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(54) Title: ALKYLPYRAZOLYL GUANIDINE F1F0-ATPASE INHIBITORS AND THERAPEUTIC USES THEREOF

(57) Abstract: The invention provides pyrazolyl guanidine compounds that inhibit F_1F_o-ATPase, and methods of using pyrazolyl guanidine compounds as therapeutic agents to treat medical disorders, such as an immune disorder, inflammatory condition, or cancer.

ALKYLPYRAZOLYL GUANIDINE F_1F_0-ATPASE INHIBITORS AND THERAPEUTIC USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to United States Provisional Patent Application serial number 61/914,088, filed December 10, 2013, the contents of which are hereby incorporated by reference.

FIELD OF THE INVENTION

[0002] The invention provides inhibitors of F_1F_0-ATPases (e.g., mitochondrial F_1F_0-ATPases) and their therapeutic use. In particular, the invention provides pyrazolyl guanidine compounds that inhibit F_1F_0-ATPase, and methods of using pyrazolyl guanidine compounds as therapeutic agents to treat a number of medical conditions.

BACKGROUND

[0003] Multicellular organisms exert precise control over cell number. A balance between cell proliferation and cell death achieves this homeostasis. Cell death occurs in nearly every type of vertebrate cell via necrosis or through a suicidal form of cell death, known as apoptosis. Apoptosis is triggered by a variety of extracellular and intracellular signals that engage a common, genetically programmed death mechanism.

[0004] Multicellular organisms use apoptosis to instruct damaged or unnecessary cells to destroy themselves for the good of the organism. Control of the apoptotic process therefore is very important to normal development, for example, fetal development of fingers and toes requires the controlled removal, by apoptosis, of excess interconnecting tissues, as does the formation of neural synapses within the brain. Similarly, controlled apoptosis is responsible for the sloughing off of the inner lining of the uterus (the endometrium) at the start of menstruation. While apoptosis plays an important role in tissue sculpting and normal cellular maintenance, it is also a component of the primary defense against cells and invaders (*e.g.*, viruses) which threaten the well being of the organism.

[0005] Not surprisingly many diseases are associated with dysregulation of apoptotic cell death. Experimental models have established a cause-effect relationship between aberrant apoptotic regulation and the pathogenicity of various neoplastic, autoimmune and viral diseases. For instance, in the cell-mediated immune response, effector cells (*e.g.*, cytotoxic T lymphocytes "CTLs") destroy virus-infected cells by inducing the infected cells to undergo apoptosis. The organism subsequently relies on the apoptotic process to destroy the effector cells when they are no longer needed. Autoimmunity is normally prevented by the CTLs inducing apoptosis in each other and even in themselves. Defects in this process are associated with a variety of immune diseases such as lupus erythematosus and rheumatoid arthritis.

[0006] Multicellular organisms also use apoptosis to instruct cells with damaged nucleic acids (*e.g.*, DNA) to destroy themselves prior to becoming cancerous. Some cancer-causing viruses overcome this safeguard by reprogramming infected (transformed) cells to abort the normal apoptotic process. For example, several human papilloma viruses (HPVs) have been implicated in causing cervical cancer by suppressing the apoptotic removal of transformed cells by producing a protein (E6) which inactivates the p53 apoptosis promoter. Similarly, the Epstein-Barr virus (EBV), the causative agent of mononucleosis and Burkitt's lymphoma, reprograms infected cells to produce proteins that prevent normal apoptotic removal of the aberrant cells thus allowing the cancerous cells to proliferate and to spread throughout the organism.

[0007] Still other viruses destructively manipulate a cell's apoptotic machinery without directly resulting in the development of a cancer. For example, destruction of the immune system in individuals infected with the human immunodeficiency virus (HIV) is thought to progress through infected $CD4^+$ T cells (about 1 in 100,000) instructing uninfected sister cells to undergo apoptosis.

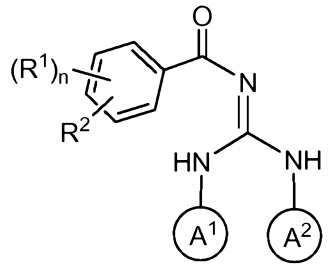
[0008] Some cancers that arise by non-viral means have also developed mechanisms to escape destruction by apoptosis. Melanoma cells, for instance, avoid apoptosis by inhibiting the expression of the gene encoding Apaf-1. Other cancer cells, especially lung and colon cancer cells, secrete high levels of soluble decoy molecules that inhibit the initiation of CTL mediated clearance of aberrant cells. Faulty regulation of the apoptotic machinery has also been implicated in various degenerative conditions and vascular diseases.

[0009] Controlled regulation of the apoptotic process and its cellular machinery is important to the survival of multicellular organisms. Typically, the biochemical changes that occur in a cell instructed to undergo apoptosis occur in an orderly procession. However, as shown above, flawed regulation of apoptosis can cause serious deleterious effects in the organism.

[0010] The need exists for improved compositions and methods for regulating the apoptotic processes in subjects afflicted with diseases and conditions characterized by faulty regulation of these processes (*e.g.*, viral infections, hyperproliferative autoimmune disorders, chronic inflammatory conditions, and cancers). The present invention addresses this need and provides other related advantages.

SUMMARY

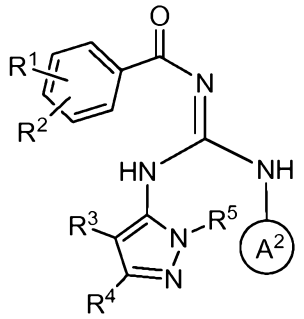
[0011] The invention provides pyrazolyl guanidine compounds that inhibit F_1F_0-ATPase (e.g., mitochondrial F_1F_0-ATPase), pharmaceutical compositions comprising pyrazolyl guanidine compounds, and methods of using such compounds and pharmaceutical compositions to treat a number of medical conditions. Accordingly, one aspect of the invention provides a family of compounds represented by Formula I:



(I)

including all stereoisomers, geometric isomers, and tautomers; or a pharmaceutically acceptable salt or solvate of any of the foregoing; wherein the variables are as defined in the detailed description. Variable A^1 is a multiply substituted pyrazolyl group, where it has been discovered that the substitution pattern on the pyrazolyl can significantly impact properties of the compound, such as illustrated by experimental results presented herein showing a substantially increased blood plasma concentration of compound when the compound has, in addition to a haloalkyl group attached to a carbon atom of the pyrazolyl, an alkyl group attached to a carbon atom vicinal to the carbon atom bearing the haloalkyl group.

[0012] Another aspect of the invention provides a family of compounds represented by Formula I-A:



(I-A)

including all stereoisomers, geometric isomers, and tautomers; or a pharmaceutically acceptable salt thereof; wherein the variables are as defined in the detailed description.

[0013] The foregoing compounds can be present in a pharmaceutical composition comprising a compound described herein and a pharmaceutically acceptable carrier.

[0014] Another aspect of the invention provides a method of treating a subject suffering from a medical disorder. The method comprises administering to the subject a therapeutically effective amount of one or more pyrazolyl guanidine compounds described herein, e.g., a compound of Formula I or I-A, in order to ameliorate a symptom of the disorder. A large number of disorders can be treated using the pyrazolyl guanidine compounds described herein. For example, the compounds described herein can be used to treat an immune disorder or inflammatory disorder, such as rheumatoid arthritis, psoriasis, chronic graft-versus-host disease, acute graft-versus-host disease, Crohn's disease, inflammatory bowel disease, multiple sclerosis, systemic lupus erythematosus, Celiac Sprue, idiopathic thrombocytopenic thrombotic purpura, myasthenia gravis, Sjogren's syndrome, scleroderma, ulcerative colitis, asthma, epidermal hyperplasia, and other medical disorders described herein. The compounds described herein can also be used to treat a cardiovascular disease, myeloma, lymphoma, cancer, or bacterial infection.

[0015] Another aspect of the invention provides a method of inhibiting an F_1F_0-ATPase, for example, a mitochondrial F_1F_0-ATPase. The method comprises exposing the F_1F_0-ATPase to a compound described herein, such as a compound of Formula I or I-A, to inhibit said F_1F_0-ATPase.

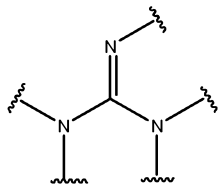
DETAILED DESCRIPTION OF THE INVENTION

[0016] The invention provides pyrazolyl guanidine compounds that inhibit F_1F_0-ATPase (e.g., mitochondrial F_1F_0-ATPase), pharmaceutical compositions comprising the pyrazolyl guanidine compounds, and methods of using the pyrazolyl guanidine compounds and pharmaceutical compositions in therapy.

[0017] Exemplary compositions and methods of the present invention are described in more detail in the following sections: I. Modulators of F_1F_0-ATPase Activity; II. Pyrazolyl Guanidine Compounds; III. Therapeutic Applications of Pyrazolyl Guanidine Compounds, and IV. Pharmaceutical Compositions, Formulations, and Exemplary Administration Routes and Dosing Considerations. Aspects of the invention described in one particular section are not to be limited to any particular section.

[0018] The practice of the present invention employs, unless otherwise indicated, conventional techniques of organic chemistry, pharmacology, molecular biology (including recombinant techniques), cell biology, biochemistry, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as "Comprehensive Organic Synthesis" (B.M. Trost & I. Fleming, eds., 1991-1992); "Molecular cloning: a laboratory manual" Second Edition (Sambrook *et al.*, 1989); "Oligonucleotide synthesis" (M.J. Gait, ed., 1984); "Animal cell culture" (R.I. Freshney, ed., 1987); the series "Methods in enzymology" (Academic Press, Inc.); "Handbook of experimental immunology" (D.M. Weir & C.C. Blackwell, eds.); "Gene transfer vectors for mammalian cells" (J.M. Miller & M.P. Calos, eds., 1987); "Current protocols in molecular biology" (F.M. Ausubel *et al.*, eds., 1987, and periodic updates); "PCR: the polymerase chain reaction" (Mullis *et al.*, eds., 1994); and "Current protocols in immunology" (J.E. Coligan *et al.*, eds., 1991), each of which is herein incorporated by reference in its entirety.

[0019] To facilitate an understanding of the present invention, a number of terms and phrases are defined below.

[0020] As used herein, the term "guanidine" refers to a compound having the following core structure:  , including pharmaceutically acceptable salt forms.

[0021] The term "alkyl" refers to a saturated straight or branched hydrocarbon, such as a straight or branched group of 1-12, 1-10, or 1-6 carbon atoms, referred to herein as C_1-C_{12}alkyl, C_1-C_{10}alkyl, and C_1-C_6alkyl, respectively. Exemplary alkyl groups include, but are not limited to, methyl, ethyl, propyl, isopropyl, 2-methyl-1-propyl, 2-methyl-2-propyl, 2-methyl-1-butyl, 3-methyl-1-butyl, 2-methyl-3-butyl, 2,2-dimethyl-1-propyl, 2-methyl-1-pentyl, 3-methyl-1-pentyl, 4-methyl-1-pentyl, 2-methyl-2-pentyl, 3-methyl-2-pentyl, 4-methyl-2-pentyl, 2,2-dimethyl-1-butyl, 3,3-dimethyl-1-butyl, 2-ethyl-1-butyl, butyl, isobutyl, t-butyl, pentyl, isopentyl, neopentyl, hexyl, heptyl, octyl, etc.

[0022] The term "alkylene" refers to a diradical of an alkyl group. Exemplary alkylene groups include $-CH_2-$ and $-CH_2CH_2-$.

[0023] The term "haloalkyl" refers to an alkyl group that is substituted with at least one halogen. For example, $-CH_2F$, $-CHF_2$, $-CF_3$, $-CH_2CF_3$, $-CF_2CF_3$, and the like.

[0024] The term "cycloalkyl" refers to a monovalent saturated cyclic, bicyclic, or bridged cyclic (e.g., adamantyl) hydrocarbon group of 3-12, 3-8, 4-8, or 4-6 carbons, referred to herein, e.g., as "C_{4-8}cycloalkyl," derived from a cycloalkane. Exemplary cycloalkyl groups include cyclohexyl, cyclopentyl, cyclobutyl, and cyclopropyl.

[0025] The terms *ortho*, *meta* and *para* are art-recognized and refer to 1,2-, 1,3- and 1,4-disubstituted benzenes, respectively. For example, the names 1,2-dimethylbenzene and ortho-dimethylbenzene are synonymous.

[0026] The terms "alkoxyl" and "alkoxy" are art-recognized and refer to an alkyl group, as defined above, having an oxygen radical attached thereto. Representative alkoxyl groups include methoxy, ethoxy, propyloxy, tert-butoxy and the like.

[0027] The symbol "〰" indicates a point of attachment.

[0028] The compounds of the disclosure may contain one or more chiral centers and/or double bonds and, therefore, exist as stereoisomers, such as geometric isomers, enantiomers or

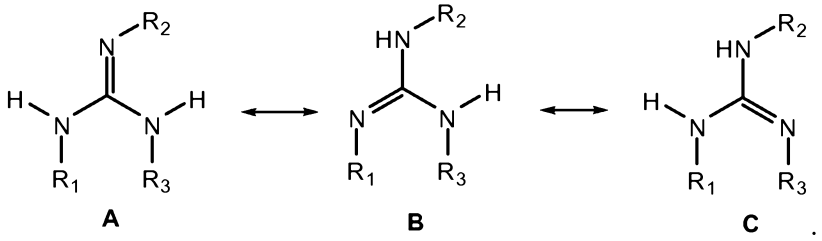
diastereomers. The term "stereoisomers" when used herein consist of all geometric isomers, enantiomers or diastereomers. These compounds may be designated by the symbols "R" or "S," depending on the configuration of substituents around the stereogenic carbon atom. Stereoisomers include enantiomers and diastereomers. Mixtures of enantiomers or diastereomers may be designated "(±)" in nomenclature, but the skilled artisan will recognize that a structure may denote a chiral center implicitly. Unless indicated otherwise, generic chemical structures and graphical representations of specific compounds encompass all stereoisomers.

[0029] Individual stereoisomers of compounds of the present invention can be prepared synthetically from commercially available starting materials that contain asymmetric or stereogenic centers, or by preparation of racemic mixtures followed by resolution methods well known to those of ordinary skill in the art. These methods of resolution are exemplified by (1) attachment of a mixture of enantiomers to a chiral auxiliary, separation of the resulting mixture of diastereomers by recrystallization or chromatography and liberation of the optically pure product from the auxiliary, (2) salt formation employing an optically active resolving agent, or (3) direct separation of the mixture of optical enantiomers on chiral chromatographic columns. Stereoisomeric mixtures can also be resolved into their component stereoisomers by well known methods, such as chiral-phase gas chromatography, chiral-phase high performance liquid chromatography, crystallizing the compound as a chiral salt complex, or crystallizing the compound in a chiral solvent. Stereoisomers can also be obtained from stereomerically-pure intermediates, reagents, and catalysts by well known asymmetric synthetic methods.

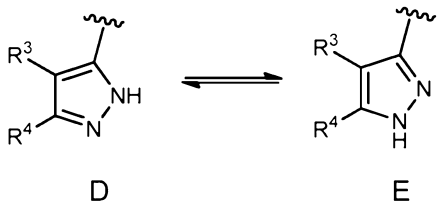
[0030] Geometric isomers can also exist in the compounds of the present invention. The present invention encompasses the various geometric isomers and mixtures thereof resulting from the arrangement of substituents around a carbon-carbon double bond or arrangement of substituents around a carbocyclic ring. Substituents around a carbon-carbon double bond are designated as being in the "*Z*" or "*E*" configuration wherein the terms "*Z*" and "*E*" are used in accordance with IUPAC standards. Unless otherwise specified, structures depicting double bonds encompass both the "*E*" and "*Z*" isomers.

[0031] Certain compounds described herein may exist as a single tautomer or as a mixture of tautomers. For example, certain guanidine compounds having a hydrogen atom attached to at least one of the guanidine nitrogen atoms can exist as a single tautomer or a mixture of

tautomers. For example, depending upon the substituents attached at the R^1, R^2 and R^3 positions, the guanidine compound may exist as a single tautomer represented by **A**, **B**, or **C**, or as mixture of two or more of **A**, **B**, and **C**.



[0032] Similarly, certain guanidine compounds having a hydrogen atom attached to a pyrazole ring nitrogen atom can exist as a single tautomer (**D** or **E**) or a mixture of tautomers **D** and **E**.



[0033] The compounds disclosed herein can exist in solvated as well as unsolvated forms with pharmaceutically acceptable solvents such as water, ethanol, and the like, and it is intended that the invention embrace both solvated and unsolvated forms.

[0034] The invention also embraces isotopically labeled compounds of the invention which are identical to those recited herein, except that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into compounds of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, sulfur, fluorine and chlorine, such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F, and ^{36}Cl, respectively.

[0035] Certain isotopically-labeled disclosed compounds (*e.g.*, those labeled with ^{3}H and 14C) are useful in compound and/or substrate tissue distribution assays. Tritiated (i.e., 3H) and carbon-14 (i.e., 14C) isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium (*i.e.*, ^{2}H) may afford certain therapeutic advantages resulting from greater metabolic stability (*e.g.*, increased *in vivo* half-life or reduced dosage requirements) and hence may be preferred in some circumstances.

Isotopically labeled compounds of the invention can generally be prepared by following procedures analogous to those disclosed in the e.g., Examples herein, by substituting an isotopically labeled reagent for a non-isotopically labeled reagent.

[0036] The term "IC_{50}" is art-recognized and refers to the concentration of a compound that is required for 50% inhibition of its target.

[0037] The term "EC_{50}" is art-recognized and refers to the concentration of a compound at which 50% of its maximal effect is observed.

[0038] The terms "subject" and "patient" refer to organisms to be treated by the methods of the present invention. Such organisms preferably include, but are not limited to, mammals (*e.g.*, murines, simians, equines, bovines, porcines, canines, felines, and the like), and most preferably includes humans. In the context of the invention, the terms "subject" and "patient" generally refer to an individual who will receive or who has received treatment (*e.g.*, administration of a compound of the present invention and optionally one or more other agents) for a condition characterized by the dysregulation of apoptotic processes.

[0039] As used herein, the term "effective amount" refers to the amount of a compound sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages and is not intended to be limited to a particular formulation or administration route. As used herein, the term "treating" includes any effect, *e.g.*, lessening, reducing, modulating, ameliorating or eliminating, that results in the improvement of the condition, disease, disorder, and the like, or ameliorating a symptom thereof.

[0040] The phrase "pathologically proliferating or growing cells" refers to a localized population of proliferating cells in an animal that is not governed by the usual limitations of normal growth.

[0041] As used herein, the term "un-activated target cell" refers to a cell that is either in the G_o phase or one to which a stimulus has not been applied.

[0042] As used herein, the term "activated target lymphoid cell" refers to a lymphoid cell that has been primed with an appropriate stimulus to cause a signal transduction cascade, or alternatively, a lymphoid cell that is not in G_o phase. Activated lymphoid cells may proliferate, undergo activation induced cell death, or produce one or more cytotoxins, cytokines, or other

related membrane-associated proteins characteristic of the cell type (*e.g.*, $CD8^+$ or $CD4^+$). They are also capable of recognizing and binding any target cell that displays a particular antigen on its surface, and subsequently releasing its effector molecules.

[0043] As used herein, the term "activated cancer cell" refers to a cancer cell that has been primed with an appropriate stimulus to cause signal transduction. An activated cancer cell may or may not be in the G_O phase.

