Compounds and methods of treating insulin resistance and cardiomyopathy

Novel compounds, compositions comprising compounds, and methods for methods for preparing and using compounds are described herein. Methods of treating or ameliorating various conditions, including insulin resistance, pancreatic beta cell apoptosis, obesity, pro-thrombotic conditions, myocardial infarction, hypertension, dyslipidemia, manifestations of Syndrome X, congestive heart failure, inflammatory disease of the cardiovascular system, atherosclerosis, sepsis, type 1 diabetes, liver damage, and cachexia, by administering compounds described herein. Compounds presented herein may be used to modulate serine palmitoyl transferase activity.
Compounds and Methods of Treating Insulin Resistance and Cardiomyopathy

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present PCT application is a continuation-in-part of U.S. patent application No. 11/618,120, filed December 29, 2006, which is a continuation of U.S. patent application No. 11/248,491, filed October 12, 2005, which claims the benefit of US provisional patent application 60/617,911, filed October 12, 2004; 60/664,835, filed March 23, 2005; 60/664,919, filed March 23, 2005; and 60/693,463, filed June 23, 2005, which are incorporated herein by reference in their entirety and to which applications we claim priority.

BACKGROUND

[0002] All publications mentioned herein are cited for the purpose of familiarizing the reader with the background of the invention. Nothing herein is to be construed as an admission that these references are prior art in relation to the inventions described herein.

[0003] Although Type 2 Diabetes (i.e., T2D, diabetes mellitus, non-insulin dependent diabetes mellitus, adult onset diabetes) is frequently thought of as a disease caused by high blood sugar, modern thinking has regarded blood glucose levels as mainly a symptom of an underlying disease related to dysregulated fat metabolism. Thus high fatty acid levels lead to a range of lipotoxicities: insulin resistance, pancreatic beta cell apoptosis, and a disorder termed “metabolic syndrome.” In addition, and as discussed below, there is increasing recognition that these lipotoxicities are part of and encompass a broader range of inflammatory syndromes (Unger R.H. Annu Rev Med 53: 319-36 (2002)). Insulin resistance can be detected by the following indications: as an increased level of blood insulin, increased blood levels of glucose in response to oral glucose tolerance test (OGTT), decreased levels of phosphorylated protein kinase B (AKT) in response to insulin administration, and the like. Insulin resistance can be detected by the following indications: as an increased level of blood insulin, increased blood levels of glucose in response to oral glucose tolerance test (OGTT), decreased levels of phosphorylated protein kinase B (AKT) in response to insulin administration, and the like. Insulin resistance may be caused by decreased sensitivity of the insulin receptor-related signaling system in cells and/or by loss of beta cells in the pancreas through apoptosis. There is also evidence that insulin resistance can be characterized as having an underlying inflammatory component (Grundy, S.M., et al. Circulation 109: 433-8 (2004)).

[0004] Sedentary lifestyle and obesity have contributed to the increased occurrence of T2D. Therapeutic intervention has been aimed at people with impaired glucose tolerance (IGT). IGT
is defined as hyperglycaemia (with glucose values intermediate between normal and diabetes) following a glucose load, and affects at least 200 million people worldwide. People afflicted with IGT possess a higher future risk than the general population for developing diabetes. Approximately 40% of people with IGT progress to diabetes in 5-10 years, but some revert to normal or remain IGT.

Moreover, people with IGT also have a heightened risk of developing cardiovascular disease, such as hypertension, dyslipidaemia and central obesity. Thus, the diagnosis of IGT, particularly in apparently healthy and ambulatory individuals, has important prognostic implications. For a more detailed review, see Zimmet P, et al., Nature, 414:783-7 (2001), the disclosure of which is incorporated herein by reference.

Recently, impaired fasting glucose (IFG) was introduced as another category of abnormal glucose metabolism. IGF is defined on the basis of fasting glucose concentration and, like IGT, it is also associated with risk of cardiovascular disease and future diabetes.

T2D may be caused by a variety of factors. Additionally, the disease also manifests heterogeneous symptoms. Previously, T2D was regarded as a relatively distinct disease entity, but current understanding has revealed that T2D (and its associated hyperglycaemia or dysglycaemia) is often a manifestation of a much broader underlying disorder, which includes the metabolic syndrome. This syndrome is sometimes referred to as Syndrome X, and is a cluster of cardiovascular disease risk factors that, in addition to glucose intolerance, includes hyperinsulinaemia, dyslipidaemia, hypertension, visceral obesity, hypercoagulability, and microalbuminuria.

Recent understanding of the factors leading to T2D has influenced contemporary therapy for the disease. More aggressive approaches to treating hyperglycaemia as well as other risk factors such as hypertension, dyslipidaemia and central obesity in type 2 diabetics have been pursued. In addition, more simplistic and comprehensive screening of at-risk individuals has been advocated by health organizations, such as the American Diabetes Association.

Ceramide has been reported as showing activity in some of the factors relating to T2D, such as insulin resistance and beta cell apoptosis. For example, Schmitz-Peiffer et al. report that feeding cells with palmitic acid or ceramide leads to insulin resistance (Schmitz-Peiffer C, et al., J. Biol. Chem., 274: 24202-10 (1999)). Increased levels of palmitic acid in cells leads directly to increased levels of ceramide through an increase in levels of Palmitoyl-CoA which feeds into the de novo ceramide synthesis pathway. Studies suggest that de novo ceramide synthesis of ceramide is an important factor, since inhibition of ceramide synthase with fuminosin blocks beta
cell apoptosis (Shimabukuro M., et al, Proc. Natl. Acad. Sci. USA, 95: 2498-2502 (1998)). Similarly, it has been recognized that the enzyme involved in the rate limiting step for the de novo pathway for ceramide synthase, serine palmitoyl transferase (SPT), may be a viable target for blockade of beta cell apoptosis. For example, Shimabukuro et al. report that inhibition of SPT with cycloserine has a partial beta cell protective effect (≈ 50% activity) in the diabetic Zucker fatty rat model (Shimabukuro, et al, J. Biol. Chem., 273: 32487-90 (1998), the disclosure of which is incorporated herein by reference).

[0010] As mentioned above, atherogenic dyslipidemia is part of the metabolic syndrome and atherosclerosis is a major human disease. It is now recognized that atherosclerosis has an important inflammatory component. In an intriguing series of studies with the SPT inhibitor myriocin the observation was made that a dramatic reduction in atherosclerotic plaque was observed (Park, et al. Circulation 110: 3456-71 (2004); Hojjati, et al. J. Biol. Chem. 280: 10284-9 (2005); Park, TS, Panek, R.L., Rekhter, M.D., Mueller, S.B., Rosebury, W.S., Robertson, A.W, Hanselman, J.C. (2006). Modulation of lipoprotein metabolism by inhibition of sphingomyelin synthesis in ApoE knockout mice. Atherosclerosis, epub ahead of print (2006)). While the authors are tempted to ascribe the observed plaque reduction to inhibition of SPT, these studies with myriocin do not convincingly demonstrate this result, as acknowledged by the authors. This is due to another major biological activity of myriocin, inhibition of lymphocyte chemotaxis. This latter effect is the cause of the known potent immunosuppressive activity of myriocin. This activity is caused by the phosphorylation of myriocin in vivo to generate a structure that mimics the structure and activity of sphingosine-1-phosphate (SIP). This structure binds to Edg receptors to inhibit release of lymphocytes from the spleen. These activities are mimicked by the immunosuppressive FTY720 and much of the mechanism has been clarified using FTY720 and its analogs (Rosen, H. and Liao, J. Curr. Opin. Chem. Biol. 7: 461-8 (2003)). The compounds of the invention may or may not have this latter immunosuppressive activity, on a case by case basis. Such activity also may lead to valuable clinical profile for individual compounds.

[0011] Treatment of mice with the SPT inhibitor myriocin in an accepted model of emphysema (Vascular Endothelial Growth Factor Receptor blockade) showed very strong protective effect (Petrache, L, et al, Nature Medicine 11: 491-8 (2005)). Prevention of progression of emphysema in this animal model was also demonstrated by another inhibitor of de novo ceramide synthesis, fumonisin B1, although it was less effective and showed some toxicity at higher doses. Chronic obstructive pulmonary disease (COPD) is another progressive inflammatory lung disease where there is disruption of lung tissue structure and function (Barnes,
PJ., COPD 1: 59-70 (2005)). Currently there are no effective therapeutics to prevent the progression of COPD. Thus there is evidence that compounds of the invention will have beneficial therapeutic effects in both emphysema and COPD.

the opportunity to employ an novel approach to the suppression of side effects in drug eluting stents.

[0013] A well known proinflammatory signal, Tumor Necrosis Factor alpha (TNF), has been shown to raise ceramide levels in cells in culture (Sawada, M, *et al*, Cell Death Differ., 11:997-1008 (2004); Meyer, SG, *et al*, Biochim Biophys Acta. 1643(1-3):1-4(2003)). TNF administration reduces PPAR-gamma levels in adipocytes and this has been shown to implicate ceramide (Kajita, K, *et al*. Diabetes. Res. Clin. Pract, 66 Suppl 1:S79-83 (2004)). TNF also induces apoptosis in liver cells and has been implicated in injury due to viral hepatitis, alcoholism, ischemia, and fulminant hepatic failure (Ding, WX and Yin, XM, J. Cell. Mol. Med. 8:445-54 (2004); Kanzler S., *et al*. Semin Cancer Biol. 10(3): 173-84 (2000)). Similarly, TNF and IL-6 are implicated in cachexia, another syndrome with strong evidence of an inflammatory component, implicating ceramide as an effector. It is known that atherosclerosis has an inflammatory component. Induction of oxidative stress by amyloid involves induction of a cascade that increases ceramide levels in neuronal cells (Ayasolla K., *et al*, Free Radic. Biol. Med., 37(3):325-38(2004)). Thus altered ceramide levels may be causative in dementias such as Alzheimer's disease and HIV dementia and modulation of these levels with an SPT inhibitor is conceived as having promise as a treatment (Cutler RG, *et al*, Proc Natl. Acad. ScL, 101:2070-5 (2004)). TNF is known to be involved in sepsis and insulin has protective effects (Esmon, CT. Crosstalk between inflammation and thrombosis, Maturitas, 47:305-14 (2004)). De novo ceramide levels possibly serve as a central effector mechanism in the inflammatory processes central to many diseases and conditions. However, the potential for modulators of SPT to be used as therapeutic agents for diseases and conditions related to ceramide’s involvement, as an effector in inflammatory processes, has not previously been shown.

[0014] Elevated levels of fatty acids can induce a syndrome that mimics the pathology of cardiomyopathy (*i.e.*, heart failure). The pathogenesis of this lethal condition is poorly understood, but appears to be related to lipotoxicities. Studies indicate that lipid overload in cardiac myocytes may well be an underlying cause for cardiomyopathy. In addition, recent studies have identified low levels of myocyte apoptosis (80-250 myocytes per 10⁵ nuclei) in failing human hearts. It remains unclear, however, whether this cell death is a coincidental finding, a protective process, or a causal component in disease pathogenesis (*See, e.g.*, Wencker D., *et al*, J. Clin. Invest, 111:1497-1504 (2003), the disclosure of which is incorporated herein by reference). Increases in fatty acid levels in cells directly lead to elevated rates of de novo ceramide synthesis. TNF has been implicated in CHF, and thereby ceramide, an associated
effector for TNF signaling, is implicated through an independent direction (McTiernan, CF, et al, Curr Cardiol Rep. 2(3): 189-97 (2000)). However, the utility of de novo ceramide synthesis modulators, as agents to block progression of and allow healing of heart muscles in cardiomyopathy, has not been demonstrated.

Cachexia is a progressive wasting syndrome with loss of skeletal muscle mass (Frost RA and Lang CH.; Curr. Opin. Clin. Nutrit. Metab. Care., 255-263 (2005)) and adipose tissue. This syndrome is found in response to infection, inflammation, cancer (Tisdale MJ; Langenbecks Arch Surg., 389:299-305 (2004)) or some chronic diseases like rheumatoid arthritis (Rail LC and Roubenoff, R, Rheumatol 43:1219-23 (2004)). Release of various cytokines has been implicated in this syndrome and both TNF and IL-6 are recognized as central players. Thus cachexia can be looked at as a chronic inflammatory state. Ceramide is a well-known central effector of TNF signaling. In addition, ceramide is known to modulate the expression of IL-6 (Shinoda J, Kozawa O, Tokuda H, Uematsu, T. Cell Signal, 11:435-41 (1999)); Coroneos, E; Wang, Y; Panuska, JR; Templeton, DJ; Kester, M.; Biochem J; 316:13-7 (1996)). Existing data lead us to believe that de novo ceramide synthesis is playing a central role as a signal for this inflammatory state as well. We therefore believe that inhibition of TNF and/or IL-6 signaling through ceramide will provide a clinical benefit to patients with this wasting syndrome.


Beattie, et al have reported that various treatments (e.g. trehalose, removal of Arg from culture medium, and the like) may improve the yield of transplantable islets but substantial cell death remains (Beattie GM, Leibowitz G, Lopez AD, Levine F, Hayek A, Cell Transplant. 9:431-38) (2000)). Treatment of cells and tissues by caspase inhibitors leads to a partial block of apoptosis in response to various metabolic insults, but apoptosis may be driven by many mechanisms, and caspase inhibition may have useful or marginal effects depending on the specific instance being studied. Study of caspase inhibitors for limiting death in mammalian cell culture. Sauerwald TM, Oyler GA, Betenbaugh MJ.) (Biotechnol. Bioeng., 81:329-40 (2003)).
Studies of inhibition of de novo synthesis of ceramide have shown that such inhibition appears to have anti-apoptotic effects in a number of important situations. Beta cell apoptosis in response to treatment with free palmitic acid and/or in combination with high levels of glucose can be blocked by treatment with fumonisin B1 (inhibitor of ceramide synthase), for example (Maedler, K. Diabetes, 52:726-33 (2003). It is thus possible that the inhibition or de novo ceramide synthesis can be applied to prevention of apoptotic events. However, treatment with agents that inhibit ceramide synthase have been shown to result in toxic effects, as seen with ingestion of fumonisin B1 (Bennett JW and Klich M., Clin. Microbiol. Rev., 16:497-516 (2003)). Inhibition of SPT provides an alternate method for preventing apoptosis of pancreatic beta cells, however, modulators of SPT have not been shown to prevent the loss of pancreatic beta cells in culture prior to transplant.

