenoxy)-pyrimidine-2,4,6-trione and prinomistat.

Poly(ADP ribose) polymerase (PARP) is an abundant nuclear enzyme which is activated by DNA strand single breaks to synthesize poly (ADP ribose) from
10 NAD. Under normal conditions, PARP is involved in base excision repair caused by oxidative stress via the activation and recruitment of DNA repair enzymes in the nucleus. Thus, PARP plays a role in cell necrosis and DNA repair. PARP also participates in regulating cytokine expression that mediates inflammation. Under conditions where DNA damage is excessive (such as by acute excessive exposure to
15 a pathological insult), PARP is over-activated, resulting in cell-based energetic failure characterized by NAD depletion and leading to ATP consumption, cellular necrosis, tissue injury, and organ damage/failure. PARP is thought to contribute to neurodegeneration by depleting nicotinamide adenine dinucleotide (NAD⁺) which then reduces adenosine triphosphate (ATP; Cosi and Marien, Ann. N.Y. Acad. Sci.,
20 890:227, 1999) contributing to cell death which can be prevented by PARP inhibitors. Exemplary PARP inhibitors can be found in Southan and Szabo, Current Medicinal Chemistry, 10:321, 2003.

Exemplary inhibitors of p38 MAP kinase and c-jun-N-terminal kinases include pyridyl imidazoles, such as PD 169316, isomeric PD 169316, SB 203580,
25 SB 202190, SB 220026, and RWJ 67657. Others are described in US Patent 6,288,089, and incorporated by reference herein.

In an exemplary embodiment, a combination therapy for treating or preventing MS comprises a therapeutically effective amount of one or more CLK-inhibiting compounds and one or more of Avonex[®] (interferon beta-1a), Tysabri[®]
30 (natalizumab), or Fumaderm[®] (BG-12/Oral Fumarate).

In another embodiment, a combination therapy for treating or preventing diabetic neuropathy or conditions associated therewith comprises a therapeutically

effective amount of one or more CLK-inhibiting compounds and one or more of tricyclic antidepressants (TCAs) (including, for example, imipramine, amitriptyline, desipramine and nortriptyline), serotonin reuptake inhibitors (SSRIs) (including, for example, fluoxetine, paroxetine, sertralene, and citalopram) and antiepileptic drugs (AEDs) (including, for example, gabapentin, carbamazepine, and topiramate).

In another embodiment, the invention provides a method for treating or preventing a polyglutamine disease using a combination comprising at least one CLK-inhibiting compound and at least one HDAC I/II inhibitor. Examples of HDAC I/II inhibitors include hydroxamic acids, cyclic peptides, benzamides, short-chain fatty acids, and depudecin.

Examples of hydroxamic acids and hydroxamic acid derivatives, but are not limited to, trichostatin A (TSA), suberoylanilide hydroxamic acid (SAHA), oxamflatin, suberic bishydroxamic acid (SBHA), m-carboxy-cinnamic acid bishydroxamic acid (CBHA), valproic acid and pyroxamide. TSA was isolated as an antifungal antibiotic (Tsuji et al (1976) J. Antibiot (Tokyo) 29:1-6) and found to be a potent inhibitor of mammalian HDAC (Yoshida et al. (1990) J. Biol. Chem. 265:17174-17179). The finding that TSA-resistant cell lines have an altered HDAC evidences that this enzyme is an important target for TSA. Other hydroxamic acid-based HDAC inhibitors, SAHA, SBHA, and CBHA are synthetic compounds that are able to inhibit HDAC at micromolar concentration or lower in vitro or in vivo. Glick et al. (1999) Cancer Res. 59:4392-4399. These hydroxamic acid-based HDAC inhibitors all possess an essential structural feature: a polar hydroxamic terminal linked through a hydrophobic methylene spacer (e.g. 6 carbon at length) to another polar site which is attached to a terminal hydrophobic moiety (e.g., benzene ring). Compounds developed having such essential features also fall within the scope of the hydroxamic acids that may be used as HDAC inhibitors.

Cyclic peptides used as HDAC inhibitors are mainly cyclic tetrapeptides. Examples of cyclic peptides include, but are not limited to, trapoxin A, apicidin and depsipeptide. Trapoxin A is a cyclic tetrapeptide that contains a 2-amino-8-oxo-9,10-epoxy-decanoyl (AOE) moiety. Kijima et al. (1993) J. Biol. Chem. 268:22429-22435. Apicidin is a fungal metabolite that exhibits potent, broad-spectrum antiprotozoal activity and inhibits HDAC activity at nanomolar concentrations.

Darkin-Rattray et al. (1996) Proc. Natl. Acad. Sci. USA. 93:13143-13147.

Depsipeptide is isolated from *Chromobacterium violaceum*, and has been shown to inhibit HDAC activity at micromolar concentrations.

Examples of benzamides include but are not limited to MS-27-275. Saito et al. (1990) Proc. Natl. Acad. Sci. USA. 96:4592-4597. Examples of short-chain fatty acids include but are not limited to butyrates (e.g., butyric acid, arginine butyrate and phenylbutyrate (PB)). Newmark et al. (1994) Cancer Lett. 78:1-5; and Carducci et al. (1997) Anticancer Res. 17:3972-3973. In addition, depudecin which has been shown to inhibit HDAC at micromolar concentrations (Kwon et al. (1998) Proc. Natl. Acad. Sci. USA. 95:3356-3361) also falls within the scope of histone deacetylase inhibitor as described herein.

v. Blood Coagulation Disorders

In other aspects, CLK-inhibiting compounds can be used to treat or prevent blood coagulation disorders (or hemostatic disorders). As used interchangeably herein, the terms “hemostasis”, “blood coagulation,” and “blood clotting” refer to the control of bleeding, including the physiological properties of vasoconstriction and coagulation. Blood coagulation assists in maintaining the integrity of mammalian circulation after injury, inflammation, disease, congenital defect, dysfunction or other disruption. After initiation of clotting, blood coagulation proceeds through the sequential activation of certain plasma proenzymes to their enzyme forms (see, for example, Coleman, R. W. et al. (eds.) *Hemostasis and Thrombosis, Second Edition*, (1987)). These plasma glycoproteins, including Factor XII, Factor XI, Factor IX, Factor X, Factor VII, and prothrombin, are zymogens of serine proteases. Most of these blood clotting enzymes are effective on a physiological scale only when assembled in complexes on membrane surfaces with protein cofactors such as Factor VIII and Factor V. Other blood factors modulate and localize clot formation, or dissolve blood clots. Activated protein C is a specific enzyme that inactivates procoagulant components. Calcium ions are involved in many of the component reactions. Blood coagulation follows either the intrinsic pathway, where all of the protein components are present in blood, or the extrinsic pathway, where the cell-membrane protein tissue factor plays a critical role. Clot

formation occurs when fibrinogen is cleaved by thrombin to form fibrin. Blood clots are composed of activated platelets and fibrin.

Further, the formation of blood clots does not only limit bleeding in case of an injury (hemostasis), but may lead to serious organ damage and death in the context of atherosclerotic diseases by occlusion of an important artery or vein. Thrombosis is thus blood clot formation at the wrong time and place. It involves a cascade of complicated and regulated biochemical reactions between circulating blood proteins (coagulation factors), blood cells (in particular platelets), and elements of an injured vessel wall.

Accordingly, the present invention provides anticoagulation and antithrombotic treatments aiming at inhibiting the formation of blood clots in order to prevent or treat blood coagulation disorders, such as myocardial infarction, stroke, loss of a limb by peripheral artery disease or pulmonary embolism.

As used interchangeably herein, “modulating or modulation of hemostasis” and “regulating or regulation of hemostasis” includes the induction (e.g., stimulation or increase) of hemostasis, as well as the inhibition (e.g., reduction or decrease) of hemostasis.

In one aspect, the invention provides a method for reducing or inhibiting hemostasis in a subject by administering a CLK-inhibiting compound. The compositions and methods disclosed herein are useful for the treatment or prevention of thrombotic disorders. As used herein, the term “thrombotic disorder” includes any disorder or condition characterized by excessive or unwanted coagulation or hemostatic activity, or a hypercoagulable state. Thrombotic disorders include diseases or disorders involving platelet adhesion and thrombus formation, and may manifest as an increased propensity to form thromboses, e.g., an increased number of thromboses, thrombosis at an early age, a familial tendency towards thrombosis, and thrombosis at unusual sites. Examples of thrombotic disorders include, but are not limited to, thromboembolism, deep vein thrombosis, pulmonary embolism, stroke, myocardial infarction, miscarriage, thrombophilia associated with anti-thrombin III deficiency, protein C deficiency, protein S deficiency, resistance to activated protein C, dysfibrinogenemia, fibrinolytic disorders, homocystinuria, pregnancy, inflammatory disorders, myeloproliferative disorders, arteriosclerosis,

angina, e.g., unstable angina, disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, cancer metastasis, sickle cell disease, glomerular nephritis, and drug induced thrombocytopenia (including, for example, heparin induced thrombocytopenia). In addition, CLK-inhibiting compounds may be
5 administered to prevent thrombotic events or to prevent re-occlusion during or after therapeutic clot lysis or procedures such as angioplasty or surgery.

In another embodiment, a combination drug regimen may include drugs or compounds for the treatment or prevention of blood coagulation disorders or secondary conditions associated with these conditions. Thus, a combination drug
10 regimen may include one or more CLK-inhibiting compounds and one or more anti-coagulation or anti-thrombosis agents. For example, one or more CLK-inhibiting compounds can be combined with an effective amount of one or more of: aspirin, heparin, and oral Warfarin that inhibits Vit K-dependent factors, low molecular weight heparins that inhibit factors X and II, thrombin inhibitors, inhibitors of
15 platelet GP IIb/IIIa receptors, inhibitors of tissue factor (TF), inhibitors of human von Willebrand factor, inhibitors of one or more factors involved in hemostasis (in particular in the coagulation cascade). In addition, CLK-inhibiting compounds can be combined with thrombolytic agents, such as t-PA, streptokinase, reptilase, TNK-t-PA, and staphylokinase.

20 *vi. Weight Control*

In another aspect, CLK-inhibiting compounds may be used for treating or preventing weight gain or obesity in a subject. For example, CLK-inhibiting compounds may be used, for example, to treat or prevent hereditary obesity, dietary obesity, hormone related obesity, obesity related to the administration of medication,
25 to reduce the weight of a subject, or to reduce or prevent weight gain in a subject. A subject in need of such a treatment may be a subject who is obese, likely to become obese, overweight, or likely to become overweight. Subjects who are likely to become obese or overweight can be identified, for example, based on family history, genetics, diet, activity level, medication intake, or various combinations thereof.

30 In yet other embodiments, CLK-inhibiting compounds may be administered to subjects suffering from a variety of other diseases and conditions that may be treated or prevented by promoting weight loss in the subject. Such diseases include,

for example, high blood pressure, hypertension, high blood cholesterol, dyslipidemia, type 2 diabetes, insulin resistance, glucose intolerance, hyperinsulinemia, coronary heart disease, angina pectoris, congestive heart failure, stroke, gallstones, cholecystitis and cholelithiasis, gout, osteoarthritis, obstructive
5 sleep apnea and respiratory problems, some types of cancer (such as endometrial, breast, prostate, and colon), complications of pregnancy, poor female reproductive health (such as menstrual irregularities, infertility, irregular ovulation), bladder control problems (such as stress incontinence); uric acid nephrolithiasis; psychological disorders (such as depression, eating disorders, distorted body image,
10 and low self esteem). Stunkard AJ, Wadden TA. (Editors) Obesity: theory and therapy, Second Edition. New York: Raven Press, 1993. Finally, patients with AIDS can develop lipodystrophy or insulin resistance in response to combination therapies for AIDS.

In another embodiment, CLK-inhibiting compounds may be used for
15 inhibiting adipogenesis or fat cell differentiation, whether in vitro or in vivo. In particular, high circulating levels of insulin and/or insulin like growth factor (IGF) 1 will be prevented from recruiting preadipocytes to differentiate into adipocytes. Such methods may be used for treating or preventing obesity.

In other embodiments, CLK-inhibiting compounds may be used for reducing
20 appetite and/or increasing satiety, thereby causing weight loss or avoidance of weight gain. A subject in need of such a treatment may be a subject who is overweight, obese or a subject likely to become overweight or obese. The method may comprise administering daily or, every other day, or once a week, a dose, e.g., in the form of a pill, to a subject. The dose may be an "appetite reducing dose."

25 A method for modulating weight may further comprise monitoring the weight of the subject and/or the level of modulation of CLKs, for example, in adipose tissue.

In an exemplary embodiment, CLK-inhibiting compounds may be administered as a combination therapy for treating or preventing weight gain or
30 obesity. For example, one or more CLK-inhibiting compounds may be administered in combination with one or more anti-obesity agents. Exemplary anti-obesity agents include, for example, phenylpropanolamine, ephedrine, pseudoephedrine,

phentermine, a cholecystokinin-A agonist, a monoamine reuptake inhibitor (such as sibutramine), a sympathomimetic agent, a serotonergic agent (such as dexfenfluramine or fenfluramine), a dopamine agonist (such as bromocriptine), a melanocyte-stimulating hormone receptor agonist or mimetic, a melanocyte-stimulating hormone analog, a cannabinoid receptor antagonist, a melanin concentrating hormone antagonist, the OB protein (leptin), a leptin analog, a leptin receptor agonist, a galanin antagonist or a GI lipase inhibitor or decreaser (such as orlistat). Other anorectic agents include bombesin agonists, dehydroepiandrosterone or analogs thereof, glucocorticoid receptor agonists and antagonists, orexin receptor antagonists, urocortin binding protein antagonists, agonists of the glucagon-like peptide-1 receptor such as Exendin and ciliary neurotrophic factors such as Axokine.

In another embodiment, CLK-inhibiting compounds may be administered to reduce drug-induced weight gain. For example, a CLK-inhibiting compound may be administered as a combination therapy with medications that may stimulate appetite or cause weight gain, in particular, weight gain due to factors other than water retention. Examples of medications that may cause weight gain, include for example, diabetes treatments, including, for example, sulfonylureas (such as glipizide and glyburide), thiazolidinediones (such as pioglitazone and rosiglitazone), meglitinides, nateglinide, repaglinide, sulphonylurea medicines, and insulin; antidepressants, including, for example, tricyclic antidepressants (such as amitriptyline and imipramine), irreversible monoamine oxidase inhibitors (MAOIs), selective serotonin reuptake inhibitors (SSRIs), bupropion, paroxetine, and mirtazapine; steroids, such as, for example, prednisone; hormone therapy; lithium carbonate; valproic acid; carbamazepine; chlorpromazine; thiothixene; beta blockers (such as propranolol); alpha blockers (such as clonidine, prazosin and terazosin); and contraceptives including oral contraceptives (birth control pills) or other contraceptives containing estrogen and/or progesterone (Depo-Provera, Norplant, Ortho), testosterone or Megestrol. In another exemplary embodiment, CLK-inhibiting compounds may be administered as part of a smoking cessation program to prevent weight gain or reduce weight already gained.

In certain embodiments, the CLK-inhibiting compounds may be used to reduce the weight or prevent weight gain in a subject, wherein said subject does not

reduce calorie consumption, increase activity or a combination thereof to an extent sufficient to cause weight loss in the absence of the CLK-inhibiting compound.

vii. Metabolic Disorders/Diabetes

In another aspect, CLK-inhibiting compounds may be used for treating or
5 preventing a metabolic disorder, such as insulin-resistance, a pre-diabetic state, type II diabetes, and/or complications thereof. Administration of a CLK-inhibiting compound may increase insulin sensitivity and/or decrease insulin levels in a subject. A subject in need of such a treatment may be a subject who has insulin resistance or other precursor symptom of type II diabetes, who has type II diabetes,
10 or who is likely to develop any of these conditions. For example, the subject may be a subject having insulin resistance, e.g., having high circulating levels of insulin and/or associated conditions, such as hyperlipidemia, dyslipogenesis, hypercholesterolemia, impaired glucose tolerance, high blood glucose sugar level, other manifestations of syndrome X, hypertension, atherosclerosis and
15 lipodystrophy.

In an exemplary embodiment, CLK-inhibiting compounds may be administered as a combination therapy for treating or preventing a metabolic disorder. For example, one or more CLK-inhibiting compounds may be administered in combination with one or more anti-diabetic agents. Exemplary anti-
20 diabetic agents include, for example, an aldose reductase inhibitor, a glycogen phosphorylase inhibitor, a sorbitol dehydrogenase inhibitor, a protein tyrosine phosphatase 1B inhibitor, a dipeptidyl protease inhibitor, insulin (including orally bioavailable insulin preparations), an insulin mimetic, metformin, acarbose, a peroxisome proliferator-activated receptor- γ (PPAR- γ) ligand such as troglitazone, rosaglitazone, pioglitazone or GW-1929, a sulfonylurea, glipazide, glyburide, or
25 chlorpropamide wherein the amounts of the first and second compounds result in a therapeutic effect. Other anti-diabetic agents include a glucosidase inhibitor, a glucagon-like peptide-1 (GLP-1), insulin, a PPAR α/γ dual agonist, a meglitimide and an $\alpha P2$ inhibitor. In an exemplary embodiment, an anti-diabetic agent may be a
30 dipeptidyl peptidase IV (DP-IV or DPP-IV) inhibitor, such as, for example LAF237 from Novartis (NVP DPP728; 1-[[[2-[(5-cyanopyridin-2-yl)amino]

ethyl]amino]acetyl]-2- cyano-(S)- pyrrolidine) or MK-04301 from Merck (see e.g., Hughes et al., Biochemistry 38: 11597-603 (1999)).

viii. Inflammatory Diseases

In other aspects, CLK-inhibiting compounds can be used to treat or prevent
5 a disease or disorder associated with inflammation. CLK-inhibiting compounds may be administered prior to the onset of, at, or after the initiation of inflammation. When used prophylactically, the compounds are preferably provided in advance of any inflammatory response or symptom. Administration of the compounds may prevent or attenuate inflammatory responses or symptoms.

10 Exemplary inflammatory conditions include, for example, multiple sclerosis, rheumatoid arthritis, psoriatic arthritis, degenerative joint disease, spondouloarthropathies, gouty arthritis, systemic lupus erythematosus, juvenile arthritis, rheumatoid arthritis, osteoarthritis, osteoporosis, diabetes (e.g., insulin dependent diabetes mellitus or juvenile onset diabetes), menstrual cramps, cystic
15 fibrosis, inflammatory bowel disease, irritable bowel syndrome, Crohn's disease, mucous colitis, ulcerative colitis, gastritis, esophagitis, pancreatitis, peritonitis, Alzheimer's disease, shock, ankylosing spondylitis, gastritis, conjunctivitis, pancreatitis (acute or chronic), multiple organ injury syndrome (e.g., secondary to septicemia or trauma), myocardial infarction, atherosclerosis, stroke, reperfusion
20 injury (e.g., due to cardiopulmonary bypass or kidney dialysis), acute glomerulonephritis, vasculitis, thermal injury (i.e., sunburn), necrotizing enterocolitis, granulocyte transfusion associated syndrome, and/or Sjogren's syndrome. Exemplary inflammatory conditions of the skin include, for example, eczema, atopic dermatitis, contact dermatitis, urticaria, schleroderma, psoriasis, and
25 dermatosis with acute inflammatory components.

In another embodiment, CLK-inhibiting compounds may be used to treat or prevent allergies and respiratory conditions, including asthma, bronchitis, pulmonary fibrosis, allergic rhinitis, oxygen toxicity, emphysema, chronic bronchitis, acute respiratory distress syndrome, and any chronic obstructive
30 pulmonary disease (COPD). The compounds may be used to treat chronic hepatitis infection, including hepatitis B and hepatitis C.

Additionally, CLK-inhibiting compounds may be used to treat autoimmune diseases and/or inflammation associated with autoimmune diseases such as organ-tissue autoimmune diseases (e.g., Raynaud's syndrome), scleroderma, myasthenia gravis, transplant rejection, endotoxin shock, sepsis, psoriasis, eczema, dermatitis, multiple sclerosis, autoimmune thyroiditis, uveitis, systemic lupus erythematosus, Addison's disease, autoimmune polyglandular disease (also known as autoimmune polyglandular syndrome), and Grave's disease.

In certain embodiments, one or more CLK-inhibiting compounds may be taken alone or in combination with other compounds useful for treating or preventing inflammation. Exemplary anti-inflammatory agents include, for example, steroids (e.g., cortisol, cortisone, fludrocortisone, prednisone, 6 α -methylprednisone, triamcinolone, betamethasone or dexamethasone), nonsteroidal antiinflammatory drugs (NSAIDS (e.g., aspirin, acetaminophen, tolmetin, ibuprofen, mefenamic acid, piroxicam, nabumetone, rofecoxib, celecoxib, etodolac or nimesulide). In another embodiment, the other therapeutic agent is an antibiotic (e.g., vancomycin, penicillin, amoxicillin, ampicillin, cefotaxime, ceftriaxone, cefixime, rifampin, metronidazole, doxycycline or streptomycin). In another embodiment, the other therapeutic agent is a PDE4 inhibitor (e.g., roflumilast or rolipram). In another embodiment, the other therapeutic agent is an antihistamine (e.g., cyclizine, hydroxyzine, promethazine or diphenhydramine). In another embodiment, the other therapeutic agent is an anti-malarial (e.g., artemisinin, artemether, artesunate, chloroquine phosphate, mefloquine hydrochloride, doxycycline hyclate, proguanil hydrochloride, atovaquone or halofantrine). In one embodiment, the other therapeutic agent is drotrecogin alfa.

Further examples of anti-inflammatory agents include, for example, aceclofenac, acetaminophen, e-acetamidocaproic acid, acetaminophen, acetaminosalol, acetanilide, acetylsalicylic acid, S-adenosylmethionine, alclofenac, alclometasone, alfentanil, algestone, allylprodine, alminoprofen, aloxiprin, alphaprodine, aluminum bis(acetylsalicylate), amcinonide, amfenac, aminochlorthenoxazin, 3-amino-4-hydroxybutyric acid, 2-amino-4-picoline, aminopropyl, aminopyrine, amixetrine, ammonium salicylate, ampiroxicam, amtolmetin guacil, anileridine, antipyrine, antrafenine, apazone, beclomethasone, bendazac, benorylate,

- benoxaprofen, benzpiperylon, benzydamine, benzylmorphine, bermoprofen,
 betamethasone, betamethasone-17-valerate, bezitramide, α -bisabolol, bromfenac,
 p-bromoacetanilide, 5-bromosalicylic acid acetate, bromosaligenin, buccetin,
 bucloxic acid, bucolome, budesonide, bufexamac, bumadizon, buprenorphine,
 5 butacetin, butibufen, butorphanol, carbamazepine, carbiphen, carprofen, carsalam,
 chlorobutanol, chloroprednisone, chlorthenoxazin, choline salicylate, cinchophen,
 cinmetacin, ciramadol, clidanac, clobetasol, clocortolone, clometacin, clonitazene,
 clonixin, clopirac, cloprednol, clove, codeine, codeine methyl bromide, codeine
 phosphate, codeine sulfate, cortisone, cortivazol, cropropamide, crotethamide,
 10 cyclazocine, deflazacort, dehydrotestosterone, desomorphine, desonide,
 desoximetasone, dexamethasone, dexamethasone-21-isonicotinate, dexoadrol,
 dextromoramide, dextropropoxyphene, deoxycorticosterone, dezocine,
 diampromide, diamorphine, diclofenac, difenamizole, difenpiramide, diflorasone,
 diflucortolone, diflunisal, difluprednate, dihydrocodeine, dihydrocodeinone enol
 15 acetate, dihydromorphine, dihydroxyaluminum acetylsalicylate, dimenoxadol,
 dimepheptanol, dimethylthiambutene, dioxaphetyl butyrate, dipipanone, diprocetyl,
 dipyrone, ditazol, droxicam, emorfazone, enfenamic acid, enoxolone, epirizole,
 eptazocine, etersalate, ethenzamide, ethoheptazine, ethoxazene,
 ethylmethylthiambutene, ethylmorphine, etodolac, etofenamate, etonitazene,
 20 eugenol, felbinac, fenbufen, fenclozic acid, fendosal, fenoprofen, fentanyl,
 fentiazac, fepradinol, feprazone, floctafenine, fluazacort, flucloronide, flufenamic
 acid, flumethasone, flunisolide, flunixin, flunoxaprofen, fluocinolone acetonide,
 fluocinonide, fluocinolone acetonide, fluocortin butyl, fluocortolone, fluoresone,
 fluorometholone, fluperolone, flupirtine, fluprednidene, fluprednisolone,
 25 fluproquazone, flurandrenolide, flurbiprofen, fluticasone, formocortal, fosfosal,
 gentisic acid, glafenine, glucametacin, glycol salicylate, guaiazulene, halcinonide,
 halobetasol, halometasone, haloprednone, heroin, hydrocodone, hydrocortamate,
 hydrocortisone, hydrocortisone acetate, hydrocortisone succinate, hydrocortisone
 hemisuccinate, hydrocortisone 21-lysinate, hydrocortisone cypionate,
 30 hydromorphone, hydroxypethidine, ibufenac, ibuprofen, ibuproxam, imidazole
 salicylate, indomethacin, indoprofen, isofezolac, isoflupredone, isoflupredone
 acetate, isoladol, isomethadone, isonixin, isoxepac, isoxicam, ketobemidone,

ketoprofen, ketorolac, p-lactophenetide, lefetamine, levallorphan, levorphanol, levophenacyl-morphan, lofentanil, lonazolac, lornoxicam, loxoprofen, lysine acetylsalicylate, mazipredone, meclofenamic acid, medrysone, mefenamic acid, meloxicam, meperidine, meprednisone, meptazinol, mesalamine, metazocine, methadone, methotrimeprazine, methylprednisolone, methylprednisolone acetate, methylprednisolone sodium succinate, methylprednisolone suleptnate, metiazinic acid, metofoline, metopon, mofebutazone, mofezolac, mometasone, morazone, morphine, morphine hydrochloride, morphine sulfate, morpholine salicylate, myrophine, nabumetone, nalbuphine, nalorphine, 1-naphthyl salicylate, naproxen, narceine, nefopam, nicomorphine, nifenazone, niflumic acid, nimesulide, 5'-nitro-2'-propoxyacetanilide, norlevorphanol, normethadone, normorphine, norpipanone, olsalazine, opium, oxaceprol, oxametacine, oxaprozin, oxycodone, oxymorphone, oxyphenbutazone, papaveretum, paramethasone, paranyline, parsalimide, pentazocine, perisoxal, phenacetin, phenadoxone, phenazocine, phenazopyridine hydrochloride, phenocoll, phenoperidine, phenopyrazone, phenomorphan, phenyl acetylsalicylate, phenylbutazone, phenyl salicylate, phenylramidol, piketoprofen, piminodine, pipebuzone, piperylone, pirazolac, piritramide, piroxicam, pirprofen, pranoprofen, prednicarbate, prednisolone, prednisone, prednival, prednylidene, proglumetacin, proheptazine, promedol, propacetamol, properidine, propiram, propoxyphene, propyphenazone, proquazone, protizinic acid, proxazole, ramifenazone, remifentanil, rimazolium metilsulfate, salacetamide, salicin, salicylamide, salicylamide o-acetic acid, salicylic acid, salicylsulfuric acid, salsalate, salverine, simetride, sufentanil, sulfasalazine, sulindac, superoxide dismutase, suprofen, suxibuzone, talniflumate, tenidap, tenoxicam, terofenamate, tetrandrine, thiazolinobutazone, tiaprofenic acid, tiaramide, tilidine, tinoridine, tixocortol, tolfenamic acid, tolmetin, tramadol, triamcinolone, triamcinolone acetone, tropesin, viminol, xenbucin, ximoprofen, zaltoprofen and zomepirac.

In an exemplary embodiment, a CLK-inhibiting compound may be administered with a selective COX-2 inhibitor for treating or preventing inflammation. Exemplary selective COX-2 inhibitors include, for example, deracoxib, parecoxib, celecoxib, valdecoxib, rofecoxib, etoricoxib, lumiracoxib, 2-(3,5-difluorophenyl)-3-[4-(methylsulfonyl)phenyl]-2-cyclopenten-1-one, (S)-6,8-

dichloro-2-(trifluoromethyl)-2H-1-benzopyran-3-carboxylic acid, 2-(3,4-difluorophenyl)-4-(3-hydroxy-3-methyl-1-butoxy)-5-[4-(methylsulfonyl)phenyl]-3-(2H)-pyridazinone, 4-[5-(4-fluorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide, tert-butyl 1-benzyl-4-[(4-oxopiperidin-1-yl)sulfonyl]piperidine-4-carboxylate, 4-[5-(phenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide, salts and prodrugs thereof.

ix. Flushing

In another aspect, CLK-inhibiting compounds may be used for reducing the incidence or severity of flushing and/or hot flashes which are symptoms of a disorder. For instance, the subject method includes the use of CLK-inhibiting compounds, alone or in combination with other agents, for reducing incidence or severity of flushing and/or hot flashes in cancer patients. In other embodiments, the method provides for the use of CLK-inhibiting compounds to reduce the incidence or severity of flushing and/or hot flashes in menopausal and post-menopausal woman.

In another aspect, CLK-inhibiting compounds may be used as a therapy for reducing the incidence or severity of flushing and/or hot flashes which are side-effects of another drug therapy, e.g., drug-induced flushing. In certain embodiments, a method for treating and/or preventing drug-induced flushing comprises administering to a patient in need thereof a formulation comprising at least one flushing inducing compound and at least one CLK-inhibiting compound. In other embodiments, a method for treating drug induced flushing comprises separately administering one or more compounds that induce flushing and one or more CLK-inhibiting compounds, e.g., wherein the CLK-inhibiting compound and flushing inducing agent have not been formulated in the same compositions. When using separate formulations, the CLK-inhibiting compound may be administered (1) at the same as administration of the flushing inducing agent, (2) intermittently with the flushing inducing agent, (3) staggered relative to administration of the flushing inducing agent, (4) prior to administration of the flushing inducing agent, (5) subsequent to administration of the flushing inducing agent, and (6) various combination thereof. Exemplary flushing inducing agents include, for example,

niacin, faloixifene, antidepressants, anti-psychotics, chemotherapeutics, calcium channel blockers, and antibiotics.

In one embodiment, CLK-inhibiting compounds may be used to reduce flushing side effects of a vasodilator or an antilipemic agent (including
5 anticholesteremic agents and lipotropic agents). In an exemplary embodiment, a CLK-inhibiting compound may be used to reduce flushing associated with the administration of niacin.

Nicotinic acid, 3-pyridinecarboxylic acid or niacin, is an antilipidemic agent that is marketed under, for example, the trade names Nicolar[®], SloNiacin[®], Nicobid[®]
10 and Time Release Niacin[®]. Nicotinic acid has been used for many years in the treatment of lipidemic disorders such as hyperlipidemia, hypercholesterolemia and atherosclerosis. This compound has long been known to exhibit the beneficial effects of reducing total cholesterol, low density lipoproteins or "LDL cholesterol," triglycerides and apolipoprotein a (Lp(a)) in the human body, while increasing
15 desirable high density lipoproteins or "HDL cholesterol".

Typical doses range from about 1 gram to about 3 grams daily. Nicotinic acid is normally administered two to four times per day after meals, depending upon the dosage form selected. Nicotinic acid is currently commercially available in two
20 dosage forms. One dosage form is an immediate or rapid release tablet which should be administered three or four times per day. Immediate release ("IR") nicotinic acid formulations generally release nearly all of their nicotinic acid within about 30 to 60 minutes following ingestion. The other dosage form is a sustained release form which is suitable for administration two to four times per day. In contrast to IR formulations, sustained release ("SR") nicotinic acid formulations are designed to
25 release significant quantities of drug for absorption into the blood stream over specific timed intervals in order to maintain therapeutic levels of nicotinic acid over an extended period such as 12 or 24 hours after ingestion.

As used herein, the term "nicotinic acid" is meant to encompass nicotinic acid or a compound other than nicotinic acid itself which the body metabolizes into
30 nicotinic acid, thus producing essentially the same effect as nicotinic acid. Exemplary compounds that produce an effect similar to that of nicotinic acid include, for example, nicotinyl alcohol tartrate, d-glucitol hexanicotinate, aluminum

nicotinate, niceritrol and d,1-alpha-tocopheryl nicotinate. Each such compound will be collectively referred to herein as "nicotinic acid."

In another embodiment, the invention provides a method for treating and/or preventing hyperlipidemia with reduced flushing side effects. The method comprises
5 the steps of administering to a subject in need thereof a therapeutically effective amount of nicotinic acid and a CLK-inhibiting compound in an amount sufficient to reduce flushing. In an exemplary embodiment, the nicotinic acid and/or CLK-inhibiting compound may be administered nocturnally.

In another representative embodiment, the method involves the use of CLK-inhibiting compounds to reduce flushing side effects of raloxifene. Raloxifene acts
10 like estrogen in certain places in the body, but is not a hormone. It helps prevent osteoporosis in women who have reached menopause. Osteoporosis causes bones to gradually grow thin, fragile, and more likely to break. Evista slows down the loss of bone mass that occurs with menopause, lowering the risk of spine fractures due to
15 osteoporosis. A common side effect of raloxifene is hot flashes (sweating and flushing). This can be uncomfortable for women who already have hot flashes due to menopause.

In another representative embodiment, the method involves the use of CLK-inhibiting compounds to reduce flushing side effects of antidepressants or anti-
20 psychotic agent. For instance, CLK-inhibiting compounds can be used in conjunction (administered separately or together) with a serotonin reuptake inhibitor, a 5HT2 receptor antagonist, an anticonvulsant, a norepinephrine reuptake inhibitor, an α -adrenoreceptor antagonist, an NK-3 antagonist, an NK-1 receptor antagonist, a PDE4 inhibitor, an Neuropeptide Y5 Receptor Antagonists, a D4
25 receptor antagonist, a 5HT1A receptor antagonist, a 5HT1D receptor antagonist, a CRF antagonist, a monoamine oxidase inhibitor, or a sedative-hypnotic drug.

In certain embodiments, CLK-inhibiting compounds may be used as part of a treatment with a serotonin reuptake inhibitor (SRI) to reduce flushing. In certain preferred embodiments, the SRI is a selective serotonin reuptake inhibitor (SSRI),
30 such as a fluoxetine (fluoxetine, norfluoxetine) or a nefazodonoid (nefazodone, hydroxynefazodone, oxonefazodone). Other exemplary SSRI's include duloxetine, venlafaxine, milnacipran, citalopram, fluvoxamine, paroxetine and sertraline. The

CLK-inhibiting compound can also be used as part of a treatment with sedative-hypnotic drug, such as selected from the group consisting of a benzodiazepine (such as alprazolam, chlordiazepoxide, clonazepam, chlorazepate, clobazam, diazepam, halazepam, lorazepam, oxazepam and prazepam), zolpidem, and barbiturates. In still other embodiments, a CLK-inhibiting compound may be used as part of a treatment with a 5-HT_{1A} receptor partial agonist, such as selected from the group consisting of buspirone, flesinoxan, gepirone and ipsapirone. CLK-inhibiting compounds can also be used as part of a treatment with a norepinephrine reuptake inhibitor, such as selected from tertiary amine tricyclics and secondary amine tricyclics. Exemplary tertiary amine tricyclic include amitriptyline, clomipramine, doxepin, imipramine and trimipramine. Exemplary secondary amine tricyclic include amoxapine, desipramine, maprotiline, nortriptyline and protriptyline. In certain embodiments, CLK-inhibiting compounds may be used as part of a treatment with a monoamine oxidase inhibitor, such as selected from the group consisting of isocarboxazid, phenelzine, tranylcypromine, selegiline and moclobemide.

In still another representative embodiment, CLK-inhibiting compounds may be used to reduce flushing side effects of chemotherapeutic agents, such as cyclophosphamide, tamoxifen.

In another embodiment, CLK-inhibiting compounds may be used to reduce flushing side effects of calcium channel blockers, such as amlodipine.

In another embodiment, CLK-inhibiting compounds may be used to reduce flushing side effects of antibiotics. For example, CLK-inhibiting compounds can be used in combination with levofloxacin. Levofloxacin is used to treat infections of the sinuses, skin, lungs, ears, airways, bones, and joints caused by susceptible bacteria. Levofloxacin also is frequently used to treat urinary infections, including those resistant to other antibiotics, as well as prostatitis. Levofloxacin is effective in treating infectious diarrheas caused by *E. coli*, *campylobacter jejuni*, and *shigella* bacteria. Levofloxacin also can be used to treat various obstetric infections, including mastitis.

x. Ocular Disorders

One aspect of the present invention is a method for inhibiting, reducing or otherwise treating vision impairment by administering to a patient a therapeutic

dosage of a CLK-inhibiting compound, or a pharmaceutically acceptable salt, prodrug or a metabolic derivative thereof.

In certain aspects of the invention, the vision impairment is caused by damage to the optic nerve or central nervous system. In particular embodiments, 5 optic nerve damage is caused by high intraocular pressure, such as that created by glaucoma. In other particular embodiments, optic nerve damage is caused by swelling of the nerve, which is often associated with an infection or an immune (e.g., autoimmune) response such as in optic neuritis.

Glaucoma describes a group of disorders which are associated with a visual 10 field defect, cupping of the optic disc, and optic nerve damage. These are commonly referred to as glaucomatous optic neuropathies. Most glaucomas are usually, but not always, associated with a rise in intraocular pressure. Exemplary forms of glaucoma include Glaucoma and Penetrating Keratoplasty, Acute Angle Closure, Chronic Angle Closure, Chronic Open Angle, Angle Recession, Aphakic and Pseudophakic, 15 Drug-Induced, Hyphema, Intraocular Tumors, Juvenile, Lens-Particle, Low Tension, Malignant, Neovascular, Phacolytic, Phacomorphic, Pigmentary, Plateau Iris, Primary Congenital, Primary Open Angle, Pseudoexfoliation, Secondary Congenital, Adult Suspect, Unilateral, Uveitic, Ocular Hypertension, Ocular Hypotony, Posner-Schlossman Syndrome and Scleral Expansion Procedure in 20 Ocular Hypertension & Primary Open-angle Glaucoma.

Intraocular pressure can also be increased by various surgical procedures, such as phacoemulsification (i.e., cataract surgery) and implananation of structures such as an artificial lens. In addition, spinal surgeries in particular, or any surgery in which the patient is prone for an extended period of time can lead to increased 25 interocular pressure.

