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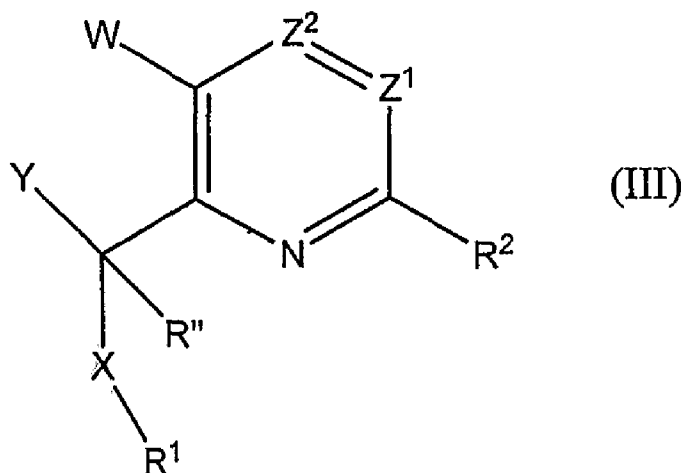
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(54) Title: PYRIDINE, BICYCLIC PYRIDINE AND RELATED ANALOGS AS SIRTUIN MODULATORS



(57) Abstract: The present invention provides novel sirtuin-modulating compounds and methods of use thereof. The sirtuin-modulating compounds may be used for increasing the lifespan of a cell, and treating and/or preventing a wide variety of diseases and disorders relating to aging or stress, diabetes, obesity, neurodegenerative disease, cardiovascular disease, blood clotting disorders, inflammation, cancer, and/or flushing as well as diseases or disorder that would benefit from increased mitochondrial activity. Also provided are compositions comprising a sirtuin-modulating compound in combination with another therapeutic agent. The compounds are of general formula (III) wherein R¹, R², R'', X, Y, W, Z¹ and Z² are as defined in the specification.



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PYRIDINE, BICYCLIC PYRIDINE AND RELATED ANALOGS AS SIRTUIN MODULATORS

REFERENCE TO RELATED APPLICATION

5 This application claims the benefit of U.S. Provisional Application No. 61/197,595, filed October 29, 2008, the disclosure of which is incorporated herein by reference thereto.

BACKGROUND

10 The Silent Information Regulator (SIR) family of genes represents a highly conserved group of genes present in the genomes of organisms ranging from archaeobacteria to eukaryotes. The encoded SIR proteins are involved in diverse processes from regulation of gene silencing to DNA repair. The proteins encoded by members of the SIR gene family show high sequence conservation in a 250
15 amino acid core domain. A well-characterized gene in this family is *S. cerevisiae* SIR2, which is involved in silencing HM loci that contain information specifying yeast mating type, telomere position effects and cell aging. The yeast Sir2 protein belongs to a family of histone deacetylases. The Sir2 homolog, CobB, in *Salmonella typhimurium*, functions as an NAD (nicotinamide adenine dinucleotide)-
20 dependent ADP-ribosyl transferase.

 The Sir2 protein is a class III deacetylase which uses NAD as a cosubstrate. Unlike other deacetylases, many of which are involved in gene silencing, Sir2 is insensitive to class I and II histone deacetylase inhibitors like trichostatin A (TSA).

 Deacetylation of acetyl-lysine by Sir2 is tightly coupled to NAD hydrolysis,
25 producing nicotinamide and a novel acetyl-ADP ribose compound. The NAD-dependent deacetylase activity of Sir2 is essential for its functions which can connect its biological role with cellular metabolism in yeast. Mammalian Sir2 homologs have NAD-dependent histone deacetylase activity.

 Biochemical studies have shown that Sir2 can readily deacetylate the amino-
30 terminal tails of histones H3 and H4, resulting in the formation of 1-O-acetyl-ADP-ribose and nicotinamide. Strains with additional copies of SIR2 display increased rDNA silencing and a 30% longer life span. It has recently been shown that

additional copies of the *C. elegans* SIR2 homolog, sir-2.1, and the *D. melanogaster* dSir2 gene greatly extend life span in those organisms. This implies that the SIR2-dependent regulatory pathway for aging arose early in evolution and has been well conserved. Today, Sir2 genes are believed to have evolved to enhance an

5 organism's health and stress resistance to increase its chance of surviving adversity.

In humans, there are seven Sir2-like genes (SIRT1-SIRT7) that share the conserved catalytic domain of Sir2. SIRT1 is a nuclear protein with the highest degree of sequence similarity to Sir2. SIRT1 regulates multiple cellular targets by deacetylation including the tumor suppressor p53, the cellular signaling factor NF- κ B, and the FOXO transcription factor.

SIRT3 is a homolog of SIRT1 that is conserved in prokaryotes and eukaryotes. The SIRT3 protein is targeted to the mitochondrial cristae by a unique domain located at the N-terminus. SIRT3 has NAD⁺-dependent protein deacetylase activity and is ubiquitously expressed, particularly in metabolically active tissues.

15 Upon transfer to the mitochondria, SIRT3 is believed to be cleaved into a smaller, active form by a mitochondrial matrix processing peptidase (MPP).

Caloric restriction has been known for over 70 years to improve the health and extend the lifespan of mammals. Yeast life span, like that of metazoans, is also extended by interventions that resemble caloric restriction, such as low glucose. The discovery that both yeast and flies lacking the SIR2 gene do not live longer when calorically restricted provides evidence that SIR2 genes mediate the beneficial health effects of a restricted calorie diet. Moreover, mutations that reduce the activity of the yeast glucose-responsive cAMP (adenosine 3',5'-monophosphate)-dependent (PKA) pathway extend life span in wild type cells but not in mutant sir2 strains, demonstrating that SIR2 is likely to be a key downstream component of the caloric restriction pathway.

SUMMARY

Provided herein are novel sirtuin-modulating compounds and methods of use thereof.

In one aspect, the invention provides sirtuin-modulating compounds of Structural Formulas (I) to (VI) as are described in detail below.

In another aspect, the invention provides methods for using sirtuin-modulating compounds, or compositions comprising sirtuin-modulating compounds. In certain embodiments, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used 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, chemotherapeutic induced neuropathy, neuropathy associated with an ischemic event, ocular diseases and/or disorders, cardiovascular disease, blood clotting disorders, inflammation, and/or flushing, etc. Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein 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. In other embodiments, sirtuin-modulating compounds that decrease the level and/or activity of a sirtuin protein may be used for a variety of therapeutic applications including, for example, increasing cellular sensitivity to stress, increasing apoptosis, treatment of cancer, stimulation of appetite, and/or stimulation of weight gain, etc. As described further below, the methods comprise administering to a subject in need thereof a pharmaceutically effective amount of a sirtuin-modulating compound.

In certain aspects, the sirtuin-modulating compounds may be administered alone or in combination with other compounds, including other sirtuin-modulating compounds, or other therapeutic agents.

25 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.

30 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

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.

5 The term “bioavailable” when referring to a compound is art-recognized and 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 into, or otherwise physiologically available to a subject or patient to whom it is administered.

 “Biologically active portion of a sirtuin” refers to a portion of a sirtuin
10 protein having a biological activity, such as the ability to deacetylate. Biologically active portions of a sirtuin may comprise the core domain of sirtuins. Biologically active portions of SIRT1 having GenBank Accession No. NP_036370 that encompass the NAD⁺ binding domain and the substrate binding domain, for example, may include without limitation, amino acids 62-293 of GenBank
15 Accession No. NP_036370, which are encoded by nucleotides 237 to 932 of GenBank Accession No. NM_012238. Therefore, this region is sometimes referred to as the core domain. Other biologically active portions of SIRT1, also sometimes referred to as core domains, include about amino acids 261 to 447 of GenBank
20 Accession No. NP_036370, which are encoded by nucleotides 834 to 1394 of GenBank Accession No. NM_012238; about amino acids 242 to 493 of GenBank Accession No. NP_036370, which are encoded by nucleotides 777 to 1532 of GenBank Accession No. NM_012238; or about amino acids 254 to 495 of GenBank Accession No. NP_036370, which are encoded by nucleotides 813 to 1538 of GenBank Accession No. NM_012238.

25 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,
30 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” refers to the art-recognized measure of effective dose. 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” refers to the art-recognized measure of lethal dose. In certain embodiments, LD₅₀ means
10 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.

15 The term “insulin resistance” refers to a state in which a normal amount of 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, atherosclerotic disease including stroke, coronary artery disease or myocardial infarction, hyperglycemia, hyperinsulinemia and/or hyperproinsulinemia, impaired glucose tolerance, delayed insulin release,
25 diabetic complications, including coronary heart disease, angina 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
30 health (such as menstrual irregularities, 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 bone loss, e.g. osteoporosis in particular.

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 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) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

The term "preventing" is art-recognized, and when used in relation to a condition, such as a local recurrence (e.g., pain), a disease such as cancer, a syndrome complex such as heart failure or any other medical condition, is well understood in the art, and includes administration of a composition which reduces the frequency of, or delays the onset of, symptoms of a medical condition in a subject relative to a subject which does not receive the composition. Thus, prevention of cancer includes, for example, reducing the number of detectable cancerous growths in a population of patients receiving a prophylactic treatment relative to an untreated control population, and/or delaying the appearance of detectable cancerous growths in a treated population versus an untreated control population, e.g., by a statistically and/or clinically significant amount. Prevention of an infection includes, for example, reducing the number of diagnoses of the infection in a treated population versus an untreated control population, and/or delaying the onset of symptoms of the infection in a treated population versus an untreated control population. Prevention of pain includes, for example, reducing the magnitude of, or alternatively delaying, pain sensations experienced by subjects in a treated population versus an untreated control population.

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

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).

5 The term “pyrogen-free”, with reference to a composition, refers to a 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
10 substantially free of, an endotoxin such as, for example, a lipopolysaccharide (LPS).

 “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
15 a cell” or “extending the lifespan of a cell,” as applied to cells or organisms, refers to 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
20 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 10%, 20%, 30%, 40%, 50%, 60% or between 20% and 70%, 30% and 60%, 40% and 60% or more using methods described herein.

25 “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 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
30 deacetylation, e.g., of histones and p53; extending lifespan; increasing genomic

stability; silencing transcription; and controlling the segregation of oxidized proteins between mother and daughter cells.

“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), 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, 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.

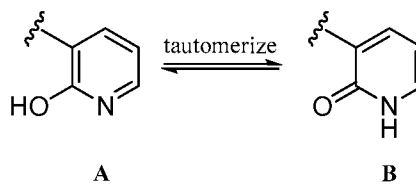
“SIRT1 protein” refers to a member of the sir2 family of sirtuin deacetylases. In one embodiment, a SIRT1 protein includes yeast Sir2 (GenBank Accession No. P53685), *C. elegans* Sir-2.1 (GenBank Accession No. NP_501912), human SIRT1 (GenBank Accession No. NM_012238 or NP_036370 (or AF083106)), and equivalents and fragments thereof. In another embodiment, a SIRT1 protein includes a polypeptide comprising a sequence 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 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. 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.

As used herein “SIRT2 protein”, “SIRT3 protein”, “SIRT4 protein”, SIRT 5 protein”, “SIRT6 protein”, and “SIRT7 protein” refer to other mammalian, e.g. human, sirtuin deacetylase proteins that are homologous to SIRT1 protein, particularly in the approximately 275 amino acid conserved catalytic domain. For example, “SIRT3 protein” refers to a member of the sirtuin deacetylase protein family that is homologous to SIRT1 protein. In one embodiment, a SIRT3 protein includes human SIRT3 (GenBank Accession No. AAH01042, NP_036371, or NP_001017524) and mouse SIRT3 (GenBank Accession No. NP_071878) proteins, and equivalents and fragments thereof. In another embodiment, a SIRT3 protein includes a polypeptide comprising a sequence consisting of, or consisting essentially of, the amino acid sequence set forth in GenBank Accession Nos. AAH01042, NP_036371, NP_001017524, or NP_071878. SIRT3 proteins include polypeptides comprising all or a portion of the amino acid sequence set forth in GenBank Accession AAH01042, NP_036371, NP_001017524, or NP_071878; the amino acid sequence set forth in GenBank Accession Nos. AAH01042, NP_036371, NP_001017524, or NP_071878 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. AAH01042, NP_036371, NP_001017524, or NP_071878, and functional fragments thereof. Polypeptides of the invention also include homologs (e.g., orthologs and paralogs), variants, or fragments, of GenBank Accession Nos. AAH01042, NP_036371, NP_001017524, or NP_071878. In one embodiment, a SIRT3 protein includes a fragment of SIRT3 protein that is produced by cleavage with a mitochondrial matrix processing peptidase (MPP) and/or a mitochondrial intermediate peptidase (MIP).

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.

The term "tautomer" as used herein is art-recognized and refers to the formal migration of a hydrogen atom, i.e., proton, accompanied by a switch of a single bond and adjacent double bond. When used herein to describe a compound or genus of compounds, tautomer includes any portion of a compound or the entire compound such as a single substituent of a compound, multiple substituents of a compound or, for example, the entire compound. For example, the tautomer of a compound that includes a hydroxyl-substituted pyridine ring (A) is a compound that includes the keto-enol substituted ring (B):



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 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 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 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.

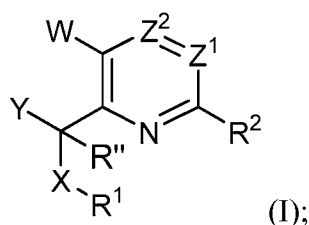
“Treating” a condition or disease refers to curing as well as ameliorating at least one symptom of the condition or disease.

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
 5 corrective lenses, or a visual field of less than 20 degrees diameter (10 degrees radius).

2. Sirtuin Modulators

In one aspect, the invention provides novel sirtuin-modulating compounds for treating and/or preventing a wide variety of diseases and disorders including, for
 10 example, diseases or disorders related to aging or stress, diabetes, obesity, neurodegenerative diseases, ocular diseases and disorders, cardiovascular disease, blood clotting disorders, inflammation, cancer, and/or flushing, etc. Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may also be used for treating a disease or disorder in a subject that would benefit
 15 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.

20 In another embodiment, sirtuin-modulating compounds of the invention are represented by Structural Formula (I):



or a salt thereof, wherein:

- 25 each of Z¹ and Z² is independently selected from N and CR, wherein:
 at least one of Z¹ and Z² is CR; and
 each R is independently selected from hydrogen, halo, -OH, -C≡N, fluoro-substituted C₁-C₂ alkyl, -O-(C₁-C₂) fluoro-substituted alkyl,

-S-(C₁-C₂) fluoro-substituted alkyl, C₁-C₄ alkyl, -O-(C₁-C₄) alkyl, -S-(C₁-C₄) alkyl and C₃-C₇ cycloalkyl;

R" is selected from hydrogen and C₁-C₄ alkyl optionally substituted with one or more substituents independently selected from halo, -C≡N, C₁-C₄ alkyl, =O,
 5 C₃-C₇ cycloalkyl, fluoro-substituted C₁-C₂ alkyl, -O-R³, -S-R³, -(C₁-C₄ alkyl)-N(R³)(R³), -N(R³)(R³), -O-(C₁-C₄ alkyl)-N(R³)(R³), -(C₁-C₄ alkyl)-O-(C₁-C₄ alkyl)-N(R³)(R³), -C(O)-N(R³)(R³), and -(C₁-C₄ alkyl)-C(O)-N(R³)(R³);

R¹ is selected from a carbocycle and a heterocycle, wherein R¹ is optionally substituted with one to two substituents independently selected from halo, -C≡N,
 10 C₁-C₄ alkyl, =O, C₃-C₇ cycloalkyl, fluoro-substituted C₁-C₂ alkyl, -O-R³, -S-R³, -(C₁-C₄ alkyl)-N(R³)(R³), -N(R³)(R³), -O-(C₁-C₄ alkyl)-N(R³)(R³), -(C₁-C₄ alkyl)-O-(C₁-C₄ alkyl)-N(R³)(R³), -C(O)-N(R³)(R³), and -(C₁-C₄ alkyl)-C(O)-N(R³)(R³), and when R¹ is phenyl, R¹ is also optionally substituted with O-(saturated heterocycle), fluoro-substituted-O-(saturated heterocycle), C₁-C₄
 15 alkyl-substituted-O-(saturated heterocycle), 3,4-methylenedioxy, fluoro-substituted 3,4-methylenedioxy, 3,4-ethylenedioxy, or fluoro-substituted 3,4-ethylenedioxy, wherein

each R³ is independently selected from hydrogen, and -C₁-C₄ alkyl; or two R³ are taken together with the nitrogen atom to which they are bound to
 20 form a 4- to 8-membered saturated heterocycle optionally comprising one additional heteroatom selected from N, S, S(=O), S(=O)₂, and O, wherein the alkyl is optionally substituted with one or more -OH, fluoro, -NH₂, -NH(C₁-C₄ alkyl), -N(C₁-C₄ alkyl)₂, -NH(CH₂CH₂OCH₃), or -N(CH₂CH₂OCH₃)₂ and the saturated heterocycle is optionally substituted at
 25 a carbon atom with -OH, -C₁-C₄ alkyl, fluoro, -NH₂, -NH(C₁-C₄ alkyl), -N(C₁-C₄ alkyl)₂, -NH(CH₂CH₂OCH₃), or -N(CH₂CH₂OCH₃)₂;

R² is selected from a carbocycle and a heterocycle, wherein R² is optionally substituted with one to two substituents independently selected from halo, -C≡N, C₁-C₄ alkyl, C₃-C₇ cycloalkyl, C₁-C₂ fluoro-substituted alkyl, -O-R³, -S-R³, -(C₁-C₄ alkyl)-N(R³)(R³), -N(R³)(R³), -O-(C₁-C₄ alkyl)-N(R³)(R³), -(C₁-C₄ alkyl)-O-(C₁-C₄ alkyl)-N(R³)(R³), -C(O)-N(R³)(R³), -(C₁-C₄ alkyl)-C(O)-N(R³)(R³), -O-phenyl,
 30 phenyl, and a second heterocycle, and when R² is phenyl, R² is also optionally

substituted with -O-(saturated heterocycle), 3,4-methylenedioxy, fluoro-substituted 3,4-methylenedioxy, 3,4-ethylenedioxy, or fluoro-substituted 3,4-ethylenedioxy, wherein any phenyl, saturated heterocycle, or second heterocycle substituent of R² is optionally substituted with halo; -C≡N, C₁-C₄ alkyl, fluoro-substituted C₁-C₂ alkyl, 5 -O-(C₁-C₂) fluoro-substituted alkyl, -O-(C₁-C₄) alkyl, -S-(C₁-C₄) alkyl, -S-(C₁-C₂) fluoro-substituted alkyl, -NH-(C₁-C₄) alkyl, and -N-(C₁-C₄)₂ alkyl;

X is selected from -NH-C(=O)-†, -C(=O)-NH-†, -NH-C(=S)-†, -C(=S)-NH-†, -NH-S(=O)-†, -S(=O)-NH-†, -S(=O)₂-NH-†, -NH-S(=O)₂-†, -NH-S(O)₂-NR⁴-†, -NR⁴-S(O)₂-NH-†, -NH-C(=O)O-†, -OC(=O)NH-†, 10 -NH-C(=O)NR⁴-†, -NR⁴-C(=O)NH-†, -NH-NR⁴-†, -NR⁴-NH-†, -O-NH-†, -NH-O-†, -NH-CR⁴R⁵-†, -CR⁴R⁵-NH-†, -NH-C(=NR⁴)-†, -C(=NR⁴)-NH-†, -C(=O)-NH-CR⁴R⁵-†, -NH-C(=O)-CR⁴R⁵-†, -CR⁴R⁵-NH-C(O)-†, -NH-C(=S)-CR⁴R⁵-†, -CR⁴R⁵-C(=S)-NH-†, -NH-S(O)-CR⁴R⁵-†, -CR⁴R⁵-S(O)-NH-†, -NH-S(O)₂-CR⁴R⁵-†, -CR⁴R⁵-S(O)₂-NH-†, 15 -NH-C(=O)-O-CR⁴R⁵-†, -CR⁴R⁵-O-C(=O)-NH-†, -NH-C(=O)-NR⁴-CR⁴R⁵-†, and -CR⁴R⁵-NH-C(=O)-O-†, wherein:

† represents where X is bound to R¹; and

each R⁴ and R⁵ is independently selected from hydrogen, C₁-C₄ alkyl, -CF₃ and (C₁-C₃ alkyl)-CF₃; and

20 W is R⁶; and

Y is selected from C₁-C₄ alkyl and fluoro-substituted C₁-C₄ alkyl;

or

W and Y are bound to one another to form a 5- or 6-membered saturated ring, wherein:

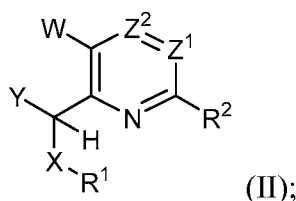
25 W is selected from -O-, -S-, -S(O)-, -S(O)₂ and -C(R⁶)(R⁶)-, and

Y is selected from -C(R⁶)(R⁶)- and -C(R⁶)(R⁶)-C(R⁶)(R⁶)-, and

each R⁶ is independently selected from hydrogen, C₁-C₄ alkyl and fluoro-substituted C₁-C₄ alkyl.

In a particular embodiment, the compound is not: 2-pyridinemethanol, α-(1,1-dimethylethyl)-6-phenyl, phenylcarbamate ester. 30

In certain embodiments, sirtuin-modulating compounds of the invention are represented by Structural Formula (II):



or a salt thereof, wherein variables W, Z¹, Z², Y, X, R¹ and R² are as defined for Structural Formula (I). In certain such embodiments, the compound is not: 2-pyridinemethanol, α -(1,1-dimethylethyl)-6-phenyl, phenylcarbamate ester.

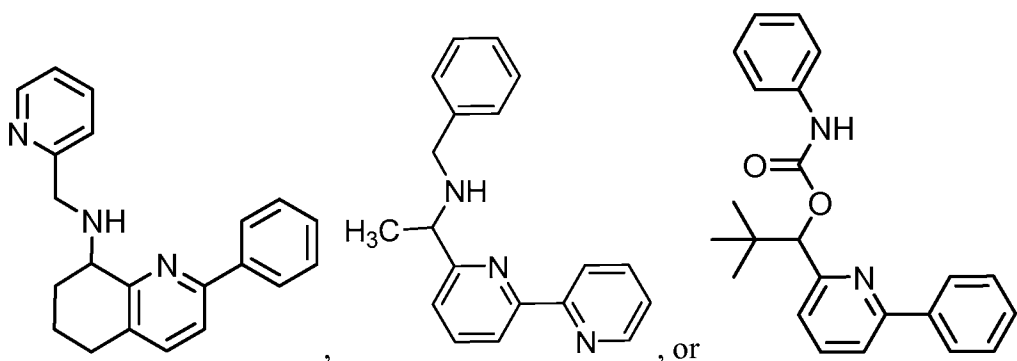
The embodiments described below apply to both Structural Formula (I) and Structural Formula (II).

In certain embodiments, compounds represented by Structural Formula (I) or (II) may be characterized by one or more of the following features:

when each of Z¹ and Z² is -CH-, W is hydrogen, Y is C₁-C₄ alkyl, X is -NH-CR⁴R⁵- and R² is unsubstituted phenyl, R¹ is other than unsubstituted phenyl, or unsubstituted pyridin-2-yl;

when each of Z¹ and Z² is -CH-, W is hydrogen, Y is C₁-C₄ alkyl, X is -NH-S(O)- and R is 4-methylphenyl, then R² is not unsubstituted phenyl or unsubstituted morpholin-4-yl; and

the compound of Structural Formula (I) or (II) is not:

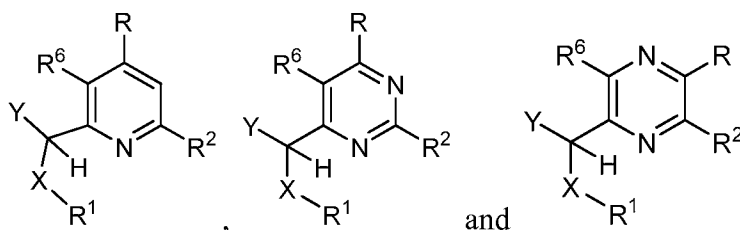


In certain embodiments, W is R⁶. In certain such embodiments, R⁶ is selected from hydrogen and C₁-C₄ alkyl. In certain embodiments, R⁶ is hydrogen.

In certain embodiments, Y is selected from C₁-C₄ alkyl and fluoro-substituted C₁-C₄ alkyl. In certain embodiments, Y is C₁-C₄ alkyl, such as methyl, ethyl, propyl, butyl, *iso*-propyl and *tert*-butyl. In certain embodiments, Y is fluoro-substituted C₁-C₄ alkyl such as -CF₂CHF₂, -CH₂CHF₂CF₃, and -CF(CH₃)₂, up

to perfluoro substituted alkyl. In certain embodiments, wherein Z^1 and Z^2 are each CR, R in both occurrences is hydrogen, X is $-\text{NH}-\text{C}(=\text{O})-\dagger$ or $-\text{C}(=\text{O})-\text{NH}-\dagger$, W is R^6 and R^6 is hydrogen, and Y is $\text{C}_1\text{-C}_4$ alkyl (e.g., methyl).

In certain embodiments, the compound of Structural Formula (II) may be a
5 formula selected from:

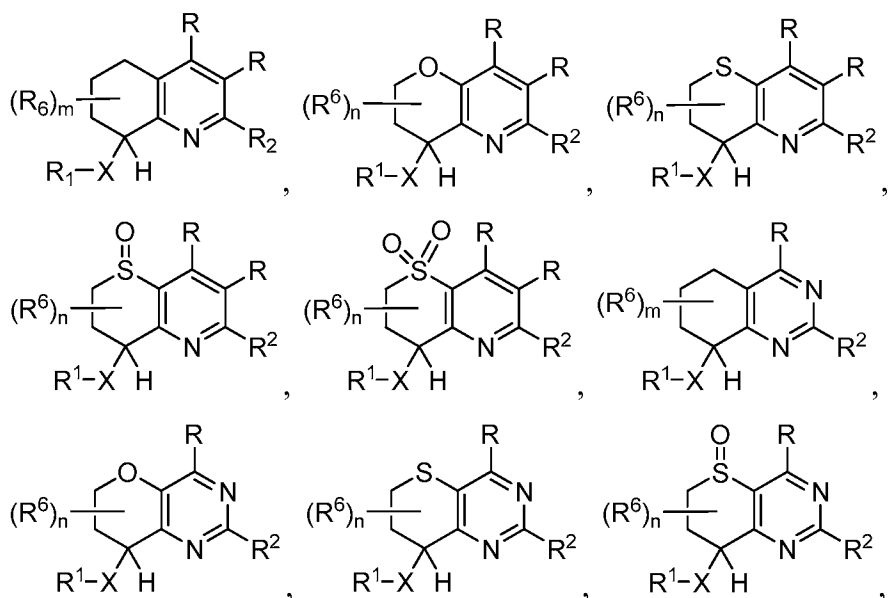


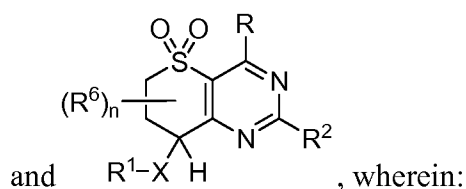


 and
 
 . In certain embodiments, wherein the compound is represented by one of the preceding monocyclic structures, R is selected from hydrogen, halo and hydroxyl. In particular embodiments, R is hydrogen. In certain exemplary embodiments, wherein Structural Formula (II) is selected from the preceding structures, R is hydrogen, X is selected from –

10 NH-C(=O)-† and -C(=O)-NH-†, R⁶ is hydrogen and Y is selected from C₁-C₄ alkyl.

In certain embodiments, W and Y are bound to one another to form a 6-membered saturated ring, wherein W is selected from -O-, -S-, -S(O)₂- and -C(R⁶)(R⁶)- and Y is -C(R⁶)(R⁶)-C(R⁶)(R⁶)-. In certain embodiments, the compound of Structural Formula (II) is of a formula selected from:



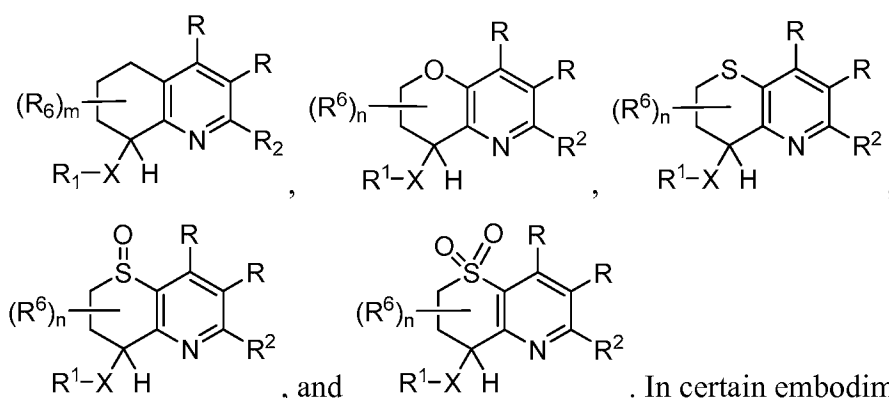


n is 4;

m is 6; and

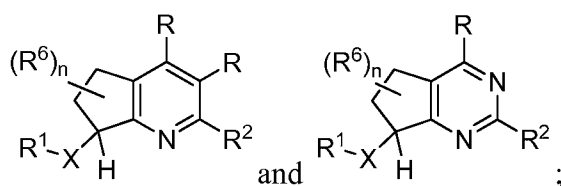
each R⁶ is independently selected from hydrogen, C₁-C₄ alkyl and

- 5 fluoro-substituted C₁-C₄ alkyl. In certain such embodiments, the compound of Structural Formula (II) is of a formula selected from:



- 10 Structural Formula (II) is selected from any of the preceding 6-6 bicyclic structures, i.e., two fused 6-membered rings, R is selected at each occurrence from hydrogen, halo and hydroxyl. In certain embodiments, R is hydrogen at each occurrence. In certain exemplary embodiments, wherein Structural Formula (II) is selected from the preceding bicyclic structures, R is hydrogen at each occurrence, X is selected from -NH-C(=O)-† and -C(=O)-NH-† and R⁶ is hydrogen at each occurrence.

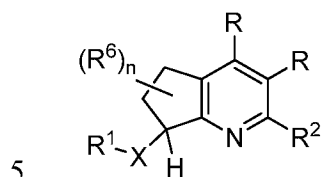
- 15 In certain embodiments, W and Y are bound to one another to form a 5-membered saturated ring, W is -C(R⁶)(R⁶)- and Y is -C(R⁶)(R⁶)-. In certain embodiments, the compound of Structural Formula (II) is of a formula selected from:



- 20 wherein:

n is 4; and

each R^6 is independently selected from hydrogen, C_1 - C_4 alkyl and fluoro-substituted C_1 - C_4 alkyl. In certain embodiments, the compound of Structural Formula (II) is of the formula:



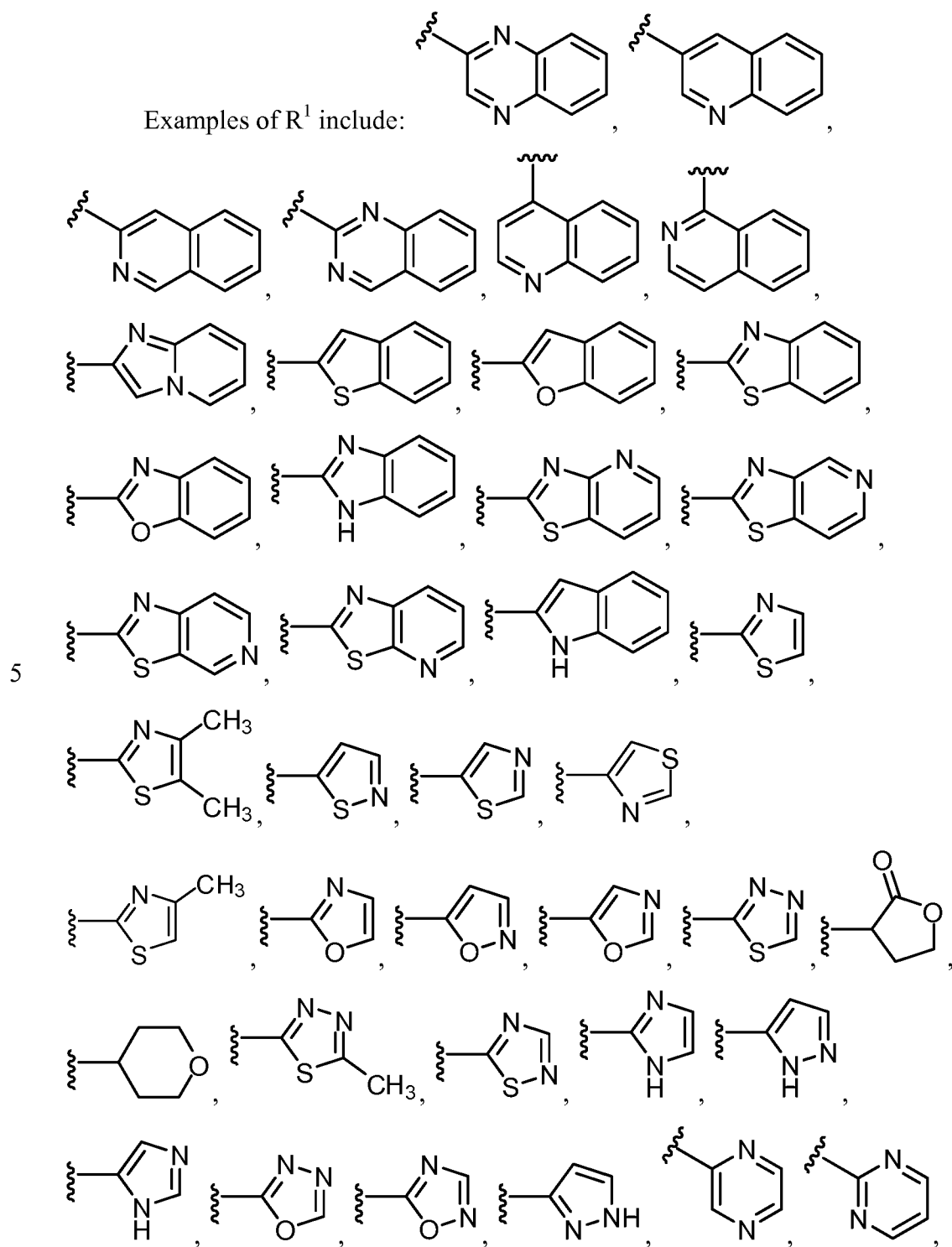
. In certain embodiments, wherein Structural Formula (II) is selected from the preceeding 5-6 bicyclic structures, i.e., two fused rings, one 5-membered the other 6-membered, R is selected at each occurrence from hydrogen, halo and hydroxyl. In certain embodiments, R is hydrogen at each occurrence. In exemplary embodiments, wherein Structural Formula (II) is selected from the preceeding 5-6 bicyclic structures, R is hydrogen at each occurrence, X is selected from $-NH-C(=O)-\dagger$ and $-C(=O)-NH-\dagger$ and R^6 is hydrogen at each occurrence.

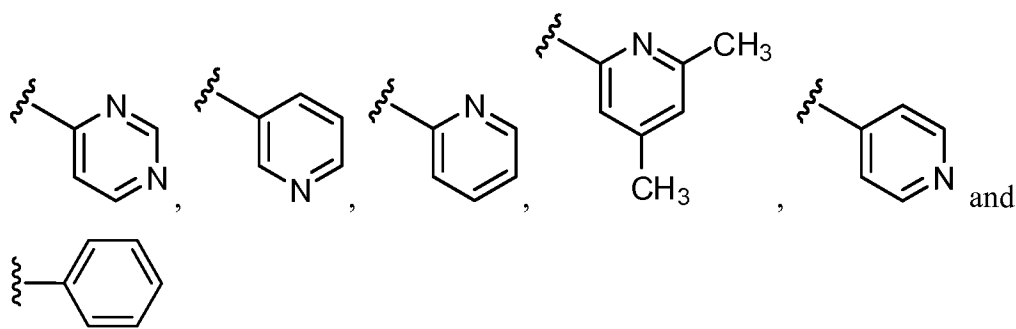
In certain embodiments, such as any of those described above, each of Z^1 and Z^2 are CR. In such embodiments, R may at each occurrence be selected from hydrogen, halo, and -OH. In particular embodiments, wherein Z^1 and Z^2 are both CR, R in both occurrences is hydrogen.

In certain embodiments, such as any of those described above, X is $-NH-C(=O)-\dagger$. In other embodiments, such as any of those described above, X is $-C(=O)-NH-\dagger$. In an exemplary embodiment, X is $-NH-C(=O)-\dagger$, each of Z^1 and Z^2 are CR and R in both occurrences is hydrogen. In another exemplary embodiment, X is $-C(=O)-NH-\dagger$, each of Z^1 and Z^2 are CR and R in both occurrences is hydrogen.

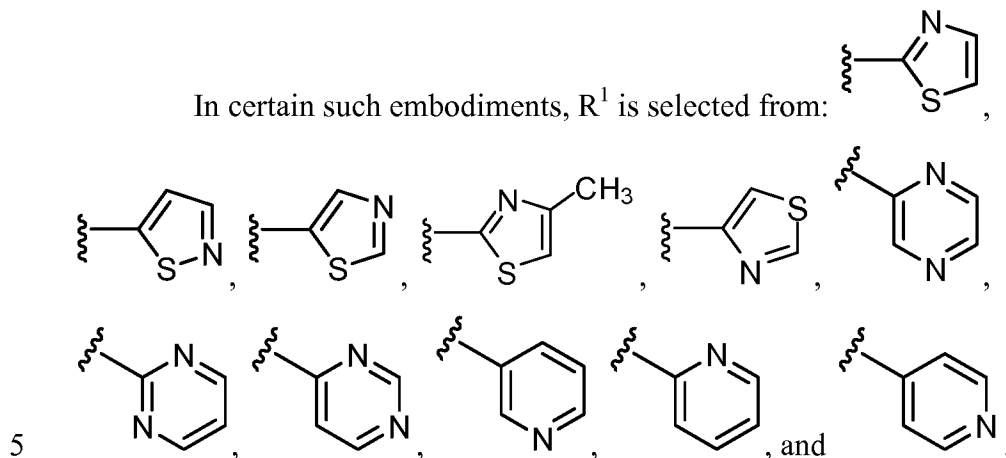
In certain embodiments, R^1 is selected from heterocycles (e.g., heteroaryls) comprising one or more heteroatoms selected from N, O and S. In particular embodiments, R^1 is selected from heterocycles (e.g., heteroaryls) comprising one or two nitrogens. In particular embodiments, R^1 is selected from heterocycles (e.g., heteroaryls) comprising up to three heteroatoms selected from S and N. In other embodiments, R^1 is selected from heterocycles (e.g., heteroaryls) comprising up to three heteroatoms selected from O and N.