Thus, modulators of de novo ceramide synthesis could provide important new therapeutic agents for a range of human and veterinary diseases that entail an inflammatory component making use of ceramide as an effector agent. However, interference with the de novo ceramide synthesis pathway at several points (e.g., as with Fumonisin B1) is known to lead to toxicities. Inhibition at the level of Serine Palmitoyl Transferase, however, leads to the build up of innocuous cellular components serine and Palmitoyl CoA.

There are several potent natural product inhibitors of SPT. Myriocin is perhaps the best known, and it shows sub-nanomolar IC$_{50}$ for inhibition of SPT (Kluepfel, D., et ah, J. Antibiot. 25: 109-115 (1972); Miyaki, Y., et ah, Biochem Biophys Res Commun. 211: 396-403 (1995); Hanada, K. Biochem Biophys Acta 1632: 16-30(2003)). Mycetinillicins also comprise a family of potent immunosuppressive natural products. They are structurally related to myriocin and have potent inhibitory activity on SPT (Sasaki, S, et ah, J. Antibiot. 47: 420-33 (1994)). Another class of potent natural product inhibitors of SPT is the sphingofungins (VanMiddlesworth F., et ah, J. Antibiotics 45: 861-7 (1992)).
Known inhibitors of SPT include cycloserine, D-serine, myriocin, sphingofungin B, viridiofungin A, and lipoxamycin. A number of these natural products, such as myriocin, have been shown to have unacceptable toxicities. Furthermore, these ceramides impart only partially protective activity. In addition, some SPT inhibitors, such as cycloserine, show weak inhibition and exhibit low specificity. Structural studies suggest that natural ceramides mimic the active site bound form of the starting materials or products (Hanada K., et al, Biochem. Biophys. Acta, 1632:16-30 (2003)).

The SPT inhibitor myriocin is known to be a powerful immunosuppressive molecule. A number of analogs have been designed based on its structure. Structures that have the immunosuppressive activity of myriocin, such as those related to compound FTY720, illustrated below, do not inhibit SPT. Additionally, the carboxylic derivative of FTY720, shown below as compound 2, did not exhibit activity against SPT, as demonstrated in an immunosuppressive assay for FTY720-like activity (Kiuchi M. et al, J. Med. Chem., 43:2946-61 (2000)) and was suggested to be inactive due to extremely low solubility if not lack of binding affinity, per se.
Work with FTY720 has demonstrated that it undergoes phosphorylation by sphingosine kinase and that the resulting phosphorylated species (FTY720-PO4) is the active molecule in vivo (Mandala S. et al, Science 296: 346-9 (2002); Brinkmann V. et al. J. Biol. Chem. 277: 21453-7 (2002); Rosen H and Liao, J. Curr. Opin. Chem. Biol. 7: 461-8 (2003)). Thus the source of the immunomodulatory activity inherent in the structure of myriocin is the hydroxymethyl function on the head group which can be phosphorylated to yield a sphingosine-1-phosphate (SIP) like structure.

Modulation of SPT presents an attractive means to attenuate insulin resistance and prevent loss of pancreatic beta cells. Inhibitors of SPT, in particular, may offer new therapeutics for the treatment of T2D. These agents could be beneficial for the protection of tissue for transplantation such as in islet transplantation and liver transplantation. As outlined above, such inhibitors could also have beneficial uses in the treatment of cardiomyopathy, sepsis, cachexia, atherosclerosis, liver damage, reperfusion injury, Alzheimer's Disease, Type 1 diabetes, in which apoptosis plays a role, as well as other inflammatory diseases. Bioavailable agents that are highly potent and selective inhibitors of SPT were heretofore not available. Nontoxic, bioavailable, potent and selective modulators of SPT could prove to be important new agents for the treatment of the diseases and conditions as disclosed herein and other diseases and conditions involving apoptosis and in which TNF is known, to those of skill in the art, to play a role. The generation of such compounds and their usefulness for treating these indications has not been previously shown.

SUMMARY OF THE INVENTION

Presented herein are novel compounds and methods of use. In a preferred embodiment, compounds provided herein exhibit activity on the enzyme, serine palmitoyl transferase (SPT), the first committed step of an enzymatic pathway known to have a broad pro-inflammatory role, for example, as an effector of TNFα signaling.

Compounds provided herein may be employed in the treatment of a variety of human diseases or conditions. In a preferred embodiment, compounds are used to treat diseases such as T2D, insulin resistance, pancreatic beta cell apoptosis, or obesity. In another preferred embodiment, compounds are used to treat pro-thrombotic conditions, congestive heart failure, myocardial infarction, hypertension, dyslipidemia, or other symptoms of Metabolic Syndrome (i.e., Syndrome X). In yet another preferred embodiment, compounds are used to treat inflammatory diseases, such as inflammatory diseases of the cardiovascular system, sepsis and cachexia. Exemplary inflammatory diseases of the cardiovascular system include
atherosclerosis. In yet another preferred embodiment, these compounds are used to prevent liver
damage from viral, alcohol related, reperfusion injuries as outlined above. In yet another
preferred embodiment, these compounds are used to protect and enhance the yield for
transplantation of pancreatic liver cells and or livers, either alone or in combination with the
currently approved cocktails and/or caspase inhibitors. In yet another preferred embodiment,
these compounds are used to treat inflammatory lung diseases such as emphysema and COPD.

[0027] Also provided are compositions comprising compounds presented herein, in
combination with a therapeutically effective amount of another active agent. Exemplary agents
include insulin, insulin analogs, incretin, incretin analogs, glucagon-like peptide, glucagon-like
peptide analogs, exendin, exendin analogs, PACAP and VIP analogs, sulfonylureas, biguanides,
α-glucosidase inhibitors, Acetyl-CoA Carboxylase inhibitors, caspase inhibitors, delta 3
unsaturated fatty acids, polyunsaturated fatty acids, inhaled corticosteroids, beta2 adrenoceptor
agonists and PPAR ligands. Accordingly, embodiments of methods for treating various diseases
include co-administering compounds presented herein and a therapeutically effective amount of
another active agent, or administration of combination compositions provided herein.

DETAILED DESCRIPTION

[0028] As described above, the compounds of the invention inhibit SPT, the first committed
step of an enzymatic pathway known to have a broad pro-inflammatory role as an effector of
TNFα signaling. Therefore, modulation of this pathway has great importance for the treatment
of a number of inflammatory diseases, for example - the Metabolic Syndrome (Syndrome X) and
its components (atherosclerosis, insulin resistance, prothrombotic state, hypertension), diabetes
(beta cell apoptosis; in vitro and in vivo), congestive heart failure, sepsis, cachexia, liver damage
(inflammatory or viral), restenosis, drug eluting stents, and the like.

[0029] Furthermore, the agents of the invention can be used advantageously in combination
with other known therapeutics for these diseases for even greater beneficial effect. This includes
use in conjunction with 1. insulin or insulin analogs (human, hog, beef, lispro, aspart, glargine,
detemir), 2. oral hypoglycemic agents such as the sulfonylureas and the agents having similar
effect (Glipizide, Gliclazide, Glibenclamide, Glimepiride, Repaglinide, Nateglinide and the
generic chemical forms thereof), 3. Biguanides (metformin, buformin, phenformin, and the like),
4. alphaglucosidase inhibitors (Acarbose, miglitol, and the like), 5. caspase inhibitors (VX-765,
IDN-6556, and the like), 6. PPAR ligands (pioglitazone, rosiglitazone, and the like, including
ligands of all PPAR receptor classes), 7. Incretin/GIPI analogs (exenatide, Liraglutide, ZP-
10A/AVE-OIO, Albugon, BIM-51077 and the like), 8. PACAP or VIP analogs (Ro 25-1555, Bay
55-9837, and the like), 9. Acetyl-CoA inhibitors and 10. pulmonary disease therapeutics like beta2 adrenoceptor agonists (formoterol, salmeterol, albuterol, and the like) and inhaled corticosteroids (beclomethasone, fluticasone, mometasone, and the like). These examples are meant to be illustrative and not limit the scope of the combinations of therapeutics contemplated by the invention.

[0030] As mentioned above, another major biological activity of myriocin is immunosuppression caused by inhibition of lymphocyte chemotaxis. This activity is thought to be caused by the phosphorylation of myriocin in vivo on the hydroxymethyl function on the quaternary head group to generate a structure that mimics the structure and activity of SIP. This structure binds to Edg receptors to interfere with the release of lymphocytes from the spleen. This immunosuppressive activity of myriocin and its analogs may be a desirable attribute for some of the uses described herein. The compounds of the invention are differentiated from myriocin by being designed to have favorable pharmaceutical properties and inhibit SPT activity. In addition individual structures may have strongly diminished immunosuppressive activity.

[0031] There are a number of assays that can be used to determine whether a molecule has potent immunosuppressive activity through the mode of action used by myriocin and FTY720 (Chiba, K., et al. Role of Sphingosine 1-Phosphate Receptor Type 1 in Lymphocyte Egress from Secondary Lymphoid Tissues and Thymus. Cell. Molec. Immunol. 3: 11-19 (2006)). A simple in vivo assay uses the quantitation of lymphocytes 24 hr after treatment of normal rats and makes use of flow cytometry to determine amounts of T-cells and B-cells in the peripheral blood (Kiuchi, M., et al. Synthesis and Immunosuppressive Activity of 2-Substituted 2-Aminopropane-1,3-diols and 2-Aminoethanols. J. Med. Chem. 43: 2946-61 (2000)). Kiuchi, et al (2000) also report the use of a rat skin allograft model and popliteal lymph node gain assays. FTY720 may be used as a positive control and less than 10% or preferably less than 1% of the activity of FTY720, is indicative of weak immunosuppressive activity, which also may be desirable for some applications of the compounds of the invention.

[0032] As used in the specification, "a" or "an" means one or more. As used in the claim(s), when used in conjunction with the word "comprising," the words "a" or "an" mean one or more. As used herein, "another" means at least a second or more.

[0033] Reference now will be made in detail to various embodiments and particular applications of the invention. While the invention will be described in conjunction with the various embodiments and applications, it will be understood that such embodiments and applications are not intended to limit the invention. On the contrary, the invention is intended to
cover alternatives, modifications and equivalents that may be included within the spirit and scope of the invention. In addition, throughout this disclosure various patents, patent applications, websites and publications are referenced, and unless otherwise indicated, each is incorporated by reference in its entirety for all purposes. All publications mentioned herein are cited for the purpose of describing and disclosing reagents, methodologies and concepts with the present invention. Nothing herein is to be construed as an admission that these references are prior art in relation to the inventions described herein.

1. Compounds

Presented herein are novel compounds, and pharmaceutically acceptable salts thereof, corresponding to Formula (I):

wherein:

- $R_1$ is H or optionally substituted lower alkyl, aryl, aralkyl, or alkylxyalkyl;
- each $R_2$ is independently H, protecting group, or $-\text{C} (=\text{O})-\text{CHR}_a-\text{NHR}_b$ where:
  - $R_a$ is selected from the group consisting of alkyl, aryl, acyl, keto, azido, hydroxyl, hydrazine, cyano, halo, hydrazide, alkenyl, alkynyl, ether, thiol, seleno, sulfonyl, borate, boronate, phospho, phosphono, phosphine, heterocyclic, enone, imine, aldehyde, ester, thioacid, hydroxylamine, amino group, and combinations thereof; and
  - $R_b$ is H or amino protecting group;
- each $V$ and $Z$ is independently $(\text{CR}_eR_d)_n, \text{O}, \text{NR}_e, \text{S}$, optionally substituted alkene (cis or trans), Ar, CR$_e$RaAr, OAr, NR$_d$Ar, SAr, or Ar where:
  - each $R_0$ and $R_d$ is independently H, X, lower alkyl, OH, 0-lower alkyl, or
  - $R_0$ and $Ra$, taken together, is $=\text{O}, =\text{N}-\text{OH}, =\text{N}-\text{O-lower alkyl}$, or
  - $=\text{N}-\text{O-CH}_2\text{CH}_2\text{-O-CH}_3$;
- $R_e$ is H, lower alkyl, or $-\text{CH}_2\text{CH}_2\text{-O-CH}_3$; and
- $n$ is 1 to 7;
q is 0 to 3;
Ar is an optionally substituted aryl or heteroaryl;
u is 0 or 1;
each X is independently H or halogen; and
m is 4 to 12.

[0035] In some embodiments of the invention, compounds of Formula (I) do not include:

![Chemical structures]

[0036] Preferred compounds of Formula (I) include those where R₁ is lower alkyl, such as methyl, ethyl, isopropyl, and the like. Additionally preferred embodiments include those compounds where Rᵢ is alkylxyalkyl, such as CH₃-O-CH₂-CH₂-, HO-CH₂-CH₂-O-, HO-(CH₂-CH₂-O-)ᵢ-, hydroxyethyl alcohol, hydroxypropyl alcohol, hydroxyethoxyethyl alcohol, and polyethylene glycol or derivatives there. Other preferred compounds of Formula (I) include those where X is halogen, such as fluorine. Additional preferred compounds of Formula (I) include those where Z is NRᵣ₄, O, or S. Another preferred embodiment includes compounds of Formula (I) where Ar is an optionally substituted heteroaryl. Another preferred embodiment includes compounds of Formula (I) where Ar is an optionally substituted fused ring system, such as a 5-5, 5-6, or 6-6 ring system.

[0037] In an embodiment, compounds of Formula (I) correspond to Formula (II):

![Chemical structures]

(H)

wherein:
L is CH₂, CHRᵣ, CRᵣᵣ, O, NRᵣ₄, S, Ar, CH₂Ar, CHRᵣAr, CRᵣᵣAr, OAr, NRᵣᵣAr, SAR, or ArAr, where
Rᵣ is H, lower alkyl, OH, 0-lower alkyl,
Rᵣᵣ is H, or
R_f and R_g, taken together, is =0, =N-0H, =N-O-lower alkyl, or =N-O-CH_2CH_2-O-CH_3, and
Rh is H, lower alkyl, or -CH_2CH_2-O-CH_3.

[0038] In an embodiment, compounds of Formula (I) correspond to Formula (HA):

(HA)

wherein each Y is independently C, CH, O, S, N, or NH.

[0039] In another embodiment, compounds of Formula (I) correspond to Formula (HB):

(HB)

wherein each W is independently C, CH, N, or NH.