[0044] An activating agent is a stimulus that upon interaction with a target cell results in a signal transduction cascade. Examples of activating stimuli include, but are not limited to, small molecules, radiant energy, and molecules that bind to cell activation cell surface receptors. Responses induced by activation stimuli can be characterized by changes in, among others, intracellular Ca^{2+}, superoxide, or hydroxyl radical levels; the activity of enzymes like kinases or phosphatases; or the energy state of the cell. For cancer cells, activating agents also include transforming oncogenes.

[0045] As used herein, the term "dysregulation of the process of cell death" refers to any aberration in the ability (*e.g.*, predisposition) of a cell to undergo cell death via either necrosis or apoptosis. Dysregulation of cell death is associated with or induced by a variety of conditions, including for example, immune disorders (*e.g.*, systemic lupus erythematosus, autoimmune disorders, rheumatoid arthritis, graft-versus-host disease, myasthenia gravis, Sjögren's syndrome, *etc.*), chronic inflammatory conditions (*e.g.*, psoriasis, asthma and Crohn's disease), hyperproliferative disorders (*e.g.*, tumors, B cell lymphomas, T cell lymphomas, *etc.*), viral infections (*e.g.*, herpes, papilloma, HIV), and other conditions such as osteoarthritis and atherosclerosis.

[0046] It should be noted that when the dysregulation is induced by or associated with a viral infection, the viral infection may or may not be detectable at the time dysregulation occurs or is observed. That is, viral-induced dysregulation can occur even after the disappearance of symptoms of viral infection.

[0047] A "hyperproliferative disorder," as used herein refers to any condition in which a localized population of proliferating cells in an animal is not governed by the usual limitations of normal growth. Examples of hyperproliferative disorders include tumors, neoplasms, lymphomas and the like. A neoplasm is said to be benign if it does not undergo invasion or metastasis and malignant if it does either of these. A metastatic cell or tissue means that the

cell can invade and destroy neighboring body structures. Hyperplasia is a form of cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. Metaplasia is a form of controlled cell growth in which one type of fully differentiated cell substitutes for another type of differentiated cell. Metaplasia can occur in epithelial or connective tissue cells. A typical metaplasia involves a somewhat disorderly metaplastic epithelium.

[0048] The pathological growth of activated lymphoid cells often results in an immune disorder or a chronic inflammatory condition. As used herein, the term "immune disorder" refers to any condition in which an organism produces antibodies or immune cells which recognize the organism's own molecules, cells or tissues. Non-limiting examples of immune disorders include autoimmune disorders, immune hemolytic anemia, immune hepatitis, Berger's disease or IgA nephropathy, Celiac Sprue, chronic fatigue syndrome, Crohn's disease, dermatomyositis, fibromyalgia, graft-versus-host disease, Grave's disease, Hashimoto's thyroiditis, idiopathic thrombocytopenia purpura, lichen planus, multiple sclerosis, myasthenia gravis, psoriasis, rheumatic fever, rheumatic arthritis, scleroderma, Sjorgren syndrome, systemic lupus erythematosus, type 1 diabetes, ulcerative colitis, vitiligo, tuberculosis, and the like.

[0049] As used herein, the term "chronic inflammatory condition" refers to a condition wherein the organism's immune cells are activated. Such a condition is characterized by a persistent inflammatory response with pathologic sequelae. This state is characterized by infiltration of mononuclear cells, proliferation of fibroblasts and small blood vessels, increased connective tissue, and tissue destruction. Examples of chronic inflammatory diseases include, but are not limited to, Crohn's disease, psoriasis, chronic obstructive pulmonary disease, inflammatory bowel disease, multiple sclerosis, and asthma. Immune diseases such as rheumatoid arthritis and systemic lupus erythematosus can also result in a chronic inflammatory state.

[0050] As used herein, the term "co-administration" refers to the administration of at least two agent(s) (*e.g.*, a compound of the present invention) or therapies to a subject. In some embodiments, the co-administration of two or more agents/therapies is concurrent. In other embodiments, a first agent/therapy is administered prior to a second agent/therapy. Those of skill in the art understand that the formulations and/or routes of administration of the various

agents/therapies used may vary. The appropriate dosage for co-administration can be readily determined by one skilled in the art. In some embodiments, when agents/therapies are co-administered, the respective agents/therapies are administered at lower dosages than appropriate for their administration alone. Thus, co-administration is especially desirable in embodiments where the co-administration of the agents/therapies lowers the requisite dosage of a known potentially harmful (*e.g.*, toxic) agent(s).

[0051] As used herein, the term "pharmaceutical composition" refers to the combination of an active agent with a carrier, inert or active, making the composition especially suitable for diagnostic or therapeutic use *in vivo* or *ex vivo*.

[0052] As used herein, the term "pharmaceutically acceptable carrier" refers to any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions (*e.g.*, such as an oil/water or water/oil emulsions), and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, see e.g., Martin, Remington's Pharmaceutical Sciences, 15th Ed., Mack Publ. Co., Easton, PA [1975].

[0053] As used herein, the term "pharmaceutically acceptable salt" refers to any pharmaceutically acceptable salt (*e.g.*, acid or base) of a compound of the present invention which, upon administration to a subject, is capable of providing a compound of this invention or an active metabolite or residue thereof. As is known to those of skill in the art, "salts" of the compounds of the present invention may be derived from inorganic or organic acids and bases. Examples of acids include, but are not limited to, hydrochloric, hydrobromic, sulfuric, nitric, perchloric, fumaric, maleic, phosphoric, glycolic, lactic, salicylic, succinic, toluene-p-sulfonic, tartaric, acetic, citric, methanesulfonic, ethanesulfonic, formic, benzoic, malonic, naphthalene-2-sulfonic, benzenesulfonic acid, and the like. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts.

[0054] Examples of bases include, but are not limited to, alkali metals (*e.g.*, sodium) hydroxides, alkaline earth metals (*e.g.*, magnesium), hydroxides, ammonia, and compounds of formula NW_4^+, wherein W is C_{1-4} alkyl, and the like.

[0055] Examples of salts include, but are not limited to: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, flucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, palmoate, pectinate, persulfate, phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, undecanoate, and the like. Other examples of salts include anions of the compounds of the present invention compounded with a suitable cation such as Na^+, NH_4^+, and NW_4^+ (wherein W is a C_{1-4} alkyl group), and the like.

[0056] For therapeutic use, salts of the compounds of the present invention are contemplated as being pharmaceutically acceptable. However, salts of acids and bases that are non-pharmaceutically acceptable may also find use, for example, in the preparation or purification of a pharmaceutically acceptable compound.

[0057] As used herein, the term "modulate" refers to the activity of a compound (*e.g.*, a compound of the present invention) to affect (*e.g.*, to promote or retard) an aspect of cellular function, including, but not limited to, cell growth, proliferation, apoptosis, and the like.

[0058] The phrase "$CDCl_3$ with TFA-d" refers to a solution of $CDCl_3$ containing CF_3CO_2D.

[0059] Throughout the description, where compositions are described as having, including, or comprising specific components, or where processes and methods are described as having, including, or comprising specific steps, it is contemplated that, additionally, there are compositions of the present invention that consist essentially of, or consist of, the recited components, and that there are processes and methods according to the present invention that consist essentially of, or consist of, the recited processing steps.

[0060] As a general matter, compositions specifying a percentage are by weight unless otherwise specified. Further, if a variable is not accompanied by a definition, then the previous definition of the variable controls.

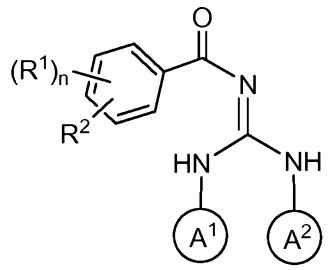
I. Modulators of F_1F_0-ATPase Activity

[0061] In some embodiments, the present invention regulates F_1F_0-ATPase activity (e.g., mitochondrial F_1F_0-ATPase activity) through the exposure of cells to compounds of the present invention. In some embodiments, the compounds inhibit ATP synthesis and ATP hydrolysis. The effect of the compounds can be measured by detecting any number of cellular changes. For example, mitochondrial F_1F_0-ATPase activity and/or cell death may be assayed as described herein and in the art. In some embodiments, cell lines are maintained under appropriate cell culturing conditions (*e.g.*, gas (CO_2), temperature and media) for an appropriate period of time to attain exponential proliferation without density dependent constraints. Cell number and or viability are measured using standard techniques, such as trypan blue exclusion/hemo-cytometry, or an Alamar Blue or MTT dye conversion assay. Alternatively, the cell may be analyzed for the expression of genes or gene products associated with aberrations in apoptosis or necrosis.

[0062] In some embodiments, exposing the compounds of the present invention to a cell induces apoptosis. In certain other embodiments, the present invention induces apoptosis or arrest of cell proliferation through interacting with the mitochondrial F_1F_0-ATPase. In yet other embodiments, compounds of the present invention cause an initial increase in cellular ROS levels (*e.g.*, O_2^-) when administered to a subject.

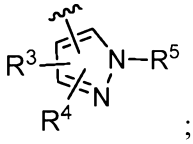
II. Pyrazolyl Guanidine Compounds

[0063] One aspect of the invention provides a family of compounds represented by Formula I:



(I)

including all stereoisomers, geometric isomers, and tautomers; or a pharmaceutically acceptable salt or solvate thereof; wherein:

A^1 is  ;

A^2 is phenyl substituted with 1, 2, or 3 substituents independently selected from the group consisting of halogen, C_1-C_6 alkyl, and C_1-C_3 haloalkyl;

R^1 represents independently for each occurrence chloro, fluoro, C_1-C_6 alkoxy, trifluoromethyl, or cyano;

R^2 is hydrogen, chloro, fluoro, C_1-C_6 alkoxy, or trifluoromethyl;

R^3 is C_1-C_6 alkyl or C_3-C_6 cycloalkyl;

R^4 is C_1-C_3 haloalkyl;

R^5 is hydrogen, C_1-C_6 alkyl, or C_3-C_6 cycloalkyl; and

n is 1 or 2.

[0064] Definitions of the variables in Formula I above encompass multiple chemical groups. The application contemplates embodiments where, for example, i) the definition of a variable is a single chemical group selected from those chemical groups set forth above, ii) the definition is a collection of two or more of the chemical groups selected from those set forth above, and iii) the compound is defined by a combination of variables in which the variables are defined by (i) or (ii), e.g., such as where R^1 and R^2 are fluoro, and R^5 is hydrogen or C_1-C_6 alkyl.

[0065] As described above, it has been discovered that the substitution pattern on the pyrazolyl can significantly impact properties of the compound, such as illustrated by experimental results presented herein showing a substantially increased blood plasma concentration of compound when the compound has, in addition to a haloalkyl group attached to a carbon atom of the pyrazolyl, an alkyl group attached to a carbon atom vicinal to the carbon atom bearing the haloalkyl group. Accordingly, pyrazolyl guanidine compounds herein, e.g., compounds of Formula I, may be further characterized according to the physical property of having a greater (e.g., at least 1.5-fold, 2-fold, 2.5-fold, or 3-fold greater) Cmax and/or AUC when administered orally to a subject (e.g., mouse or rat) than the corresponding compound lacking an alkyl group attached to a carbon atom of the pyrazolyl vicinal to the carbon atom bearing the haloalkyl group (e.g., where R^3 is hydrogen).

[0066] In certain embodiments, the compound is a compound of Formula I or a stereoisomer, geometric isomer, or tautomer; or a pharmaceutically acceptable salt of any of the foregoing.

[0067] In certain embodiments, R^1 is chloro or fluoro. In certain embodiments, R^1 is fluoro, e.g., *meta*-fluoro. In other embodiments, R^1 is chloro, e.g., *ortho*-chloro or *meta*-chloro.

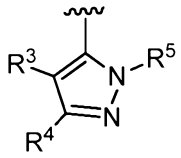
[0068] In certain embodiments, R^2 is hydrogen, chloro, or fluoro. In certain embodiments, R^2 is hydrogen. In certain embodiments, R^2 is chloro or fluoro. In certain embodiments, R^2 is fluoro, e.g., *para*-fluoro. In other embodiments, R^2 is chloro, e.g., *para*-chloro.

[0069] In certain embodiments, R^1 is fluoro, and R^2 is fluoro, e.g., R^1 is *meta*-fluoro, and R^2 is *para*-fluoro. In other embodiments, R^1 is chloro, and R^2 is chloro, e.g., R^1 is *ortho*-chloro, and R^2 is *para*-chloro. In still other embodiments, R^1 is chloro (e.g., *meta*-chloro), and R^2 is hydrogen.

[0070] In certain embodiments, R^3 is C_1-C_6 alkyl, such as methyl or ethyl.

[0071] In certain embodiments, R^4 is trifluoromethyl.

[0072] In certain embodiments, R^5 is hydrogen or C_1-C_6 alkyl. In certain embodiments, R^5 is C_1-C_6 alkyl, such as methyl or ethyl. In other embodiments, R^5 is hydrogen.

[0073] In certain embodiments, A^1 is 

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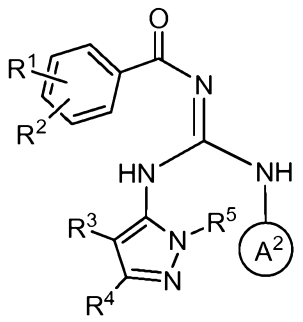
[0074] In certain embodiments, A^2 is phenyl substituted with 1, 2, or 3 substituents independently selected from the group consisting of halogen and C_1-C_3 haloalkyl. In certain embodiments, A^2 is phenyl substituted with 1, 2, or 3 substituents independently selected from the group consisting of chloro, fluoro, and C_1-C_3 haloalkyl. In certain embodiments, A^2 is phenyl substituted with 1 or 2 substituents independently selected from the group consisting of chloro, fluoro, and C_1-C_3 haloalkyl. In certain embodiments, A^2 is phenyl substituted with 1 or 2 substituents independently selected from the group consisting of chloro and fluoro. In certain embodiments, A^2 is 3-chloro-5-fluorophenyl.

[0075] In certain embodiments, n is 1. In certain other embodiments, n is 2.

[0076] All combinations of variable definitions are encompassed. For example, in certain embodiments, R^1 is chloro or fluoro; R^2 is hydrogen, chloro, or fluoro; R^3 is C_1-C_6 alkyl (e.g., methyl or ethyl); R^4 is trifluoromethyl; and A^2 is phenyl substituted with 1 or 2 substituents independently selected from the group consisting of chloro and fluoro (e.g., A^2 is 3-chloro-5-fluorophenyl). In certain embodiments, n is 1, R^1 is fluoro, and R^2 is fluoro, e.g., R^1 is *meta*-fluoro, and R^2 is *para*-fluoro. In other embodiments, n is 1, R^1 is chloro, and R^2 is chloro, e.g., R^1 is *ortho*-chloro, and R^2 is *para*-chloro. In still other embodiments, n is 1, R^1 is chloro (e.g., *meta*-chloro), and R^2 is hydrogen. In certain embodiments, n is 1, R^1 is chloro or fluoro; R^2 is hydrogen, chloro, or fluoro; R^3 is C_1-C_6 alkyl (e.g., methyl or ethyl); R^4 is trifluoromethyl; A^2 is phenyl substituted with 1 or 2 substituents independently selected from the group consisting of chloro and fluoro (e.g., A^2 is 3-chloro-5-fluorophenyl); and R^5 is C_1-C_6 alkyl (e.g., methyl or ethyl). In certain embodiments, n is 1, R^1 is fluoro, and R^2 is fluoro, e.g., R^1 is *meta*-fluoro, and R^2 is *para*-fluoro. In other embodiments, n is 1, R^1 is chloro, and R^2 is chloro, e.g., R^1 is *ortho*-chloro, and R^2 is *para*-chloro. In still other embodiments, n is 1, R^1 is chloro (e.g., *meta*-chloro), and R^2 is hydrogen. In certain embodiments, n is 1, R^1 is chloro or fluoro; R^2 is hydrogen, chloro, or fluoro; R^3 is C_1-C_6 alkyl (e.g., methyl or ethyl); R^4 is trifluoromethyl; A^2 is phenyl substituted with 1 or 2 substituents independently selected from the group consisting of chloro and fluoro (e.g., A^2 is 3-chloro-5-fluorophenyl); and R^5 is hydrogen. In certain embodiments, n is 1, R^1 is fluoro, and R^2 is fluoro, e.g., R^1 is *meta*-fluoro, and R^2 is *para*-fluoro. In other embodiments, n is 1, R^1 is chloro, and R^2 is chloro, e.g., R^1 is *ortho*-chloro, and R^2 is *para*-chloro. In still other embodiments, n is 1, R^1 is chloro (e.g., *meta*-chloro), and R^2 is hydrogen.

[0077] Another aspect of the invention provides a compound of Formula I-A:



(I-A)

including all stereoisomers, geometric isomers, and tautomers; or a pharmaceutically acceptable salt thereof; wherein:

R^1 is chloro or fluoro;

R^2 is hydrogen, chloro, or fluoro;

R^3 is C_1-C_6 alkyl;

R^4 is trifluoromethyl;

R^5 is hydrogen or C_1-C_6 alkyl; and

A^2 is phenyl substituted with 1 or 2 substituents independently selected from the group consisting of chloro and fluoro.

[0078] Definitions of the variables in Formula I-A above encompass multiple chemical groups. The application contemplates embodiments where, for example, i) the definition of a variable is a single chemical group selected from those chemical groups set forth above, ii) the definition is a collection of two or more of the chemical groups selected from those set forth above, and iii) the compound is defined by a combination of variables in which the variables are defined by (i) or (ii), e.g., such as where R^1 and R^2 are fluoro, and R^5 is hydrogen or C_1-C_6 alkyl.

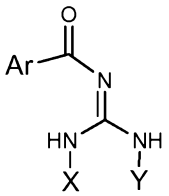
[0079] In certain embodiments, R^1 and R^2 are fluoro. In certain other embodiments, R^1 is *meta*-fluoro, and R^2 is *para*-fluoro. In certain embodiments, R^1 and R^2 are chloro. In certain embodiments, R^1 is *ortho*-chloro, and R^2 is *para*-chloro. In certain embodiments, R^1 is chloro, and R^2 is hydrogen. In certain embodiments, R^1 is *meta*-chloro.

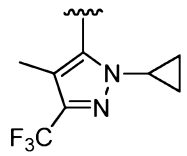
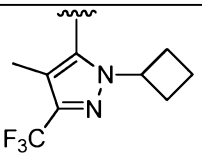
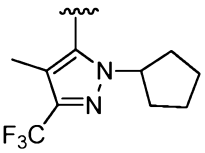
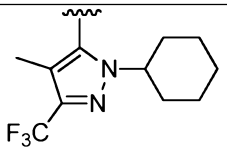
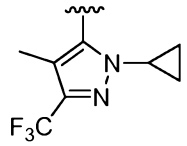
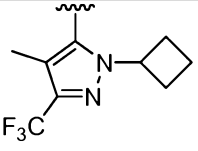
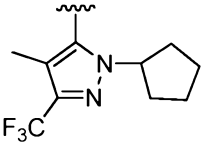
[0080] In certain embodiments, R^3 is methyl or ethyl. In certain embodiments, R^5 is hydrogen. In certain embodiments, R^5 is C_1-C_6 alkyl. In certain embodiments, R^5 is methyl or ethyl.