Optic neuritis (ON) is inflammation of the optic nerve and causes acute loss of vision. It is highly associated with multiple sclerosis (MS) as 15-25% of MS patients initially present with ON, and 50-75% of ON patients are diagnosed with MS. ON is also associated with infection (e.g., viral infection, meningitis, syphilis), 30 inflammation (e.g., from a vaccine), infiltration and ischemia.

Another condition leading to optic nerve damage is anterior ischemic optic neuropathy (AION). There are two types of AION. Arteritic AION is due to giant

cell arteritis (vasculitis) and leads to acute vision loss. Non-arteritic AION encompasses all cases of ischemic optic neuropathy other than those due to giant cell arteritis. The pathophysiology of AION is unclear although it appears to incorporate both inflammatory and ischemic mechanisms.

5 Other damage to the optic nerve is typically associated with demyelination, inflammation, ischemia, toxins, or trauma to the optic nerve. Exemplary conditions where the optic nerve is damaged include Demyelinating Optic Neuropathy (Optic Neuritis, Retrobulbar Optic Neuritis), Optic Nerve Sheath Meningioma, Adult Optic Neuritis, Childhood Optic Neuritis, Anterior Ischemic Optic Neuropathy, Posterior
10 Ischemic Optic Neuropathy, Compressive Optic Neuropathy, Papilledema, Pseudopapilledema and Toxic/Nutritional Optic Neuropathy.

 Other neurological conditions associated with vision loss, albeit not directly associated with damage to the optic nerve, include Amblyopia, Bells Palsy, Chronic Progressive External Ophthalmoplegia, Multiple Sclerosis, Pseudotumor Cerebri
15 and Trigeminal Neuralgia.

 In certain aspects of the invention, the vision impairment is caused by retinal damage. In particular embodiments, retinal damage is caused by disturbances in blood flow to the eye (e.g., arteriosclerosis, vasculitis). In particular embodiments, retinal damage is caused by disruption of the macula (e.g., exudative or non-
20 exudative macular degeneration).

 Exemplary retinal diseases include Exudative Age Related Macular Degeneration, Nonexudative Age Related Macular Degeneration, Retinal Electronic Prosthesis and RPE Transplantation Age Related Macular Degeneration, Acute Multifocal Placoid Pigment Epitheliopathy, Acute Retinal Necrosis, Best Disease,
25 Branch Retinal Artery Occlusion, Branch Retinal Vein Occlusion, Cancer Associated and Related Autoimmune Retinopathies, Central Retinal Artery Occlusion, Central Retinal Vein Occlusion, Central Serous Chorioretinopathy, Eales Disease, Epimacular Membrane, Lattice Degeneration, Macroaneurysm, Diabetic Macular Edema, Irvine-Gass Macular Edema, Macular Hole, Subretinal Neovascular
30 Membranes, Diffuse Unilateral Subacute Neuroretinitis, Nonpseudophakic Cystoid Macular Edema, Presumed Ocular Histoplasmosis Syndrome, Exudative Retinal Detachment, Postoperative Retinal Detachment, Proliferative Retinal Detachment,

Rhegmatogenous Retinal Detachment, Tractional Retinal Detachment, Retinitis Pigmentosa, CMV Retinitis, Retinoblastoma, Retinopathy of Prematurity, Birdshot Retinopathy, Background Diabetic Retinopathy, Proliferative Diabetic Retinopathy, Hemoglobinopathies Retinopathy, Purtscher Retinopathy, Valsalva Retinopathy, 5 Juvenile Retinoschisis, Senile Retinoschisis, Terson Syndrome and White Dot Syndromes.

Other exemplary diseases include ocular bacterial infections (e.g. conjunctivitis, keratitis, tuberculosis, syphilis, gonorrhea), viral infections (e.g. Ocular Herpes Simplex Virus, Varicella Zoster Virus, Cytomegalovirus retinitis, 10 Human Immunodeficiency Virus (HIV)) as well as progressive outer retinal necrosis secondary to HIV or other HIV-associated and other immunodeficiency-associated ocular diseases. In addition, ocular diseases include fungal infections (e.g. Candida choroiditis, histoplasmosis), protozoal infections (e.g. toxoplasmosis) and others such as ocular toxocariasis and sarcoidosis.

15 One aspect of the invention is a method for inhibiting, reducing or treating vision impairment in a subject undergoing treatment with a chemotherapeutic drug (e.g., a neurotoxic drug, a drug that raises intraocular pressure such as a steroid), by administering to the subject in need of such treatment a therapeutic dosage of a CLK-inhibiting compound.

20 Another aspect of the invention is a method for inhibiting, reducing or treating vision impairment in a subject undergoing surgery, including ocular or other surgeries performed in the prone position such as spinal cord surgery, by administering to the subject in need of such treatment a therapeutic dosage of a CLK-inhibiting compound disclosed herein. Ocular surgeries include cataract, 25 iridotomy and lens replacements.

Another aspect of the invention is the treatment, including inhibition and prophylactic treatment, of age related ocular diseases include cataracts, dry eye, retinal damage and the like, by administering to the subject in need of such treatment a therapeutic dosage of a CLK-inhibiting compound.

30 The formation of cataracts is associated with several biochemical changes in the lens of the eye, such as decreased levels of antioxidants ascorbic acid and glutathione, increased lipid, amino acid and protein oxidation, increased sodium and

calcium, loss of amino acids and decreased lens metabolism. The lens, which lacks blood vessels, is suspended in extracellular fluids in the anterior part of the eye. Nutrients, such as ascorbic acid, glutathione, vitamin E, selenium, bioflavonoids and carotenoids are required to maintain the transparency of the lens. Low levels of
5 selenium results in an increase of free radical-inducing hydrogen peroxide, which is neutralized by the selenium-dependent antioxidant enzyme glutathione peroxidase. Lens-protective glutathione peroxidase is also dependent on the amino acids methionine, cysteine, glycine and glutamic acid.

Cataracts can also develop due to an inability to properly metabolize
10 galactose found in dairy products that contain lactose, a disaccharide composed of the monosaccharide galactose and glucose. Cataracts can be prevented, delayed, slowed and possibly even reversed if detected early and metabolically corrected.

Retinal damage is attributed, *inter alia*, to free radical initiated reactions in glaucoma, diabetic retinopathy and age-related macular degeneration (AMD). The
15 eye is a part of the central nervous system and has limited regenerative capability. The retina is composed of numerous nerve cells which contain the highest concentration of polyunsaturated fatty acids (PFA) and subject to oxidation. Free radicals are generated by UV light entering the eye and mitochondria in the rods and cones, which generate the energy necessary to transform light into visual impulses.
20 Free radicals cause peroxidation of the PFA by hydroxyl or superoxide radicals which in turn propagate additional free radicals. The free radicals cause temporary or permanent damage to retinal tissue.

Glaucoma is usually viewed as a disorder that causes an elevated intraocular pressure (IOP) that results in permanent damage to the retinal nerve fibers, but a
25 sixth of all glaucoma cases do not develop an elevated IOP. This disorder is now perceived as one of reduced vascular perfusion and an increase in neurotoxic factors. Recent studies have implicated elevated levels of glutamate, nitric oxide and peroxynitrite in the eye as the causes of the death of retinal ganglion cells. Neuroprotective agents may be the future of glaucoma care. For example, nitric
30 oxide synthase inhibitors block the formation of peroxynitrite from nitric oxide and superoxide. In a recent study, animals treated with aminoguanidine, a nitric oxide synthase inhibitor, had a reduction in the loss of retinal ganglion cells. It was

concluded that nitric oxide in the eye caused cytotoxicity in many tissues and neurotoxicity in the central nervous system.

Diabetic retinopathy occurs when the underlying blood vessels develop microvascular abnormalities consisting primarily of microaneurysms and intraretinal hemorrhages. Oxidative metabolites are directly involved with the pathogenesis of diabetic retinopathy and free radicals augment the generation of growth factors that lead to enhanced proliferative activity. Nitric oxide produced by endothelial cells of the vessels may also cause smooth muscle cells to relax and result in vasodilation of segments of the vessel. Ischemia and hypoxia of the retina occur after thickening of the arterial basement membrane, endothelial proliferation and loss of pericytes. The inadequate oxygenation causes capillary obliteration or nonperfusion, arteriolar-venular shunts, sluggish blood flow and an impaired ability of RBCs to release oxygen. Lipid peroxidation of the retinal tissues also occurs as a result of free radical damage.

The macula is responsible for our acute central vision and composed of light-sensing cells (cones) while the underlying retinal pigment epithelium (RPE) and choroid nourish and help remove waste materials. The RPE nourishes the cones with the vitamin A substrate for the photosensitive pigments and digests the cones shed outer tips. RPE is exposed to high levels of UV radiation, and secretes factors that inhibit angiogenesis. The choroid contains a dense vascular network that provides nutrients and removes the waste materials.

In AMD, the shed cone tips become indigestible by the RPE, where the cells swell and die after collecting too much undigested material. Collections of undigested waste material, called drusen, form under the RPE. Phototoxic damage also causes the accumulation of lipofuscin in RPE cells. The intracellular lipofuscin and accumulation of drusen in Bruch's membrane interferes with the transport of oxygen and nutrients to the retinal tissues, and ultimately leads to RPE and photoreceptor dysfunction. In exudative AMD, blood vessels grow from the choriocapillaris through defects in Bruch's membrane and may grow under the RPE, detaching it from the choroid, and leaking fluid or bleeding.

Macular pigment, one of the protective factors that prevent sunlight from damaging the retina, is formed by the accumulation of nutritionally derived

carotenoids, such as lutein, the fatty yellow pigment that serves as a delivery vehicle for other important nutrients and zeaxanthin. Antioxidants such as vitamins C and E, beta-carotene and lutein, as well as zinc, selenium and copper, are all found in the healthy macula. In addition to providing nourishment, these antioxidants protect
5 against free radical damage that initiates macular degeneration.

Another aspect of the invention is the prevention or treatment of damage to the eye caused by stress, chemical insult or radiation, by administering to the subject in need of such treatment a therapeutic dosage of a CLK modulator, and in particular a CLK-inhibiting compound, disclosed herein. Radiation or electromagnetic damage
10 to the eye can include that caused by CRT's or exposure to sunlight or UV.

In one embodiment, a combination drug regimen may include drugs or compounds for the treatment or prevention of ocular disorders or secondary conditions associated with these conditions. Thus, a combination drug regimen may include one or more CLK inhibitors and one or more therapeutic agents for the
15 treatment of an ocular disorder. For example, one or more CLK-inhibiting compounds can be combined with an effective amount of one or more of: an agent that reduces intraocular pressure, an agent for treating glaucoma, an agent for treating optic neuritis, an agent for treating CMV Retinopathy, an agent for treating multiple sclerosis, and/or an antibiotic, etc.

In one embodiment, a CLK-inhibiting compound can be administered in conjunction with a therapy for reducing intraocular pressure. One group of therapies involves blocking aqueous production. For example, topical beta-adrenergic antagonists (timolol and betaxolol) decrease aqueous production. Topical timolol causes IOP to fall in 30 minutes with peak effects in 1-2 hours. A reasonable
20 regimen is Timoptic 0.5%, one drop every 30 minutes for 2 doses. The carbonic anhydrase inhibitor, acetazolamide, also decreases aqueous production and should be given in conjunction with topical beta-antagonists. An initial dose of 500 mg is administered followed by 250 mg every 6 hours. This medication may be given orally, intramuscularly, or intravenously. In addition, alpha 2-agonists (e.g.,
25 Apraclonidine) act by decreasing aqueous production. Their effects are additive to topically administered beta-blockers. They have been approved for use in controlling an acute rise in pressure following anterior chamber laser procedures, but has been
30

reported effective in treating acute closed-angle glaucoma. A reasonable regimen is 1 drop every 30 minutes for 2 doses.

A second group of therapies for reducing intraocular pressure involve reducing vitreous volume. Hyperosmotic agents can be used to treat an acute attack.

- 5 These agents draw water out of the globe by making the blood hyperosmolar. Oral glycerol in a dose of 1 mL/kg in a cold 50% solution (mixed with lemon juice to make it more palatable) often is used. Glycerol is converted to glucose in the liver; persons with diabetes may need additional insulin if they become hyperglycemic after receiving glycerol. Oral isosorbide is a metabolically inert alcohol that also can
- 10 be used as an osmotic agent for patients with acute angle-closure glaucoma. Usual dose is 100 g taken p.o. (220 cc of a 45% solution). This inert alcohol should not be confused with isosorbide dinitrate, a nitrate-based cardiac medication used for angina and for congestive heart failure. Intravenous mannitol in a dose of 1.0-1.5 mg/kg also is effective and is well tolerated in patients with nausea and vomiting.
- 15 These hyperosmotic agents should be used with caution in any patient with a history of congestive heart failure.

- A third group of therapies involve facilitating aqueous outflow from the eye. Miotic agents pull the iris from the iridocorneal angle and may help to relieve the obstruction of the trabecular meshwork by the peripheral iris. Pilocarpine 2% (blue
- 20 eyes)-4% (brown eyes) can be administered every 15 minutes for the first 1-2 hours. More frequent administration or higher doses may precipitate a systemic cholinergic crisis. NSAIDs are sometimes used to reduce inflammation.

- Exemplary therapeutic agents for reducing intraocular pressure include ALPHAGAN® P (Allergan) (brimonidine tartrate ophthalmic solution), AZOPT®
- 25 (Alcon) (brinzolamide ophthalmic suspension), BETAGAN® (Allergan) (levobunolol hydrochloride ophthalmic solution, USP), BETIMOL® (Vistakon) (timolol ophthalmic solution), BETOPTIC S® (Alcon) (betaxolol HCl), BRIMONIDINE TARTRATE (Bausch & Lomb), CARTEOLOL HYDROCHLORIDE (Bausch & Lomb), COSOPT® (Merck) (dorzolamide
- 30 hydrochloride-timolol maleate ophthalmic solution), LUMIGAN® (Allergan) (bimatoprost ophthalmic solution), OPTIPRANOLOL® (Bausch & Lomb) (metipranolol ophthalmic solution), TIMOLOL GFS (Falcon) (timolol maleate

ophthalmic gel forming solution), TIMOPTIC® (Merck) (timolol maleate ophthalmic solution), TRAVATAN® (Alcon) (travoprost ophthalmic solution), TRUSOPT® (Merck) (dorzolamide hydrochloride ophthalmic solution) and XALATAN® (Pharmacia & Upjohn) (latanoprost ophthalmic solution).

5 In one embodiment, a CLK-inhibiting compound can be administered in conjunction with a therapy for treating and/or preventing glaucoma. An example of a glaucoma drug is DARANIDE® Tablets (Merck) (Dichlorphenamide).

 In one embodiment, a CLK-inhibiting compound can be administered in conjunction with a therapy for treating and/or preventing optic neuritis. Examples of
10 drugs for optic neuritis include DECADRON® Phosphate Injection (Merck) (Dexamethasone Sodium Phosphate), DEPO-MEDROL® (Pharmacia & Upjohn)(methylprednisolone acetate), HYDROCORTONE® Tablets (Merck) (Hydrocortisone), ORAPRED® (Biomarin) (prednisolone sodium phosphate oral solution) and PEDIAPRED® (Celltech) (prednisolone sodium phosphate, USP).

15 In one embodiment, a CLK-inhibiting compound can be administered in conjunction with a therapy for treating and/or preventing CMV Retinopathy. Treatments for CMV retinopathy include CYTOVENE® (ganciclovir capsules) and VALCYTE® (Roche Laboratories) (valganciclovir hydrochloride tablets).

 In one embodiment, a CLK-inhibiting compound can be administered in
20 conjunction with a therapy for treating and/or preventing multiple sclerosis. Examples of such drugs include DANTRIUM® (Procter & Gamble Pharmaceuticals) (dantrolene sodium), NOVANTRONE® (Serono) (mitoxantrone), AVONEX® (Biogen Idec) (Interferon beta-1a), BETASERON® (Berlex) (Interferon beta-1b), COPAXONE® (Teva Neuroscience) (glatiramer acetate
25 injection) and REBIF® (Pfizer) (interferon beta-1a).

 In addition, macrolide and/or mycophenolic acid, which has multiple activities, can be co-administered with a CLK-inhibiting compound. Macrolide antibiotics include tacrolimus, cyclosporine, sirolimus, everolimus, ascomycin, erythromycin, azithromycin, clarithromycin, clindamycin, lincomycin,
30 dirithromycin, josamycin, spiramycin, diacetyl-midecamycin, tylosin, roxithromycin, ABT-773, telithromycin, leucomycins, and lincosamide.

xi. Mitochondrial-Associated Diseases and Disorders

In certain embodiments, the invention provides methods for treating diseases or disorders that would benefit from increased mitochondrial activity. The methods involve administering to a subject in need thereof a therapeutically effective amount
5 of a CLK-inhibiting compound. Increased mitochondrial activity refers to increasing activity of the mitochondria while maintaining the overall numbers of mitochondria (e.g., mitochondrial mass), increasing the numbers of mitochondria thereby increasing mitochondrial activity (e.g., by stimulating mitochondrial biogenesis), or combinations thereof. In certain embodiments, diseases and
10 disorders that would benefit from increased mitochondrial activity include diseases or disorders associated with mitochondrial dysfunction.

In certain embodiments, methods for treating diseases or disorders that would benefit from increased mitochondrial activity may comprise identifying a subject suffering from a mitochondrial dysfunction. Methods for diagnosing a
15 mitochondrial dysfunction may involve molecular genetic, pathologic and/or biochemical analysis and are summarized in Cohen and Gold, Cleveland Clinic Journal of Medicine, 68: 625-642 (2001). One method for diagnosing a mitochondrial dysfunction is the Thor-Byrne-ier scale (see e.g., Cohen and Gold, *supra*; Collin S. et al., Eur Neurol. 36: 260-267 (1996)). Other methods for
20 determining mitochondrial number and function include, for example, enzymatic assays (e.g., a mitochondrial enzyme or an ATP biosynthesis factor such as an ETC enzyme or a Krebs cycle enzyme), determination of mitochondrial mass, mitochondrial volume, and/or mitochondrial number, quantification of mitochondrial DNA, monitoring intracellular calcium homeostasis and/or cellular
25 responses to perturbations of this homeostasis, evaluation of response to an apoptogenic stimulus, determination of free radical production. Such methods are known in the art and are described, for example, in U.S. Patent Publication No. 2002/0049176 and references cited therein.

Mitochondria are critical for the survival and proper function of almost all
30 types of eukaryotic cells. Mitochondria in virtually any cell type can have congenital or acquired defects that affect their function. Thus, the clinically significant signs and symptoms of mitochondrial defects affecting respiratory chain function are

heterogeneous and variable depending on the distribution of defective mitochondria among cells and the severity of their deficits, and upon physiological demands upon the affected cells. Nondividing tissues with high energy requirements, e.g. nervous tissue, skeletal muscle and cardiac muscle are particularly susceptible to

5 mitochondrial respiratory chain dysfunction, but any organ system can be affected.

Diseases and disorders associated with mitochondrial dysfunction include diseases and disorders in which deficits in mitochondrial respiratory chain activity contribute to the development of pathophysiology of such diseases or disorders in a mammal. This includes 1) congenital genetic deficiencies in activity of one or more
10 components of the mitochondrial respiratory chain; and 2) acquired deficiencies in the activity of one or more components of the mitochondrial respiratory chain, wherein such deficiencies are caused by a) oxidative damage during aging; b) elevated intracellular calcium; c) exposure of affected cells to nitric oxide; d) hypoxia or ischemia; e) microtubule-associated deficits in axonal transport of
15 mitochondria, or f) expression of mitochondrial uncoupling proteins.

Diseases or disorders that would benefit from increased mitochondrial activity generally include for example, diseases in which free radical mediated oxidative injury leads to tissue degeneration, diseases in which cells inappropriately undergo apoptosis, and diseases in which cells fail to undergo apoptosis. Exemplary
20 diseases or disorders that would benefit from increased mitochondrial activity include, for example, AD (Alzheimer's Disease), ADPD (Alzheimer's Disease and Parkinson's Disease), AMDF (Ataxia, Myoclonus and Deafness), auto-immune disease, cancer, CIPO (Chronic Intestinal Pseudoobstruction with myopathy and Ophthalmoplegia), congenital muscular dystrophy, CPEO (Chronic Progressive
25 External Ophthalmoplegia), DEAF (Maternally inherited DEAFness oraminoglycoside-induced DEAFness), DEMCHO (Dementia and Chorea), diabetes mellitus (Type I or Type II), DIDMOAD (Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy, Deafness), DMDF (Diabetes Mellitus and Deafness), dystonia, Exercise Intolerance, ESOC (Epilepsy, Strokes, Optic atrophy, and Cognitive
30 decline), FBSN (Familial Bilateral Striatal Necrosis), FICP (Fatal Infantile Cardiomyopathy Plus, a MELAS-associated cardiomyopathy), GER (Gastrointestinal Reflux), HD (Huntington's Disease), KSS (Kearns Sayre

- Syndrome), "later-onset" myopathy, LDYT (Leber's hereditary optic neuropathy and DYsTonia), Leigh's Syndrome, LHON (Leber Hereditary Optic Neuropathy), L IMM (Lethal Infantile Mitochondrial Myopathy), MDM (Myopathy and Diabetes Mellitus), MELAS (Mitochondrial Encephalomyopathy, Lactic Acidosis, and
- 5 Stroke-like episodes), MEPR (Myoclonic Epilepsy and Psychomotor Regression), MERME (MERRF/MELAS overlap disease), MERRF (Myoclonic Epilepsy and Ragged Red Muscle Fibers), MHCM (Maternally Inherited Hypertrophic CardioMyopathy), MICM (Maternally Inherited Cardiomyopathy), MILS (Maternally Inherited Leigh Syndrome), Mitochondrial Encephalocardiomyopathy,
- 10 Mitochondrial Encephalomyopathy, MM (Mitochondrial Myopathy), MMC (Maternal Myopathy and Cardiomyopathy), MNGIE (Myopathy and external ophthalmoplegia, Neuropathy, Gastro-Intestinal, Encephalopathy), Multisystem Mitochondrial Disorder (myopathy, encephalopathy, blindness, hearing loss, peripheral neuropathy), NARP (Neurogenic muscle weakness, Ataxia, and Retinitis
- 15 Pigmentosa; alternate phenotype at this locus is reported as Leigh Disease), PD (Parkinson's Disease), Pearson's Syndrome, PEM (Progressive Encephalopathy), PEO (Progressive External Ophthalmoplegia), PME (Progressive Myoclonus Epilepsy), PMPS (Pearson Marrow-Pancreas Syndrome), psoriasis, RTT (Rett Syndrome), schizophrenia, SIDS (Sudden Infant Death Syndrome), SNHL
- 20 (Sensorineural Hearing Loss), Varied Familial Presentation (clinical manifestations range from spastic paraparesis to multisystem progressive disorder & fatal cardiomyopathy to truncal ataxia, dysarthria, severe hearing loss, mental regression, ptosis, ophthalmoparesis, distal cyclones, and diabetes mellitus), or Wolfram syndrome.
- 25 Other diseases and disorders that would benefit from increased mitochondrial activity include, for example, Friedreich's ataxia and other ataxias, amyotrophic lateral sclerosis (ALS) and other motor neuron diseases, macular degeneration, epilepsy, Alpers syndrome, Multiple mitochondrial DNA deletion syndrome, MtDNA depletion syndrome, Complex I deficiency, Complex II (SDH) deficiency,
- 30 Complex III deficiency, Cytochrome c oxidase (COX, Complex IV) deficiency, Complex V deficiency, Adenine Nucleotide Translocator (ANT) deficiency, Pyruvate dehydrogenase (PDH) deficiency, Ethylmalonic aciduria with lactic

acidemia, 3-Methyl glutaconic aciduria with lactic acidemia, Refractory epilepsy with declines during infection, Asperger syndrome with declines during infection, Autism with declines during infection, Attention deficit hyperactivity disorder (ADHD), Cerebral palsy with declines during infection, Dyslexia with declines
 5 during infection, materially inherited thrombocytopenia and leukemia syndrome, MARIAHS syndrome (Mitochondrial ataxia, recurrent infections, aphasia, hypouricemia/hypomyelination, seizures, and dicarboxylic aciduria), ND6 dystonia, Cyclic vomiting syndrome with declines during infection, 3-Hydroxy isobutyric aciduria with lactic acidemia, Diabetes mellitus with lactic acidemia, Uridine
 10 responsive neurologic syndrome (URNS), Dilated cardiomyopathy, Splenic Lymphoma, and Renal Tubular Acidosis/Diabetes/Ataxia syndrome.

In other embodiments, the invention provides methods for treating a subject suffering from mitochondrial disorders arising from, but not limited to, Post-traumatic head injury and cerebral edema, Stroke (invention methods useful for
 15 preventing or preventing reperfusion injury), Lewy body dementia, Hepatorenal syndrome, Acute liver failure, NASH (non-alcoholic steatohepatitis), Anti-metastasis/prodiferentiation therapy of cancer, Idiopathic congestive heart failure, Atrial fibrillation (non-valvular), Wolff-Parkinson-White Syndrome, Idiopathic heart block, Prevention of reperfusion injury in acute myocardial infarctions, Familial
 20 migraines, Irritable bowel syndrome, Secondary prevention of non-Q wave myocardial infarctions, Premenstrual syndrome, Prevention of renal failure in hepatorenal syndrome, Anti-phospholipid antibody syndrome, Eclampsia/pre-eclampsia, Oopause infertility, Ischemic heart disease/Angina, and Shy-Drager and unclassified dysautonomia syndromes.

In still another embodiment, there are provided methods for the treatment of mitochondrial disorders associated with pharmacological drug-related side effects. Types of pharmaceutical agents that are associated with mitochondrial disorders include reverse transcriptase inhibitors, protease inhibitors, inhibitors of DHOD, and the like. Examples of reverse transcriptase inhibitors include, for example,
 30 Azidothymidine (AZT), Stavudine (D4T), Zalcitabine (ddC), Didanosine (DDI), Fluoriodoauracil (FIAU), and the like. Examples of protease inhibitors include, for example, Ritonavir, Indinavir, Saquinavir, Nelfinavir and the like. Examples of

inhibitors of dihydroorotate dehydrogenase (DHOD) include, for example, Leflunomide, Brequinar and the like.

Common symptoms of mitochondrial diseases include cardiomyopathy, muscle weakness and atrophy, developmental delays (involving motor, language,
5 cognitive or executive function), ataxia, epilepsy, renal tubular acidosis, peripheral neuropathy, optic neuropathy, autonomic neuropathy, neurogenic bowel dysfunction, sensorineural deafness, neurogenic bladder dysfunction, dilating cardiomyopathy, migraine, hepatic failure, lactic acidemia, and diabetes mellitus.

In certain embodiments, the invention provides methods for treating a
10 disease or disorder that would benefit from increased mitochondrial activity that involves administering to a subject in need thereof one or more CLK-inhibiting compounds in combination with another therapeutic agent such as, for example, an agent useful for treating mitochondrial dysfunction (such as antioxidants, vitamins, or respiratory chain cofactors), an agent useful for reducing a symptom associated
15 with a disease or disorder involving mitochondrial dysfunction (such as, an anti-seizure agent, an agent useful for alleviating neuropathic pain, an agent for treating cardiac dysfunction), a cardiovascular agent (as described further below), a chemotherapeutic agent (as described further below), or an anti-neurodegeneration agent (as described further below). In an exemplary embodiment, the invention
20 provides methods for treating a disease or disorder that would benefit from increased mitochondrial activity that involves administering to a subject in need thereof one or more CLK-inhibiting compounds in combination with one or more of the following: coenzyme Q₁₀, L-carnitine, thiamine, riboflavin, niacinamide, folate, vitamin E, selenium, lipoic acid, or prednisone. Compositions comprising such combinations
25 are also provided herein.

In exemplary embodiments, the invention provides methods for treating diseases or disorders that would benefit from increased mitochondrial activity by administering to a subject a therapeutically effective amount of a CLK-inhibiting compound. Exemplary diseases or disorders include, for example, neuromuscular
30 disorders (e.g., Friedreich's Ataxia, muscular dystrophy, multiple sclerosis, etc.), disorders of neuronal instability (e.g., seizure disorders, migraine, etc.), developmental delay, neurodegenerative disorders (e.g., Alzheimer's Disease,

Parkinson's Disease, amyotrophic lateral sclerosis, etc.), ischemia, renal tubular acidosis, age-related neurodegeneration and cognitive decline, chemotherapy fatigue, age-related or chemotherapy-induced menopause or irregularities of menstrual cycling or ovulation, mitochondrial myopathies, mitochondrial damage
5 (e.g., calcium accumulation, excitotoxicity, nitric oxide exposure, hypoxia, etc.), and mitochondrial deregulation.

A gene defect underlying Friedreich's Ataxia (FA), the most common hereditary ataxia, was recently identified and is designated "frataxin". In FA, after a period of normal development, deficits in coordination develop which progress to
10 paralysis and death, typically between the ages of 30 and 40. The tissues affected most severely are the spinal cord, peripheral nerves, myocardium, and pancreas. Patients typically lose motor control and are confined to wheel chairs, and are commonly afflicted with heart failure and diabetes. The genetic basis for FA involves GAA trinucleotide repeats in an intron region of the gene encoding
15 frataxin. The presence of these repeats results in reduced transcription and expression of the gene. Frataxin is involved in regulation of mitochondrial iron content. When cellular frataxin content is subnormal, excess iron accumulates in mitochondria, promoting oxidative damage and consequent mitochondrial degeneration and dysfunction. When intermediate numbers of GAA repeats are
20 present in the frataxin gene intron, the severe clinical phenotype of ataxia may not develop. However, these intermediate-length trinucleotide extensions are found in 25 to 30% of patients with non-insulin dependent diabetes mellitus, compared to about 5% of the nondiabetic population. In certain embodiments, CLK-inhibiting compounds may be used for treating patients with disorders related to deficiencies or
25 defects in frataxin, including Friedreich's Ataxia, myocardial dysfunction, diabetes mellitus and complications of diabetes like peripheral neuropathy.

Muscular dystrophy refers to a family of diseases involving deterioration of neuromuscular structure and function, often resulting in atrophy of skeletal muscle and myocardial dysfunction. In the case of Duchenne muscular dystrophy, mutations
30 or deficits in a specific protein, dystrophin, are implicated in its etiology. Mice with their dystrophin genes inactivated display some characteristics of muscular dystrophy, and have an approximately 50% deficit in mitochondrial respiratory

chain activity. A final common pathway for neuromuscular degeneration in most cases is calcium-mediated impairment of mitochondrial function. In certain embodiments, CLK-inhibiting compounds may be used for reducing the rate of decline in muscular functional capacities and for improving muscular functional status in patients with muscular dystrophy.

Multiple sclerosis (MS) is a neuromuscular disease characterized by focal inflammatory and autoimmune degeneration of cerebral white matter. Periodic exacerbations or attacks are significantly correlated with upper respiratory tract and other infections, both bacterial and viral, indicating that mitochondrial dysfunction plays a role in MS. Depression of neuronal mitochondrial respiratory chain activity caused by Nitric Oxide (produced by astrocytes and other cells involved in inflammation) is implicated as a molecular mechanism contributing to MS. In certain embodiments, CLK-inhibiting compounds may be used for treatment of patients with multiple sclerosis, both prophylactically and during episodes of disease exacerbation.

Epilepsy is often present in patients with mitochondrial cytopathies, involving a range of seizure severity and frequency, e.g. absence, tonic, atonic, myoclonic, and status epilepticus, occurring in isolated episodes or many times daily. In certain embodiments, CLK-inhibiting compounds may be used for treating patients with seizures secondary to mitochondrial dysfunction, including reducing frequency and severity of seizure activity.

Metabolic studies on patients with recurrent migraine headaches indicate that deficits in mitochondrial activity are commonly associated with this disorder, manifesting as impaired-oxidative phosphorylation and excess lactate production. Such deficits are not necessarily due to genetic defects in mitochondrial DNA. Migraineurs are hypersensitive to nitric oxide, an endogenous inhibitor of Cytochrome c Oxidase. In addition, patients with mitochondrial cytopathies, e.g. MELAS, often have recurrent migraines. In certain embodiments, CLK-inhibiting compounds may be used for treating patients with recurrent migraine headaches, including headaches refractory to ergot compounds or serotonin receptor antagonists.

Delays in neurological or neuropsychological development are often found in children with mitochondrial diseases. Development and remodeling of neural connections requires intensive biosynthetic activity, particularly involving synthesis of neuronal membranes and myelin, both of which require pyrimidine nucleotides as cofactors. Uridine nucleotides are involved inactivation and transfer of sugars to glycolipids and glycoproteins. Cytidine nucleotides are derived from uridine nucleotides, and are crucial for synthesis of major membrane phospholipid constituents like phosphatidylcholine, which receives its choline moiety from cytidine diphosphocholine. In the case of mitochondrial dysfunction (due to either mitochondrial DNA defects or any of the acquired or conditional deficits like excitotoxic or nitric oxide-mediated mitochondrial dysfunction) or other conditions resulting in impaired pyrimidine synthesis, cell proliferation and axonal extension is impaired at crucial stages in development of neuronal interconnections and circuits, resulting in delayed or arrested development of neuropsychological functions like language, motor, social, executive function, and cognitive skills. In autism for example, magnetic resonance spectroscopy measurements of cerebral phosphate compounds indicates that there is global undersynthesis of membranes and membrane precursors indicated by reduced levels of uridine diphospho-sugars, and cytidine nucleotide derivatives involved in membrane synthesis. Disorders characterized by developmental delay include Rett's Syndrome, pervasive developmental delay (or PDD-NOS "pervasive developmental delay not otherwise specified" to distinguish it from specific subcategories like autism), autism, Asperger's Syndrome, and Attention Deficit/Hyperactivity Disorder (ADHD), which is becoming recognized as a delay or lag in development of neural circuitry underlying executive functions. In certain embodiments, CLK-inhibiting compounds may be useful for treating treating patients with neurodevelopmental delays (e.g., involving motor, language, executive function, and cognitive skills), or other delays or arrests of neurological and neuropsychological development in the nervous system and somatic development in non-neural tissues like muscle and endocrine glands.

The two most significant severe neurodegenerative diseases associated with aging, Alzheimer's Disease (AD) and Parkinson's Disease (PD), both involve

mitochondrial dysfunction in their pathogenesis. Complex I deficiencies in particular are frequently found not only in the nigrostriatal neurons that degenerate in Parkinson's disease, but also in peripheral tissues and cells like muscle and platelets of Parkinson's Disease patients. In Alzheimer's Disease, mitochondrial respiratory chain activity is often depressed, especially Complex IV (Cytochrome c Oxidase). Moreover, mitochondrial respiratory function altogether is depressed as a consequence of aging, further amplifying the deleterious sequelae of additional molecular lesions affecting respiratory chain function. Other factors in addition to primary mitochondrial dysfunction underlie neurodegeneration in AD, PD, and related disorders. Excitotoxic stimulation and nitric oxide are implicated in both diseases, factors which both exacerbate mitochondrial respiratory chain deficits and whose deleterious actions are exaggerated on a background of respiratory chain dysfunction. Huntington's Disease also involves mitochondrial dysfunction in affected brain regions, with cooperative interactions of excitotoxic stimulation and mitochondrial dysfunction contributing to neuronal degeneration. In certain embodiments, CLK-inhibiting compounds may be useful for treating and attenuating progression of age-related neurodegenerative disease including AD and PD.

One of the major genetic defects in patients with Amyotrophic Lateral Sclerosis (ALS or Lou Gehrig's Disease) is mutation or deficiency in Copper-Zinc Superoxide Dismutase (SOD 1), an antioxidant enzyme. Mitochondria both produce and are primary targets for reactive oxygen species. Inefficient transfer of electrons to oxygen in mitochondria is the most significant physiological source of free radicals in mammalian systems. Deficiencies in antioxidants or antioxidant enzymes can result in or exacerbate mitochondrial degeneration. Mice transgenic for mutated SOD1 develop symptoms and pathology similar to those in human ALS. The development of the disease in these animals has been shown to involve oxidative destruction of mitochondria followed by functional decline of motor neurons and onset of clinical symptoms. Skeletal muscle from ALS patients has low mitochondrial Complex I activity. In certain embodiments, CLK-inhibiting compounds may be useful for treating ALS, for reversing or slowing the progression of clinical symptoms.

Oxygen deficiency results in both direct inhibition of mitochondrial respiratory chain activity by depriving cells of a terminal electron acceptor for Cytochrome c reoxidation at Complex IV, and indirectly, especially in the nervous system, via secondary post-anoxic excitotoxicity and nitric oxide formation. In conditions like cerebral anoxia, angina or sickle cell anemia crises, tissues are relatively hypoxic. In such cases, compounds that increase mitochondrial activity provide protection of affected tissues from deleterious effects of hypoxia, attenuate secondary delayed cell death, and accelerate recovery from hypoxic tissue stress and injury. In certain embodiments, CLK-inhibiting compounds may be useful for preventing delayed cell death (apoptosis in regions like the hippocampus or cortex occurring about 2 to 5 days after an episode of cerebral ischemia) after ischemic or hypoxic insult to the brain.

Acidosis due to renal dysfunction is often observed in patients with mitochondrial disease, whether the underlying respiratory chain dysfunction is congenital or induced by ischemia or cytotoxic agents like cisplatin. Renal tubular acidosis often requires administration of exogenous sodium bicarbonate to maintain blood and tissue pH. In certain embodiments, CLK-inhibiting compounds may be useful for treating renal tubular acidosis and other forms of renal dysfunction caused by mitochondrial respiratory chain deficits.