[0040] In yet another embodiment, compounds of Formula (I) correspond to Formula (IIC):

(HC)

wherein each Y is independently C, CH, O, S, N, or NH.

[0041] In another embodiment, compounds of Formula (I) correspond to Formula (HD):

(HD)

[0042] In another embodiment, compounds of Formula (I) correspond to Formula (HE):
[0043] In another embodiment, compounds of Formula (I) correspond to Formula (HF):

(HF).

[0044] In an additional embodiment, compounds of Formula (I) correspond to Formula (III):

(HI).

[0045] In another embodiment, compounds of Formula (I) correspond to Formula (IIIA):

(IIIA).

[0046] In another embodiment, compounds of Formula (I) correspond to Formula (IIIB):

(IIIB).

[0047] In another embodiment, compounds of Formula (I) correspond to Formula (IIIC):
wherein each Y is independently C, CH, O, S, N, or NH.

[0048] In another embodiment, compounds of Formula (I) correspond to Formula (HID):

(HID)

wherein each Y is independently C, CH, O, S, N, or NH.

[0049] In another embodiment, compounds of Formula (I) correspond to Formula (HIE):

(HIE)

wherein each Y is independently C, CH, O, S, N, or NH.

[0050] In another embodiment, compounds of Formula (I) correspond to Formula (IIIF):

(IIIF)

wherein each W is independently C, CH, N, or NH.

[0051] In another embodiment, compounds of Formula (I) correspond to Formula (IIIG):
wherein each \( W \) is independently \( C \), \( CH \), \( N \), or \( NH \).

[0052] In another embodiment, compounds of Formula (I) correspond to Formula (IIIH):

(IIIH)

wherein each \( W \) is independently \( C \), \( CH \), \( N \), or \( NH \).

[0053] In another embodiment, compounds of Formula (I) correspond to Formula (IILJ):

(IILJ)

wherein each \( Y \) is independently \( C \), \( CH \), \( O \), \( S \), \( N \), or \( NH \).

[0054] In another embodiment, compounds of Formula (I) correspond to Formula (IIIK):

(IIIK)

wherein each \( Y \) is independently \( C \), \( CH \), \( O \), \( S \), \( N \), or \( NH \).

[0055] In another embodiment, compounds of Formula (I) correspond to Formula (IIIL):

(IIIL)

wherein each \( Y \) is independently \( C \), \( CH \), \( O \), \( S \), \( N \), or \( NH \).

[0056] In another embodiment, compounds of Formula (I) correspond to Formula (HIM):
wherein \( q + m \) is less than 12.

[0057] In another embodiment, compounds of Formula (I) correspond to Formula (IIIN):

\[
\text{(IIIN)}
\]

[0058] In yet another embodiment, prodrug forms of compounds of Formula (I) are presented. Prodrug forms of compounds are optimal for oral administration, and typically correspond to the ester of the acid active species. Active species of the prodrugs can be used to prepare active drug compounds.

[0059] In an embodiment, prodrug compounds correspond to Formula (IIIO):

\[
\text{(IIIO)}
\]

wherein \( R_a \) is the side chain of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, pyrolysine and selenocysteine.

[0060] Representative prodrug compounds corresponding to Formula (IIIO) include compounds corresponding to Formula (HIP):
In another embodiment, prodrug compounds correspond to Formula (IIIQ):

wherein \( R_a \) is the side chain of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, pyrolysine and selenocysteine.

Representative prodrug compounds corresponding to Formula (HIP) include compounds corresponding to Formula (MR):

wherein \( Y \) is \( C, \ CH, \ O, \ S, \ N, \) or \( \text{NH} \).

In another embodiment, compounds of Formula (I) correspond to Formula (IVA):

wherein \( Y \) is \( C, \ CH, \ O, \ S, \ N, \) or \( \text{NH} \).

In another embodiment, compounds of Formula (I) correspond to Formula (IVB):
In another embodiment, compounds of Formula (I) correspond to Formula (IVC): (IVC).

In another embodiment, compounds of Formula (I) correspond to Formula (V): (VD).

In another embodiment, compounds of Formula (I) correspond to Formula (VE): (VE).

In another embodiment, compounds of Formula (I) correspond to Formula (VF): (VF).

In another embodiment, compounds of Formula (I) correspond to Formula (VG): (VG).
In another embodiment, compounds of Formula (I) correspond to Formula (VH):

(VH).

In another embodiment, compounds of Formula (I) correspond to Formula (VJ):

(VJ).

In another embodiment, compounds of Formula (I) correspond to Formula (VK):

(VK).

In another embodiment, compounds of Formula (I) correspond to Formula (VL):

(VL).

In another embodiment, compounds of Formula (I) correspond to Formula (IVM):
In another embodiment, compounds of Formula (I) correspond to Formula (VN):

\[
\text{(VN).}
\]

Exemplary compounds provided herein are listed below in Table 1.

**TABLE 1 - Representative Compounds**

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\] | \[
\text{HO} \overset{\text{NH}_2}{\text{C}} \overset{\text{O}}{\text{C}} \overset{\text{OH}}{\text{O}} \overset{\text{CF}_3}{\text{F}}
\] |
|   | 63 | 64 |
|   | \[
\text{EIO} \overset{\text{NH}_2}{\text{C}} \overset{\text{O}}{\text{C}} \overset{\text{CH}}{\text{CH}} \overset{\text{CH}_2\text{CF}_3}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{OH}_2\text{CF}_3}{\text{F}}
\] | \[
\text{HO} \overset{\text{NH}_2}{\text{C}} \overset{\text{O}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{CH}_2\text{OF}_2\text{CF}_3}{\text{C}} \overset{\text{OH}}{\text{F}}
\] |
|   | 65 | 66 |
|   | \[
\text{HO} \overset{\text{NH}_2}{\text{C}} \overset{\text{O}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{CH}_2\text{OF}_2\text{CF}_3}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{CH}_2\text{OF}_2\text{CF}_3}{\text{C}} \overset{\text{F}}{\text{F}}
\] | \[
\text{HO} \overset{\text{NH}_2}{\text{C}} \overset{\text{O}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{CH}_2\text{OF}_2\text{CF}_3}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{CH}_2\text{OF}_2\text{CF}_3}{\text{C}} \overset{\text{F}}{\text{F}}
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|   | 67 | 68 |
|   | \[
\text{HO} \overset{\text{NH}_2}{\text{C}} \overset{\text{O}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{CH}_2\text{OF}_2\text{CF}_3}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{CH}_2\text{OF}_2\text{CF}_3}{\text{C}} \overset{\text{F}}{\text{F}}
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\text{HO} \overset{\text{NH}_2}{\text{C}} \overset{\text{O}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{CH}_2\text{OF}_2\text{CF}_3}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{CH}_2\text{OF}_2\text{CF}_3}{\text{C}} \overset{\text{F}}{\text{F}}
\] |
|   | 69 | 70 |
|   | \[
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\text{HO} \overset{\text{NH}_2}{\text{C}} \overset{\text{O}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{F}}{\text{F}}
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|   | 71 | 72 |
|   | \[
\text{HO} \overset{\text{NH}_2}{\text{C}} \overset{\text{O}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{F}}{\text{F}}
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\text{HO} \overset{\text{NH}_2}{\text{C}} \overset{\text{O}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{F}}{\text{F}}
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|   | 73 | 74 |
|   | \[
\text{HO} \overset{\text{NH}_2}{\text{C}} \overset{\text{O}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{F}}{\text{F}}
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\text{HO} \overset{\text{NH}_2}{\text{C}} \overset{\text{O}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{F}}{\text{F}}
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|   | 75 | 76 |
|   | \[
\text{HO} \overset{\text{NH}_2}{\text{C}} \overset{\text{O}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{F}}{\text{F}}
\] | \[
\text{HO} \overset{\text{NH}_2}{\text{C}} \overset{\text{O}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{OH}}{\text{C}} \overset{\text{F}}{\text{F}}
\] |
II. Definitions
Compounds presented herein embrace isotopically-labelled compounds, which are identical to those recited in Formula I, but for the fact 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 the present compounds include isotopes of hydrogen, carbon, nitrogen, oxygen, fluorine and chlorine, such as $^2$H, $^3$H, $^{13}$C, $^{14}$C, $^{15}$N, $^{17}$O, $^{18}$O, $^{35}$S, $^{18}$F, $^{35}$Cl, respectively. Compounds presented herein, prodrugs thereof, and pharmaceutically acceptable salts of said compounds or of said prodrugs which contain the aforementioned isotopes and/or other isotopes of other atoms are within the scope of this invention. Certain isotopically-labelled compounds of the present invention, for example those into which radioactive isotopes such as $^3$H and $^{14}$C are incorporated, are useful in drug and/or substrate tissue distribution assays. $^3$H and $^{14}$C isotopes are preferred for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium, $i.e.$, $^2$H, can afford certain therapeutic advantages resulting from greater metabolic stability, for example increased in vivo half-life or reduced dosage requirements and, hence, may be preferred in some circumstances. Isotopically labeled compounds herein and prodrugs thereof can generally be prepared by carrying out the procedures disclosed in the Schemes and/or in the Examples below, by substituting a readily available isotopically labelled reagent for a non-isotopically labelled reagent.

Some of the compounds herein have asymmetric carbon atoms and can therefore exist as enantiomers or diastereomers. Diastereomeric mixtures can be separated into their individual diastereomers on the basis of their physical chemical differences by methods known, for example, by chromatography and/or fractional crystallization. Enantiomers can be separated by converting the enantiomeric mixture into a diastereomeric mixture by reaction with an appropriate optically active compound ($e.g.$, alcohol), separating the diastereomers and converting ($e.g.$, hydrolyzing) the individual diastereomers to the corresponding pure enantiomers. Enantiomers can also be synthesized using asymmetric reagents, for example to prepare the alpha alkyl amino acid head group of myriocin and its analogs ($e.g.$, Seebach, D., $et$ $al.$, Helv. Chim. Acta., 70: 1194-1216 (1987)); Hale, JJ, $et$ $al.$ Bio-org. Med.Chem. Lett., 12:4803-07 (2004)); Kobayashi, S., $et$ $al.$, J. Am. Chem. Soc, 120:908-19 (1998)). Alternatively, chiral synthesis of enantiomeric centers using chiral synthons from natural products is a facile approach to such syntheses, for example the synthesis of myriocin from d-mannose (Oishi, T., $et$ $al.$ Chemical Commun. 1932-33 (2001)); and references to myriocin synthesis therein) and of myriocin analogs from isolated, natural myriocin (Chen, JK, $et$ $al.$ Chem Biol. 6, 221-35 (1999)); Fujita, T, $et$ $al.$ J. Med. Chem.
In addition, use of enzymes (free or supported) to preferentially modify one of the enantiomeric centers and thus allow separation or interconversion of enantiomers is well-known to the art (for example Wang, Y.-F., et al. (1988). J. Am. Chem. Soc. 110, 7200-5) and has great usefulness in production of pharmaceuticals. All such isomers, including diastereomers, enantiomers, and mixtures thereof are considered as part of this invention.

Those skilled in the art will recognize that some of the compounds herein can exist in several tautomeric forms. All such tautomeric forms are considered as part of this invention. Also, for example all enol-keto forms of any compounds herein are included in this invention.

Some of the compounds of this invention are acidic and may form a salt with a pharmaceutically acceptable cation. Some of the compounds of this invention can be basic and accordingly, may form a salt with a pharmaceutically acceptable anion. All such salts, including di-salts are within the scope of this invention and they can be prepared by conventional methods. For example, salts can be prepared simply by contacting the acidic and basic entities, in either an aqueous, non-aqueous or partially aqueous medium. The salts are recovered either by filtration, by precipitation with a non-solvent followed by filtration, by evaporation of the solvent, or, in the case of aqueous solutions, by lyophilization, as appropriate.

In addition, compounds herein embrace metabolites, hydrates, or solvates thereof and all of which are within the scope of the invention.

The term "substituted" refers to substitution on any carbon or heteroatom with any chemically feasible substituent. Representative substitutions include halogen substitution or substitution with any heteroatom containing group, e.g., alkoxy, phosphoryl, sulphydryl, etc.

The term "alkyl" refers to straight chain, branched, or cyclic hydrocarbons. Exemplary of such alkyl groups (assuming the designated length encompasses the particular example) are methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tertiary butyl, pentyl, isopentyl, neopentyl, tertiary pentyl, 1-methylbutyl, 2-methylbutyl, 3-methylbutyl, hexyl, isohexyl, heptyl and octyl. The term "lower alkyl" refers to alkyl as defined above comprising C₁-C₅. Substituted alkyl refers to alkyl groups which are substituted as defined above and are exemplified by haloalkyl, e.g., CF₃, CHF₂, CH₂F, etc.

The term "aryl" refers to any aromatic group comprising C₃-C₂₀. Aryl groups also embrace fused ring systems, such as 5-5, 5-6, and 6-6 ring systems. Representative aryl groups include phenyl, biphenyl, anthracyl, norbornyl, and the like. Aryl groups may be substituted according to the definition provided above.
The term "heteroaryl" refers to any aryl group comprising at least one heteroatom within the aromatic ring. Heteroaryl groups also embrace fused ring systems, such as 5-5, 5-6, and 6-6 ring systems. Representative heteroaryl groups include imidazole, thiazole, oxazole, phenyl, pyridinyl, pyrimidyl, imidazolyl, benzimidazolyl, thiazolyl, oxazolyl, isoxazolyl, benzthiazolyl, or benzoazolyl. Heteroaryl groups may be substituted according to the definition provided above.

The term "aralkyl" or "arylalkyl" refers to an aryl group comprising an alkyl group as defined above. Aralkyl or arylalkyl groups may be appended from the aryl or the alkyl moiety.

The term "alkoxy" refers to alkyl groups bonded through an oxygen. Exemplary alkoxy groups (assuming the designated length encompasses the particular example) are methoxy, ethoxy, propoxy, isoproxy, butoxy, isobutoxy, tertiary butoxy, pentoxy, isopentoxo, neopentoxy, tertiary pentoxy, hexoxy, isohexoxy, heptoxy and octoxy. Alkoxy may be substituted according to the definition provided above.

The term "alkoxyalkyl" refers to an alkoxy group comprising an alkyl group as defined above. Alkoxyalkyl groups may be substituted according to the definition provided above.