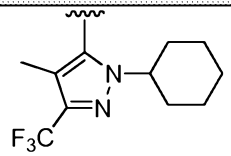
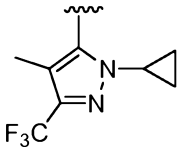
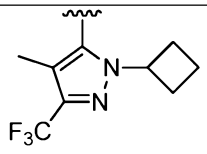
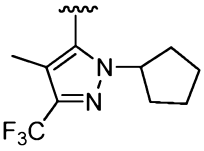
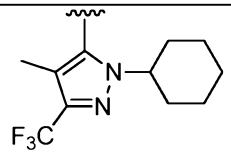
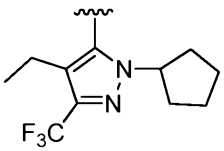
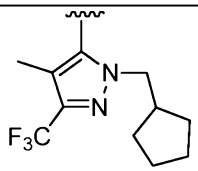
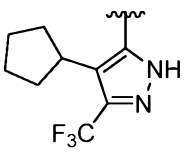
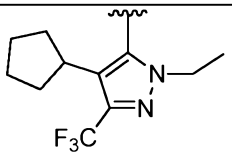
[0081] In certain embodiments, A^2 is 3-chloro-5-fluorophenyl.

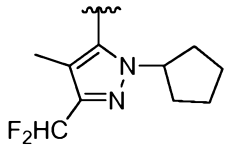
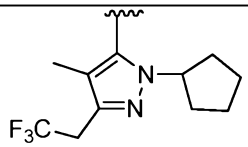
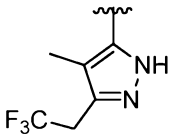
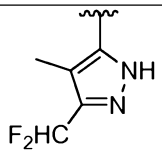
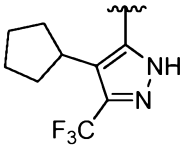
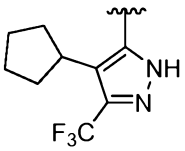
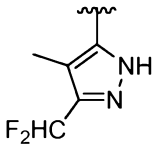
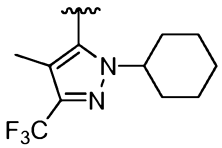
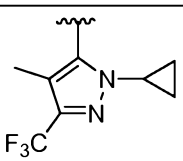
[0082] In certain embodiments, the compound is one of the compounds listed in any one of Tables 1-4 below, or a pharmaceutically acceptable salt thereof. In certain other embodiments, the compound is one of the compounds listed in Table 1 below, or a pharmaceutically acceptable salt thereof. It is understood that the foregoing compounds can be combined with a pharmaceutically acceptable carrier to produce a pharmaceutical composition.

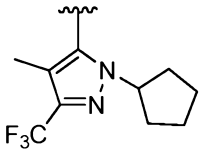
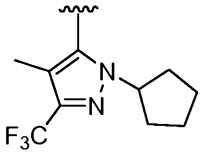
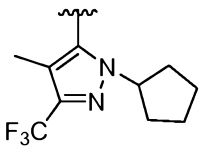
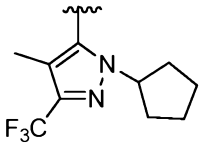
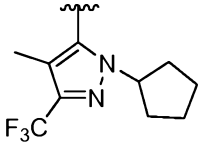
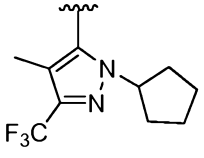
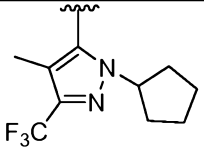
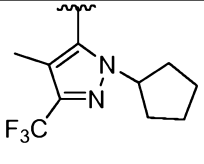
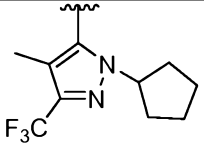
TABLE 1

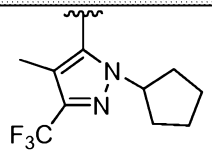
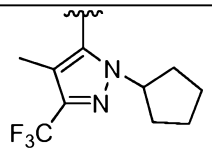
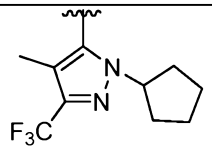
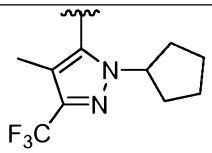
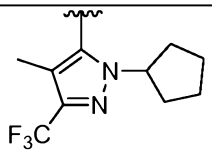


No.	Ar	X	Y
I-1	3-chlorophenyl		3-chloro-5-fluorophenyl
I-2	3-chlorophenyl		3-chloro-5-fluorophenyl
I-3	3-chlorophenyl		3-chloro-5-fluorophenyl
I-4	3-chlorophenyl		3-chloro-5-fluorophenyl
I-5	3,4-difluorophenyl		3-chloro-5-fluorophenyl
I-6	3,4-difluorophenyl		3-chloro-5-fluorophenyl
I-7	3,4-difluorophenyl		3-chloro-5-fluorophenyl

No.	Ar	X	Y
I-8	3,4-difluorophenyl		3-chloro-5-fluorophenyl
I-9	2,4-dichlorophenyl		3-chloro-5-fluorophenyl
I-10	2,4-dichlorophenyl		3-chloro-5-fluorophenyl
I-11	2,4-dichlorophenyl		3-chloro-5-fluorophenyl
I-12	2,4-dichlorophenyl		3-chloro-5-fluorophenyl
I-13	3-chlorophenyl		3-chloro-5-fluorophenyl
I-14	3-chlorophenyl		3-chloro-5-fluorophenyl
I-15	3-chlorophenyl		3-chloro-5-fluorophenyl
I-16	3-chlorophenyl		3-chloro-5-fluorophenyl

No.	Ar	X	Y
I-17	3-chlorophenyl		3-chloro-5-fluorophenyl
I-18	3-chlorophenyl		3-chloro-5-fluorophenyl
I-19	3-chlorophenyl		3-chloro-5-fluorophenyl
I-20	3-chlorophenyl		3-chloro-5-fluorophenyl
I-21	3-chlorophenyl		3-chloro-5-fluorophenyl
I-22	3,4-difluorophenyl		3-chloro-5-fluorophenyl
I-23	3,4-difluorophenyl		3-chloro-5-fluorophenyl
I-24	3-cyanophenyl		3-chloro-5-fluorophenyl
I-25	3-cyanophenyl		3-chloro-5-fluorophenyl

No.	Ar	X	Y
I-26	3-cyanophenyl		3-chloro-5-fluorophenyl
I-27	3-cyano-4-fluorophenyl		3-chloro-5-fluorophenyl
I-28	3-cyano-4-trifluoromethylphenyl		3-chloro-5-fluorophenyl
I-29	3-cyano-4-methylphenyl		3-chloro-5-fluorophenyl
I-30	2-chloro-4-fluorophenyl		3-chloro-5-fluorophenyl
I-31	2-chloro-4-cyanophenyl		3-chloro-5-fluorophenyl
I-32	2-chloro-3-cyanophenyl		3-chloro-5-fluorophenyl
I-33	2-chloro-4-fluorophenyl		3-chlorophenyl
I-34	3,4-difluorophenyl		3-chlorophenyl

No.	Ar	X	Y
I-35	3-fluoro-4-methoxyphenyl		3-chlorophenyl
I-36	3-methoxy-4-fluorophenyl		3-chlorophenyl
I-37	2-chloro-3-methoxy-4-fluorophenyl		3-chlorophenyl
I-38	3-chlorophenyl		3-chloro-5-fluorophenyl
I-39	3,4-difluorophenyl		3-chloro-5-fluorophenyl

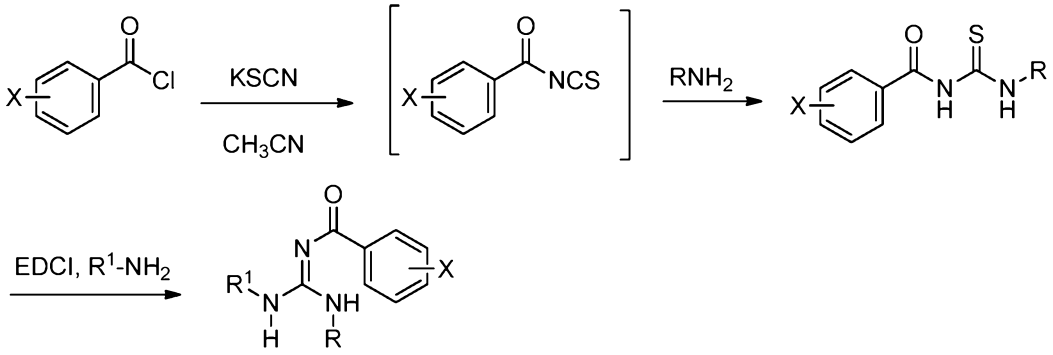
[0083] In certain embodiments, the compound is one of the compounds listed in Table 2 or 4 in the Examples section, or a pharmaceutically acceptable salt thereof. It is understood that the foregoing compounds can be combined with a pharmaceutically acceptable carrier to produce a pharmaceutical composition.

[0084] Exemplary methods for preparing compounds described herein are provided in the examples. Further exemplary procedures for making various compounds described herein are described in Schemes 1A and 1B below. The synthetic scheme is provided for the purpose of illustrating the invention, but not for limiting the scope or spirit of the invention. Starting materials can be obtained from commercial sources or be prepared based on procedures described in the literature.

[0085] The synthetic route in Scheme 1-A involves reacting an optionally substituted benzoylchloride with potassium thiocyanate in an organic solvent to form an acyl isothiocyanate intermediate. This acyl isothiocyanate intermediate is treated with an amine to

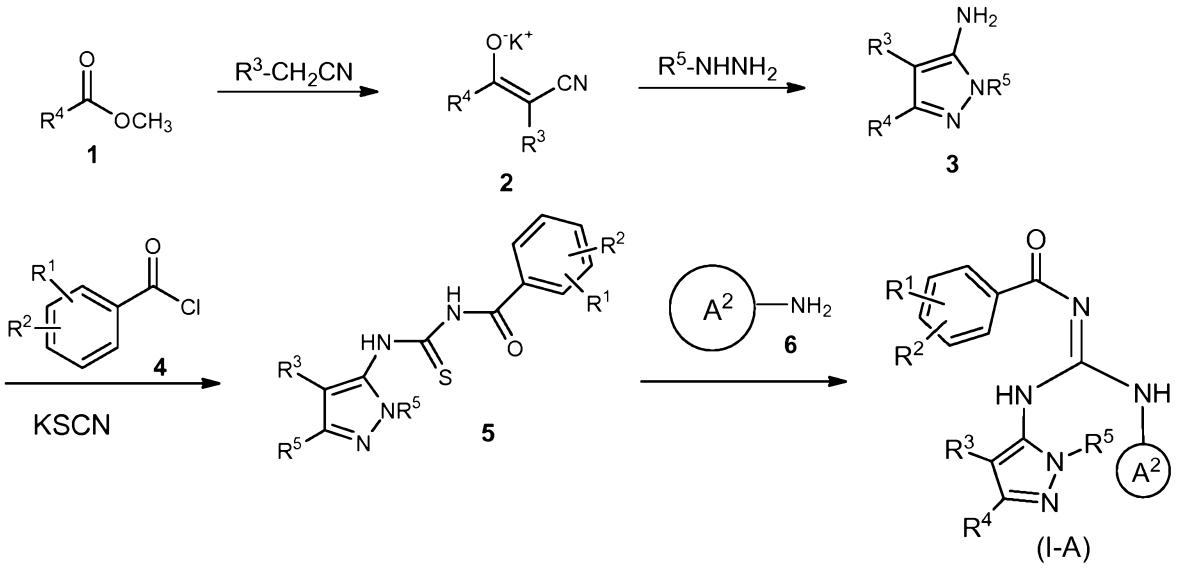
form an acyl thiourea, which is isolated by filtration or extraction. The acyl thiourea is coupled with a second amine using a coupling agent such as 1-ethyl-2',2'-dimethylaminopropylcarbodiimide hydrochloride salt (EDCI) to form the desired pyrazolyl-containing acyl-guanidine product. To the extent that either amine compound contains further functionality that may undergo reaction under the conditions illustrated in Scheme 1, standard protecting group strategies for protection and deprotection may be employed. See, for example, Greene, T.W.; Wuts, P.G.M. *Protective Groups in Organic Synthesis*, 2nd ed.; Wiley: New York, 1991.

SCHEME 1-A



[0086] A more specific synthetic route is provided in Scheme 1-B, which involves forming an enolate salt **2** from ester **1** (e.g., R^4 is CF_3) and a nitrile, such as propionitrile (R^3 is CH_3). The enolate salt **2** is then reacted with hydrazine (for formula (I-A) compounds in which R^5 is hydrogen) or an alkyl or cycloalkyl-substituted hydrazine to form pyrazole **3**, which in turn is treated with potassium thiocyanate and a substituted benzoylchloride **4** and to form thiourea **5**. The thiourea is reacted with a reagent, such as *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride, and aniline **6** to form the desired pyrazolyl guanidine compound.

SCHEME 1-B



III. Therapeutic Applications of Pyrazolyl Guanidine Compounds

[0087] It is contemplated that the guanidine compounds described herein, such as the guanidine compounds of Formula I or I-A, provide therapeutic benefits to patients suffering from any one or more of a number of conditions, *e.g.*, diseases characterized by dysregulation of F_1F_0-ATPase activity, diseases characterized by dysregulation of necrosis and/or apoptosis processes in a cell or tissue, and diseases characterized by aberrant cell growth and/or hyperproliferation. The compounds described herein can also be used to treat a variety of dysregulatory disorders related to cellular death as described elsewhere herein. Additionally, the compounds described herein can be used to inhibit ATP synthesis.

[0088] Accordingly, one aspect of the invention provides a method of treating a subject suffering from a medical disorder. The method comprises administering to the subject a therapeutically effective amount of one or more pyrazolyl guanidine compounds described herein, e.g., a compound of Formula I or I-A, as described in Section II above, in order to ameliorate a symptom of the disorder.

[0089] A large number of medical disorders can be treated using the guanidine compounds described herein. For example, the compounds described herein can be used to treat medical disorders characterized by dysregulation of necrosis and/or apoptosis processes in a cell or tissue, diseases characterized by aberrant cell growth and/or hyperproliferation, *etc.*, or lupus,

rheumatoid arthritis, psoriasis, graft-versus-host disease, Crohn's disease, inflammatory bowel disease, multiple sclerosis, cardiovascular disease, myeloma, lymphoma, cancer, and bacterial infection. In certain embodiments, the cancer is a solid tumor, leukemia, colon cancer, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, lung cancer, small cell lung cancer, non-small cell lung cancer, bladder cancer, stomach cancer, cervical cancer, testicular tumor, skin cancer, rectal cancer, thyroid cancer, kidney cancer, uterus cancer, espophagus cancer, liver cancer, an acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, or retinoblastoma.

[0090] Although not wishing to be bound to a particular theory, it is believed that the compounds impart therapeutic benefit by modulating (*e.g.*, inhibiting or promoting) the activity of the F_1F_0-ATPase complexes (e.g., mitochondrial F_1F_0-ATPase complexes) in affected cells or tissues. In some embodiments, the compositions of the present invention are used to treat immune/chronic inflammatory conditions (*e.g.,* psoriasis, autoimmune disorders, organ-transplant rejection, and epidermal hyperplasia). In further embodiments, the compositions of the present invention are used in conjunction with stenosis therapy to treat compromised (*e.g.,* occluded) vessels.

[0091] In certain embodiments, a composition comprising a guanidine compound is administered under conditions (e.g., timing, dose, co-administration with other agent, mode of administration, selection of subject, use of targeting agents, etc.) that maximize desired effects directed at the F_1F_0-ATPase.

[0092] In certain embodiments, the medical disorder is an immune disorder. In certain other embodiments, the medical disorder is an inflammatory disorder. In certain other embodiments, the medical disorder is an autoimmune disorder. In certain other embodiments, the medical disorder is rheumatoid arthritis, psoriasis, chronic graft-versus-host disease, acute graft-versus-host disease, Crohn's disease, inflammatory bowel disease, multiple sclerosis, systemic lupus erythematosus, Celiac Sprue, idiopathic thrombocytopenic thrombotic purpura, myasthenia gravis, Sjogren's syndrome, scleroderma, ulcerative colitis, asthma, uveitis, or epidermal hyperplasia.

[0093] In certain embodiments, the disorder is Crohn's disease or ulcerative colitis.

[0094] In certain other embodiments, the medical disorder is cartilage inflammation, bone degradation, arthritis, juvenile arthritis, juvenile rheumatoid arthritis, pauciarticular juvenile rheumatoid arthritis, polyarticular juvenile rheumatoid arthritis, systemic onset juvenile rheumatoid arthritis, juvenile ankylosing spondylitis, juvenile enteropathic arthritis, juvenile reactive arthritis, juvenile Reter's Syndrome, SEA Syndrome, juvenile dermatomyositis, juvenile psoriatic arthritis, juvenile scleroderma, juvenile systemic lupus erythematosus, juvenile vasculitis, pauciarticular rheumatoid arthritis, polyarticular rheumatoid arthritis, systemic onset rheumatoid arthritis, ankylosing spondylitis, enteropathic arthritis, reactive arthritis, Reter's Syndrome, dermatomyositis, psoriatic arthritis, vasculitis, myolitis, polymyolitis, dermatomyolitis, osteoarthritis, polyarteritis nodossa, Wegener's granulomatosis, arteritis, polymyalgia rheumatica, sarcoidosis, sclerosis, primary biliary sclerosis, sclerosing cholangitis, dermatitis, atopic dermatitis, atherosclerosis, Still's disease, chronic obstructive pulmonary disease, Guillain-Barre disease, Type I diabetes mellitus, Graves' disease, Addison's disease, Raynaud's phenomenon, or autoimmune hepatitis. In certain embodiments, the psoriasis is plaque psoriasis, guttate psoriasis, inverse psoriasis, pustular psoriasis, or erythrodermic psoriasis.

[0095] In certain other embodiments, the medical disorder is Crohn's disease, inflammatory bowel disease, multiple sclerosis, graft-versus-host disease, lupus, rheumatoid arthritis, or psoriasis. In certain other embodiments, the medical disorder is cardiovascular disease, myeloma, lymphoma, or cancer. In certain other embodiments, the medical disorder is lupus, rheumatoid arthritis, psoriasis, graft-versus-host disease, myeloma, or lymphoma. In certain other embodiments, the medical disorder is cardiovascular disease or cancer. In certain other embodiments, the medical disorder is Crohn's disease, inflammatory bowel disease, or multiple sclerosis. In certain other embodiments, the medical disorder is graft-versus-host disease. In further embodiments, the medical disorder is a bacterial infection. In certain embodiments, the patient (or subject) is a human.