Examples of $\mathbb{R}^1$ include:





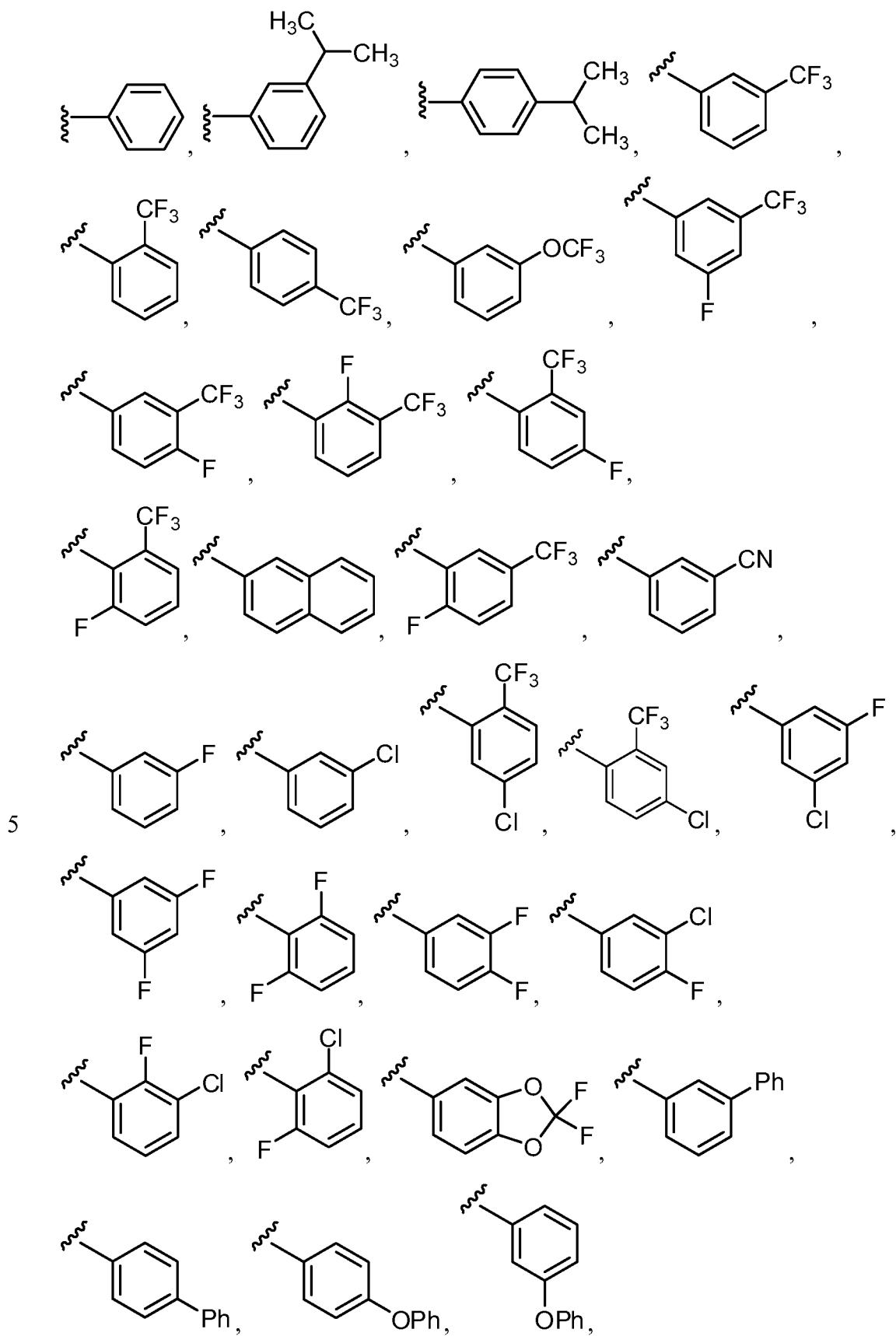
In certain such embodiments, R^1 is selected from:

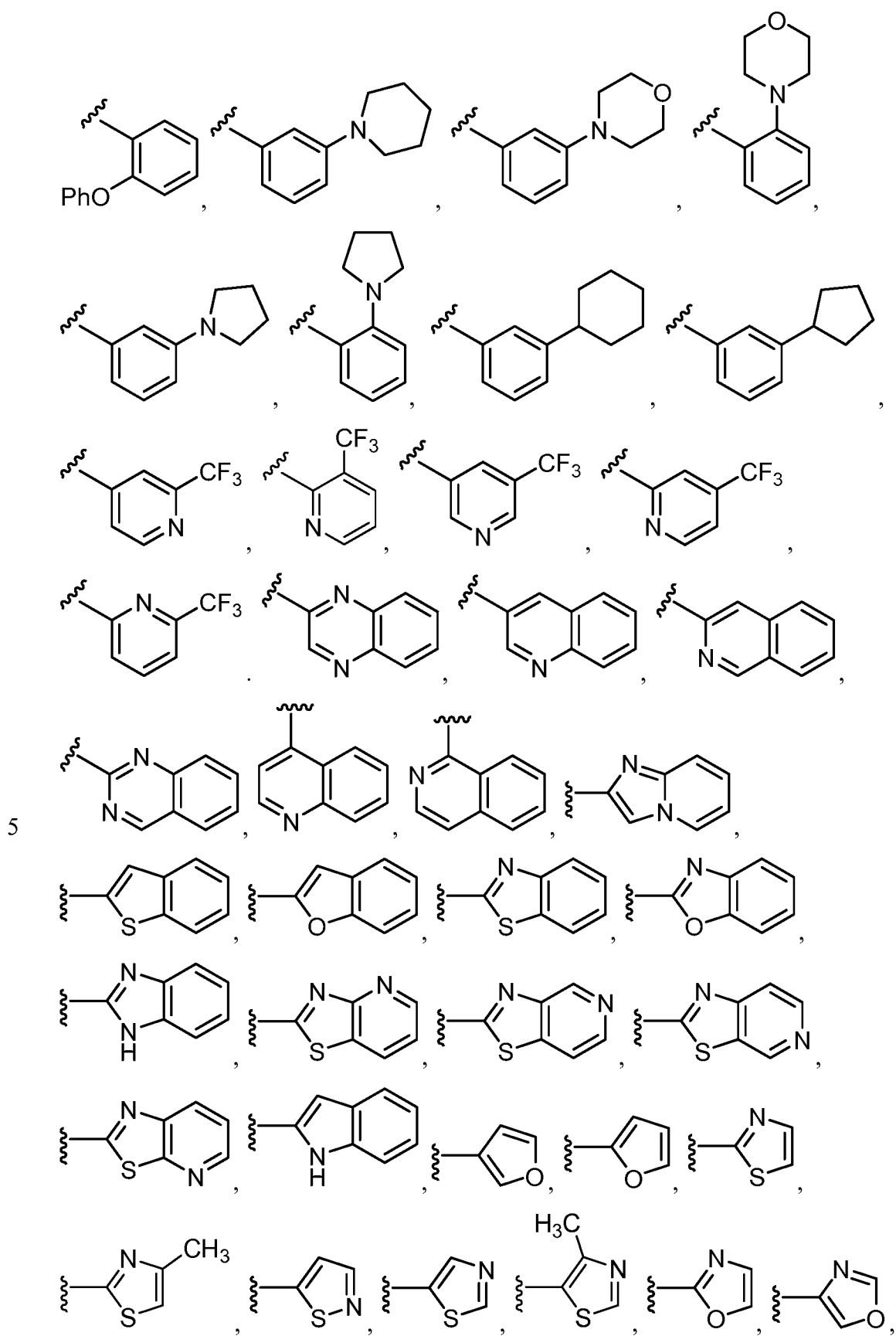


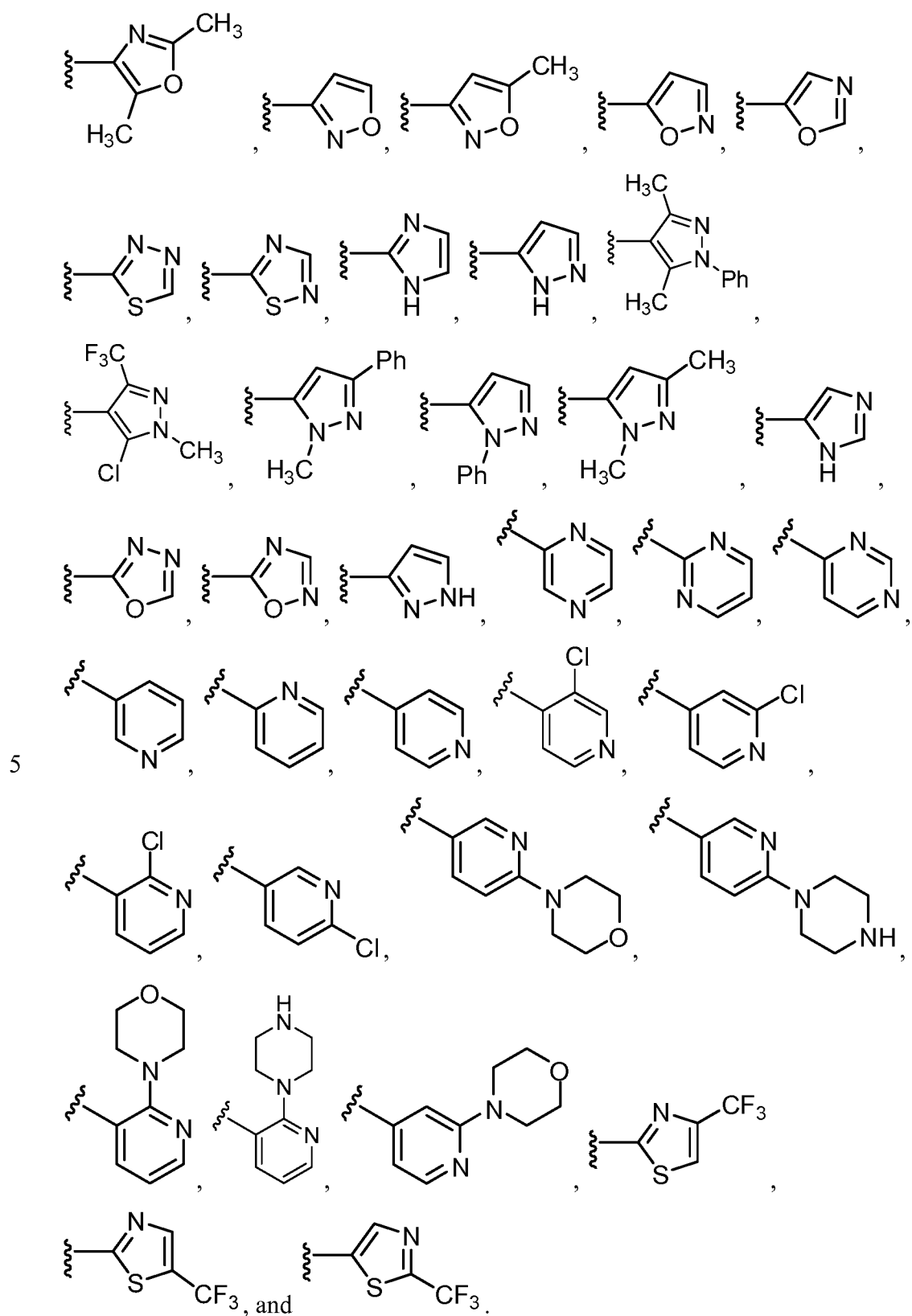
In the embodiments above, R^1 is optionally substituted by 1 or 2 substituents independently selected from halo, (C_1-C_4) alkyl and $=O$. In certain embodiments, R^1 is thiazole or pyrazine optionally substituted with one or more substituents selected from halo and (C_1-C_4) alkyl. In certain embodiments, R^1 is optionally substituted thiazole and X is $-NH-C(=O)-\dagger$. In other embodiments, R^1 is optionally substituted pyrazine and X is $-NH-C(=O)-\dagger$.

In certain embodiments, R^2 is selected from aryl and heteroaryl. In certain embodiments, R^2 is optionally substituted with one to two substituents independently selected from halo, $-C\equiv N$, C_1-C_4 alkyl, C_1-C_2 fluoro-substituted alkyl, $-OR^3$ wherein R^3 is alkyl optionally substituted with one or more halo substituents. In certain embodiments, R^2 is phenyl optionally substituted by one or more substituents independently selected from $-Cl$, $-Br$, $-F$, $-C\equiv N$, $-CF_3$ and $-OCF_3$.

Examples of R^2 include:

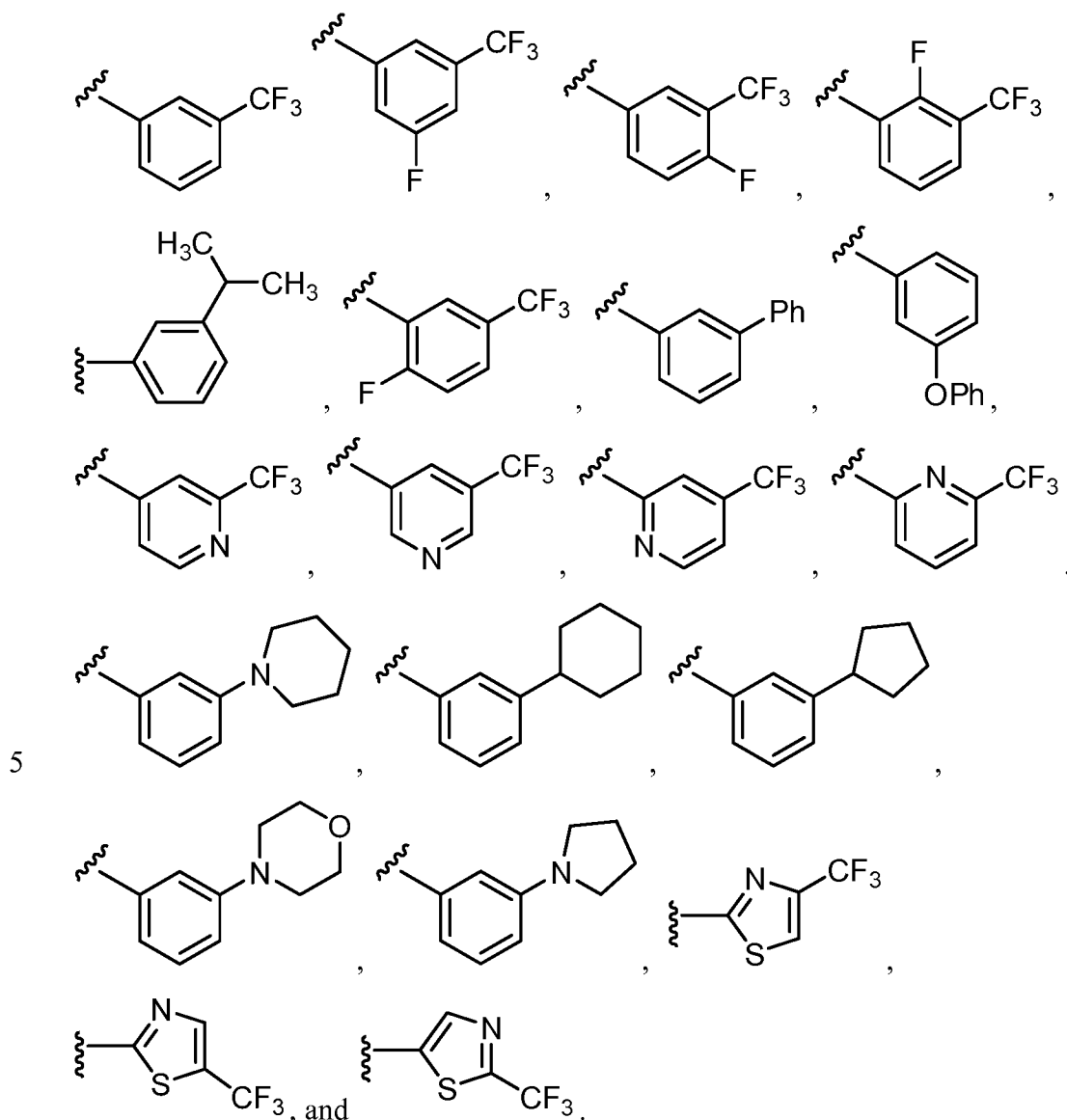






In particular embodiments, R^2 is meta-substituted relative to the attachment of R^2 to the rest of the compound, and wherein R^2 is optionally further substituted as

described above. In certain embodiments, R^2 is selected from:



In certain embodiments, R^1 is thiazole or pyrazine optionally substituted with one or more substituents selected from halo and (C_1-C_4) alkyl and R^2 is phenyl optionally substituted by one or more substituents independently selected from $-Cl$, $-Br$, $-F$, $-C\equiv N$, $-CF_3$ and $-OCF_3$. In certain embodiments, R^1 is thiazole or pyrazine, R^2 is phenyl, X is selected from $-NH-C(=O)-\dagger$ and $-C(=O)-NH-\dagger$, Z^1 and Z^2 are CR , Y is selected from C_1-C_4 alkyl, W is R^6 and R^6 is hydrogen. In certain embodiments, R^1 is thiazole or pyrazine, R^2 is phenyl, X is selected from $-NH-C(=O)-\dagger$ and $-C(=O)-NH-\dagger$, Z^1 and Z^2 are CR , and W and Y are bound to one another to form a 5 or 6-membered ring. In certain embodiments, W and Y are

bound to one another to form a 6-membered ring, wherein W is $-C(R^6)(R^6)-$ and Y is $-C(R^6)(R^6)-C(R^6)(R^6)-$ and R^6 in each occurrence is hydrogen.

In certain embodiments, sirtuin-modulating compounds of the invention are represented by Structural Formula (II), or a salt thereof, wherein:

5 W is R^6 ; and

Y is selected from C_1-C_3 alkyl and fluoro-substituted C_1-C_4 alkyl;

or

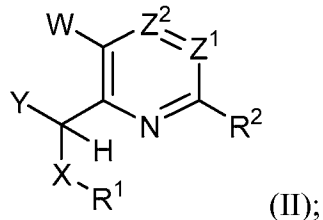
W and Y are bound to one another to form a 5- or 6-membered saturated ring, wherein:

10 W is selected from O, S, $-S(O)-$, $-S(O)_2$ and $-C(R^6)(R^6)-$, and

Y is selected from $-C(R^6)(R^6)-$ and $-C(R^6)(R^6)-C(R^6)(R^6)-$, and

each R^6 is independently selected from hydrogen, C_1-C_4 alkyl and fluoro-substituted C_1-C_4 alkyl.

In certain embodiments, the sirtuin modulating pharmaceutical compositions of the invention comprise a pharmaceutically acceptable carrier or diluent and a compound represented by Structural Formula (II):



or a salt thereof, wherein:

each of Z^1 and Z^2 is independently selected from N and CR, wherein:

20 at least one of Z^1 and Z^2 is CR; and

each R is independently selected from hydrogen, halo, $-OH$, $-C\equiv N$, fluoro-substituted C_1-C_2 alkyl, $-O-(C_1-C_2)$ fluoro-substituted alkyl, $-S-(C_1-C_2)$ fluoro-substituted alkyl, C_1-C_4 alkyl, $-O-(C_1-C_4)$ alkyl, $-S-(C_1-C_4)$ alkyl and C_3-C_7 cycloalkyl;

25 R^1 is selected from a carbocycle and a heterocycle, wherein R^1 is optionally substituted with one to two substituents independently selected from halo, $-C\equiv N$, C_1-C_4 alkyl, $=O$, C_3-C_7 cycloalkyl, fluoro-substituted C_1-C_2 alkyl, $-O-R^3$, $-S-R^3$, $-(C_1-C_4 \text{ alkyl})-N(R^3)(R^3)$, $-N(R^3)(R^3)$, $-O-(C_1-C_4 \text{ alkyl})-N(R^3)(R^3)$, $-(C_1-C_4$

alkyl)-O-(C₁-C₄ alkyl)-N(R³)(R³), -C(O)-N(R³)(R³), and -(C₁-C₄
 alkyl)-C(O)-N(R³)(R³), and when R¹ is phenyl, R¹ is also optionally substituted with
 -O-(saturated heterocycle), fluoro-substituted -O-(saturated heterocycle), C₁-C₄
 alkyl-substituted O-(saturated heterocycle), 3,4-methylenedioxy, fluoro-substituted
 5 3,4-methylenedioxy, 3,4-ethylenedioxy, or fluoro-substituted 3,4-ethylenedioxy,
 wherein

each R³ is independently selected from hydrogen, and -C₁-C₄ alkyl; or
 two R³ are taken together with the nitrogen atom to which they are bound to
 form a 4- to 8-membered saturated heterocycle optionally comprising one
 10 additional heteroatom selected from N, S, S(=O), S(=O)₂, and O, wherein the
 alkyl is optionally substituted with one or more -OH, fluoro, -NH₂,
 -NH(C₁-C₄ alkyl), -N(C₁-C₄ alkyl)₂, -NH(CH₂CH₂OCH₃), or
 -N(CH₂CH₂OCH₃)₂ and the saturated heterocycle is optionally substituted at
 a carbon atom with -OH, -C₁-C₄ alkyl, fluoro, -NH₂, -NH(C₁-C₄ alkyl),
 15 -N(C₁-C₄ alkyl)₂, -NH(CH₂CH₂OCH₃), or -N(CH₂CH₂OCH₃)₂;

R² is selected from a carbocycle and a heterocycle, wherein R² is optionally
 substituted with one to two substituents independently selected from halo, -C≡N,
 C₁-C₄ alkyl, C₃-C₇ cycloalkyl, C₁-C₂ fluoro-substituted alkyl, -O-R³, -S-R³, -(C₁-C₄
 alkyl)-N(R³)(R³), -N(R³)(R³), -O-(C₁-C₄ alkyl)-N(R³)(R³), -(C₁-C₄ alkyl)-O-(C₁-C₄
 20 alkyl)-N(R³)(R³), -C(O)-N(R³)(R³), -(C₁-C₄ alkyl)-C(O)-N(R³)(R³), -O-phenyl,
 phenyl, and a second heterocycle, and when R² is phenyl, R² is also optionally
 substituted with -O-(saturated heterocycle), 3,4-methylenedioxy, fluoro-substituted
 3,4-methylenedioxy, 3,4-ethylenedioxy, or fluoro-substituted 3,4-ethylenedioxy,
 wherein any phenyl, saturated heterocycle, or second heterocycle substituent of R²
 25 is optionally substituted with halo; -C≡N, C₁-C₄ alkyl, fluoro-substituted C₁-C₂
 alkyl, -O-(C₁-C₂) fluoro-substituted alkyl, -O-(C₁-C₄) alkyl, -S-(C₁-C₄) alkyl,
 -S-(C₁-C₂) fluoro-substituted alkyl, -NH-(C₁-C₄) alkyl, and -N-(C₁-C₄)₂ alkyl;

X is selected from -NH-C(=O)-†, -C(=O)-NH-†, -NH-C(=S)-†,
 -C(=S)-NH-†, -NH-S(=O)-†, -S(=O)-NH-†, -S(=O)₂-NH-†, -NH-S(=O)₂-†,
 30 -NH-S(O)₂-NR⁴-†, -NR⁴-S(O)₂-NH-†, -NH-C(=O)O-†, -OC(=O)NH-†,
 -NH-C(=O)NR⁴-†, -NR⁴-C(=O)NH-†, -NH-NR⁴-†, -NR⁴-NH-†, -O-NH-†, -NH-O-†,
 -NH-CR⁴R⁵-†, -CR⁴R⁵-NH-†, -NH-C(=NR⁴)-†, -C(=NR⁴)-NH-†,

- C(=O)-NH-CR⁴R⁵-†, -NH-C(=O)-CR⁴R⁵-†, -CR⁴R⁵-NH-C(O)-†,
 -NH-C(=S)-CR⁴R⁵-†, -CR⁴R⁵-C(=S)-NH-†, -NH-S(O)-CR⁴R⁵-†,
 -CR⁴R⁵-S(O)-NH-†, -NH-S(O)₂-CR⁴R⁵-†, -CR⁴R⁵-S(O)₂-NH-†,
 -NH-C(=O)-O-CR⁴R⁵-†, -CR⁴R⁵-O-C(=O)-NH-†, -NH-C(=O)-NR⁴-CR⁴R⁵-†, and
 5 -CR⁴R⁵-NH-C(=O)-O-†, wherein:

† represents where X is bound to R¹; and

each R⁴ and R⁵ is independently selected from hydrogen, C₁-C₄ alkyl,
 -CF₃ and (C₁-C₃ alkyl)-CF₃; and

W is R⁶; and

- 10 Y is selected from C₁-C₄ alkyl and fluoro-substituted C₁-C₄ alkyl;

or

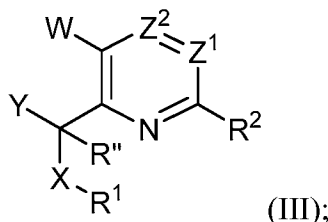
W and Y are bound to one another to form a 5- or 6-membered saturated
 ring, wherein:

W is selected from -O-, -S-, -S(O)-, -S(O)₂ and -C(R⁶)(R⁶)-, and

- 15 Y is selected from -C(R⁶)(R⁶)- and -C(R⁶)(R⁶)-C(R⁶)(R⁶)-, and

each R⁶ is independently selected from hydrogen, C₁-C₄ alkyl and fluoro-substituted
 C₁-C₄ alkyl.

In one embodiment, sirtuin-modulating compounds of the invention are
 represented by Structural Formula (III):



20

(III);

a tautomer or a salt thereof, wherein:

each of Z¹ and Z² is independently selected from N and CR, wherein:

at least one of Z¹ and Z² is CR; and

each R is independently selected from hydrogen, halo, -OH, -C≡N,

- 25 fluoro-substituted C₁-C₂ alkyl, -O-(C₁-C₂) fluoro-substituted alkyl,
 -S-(C₁-C₂) fluoro-substituted alkyl, C₁-C₄ alkyl, -O-(C₁-C₄) alkyl, -S-(C₁-C₄)
 alkyl; C₃-C₇ cycloalkyl -(C₁-C₂) alkyl-N(R³)(R³), -O-CH₂CH(OH)CH₂OH,
 -O-(C₁-C₃) alkyl-N(R³)(R³), and -N(R³)(R³);

R" is selected from hydrogen and C₁-C₄ alkyl optionally substituted with one or more substituents independently selected from halo, -C≡N, C₁-C₄ alkyl, =O, C₃-C₇ cycloalkyl, fluoro-substituted C₁-C₂ alkyl, -O-R³, -S-R³, -(C₁-C₄ alkyl)-N(R³)(R³), -N(R³)(R³), -O-(C₁-C₄ alkyl)-N(R³)(R³), -(C₁-C₄ alkyl)-O-(C₁-C₄ alkyl)-N(R³)(R³), -C(O)-N(R³)(R³), and -(C₁-C₄ alkyl)-C(O)-N(R³)(R³);

R¹ is selected from a carbocycle and a heterocycle, wherein R¹ is optionally substituted with one or more substituents independently selected from halo, -C≡N, C₁-C₄ alkyl, =O, C₃-C₇ cycloalkyl, fluoro-substituted C₁-C₄ alkyl, -O-R³, -S-R³, -(C₁-C₄ alkyl)-N(R³)(R³), -N(R³)(R³), -O-(C₁-C₄ alkyl)-N(R³)(R³), -(C₁-C₄ alkyl)-O-(C₁-C₄ alkyl)-N(R³)(R³), -C(O)-N(R³)(R³), -(C₁-C₄ alkyl)-C(O)-N(R³)(R³), and a 5- or 6-membered saturated heterocycle and when R¹ is phenyl, R¹ is also optionally substituted with O-(saturated heterocycle), -O-(fluoro-substituted saturated heterocycle), C₁-C₄ alkyl-substituted saturated heterocycle, 3,4-methylenedioxy, fluoro-substituted 3,4-methylenedioxy, 3,4-ethylenedioxy, or fluoro-substituted 3,4-ethylenedioxy, wherein

each R³ is independently selected from hydrogen, and -C₁-C₄ alkyl; or two R³ are taken together with the nitrogen atom to which they are bound to form a 4- to 8-membered saturated heterocycle optionally comprising one additional heteroatom selected from NH, S, S(=O), S(=O)₂, and O, wherein:

when R³ is alkyl, the alkyl is optionally substituted with one or more substituents selected from -OH, fluoro, -NH₂, -NH(C₁-C₄ alkyl), -N(C₁-C₄ alkyl)₂, -NH(CH₂CH₂OCH₃), and -N(CH₂CH₂OCH₃)₂ and

when two R³ are taken together with the nitrogen atom to which they are bound to form a 4- to 8-membered saturated heterocycle, the saturated heterocycle is optionally substituted at any carbon atom with -OH, -C₁-C₄ alkyl, fluoro, -NH₂, -NH(C₁-C₄ alkyl), -N(C₁-C₄ alkyl)₂, -NH(CH₂CH₂OCH₃), or -N(CH₂CH₂OCH₃)₂; and optionally substituted at any substitutable nitrogen atom with -C₁-C₄ alkyl, fluoro-substituted C₁-C₄ alkyl, or -(CH₂)₂-O-CH₃;

R² is selected from a carbocycle and a heterocycle, wherein R² is optionally substituted with one or more substituents independently selected from halo, -C≡N, C₁-C₄ alkyl, C₃-C₇ cycloalkyl, C₁-C₂ fluoro-substituted alkyl, -O-R³, -S-R³,

-S(O)-R³, -S(O)₂-R³, -(C₁-C₄ alkyl)-N(R³)(R³), -N(R³)(R³), -O-(C₁-C₄ alkyl)-N(R³)(R³), -(C₁-C₄ alkyl)-O-(C₁-C₄ alkyl)-N(R³)(R³), -C(O)-N(R³)(R³), -(C₁-C₄ alkyl)-C(O)-N(R³)(R³), -O-phenyl, phenyl, and a second heterocycle, and when R² is phenyl, R² is also optionally substituted with -O-(saturated heterocycle),
 5 3,4-methylenedioxy, fluoro-substituted 3,4-methylenedioxy, 3,4-ethylenedioxy, or fluoro-substituted 3,4-ethylenedioxy, wherein any phenyl, saturated heterocycle, or second heterocycle substituent of R² is optionally substituted with halo; -C≡N, C₁-C₄ alkyl, fluoro-substituted C₁-C₂ alkyl, -O-(C₁-C₂) fluoro-substituted alkyl, -O-(C₁-C₄) alkyl, -S-(C₁-C₄) alkyl, -S-(C₁-C₂) fluoro-substituted alkyl, -NH-(C₁-C₄) alkyl, and
 10 -N-(C₁-C₄)₂ alkyl;

X is selected from -NH-C(=O)-†, -C(=O)-NH-†, -NH-C(=S)-†, -C(=S)-NH-†, -NH-S(=O)-†, -S(=O)-NH-†, -S(=O)₂-NH-†, -NH-S(=O)₂-†, -NH-S(O)₂-NR⁴-†, -NR⁴-S(O)₂-NH-†, -NH-C(=O)O-†, -OC(=O)NH-†, -NH-C(=O)NR⁴-†, -NR⁴-C(=O)NH-†, -NH-NR⁴-†, -NR⁴-NH-†, -O-NH-†, -NH-O-†,
 15 -NH-CR⁴R⁵-†, -CR⁴R⁵-NH-†, -NH-C(=NR⁴)-†, -C(=NR⁴)-NH-†, -C(=O)-NH-CR⁴R⁵-†, -NH-C(=O)-CR⁴R⁵-†, -CR⁴R⁵-NH-C(O)-†, -NH-C(=S)-CR⁴R⁵-†, -CR⁴R⁵-C(=S)-NH-†, -NH-S(O)-CR⁴R⁵-†, -CR⁴R⁵-S(O)-NH-†, -NH-S(O)₂-CR⁴R⁵-†, -CR⁴R⁵-S(O)₂-NH-†, -NH-C(=O)-O-CR⁴R⁵-†, -CR⁴R⁵-O-C(=O)-NH-†, -NH-C(=O)-NR⁴-CR⁴R⁵-†, and
 20 -CR⁴R⁵-NH-C(=O)-O-†, wherein:

† represents where X is bound to R¹; and

each R⁴ and R⁵ is independently selected from hydrogen, C₁-C₄ alkyl, -CF₃ and (C₁-C₃ alkyl)-CF₃; and

W is selected from hydrogen, C₁-C₄ alkyl and fluoro-substituted C₁-C₄ alkyl;

25 and

Y is selected from C₁-C₄ alkyl and fluoro-substituted C₁-C₄ alkyl;

or

W and Y are bound to one another to form a 5- to 7-membered ring, wherein:

W is selected from -O-, -NH-, -N(C₁-C₄ alkyl)-, -S-, -S(O)-, -S(O)₂ and

30 -C(R⁶)(R⁶)-, and

Y is (-C(R⁶)(R⁶)-)₁₋₃, and

each R^6 is independently selected from hydrogen, C_1 - C_4 alkyl and fluoro-substituted C_1 - C_4 alkyl, or two R^6 bound to the same carbon atom are taken together to form $=O$.

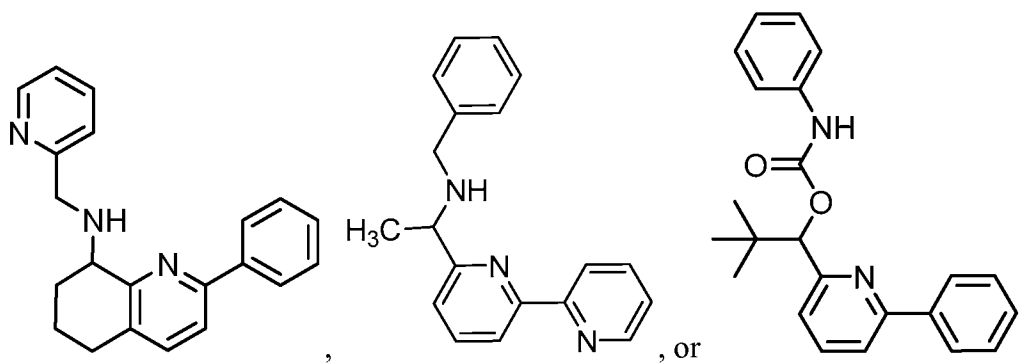
In certain embodiments, compounds represented by Structural Formula (III)

5 may be characterized by one or more of the following features:

when each of Z^1 and Z^2 is $-CH-$, W is hydrogen, Y is C_1 - C_4 alkyl, X is $-NH-CR^4R^5-$ and R^2 is unsubstituted phenyl, R^1 is other than unsubstituted phenyl, or unsubstituted pyridin-2-yl;

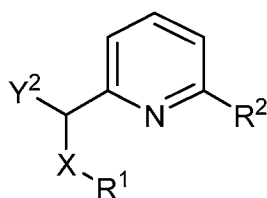
10 when each of Z^1 and Z^2 is $-CH-$, W is hydrogen, Y is C_1 - C_4 alkyl, X is $-NH-S(O)-$ and R is 4-methylphenyl, then R^2 is not unsubstituted phenyl or unsubstituted morpholin-4-yl; and

the compound of Structural Formula (III) is not:



In certain embodiments, R'' is hydrogen.

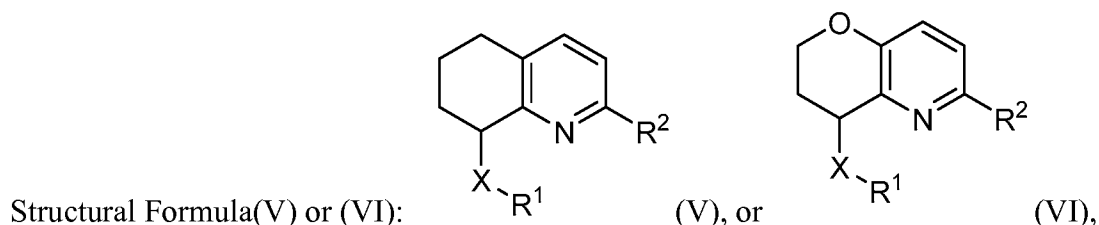
15 In certain embodiments, a compound of Structural Formula (III) has



Structural Formula (IV): (IV),

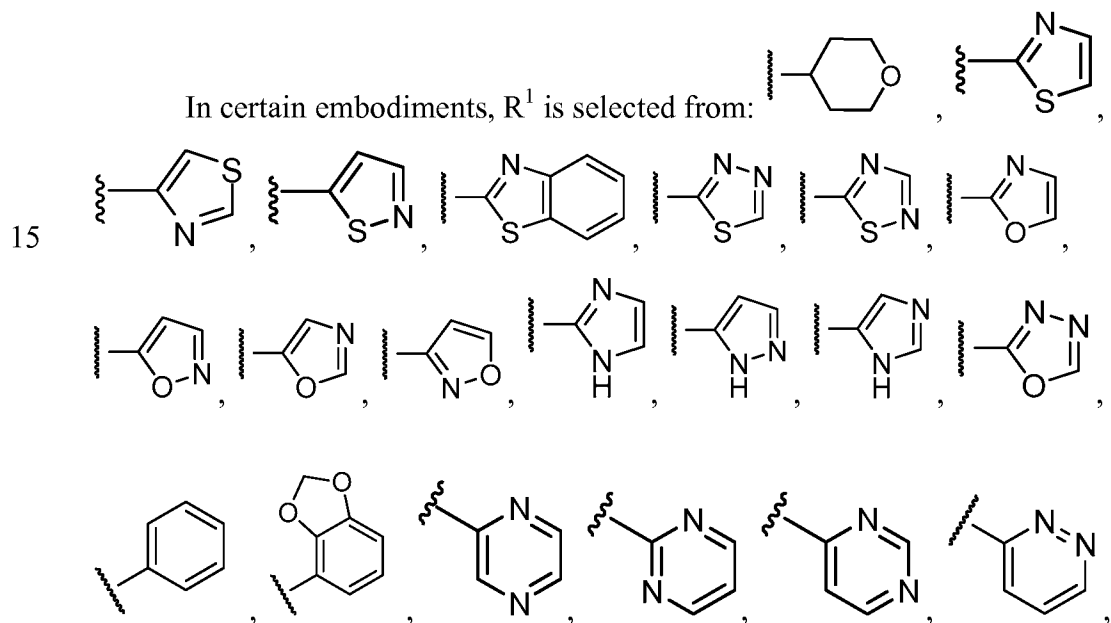
wherein X , R^1 and R^2 are as defined for a compound of Structural Formula (III); and Y^2 is methyl;

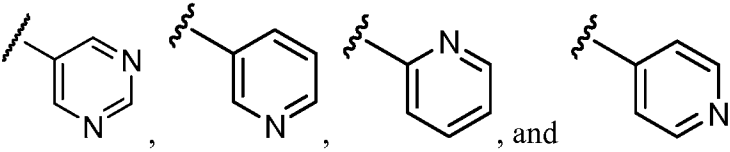
In certain embodiments, a compound of Structural Formula (III) has

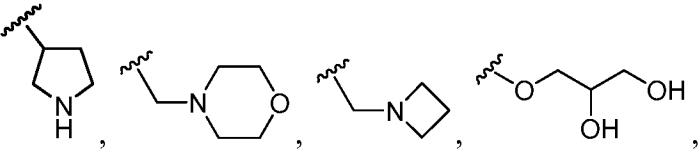
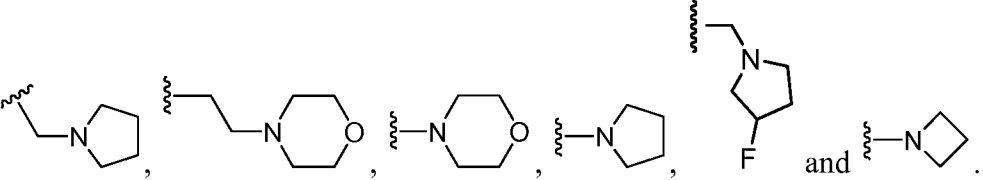
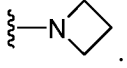


wherein X, R¹ and R² are as defined for a compound of Structural Formula (III).