During normal aging, there is a progressive decline in mitochondrial respiratory chain function. Beginning about age 40, there is an exponential rise in accumulation of mitochondrial DNA defects in humans, and a concurrent decline in nuclear-regulated elements of mitochondrial respiratory activity. Many mitochondrial DNA lesions have a selection advantage during mitochondrial turnover, especially in postmitotic cells. The proposed mechanism is that mitochondria with a defective respiratory chain produce less oxidative damage to themselves than do mitochondria with intact functional respiratory chains (mitochondrial respiration is the primary source of free radicals in the body). Therefore, normally-functioning mitochondria accumulate oxidative damage to membrane lipids more rapidly than do defective mitochondria, and are therefore "tagged" for degradation by lysosomes. Since mitochondria within cells have a half life of about 10 days, a selection advantage can result in rapid replacement of

functional mitochondria with those with diminished respiratory activity, especially in slowly dividing cells. The net result is that once a mutation in a gene for a mitochondrial protein that reduces oxidative damage to mitochondria occurs, such defective mitochondria will rapidly populate the cell, diminishing or eliminating its respiratory capabilities. The accumulation of such cells results in aging or degenerative disease at the organismal level. This is consistent with the progressive mosaic appearance of cells with defective electron transport activity in muscle, with cells almost devoid of Cytochrome c Oxidase (COX) activity interspersed randomly amidst cells with normal activity, and a higher incidence of COX-negative cells in biopsies from older subjects. The organism, during aging, or in a variety of mitochondrial diseases, is thus faced with a situation in which irreplaceable postmitotic cells (e.g. neurons, skeletal and cardiac muscle) must be preserved and their function maintained to a significant degree, in the face of an inexorable progressive decline in mitochondrial respiratory chain function. Neurons with dysfunctional mitochondria become progressively more sensitive to insults like excitotoxic injury. Mitochondrial failure contributes to most degenerative diseases (especially neurodegeneration) that accompany aging. Congenital mitochondrial diseases often involve early-onset neurodegeneration similar in fundamental mechanism to disorders that occur during aging of people born with normal mitochondria. In certain embodiments, CLK-inhibiting compounds may be useful for treating or attenuating cognitive decline and other degenerative consequences of aging.

Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in cells subjected to oxidative stress or cancer chemotherapy agents like cisplatin due to both greater vulnerability and less efficient repair of mitochondrial DNA. Although mitochondrial DNA may be more sensitive to damage than nuclear DNA, it is relatively resistant, in some situations, to mutagenesis by chemical carcinogens. This is because mitochondria respond to some types of mitochondrial DNA damage by destroying their defective genomes rather than attempting to repair them. This results in global mitochondrial dysfunction for a period after cytotoxic chemotherapy. Clinical use of chemotherapy agents like cisplatin, mitomycin, and cytoxan is often accompanied by debilitating

"chemotherapy fatigue", prolonged periods of weakness and exercise intolerance which may persist even after recovery from hematologic and gastrointestinal toxicities of such agents. In certain embodiments, CLK-inhibiting compounds may be useful for treatment and prevention of side effects of cancer chemotherapy related to mitochondrial dysfunction.

A crucial function of the ovary is to maintain integrity of the mitochondrial genome in oocytes, since mitochondria passed onto a fetus are all derived from those present in oocytes at the time of conception. Deletions in mitochondrial DNA become detectable around the age of menopause, and are also associated with abnormal menstrual cycles. Since cells cannot directly detect and respond to defects in mitochondrial DNA, but can only detect secondary effects that affect the cytoplasm, like impaired respiration, redox status, or deficits in pyrimidine synthesis, such products of mitochondrial function participate as a signal for oocyte selection and follicular atresia, ultimately triggering menopause when maintenance of mitochondrial genomic fidelity and functional activity can no longer be guaranteed. This is analogous to apoptosis in cells with DNA damage, which undergo an active process of cellular suicide when genomic fidelity can no longer be achieved by repair processes. Women with mitochondrial cytopathies affecting the gonads often undergo premature menopause or display primary cycling abnormalities. Cytotoxic cancer chemotherapy often induces premature menopause, with a consequent increased risk of osteoporosis. Chemotherapy-induced amenorrhea is generally due to primary ovarian failure. The incidence of chemotherapy-induced amenorrhea increases as a function of age in premenopausal women receiving chemotherapy, pointing toward mitochondrial involvement. Inhibitors of mitochondrial respiration or protein synthesis inhibit hormone-induced ovulation, and furthermore inhibit production of ovarian steroid hormones in response to pituitary gonadotropins. Women with Downs syndrome typically undergo menopause prematurely, and also are subject to early onset of Alzheimer-like dementia. Low activity of cytochrome oxidase is consistently found in tissues of Downs patients and in late-onset Alzheimer's Disease. Appropriate support of mitochondrial function or compensation for mitochondrial dysfunction therefore is useful for protecting against age-related or chemotherapy-induced menopause or

irregularities of menstrual cycling or ovulation. In certain embodiments, CLK-inhibiting compounds may be useful for treating and preventing amenorrhea, irregular ovulation, menopause, or secondary consequences of menopause.

In certain embodiments, CLK modulating compounds, and in particular a CLK-inhibiting compound, may be useful for treatment mitochondrial myopathies. Mitochondrial myopathies range from mild, slowly progressive weakness of the extraocular muscles to severe, fatal infantile myopathies and multisystem encephalomyopathies. Some syndromes have been defined, with some overlap between them. Established syndromes affecting muscle include progressive external ophthalmoplegia, the Kearns-Sayre syndrome (with ophthalmoplegia, pigmentary retinopathy, cardiac conduction defects, cerebellar ataxia, and sensorineural deafness), the MELAS syndrome (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes), the MERFF syndrome (myoclonic epilepsy and ragged red fibers), limb-girdle distribution weakness, and infantile myopathy (benign or severe and fatal). Muscle biopsy specimens stained with modified Gomori's trichrome stain show ragged red fibers due to excessive accumulation of mitochondria. Biochemical defects in substrate transport and utilization, the Krebs cycle, oxidative phosphorylation, or the respiratory chain are detectable. Numerous mitochondrial DNA point mutations and deletions have been described, transmitted in a maternal, nonmendelian inheritance pattern. Mutations in nuclear-encoded mitochondrial enzymes occur.

In certain embodiments, CLK-inhibiting compounds may be useful for treating patients suffering from toxic damage to mitochondria, such as, toxic damage due to calcium accumulation, excitotoxicity, nitric oxide exposure, or hypoxia.

A fundamental mechanism of cell injury, especially in excitable tissues, involves excessive calcium entry into cells, as a result of either leakage through the plasma membrane or defects in intracellular calcium handling mechanisms. Mitochondria are major sites of calcium sequestration, and preferentially utilize energy from the respiratory chain for taking up calcium rather than for ATP synthesis, which results in a downward spiral of mitochondrial failure, since calcium uptake into mitochondria results in diminished capabilities for energy transduction.

Excessive stimulation of neurons with excitatory amino acids is a common mechanism of cell death or injury in the central nervous system. Activation of glutamate receptors, especially of the subtype designated NMDA receptors, results in mitochondrial dysfunction, in part through elevation of intracellular calcium
5 during excitotoxic stimulation. Conversely, deficits in mitochondrial respiration and oxidative phosphorylation sensitizes cells to excitotoxic stimuli, resulting in cell death or injury during exposure to levels of excitotoxic neurotransmitters or toxins that would be innocuous to normal cells.

Nitric oxide (about 1 micromolar) inhibits cytochrome oxidase (Complex IV)
10 and thereby inhibits mitochondrial respiration; moreover, prolonged exposure to nitric oxide (NO) irreversibly reduces Complex I activity. Physiological or pathophysiological concentrations of NO thereby inhibit pyrimidine biosynthesis. Nitric oxide is implicated in a variety of neurodegenerative disorders including inflammatory and autoimmune diseases of the central nervous system, and is
15 involved in mediation of excitotoxic and post-hypoxic damage to neurons.

Oxygen is the terminal electron acceptor in the respiratory chain. Oxygen deficiency impairs electron transport chain activity, resulting in diminished pyrimidine synthesis as well as diminished ATP synthesis via oxidative phosphorylation. Human cells proliferate and retain viability under virtually
20 anaerobic conditions if provided with uridine and pyruvate (or a similarly effective agent for oxidizing NADH to optimize glycolytic ATP production).

In certain embodiments, CLK-inhibiting compounds may be useful for treating diseases or disorders associated with mitochondrial deregulation.

Transcription of mitochondrial DNA encoding respiratory chain components
25 requires nuclear factors. In neuronal axons, mitochondria must shuttle back and forth to the nucleus in order to maintain respiratory chain activity. If axonal transport is impaired by hypoxia or by drugs like taxol which affect microtubule stability, mitochondria distant from the nucleus undergo loss of cytochrome oxidase activity. Accordingly, treatment with a CLK-inhibiting compound may be useful for
30 promoting nuclear-mitochondrial interactions.

Mitochondria are the primary source of free radicals and reactive oxygen species, due to spillover from the mitochondrial respiratory chain, especially when

defects in one or more respiratory chain components impairs orderly transfer of electrons from metabolic intermediates to molecular oxygen. To reduce oxidative damage, cells can compensate by expressing mitochondrial uncoupling proteins (UCP), of which several have been identified. UCP-2 is transcribed in response to oxidative damage, inflammatory cytokines, or excess lipid loads, e.g. fatty liver and steatohepatitis. UCPs reduce spillover of reactive oxygen species from mitochondria by discharging proton gradients across the mitochondrial inner membrane, in effect wasting energy produced by metabolism and rendering cells vulnerable to energy stress as a trade-off for reduced oxidative injury.

10 ***xii. Muscle Performance***

In other embodiments, the invention provides methods for enhancing muscle performance by administering a therapeutically effective amount of a CLK-inhibiting compound. For example, CLK-inhibiting compounds may be useful for improving physical endurance (e.g., ability to perform a physical task such as exercise, physical labor, sports activities, etc.), inhibiting or retarding physical fatigues, enhancing blood oxygen levels, enhancing energy in healthy individuals, enhance working capacity and endurance, reducing muscle fatigue, reducing stress, enhancing cardiac and cardiovascular function, improving sexual ability, increasing muscle ATP levels, and/or reducing lactic acid in blood. In certain embodiments, the methods involve administering an amount of a CLK inhibiting compound that increase mitochondrial activity, increase mitochondrial biogenesis, increase mitochondrial mass, or a high dose of a CLK-inhibiting compound.

Sports performance refers to the ability of the athlete's muscles to perform when participating in sports activities. Enhanced sports performance, strength, speed and endurance are measured by an increase in muscular contraction strength, increase in amplitude of muscle contraction, shortening of muscle reaction time between stimulation and contraction. Athlete refers to an individual who participates in sports at any level and who seeks to achieve an improved level of strength, speed and endurance in their performance, such as, for example, body builders, bicyclists, long distance runners, short distance runners, etc. An athlete may be hard training, that is, performs sports activities intensely more than three days a week or for competition. An athlete may also be a fitness enthusiast who

seeks to improve general health and well-being, improve energy levels, who works out for about 1-2 hours about 3 times a week. Enhanced sports performance is manifested by the ability to overcome muscle fatigue, ability to maintain activity for longer periods of time, and have a more effective workout.

5 In the arena of athlete muscle performance, it is desirable to create conditions that permit competition or training at higher levels of resistance for a prolonged period of time. However, acute and intense anaerobic use of skeletal muscles often results in impaired athletic performance, with losses in force and work output, and increased onset of muscle fatigue, soreness, and dysfunction. It is now recognized
10 that even a single exhaustive exercise session, or for that matter any acute trauma to the body such as muscle injury, resistance or exhaustive muscle exercise, or elective surgery, is characterized by perturbed metabolism that affects muscle performance in both short and long term phases. Both muscle metabolic/enzymatic activity and gene expression are affected. For example, disruption of skeletal muscle nitrogen
15 metabolism as well as depletion of sources of metabolic energy occur during extensive muscle activity. Amino acids, including branched-chain amino acids, are released from muscles followed by their deamination to elevate serum ammonia and local oxidation as muscle fuel sources, which augments metabolic acidosis. In addition, there is a decline in catalytic efficiency of muscle contraction events, as
20 well as an alteration of enzymatic activities of nitrogen and energy metabolism. Further, protein catabolism is initiated where rate of protein synthesis is decreased coupled with an increase in the degradation of non-contractile protein. These metabolic processes are also accompanied by free radical generation which further damages muscle cells.

25 Recovery from fatigue during acute and extended exercise requires reversal of metabolic and non-metabolic fatiguing factors. Known factors that participate in human muscle fatigue, such as lactate, ammonia, hydrogen ion, etc., provide an incomplete and unsatisfactory explanation of the fatigue/recovery process, and it is likely that additional unknown agents participate (Baker et al., J. Appl. Physiol.
30 74:2294-2300, 1993; Bazzarre et al., J Am. Coll. Nutr. 11:505-511, 1992; Dohm et al., Fed. Proc. 44:348-352, 1985; Edwards In: Biochemistry of Exercise, Proceedings of the Fifth International Symposium on the Biochemistry of Exercise

(Kutrgen, Vogel, Poormans, eds.), 1983; MacDougall et al., *Acta Physiol. Scand.* 146:403-404, 1992; Walser et al., *Kidney Int.* 32:123-128, 1987). Several studies have also analyzed the effects of nutritional supplements and herbal supplements in enhancing muscle performance.

5 Aside from muscle performance during endurance exercise, free radicals and oxidative stress parameters are affected in pathophysiological states. A substantial body of data now suggests that oxidative stress contributes to muscle wasting or atrophy in pathophysiological states (reviewed in Clarkson, P. M. Antioxidants and physical performance. *Crit. Rev. Food Sci. Nutr.* 35: 31-41; 1995; Powers, S. K.;
10 Lennon, S. L. Analysis of cellular responses to free radicals: Focus on exercise and skeletal muscle. *Proc. Nutr. Soc.* 58: 1025-1033; 1999). For example, with respect to muscular disorders where both muscle endurance and function are compensated, the role of nitric oxide (NO), has been implicated. In muscular dystrophies, especially those due to defects in proteins that make up the dystrophin-glycoprotein complex
15 (DGC), the enzyme that synthesizes NO, nitric oxide synthase (NOS), has been associated. Recent studies of dystrophies related to DGC defects suggest that one mechanism of cellular injury is functional ischemia related to alterations in cellular NOS and disruption of a normal protective action of NO. This protective action is the prevention of local ischemia during contraction-induced increases in sympathetic
20 vasoconstriction. Rando (*Microsc Res Tech* 55(4):223-35, 2001), has shown that oxidative injury precedes pathologic changes and that muscle cells with defects in the DGC have an increased susceptibility to oxidant challenges. Excessive lipid peroxidation due to free radicals has also been shown to be a factor in myopathic diseases such as McArdle's disease (Russo et al., *Med Hypotheses.* 39(2):147-51,
25 1992). Furthermore, mitochondrial dysfunction is a well-known correlate of age-related muscle wasting (sarcopenia) and free radical damage has been suggested, though poorly investigated, as a contributing factor (reviewed in Navarro, A.; Lopez-Cepero, J. M.; Sanchez del Pino, M. L. *Front. Biosci.* 6: D26-44; 2001). Other indications include acute sarcopenia, for example muscle atrophy and/or
30 cachexia associated with burns, bed rest, limb immobilization, or major thoracic, abdominal, and/or orthopedic surgery. It is contemplated that the methods of the

present invention will also be effective in the treatment of muscle related pathological conditions.

In certain embodiments, the invention provides novel dietary compositions comprising CLK-inhibiting compounds, a method for their preparation, and a
5 method of using the compositions for improvement of sports performance. Accordingly, provided are therapeutic compositions, foods and beverages that have actions of improving physical endurance and/or inhibiting physical fatigues for those people involved in broadly-defined exercises including sports requiring endurance and labors requiring repeated muscle exertions. Such dietary compositions may
10 additional comprise electrolytes, caffeine, vitamins, carbohydrates, etc.

xiii. Splicing-Related Disorders

In certain embodiments, CLK-inhibiting compounds may be used for treating or preventing disorders related to abnormal splicing associated with excessive kinase induction. In other embodiments, the invention specifically
15 excludes such disorders. Such disorders include FTDP-17, NF2, FRASIER, Wilms tumor, breast cancer, ovarian cancer, renal cancer, lung cancer, urothelial cancer, gastric cancer, papillary thyroid cancer, HNSCC, invasive breast cancer, glant cell tumors of bone, prostate cancer, melanoma, lymphoma, oral cancer, pharyngeal cancer, progeria, and neurodegenerative diseases such as Alzheimer's disease,
20 Parkinson's disease, amyotrophic lateral sclerosis (ALS), Huntington disease, spinocerebellar ataxia, spinal and bulbar muscular atrophy (SBMA), epilepsy, progressive supranuclear palsy, and Pick's disease.

xiv. Other Uses

CLK-inhibiting compounds may be used for treating or preventing viral
25 infections (such as infections by influenza, herpes or papilloma virus) or as antifungal agents. In certain embodiments, CLK-inhibiting compounds may be administered as part of a combination drug therapy with another therapeutic agent for the treatment of viral diseases, including, for example, acyclovir, ganciclovir and zidovudine. In another embodiment, CLK-inhibiting compounds may be
30 administered as part of a combination drug therapy with another anti-fungal agent including, for example, topical anti-fungals such as ciclopirox, clotrimazole, econazole, miconazole, nystatin, oxiconazole, terconazole, and tolnaftate, or

systemic anti-fungal such as fluconazole (Diflucan), itraconazole (Sporanox), ketoconazole (Nizoral), and miconazole (Monistat I.V.).

Subjects that may be treated as described herein include eukaryotes, such as mammals, e.g., humans, ovines, bovines, equines, porcines, canines, felines, non-
5 human primate, mice, and rats. Cells that may be treated include eukaryotic cells, e.g., from a subject described above, or plant cells, yeast cells and prokaryotic cells, e.g., bacterial cells. For example, modulating compounds may be administered to farm animals to improve their ability to withstand farming conditions longer.

CLK-inhibiting compounds may also be used to increase lifespan, stress
10 resistance, and resistance to apoptosis in plants. In one embodiment, a compound is applied to plants, e.g., on a periodic basis, or to fungi. In another embodiment, plants are genetically modified to produce a compound. In another embodiment, plants and fruits are treated with a compound prior to picking and shipping to increase resistance to damage during shipping. Plant seeds may also be contacted
15 with compounds described herein, e.g., to preserve them.

In other embodiments, CLK-inhibiting compounds may be used for modulating lifespan in yeast cells. Situations in which it may be desirable to extend the lifespan of yeast cells include any process in which yeast is used, e.g., the making of beer, yogurt, and bakery items, e.g., bread. Use of yeast having an
20 extended lifespan can result in using less yeast or in having the yeast be active for longer periods of time. Yeast or other mammalian cells used for recombinantly producing proteins may also be treated as described herein.

CLK-inhibiting compounds may also be used to increase lifespan, stress resistance and resistance to apoptosis in insects. In this embodiment, compounds
25 would be applied to useful insects, e.g., bees and other insects that are involved in pollination of plants. In a specific embodiment, a compound would be applied to bees involved in the production of honey. Generally, the methods described herein may be applied to any organism, e.g., eukaryote, that may have commercial importance. For example, they can be applied to fish (aquaculture) and birds (e.g.,
30 chicken and fowl).

Higher doses of CLK-inhibiting compounds may also be used as a pesticide by interfering with the regulation of silenced genes and the regulation of apoptosis

during development. In this embodiment, a compound may be applied to plants using a method known in the art that ensures the compound is bio-available to insect larvae, and not to plants.

In other embodiments, CLK-inhibiting compounds can be applied to affect the reproduction of organisms such as insects, animals and microorganisms.

4. Assays

Yet other methods contemplated herein include screening methods for identifying compounds or agents that modulate CLK proteins. Assays may be conducted in a cell based or cell free format. For example, an assay may comprise incubating (or contacting) a CLK with a test agent under conditions in which a CLK can be modulated by an agent known to modulate the CLK, and monitoring or determining the level of modulation of the CLK in the presence of the test agent relative to the absence of the test agent. The level of modulation of a CLK can be determined by determining its ability to phosphorylate a substrate.

Methods for identifying an agent that modulates, e.g., stimulates or inhibits, CLKs *in vivo* may comprise (i) contacting a cell with a test agent and a substrate that is capable of entering a cell under conditions appropriate for the CLK to phosphorylate the substrate in the absence of the test agent; and (ii) determining the level of phosphorylation of the substrate, wherein (i) a lower level of phosphorylation of the substrate in the presence of the test agent relative to the level of phosphorylation in the absence of the test agent indicates that the test agent inhibits phosphorylation by the CLK, or (ii) wherein a higher level of phosphorylation of the substrate in the presence of the test agent relative to the level of phosphorylation in the absence of the test agent indicates that the test agent activates phosphorylation by the CLK.

In yet other embodiments, provided are methods (e.g., assays such as screening assays or high throughput screens) for identifying agents, such as CLK modulating compounds, that are useful for modulating mitochondrial mass and/or mitochondrial function in cells of an animal or human subject. In certain embodiments, candidate agents are screened for their ability to increase mitochondrial mass and/or improve mitochondrial function. In an exemplary embodiment, the methods described herein may be used to identify an agent that

increases mitochondrial mass and/or improves mitochondrial function in cells, such as, for example, a CLK-inhibiting compound.

5. Pharmaceutical Compositions

The CLK-inhibiting compounds described herein may be formulated in a conventional manner using one or more physiologically acceptable carriers or excipients. For example, CLK-inhibiting compounds and their physiologically acceptable salts and solvates may be formulated for administration by, for example, injection (e.g. SubQ, IM, IP), inhalation or insufflation (either through the mouth or the nose) or oral, buccal, sublingual, transdermal, nasal, parenteral or rectal administration. In one embodiment, a CLK-inhibiting compound may be administered locally, at the site where the target cells are present, i.e., in a specific tissue, organ, or fluid (e.g., blood, cerebrospinal fluid, etc.).

CLK-inhibiting compounds can be formulated for a variety of modes of administration, including systemic and topical or localized administration. Techniques and formulations generally may be found in Remington's Pharmaceutical Sciences, Meade Publishing Co., Easton, PA. For parenteral administration, injection is preferred, including intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the compounds can be formulated in liquid solutions, preferably in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the compounds may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets, lozenges, or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other

suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., ationd oil,
5 oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

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

CLK-inhibiting compounds may be formulated for parenteral administration
20 by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.
25 Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

CLK-inhibiting compounds may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

30 In addition to the formulations described previously, CLK-inhibiting compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or

intramuscularly) or by intramuscular injection. Thus, for example, CLK-inhibiting compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt. Controlled
5 release formula also includes patches.

In certain embodiments, the compounds described herein can be formulated for delivery to the central nervous system (CNS) (reviewed in Begley, Pharmacology & Therapeutics 104: 29-45 (2004)). Conventional approaches for drug delivery to the CNS include: neurosurgical strategies (e.g., intracerebral
10 injection or intracerebroventricular infusion); molecular manipulation of the agent (e.g., production of a chimeric fusion protein that comprises a transport peptide that has an affinity for an endothelial cell surface molecule in combination with an agent that is itself incapable of crossing the blood-brain-barrier (BBB)) in an attempt to exploit one of the endogenous transport pathways of the BBB; pharmacological
15 strategies designed to increase the lipid solubility of an agent (e.g., conjugation of water-soluble agents to lipid or cholesterol carriers); and the transitory disruption of the integrity of the BBB by hyperosmotic disruption (resulting from the infusion of a mannitol solution into the carotid artery or the use of a biologically active agent such as an angiotensin peptide).

20 One possibility to achieve sustained release kinetics is embedding or encapsulating the active compound into nanoparticles. Nanoparticles can be administrated as powder, as a powder mixture with added excipients or as suspensions. Colloidal suspensions of nanoparticles can easily be administrated through a cannula with small diameter.

25 Nanoparticles are particles with a diameter from about 5 nm to up to about 1000 nm. The term "nanoparticles" as it is used hereinafter refers to particles formed by a polymeric matrix in which the active compound is dispersed, also known as "nanospheres", and also refers to nanoparticles which are composed of a core containing the active compound which is surrounded by a polymeric membrane, also
30 known as "nanocapsules". In certain embodiments, nanoparticles are preferred having a diameter from about 50 nm to about 500 nm, in particular from about 100 nm to about 200 nm.

Further description on preparing nanoparticles can be found, for example, in US Patent No. 6,264,922, the contents of which are incorporated herein by reference.

Liposomes are a further drug delivery system which is easily injectable.

5 Accordingly, in the method of invention the active compounds can also be administered in the form of a liposome delivery system. Liposomes are well-known by a person skilled in the art. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines. Liposomes being usable for the method of invention encompass all types of liposomes
10 including, but not limited to, small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles.

Another way to produce a formulation, particularly a solution, of a CLK modulator, is through the use of cyclodextrin. By cyclodextrin is meant α -, β -, or γ -cyclodextrin. Cyclodextrins are described in detail in Pitha et al., U.S. Pat. No.
15 4,727,064, which is incorporated herein by reference. Cyclodextrins are cyclic oligomers of glucose; these compounds form inclusion complexes with any drug whose molecule can fit into the lipophile-seeking cavities of the cyclodextrin molecule.

Additional description of the use of cyclodextrin for solubilizing compounds
20 can be found in US 2005/0026849, the contents of which are incorporated herein by reference.

Rapidly disintegrating or dissolving dosage forms are useful for the rapid absorption, particularly buccal and sublingual absorption, of pharmaceutically active agents. Fast melt dosage forms are beneficial to patients, such as aged and pediatric
25 patients, who have difficulty in swallowing typical solid dosage forms, such as caplets and tablets. Additionally, fast melt dosage forms circumvent drawbacks associated with, for example, chewable dosage forms, wherein the length of time an active agent remains in a patient's mouth plays an important role in determining the amount of taste masking and the extent to which a patient may object to throat
30 grittiness of the active agent.

To overcome such problems manufacturers have developed a number of fast melt solid dose oral formulations. These are available from manufacturers including

Cima Labs, Fuisz Technologies Ltd., Prographarm, R. P. Scherer, Yamanouchi-Shaklee, and McNeil-PPC, Inc. All of these manufacturers market different types of rapidly dissolving solid oral dosage forms. See e.g., patents and publications by Cima Labs such as U.S. Pat. No. 5,607,697, 5,503,846, 5,223,264, 5,401,513, 5,219,574, and 5,178,878, WO 98/46215, WO 98/14179; patents to Fuisz Technologies, now part of BioVail, such as U.S. Pat. No. 5,871,781, 5,869,098, 5,866,163, 5,851,553, 5,622,719, 5,567,439, and 5,587,172; U.S. Pat. No. 5,464,632 to Prographarm; patents to R. P. Scherer such as U.S. Pat. No. 4,642,903, 5,188,825, 5,631,023 and 5,827,541; patents to Yamanouchi-Shaklee such as U.S. Pat. No. 5,576,014 and 5,446,464; patents to Janssen such as U.S. Pat. No. 5,807,576, 5,635,210, 5,595,761, 5,587,180 and 5,776,491; U.S. Pat. Nos. 5,639,475 and 5,709,886 to Eurand America, Inc.; U.S. Pat. Nos. 5,807,578 and 5,807,577 to L.A.B. Pharmaceutical Research; patents to Schering Corporation such as U.S. Pat. Nos. 5,112,616 and 5,073,374; U.S. Pat. No. 4,616,047 to Laboratoire L. LaFon; U.S. Pat. No. 5,501,861 to Takeda Chemicals Inc., Ltd.; and U.S. Pat. No. 6,316,029 to Elan.

Additional description of the preparation of fast melt tablets can be found, for example, in U.S. Pat. No. 5,939,091, the contents of which are incorporated herein by reference.

Pharmaceutical compositions (including cosmetic preparations) may comprise from about 0.00001 to 100% such as from 0.001 to 10% or from 0.1% to 5% by weight of one or more CLK-inhibiting compounds described herein.

In one embodiment, a CLK-inhibiting compound described herein, is incorporated into a topical formulation containing a topical carrier that is generally suited to topical drug administration and comprising any such material known in the art. The topical carrier may be selected so as to provide the composition in the desired form, e.g., as an ointment, lotion, cream, microemulsion, gel, oil, solution, or the like, and may be comprised of a material of either naturally occurring or synthetic origin. It is preferable that the selected carrier not adversely affect the active agent or other components of the topical formulation. Examples of suitable topical carriers for use herein include water, alcohols and other nontoxic organic

solvents, glycerin, mineral oil, silicone, petroleum jelly, lanolin, fatty acids, vegetable oils, parabens, waxes, and the like.

Formulations may be colorless, odorless ointments, lotions, creams, microemulsions and gels.

5 CLK-inhibiting compounds may be incorporated into ointments, which generally are semisolid preparations which are typically based on petrolatum or other petroleum derivatives. The specific ointment base to be used, as will be appreciated by those skilled in the art, is one that will provide for optimum drug delivery, and, preferably, will provide for other desired characteristics as well, e.g.,
10 emolliency or the like. As with other carriers or vehicles, an ointment base should be inert, stable, nonirritating and nonsensitizing. As explained in Remington's (*supra*) ointment bases may be grouped in four classes: oleaginous bases; emulsifiable bases; emulsion bases; and water-soluble bases. Oleaginous ointment bases include, for example, vegetable oils, fats obtained from animals, and
15 semisolid hydrocarbons obtained from petroleum. Emulsifiable ointment bases, also known as absorbent ointment bases, contain little or no water and include, for example, hydroxystearin sulfate, anhydrous lanolin and hydrophilic petrolatum. Emulsion ointment bases are either water-in-oil (W/O) emulsions or oil-in-water (O/W) emulsions, and include, for example, cetyl alcohol, glyceryl monostearate,
20 lanolin and stearic acid. Exemplary water-soluble ointment bases are prepared from polyethylene glycols (PEGs) of varying molecular weight; again, reference may be had to Remington's, *supra*, for further information.

CLK-inhibiting compounds may be incorporated into lotions, which generally are preparations to be applied to the skin surface without friction, and are
25 typically liquid or semiliquid preparations in which solid particles, including the active agent, are present in a water or alcohol base. Lotions are usually suspensions of solids, and may comprise a liquid oily emulsion of the oil-in-water type. Lotions are preferred formulations for treating large body areas, because of the ease of applying a more fluid composition. It is generally necessary that the insoluble
30 matter in a lotion be finely divided. Lotions will typically contain suspending agents to produce better dispersions as well as compounds useful for localizing and holding the active agent in contact with the skin, e.g., methylcellulose, sodium

carboxymethylcellulose, or the like. An exemplary lotion formulation for use in conjunction with the present method contains propylene glycol mixed with a hydrophilic petrolatum such as that which may be obtained under the trademark Aquaphor™ from Beiersdorf, Inc. (Norwalk, CT).

5 CLK-inhibiting compounds may be incorporated into creams, which generally are viscous liquid or semisolid emulsions, either oil-in-water or water-in-oil. Cream bases are water-washable, and contain an oil phase, an emulsifier and an aqueous phase. The oil phase is generally comprised of petrolatum and a fatty alcohol such as cetyl or stearyl alcohol; the aqueous phase usually, although not
10 necessarily, exceeds the oil phase in volume, and generally contains a humectant. The emulsifier in a cream formulation, as explained in Remington's, *supra*, is generally a nonionic, anionic, cationic or amphoteric surfactant.

CLK-inhibiting compounds may be incorporated into microemulsions, which generally are thermodynamically stable, isotropically clear dispersions of
15 two immiscible liquids, such as oil and water, stabilized by an interfacial film of surfactant molecules (Encyclopedia of Pharmaceutical Technology (New York: Marcel Dekker, 1992), volume 9). For the preparation of microemulsions, surfactant (emulsifier), co-surfactant (co-emulsifier), an oil phase and a water phase are necessary. Suitable surfactants include any surfactants that are useful in the
20 preparation of emulsions, e.g., emulsifiers that are typically used in the preparation of creams. The co-surfactant (or "co-emulsifier") is generally selected from the group of polyglycerol derivatives, glycerol derivatives and fatty alcohols. Preferred emulsifier/co-emulsifier combinations are generally although not necessarily selected from the group consisting of: glyceryl monostearate and polyoxyethylene
25 stearate; polyethylene glycol and ethylene glycol palmitostearate; and caprylic and capric triglycerides and oleoyl macrogolglycerides. The water phase includes not only water but also, typically, buffers, glucose, propylene glycol, polyethylene glycols, preferably lower molecular weight polyethylene glycols (e.g., PEG 300 and PEG 400), and/or glycerol, and the like, while the oil phase will generally
30 comprise, for example, fatty acid esters, modified vegetable oils, silicone oils, mixtures of mono- di- and triglycerides, mono- and di-esters of PEG (e.g., oleoyl macrogol glycerides), etc.

CLK-inhibiting compounds may be incorporated into gel formulations, which generally are semisolid systems consisting of either suspensions made up of small inorganic particles (two-phase systems) or large organic molecules distributed substantially uniformly throughout a carrier liquid (single phase gels).

- 5 Single phase gels can be made, for example, by combining the active agent, a carrier liquid and a suitable gelling agent such as tragacanth (at 2 to 5%), sodium alginate (at 2-10%), gelatin (at 2-15%), methylcellulose (at 3-5%), sodium carboxymethylcellulose (at 2-5%), carbomer (at 0.3-5%) or polyvinyl alcohol (at 10-20%) together and mixing until a characteristic semisolid product is produced.
- 10 Other suitable gelling agents include methylhydroxycellulose, polyoxyethylene-polyoxypropylene, hydroxyethylcellulose and gelatin. Although gels commonly employ aqueous carrier liquid, alcohols and oils can be used as the carrier liquid as well.

- Various additives, known to those skilled in the art, may be included in
- 15 formulations, e.g., topical formulations. Examples of additives include, but are not limited to, solubilizers, skin permeation enhancers, opacifiers, preservatives (e.g., anti-oxidants), gelling agents, buffering agents, surfactants (particularly nonionic and amphoteric surfactants), emulsifiers, emollients, thickening agents, stabilizers, humectants, colorants, fragrance, and the like. Inclusion of solubilizers and/or skin
- 20 permeation enhancers is particularly preferred, along with emulsifiers, emollients and preservatives. An optimum topical formulation comprises approximately: 2 wt. % to 60 wt. %, preferably 2 wt. % to 50 wt. %, solubilizer and/or skin permeation enhancer; 2 wt. % to 50 wt. %, preferably 2 wt. % to 20 wt. %, emulsifiers; 2 wt. % to 20 wt. % emollient; and 0.01 to 0.2 wt. % preservative, with the active agent and
- 25 carrier (e.g., water) making of the remainder of the formulation.

- A skin permeation enhancer serves to facilitate passage of therapeutic levels of active agent to pass through a reasonably sized area of unbroken skin. Suitable enhancers are well known in the art and include, for example: lower alkanols such as methanol ethanol and 2-propanol; alkyl methyl sulfoxides such as
- 30 dimethylsulfoxide (DMSO), decylmethylsulfoxide (C₁₀ MSO) and tetradecylmethyl sulfoxide; pyrrolidones such as 2-pyrrolidone, N-methyl-2-pyrrolidone and N-(-hydroxyethyl)pyrrolidone; urea; N,N-diethyl-m-toluamide; C₂-C₆ alkanediols;

miscellaneous solvents such as dimethyl formamide (DMF), N,N-dimethylacetamide (DMA) and tetrahydrofurfuryl alcohol; and the 1-substituted azacycloheptan-2-ones, particularly 1-n-dodecylcyclazacycloheptan-2-one (laurocapram; available under the trademark Azone^{RTM} from Whitby Research Incorporated, Richmond, Va.).

Examples of solubilizers include, but are not limited to, the following: hydrophilic ethers such as diethylene glycol monoethyl ether (ethoxydiglycol, available commercially as Transcutol^{RTM}) and diethylene glycol monoethyl ether oleate (available commercially as Softcutol^{RTM}); polyethylene castor oil derivatives such as polyoxy 35 castor oil, polyoxy 40 hydrogenated castor oil, etc.; polyethylene glycol, particularly lower molecular weight polyethylene glycols such as PEG 300 and PEG 400, and polyethylene glycol derivatives such as PEG-8 caprylic/capric glycerides (available commercially as Labrasol^{RTM}); alkyl methyl sulfoxides such as DMSO; pyrrolidones such as 2-pyrrolidone and N-methyl-2-pyrrolidone; and DMA. Many solubilizers can also act as absorption enhancers. A single solubilizer may be incorporated into the formulation, or a mixture of solubilizers may be incorporated therein.

Suitable emulsifiers and co-emulsifiers include, without limitation, those emulsifiers and co-emulsifiers described with respect to microemulsion formulations. Emollients include, for example, propylene glycol, glycerol, isopropyl myristate, polypropylene glycol-2 (PPG-2) myristyl ether propionate, and the like.

Other active agents may also be included in formulations, e.g., other anti-inflammatory agents, analgesics, antimicrobial agents, antifungal agents, antibiotics, vitamins, antioxidants, and sunblock agents commonly found in sunscreen formulations including, but not limited to, anthranilates, benzophenones (particularly benzophenone-3), camphor derivatives, cinnamates (e.g., octyl methoxycinnamate), dibenzoyl methanes (e.g., butyl methoxydibenzoyl methane), p-aminobenzoic acid (PABA) and derivatives thereof, and salicylates (e.g., octyl salicylate).

In certain topical formulations, the active agent is present in an amount in the range of approximately 0.25 wt. % to 75 wt. % of the formulation, preferably in

the range of approximately 0.25 wt. % to 30 wt. % of the formulation, more preferably in the range of approximately 0.5 wt. % to 15 wt. % of the formulation, and most preferably in the range of approximately 1.0 wt. % to 10 wt. % of the formulation.

5 Topical skin treatment compositions can be packaged in a suitable container to suit its viscosity and intended use by the consumer. For example, a lotion or cream can be packaged in a bottle or a roll-ball applicator, or a propellant-driven aerosol device or a container fitted with a pump suitable for finger operation. When the composition is a cream, it can simply be stored in a non-deformable bottle or
10 squeeze container, such as a tube or a lidded jar. The composition may also be included in capsules such as those described in U.S. Pat. No. 5,063,507. Accordingly, also provided are closed containers containing a cosmetically acceptable composition as herein defined.

 In an alternative embodiment, a pharmaceutical formulation is provided for
15 oral or parenteral administration, in which case the formulation may comprises a modulating compound-containing microemulsion as described above, but may contain alternative pharmaceutically acceptable carriers, vehicles, additives, etc. particularly suited to oral or parenteral drug administration. Alternatively, a modulating compound-containing microemulsion may be administered orally or
20 parenterally substantially as described above, without modification.