[0096] As indicated above, the guanidine compounds described herein can be used in the treatment of a bacterial infection. A variety of bacteria are contemplated to be susceptible to the guanidine compounds. Representative bacteria include *Staphylococci* species, e.g., *S. aureus*; *Enterococci* species, e.g., *E. faecalis* and *E. faecium*; *Streptococci* species, e.g., *S. pyogenes* and *S. pneumoniae*; *Escherichia* species, e.g., *E. coli*, including enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic and enteroaggregative *E. coli* strains;

Haemophilus species, e.g., *H. influenza*; and *Moraxella* species, e.g., *M. catarrhalis*. Other examples include *Mycobacteria* species, e.g., *M. tuberculosis*, *M. avian-intracellulare*, *M. kansasii*, *M. bovis*, *M. africanum*, *M. genavense*, *M. leprae*, *M. xenopi*, *M. simiae*, *M. scrofulaceum*, *M. malmoense*, *M. celatum*, *M. abscessus*, *M. chelonae*, *M. szulgai*, *M. gordonae*, *M. haemophilum*, *M. fortuni* and *M. marinum*; *Corynebacteria* species, e.g., *C. diphtheriae*; *Vibrio* species, e.g., *V. cholerae*; *Campylobacter* species, e.g., *C. jejuni*; *Helicobacter* species, e.g., *H. pylori*; *Pseudomonas species*, e.g., P. *aeruginosa*; *Legionella* species, e.g., *L. pneumophila*; *Treponema* species, e.g., *T. pallidum*; *Borrelia* species, e.g., *B. burgdorferi*; *Listeria* species, e.g., *L monocytogenes*; *Bacillus* species, e.g., *B. cereus*; *Bordatella* species, e.g., *B. pertussis*; *Clostridium* species, e.g., *C. perfringens*, *C. tetani*, *C. difficile* and *C. botulinum*; *Neisseria* species, e.g., *N. meningitidis* and *N. gonorrhoeae*; *Chlamydia* species, e.g., *C. psittaci*, *C. pneumoniae* and *C. trachomatis*; *Rickettsia* species, e.g., *R. rickettsii* and *R. prowazekii*; *Shigella* species, e.g., *S. sonnei*; *Salmonella* species, e.g., *S. typhimurium*; *Yersinia* species, e.g., *Y. enterocolitica* and *Y. pseudotuberculosis*; *Klebsiella* species, e.g., *K. pneumoniae*; *Mycoplasma* species, e.g., *M. pneumoniae*; and *Trypanosoma brucei*. In certain embodiments, the guanidine compounds described herein are used to treat a subject suffering from a bacterial infection selected from the group consisting of *S. aureus*, *E. faecalis*, *E. faecium*, *S. pyogenes*, *S. pneumonia*, and *P. aeruginosa*. In certain embodiments, the guanidine compounds described herein are used to treat a subject suffering from a *Trypanosoma brucei* infection.

[0097] The antibacterial activity of the compounds described herein may be evaluated using standard assays known in the art, such as the microbroth dilution minimum inhibition concentration (MIC) assay, as further described in National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Susceptibility Testing; Fourteenth Informational Supplement. NCCLS document M100-S14 {ISBN 1-56238-516-X}. This assay may be used to determine the minimum concentration of a compound necessary to prevent visible bacterial growth in a solution. In general, the drug to be tested is serially diluted into wells, and aliquots of liquid bacterial culture are added. This mixture is incubated under appropriate conditions, and then tested for growth of the bacteria. Compounds with low or no antibiotic activity (a high MIC) will allow growth at high concentrations of compound, while compounds with high antibiotic activity will allow bacterial growth only at lower concentrations (a low MIC).

[0098] The assay uses stock bacterial culture conditions appropriate for the chosen strain of bacteria. Stock cultures from the permanent stock culture collection can be stored as frozen suspensions at -70°C. Cultures may be suspended in 10% skim milk (BD) prior to snap freezing in dry ice/ethanol and then placed in a -70°C freezer. Cultures may be maintained on Tryptic Soy Agar containing 5% Sheep Blood at room temperature (20°C), and each culture may be recovered from frozen form and transferred an additional time before MIC testing. Fresh plates are inoculated the day before testing, incubated overnight, and checked to confirm purity and identity.

[0099] The identity and purity of the cultures recovered from the stock culture can be confirmed to rule out the possibility of contamination. The identity of the strains may be confirmed by standard microbiological methods (See, e.g., Murray *et al.,* Manual of Clinical Microbiology, Eighth Edition. ASM Press {ISBN 1-55581-255-4}). In general, cultures are streaked onto appropriate agar plates for visualization of purity, expected colony morphology, and hemolytic patterns. Gram stains can also be utilized. The identities are confirmed using a MicroScan WalkAway 40 SI Instrument (Dade Behring, West Sacramento, California). This device utilizes an automated incubator, reader, and computer to assess for identification purposes the biochemical reactions carried out by each organism. The MicroScan WalkAway can also be used to determine a preliminary MIC, which may be confirmed using the method described below.

[0100] Frozen stock cultures may be used as the initial source of organisms for performing microbroth dilution minimum inhibition concentration (MIC) testing. Stock cultures are passed on their standard growth medium for at least 1 growth cycle (18-24 hours) prior to their use. Most bacteria may be prepared directly from agar plates in 10 mL aliquots of the appropriate broth medium. Bacterial cultures are adjusted to the opacity of a 0.5 McFarland Standard (optical density value of 0.28-0.33 on a Perkin-Elmer Lambda EZ150 Spectrophotometer, Wellesley, Massachusetts, set at a wavelength of 600nm). The adjusted cultures are then diluted 400 fold (0.25 mL inoculum + 100 mL broth) in growth media to produce a starting suspension of approximately 5 x 105 colony forming units (CFU)/mL. Most bacterial strains may be tested in cation adjusted Mueller Hinton Broth (CAMHB).

[0101] Test compounds ("drugs") are solubilized in a solvent suitable for the assay, such as DMSO. Drug stock solutions may be prepared on the day of testing. Microbroth dilution stock

plates may be prepared in two dilution series, 64 to 0.06 μg drug/mL and 0.25 to 0.00025 μg drug/mL. For the high concentration series, 200 μL of stock solution (2 mg/mL) is added to duplicate rows of a 96-well microtiter plate. This is used as the first well in the dilution series. Serial two-fold decremental dilutions are made using a BioMek FX robot (Beckman Coulter Inc., Fullerton, CA) with 10 of the remaining 11 wells, each of which will contain 100 μL of the appropriate solvent/diluent. Row 12 contains solvent/diluent only and serves as the control. For the first well of the low concentration series, 200 μL of an 8 μg/mL stock are added to duplicate rows of a 96-well plate. Serial two-fold dilutions are made as described above.

[0102] Daughter 96-well plates may be spotted (3.2 μL/well) from the stock plates listed above using the BioMek FX robot and used immediately or frozen at -70°C until use. Aerobic organisms are inoculated (100 μL volumes) into the thawed plates using the BioMek FX robot. The inoculated plates are be placed in stacks and covered with an empty plate. These plates are then incubated for 16 to 24 hours in ambient atmosphere according to CLSI guidelines (National Committee for Clinical Laboratory Standards, Methods for Dilution, Antimicrobial Tests for Bacteria that Grow Aerobically; Approved Standard-Sixth Edition. NCCLS document M7-A6 {ISBN 1-56238-486-4}).

[0103] After inoculation and incubation, the degree of bacterial growth can be estimated visually with the aid of a Test Reading Mirror (Dynex Technologies 220 16) in a darkened room with a single light shining directly through the top of the microbroth tray. The MIC is the lowest concentration of drug that prevents macroscopically visible growth under the conditions of the test.

[0104] Additionally, any one or more of the pyrazolyl guanidine compounds described herein can be used to treat a F_1F_0-ATP hydrolase associated disorder (e.g., myocardial infarction, ventricular hypertrophy, coronary artery disease, non-Q wave MI, congestive heart failure, cardiac arrhythmias, unstable angina, chronic stable angina, Prinzmetal's angina, high blood pressure, intermittent claudication, peripheral occlusive arterial disease, thrombotic or thromboembolic symptoms of thromboembolic stroke, venous thrombosis, arterial thrombosis, cerebral thrombosis, pulmonary embolism, cerebral embolism, thrombophilia, disseminated intravascular coagulation, restenosis, atrial fibrillation, ventricular enlargement, atherosclerotic vascular disease, atherosclerotic plaque rupture, atherosclerotic plaque formation, transplant atherosclerosis, vascular remodeling atherosclerosis, cancer, surgery, inflammation, systematic

infection, artificial surfaces, interventional cardiology, immobility, medication, pregnancy and fetal loss, and diabetic complications comprising retinopathy, nephropathy and neuropathy) in a subject.

Combination Therapy

[0105] Additionally, the guanidine compounds described herein can be used in combination with at least one other therapeutic agent, such as Bz-423 (a benzodiazepine compound as described in U.S. Patent Nos. 7,144,880 and 7,125,866, U.S. Patent Application Serial Nos. 11/586,097, 11/585,492, 11/445,010, 11/324,419, 11/176,719, 11/110,228, 10/935,333, 10/886,450, 10/795,535, 10/634,114, 10/427, 211, 10/217,878, and 09/767,283, and U.S. Provisional Patent Nos. 60/878,519, 60/812,270, 60/802,394, 60/732,045, 60/730,711, 60/704,102, 60/686,348, 60/641,040, 60/607,599, and 60/565,788), potassium channel openers, calcium channel blockers, sodium hydrogen exchanger inhibitors, antiarrhythmic agents, antiatherosclerotic agents, anticoagulants, antithrombotic agents, prothrombolytic agents, fibrinogen antagonists, diuretics, antihypertensive agents, ATPase inhibitors, mineralocorticoid receptor antagonists, phospodiesterase inhibitors, antidiabetic agents, anti-inflammatory agents, antioxidants, angiogenesis modulators, antiosteoporosis agents, hormone replacement therapies, hormone receptor modulators, oral contraceptives, antiobesity agents, antidepressants, antianxiety agents, antipsychotic agents, antiproliferative agents, antitumor agents, antiulcer and gastroesophageal reflux disease agents, growth hormone agents and/or growth hormone secretagogues, thyroid mimetics, anti-infective agents, antiviral agents, antibacterial agents, antifungal agents, cholesterol/lipid lowering agents and lipid profile therapies, and agents that mimic ischemic preconditioning and/or myocardial stunning, antiatherosclerotic agents, anticoagulants, antithrombotic agents, antihypertensive agents, antidiabetic agents, and antihypertensive agents selected from ACE inhibitors, AT-1 receptor antagonists, ET receptor antagonists, dual ET/AII receptor antagonists, vasopepsidase inhibitors, an antiplatelet agent selected from GPIIb/IIIa blockers, $P2Y_1$ and $P2Y_{12}$ antagonists, thromboxane receptor antagonists, or aspirin, along with a pharmaceutically-acceptable carrier or diluent in a pharmaceutical composition.

IV. Pharmaceutical Compositions, Formulations, and Exemplary Administration Routes and Dosing Considerations

[0106] Exemplary embodiments of various contemplated medicaments and pharmaceutical compositions are provided below.

A. Preparing Medicaments

[0107] Compounds of the present invention are useful in the preparation of medicaments to treat a variety of conditions, such as conditions associated with dysregulation of cell death, aberrant cell growth and hyperproliferation. One of skill in the art will appreciate that any one or more of the compounds described herein, including the many specific embodiments, are prepared by applying standard pharmaceutical manufacturing procedures. Such medicaments can be delivered to the subject by using delivery methods that are well-known in the pharmaceutical arts.

B. Exemplary Pharmaceutical Compositions and Formulation

[0108] In some embodiments of the present invention, the compositions are administered alone, while in some other embodiments, the compositions are preferably present in a pharmaceutical formulation comprising at least one active ingredient/agent, as discussed above, together with a solid support or alternatively, together with one or more pharmaceutically acceptable carriers and optionally other therapeutic agents (*e.g.,* those described in section III hereinabove). Each carrier should be "acceptable" in the sense that it is compatible with the other ingredients of the formulation and not injurious to the subject.

[0109] Contemplated formulations include those suitable for oral, rectal, nasal, topical (including transdermal, buccal and sublingual), vaginal, parenteral (including subcutaneous, intramuscular, intravenous and intradermal) and pulmonary administration. In some embodiments, formulations are conveniently presented in unit dosage form and are prepared by any method known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association (*e.g.*, mixing) the active ingredient with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

[0110] Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets, wherein each preferably contains a predetermined amount of the active ingredient; as a powder or granules; as a solution or suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. In other embodiments, the active ingredient is presented as a bolus, electuary, or paste, *etc.*

[0111] In some embodiments, tablets comprise at least one active ingredient and optionally one or more accessory agents/carriers are made by compressing or molding the respective agents. In some embodiments, compressed tablets are prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (*e.g.*, povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (*e.g.*, sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose) surface-active or dispersing agent. Molded tablets are made by molding in a suitable machine a mixture of the powdered compound (*e.g.*, active ingredient) moistened with an inert liquid diluent. Tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

[0112] Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

[0113] Pharmaceutical compositions for topical administration according to the present invention are optionally formulated as ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, sprays, aerosols or oils. In alternative embodiments, topical formulations comprise patches or dressings such as a bandage or adhesive plasters impregnated with active ingredient(s), and optionally one or more excipients or diluents. In some embodiments, the topical formulations include a compound(s) that enhances absorption or

penetration of the active agent(s) through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethylsulfoxide (DMSO) and related analogues.

[0114] If desired, the aqueous phase of a cream base includes, for example, at least about 30% w/w of a polyhydric alcohol, *i.e.*, an alcohol having two or more hydroxyl groups such as propylene glycol, butane-1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol and mixtures thereof.

[0115] In some embodiments, oily phase emulsions of this invention are constituted from known ingredients in a known manner. This phase typically comprises a lone emulsifier (otherwise known as an emulgent), it is also desirable in some embodiments for this phase to further comprise a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil.

[0116] Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier so as to act as a stabilizer. In some embodiments it is also preferable to include both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make up the so-called emulsifying wax, and the wax together with the oil and/or fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations.

[0117] Emulgents and emulsion stabilizers suitable for use in the formulation of the present invention include Tween 60, Span 80, cetostearyl alcohol, myristyl alcohol, glyceryl monostearate and sodium lauryl sulfate.

[0118] The choice of suitable oils or fats for the formulation is based on achieving the desired properties (*e.g.*, cosmetic properties), since the solubility of the active compound/agent in most oils likely to be used in pharmaceutical emulsion formulations is very low. Thus creams should preferably be non-greasy, non-staining and washable products with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP may be used, the last three being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

[0119] Formulations suitable for topical administration to the eye also include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent for the agent.

[0120] Formulations for rectal administration may be presented as a suppository with suitable base comprising, for example, cocoa butter or a salicylate.

[0121] Formulations suitable for vaginal administration may be presented as pessaries, creams, gels, pastes, foams or spray formulations containing in addition to the agent, such carriers as are known in the art to be appropriate.

[0122] Formulations suitable for nasal administration, wherein the carrier is a solid, include coarse powders having a particle size, for example, in the range of about 20 to about 500 microns which are administered in the manner in which snuff is taken, *i.e.*, by rapid inhalation (*e.g.*, forced) through the nasal passage from a container of the powder held close up to the nose. Other suitable formulations wherein the carrier is a liquid for administration include, but are not limited to, nasal sprays, drops, or aerosols by nebulizer, and include aqueous or oily solutions of the agents.

[0123] Formulations suitable for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain antioxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. In some embodiments, the formulations are presented/formulated in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

[0124] Preferred unit dosage formulations are those containing a daily dose or unit, daily subdose, as herein above-recited, or an appropriate fraction thereof, of an agent.

[0125] It should be understood that in addition to the ingredients particularly mentioned above, the formulations of this invention may include other agents conventional in the art

having regard to the type of formulation in question, for example, those suitable for oral administration may include such further agents as sweeteners, thickeners and flavoring agents. It also is intended that the agents, compositions and methods of this invention be combined with other suitable compositions and therapies. Still other formulations optionally include food additives (suitable sweeteners, flavorings, colorings, *etc.*), phytonutrients (*e.g.*, flax seed oil), minerals (*e.g.*, Ca, Fe, K, *etc.*), vitamins, and other acceptable compositions (*e.g.*, conjugated linoelic acid), extenders, and stabilizers, *etc.*

C. Exemplary Administration Routes and Dosing Considerations

[0126] Various delivery systems are known and can be used to administer therapeutic agents (*e.g.,* exemplary compounds as described above) of the present invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, receptor-mediated endocytosis, and the like. Methods of delivery include, but are not limited to, intra-arterial, intra-muscular, intravenous, intranasal, and oral routes. In specific embodiments, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, injection, or by means of a catheter.

[0127] The agents identified can be administered to subjects or individuals susceptible to or at risk of developing pathological growth of target cells and correlated conditions. When the agent is administered to a subject such as a mouse, a rat or a human patient, the agent can be added to a pharmaceutically acceptable carrier and systemically or topically administered to the subject. To identify patients that can be beneficially treated, a tissue sample is removed from the patient and the cells are assayed for sensitivity to the agent.

[0128] Therapeutic amounts are empirically determined and vary with the pathology being treated, the subject being treated and the efficacy and toxicity of the agent. When delivered to an animal, the method is useful to further confirm efficacy of the agent. One example of an animal model is MLR/MpJ-*lpr/lpr* ("MLR-*lpr*") (available from Jackson Laboratories, Bar Harbor, Maine). MLR-*lpr* mice develop systemic autoimmune disease. Alternatively, other animal models can be developed by inducing tumor growth, for example, by subcutaneously inoculating nude mice with about 10^5 to about 10^9 hyperproliferative, cancer or target cells as defined herein. When the tumor is established, the compounds described herein are administered, for example, by subcutaneous injection around the tumor. Tumor measurements

to determine reduction of tumor size are made in two dimensions using venier calipers twice a week. Other animal models may also be employed as appropriate. Such animal models for the above-described diseases and conditions are well-known in the art.

[0129] In some embodiments, *in vivo* administration is effected in one dose, continuously or intermittently throughout the course of treatment. Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and vary with the composition used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations are carried out with the dose level and pattern being selected by the treating physician.

[0130] Suitable dosage formulations and methods of administering the agents are readily determined by those of skill in the art. Preferably, the compounds are administered at about 0.01 mg/kg to about 200 mg/kg, more preferably at about 0.1 mg/kg to about 100 mg/kg, even more preferably at about 0.5 mg/kg to about 50 mg/kg. When the compounds described herein are co-administered with another agent (*e.g.*, as sensitizing agents), the effective amount may be less than when the agent is used alone.

[0131] The pharmaceutical compositions can be administered orally, intranasally, parenterally or by inhalation therapy, and may take the form of tablets, lozenges, granules, capsules, pills, ampoules, suppositories or aerosol form. They may also take the form of suspensions, solutions and emulsions of the active ingredient in aqueous or non-aqueous diluents, syrups, granulates or powders. In addition to an agent of the present invention, the pharmaceutical compositions can also contain other pharmaceutically active compounds or a plurality of compounds of the invention.

[0132] More particularly, an agent of the present invention also referred to herein as the active ingredient, may be administered for therapy by any suitable route including, but not limited to, oral, rectal, nasal, topical (including, but not limited to, transdermal, aerosol, buccal and sublingual), vaginal, parental (including, but not limited to, subcutaneous, intramuscular, intravenous and intradermal) and pulmonary. It is also appreciated that the preferred route varies with the condition and age of the recipient, and the disease being treated.

[0133] Ideally, the agent should be administered to achieve peak concentrations of the active compound at sites of disease. This may be achieved, for example, by the intravenous

injection of the agent, optionally in saline, or by oral administration, for example, as a tablet, capsule or syrup containing the active ingredient.

[0134] Desirable blood levels of the agent may be maintained by a continuous infusion to provide a therapeutic amount of the active ingredient within disease tissue. The use of operative combinations is contemplated to provide therapeutic combinations requiring a lower total dosage of each component than may be required when each individual therapeutic compound or drug is used alone, thereby reducing adverse effects.

D. Exemplary Co-administration Routes and Dosing Considerations

[0135] The invention also includes methods involving co-administration of the compounds described herein with one or more additional active agents. Indeed, it is a further aspect of this invention to provide methods for enhancing prior art therapies and/or pharmaceutical compositions by co-administering a compound of this invention. In co-administration procedures, the agents may be administered concurrently or sequentially. In one embodiment, the compounds described herein are administered prior to the other active agent(s). The pharmaceutical formulations and modes of administration may be any of those described above. In addition, the two or more co-administered chemical agents, biological agents or radiation may each be administered using different modes or different formulations.

[0136] The agent or agents to be co-administered depend on the type of condition being treated. For example, when the condition being treated is cancer, the additional agent can be a chemotherapeutic agent or radiation. When the condition being treated is an immune disorder, the additional agent can be an immunosuppressant or an anti-inflammatory agent. When the condition being treated is chronic inflammation, the additional agent can be an anti-inflammatory agent. The additional agents to be co-administered, such as anticancer, immunosuppressant, anti-inflammatory, can be any of the well-known agents in the art, including, but not limited to, those that are currently in clinical use. The determination of appropriate type and dosage of radiation treatment is also within the skill in the art or can be determined with relative ease.