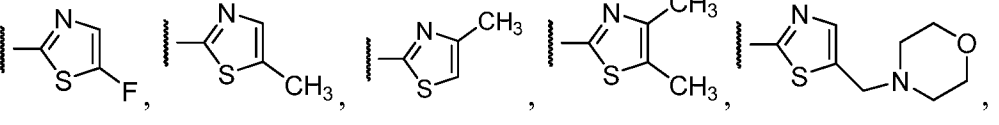
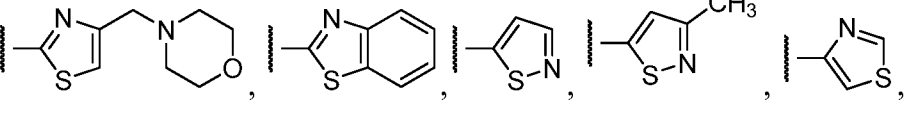
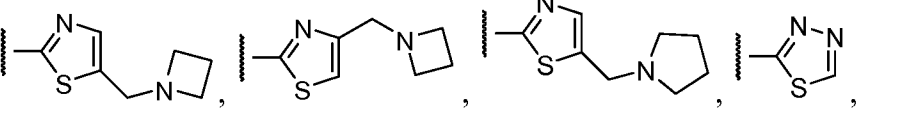
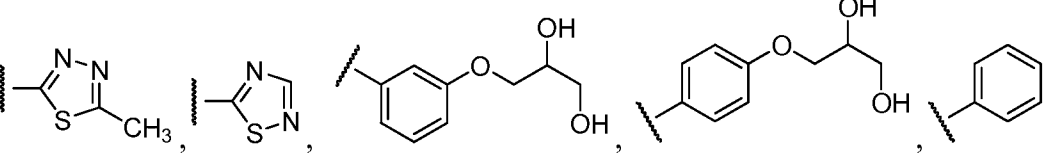
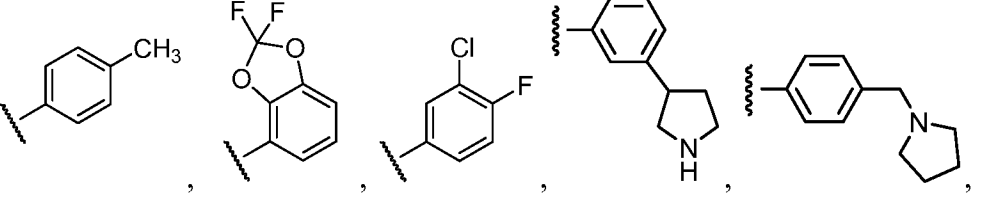
In certain embodiments, X is selected from -NH-C(=O)- $\dagger$, -C(=O)-NH- $\dagger$,
5 -NH-C(=S)- $\dagger$, -C(=S)-NH- $\dagger$, -S(=O)-NH- $\dagger$, -S(=O)₂-NH- $\dagger$, -NH-S(=O)₂- $\dagger$,
-NH-S(O)₂-NR⁴- $\dagger$, -NR⁴-S(O)₂-NH- $\dagger$, -NH-C(=O)O- $\dagger$, -NH-C(=O)NR⁴- $\dagger$,
-NR⁴-C(=O)NH- $\dagger$, -NH-NR⁴- $\dagger$, -NR⁴-NH- $\dagger$, -O-NH- $\dagger$, -NH-O- $\dagger$, -CR⁴R⁵-NH- $\dagger$,
-NH-C(=NR⁴)- $\dagger$, -C(=NR⁴)-NH- $\dagger$, -C(=O)-NH-CR⁴R⁵- $\dagger$, -NH-C(=O)-CR⁴R⁵- $\dagger$,
-CR⁴R⁵-NH-C(O)- $\dagger$, -NH-C(=S)-CR⁴R⁵- $\dagger$, -CR⁴R⁵-C(=S)-NH- $\dagger$,
10 -NH-S(O)-CR⁴R⁵- $\dagger$, -CR⁴R⁵-S(O)-NH- $\dagger$, -NH-S(O)₂-CR⁴R⁵- $\dagger$, -CR⁴R⁵-S(O)₂-NH- $\dagger$,
-NH-C(=O)-O-CR⁴R⁵- $\dagger$, -CR⁴R⁵-O-C(=O)-NH- $\dagger$, -NH-C(=O)-NR⁴-CR⁴R⁵- $\dagger$, and
-CR⁴R⁵-NH-C(=O)-O- $\dagger$ In certain such embodiments, X is selected from
-NH-C(=O)- $\dagger$ and -C(=O)-NH- $\dagger$.

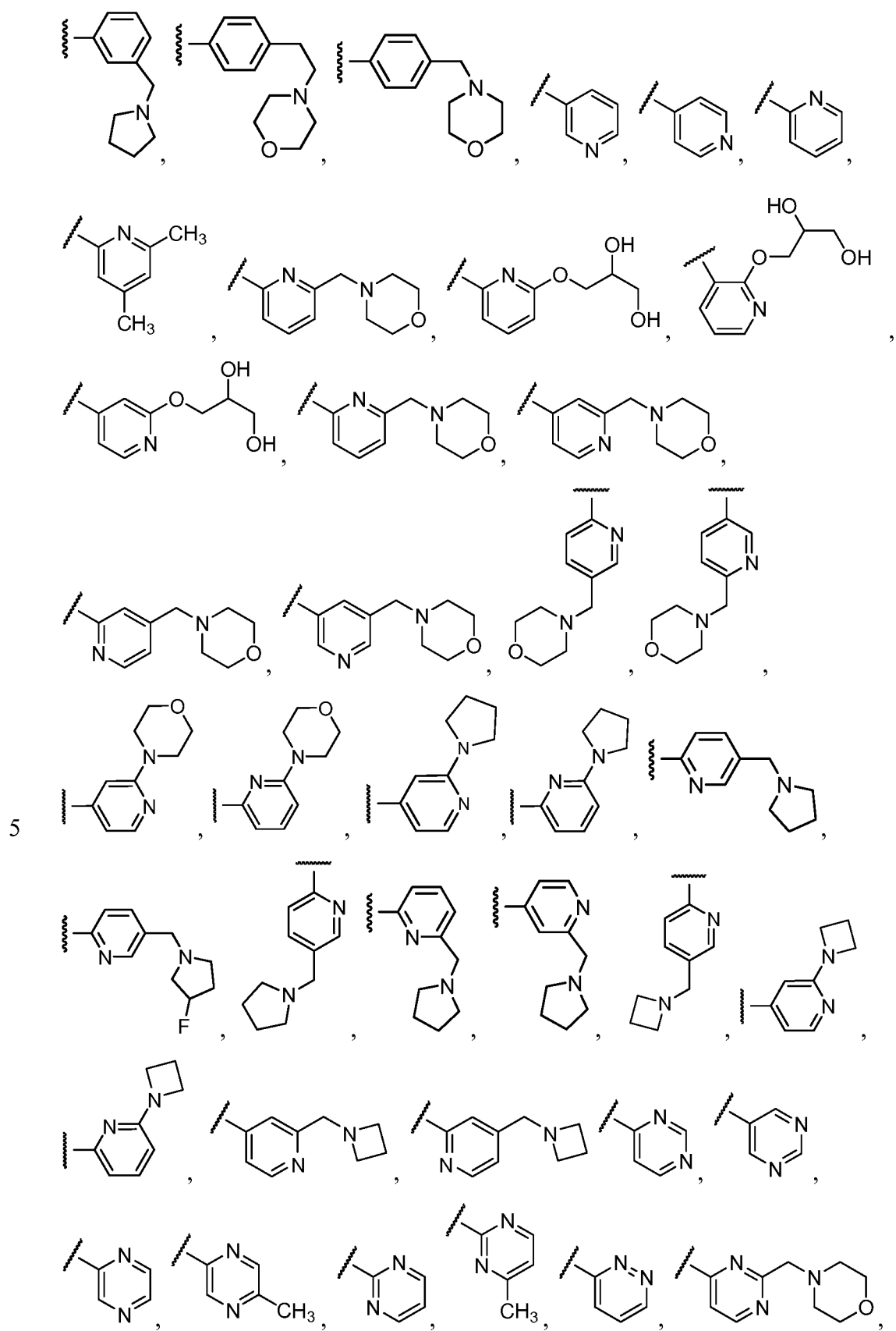


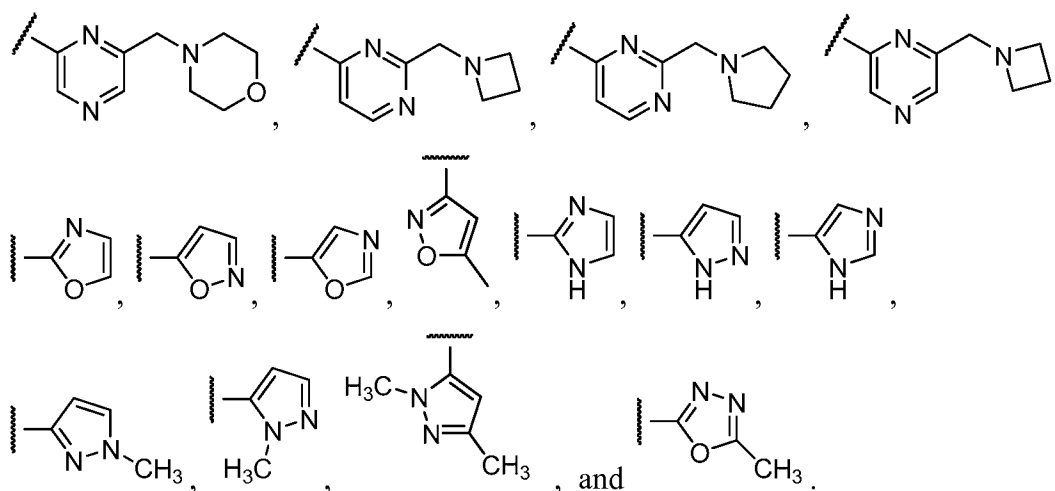

 , wherein R¹ is optionally substituted with one or more substituents independently selected from halo, C₁-C₄ alkyl, -(C₁-C₄ alkyl)-N(R³)(R³), -N(R³)(R³), =O, -O-R³, and pyrrolidinyl. In certain embodiments, R¹ is substituted with one or more groups independently selected

5 from -F, -Cl, -CH₃, 
 , 
 and 
 .

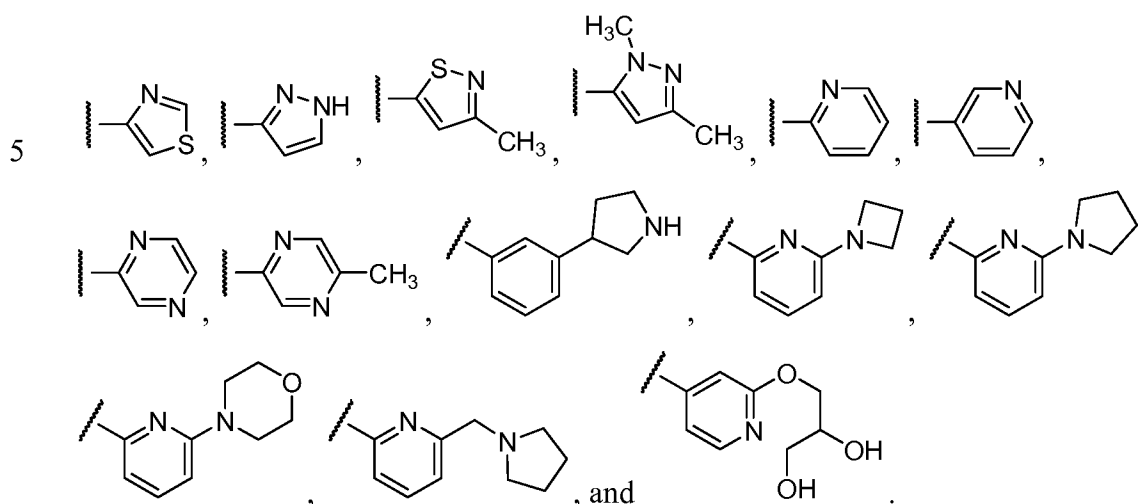
In particular embodiments, R¹ is selected from:


 , 
 , 
 , 
 , 
 ,

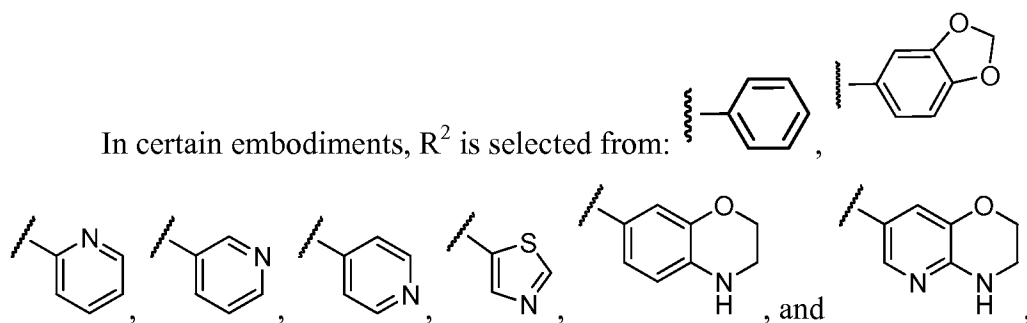




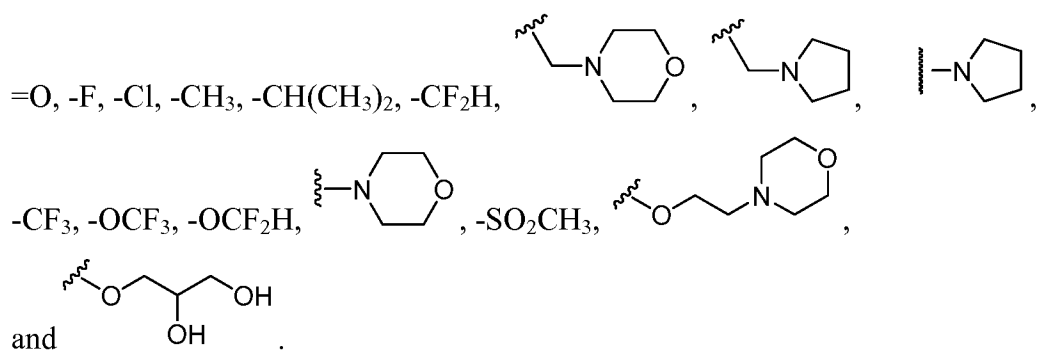
In even more particular embodiments, R^1 is selected from



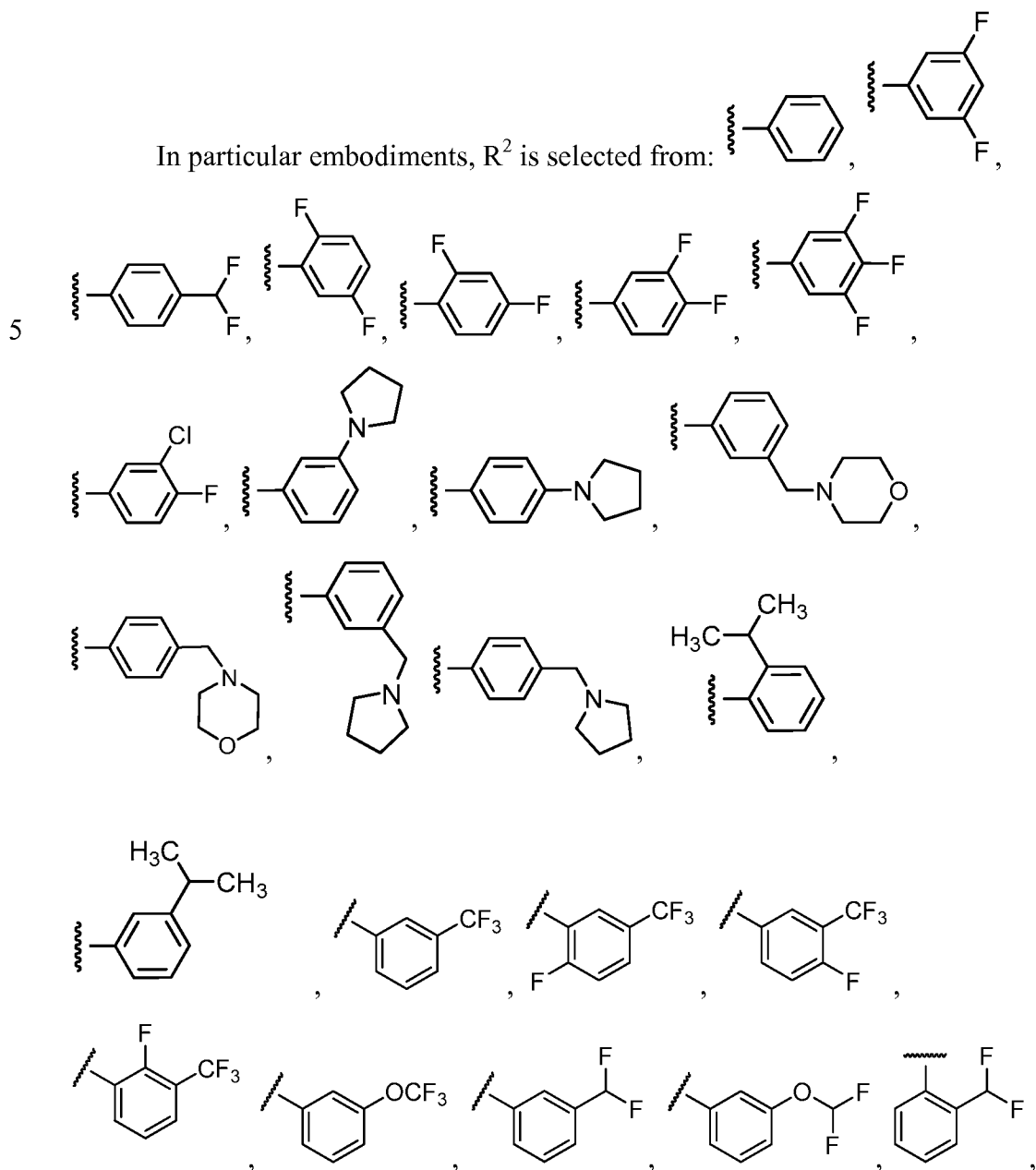
In certain embodiments, R^2 is selected from:

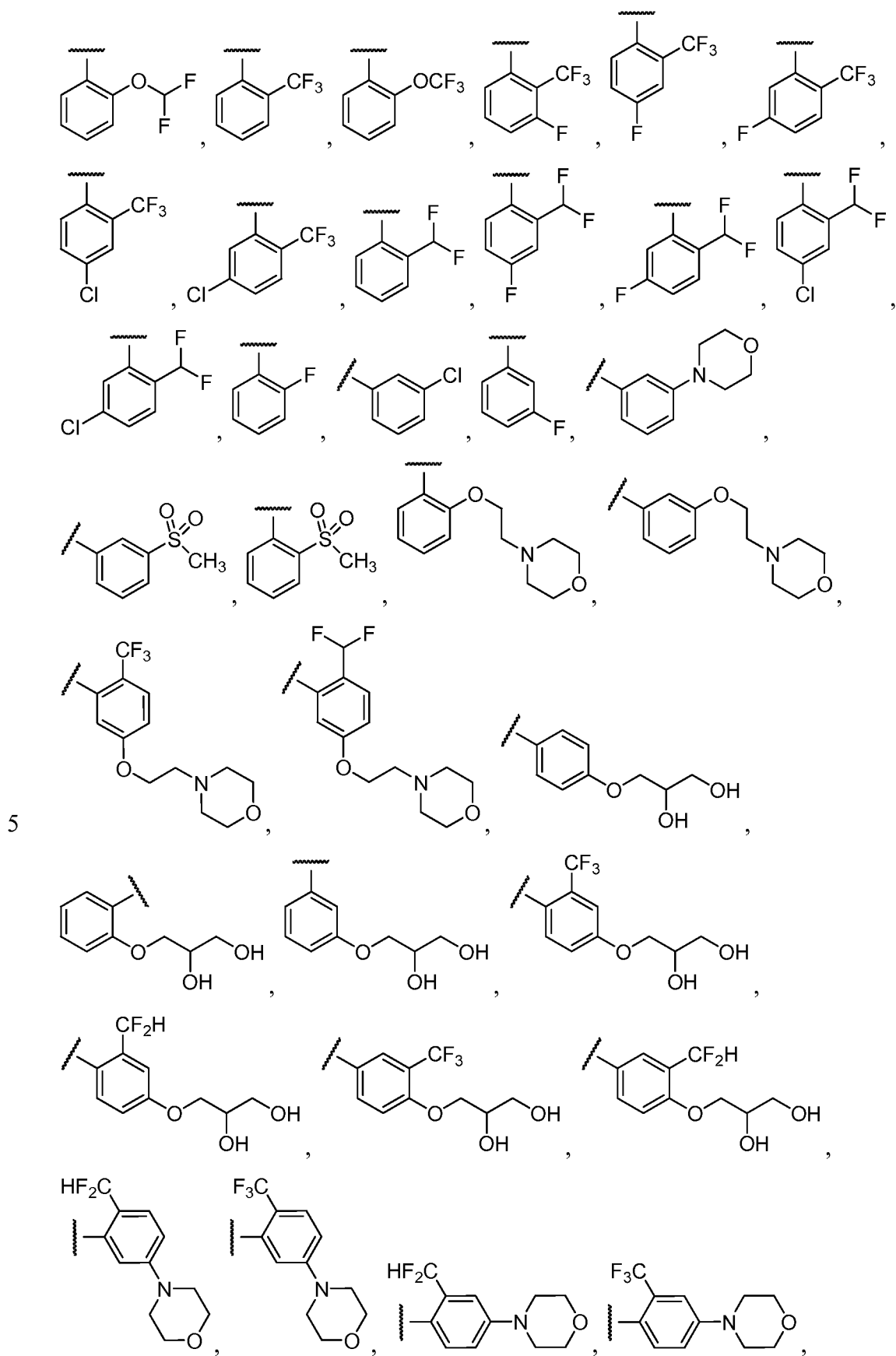


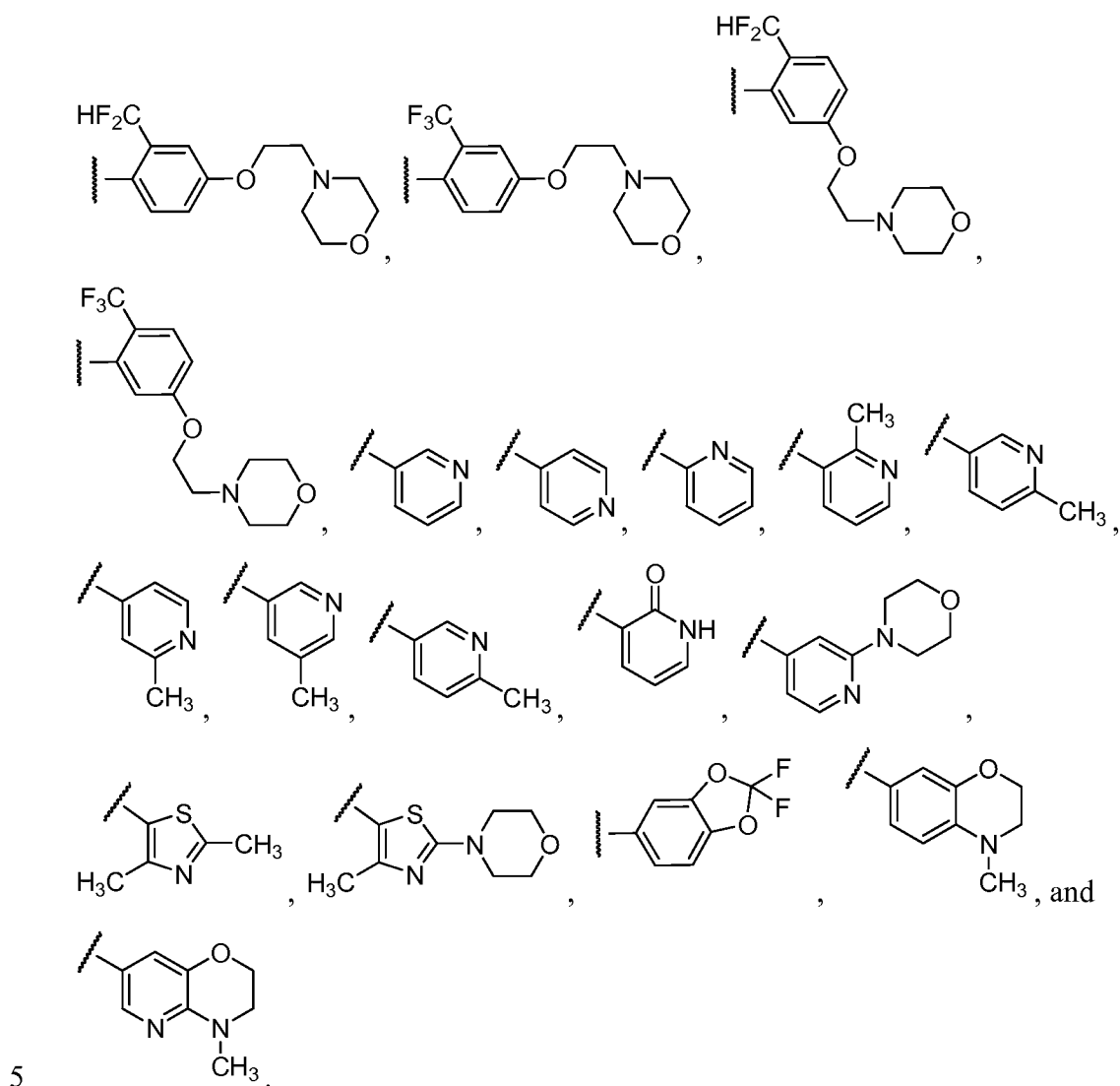
10 wherein R² is optionally substituted with one or two groups independently selected from halo, C₁-C₄ alkyl, -(C₁-C₄ alkyl)-N(R³)(R³), C₁-C₂ fluoro-substituted alkyl, -O-R³, -S(O)₂-R³, -N(R³)(R³), and -O-(C₁-C₄ alkyl)-N(R³)(R³). In certain such embodiments, R² is optionally substituted with one or more groups selected from



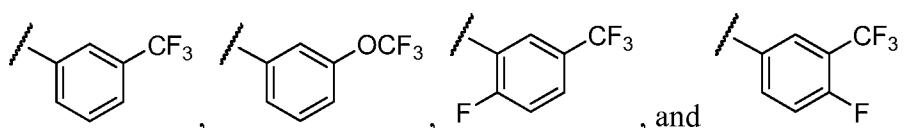
In particular embodiments, R^2 is selected from:







In even more particular embodiments, R^2 is selected from:



In certain embodiments, W is selected from C₁-C₄ alkyl and fluoro-substituted C₁-C₄ alkyl; and Y is selected from C₁-C₄ alkyl and fluoro-substituted C₁-C₄ alkyl; or

W and Y are bound to one another to form a 5- to 7-membered ring, where W is selected from -O-, -NH-, -N(C₁-C₄ alkyl)-, -S-, -S(O)-, and -S(O)₂, and Y is (-C(R⁶)(R⁶)-)₁₋₃.

In certain embodiments, R^1 , R^2 , R'' , W , X , Y , Z^1 and Z^2 are chosen to have
15 one, two, three, four, five, six, seven or eight of the particular values described

above. For example, Rⁿ, W, Y, Z¹ and Z² may be chosen to have one of Structural Formulas (IV)-(VI) in combination with X as -C(=O)-NH-† or -NH-C(=O)-† and any of the particular structures shown for R¹ and R² above.

Compounds of the invention, including novel compounds of the invention,
5 can also be used in the methods described herein.

The compounds and salts thereof described herein can also be present as their corresponding hydrates (e.g., hemihydrate, monohydrate, dihydrate, trihydrate, tetrahydrate) and solvates. Suitable solvents for preparation of solvates and hydrates can generally be selected by a skilled artisan.

10 The compounds and salts thereof can be present in amorphous or crystalline (including co-crystalline and polymorph) forms.

Sirtuin-modulating compounds of the invention advantageously modulate the level and/or activity of a sirtuin protein, particularly the deacetylase activity of the sirtuin protein.

15 Separately or in addition to the above properties, certain sirtuin-modulating compounds of the invention do not substantially have one or more of the following activities: inhibition of PI3-kinase, inhibition of aldoreductase, inhibition of tyrosine kinase, transactivation of EGFR tyrosine kinase, coronary dilation, or spasmolytic activity, at concentrations of the compound that are effective for modulating the
20 deacetylation activity of a sirtuin protein (e.g., such as a SIRT1 and/or a SIRT3 protein).

The terms “carbocycle”, and “carbocyclic”, as used herein, refers to a saturated or unsaturated ring in which each atom of the ring is carbon. Carbocyclic includes 5-7 membered monocyclic and 8-12 membered bicyclic rings. Each ring of
25 a bicyclic carbocycle may be selected from saturated, unsaturated and aromatic rings. In an exemplary embodiment, an aromatic ring, e.g., phenyl, may be fused to a saturated or unsaturated ring, e.g., cyclohexane, cyclopentane, or cyclohexene. Any combination of saturated, unsaturated and aromatic bicyclic rings, as valence permits, are included in the definition of carbocyclic. Exemplary carbocycles
30 include cyclopentyl, cyclohexyl, cyclohexenyl, adamantyl, phenyl and naphthyl.

A cycloalkyl group is a carbocycle which is completely saturated. Exemplary cycloalkyl groups include cyclopentyl, cyclohexyl, bicyclo[2,2,1]heptanyl and adamantyl.

The terms "heterocycle", and "heterocyclic", as used herein, refers to a saturated or unsaturated ring comprising one or more heteroatoms selected from, for example, N, O, and S atoms. Heterocycles include 4-7 membered monocyclic and 8-12 membered bicyclic rings. Each ring of a bicyclic heterocycle may be selected from saturated, unsaturated and aromatic rings. In an exemplary embodiment, an aromatic ring, e.g., pyridyl, may be fused to a saturated or unsaturated ring, e.g., cyclohexane, cyclopentane, or cyclohexene. The terms "heterocyclyl" and "heterocyclic" also include polycyclic ring systems having two or more cyclic rings in which two or more carbons or heteroatoms are common to two adjoining rings wherein at least one of the rings is heterocyclic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryl, and/or heterocycloalkyls. Heterocyclyl groups include, for example, piperidine, piperazine, pyrrolidine, morpholine, lactones, and lactams.

The term "heteroaryl" includes substituted or unsubstituted aromatic single ring structures, preferably 5- to 7-membered rings, more preferably 5- to 6-membered rings, whose ring structures include at least one heteroatom, preferably one to four heteroatoms, more preferably one or two heteroatoms. The terms "heteroaryl" also include polycyclic ring systems having two or more cyclic rings in which two or more carbons or heteroatoms are common to two adjoining rings wherein at least one of the rings is heteroaromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryl, and/or heterocyclyls. Heteroaryl groups include, for example, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrazine, pyridazine, and pyrimidine, and the like.

Monocyclic rings include 5-7 membered aryl or heteroaryl, 3-7 membered cycloalkyl, and 5-7 membered non-aromatic heterocyclyl. Exemplary monocyclic groups include substituted or unsubstituted heterocycles or carbocycles such as thiazolyl, oxazolyl, oxazinyl, thiazinyl, dithianyl, dioxanyl, isoxazolyl, isothiazolyl,

triazolyl, furanyl, tetrahydrofuranyl, dihydrofuranyl, pyranyl, tetrazolyl, pyrazolyl, pyrazinyl, pyridazinyl, imidazolyl, pyridinyl, pyrrolyl, dihydropyrrolyl, pyrrolidinyl, piperidinyl, piperazinyl, pyrimidinyl, morpholinyl, tetrahydrothiophenyl, thiophenyl, cyclohexyl, cyclopentyl, cyclopropyl, cyclobutyl, cycloheptanyl, azetidiny, 5 oxetanyl, thiiranyl, oxiranyl, aziridinyl, and thiomorpholinyl.

Aromatic (aryl) groups include carbocyclic aromatic groups such as phenyl, naphthyl, and anthracyl, and heteroaryl groups such as imidazolyl, thienyl, furyl, pyridyl, pyrimidyl, pyranyl, pyrazolyl, pyrrolyl, pyrazinyl, thiazolyl, oxazolyl, and tetrazolyl.

10 Aromatic 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.

Azabicyclo refers to a bicyclic molecule that contains a nitrogen atom in the 15 ring skeleton. The two rings of the bicycle may be fused, at two mutually bonded atoms, e.g., indole, across a sequence of atoms, e.g., azabicyclo[2.2.1]heptane, or at a single atom, e.g., spirocycle.

Bridged azabicyclo refers to a bicyclic molecule that contains a nitrogen atom and two fused rings wherein the fusion occurs across a sequence of atoms, i.e., 20 bridgehead atoms. Bridged bicyclo compounds comprise at least one bridge of one or more atoms connecting two bridgehead atoms.

Fluoro-substituted includes from one fluoro substituent up to per-fluoro-substitution. Exemplary fluoro-substituted C₁-C₂ alkyl includes -CFH₂, CF₂H, -CF₃, -CH₂CH₂F, -CH₂CHF₂, -CHFCH₃, and -CF₂CHF₂. Per-fluoro-substituted C₁-C₂ 25 alkyl, for example, includes -CF₃ and -CF₂CF₃.

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 30 of time to be useful for the purposes detailed herein.

The compounds disclosed herein also include partially and fully deuterated variants. In certain embodiments, deuterated variants may be used for kinetic

studies. One of ordinary skill in the art can select the sites at which such deuterium atoms are present.

Also included in the present invention are salts, particularly pharmaceutically acceptable salts, of the sirtuin-modulating 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 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 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, 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, 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 sirtuin-modulating compounds. The compounds

may be synthesized using conventional techniques. Advantageously, these compounds are conveniently synthesized from readily available starting materials.

Synthetic chemistry transformations and methodologies useful in synthesizing the sirtuin-modulating compounds described herein are known in the art and include, for example, those described in R. Larock, *Comprehensive Organic Transformations* (1989); T. W. Greene and P. G. M. Wuts, *Protective Groups in Organic Synthesis*, 2d. Ed. (1991); L. Fieser and M. Fieser, *Fieser and Fieser's Reagents for Organic Synthesis* (1994); and L. Paquette, ed., *Encyclopedia of Reagents for Organic Synthesis* (1995).

In an exemplary embodiment, a sirtuin-modulating compound may traverse the cytoplasmic membrane of a cell. For example, a compound may have a cell-permeability of at least about 20%, 50%, 75%, 80%, 90% or 95%.

Sirtuin-modulating compounds described herein may also have one or more of the following characteristics: the compound may be essentially non-toxic to a cell or subject; the sirtuin-modulating compound may be an organic molecule or a small molecule of 2000 amu or less, 1000 amu or less; a compound may have a half-life under normal atmospheric conditions of at least about 30 days, 60 days, 120 days, 6 months or 1 year; the compound may have a half-life in solution of at least about 30 days, 60 days, 120 days, 6 months or 1 year; a sirtuin-modulating compound may be more stable in solution than resveratrol by at least a factor of about 50%, 2 fold, 5 fold, 10 fold, 30 fold, 50 fold or 100 fold; a sirtuin-modulating compound may promote deacetylation of the DNA repair factor Ku70; a sirtuin-modulating compound may promote deacetylation of RelA/p65; a compound may increase general turnover rates and enhance the sensitivity of cells to TNF-induced apoptosis.

In certain embodiments, a sirtuin-modulating compound does not have any substantial ability to inhibit a histone deacetylase (HDACs) class I, a HDAC class II, or HDACs I and II, at concentrations (e.g., in vivo) effective for modulating the deacetylase activity of the sirtuin. For instance, in preferred embodiments the sirtuin-modulating compound is a sirtuin-activating compound and is chosen to have an EC₅₀ for activating sirtuin deacetylase activity that is at least 5 fold less than the EC₅₀ for inhibition of an HDAC I and/or HDAC II, and even more

preferably at least 10 fold, 100 fold or even 1000 fold less. Methods for assaying HDAC I and/or HDAC II activity are well known in the art and kits to perform such assays may be purchased commercially. See e.g., BioVision, Inc. (Mountain View, CA; world wide web at biovision.com) and Thomas Scientific (Swedesboro, NJ; world wide web at tomassci.com).

In certain embodiments, a sirtuin-modulating compound does not have any substantial ability to modulate sirtuin homologs. In one embodiment, an activator of a human sirtuin protein may not have any substantial ability to activate a sirtuin protein from lower eukaryotes, particularly yeast or human pathogens, at concentrations (e.g., in vivo) effective for activating the deacetylase activity of human sirtuin. For example, a sirtuin-activating compound may be chosen to have an EC_{50} for activating a human sirtuin, such as SIRT1 and/or SIRT3, deacetylase activity that is at least 5 fold less than the EC_{50} for activating a yeast sirtuin, such as Sir2 (such as *Candida*, *S. cerevisiae*, etc.), and even more preferably at least 10 fold, 100 fold or even 1000 fold less. In another embodiment, an inhibitor of a sirtuin protein from lower eukaryotes, particularly yeast or human pathogens, does not have any substantial ability to inhibit a sirtuin protein from humans at concentrations (e.g., in vivo) effective for inhibiting the deacetylase activity of a sirtuin protein from a lower eukaryote. For example, a sirtuin-inhibiting compound may be chosen to have an IC_{50} for inhibiting a human sirtuin, such as SIRT1 and/or SIRT3, deacetylase activity that is at least 5 fold less than the IC_{50} for inhibiting a yeast sirtuin, such as Sir2 (such as *Candida*, *S. cerevisiae*, etc.), and even more preferably at least 10 fold, 100 fold or even 1000 fold less.

In certain embodiments, a sirtuin-modulating compound may have the ability to modulate one or more sirtuin protein homologs, such as, for example, one or more of human SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, or SIRT7. In one embodiment, a sirtuin-modulating compound has the ability to modulate both a SIRT1 and a SIRT3 protein.

In other embodiments, a SIRT1 modulator does not have any substantial ability to modulate other sirtuin protein homologs, such as, for example, one or more of human SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, or SIRT7, at concentrations (e.g., in vivo) effective for modulating the deacetylase activity of human SIRT1.

For example, a sirtuin-modulating compound may be chosen to have an ED₅₀ for modulating human SIRT1 deacetylase activity that is at least 5 fold less than the ED₅₀ for modulating one or more of human SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, or SIRT7, and even more preferably at least 10 fold, 100 fold or even 1000 fold less. In one embodiment, a SIRT1 modulator does not have any substantial ability to modulate a SIRT3 protein.

In other embodiments, a SIRT3 modulator does not have any substantial ability to modulate other sirtuin protein homologs, such as, for example, one or more of human SIRT1, SIRT2, SIRT4, SIRT5, SIRT6, or SIRT7, at concentrations (e.g., in vivo) effective for modulating the deacetylase activity of human SIRT3. For example, a sirtuin-modulating compound may be chosen to have an ED₅₀ for modulating human SIRT3 deacetylase activity that is at least 5 fold less than the ED₅₀ for modulating one or more of human SIRT1, SIRT2, SIRT4, SIRT5, SIRT6, or SIRT7, and even more preferably at least 10 fold, 100 fold or even 1000 fold less. In one embodiment, a SIRT3 modulator does not have any substantial ability to modulate a SIRT1 protein.

In certain embodiments, a sirtuin-modulating compound may have a binding affinity for a sirtuin protein of about 10⁻⁹M, 10⁻¹⁰M, 10⁻¹¹M, 10⁻¹²M or less. A sirtuin-modulating compound may reduce (activator) or increase (inhibitor) the apparent Km of a sirtuin protein for its substrate or NAD⁺ (or other cofactor) by a factor of at least about 2, 3, 4, 5, 10, 20, 30, 50 or 100. In certain embodiments, Km values are determined using the mass spectrometry assay described herein. Preferred activating compounds reduce the Km of a sirtuin for its substrate or cofactor to a greater extent than caused by resveratrol at a similar concentration or reduce the Km of a sirtuin for its substrate or cofactor similar to that caused by resveratrol at a lower concentration. A sirtuin-modulating compound may increase the Vmax of a sirtuin protein by a factor of at least about 2, 3, 4, 5, 10, 20, 30, 50 or 100. A sirtuin-modulating compound may have an ED50 for modulating the deacetylase activity of a SIRT1 and/or SIRT3 protein of less than about 1 nM, less than about 10 nM, less than about 100 nM, less than about 1 μM, less than about 10 μM, less than about 100 μM, or from about 1-10 nM, from about 10-100 nM, from about 0.1-1 μM, from about 1-10 μM or from about 10-100 μM. A sirtuin-

modulating compound may modulate the deacetylase activity of a SIRT1 and/or SIRT3 protein by a factor of at least about 5, 10, 20, 30, 50, or 100, as measured in a cellular assay or in a cell based assay. A sirtuin-activating compound may cause at least about 10%, 30%, 50%, 80%, 2 fold, 5 fold, 10 fold, 50 fold or 100 fold greater induction of the deacetylase activity of a sirtuin protein relative to the same concentration of resveratrol. A sirtuin-modulating compound may have an ED50 for modulating SIRT5 that is at least about 10 fold, 20 fold, 30 fold, 50 fold greater than that for modulating SIRT1 and/or SIRT3.

3. Exemplary Uses

In certain aspects, the invention provides methods for modulating the level and/or activity of a sirtuin protein and methods of use thereof.

In certain embodiments, the invention provides methods for using sirtuin-modulating compounds wherein the sirtuin-modulating compounds activate a sirtuin protein, e.g., increase the level and/or activity of a sirtuin protein. Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein 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. The methods comprise administering to a subject in need thereof a pharmaceutically effective amount of a sirtuin-modulating compound, e.g., a sirtuin-activating compound.

Without wishing to be bound by theory, it is believed that activators of the instant invention may interact with a sirtuin at the same location within the sirtuin protein (e.g., active site or site affecting the K_m or V_{max} of the active site). It is believed that this is the reason why certain classes of sirtuin activators and inhibitors can have substantial structural similarity.