 Conditions of the eye can be treated or prevented by, e.g., systemic, topical, intraocular injection of a CLK-inhibiting compound, or by insertion of a sustained release device that releases a CLK-inhibiting compound. A CLK-inhibiting compound that increases or decreases the level and/or activity of a CLK protein
25 may be delivered in a pharmaceutically acceptable ophthalmic vehicle, such that the compound is maintained in contact with the ocular surface for a sufficient time period to allow the compound to penetrate the corneal and internal regions of the eye, as for example the anterior chamber, posterior chamber, vitreous body, aqueous humor, vitreous humor, cornea, iris/ciliary, lens, choroid/retina and sclera.
30 The pharmaceutically-acceptable ophthalmic vehicle may, for example, be an ointment, vegetable oil or an encapsulating material. Alternatively, the compounds of the invention may be injected directly into the vitreous and aqueous humour. In

a further alternative, the compounds may be administered systemically, such as by intravenous infusion or injection, for treatment of the eye.

CLK-inhibiting compounds described herein may be stored in oxygen free environment according to methods in the art.

5 Cells, e.g., treated *ex vivo* with a CLK-inhibiting compound, can be administered according to methods for administering a graft to a subject, which may be accompanied, e.g., by administration of an immunosuppressant drug, e.g., cyclosporin A. For general principles in medicinal formulation, the reader is referred to Cell Therapy: Stem Cell Transplantation, Gene Therapy, and Cellular
10 Immunotherapy, by G. Morstyn & W. Sheridan eds, Cambridge University Press, 1996; and Hematopoietic Stem Cell Therapy, E. D. Ball, J. Lister & P. Law, Churchill Livingstone, 2000.

Toxicity and therapeutic efficacy of CLK-inhibiting compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental
15 animals. The LD₅₀ is the dose lethal to 50% of the population. The ED₅₀ is the dose therapeutically effective in 50% of the population. The dose ratio between toxic and therapeutic effects (LD₅₀/ED₅₀) is the therapeutic index. CLK-inhibiting compounds that exhibit large therapeutic indexes are preferred. While CLK-inhibiting compounds that exhibit toxic side effects may be used, care should be
20 taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds
25 may lie within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma
30 concentration range that includes the IC₅₀ (i.e., the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful

doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

6. Kits

Also provided herein are kits, e.g., kits for therapeutic purposes or kits for
5 modulating the lifespan of cells or modulating apoptosis. A kit may comprise one or more CLK-inhibiting compounds, e.g., in premeasured doses. A kit may optionally comprise devices for contacting cells with the compounds and instructions for use. Devices include syringes, stents and other devices for introducing a CLK-inhibiting compound into a subject (e.g., the blood vessel of a
10 subject) or applying it to the skin of a subject.

Another type of kit contemplated by the invention are kits for identifying CLK-inhibiting compounds. Such kits contain (1) a CLK protein and (2) a CLK-inhibiting compound of the invention, which are in separate vessels. Such kits can be used, for example, to perform a competition-type assay to test other compounds
15 (typically provided by the user) for CLK-inhibiting activity. In certain embodiments, these kits further comprise means for determining CLK activity (e.g., a peptide substrate).

In yet another embodiment, the invention provides a composition of matter comprising a CLK-inhibiting compound described herein and another therapeutic
20 agent in separate dosage forms, but associated with one another. The term “associated with one another” as used herein means that the separate dosage forms are packaged together or otherwise attached to one another such that it is readily apparent that the separate dosage forms are intended to be sold and administered as part of the same regimen. The agent and the CLK-inhibiting compound are
25 preferably packaged together in a blister pack or other multi-chamber package, or as connected, separately sealed containers (such as foil pouches or the like) that can be separated by the user (e.g., by tearing on score lines between the two containers).

In still another embodiment, the invention provides a kit comprising in
30 separate vessels, a) a CLK-inhibiting compound of this invention; and b) another therapeutic agent such as those described elsewhere in the specification.

The practice of the present methods will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Molecular Cloning A Laboratory Manual, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989); DNA Cloning, Volumes I and II (D. N. Glover ed., 1985); Oligonucleotide Synthesis (M. J. Gait ed., 1984); Mullis et al. U.S. Patent No: 4,683,195; Nucleic Acid Hybridization (B. D. Hames & S. J. Higgins eds. 1984); Transcription And Translation (B. D. Hames & S. J. Higgins eds. 1984); Culture Of Animal Cells (R. I. Freshney, Alan R. Liss, Inc., 1987); Immobilized Cells And Enzymes (IRL Press, 1986); B. Perbal, A Practical Guide To Molecular Cloning (1984); the treatise, Methods In Enzymology (Academic Press, Inc., N.Y.); Gene Transfer Vectors For Mammalian Cells (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); Methods In Enzymology, Vols. 154 and 155 (Wu et al. eds.), Immunochemical Methods In Cell And Molecular Biology (Mayer and Walker, eds., Academic Press, London, 1987); Handbook Of Experimental Immunology, Volumes I-IV (D. M. Weir and C. C. Blackwell, eds., 1986); Manipulating the Mouse Embryo, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986).

EXEMPLIFICATION

The invention now being generally described, it will be more readily understood by reference to the following examples which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the invention in any way.

EXAMPLE 1: Procedures for the Preparation of CLK inhibitors and precursors thereof

General Procedure A: Alkylation of benzothiazoles. To 1 equivalent of the thiazole was added 10 equivalents of the appropriate alkyl iodide, or 3 equivalents of the appropriate benzyl bromide. Methyl iodide and benzyl bromide mixtures were heated at reflux, or at 70 °C respectively for 24 h unless otherwise specified. Ethyl iodide mixtures were heated at reflux for 41 h. The precipitates were filtered, washed with ethyl acetate, and dried *in vacuo* to give the thiazolium salts.

General Procedure B: Acylation of thiazolium salts. To a suspension of 1.5 mmol of the appropriate thiazolium salt in 10 mL of acetonitrile was added 3.7 mmol of the appropriate anhydride, and 4.5 mmol of triethylamine. The reaction was heated at reflux for 2 h, then concentrated *in vacuo*. The residue was dissolved in 50 mL of water, and extracted with ethyl acetate or dichloromethane. (3 x 15 mL). The combined organic layers were washed with brine (1 x 30 mL), dried over Na₂SO₄, filtered, and concentrated. If indicated, the product was purified via silica gel chromatography, eluting with an appropriate solvent mixture. Alternatively, the crude product solution was treated with decolorizing charcoal and filtered through a short silica gel pad, washing with CH₂Cl₂. After removal of the solvent *in vacuo*, the residue was triturated with 1:3 petroleum ether: ethyl acetate, then filtered to give the desired product.

General Procedure C: Hydrolysis of phenyl acetates. To a mixture of 1.0 mmol of the ester, and 1.0 mmol of NaOH was added 6 mL of methanol. The reaction was stirred and heated at 60 °C for 30 min, then cooled to ambient temperature. Next, 1 M HCl was added until pH = 7, then the methanol was removed *in vacuo*. The residue was dissolved in 20 mL of water, then the precipitate was filtered and rinsed with water. The solid was dissolved in 10:1 CH₂Cl₂:methanol, filtered, and concentrated *in vacuo*. The residue was triturated with methanol, filtered, and rinsed with methanol to give the product.

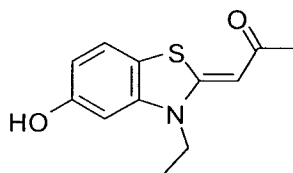
General Procedure D: Palladium catalyzed coupling of amines with aryl bromides. To a solution of 1.0 mmol of the aryl bromide 9.0 mmol of the alkylamine, 12 mg (0.054 mmol) of Pd(OAc)₂, 22.5 mg (0.1 mmol) of 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (BINAP), and 975 mg (3.0 mmol) of cesium carbonate, was added 20 mL of 1:1 (v/v) dioxane: toluene. The reaction was stirred under N₂ at 85 °C overnight. The mixture was filtered and concentrated *in vacuo*. The residue was purified via silica gel chromatography, eluting with 5:2 petroleum ether: ethyl acetate. Alternatively, the product could be crystallized from a mixture of 1:1:1 CH₂Cl₂: petroleum ether: ethyl acetate. Products were isolated as yellow solids.

General Procedure E: Hydrolysis of acetanilides. To 1.0 mmol of the amide was added 6 mL of 1.7M NaOH in methanol. The solution was heated at reflux for 16 h,

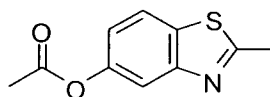
then the solvent was removed *in vacuo*. The residue was dissolved in 20 mL of water, and 1 M HCl was added until pH = 6-7. The precipitate was filtered and rinsed with water, then dissolved in 10:1 CH₂Cl₂: methanol, filtered, and concentrated *in vacuo*. The residue was triturated with methanol, filtered, and rinsed with methanol to give the aniline.

General Procedure F: Preparative HPLC methods. Compounds were eluted over a C₁₈ column with a CH₃CN: water mixture. 20-95 CH₃CN gradient protocol: mobile phase: 20% CH₃CN and 80% H₂O for 5 min, then from 20% CH₃CN and 80% H₂O to 95 % CH₃CN and 5% H₂O over 18 min, then from 95% CH₃CN and 5% H₂O to 20 % CH₃CN and 80% H₂O over 3 min, finally 20% CH₃CN and 80% H₂O for 5 min. 40-95 CH₃CN gradient protocol: 40% CH₃CN and 60% H₂O for 5 min, then from 40% CH₃CN and 60% H₂O to 95 % CH₃CN and 5% H₂O over 18 min, then from 95% CH₃CN and 5% H₂O to 40 % CH₃CN and 60% H₂O over 3 min, finally 40% CH₃CN and 60% H₂O for 5 min.

Preparation of (Z)-1-(3-ethyl-5-hydroxybenzo[d]thiazol-2(3H)-ylidene)propan-2-one (2):

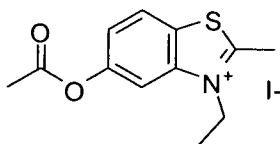


Preparation of 2-methylbenzothiazol-5-yl acetate:



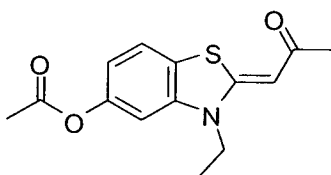
A mixture of 1.18 g (7.1 mmol) of 2-methylbenzo[d]thiazol-5-ol and 0.8 mL (8.5 mmol) of acetic anhydride in 7 mL of pyridine was stirred at ambient temperature for 1 h. The solvent was removed *in vacuo*, then the residue was taken up in ethyl acetate (30 mL). The organic suspension was washed with water (1 x 30 mL), dried over Na₂SO₄, filtered, and concentrated *in vacuo* to give 1.49 g (100%) of a white solid.

Preparation of 5-acetoxy-3-ethyl-2-methylbenzo[d]thiazol-3-ium iodide:



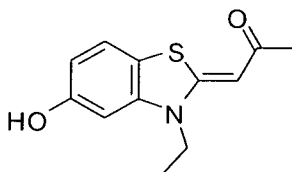
A mixture of 1.49 g (7.17 mmol) of 2-methylbenzothiazol-5-yl acetate and 15 mL (188 mmol) of ethyl iodide was heated at reflux for 41 h. The reaction mixture was cooled to ambient temperature, then the precipitate was filtered, rinsed with ethyl acetate (50 mL), and dried *in vacuo* to give 729 mg (28%) of a blue solid.

Preparation of (Z)-3-ethyl-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl acetate:



To a suspension of 508 mg (1.4 mmol) of 5-acetoxy-3-ethyl-2-methylbenzo[d]thiazol-3-ium iodide in 4 mL of acetonitrile was added 0.3 mL (3.2 mmol) of acetic anhydride, and 0.58 mL (4.2 mmol) of triethylamine. The reaction was heated at reflux for 2 h, then cooled to ambient temperature and concentrated *in vacuo*. The residue was suspended in 50 mL of water, then the mixture was extracted with ethyl acetate (3 x 20 mL). The combined ethyl acetate layers were back extracted with brine (1 x 40 mL), dried over Na₂SO₄, filtered, and concentrated *in vacuo*. The residue was purified via silica gel chromatography, eluting with 4:1 CH₂Cl₂: ethyl acetate to give 284 mg (73%) of a pale yellow solid.

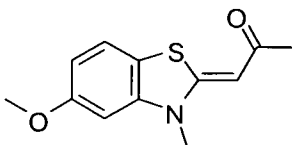
Preparation of (Z)-1-(3-ethyl-5-hydroxybenzo[d]thiazol-2(3H)-ylidene)propan-2-one:



To a solution of 161 mg (0.58 mmol) of (Z)-3-ethyl-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl acetate in 10 mL of methanol was added 28 mg (0.58 mmol) of NaOH_(s). The mixture was heated at reflux for 20 min, then cooled and concentrated to about 4 mL. Next, HCl_(aq.) was added to pH=6-7, then the precipitate

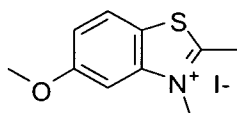
was filtered, and dried *in vacuo* to give 115 mg (85%) of a pale yellow solid. MS (ESI⁺) Calc'd for C₁₂H₁₃NO₂S: 235.07. Found: *m/z* = 236 (M+H⁺).

Preparation of (Z)-1-(5-methoxy-3-methylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one (3):



5

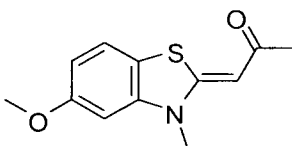
Preparation of 5-methoxy-2,3-dimethylbenzo[d]thiazol-3-ium iodide:



A mixture of 757 mg (4.22 mmol) of 5-methoxy-2-methylbenzo[d]thiazole and 8 mL (128 mmol) of methyl iodide was heated at reflux for 26 h. The precipitate was filtered, rinsed with 50 mL of ethyl acetate and dried *in vacuo* to give 1.1 g (82%) of a blue solid.

10

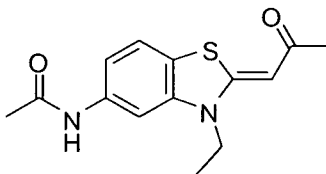
Preparation of (Z)-1-(5-methoxy-3-methylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one:



Prepared according to general procedure B from 5-methoxy-2,3-dimethylbenzo[d]thiazol-3-ium iodide and acetic anhydride. Yield 310 mg (73%), yellow solid. MS (ESI⁺) Calc'd for C₁₂H₁₃NO₂S: 235.07. Found: *m/z* = 236 (M+H⁺).

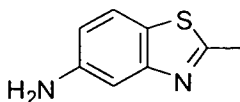
15

Preparation of (Z)-N-(3-ethyl-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)acetamide (72):



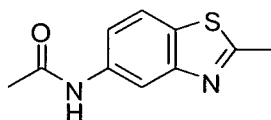
20

Preparation of 2-methylbenzo[d]thiazol-5-amine:



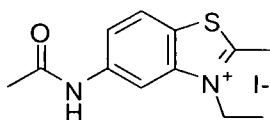
To a mixture of 3.08 g (15.9 mmol) of 2-methyl-5-nitrobenzo[d]thiazole, 4.87 g (87.2 mmol) of powdered iron, and 6.79 g (126 mmol) of ammonium chloride was added 120 mL of methanol, and 30 mL of water. The mixture was stirred and heated at reflux for 2 h, then cooled to ambient temperature and filtered. The filtrate was concentrated *in vacuo*, then the residue was partitioned between 60 mL of ethyl acetate and 40 mL of 10% Na₂CO₃. The combined ethyl acetate layers were dried over Na₂SO₄, filtered, and concentrated *in vacuo*. The crude product was purified via silica gel chromatography, eluting with 2:1 petroleum ether: ethyl acetate to give 2.55 g (98%) of a pale yellow solid.

10 **Preparation of N-(2-methylbenzo[d]thiazol-5-yl)acetamide:**



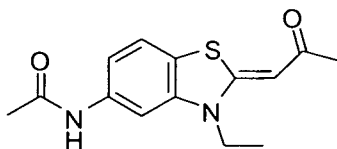
To 1.49 g (9.1 mmol) of 2-methylbenzo[d]thiazol-5-amine in 20 mL of pyridine was added 0.94 mL (10 mmol) of acetic anhydride, dropwise. The reaction was stirred at ambient temperature for 2 h, then concentrated *in vacuo*. The residue was diluted with 30 mL of water, then the precipitate was filtered and dried to give 1.83 g (98%) of a pale yellow solid.

Preparation of 5-acetamido-3-ethyl-2-methylbenzo[d]thiazol-3-ium iodide:



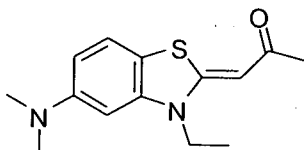
To 1.83 g (8.85 mmol) of N-(2-methylbenzo[d]thiazol-5-yl)acetamide was added 40 mL (500 mmol) of ethyl iodide. The mixture was heated at reflux for 24 h, then cooled to ambient temperature and concentrated *in vacuo*. The residue was purified via silica gel chromatography, eluting with 40:1 CH₂Cl₂:CH₃OH, then with CH₃OH, to give 0.9 g (28%) of a gray solid.

Preparation of (Z)-N-(3-ethyl-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)acetamide:

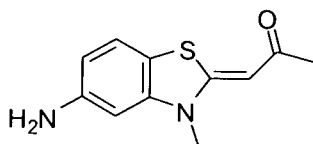


Prepared according to General Procedure B. Yield 151 mg (58%), white solid. MS (ESI⁺) Calc'd for C₁₄H₁₆N₂O₂S: 276.09. Found: *m/z* = 277 (M+H⁺).

Preparation of (Z)-1-(5-(dimethylamino)-3-ethylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one (15):

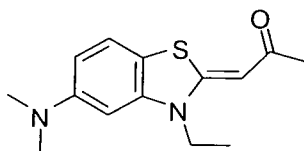


Preparation of (Z)-1-(5-amino-3-ethylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one:



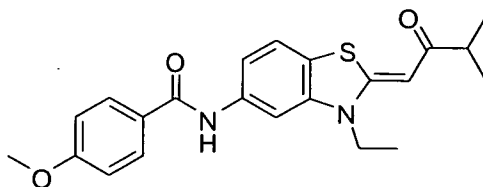
To a solution of 130 mg (0.47 mmol) of (Z)-N-(3-ethyl-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)acetamide in 30 mL of methanol was added 282 mg (7.05 mmol) of NaOH_(s). The reaction was heated at reflux for 24 h, then cooled to ambient temperature and concentrated *in vacuo*. The residue was diluted with 10 mL of water, then 12M HCl was added until pH = 6-7. The resulting precipitate was filtered, then dried *in vacuo* to give 119 mg (94%) of a yellow solid.

Preparation of (Z)-1-(5-(dimethylamino)-3-ethylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one:



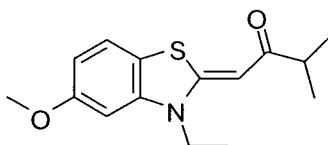
To a mixture of 85 mg (0.36 mmol) of (Z)-1-(5-amino-3-ethylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one and 121 mg (0.94 mmol) of Na₂CO₃ in 3 mL of methanol was added 119 mL (1.26 mmol) of dimethyl sulfate. The reaction was stirred at ambient temperature for 5 h, then the solids were filtered away, and the filtrate was concentrated *in vacuo*. The residue was purified via preparative TLC, eluting with 4:1 CH₂Cl₂: ethyl acetate to give 32 mg (34%) of a yellow solid. MS (ESI⁺) Calc'd for C₁₄H₁₈N₂OS: 262.11. Found: *m/z* = 263 (M+H⁺).

Preparation of (Z)-N-(3-ethyl-2-(3-methyl-2-oxobutylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)-4-methoxybenzamide (88):

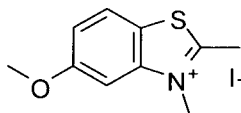


To a solution of 15 mg (0.064 mmol) of (Z)-1-(5-amino-3-ethylbenzo[d]thiazol-2(3H)-ylidene)-3-methylbutan-2-one in 2 mL of pyridine was added a solution of 11 mg (0.064 mmol) of 4-methoxybenzoyl chloride in 1.0 mL of CH₂Cl₂, while cooling with an ice bath. The ice bath was removed, then the reaction was stirred at ambient temperature for 20 h. The solvent was removed *in vacuo*, then the residue was purified via silica gel chromatography, eluting with 3:2 petroleum ether: ethyl acetate to give 18 mg (76%) of a pale yellow solid. MS (ESI⁺) Calc'd for C₂₂H₂₄N₂O₃S: 396.15. Found: *m/z* = 397 (M+H⁺).

10 Preparation of (Z)-1-(3-ethyl-5-methoxybenzo[d]thiazol-2(3H)-ylidene)-3-methylbutan-2-one (1)

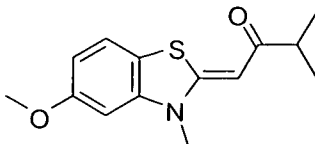


Preparation of 3-ethyl-5-methoxy-2-methylbenzo[d]thiazol-3-ium iodide:



15 .To 5.08 g (28.4 mmol) of 5-methoxy-2-methylbenzo[d]thiazole was added 44 mL (850 mmol) of ethyl iodide. The mixture was heated at reflux for 20 h, then cooled to ambient temperature. The precipitate was filtered, rinsed with 50 mL of ethyl acetate, and dried *in vacuo* to give 4.42 g (46%) of a pale green solid.

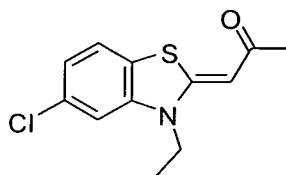
20 Preparation of (Z)-1-(3-ethyl-5-methoxybenzo[d]thiazol-2(3H)-ylidene)-3-methylbutan-2-one:



Prepared according to General Procedure B from 3-ethyl-5-methoxy-2-methylbenzo[d]thiazol-3-ium iodide and isobutyric anhydride. The crude product was purified via silica gel chromatography, eluting with 4:1 CH₂Cl₂: ethyl acetate to

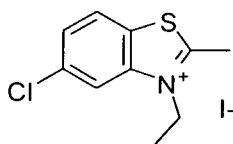
give 281 mg (74%) of a yellow solid. MS (ESI⁺) Calc'd for C₁₅H₁₉NO₂S: 277.11. Found: *m/z* = 278 (M+H⁺).

Preparation of (Z)-1-(5-chloro-3-ethylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one (90):



5

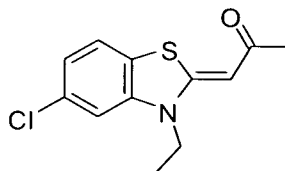
Preparation of 5-chloro-3-ethyl-2-methylbenzo[d]thiazol-3-ium iodide:



To 2.50 g (13.6 mmol) of 5-chloro-2-methylbenzo[d]thiazole was added 50 mL (500 mmol) of ethyl iodide. The mixture was heated at reflux for 72 h. The precipitate was filtered, washed with 50 mL of ethyl acetate, and dried *in vacuo* to give 1.02 g (22%) of a green solid.

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Preparation of (Z)-1-(5-chloro-3-ethylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one:

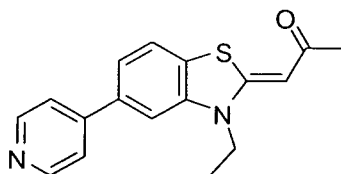


Prepared according to General Procedure B from 5-chloro-3-ethyl-2-methylbenzo[d]thiazol-3-ium iodide and acetic anhydride. Yield 371 mg (84%), pale yellow solid. MS (ESI⁺) Calc'd for C₁₂H₁₂ClNOS: 253.03. Found: *m/z* = 254 (M+H⁺).

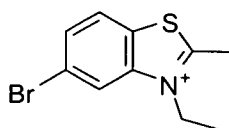
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Preparation of (Z)-1-(3-ethyl-5-(pyridin-4-yl)benzo[d]thiazol-2(3H)-ylidene)propan-2-one (89):

20

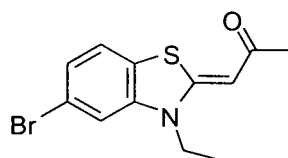


Preparation of 5-bromo-3-ethyl-2-methylbenzo[d]thiazol-3-ium iodide:



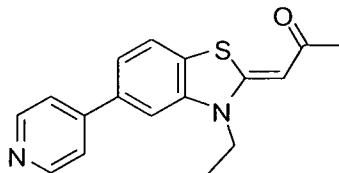
To 4.0 g (17.5 mmol) of 5-bromo-2-methylbenzo[d]thiazole was added 40 mL (500 mmol) of ethyl iodide. The reaction was heated at reflux for 45 h, then the precipitate was filtered, washed with 50 mL of ethyl acetate, and dried *in vacuo* to give 1.12 g (17%) of a pale green solid.

Preparation of (Z)-1-(5-bromo-3-ethylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one:



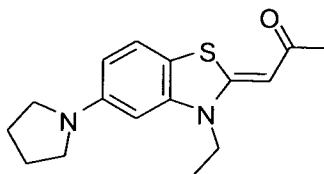
Prepared according to General Procedure B, starting with 5-bromo-3-ethyl-2-methylbenzo[d]thiazol-3-ium iodide and acetic anhydride. The crude product was purified via silica gel chromatography, eluting with 4:1 petroleum ether: ethyl acetate to give 778 mg (90%) of a pale yellow solid.

Preparation of (Z)-1-(3-ethyl-5-(pyridin-4-yl)benzo[d]thiazol-2(3H)-ylidene)propan-2-one:



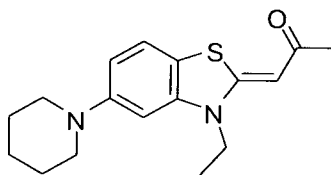
To 153 mg (0.51 mmol) of (Z)-1-(5-bromo-3-ethylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one, 63 mg (0.51 mmol) of pyridine-4-ylboronic acid, 272 mg (1.2 mmol) of $K_3PO_4 \cdot 3H_2O$, and 29 mg (0.026 mmol) of $Pd(PPh_3)_4$ was added 5 mL of DMF. The flask was flushed with argon, then heated at 85 °C for 14 h. Next, the reaction was diluted with 30 mL of water, then the suspension was extracted with ethyl acetate (3 x 20 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 , filtered, and concentrated *in vacuo*. The residue was purified via silica gel chromatography, eluting with 1:2 n-hexane: ethyl acetate to give 48 mg (32%) of the product as a pale yellow solid. MS (ESI⁺) Calc'd for $C_{17}H_{16}N_2OS$: 296.10. Found: $m/z = 297$ (M+H⁺).

Preparation of (Z)-1-(3-ethyl-5-(pyrrolidin-1-yl)benzo[d]thiazol-2(3H)-ylidene)propan-2-one (28):



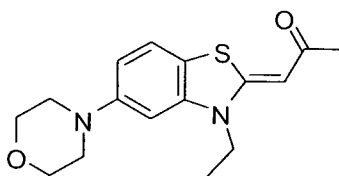
Prepared according to General Procedure D from (Z)-1-(5-bromo-3-ethylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one and pyrrolidine. The product was purified via silica gel chromatography, eluting with 5:2 petroleum ether: ethyl acetate to give 82 mg (28%) of a yellow solid. MS (ESI⁺) Calc'd for C₁₆H₂₀N₂OS: 288.13. Found: m/z = 289 (M+H⁺).

Preparation of (Z)-1-(3-ethyl-5-(piperidin-1-yl)benzo[d]thiazol-2(3H)-ylidene)propan-2-one (93):

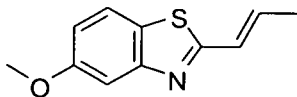
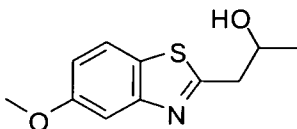


Prepared according to General Procedure D from (Z)-1-(5-bromo-3-ethylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one and piperidine. The product was purified via silica gel chromatography, eluting with 5:2 petroleum ether: ethyl acetate to give 53 mg (18%) of a yellow solid. MS (ESI⁺) Calc'd for C₁₇H₂₂N₂OS: 302.15. Found: m/z = 303 (M+H⁺).

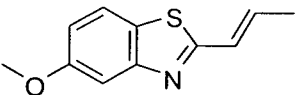
Preparation of (Z)-1-(3-ethyl-5-morpholinobenzo[d]thiazol-2(3H)-ylidene)propan-2-one (94):



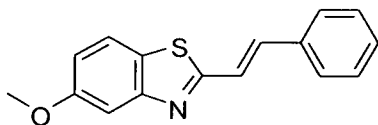
Prepared according to General Procedure D from (Z)-1-(5-bromo-3-ethylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one and morpholine. The product was purified via silica gel chromatography, eluting with 5:2 petroleum ether: ethyl acetate to give 185 mg (61%) of a yellow solid. MS (ESI⁺) Calc'd for C₁₆H₂₀N₂O₂S: 304.12. Found: m/z = 305 (M+H⁺).

Preparation of (E)-5-methoxy-2-(prop-1-enyl)benzo[d]thiazole (91):**Preparation of 1-(5-methoxybenzo[d]thiazol-2-yl)propan-2-ol:**

- 5 To a solution of 800 mg (4.5 mmol) of 5-methoxy-2-methylbenzo[d]thiazole in 8 mL of THF at -78 °C was added dropwise, 2 mL (5 mmol) of 2.5 M n-BuLi in hexanes. The mixture was stirred at -78 °C for 30 min, then 0.55 mL (9.0 mmol) of acetaldehyde in 2 mL of THF was added dropwise. The reaction was stirred at -78 °C for 1 h, then allowed to warm to ambient temperature and stirred overnight.
- 10 Next, 0.2 mL of acetic acid was added, and the mixture was diluted with 50 mL of CH₂Cl₂. The solution was dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified via silica gel chromatography, eluting with 10:1 petroleum ether: ethyl acetate, then with 20:1 CH₂Cl₂: methanol to give 456 mg (45%) of the product as an orange oil.

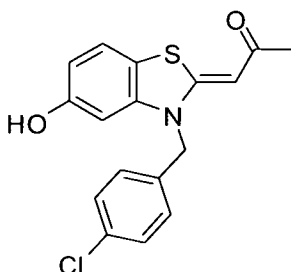
15 Preparation of (E)-5-methoxy-2-(prop-1-enyl)benzo[d]thiazole:

- To a mixture of 456 mg (2.0 mmol) of 1-(5-methoxybenzo[d]thiazol-2-yl)propan-2-ol and 40 mg (0.20 mmol) of p-toluenesulfonic acid monohydrate was added 20 mL of toluene. The reaction was heated at reflux for 3 h, then cooled to ambient
- 20 temperature and concentrated *in vacuo*. The crude product was purified via silica gel chromatography, eluting with 10:1 petroleum ether: ethyl acetate to give 172 mg (41%) of the product as an orange oil. MS (ESI⁺) Calc'd for C₁₁H₁₁NOS: 205.06. Found: *m/z* = 206 (M+H⁺).

Preparation of (E)-5-methoxy-2-styrylbenzo[d]thiazole (92):

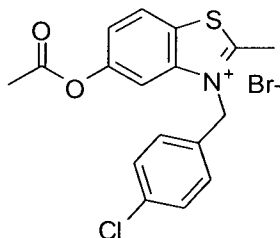
To a solution of 500 mg (0.28 mmol) of 5-methoxy-2-methylbenzo[d]thiazole in 20 mL of methanol was added 0.6 mL (5.6 mmol) of benzaldehyde, then 1.2 g (30 mmol) of NaOH_(s). The reaction was stirred and heated at reflux for 18 h. Next, about 5 mL of methanol was removed *in vacuo*, then the light yellow precipitate was
 5 filtered. The product was recrystallized from ethanol to give 170 mg (23%) of light yellow crystals. MS (ESI⁺) Calc'd for C₁₆H₁₃NOS: 267.07. Found: *m/z* = 268 (M+H⁺).

Preparation of (Z)-1-(3-(4-chlorobenzyl)-5-hydroxybenzo[d]thiazol-2(3H)-ylidene)propan-2-one (95):



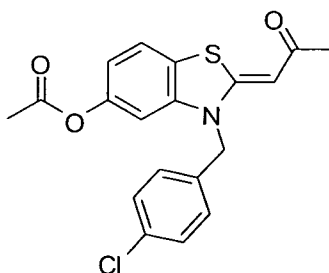
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Preparation of 5-acetoxy-3-(4-chlorobenzyl)-2-methylbenzo[d]thiazol-3-ium bromide:



Prepared according to General Procedure A from 2-methylbenzo[d]thiazol-5-yl
 15 acetate and 4-chlorobenzyl bromide. Yield 200 mg (48%), yellow solid.

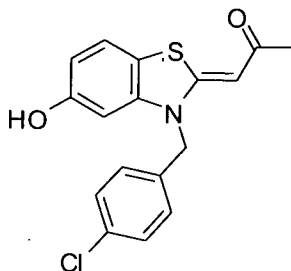
Preparation of (Z)-3-(4-chlorobenzyl)-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl acetate:



Prepared according to General Procedure B from 5-acetoxy-3-(4-chlorobenzyl)-2-methylbenzo[d]thiazol-3-ium bromide and acetic anhydride. The product was
 20

extracted with CH₂Cl₂. Purification via a short silica gel pad gave 136 mg (75%) of a yellow solid.

Preparation of (Z)-1-(3-(4-chlorobenzyl)-5-hydroxybenzo[d]thiazol-2(3H)-ylidene)propan-2-one:

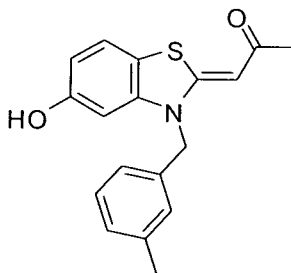


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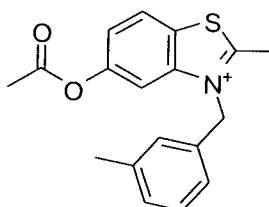
Prepared according to General Procedure C from (Z)-3-(4-chlorobenzyl)-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl acetate. Yield 68 mg (57%). MS (ESI⁺) Calc'd for C₁₇H₁₄ClNO₂S: 331.04. Found: *m/z* = 332 (M+H⁺).

Preparation of (Z)-1-(5-hydroxy-3-(3-methylbenzyl)benzo[d]thiazol-2(3H)-ylidene)propan-2-one (6):

10

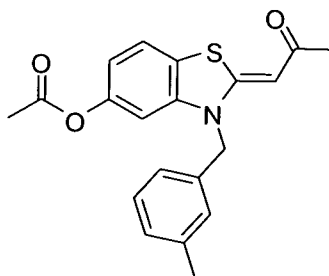


Preparation of 5-acetoxy-2-methyl-3-(3-methylbenzyl)benzo[d]thiazol-3-ium bromide:



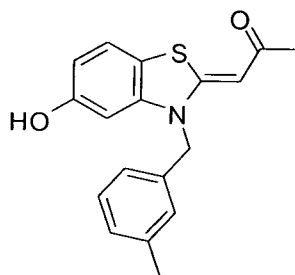
15 Prepared according to General Procedure A from 2-methylbenzo[d]thiazol-5-yl acetate and 3-methylbenzyl bromide. Yield 203 mg (52%).

Preparation of (Z)-3-(3-methylbenzyl)-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl acetate:



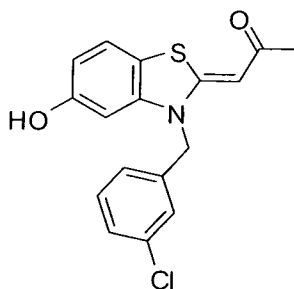
Prepared according to General Procedure B from 5-acetoxy-2-methyl-3-(3-methylbenzyl)benzo[d]thiazol-3-ium bromide and acetic anhydride. The product was extracted with CH₂Cl₂. Purification via a short silica gel pad gave 133 mg
 5 (77%) of a yellow solid.

Preparation of (Z)-1-(5-hydroxy-3-(3-methylbenzyl)benzo[d]thiazol-2(3H)-ylidene)propan-2-one:

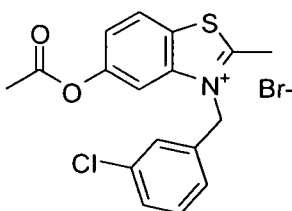


Prepared according to General Procedure C from (Z)-3-(3-methylbenzyl)-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl acetate. Yield 80 mg (67%) of a
 10 yellow solid. MS (ESI⁺) Calc'd for C₁₈H₁₇NO₂S: 311.10. Found: *m/z* = 312 (M+H⁺).

Preparation of (Z)-1-(3-(3-chlorobenzyl)-5-hydroxybenzo[d]thiazol-2(3H)-ylidene)propan-2-one (7):



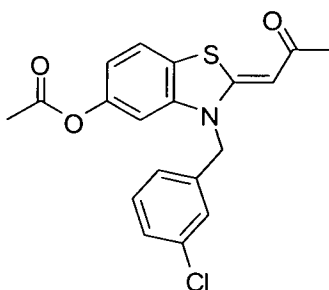
15 **Preparation of 5-acetoxy-3-(3-chlorobenzyl)-2-methylbenzo[d]thiazol-3-ium bromide:**



Prepared according to General Procedure A from 2-methylbenzo[d]thiazol-5-yl acetate and 3-chlorobenzyl bromide. Yield 140 mg (34%), yellow solid.

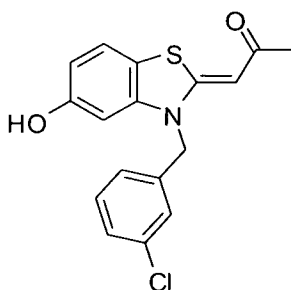
Preparation of (Z)-3-(3-chlorobenzyl)-2-(2-oxopropylidene)-2,3-

5 dihydrobenzo[d]thiazol-5-yl acetate:



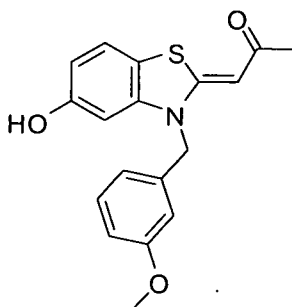
Prepared according to General Procedure B from 5-acetoxy-3-(3-chlorobenzyl)-2-methylbenzo[d]thiazol-3-ium bromide and acetic anhydride. The product was extracted with CH₂Cl₂. Purification via a short silica gel pad gave 60 mg (45%) of a yellow solid.