The term "halogen" refers to chloro, bromo, iodo, or fluoro.

The term "modulator" means a molecule that interacts with a target either directly or indirectly. The interactions include, but are not limited to, agonist, antagonist, and the like.

The term "agonist" means a molecule such as a compound, a drug, an enzyme activator or a hormone that enhances the activity of another molecule or the activity of a receptor site.

The term "antagonist" means a molecule such as a compound, a drug, an enzyme inhibitor, or a hormone, that diminishes or prevents the action of another molecule or the activity of a receptor site.

The terms "effective amount" or "therapeutically effective amount" refer to a sufficient amount of the agent to provide the desired biological result. That result can be reduction and/or alleviation of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system. For example, an "effective amount" for therapeutic use is the amount of the composition comprising a compound as disclosed herein required to provide a clinically significant decrease in a disease. An appropriate "effective" amount in any individual case may be determined by one of ordinary skill in the art using routine experimentation.

As used herein, the terms "treat" or "treatment" are used interchangeably and are meant to indicate a postponement of development of diseases and/or a reduction in the severity of such symptoms that will or are expected to develop. The terms further include ameliorating existing
disease symptoms, preventing additional symptoms, and ameliorating or preventing the underlying metabolic causes of symptoms.

By "pharmacologically acceptable" or "pharmacologically acceptable" is meant a material which is not biologically or otherwise undesirable, i.e., the material may be administered to an individual without causing any undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

III. Preparation of Compounds


[0098] Kiuchi et al. (Kiuchi M, et al, J. Med. Chem., 43:2946-61 (2000)) discusses procedures for the synthesis of compound 2. Representative methods for preparing compounds herein may include synthetic precursors reported therein. The general sequence (Scheme 1) for preparing Cα-substituted serine moieties from alkyl halides (or from the corresponding hydroxyl or aldehyde structures by conversion to alkyl halides) can be used broadly to prepare the present compounds. Illustrated in the synthetic schemes below are exemplary methods for preparing the present compounds.

[0099] Scheme 1 below illustrates a preparative route reported in Kiuchi et al. for preparing compound 2.

![Scheme 1](image)

**SCHEME 1**
[00100] Scheme 2 illustrates a similar synthetic procedure for preparing of an analog having increased water solubility.

\[
\begin{align*}
\text{HO} & \quad \xrightarrow{n-C_6H_{13}Br} \quad \xrightarrow{NaOH/EtOH} \quad \text{EtOH} \\
\text{HO} & \quad \xrightarrow{\text{n-C}_6\text{H}_{13}^\text{Br}} \quad \xrightarrow{\text{NaOH/EtOH}} \quad \text{77.6\%} \\
\text{n-C}_6\text{H}_{13} & \quad \xrightarrow{\text{MsCl/Et}_3\text{N}} \\
\text{EtCOMe} & \quad \xrightarrow{\text{64\%, 2 steps}} \quad \text{EtCOMe} \\
\text{NC}^\text{NHAc} & \quad \xrightarrow{\text{EtCOMe}} \quad \text{79\%} \\
\text{LiBH}_4 & \quad \xrightarrow{\text{THF}} \quad \text{LiBH}_4 \\
\text{HCl} & \quad \xrightarrow{\text{AcOH/H}_2\text{O}} \quad \text{HCl} \\
\text{EtOH} & \quad \xrightarrow{\text{H}_2\text{SO}_4} \quad \text{EtOH} \\
\end{align*}
\]

SCHEME 2

[00101] As illustrated in Scheme 3 below, analogs of myriocin which contain two hydroxyl functional groups alpha and beta to the head group, can be prepared from native myriocin using a variation of the approach reported by Chen, JK, et al. (1999). Shown below is an exemplary synthetic procedure using starting material reported in Chen et al. to obtain a range of analogs having various functionalities in R’ by employing a Wittig-type reaction with iodoalkyl compounds. For example, R’ can be alkyl, haloalkyl, aryl, aralkyl, and the like. Scheme 3 is a chiral preparation and corresponding enantiomers can be produced using this procedure by protecting the primary OH and NH/CO$_2$H functional groups, followed by inversion chemistry on the secondary OH groups. Exemplary compounds 13, 17, 18, and 19 are readily prepared from the corresponding iodoalkyl compounds using the procedure illustrated below.
Compounds having a single hydroxyl function alpha to the serine head group can be prepared in the synthetic method illustrated below in Scheme 4. Similar reagents may be used to carry out these synthetic steps with greater or lesser yields, depending on the actual substrates used. Exemplary compounds 14, 19, and 20 are readily prepared using Scheme 4.

[00103] Similarly, compounds with a single hydroxyl function, beta to the serine like head group (e.g. compound 20) are prepared through a route starting from the corresponding, readily available alpha-haloketones or alpha-hydroxyketones according to Scheme 5.
[00104] Similarly, compounds with a single hydroxyl or ketone function, beta to the amino acid-like head group (e.g. compound 16) are prepared through a route starting from the corresponding, readily available alpha-haloketones or alpha-hydroxyketones according to Scheme 5. Elements of this scheme have been illustrated by Sano, S., et al., Tetrahedron Letters, 36: 2097-2100 (1995). The bis-lactim reagent is generated according to Sano, S., et al., Tetrahedron Letters 36: 4101-4 (1995) and the typical coupling conditions with reagents are examined in detail in that publication, incorporated herein by reference.

Additional routes to the compounds of the invention are variations from previous synthetic routes toward the natural products inhibitors of SPT (Liao, J. et al. Tetrahedron 61: 4715-33 (2005)), which are unsuitable as pharmaceutical agents. Thus work by Trost (Trost, B.M. and Lee, C. J. Am. Chem. Soc. 123: 12191-201 (2001)) and Kobayashi (Kobayashi, S. and Furuta, T. J. Am. Chem. Soc. 120: 908-19 (1998) offer a fertile background for the design of syntheses of analogs such as the compounds of the invention. Examples are given in Schemes 7 and 8. Thus in Scheme 7, the illustrated route (Trost, B.M. and Lee, C., 2001), the content of which is incorporated by reference, begins with intermediate 21 from that reference and uses reactions illustrated therein. Although the stereochemistry at position 2 is important, that the stereochemistry at other positions is less so. Stereochemistry at all positions is easily modified by routes illustrated in this reference. For example, 2-position stereochemistry is inverted by using an amino acid of the opposite configuration to begin the synthesis. Additionally, with reference to the above Trost publication, stereochemistry at position 3 can be inverted by transesterification/saponification of the Ac group, activation to the triflate and inversion by rearrangement of the benzoate group. The compounds of the invention will make use of
intermediates that have a protected hydroxymethyl group on the azlactone ring, and will make use of similar reactions. This Scheme is merely illustrative of the route for assembly of the aliphatic chain.

SCHEME 7

[00107] Similarly, as illustrated in Scheme 8, an approach used by Kobayashi and Furuta J. Am. Chem. Soc. 120: 908-19 (1998) provides a precedent for the use of a very versatile bis-lactim intermediate (Schöllkopf, U. Pure Appl Chem. 55: 1799 (1983); See also Scheme 5). The modification by Sano S, et al. makes use of a 2-carboxylate form of the bis-lactim to generate a very wide range of α-alkylated serines and is very convenient for the preparation of the compounds of the invention (Sano, S, et al. 1995a, b; 1998). This route provides multihydroxyl analogs or a saturated alkyl chain, depending on whether reduction or dihydroxylation of the double bond is pursued. This route again offers great flexibility in the synthesis of analogs, depending on which aldehyde is used to react with the bis-lactim intermediate.

[00109] An example of the use of a natural product starting material for a chiral synthesis is shown in Scheme 9, wherein N-acetyl-D-mannosamine is used to provide the correct chirality at the amino acid and hydroxyl positions (Mori, K. and Otaka, K., Tetrahedron Lett. 35: 9207-10 (1994)). This is an extremely versatile synthesis route that allows incorporation of a wide variety of R groups with the correct chirality for the critical functional groups of the amino acid like head group. Alkylation of the initial lactone intermediate can be used to introduce a protected hydroxymethyl function, for example as illustrated above, in order to yield the compounds of the invention.
[00110] Another flexible approach to the synthesis of sphingofungin analogs or compounds of the present invention is that shown in Scheme 10. This approach is preceded by the synthesis of sphingofungin B Kobayashi, S. and Furuta, T., Tetrahedron 54: 10275-94 (1998) and allows great flexibility with respect to the types of R groups that can be incorporated. In this case, a precursor to the final hydroxymethyl functional group is introduced, as illustrated above (Sano, S., et al. 1995a, b; 1998). The R group diversity is important since the appropriate R groups will lend the final product the physical properties that provide it with the pharmaceutical, pharmacokinetic and pharmacodynamic properties required of a successful drug candidate.
A specific example of the use of the bis-lactim route to the synthesis of compounds of the invention is illustrated in SCHEME 11. This route illustrates the use of Compound 106 as a common intermediate for the rapid and convenient synthesis of a wide variety of SPT inhibitor structures from readily available olefins. The readily available 4-(tert-butyldimethylsilyloxy)-butanal was subjected to iodomethylenation in the manner of Takai, T, et al. (1986) as illustrated by Trost and Lee (2001), deprotected with F, and oxidized by the DessMartin reagent. A detailed study of the iodomethylenation reaction has been carried out by Evans and Black (1993) and individual reactions may be further optimized by small changes to the reaction solvent conditions as outlined therein. The resultant vinyl iodide was condensed with the modified Schollkopf bis-lactim (Schölklkopf U, 1983; Sano S, et al. 1995a, b; 1998) under conditions used by Kobayashi and Furuta (1998) to yield the intermediate compound 105 in moderate to good yield and as a mixture of two products isomeric at the alcohol position. The two diastereomers were separated by chromatography on silica gel and then the alcohol was protected as the TBS ether using TBS-triflate reagent. This product, Compound 106 could be converted into a wide variety of the compounds of the invention by 5-alkyl Suzuki coupling with
the corresponding organoboranes to form the final products. A further specific example, meant to be illustrative and not to limit the scope of the invention in any way is shown in SCHEME 12.

SCHEME 11

[00112] The strategic intermediate Compound 106 was subjected to 5-alkyl Suzuki coupling conditions in the manner of Trost and Lee (2001). The required organoborane is generated in situ by reaction of the corresponding olefin (Compound 107) with 9-BBN-H. Following the work-up, the product, Compound 108 is hydrolyzed in a two step process to yield a compound of the invention, Compound 69. In a similar manner are prepared a wide range of the compounds of the invention.

SCHEME 12

[00113] A proof of concept of this reaction scheme is shown in SCHEME 13, wherein 1-heptene is used as a model reactant. Hydroboration with 9-BBN-H was used to generate the required
organoborane intermediate immediately prior to the coupling. Catalyst mixtures for this coupling have been investigated and optimized conditions reported by Johnson CR and Braun MP (1993) and by Ohba, M, et al (1996). In general a mixture of bis(diphenylphosphino)ferrocene palladium(II) chloride (PdCl$_2$(dppf)) and triphenylarsine as coligand was found to be optimal and catalyst loading of 5-20 mole% can be used. The solvent was optimized as a mixture of DMF/THF/H$_2$O with added Cs$_2$CO$_3$ as base. These conditions allow the use of a wide variety of functional groups. Trost and Lee (2001) use a slight variant wherein the water is added to the organoborane prep prior to addition to the coupling reaction.

**SCHEME 13**

[00114] For compounds where a 3,4 diol structure is desired, such as Compound 42, an alternate strategic intermediate can be used (Compound 117). Starting from the chiral (R)-1,2,4-butanetriol (SigmaAldrich), selective protection as the 1,2-acetonide (Kocienski, et al. (1987)) is followed by elaboration as demonstrated above and as shown in SCHEME 14. Selective oxidation of the diol intermediate prior to Compound 115 is by 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO)-mediated selective oxidation of the primary alcohol (Einhorn, et al. (1996)). The aldol reaction with the modified Schollkopf bis-lactim (Sano S., et al. 1995a,b; 1998) follows the procedure of Kobayashi, et al. (1998). Separation of the small amounts of diasteromer at the alcohol position, so formed, is done by silica gel or other chromatography (compound dependent) and is followed by TBS protection using the triflate reagent. Compound 777 is then coupled with a variety of organoborane intermediates (generated "in situ" from 9-BBN-H and the corresponding olefins) representing the tail region of the compounds of the invention. Deprotection of the silyl protecting group and hydrolysis of the bis-lactim is followed by chromatographic purification (silica gel or reversed-phase) to yield the compounds of the invention.
Hydrolysis of the intermediate bis-lactim products can take place by various related routes. Acid hydrolysis under mild conditions (typically HCl or trifluoroacetic acid in aqueous, acetonitrile or MeOH mixtures) can yield the final amino acid or mixtures containing ester or amide hydrolysis intermediates. Final saponification optionally can be used to effect the full hydrolysis to the amino acid (Schollkopf (1983, 1988); Kobayashi, et al. (1996)). An example is shown in SCHEME 15.

An alternative route to strategic intermediates that allow a convergent synthesis entails the formation of the tail portion followed by coupling to various head groups. An illustrative example, not meant to limit the scope in any fashion, is shown in SCHEME 16. In this illustration, the 5-alkyl Suzuki coupling is performed to yield an aldehyde precursor (Compound 126) which is then deprotected, oxidized and coupled to the bis-lactim to yield the assembled,
protected final product. Mild hydrolysis follows, as illustrated above, to yield the final product, Compound 69, in this case. This is a very general and convergent route to the compounds of the invention.

**Scheme 16**

**IV. Pharmaceutical Compositions**

[00117] Compositions presented herein include compounds provided herein and a pharmaceutically acceptable carrier.

**A. Formulations**

[00118] Pharmaceutically useful compositions comprising the compounds of the present invention may be formulated according to known methods such as by the admixture of a pharmaceutically acceptable carrier. Examples of such carriers and methods of formulation may be found in Remington's Pharmaceutical Sciences. To form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of the compound, e.g., a prodrug or an active species (e.g., the corresponding acid of the ester or prodrug), of the present invention.
[00119] Suitable formulations for administering the present compounds include topical, transdermal, oral, systemic, and parenteral pharmaceutical formulations. Compositions containing compounds herein can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compounds or modulators can be administered in such oral dosage forms as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by transdermal delivery or injection. Likewise, they may also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, transdermal, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts. The present compounds may be delivered by a wide variety of mechanisms, including but not limited to, transdermal delivery, or injection by needle or needle-less injection means.