In certain embodiments, the sirtuin-modulating compounds described herein may be taken alone or in combination with other compounds. In one embodiment, a mixture of two or more sirtuin-modulating compounds may be administered to a subject in need thereof. In another embodiment, a sirtuin-modulating compound that

increases the level and/or activity of a sirtuin protein may be administered with one or more of the following compounds: resveratrol, butein, fisetin, piceatannol, or quercetin. In an exemplary embodiment, a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein may be administered in combination with nicotinic acid. In another embodiment, a sirtuin-modulating compound that decreases the level and/or activity of a sirtuin protein may be administered with one or more of the following compounds: nicotinamide (NAM), suramin; NF023 (a G-protein antagonist); NF279 (a purinergic receptor antagonist); Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid); (-)-epigallocatechin (hydroxy on sites 3,5,7,3',4', 5'); (-)-epigallocatechin gallate (Hydroxy sites 5,7,3',4',5' and gallate ester on 3); cyanidin chloride (3,5,7,3',4'-pentahydroxyflavylium chloride); delphinidin chloride (3,5,7,3',4',5'-hexahydroxyflavylium chloride); myricetin (cannabiscetin; 3,5,7,3',4',5'-hexahydroxyflavone); 3,7,3',4',5'-pentahydroxyflavone; gossypetin (3,5,7,8,3',4'-hexahydroxyflavone), sirtinol; and splitomicin. In yet another embodiment, one or more sirtuin-modulating compounds may be administered with one or more therapeutic agents for the treatment or prevention of various diseases, including, for example, cancer, diabetes, neurodegenerative diseases, cardiovascular disease, blood clotting, inflammation, flushing, obesity, aging, stress, etc. In various embodiments, combination therapies comprising a sirtuin-modulating compound may refer to (1) pharmaceutical compositions that comprise one or more sirtuin-modulating compounds in combination with one or more therapeutic agents (e.g., one or more therapeutic agents described herein); and (2) co-administration of one or more sirtuin-modulating compounds with one or more therapeutic agents wherein the sirtuin-modulating compound and therapeutic agent have not been formulated in the same compositions (but may be present within the same kit or package, such as a blister pack or other multi-chamber package; connected, separately sealed containers (e.g., foil pouches) that can be separated by the user; or a kit where the sirtuin modulating compound(s) and other therapeutic agent(s) are in separate vessels). When using separate formulations, the sirtuin-modulating compound may be administered at the same, intermittent, staggered, prior to, subsequent to, or combinations thereof, with the administration of another therapeutic agent.

In certain embodiments, methods for reducing, preventing or treating diseases or disorders using a sirtuin-modulating compound may also comprise increasing the protein level of a sirtuin, such as human SIRT1, SIRT2 and/or SIRT3, or homologs thereof. Increasing protein levels can be achieved by introducing into a cell one or more copies of a nucleic acid that encodes a sirtuin. For example, the level of a sirtuin can be increased in a mammalian cell by introducing into the mammalian cell a nucleic acid encoding the sirtuin, e.g., increasing the level of SIRT1 by introducing a nucleic acid encoding the amino acid sequence set forth in GenBank Accession No. NP_036370 and/or increasing the level of SIRT3 by introducing a nucleic acid encoding the amino acid sequence set forth in GenBank Accession No. AAH01042.

A nucleic acid that is introduced into a cell to increase the protein level of a sirtuin may encode a protein that is at least about 80%, 85%, 90%, 95%, 98%, or 99% identical to the sequence of a sirtuin, e.g., SIRT1 and/or SIRT3 protein. For example, the nucleic acid encoding the protein may be at least about 80%, 85%, 90%, 95%, 98%, or 99% identical to a nucleic acid encoding a SIRT1 (e.g. GenBank Accession No. NM_012238) and/or SIRT3 (e.g., GenBank Accession No. BC001042) protein. The nucleic acid may also be a nucleic acid that hybridizes, preferably under stringent hybridization conditions, to a nucleic acid encoding a wild-type sirtuin, e.g., SIRT1 and/or SIRT3 protein. Stringent hybridization conditions may include hybridization and a wash in 0.2 x SSC at 65 °C. When using a nucleic acid that encodes a protein that is different from a wild-type sirtuin protein, such as a protein that is a fragment of a wild-type sirtuin, the protein is preferably biologically active, e.g., is capable of deacetylation. It is only necessary to express in a cell a portion of the sirtuin that is biologically active. For example, a protein that differs from wild-type SIRT1 having GenBank Accession No. NP_036370, preferably contains the core structure thereof. The core structure sometimes refers to amino acids 62-293 of GenBank Accession No. NP_036370, which are encoded by nucleotides 237 to 932 of GenBank Accession No. NM_012238, which encompasses the NAD binding as well as the substrate binding domains. The core domain of SIRT1 may also refer to about amino acids 261 to 447 of GenBank Accession No. NP_036370, which are encoded by nucleotides 834 to 1394 of

GenBank Accession No. NM_012238; to about amino acids 242 to 493 of GenBank Accession No. NP_036370, which are encoded by nucleotides 777 to 1532 of GenBank Accession No. NM_012238; or to about amino acids 254 to 495 of GenBank Accession No. NP_036370, which are encoded by nucleotides 813 to 1538 of GenBank Accession No. NM_012238. Whether a protein retains a biological function, e.g., deacetylation capabilities, can be determined according to methods known in the art.

In certain embodiments, methods for reducing, preventing or treating diseases or disorders using a sirtuin-modulating compound may also comprise decreasing the protein level of a sirtuin, such as human SIRT1, SIRT2 and/or SIRT3, or homologs thereof. Decreasing a sirtuin protein level can be achieved according to methods known in the art. For example, an siRNA, an antisense nucleic acid, or a ribozyme targeted to the sirtuin can be expressed in the cell. A dominant negative sirtuin mutant, e.g., a mutant that is not capable of deacetylating, may also be used. For example, mutant H363Y of SIRT1, described, e.g., in Luo et al. (2001) Cell 107:137 can be used. Alternatively, agents that inhibit transcription can be used.

Methods for modulating sirtuin protein levels also include methods for modulating the transcription of genes encoding sirtuins, methods for stabilizing/destabilizing the corresponding mRNAs, and other methods known in the art.

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 sirtuin-modulating compound of the invention that increases the level and/or activity of a sirtuin protein. In an exemplary embodiment, the methods comprise contacting the cell with a sirtuin-activating 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 sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein to keep the cells, or progeny thereof, in culture for longer periods of time.

- 5 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 sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein. The cells may be in suspension (e.g., blood
10 cells, serum, biological growth media, etc.) or in tissues or organs. For example, blood collected from an individual for purposes of transfusion may be treated with a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein to preserve the blood cells for longer periods of time. Additionally, blood to be used for forensic purposes may also be preserved using a sirtuin-modulating
15 compound that increases the level and/or activity of a sirtuin protein. 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 (such as vegetables).

Sirtuin-modulating compounds that increase the level and/or activity of a
20 sirtuin protein may also be applied during developmental and growth phases in mammals, plants, insects or microorganisms, in order to, e.g., alter, retard or accelerate the developmental and/or growth process.

In another embodiment, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used to treat cells useful for
25 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 sirtuin-modulating compound prior to
administration/implantation, concurrently with administration/implantation, and/or
30 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 sirtuin-modulating compound or may have a subset of cells/tissue treated locally with a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein. 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 sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein *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 sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein. In an exemplary embodiment, skin is contacted with a pharmaceutical or cosmetic composition comprising a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein. 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 damage or natural aging. For example, the compositions 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 pemphigus), 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, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used for the treatment of wounds and/or burns to promote healing, 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.

Topical formulations comprising one or more sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein 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.

5 Sirtuin-modulating compounds may be delivered locally or systemically to a subject. In one embodiment, a sirtuin-modulating compound is delivered locally to a tissue or organ of a subject by injection, topical formulation, etc.

10 In another embodiment, a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein 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, 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
15 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 the lethality caused by a disease, such as cancer.

20 In yet another embodiment, a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein 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 hormesis, i.e., mild stress that is beneficial to organisms and may extend their lifespan.

25 Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein 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 conditions that can be treated include ocular disorders, e.g., associated with the aging of the eye, such as
30 cataracts, glaucoma, and macular degeneration. Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein 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 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; 5 diseases linked to degeneration of the brain, such as Creutzfeld-Jakob disease, retinitis pigmentosa and cerebellar degeneration; myelodysplasia such as aplastic anemia; ischemic 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; 10 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.

Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein 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 15 myocardial infarction or a subject suffering from a spinal cord injury. Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may also be used to repair an alcoholic's liver.

Cardiovascular Disease

In another embodiment, the invention provides a method for treating and/or 20 preventing a cardiovascular disease by administering to a subject in need thereof a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein.

Cardiovascular diseases that can be treated or prevented using the sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein 25 include cardiomyopathy or myocarditis; such as idiopathic cardiomyopathy, metabolic cardiomyopathy, alcoholic cardiomyopathy, drug-induced cardiomyopathy, ischemic cardiomyopathy, and hypertensive cardiomyopathy. Also treatable or preventable using compounds and methods described herein are atheromatous disorders of the major blood vessels (macrovascular disease) such as 30 the aorta, the coronary arteries, the carotid arteries, the cerebrovascular arteries, the renal arteries, the iliac arteries, the 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 sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may also be
5 used for increasing HDL levels in plasma of an individual.

Yet other disorders that may be treated with sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein include restenosis, e.g., following coronary intervention, and disorders relating to an abnormal level of high density and low density cholesterol.

10 In one embodiment, a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein may be administered as part of a combination therapeutic with another cardiovascular agent. In one embodiment, a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein may be administered as part of a combination therapeutic with an anti-arrhythmia
15 agent. In another embodiment, a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein may be administered as part of a combination therapeutic with another cardiovascular agent.

Cell Death/Cancer

Sirtuin-modulating compounds that increase the level and/or activity of a
20 sirtuin protein 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., administered as a prophylactic measure. In another embodiment, the radiation or toxin exposure is received unintentionally. In such a case, the compound is
25 preferably administered as soon as possible after the exposure to inhibit apoptosis and the subsequent development of acute radiation syndrome.

Sirtuin-modulating compounds may also be used for treating and/or preventing cancer. In certain embodiments, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used for treating and/or
30 preventing cancer. Calorie restriction has been linked to a reduction in the incidence of age-related disorders including cancer. Accordingly, an increase in the level and/or activity of a sirtuin protein may be useful for treating and/or preventing

the incidence of age-related disorders, such as, for example, cancer. Exemplary cancers that may be treated using a sirtuin-modulating compound are those of the brain and kidney; hormone-dependent cancers including breast, prostate, testicular, and ovarian cancers; lymphomas, and leukemias. In cancers associated with solid tumors, a modulating compound may be administered directly into the tumor. Cancer of blood cells, e.g., leukemia, can be treated by administering a modulating compound into the blood stream or into the bone marrow. Benign cell growth, e.g., warts, can also be treated. Other diseases that can be treated include autoimmune diseases, e.g., systemic lupus erythematosus, scleroderma, and arthritis, in which autoimmune cells should be removed. Viral infections such as herpes, HIV, adenovirus, and HTLV-1 associated malignant and benign disorders can also be treated by administration of sirtuin-modulating compound. Alternatively, cells can be obtained from a subject, treated ex vivo to remove certain undesirable cells, e.g., cancer cells, and administered back to the same or a different subject.

Chemotherapeutic agents may be co-administered with modulating compounds described herein as having anti-cancer activity, e.g., compounds that induce apoptosis, compounds that reduce lifespan or compounds that render cells sensitive to stress. Chemotherapeutic agents may be used by themselves with a sirtuin-modulating compound described herein as inducing cell death or reducing lifespan or increasing sensitivity to stress and/or in combination with other chemotherapeutics agents. In addition to conventional chemotherapeutics, the sirtuin-modulating compounds described herein may also be used with antisense RNA, RNAi or other polynucleotides to inhibit the expression of the cellular components that contribute to unwanted cellular proliferation.

Combination therapies comprising sirtuin-modulating compounds and a conventional chemotherapeutic agent may be advantageous over combination therapies known in the art because the combination allows the conventional chemotherapeutic agent to exert greater effect at lower dosage. In a preferred embodiment, the effective dose (ED₅₀) for a chemotherapeutic agent, or combination of conventional chemotherapeutic agents, when used in combination with a sirtuin-modulating compound is at least 2 fold less than the ED₅₀ for the chemotherapeutic agent alone, and even more preferably at 5 fold, 10 fold or even

25 fold less. Conversely, the therapeutic index (TI) for such chemotherapeutic agent or combination of such chemotherapeutic agent when used in combination with a sirtuin-modulating compound described herein can be at least 2 fold greater than the TI for conventional chemotherapeutic regimen alone, and even more preferably at 5 fold, 10 fold or even 25 fold greater.

Neuronal Diseases/Disorders

In certain aspects, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein 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). 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 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 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. Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein can be used to treat these disorders and others as described below.

AD is a CNS disorder that results 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 CNS disorder that results in uncontrolled body movements, rigidity, tremor, and

dyskinesia, and is associated with the death of brain cells in an area of the brain that produces dopamine. ALS (motor neuron disease) is a CNS disorder that attacks the motor neurons, components of the CNS that connect the brain to the skeletal muscles.

5 HD is another neurodegenerative disease that causes uncontrolled movements, loss of intellectual faculties, and emotional disturbance. Tay-Sachs disease and Sandhoff disease are glycolipid storage diseases where GM2 ganglioside and related glycolipids substrates for β -hexosaminidase accumulate in the nervous system and trigger acute neurodegeneration.

10 It is well-known that apoptosis plays a role in AIDS pathogenesis in the immune system. However, HIV-1 also induces neurological disease, which can be treated with sirtuin-modulating compounds of the invention.

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
15 and goats, and feline spongiform encephalopathy (FSE) in cats. Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be useful for treating or preventing neuronal loss due to these prior diseases.

In another embodiment, a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein may be used to treat or prevent any disease
20 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
25 alcoholism, or the effects of toxins or drugs. 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
30 diabetes mellitus. 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.

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
5 from the side-effects of systemic illness. Major causes of peripheral neuropathy include seizures, nutritional deficiencies, and HIV, though diabetes is the most likely cause.

In an exemplary embodiment, a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein may be used to treat or prevent multiple
10 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.

In yet another embodiment, a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein may be used to treat trauma to the
15 nerves, including, trauma due to disease, injury (including surgical intervention), or environmental trauma (e.g., neurotoxins, alcoholism, etc.).

Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may also be useful to prevent, treat, and alleviate symptoms of various PNS disorders. The term “peripheral neuropathy” encompasses a wide
20 range of disorders in which the nerves outside of the brain and spinal cord—peripheral nerves—have 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.

PNS diseases treatable with sirtuin-modulating compounds that increase the
25 level and/or activity of a sirtuin protein include: diabetes, leprosy, Charcot-Marie-Tooth disease, Guillain-Barré syndrome and Brachial Plexus Neuropathies (diseases of the cervical and first thoracic roots, nerve trunks, cords, and peripheral nerve components of the brachial plexus).

In another embodiment, a sirtuin activating compound may be used to treat
30 or prevent a polyglutamine disease. Exemplary polyglutamine diseases include Spinobulbar muscular atrophy (Kennedy disease), Huntington’s Disease (HD), Dentatorubral-pallidoluysian atrophy (Haw River syndrome), Spinocerebellar ataxia

type 1, Spinocerebellar ataxia type 2, Spinocerebellar ataxia type 3 (Machado-Joseph disease), Spinocerebellar ataxia type 6, Spinocerebellar ataxia type 7, and Spinocerebellar ataxia type 17.

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 reduction. In one 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 sirtuin activating compound to a subject to treat a central nervous system ischemic condition. A number of central nervous system ischemic conditions may be treated by the sirtuin activating 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. In another alternative of this embodiment, the stroke is a cerebellar stroke. In still another embodiment, the stroke is an embolic stroke. In yet another alternative, the stroke may be a hemorrhagic stroke. In a further embodiment, the stroke is a thrombotic stroke.

In yet another aspect, a sirtuin activating compound may be administered to reduce infarct size of the ischemic core following a central nervous system ischemic condition. Moreover, a sirtuin activating compound may also be beneficially 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

regimen may include one or more sirtuin activators and one or more anti-neurodegeneration agents.

Blood Coagulation Disorders

In other aspects, sirtuin-modulating compounds that increase the level and/or
5 activity of a sirtuin protein 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
10 injury, inflammation, disease, congenital defect, dysfunction or other disruption. 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.

15 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”
20 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 sirtuin-modulating compound that
25 increases the level and/or activity of a sirtuin protein. 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
30 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.

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 regimen may include one or more sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein and one or more anti-coagulation or anti-thrombosis agents.

Weight Control

In another aspect, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used for treating or preventing weight gain or obesity in a subject. For example, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used, for example, to treat or prevent hereditary obesity, dietary obesity, hormone related obesity, obesity related to the administration of medication, 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.

In yet other embodiments, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein 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 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, and low self esteem). Finally, patients with AIDS can develop lipodystrophy or insulin resistance in response to combination therapies for AIDS.

5 In another embodiment, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used for inhibiting adipogenesis or fat cell differentiation, whether in vitro or in vivo. Such methods may be used for treating or preventing obesity.

10 In other embodiments, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used for reducing 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."

15 In an exemplary embodiment, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be administered as a combination therapy for treating or preventing weight gain or obesity. For example, one or more sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be administered in combination with one or more anti-obesity agents.

20 In another embodiment, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be administered to reduce drug-induced weight gain. For example, a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein 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.

Metabolic Disorders/Diabetes

25 In another aspect, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used for treating or preventing a metabolic disorder, such as insulin-resistance, a pre-diabetic state, type II diabetes, and/or complications thereof. Administration of a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein 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, 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

5 hyperlipidemia, dyslipogenesis, hypercholesterolemia, impaired glucose tolerance, high blood glucose sugar level, other manifestations of syndrome X, hypertension, atherosclerosis and lipodystrophy.

In an exemplary embodiment, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be administered as a combination

10 therapy for treating or preventing a metabolic disorder. For example, one or more sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be administered in combination with one or more anti-diabetic agents.

Inflammatory Diseases

In other aspects, sirtuin-modulating compounds that increase the level

15 and/or activity of a sirtuin protein can be used to treat or prevent a disease or disorder associated with inflammation. Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein 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

20 symptom. Administration of the compounds may prevent or attenuate inflammatory responses or symptoms.

In another embodiment, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used to treat or prevent allergies and respiratory conditions, including asthma, bronchitis, pulmonary fibrosis, allergic

25 rhinitis, oxygen toxicity, emphysema, chronic bronchitis, acute respiratory distress syndrome, and any chronic obstructive pulmonary disease (COPD). The compounds may be used to treat chronic hepatitis infection, including hepatitis B and hepatitis C.

Additionally, sirtuin-modulating compounds that increase the level and/or

30 activity of a sirtuin protein may be used to treat autoimmune diseases, and/or inflammation associated with autoimmune diseases, such as arthritis, including rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis, as well as

organ-tissue autoimmune diseases (e.g., Raynaud's syndrome), ulcerative colitis, Crohn's disease, oral mucositis, scleroderma, myasthenia gravis, transplant rejection, endotoxin shock, sepsis, psoriasis, eczema, dermatitis, multiple sclerosis, autoimmune thyroiditis, uveitis, systemic lupus erythematosus, Addison's disease,
5 autoimmune polyglandular disease (also known as autoimmune polyglandular syndrome), and Grave's disease.

In certain embodiments, one or more sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be taken alone or in combination with other compounds useful for treating or preventing inflammation.

10 ***Flushing***

In another aspect, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein 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 sirtuin-modulating compounds that increase the
15 level and/or activity of a sirtuin protein, 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 sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein to reduce the incidence or severity of flushing and/or hot flashes in menopausal and post-
20 menopausal woman.

In another aspect, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein 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
25 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 sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein. In other embodiments, a method for treating drug induced flushing comprises separately administering one or more compounds that
30 induce flushing and one or more sirtuin-modulating compounds, e.g., wherein the sirtuin-modulating compound and flushing inducing agent have not been formulated in the same compositions. When using separate formulations, the sirtuin-modulating

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

In one embodiment, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used to reduce flushing side effects of a vasodilator or an antilipemic agent (including anticholesteremic agents and lipotropic agents). In an exemplary embodiment, a sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein may be used to reduce flushing associated with the administration of niacin.

In another embodiment, the invention provides a method for treating and/or preventing hyperlipidemia with reduced flushing side effects. In another representative embodiment, the method involves the use of sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein to reduce flushing side effects of raloxifene. In another representative embodiment, the method involves the use of sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein to reduce flushing side effects of antidepressants or anti-psychotic agent. For instance, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein can be used in conjunction (administered separately or together) with a serotonin reuptake inhibitor, or a 5HT2 receptor antagonist.

In certain embodiments, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used as part of a treatment with a serotonin reuptake inhibitor (SRI) to reduce flushing. In still another representative embodiment, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used to reduce flushing side effects of chemotherapeutic agents, such as cyclophosphamide and tamoxifen.

In another embodiment, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used to reduce flushing side effects of calcium channel blockers, such as amlodipine.

5 In another embodiment, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used to reduce flushing side effects of antibiotics. For example, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein can be used in combination with levofloxacin.

Ocular Disorders

10 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 sirtuin modulator selected from a compound disclosed herein, 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, 15 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.

In certain aspects of the invention, the vision impairment is caused by retinal 20 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-exudative macular degeneration).

Exemplary retinal diseases include Exudative Age Related Macular 25 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, Branch Retinal Artery Occlusion, Branch Retinal Vein Occlusion, Cancer Associated and Related Autoimmune Retinopathies, Central Retinal Artery 30 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

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

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

10 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, Human Immunodeficiency Virus (HIV)) as well as progressive outer retinal necrosis secondary to HIV or other HIV-associated and other immunodeficiency-associated

15 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.

One aspect of the invention is a method for inhibiting, reducing or treating vision impairment in a subject undergoing treatment with a chemotherapeutic drug

20 (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 sirtuin modulator disclosed herein.

Another aspect of the invention is a method for inhibiting, reducing or treating vision impairment in a subject undergoing surgery, including ocular or other

25 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 sirtuin modulator disclosed herein. Ocular surgeries include cataract, iridotomy and lens replacements.

Another aspect of the invention is the treatment, including inhibition and

30 prophylactic treatment, of age related ocular diseases include cataracts, dry eye, age-related macular degeneration (AMD), retinal damage and the like, by administering

to the subject in need of such treatment a therapeutic dosage of a sirtuin modulator disclosed herein.

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
5 in need of such treatment a therapeutic dosage of a sirtuin modulator disclosed herein. Radiation or electromagnetic damage 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
10 conditions associated with these conditions. Thus, a combination drug regimen may include one or more sirtuin activators and one or more therapeutic agents for the treatment of an ocular disorder.

In one embodiment, a sirtuin modulator can be administered in conjunction with a therapy for reducing intraocular pressure. In another embodiment, a sirtuin
15 modulator can be administered in conjunction with a therapy for treating and/or preventing glaucoma. In yet another embodiment, a sirtuin modulator can be administered in conjunction with a therapy for treating and/or preventing optic neuritis. In one embodiment, a sirtuin modulator can be administered in conjunction with a therapy for treating and/or preventing CMV Retinopathy. In another
20 embodiment, a sirtuin modulator can be administered in conjunction with a therapy for treating and/or preventing multiple sclerosis.

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
25 involve administering to a subject in need thereof a therapeutically effective amount of a sirtuin activating 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
30 biogenesis), or combinations thereof. In certain embodiments, diseases and 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 mitochondrial dysfunction may involve molecular genetic, pathologic and/or
5 biochemical analyses. 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. Diseases or disorders that would benefit from increased mitochondrial activity generally include for example, diseases in which
10 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.

In certain embodiments, the invention provides methods for treating a disease or disorder that would benefit from increased mitochondrial activity that
15 involves administering to a subject in need thereof one or more sirtuin activating compounds in combination with another therapeutic agent such as, for example, an agent useful for treating mitochondrial dysfunction or an agent useful for reducing a symptom associated with a disease or disorder involving mitochondrial dysfunction.

In exemplary embodiments, the invention provides methods for treating
20 diseases or disorders that would benefit from increased mitochondrial activity by administering to a subject a therapeutically effective amount of a sirtuin activating compound. Exemplary diseases or disorders include, for example, neuromuscular disorders (e.g., Friedreich's Ataxia, muscular dystrophy, multiple sclerosis, etc.), disorders of neuronal instability (e.g., seizure disorders, migraine, etc.),
25 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
30 (e.g., calcium accumulation, excitotoxicity, nitric oxide exposure, hypoxia, etc.), and mitochondrial deregulation.

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, such as Duchenne muscular dystrophy. In certain embodiments, sirtuin activating 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.

In certain embodiments, sirtuin modulating compounds 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).

In certain embodiments, sirtuin activating 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, drug induced toxic damage, or hypoxia.

In certain embodiments, sirtuin activating compounds may be useful for treating diseases or disorders associated with mitochondrial deregulation.

Muscle Performance

In other embodiments, the invention provides methods for enhancing muscle performance by administering a therapeutically effective amount of a sirtuin activating compound. For example, sirtuin activating 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 sirtuin activating compound that increase mitochondrial activity, increase mitochondrial biogenesis, and/or increase mitochondrial mass.

5 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
10 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. 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.

15 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.

 It is contemplated that the methods of the present invention will also be effective in the treatment of muscle related pathological conditions, including acute
20 sarcopenia, for example, muscle atrophy and/or cachexia associated with burns, bed rest, limb immobilization, or major thoracic, abdominal, and/or orthopedic surgery.

 In certain embodiments, the invention provides novel dietary compositions comprising sirtuin modulators, a method for their preparation, and a method of using the compositions for improvement of sports performance. Accordingly, provided
25 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 additionally comprise electrolytes, caffeine, vitamins, carbohydrates, etc.

30 ***Other Uses***

 Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used for treating or preventing viral infections (such as

infections by influenza, herpes or papilloma virus) or as antifungal agents. In certain embodiments, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be administered as part of a combination drug therapy with another therapeutic agent for the treatment of viral diseases. In
5 another embodiment, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be administered as part of a combination drug therapy with another anti-fungal agent.

Subjects that may be treated as described herein include eukaryotes, such as mammals, e.g., humans, ovines, bovines, equines, porcines, canines, felines, non-
10 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.

Sirtuin-modulating compounds that increase the level and/or activity of a
15 sirtuin protein may also be used to increase lifespan, stress 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
20 resistance to damage during shipping. Plant seeds may also be contacted with compounds described herein, e.g., to preserve them.

In other embodiments, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may be used for modulating lifespan in yeast cells. Situations in which it may be desirable to extend the lifespan of yeast cells
25 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 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.

30 Sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may also be used to increase lifespan, stress resistance and resistance to apoptosis in insects. In this embodiment, compounds 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, which may have commercial importance. For
5 example, they can be applied to fish (aquaculture) and birds (e.g., chicken and fowl).

Higher doses of sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein may also be used as a pesticide by interfering with the regulation of silenced genes and the regulation of apoptosis during development. In
10 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.

At least in view of the link between reproduction and longevity, sirtuin-modulating compounds that increase the level and/or activity of a sirtuin protein can be applied to affect the reproduction of organisms such as insects, animals and
15 microorganisms.

4. Assays

Yet other methods contemplated herein include screening methods for identifying compounds or agents that modulate sirtuins. An agent may be a nucleic
20 acid, such as an aptamer. Assays may be conducted in a cell based or cell free format. For example, an assay may comprise incubating (or contacting) a sirtuin with a test agent under conditions in which a sirtuin can be modulated by an agent known to modulate the sirtuin, and monitoring or determining the level of modulation of the sirtuin in the presence of the test agent relative to the absence of
25 the test agent. The level of modulation of a sirtuin can be determined by determining its ability to deacetylate a substrate. Exemplary substrates are acetylated peptides which can be obtained from BIOMOL (Plymouth Meeting, PA). Preferred substrates include peptides of p53, such as those comprising an acetylated K382. A particularly preferred substrate is the Fluor de Lys-SIRT1 (BIOMOL), i.e.,
30 the acetylated peptide Arg-His-Lys-Lys. Other substrates are peptides from human histones H3 and H4 or an acetylated amino acid. Substrates may be fluorogenic. The sirtuin may be SIRT1, Sir2, SIRT3, or a portion thereof. For example,

recombinant SIRT1 can be obtained from BIOMOL. The reaction may be conducted for about 30 minutes and stopped, e.g., with nicotinamide. The HDAC fluorescent activity assay/drug discovery kit (AK-500, BIOMOL Research Laboratories) may be used to determine the level of acetylation. Similar assays are described in Bitterman et al. (2002) J. Biol. Chem. 277:45099. The level of modulation of the sirtuin in an assay may be compared to the level of modulation of the sirtuin in the presence of one or more (separately or simultaneously) compounds described herein, which may serve as positive or negative controls. Sirtuins for use in the assays may be full length sirtuin proteins or portions thereof. Since it has been shown herein that activating compounds appear to interact with the N-terminus of SIRT1, proteins for use in the assays include N-terminal portions of sirtuins, e.g., about amino acids 1-176 or 1-255 of SIRT1; about amino acids 1-174 or 1-252 of Sir2.

In one embodiment, a screening assay comprises (i) contacting a sirtuin with a test agent and an acetylated substrate under conditions appropriate for the sirtuin to deacetylate the substrate in the absence of the test agent ; and (ii) determining the level of acetylation of the substrate, wherein a lower level of acetylation of the substrate in the presence of the test agent relative to the absence of the test agent indicates that the test agent stimulates deacetylation by the sirtuin, whereas a higher level of acetylation of the substrate in the presence of the test agent relative to the absence of the test agent indicates that the test agent inhibits deacetylation by the sirtuin.

Methods for identifying an agent that modulates, e.g., stimulates, sirtuins *in vivo* may comprise (i) contacting a cell with a test agent and a substrate that is capable of entering a cell in the presence of an inhibitor of class I and class II HDACs under conditions appropriate for the sirtuin to deacetylate the substrate in the absence of the test agent; and (ii) determining the level of acetylation of the substrate, wherein a lower level of acetylation of the substrate in the presence of the test agent relative to the absence of the test agent indicates that the test agent stimulates deacetylation by the sirtuin, whereas a higher level of acetylation of the substrate in the presence of the test agent relative to the absence of the test agent indicates that the test agent inhibits deacetylation by the sirtuin. A preferred

substrate is an acetylated peptide, which is also preferably fluorogenic, as further described herein. The method may further comprise lysing the cells to determine the level of acetylation of the substrate. Substrates may be added to cells at a concentration ranging from about 1 μ M to about 10mM, preferably from about 10 μ M to 1mM, even more preferably from about 100 μ M to 1mM, such as about 200 μ M. A preferred substrate is an acetylated lysine, e.g., ϵ -acetyl lysine (Fluor de Lys, FdL) or Fluor de Lys-SIRT1. A preferred inhibitor of class I and class II HDACs is trichostatin A (TSA), which may be used at concentrations ranging from about 0.01 to 100 μ M, preferably from about 0.1 to 10 μ M, such as 1 μ M. Incubation of cells with the test compound and the substrate may be conducted for about 10 minutes to 5 hours, preferably for about 1-3 hours. Since TSA inhibits all class I and class II HDACs, and that certain substrates, e.g., Fluor de Lys, is a poor substrate for SIRT2 and even less a substrate for SIRT3-7, such an assay may be used to identify modulators of SIRT1 *in vivo*.

15

5. Pharmaceutical Compositions

The sirtuin-modulating compounds described herein may be formulated in a conventional manner using one or more physiologically or pharmaceutically acceptable carriers or excipients. For example, sirtuin-modulating compounds and their pharmaceutically 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 sirtuin-modulating 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.).

Sirtuin-modulating 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

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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.

5 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., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g.,
10 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
15 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., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g.,
20 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.

 For administration by inhalation (e.g., pulmonary delivery), sirtuin-
25 modulating 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 pressurized aerosol the dosage unit may be determined by providing a valve to
30 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.

Sirtuin-modulating compounds may be formulated for parenteral administration 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
5 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. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

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

In addition to the formulations described previously, sirtuin-modulating compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or
15 intramuscularly) or by intramuscular injection. Thus, for example, sirtuin-modulating 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 release formula also includes patches.

20 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 injection or intracerebroventricular infusion); molecular manipulation of the agent
25 (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 BBB) in an attempt to exploit one of the endogenous transport pathways of the BBB; pharmacological strategies designed to increase the lipid solubility of an agent (e.g., conjugation of water-soluble agents to
30 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).

Liposomes are a further drug delivery system which is easily injectable. 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 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 sirtuin modulator such as resveratrol or a derivative thereof, 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. 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.

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 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 grittiness of the active agent.

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 sirtuin-modulating compounds described herein. In other embodiments, the pharmaceutical composition comprises: (i) 0.05 to 1000 mg

of the compounds of the invention, or a pharmaceutically acceptable salt thereof, and (ii) 0.1 to 2 grams of one or more pharmaceutically acceptable excipients.

In one embodiment, a sirtuin-modulating 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.

Sirtuin-modulating 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., emolliency or the like. As with other carriers or vehicles, an ointment base should be inert, stable, nonirritating and nonsensitizing.

Sirtuin-modulating compounds may be incorporated into lotions, which generally are preparations to be applied to the skin surface without friction, and are 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.

Sirtuin-modulating 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

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.

Sirtuin-modulating compounds may be incorporated into microemulsions, which generally are thermodynamically stable, isotropically clear dispersions of 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).

Sirtuin-modulating 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). Although gels commonly employ aqueous carrier liquid, alcohols and oils can be used as the carrier liquid as well.

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.

Conditions of the eye can be treated or prevented by, e.g., systemic, topical, intraocular injection of a sirtuin-modulating compound, or by insertion of a sustained release device that releases a sirtuin-modulating compound. A sirtuin-modulating compound that increases the level and/or activity of a sirtuin protein

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. 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.

Sirtuin-modulating compounds described herein may be stored in oxygen free environment. For example, resveratrol or analog thereof can be prepared in an airtight capsule for oral administration, such as Capsugel from Pfizer, Inc.

Cells, e.g., treated *ex vivo* with a sirtuin-modulating 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 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 sirtuin-modulating compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental 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. Sirtuin-modulating compounds that exhibit large therapeutic indexes are preferred. While sirtuin-modulating compounds that exhibit toxic side effects may be used, care should be 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 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 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 modulating the lifespan of cells or modulating apoptosis. A kit may comprise one or more sirtuin-modulating 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 sirtuin-modulating compound into a subject (e.g., the blood vessel of a subject) or applying it to the skin of a subject.

In yet another embodiment, the invention provides a composition of matter comprising a sirtuin modulator of this invention and another therapeutic agent (the same ones used in combination therapies and combination compositions) 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 sirtuin modulator are 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 separate vessels, a) a sirtuin modulator of this invention; and b) another therapeutic agent such as those described elsewhere in the specification.

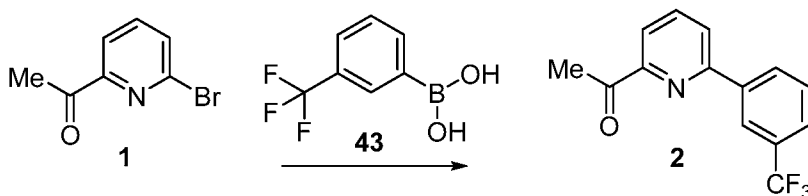
The practice of the present methods will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. 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.

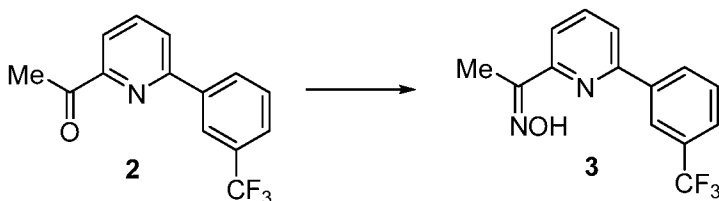
Example 1. Preparation of N-(1-(6-(3-(trifluoromethyl)phenyl)pyridin-2-yl)ethyl)pyrazine-2-carboxamide (Compound 204):

Step 1. Synthesis of 1-(6-(3-(trifluoromethyl)phenyl)pyridin-2-yl)ethanone (2):



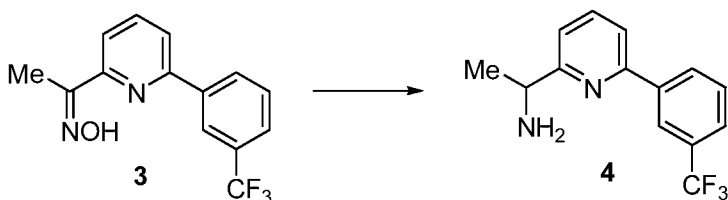
A mixture of 3-(trifluoromethyl)phenylboronic acid (**43**; 500 mg, 2.63 mmol), 1-(6-bromopyridin-2-yl)ethanone (**1**; 438 mg, 2.19 mmol), Pd[Ph₃P]₄ (100 mg) and 2 M aqueous K₂CO₃ (3 mL) in 25 mL of toluene was stirred at 90 °C for 1.5 h. LC showed the reaction was complete. The solution was cooled, extracted with EtOAc (50 mL), washed with 25 mL of 2 mol/L NaOH(aq.), 25 mL of brine, dried with anhydrous MgSO₄ and concentrated. The resultant residue was purified by chromatography (EtOAc/petroleum ether = 1:30) to afford 1-(6-(3-(trifluoromethyl)phenyl)pyridin-2-yl)ethanone **2** as a white solid (538 mg, yield: 92%). MS (ESI) calcd for C₁₄H₁₀F₃NO: 265.07; found: 266 [M+H].

Step 2. Synthesis of 1-(6-(3-(trifluoromethyl)phenyl)pyridin-2-yl)ethanone oxime (3):



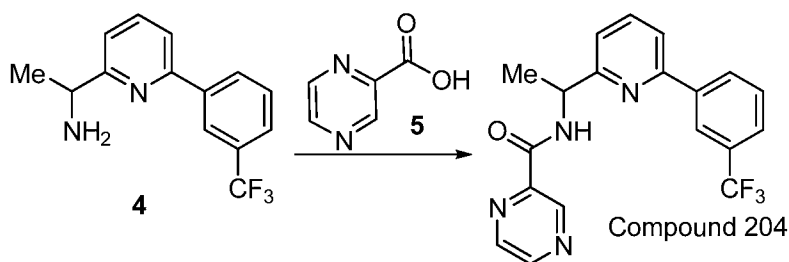
A mixture of 1-(6-(3-(trifluoromethyl)phenyl)pyridin-2-yl)ethanone (**2**; 528 mg, 1.99 mmol), hydroxylamine hydrochloride (168 mg, 2.41 mmol) and Et₃N (302 mg, 2.98 mmol) in 10 mL absolute EtOH was stirred at room temperature for 18 h. The reaction mixture was diluted with water (15 mL). The precipitated white solids were collected by filtration, washed with H₂O, and dried. Purification by chromatography (EtOAc/petroleum ether = 1:20) afforded 1-(6-(3-(trifluoromethyl)phenyl)pyridin-2-yl)ethanone oxime **3** as a white solid (398 mg, yield: 71%). MS (ESI) calcd for C₁₄H₁₁F₃N₂O: 280.08; found: 281 [M+H].

Step 3. Synthesis of 1-(6-(3-(trifluoromethyl)phenyl)pyridin-2-yl)ethanamine (4):



1-(6-(3-(Trifluoromethyl)phenyl)pyridin-2-yl)ethanone oxime (**3**; 1.77 g, 6.31 mmol) was taken up in glacial acetic acid (5 mL) and EtOH (40 mL), along with Palladium on carbon (0.53 g, wet, 30%). The system was evacuated and purged completely with hydrogen. This process was performed three times to ensure complete saturation of hydrogen to the system. The reaction mixture was then stirred at 40 °C for 4 h under 1 atm of hydrogen. LC showed the reaction was complete. The mixture was filtered through a pad of Celite and the filtrate was concentrated under reduced pressure. The residue was diluted with EtOAc (50 mL), washed with 2M NaOH(aq) (10 mL), dried (MgSO₄) and concentrated under reduced pressure. The resulting residue was purified by chromatography (CH₂Cl₂:CH₃OH = 40:1) to afford 1-(6-(3-(trifluoromethyl)phenyl)pyridin-2-yl)ethanamine **4** as a light yellow solid (797 mg, yield: 47%). MS (ESI) calcd for C₁₄H₁₁F₃N₂O: 280.08; found: 281 [M+H].