Preparation of (Z)-1-(3-(3-chlorobenzyl)-5-hydroxybenzo[d]thiazol-2(3H)-ylidene)propan-2-one:

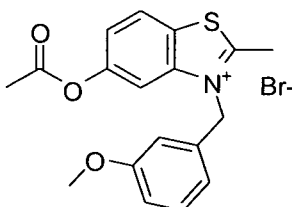


Prepared according to General Procedure C from (Z)-3-(3-chlorobenzyl)-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl acetate. Yield 35 mg (66%) of a yellow solid. MS (ESI⁺) Calc'd for C₁₇H₁₄ClNO₂S: 331.04. Found: *m/z* = 332 (M+H⁺).

Preparation of (Z)-1-(5-hydroxy-3-(3-methoxybenzyl)benzo[d]thiazol-2(3H)-ylidene)propan-2-one (8):

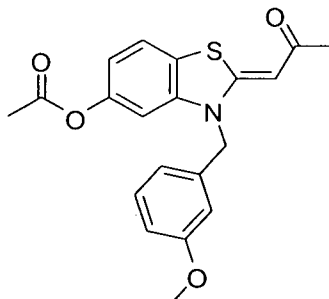


Preparation of 5-acetoxy-3-(3-methoxybenzyl)-2-methylbenzo[d]thiazol-3-ium bromide:



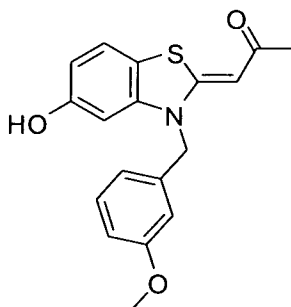
- 5 Prepared according to General Procedure A from 2-methylbenzo[d]thiazol-5-yl acetate and 3-methoxybenzyl bromide. Yield 300 mg (74%).

Preparation of (Z)-3-(3-methoxybenzyl)-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl acetate:



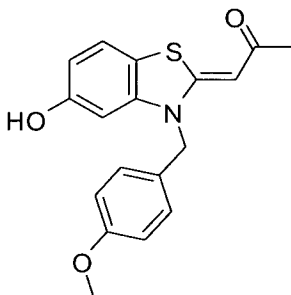
- 10 Prepared according to General Procedure B from 5-acetoxy-3-(3-methoxybenzyl)-2-methylbenzo[d]thiazol-3-ium bromide and acetic anhydride. The product was extracted with CH₂Cl₂. Purification via a short silica gel pad gave 238 mg (88%) of a yellow solid.

- 15 **Preparation of (Z)-1-(5-hydroxy-3-(3-methoxybenzyl)benzo[d]thiazol-2(3H)-ylidene)propan-2-one:**

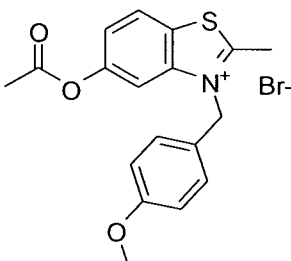


Prepared according to General Procedure C from (Z)-3-(3-methoxybenzyl)-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl acetate. Yield 152 mg (73%) of a yellow solid. MS (ESI⁺) Calc'd for C₁₈H₁₇NO₃S: 327.09. Found: *m/z* = 328 (M+H⁺).

5 **Preparation of (Z)-1-(5-hydroxy-3-(4-methoxybenzyl)benzo[d]thiazol-2(3H)-ylidene)propan-2-one (9):**



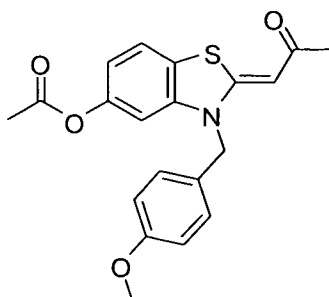
Preparation of 5-acetoxy-3-(4-methoxybenzyl)-2-methylbenzo[d]thiazol-3-ium bromide:



10

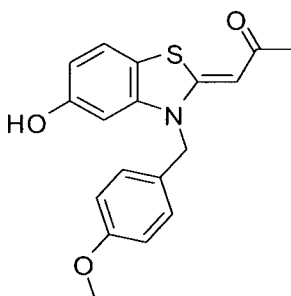
Prepared according to General Procedure A from 2-methylbenzo[d]thiazol-5-yl acetate and 4-methoxybenzyl bromide. Yield 400 mg (98%) of a yellow solid.

Preparation of (Z)-3-(4-methoxybenzyl)-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl acetate:



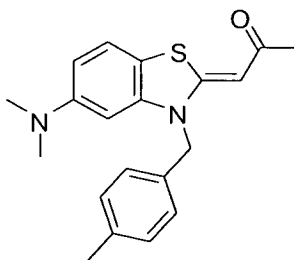
Prepared according to General Procedure B from 5-acetoxy-3-(4-methoxybenzyl)-2-methylbenzo[d]thiazol-3-ium bromide and acetic anhydride. The product was extracted with CH₂Cl₂. Purification via a short silica gel pad gave 260 mg (66%) of a yellow solid.

Preparation of (Z)-1-(5-hydroxy-3-(4-methoxybenzyl)benzo[d]thiazol-2(3H)-ylidene)propan-2-one:

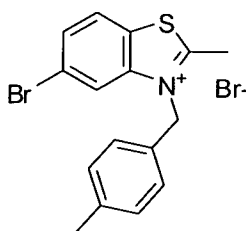


Prepared according to General Procedure C from (Z)-3-(4-methoxybenzyl)-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl acetate. Yield 145 mg (63%) of a yellow solid. MS (ESI⁺) Calc'd for C₁₈H₁₇NO₃S: 327.09. Found: *m/z* = 328 (M+H⁺).

Preparation of (Z)-1-(5-(dimethylamino)-3-(4-methylbenzyl)benzo[d]thiazol-2(3H)-ylidene)propan-2-one (17):

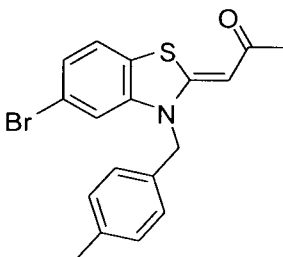


Preparation of 5-bromo-2-methyl-3-(4-methylbenzyl)benzo[d]thiazol-3-ium bromide:



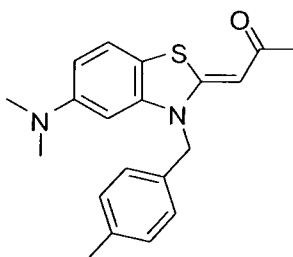
Prepared according to General Procedure A from 5-bromo-2-methylbenzo[d]thiazole and 4-methyl benzyl bromide. The reaction was heated at 140 °C for 18 h. Yield 1.48 g (72%) of a white solid.

5 **Preparation of (Z)-1-(5-bromo-3-(4-methylbenzyl)benzo[d]thiazol-2(3H)-ylidene)propan-2-one:**



Prepared according to General Procedure B from 5-bromo-2-methyl-3-(4-methylbenzyl)benzo[d]thiazol-3-ium bromide and acetic anhydride. The product was extracted with CH₂Cl₂. Trituration with 8:1 ethyl acetate; petroleum ether followed by filtration gave 0.89 g (66%) of a white solid.

Preparation of (Z)-1-(5-(dimethylamino)-3-(4-methylbenzyl)benzo[d]thiazol-2(3H)-ylidene)propan-2-one:

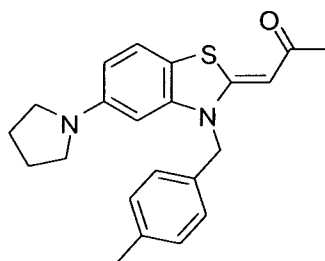


15 A microwave tube was charged with 300 mg (0.80 mmol) of (Z)-1-(5-bromo-3-(4-methylbenzyl)benzo[d]thiazol-2(3H)-ylidene)propan-2-one, 300 mg (3.68 mmol) of dimethylamine hydrochloride, 73 mg (0.08 mol) of tris(dibenzylideneacetone)dipalladium(0), 139 mg (0.24 mmol) of 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (xant phos), 480 mg (5.0 mmol) of sodium tert-butoxide, and 15 mL of 1:1 (v:v) dioxane: toluene. The tube was sealed

20

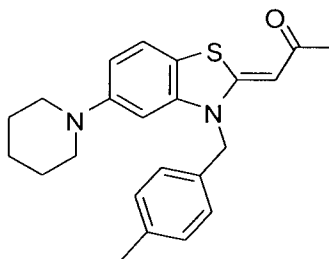
and heated at 100 °C in a microwave reactor for 1 h. The reaction mixture was filtered, then the filtrate was concentrated and purified via silica gel chromatography, eluting with 4:1 petroleum ether: ethyl acetate to give 107 mg (40%) of a yellow solid. MS (ESI⁺) Calc'd for C₂₀H₂₂N₂OS: 338.15. Found: *m/z* = 339 (M+H⁺).

Preparation of (Z)-1-(3-(4-methylbenzyl)-5-(pyrrolidin-1-yl)benzo[d]thiazol-2(3H)-ylidene)propan-2-one (30):



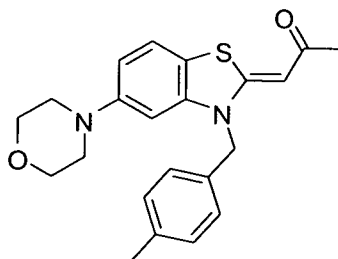
Prepared according to General Procedure D from (Z)-1-(5-bromo-3-(4-methylbenzyl)benzo[d]thiazol-2(3H)-ylidene)propan-2-one and pyrrolidine. The crude product was recrystallized from 1:1:1 CH₂Cl₂: petroleum ether: ethyl acetate to give 120 mg (62%) of a yellow solid. MS (ESI⁺) Calc'd for C₂₂H₂₄N₂OS: 364.16. Found: *m/z* = 365 (M+H⁺).

Preparation of (Z)-1-(3-(4-methylbenzyl)-5-(piperidin-1-yl)benzo[d]thiazol-2(3H)-ylidene)propan-2-one (100):



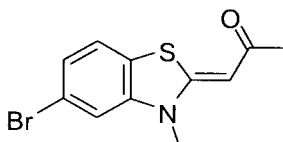
Prepared according to General Procedure D from (Z)-1-(5-bromo-3-(4-methylbenzyl)benzo[d]thiazol-2(3H)-ylidene)propan-2-one and piperidine. The crude product was recrystallized from 1:1:1 CH₂Cl₂: petroleum ether: ethyl acetate to give 97 mg (48%) of a yellow solid. MS (ESI⁺) Calc'd for C₂₃H₂₆N₂OS: 378.18. Found: *m/z* = 379 (M+H⁺).

Preparation of (Z)-1-(3-(4-methylbenzyl)-5-morpholinobenzo[d]thiazol-2(3H)-ylidene)propan-2-one (101):

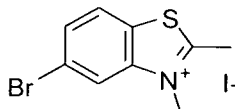


Prepared according to General Procedure D from (Z)-1-(5-bromo-3-(4-methylbenzyl)benzo[d]thiazol-2(3H)-ylidene)propan-2-one and morpholine. The crude product was recrystallized from 1:1:1 CH₂Cl₂: petroleum ether: ethyl acetate to give 104 mg (51%) of a yellow solid. MS (ESI⁺) Calc'd for C₂₂H₂₄N₂O₂S: 380.16. Found: *m/z* = 381 (M+H⁺).

Preparation of (Z)-1-(5-(dimethylamino)-3-methylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one (120):

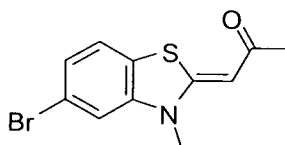


Preparation of 5-bromo-2,3-dimethylbenzo[d]thiazol-3-ium iodide:



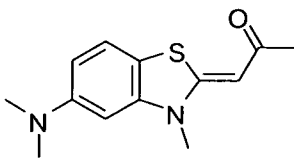
Prepared according to General Procedure A from 5-bromo-2-methylbenzo[d]thiazole and methyl iodide. Yield 3.88 g (95%), white solid.

Preparation of (Z)-1-(5-bromo-3-methylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one:



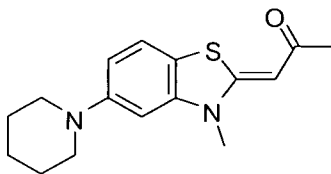
Prepared according to General Procedure B from 5-bromo-2,3-dimethylbenzo[d]thiazol-3-ium iodide and acetic anhydride. The product was extracted with CH₂Cl₂. Trituration with 4:1 ethyl acetate: petroleum ether followed by filtration gave 2.45 g (81%) of a white solid.

Preparation of (Z)-1-(5-(dimethylamino)-3-methylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one (16):

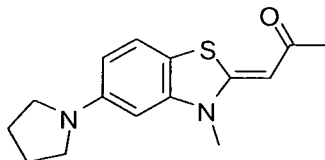


- A microwave tube was charged with 322 mg (0.80 mmol) of (Z)-1-(5-bromo-3-methylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one, 290 mg (3.45 mmol) of dimethylamine hydrochloride, 100 mg (0.11 mol) of
- 5 tris(dibenzylideneacetone)dipalladium(0), 225 mg (0.39 mmol) of 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (xant phos), 530 mg (5.52 mmol) of sodium tert-butoxide, and 15 mL of 1:1 (v:v) dioxane: toluene. The tube was sealed and heated at 100 °C in a microwave reactor for 1 h. The reaction mixture was filtered, then the filtrate was concentrated and purified via silica gel
- 10 chromatography, eluting with 5:2 petroleum ether: ethyl acetate to give 80 mg (30%) of a yellow solid. MS (ESI⁺) Calc'd for C₁₃H₁₆N₂OS: 248.10. Found: *m/z* = 249 (M+H⁺).

Preparation of (Z)-1-(3-methyl-5-(piperidin-1-yl)benzo[d]thiazol-2(3H)-ylidene)propan-2-one (87):

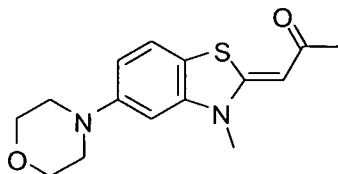


- 15 Prepared according to General Procedure D from (Z)-1-(5-bromo-3-methylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one and piperidine. Yield 72 mg (25%) of a yellow solid. MS (ESI⁺) Calc'd for C₁₆H₂₀N₂OS: 288.13. Found: *m/z* = 289 (M+H⁺).
- 20 **Preparation of (Z)-1-(3-methyl-5-(pyrrolidin-1-yl)benzo[d]thiazol-2(3H)-ylidene)propan-2-one (102):**



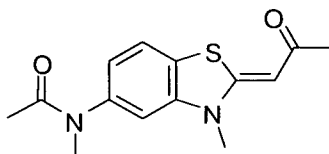
- Prepared according to General Procedure D from (Z)-1-(5-bromo-3-methylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one and pyrrolidine. Yield 104 mg
- 25 (38%). MS (ESI⁺) Calc'd for C₁₅H₁₈N₂OS: 274.11. Found: *m/z* = 275 (M+H⁺).

Preparation of (Z)-1-(3-methyl-5-morpholinobenzo[d]thiazol-2(3H)-ylidene)propan-2-one (103):

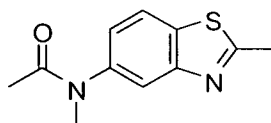


Prepared according to General Procedure D from (Z)-1-(5-bromo-3-methylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one and morpholine. Yield 200 mg (66%). MS (ESI⁺) Calc'd for C₁₅H₁₈N₂O₂S: 290.11. Found: *m/z* = 291 (M+H⁺).

Preparation of (Z)-N-methyl-N-(3-methyl-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)acetamide (108):

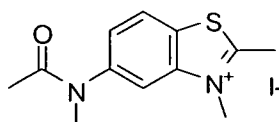


Preparation of N-methyl-N-(2-methylbenzo[d]thiazol-5-yl)acetamide:



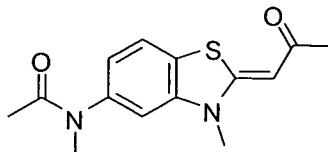
A mixture of 4.12 g (20 mmol) of N-(2-methylbenzo[d]thiazol-5-yl)acetamide, 4.48 g (40 mmol) of potassium tert-butoxide, 4 mL (64 mmol) of methyl iodide, and 70 mL of tetrahydrofuran were stirred at ambient temperature for 1 h. The reaction was concentrated *in vacuo*, then the residue was taken up in CH₂Cl₂. The solution was extracted with water, then the organic layer was treated with activated charcoal and Na₂SO₄, and filtered through a silica gel pad. The pad was washed with CH₂Cl₂, then the combined CH₂Cl₂ solutions were concentrated *in vacuo*. The residue was triturated with 10:1 ethyl acetate: petroleum ether and filtered to give 3.06 g (69%) of a white solid.

Preparation of 2,3-dimethyl-5-(N-methylacetamido)benzo[d]thiazol-3-ium iodide:



Prepared according to General Procedure A from N-(2-methylbenzo[d]thiazol-5-yl)acetamide and methyl iodide. Yield: 795 mg (76%), white solid.

Preparation of (Z)-N-methyl-N-(3-methyl-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)acetamide:

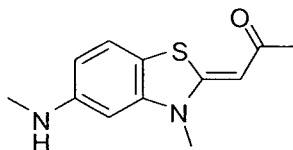


5

Prepared according to General Procedure B from 2,3-dimethyl-5-(N-methylacetamido)benzo[d]thiazol-3-ium iodide and acetic anhydride. The product was extracted with CH₂Cl₂. Trituration with 4:1 ethyl acetate: petroleum ether followed by filtration gave 486 mg (80%) of a white solid. MS (ESI⁺) Calc'd for

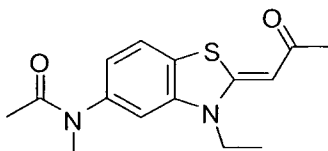
10 C₁₄H₁₆N₂O₂S: 276.09. Found: m/z = 277 (M+H⁺).

Preparation of (Z)-1-(3-methyl-5-(methylamino)benzo[d]thiazol-2(3H)-ylidene)propan-2-one (104):

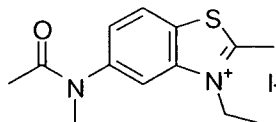


15 Prepared according to General Procedure E from (Z)-N-methyl-N-(3-methyl-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)acetamide. Yield: 118 mg (57%), yellow solid. MS (ESI⁺) Calc'd for C₁₂H₁₄N₂OS: 234.08. Found: m/z = 235 (M+H⁺).

Preparation of (Z)-N-(3-ethyl-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)-N-methylacetamide (109):

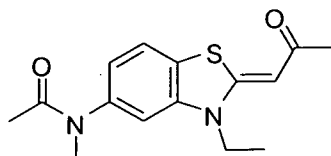


20 **Preparation of 3-ethyl-2-methyl-5-(N-methylacetamido)benzo[d]thiazol-3-ium iodide:**



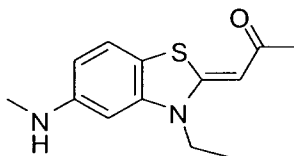
Prepared according to General Procedure A from N-(2-methylbenzo[d]thiazol-5-yl)acetamide and ethyl iodide. Yield: 431 mg (42%), white solid.

Preparation of (Z)-N-(3-ethyl-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)-N-methylacetamide:



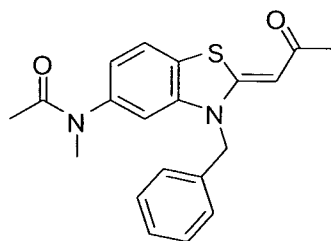
Prepared according to General Procedure B from 3-ethyl-2-methyl-5-(N-methylacetamido)benzo[d]thiazol-3-ium iodide and acetic anhydride. The product was extracted with CH_2Cl_2 . Trituration with 4:1 ethyl acetate: petroleum ether followed by filtration gave 257 mg (77%) of a white solid. MS (ESI^+) Calc'd for $\text{C}_{15}\text{H}_{18}\text{N}_2\text{O}_2\text{S}$: 290.11. Found: $m/z = 291$ ($\text{M}+\text{H}^+$).

Preparation of (Z)-1-(3-ethyl-5-(methylamino)benzo[d]thiazol-2(3H)-ylidene)propan-2-one (105):

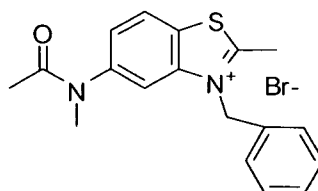


Prepared according to General Procedure E from (Z)-N-(3-ethyl-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)-N-methylacetamide. Yield 78 mg (57%), yellow solid. MS (ESI^+) Calc'd for $\text{C}_{13}\text{H}_{16}\text{N}_2\text{OS}$: 248.10. Found: $m/z = 249$ ($\text{M}+\text{H}^+$).

Preparation of (Z)-N-(3-benzyl-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)-N-methylacetamide (110):

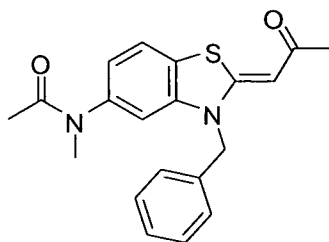


Preparation of 3-benzyl-2-methyl-5-(N-methylacetamido)benzo[d]thiazol-3-ium bromide:



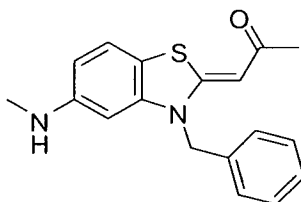
Prepared according to General Procedure A from N-methyl-N-(2-methylbenzo[d]thiazol-5-yl)acetamide and benzyl bromide. Yield 1.1 g (100%), yellow solid.

Preparation of (Z)-N-(3-benzyl-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)-N-methylacetamide:



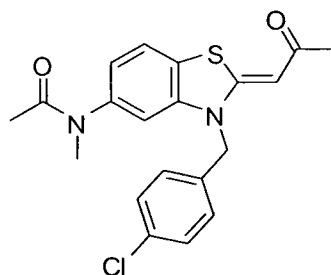
Prepared according to General Procedure B from 3-benzyl-2-methyl-5-(N-methylacetamido)benzo[d]thiazol-3-ium bromide and acetic anhydride. The product was extracted with CH_2Cl_2 . Purification via silica gel chromatography, eluting with a 50 to 85% CH_2Cl_2 : ethyl acetate gradient gave 70 mg (7%) of a white solid. MS (ESI⁺) Calc'd for $\text{C}_{20}\text{H}_{20}\text{N}_2\text{O}_2\text{S}$: 352.12. Found: m/z = 353 ($\text{M}+\text{H}^+$).

Preparation of (Z)-1-(3-benzyl-5-(methylamino)benzo[d]thiazol-2(3H)-ylidene)propan-2-one (106):

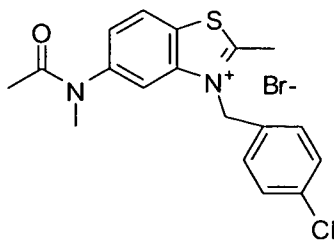


Prepared according to General Procedure E from (Z)-N-(3-benzyl-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)-N-methylacetamide. Yield 27 mg (55%), yellow solid. MS (ESI⁺) Calc'd for $\text{C}_{18}\text{H}_{18}\text{N}_2\text{OS}$: 310.11. Found: m/z = 311 ($\text{M}+\text{H}^+$).

Preparation of (Z)-N-(3-(4-chlorobenzyl)-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)-N-methylacetamide (111):

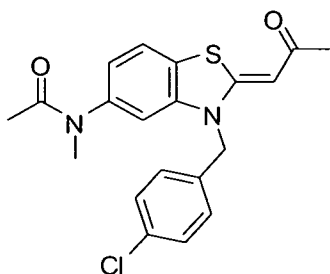


Preparation of 3-(4-chlorobenzyl)-2-methyl-5-(N-methylacetamido)benzo[d]thiazol-3-ium bromide:



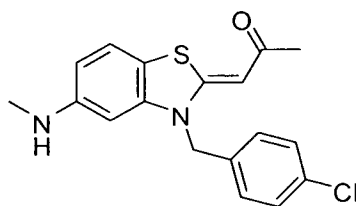
Prepared according to General Procedure A from N-methyl-N-(2-methylbenzo[d]thiazol-5-yl)acetamide and 4-chlorobenzyl chloride. Yield 1.88 g (97%), yellow solid.

Preparation of (Z)-N-(3-(4-chlorobenzyl)-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)-N-methylacetamide:



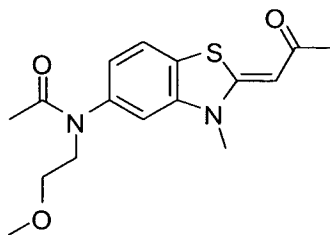
Prepared according to General Procedure B from 3-(4-chlorobenzyl)-2-methyl-5-(N-methylacetamido)benzo[d]thiazol-3-ium bromide and acetic anhydride. The product was extracted with CH_2Cl_2 , then purified via silica gel chromatography, eluting with a 50 to 85% CH_2Cl_2 in ethyl acetate gradient, to give 260 mg (19%) of a white solid. MS (ESI^+) Calc'd for $\text{C}_{20}\text{H}_{19}\text{ClN}_2\text{O}_2\text{S}$: 386.09. Found: $m/z = 387$ ($\text{M}+\text{H}^+$).

Preparation of (Z)-1-(3-(4-chlorobenzyl)-5-(methylamino)benzo[d]thiazol-2(3H)-ylidene)propan-2-one (107):

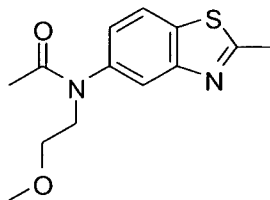


Prepared according to General Procedure E from (Z)-N-(3-(4-chlorobenzyl)-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)-N-methylacetamide. Yield 97 mg (78%). MS (ESI^+) Calc'd for $\text{C}_{18}\text{H}_{17}\text{ClN}_2\text{OS}$: 344.08. Found: $m/z = 345$ ($\text{M}+\text{H}^+$).

Preparation of (Z)-N-(2-methoxyethyl)-N-(3-methyl-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)acetamide (112):



Preparation of N-(2-methoxyethyl)-N-(2-methylbenzo[d]thiazol-5-yl)acetamide:



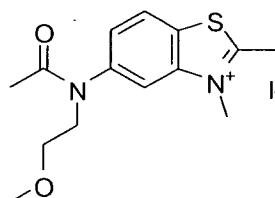
5

A mixture of 4.12 g (20.0 mmol) of N-(2-methylbenzo[d]thiazol-5-yl)acetamide, 4.48 g (40.0 mmol) of potassium tert-butoxide, and 80 mL of tetrahydrofuran were stirred at ambient temperature for 30 min. Next, 5.56 g (40 mmol) of 2-methoxy-1-bromoethane was added, then the mixture was stirred at 50 °C for 16 h. The reaction was concentrated *in vacuo*, then the residue was suspended in 600 mL of water, and extracted with CH₂Cl₂ (3 x 250 mL). The combined organic layers were washed with brine, treated with activated carbon and Na₂SO₄, then filtered and concentrated *in vacuo* to give 4.7 g (89%) of a yellow oil.

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Preparation of 5-(N-(2-methoxyethyl)acetamido)-2,3-dimethylbenzo[d]thiazol-3-ium iodide:

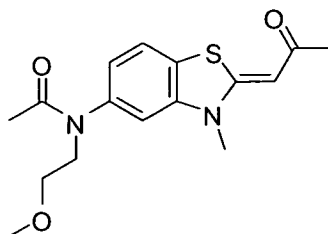
15



Prepared according to General Procedure A from N-(2-methoxyethyl)-N-(2-methylbenzo[d]thiazol-5-yl)acetamide and methyl iodide. The ethyl acetate wash step was omitted, giving a semisolid product that was used without purification in the next step.

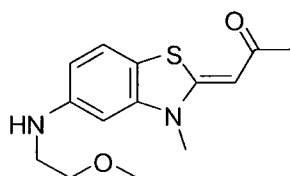
20

Preparation of (Z)-N-(2-methoxyethyl)-N-(3-methyl-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)acetamide:

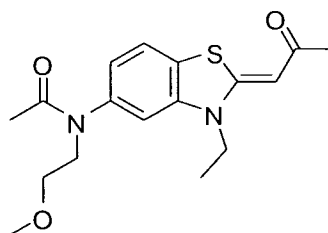


- Prepared according to General Procedure B from 5-(N-(2-methoxyethyl)acetamido)-2,3-dimethylbenzo[d]thiazol-3-ium iodide and acetic anhydride. The product was extracted with CH₂Cl₂ to give 390 mg (41%, 2 steps) of a white solid. Preparative TLC, eluting with 3:1 CH₂Cl₂: ethyl acetate gave pure product. MS (ESI⁺) Calc'd for C₁₆H₂₀N₂O₃S: 320.12. Found: *m/z* = 321 (M+H⁺).

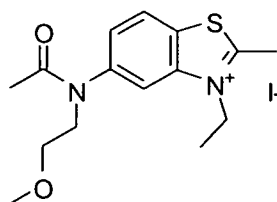
Preparation of (Z)-1-(5-(2-methoxyethylamino)-3-methylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one (116):



- 10 Prepared according to General Procedure E from (Z)-N-(2-methoxyethyl)-N-(3-methyl-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)acetamide. The product was purified via preparative TLC, eluting with 3:1 CH₂Cl₂: ethyl acetate to give 102 mg (58%) of a yellow solid. MS (ESI⁺) Calc'd for C₁₄H₁₈N₂O₂S: 278.11. Found: *m/z* = 279 (M+H⁺).
- 15 Preparation of (Z)-N-(3-ethyl-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)-N-(2-methoxyethyl)acetamide (113):

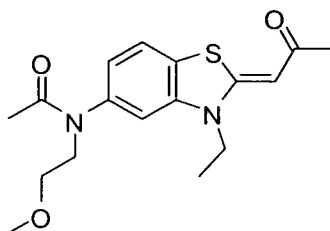


Preparation of 3-ethyl-5-(N-(2-methoxyethyl)acetamido)-2-methylbenzo[d]thiazol-3-ium iodide:



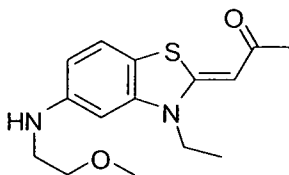
Prepared according to General Procedure A from N-(2-methoxyethyl)-N-(2-methylbenzo[d]thiazol-5-yl)acetamide and ethyl iodide. The ethyl acetate wash was omitted, and the semi-solid product was used in the next step without further purification.

Preparation of (Z)-N-(3-ethyl-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)-N-(2-methoxyethyl)acetamide:



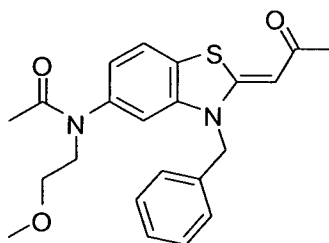
Prepared according to General Procedure B from 3-ethyl-5-(N-(2-methoxyethyl)acetamido)-2-methylbenzo[d]thiazol-3-ium iodide and acetic anhydride. The product was extracted with CH₂Cl₂. The product was purified via HPLC, (General Procedure F, 20-95 CH₃CN) to give 250 mg (23%, 2 steps) of a white solid. MS (ESI⁺) Calc'd for C₁₇H₂₂N₂O₃S: 334.14. Found: *m/z* = 335 (M+H⁺).

Preparation of (Z)-1-(3-ethyl-5-(2-methoxyethylamino)benzo[d]thiazol-2(3H)-ylidene)propan-2-one (117):

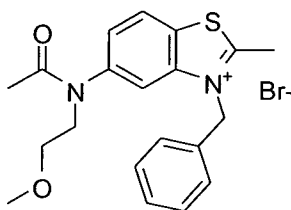


Prepared according to General Procedure E from (Z)-N-(3-ethyl-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)-N-(2-methoxyethyl)acetamide. The product was purified by preparative TLC, eluting with 3:1 CH₂Cl₂: ethyl acetate to give 69 mg (53%) of a yellow solid. MS (ESI⁺) Calc'd for C₁₅H₂₀N₂O₂S: 292.12. Found: *m/z* = 293 (M+H⁺).

Preparation of (Z)-N-(3-benzyl-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)-N-(2-methoxyethyl)acetamide (114):

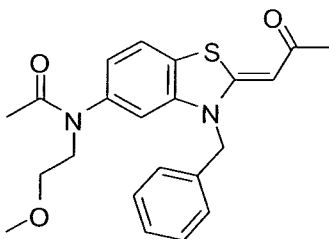


Preparation of 3-benzyl-5-(N-(2-methoxyethyl)acetamido)-2-methylbenzo[d]thiazol-3-ium bromide:



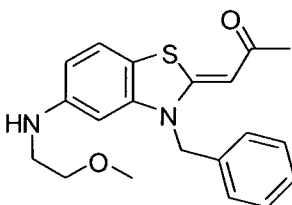
- 5 Prepared according to General Procedure A from N-(2-methoxyethyl)-N-(2-methylbenzo[d]thiazol-5-yl)acetamide and benzyl bromide, heating at 60 °C for 24 h. The product was washed with 2:1 ethyl acetate: acetonitrile to give 500 mg (49%) of a brown solid.

Preparation of (Z)-N-(3-benzyl-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)-N-(2-methoxyethyl)acetamide:



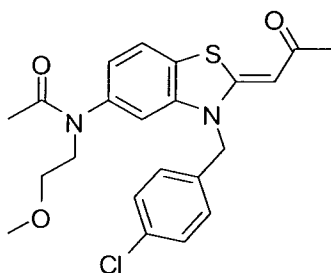
- 15 Prepared according to General Procedure B from 3-benzyl-5-(N-(2-methoxyethyl)acetamido)-2-methylbenzo[d]thiazol-3-ium bromide and acetic anhydride. The product was extracted with CH₂Cl₂. Purification via HPLC (General Procedure F, 20-95 CH₃CN) gave 196 mg (43%) of a yellow solid. MS (ESI⁺) Calc'd for C₂₂H₂₄N₂O₃S: 396.15. Found: *m/z* = 397 (M+H⁺).

Preparation of (Z)-1-(3-benzyl-5-(2-methoxyethylamino)benzo[d]thiazol-2(3H)-ylidene)propan-2-one (118):



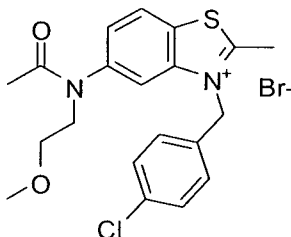
Prepared according to General Procedure E from (Z)-N-(3-benzyl-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)-N-(2-methoxyethyl)acetamide. After precipitation from water, the product was dried, triturated with 1:1 CH₂Cl₂: ethyl acetate, filtered, and rinsed with 1:1 petroleum ether: ethyl acetate to give 79 mg (59%) of a yellow solid. MS (ESI⁺) Calc'd for C₂₂H₂₄N₂O₃S: 396.15. Found: *m/z* = 397 (M+H⁺).

Preparation of (Z)-N-(3-(4-chlorobenzyl)-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)-N-(2-methoxyethyl)acetamide (115):



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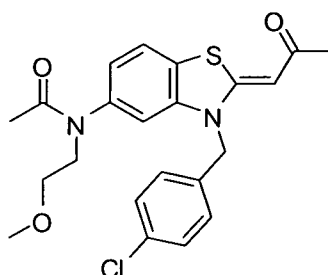
Preparation of 3-(4-chlorobenzyl)-5-(N-(2-methoxyethyl)acetamido)-2-methylbenzo[d]thiazol-3-ium bromide:



Prepared according to General Procedure A from N-(2-methoxyethyl)-N-(2-methylbenzo[d]thiazol-5-yl)acetamide and 4-chlorobenzyl bromide. The reaction was heated at 60 °C for 24 h. The reaction was diluted with 2:1 ethyl acetate: acetonitrile, then the precipitate was collected to give 545 mg (36%) of a brown solid.

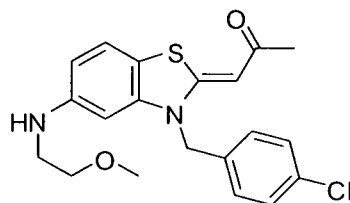
Preparation of (Z)-N-(3-(4-chlorobenzyl)-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)-N-(2-methoxyethyl)acetamide:

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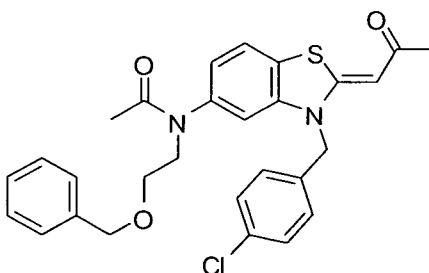
- Prepared according to General Procedure B from 3-(4-chlorobenzyl)-5-(N-(2-methoxyethyl)acetamido)-2-methylbenzo[d]thiazol-3-ium bromide and acetic anhydride. The product was extracted with CH₂Cl₂. Purification via HPLC (General Procedure F, 20-95 CH₃CN) gave 180 mg (36%) of a yellow solid. MS (ESI⁺) Calc'd for C₂₂H₂₃ClN₂O₃S: 430.11. Found: *m/z* = 431 (M+H⁺).

Preparation of (Z)-1-(3-(4-chlorobenzyl)-5-(2-methoxyethylamino)benzo[d]thiazol-2(3H)-ylidene)propan-2-one (119):

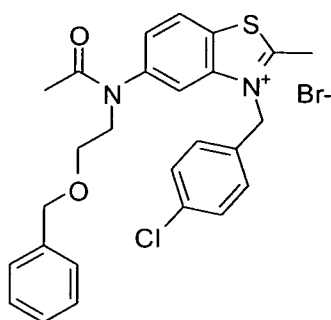


- Prepared according to General Procedure E from (Z)-N-(3-(4-chlorobenzyl)-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)-N-(2-methoxyethyl)acetamide. The precipitate from water was dried, triturated with 1:1 CH₂Cl₂: ethyl acetate, filtered, and rinsed with 1:1 petroleum ether: ethyl acetate to give 75 mg (69%) of a yellow solid. MS (ESI⁺) Calc'd for C₂₀H₂₁ClN₂O₂S: 388.10. Found: *m/z* = 389 (M+H⁺).