B. Dosages

[00120] Embodiments include pharmaceutical compositions comprising an effective amount of compounds presented herein. Effective dosages of compounds disclosed herein may be defined by routine testing in order to obtain optimal inhibition of serine palmitoyl transferase while minimizing any potential toxicity.

[00121] As is well known to one of skill in the art, effective amounts can be routinely determined and vary according to a variety of factors such as the individual's condition, weight, sex, age, medical condition of the patient, severity of the condition to be treated, route of administration, renal and hepatic function of the patient, and the particular compound thereof employed. A physician or veterinarian of ordinary skill can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving concentrations of drug within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a drug.

[00122] An effective but non-toxic amount of the compound desired can be employed as a serine palmitoyl transferase-modulating agent. Dosages contemplated for administration of the present compounds range from 0.01 to 1,000 mg per patient, per day. For oral administration, the compositions are preferably provided in the form of scored or un-scored tablets containing 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, and 50.0 milligrams of the active ingredient for the symptomatic adjustment of the dosage to the patient to be treated. Dosage amounts may also
vary by body weight and can range, for example, from about 0.0001 mg/kg to about 100 mg/kg of body weight per day, preferably from about 0.001 mg/kg to 10 mg/kg of body weight per day.

[00123] Compounds may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three, or four times daily. To be administered in the form of a transdermal delivery system, the dosage administration will be continuous rather than intermittent throughout the dosage regimen.

[00124] The dosages of the compounds of the present invention are adjusted when combined with other therapeutic agents. Dosages of these various agents may be independently optimized and combined to achieve a synergistic result wherein the pathology is reduced more than it would be if either agent were used alone. In addition, co-administration or sequential administration of other agents may be desirable.

C. Derivatives

[00125] Embodiments of compounds presented herein include "chemical derivatives." Chemical derivatives comprise compounds herein and additional moieties that improve the solubility, half-life, absorption, etc. of the compound. Chemical derivatives may also comprise moieties that attenuate undesirable side effects or decrease toxicity. Examples of such moieties are described in a variety of texts, such as Remington's Pharmaceutical Sciences, and are well known to one of skill in the art.

D. Carriers and Excipients

[00126] Compounds herein can be administered in admixture with suitable pharmaceutical diluents, excipients, or carriers (collectively referred to herein as "carrier" materials) suitably selected with respect to the intended form of administration, that is, oral tablets, capsules, elixirs, syrups and the like, and consistent with conventional pharmaceutical practices.

[00127] For oral administration in the form of a tablet or capsule, the active drug component can be combined with an oral, non-toxic pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents and coloring agents can also be incorporated into the mixture. Suitable binders include, without limitation, starch, gelatin, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes and the like. Lubricants used in these dosage forms include, without limitation, sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum and the like.
For liquid forms the active drug component can be combined in suitably flavored suspending or dispersing agents such as the synthetic and natural gums, for example, tragacanth, acacia, methyl-cellulose and the like. Other dispersing agents which may be employed include glycerin and the like.

For parenteral administration, sterile suspensions and solutions are desired. Isotonic preparations which generally contain suitable preservatives are employed when intravenous administration is desired.

Topical preparations comprising the present compounds can be admixed with a variety of carrier materials well known in the art, such as alcohols, aloe vera gel, allantoin, glycerine, vitamin A and E oils, mineral oil, PPG2 myristyl propionate, and the like, to form, for example, alcoholic solutions, topical cleansers, cleansing creams, skin gels, skin lotions, and shampoos in cream or gel formulations.

Compounds can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines.

Compounds presented herein may also be delivered by the use of monoclonal antibodies as individual carriers to which the compound molecules are coupled. Compounds may be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinyl-pyrrolidone, pyran copolymer, polyhydroxypropylmethacryl-amidephenol, polyhydroxy-ethylaspartamidephenol, or polyethyl-eneoxide polylysine substituted with palmitoyl residues. Furthermore, compounds may be coupled to biodegradable polymers useful in achieving controlled release of a drug, such as polylactic acid, polyepson caprolactone, polyhydroxy butyric acid, polyorthoeesters, polyacetals, polydihydro-pyrans, polycyanoacrylates, cross-linked or amphipathic block copolymers of hydrogels, and other suitable polymers known to those skilled in the art.

For oral administration, compounds may be administered in capsule, tablet, or bolus form. The capsules, tablets, and boluses comprise an appropriate carrier vehicle, such as starch, talc, magnesium stearate, or di-calcium phosphate.

Unit dosage forms are prepared by intimately mixing compounds with suitable finely-powdered inert ingredients including diluents, fillers, disintegrating agents, and/or binders such that a uniform mixture is obtained. An inert ingredient is one that will not adversely react with the compounds. Suitable inert ingredients include starch, lactose, talc, magnesium stearate,
vegetable gums and oils, and the like. Compounds can be intimately mixed with inert carriers by grinding, stirring, milling, or tumbling.

[00135] Injectable formulations comprise compounds herein mixed with an appropriate inert liquid carrier. Acceptable liquid carriers include the vegetable oils such as peanut oil, cottonseed oil, sesame oil and the like as well as organic solvents such as solketal, glycerol formal and the like. As an alternative, aqueous parenteral formulations may also be used. The vegetable oils are the preferred liquid carriers. The formulations are prepared by dissolving or suspending the compound in a liquid carrier.

[00136] Topical application of compounds is possible through the use of, for example, a liquid drench or a shampoo containing the instant compounds or in modulators as an aqueous solution or suspension. These formulations may comprise a suspending agent such as bentonite and optionally, an antifoaming agent.

E. Modes of Administration

[00137] Other factors affecting dosage amounts are the modes of administration. The pharmaceutical compositions of the present invention may be provided to the individual by a variety of routes including, but not limited to subcutaneous, intramuscular, intra-venous, topical, transdermal, oral and any other parenteral or non-parenteral route. Furthermore, compounds can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art.

[00138] The compounds or modulators may alternatively be administered parenterally via injection of a formulation consisting of the active ingredient dissolved in an inert liquid carrier. Injection may be either intramuscular, intraruminal, intratracheal, or subcutaneous, either by needle or needle-less means.

F. Pharmaceutically Acceptable Salts

[00139] Embodiments include compounds presented herein in the form of a free base or as a pharmaceutically acceptable salt. Exemplary pharmaceutically acceptable salts include hydrobromic, hydroiodic, hydrochloric, perchloric, sulfuric, maleic, fumaric, malic, tartaric, citric, benzoic, mandelic, methanesulfonic, hydroethanesulfonic, benzenesulfonic, oxalic, pamoic, 2-naphthalenesulfonic, p-toluenesulfonic, cyclohexanesulfamic and saccharic. Ion exchange, metathesis or neutralization steps may be used to form the desired salt form.

G. Combinations
[00140] Embodiments include compositions comprising compounds presented herein in combination with another active agent. Exemplary active agents which may be employed include insulin, insulin analogs, incretin, incretin analogs, glucagon-like peptide, glucagon-like peptide analogs, exendin, exendin analogs, PACAP and VIP analogs, sulfonylureas, biguanides, α-glucosidase inhibitors, and ligands for the Peroxisome Proliferator-Activated Receptors (PPARs) of all classes.

[00141] For combination treatment with more than one active agent, where the active agents are in separate dosage formulations, the active agents can be administered concurrently, or they each can be administered at separately staggered times.

[00142] The dosages of the compounds of the present invention are adjusted when combined with other therapeutic agents. Dosages of these various agents may be independently optimized and combined to achieve a synergistic result wherein the pathology is reduced more than it would be if either agent were used alone. In addition, co-administration or sequential administration of other agents may be desirable.

H. Kits

[00143] In a preferred embodiment, compounds herein are packaged in a kit. An example of such a kit is a so-called blister pack. Blister packs are well known in the packaging industry and are being widely used for the packaging of pharmaceutical unit dosage forms (tablets, capsules, and the like). Blister packs generally consist of a sheet of relatively stiff material covered with a foil of a preferably transparent plastic material. During the packaging process recesses are formed in the plastic foil. The recesses have the size and shape of the tablets or capsules to be packed. Next, the tablets or capsules are placed in the recesses and the sheet of relatively stiff material is sealed against the plastic foil at the face of the foil which is opposite from the direction in which the recesses were formed. As a result, the tablets or capsules are sealed in the recesses between the plastic foil and the sheet. Preferably the strength of the sheet is such that the tablets or capsules can be removed from the blister pack by manually applying pressure on the recesses whereby an opening is formed in the sheet at the place of the recess. The tablet or capsule can then be removed via said opening.

[00144] It may be desirable to provide a memory aid on the kit, e.g., in the form of numbers next to the tablets or capsules whereby the numbers correspond with the days of the regimen which the tablets or capsules so specified should be ingested. Another example of such a memory aid is a calendar printed on the card, e.g., as follows "First Week, Monday, Tuesday, . . . etc . . . Second Week, Monday, Tuesday, . . . " etc. Other variations of memory aids will be
readily apparent. A "daily dose" can be a single tablet or capsule or several pills or capsules to be taken on a given day. Also, a daily dose of Formula I compound can consist of a single tablet or capsule while a daily dose of the second compound can consist of several tablets or capsules and vice versa. The memory aid should reflect this.

[00145] In another specific embodiment of the invention, a dispenser designed to dispense the daily doses one at a time in the order of their intended use is provided. Preferably, the dispenser is equipped with a memory aid, so as to further facilitate compliance with the regimen. An example of such a memory aid is a mechanical counter which indicates the number of daily doses that has been dispensed. Another example of such a memory aid is a battery powered microchip memory coupled with a liquid crystal readout, or audible reminder signal which, for example, reads out the date that the last daily dose has been taken and/or reminds one when the next dose is to be taken.

V. Methods of Treatment

[00146] An important feature of the present invention relates to the involvement of ceramide as a signaling molecule in inflammatory processes. In addition to its effect on the apoptosis of beta cells relevant to T2D, de novo ceramide can have broader apoptotic effects in human health. Influencing the levels of ceramide can lead to novel treatments of human islets, or islets from other commercially or medicinally important sources, in culture during isolation for transplant with the intent of improving survival of islets in vitro and post transplant. SPT inhibitors can be added to currently used or accepted treatment protocols in order to inhibit, either alone and/or in a synergistic fashion, the loss of islets and beta cells due to apoptotic and/or necrotic processes.


[00148] Blockade of de novo ceramide synthesis shows a synergistic improvement in cell survival when comprising addition of compounds of the present invention, e.g., SPT inhibitors, to the protocols enumerated above, and their like. Loss of pancreatic islets in Type 1 Diabetes also shows evidence of inflammatory processes leading to apoptosis and necrosis.

[00149] Embodiments of the invention include methods for treating developing Type 1 Diabetes and / or the further loss of islets following transplantation (human or xenobiotic islet cell transplantation) comprising the addition of compounds of the present invention, e.g., SPT inhibitors, to current treatment protocols (IUBMB Life. 2004 Jul, 56:387-94. Protecting pancreatic beta-cells. Pileggi A, Fenjves ES, Klein D, Ricordi C, Pastori RL.). Xenobiotic cells contemplated for use in the methods of the present invention include, but are not limited to, porcine, bovine, murine, and other mammalian cell types. The inhibition of de novo ceramide synthesis shows beneficial effects when used alone or as an addition to existing protocols. Such treatment may commence immediately upon detection of loss of beta cell mass or function, and be used alone or in conjunction with immunosuppressive regimens (cyclosporine, mycophenolic acid agents, FTY720, and the like, for example). This is a broadly based mechanism to protect beta cells from a wide array of insults that result in apoptosis and necrosis.

[00150] In additional embodiments of this invention, the compounds of the invention are used for the blockade of apoptosis of neuronal cells following spinal injury, and in loss of CNS neurons, e.g. in Alzheimer's disease or stroke. This treatment with an inhibitor of SPT may be
used effectively alone or in combination with other treatments such as antioxidants, caspase inhibitors (Neurochem Res., 28:143-52 (2003). Protection of mature oligodendrocytes by inhibitors of caspases and calpains. Benjamins JA, Nedelkoska L, George EB) and/or other treatments for protection from the late effects of stroke that are well known to those skilled in the art.

[00151] Compounds and compositions presented herein may be administered to patients in the treatment of a variety of diseases. Preferably, methods of treatment presented herein are directed to patients (i.e., humans and other mammals) with disorders or conditions associated with the activity or hyperactivity of serine palmitoyl transferase (SPT). Accordingly, methods of treating insulin resistance and cardiomyopathy are provided. Compounds effective in treating cardiomyopathy may interfere with the process of cardiomyopathy development. Compounds of the invention may also be used to treat cachexia and sepsis.

[00152] Preferred compounds employed in methods of treatment possess desirable bioavailability characteristics. Exemplary compounds are esters which can function as a pro-drug form having improved solubility, duration of action, and in vivo potency. Preferred compounds employed in treatment methods exhibit improved solubility in water and less potential to cross the blood brain barrier to cause side effects, such as altered feeding behavior.

[00153] Pharmaceutical compositions are administered to an individual in amounts sufficient to treat or diagnose disorders in which modulation of serine palmitoyl transferase activity is indicated. Examples of diseases or conditions known to be, or suspected of being mediated by serine palmitoyl transferase include, but are not limited to, insulin resistance, type 2 diabetes and its complications, obesity, pro thrombotic conditions, myocardial infarction, congestive heart failure, hypertension, dyslipidemia, and other manifestations of the commonly accepted "Metabolic Syndrome" and "Syndrome X." Compounds effective in treatment methods herein potently and specifically modulate the enzyme Serine Palmitoyl Transferase.

[00154] It is to be understood that the above description is intended to be illustrative and not restrictive. The scope of the invention should, therefore, be determined not with reference to the above description, but instead with reference to the appended claims along with the full scope of equivalents thereto.

**EXAMPLES**

[00155] In order to illustrate the invention the following examples are included. These examples do not limit the invention. They are meant to illustrate only exemplary methods and compounds presented herein. Those knowledgeable in chemical synthesis and the treatment of
serine palmitoyl transferase related disorders may find other methods of practicing the invention. However those methods are deemed to be within the scope of this invention.