Step 4. Synthesis of N-(1-(6-(3-(trifluoromethyl)phenyl)pyridin-2-yl)ethyl)pyrazine-2-carboxamide (Compound 204):

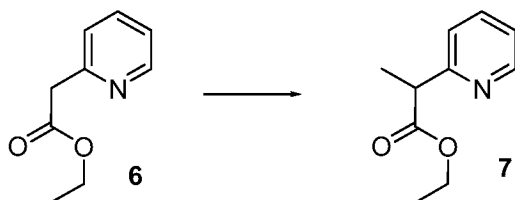


1-(6-(3-(Trifluoromethyl)phenyl)pyridin-2-yl)ethanamine (**4**; 110 mg, 0.41 mmol) was taken up in 4 mL of DMF along with pyrazine-2-carboxylic acid (55.8 mg, 0.45 mmol), HATU (232 mg, 0.61 mmol) and DIPEA (159 mg, 1.23 mmol). The reaction mixture was stirred at room temperature for 18 h and then diluted with H₂O (10 mL). The mixture was extracted with EtOAc (3 x 20 mL). The organic phase was dried (MgSO₄) and concentrated under reduced pressure. The resulting residue was purified by preparative TLC (CH₂Cl₂:CH₃OH=20:1) to afford N-(1-(6-(3-(trifluoromethyl)phenyl)pyridin-2-yl)ethyl)pyrazine-2-carboxamide (Compound 204) as a white solid (76 mg, yield: 50%). MS (ESI) calcd for C₁₉H₁₅F₃N₄O: 372.12; found: 373 [M+H].

This general amide coupling procedure is used to prepare a variety of N-(1-(6-aryl-pyridin-2-yl)ethyl)amide derivatives by substituting the appropriate carboxylic acid for pyrazine carboxylic acid 5.

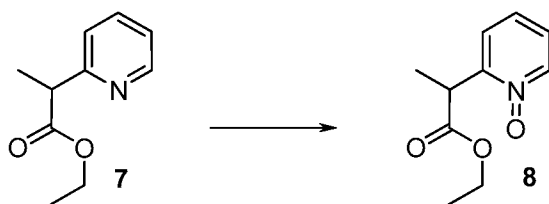
Example 2. Preparation of N-2-pyrazinyl-2-{6-[3-(trifluoromethyl)phenyl]-2-pyridinyl}propanamide (Compound 214):

Step 1. Synthesis of ethyl 2-(2-pyridinyl)propanoate (7):



The procedure detailed here is analogous to that described in WO2005/051919. A solution of n-butyllithium in hexanes (24 ml, 60 mmol) was added to a solution of diisopropylamine (8.56 ml, 60.0 mmol) in tetrahydrofuran (20 mL) and the resulting solution was stirred at -78 °C for 15 min. Ethyl 2-pyridinylacetate (**6**; 3 ml, 19.69 mmol) was added and the mixture was stirred at -78 °C for 30 min, before iodomethane (6.15 ml, 98 mmol) was added. The reaction mixture was stirred at -78 °C for 15 min and then at room temperature for 3h. The reaction mixture was cooled in an ice bath and 20 mL water was added. This was extracted with EtOAc (3x50 ml). The combined organics were washed with brine, dried over sodium sulfate and concentrated to afford a red colored oil **7**. This was purified by silica gel chromatography eluting with isohexane/EtOAc (0-50%). (1.5g, yield: 42.5%). MS (ESI) calcd for C₁₀H₁₃NO₂: 179.22; found: 180 [M+H].

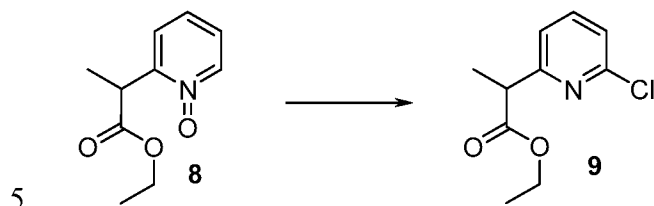
Step 2. Synthesis of ethyl 2-(1-oxido-2-pyridinyl)propanoate (8):



To a solution of ethyl 2-(2-pyridinyl)propanoate (**7**; 1.5 g, 8.37 mmol) in dichloromethane (50 mL) was added mCPBA (1.878 g, 10.88 mmol) portionwise over a period of ten minutes and the reaction mixture was stirred at room temperature for 2h. The reaction was found to be complete by LCMS analysis. The reaction mixture was washed with sat. aqueous NaHCO₃ (2x20 ml), then with brine,

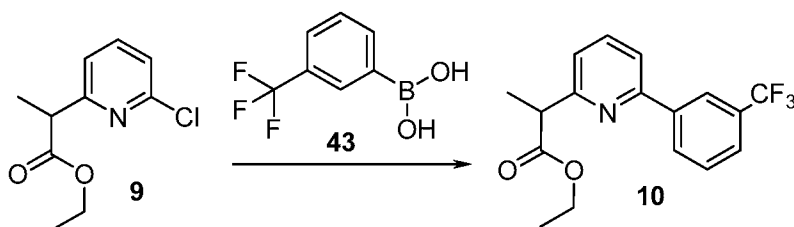
dried over sodium sulfate and concentrated to afford an oil **8** (1.4g). This crude material was carried through to the next step. MS (ESI) calcd for $C_{10}H_{13}NO_3$: 195.22; found: 196 [M+H].

Step 3. Synthesis of ethyl 2-(6-chloro-2-pyridinyl)propanoate (9):



Ethyl 2-(1-oxido-2-pyridinyl)propanoate (**8**; 1.4 g, 7.17 mmol) was dissolved in $POCl_3$ (5 ml, 53.6 mmol) and this was heated at 100 °C for 2 hours. $POCl_3$ was removed in vacuo and the reaction mixture cooled to room temperature. Ice cold water (30 ml) was then added to the flask, neutralized to pH 8-9 and extracted with EtOAc (3x20 ml). The combined organics were washed with brine, dried over sodium sulfate and concentrated. The residue was purified by silica gel chromatography eluting with 0-15% hexane:EtOAc to afford the title compound **9** (230 mg, yield: 15% yield). MS (ESI) calcd for $C_{10}H_{12}ClNO_2$: 213/215; found: 214/216 [M+H].

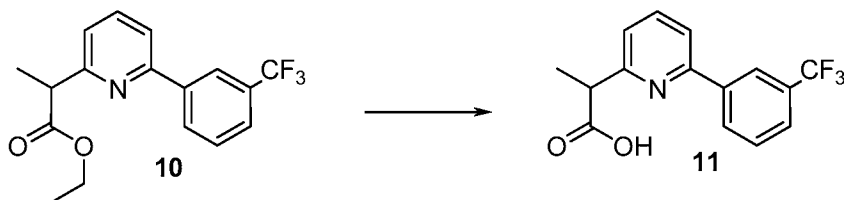
15 **Step 4. Synthesis of ethyl 2-{6-[3-(trifluoromethyl)phenyl]-2-pyridinyl}propanoate (10):**



Ethyl 2-(6-chloro-2-pyridinyl)propanoate (**9**; 230 mg, 1.076 mmol) was dissolved in N,N-dimethylformamide (6 mL) and tetrakis(triphenylphosphine)palladium(0) (249 mg, 0.215 mmol) was added and the reaction was stirred under nitrogen followed by addition of 3-trifluoromethyl phenyl boronic acid (**43**; 204 mg, 1.076 mmol) and Cs_2CO_3 (701 mg, 2.153 mmol). The reaction mixture was heated at 90 °C under nitrogen for 2 hours. The reaction mixture turns red. LCMS analysis shows reaction to be complete. This was cooled to room temperature, water (50 ml) was added and extracted with EtOAc (3x30ml). The combined organic washings were washed with

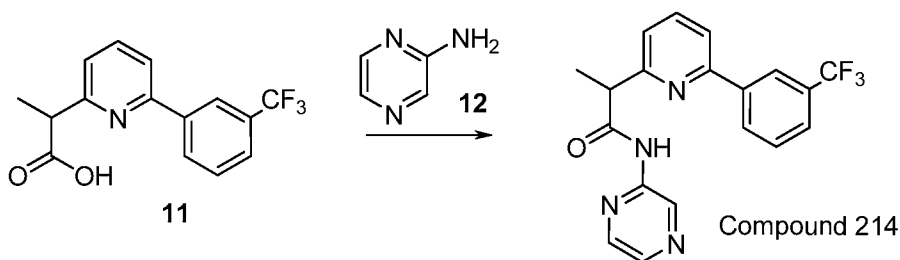
brine, dried over Na₂SO₄ and concentrated. The residue was purified by silica gel chromatography eluting with isohexane/EtOAc (0-20%) to afford the title compound **10** (170 mg, yield: 48.8%). MS (ESI) calcd for C₁₇H₁₆F₃NO₂: 323.31; found: 324 [M+H].

5 **Step 5. Synthesis of 2-{6-[3-(trifluoromethyl)phenyl]-2-pyridinyl}propanoic acid (11):**



To a solution of ethyl 2-{6-[3-(trifluoromethyl)phenyl]-2-pyridinyl}propanoate (**10**; 150 mg, 0.464 mmol) in tetrahydrofuran (5 mL) and water (1 mL) was added LiOH (22.22 mg, 0.928 mmol) and the reaction mixture was stirred at room temperature for 6 hours. The reaction was found to be complete by LCMS analysis. The solvent was evaporated and resulting residue was re-dissolved in 1M HCl in MeOH, and then purified by prep-HPLC, to obtain the title compound **11** (109 mg, yield: 80%). MS (ESI) calcd for C₁₅H₁₂F₃NO₂: 295.26; found: 296 [M+H].

15 **Step 6. Synthesis of N-2-pyrazinyl-2-{6-[3-(trifluoromethyl)phenyl]-2-pyridinyl}propanamide (Compound 214):**



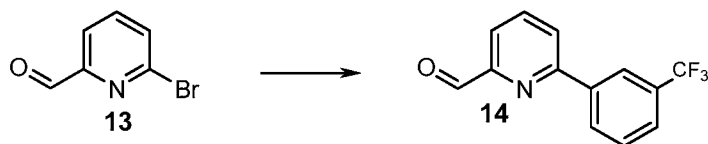
To a solution of 2-{6-[3-(trifluoromethyl)phenyl]-2-pyridinyl}propanoic acid (**11**; 109 mg, 0.369 mmol) and 2-pyrazinamine (**12**; 35.1 mg, 0.369 mmol) in dichloromethane (5 mL) was added DIPEA (0.129 mL, 0.738 mmol) and HATU (211 mg, 0.554 mmol) and the reaction mixture was stirred at room temperature for 4 hours. The reaction was found to be complete by LCMS analysis. The mixture was washed with water, brine and concentrated to afford an oil. This was purified by prep-HPLC to afford the title compound (33 mg, yield: 21.87 %). 2M HCl in diethylether was then added followed by removal of volatiles, to give the title

compound (Compound 214) as the HCl salt (33 mg). MS (ESI) calcd for $C_{19}H_{15}F_3N_4O$: 372.35; found: 373[M+H].

This general amide coupling procedure could be used to prepare a variety of 2-(6-arylpyridin-2-yl)propanamide derivatives by substituting the appropriate amine for 2-pyrazinamine (12).

Example 3. Preparation of *N*-({6-[3-(trifluoromethyl)phenyl]-2-pyridinyl}methyl)benzamide (Compound 208):

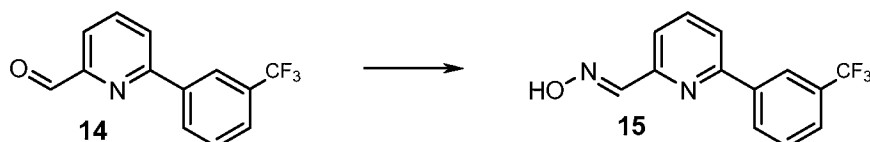
Step 1. Synthesis of 6-[3-(trifluoromethyl)phenyl]-2-pyridinecarbaldehyde (14):



6-Bromo-2-pyridinecarbaldehyde (**13**; 1 g, 5.38 mmol) was dissolved in toluene (50 mL), then [3-(trifluoromethyl)phenyl]boronic acid (1.123 g, 5.91 mmol), tetrakis (0.217 g, 0.188 mmol) and 2M potassium carbonate (aq.) (6.45 mL, 12.90 mmol) were added. The reaction mixture was stirred at 90 °C overnight. Water was added and the mixture was extracted with EtOAc (100ml). Organics were separated and solvent was removed.

Purification by silica gel chromatography (0 to 20% gradient of ethyl acetate in hexane) afforded the title compound **14** as a yellow oil (880 mg, yield: 32.6%). This product was taken as such for the next step. MS (ESI) calcd for $C_{13}H_8F_3NO$: 251.20; found: 252.1 [M+H].

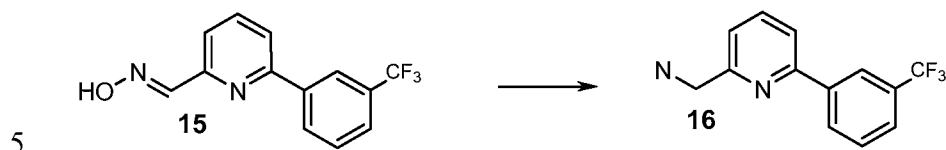
Step 2. Synthesis of 6-[3-(trifluoromethyl)phenyl]-2-pyridinecarbaldehyde oxime (15):



6-[3-(Trifluoromethyl)phenyl]-2-pyridinecarbaldehyde (**14**; 880 mg, 2.98 mmol) was dissolved in ethanol (20 mL), then hydroxylamine hydrochloride (260 mg, 3.74 mmol) and triethylamine (0.623 mL, 4.47 mmol) were added in. The reaction mixture was stirred at room temperature for 60 hr. Water (~50ml) was added to the reaction mixture and the resulting solid was then filtered and oven dried, to give the

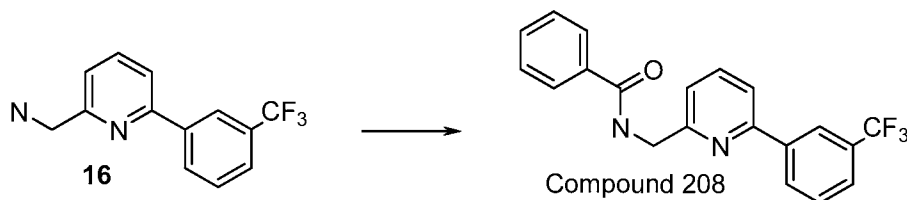
title compound **15** (778 mg, yield: 98%). MS (ESI) calcd for $C_{13}H_9F_3N_2O$: 266.22; found: 267.1 [M+H].

Step 3. Synthesis of ({6-[3-(trifluoromethyl)phenyl]-2-pyridinyl}methyl)amine (16):



6-[3-(Trifluoromethyl)phenyl]-2-pyridinecarbaldehyde oxime (**15**; 778 mg, 2.92 mmol) was dissolved in ethanol (22 mL), then acetic acid (2.3 mL, 40.2 mmol) and Pd/C (737 mg, 0.693 mmol) were added in. The reaction mixture was stirred under hydrogen at room temperature overnight. The reaction mixture was then filtered through celite and solvent was removed. The residue was dissolved in MeOH and passed through a SCX cartridge, eluting with MeOH, then 2M NH_3 in methanol. The relevant fractions were combined and solvent was removed to give the title compound **16** as a yellow oil (525 mg, yield: 57%). MS (ESI) calcd for $C_{13}H_{11}F_3N_2$: 252.24; found: 253.1 [M+H].

15 **Step 4. Synthesis of N-({6-[3-(trifluoromethyl)phenyl]-2-pyridinyl}methyl)benzamide (Compound 208):**

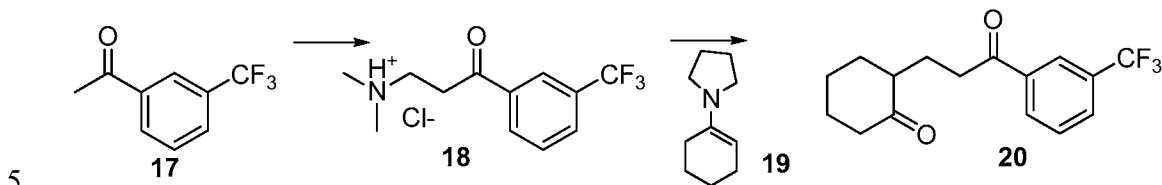


20 ({6-[3-(Trifluoromethyl)phenyl]-2-pyridinyl}methyl)amine (**16**; 150 mg, 0.595 mmol) was dissolved in dichloromethane (8 mL), then benzoic acid (80 mg, 0.654 mmol), HATU (339 mg, 0.892 mmol) and DIPEA (0.312 mL, 1.784 mmol) were added in. The reaction mixture was stirred at room temperature overnight, diluted with water (20ml) and extracted with EtOAc (3x40ml). The organics were dried through a hydrophobic frit and solvent was evaporated. Purification by silica gel chromatography (0 to 50% gradient of ethyl acetate in hexane), gave a white solid.

25 This was further purified by prep-HPLC to give the title compound (Compound 208) (90 mg, yield: 42%). MS (ESI) calcd for $C_{20}H_{15}F_3N_2O$: 356.34; found: 357.2 [M+H].

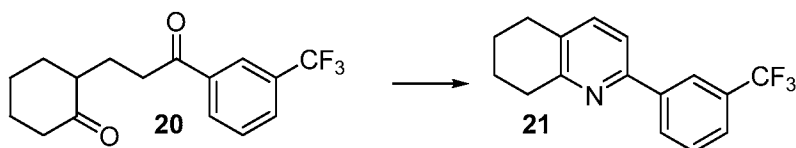
Example 4. Preparation of N-(2-(3-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydroquinolin-8-yl)pyrazine-2-carboxamide (Compound 206):

Step 1. Synthesis of 2-(3-oxo-3-(3-(trifluoromethyl)phenyl)propyl)cyclohexanone 20:



The procedure detailed here is analogous to that described by Kaiser et al (*Angew. Chemie, Int. Ed.* **2006**, p. 5194). 3-Trifluoromethylacetophenone (**17**; 10 g, 0.053 mol) was taken up in 120 mL of anhydrous CH₃CN along with N,N-dimethylmethyleammonium chloride (5.0 g, 0.053 mol) and stirred under reflux for 1 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure to afford the crude dimethylammonium chloride salt **18**. This material **18** was taken up in 100 mL of anhydrous 1,4-dioxane along with cyclohexanone pyrrolidine enamine (**19**; 8.50 mL, 0.053 mol) and stirred under reflux for 18 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was taken up in EtOAc and washed with dilute 1 N HCl, brine, dried (Na₂SO₄) and concentrated under reduced pressure. Purification by chromatography (9:1 pentane/EtOAc) afforded 3.5 g of 2-(3-oxo-3-(3-(trifluoromethyl)phenyl)propyl)cyclohexanone **20** as a clear, light yellow oil. MS (ESI) calcd for C₁₆H₁₇F₃O₂: 298.17; found: 299 [M+H].

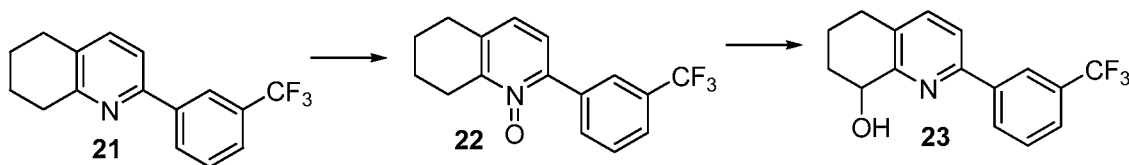
Step 2. Synthesis of 2-(3-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydroquinoline (21):



2-(3-Oxo-3-(3-(trifluoromethyl)phenyl)propyl)cyclohexanone (**20**; 5.30 g, 0.0178 mol) was taken up in 60 mL of absolute EtOH along with hydroxylamine hydrochloride (1.24 g, 0.0178 mo) and stirred under reflux for 3 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure.

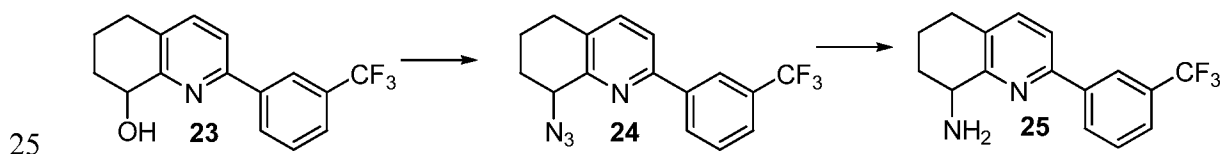
The residue was taken up in EtOAc and washed with dilute NaHCO₃, brine, dried (Na₂SO₄) and concentrated under reduced pressure. Purification by chromatography (9:1 pentane/EtOAc) afforded 1.20 g of 2-(3-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydroquinoline **21**. MS (ESI) calcd for C₁₆H₁₄F₃N: 277.11; found: 278 [M+H].

5 **Step 3. Synthesis of 2-(3-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydroquinolin-8-ol (23):**



2-(3-(Trifluoromethyl)phenyl)-5,6,7,8-tetrahydroquinoline (**21**; 1.20 g, 4.33 mmol)
 10 was dissolved in 30 mL of CH₂Cl₂ along with mCPBA (Aldrich, 77%, 1.5g, 6.5 mmol). The reaction mixture was stirred at room temperature for 1 h and partitioned between dilute aqueous NaHCO₃. The organic layer was separated, dried (Na₂SO₄) and concentrated under reduced pressure to afford the crude pyridine N-oxide derivative **22**. This material **22** was taken up in 30 mL of CH₂Cl₂ and cooled to 0
 15 °C. Trifluoroacetic anhydride (1.50 mL, 10.8 mmol) was added. The resulting reaction mixture was stirred at 0 °C for 30 min and warmed to room temperature and stirred for an additional 4 h. A solution of 2 N LiOH (10 mL) was added and the resulting mixture was stirred vigorously at room temperature for 3 h. The two layers were separated; the organic layer was dried (Na₂SO₄) and concentrated under
 20 reduced pressure. Purification by chromatography (8:1 pentane/EtOAc) afforded 450 mg of 2-(3-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydroquinolin-8-ol **23**. MS (ESI) calcd for C₁₆H₁₄F₃NO: 293.10; found: 294 [M+H].

Step 4. Synthesis of 2-(3-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydroquinolin-8-amine (25):

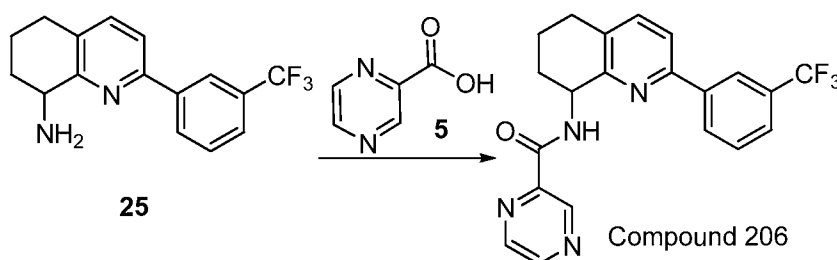


2-(3-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydroquinolin-8-ol (**23**; 450 mg, 1.53 mmol) was dissolved in 20 mL of CH₂Cl₂ along with triethylamine (318 μL, 2.3

mmol) and cooled to 0 °C. Methanesulfonyl chloride (143 µL, 1.84 mmol) was added and the resulting reaction mixture was warmed to room temperature. After stirring for 1 h at room temperature, the reaction mixture was quenched with brine and the two layers were separated. The organic layer was dried (Na₂SO₄) and concentrated under reduced pressure to afford the crude mesylate. This material was taken up in 10 mL of DMSO along with sodium azide (500 mg, 7.65 mmol). The resulting reaction mixture was stirred at 50 °C for 3 h. It was then cooled to room temperature and diluted with EtOAc (50 mL). The resulting organic layer was washed with H₂O (5 x 5 mL), brine, dried (Na₂SO₄) and concentrated under reduced pressure. Purification by chromatography (9:1 pentane/EtOAc) afforded 210 mg of 8-azido-2-(3-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydroquinoline **24**. MS (ESI) calcd for C₁₆H₁₃F₃N₄: 318.11; found: 319 [M+H].

8-Azido-2-(3-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydroquinoline (**24**; 210 mg) was dissolved in 50 mL of MeOH. After 25 mg of 10% Pd/C was added, the reaction mixture was stirred under 1 atm of hydrogen at room temperature for 18 h. The reaction mixture was filtered through a pad of Celite and the filtrate was concentrated under reduced pressure to afford 140 mg of 2-(3-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydroquinolin-8-amine **25**. MS (ESI) calcd for C₁₆H₁₅F₃N₂: 292.11; found: 293 [M+H].

Step 5. Synthesis of N-(2-(3-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydroquinolin-8-yl)pyrazine-2-carboxamide (Compound 206):

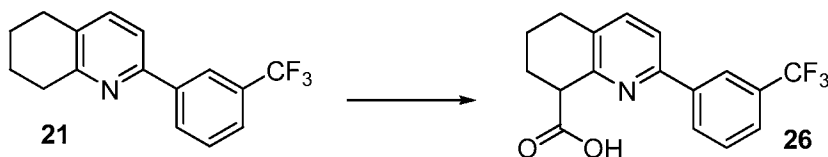


2-(3-(Trifluoromethyl)phenyl)-5,6,7,8-tetrahydroquinolin-8-amine (**25**; 67 mg, 0.23 mmol) was taken up in 2 mL of DMF along with pyrazine-2-carboxylic acid (**5**; 29 mg, 0.23 mmol), HATU (175 mg, 0.46 mmol) and DIPEA (80 µL, 0.46 mmol). The reaction mixture was stirred at room temperature for 18 h. It was then diluted with EtOAc (15 mL) and washed with water (3 x 5 mL). The organic layer was dried

(Na₂SO₄) and concentrated under reduced pressure. The resulting residue was purified by chromatography (gradient elution, 9:1 pentane/EtOAc >> 1:1 pentane/EtOAc) to afford 16 mg of N-(2-(3-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydroquinolin-8-yl)pyrazine-2-carboxamide (Compound 206). MS (ESI) calcd for C₂₁H₁₇F₃N₄O: 398.14; found: 399 [M+H].

This general amide coupling procedure could be used to prepare a variety of N-(2-aryl-5,6,7,8-tetrahydroquinolin-8-yl)-amides by substituting the appropriate carboxylic acid for pyrazine-2-carboxylic acid **5**.

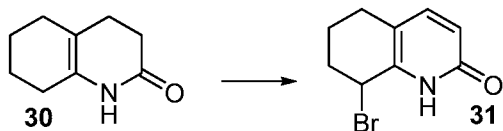
Example 5. Preparation of 2-(3-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydroquinoline-8-carboxylic acid (26):



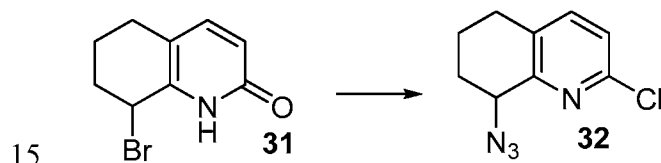
2-(3-(Trifluoromethyl)phenyl)-5,6,7,8-tetrahydroquinoline (**21**; 8.32 g, 30 mmol) was taken up in 100 mL of anhydrous ether along with diisopropylamine (3.04 g, 30 mmol). The flask was filled with N₂, cooled to -15 °C. Then nBuLi (24 mL, 2.5 M in hexane, 60 mmol) was added dropwise in 15 min at -15 °C. The reaction mixture was stirred at this temperature for 2 hours. Then CO₂ gas was bubbled into the solution for 3 hours and the color of the solution was changed from dark red to yellow. The mixture was poured into water and split to two layers. The aqueous layer was neutralized to pH 5-6 and was extracted with DCM. The organic layer was dried and concentrated. Purification by column chromatography afforded 3.65 g (yield 38%) of 2-(3-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydroquinoline-8-carboxylic acid **26** as a yellow solid.

This carboxylic acid **26** was reacted with various appropriate amines in a method analogous to the preparation of N-(2-(3-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydroquinolin-8-yl)pyrazine-2-carboxamide (Compound 206) as described above to generate other N-aryl-2-(3-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydroquinoline-8-carboxamides within the scope of this invention.

Example 6. Preparation of N-(2-(5-methylpyridin-3-yl)-5,6,7,8-tetrahydroquinolin-8-yl)pyrazine-2-carboxamide (Compound 200):

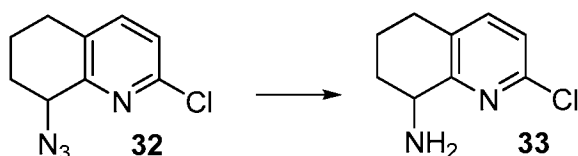
Step 1. Synthesis of 8-bromo-5,6,7,8-tetrahydroquinolin-2(1H)-one (31):

The procedure detailed here is similar to that described in US 4,226,997. To a solution of 3,4,5,6,7,8-hexahydroquinolin-2(1H)-one (**30**; 22.4 g, 148 mmol) in acetonitrile (250 mL) was added bromine (15.2 mL) dropwise over 1 hour while maintaining the temperature below 30 °C. The mixture was stirred at room temperature for 3 hours, heated to reflux for 15 minutes and cooled the room temperature. The solids were filtered, washed thoroughly with anhydrous acetonitrile and dried to obtain 22.1 grams of 8-bromo-5,6,7,8-tetrahydroquinolin-2(1H)-one HBr salt. The HBr salt was suspended in DCM and neutralized with aqueous NaHCO₃ (sat.). The solids were collected by filtration and dried thoroughly to obtain 8-bromo-5,6,7,8-tetrahydroquinolin-2(1H)-one **31** as a white solid (13.35 grams, 40% yield). MS (ESI) calcd for C₉H₁₀BrNO: 226.99; found: 228 [M+H].

Step 2. Synthesis of 8-azido-2-chloro-5,6,7,8-tetrahydroquinoline (32):

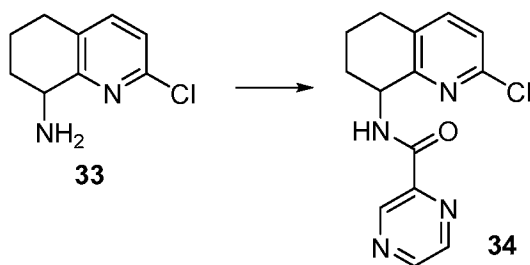
A solution of 8-bromo-5,6,7,8-tetrahydroquinolin-2(1H)-one (**31**; 1.0 g, 4.38 mmol), DMF (1 drop) and POCl₃ (20 mL) was heated to 110°C for 18 hours. The mixture was concentrated, added to ice, and neutralized with solid NaHCO₃. The solution was extracted with CH₂Cl₂ (3x), washed with brine, dried (Na₂SO₄) and concentrated. The residue was stirred with NaN₃ (712 mg, 10.9 mmol) in DMF (20 mL) at room temperature for 3 hours, and 1 hour at 60 °C. The reaction mixture was cooled to room temperature, diluted with water (20 mL) and brine (150 mL), then extracted with ethyl acetate (80 mL). The organic layer was dried (Na₂SO₄) and concentrated. Purification by silica gel (0 to 40% gradient of ethyl acetate in pentane) gave 8-azido-2-chloro-5,6,7,8-tetrahydroquinoline **32** as a clear oil (751 mg, 82% yield). MS (ESI) calcd for C₉H₉ClN₄: 208.05; found: 209 [M+H].

Step 3. Synthesis of 2-chloro-5,6,7,8-tetrahydroquinolin-8-amine (33):



A solution of 8-azido-2-chloro-5,6,7,8-tetrahydroquinoline (**32**; 751 mg, 3.6 mmol), PPh₃ (1.89 g, 7.2 mmol) in 10:1 THF/water (22 mL) was heated to 40 °C for 4 hours. The solution was concentrated, chased with toluene and diethyl ether (20 mL) was added. The precipitate (mostly POPh₃) was removed by filtration and the mother liquor was concentrated. The residue was purified on silica gel (0 to 10% gradient of MeOH in CH₂Cl₂) to obtain 2-chloro-5,6,7,8-tetrahydroquinolin-8-amine **33** as an oil (0.58 grams, 88% yield). MS (ESI) calcd for C₉H₁₁ClN₂: 182.06; found: 183 [M+H].

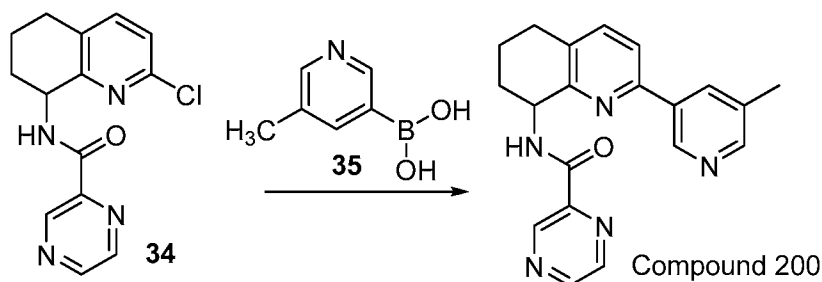
Step 4. Synthesis of N-(2-chloro-5,6,7,8-tetrahydroquinolin-8-yl)pyrazine-2-carboxamide (34):



To a mixture of pyrazine-2-carboxylic acid (**5**; 0.47 grams, 3.78 mmol), DIPEA (0.825 mL, 4.76 mmol), and HATU (1.51 grams, 3.97 mmol) in DMF (15 mL) was added a solution of 2-chloro-5,6,7,8-tetrahydroquinolin-8-amine (**33**; 0.58 grams, 3.18 mmol) in DMF (5 mL). The mixture was stirred at room temperature until the reaction was complete, diluted with aqueous NaHCO₃ and extracted with ethyl acetate (2x). The organic layer was washed with water (3x), brine, dried (Na₂SO₄) and concentrated. The product was purification on silica gel (gradient 0 to 100% ethyl acetate in pentane) to obtain N-(2-chloro-5,6,7,8-tetrahydroquinolin-8-yl)pyrazine-2-carboxamide **34** as a white solid (0.68 grams, 74% yield). MS (ESI) calcd for C₁₄H₁₃ClN₄O: 288.08; found: 289 [M+H].

This general amide coupling procedure could be used to prepare a variety of N-(2-chloro-5,6,7,8-tetrahydroquinolin-8-yl) amides by substituting the appropriate carboxylic acid for pyrazine-2-carboxylic acid **5**.

Step 5. Synthesis of N-(2-(5-methylpyridin-3-yl)-5,6,7,8-tetrahydroquinolin-8-yl)pyrazine-2-carboxamide (Compound 200):

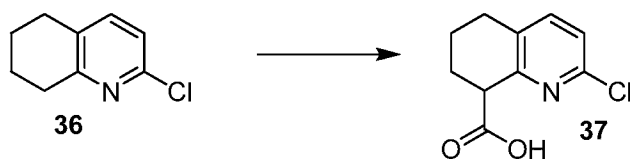


A solution of N-(2-chloro-5,6,7,8-tetrahydroquinolin-8-yl)pyrazine-2-carboxamide (34; 57 mg, 0.200 mmol), 5-methylpyridin-3-ylboronic acid (35; 41 mg, 0.300 mmol), K₃PO₄ (63 mg, 0.300 mmol), and Pd(dppf)Cl₂.DCM (8 mg, 0.01 mmol) in DME (4 mL) was microwave heated (110 °C x 30 min, and 165 °C x 30 min), and concentrated to dryness. The residue was diluted with CH₂Cl₂, washed with water, aqueous NaHCO₃ (sat.), brine and concentrated. The product was purification on prep-HPLC (15 to 95% CH₃CN gradient in water modified with 0.1% TFA). The fractions were concentrated. The residue was dissolved in CH₂Cl₂ washed with NaHCO₃ (sat), dried (Na₂SO₄) and concentrated to an oil. Trituration with pentane gave the product (Compound 200) as a solid (10 mg, 14% yield). MS (ESI) calcd for C₂₀H₁₉N₅O: 345.16; found: 346 [M+H].

This general Suzuki coupling procedure could be used to prepare a variety of 2-aryl-5,6,7,8-tetrahydroquinoline derivatives by substituting the appropriate boronic acid for 5-methylpyridin-3-ylboronic acid 35.

Example 7. Preparation of Compounds Having a 5,6,7,8-tetrahydroquinoline-8-carboxamide Core:

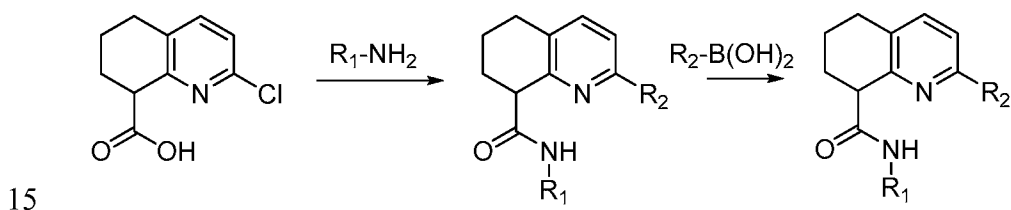
Step 1. Synthesis of 2-chloro-5,6,7,8-tetrahydroquinoline-8-carboxylic acid (37).



A solution of 2-chloro-5,6,7,8-tetrahydroquinoline (36; 9.0 g) and diisopropylamine (5.4 g, 1 equiv) in dry Et₂O (20 ml) was stirred for 10 min under N₂ atmosphere. The solution was cooled to between -15 °C to -30 °C. A solution of n-BuLi in hexane (2 equiv.) was added over 10 minutes at -15 °C. The mixture was stirred at -15 °C for 1

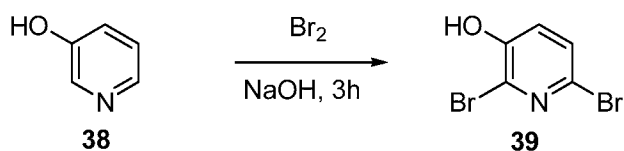
hr, then dry CO₂(g) was added until the color of mixture changed from red to a white-yellow suspension. The solution was stirred for 1 hour, and water was added. The biphasic mixture was warmed to room temperature and the layer was separated. The aqueous layer was washed with ethyl acetate (3x), and concentrated to one half
 5 volume under reduced pressure. The aqueous layer was cooled to 0 °C, neutralized to pH = 5-6 with HCl (4 N). The resulting precipitate was dissolved into ethyl acetate and the layers were split. The organic layer was purified by silica gel column chromatography using ethyl acetate as the eluent. The aqueous fraction was concentrated and purified by column chromatography. 5.3 grams (46% yield) of 2-
 10 chloro-5,6,7,8-tetrahydroquinoline-8-carboxylic acid **37** was obtained.

The resulting carboxylic acid **37** is then reacted sequentially with an appropriate amine and an appropriate boronic acid as depicted below in a manner analogous to that described in the previous examples to generate 5,6,7,8-tetrahydroquinoline-8-carboxamide of this invention (e.g., Compounds 262-301).

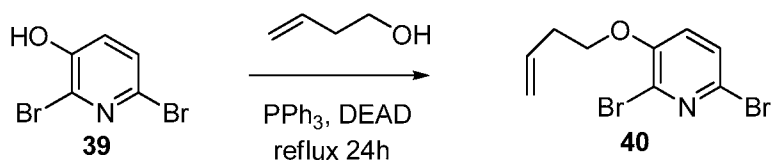


Example 8. Preparation of 6-morpholino-N-(6-(3-(trifluoromethyl)phenyl)-3,4-dihydro-2H-pyrano[3,2-b]pyridin-4-yl)picolinamide (Compound 222):

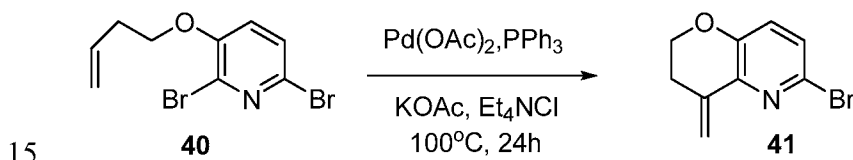
Step 1. Synthesis of 2,6-dibromopyridin-3-ol (39):



20 To a 10% aqueous NaOH solution (300ml) was added bromine (48.6 ml, 946 mmol) at 0 °C. An ice cooled solution of pyridin-3-ol (**38**; 30 g, 315 mmol) in 10ml 10% NaOH solution was then added slowly. The reaction mixture was stirred for 15 min at 0 °C and 45 min at room temperature. The resulting precipitate was filtered and the filtrate was acidified with aqueous HCl (pH = 4) upon which the crude product
 25 precipitated out. The resulting precipitate was filtered and purified by chromatography (EtOAc/petroleum ether = 1:20) to give the target compound **39** (30g, 43.9% yield). MS (ESI) calcd for C₅H₃Br₂NO 251.86, found 251.85 [M+H].