[0137] Treatment of the various conditions associated with abnormal apoptosis is generally limited by the following two major factors: (1) the development of drug resistance and (2) the toxicity of known therapeutic agents. In certain cancers, for example, resistance to chemicals and radiation therapy has been shown to be associated with inhibition of apoptosis. Some

therapeutic agents have deleterious side effects, including non-specific lymphotoxicity, renal and bone marrow toxicity.

[0138] The methods described herein address both these problems. Drug resistance, where increasing dosages are required to achieve therapeutic benefit, is overcome by co-administering the compounds described herein with the known agent. The compounds described herein sensitize target cells to known agents (and vice versa) and, accordingly, less of these agents are needed to achieve a therapeutic benefit.

[0139] The sensitizing function of the claimed compounds also addresses the problems associated with toxic effects of known therapeutics. In instances where the known agent is toxic, it is desirable to limit the dosages administered in all cases, and particularly in those cases where drug resistance has increased the requisite dosage. When the claimed compounds are co-administered with the known agent, they reduce the dosage required which, in turn, reduces the deleterious effects.

EXAMPLES

[0140] The invention now being generally described, 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. Various compounds were characterized by high performance liquid chromatography (HPLC). HPLC methods used are as follows: Method A1 conditions were Agilent Zorbax C-18 column, 4.6 x 50 mm, 1.8 micron, 28 °C, 2.0 mL/min, 5 min gradient of 5% to 95% MeCN (0.05% TFA) in H_2O (0.1% TFA), then 95% MeCN (0.05% TFA) in H_2O (0.1% TFA) for 1.5 min; Method A2 conditions were Waters Symmetry C-18 column, 4.6 x 150 mm, 3.5 micron, 26 °C, 2.0 mL/min, 5 min 50% MeCN (0.05% TFA) in H_2O (0.05% TFA), 5 min gradient of 50% to 95% MeCN (0.05% TFA) in H_2O (0.05% TFA), then 95% MeCN (0.05% TFA) in H_2O (0.05% TFA) for 5 min; Method A4 conditions were Shim-pack XR-ODS Column, 3.0 x 50 mm, 2.2 micron, 40 °C, 1.0 mL/min, 4.2 min gradient of 5% to 100% MeCN (0.05% TFA) in H_2O (0.05% TFA), then 100% MeCN (0.05% TFA) for 1 min; Method A5 conditions were Shim-pack XR-ODS Column, 3.0 x 50 mm, 2.2 micron, 40 °C, 1.0 mL/min, 2.2 min gradient of 5% to 100% MeCN (0.05% TFA) in H_2O (0.05% TFA), then 100% MeCN (0.05% TFA) for 1 min; Method A6 conditions were Shim-pack XR-ODS Column, 3.0 x 50 mm, 2.2 micron, 40 °C, 1.0 mL/min, 5.3 min gradient of 5% to 80% MeCN (0.05% TFA) in H_2O (0.05% TFA),

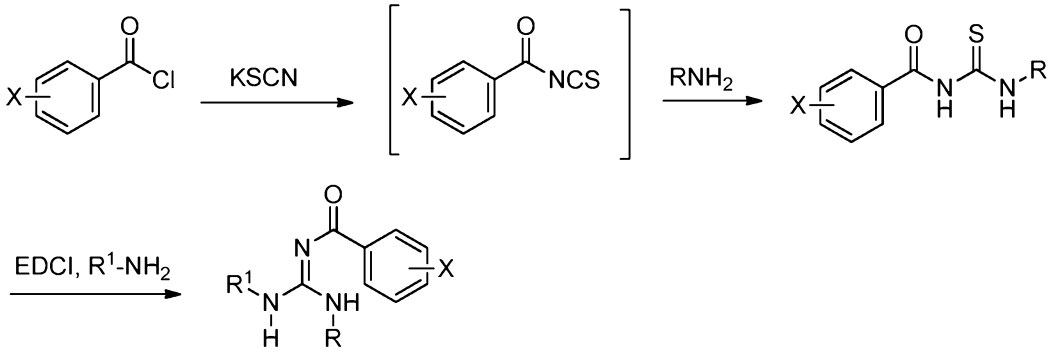
then 80% MeCN (0.05% TFA) in H_2O (0.05% TFA) for 1.7 min; Method A7 conditions were Phenomenex Kinetext Column, 3.0 x 50 mm, 2.6 micron, 40 °C, 1.5 mL/min, 2.8 min gradient of 50% to 100% MeCN (0.1% TFA) in H_2O (0.1% TFA), then 100% MeCN (0.1% TFA) for 1 min; Method A8 conditions were Phenomenex Kinetext Column, 3.0 x 50 mm, 2.6 micron, 40 °C, 1.5 mL/min, 2 min gradient of 10% to 100% MeCN (0.1% TFA) in H_2O (0.1% TFA), then 100% MeCN (0.1% TFA) for 0.6 min; and Method A9 conditions were Phenomenex Kinetext Column, 3.0 x 50 mm, 2.6 micron, 40 °C, 1.5 mL/min, 2.5 min gradient of 30% to 100% MeCN (0.1% TFA) in H_2O (0.1% TFA), then 100% MeCN (0.1% TFA) for 1 min. The phrase "MeCN (0.05% TFA)" is art-recognized and refers to acetonitrile containing 0.05% v/v trifluoroacetic acid. The phrase "MeCN (0.1% TFA)" is art-recognized and refers to acetonitrile containing 0.1% v/v trifluoroacetic acid. The phrase "H_2O (0.1% TFA)" is art-recognized and refers to water containing 0.1% v/v trifluoroacetic acid. The phrase "H_2O (0.05% TFA)" is art-recognized and refers to water containing 0.05% v/v trifluoroacetic acid.

EXAMPLE 1 – PREPARATION OF PYRAZOLYL GUANIDINE COMPOUNDS

[0141] Described below are exemplary, general synthetic procedures for making pyrazolyl guanidine compounds, along with an exemplary synthetic procedure for making the specific pyrazolyl guanidine compound *N*-(((3-chloro-5-fluorophenyl)amino)((4-methyl-5-(trifluoromethyl)-1*H*-pyrazol-3-yl)amino)methylene)-3,4-difluorobenzamide.

Part I: General Method for Making Pyrazolyl Guandine Compounds

SCHEME 2



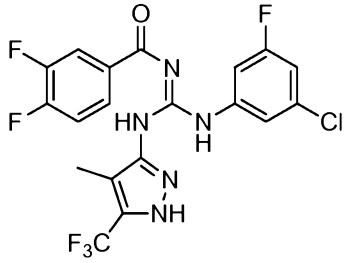
[0142] In the first step, the requisite acid chloride is combined with potassium thiocyanate in an organic solvent, and this mixture is stirred at ambient temperature for about 30 to 240

minutes. A first amine is added (either neat or as a solution in an organic solvent) and stirring is continued until the reaction is complete or nearly complete (typically 30 minutes to 18 hours). The resulting thiourea maybe obtained by concentration of the organic layer, but more commonly is precipitated by the addition of water to the reaction mixture and collected by filtration.

[0143] The thiourea and an appropriate second amine (R^1-NH_2) are dissolved in a polar organic solvent such as tetrahydrofuran, acetonitrile or dimethylformamide at ambient temperature to form a mixture. To this mixture, 1-ethyl-2',2'-dimethylaminopropylcarbodiimide hydrochloride salt is added and the resulting mixture is stirred until the reaction appears complete by HPLC analysis of aliquots of the reaction mixture. Typical reaction times range from 30 minutes to 12 hours, and the reaction mixture may be heated (e.g., to approximately 50-60 °C) to accelerate the reaction. Once the reaction appears to be complete by HPLC analysis, the reaction mixture is diluted with an organic solvent (such as ethylacetate), washed with water, washed with brine, and the organic layer is dried over an appropriate drying agent, filtered, and the solvents removed under reduced pressure. The desired product can be purified by chromatography if necessary. In some cases, the crude reaction mixture may be concentrated directly onto silica gel omitting the extractive work-up, and the product isolated by chromatography.

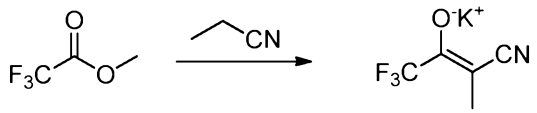
[0144] Many amines, including anilines are readily available from commercial sources or may be prepared using synthetic procedures described in the literature. The requisite hydrazines for the preparation of *N*-substituted pyrazolyl-amines may be prepared following literature methods such as those described in, for example, International Patent Application Publication Nos. WO 2011/146594 and WO 2011/124930. Additional procedures for preparing acyl guanidine compounds are described in, for example, International Patent Application Publication Nos. WO 2009/036175, WO 2010/030891, WO 2012/078867, WO 2012/078869, and WO 2012/078874.

Part II: Exemplary Synthetic Procedure for Preparing Pyrazolyl Guandine Compound N-(((3-Chloro-5-fluorophenyl)amino)((4-methyl-5-(trifluoromethyl)-1H-pyrazol-3-yl)amino)methylene)-3,4-difluorobenzamide (Compound 1)



(**1**)

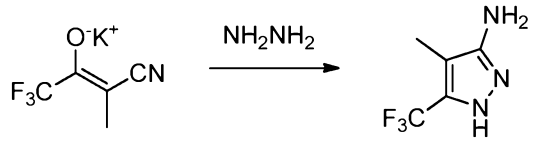
Step A: Representative Procedure for Preparing an Enolate Salt



Potassium (*Z*)-3-cyano-1,1,1-trifluorobut-2-en-2-olate

[0145] To a 1M solution of potassium *tert*-butoxide in THF (164 mL, 164 mmol) was added ethyl 2,2,2-trifluoroacetate (15.7 mL, 156 mmol) at 0 °C. Propionitrile was added to the reaction mixture dropwise at 0 °C. The resulting solution was stirred overnight at room temperature then concentrated under vacuum. The resultant product was used without further purification.

Step B: Representative Procedure for Pyrazole Formation.

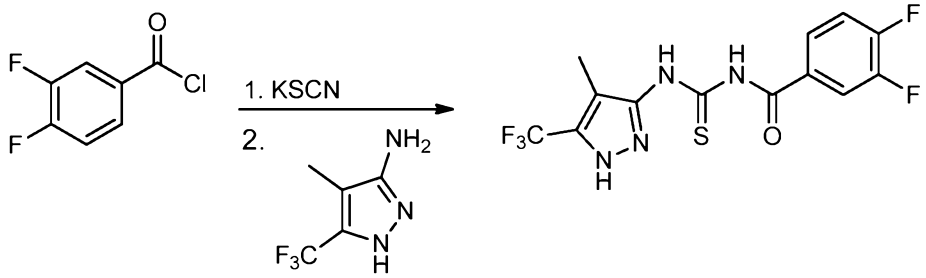


4-Methyl-5-(trifluoromethyl)-1*H*-pyrazol-3-amine

[0146] To potassium (*Z*)-3-cyano-1,1,1-trifluoro-but-2-en-2-olate (29.5 g, 156 mmol) in ethanol (240 mL) was added acetic acid (11.2 g, 187 mmol) followed by hydrazine (1.0 g, 31.4 mmol) at room temperature. This solution was warmed to 70 °C for 1 day then concentrated to a light brown sludge. This residue was re-dissolved in ethyl acetate and washed with dilute

aqueous sodium bicarbonate followed by brine. The organic layer was dried over sodium sulfate then re-concentrated and dissolved in a minimum quantity of diethyl ether (~70 mL) and diluted slowly with hexanes (~250 mL). This mixture was stirred at room temperature overnight then filtered to give the title compound as a brown solid. HPLC: Retention time 2.42 minutes; (254 nm) (25 to 95% MeCN/water; 2 mL/min.; 0.1% TFA; Symmetry C18 2.5 μm; 4.6 x 150 mm).

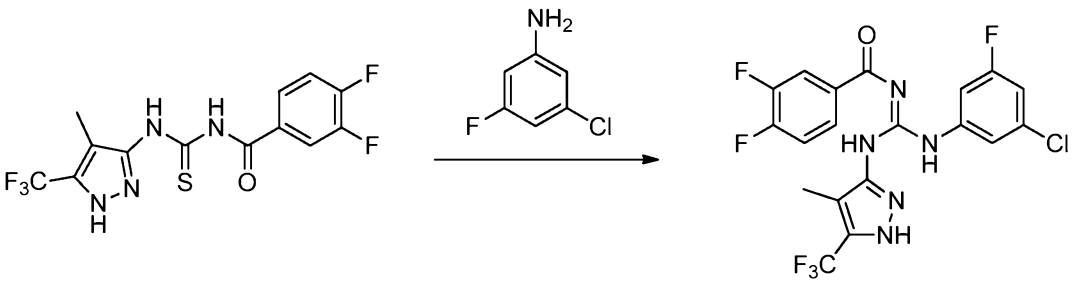
Step C: Representative Procedure for Thiourea Formation



3,4-Difluoro-*N*-((4-methyl-5-(trifluoromethyl)-1*H*-pyrazol-3-yl)carbamothioyl)benzamide

[0147] 3,4-Difluorobenzoyl chloride (2 g, 11.33 mmol) was dissolved in acetonitrile (30 mL), and potassium thiocyanate (1.21 g, 12.46 mmol) was added in one portion. A precipitate formed. The mixture was stirred at room temperature for 50 minutes, then 4-methyl-5-(trifluoromethyl)-1*H*-pyrazol-3-amine (1.87 g, 11.33 mmol) was added. This solution was stirred for another 45 minures at room temperature. Water (90 mL) was added and the mixture was stirred for 5 minutes, then filtered, rinsing the pad with water to give a tan colored solid that was dried *in vacuo*. The resulting solid was chromatographed on silica gel to give the title compound as a light tan colored solid (3.84 g).

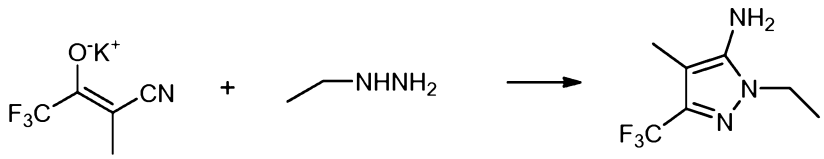
Step D: Representative Procedure for Coupling of Substituted Aniline to a Thiourea



***N*-(((3-Chloro-5-fluorophenyl)amino)((4-methyl-5-(trifluoromethyl)-1*H*-pyrazol-3-yl)amino)methylene)-3,4-difluorobenzamide**.

[0148] 3,4-Difluoro-*N*-((4-methyl-5-(trifluoromethyl)-1*H*-pyrazol-3-yl)carbamothioyl) benzamide (1.9 g, 5.2 mmol) and 3-chloro-5-fluoroaniline (0.84 g, 5.73 mmol) were combined in THF (20 mL) and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (1.1 g, 5.73 mmol) was added. This mixture was warmed to 50 °C for 2 h, then allowed to cool. The cooled reaction mixture was diluted with ethyl acetate and washed twice with water then once with brine and dried over anhydrous sodium sulfate. Chromatography on silica gel eluting with 5-15% ethyl acetate in hexanes gave a yellow oil that was dried in a 50 °C vacuum oven to give a yellow solid. This solid was stirred in diethyl ether (~20 mL) at room termperature then filtered and rinsed with 20% ether in hexanes to give the product as a white solid (1 g, 2.1 mmol). HPLC (Method A1): retention time was 7.01 minutes; MS: calc. = 475.78; obs. 474.21 (neg mode).

EXAMPLE 2 – PREPARATION OF 1-ETHYL-4-METHYL-3-(TRIFLUOROMETHYL)-1H-PYRAZOL-5-AMINE



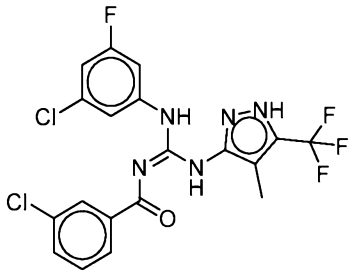
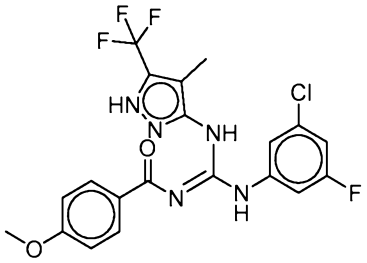
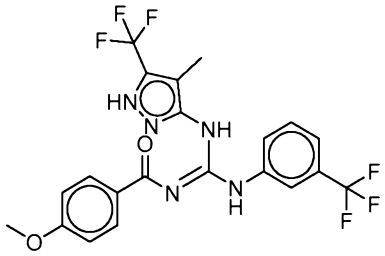
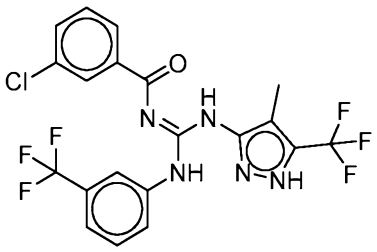
[0149] Potassium (*Z*)-3-cyano-1,1,1-trifluoro-but-2-en-2-olate (1 g, 5.29 mmol) and ethyl hydrazine oxalate (0.79 g, 5.29 mmol) were suspended in ethanol (18 mL) and stirred at room temperature for 7 days. The resulting mixture was filtered and the filtrate was concentrated onto silica gel and chromatographed to give the title compound (0.38 g).