Example 1

Synthesis of Methyl Ester of Compound 2

[00156] In a round-bottomed flask, 500mL of MeOH is cooled to -5 °C and treated with 0.11 mol of SOCl₂ in a dropwise fashion with stirring. Powdered compound 2 (0.1 mol) is added immediately with cooling and stirring. The solution is allowed to warm slowly to room temperature over a period of 2 hrs. Evaporation of the excess MeOH provides the desired compound (R₁ = Me) as the HCl salt in high yield as a white powder. Recrystallization from a suitable solvent (MeOH/Et₂O) provides the desired compound in high purity as a white, waxy solid. In a like manner, additional ester forms of compound herein can be prepared. Synthesis of compound 2 is described in Kiuchi et al. 2000 (supra).

Example 2

Synthesis of Ethyl Ester of Compound 2

[00157] In a round-bottomed flask, 500mL of EtOH is treated with 0.01 mol of HCl in EtOAc and powdered compound 2 (0.1 mol) is added immediately with cooling and stirring. The solution is warmed to reflux and heated for a period of 24 hrs. Evaporation of the excess EtOH provides the desired compound (R₁ = Me) as the HCl salt in high yield as a white powder. Recrystallization from a suitable solvent (EtOH/Et₂O) provides the desired compound in high purity as a colorless oil which slowly forms a waxy solid. In a like manner, additional ester forms of compound herein can be prepared. Alternatively, addition of an equivalent amount of HCl and H₂SO₄ in EtOH and refluxing for 2 days provides a high yield of product.

Example 3

Synthesis of Compound 23

[00158] Compound 23 was prepared using the route outlined in Scheme 2, starting with 4-(3-hydroxypropyl)phenol (Aldrich Chemical Company). Yields obtained are reported in Scheme 2. Compound 23 was obtained as an off white solid and melting point was broad.

[00159] (M-I) molecular ion is 322.3 a.m.u. ¹H NMR (CD₃OD) δ: 0.95 (3H, tr), 1.37 (4H, m), 1.45 (2H, m), 1.75 (6H, m), 2.55 (2H, m), 3.7 (2H, dd), 3.9 (2H, t), 6.9 (4H, dd).
**Example 4**

*Synthesis of Compound 24*

[00160] Compound 24 was prepared using the route outlined in Scheme 2 starting with \( A-(A\text{-hydroxybutyl})\text{phenol} \). Compound 24 was obtained as an off white solid and melting point was broad.

[00161] (M-I) molecular ion is 336.3 a.m.u. \(^1H\) NMR (D\(_6\)-DMSO) \( \delta \): 0.93 (3H, t), 1.4 (12H, broad m), 2.45 (2H, d), 3.5 (2H, q), 3.9 (2H, t), 6.9 (4H, dd).

**Example 5**

*Synthesis of Compound 107*

[00162] **Hept-6-enal.** To an emulsion of 7-octene-1,2-diol (5.0 g, 34.7 mmol) and water (20 mL) was added a solution of NaIO\(_4\) (8.14 g, 38.2 mmol) in water (47.5 mL) over 30 min. After the reaction mixture was stirred at r.t. for 2 h, the solution was saturated with NaCl, and the organic phase was separated and dried over Na\(_2\)SO\(_4\) to give the product as a colorless oil (2.5 g) without further purification. The aqueous solution was extracted with CH\(_2\)Cl\(_2\), dried over Na\(_2\)SO\(_4\). Solvent was removed under reduced pressure (without heating due to low boiling point of the product) to give a colorless oil (1.0 g). Total yield 3.5 g, 90%. \(^1H\) NMR (500 MHz, CDCl\(_3\) \( \delta \) 9.75 (s, 1 H), 5.82-5.74 (m, 1 H), 5.02 (m, 0.5 H), 4.99 (m, 0.5 H), 4.96 (m, 0.5 H), 4.94 (m, 0.5 H), 2.43 (td, \( J = 7.36, 1.75 \text{, 2 H} \)), 2.09-2.05 (m, 2 H), 1.67-1.61 (m, 2 H), 1.46-1.40 (m, 2 H). ESIMS (M-H\(^-\)) \( m/z \) 111.4.

[00163] **7,7-difluorohept-l-ene.** A solution of hept-6-enal (1.74 g, 15.5 mmol) in CH\(_2\)Cl\(_2\) (10 mL) in a plastic bottle was cooled to 0 °C. DAST (5.0 g, 31.1 mmol) was added slowly and the reaction mixture was stirred at r.t. under Ar for 12 h. After cooling to 0 °C, water (5.0 mL) was added very slowly. CH\(_2\)Cl\(_2\) (20 mL) was added and a saturated NaHCO\(_3\) solution was added very slowly, until no additional CO\(_2\) bubbles formed. The CH\(_2\)C\(_12\) layer was washed with brine and dried over Na\(_2\)SO\(_4\). The organic layer was filtered through a silica column (1x10 cm, CH\(_2\)Cl\(_2\)) and concentrated. The product, **Compound 107**, was used without further purification. Based on TLC, the yield was 75% (due to the low boiling point of the product, the solvent was not thoroughly removed to determine the yield.) \(^1H\) NMR (500 MHz, CDCl\(_3\) \( \delta \) 5.80 (tt, \( J = 57.1, 4.6 \text{ Hz, 1 H} \)), 5.81-5.76 (m, 1 H), 5.02 (m, 0.5 H), 4.99 (m, 0.5 H), 4.97 (m, 0.5 H), 4.94 (m, 0.5 H), 2.08-2.03 (m, 2 H), 1.84-1.80 (m, 2 H), 1.48-1.41 (m, 4 H). ESIMS (M+H\(^+\)) \( m/z \) 135.

**Example 6**
**Synthesis of Compound 104**

[00164] (E)-(5-iodopent-4-enyloxy) tert-butyl dimethylsilane. To a suspension of Dess Martin periodinane (8.5 g, 20.0 mmol) in CH₂Cl₂ (150 mL) was added NaHCO₃ (1.68 g, 20.0 mmol) and a solution of 4-(t-tert-butyldimethylsilyloxy)butan-1-ol (5.3 g, 5.97 mL, 13.3 mmol) in CH₂Cl₂ (20 mL) at 0 °C. After the reaction was stirred at 0 °C for 2 h, it was warmed to r.t. It was filtered through a silica column (2x15 cm, CH₂Cl₂). The aldehyde solution and a solution of iodoform (10.5 g, 26.7 mmol) was added to a suspension of anhydrous CrCl₃ (10.0 g, 81.3 mmol) in THF (50 mL) at 0 °C. After it was stirred at 0 °C for 4 h and at r.t. for 8 h, it was poured into ice/water (100 mL), and extracted with EtOAc (4x75 mL). The organic layer was separated, washed with brine, and dried over anhyd Na₂SO₄. It was filtered, concentrated, and cooled to -20 °C. The solid (iodoform) was removed by filtration, and washed with hexanes. The solution was concentrated again, and cooled to -20 °C. The solid (iodoform) was removed. The solution was purified by silica gel column chromatography (4 x 20 cm, first eluted with hexanes to remove residual iodoform, then 1% EtOAc in hexanes) to give the product, **Compound 104**, as a colorless oil, 2.41 g (56% over 2 steps). ¹H NMR (500 MHz, CDCl₃) δ 6.55-6.49 (m, 1 H), 5.99 (dd, J = 14.4, 1.4 Hz, 1 H), 3.60 (t, J = 6.2 Hz, 2 H), 2.15-2.10 (m, 2 H), 1.64-1.57 (2 H), 0.88 (s, 9 H), 0.04 (s, 6 H). ESIMS (MNa⁺) m/z 349.0.

**Example 7**

**Synthesis of Compound 105**

[00165] To a solution of (E)-tert-butyl(5-iodopent-4-enyloxy)dimethylsilane (2.50 g, 7.67 mmol) in THF (7.0 mL) was added a solution of TBAF in THF (1.0 M, 15.34 mL) at 0°C under Ar. After it was stirred for 4 h, THF was removed and water (30 mL) was added. It was extracted with EtOAc (4 x 30 mL), washed with brine and dried over Na₂SO₄. Solvent was removed and the residue was purified by silica gel column chromatography (3 x 15 cm, Hexanes: EtOAc 9:1) to give (E)-5-iodopent-4-en-l-ol as a colorless viscous oil (1.26 g, 77%). ¹H NMR (500 MHz, CDC13) δ 6.55-6.50 (1 H), 6.04 (dd, J = 14.2, 1.3 Hz, 1 HHHH), 3.65 (t, J = 6.4 Hz, 2 H), 2.18-2.13 (m, 2 H), 1.72-1.63 (m, 2 H). ESIMS (MNa⁺) m/z 234.9.

[00166] To a suspension of Dess-Martin periodinane (2.13 g, 5.03 mmol) in CH₂Cl₂ was added NaHCO₃ (0.423 g, 5.03 mmol), then (E)-5-iodopent-4-en-l-ol at 0°C. After it was stirred at 0°C for 2 h, and r.t. for 1 h, it was filtered through a silica column (1 x 15 cm, CH₂Cl₂). The aldehyde
fractions were concentrated, and dissolved in hexanes, and dried over Na₂SO₄. Solvent was removed and the residue was dissolved in THF (5.0 mL) for immediate use. To a solution of (5i?)-ethyl-3,6-dimethoxy-5-isopropyl-2,5-dihydropyrazine-2-carboxylate (1.8 g, 6.7 mmol; Sano, S., et al. 1995b) in THF (10 mL) is added a solution of w-BuLi (1.6 M, 4.19 mL, 6.7 mmol) at -78 °C under Ar. The solution is warmed to 0°C for 15 min, a solution of anhydrous ZnCl₂ (0.5 M, 13.4 mL, 6.7 mmol) in THF is added and stirred at 0°C for 15 min. After the solution is cooled to -78°C, the aldehyde solution in THF is added slowly. After the mixture is stirred at -78°C for 1 h, a phosphate buffer (pH 7.0, 0.10 M, 70 mL) is added and it is extracted with EtOAc (4x70 mL), organic layer washed with brine, and dried over Na₂SO₄. Solvent is removed and the residue is purified by silica gel column chromatography (2 x 30 cm, Hexanes: EtOAc 9:1) to give the product, (1,i?,S;4£)-5-iodo-l-((2S,5R)-ethyl-3,6-dimethoxy-5-isopropyl-2,5-dihydropyrazincarboxylato-2-yl)pent-4-en-l-ol (Compound 105), a mixture of diastereomeric compounds (alcohol position) as colorless oils.

**Example 8**

*Synthesis of Compound 106*

[00167] To a solution of Compound 105 (2.5 mmol) in CH₂Cl₂ (10 mL) is added 2,6-lutidine (0.318 mL, 0.293 g, 2.74 mmol). The reaction mixture is cooled to -78 °C and TBSOTf (0.472 mL, 0.543 g, 2.06 mmol) is added dropwise. After stirring at -78 °C under Ar for 2 h, a solution of NH₄Cl in water (5.0 mL) is added and it is extracted with CH₂Cl₂ (3x5 mL). The organic layer is washed with water (5 mL) and brine, and dried over anhyd Na₂SO₄. The organic layer is filtered, concentrated in vacuo and the residue is purified by silica gel column chromatography (2 x 20 cm, hexanes: EtOAc 95:5) to give (2S,5R)-ethyl-2-((S,E)-l-(tert-butyldimethylsilyloxy)-5-iodopent-4-enyl)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine-2-carboxylate as a colorless oil. In an identical manner is prepared the other diastemer at position 1.

**Example 9**

*Synthesis of Compound 108*

[00168] To a solution of 7,7-difluorohept-l-ene, Compound 107, (0.254 g, 1.90 mmol) in THF (15 mL) is added a solution of 9-BBN-H (0.50 M, 4.23 mL, 2.12 mmol) in THF at r.t. under Ar. After it was stirred at r.t. for 1 h, degassed water (1.08 mL, 60.3 mmol) is added and the reaction mixture is stirred at r.t. for 30 min. The solution is then transferred to a mixture of (2S,5R)-ethyl-2-((5£,E)-l-(tert-butyldimethylsilyloxy)-5-iodopent-4-enyl)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine-2-carboxylate, Compound 106 (0.8 g, 1.4 mmol), Pd(dppf)Cl₂·CH₂Cl₂ (59.61
mg, 0.073 mmol), Ph₃As (22.35 mg, 0.073 mmol) and Cs₂CO₃ (618.4 mg, 1.90 mmol) in DMF (22.5 mL) under Ar. After stirring at r.t. for 4 h, water (50 mL) is added and it is extracted with hexanes (5x50 mL), washed with brine, and dried over Na₂SO₄. Solvent is removed and the residue is purified by silica gel column chromatography (2 x 25 cm, hexanes: EtOAc 97:3 to 95:5) to give Compound 108 as a colorless oil.

**Example 10**  
**Synthesis of Compound 69**

[00169] To a solution of Compound 108 (1.2 g, 2 mmol) in THF (5 mL) is added a solution of TBAF in THF (1.0 M, 4 mL, 4 mmol) at 0°C under Ar. After it is stirred for 4 h, THF is removed and water (10 mL) is added. It is extracted with EtOAc (4x 20 mL), washed with brine and dried over Na₂SO₄. Solvent is removed and the residue is purified by silica gel column chromatography (2 x 15 cm, Hexanes: EtOAc 9:1) to give the desired product, (2S,5R)-ethyl 2-(S,E)-12,12-difluoro-l-hydroxydodec-4-enyl)— 5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-carboxylate, as a colorless oil.

[00170] To a solution of the deprotected bislactim above (0.40 g, 1.0 mmol) in CH₂Cl₂ at 0°C is added DIBAL-H (1M solution in hexane; 4 mL, 4mmol). The solution is allowed to warm to rt and stirred until the reduction is complete by TLC. The mixture is cooled to -20°C and quenched with a 1:2 mixture of Celite and NaSO₄ decahydrate with slow warming to rt for 4 hr. Additional CH₂Cl₂ is added and the solid is filtered and washed with additional CH₂Cl₂. The combined organic layers are concentrated to dryness and taken up in a mixture of 3N HCl/CH₃CN. After stirring for 2 days the solution is extracted with ether and the aqueous layer is dried under vacuum and purified by silica gel chromatography to yield Compound 69 as a white solid. It is further purified by reversed-phase column chromatography to yield (2S,3S,E)-2-amino- 14,14-difluoro-3-hydroxy-2-hydroxymethyl)tetradec-6-enoic acid, Compound 69.