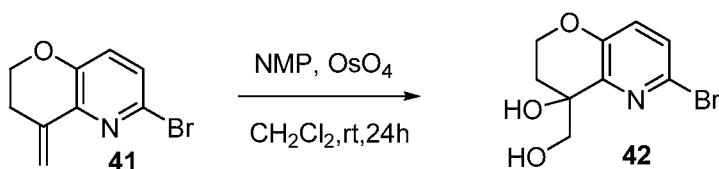
Step 2. Synthesis of 2,6-dibromo-3-(but-3-enyloxy)pyridine (40):

To a stirred mixture of 2,6-dibromopyridin-3-ol (**39**; 35 g, 138 mmol) and but-3-en-1-ol (12.15 ml, 141 mmol) in anhydrous THF (10 ml) at 0 °C was added
 5 triphenylphosphine (43.6 g, 166 mmol), followed by diethylazodicarboxylate (23.97 ml, 152 mmol). The mixture was heated at reflux for 1 hour and then concentrated in vacuo to give a dark brown oil. The oil was dissolved in EtOAc, washed with a saturated NaHCO_3 solution and brine, dried with Na_2SO_4 , and concentrated in vacuo. The crude product mixture was dissolved in dichloromethane. The white solid was
 10 removed by filtration, and the filtrate was purified by silica gel chromatography to afford the title compound **40** (30g, 70.6% yield). MS (ESI) calcd for $\text{C}_9\text{H}_9\text{Br}_2\text{NO}$ 305.91, found 305.82[M+H].

Step 3. Synthesis of 6-bromo-4-methylene-3,4-dihydro-2H-pyrano[3,2-b]pyridine (41):

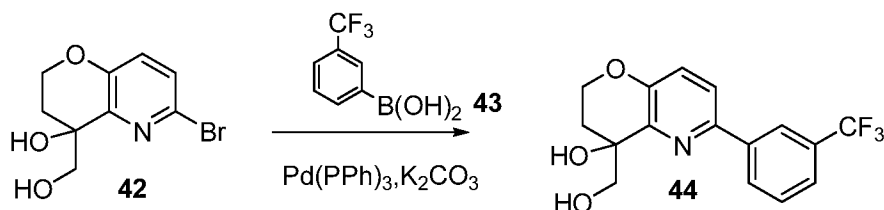
To a stirred mixture of triphenylphosphine (2.82 g, 10.75 mmol), potassium acetate (17.58 g, 179 mmol), palladium acetate (0.804 g, 3.58 mmol), and Et_4NCl (11.88 g, 71.7 mmol), was added 2,6-dibromo-3-(but-3-enyloxy)pyridine (**40**; 11 g, 35.8 mmol) in anhydrous DMF. The reaction mixture was heated at 105 °C overnight.
 20 After cooling to room temperature, the mixture was dissolved in EtOAc, washed with brine, dried over Na_2SO_4 , and concentrated in vacuo. The crude product was purified by silica gel chromatography to afford the target compound **41** (3.2g, 49.8% yield). MS (ESI) calcd for $\text{C}_9\text{H}_8\text{BrNO}$ 225.98, found 225.84[M+H].

Step 4. Synthesis of 6-bromo-4-(hydroxymethyl)-3,4-dihydro-2H-pyrano[3,2-b]pyridin-4-ol (42):



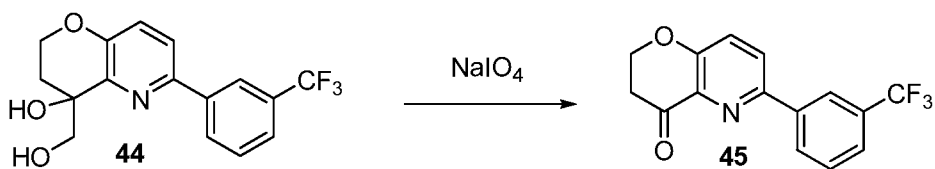
To 6-bromo-4-methylene-3,4-dihydro-2H-pyrano[3,2-b]pyridine (**41**; 4 g, 17.69 mmol) was added N-methylmorpholine N-oxide (2.073 g, 17.69 mmol) in dichloromethane. A 5% solution of osmium(VIII) oxide in t-butanol (0.5 ml, 17.69 mmol) was added dropwise. The reaction mixture was stirred at room temperature overnight. Aqueous saturated NaHSO₃ was added. The mixture was extracted with dichloromethane. The organic extracts were washed with brine, dried over Na₂SO₄, concentrated, and purified by silica gel chromatography to afford the title compound **42** (2.3g, 50.0% yield). MS (ESI) calcd for C₉H₁₀BrNO₃ 259.98, found 259.76 [M+H].

Step 5. Synthesis of 4-(hydroxymethyl)-6-(3-(trifluoromethyl)phenyl)-3,4-dihydro-2H-pyrano[3,2-b]pyridin-4-ol (44**):**



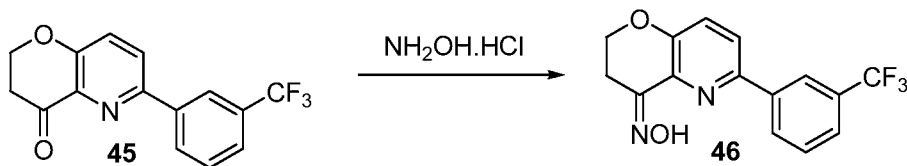
A mixture of 6-bromo-4-(hydroxymethyl)-3,4-dihydro-2H-pyrano[3,2-b]pyridin-4-ol (2.8 g, 10.77 mmol), 3-(trifluoromethyl)phenylboronic acid (**43**; 2.454 g, 12.92 mmol), Cs₂CO₃ (7.02 g, 21.53 mmol), and Pd(PPh₃)₄ (0.622 g, 0.538 mmol) was heated in toluene (9 ml) at 105 °C in a microwave for about 2 hours. The mixture was extracted with EtOAc. The combined organic layers were dried over Na₂SO₄, concentrated, and purified by silica gel chromatography to afford the target compound **44** (2.2g, 62.8% yield). MS (ESI) C₁₆H₁₄F₃NO₃ 326.09, found 325.94 [M+H].

Step 6. Synthesis of 6-(3-(trifluoromethyl)phenyl)-2H-pyrano[3,2-b]pyridin-4(3H)-one (45**):**



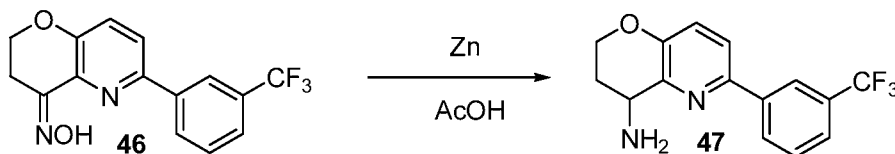
To a solution of 4-(hydroxymethyl)-6-(3-(trifluoromethyl)phenyl)-3,4-dihydro-2H-pyrano[3,2-b]pyridin-4-ol (**44**; 0.55 g, 1.691 mmol) in H₂O (8 ml) and THF (8.00 ml) was added sodium periodate (1.085 g, 5.07 mmol). The reaction mixture was stirred at RT overnight. Aqueous saturated NaHCO₃ solution was added and the mixture was extracted with dichloromethane. The combined organic extracts were washed with brine, dried (Na₂SO₄), concentrated, and purified by vacuum distillation to afford the title compound **45** (450mg, 91% yield). MS (ESI) C₁₅H₁₀F₃NO₂ 294.07, found 293.79 [M+H].

Step 7. Synthesis of 6-(3-(trifluoromethyl)phenyl)-2H-pyrano[3,2-b]pyridin-4(3H)-one oxime (46):



To a solution of 6-(3-(trifluoromethyl)phenyl)-2H-pyrano[3,2-b]pyridin-4(3H)-one (**45**; 0.45 g, 1.535 mmol) in methanol (20 ml) was added hydroxylamine hydrochloride (0.213 g, 3.07 mmol). The reaction mixture was stirred at RT overnight. Aqueous saturated NaHCO₃ was added and the aqueous layer was extracted with dichloromethane. The organic extracts were washed with brine, dried over Na₂SO₄, and concentrated in vacuo to afford the title compound **46** (430mg, 91% yield). MS (ESI) C₁₅H₁₁F₃N₂O₂ 309.08, found 308.8 [M+H].

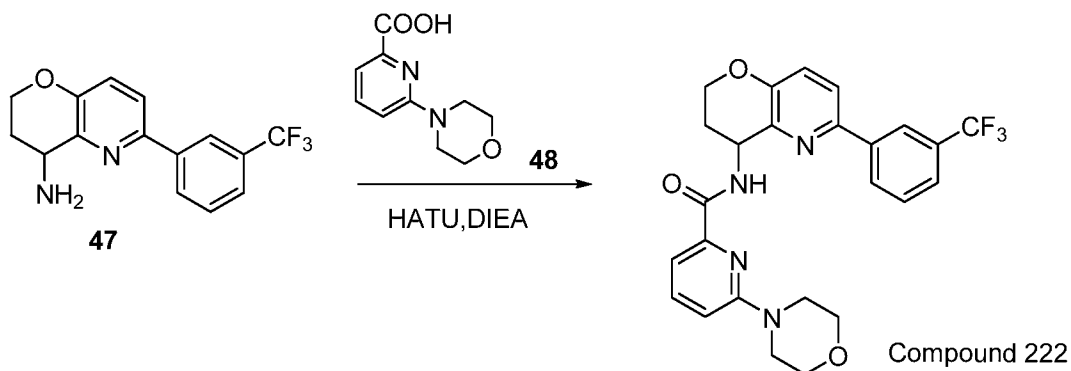
Step 8. Synthesis of 6-(3-(trifluoromethyl)phenyl)-3,4-dihydro-2H-pyrano[3,2-b]pyridin-4-amine (47):



To a solution of 6-(3-(trifluoromethyl)phenyl)-3,4-dihydro-2H-pyrano[3,2-b]pyridin-4-amine (**46**, 0.43 g, 1.395 mmol) in acetic acid (5 ml) was added zinc

(0.182 g, 2.79 mmol). The reaction mixture was stirred at RT for 2 hours. The precipitate was filtered and the filtrate was concentrated in vacuo. The crude material was purified by vacuum distillation to afford the title compound (**47**, 260mg, 63.3% yield). MS (ESI) $C_{15}H_{13}F_3N_2O$ 295.1, found 294.8 [M+H].

5 **Step 9. Synthesis of 6-morpholino-N-(6-(3-(trifluoromethyl)phenyl)-3,4-dihydro-2H-pyrano[3,2-b]pyridin-4-yl)picolinamide (Compound 222):**



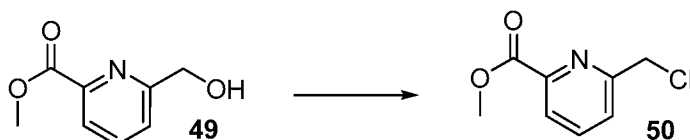
6-morpholinopicolinic acid (**48**; 29.2 mg, 0.163 mmol) was taken up in DMF (5 ml) along with 6-(3-(trifluoromethyl)phenyl)-3,4-dihydro-2H-pyrano[3,2-b]pyridin-4-amine (**47**; 40 mg, 0.136 mmol), HATU (103 mg, 0.272 mmol) and DIEA (35.1 mg, 0.272 mmol). The resulting reaction mixture was stirred at 60°C for 12 hours. Water was added and the solid was purified by silica gel chromatography to give the title compound (**Compound 222**; 17% yield) MS (ESI) calcd for $C_{25}H_{23}F_3N_4O_3$: 484.2; found: 485 [M+H].

15 This general amide coupling procedure could be used to prepare a variety of N-(6-aryl-3,4-dihydro-2H-pyrano[3,2-b]pyridin-4-yl) amide derivatives by substituting the appropriate carboxylic acid for 6-morpholinopicolinic acid **48**.

The syntheses of certain specific carboxylic acids are set forth in Examples 9-13. These carboxylic acids are useful in coupling to various amides to produce additional compounds of the invention by the methods described in the preceding examples.

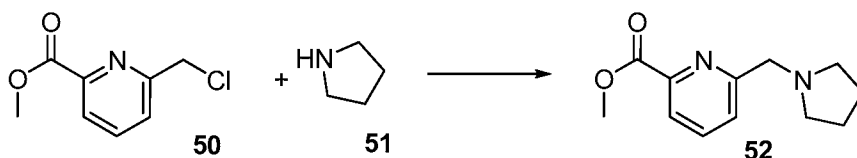
Example 9. Preparation of 6-(pyrrolidin-1-ylmethyl)picolinic acid (53):

Step 1. Synthesis of methyl 6-(chloromethyl)picolinate (50):



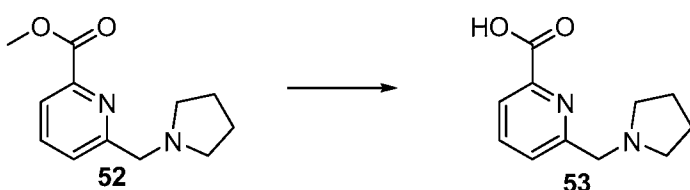
SOCI₂ (57 g, 0.48 mol) was added to a solution of methyl 6-(hydroxymethyl)picolinate (**49**; 40.0 g, 0.239 mol) (*Chem. Eur. J.* **2006**, *12*, 6393-6402) in dichloromethane (500 mL) at room temperature. The mixture was stirred at
 5 40°C for 1 h and sat. aq K₂CO₃ was added to adjust the pH to 9. The mixture was extracted with CH₂Cl₂ and the combined organics were washed with brine, dried (Na₂SO₄), and concentrated *in vacuo* to give (45 g).

Step 2. Synthesis of methyl 6-(pyrrolidin-1-ylmethyl)picolinate (52):



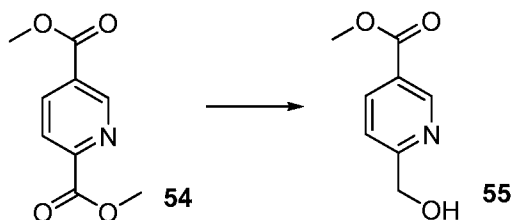
K₂CO₃ (66 g, 0.48 mol) was added to a solution of methyl 6-(chloromethyl)picolinate (**50**; 45.0 g) and pyrrolidine (**51**; 34 g, 0.48 mol) in DMF
 10 (300 mL). The reaction mixture was heated at 80°C for 12 h. H₂O (300 mL) was added and the mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried (Na₂SO₄) and concentrated *in vacuo* to give methyl 6-(pyrrolidin-1-ylmethyl)picolinate **52** (36 g).
 15

Step 3. Synthesis of 6-(pyrrolidin-1-ylmethyl)picolinic acid (53):

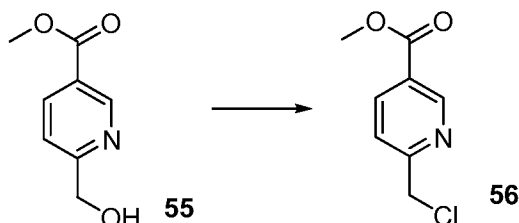


A mixture of methyl 6-(pyrrolidin-1-ylmethyl)picolinate (**52**; 36 g) and NaOH (40 g, 1.0 mol) in ethanol/ H₂O (320 mL) was stirred at 75°C for 16 h. The pH was
 20 adjusted to 7 with 3N HCl and extracted with EtOAc. The aqueous layer was concentrated to dryness and extracted with dichloromethane/methanol (v:v=3:1), The organic layer was dried to give 6-(pyrrolidin-1-ylmethyl)picolinic acid **53** (27 g, 55 % yield).

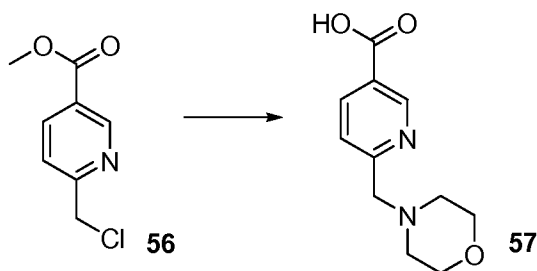
Example 10. Preparation of 6-(morpholinomethyl)nicotinic acid (57):

Step 1. Synthesis of methyl 6-(hydroxymethyl)nicotinate (55):

A mixture of dimethyl pyridine-2,5-dicarboxylate (**54**; 100.0 g, 0.51 mol), CaCl_2 (227.4 g, 2.05 mol), THF (1100 mL) and EtOH (1200 mL) was stirred for 30 min, NaBH_4 (48.6 g, 1.28 mol) was added portionwise at 0°C . The mixture was stirred for 18 hours. Saturated NH_4Cl solution (1.5 L) and water (2.0L) was added slowly, and the resulting mixture was extracted with dichloromethane (3 x 3.0 L). The combined organic solvent was dried over MgSO_4 and concentrated to give methyl 6-(hydroxymethyl)nicotinate **55** (82.0 g, 96%).

Step 2. Synthesis of Methyl 6-(chloromethyl)nicotinate (56):

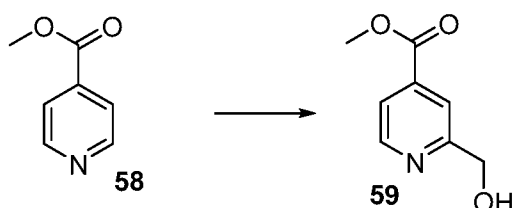
To a solution of methyl 6-(hydroxymethyl)nicotinate (**55**; 83.0 g, 0.497 mol) in dichloromethane (400 mL) at 0°C was added SOCl_2 (119.0 g, 1.0 mol). The mixture was stirred at room temperature for 2 hours, concentrated, and the residue was neutralized with aqueous NaHCO_3 (sat). The mixture was extracted with ethyl acetate (3 x 250 mL), dried over Na_2SO_4 , concentrated and purified by chromatography on silica gel to give Methyl 6-(chloromethyl)nicotinate **56** (70.0 g)

Step 3. Synthesis of 6-(morpholinomethyl)nicotinic acid (57):

6-(morpholinomethyl)nicotinic acid **57** was prepared in two steps from methyl 6-(chloromethyl)nicotinate **56** by a procedure similar to that reported for 6-(pyrrolidin-1-ylmethyl)picolinic acid **52** in Example 9.

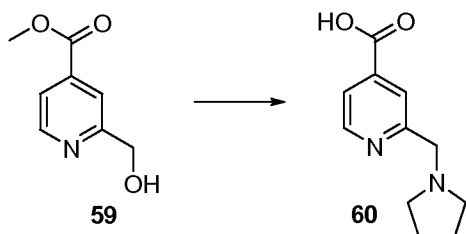
Example 11. Preparation of 2-(pyrrolidin-1-ylmethyl)isonicotinic acid (60):

5 **Step 1. Synthesis of methyl 2-(hydroxymethyl)isonicotinate (59):**



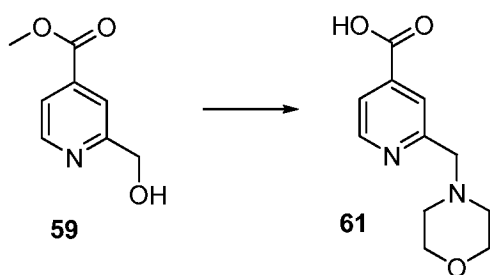
A solution of methyl isonicotinate (**58**; 70 grams 0.5 mol), ammonium peroxodisulfate (233 grams, 1.02 mol), and conc. sulfuric acid (5 mL) in methanol (600 mL) was refluxed until starting material was consumed. The reaction was
10 concentrated, water was added and the solution was neutralized to pH=9 with K₂CO₃. The resulting aqueous solution was extracted with ethyl acetate, and the extracts were concentrated, stirred with petroleum ether for 1 hour and the solids were collected by filtration to obtain 15 grams (18% yield) of 2-(hydroxymethyl)isonicotinate **59**.

15 **Step 2. Synthesis of 2-(pyrrolidin-1-ylmethyl)isonicotinic acid (60):**



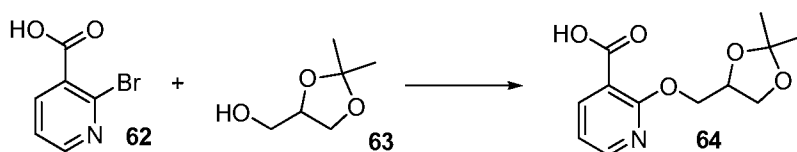
2-(pyrrolidin-1-ylmethyl)isonicotinic acid **60** was prepared in three steps from methyl 2-(hydroxymethyl)isonicotinate by a procedure similar to that reported for 6-(pyrrolidin-1-ylmethyl)picolinic acid **52** in Example 9.

20 **Example 12. Preparation of 2-(morpholinomethyl)isonicotinic acid (61):**



2-(morpholinomethyl)isonicotinic acid **61** was prepared in three steps from methyl 2-(hydroxymethyl)isonicotinate **59** by a procedure similar to that reported for 6-(pyrrolidin-1-ylmethyl)picolinic acid **52** in Example 9.

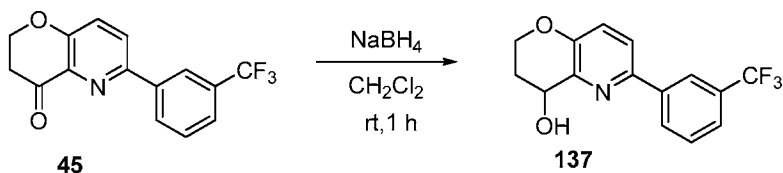
5 Example 13. Preparation of 2-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)nicotinic acid (64):



Solketal **63** (1.95 g, 15 mmol) was added to a room temperature suspension of 60% NaH (0.6 g, 15 mmol) in 1,4-dioxane (100 mL) at 0 °C. The reaction mixture was stirred at room temperature for 45 min and 2-bromo-nicotinic acid (**62**; 1 g, 5 mmol) was added. The reaction mixture was heated at reflux until the starting material was consumed. After cooling to room temperature, the solution was filtered. The filter cake was dissolved in water (100 mL), and acidified to pH 2-3. The resulting white solids were filtered, washed with water and dried to obtain the product **64** as a white solid (0.6 grams, 48% yield).

Example 14. Preparation of N-(6-morpholinopyridin-2-yl)-6-(3-(trifluoromethyl)phenyl)-3,4-dihydro-2H-pyrano[3,2-b]pyridine-4-carboxamide (Compound 302)

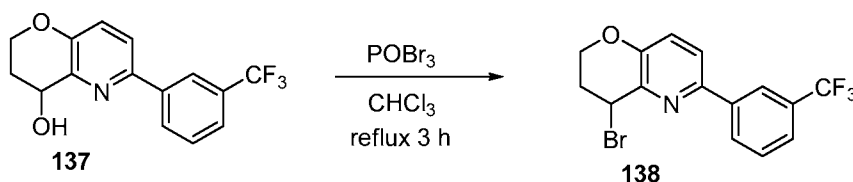
20 Step 1. Synthesis of 6-(3-(trifluoromethyl)phenyl)-3,4-dihydro-2H-pyrano[3,2-b]pyridin-4-ol (137):



Sodium borohydride (484mg, 12.79 mmol) was added to a stirred solution of 6-(3-(trifluoromethyl)phenyl)-2H-pyrano[3,2-b]pyridin-4(3H)-one (**45**, 2.5g, 8.53 mmol)

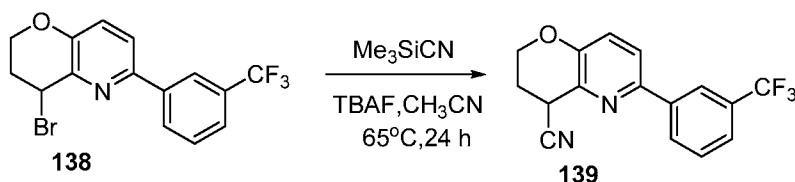
in 50 mL of methanol at room temperature for 1 hour. The mixture was concentrated in vacuo, and extracted with CH_2Cl_2 . The combined organic extracts were washed with brine, dried over Na_2SO_4 , and concentrated in vacuo to obtain 6-(3-(trifluoromethyl)phenyl)-3,4-dihydro-2H-pyrano[3,2-b]pyridin-4-ol (**137**) an oil, used in next step without any purification. MS (ESI) calcd for $\text{C}_{15}\text{H}_{12}\text{F}_3\text{NO}_2$ (m/z) 295.08, found: 296 [M+H].

Step 2. Synthesis of 4-bromo-6-(3-(trifluoromethyl)phenyl)-3,4-dihydro-2H-pyrano[3,2-b]pyridine (138**):**



Phosphorous tribromide (5.24 g, 18.29 mmol) was added dropwise to a solution of 6-(3-(trifluoromethyl)phenyl)-3,4-dihydro-2H-pyrano[3,2-b]pyridin-4-ol (**137**, 2.7 g, 9.14 mmol) in 50 mL of CHCl_3 . The mixture was heated at reflux for 3 hours, cooled to room temperature, poured into a sat. aqueous NaHCO_3 solution. The aqueous mixture was separated and the aqueous layer was extracted with CH_2Cl_2 . The combined organic extracts were washed with water, sat. aqueous NaHCO_3 , and brine. The organic solution was dried with Na_2SO_4 , filtered, concentrated in vacuo and purified by column chromatography (1:5 EtOAc/pet. ether) to give 2.5 g of 4-bromo-6-(3-(trifluoromethyl)phenyl)-3,4-dihydro-2H-pyrano[3,2-b]pyridine (**138**) as a colorless oil. MS (ESI) calcd for $\text{C}_{15}\text{H}_{11}\text{BrF}_3\text{NO}$ (m/z) 357.00, found 358 [M+H].

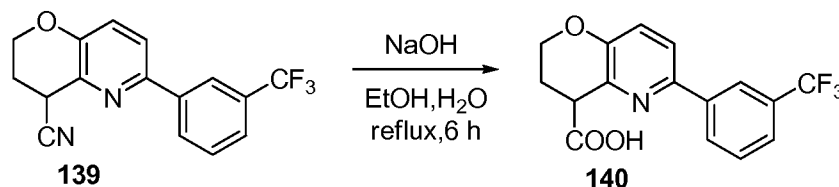
Step 3. Synthesis of 6-(3-(trifluoromethyl)phenyl)-3,4-dihydro-2H-pyrano[3,2-b]pyridine-4-carbonitrile (139**):**



A solution of Trimethylsilanecarbonitrile (1.385 g, 13.96 mmol) and TBAF (3.65 g, 13.96 mmol), and 4-bromo-6-(3-(trifluoromethyl)phenyl)-3,4-dihydro-2H-pyrano[3,2-b]pyridine (**138**, 2.5 g, 6.98 mmol) in 100 mL CH_3CN was heated to 65°C overnight. The reaction mixture was concentrated in vacuo, and the resulting

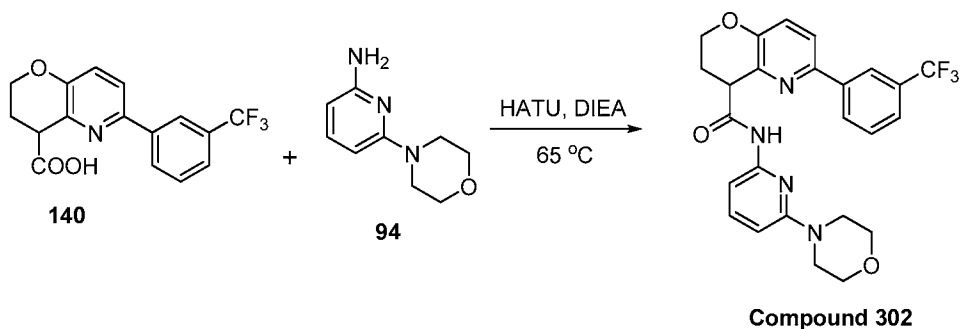
residue was purified by chromatography (1:5, EtOAc/Pet. ether) to afford 1.1 g of 6-(3-(trifluoromethyl)phenyl)-3,4-dihydro-2H-pyrano[3,2-b]pyridine-4-carbonitrile (**139**) as a white solid. MS (ESI) calcd for $C_{16}H_{11}F_3N_2O$ (m/z) 304.08, found 305 [M+H].

5 **Step 4. Synthesis of 6-(3-(trifluoromethyl)phenyl)-3,4-dihydro-2H-pyrano[3,2-b]pyridine-4-carboxylic acid (**140**):**



To a solution of 6-(3-(trifluoromethyl)phenyl)-3,4-dihydro-2H-pyrano[3,2-b]pyridine-4-carbonitrile (**139**, 1 g, 3.29 mmol) in 20 mL EtOH and 20 mL water, was added NaOH (1.315 g, 32.9 mmol). The reaction mixture was heated to reflux for 6 h. The EtOH was removed in vacuo, water (10 mL) was added, and the resulting mixture was adjusted to pH = 4 with ice cold 6 N HCl. The solid was collected by filtration and dried to obtain 720 mg of 6-(3-(trifluoromethyl)phenyl)-3,4-dihydro-2H-pyrano[3,2-b]pyridine-4-carboxylic acid (**140**). MS (ESI) calcd for $C_{16}H_{12}F_3NO_3$ (m/z) 323.08, found 324 [M+H].

Step 5. Preparation of N-(6-morpholinopyridin-2-yl)-6-(3-(trifluoromethyl)phenyl)-3,4-dihydro-2H-pyrano[3,2-b]pyridine-4-carboxamide (Compound **302)**



6-Morpholinopyridin-2-amine (**94**, 33.3 mg, 0.186 mmol) was dissolved in DMF (1 mL) along with 6-(3-(trifluoromethyl)phenyl)-3,4-dihydro-2H-pyrano[3,2-b]pyridine-4-carboxylic acid (**140**, 50 mg, 0.155 mmol), HATU (118 mg, 0.309 mmol) and DIPEA (40.0 mg, 0.309 mmol). The resulting reaction mixture was stirred at 65 °C for overnight. Water (10mL) was added and the solid was filtered to

give compound 302 as pale brown solid. MS (ESI) calcd for $C_{25}H_{23}F_3N_4O_3$ (m/z) 484.17, found 485 [M+H].

Example 15 Biological activity

A mass spectrometry based assay was used to identify modulators of SIRT1 activity. The mass spectrometry based assay utilizes a peptide having 20 amino acid residues as follows: Ac-EE-K(biotin)-GQSTSSHSK(Ac)NleSTEG-K(5TMR)-EE-NH₂ (SEQ ID NO: 1) wherein K(Ac) is an acetylated lysine residue and Nle is a norleucine. The peptide is labeled with the fluorophore 5TMR (excitation 540 nm/emission 580 nm) at the C-terminus. The sequence of the peptide substrate is based on p53 with several modifications. In addition, the methionine residue naturally present in the sequence was replaced with the norleucine because the methionine may be susceptible to oxidation during synthesis and purification.

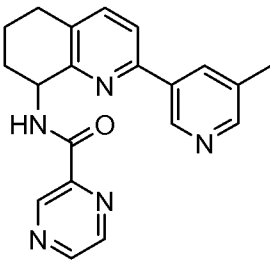
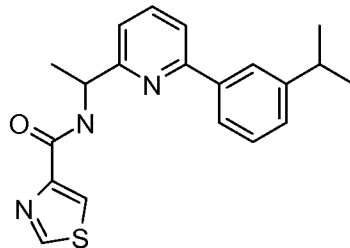
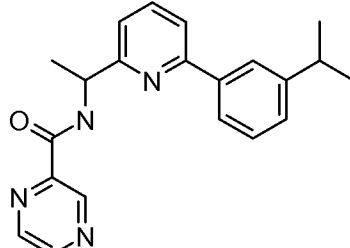
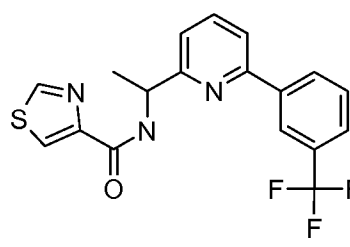
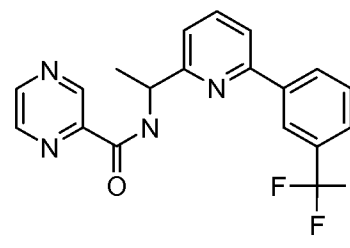
The mass spectrometry assay is conducted as follows: 0.5 μ M peptide substrate and 120 μ M β NAD⁺ is incubated with 10 nM SIRT1 for 25 minutes at 25°C in a reaction buffer (50 mM Tris-acetate pH 8, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂, 5 mM DTT, 0.05% BSA). Test compounds may be added to the reaction as described above. The Sirt1 gene is cloned into a T7-promoter containing vector and transformed into BL21(DE3). After the 25 minute incubation with SIRT1, 10 μ L of 10% formic acid is added to stop the reaction. Reactions are sealed and frozen for later mass spec analysis. Determination of the mass of the substrate peptide allows for precise determination of the degree of acetylation (i.e. starting material) as compared to deacetylated peptide (product).

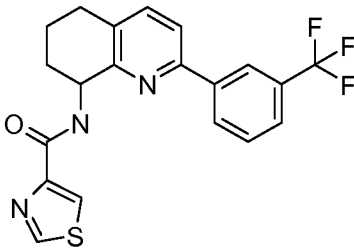
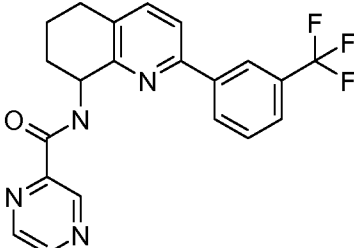
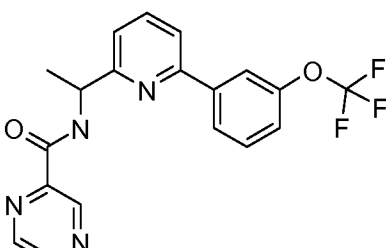
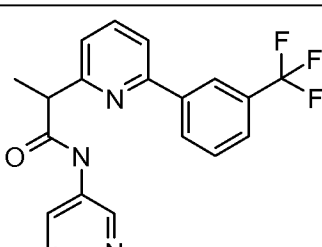
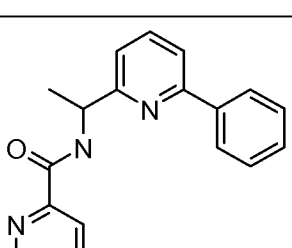
A control for inhibition of sirtuin activity is conducted by adding 1 μ L of 500 mM nicotinamide as a negative control at the start of the reaction (e.g., permits determination of maximum sirtuin inhibition). A control for activation of sirtuin activity is conducted using 10 nM of sirtuin protein, with 1 μ L of DMSO in place of compound, to determine the amount of deacetylation of the substrate at a given timepoint within the linear range of the assay. This timepoint is the same as that used for test compounds and, within the linear range, the endpoint represents a change in velocity.

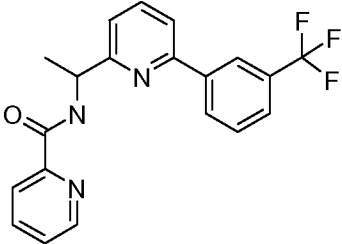
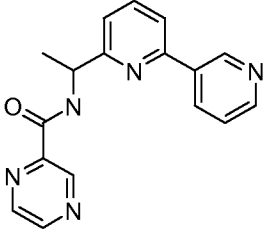
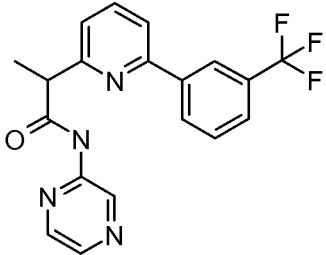
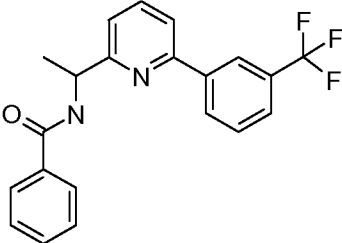
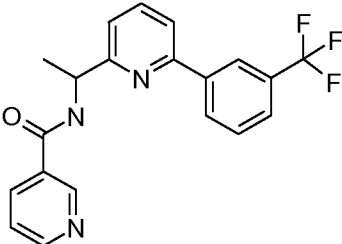
For the above assay, SIRT1 protein was expressed and purified as follows. The SirT1 gene was cloned into a T7-promoter containing vector and transformed into BL21(DE3). The protein was expressed by induction with 1 mM IPTG as an N-terminal His-tag fusion protein at 18°C overnight and harvested at 30,000 x g. Cells
5 were lysed with lysozyme in lysis buffer (50 mM Tris-HCl, 2 mM Tris[2-carboxyethyl] phosphine (TCEP), 10 μ M ZnCl₂, 200 mM NaCl) and further treated with sonication for 10 min for complete lysis. The protein was purified over a Ni-NTA column (Amersham) and fractions containing pure protein were pooled, concentrated and run over a sizing column (Sephadex S200 26/60 global). The peak
10 containing soluble protein was collected and run on an Ion-exchange column (MonoQ). Gradient elution (200 mM - 500 mM NaCl) yielded pure protein. This protein was concentrated and dialyzed against dialysis buffer (20 mM Tris-HCl, 2 mM TCEP) overnight. The protein was aliquoted and frozen at -80°C until further use.

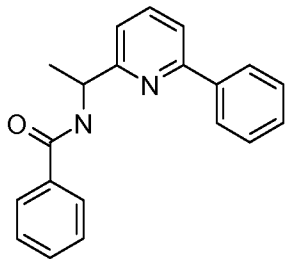
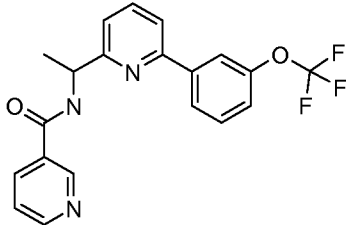
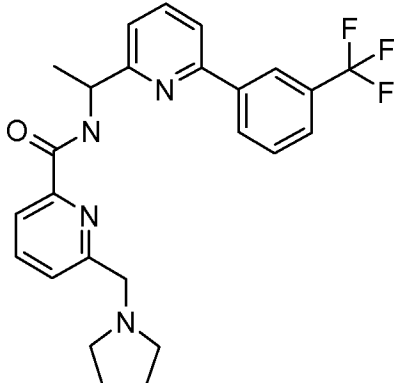
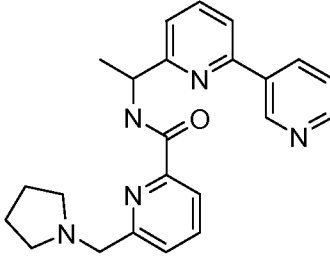
15 Sirtuin modulating compounds that activated SIRT1 were identified using the assay described above and are shown below in Table 1 for compounds of Formula (III)). The EC_{1.5} values represent the concentration of test compounds that result in 150% activation of SIRT1. The EC_{1.5} values for the activating compounds in Table 1 are represented by A (EC_{1.5} <10.0 μ M), B (EC_{1.5} 10-25 μ M), C (EC_{1.5}
20 >25 μ M). The percent maximum fold activation is represented by A (Fold activation >200%) or B (Fold Activation <200%); "NT" means not tested; "ND" mean not determinable.

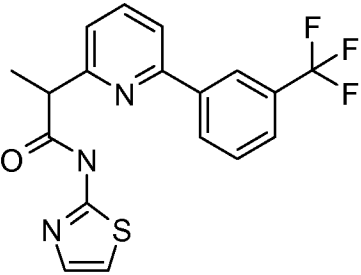
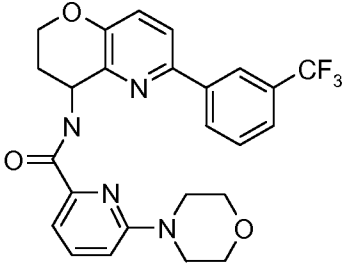
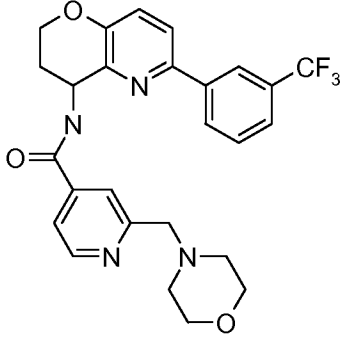
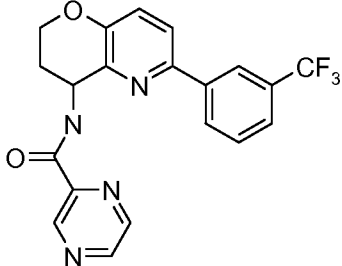
Table 1. Compounds of Formula (III).