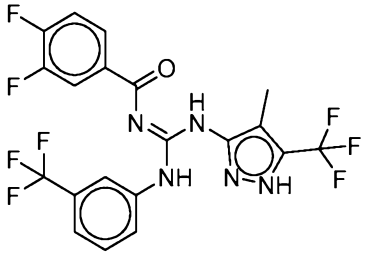
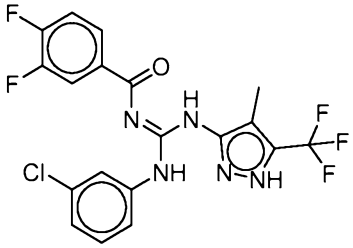
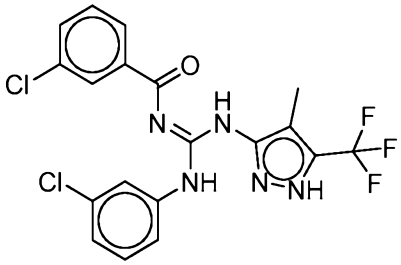
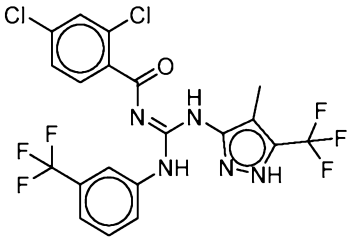
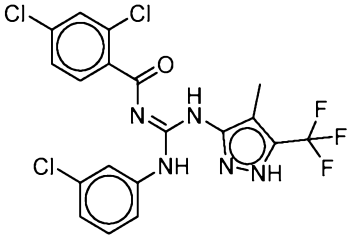
EXAMPLE 3 – ADDITIONAL PYRAZOLYL GUANIDINE COMPOUNDS & CHARACTERIZATION DATA

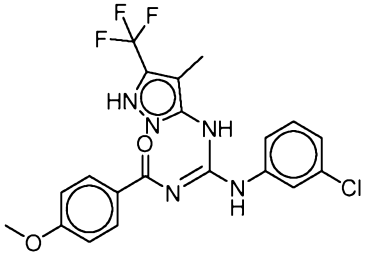
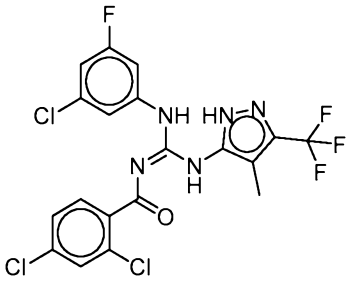
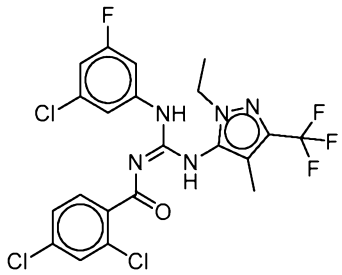
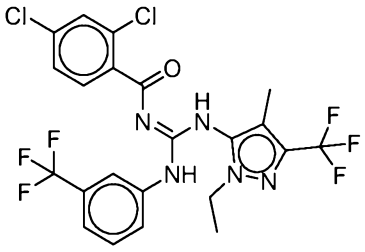
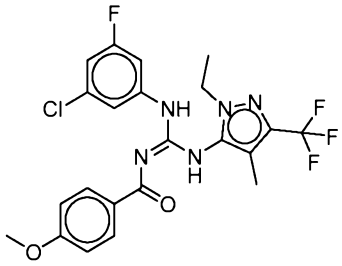
[0150] Compounds in Table 2 below were prepared based on the procedures described above. Starting materials can be obtained from commercial sources or readily prepared from commercially available materials. Furthermore, exemplary compounds were characterized by high performance liquid chromatography (HPLC) and/or mass spectrometry (MS). Unless indicated otherwise, mass spectral data in Table 2 was collected using electrospray ionization in the positive ion mode. The HPLC method and retention time, along with mass spectral data are

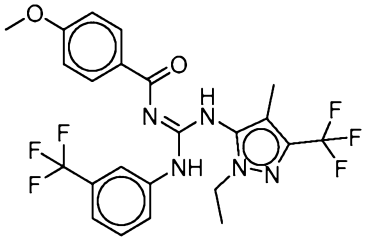
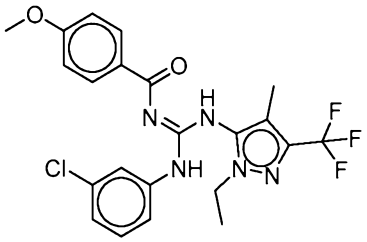
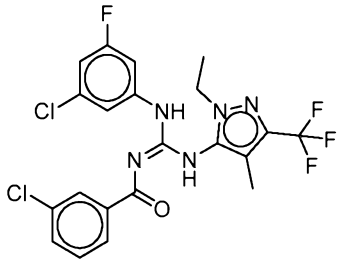
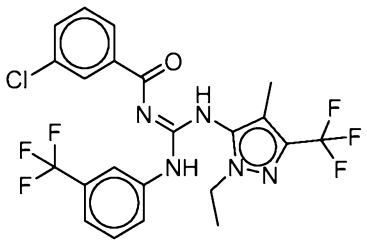
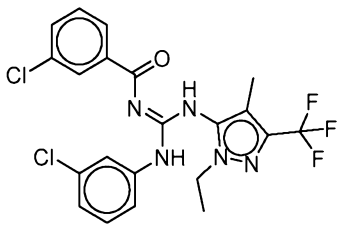
provided in Table 2 below. ^{1}H nuclear magnetic resonance data for exemplary compounds is provided in Table 3 below. The symbol "NA" indicates that no data was available.

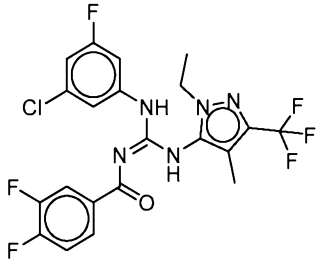
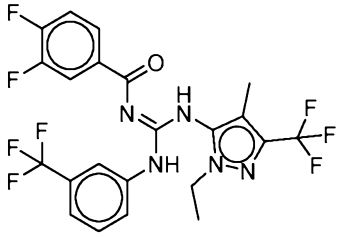
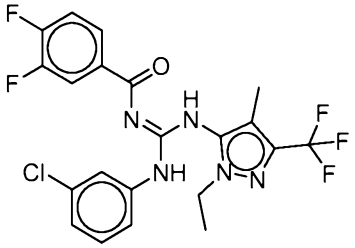
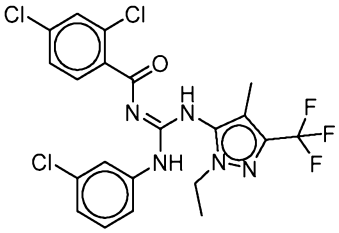
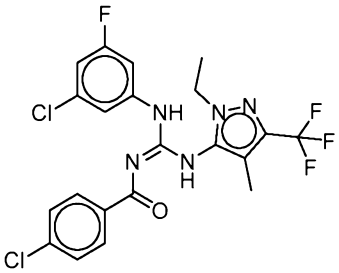
TABLE 2

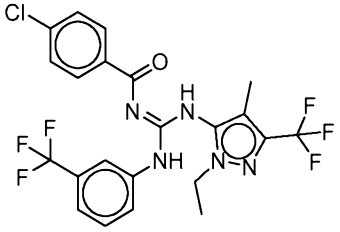
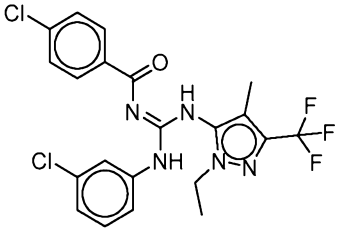
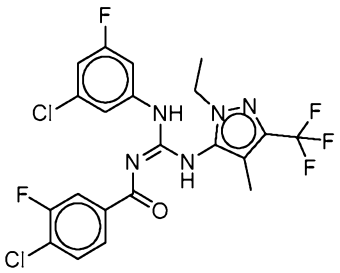
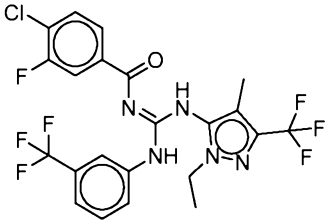
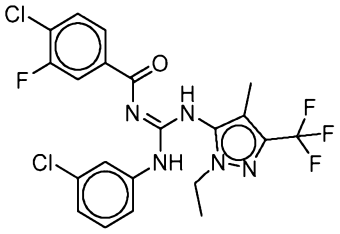
Compound No.	Chemical Structure	Calculated MW (g/mol)	MS (m/z)	HPLC Method	HPLC Retention Time (min)
A-1		474.24	474, 476	A2	7.84
A-2		469.82	470, 472	A4	4.530
A-3		485.38	486	A5	2.759
A-4		489.8	490, 492	A4	4.480

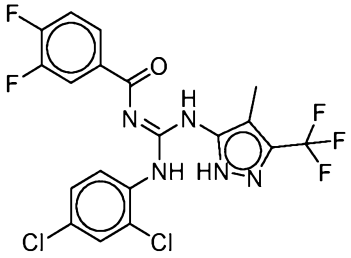
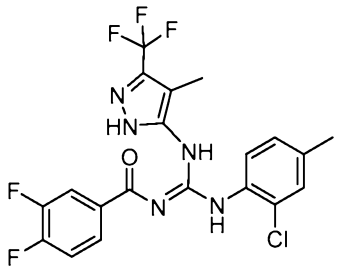
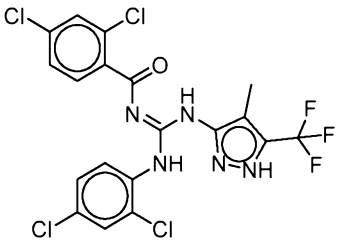
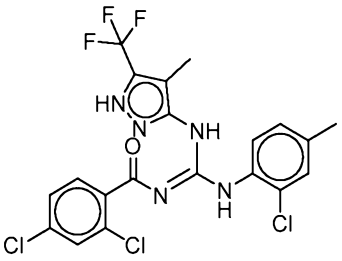
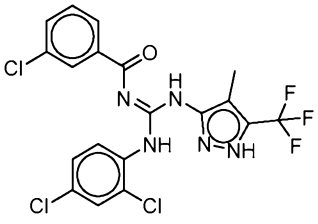
Compound No.	Chemical Structure	Calculated MW (g/mol)	MS (m/z)	HPLC Method	HPLC Retention Time (min)
A-5		491.34	492	A5	2.824
A-6		457.78	458, 460	A5	2.827
A-7		456.25	456, 458, 460	A4	4.465
A-8		524.25	524, 526	A5	2.263
A-9		490.69	490, 492, 494	A6	6.072

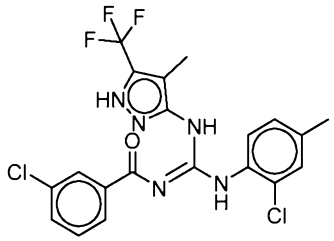
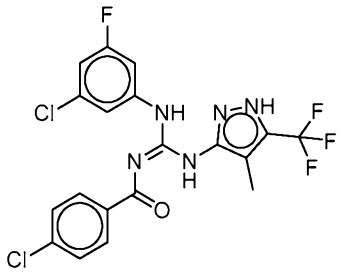
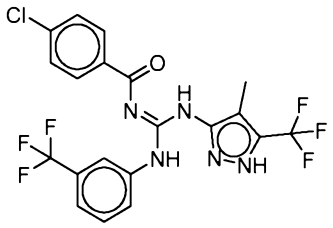
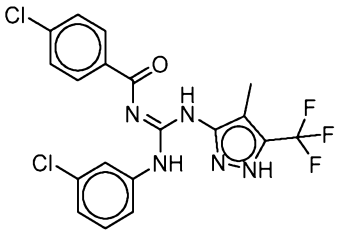
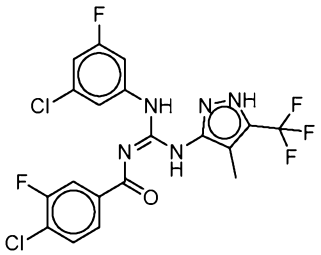
Compound No.	Chemical Structure	Calculated MW (g/mol)	MS (m/z)	HPLC Method	HPLC Retention Time (min)
A-10		451.83	452, 454	A5	2.712
A-11		508.68	508, 510, 512	A5	2.574
A-12		536.74	536, 538, 540	A4	4.116
A-13		552.3	552, 554	A5	2.380
A-14		497.87	498, 500	A5	2.370

Compound No.	Chemical Structure	Calculated MW (g/mol)	MS (m/z)	HPLC Method	HPLC Retention Time (min)
A-15		513.44	514	A5	2.364
A-16		479.88	480, 482	A5	2.352
A-17		502.29	502, 504	A5	2.531
A-18		517.85	518, 520	A5	2.475
A-19		484.3	484, 486	A5	2.371

Compound No.	Chemical Structure	Calculated MW (g/mol)	MS (m/z)	HPLC Method	HPLC Retention Time (min)
A-20		503.83	504, 506	A5	1.629
A-21		519.39	520	A5	2.327
A-22		485.84	486, 488	A5	2.310
A-23		518.75	518, 520, 522	A5	2.480
A-24		502.29	502, 504	A5	2.760

Compound No.	Chemical Structure	Calculated MW (g/mol)	MS (m/z)	HPLC Method	HPLC Retention Time (min)
A-25		517.85	518, 520	A5	2.464
A-26		484.3	484, 486	A5	2.718
A-27		520.28	520, 522	A5	2.849
A-28		535.85	536, 538	A5	2.504
A-29		502.29	502, 504	A5	2.820

Compound No.	Chemical Structure	Calculated MW (g/mol)	MS (m/z)	HPLC Method	HPLC Retention Time (min)
A-30		492.23	492, 494	A5	2.605
A-31		471.81	472, 474	A7	1.970
A-32		525.14	524, 526, 528	A7	2.744
A-33		504.72	504, 506	A8	2.092
A-34		490.69	490, 492, 494	A5	2.704

Compound No.	Chemical Structure	Calculated MW (g/mol)	MS (m/z)	HPLC Method	HPLC Retention Time (min)
A-35		470.28	470, 472	A9	2.341
A-36		474.24	474, 476	A5	2.909
A-37		489.8	490, 492	A5	2.408
A-38		456.25	456, 458	A5	2.567
A-39		492.23	492, 494	A5	2.912

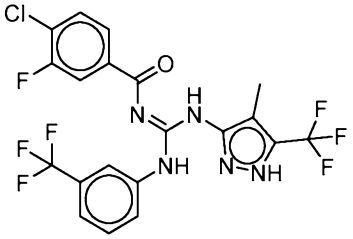
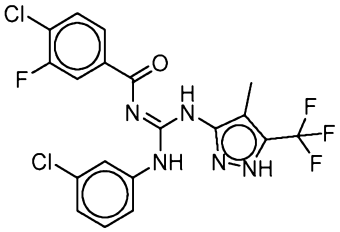
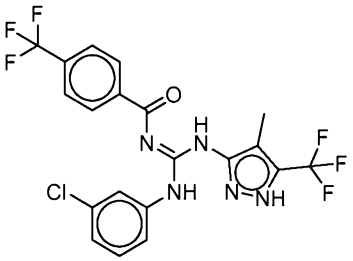
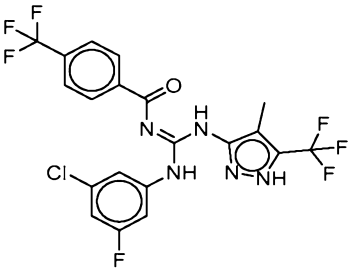
Compound No.	Chemical Structure	Calculated MW (g/mol)	MS (m/z)	HPLC Method	HPLC Retention Time (min)
A-40		507.79	508, 510	A6	3.790
A-41		474.24	474, 476	A8	2.124
A-42		489.8	488 (neg mode)	A2	6.75
A-43		507.79	506	A2	7.4

TABLE 3

Compound No.	NMR Solvent	^{1}H NMR Resonance Data (δ)
A-1	DMSO-d_6	12.84 (s, 1H), 10.58 (s,1H), 9.93 (s, 1H), 7.93 (m, 1H), 7.8-7.5 (m, 5H), 7.05 (m,1H), 1.79 (s, 3H).
A-2	$CDCl_3$ with TFA-d	11.47 (s, 3H), 7.85 (br s, 2H), 7.24-6.99 (m, 5H), 3.90 (s, 3H), 2.09 (br s, 3H)
A-3	$CDCl_3$ with TFA-d	11.37 (br s, 3H), 8.00-7.49 (m, 6H), 7.40-7.01 (m, 2H), 3.92 (s, 3H), 2.14 (br s, 3H)
A-4	$CDCl_3$ with TFA-d	11.64 (br s, 3H), 8.15-7.37 (m, 8H), 2.00 (vbr s, 3H)
A-5	$CDCl_3$ with TFA-d	11.10 (br s, 3H), 8.13-7.29 (m, 7H), 2.03 (br s, 3H)
A-6	$CDCl_3$ with TFA-d	10.64 (br s, 3H), 8.06-7.05 (m, 7H), 2.04 (br s, 3H)
A-7	$CDCl_3$ with TFA-d	8.20-7.03 (m, 8H), 2.02 (br s, 3H)
A-8	$CDCl_3$ with TFA-d	11.19 (br s, 1H), 10.11 (br s, 2H), 7.93-7.41 (m, 7H), 2.25 (br s, 3H)
A-9	$CDCl_3$ with TFA-d	10.37 (br s, 3H), 7.85-7.27 (m, 7H), 2.26 (br s, 3H)
A-10	$CDCl_3$ with TFA-d	11.88 (br s, 3H), 8.01-6.90 (m, 8H), 3.88 (s, 3H), 2.02 (br s, 3H)
A-11	$CDCl_3$ with TFA-d	10.32 (br s, 3H), 7.80-7.01 (m, 6H), 2.22 (br s, 3H)
A-12	$CDCl_3$ with TFA-d	10.49 (br s, 2H), 7.70-6.75 (m, 6H), 4.20-4.09 (m, 2H), 1.99 (br s, 3H), 1.45 (t, 3H)
A-13	$CDCl_3$ with TFA-d	10.70 (br s, 2H), 7.68-7.25 (m, 6H), 4.15-4.07 (m, 2H), 1.94 (br s, 3H), 1.27 (t, 3H)
A-14	$CDCl_3$ with TFA-d	11.64 (br s, 2H), 8.03 (d, 2H), 7.08-6.72 (m, 5H), 4.19-4.07 (m, 2H), 3.92 (s, 3H), 1.97 (s, 3H), 1.29 (t, 3H)

Compound No.	NMR Solvent	^{1}H NMR Resonance Data (δ)
A-15	$CDCl_3$ with TFA-d	11.76 (br s, 2H), 8.05 (d, 2H), 7.57-7.23 (m, 4H), 7.05 (d, 2H), 4.12-4.05 (m, 2H), 3.94 (s, 3H), 1.91 (s, 3H), 1.41 (t, 3H)
A-16	$CDCl_3$ with TFA-d	11.95 (br s, 2H), 8.08 (d, 2H), 7.26-6.90 (m, 6H), 4.10-4.02 (m, 2H), 3.94 (s, 3H), 1.93 (s, 3H), 1.44 (t, 3H)
A-17	$CDCl_3$ with TFA-d	11.48 (br s, 2H), 8.04-7.52 (m, 4H), 7.07-6.75 (m, 3H), 4.16-4.09 (m, 2H), 1.98 (s, 3H), 1.47 (t, 3H)
A-18	$CDCl_3$ with TFA-d	11.08 (br s, 2H), 8.03-7.25 (m, 8H), 4.13 (q, 2H), 1.93 (s, 3H), 1.42 (t, 3H)
A-19	$CDCl_3$ with TFA-d	11.04 (br s, 2H), 8.03-6.94 (m, 8H), 4.10 (q, 2H), 1.95 (s, 3H), 1.43 (t, 3H)
A-20	$CDCl_3$ with TFA-d	11.48 (br s, 2H), 7.98-6.73 (m, 6H), 4.10 (q, 2H), 1.97 (s, 3H), 1.46 (t, 3H)
A-21	$CDCl_3$ with TFA-d	10.33 (br s, 2H), 8.00-7.23 (m, 7H), 4.08 (q, 2H), 1.91 (s, 3H), 1.44 (s, 3H)
A-22	$CDCl_3$ with TFA-d	10.88 (br s, 2H), 7.97-7.87 (m, 2H), 7.45-6.93 (m, 5H), 4.10 (q, 2H), 1.94 (s, 3H), 1.43 (t, 3H)
A-23	$CDCl_3$ with TFA-d	10.49 (br s, 2H), 7.69-6.92 (m, 7H), 4.12 (q, 2H), 1.97 (s, 3H), 1.44 (t, 3H)
A-24	$CDCl_3$ with TFA-d	11.81 (br s, 2H), 8.01-6.73 (m, 7H), 4.09 (q, 2H), 1.97 (s, 3H), 1.46 (t, 3H)
A-25	$CDCl_3$ with TFA-d	11.39 (br s, 2H), 8.05-7.22 (m, 8H), 4.06 (q, 2H), 1.90 (s, 3H), 1.43 (t, 3H)
A-26	$CDCl_3$ with TFA-d	11.89 (br s, 2H), 8.08-6.91 (m, 8H), 4.06 (q, 2H), 1.93 (s, 3H), 1.45 (t, 3H)
A-27	$CDCl_3$ with TFA-d	9.02 (br s, 2H), 7.90-6.76 (m, 6H), 4.10 (q, 2H), 1.98 (s, 3H), 1.47 (t, 3H)