Preparation of (Z)-N-(2-(benzyloxy)ethyl)-N-(3-(4-chlorobenzyl)-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)acetamide (133):

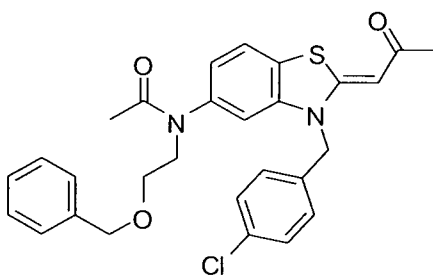


- Preparation of 5-(N-(2-(benzyloxy)ethyl)acetamido)-3-(4-chlorobenzyl)-2-methylbenzo[d]thiazol-3-ium bromide:**



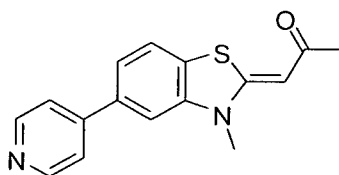
A mixture of 1.01 g (3.82 mmol) of N-(2-methoxyethyl)-N-(2-methylbenzo[d]thiazol-5-yl)acetamide and 12 mL of benzyl bromide were heated at 90 °C under N₂ for 3 days. Ethyl acetate was added, and the suspension was stirred for 10 min. The precipitate was filtered, washed with ethyl acetate, and dried to give 1.75 g (76%) of a brown solid.

Preparation of (Z)-N-(2-(benzyloxy)ethyl)-N-(3-(4-chlorobenzyl)-2-(2-oxopropylidene)-2,3-dihydrobenzo[d]thiazol-5-yl)acetamide:



Prepared according to General Procedure B from 5-(N-(2-(benzyloxy)ethyl)acetamido)-3-(4-chlorobenzyl)-2-methylbenzo[d]thiazol-3-ium bromide and acetic anhydride. The product was extracted with CH₂Cl₂. Purification via HPLC (General Procedure F, 40-95 CH₃CN) gave 200 mg (15%) of a yellow solid. MS (ESI⁺) Calc'd for C₂₈H₂₈N₂O₃S: 472.18. Found: *m/z* = 473 (M+H⁺).

Preparation of (Z)-1-(3-methyl-5-(pyridin-4-yl)benzo[d]thiazol-2(3H)-ylidene)propan-2-one (122):

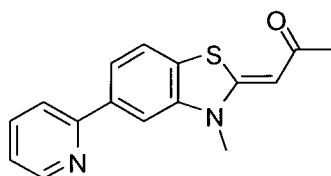


A mixture of 284 mg (1.0 mmol) of (Z)-1-(5-bromo-3-methylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one, 184 mg (1.5 mmol) of 4-pyridylboronic acid, 83 mg (0.1 mmol) of [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II), 420 mg (3.0

mmol) of potassium carbonate, and 20 mL of 5:1 (v/v) dioxane/H₂O was stirred at 70 °C under N₂ overnight. The reaction was filtered, concentrated and purified by prepTLC, eluting with 15:1 CH₂Cl₂: methanol, then recrystallized from MeOH to give 132mg (47%) of a yellow solid. MS (ESI⁺) Calc'd for C₁₆H₁₄N₂OS: 282.08.

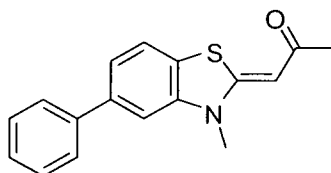
5 Found: $m/z = 283$ (M+H⁺).

Preparation of (Z)-1-(3-methyl-5-(pyridin-4-yl)benzo[d]thiazol-2(3H)-ylidene)propan-2-one (123):



A microwave tube was charged with 284 mg (1.0 mmol) of (Z)-1-(5-bromo-3-methylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one, 250 mg (2.0 mmol) of 2-pyridylboronic acid, 100 mg (0.12 mmol) of [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II), 420 mg (3.0 mmol) of potassium carbonate, and 15 mL of dioxane. The reaction was heated at 150 °C for 3 h in a microwave reactor, then it was filtered, and concentrated. The residue was purified via preparative TLC, eluting with 15:1 CH₂Cl₂: methanol. The product was then recrystallized from methanol to give 34 mg (11%) of a yellow solid. MS (ESI⁺) Calc'd for C₁₆H₁₄N₂OS: 282.08. Found: $m/z = 283$ (M+H⁺).

Preparation of (Z)-1-(3-methyl-5-phenylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one (121):



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A mixture of 142 mg (0.50 mmol) of (Z)-1-(5-bromo-3-methylbenzo[d]thiazol-2(3H)-ylidene)propan-2-one, 92 mg (0.75 mmol) of phenylboronic acid, 58 mg (0.05 mmol) of tetrakis(triphenylphosphino)palladium, 1.0 mL of 2M Na₂CO_{3(aq.)}, and 5 mL of 1,2-dimethoxyethane was stirred at 90 °C for 16 h. The reaction was filtered, then concentrated *in vacuo*. The residue was purified via silica gel chromatography, eluting with 20:1 CH₂Cl₂: methanol. The product was then

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recrystallized from 2:1 petroleum ether: ethyl acetate to give 96 mg (69%) of a yellow solid. MS (ESI⁺) Calc'd for C₁₇H₁₅NOS: 281.09. Found: *m/z* = 282 (M+H⁺).

EXAMPLE 2: Expression and purification of CLK1 and 2

The expression protocol is partly based on the purification protocol for protein for human CLK1 and crystal structure in complex with 10Z- 2 hymenialdisine at 1.7 Å as reported in the pdb data base (1Z57). Briefly, a T7 promoter based vector (Novagen) for expression of CLK1 and CLK2 is transformed into BL21(DE3), BL21(DE3) RIL, BL21(DE3) RP or BL21(DE3) pLys cells (Invitrogen) and plated onto an LB agar plate. One of the freshly grown colonies is picked and grown in a small culture (5 ml, 100 mg/mL ampicillin (AMP) of either LB, Terrific broth, Super broth (vendor all: RPI) or M9 media (vendor Tecknova) at 37°C over night. The culture is 100-fold diluted into new media containing AMP (final concentration 1mM) and grown at 37°C to an OD₆₀₀ of 0.8. Cultures are iced to a temperature of 18°C prior to induction with IPTG (final concentration 1mM). Cultures are harvested after 12 hours of induction time at 18°C.

Cells are harvested at 8000 x g for 6 minutes and resuspended in lysis buffer (50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol) and lyzed with lysozyme (5 mg/g cell paste) for 30 minutes following sonication for 10 minutes. Cells are then centrifuged at 30,000 x g for 45 minutes and the supernatant loaded onto a DE52 column (Whatman) for nucleic acid removal. The flow through is collected and loaded onto a Ni-chelating column for affinity chromatography. The column is washed with wash buffer (20 mM Imidazole, 300 mM NaCl, 50mM KH₂PO₄, pH 8.0) to remove endogenous bound protein. The protein is cleaved from the column with either TEV or Pre scission protease (GE Healthcare) over night as well as dephosphorylated with GST-tagged Lambda phosphatase (New England Biolabs, Beverly, MA). The supernatant is collected and concentrated to 12 mg/mL for size exclusion chromatography. 2 mL fractions of the concentrated protein are loaded onto a S200 16/60 global sizing column (GE Healthcare) and protein peaks collected and analyzed for solubility by SDS and native polyacrylamide gels (Invitrogen). Additional purification with Ion exchange chromatography (GE Healthcare) is optional.

The protein is concentrated to 15 mg/mL, dialyzed against storage buffer (100 mM NaCl, 20% Glycerol, 20mM Tris-HCl, pH 8.0) and stored in aliquots at -80°C.

EXAMPLE 3: CLK In Vitro Kinase Assay

5 CLKs are assayed in a reaction mixture containing 200 mM Tris-HCl (pH 7.5), 12.5 mM MgCl₂, 8 mM dithiothreitol, 4 mM EGTA, 1-20 µM ATP, 1 µCi of [gamma-³²P]ATP, 1 µg of synthetic peptide of SF2/ASF RS domain (NH₂-RSPSYGRSRSRSRSRSRSRSRSNSRSRSY-OH)(SEQ ID NO: 1), and 0.1-1 µg of purified kinases in a final volume of 40 µL. The final concentration of DMSO is
10 adjusted to 1% regardless of inhibitor concentration. The reaction mixture is incubated at 30 C for 10 min, and a half-portion is spotted on P81 phosphocellulose membrane (Whatman). The kinase assay conditions, including the incubation period and concentration of kinases and substrates, are optimized to maintain the linearity during incubation. The membrane is washed with 5% phosphoric acid solution for at
15 least 15 min. The radioactivity is measured using a liquid scintillation counter. The net radioactivity is deduced by subtracting the background count from the reaction mixture without kinase, and the data are expressed as the percentage to the control sample containing the solvent.

EXAMPLE 4: CLK Cell-Based Assays

20 i. *Fat mobilization assay.* 3T3 L1 cells are plated with 2 ml of 30,000 cells/ml in Dulbecco's Modified Eagle Medium (DMEM)/10% newborn calf serum in 24-well plates. Individual wells are then allowed to differentiate by addition of 100 nM Rosiglitazone. Undifferentiated control cells are maintained in fresh DMEM/10% newborn calf serum throughout the duration of the assay. At 48 hours
25 (2 days), adipogenesis is initiated by addition of DMEM/10% fetal calf serum/0.5 mM 3-isobutyl-1-methylxanthine (IBMX)/1 µM dexamethasone. At 96 hours (4 days), adipogenesis is allowed to progress by removal of the media and adding 2 ml of DMEM/10% fetal calf serum to each well along with either 10 µg/mL insulin or 100 nM Rosiglitazone. At 144 hours (6 days) and 192 hours (8 days), all wells are
30 changed to DMEM/10% fetal calf serum.

At 240 hours (10 days from the original cell plating), test compounds at a range of concentrations are added to individual wells in triplicate along with 100 nM

Rosiglitazone. Three wells of undifferentiated cells are maintained in DMEM/10% newborn calf serum and three wells of differentiated control cells are maintained in fresh DMEM/10% newborn calf serum with 100 nM Rosiglitazone. As a positive control for fat mobilization, resveratrol (a SIRT1 activator) is used at concentrations ranging in three fold dilutions from 100 μ M to 0.4 μ M.

At 312 hours (13 days), the media is removed and cells are washed twice with PBS. 0.5 mL of Oil Red O solution (supplied in Adipogenesis Assay Kit, Cat.# ECM950, Chemicon International, Temecula, CA) is added per well, including wells that have no cells as background control. Plates are incubated for 15 minutes at room temperature, and then the Oil Red O staining solution is removed and the wells are washed 3 times with 1 mL wash solution (Adipogenesis Assay Kit). After the last wash is removed, stained plates are visualized, scanned or photographed. Dye is extracted (Adipogenesis Assay Kit) and quantified in a plate reader at 520 nM. Quantitative results are shown in Figure 16.

II. Primary dorsal root ganglion (DRG) cell protection assay. CLK modulators are tested in an axon protection assay as described (Araki et al. (2004) Science 305(5686):1010-3). Briefly, mouse DRG explants from E12.5 embryos are cultured in the presence of 1 nM nerve growth factor. Non-neuronal cells are removed from the cultures by adding 5-fluorouracil to the culture medium. CLK modulators are added 12 to 24 hours prior to axon transections. Transection of neurites was performed at 10-20 days *in vitro* (DIV) using an 18-gauge needle to remove the neuronal cell bodies.

EXAMPLE 5: Effects of CLK Modulators in Normal Mice

C57BL6 mice (male, 6 weeks old) are allowed to acclimatise for 48 hours. Mice are divided in to 4 groups (n = 10) and receive a single, daily intraperitoneal injection of a CLK modulator (10, 30 or 100 mg/kg) or vehicle for 7 days. Daily body weights and visual observations are taken. At the end of the dosing period, mice are sacrificed by CO₂ asphyxiation and blood, brain, a leg muscle and the liver collected. Blood is processed for collection of plasma, white and red blood cells. All tissues are snap frozen for storage prior to assay.

EXAMPLE 6: Treatment of Amyotrophic Lateral Sclerosis (ALS) (Murine Model) using CLK Modulators

ALS is a rapidly progressive motor neuron disease that invariably leads to death. In the United States alone, as many as 20,000 people are affected, and an estimated additional 5,000 people are diagnosed with the disease each year. ALS most commonly affects people between 40 and 60 years of age. In the vast majority of patients, ALS is sporadic and occurs apparently at random with no clearly associated risk factors. A particularly devastating effect of ALS is that a person's mind, personality, intelligence or memory is not affected, but their ability to react, communicate, and to control voluntary and involuntary muscles is lost.

CNS Penetration and Distribution of Radiolabeled Compound. For a compound to exhibit efficacy in an animal model of ALS, it must achieve therapeutic concentrations within the CNS and reach the sites within the CNS that are relevant to the degeneration observed. In the mouse models of ALS, the primary site of neuronal loss is the lumbar spinal cord that innervates the hind limbs and tail. To confirm that the compound of interest reaches the CNS, brain and spinal cord penetration and distribution are studied. The compound of interest is radiolabeled and administered to mice. Distribution of the compound within the CNS is determined by autoradiography and extraction.

Briefly, male Swiss Webster mice weighing 20-25 g at the time of the experiment are maintained under a light-dark cycle of 12 h - 12 h at a room temperature of 21 ± 2 °C, with $50 \pm 15\%$ humidity. The mice have free access to commercial mouse food and tap water.

The ^{14}C -labeled CLK modulator is administered as intraperitoneal (i.p.) injections to mice every 12 h for 2 days. The amount of ^{14}C -labeled compound injected is determined based on its specific activity and *in vitro* activity.

Following administration, animals are sacrificed at 30 minutes, 3 hours and 6 hours. The brains and spinal cords are rapidly removed and frozen in 2-methylbutane at -20°C, then kept below -70°C until sectioning or solid phase extraction.

Frozen brains are mounted on cryostat chucks and cut into 20 µm thick coronal sections at -20°C in a Microm HM 500 O microtome cryostat. Sections are thaw-mounted near the edge of slides and dried overnight under a gentle stream of air. The slides are exposed to ^{14}C -sensitive film (Hyperfilm MP, Amersham

Biosciences) at 5°C for 3 days. Images are analyzed using an HP Scanjet 8200C scanner and analyzed using an image analysis software package (Image, NIH software). ^{14}C standards (^{14}C -microscales) (30-860 nCi/g) are used for quantifying the autoradiograms. Density readings for standards of known radioactivity are taken
5 for comparison of optical density to isotope levels on each sheet of film. Standard curves for converting optical density to nCi/g values are best-fit by linear transformation. Background readings of optical density are used in determining the relative amount of drug bound to each section. Different regions of the brain selected are examined for labeling with ^{14}C -labeled compound. Regions are
10 identified using an atlas of the brain (Paxinos G., Franklin K. B. J., The mouse brain in stereotaxic coordinates Academic Press, New-York, 2003). The amount of ^{14}C -labeled compound bound to each area is expressed as the mean for each slide (3 sections per slide). Data taken from areas found in both the left and right hemispheres are pooled from each section to determine the overall mean for that
15 region of brain.

To determine compound exposure to the spinal cord, the spinal cord is homogenized and centrifuged to remove any solids from the sample. An aliquot of the sample is combined with 1% phosphoric acid with water in a 96-well plate and mixed. The sample is added to a Phenomenex StrataX extraction plate that has been
20 equilibrated with methanol and water. Following washing, the sample is eluted with 100 % acetonitrile into a clean 96-well plate. The samples are evaporated under a stream of N_2 and the residue reconstituted in solvent. The quantity of compound is assessed by mass spectrometry (LC-MS/MS).

Data are analyzed for statistical significance by ANOVA and Dunnett's t-test
25 using the software Statview (BrainPower, Calabasas, CA, U.S.A.). Statistical significance is taken as $p < 0.05$.

Compound Efficacy in an Animal Model of Progressive Motor Neuron Disease (pmn/pmn). The *pmn* mouse model is a widely used genetic animal model for studying degeneration of motor neurons. The mice carry a spontaneous
30 autosomal recessive mutation that leads to progressive motor neuronopathy (Schmalbruch, H., et al. J Neuropathol Exp Neurol, 1991. 50(3): p. 192-204). *pmn* homozygous mice develop weakness in the hind limbs during the third week of life

and die at approximately 6 weeks of age. At this latter age, the animals show a severe muscle wasting particularly in those muscles of the thoracic and pelvic regions. Heterozygous *pmn* mice are phenotypically normal. Histological studies have revealed that the sciatic and phrenic nerves of *pmn* animals are severely
5 affected (Schmalbruch, H., et al., *supra*; Sagot, Y., et al. Eur J Neurosci, 1995. 7(6): p. 1313-22; Sagot, Y., et al. J Neurosci, 1995. 15(11): p. 7727-33; and Sagot, Y., et al. J Neurosci, 1996. 16(7): p. 2335-41) and that 30% of the facial nucleus motor neurons degenerate (Sendtner, M., et al. Nature, 1992. 358(6386): p. 502-4). The *pmn* mouse model of motor neuron disease is used to examine the potential
10 neuroprotective properties of CLK modulators. The effects of CLK modulators on disease onset, motor function, motor neuron loss, and survival of the *pmn/pmn* mouse are determined.

Heterozygous *pmn* mice are obtained from the laboratory of Dr. Ann Kato from the Centre Medical Universitaire (Geneva, Switzerland). A large colony of
15 *pmn* mice is generated; *pmn/pmn* homozygotes are infertile and are obtained from double heterozygous crosses at the Mendelian ratio of 25%. Starting at 12 days of age, the mice are examined for grasp activity of the hind limb paws. The first clinical signs of weakness usually appear between days 14 and 16. Animals are divided into groups at two weeks of age. Controls and treated *pmn* mice have access
20 to commercial food and tap water *ad libitum* throughout the study. When it is determined by examiners that the mice are unable to reach dry food and/or water, a water-based nutrient gel will be placed on the bottom of the cage, and a longer spout will be attached to the water bottle.

The mice are divided into four test groups: Group A: negative-control
25 animals (heterozygote and wild type mice) treated with vehicle; group B: positive-control animals (*pmn/pmn* homozygotes) treated with vehicle; group C: *pmn/pmn* homozygotes treated with CLK modulator (dose 1); and group D: *pmn/pmn* homozygotes treated with CLK modulator (dose 2).

Briefly, Group A serves as negative-control animals that do not exhibit
30 motor neuron loss (heterozygote and wild type mice). Group A is treated with vehicle daily throughout the study. Group B is the positive-control animals and is dosed with vehicle daily throughout the study. Groups C and D are treated with the

CLK modulating compound at 2 different doses. The dose is determined based on compound activity *in vitro* and CNS penetration determined using radiolabeled compound as described above. For these studies, test compounds or vehicle is administered i.p. twice a day with 10 to 12 hours between injections. The treatment is administered from two weeks of age throughout the study. Animals from each group are used for histological evaluation. These mice are sacrificed at a late disease stage (35 days) to assess the extent of motor neuron loss and the extent of gliosis.

The parameters followed for this study are body weight, behavior, motor neuron loss, gliosis, and life span. Throughout the study, body weight is determined daily by weighing the animals at the same time each morning prior to the administration of the CLK modulator or vehicle. The body weight evolution is expressed as the cumulative sum of the variation in the percentage of the initial body weight.

For the behavioural assessment, the mice are tested for their ability to execute the following behavioural tests: back leg grasping, bar crossing, inclined plane test and grip test.

Back leg grasping. This test measures the ability of *pmn* mice to hold onto the side of their cage with their hind limbs. The mice, held head-down by the tail, will be allowed to grasp the cage and remain suspended. As early as day 15, *pmn* homozygous animals can be diagnosed by their inability to grasp onto the side of the cage. The mice are tested every 2 days.

Bar crossing. In this test, the time to cross a 25 cm long cylindrical bar is measured. If the mice fall from the bar, the test is considered unsuccessful and is repeated three times. The mice are tested every 2 days.

Inclined plane test. The mice are tested 1 time per week for their ability to stay on an inclined plane within a maximum of 5 seconds. The slope that each animal remains on the plane is recorded.

Grip test. The mice are tested 1 time per week for their ability to hold a horizontal bar two times, within a maximum of 30 seconds. The time each animal remains on the bar is recorded.

For histological and stereological analysis, mice are perfused with phosphate buffered saline followed by paraformaldehyde. The spinal cords are dissected and the lumbar segments identified. Tissues are postfixed and blocks will be cryoprotected. To quantify motor neurons numbers, high-precision stereological analysis are performed. Serial coronal sections are cut through the lumbar (L1 to L4) spinal cord. The sections are mounted onto slides and stained for Nissl substance using cresyl violet. A separate set of sections are collected as free-floating sections and processed for immunohistochemistry, which is aimed at determining the extent of gliosis or astrocyte and microglial involvement. The sections are immunostained with CD40 (microglial marker) and GFAP (astrocyte marker) antibodies using double label immunofluorescence.

Life span is determined for each test group. In order to reduce animal suffering, new guidelines have been established to determine endpoint (survival); animals are euthanized when they are unable to do any of the following: right themselves within 15 seconds when placed on their sides, groom their faces (as determined by infection in one or both eyes), or move around the cage, even by use of front limbs, to reach food placed at the bottom of the cage. Negative control animals are euthanized at the end of the study by CO₂ inhalation.

For statistical evaluation of the data, the life span results are submitted to a Kaplan-Meier test. Two different tests of measuring statistical significance are used; the Log-Rank test and the Wilcoxon test. Data related to quantitative behavioral assessments are analyzed with Kruskal-Wallis followed by non parametric Mann-Whitney U-test. Significance is considered as $p < 0.05$.

Compound Efficacy in an Animal Model of ALS Disease (SOD1^{G93A}). The SOD1^{G93A} mice are obtained from the Jackson Laboratories (Gurney, M.E., et al. Science, 1994. 264(5166): p. 1772-5). The mice express high levels of human SOD1 containing a substitution of glycine to alanine at position 93. This mutation is found mutated in 20% of familial ALS patients and thus represents a useful and relevant model for studying the efficacy of CLK modulators. The effects of the CLK modulator across standard experimental parameters are examined: disease onset, motor function, motor neuron loss, gliosis, and survival of the SOD^{G93A} mouse.

The specific mouse strain, designated G1H, is maintained as a heterozygous hybrid line which is a cross between C57B6/J and SJL mice. Transgenic males are crossed with nontransgenic B6SJLF1 females. Animals are genotyped at weaning, approximately 21-30 days of age by PCR amplification from DNA extracted from tail biopsies while the animals are temporarily anesthetized by inhalation of isoflurane. For the DNA extraction, a QIAamp Tissue Kit from Qiagen is used. PCR amplification is performed using a primer pair specific for exon 4 of the human SOD1 gene. At 30 days of age, the mice are randomized into three different treatment arms. All animals have access to commercial food and tap water *ad libitum* throughout the study. When it is determined by examiners that the mice are unable to reach dry food and/or water, a water-based nutrient gel will be placed on the bottom of the cage and a longer spout will be attached to the water bottle.

The following three test groups are studied: Group A: SOD1^{G93A} mice treated with vehicle serve as the positive control group; Group B: SOD1^{G93A} mice treated with the CLK modulator (dose 1); and Group C: SOD1^{G93A} mice treated with the CLK modulator (dose 2).

Briefly, Group A serves as positive-control animals that exhibit motor neuron loss. Group A is treated with vehicle daily throughout the study. Groups B and C are treated with the CLK modulating compound at 2 different doses. The dose is determined based on compound activity *in vitro* and CNS penetration. For these studies, test compounds or vehicle are administered i.p. twice daily with 10 to 12 hours between injections. The treatment is initiated on day 30 and continues throughout the study. Animals from each group will be used for histological evaluation. These mice are sacrificed at a late stage in the disease (120 days) to assess the extent of motor neurons loss and the extent of gliosis.

The parameters followed for this study are body weight, disease onset, gait, life span, motor neuron loss, and gliosis. Throughout the study body weight is determined daily by weighing the animals at the same time each morning prior to the administration of the test compound or vehicle. The body weight evolution is expressed as the cumulative sum of the variation in the percentage of the initial body weight.

The mice are examined twice weekly to determine disease onset. Onset is defined as the day of the first appearance of limb tremor when the animals are held suspended briefly by their tails. This usually begins unilaterally, followed by bilateral tremulousness and weakness in the affected limb(s). Following initial
5 diagnosis, animals are examined daily for early stages of hind-limb paralysis.

Gait analysis is performed to assess motor functioning of the test groups. Briefly, footprint patterns are studied using mouse fore- and hindpaws dipped in blue and red non-toxic, water based paint, respectively. The mice are placed in a clear Perspex runway that has a black goal box fixed to one of the distal ends.
10 White paper is used to line the runway floor. Mice are permitted to walk to the goal box from the opposite end of the runway thus allowing their footprints to leave patterns on the paper. Five separate parameters are measured; stride length, hind- and forepaw base width, overlap between fore and hindpaws, and latency to travel the runway.

15 Life span determination, histological analysis, stereological analysis and statistical evaluation are carried out as described above.

EXAMPLE 7: Treatment of Multiple Sclerosis (MS) (Murine Modulator) using CLK Modulators

Multiple Sclerosis (MS) is the most common cause of non-traumatic
20 neurological disability affecting young adults. An estimated 2.5 million people have MS worldwide and approximately 400,000 in the U.S (source: NINDS). MS is an inflammatory disease of the central nervous system (CNS) in which demyelination and axonal injury result in a permanent neurological disability. The disease can present in different forms, such as primary progressive (accumulation of disability
25 without remission) or relapsing remitting (acute attacks followed by periods of recovery). About 40% of patients enter a secondary progressive stage (attacks with incomplete recovery that lead to progressive disability between exacerbations). There is no cure for MS. Recently approved drugs focus on the inflammatory autoimmune components of the disease, and they appear to control relapses and may
30 be effective in slowing progression from relapsing-remitting to secondary progressive. However, these immunomodulatory interventions do not address the

underlying axonal injuries, and therefore do not impact the neurological damage resulting from acute demyelinating events, acute axonal transection and axonal loss.

Experimental autoimmune encephalomyelitis (EAE) is an animal model of MS induced by immunization with proteolipid protein (PLP). Animals mount an
5 immune response resulting in inflammation, demyelination, and neuronal damage in the brain, spinal cord, and optic nerve, similar to MS patients. Assessment of clinical/neurological symptoms, and histological analysis of demyelination and axonal damage in the thoracic spinal cord are examined.

Chronic relapsing EAE is induced in 8-12 week old female SJL mice by
10 subcutaneous (s.c.) injection with an emulsion containing PLP 139-151 peptide and complete Freund's adjuvant containing 150 µg of peptide and 200 µg of *Mycobacterium tuberculosis* in a total volume of 0.2 ml. In addition, mice are injected intraperitoneally (i.p.) with 200 ng pertussis toxin (List Biological, Campbell, CA) in 0.1 ml PBS on day 0 (day of immunization) and again on day 2.
15 The animals are housed in standard conditions: constant temperature (22±1 °C), humidity (relative, 25%) and a 12-h light/12-h dark cycle, and are allowed free access to food and water. Animals are assessed daily for weight and clinical signs of EAE, beginning 11 days after immunization. Clinical assessment is on a scale from 0-5 (with "5" being moribund, "4" being quadriplegic through to "0" which is an
20 apparently healthy animal). Assessment continues until day 40 after the initial inoculation. During this time animals undergo an initial phase of EAE, followed by recovery. A relapse of EAE typically occurs 20-30 days post-immunization. Mice are considered to have had a relapse if they have an increase by 1 on the clinical scale for two or more days after a period of five or more days of stable or improved
25 appearance.

In order to assess the effect of CLK modulators on neurodegeneration, it is critical not to interfere with the lymphoid development of effector cells early in the disease process. Therefore, the CLK modulator is administered at the onset of clinical EAE. At the onset of clinical EAE, mice are divided randomly into groups
30 and treated with CLK modulator (50, 100, and 200 mg/kg) or vehicle. All treatments are given by daily i.p. injection until the termination of the study.

At day 40 post-immunization, mice from each group are sacrificed with an overdose of ketamine/xylazine. Spinal cords are dissected, fixed in 10% buffered formalin, and embedded in paraffin. Five micron thick sections are stained with Hematoxylin and Eosin (H&E) and Luxol Fast Blue (LFB) to assess myelin loss.

5 Bielshovesky's silver impregnation is used to evaluate axonal integrity. To assess the amount of axonal loss, paraffin sections are exposed to monoclonal antibodies against mouse non-phosphorylated neurofilament H (Clone SMI-32, Sternberger Monoclonals, Baltimore, USA) and monoclonal antibodies against APP (Clone 22C11, Chemicon). SMI-32 is detected with a Cy3-labeled antibody and visualized

10 by fluorescence microscopy. Anti-APP antibodies are detected by incubation with ColonoPAP, and APP-positive axons are visualized with 3,3'-diaminobenzidine (DAB).

To evaluate the extent of axonal loss, images of slides are captured and the areas stained by immunohistochemistry are quantified blinded to treatment status.

15 Axonal integrity and demyelination are assessed qualitatively.

Even though immunosuppression is responsible for reducing the clinical severity of the initial phase of EAE, a recent study suggests that a combination of immunosuppression and neuroprotection may be critical to effectively inhibit relapses, demyelination and axonal injury, and that chronic immunosuppression in

20 the absence of effective neuroprotection may worsen the clinical outcome in EAE and, perhaps, MS. This issue is addressed by evaluating the effect of immunosuppression (by Copaxone (glatiramer acetate)) in combination with neuroprotection (by CLK modulators) in the PLP-induced EAE mouse model.

Chronic relapsing EAE is induced as described above. Mice are divided into

25 three treatment groups: Group 1: vehicle control, daily i.p. injections of cyclodextrin (days 12-39); Group 2: Copaxone treatment, daily s.c. injection (days 0-9); and Group 3: Copaxone (days 0-9) and CLK modulator (days 12-39). As described above, disease progression is monitored, and mice from each group are sacrificed, the spinal cords harvested and analyzed for demyelination, axonal integrity and

30 axonal damage.

EXAMPLE 8: Treatment of Huntington's Disease (Murine Model) using CLK Modulators

The R6/2 mutant mouse model of Huntington's disease (HD) is used to test the efficacy of CLK modulating compounds to attenuate HD disease-related symptoms.

5 R6/2 mice are treated with a CLK modulating compound for at least 12 weeks. The mice are evaluated at 4, 6, 8 and 12 weeks of age (except for Grip Strength which will only be tested 12 weeks of age) using the Rotarod, grip strength, rearing/climbing, open field, and body weight/survival test.

During the course of the study, 12/12 light/dark cycles are maintained. The room temperature is maintained between 20 and 23°C with a relative humidity
10 maintained around 50%. Chow and water are provided *ad libitum* for the duration of the study. Each mouse is randomly assigned across the dose groups and balanced by cage numbers. The test is performed during the animal's light cycle phase unless otherwise specified.

Rotarod. Motor coordination and exercise capacity are assessed by rotarod
15 at 4, 6, 8 and 12 weeks of age. Tests are performed on three separate days, with four trials per day. Animals are loaded on the continuous rotating rod (Accuscan, Columbus, OH) 8 animals at a time. They are given a 5-min training period at a slow speed of 4 rpm. If an animal falls off the rod it is placed back on the rod for the duration of the 5-min training period. Animals are then placed back into the home or
20 test cage for at least one hour prior to actual testing. The mice are then placed on the rotarod and the speed is gradually and uniformly increased to a speed of 40 rpm by 300 s. The time that each mouse remains on the rotating rod before falling 20 cm onto a foam pad is recorded. Any abnormal behavior is also noted, i.e., looping behavior recording the number of rotation times per session trial, walking forward
25 against the rod direction, and number of fecal boli. After rotarod testing animals are placed back into the test or home cage

Grip-strength test. Grip strength is used to assess muscular strength in limb muscles and mice are tested at 12 weeks of age. Mice are held by the tail and lowered towards the mesh grip piece on the push-pull gauge (San Diego
30 Instruments, San Diego, CA) until the animal grabs with both front paws. The animal is lowered toward the platform and gently pulled backwards with consistent force by the experimenter until it releases its grip. The forelimb grip force is

recorded on the strain gauge. The experimenter continues to pull the animal backwards along the platform until the animal's hind paws grab the mesh grip piece on the push-pull gauge. The animal is gently pulled backwards with consistent force by the experimenter until it releases its grip. The hind limb grip force is recorded on
5 the strain gauge. After testing animals are placed back into the test or home cage.

Rearing-Climbing. Rearing-climbing behavior is used to assess motor movement and coordination. The mouse is placed on a flat surface and a closed-top wire mesh cylinder 15 cm X 20 cm tall is placed over the mouse. The animal's behavior is videotaped. The following parameters are then measured over a 5 min
10 period: number of free rears, the number of times the animal rears in contact with the wall, number of times the animal lifts either 1, 2 or 3 paws from the floor, the number of climbing episodes (lifting 4 paws), the number of hanging episodes (from the mesh), and the time spent hanging and climbing. After the 5-min session animals are placed back into the home cage.

15 Open field - locomotor activity. Mice are acclimated to the test room at least 1 hour prior to the commencing the test. The open field test (OF) is used to assess both anxiety-like behavior and motor activity. The open field chambers are plexi-glass square chambers (27.3 x 27.3 x 20.3 cm; Med Associates Inc., St Albans, VT) surrounded by infrared photobeam sources (16 x 16 x 16). The enclosure is
20 configured to split the open field into a center and periphery zone and the photocell beams are set to measure activity in the center and in the periphery of the OF chambers. Animals having higher levels of anxiety or lower levels of activity tend to stay in the corners of the OF enclosures. On the other hand, mice that have high levels of activity and low levels of anxiety tend to spend more time in the center of
25 the enclosure. Horizontal activity (distance traveled) and vertical activity (rearing) are measured from consecutive beam breaks. Animals will be placed in the OF chambers for 30 minutes. Ambulatory distance in center and periphery; rearing in center and periphery; the number of zone entries and average velocity are measured.

Body Weight and Survival. Body weights are measured daily. The survival
30 times of the mice tested as described above are determined. Fatalities are evaluated in the context of the other parameters measured. In our previous studies in R6/2

Huntington's disease model mice, we found no differences between survival times in experimental versus non-experimental groups.

Statistical Analysis. Data are analyzed by a one-way or two-way analysis of variance (ANOVA) followed by post-hoc comparisons. An effect is considered
5 significant if $p < 0.05$. Data are represented as the mean and standard error to the mean (s.e.m.). Animals are removed from the group if the data is two standard deviations away from the mean.

EXAMPLE 9: Treatment of Chemotherapeutic-Induced Neuropathy (Rodent Model) using CLK Modulators

10 The oncology drug Taxol (paclitaxel) is an effective treatment of ovarian, lung, breast and other cancers but its anti-microtubule activity can induce peripheral neuropathies. Taxol administration, either in a single large dose or several smaller doses, has been demonstrated to produce both sensory-motor deficits and histologically identified axonal abnormalities in rodent models. These models are
15 thought to be predictive of those neuropathies often seen in patients given Taxol for chemotherapy for various forms of cancer. Both sensory-motor behavioral testing and histological evaluation of nerve tissue in animals treated with Taxol and concomitantly treated with either vehicle or a CLK modulator are used to evaluate the effectiveness of CLK modulating compounds to attenuate the effects of Taxol on
20 the peripheral nervous system.

Male Sprague-Dawley rats (Harlan Sprague Dawley Inc., Indianapolis, Indiana, USA) are injected intra-peritoneally with Taxol at 20 mL/kg i.p. (32 mg/kg total dose) on Day 0 using a syringe and sterile needle. A first set of rats are treated with Normal Saline vehicle. The rats are dosed on Day 0 in combination with Taxol
25 and are injected sub-cutaneously using a syringe and sterile needle. This dosing procedure is repeated at 24 and 48 hours post-Taxol injection. The volume of vehicle administered is 1 mL/kg bodyweight. A second set of rats are treated with a CLK modulating compound. The rats are treated with a CLK modulating compound commence on Day 0 in combination with Taxol.

30 Behavioral tests. Behavioral tests will include thermal paw stimulation for pain assessment test and the open field test for activity.

Thermal paw stimulation is a commonly-used method to assess hyper- and hypoalgesia in rodents. Using a thermal paw stimulator (UCSD), the latency for the rat to lift its paw is recorded in response to a heat source placed beneath the hindpaw. The rat is placed on a glass surface maintained at a constant temperature
5 (30 ± 1°C) and then habituated to the apparatus for approximately 15 min prior to testing. Two measurements of paw lift latency are averaged for each animal if they are within 2 sec. of each other. If not, additional testing is performed until this criterion is met. Baseline testing is performed on Day -3. Further tests will be conducted on Days 4 and 7.

10 Necropsy. On day 14 animals are euthanized by CO₂ asphyxiation and cervical dislocation. Following euthanasia the dorsal ganglia of the lumbar vertebra, sciatic nerve and hind paw dermis are harvested and fixed overnight in 10% neutral buffered formalin.

Histology. The harvested tissue is blocked, embedded in paraffin, sectioned
15 and stained with H&E. The tissue is examined using light microscopy and scored by an evaluator blind to the treatment regimen. The tissue is ranked on a scale of 0 to 3 based on the degree and amount of axonal disruption observed in the section, with 0 being a normal appearance of the axon, 1 to 2 being a mild to moderate disruption of the axons and a 3 being a complete disruption and Wallerian
20 degeneration of the axons.

Statistics. A two-way repeated measures ANOVA is performed on the thermal paw stimulation and open field measurements (group x time) to assess the effects of time and treatment on the behavioral performance in these rats. If there are any overall significant differences, a factorial ANOVA is performed at specific time
25 points to determine where the difference occurred. The neuroanatomical evaluation is assessed for statistical significance using a non-parametric analysis of the rating scores for axonal disruption.

EXAMPLE 10: Metabolic Activities of CLK Inhibitors in a Diet Induced Obesity (DIO) Mouse Model

30 In order to define whether CLK inhibitors protect against the development of obesity and associated insulino-resistance, a CLK inhibitor is chronically administered (such as via food admix) to male C57BL6J mice that are subjected

during 16 weeks to a high fat diet. The mice undergo an extensive phenotypic and molecular analysis to define the regulatory pathways affected by CLK inhibition.