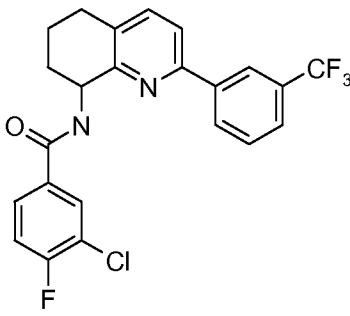
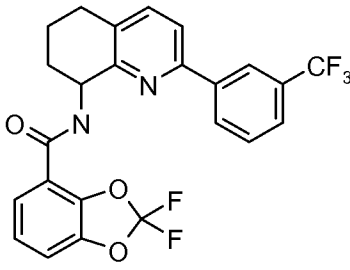
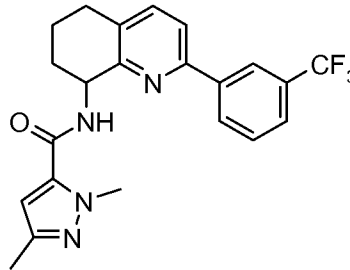
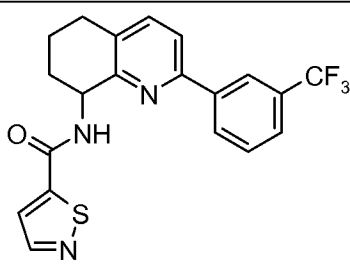
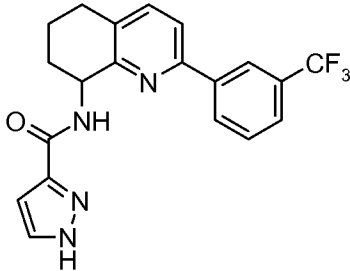
COMPOUND No.	[M+H] ⁺	STRUCTURE	EC _{1.5} μM	% FOLD ACT.
200	346		C	ND
201	352		C	B
202	347		C	B
203	378		B	B
204	373		B	A

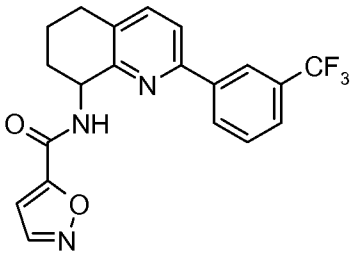
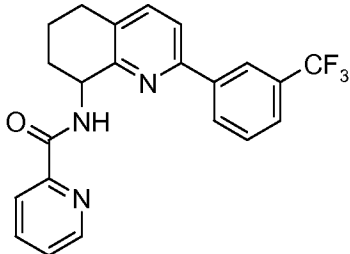
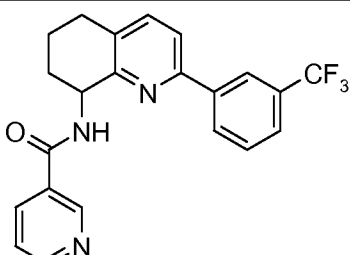
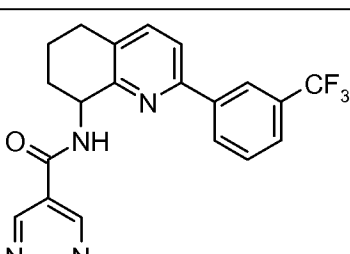
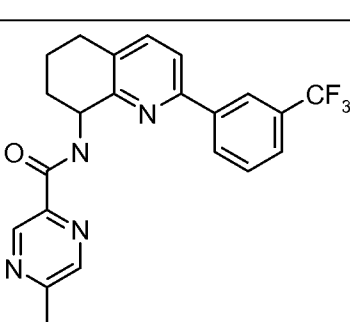
COMPOUND No.	[M+H] ⁺	STRUCTURE	EC _{1.5} uM	% FOLD ACT.
205	404		C	B
206	399		A	A
207	389		B	A
210	372		C	ND
211	305		C	ND

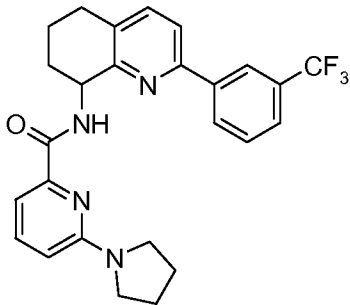
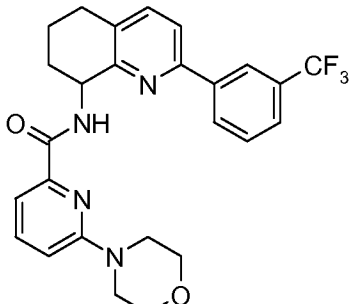
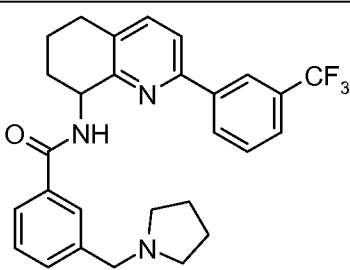
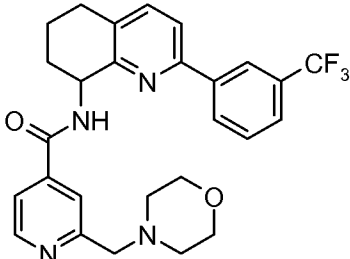
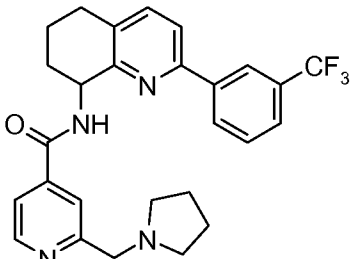
COMPOUND No.	[M+H] ⁺	STRUCTURE	EC _{1.5} uM	% FOLD ACT.
212	372		A	A
213	306		C	ND
214	373		C	ND
215	371		C	ND
216	372		C	ND

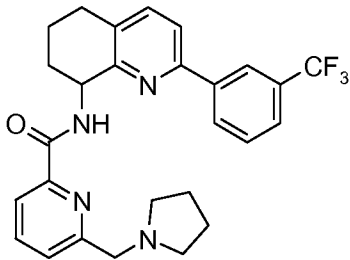
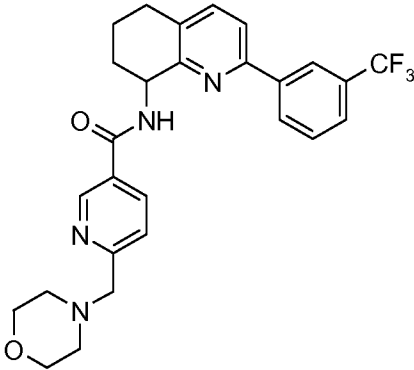
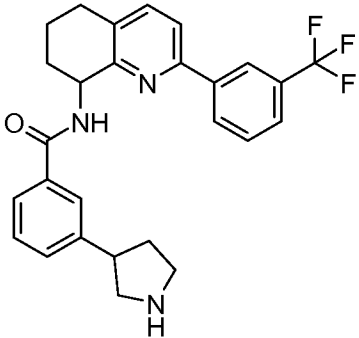
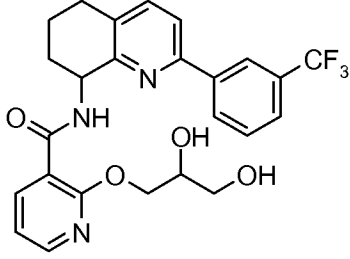
COMPOUND No.	[M+H] ⁺	STRUCTURE	EC _{1.5} uM	% FOLD ACT.
217	303		C	ND
218	388		C	ND
219	455		C	ND
220	388		C	ND

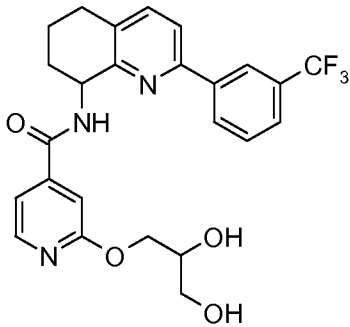
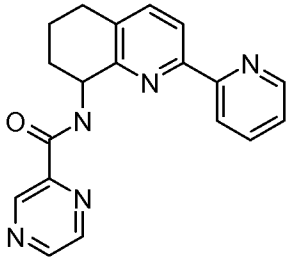
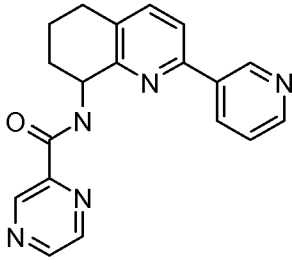
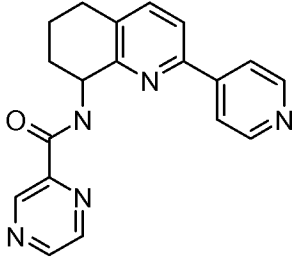
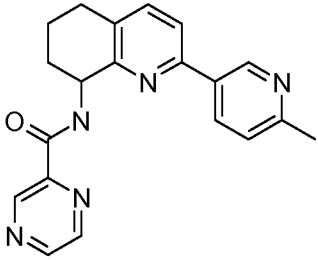
COMPOUND No.	[M+H] ⁺	STRUCTURE	EC _{1.5} uM	% FOLD ACT.
221	378		C	ND
222	485		A	A
223	499		C	ND
224	401		C	ND

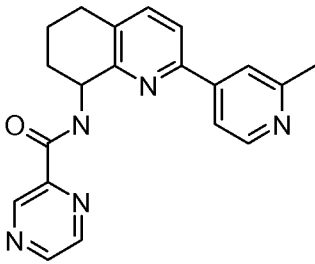
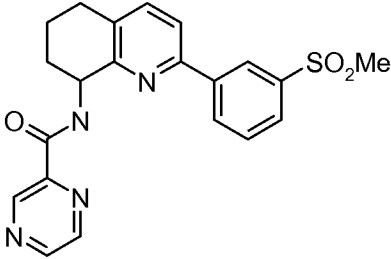
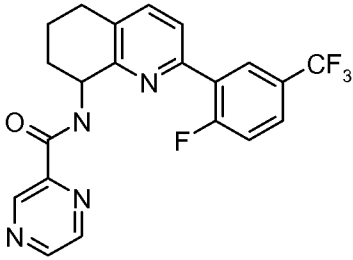
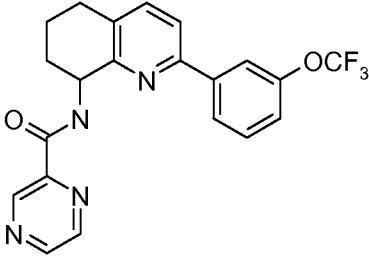
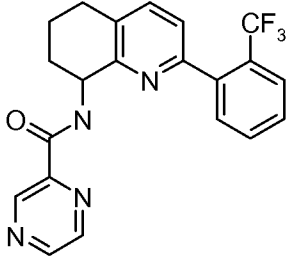
COMPOUND No.	[M+H] ⁺	STRUCTURE	EC _{1.5} uM	% FOLD ACT.
225	449		C	ND
226	477		C	ND
227	415		A	A
228	404		C	ND
229	387		B	B

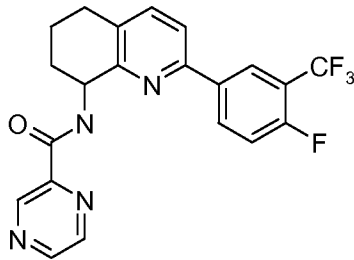
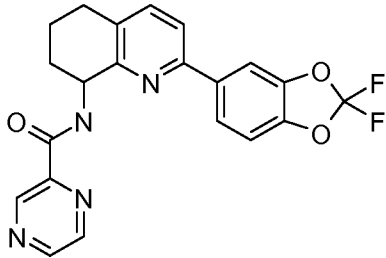
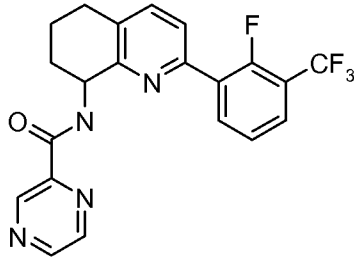
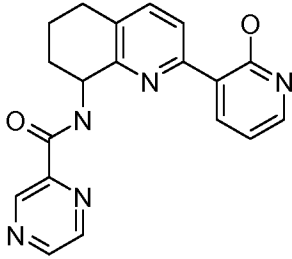
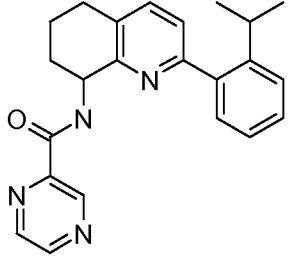
COMPOUND No.	[M+H] ⁺	STRUCTURE	EC _{1.5} uM	% FOLD ACT.
230	388		C	ND
231	398		A	A
232	398		C	B
233	399		C	ND
234	413		A	A

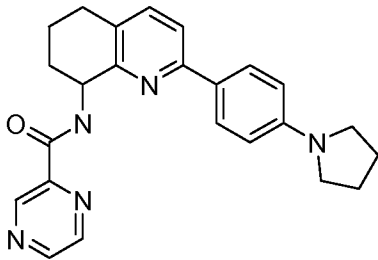
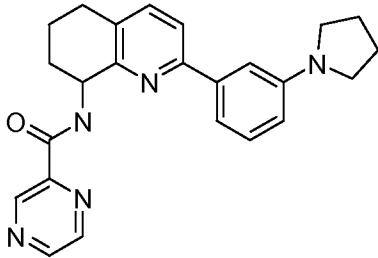
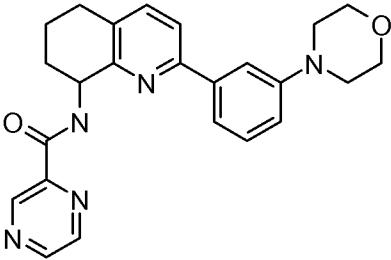
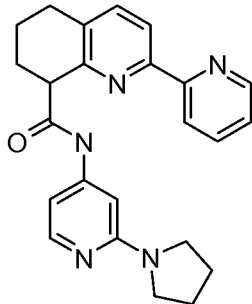
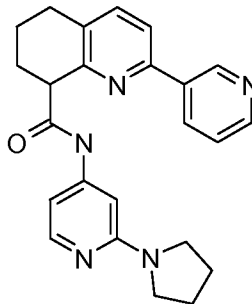
COMPOUND No.	[M+H] ⁺	STRUCTURE	EC _{1.5} uM	% FOLD ACT.
235	467		A	B
236	483		A	A
237	480		B	B
238	497		C	ND
239	481		C	ND

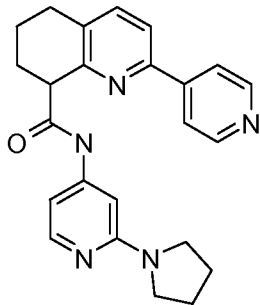
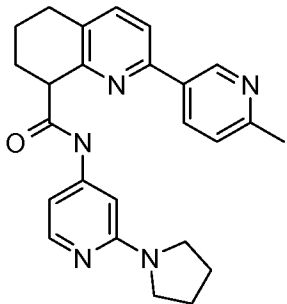
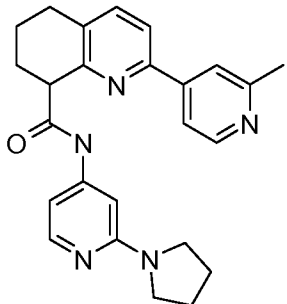
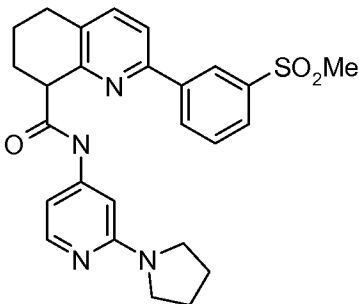
COMPOUND No.	[M+H] ⁺	STRUCTURE	EC _{1.5} uM	% FOLD ACT.
240	481		B	B
241	497		C	ND
242	466		A	A
243	488		C	ND

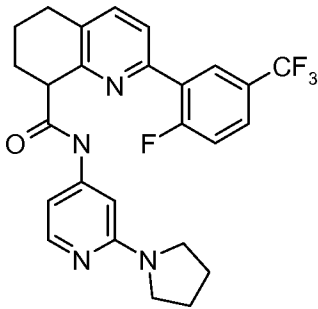
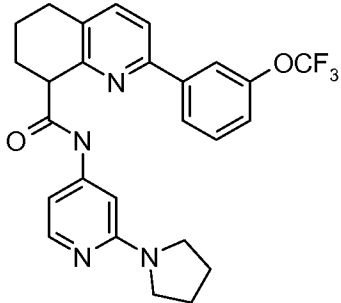
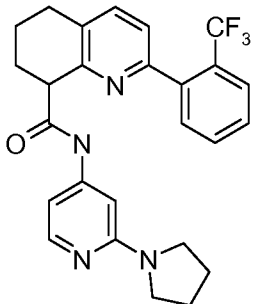
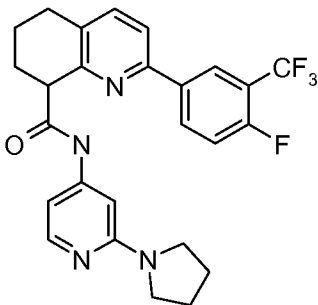
COMPOUND No.	[M+H] ⁺	STRUCTURE	EC _{1.5} uM	% FOLD ACT.
244	488		A	A
245	332		NT	NT
246	332		C	ND
247	332		C	ND
248	346		C	ND

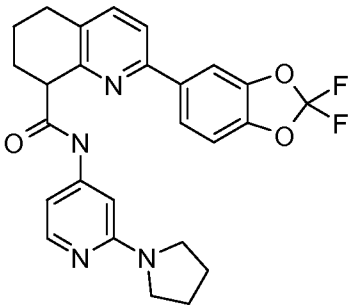
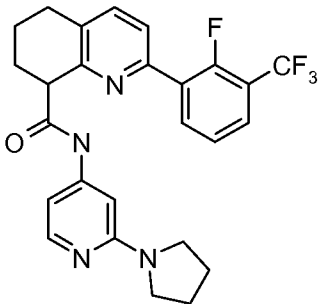
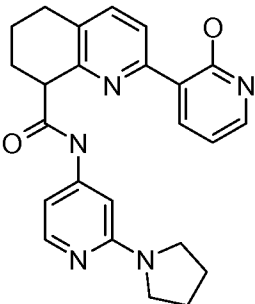
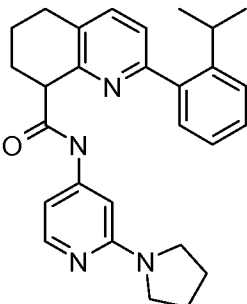
COMPOUND No.	[M+H] ⁺	STRUCTURE	EC _{1.5} uM	% FOLD ACT.
249	346		C	ND
250	409		C	ND
251	417		A	A
252	415		B	A
253	399		C	ND

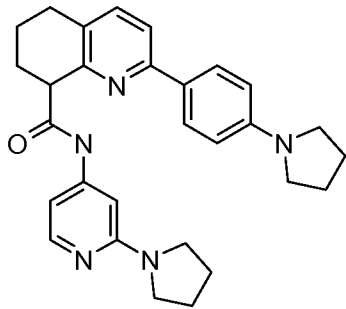
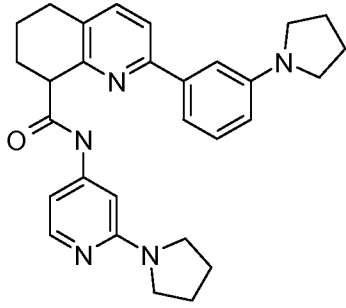
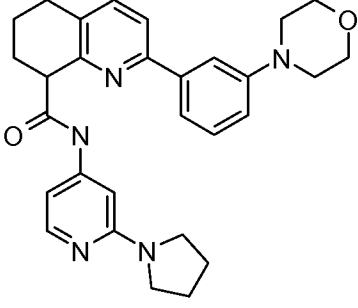
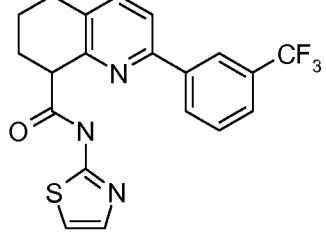
COMPOUND No.	[M+H] ⁺	STRUCTURE	EC _{1.5} uM	% FOLD ACT.
254	417		A	B
255	411		NT	NT
256	417		C	ND
257	348		C	ND
258	373		C	ND

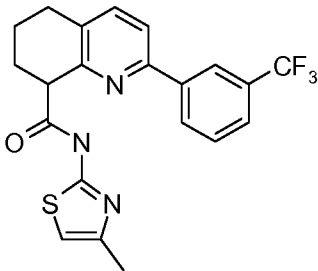
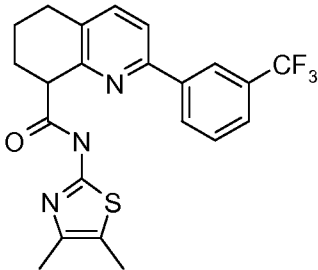
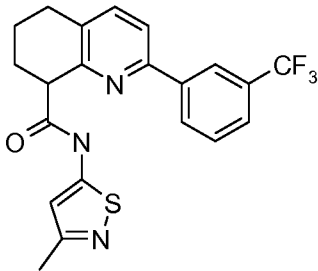
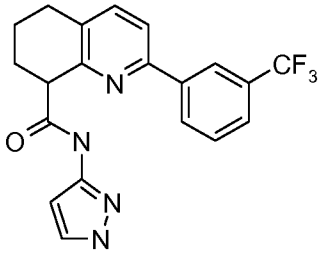
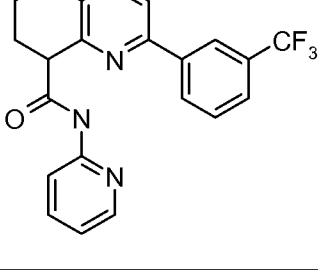
COMPOUND No.	[M+H] ⁺	STRUCTURE	EC _{1.5} uM	% FOLD ACT.
259	400		C	ND
260	400		C	ND
261	416		C	ND
262	400		NT	NT
263	400		NT	NT

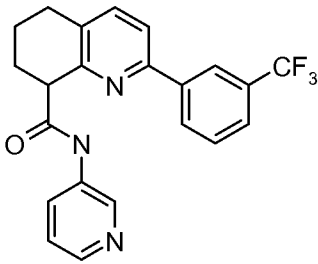
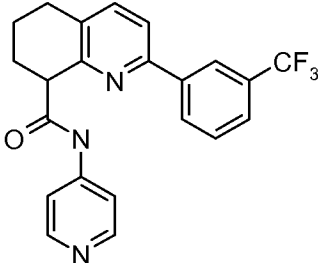
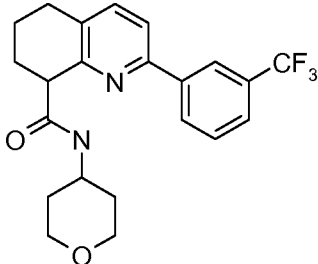
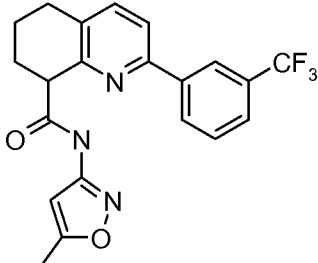
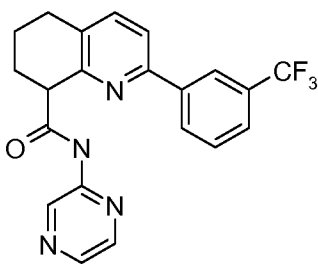
COMPOUND No.	[M+H] ⁺	STRUCTURE	EC _{1.5} uM	% FOLD ACT.
264	400		NT	NT
265	414		NT	NT
266	414		NT	NT
267	477		NT	NT

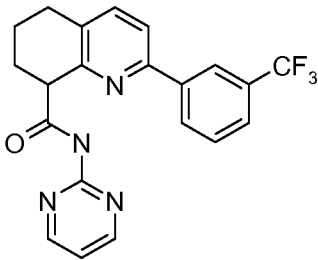
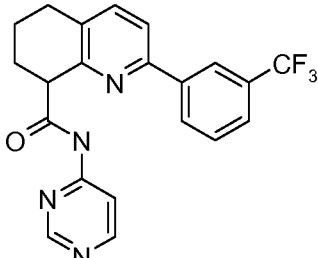
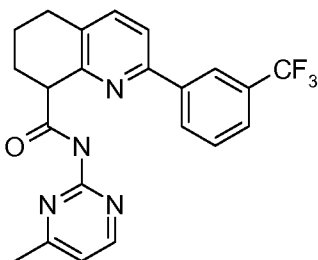
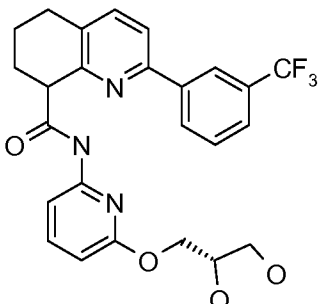
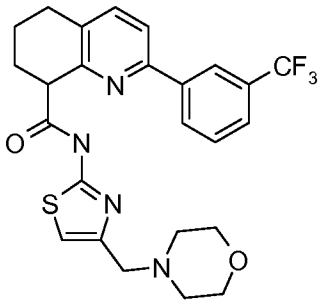
COMPOUND No.	[M+H] ⁺	STRUCTURE	EC _{1.5} uM	% FOLD ACT.
268	485		NT	NT
269	483		NT	NT
270	467		NT	NT
271	485		NT	NT

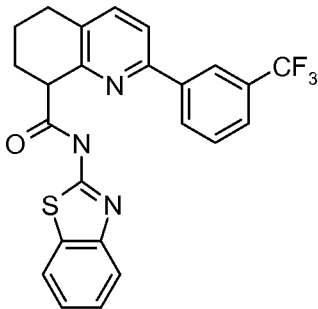
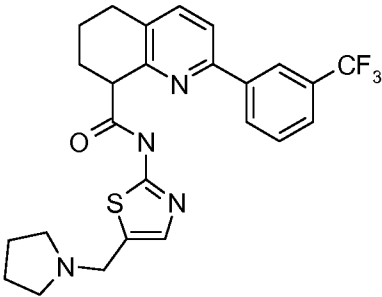
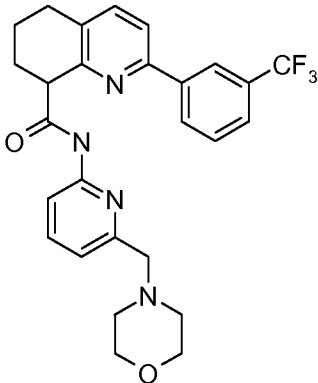
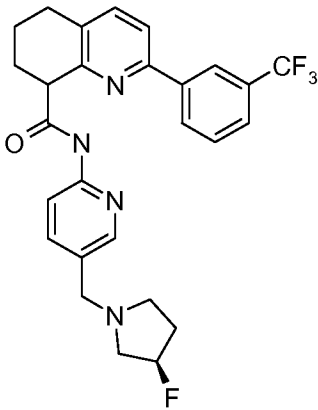
COMPOUND No.	[M+H] ⁺	STRUCTURE	EC _{1.5} uM	% FOLD ACT.
272	479		NT	NT
273	485		C	ND
274	416		NT	NT
275	441		NT	NT

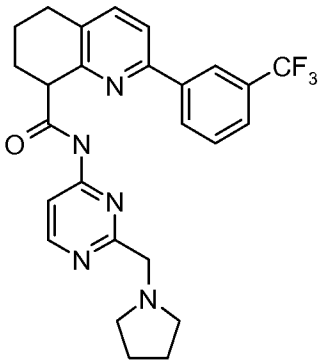
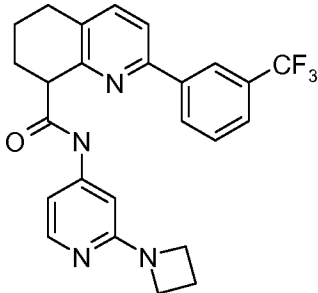
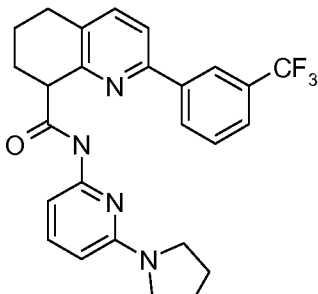
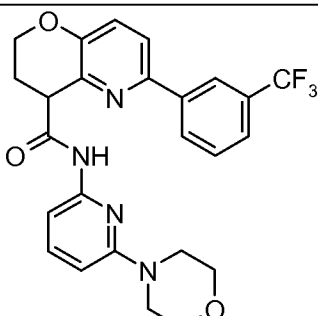
COMPOUND No.	[M+H] ⁺	STRUCTURE	EC _{1.5} uM	% FOLD ACT.
276	468		NT	NT
277	468		NT	NT
278	484		A	B
279	404		C	ND

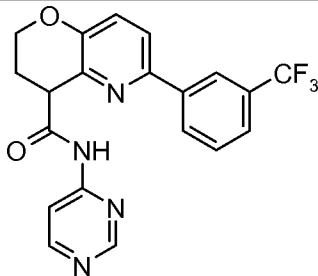
COMPOUND No.	[M+H] ⁺	STRUCTURE	EC _{1.5} uM	% FOLD ACT.
280	418		NT	NT
281	432		C	ND
282	418		B	B
283	387		NT	NT
284	398		C	ND

COMPOUND No.	[M+H] ⁺	STRUCTURE	EC _{1.5} uM	% FOLD ACT.
285	398		C	ND
286	398		C	ND
287	405		C	ND
288	402		C	ND
289	399		B	B

COMPOUND No.	[M+H] ⁺	STRUCTURE	EC _{1.5} uM	% FOLD ACT.
290	399		NT	NT
291	399		NT	NT
292	413		C	ND
293	488		NT	NT
294	503		A	A

COMPOUND No.	[M+H] ⁺	STRUCTURE	EC _{1.5} uM	% FOLD ACT.
295	454		C	ND
296	487		C	ND
297	497		C	ND
298	499		NT	NT

COMPOUND No.	[M+H] ⁺	STRUCTURE	EC _{1.5} uM	% FOLD ACT.
299	482		NT	NT
300	453		NT	NT
301	467		NT	NT
302	485		C	ND

COMPOUND No.	[M+H] ⁺	STRUCTURE	EC _{1.5} uM	% FOLD ACT.
303	401		C	ND

In certain embodiments, the compound of this invention is selected from any one of Compound Numbers 206, 212, 222, 227, 231, 234, 235, 236, 242, 244, 251, 278 and 294.

5

EQUIVALENTS

The present invention provides among other things sirtuin-activating compounds and methods of use thereof. While specific embodiments of the subject invention have been discussed, the above specification is illustrative and not
 10 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.

15 INCORPORATION BY REFERENCE

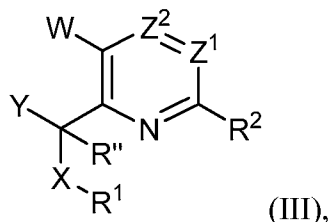
All publications and patents mentioned herein, including those items listed below, are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions
 20 herein, will control.

Also incorporated by reference in their entirety are any polynucleotide and 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 represented by Structural Formula III:



a tautomer, or a salt thereof, wherein:

each of Z^1 and Z^2 is independently selected from N and CR, wherein:

at least one of Z^1 and Z^2 is CR; and

each R is independently selected from hydrogen, halo, -OH, -C≡N, fluoro-substituted C₁-C₂ alkyl, -O-(C₁-C₂) fluoro-substituted alkyl, -S-(C₁-C₂) fluoro-substituted alkyl, C₁-C₄ alkyl, -O-(C₁-C₄) alkyl, -S-(C₁-C₄) alkyl; C₃-C₇ cycloalkyl -(C₁-C₂) alkyl-N(R³)(R³), -O-CH₂CH(OH)CH₂OH, -O-(C₁-C₃) alkyl-N(R³)(R³), and -N(R³)(R³);

R'' is selected from hydrogen and C₁-C₄ alkyl optionally substituted with one or more substituents independently selected from halo, -C≡N, C₁-C₄ alkyl, =O, C₃-C₇ cycloalkyl, fluoro-substituted C₁-C₂ alkyl, -O-R³, -S-R³, -(C₁-C₄ alkyl)-N(R³)(R³), -N(R³)(R³), -O-(C₁-C₄ alkyl)-N(R³)(R³), -(C₁-C₄ alkyl)-O-(C₁-C₄ alkyl)-N(R³)(R³), -C(O)-N(R³)(R³), and -(C₁-C₄ alkyl)-C(O)-N(R³)(R³);

R¹ is selected from a carbocycle and a heterocycle, wherein R¹ is optionally substituted with one or more substituents independently selected from halo, -C≡N, C₁-C₄ alkyl, =O, C₃-C₇ cycloalkyl, fluoro-substituted C₁-C₄ alkyl, -O-R³, -S-R³, -(C₁-C₄ alkyl)-N(R³)(R³), -N(R³)(R³), -O-(C₁-C₄ alkyl)-N(R³)(R³), -(C₁-C₄ alkyl)-O-(C₁-C₄ alkyl)-N(R³)(R³), -C(O)-N(R³)(R³), -(C₁-C₄ alkyl)-C(O)-N(R³)(R³), and a 5- or 6-membered saturated heterocycle and when R¹ is phenyl, R¹ is also optionally substituted with O-(saturated heterocycle), -O-(fluoro-substituted saturated heterocycle), C₁-C₄ alkyl-substituted saturated heterocycle, 3,4-methylenedioxy, fluoro-substituted 3,4-methylenedioxy, 3,4-ethylenedioxy, or fluoro-substituted 3,4-ethylenedioxy, wherein

each R³ is independently selected from hydrogen, and -C₁-C₄ alkyl; or two R³ are taken together with the nitrogen atom to which they are bound to form a 4- to

8-membered saturated heterocycle optionally comprising one additional heteroatom selected from NH, S, S(=O), S(=O)₂, and O, wherein:

when R³ is alkyl, the alkyl is optionally substituted with one or more substituents selected from -OH, fluoro, -NH₂, -NH(C₁-C₄ alkyl), -N(C₁-C₄ alkyl)₂, -NH(CH₂CH₂OCH₃), and -N(CH₂CH₂OCH₃)₂ and

when two R³ are taken together with the nitrogen atom to which they are bound to form a 4- to 8-membered saturated heterocycle, the saturated heterocycle is optionally substituted at any carbon atom with -OH, -C₁-C₄ alkyl, fluoro, -NH₂, -NH(C₁-C₄ alkyl), -N(C₁-C₄ alkyl)₂, -NH(CH₂CH₂OCH₃), or -N(CH₂CH₂OCH₃)₂; and optionally substituted at any substitutable nitrogen atom with -C₁-C₄ alkyl, fluoro-substituted C₁-C₄ alkyl, or -(CH₂)₂-O-CH₃;

R² is selected from a carbocycle and a heterocycle, wherein R² is optionally substituted with one or more substituents independently selected from halo, -C≡N, C₁-C₄ alkyl, C₃-C₇ cycloalkyl, C₁-C₂ fluoro-substituted alkyl, -O-R³, -S-R³, -S(O)-R³, -S(O)₂-R³, -(C₁-C₄ alkyl)-N(R³)(R³), -N(R³)(R³), -O-(C₁-C₄ alkyl)-N(R³)(R³), -(C₁-C₄ alkyl)-O-(C₁-C₄ alkyl)-N(R³)(R³), -C(O)-N(R³)(R³), -(C₁-C₄ alkyl)-C(O)-N(R³)(R³), -O-phenyl, phenyl, and a second heterocycle, and when R² is phenyl, R² is also optionally substituted with -O-(saturated heterocycle), 3,4-methylenedioxy, fluoro-substituted 3,4-methylenedioxy, 3,4-ethylenedioxy, or fluoro-substituted 3,4-ethylenedioxy, wherein any phenyl, saturated heterocycle, or second heterocycle substituent of R² is optionally substituted with halo; -C≡N, C₁-C₄ alkyl, fluoro-substituted C₁-C₂ alkyl, -O-(C₁-C₂) fluoro-substituted alkyl, -O-(C₁-C₄) alkyl, -S-(C₁-C₄) alkyl, -S-(C₁-C₂) fluoro-substituted alkyl, -NH-(C₁-C₄) alkyl, and -N-(C₁-C₄)₂ alkyl;

X is selected from -NH-C(=O)-†, -C(=O)-NH-†, -NH-C(=S)-†, -C(=S)-NH-†, -NH-S(=O)-†, -S(=O)-NH-†, -S(=O)₂-NH-†, -NH-S(=O)₂-†, -NH-S(O)₂-NR⁴-†, -NR⁴-S(O)₂-NH-†, -NH-C(=O)O-†, -OC(=O)NH-†, -NH-C(=O)NR⁴-†, -NR⁴-C(=O)NH-†, -NH-NR⁴-†, -NR⁴-NH-†, -O-NH-†, -NH-O-†, -NH-CR⁴R⁵-†, -CR⁴R⁵-NH-†, -NH-C(=NR⁴)-†, -C(=NR⁴)-NH-†, -C(=O)-NH-CR⁴R⁵-†, -NH-C(=O)-CR⁴R⁵-†, -CR⁴R⁵-NH-C(O)-†, -NH-C(=S)-CR⁴R⁵-†, -CR⁴R⁵-C(=S)-NH-†, -NH-S(O)-CR⁴R⁵-†,

$-\text{CR}^4\text{R}^5-\text{S}(\text{O})-\text{NH}-\dagger$, $-\text{NH}-\text{S}(\text{O})_2-\text{CR}^4\text{R}^5-\dagger$, $-\text{CR}^4\text{R}^5-\text{S}(\text{O})_2-\text{NH}-\dagger$,
 $-\text{NH}-\text{C}(=\text{O})-\text{O}-\text{CR}^4\text{R}^5-\dagger$, $-\text{CR}^4\text{R}^5-\text{O}-\text{C}(=\text{O})-\text{NH}-\dagger$, $-\text{NH}-\text{C}(=\text{O})-\text{NR}^4-\text{CR}^4\text{R}^5-\dagger$, and
 $-\text{CR}^4\text{R}^5-\text{NH}-\text{C}(=\text{O})-\text{O}-\dagger$, wherein:

$\dagger$ represents where X is bound to R^1 ; and

each R^4 and R^5 is independently selected from hydrogen, $\text{C}_1\text{-C}_4$ alkyl,
 $-\text{CF}_3$ and $(\text{C}_1\text{-C}_3 \text{ alkyl})-\text{CF}_3$; and

W is selected from hydrogen, $\text{C}_1\text{-C}_4$ alkyl and fluoro-substituted $\text{C}_1\text{-C}_4$ alkyl;

and

Y is selected from $\text{C}_1\text{-C}_4$ alkyl and fluoro-substituted $\text{C}_1\text{-C}_4$ alkyl;

or

W and Y are bound to one another to form a 5- to 7-membered ring, wherein:

W is selected from $-\text{O}-$, $-\text{NH}-$, $-\text{N}(\text{C}_1\text{-C}_4 \text{ alkyl})-$, $-\text{S}-$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2$ and

$-\text{C}(\text{R}^6)(\text{R}^6)-$, and

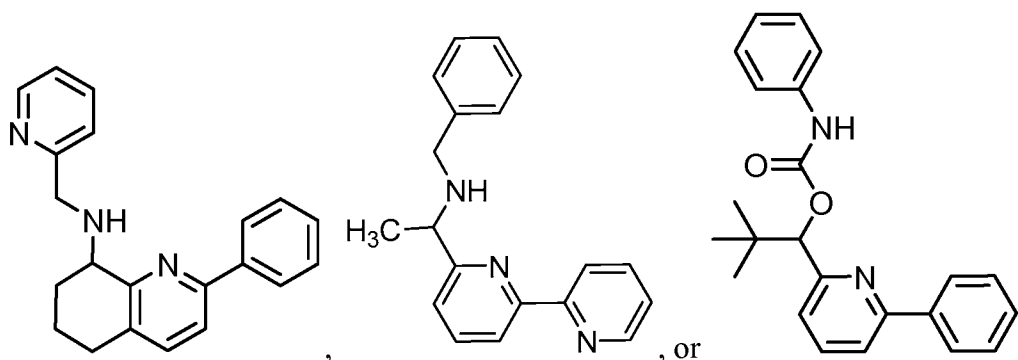
Y is $(-\text{C}(\text{R}^6)(\text{R}^6)-)_{1-3}$, and

each R^6 is independently selected from hydrogen, $\text{C}_1\text{-C}_4$ alkyl and
fluoro-substituted $\text{C}_1\text{-C}_4$ alkyl, or two R^6 bound to the same carbon atom are
taken together to form $=\text{O}$, wherein:

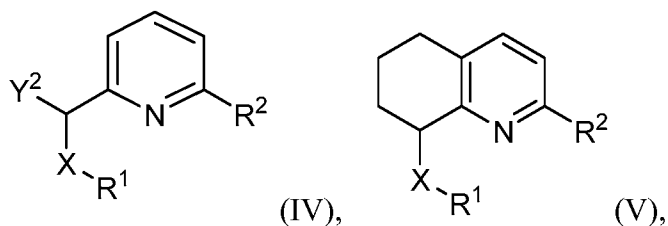
when each of Z^1 and Z^2 is $-\text{CH}-$, W is hydrogen, Y is $\text{C}_1\text{-C}_4$ alkyl, X is
 $-\text{NH}-\text{CR}^4\text{R}^5-\dagger$ and R^2 is unsubstituted phenyl, R^1 is other than unsubstituted phenyl,
or unsubstituted pyridin-2-yl;

when each of Z^1 and Z^2 is $-\text{CH}-$, W is hydrogen, Y is $\text{C}_1\text{-C}_4$ alkyl, X is
 $-\text{NH}-\text{S}(\text{O})-\dagger$ and R is 4-methylphenyl, then R^2 is not unsubstituted phenyl or
unsubstituted morpholin-4-yl; and

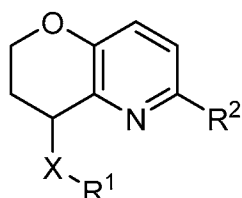
the compound is not:



2. The compound of claim 1, wherein R'' is hydrogen.
3. The compound of claim 2, selected from a compound having one of the

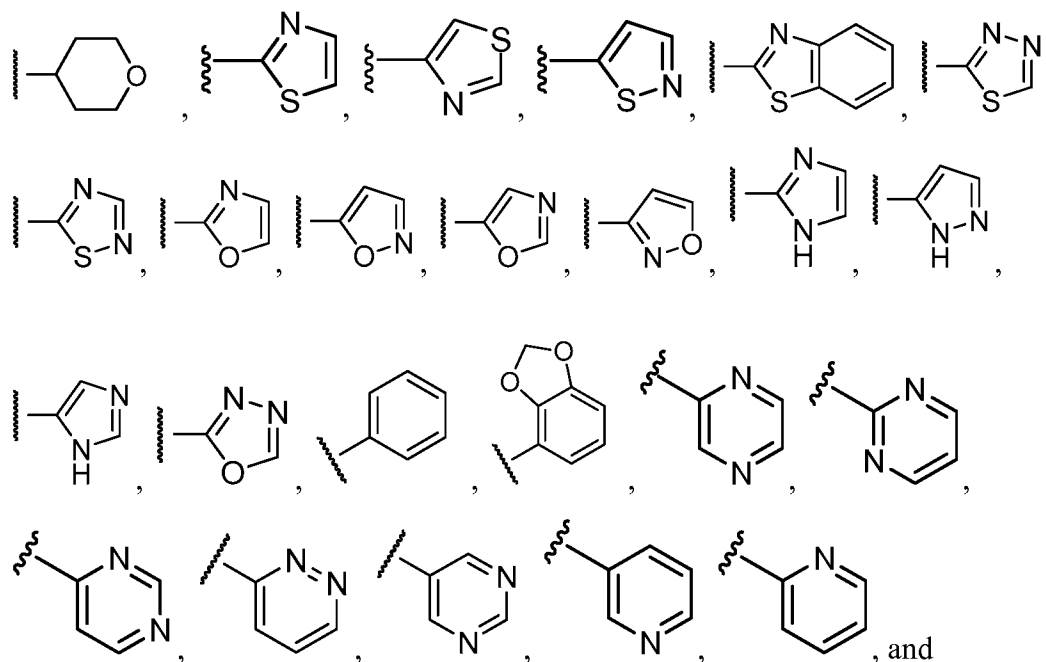


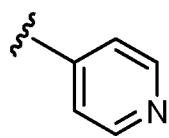
following Structural Formula:



and  (VI), wherein X, R¹ and R² are as defined for a compound of Structural Formula (III); and Y² is methyl.