Compound No.	NMR Solvent	^{1}H NMR Resonance Data (δ)
A-28	$CDCl_3$ with TFA-d	9.88 (br s, 2H), 7.89-6.91 (m, 7H), 4.10 (q, 2H), 1.91 (s, 3H), 1.27 (t, 3H)
A-29	$CDCl_3$ with TFA-d	9.60 (br s, 2H), 7.91-6.93 (m, 7H), 4.09 (q, 2H), 1.95 (s, 3H), 1.45 (t, 3H)
A-30	$CDCl_3$ with TFA-d	10.44 (br s, 1H), 10.10 (br s, 2H), 8.00-7.23 (m, 6H), 2.05 (br s, 3H)
A-31	$CDCl_3$ with TFA-d	11.09 (br s, 3H), 7.88-6.88 (m, 6H), 2.32 (s, 3H), 1.93 (br s, 3H)
A-32	$CDCl_3$ with TFA-d	9.93 (br s, 3H), 7.81-6.90 (m, 6H), 2.28 (br s, 3H)
A-33	$CDCl_3$ with TFA-d	11.39 (br s, 3H), 7.75-6.90 (m, 6H), 2.37 (s, 3H), 2.28-1.96 (m, 3H)
A-34	$CDCl_3$ with TFA-d	10.41 (br s, 3H), 8.12-7.26 (m, 7H), 2.05 (br s, 3H)
A-35	$CDCl_3$ with TFA-d	10.64 (br s, 3H), 8.05-7.16 (m, 7H), 2.39 (s, 3H), 1.99 (br s, 3H)
A-36	$CDCl_3$ with TFA-d	10.73 (br s, 3H), 7.91-6.93 (m, 7H), 2.15 (br s, 3H)
A-37	$CDCl_3$ with TFA-d	10.89 (br s, 3H), 7.90-7.37 (m, 7H), 2.01 (br s, 3H)
A-38	$CDCl_3$ with TFA-d	11.20 (br s, 3H), 7.90-7.07 (m, 8H), 2.02 (br s, 3H)
A-39	$CDCl_3$ with TFA-d	9.88 (br s, 3H), 7.89-6.91 (m, 6H), 2.11 (s, 3H)
A-40	$CDCl_3$ with TFA-d	10.15 (br s, 3H), 7.94-7.32 (m, 7H), 2.02 (br s, 3H)
A-41	$CDCl_3$ with TFA-d	11.03 (br s, 3H), 7.80-7.05 (m, 7H), 2.03 (br s, 3H)

EXAMPLE 4 – ANALYSIS OF INHIBITION OF F_1F_0-ATPASE

[0151] Exemplary compounds described in above Examples were tested for activity against F_1F_0-ATPase by measuring the ability of the compounds to inhibit ATP synthesis. In addition, the compounds were assessed for cytotoxicity in Ramos cells. Results of the biological activity tests are shown in Table 4 below. Inhibition of F_1F_0-ATPase activity in synthesizing ATP and

cytotoxicity in Ramos cells were measured according to the procedures described in K. M. Johnson *et al. Chemistry & Biology* **2005**, *12*, 485-496.

TABLE 4

Compound No.	ATP Syn IC_{50} (μM)	Ramos Cell EC_{50} (μM)
1	<10	<10
A-1	<10	<10
A-2	<10	<10
A-3	<10	<10
A-4	<10	<10
A-5	<10	<10
A-6	<10	<10
A-7	<10	<10
A-8	<10	<10
A-9	<10	<10
A-10	<10	<10
A-11	<10	<10
A-12	<10	<10
A-13	<10	<10
A-14	<10	<10
A-15	<10	<10
A-16	<10	<10
A-17	<10	<10
A-18	<10	<10
A-19	<10	<10
A-20	<10	<10
A-21	<10	<10
A-22	<10	<10

Compound No.	ATP Syn IC_{50} (μM)	Ramos Cell EC_{50} (μM)
A-23	<10	<10
A-24	<10	<10
A-25	<10	<10
A-26	<10	<10
A-27	<10	<10
A-28	<10	<10
A-29	<10	<10
A-30	<10	<10
A-31	<10	<10
A-32	<10	<10
A-33	<10	<10
A-34	<10	<10
A-35	<10	<10
A-36	<10	<10
A-37	<10	<10
A-38	<10	<10
A-39	<10	<10
A-40	<10	<10
A-41	<10	<10
A-42	<10	<10
A-43	<10	<10

EXAMPLE 5 – ANALYSIS OF BLOOD PLASMA LEVELS OF COMPOUNDS

[0152] Exemplary compounds were administered orally to animals (i.e., mice or rats), and then blood samples were taken from the animal at set time points and analyzed for the amount of test compound in the blood sample. Experimental procedures and results are described below.

Part I – Experimental Procedure

Compound Preparation

[0153] A lipid emulsion containing the test compound was prepared by first dissolving the test compound in 1:4 Labrafil M1944:Solutol, then adding 0.5% carboxymethylcellulose in water with vigorous stirring to achieve a final ratio of 5:20:75 Labrafil:Solutol:0.5% aqueous CMC. The concentration of test compound in the lipid emulsion was 2 mg/mL. Test compounds analyzed in this assay are shown in Tables 5A and 5B below.

Dosing and Blood Draws

[0154] Animals (mice or rats) were dosed by oral gavage using 5 mL/Kg of dosing solution. Animals were serially bled from the dorsal metatarsal vein (mice) or jugular vein (rats) at 30 minutes, 1 h, 2 h, 4 h, 8 h and 24 h post-dose. Test compounds **1** and **B-1** were administered to a mice. Test compounds **A-1** and **B-2** were administered to rats.

Blood Treatment and Bioanalysis

[0155] The desired serial concentrations of working solutions were achieved by diluting a stock solution of analyte with 60% acetonitrile in water solution. A 5 μL aliquot of working solutions at 10, 20, 50, 100, 500, 1000, 5000, and 10000 ng/mL were added to 50 μL of blank plasma to achieve calibration standards of 1-1000 ng/mL (1, 2, 5, 10, 50, 100, 500, 1000 ng/mL) in a total volume of 55 μL.

[0156] Four quality control samples at 3 ng/mL (low 1), 5 ng/mL (low 2), 50 ng/mL (medium), and 800 ng/mL (high) were prepared independently of those used for the calibration curves. These QC samples were prepared on the day of analysis in the same way as calibration standards.

[0157] 55 μL standards, 55 μL QC samples, and 55 μL unknown samples were added to 200 μL of acetonitrile for precipitating protein respectively. Then, the samples were vortexed for 30 seconds at 4 degree Celsius. After centrifugation at 4 degree Celsius (i.e., 15000 rpm for 5 min) a 20 μL aliquot of the supernatant was analyzed by LC/MS. Unknowns were compared to standards to obtain test compound concentrations for each sample.

Part II – Results

[0158] Results of the assay are provided in Tables 5A and 5B below. The results show that in the test animal C-methyl-pyrazolyl **1** had a Cmax of 1593 ng/mL whereas reference compound **B-1** had a Cmax of 609 ng/mL. Also, the results show that in the test animal C-methyl-pyrazolyl **A-1** had a Cmax of 1435 ng/mL whereas reference compound **B-2** had a Cmax of 541 ng/mL. Accordingly, C-methyl-pyrazolyl compounds **1** and **A-1** had a higher Cmax than reference compounds **B-1** and **B-2** which lacked the corresponding C-methyl on the pyrazolyl group. Similarly, AUC was greater for C-methyl-pyrazolyl compounds **1** and **A-1** than reference compounds **B-1** and **B-2** which lacked the corresponding C-methyl on the pyrazolyl group.

TABLE 5A

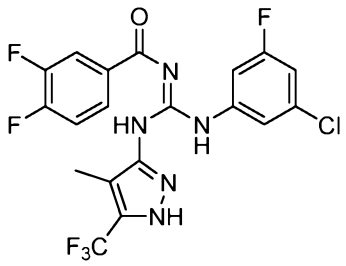
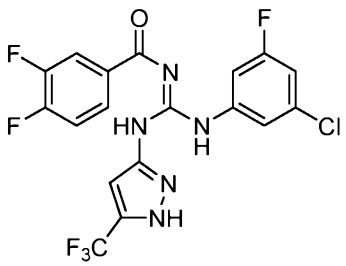
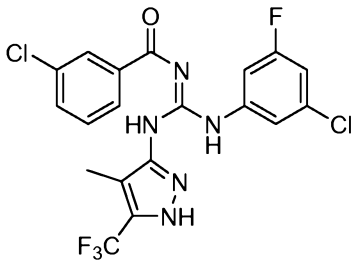
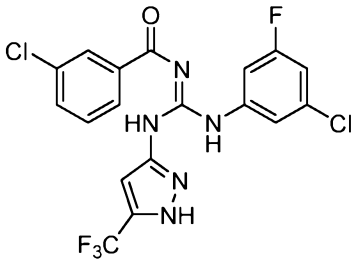
Test Compound (compound no.)	**Cmax (ng/mL)**	**AUC $_{0\text{-}24}$ (ng.h/mL)**
 (**1**)	1,593	8,755
 (reference compound **B-1**)	609	4,534

TABLE 5B

Test Compound (compound no.)	Cmax (ng/mL)	AUC $_{0\text{-}24}$ (ng.h/mL)
 (**A-1**)	1435	10,505
 (reference compound **B-2**)	541	5,480

INCORPORATION BY REFERENCE

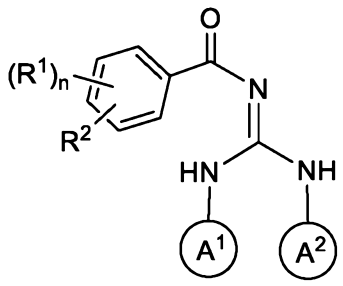
[0159] The entire disclosure of each of the patent documents and scientific articles referred to herein is incorporated by reference for all purposes.

EQUIVALENTS

[0160] The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

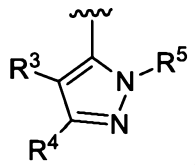
What is claimed is:

1. A compound represented by Formula I:



(I)

including all stereoisomers, geometric isomers, and tautomers; or a pharmaceutically acceptable salt or solvate thereof; wherein:

A^1 is  ;

A^2 is phenyl substituted with 1, 2, or 3 substituents independently selected from the group consisting of halogen, C_1-C_6 alkyl, and C_1-C_3 haloalkyl;

R^1 represents independently for each occurrence chloro, fluoro, C_1-C_6 alkoxy, trifluoromethyl, or cyano;

R^2 is hydrogen, chloro, fluoro, C_1-C_6 alkoxy, or trifluoromethyl;

R^3 is C_1-C_6 alkyl, C_3-C_6 cycloalkyl, or -(C_1-C_6 alkylene)-(C_3-C_6 cycloalkyl);

R^4 is C_1-C_3 haloalkyl;

R^5 is hydrogen, C_1-C_6 alkyl, or C_3-C_6 cycloalkyl; and

n is 1 or 2.

2. The compound of claim 1, wherein the compound is a compound of Formula I or or a stereoisomer, geometric isomer, or tautomer; or a pharmaceutically acceptable salt thereof.

3. The compound of claim 1 or 2, wherein n is 1.

4. The compound of any one of claims 1-3, wherein R^1 is fluoro;

   a. R^1 is *meta*-fluoro;

   b. R^1 is chloro; or

c. R^1 is *meta*-chloro.

5. The compound of any one of claims 1-4, wherein

a. R^2 is hydrogen;

b. R^2 is chloro;

c. R^2 is *para*-chloro;

d. R^2 is fluoro; or

e. R^2 is *para*-fluoro.

6. The compound of any one of claims 1-5, wherein R^3 is C_1-C_6 alkyl.

7. The compound of any one of claims 1-6, wherein R^4 is trifluoromethyl.

8. The compound of any one of claims 1-7, wherein R^5 is hydrogen.

9. The compound of any one of claims 1-7, wherein R^5 is C_1-C_6 alkyl.

10. The compound of any one of claims 1-7, wherein R^5 is C_3-C_6 cycloalkyl.

11. The compound of any one of claims 1-10, wherein

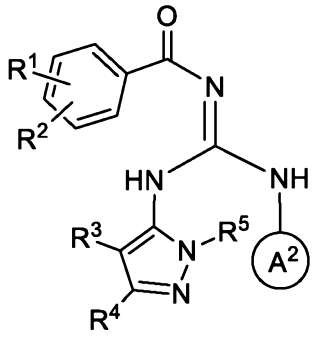
a. A^2 is phenyl substituted with 1, 2, or 3 substituents independently selected from the group consisting of chloro, fluoro, and C_1-C_3 haloalkyl;

b. A^2 is phenyl substituted with 1 or 2 substituents independently selected from the group consisting of chloro, fluoro, and trifluoromethyl;

c. A^2 is phenyl substituted with 1 or 2 substituents independently selected from the group consisting of chloro and fluoro; or

d. A^2 is 3-chloro-5-fluorophenyl.

12. The compound of claim 1, wherein the compound is represented by Formula I-A:



(I-A)

including all stereoisomers, geometric isomers, and tautomers; or a pharmaceutically acceptable salt thereof; wherein:

R^1 is chloro or fluoro;

R^2 is hydrogen, chloro, or fluoro;

R^3 is C_1-C_6 alkyl;

R^4 is trifluoromethyl;

R^5 is hydrogen or C_1-C_6 alkyl; and

A^2 is phenyl substituted with 1 or 2 substituents independently selected from the group consisting of chloro and fluoro.

13. The compound of claim 12, wherein

a. R^1 and R^2 are fluoro;

b. R^1 is *meta*-fluoro, and R^2 is *para*-fluoro;

c. R^1 and R^2 are chloro;

d. R^1 is *ortho*-chloro, and R^2 is *para*-chloro;

e. R^1 is chloro, and R^2 is hydrogen; or

f. R^1 is *meta*-chloro.

14. The compound of claim 12 or 13, wherein R^3 is methyl or ethyl.

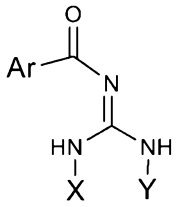
15. The compound of any one of claims 12-14, wherein R^5 is hydrogen.

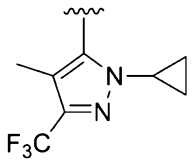
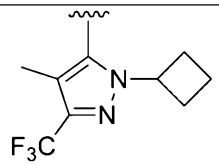
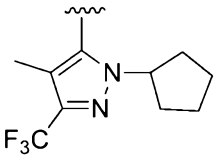
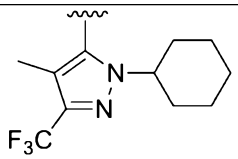
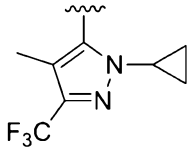
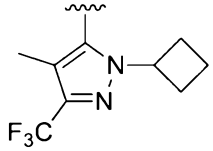
16. The compound of any one of claims 12-14, wherein R^5 is C_1-C_6 alkyl.

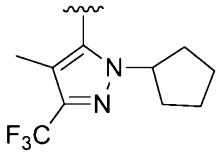
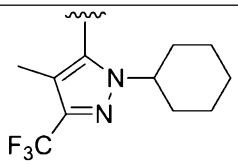
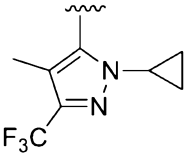
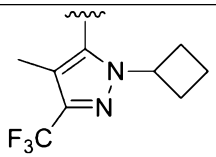
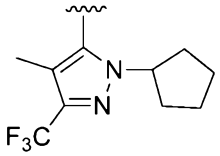
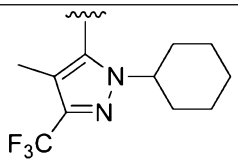
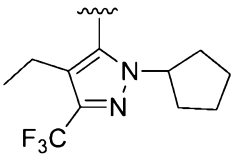
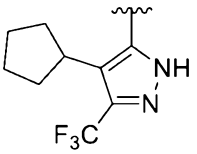
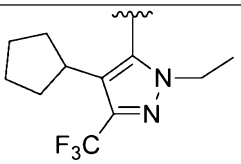
17. The compound of any one of claims 12-16, wherein A^2 is 3-chloro-5-fluorophenyl.

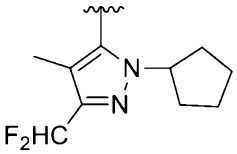
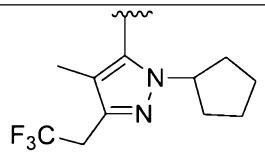
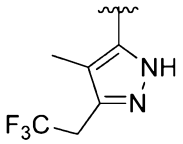
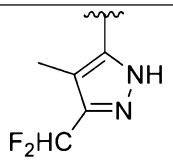
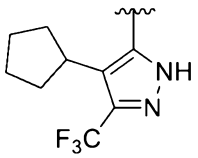
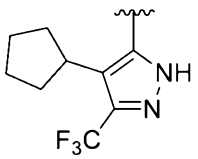
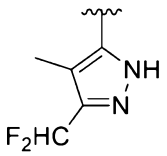
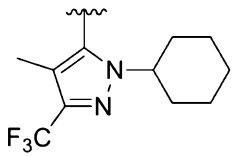
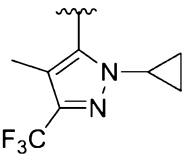
18. The compound of claim 1, wherein the compound is a compound listed in any one of Tables 1A, 2A, or 4A or a pharmaceutically acceptable salt thereof:

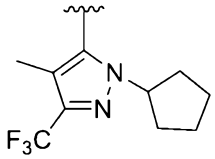
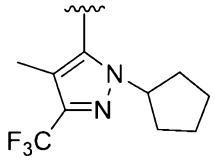
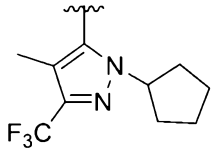
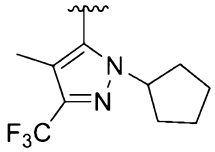
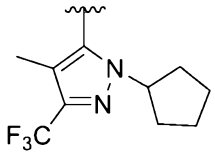
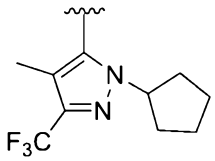
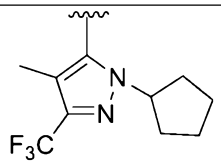
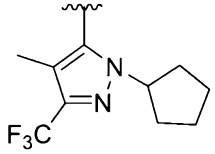
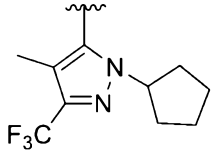
TABLE 1A



No.	Ar	X	Y
I-1	3-chlorophenyl		3-chloro-5-fluorophenyl
I-2	3-chlorophenyl		3-chloro-5-fluorophenyl
I-3	3-chlorophenyl		3-chloro-5-fluorophenyl
I-4	3-chlorophenyl		3-chloro-5-fluorophenyl
I-5	3,4-difluorophenyl		3-chloro-5-fluorophenyl
I-6	3,4-difluorophenyl		3-chloro-5-fluorophenyl

No.	Ar	X	Y
I-7	3,4-difluorophenyl		3-chloro-5-fluorophenyl
I-8	3,4-difluorophenyl		3-chloro-5-fluorophenyl
I-9	2,4-dichlorophenyl		3-chloro-5-fluorophenyl
I-10	2,4-dichlorophenyl		3-chloro-5-fluorophenyl
I-11	2,4-dichlorophenyl		3-chloro-5-fluorophenyl
I-12	2,4-dichlorophenyl		3-chloro-5-fluorophenyl
I-13	3-chlorophenyl		3-chloro-5-fluorophenyl
I-15	3-chlorophenyl		3-chloro-5-fluorophenyl
I-16	3-chlorophenyl		3-chloro-5-fluorophenyl