In this long-term study, 50 male C57BL6J mice (5 weeks of age) are analyzed during a period of 18 weeks. Five groups of 10 animals are assigned as follows:

- 1: chow diet
- 2: chow diet + CLK inhibitor (200 mg/kg/day)
- 3: high fat diet
- 4: high fat diet + CLK inhibitor (200 mg/kg/day)
- 5: high fat diet + CLK inhibitor (400 mg/kg/day)

During the entire study, body weight and food intake are monitored twice weekly.

During week 1, body composition is analyzed, for all groups, by dual energy X-ray absorptiometry (dexascan).

- During week 2, serum levels of glucose, triglycerides, cholesterol, HDL-C, LDL-C and insulin are measured in all groups after a fasting period of 12 h and mice are then placed on the diets as indicated (Day 0).

- During week 10, glucose tolerance is determined by subjecting all the animals to an intraperitoneal glucose tolerance test (IPGTT). Animals are fasted for 12 h prior to this test.

Nocturnal energy expenditure of groups 1, 3 and 5 (chow diet, high fat diet and high fat diet 400 mg) is measured by indirect calorimetry.

During week 12, body weight composition is again analysed by dexascan for all groups.

- During week 13, circadian activity of groups 3, 4 and 5 (high fat diet fed mice) is studied during a period of 30 h.

During week 14, measurement of blood pressure and heart rate is performed on groups 3, 4 and 5.

- During week 15, rectal temperature of all animals is measured at room temperature at 10:00 am.

A circadian activity measurement is performed on groups 1, 2 and 3.

During week 16, glucose tolerance is analysed by performing an oral glucose tolerance test (OGTT) on a subset of animals (n=5) of groups 3, 4 and 5, and an intraperitoneal insulin sensitivity test (IPIST) on another subset of animals (n=5). During these experiments, blood is also collected to analyze insulin levels. Animals are fasted 12 h prior these tests.

Feces are collected in all groups over a 24 h time period and fecal lipids content are measured.

During week 17, serum levels are measured on a subset of mice (n=5) at 7:00 am which corresponds to the beginning of the light cycle and on another subset of mice (n=5) three hours later (10:00 am). Moreover, thyroid hormone T3 levels are measured in the blood collected at 7:00 am and plasma lipoproteins levels are measured in the blood collected at 10:00 am.

During week 18, a cold test is performed on all animals by measuring body temperature of animals exposed to 4°C.

Three days later, animals are sacrificed.

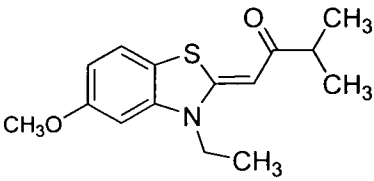
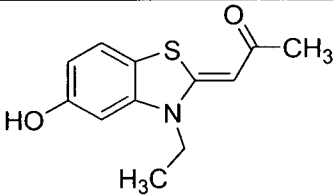
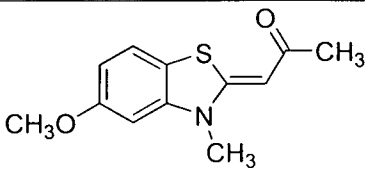
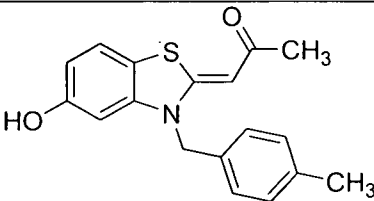
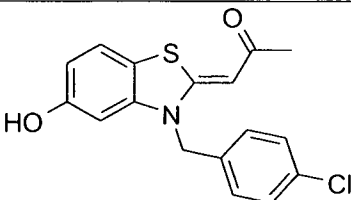
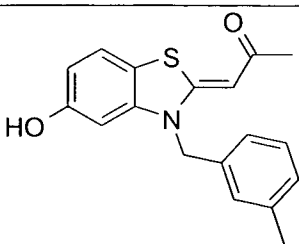
At sacrifice, blood is collected and analyzed for: plasma lipids (TC, TG, HDL-C, FFAs); liver functions (ALAT, ASAT, alkaline Pase, γ -GT); and glucose and insulin lipoprotein profiles of selected groups of plasma (size-exclusion chromatography).

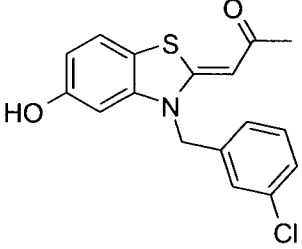
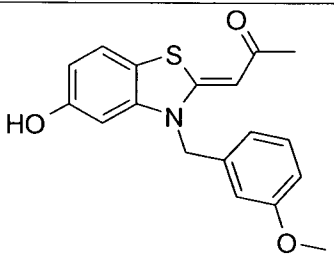
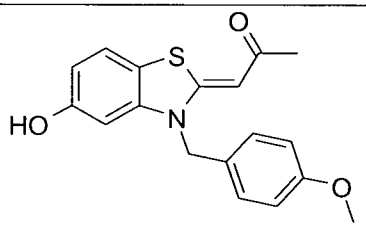
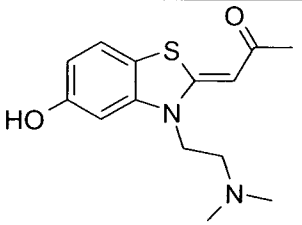
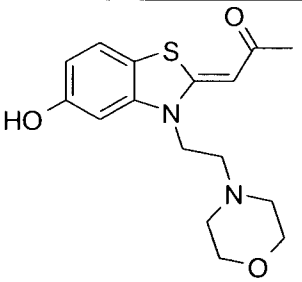
Liver, small intestine, adipose tissues (WAT and BAT), pancreas, heart and muscle are collected and weighed. These can be analyzed by standard histology (HE staining, succinate dehydrogenase staining, oil-red-O staining and cell morphology); for tissue lipid content; and by electron microscopy on BAT and muscle to analyze mitochondria. RNA isolation can be conducted for expression studies of selected genes involved in metabolism and energy homeostasis by quantitative RT-PCR. Microarray experiments can also be performed on selected tissues. In addition, protein extraction can be performed for the study of changes in protein level and post-translational modifications such as acetylation of proteins of interest (e.g. PGC-1 α).

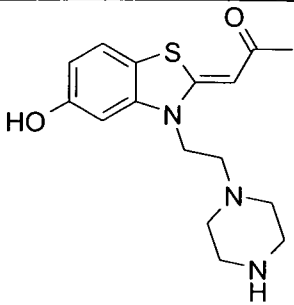
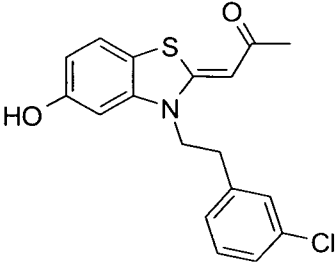
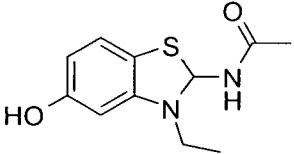
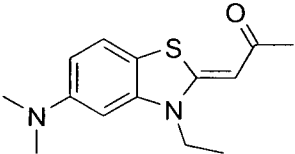
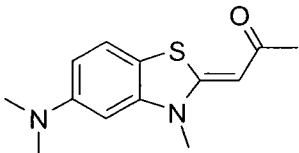
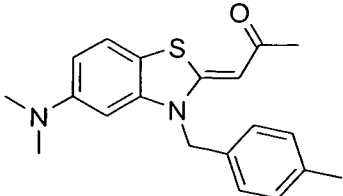
EXAMPLE 11: Exemplary Compounds

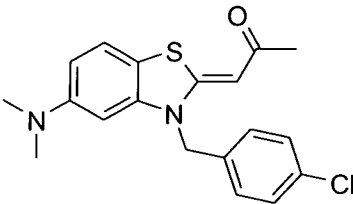
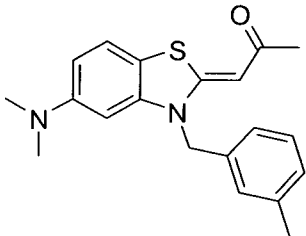
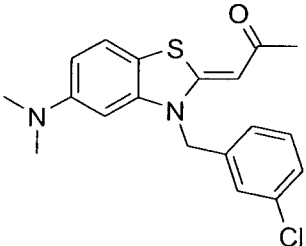
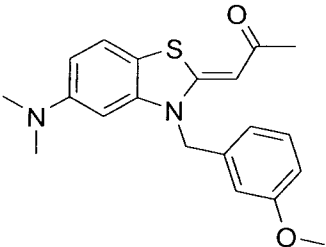
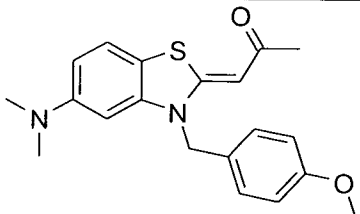
Table 2 shows a number of compounds of the invention. The activity of certain compounds was tested according to the procedure in Example 3.

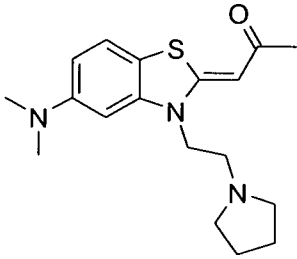
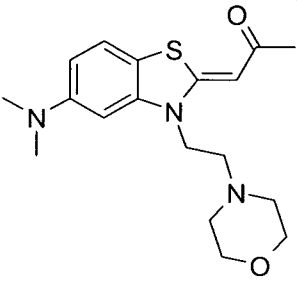
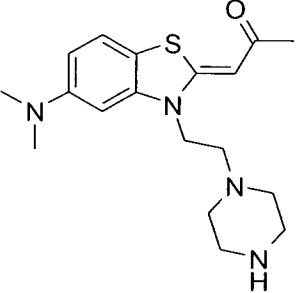
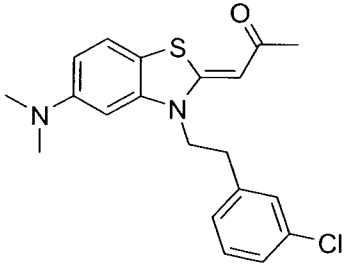
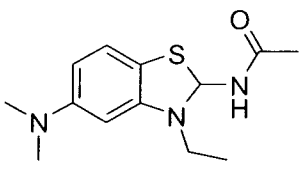
Table 2. CLK Inhibitors

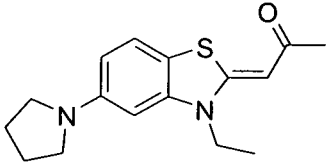
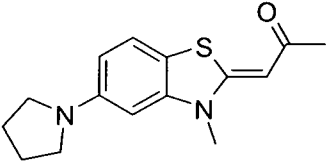
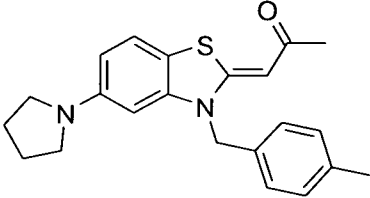
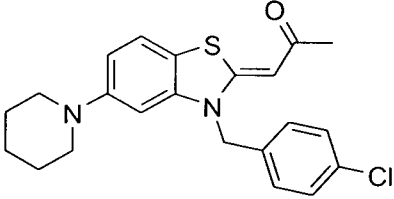
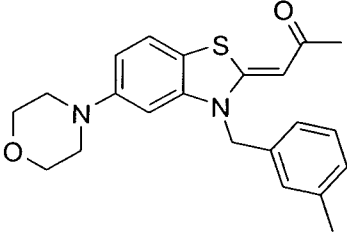
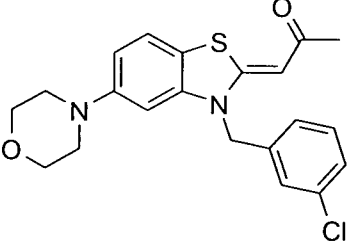
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2	236		0.078	0.064
3	236		0.193	0.263
4				
5	322			
6	312			

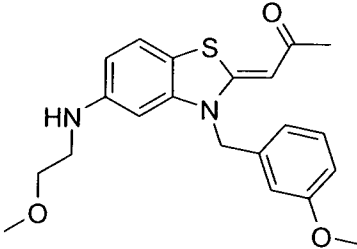
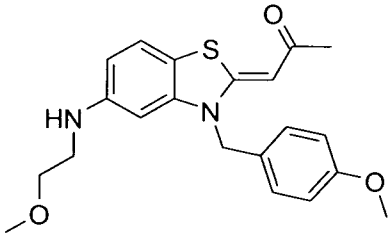
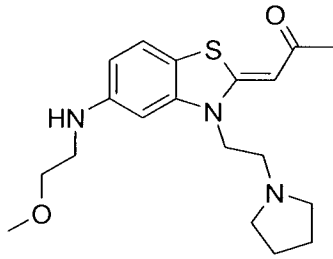
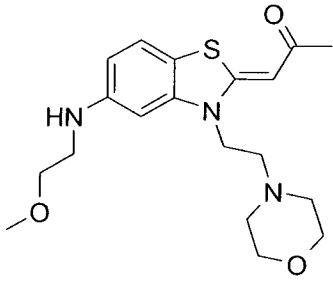
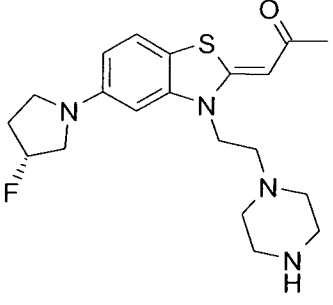
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8	328			
9	328			
10				
11				

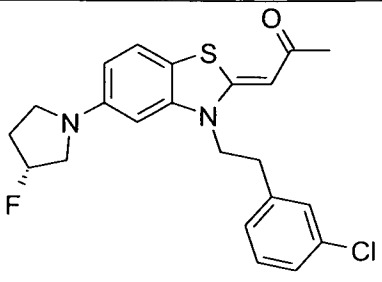
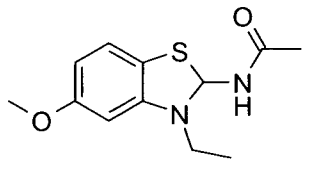
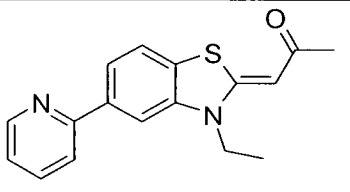
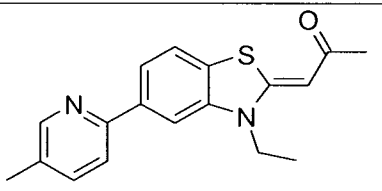
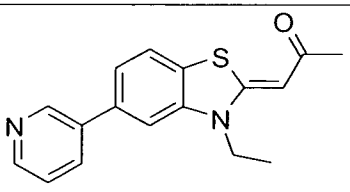
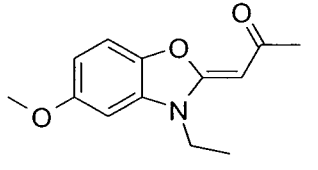
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13				
14				
15	263			
16	249			
17	339			

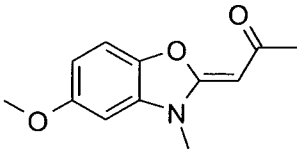
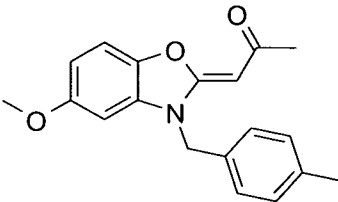
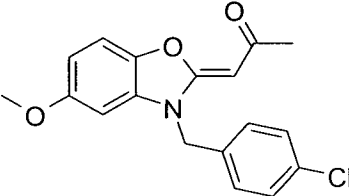
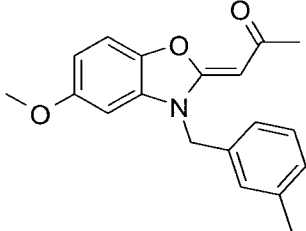
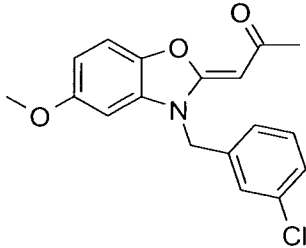
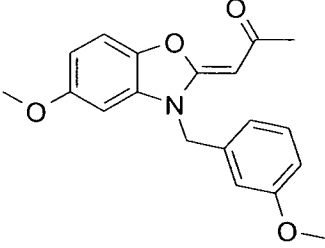
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19				
20				
21				
22				

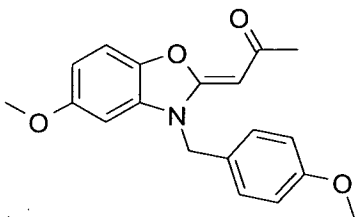
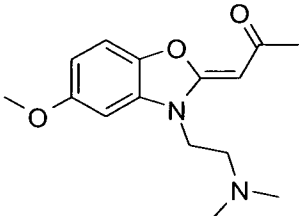
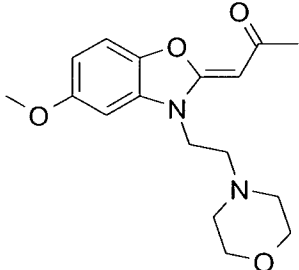
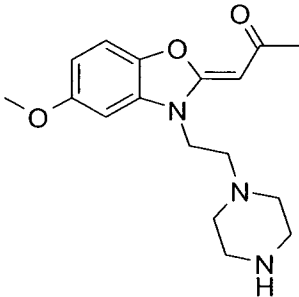
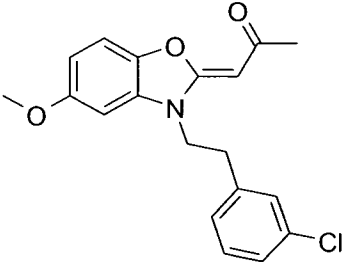
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24				
25				
26				
27				

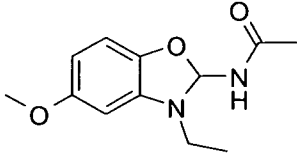
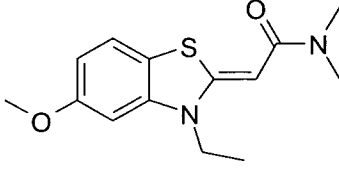
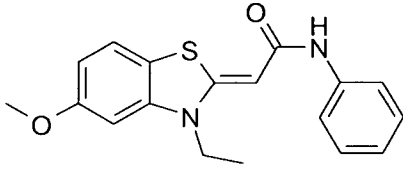
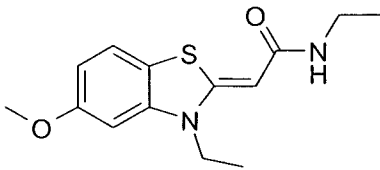
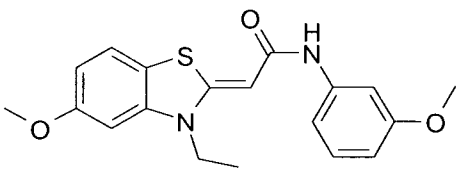
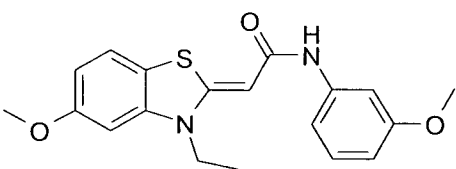
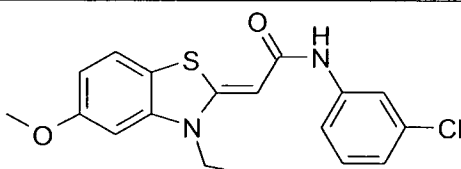
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29				
30	365			
31				
32				
33				

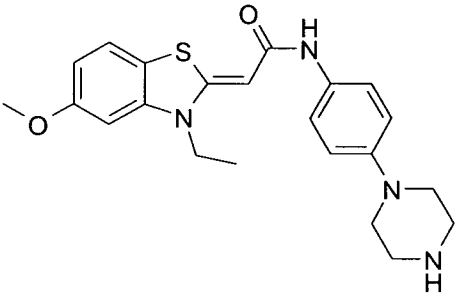
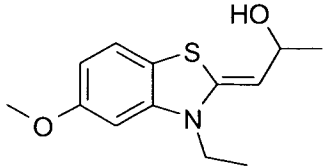
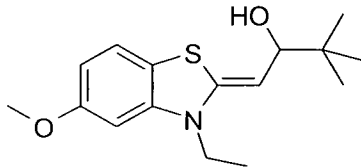
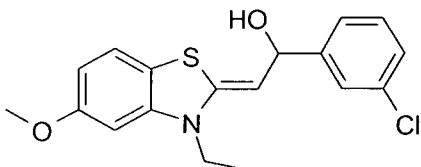
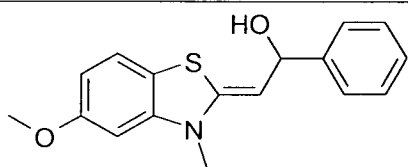
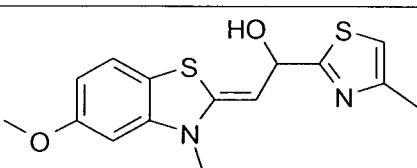
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35				
36				
37				
38				

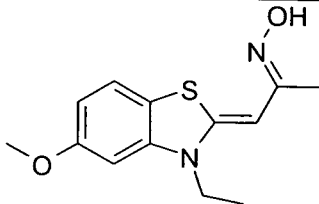
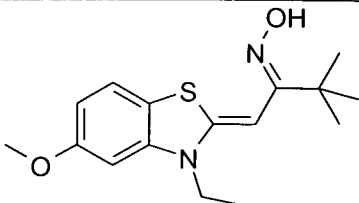
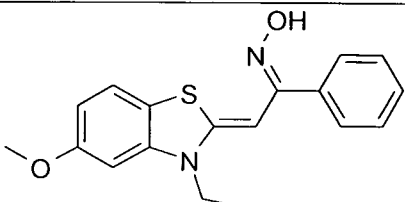
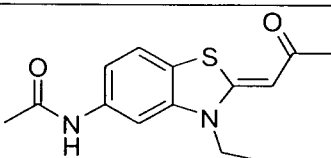
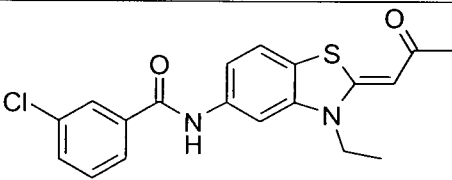
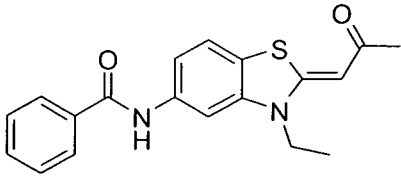
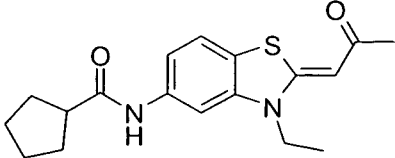
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39				
40				
41				
42				
43				
44				

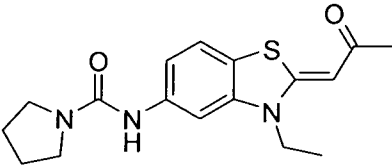
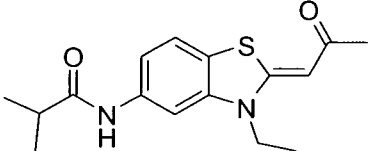
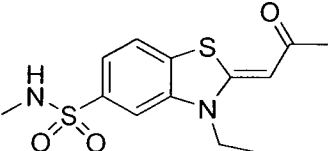
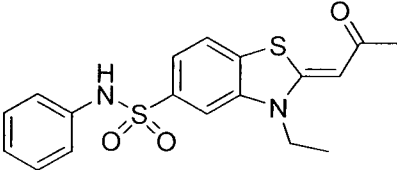
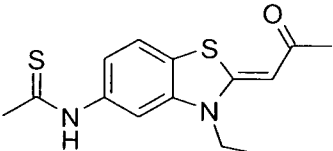
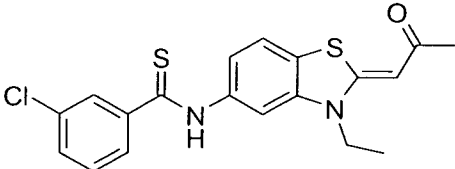
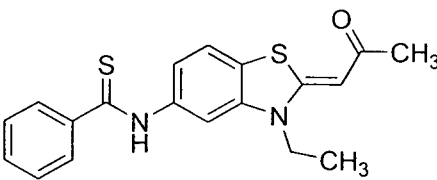
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45				
46				
47				
48				
49				
50				

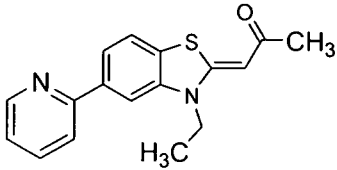
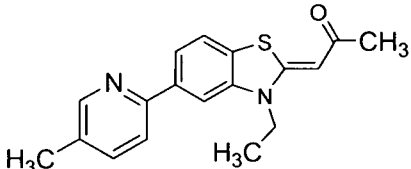
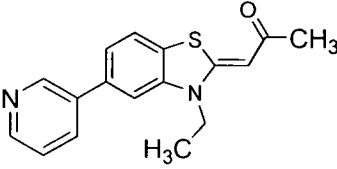
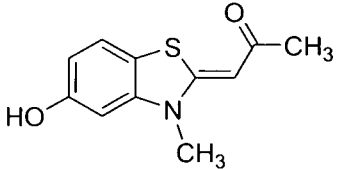
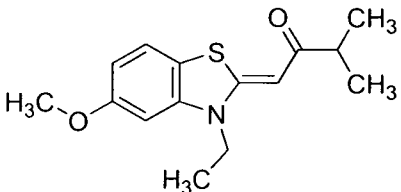
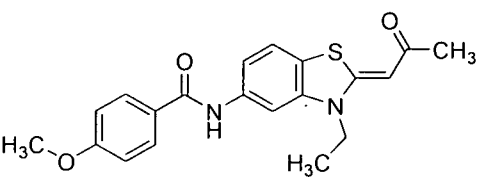
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51				
52				
53				
54				
55				

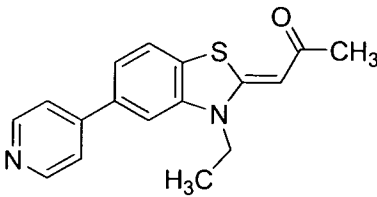
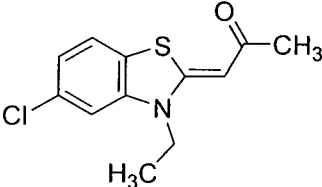
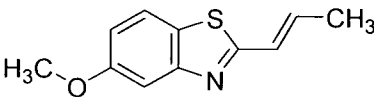
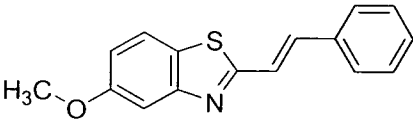
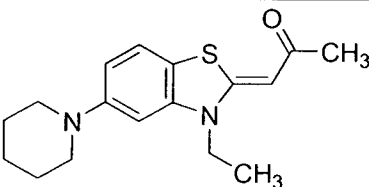
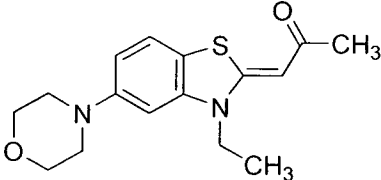
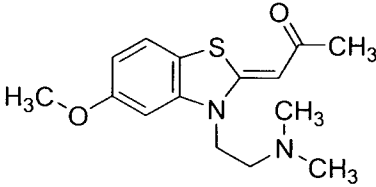
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56				
57				
58				
59				
60				
61				
62				

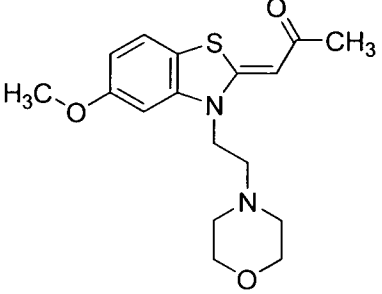
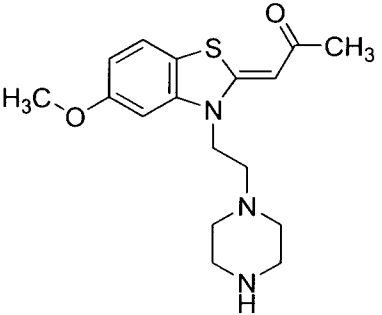
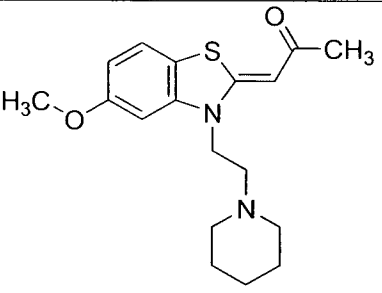
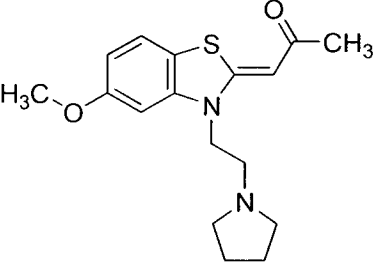
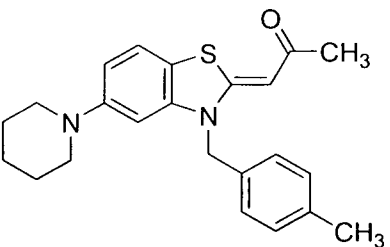
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63				
64				
65				
66				
67				
68				

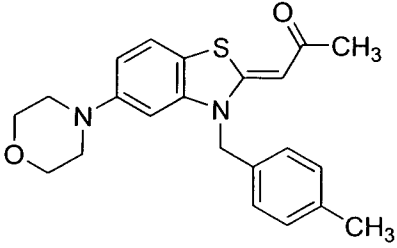
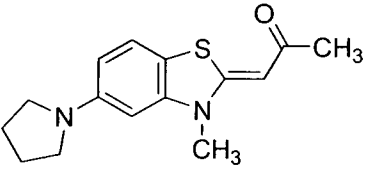
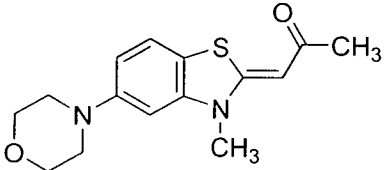
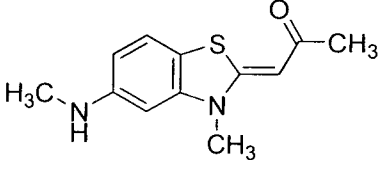
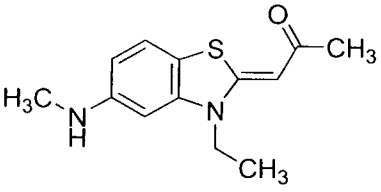
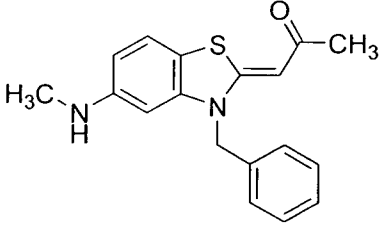
COMPOUND No	[M+H] ⁺	STRUCTURE	CLK2 IC ₅₀ (uM)	CLK1 IC ₅₀ (uM)
69				
70				
71				
72	277			
73				
74				
75				

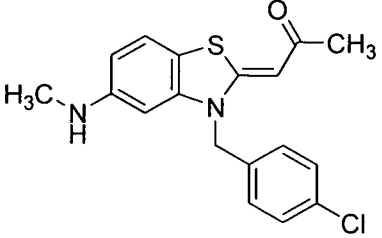
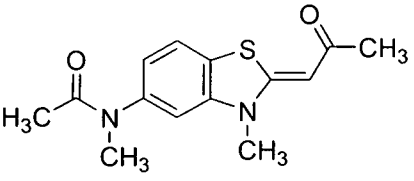
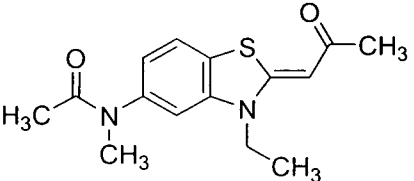
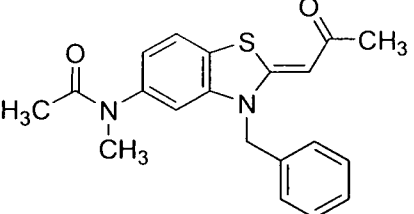
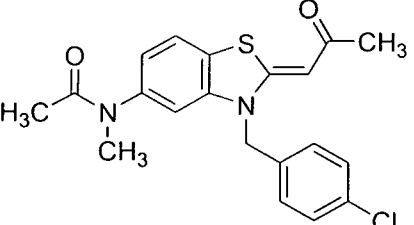
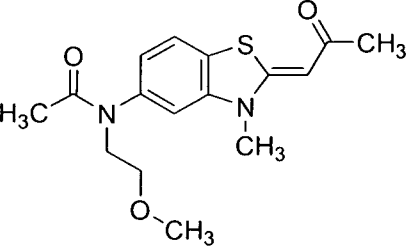
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76				
77				
78				
79				
80				
81				
82				

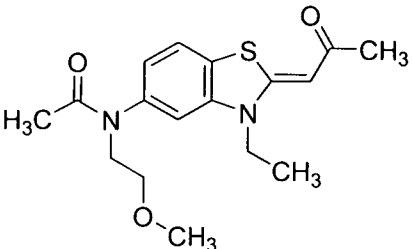
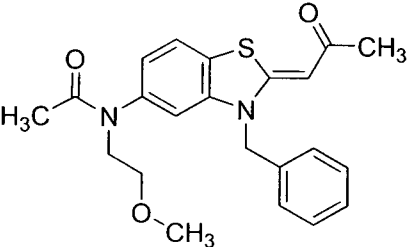
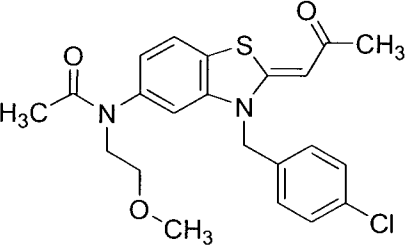
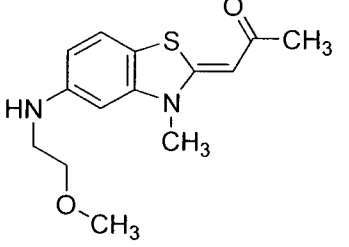
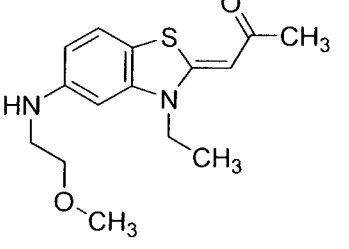
COMPOUND No	[M+H] ⁺	STRUCTURE	CLK2 IC ₅₀ (uM)	CLK1 IC ₅₀ (uM)
83				
84				
85				
86				
87	289		1.57	5.26
88	397			

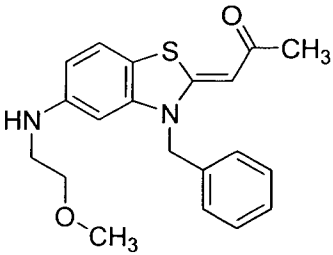
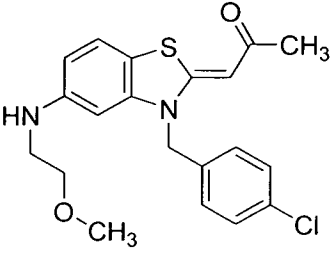
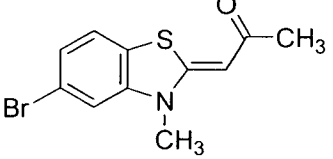
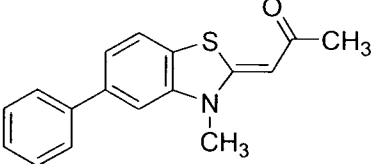
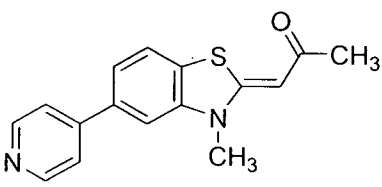
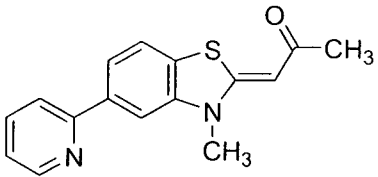
COMPOUND No	[M+H] ⁺	STRUCTURE	CLK2 IC ₅₀ (μ M)	CLK1 IC ₅₀ (μ M)
89	297			
90	254			
91	206		>25	
92	268		>25	
93	303			
94	305			
95				

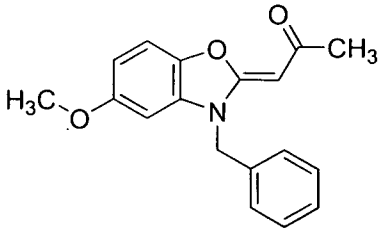
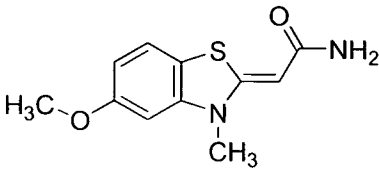
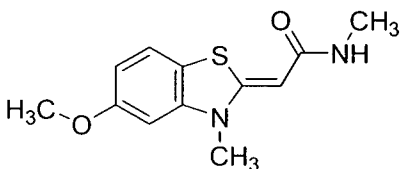
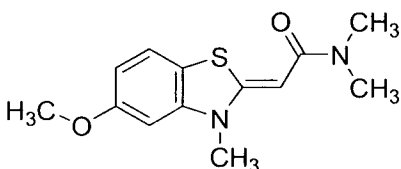
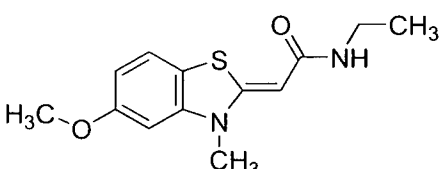
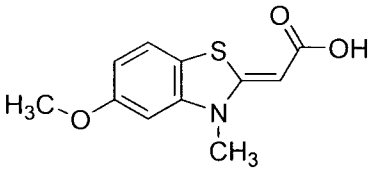
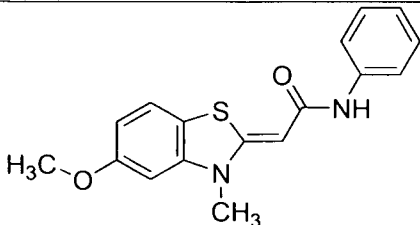
COMPOUND No	[M+H] ⁺	STRUCTURE	CLK2 IC ₅₀ (μ M)	CLK1 IC ₅₀ (μ M)
96				
97				
98				
99				
100	379			

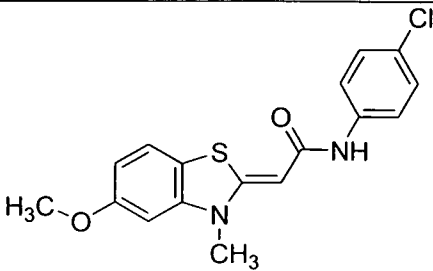
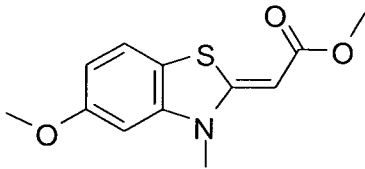
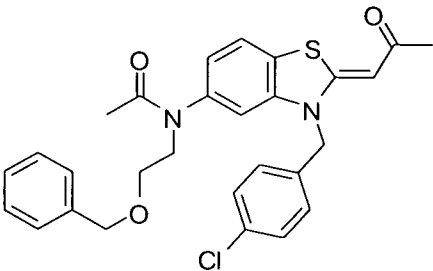
COMPOUND No	[M+H] ⁺	STRUCTURE	CLK2 IC ₅₀ (μ M)	CLK1 IC ₅₀ (μ M)
101	381			
102	275			
103	291			
104	235			
105	249			
106	311			

COMPOUND No	[M+H] ⁺	STRUCTURE	CLK2 IC ₅₀ (μ M)	CLK1 IC ₅₀ (μ M)
107	345			
108	277			
109	291			
110	353			
111	387			
112	321			

COMPOUND No	[M+H] ⁺	STRUCTURE	CLK2 IC ₅₀ (μ M)	CLK1 IC ₅₀ (μ M)
113	335			
114	397			
115	431			
116	279			
117	293			

COMPOUND No	[M+H] ⁺	STRUCTURE	CLK2 IC ₅₀ (μ M)	CLK1 IC ₅₀ (μ M)
118	397			
119	389			
120				
121	282			
122	283			
123	283			

COMPOUND No	[M+H] ⁺	STRUCTURE	CLK2 IC ₅₀ (μ M)	CLK1 IC ₅₀ (μ M)
124				
125				
126				
127				
128				
129				
130				

COMPOUND No	[M+H] ⁺	STRUCTURE	CLK2 IC ₅₀ (uM)	CLK1 IC ₅₀ (uM)
131				
132				
133	473			

Methods

Animal housing and handling. Mice are group housed (5 animals / cage) in specific pathogen-free conditions with a 12 h:12 h (on at 7:00) light-dark cycle, in a temperature (20-22°C) and humidity controlled vivarium, according to the European Community specifications. Animals are allowed free access to water and food.