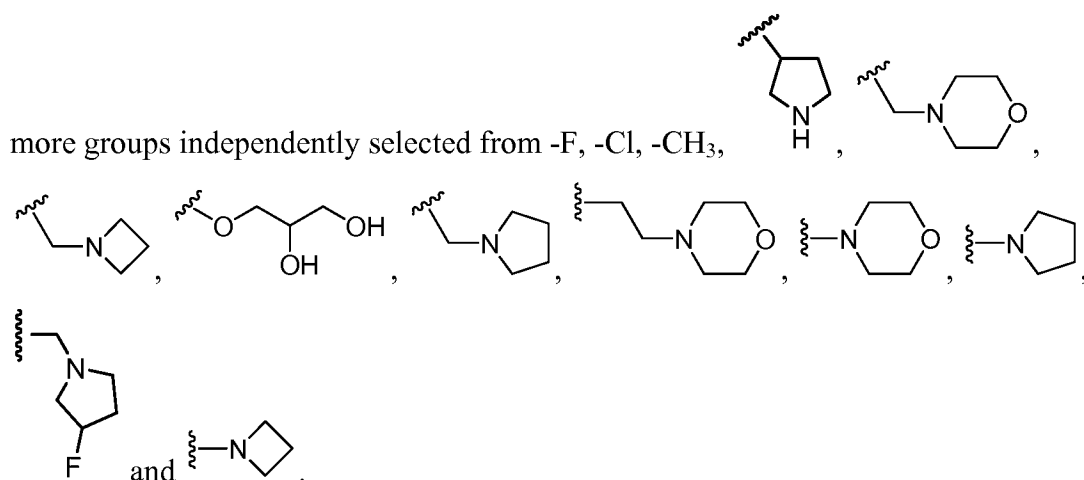
4. The compound of any one of claims 1 to 3, wherein X is selected from -NH-C(=O)-† and -C(=O)-NH-†.
5. The compound of any one of claims 1 to 4, wherein R¹ is selected from:



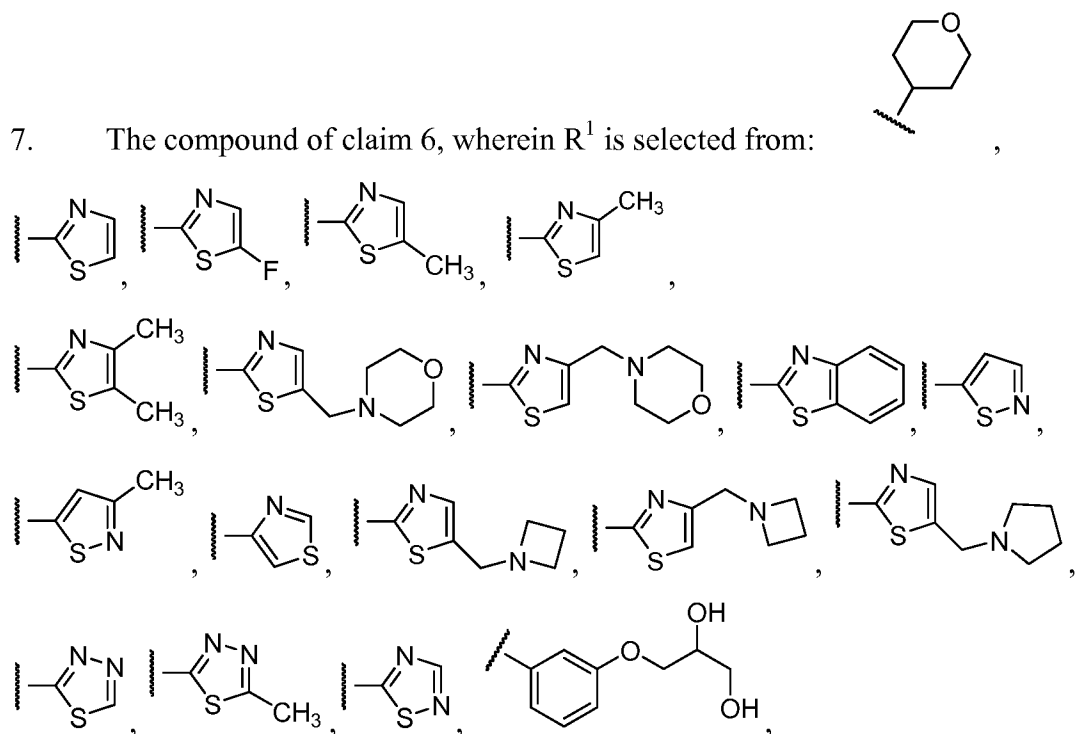


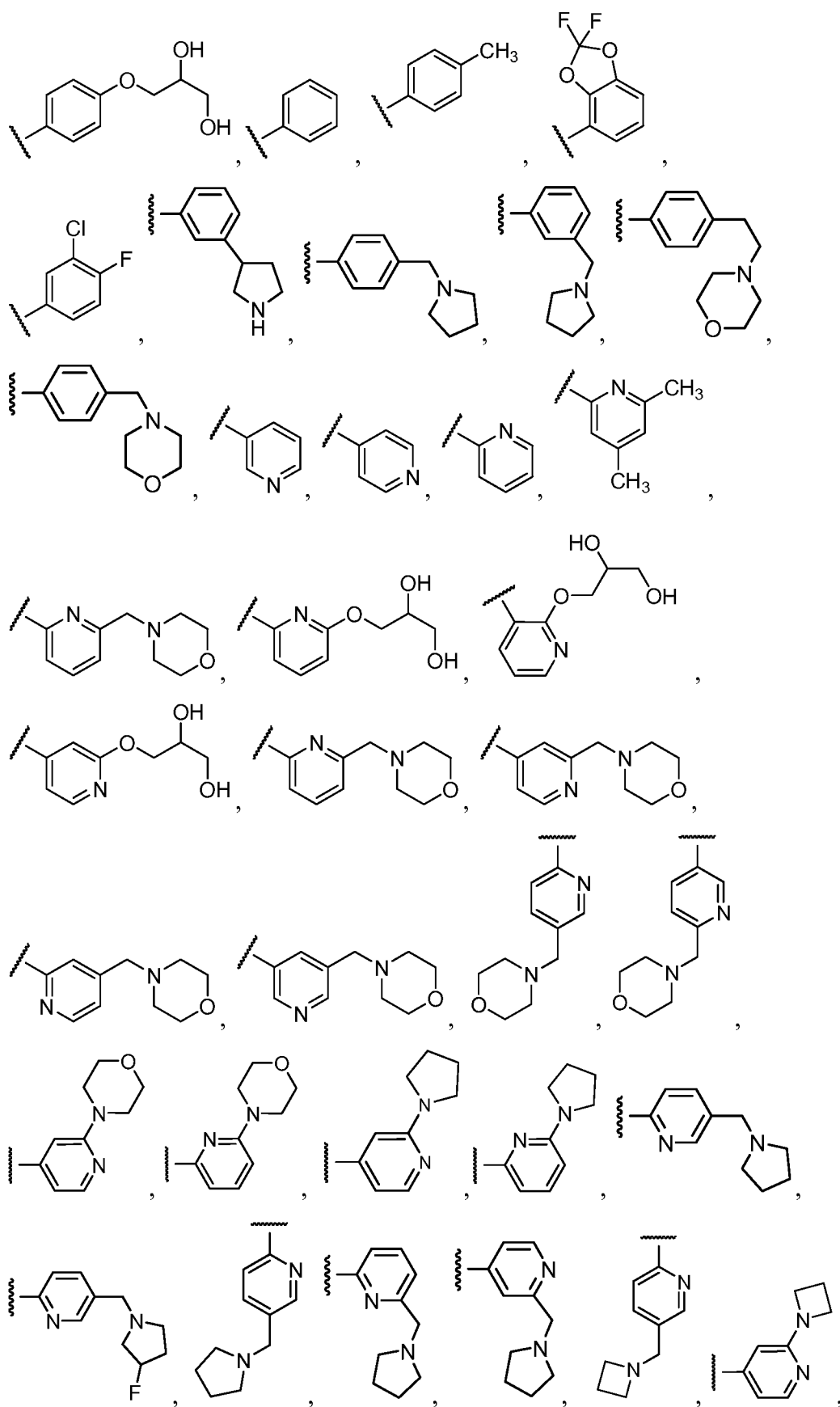
, and wherein R^1 is optionally substituted with one or more substituents independently selected from halo, C_1 - C_4 alkyl, $-(C_1$ - C_4 alkyl)- $N(R^3)(R^3)$, $-N(R^3)(R^3)$, $=O$, $-O-R^3$, and pyrrolidinyl.

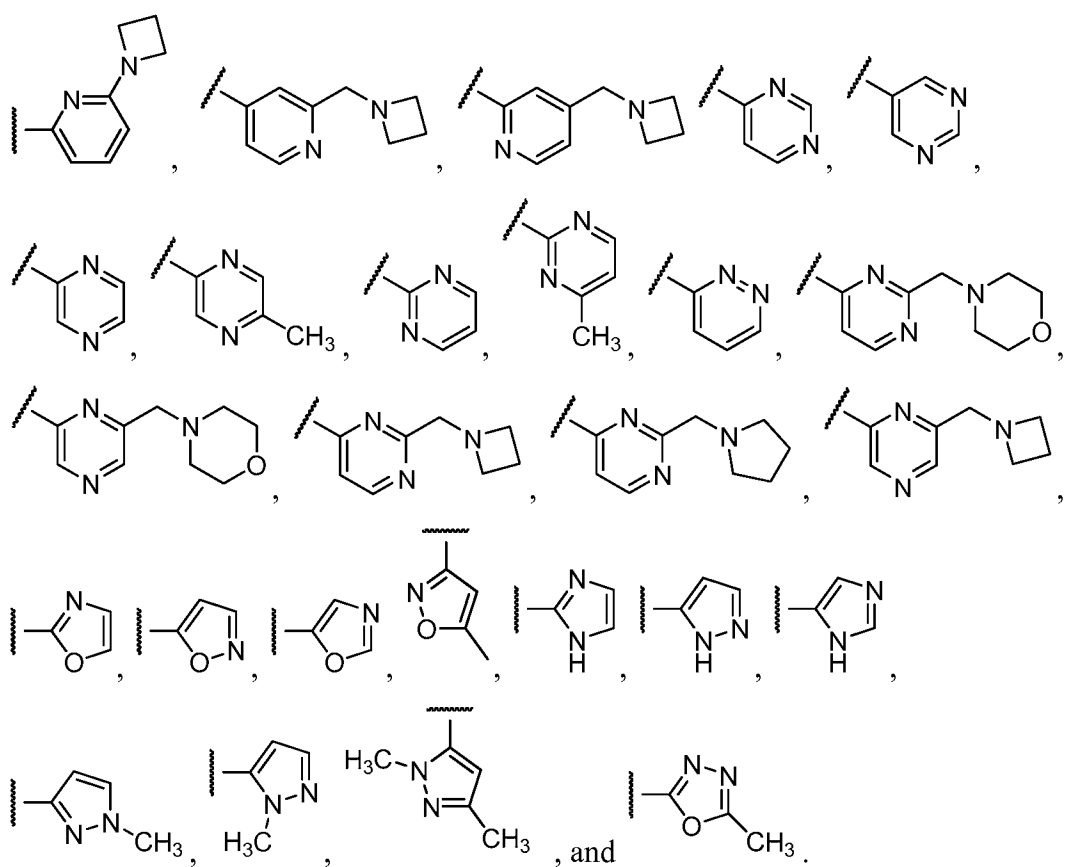
6. The compound of claim 5, wherein, R^1 is optionally substituted with one or

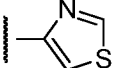


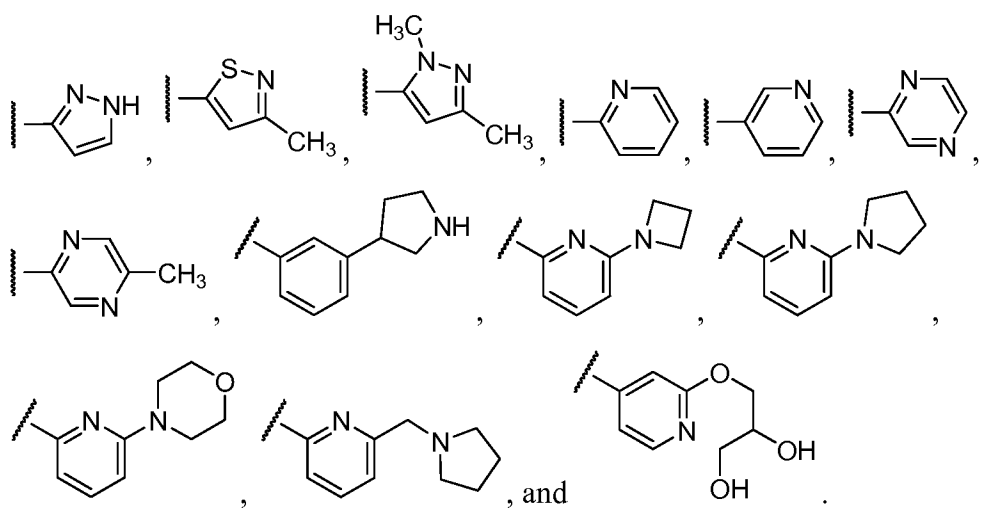
7. The compound of claim 6, wherein R^1 is selected from:



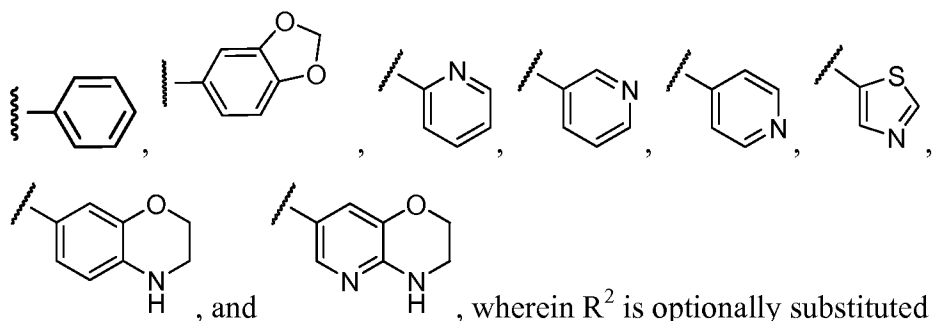




8. The compound of claim 7, wherein R^1 is selected from ,

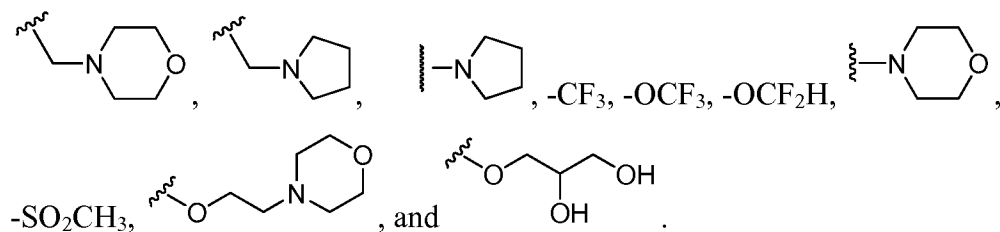


9. The compound of any one of claims 1-8, wherein R^2 is selected from:

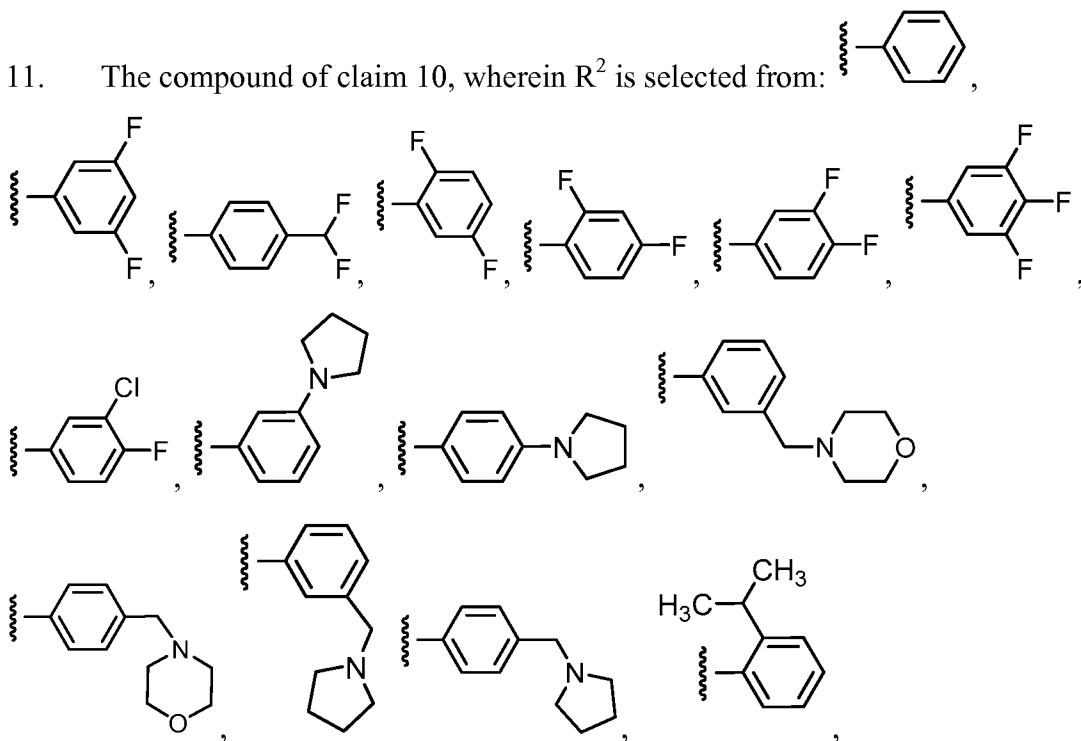


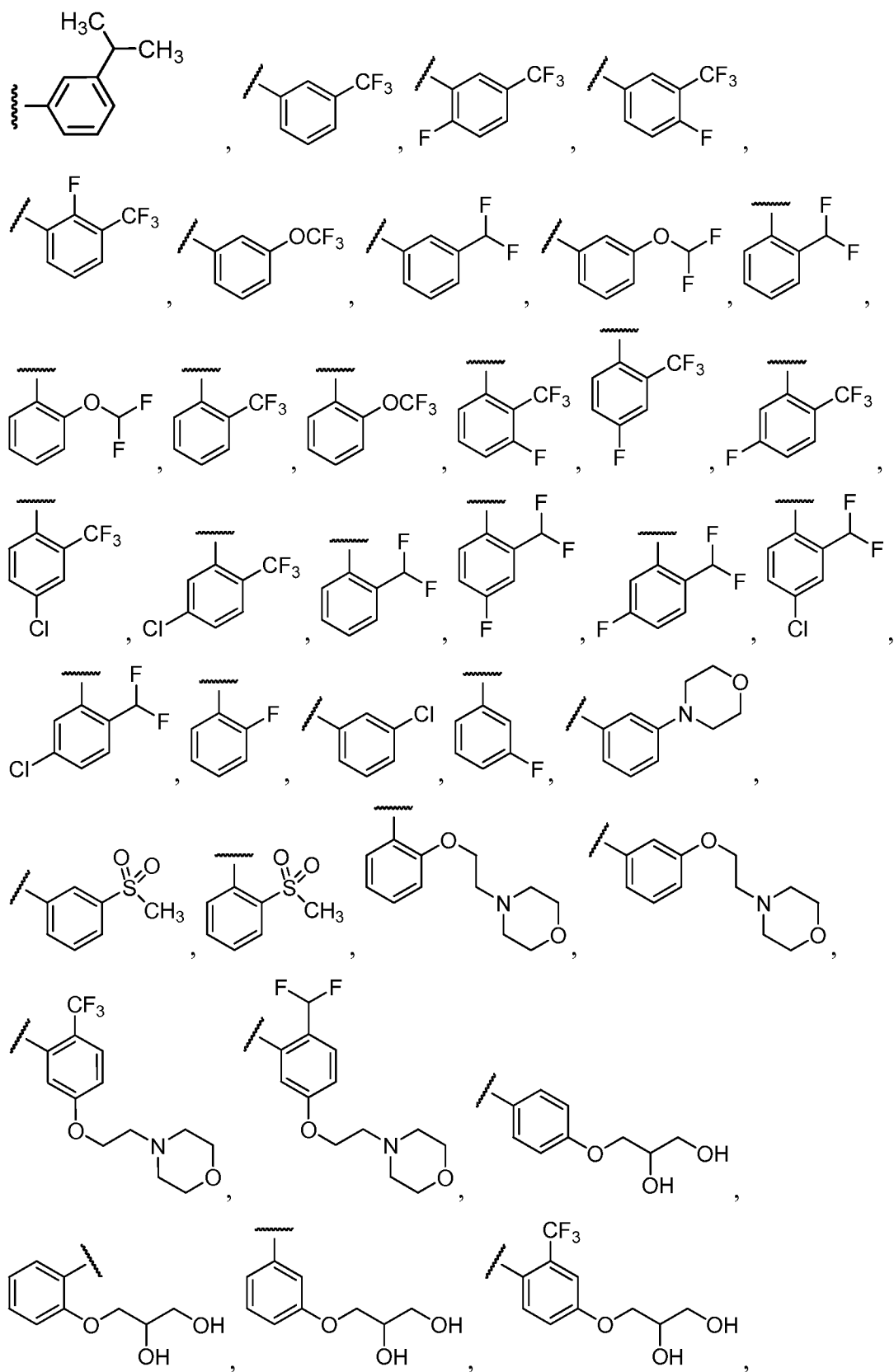
more groups independently selected from halo, C_1 - C_4 alkyl, $-(C_1$ - C_4 alkyl)- $N(R^3)(R^3)$, C_1 - C_2 fluoro-substituted alkyl, $-O-R^3$, $-SO_2-R^3$, $-N(R^3)(R^3)$, and $-O-(C_1$ - C_4 alkyl)- $N(R^3)(R^3)$.

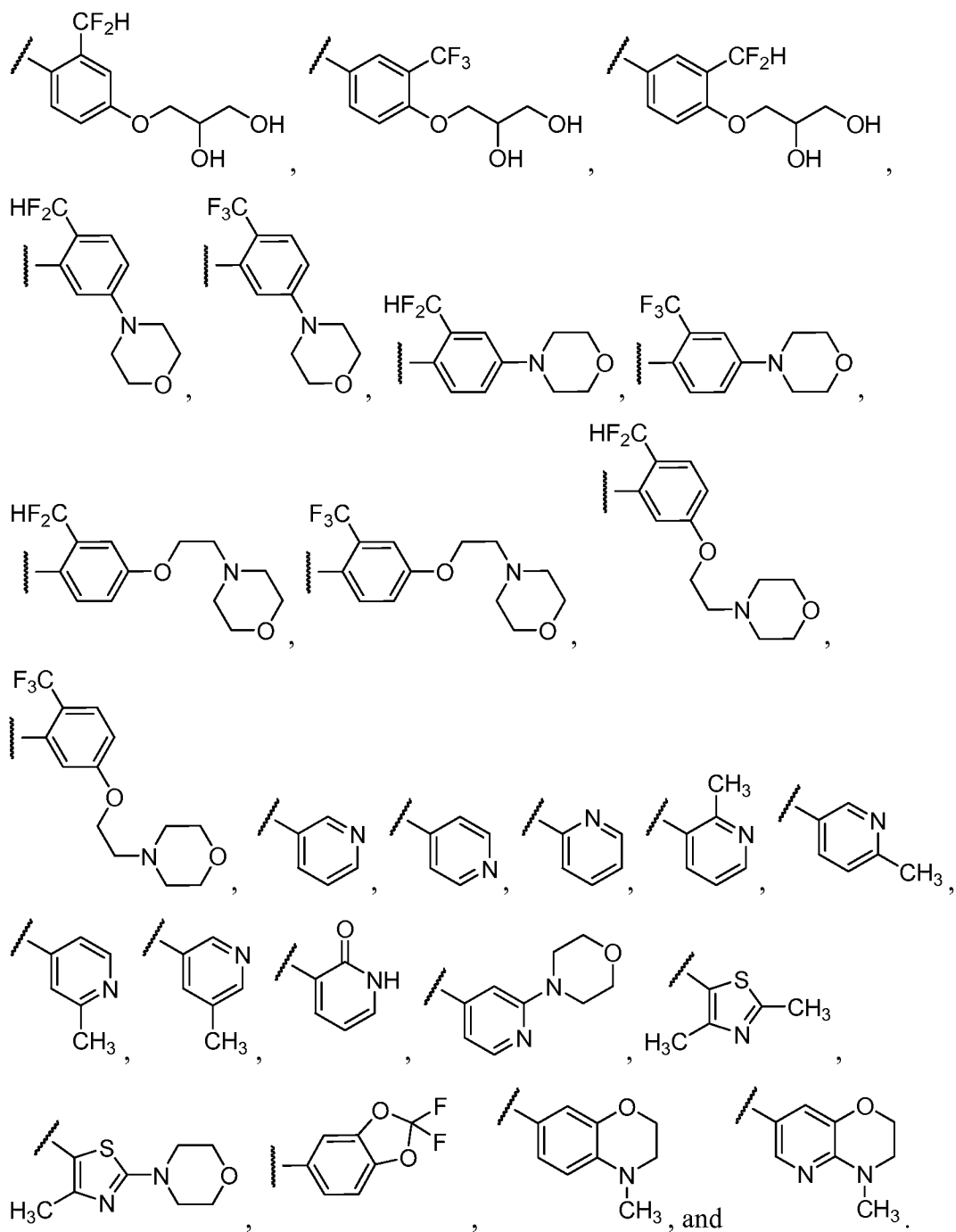
10. The compound of claim 9, wherein R^2 is optionally substituted with one or more groups independently selected from $=O$, $-F$, $-Cl$, $-CH_3$, $-CH(CH_3)_2$, $-CF_2H$,



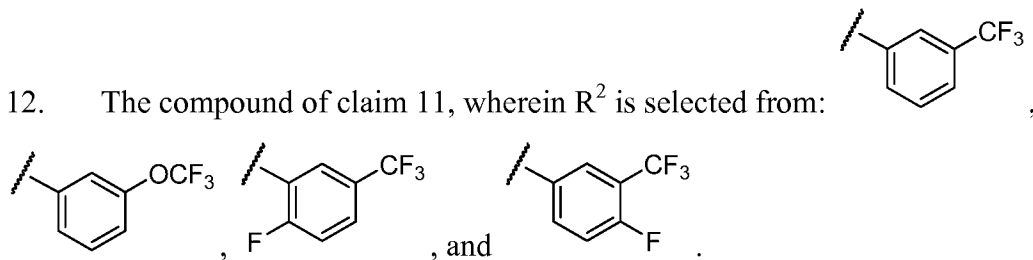
11. The compound of claim 10, wherein R^2 is selected from:







12. The compound of claim 11, wherein R² is selected from:



13. The compound of claim 1, wherein:
 W is selected from C₁-C₄ alkyl and fluoro-substituted C₁-C₄ alkyl; and
 Y is selected from C₁-C₄ alkyl and fluoro-substituted C₁-C₄ alkyl;
 or
 W and Y are bound to one another to form a 5- to 7-membered ring, wherein:
 W is selected from -O-, -NH-, -N(C₁-C₄ alkyl)-, -S-, -S(O)-, and -S(O)₂, and
 Y is (-C(R⁶)(R⁶)-)₁₋₃.
14. The compound of claim 1, wherein X is selected from -NH-C(=O)-†, -C(=O)-NH-†, -NH-C(=S)-†, -C(=S)-NH-†, -S(=O)-NH-†, -S(=O)₂-NH-†, -NH-S(=O)₂-†, -NH-S(O)₂-NR⁴-†, -NR⁴-S(O)₂-NH-†, -NH-C(=O)O-†, -NH-C(=O)NR⁴-†, -NR⁴-C(=O)NH-†, -NH-NR⁴-†, -NR⁴-NH-†, -O-NH-†, -NH-O-†, -CR⁴R⁵-NH-†, -NH-C(=NR⁴)-†, -C(=NR⁴)-NH-†, -C(=O)-NH-CR⁴R⁵-†, -NH-C(=O)-CR⁴R⁵-†, -CR⁴R⁵-NH-C(O)-†, -NH-C(=S)-CR⁴R⁵-†, -CR⁴R⁵-C(=S)-NH-†, -NH-S(O)-CR⁴R⁵-†, -CR⁴R⁵-S(O)-NH-†, -NH-S(O)₂-CR⁴R⁵-†, -CR⁴R⁵-S(O)₂-NH-†, -NH-C(=O)-O-CR⁴R⁵-†, -CR⁴R⁵-O-C(=O)-NH-†, -NH-C(=O)-NR⁴-CR⁴R⁵-†, and -CR⁴R⁵-NH-C(=O)-O-†.
15. The compound of claim 1, wherein the compound is selected from any one of Compound Numbers 206, 212, 222, 227, 231, 234, 235, 236, 242, 244, 251, 278 and 294.
16. A pharmaceutical composition comprising a compound of any one of claims 1-15 and a pharmaceutically acceptable carrier or diluent.
17. The pharmaceutical composition of claim 16, further comprising an additional active agent.
18. A method for treating a subject suffering from or susceptible to insulin resistance, a metabolic syndrome, diabetes, or complications thereof, or for increasing insulin sensitivity in a subject, comprising administering to the subject in need thereof a composition of claim 16.

19. The method of claim 18, further comprising administering to the subject in need thereof an additional therapeutic agent.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2009/062649

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

Int. Cl.

C07D 401/12 (2006.01)	A61P 5/50 (2006.01)	C07D 215/16 (2006.01)	A61K 31/44 (2006.01)
A61P 7/00 (2006.01)	C07D 401/14 (2006.01)	A61K 31/4427 (2006.01)	A61P 9/00 (2006.01)
C07D 403/14 (2006.01)	A61K 31/47 (2006.01)	A61P 17/00 (2006.01)	C07D 405/12 (2006.01)
A61K 31/4709 (2006.01)	A61P 25/00 (2006.01)	C07D 405/14 (2006.01)	A61K 31/4995 (2006.01)
A61P 27/00 (2006.01)	C07D 413/12 (2006.01)	A61K 31/506 (2006.01)	A61P 29/00 (2006.01)
C07D 413/14 (2006.01)	A61K 31/5377 (2006.01)	A61P 35/00 (2006.01)	C07D 417/12 (2006.01)
A61P 3/00 (2006.01)	C07D 213/16 (2006.01)	C07D 491/052 (2006.01)	A61P 3/10 (2006.01)
C07D 213/81 (2006.01)			

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

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

CA: Sub-structure searched based on compound of formula (III)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Baratta W. et al., "Terdentate RuX(CNN)(PP) (X = Cl, H, OR) Complexes: Synthesis, Properties, and Catalytic Activity in Fast Transfer Hydrogenation", <i>Organometallics</i> , vol. 25, pages 4611-4620 (2006) See page 4613 equation 1, and CAS Registry Number: RN 875714-60-6	

☒ Further documents are listed in the continuation of Box C☒ See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

10 February 2010

Date of mailing of the international search report

12 FEB 2010

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INTERNATIONAL SEARCH REPORT

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

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

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

[Continued in Supplemental Box 1]

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-19 (partially)

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2009/062649

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Chelucci G. et al., "Diastereoselective reduction of enantiopure <i>N</i> - <i>p</i> -toluenesulfinyl ketimines derived from pyridyl ketones", <i>Tetrahedron</i> , vol. 62, pages 619-626 (2006) See Schemes 2 and 3, compounds 6c, 6e and 11, and CAS Registry Numbers: RNs 875714-58-2, 875714-60-6 and 875714-64-0.	
A	Uenishi J. et al., "Synthesis of Chiral Nonracemic 1-(2-Pyridinyl)ethylamines: Stereospecific Introduction of Amino Function onto the 2-Pyridinylmethyl Carbon Center", <i>Journal of Organic Chemistry</i> , vol. 69, pages 6781-6789 (2004) See compounds 9c, 9e and 7, and CAS Registry Numbers: RNs 779328-58-4, 779328-61-9 and 779328-55-1	
A	Bolm C. et al., "Enantioselective Synthesis of Optically Active Pyridine Derivatives and C ₂ -Symmetric 2,2'-Bipyridines", <i>Chemische Berichte</i> , vol. 125, pages 1169-1190 (1992) See <i>N</i> -phenylcarbamate derivative of compound 26 on page 1181, and CAS Registry Number: RN 139043-00-8	
A	WO 2006/061638 A2 (ISTITUTO DI RICERCHE DI BIOLOGIA MOLECOLARE P ANGELETTI SPA) 15 June 2006 See page 62, the examples, and CAS Registry Numbers: RNs 891265-77-3, 891265-85-3 and 891265-87-5	

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Supplemental Box 1

(To be used when the space in any of Boxes I to IV is not sufficient)

Continuation of Box No: Box III**Lack of Unity continued**

The international application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept (PCT Rule 13.1).

Under PCT Rule 13.2, the requirement of unity shall be fulfilled only when there is a technical relationship among those inventions involving one or more of the same or corresponding special technical features. The expression "special technical feature" relates to those technical features that define a contribution that the claimed inventions, considered as a whole, make over the prior art.

According to Markush practice (PCT Search and Preliminary Examination Guidelines 10.17), a claim will have unity if:

- a) all alternatives have a common property or activity, and
- b) a significant structural element is shared by all of the alternatives, or the compounds belong to a recognised class of chemical compounds.

The International Searching Authority has found that there are 12 inventions:

- 1) Claims 1-19 (partially) are directed to compounds of formula (III) wherein the core structure is a pyridine ring, and their use in a composition,
- 2) Claims 1-19 (partially) are directed to compounds of formula (III) wherein the core structure is a pyridine ring fused to a 6-membered ring, and their use in a composition,
- 3) Claims 1 (partially), 2 (partially), 4-14 (partially) and 16-19 (partially) are directed to compounds of formula (III) wherein the core structure is a pyridine ring fused to a 5-membered ring, and their use in a composition,
- 4) Claims 1 (partially), 2 (partially), 4-14 (partially) and 16-19 (partially) are directed to compounds of formula (III) wherein the core structure is a pyridine ring fused to a 7-membered ring, and their use in a composition,
- 5) Claims 1 (partially), 2 (partially), 4-14 (partially) and 16-19 (partially) are directed to compounds of formula (III) wherein the core structure is a pyrazine ring, and their use in a composition,
- 6) Claims 1 (partially), 2 (partially), 4-14 (partially) and 16-19 (partially) are directed to compounds of formula (III) wherein the core structure is a pyrazine ring fused to a 6-membered ring, and their use in a composition,
- 7) Claims 1 (partially), 2 (partially), 4-14 (partially) and 16-19 (partially) are directed to compounds of formula (III) wherein the core structure is a pyrazine ring fused to a 5-membered ring, and their use in a composition,
- 8) Claims 1 (partially), 2 (partially), 4-14 (partially) and 16-19 (partially) are directed to compounds of formula (III) wherein the core structure is a pyrazine ring fused to a 7-membered ring, and their use in a composition,
- 9) Claims 1 (partially), 2 (partially), 4-14 (partially) and 16-19 (partially) are directed to compounds of formula (III) wherein the core structure is a pyrimidine ring, and their use in a composition,
- 10) Claims 1 (partially), 2 (partially), 4-14 (partially) and 16-19 (partially) are directed to compounds of formula (III) wherein the core structure is a pyrimidine ring fused to a 6-membered ring, and their use in a composition,
- 11) Claims 1 (partially), 2 (partially), 4-14 (partially) and 16-19 (partially) are directed to compounds of formula (III) wherein the core structure is a pyrimidine ring fused to a 5-membered ring, and their use in a composition, and
- 12) Claims 1 (partially), 2 (partially), 4-14 (partially) and 16-19 (partially) are directed to compounds of formula (III) wherein the core structure is a pyrimidine ring fused to a 7-membered ring, and their use in a composition.

The only feature common to the compounds defined in claims 1-19 is a 6-membered heterocyclic ring for use as a sirtuin-modulator. The use of compounds with a 6-membered heterocycle as the core structure for use as a sirtuin-modulator is already known in the art (see for example: US 2007/0037865 A1). Consequently, the common feature does not constitute a "special technical feature" since it makes no contribution over the prior art. Therefore, as there is no common special technical feature, it follows that there is no technical relationship between the identified inventions. Therefore, the claims do not satisfy the PCT requirement for unity of invention *a posteriori* within the meaning of PCT Rule 13.2.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

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This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
WO	2006/061638	AU	2005313108	CA	2590811	CN	101115742
		EP	1828171	US	2009048228		
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.							
END OF ANNEX							