**Example 11**  
**Synthesis of Compound 75**

[00171] DMSO (1.95 g, 1.64 mL, 25.0 mmol) was added drop-wise to a solution of oxalyl chloride (1.52 g, 1.05 mL, 12.0 mmol) at -78 °C. After stirring at -78 °C for 15 min, 4-phenyl-l-butanol (1.50 g, 1.52 mL, 10.0 mmol) was added dropwise. After 15 min, triethylamine (5.05 g, 6.96 mL) was added dropwise. After it was warmed up to r.t. over 2 hours, water (50 mL) was added and it was extracted with CH₂Cl₂ (4 x 50 mL). The combined organic phase was washed
with HCl (0.25 N, 3 x 50 mL), water (50 mL), saturated NaHCO₃, and brine. The organic layer was dried over Na₂SO₄, concentrated, and passed through a short silica column (2 x 3 cm), eluted with CH₂Cl₂. Solvent was removed to give 4-phenylbutanal a colorless oil (1.30 g, 87%). 1H NMR (500 MHz, CDC13) δ 9.76 (t, J = 1.5 Hz, 1H), 7.31-7.27 (m, 2H), 7.22-7.17 (m, 2H), 2.67 (t, J = 7.5 Hz, 2H), 2.46 (dt, J = 1.6, 7.3 Hz, 2H), 1.97 (m, 2H).

[00172] To a solution of (5R) ethyl 3,6-dimethoxy-5-isopropyl-2,5-dihydro-pyrazine-2-carboxylate (1.842 g, 1.792 mL, 10.0 mmol) in THF (920 mL) at -78 °C was added n-BuLi (6.25 mL, 1.6 M in hexanes, 10.0 mmol) dropwise. The solution was warmed to 0 °C. After stirring at 0 °C for 15 min, a solution of ZnCl₂ (1.36 g, 10.0 mmol) in THF (20 mL) is added and stirred at 0 °C for 15 min. After the solution was cooled to -78 °C, a solution of 4-phenylbutanal (0.74 g, 5.0 mmol) in THF (10 mL) is added slowly. After the mixture is stirred at -78°C for 1 h, a phosphate buffer (pH 7.0, 0.10 M, 50 mL) was added and it is extracted with ether (4x50 mL), washed with brine, and dried over Na₂SO₄. Solvent is removed and the residue is purified by silica gel column chromatography (2 x 30 cm, hexanes: EtOAc 9:1) to give Compound 120, (R,S)-1-((25,5R)-S-IsOpTOPyI-S,6-dimethoxy-2,5-dihydropyrazin-2-yl)-4-phenylbutan-l-ol, as a mixture of compounds as colorless oils..

[00173] To a solution of the bislactim Compound 120, (0.332 g, 1.0 mmol) in CH₂Cl₂ (12 mL) at 0°C is added a solution of DIBAL-H in hexane (1 M, 4 mL, 4 mmol). The solution is allowed to warm to rt and stirred until the reduction is complete by TLC. The mixture is cooled to -20 and quenched with a 1:2 mixture of Celite and NaSO₄ decahydrate with slow warming to rt for 4 hr. Additional CH₂Cl₂ is added and the solid is filtered and washed with additional CH₂Cl₂. The combined organic layers are concentrated to dryness and taken up in a mixture of 3N HCl/CH₃CN. After stirring for 2 days the solution is extracted with ether and the aqueous layer is dried under vacuum and purified by silica gel chromatography to yield the desired product as a white solid. It is further purified by reversed-phase column chromatography under standard conditions (aqueous CH₃CN gradient; 0.1% TFA) to yield Compound 75, (2S,3S)-2-amino-2-hydroxymethyl-3-hydroxy-6-phenylhexanoic acid.

**Example 12**

**Synthesis of Compound 126**

[00174] To a solution of 7,7-difluorohept-l-ene (0.21 g, 1.3 mmol) in THF (10 mL) was added a solution of 9-BBN-H (0.50 M, 2.9 mL, 1.45 mmol) in THF at r.t. under Ar. After it was stirred at r.t. for 1 h, degassed water (0.741 mL, 41.2 mmol) was added and stirred at r.t. for 30 min. This
solution was added to a solution of vinyl iodide (0.326 g, 1.0 mmol), Pd(dppf)Cl₂«CH₂Cl₂ (40.8 mg, 0.05 mmol), Ph₃As (15.3 mg, 0.05 mmol) and Cs₂CO₃ (422.5 mg, 1.30 mmol) in DMF (15 mL) under Ar. After it was stirred at r.t. for 4 h, water (20 mL) was added and it was extracted with hexanes (5x30 mL), washed with brine, and dried over Na₂SO₄. Solvent was removed and the residue was purified by silica gel column chromatography (2 x 15 cm, hexanes: EtOAc 98.5:1.5) to give Compound 126 as a colorless oil (0.23 g, 69%). ¹H NMR (500 MHz, CDCl₃) δ 5.78 (tt, J = 57.1, 4.6, 1 H), 5.4-5.36 (m, 2 H), 3.61-3.58 (m, 2 H), 2.05-2.00 (m, 2 h), 2.00-1.95 (m, 2 H), 1.85-1.73 (m, 2 H), 1.59-1.54 (m, 2 H), 1.45-1.44 (m, 2 H), 1.34-1.29 (m, 6 H), 0.89 (s, 9 H), 0.04 (s, 6 H). ESIMS (MNa⁺) m/z 357.6.

Compound 126 is further elaborated to the aldehyde using Dess Martin oxidation and coupled with the modified bislactim as outlined above to yield Compound 69, following DIBAL reduction and hydrolysis. In a similar manner are prepared other compounds of the invention.

Example 13
Beta Cell Apoptosis Assay

Rat Pancreatic Islets.

[00176] Biological assays are performed as according to Shimabukuro et ah, J. Biol. Chem., 273:32487-90 (1998)) with certain modifications. Zucker Diabetic Fatty rats are treated for 2 weeks by i.p. injection with compounds presented herein. Pancreatic islets are isolated and the degree of apoptosis is evaluated by electrophoresis. A significant degree of protection is noted for the treated rats in comparison to the control rats. This protection demonstrates that de novo synthesis of ceramide through the SPT pathway is inhibited specifically and results in protection of beta cells from apoptosis.

Human Pancreatic Islets.

[00177] An alternative assay for the detection of beta cell apoptosis is performed according to Maedler, K. et al, (2003). Diabetes 52, 726-33). In this assay, incubation with elevated palmitic acid or elevated glucose causes increased apoptosis and protective effects of inhibitors of ceramide synthase exhibit beneficial effects. Results from this assay demonstrate the beneficial effects of the present compounds to inhibit de novo ceramide synthesis at a different, earlier point in the enzymatic pathway, such as inhibition of SPT.

Islet isolation and culture--

[00178] Islets are isolated from pancreata of organ donors, as described in Oberholzer J, et ah Transplantation 69:1115-23 (2000)). The islet purity is >95% which is determined by dithizone

59
staining. When this degree of purity is not primarily achieved by routine isolation, islets are handpicked. The donors are typically heart-beating cadaver organ donors without a previous history of diabetes or metabolic disorders.

[00179] As reported by Maedler et al. (2003), for long-term in vitro studies, the islets are cultured on extracellular matrix-coated plates derived from bovine corneal endothelial cells (Novamed, Jerusalem, Israel), and the cells are allowed to attach to the dishes and spread, to preserve their functional integrity. The contamination by ductal cells after 4 days in culture is estimated to be between 5 and 15%, but almost all ductal cells are found in the periphery of the islets and do not co-localize with β-cells. Islets are cultured in CMRL 1066 medium containing 100 units/ml penicillin, 100 μg/ml streptomycin, and 10% FCS (Gibco, Gaithersburg, MD), hereafter referred to as culture medium.

[00180] Two days after plating, when most islets are attached and begin to flatten, the medium is changed to culture medium containing 5.5 or 33.3 mmol/l glucose supplemented with or without fatty acids (Sigma Chemical, St. Louis, MO; palmitic acid [16:0], palmitoleic acid [16:1], oleic acid [18:1], or a mixture of fatty acids [16:0/16:1, 16:0/18:1]). Fatty acids are dissolved at 10 mmol/L in culture medium containing 11% fatty acid-free BSA (Sigma) under nitrogen atmosphere, are shaken overnight at 37°C, are sonicated for 15 min, and are sterile filtered (stock solution). For control experiments, BSA in the absence of fatty acids is prepared, as described above. The effective FFA concentration may be determined after sterile filtration with a commercially available kit (Wako chemicals, Neuss, Germany). The calculated concentrations of non-albumin-bound FFA is derived from the molar ratio of total FFA (0.5 mmol/l) and albumin (0.15 mmol/l) using a stepwise equilibrium model reported in Spector AA, et al., Biochemistry, 10:3226-32 (1971). Unbound concentration of palmitic, palmitoleic, and oleic acids are of 0.832, 0.575, and 2.089 micromol/L, respectively, for a final concentration of 0.5 mmol/L FFA. In some experiments, islets are cultured with or without 15 micromol/L C2-ceramide, 15micromol/L C2-Dihydroceramide (Biomol, Plymouth Meeting, PA), 15 micromol/L fumonisin B1 (Sigma), or tested compounds at various concentrations from 10nmol/L to 100micromol/L. All of them are first dissolved in prewarmed 37°C DMSO (Fluka, Buchs, Switzerland) at 5 mmol/L. For control experiments, islets are exposed to solvent alone (0.3% DMSO).

Cell apoptosis --

[00181] As reported by Maedler, et al. (2003), the free 3-OH strand breaks resulting from DNA degradation are detected by the terminal deoxynucleotidyl transferase-mediated dUTP nick-end
labeling (TUNEL) technique (Gavrieli Y, et al, J. Cell Biol. 119:493-501 (1992)). Islet cultures are washed with PBS, fixed in 4% paraformaldehyde (30 min, room temperature) followed by permeabilization with 0.5% Triton X-100 (4 min, room temperature), followed by the TUNEL assay, performed according to the manufacturer's instructions (In Situ Cell Death Detection Kit, AP; Boehringer Mannheim, Germany). The preparations are then rinsed with Tris-buffered saline and is incubated (10 min, room temperature) with 5-bromo-4-chloro-indolyl phosphate/nitro blue tetrazolium liquid substrate system (Sigma). For staining of the activated caspase 3, after fixation and permeabilization, islets are incubated for 2 h at 37°C with a rabbit anti-cleaved caspase-3 antibody (1:50 dilution, D 175; Cell Signaling, Beverly, MA), followed by incubation (30 min, 37°C) with a Cy3-conjugated donkey anti-rabbit antibody (1:100 dilution; Jackson ImmunoResearch Laboratories, West Grove, PA). Thereafter, islets are incubated with a guinea pig anti-insulin antibody as described above, followed by detection using the streptavidin-biotin-peroxidase complex (Zymed) or by a 30-min incubation with a 1:20 dilution of fluoresceinconjugated rabbit anti-guinea pig antibody (Dako). The TUNEL assay detects DNA fragmentation associated with both apoptotic and necrotic cell death; therefore, islets are also treated with a fluorescent annexin V probe (Annexin-V-FLUOS staining kit, Boehringer Mannheim) according to the manufacturer's instructions. Double staining of cells with propidium iodide and annexin V enables the differentiation of apoptotic from necrotic cells.

**Example 14**

*Anti-Inflammatory Applications*

[00182] Zucker diabetic fatty rats are sacrificed and pancreatic islets are harvested as according to Shimabukuro et al. In culture, these islets are treated with an effective amount of Tumor Necrosis Factor alpha. De novo synthesis of ceramides is evaluated by incorporation of tritiated serine, as described in Example 8. Treatment with an effective concentration of compounds presented herein results in a significantly decreased concentration of ceramide in contrast to the control group. This demonstrates the efficacy of the compounds and specific inhibition activity against SPT in general, in anti-inflammatory applications.

**Example 15**

*Serine Palmitoyltransferase activity*

Assay A.
The assay is carried out by a minor modification of the method reported by Merrill et al., Anal. Biochem., 171:373-381 (1988).

Frozen rat or other mammalian livers are homogenized in a standard HEPES buffer system containing DTT (5 mM), sucrose (0.25 M) and EDTA at pH 7.4. The homogenate is spun at 30 kg for 0.5 hr. and the supernatant is removed. The assay is performed using the supernatant (sufficient for 50-150 µg protein) above but with the addition of 50 µM pyridoxal, 200 µM palmitoyl-CoA, and 1 mM 3H-L-serine in a buffer similar to the homogenization buffer, but at pH 8.3. The radiolabeled product, 3-ketosphinganine, is extracted in CHCl₃/CH₃OH and the radioactivity is counted in a liquid scintillation counter.

Inhibition of serine palmitoyl transferase is evaluated by incorporation of tritium label into the lipid product. Further demonstration of the activity of compounds in a CTLL-2 cell line can be performed using the assay described in Nakamura, S., et al., J. Biol. Chem., 271:1255-57 (1996).

Assay B.

An alternative assay for evaluating inhibition of SPT, the enzyme present in commonly cultured cells, is performed with CHO cells or a human cell line. Cells are washed three times with ice-cold phosphate-buffered saline (PBS). A total of 0.5 mL of lysis buffer [50 mM Hepes (pH 8.0) containing 5 mM ethylenediaminetetraacetic acid (EDTA) and 5 mM dithiothreitol (DTT)] is added to each dish. The cells are scraped using a rubber policeman, and are then transferred to a test tube on ice. The cell suspension is sonicated three times for 5 s at 1-2 min intervals on ice. Protein concentrations in cell homogenates are measured using a Bradford protein assay kit (Bio-Rad). To measure the SPT activity, 0.1 mL of cell homogenates are added to 0.1 mL of reaction buffer [20 mM Hepes (pH 8.0) containing 5 mM EDTA, 10 mM DTT, 50 µM pyridoxal-5'-phosphate, 0.4 mM palmitoyl-CoA, 2 mM L-serine, 10 µCi of [3H]serine, and test compound or standard inhibitor (myriocin). After incubation at 37 °C for 20 min with shaking, the reaction is terminated with 0.5 mL of 0.5 N NH₄OH containing 10 mM L-serine. The lipid products are extracted using the solvent system: 3 mL of chloroform/methanol (1:2), 25 µg of sphingosine (1 mg/mL in ethanol) as a carrier, 2 mL of chloroform, and 3.8 mL of 0.5 N NH₄OH. After vigorous mixing, the phases are separated by centrifugation at 2500 rpm for 5 min. The aqueous layer is removed by aspiration, and the lower chloroform layer is washed 3 times with 4.5 mL of water. The chloroform layer is transferred to a scintillation vial, and the solvent is evaporated under N₂ gas. The radioactivity is measured with a LS6000TA liquid scintillation counter (Beckman). Nonspecific conversion of [3H] serine to chloroform-soluble...
species is determined by performing the assay in the absence of palmitoyl CoA. The count of the background is about one-sixth of the count of 100% activity.