Drinking water. Chemical composition of the tap water is regularly analyzed to verify the absence of potential toxic substances. Drinking water is treated with HCl and HClO₄ to maintain pH between 5 and 5.5 and chlorine concentration between 5 and 6 ppm.

Diet. The standard rodent chow diet is obtained from UAR and the high fat diet is obtained from Research Diet. Mice are fed, either with chow diet (16% protein, 3% fat, 5% fiber, 5% ash) or with high fat diet (26,2% protein, 26,3% carbohydrate, 34,9% fat). A CLK modulators is mixed with either powdered chow

diet or powdered high fat diet and pellets are reconstituted. Control groups receive pellets as provided by the company. In case of the chow, which is harder to reconstitute, a minimal amount of water is added to the powder to reconstitute pellets, which are then air-dried. New batches of food are prepared weekly.

5 *Blood collection.* Blood is collected either from the retro-orbital sinus or from the tail vein.

Anesthesia. For the dexamethasone scanning experiment, animals are anesthetized with a mixture of ketamine (200 mg/kg) / Xylazine (10 mg/kg) administered by intra-peritoneal injection.

10 *Analysis of lipids and lipoproteins.* Serum triglycerides, total and HDL cholesterol are determined by enzymatic assays. Serum HDL cholesterol content is determined after precipitation of apo B-containing lipoproteins with phosphotungstic acid/Mg (Roche Diagnostics, Mannheim, Germany). Free fatty acids level is determined with a kit from Wako (Neuss, Germany) as specified by the provider.

15 *Metabolic and endocrine exploration.* Blood glucose concentration is measured by a Precision Q.I.D. analyzer (Medisense system), using Medisense Precis electrodes (Abbot Laboratories, Medisense products, Bedford, USA). This method has been validated, by comparing Precision Q.I.D. analyzer values with classical glucose measurements. The Precision Q.I.D. method was chosen since it
20 requires a minimal amount of blood and can hence be employed for multiple measurements such as during an IPGTT. Plasma insulin (Crystal Chem, Chicago, IL) is determined by ELISA according to the manufacturer's specifications. Plasma level of T3 is determined by standard radio-immunoassays (RIA) according to the protocol specified by the providers.

25 *Lipoprotein profiles.* Lipoprotein profiles are obtained by fast protein liquid chromatography, allowing separation of the three major lipoprotein classes VLDL, LDL, and HDL.

Intraperitoneal glucose tolerance test - Oral glucose tolerance test. IPGTT and OGTT are performed in mice which are fasted overnight (12 h). Mice are either
30 injected intraperitoneally (IPGTT) or orally gavaged (OGTT) with a solution of 20 % glucose in sterile saline (0.9% NaCl) at a dose of 2g glucose/kg body weight. Blood is collected from the tail vein, for glucose and insulin monitoring, prior to and

at 15, 30, 45, 75, 90, 120, 150, 180 min after administration of the glucose solution. The incremental area of the glucose curve is calculated as a measure of insulin sensitivity, whereas the corresponding insulin levels indicate insulin secretory reserves.

5 *Intraperitoneal insulin sensitivity test.* Fasted animals are submitted to an IP injection of regular porcine insulin (0.5-1.0 IU/kg; Lilly, Indianapolis, IN). Blood is collected at 0, 15, 30, 45, 60, and 90 min after injection and glucose analyzed as described above. Insulin sensitivity is measured as the slope of the fall in glucose over time after injection of insulin.

10 *Energy expenditure.* Energy expenditure is evaluated through indirect calorimetry by measuring oxygen consumption with the Oxymax apparatus (Columbus Instruments, Columbus, OH) during 12 h. This system consists of an open circuit with air coming in and out of plastic cages (one mouse per cage). Animals are allowed free access to food and water. A very precise CO₂ and O₂
15 sensor measures the difference in O₂ and CO₂ concentrations in both air volumes, which gives the amount of oxygen consumed in a period of time given that the air flow of air coming in the cage is constant. The data coming out of the apparatus are processed in a connected computer, analyzed, and shown in an exportable Excel file. The values are expressed as ml·kg⁻¹·h⁻¹, which is commonly known as the VO₂.

20 *Determination of body fat content by DEXA scanning.* The DEXA analyses are performed by the ultra high resolution PIXIMUS Series Densitometer (0.18 x 0.18 mm pixels, GE Medical Systems, Madison, WI, USA). Bone mineral density (BMD in g/cm²) and body composition are determined by using the PIXIMUS software (version 1.4x, GE Medical Systems).

25 *Non-invasive Blood Pressure and heart Rate measurements.* The Visitech BP-2000 Blood Pressure Analysis System is a computer-automated tail cuff system that is used for taking multiple measurements on 4 awake mice simultaneously without operator intervention. The mice are contained in individual dark chambers on a heated platform with their tails threaded through a tail cuff. The system
30 measures blood pressure by determining the cuff pressure at which the blood flow to the tail is eliminated. A photoelectric sensor detects the specimen's pulse. The system generates results that Applicants have shown correspond closely with the

mean intra-arterial pressure measured simultaneously in the carotid artery. This allows obtaining reproducible values of systolic blood pressure and heart beat rate. This requires training of the animals for one week in the system.

Circadian Activity. Spontaneous locomotor activity is measured using
5 individual boxes, each composed with a sliding floor, a detachable cage, and
equipped with infra-red captors allowing measurement of ambulatory locomotor
activity and rears. Boxes are linked to a computer using an electronic interface
(Imetronic, Pessac, France). Mice are tested for 32 h in order to measure habituation
to the apparatus as well as nocturnal and diurnal activities. The quantity of water
10 consumed is measured during the test period using an automated lickometer.

EQUIVALENTS

The present invention provides among other things CLK-modulating
compounds and methods of use thereof. While specific embodiments of the subject
invention have been discussed, the above specification is illustrative and not
15 restrictive. Many variations of the invention will become apparent to those skilled
in the art upon review of this specification. The full scope of the invention should
be determined by reference to the claims, along with their full scope of equivalents,
and the specification, along with such variations.

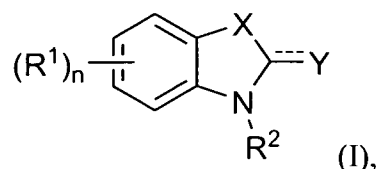
INCORPORATION BY REFERENCE

20 All publications and patents mentioned herein are hereby incorporated by
reference in their entirety as if each individual publication or patent was specifically
and individually indicated to be incorporated by reference. In case of conflict, the
present application, including any definitions herein, will control.

Also incorporated by reference in their entirety are any polynucleotide and
25 polypeptide sequences which reference an accession number correlating to an entry
in a public database, such as those maintained by The Institute for Genomic
Research (TIGR) (www.tigr.org) and/or the National Center for Biotechnology
Information (NCBI) (www.ncbi.nlm.nih.gov).

CLAIMS:

1. A compound of formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

X is selected from S or O;

Y is selected from -NH-C(O)-R^5 , $\text{=C(R}^3\text{)-C(O)-N(R}^4\text{)-R}^5$, $\text{=C(R}^3\text{)-C(O)-C(CH}_3\text{)}_3$, $\text{=C(R}^3\text{)-C(O)-CH(R}^7\text{)(R}^7\text{)}$, $\text{=C(R}^3\text{)-C(=NOH)-R}^5$, and $\text{=C(R}^3\text{)-CH(OH)-R}^5$, and when X is O, Y is additionally selected from $\text{=C(R}^3\text{)-carbocyclic}$ and $\text{=C(R}^3\text{)-heterocyclic}$;

each R^1 is independently selected from -OH , halo, -R^6 , -OR^6 , -SR^6 , -NH_2 , $\text{-NH(R}^6\text{)}$, $\text{-N(R}^6\text{)(R}^6\text{)}$, $\text{-N(R}^3\text{)C(O)R}^6$, $\text{-N(R}^3\text{)C(S)R}^6$ and $\text{-SO}_2\text{N(R}^6\text{)(R}^6\text{)}$;

R^2 is selected from $\text{-(C}_1\text{-C}_5\text{-alkyl)}$, $\text{-(C}_1\text{-C}_3\text{-alkyl)-aryl}$, $\text{-(C}_1\text{-C}_3\text{-alkyl)-NH(R}^6\text{)}$, and $\text{-(C}_1\text{-C}_3\text{-alkyl)-N(R}^6\text{)(R}^6\text{)}$;

each R^3 is independently selected from hydrogen and optionally substituted $\text{-(C}_1\text{-C}_4\text{-alkyl)}$;

R^4 is selected from hydrogen and optionally substituted $\text{-(C}_1\text{-C}_4\text{-alkyl)}$;

R^5 is selected from hydrogen, $\text{-(C}_1\text{-C}_4\text{-alkyl)}$, aryl, $\text{-(C}_1\text{-C}_4\text{-alkyl)-aryl}$, heteroaryl, and $\text{-(C}_1\text{-C}_4\text{-alkyl)-heteroaryl}$ wherein the alkyl, aryl and heteroaryl is optionally substituted;

each R^6 is independently selected from $\text{C}_1\text{-C}_6$ alkyl, $\text{C}_2\text{-C}_6$ alkenyl or alkynyl, carbocyclyl, and heterocyclyl, or wherein two R^6 together with the nitrogen atom to which they are bound are taken together to form an optionally substituted heterocyclyl; and wherein each R^6 is optionally and independently substituted;

each R^7 is independently selected from hydrogen, $\text{-(C}_1\text{-C}_4\text{-alkyl)}$, $\text{-(C}_1\text{-C}_4\text{-alkenyl)}$, $\text{-(C}_1\text{-C}_4\text{-alkynyl)}$, carbocyclyl, $\text{-(C}_1\text{-C}_4\text{-alkyl)-carbocyclyl}$, heterocyclyl, and $\text{-(C}_1\text{-C}_4\text{-alkyl)-heterocyclyl}$, wherein the alkyl, alkenyl, alkynyl, carbocyclyl and heterocyclyl are optionally substituted

n is an integer from 1 to 4; and

--- represents a single or a double bond;

wherein:

when X is O, R² is ethyl, Y is =CH-C(O)-CH₂CH₃, and n is 1, then R¹ is not methoxy;

when X is S, Y is =CH-C(O)-C(CH₃)₃, or =CH-C(O)-CH(R⁷)(R⁷); each R⁷ is independently selected from hydrogen, unsubstituted -(C₁-C₄-alkyl), unsubstituted carbocyclyl, and unsubstituted -(C₁-C₄-alkyl)-carbocyclyl; n is 1 or 2; each R¹ is independently selected from halo, -R⁶ and -OR⁶; each R⁶ is independently selected from C₁-C₆ straight or branched alkyl, C₂-C₆ straight or branched alkenyl or alkynyl, a carbocyclic moiety, and a heterocyclic moiety consisting of carbon, hydrogen and oxygen atoms; and each R⁶ is optionally substituted with one or more substituents selected from alkyl, O-alkyl and -OH; then R² is not -(C₂-C₅-alkyl), or -(C₁-C₃-alkyl)-aryl;

when X is S, Y is =CH-C(O)-CH₃, R² is -ethyl, n is 2, and one R¹ is methoxy; then the other R¹ is not NH₂ or when one R¹ is -OH, then the other R¹ is not -CH₂-N(CH₃)₂;

when X is S, Y is =CH-C(O)-CH₃, R² is ethyl, and n is 1; then R¹ is not -OH;

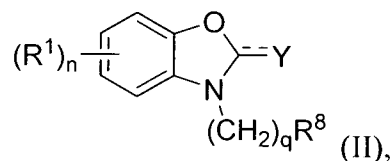
when X is S, Y is =CH-C(O)-CH₂-tetrahydrofuran-2-yl, R² is ethyl, and n is 1; then R¹ is not methyl or methoxy;

when X is S, Y is =CH-C(O)-N(CH₃)-R⁵, and R² is methyl; then R⁵ is not -CH₂-(1-methyl-1H-indol-2-yl); and

when X is S, Y is =CH-C(O)-N(H)-(4-dimethylaminophenyl), R² is ethyl, and n is 1; then R¹ is not -S-methyl.

2. The compound of claim 1, wherein R¹ is selected from -NH(R⁶), -N(R⁶)(R⁶) and a heterocyclic moiety comprising one or more heteroatoms selected from N and S
3. The compound of claim 1, wherein R² is selected from -(C₁-C₃-alkyl)-aryl, -(C₁-C₃-alkyl)-NH(R⁶), and -(C₁-C₃-alkyl)-N(R⁶)(R⁶).

4. The compound of claim 3, wherein R^2 is selected from $-(C_1-C_3\text{-alkyl})-NH(R^6)$ and $-(C_1-C_3\text{-alkyl})-N(R^6)(R^6)$.
5. The compound of claim 1, wherein Y is selected from $-NH-C(O)-R^5$, $=C(R^3)-C(=NOH)-R^5$ and $=C(R^3)-CH(OH)-R^5$, and when X is O, Y is additionally selected from $=C(R^3)$ -carbocyclic and $=C(R^3)$ -heterocyclic.
6. The compound of claim 1, wherein
 Y is $=C(R^3)-C(O)-NR^4R^5$;
 R^5 is selected from hydrogen, $-(C_1-C_4\text{-alkyl})$, aryl, $-(C_1-C_4\text{-alkyl})$ -aryl, heteroaryl, and $-(C_2-C_4\text{-alkyl})$ -heteroaryl, wherein the alkyl, aryl and heteroaryl are optionally substituted; and
 each R^1 is independently selected from $-OH$, halo, $-R^6$, $-OR^6$, and $-NH_2$.
7. A compound of formula (II):



or a pharmaceutically acceptable salt thereof, wherein:

Y is selected from $-NH-C(O)-R^5$, $=C(R^3)-C(O)-N(R^4)-R^5$, $=C(R^3)-C(O)-C(CH_3)_3$, $=C(R^3)-C(O)-CH(R^7)(R^7)$, $=C(R^3)-C(=NOH)-R^5$, $=C(R^3)-CH(OH)-R^5$, $=C(R^3)$ -carbocyclic and $=C(R^3)$ -heterocyclic;
 each R^1 is independently selected from $-OH$, halo, $-R^6$, $-OR^6$, $-SR^6$, $-NH_2$, $-NH(R^6)$, $-N(R^6)(R^6)$, $-N(R^3)C(O)R^6$, $-N(R^3)C(S)R^6$ and $-SO_2N(R^6)(R^6)$;

each R^3 is independently selected from hydrogen and optionally substituted $-(C_1-C_4\text{-alkyl})$;

R^4 is selected from hydrogen and optionally substituted $-(C_1-C_4\text{-alkyl})$;

R^5 is selected from hydrogen, $-(C_1-C_4\text{-alkyl})$, aryl, $-(C_1-C_4\text{-alkyl})$ -aryl, heteroaryl, and $-(C_1-C_4\text{-alkyl})$ -heteroaryl wherein the alkyl, aryl and heteroaryl is optionally substituted;

each R^6 is independently selected from C_1-C_6 alkyl, C_2-C_6 alkenyl or alkynyl, carbocyclyl, and heterocyclyl, or wherein two R^6 together with the

nitrogen atom to which they are bound are taken together to form an optionally substituted heterocyclyl; and wherein each R^6 is optionally and independently substituted;

each R^7 is independently selected from hydrogen, $-(C_1-C_4\text{-alkyl})$, $-(C_2-C_4\text{-alkenyl})$, $-(C_2-C_4\text{-alkynyl})$, carbocyclyl, $-(C_1-C_4\text{-alkyl})$ -carbocyclyl, heterocyclyl, and $-(C_1-C_4\text{-alkyl})$ -heterocyclyl, wherein the alkyl, alkenyl, alkynyl, carbocyclyl and heterocyclyl are optionally substituted

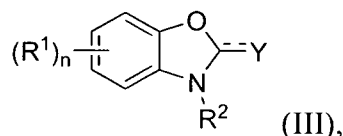
R^8 is selected from $-N(R^6)(R^6)$, carbocycle, and heterocycle, wherein carbocycle and heterocycle are optionally substituted;

n is an integer from 1 to 4;

q is an integer from 1 to 3; and

--- represents a single or a double bond.

8. A compound of formula (III):



or a pharmaceutically acceptable salt thereof, wherein:

Y is selected from $-NH-C(O)-R^5$, $=C(R^3)-C(O)-N(R^4)-R^5$, $=C(R^3)-C(O)-C(CH_3)_3$, $=C(R^3)-C(O)-CH(R^7)(R^7)$, $=C(R^3)-C(=NOH)-R^5$, $=C(R^3)-CH(OH)-R^5$, $=C(R^3)$ -carbocyclic and $=C(R^3)$ -heterocyclic;

each R^1 is independently selected from $-OH$, halo, $-R^6$, $-OR^6$, $-SR^6$, $-NH_2$, $-NH(R^6)$, $-N(R^6)(R^6)$, $-N(R^3)C(O)R^6$, $-N(R^3)C(S)R^6$ and $-SO_2N(R^6)(R^6)$;

R^2 is selected from $-(C_1-C_5\text{-alkyl})$, $-(C_1-C_3\text{-alkyl})$ -aryl, $-(C_1-C_3\text{-alkyl})$ - $NH(R^6)$, and $-(C_1-C_3\text{-alkyl})$ - $N(R^6)(R^6)$;

each R^3 is independently selected from hydrogen and optionally substituted $-(C_1-C_4\text{-alkyl})$;

R^4 is selected from hydrogen and optionally substituted $-(C_1-C_4\text{-alkyl})$;

R^5 is selected from hydrogen, $-(C_1-C_4\text{-alkyl})$, aryl, $-(C_1-C_4\text{-alkyl})$ -aryl, heteroaryl, and $-(C_1-C_4\text{-alkyl})$ -heteroaryl wherein the alkyl, aryl and heteroaryl is optionally substituted;

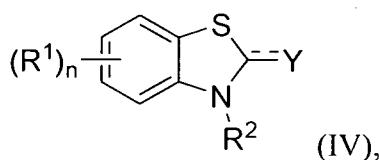
each R^6 is independently selected from C_1 - C_6 alkyl, C_2 - C_6 alkenyl or alkynyl, carbocyclyl, and heterocyclyl, or wherein two R^6 together with the nitrogen atom to which they are bound are taken together to form an optionally substituted heterocyclyl; and wherein each R^6 is optionally and independently substituted;

each R^7 is independently selected from hydrogen, $-(C_2-C_4\text{-alkyl})$, $-(C_2-C_4\text{-alkenyl})$, $-(C_3-C_4\text{-alkynyl})$, carbocyclyl, $-(C_1-C_4\text{-alkyl})$ -carbocyclyl, heterocyclyl, and $-(C_1-C_4\text{-alkyl})$ -heterocyclyl, wherein the alkyl, alkenyl, alkynyl, carbocyclyl and heterocyclyl are optionally substituted;

n is an integer from 1 to 4; and

--- represents a single or a double bond.

9. A compound of formula (IV):



or a pharmaceutically acceptable salt thereof, wherein:

Y is selected from $-NH-C(O)-R^5$, $-C(R^3)=C(R^7)(R^7)$, $=C(R^3)-C(O)-O(R^3)$, $=C(R^3)-C(O)-N(R^4)-R^5$, $=C(R^3)-C(=NOH)-R^5$, and $=C(R^3)-CH(OH)-R^5$;

each R^1 is independently selected from $-OH$, halo, $-R^6$, $-OR^6$, $-NH_2$, $-NH(R^6)$, $-N(R^6)(R^6)$, $-N(R^3)C(O)R^6$, $-N(R^3)C(S)R^6$ and $-SO_2N(R^6)(R^6)$;

R^2 is selected from $-(C_1-C_5\text{-alkyl})$, $-(C_1-C_3\text{-alkyl})$ -aryl, $-(C_1-C_3\text{-alkyl})$ - $NH(R^6)$, and $-(C_1-C_3\text{-alkyl})$ - $N(R^6)(R^6)$;

each R^3 is independently selected from hydrogen and optionally substituted $-(C_1-C_4\text{-alkyl})$;

R^4 is selected from hydrogen and optionally substituted $-(C_1-C_4\text{-alkyl})$;

R^5 is selected from hydrogen, $-(C_1-C_4\text{-alkyl})$, aryl, $-(C_1-C_4\text{-alkyl})$ -aryl, heteroaryl, and $-(C_2-C_4\text{-alkyl})$ -heteroaryl wherein the alkyl, aryl and heteroaryl is optionally substituted;

each R^6 is independently selected from C_1 - C_6 alkyl, C_2 - C_6 alkenyl or alkynyl, carbocyclyl, and heterocyclyl, or wherein two R^6 together with the

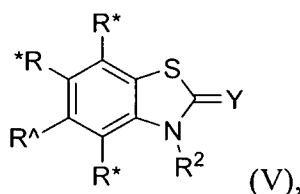
nitrogen atom to which they are bound are taken together to form an optionally substituted heterocyclyl; and wherein each R^6 is optionally and independently substituted;

each R^7 is independently selected from hydrogen, $-(C_1-C_4\text{-alkyl})$, $-(C_2-C_4\text{-alkenyl})$, $-(C_2-C_4\text{-alkynyl})$, carbocyclyl, $-(C_1-C_4\text{-alkyl})$ -carbocyclyl, heterocyclyl, and $-(C_1-C_4\text{-alkyl})$ -heterocyclyl, wherein the alkyl, alkenyl, alkynyl, carbocyclyl and heterocyclyl are optionally substituted

n is an integer from 1 to 4; and

--- represents a single or a double bond.

10. A compound of formula (V):



or a pharmaceutically acceptable salt thereof, wherein:

Y is selected from $=C(R^3)-C(O)-C(CH_3)_3$ and $=C(R^3)-C(O)-CH(R^7)(R^7)$;

each R^* at each occurrence is independently selected from OH , $-CH_3$, $-CF_3$, $-OCH_3$, $-OCH_2CH_3$, $-O(C=O)CH_3$, pyridyl, morpholinyl, pyrrolidinyl, piperidinyl, $-NH(R^6)$, $-N(R^6)(R^6)$, $-N(R^3)C(O)R^6$, $-N(R^3)C(S)R^6$, and $-SO_2N(R^6)(R^6)$; wherein when R^* is selected from pyridyl, morpholinyl, pyrrolidinyl or piperidinyl, R^* is optionally substituted; and

R^A is selected from, pyridyl, morpholinyl, pyrrolidinyl, piperidinyl, $-NH(R^6)$, $-N(R^6)(R^6)$, $-N(R^3)C(O)R^6$, $-N(R^3)C(S)R^6$, and $-SO_2N(R^6)(R^6)$; wherein when R^* is selected from pyridyl, morpholinyl, pyrrolidinyl or piperidinyl, R^* is optionally substituted;

R^2 is selected from $-(C_1-C_5\text{-alkyl})$, $-(C_1-C_3\text{-alkyl})$ -aryl, $-(C_1-C_3\text{-alkyl})$ - $NH(R^6)$, and $-(C_1-C_3\text{-alkyl})$ - $N(R^6)(R^6)$;

each R^3 is independently selected from hydrogen and optionally substituted $-(C_1-C_4\text{-alkyl})$;

R^4 is selected from hydrogen and optionally substituted-(C₁-C₄-alkyl);

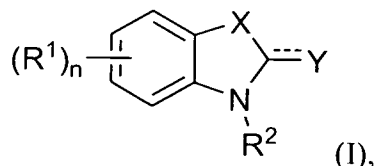
R^5 is selected from hydrogen, -(C₁-C₄-alkyl), aryl, -(C₁-C₄-alkyl)-aryl, heteroaryl, and -(C₁-C₄-alkyl)-heteroaryl wherein the alkyl, aryl and heteroaryl is optionally substituted;

each R^6 is independently selected from C₁-C₆ alkyl, C₂-C₆ alkenyl or alkynyl, carbocyclyl, and heterocyclyl, or wherein two R^6 together with the nitrogen atom to which they are bound are taken together to form an optionally substituted heterocyclyl; and wherein each R^6 is optionally and independently substituted;

each R^7 is independently selected from hydrogen, -(C₁-C₄-alkyl), -(C₂-C₄-alkenyl), -(C₂-C₄-alkynyl), carbocyclyl, -(C₁-C₄-alkyl)-carbocyclyl, heterocyclyl, and -(C₁-C₄-alkyl)-heterocyclyl, wherein the alkyl, alkenyl, alkynyl, carbocyclyl and heterocyclyl are optionally substituted; and

n is an integer from 1 to 4.

11. A pharmaceutical composition comprising a compound of any one of claims 1-10.
12. A pharmaceutical composition comprising a compound of formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

X is selected from S or O;

Y is selected from -NH-C(O)- R^5 , =C(R^3)-C(O)-N(R^4)- R^5 , =C(R^3)-C(O)-C(CH₃)₃, =C(R^3)-C(O)-CH(R^7)(R^7), =C(R^3)-C(=NOH)- R^5 , and =C(R^3)-CH(OH)- R^5 , and when X is O, Y is additionally selected from =C(R^3)-carbocyclic and =C(R^3)-heterocyclic;

each R^1 is independently selected from -OH, halo, - R^6 , -OR⁶, -SR⁶, -NH₂, -NH(R^6), -N(R^6)(R^6), -N(R^3)C(O) R^6 , -N(R^3)C(S) R^6 and -SO₂N(R^6)(R^6);

R^2 is selected from -(C₁-C₅-alkyl), -(C₁-C₃-alkyl)-aryl, -(C₁-C₃-alkyl)-NH(R^6), and -(C₁-C₃-alkyl)-N(R^6)(R^6);

each R^3 is independently selected from hydrogen and optionally substituted $-(C_1-C_4\text{-alkyl})$;

R^4 is selected from hydrogen and optionally substituted $-(C_1-C_4\text{-alkyl})$;

R^5 is selected from hydrogen, $-(C_1-C_4\text{-alkyl})$, aryl, $-(C_1-C_4\text{-alkyl})\text{-aryl}$, heteroaryl, and $-(C_1-C_4\text{-alkyl})\text{-heteroaryl}$ wherein the alkyl, aryl and heteroaryl is optionally substituted;

each R^6 is independently selected from C_1-C_6 alkyl, C_2-C_6 alkenyl or alkynyl, carbocyclyl, and heterocyclyl, or wherein two R^6 together with the nitrogen atom to which they are bound are taken together to form an optionally substituted heterocyclic moiety; and wherein each R^6 is optionally and independently substituted;

each R^7 is independently selected from hydrogen, $-(C_1-C_4\text{-alkyl})$, $-(C_1-C_4\text{-alkenyl})$, $-(C_1-C_4\text{-alkynyl})$, carbocyclyl, $-(C_1-C_4\text{-alkyl})\text{-carbocyclyl}$, heterocyclyl, and $-(C_1-C_4\text{-alkyl})\text{-heterocyclyl}$, wherein the alkyl, alkenyl, alkynyl, carbocyclyl and heterocyclyl are optionally substituted

n is an integer from 1 to 4;

--- represents a single or a double bond; and

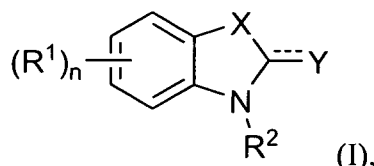
wherein:

when X is S, Y is $=CH-C(O)-C(CH_3)_3$, or $=CH-C(O)-CH(R^7)(R^7)$;

each R^7 is independently selected from hydrogen, unsubstituted $-(C_1-C_4\text{-alkyl})$, unsubstituted carbocyclyl, and unsubstituted $-(C_1-C_4\text{-alkyl})\text{-carbocyclyl}$; n is 1 or 2; each R^1 is independently selected from halo, $-R^6$ and $-OR^6$; each R^6 is independently selected from C_1-C_6 straight or branched alkyl, C_2-C_6 straight or branched alkenyl or alkynyl, a carbocyclic moiety, and a heterocyclic moiety consisting of carbon, hydrogen and oxygen atoms; and each R^6 is optionally substituted with one or more substituents selected from alkyl, O-alkyl and $-OH$, then R^2 is not $-(C_2-C_5\text{-alkyl})$, or $-(C_1-C_3\text{-alkyl})\text{-aryl}$; and

when X is S, Y is $=CH-C(O)-N(CH_3)-R^5$, and R^2 is methyl; then R^5 is not $-CH_2\text{-(1-methyl-1H-indol-2-yl)}$.

13. A method of inhibiting the activity of CLK-2 in a cell comprising the step of contacting the cell with a compound of formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

X is selected from S or O;

Y is selected from -NH-C(O)-R^5 , $\text{=C(R}^3\text{)-C(O)-N(R}^4\text{)-R}^5$, $\text{=C(R}^3\text{)-C(O)-C(CH}_3\text{)}_3$, $\text{=C(R}^3\text{)-C(O)-CH(R}^7\text{)(R}^7\text{)}$, $\text{=C(R}^3\text{)-C(=NOH)-R}^5$, and $\text{=C(R}^3\text{)-CH(OH)-R}^5$, and when X is O, Y is additionally selected from $\text{=C(R}^3\text{)-carbocyclic}$ and $\text{=C(R}^3\text{)-heterocyclic}$;

each R^1 is independently selected from -OH , halo, -R^6 , -OR^6 , -SR^6 , -NH_2 , $\text{-NH(R}^6\text{)}$, $\text{-N(R}^6\text{)(R}^6\text{)}$, $\text{-N(R}^3\text{)C(O)R}^6$, $\text{-N(R}^3\text{)C(S)R}^6$ and $\text{-SO}_2\text{N(R}^6\text{)(R}^6\text{)}$;

R^2 is selected from $\text{-(C}_1\text{-C}_5\text{-alkyl)}$, $\text{-(C}_1\text{-C}_3\text{-alkyl)-aryl}$, $\text{-(C}_1\text{-C}_3\text{-alkyl)-NH(R}^6\text{)}$, and $\text{-(C}_1\text{-C}_3\text{-alkyl)-N(R}^6\text{)(R}^6\text{)}$;

each R^3 is independently selected from hydrogen and optionally substituted $\text{-(C}_1\text{-C}_4\text{-alkyl)}$;

R^4 is selected from hydrogen and optionally substituted $\text{-(C}_1\text{-C}_4\text{-alkyl)}$;

R^5 is selected from hydrogen, $\text{-(C}_1\text{-C}_4\text{-alkyl)}$, aryl, $\text{-(C}_1\text{-C}_4\text{-alkyl)-aryl}$, heteroaryl, and $\text{-(C}_1\text{-C}_4\text{-alkyl)-heteroaryl}$ wherein the alkyl, aryl and heteroaryl is optionally substituted;

each R^6 is independently selected from $\text{C}_1\text{-C}_6$ alkyl, $\text{C}_2\text{-C}_6$ alkenyl or alkynyl, carbocyclyl, and heterocyclyl, or wherein two R^6 together with the nitrogen atom to which they are bound are taken together to form an optionally substituted heterocyclic moiety; and wherein each R^6 is optionally and independently substituted;

each R^7 is independently selected from hydrogen, $\text{-(C}_1\text{-C}_4\text{-alkyl)}$, $\text{-(C}_1\text{-C}_4\text{-alkenyl)}$, $\text{-(C}_1\text{-C}_4\text{-alkynyl)}$, carbocyclyl, $\text{-(C}_1\text{-C}_4\text{-alkyl)-carbocyclyl}$, heterocyclyl, and $\text{-(C}_1\text{-C}_4\text{-alkyl)-heterocyclyl}$, wherein the alkyl, alkenyl, alkynyl, carbocyclyl and heterocyclyl are optionally substituted

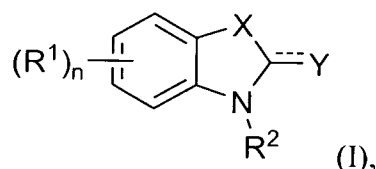
n is an integer from 1 to 4; and

--- represents a single or a double bond;

wherein:

when X is S, Y is =CH-C(O)-C(CH₃)₃, or =CH-C(O)-CH(R⁷)(R⁷); each R⁷ is independently selected from hydrogen, unsubstituted -(C₁-C₄-alkyl), unsubstituted carbocyclyl, and unsubstituted -(C₁-C₄-alkyl)-carbocyclyl; n is 1 or 2; each R¹ is independently selected from halo, -R⁶, -OR⁶; each R⁶ is independently selected from C₁-C₆ straight or branched alkyl, C₂-C₆ straight or branched alkenyl or alkynyl, a carbocyclic moiety, and a heterocyclic moiety consisting of carbon, hydrogen and oxygen atoms; and each R⁶ is optionally substituted with one or more substituents selected from alkyl, O-alkyl and -OH, then R² is not -(C₁-C₅-alkyl), or -(C₁-C₃-alkyl)-aryl.

14. A method for treating or preventing insulin resistance, a metabolic syndrome, diabetes, or complications thereof, or for increasing insulin sensitivity in a subject, comprising administering to a subject in need thereof a therapeutically effective amount of at least one compound of formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

X is selected from S or O;

Y is selected from -NH-C(O)-R⁵, =C(R³)-C(O)-N(R⁴)-R⁵, =C(R³)-C(O)-C(CH₃)₃, =C(R³)-C(O)-CH(R⁷)(R⁷), =C(R³)-C(=NOH)-R⁵, and =C(R³)-CH(OH)-R⁵, and when X is O, Y is additionally selected from =C(R³)-carbocyclic and =C(R³)-heterocyclic;

each R¹ is independently selected from -OH, halo, -R⁶, -OR⁶, -SR⁶, -NH₂, -NH(R⁶), -N(R⁶)(R⁶), -N(R³)C(O)R⁶, -N(R³)C(S)R⁶ and -SO₂N(R⁶)(R⁶);

R² is selected from -(C₁-C₅-alkyl), -(C₁-C₃-alkyl)-aryl, -(C₁-C₃-alkyl)-NH(R⁶), and -(C₁-C₃-alkyl)-N(R⁶)(R⁶);

each R³ is independently selected from hydrogen and optionally substituted -(C₁-C₄-alkyl);

R⁴ is selected from hydrogen and optionally substituted-(C₁-C₄-alkyl);

R⁵ is selected from hydrogen, -(C₁-C₄-alkyl), aryl, -(C₁-C₄-alkyl)-aryl, heteroaryl, and -(C₁-C₄-alkyl)-heteroaryl wherein the alkyl, aryl and heteroaryl is optionally substituted;

each R⁶ is independently selected from C₁-C₆ alkyl, C₂-C₆ alkenyl or alkynyl, carbocyclyl, and heterocyclyl, or wherein two R⁶ together with the nitrogen atom to which they are bound are taken together to form an optionally substituted heterocyclic moiety; and wherein each R⁶ is optionally and independently substituted;

each R⁷ is independently selected from hydrogen, -(C₁-C₄-alkyl), -(C₁-C₄-alkenyl), -(C₁-C₄-alkynyl), carbocyclyl, -(C₁-C₄-alkyl)-carbocyclyl, heterocyclyl, and -(C₁-C₄-alkyl)-heterocyclyl, wherein the alkyl, alkenyl, alkynyl, carbocyclyl and heterocyclyl are optionally substituted

n is an integer from 1 to 4; and

--- represents a single or a double bond.

15. A method of inhibiting the activity of CLK-2 in a cell comprising the step of contacting the cell with a compound of any one of claims 1-10.
16. A method for treating or preventing insulin resistance, a metabolic syndrome, diabetes, or complications thereof, or for increasing insulin sensitivity in a subject, comprising administering to a subject in need thereof a therapeutically effective amount of at least one compound of any one of claims 1-10.