No.	Ar	X	Y
I-17	3-chlorophenyl		3-chloro-5-fluorophenyl
I-18	3-chlorophenyl		3-chloro-5-fluorophenyl
I-19	3-chlorophenyl		3-chloro-5-fluorophenyl
I-20	3-chlorophenyl		3-chloro-5-fluorophenyl
I-21	3-chlorophenyl		3-chloro-5-fluorophenyl
I-22	3,4-difluorophenyl		3-chloro-5-fluorophenyl
I-23	3,4-difluorophenyl		3-chloro-5-fluorophenyl
I-24	3-cyanophenyl		3-chloro-5-fluorophenyl
I-25	3-cyanophenyl		3-chloro-5-fluorophenyl

No.	Ar	X	Y
I-26	3-cyanophenyl		3-chloro-5-fluorophenyl
I-27	3-cyano-4-fluorophenyl		3-chloro-5-fluorophenyl
I-28	3-cyano-4-trifluoromethylphenyl		3-chloro-5-fluorophenyl
I-29	3-cyano-4-methylphenyl		3-chloro-5-fluorophenyl
I-30	2-chloro-4-fluorophenyl		3-chloro-5-fluorophenyl
I-31	2-chloro-4-cyanophenyl		3-chloro-5-fluorophenyl
I-32	2-chloro-3-cyanophenyl		3-chloro-5-fluorophenyl
I-33	2-chloro-4-fluorophenyl		3-chlorophenyl
I-34	3,4-difluorophenyl		3-chlorophenyl

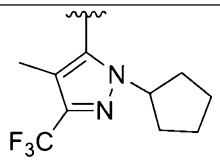
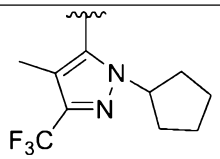
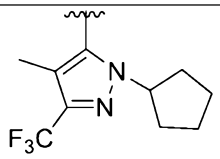
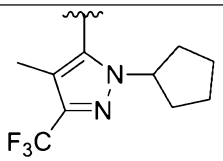
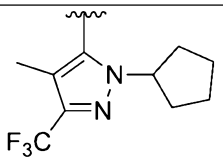
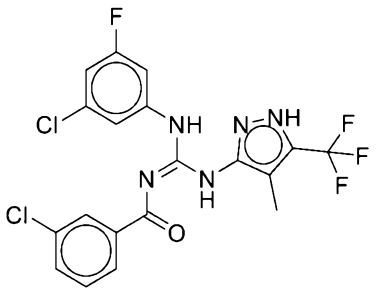
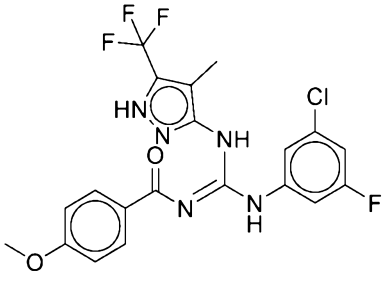
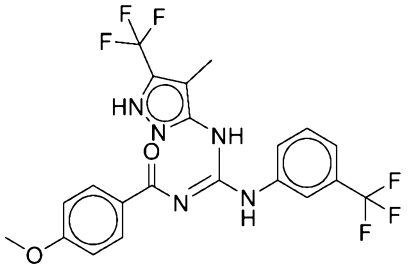
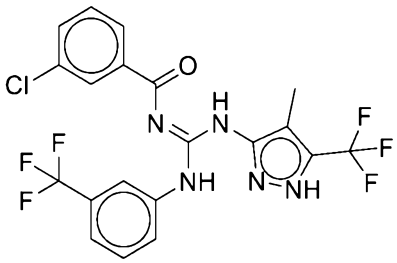
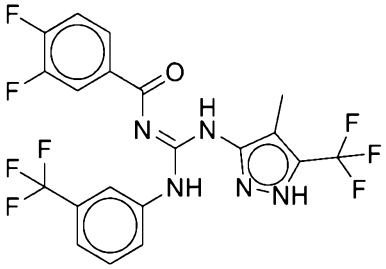
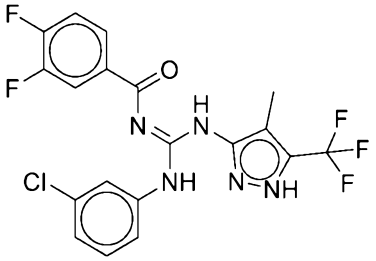
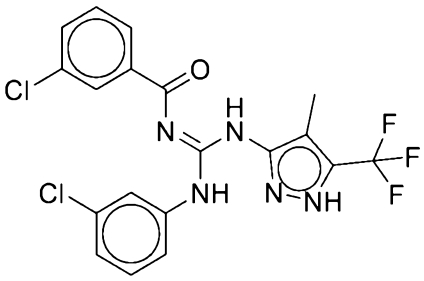
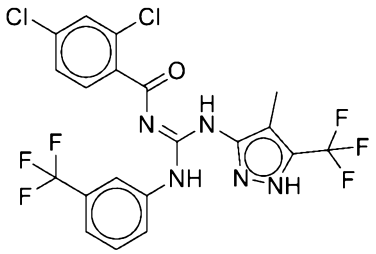
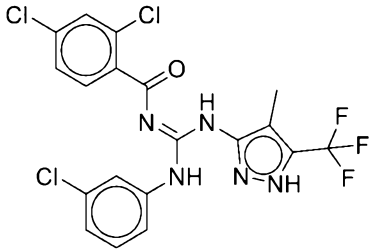
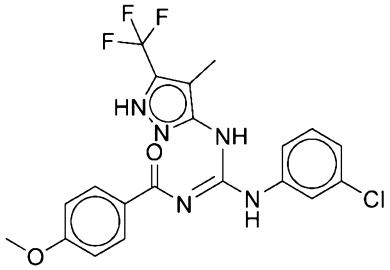
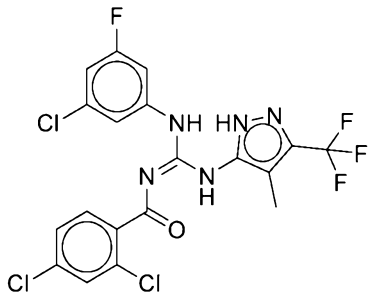
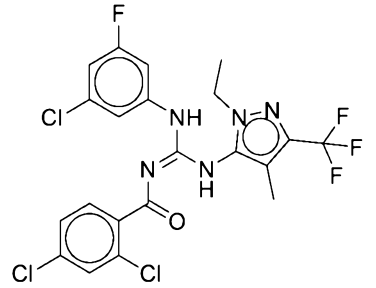
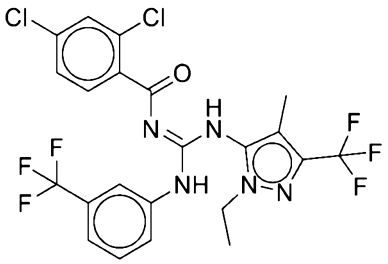
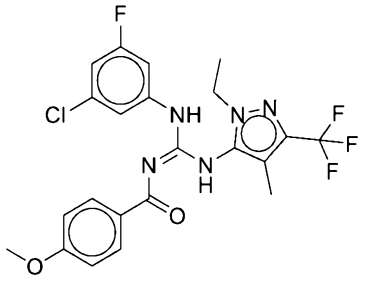
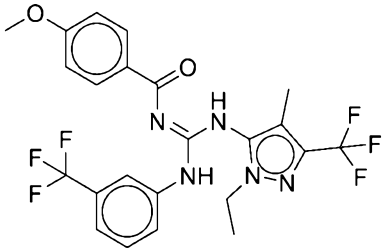
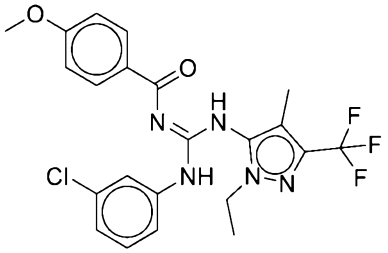
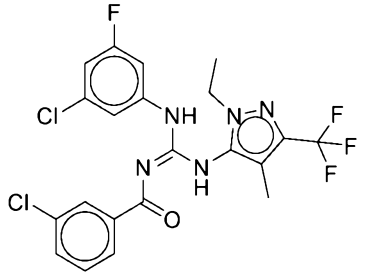
No.	Ar	X	Y
I-35	3-fluoro-4-methoxyphenyl		3-chlorophenyl
I-36	3-methoxy-4-fluorophenyl		3-chlorophenyl
I-37	2-chloro-3-methoxy-4-fluorophenyl		3-chlorophenyl
I-38	3-chlorophenyl		3-chloro-5-fluorophenyl
I-39	3,4-difluorophenyl		3-chloro-5-fluorophenyl

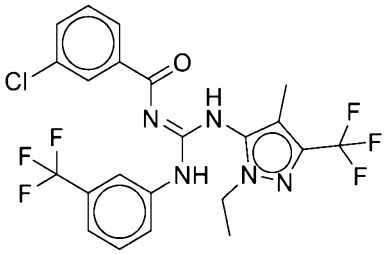
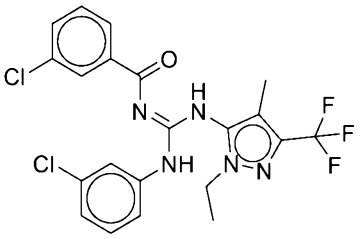
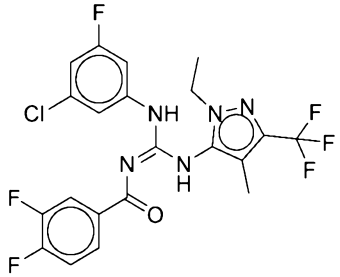
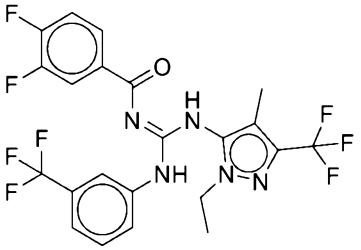
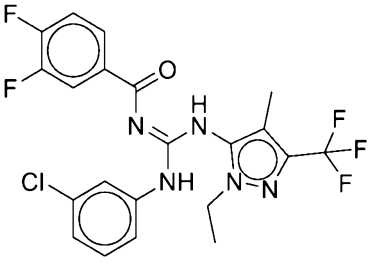
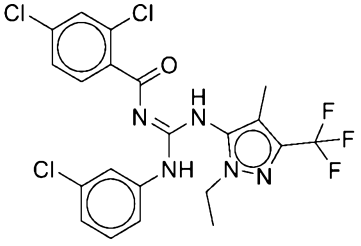
TABLE 2A

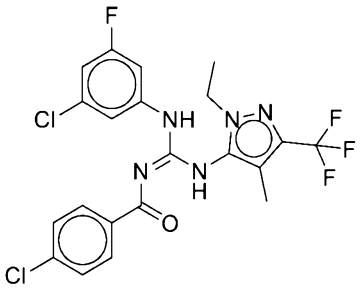
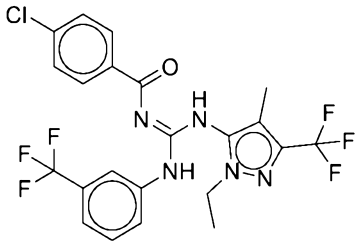
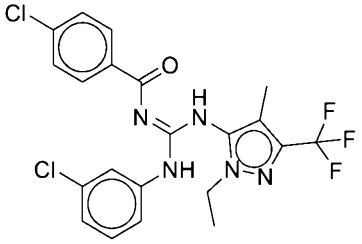
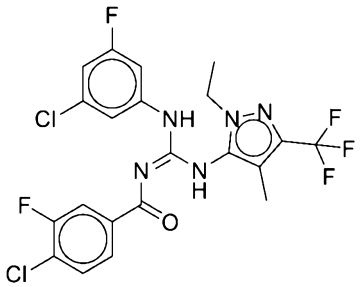
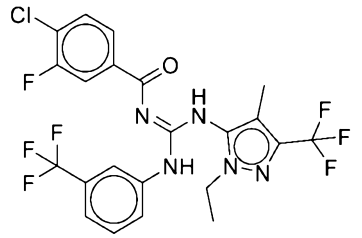
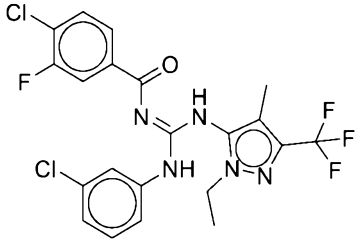
Compound No.	Chemical Structure
A-1	
A-2	

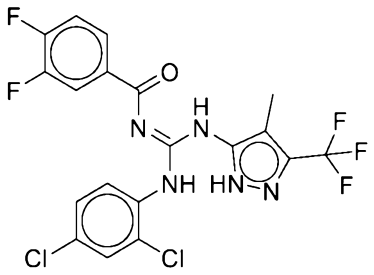
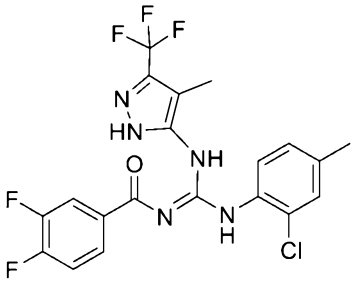
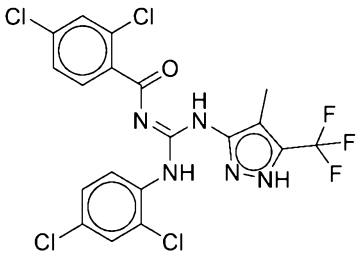
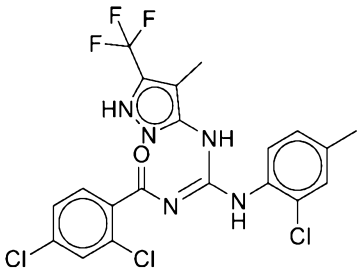
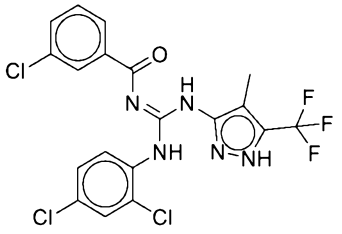
Compound No.	Chemical Structure
A-3	
A-4	
A-5	
A-6	
A-7	

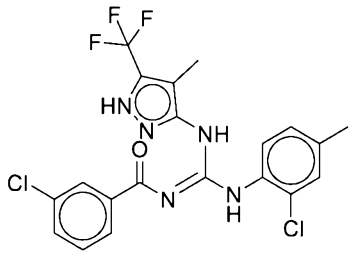
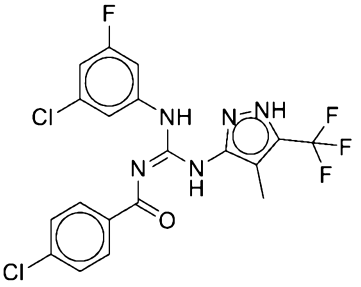
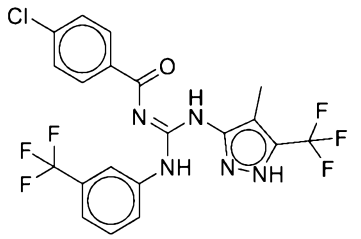
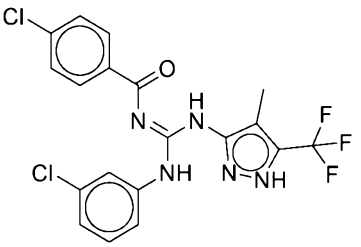
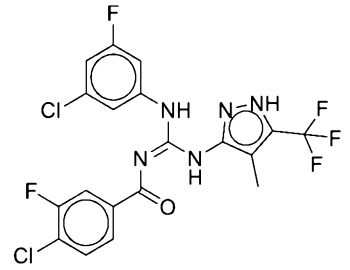
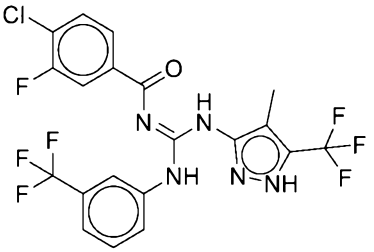
Compound No.	Chemical Structure
A-8	
A-9	
A-10	
A-11	
A-12	

Compound No.	Chemical Structure
A-13	
A-14	
A-15	
A-16	
A-17	

Compound No.	Chemical Structure
A-18	
A-19	
A-20	
A-21	
A-22	
A-23	

Compound No.	Chemical Structure
A-24	
A-25	
A-26	
A-27	
A-28	
A-29	

Compound No.	Chemical Structure
A-30	
A-31	
A-32	
A-33	
A-34	

Compound No.	Chemical Structure
A-35	
A-36	
A-37	
A-38	
A-39	
A-40	

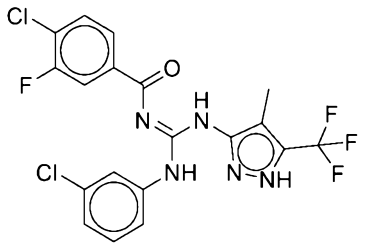
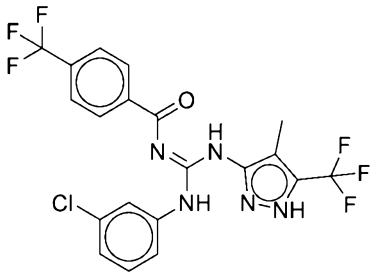
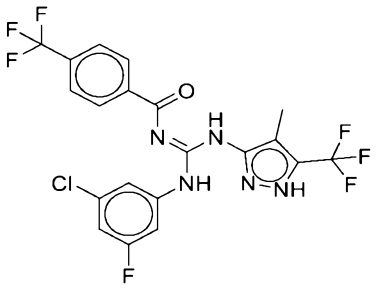
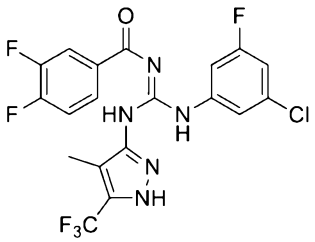
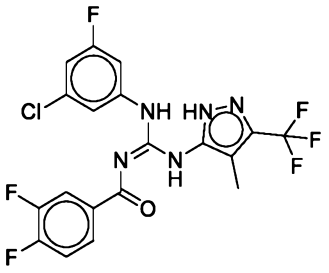
Compound No.	Chemical Structure
A-41	
A-42	
A-43	

TABLE 4A

Compound No.	Structure
1	

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



or a pharmaceutically acceptable salt thereof.

20. A pharmaceutical composition comprising a compound of any one of claims 1-19 and a pharmaceutically acceptable carrier.

21. A method of treating a disorder selected from the group consisting of rheumatoid arthritis, psoriasis, chronic graft-versus-host disease, acute graft-versus-host disease, Crohn's disease, inflammatory bowel disease, multiple sclerosis, systemic lupus erythematosus, Celiac Sprue, idiopathic thrombocytopenic thrombotic purpura, myasthenia gravis, Sjogren's syndrome, scleroderma, ulcerative colitis, asthma, uveitis, and epidermal hyperplasia by inhibition of F_1F_0-ATPase, comprising administering to a patient in need thereof a therapeutically effective amount of a compound of any one of claims 1-19 in order to ameliorate a symptom of the disorder.

22. The method of claim 21, wherein the disorder is psoriasis, chronic graft-versus-host disease, acute graft-versus-host disease, Crohn's disease, inflammatory bowel disease, or ulcerative colitis.

23. A method of inhibiting a F_1F_0-ATPase, comprising exposing a F_1F_0-ATPase to a compound of any one of claims 1-19 to inhibit said F_1F_0-ATPase.