Assay C.

[00187] An alternative assay using a non-chlorinated solvent modification of the Blye and Dyer lipid extraction method reported in Smedes (Smedes, F., Analyst 124:1711-18 (1999)) was employed to evaluate exemplary compounds. In this approach, the cells were washed three times with ice-cold phosphate-buffered saline and 0.5 mL of lysis buffer was added to each dish. The cells were scraped using a rubber policeman and transfer to a test tube on ice. The cell suspension was sonicated three times for 5 s at 1-2 min intervals on ice. A 0.1 mL sample of cell homogenates were added to 0.1 mL of reaction buffer in a test tube containing the appropriate concentration of test substance and 10 µCi of [3H] serine. The reaction mixture was incubated at 37 °C for 20 min with shaking, and the reaction was terminated with 0.5 mL of 0.05N NH₄OH stop solution containing 10mM unlabeled L-serine. Total lipids are extracted by transferring the contents of the test tube into a 15 ml centrifuge tube containing: 4.5 mL of isopropanol/cyclohexane (4:5) containing 25 µg of sphingosine (1 mg/mL in ethanol and diluted into the isopropanol/cyclohexane mixture) as a carrier. The contents were mixed vigorously and 4 mL of 0.5 N NH₄OH was added. The phases were separated by centrifugation at 2500 rpm for 5 min. An accurately measured portion of the organic layer (4.0ml) was added to a scintillation vial with 1mL of water. Ultima Gold F (5ml) was added, the vial was vortexed and allowed to settle into separate layers. The amount of [3H] serine radioactivity incorporated into lipids was quantified in a scintillation counter. Non-specific counts were determined by carrying out the assay with control samples containing no palmitoyl CoA. As shown in Table 2 below, the positive control, ISP-I (i.e., myriocin) exhibited potent but non-selective inhibition of SPT. Exemplary compound 12 was evaluated in this assay and, as shown in Table 2, exhibited moderate activity at the doses indicated.

[00188] Table 3 provides data for exemplary compounds 23 and 24 tested in this assay at 10nM and at 100nM.

### TABLE 2.

<table>
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<tr>
<th>Test group</th>
<th>Counts</th>
<th>Std Error</th>
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<td>5</td>
</tr>
<tr>
<td>No Inhibitor, t=0</td>
<td>244</td>
<td>7</td>
</tr>
<tr>
<td>No Inhibitor</td>
<td>4443</td>
<td>108</td>
</tr>
<tr>
<td>ISP (standard), 1 nM</td>
<td>2509</td>
<td>69</td>
</tr>
</tbody>
</table>
Example 16

Protection of Islets by an SPT Inhibitor

[00189] Islet protection by an exemplary compound was evaluated in an assay according to Eitel, K, et al (2002), and results obtained in this assay are reported below in Table 4. Rat pancreatic islets were cultured with control medium (RPMI 1640 supplemented with 10% fetal bovine serum, antibiotics and made 8% in glucose) or in medium supplemented with 1 millimolar sodium palmitate (Fatty Acid Medium) during a period of 3 days. The culture medium was changed after 2 days to an identical composition culture medium with fresh inhibitor in the appropriate wells. Cells were stained with propidium iodide (PI), washed and propidium staining of cells (as a measure of cellular DNA content) was assessed by flow cytometry. The percentage of cells having less than the normal amount of PI staining was considered to be apoptotic cells (Eitel, K, et al. (2002)).
[00190] In this assay, treatment with exemplary compound 12 appeared to fully protect cells from the fatty acid treatment in this assay and surprisingly imparts a benefit in comparison to treatment with the control medium.

**TABLE 4**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Apoptosis</th>
<th>Std Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control medium</td>
<td>2.40</td>
<td>0.56</td>
</tr>
<tr>
<td>Fatty Acid medium</td>
<td>17.60</td>
<td>5.52</td>
</tr>
<tr>
<td>FA plus Compound 12</td>
<td>2.33</td>
<td>0.40</td>
</tr>
<tr>
<td>myriocin -1</td>
<td>14.65</td>
<td>7.00</td>
</tr>
</tbody>
</table>
REFERENCES


5.


New York.


Rail LC and Roubenoff R Rheumatol 2004: 43, 1219-23.


Rother KI, Harlan DM ; J Clin Invest. 2004; 114: 877-83


Smith S.C , et al. (2001) ACC/AHA Guidelines for Percutaneous Coronary Intervention


CLAIMS:

1. A compound, and pharmaceutically acceptable salts thereof, corresponding to Formula (I):

   ![Chemical Structure](image)

   wherein:

   - $R_1$ is H or optionally substituted lower alkyl, aryl, aralkyl, or alkylxalkyl;
   - each $R_2$ is independently H, protecting group, or $-\text{C(=O)}-\text{CHR}-\text{NHR}_b$ where:
     - $R_a$ is selected from the group consisting of alkyl, aryl, acyl, keto, azido, hydroxyl, hydrazine, cyano, halo, hydrazide, alkenyl, alkynl, ether, thiol, seleno, sulfonyle, borate, boronate, phospho, phosphono, phosphine, heterocyclic, enone, imine, aldehyde, ester, thioacid, hydroxylamine, amino group, and combinations thereof; and
   - $R_b$ is H or amino protecting group;
   - each $V$ and $Z$ is independently $(\text{CR}_d)_{n'}, O, \text{NR}_e, S$, optionally substituted alkene (cis or trans), Ar, $\text{CR}_d\text{RaAr}, \text{OAr}, \text{NR}_d\text{Ar}, \text{SAr}$, or Ar where:
     - each $R_0$ and $R_d$ is independently H, X, lower alkyl, OH, 0-lower alkyl, or $R_0$ and $R_d$, taken together, is $=0$, $=\text{N-OH}$, $=\text{N-O}$-lower alkyl, or $=\text{N-O-CH}_2\text{CH}_2\text{O-CH}_3$;
     - $R_e$ is H, lower alkyl, or $\text{CH}_2\text{CH}_2\text{O-CH}_3$; and
     - n is 1 to 7;
   - q is 0to 3;
   - Ar is an optionally substituted aryl or heteroaryl;
   - u is 0or 1;
   - each X is independently H or halogen; and
   - m is 4 to 12.

2. The compound of claim 1, corresponding to Formula (II):
wherein:
L is CH₂, CHR, CRfRg, O, NRh, S, Ar, CH₂Ar, CHRAr, CRfRgAr, OAr, NRhAr, SAr, or ArAr, where
Rf is H, lower alkyl, OH, O-lower alkyl,
Rg is H, or
Rf and Rg, taken together, is =0, =N-0H, =N-O-lower alkyl, or =N-O-CH₂CH₂-O-CH₃, and
Rh is H, lower alkyl, or -CH₂CH₂-O-CH₃.

3. The compound of claim 1, corresponding to Formula (III):

4. The compound of claim 1, corresponding to Formula (IIIA):

5. The compound of claim 1, corresponding to Formula (MB):
6. The compound of claim 1, wherein u=0 and Z=optionally substituted alkene (cis or trans).
7. The compound of claim 1, wherein Ar is an optionally-substituted phenyl, pyridinyl, pyrimidyl, imidazolyl, benzimidazolyl, thiazolyl, oxazolyl, isoxazolyl, benzthiazolyl, or benzoazolyl.
8. The compound of claim 6, wherein Ar is phenyl, pyridinyl, or oxazolyl.
9. The compound of claim 1, wherein X is a halogen.
10. The compound of claim 8, wherein each X is fluorine.
11. The compound of claim 1, wherein R₁ is C₂-C₅.
12. The compound of claim 1, wherein R₁ is CH₃-O-CH₂-CH₂⁻, HO-CH₂-CH₂⁻, HO-CH₂⁻CH₂-O-CH₂-CH₂⁻, or CH₃-O-CH₂-CH₂-O-CH₂-CH₂⁻.
13. The compound of claim 1, wherein n is 2.
14. The compound of claim 1, wherein m is 7.
15. The compound of claim 1, wherein said compound modulates Serine Palmitoyl Transferase (SPT).
16. The compound of claim 14, wherein said compound inhibits Serine Palmitoyl Transferase (SPT).
17. A composition comprising the compound of claim 1 and a pharmaceutically acceptable carrier.
18. A composition comprising the compound of claim 1 and a therapeutically effective amount of at least one active agent selected from the group consisting of insulin, insulin analogs, incretin, incretin analogs, glucagon-like peptide, glucagon-like peptide analogs, exendin, exendin analogs, PACAP and VIP analogs, sulfonylureas, biguanides, α-glucosidase inhibitors, Acetyl-CoA Carboxylase inhibitors, caspase inhibitors, and PPAR ligands.
19. A method of treating insulin resistance, said method comprising administering the compound of claim 1 to a patient in need thereof.
20. A method of treating pancreatic beta cell apoptosis, said method comprising administering the compound of claim 1 to a patient in need thereof.
21. A method of treating obesity, said method comprising administering the compound of
claim 1 to a patient in need thereof.

22. A method of treating pro-thrombotic conditions, myocardial infarction, hypertension, dyslipidemia, or other manifestations of Syndrome X, said method comprising administering the compound of claim 1 to a patient in need thereof.

23. A method of treating congestive heart failure, said method comprising administering the compound of claim 1 to a patient in need thereof.

24. A method of treating an inflammatory disease, said method comprising administering the compound of claim 1 to a patient in need thereof, wherein said inflammatory disease is a disease of the cardiovascular system, atherosclerosis, or sepsis.

25. A method of preventing loss or death of human or xenobiotic islet cells in culture fluid, said method comprising adding a compound of claim 1 to the culture fluid.

26. A method for preserving liver tissue in culture fluid, said method comprising adding a compound of claim 1 to the culture fluid.

27. A method for treatment or prevention of type 1 diabetes, said method comprising administering the compound of claim 1 to a patient in need thereof.

28. A method for treatment or prevention of liver damage, said method comprising administering the compound of claim 1 to a patient in need thereof.

29. A method for treatment or prevention of cachexia, said method comprising administering the compound of claim 1 to a patient in need thereof.

30. A method for treatment or prevention of atherosclerosis, said method comprising administering the compound of claim 1 to a patient in need thereof.

31. A method for treating restenosis following percutaneous coronary intervention, comprising administering a therapeutically effective amount of at least one compound of claim 1 to a patient in need thereof.

32. A method for treating emphysema and chronic obstructive pulmonary disease, said method comprising administering a therapeutically effective amount of the compound of claim 1 to a patient in need thereof.

33. A device for percutaneous coronary intervention, comprising a controlled release formulation for administering a therapeutically effective amount of at least one compound of claim 1 to a patient in need thereof.

34. A method for treatment or prevention of emphysema, said method comprising administering the compound of claim 1 to a patient in need thereof.

35. A method for treatment or prevention of chronic obstructive pulmonary disease, said method
comprising administering the compound of claim 1 to a patient in need thereof.

36. A method according to claims 20-31, further comprising co-administering a therapeutically effective amount of at least one active agent selected from the group consisting of insulin, insulin analogs, incretin, incretin analogs, glucagon-like peptide, glucagon-like peptide analogs, exendin, exendin analogs, PACAP and VIP analogs, sulfonylureas, biguanides, α-glucosidase inhibitors, Acetyl-CoA Carboxylase inhibitors, caspase inhibitors, unsaturated fatty acids, polyunsaturated fatty acids, and PPAR ligands.

37. A method of any of claims 32-35, further comprising co-administering a therapeutically effective amount of at least one active agent selected from the group consisting of inhaled formulations containing bronchodilators, beta 2 adrenoceptor agonists, inhaled corticosteroids, anti-inflammatory steroids, leukotriene modifiers, leukotriene receptor antagonists, chemokine modifiers, chemokine receptor antagonists, cromolyn, nedocromil, xanthines, anticholinergic agents, immune modulating agents, other known anti-asthma medications, nitric oxide donors, prostacyclins, endothelin antagonists, adrenoceptor blockers, phosphodiesterases inhibitors, ion channel blockers and other vasodilators.
INTERNATIONAL SEARCH REPORT

International application No
PCT/US 07/89054

A CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07D 239/42, A61K 31/44, A61K 31/425 (2008.01)
USPC - 514/256 ; 514/374 , 514/365

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

B FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC- 514/256 , 514/374 , 514/365

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST DB-PGFB, USPTO, USOC, EPAB, Google Scholar, Patents, Other, palmitoyl transferase and restenosis and emphysema chronic obstructive pulmonary disease

C DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No</th>
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<tbody>
<tr>
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<td>US 2006/0079542 A1 (NESTOR) 13 Apr 2006 (13 04 2006), para [0019], [0020], [0025]-[0046], [0053]-[0055], [0078], [0079], [0083], [0107], [0100], [0121]-[0123], [0130], [0132], pg 22, claim 24 and 25</td>
<td>1-30, 31-35 and 37</td>
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<td>Y---------</td>
<td>KOLESNICK The Therapeutic Potential of Modulating the Ceramide/Sphingomyelin Pathway Journal of Clinical Investigation July 2002, 110(1) 3-8, pg 6</td>
<td>31 and 33</td>
</tr>
</tbody>
</table>

D

Further documents are listed in the continuation of Box C

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

Date of the actual completion of the international search
25 February 2008 (25 02 2008)

Date of mailing of the international search report
S W 200B

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Mail Stop PCT, Attn ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

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Authorized officer
Lee W Young

PCT I/O/RS 571 272-4300
PCT O/B 571 272-7774

Form PCT/ISA/2 to (second sheet) (April 2007)
**INTERNATIONAL SEARCH REPORT**

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

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

1. Claims Nos because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos 36 because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6(4(a))

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

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

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims, it is covered by claims Nos:

**Remark on Protest**

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

Form PCT/ISA/2 10 (continuation of first sheet (2)) (April 2007)