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(54) Title: OXIDIZED LIPIDS AND METHODS OF USE THEREOF

(57) Abstract: The present invention is directed to oxidized lipids and pharmaceutical compositions comprising the same. The present invention is also directed to methods of making an oxidized lipid of the invention and to methods of treating or preventing fibrosis or inflammatory diseases or disorders comprising an oxidized lipid of the invention.

OXIDIZED LIPIDS AND METHODS OF USE THEREOF

FIELD OF THE INVENTION

[0001] The present invention, in some embodiments thereof, relates to oxidized lipid compounds and pharmaceutical compositions comprising the same. The invention also relates to methods of making such compounds and compositions, and methods of treating or preventing fibrosis or inflammatory diseases or disorders with such compounds and compositions.

BACKGROUND OF THE INVENTION

[0002] Fibrosis is the formation of excess fibrous connective tissue in an organ or tissue, typically as the result of inflammation or damage. Fibrosis encompasses the pathological state of excess deposition of fibrous tissue, as well as the process of connective tissue deposition in healing. Fibrosis is similar to the process of scarring, in that both involve stimulated cells (e.g., fibroblasts) laying down connective tissue, including collagen and glycosaminoglycans.

[0003] Fibrosis can be considered as a scarring process in response to chronic diseases where excessive extracellular matrix (ECM) deposition leads to irreversible tissue damage and failure or disturbance of proper organ function. The pathophysiology of fibrosis has generally been studied in the context of the particular organ or tissue affected, including lung, kidney, liver, heart and skin. Loss of metabolic homeostasis and chronic low-grade inflammation may play a role in the pathogenesis of fibrosis. Fibrogenesis is a dynamic process and occurs in four phases: i) initiation, due to injury of the organ/tissue; ii) inflammation and activation of effector cells; iii) enhanced synthesis of ECM; and iv) deposition of ECM with progression to end-organ failure.

[0004] Fibrosis can occur in many tissues within the body. Examples include pulmonary fibrosis (lungs), idiopathic pulmonary fibrosis (lungs), cystic fibrosis (lungs), progressive massive fibrosis (lungs), liver fibrosis, cirrhosis (liver), steatohepatitis (fatty liver disease), nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), endomyocardial fibrosis (heart), myocardial infarction (heart), atrial fibrosis (heart),

mediastinal fibrosis (soft tissue of mediastinum), myelofibrosis (bone marrow), retroperitoneal fibrosis (soft tissue of the retroperitoneum), nephrogenic systemic fibrosis (skin), keloid (skin), Crohn's disease (intestine), scleroderma/systemic sclerosis (skin, lungs), arthrofibrosis (knee, shoulder, other joints), Peyronie's disease (penis), Dupuytren's contracture (hands, fingers), adhesive capsulitis (shoulder), kidney fibrosis, and focal and segmental glomerulosclerosis (kidney).

[0005] One of the major complications of insulin resistance and metabolic syndrome is nonalcoholic fatty liver disease (NAFLD), which can progress from fatty liver to liver inflammation (NASH) and liver fibrosis. It is believed that due to intestinal barrier leakage, accompanied by overgrowth and changes in the composition of gut flora, bacterial components travel through the portal vein into the liver, where they encounter toll like receptors (TLRs).

[0006] TLRs are a family of receptors imperative for the innate immune response against microbial invasion. TLRs can be divided into two major subgroups based on their cellular localization. Plasma membrane expressed TLRs include TLR1, TLR2, TLR4, TLR5, and TLR6, whereas the intracellular TLRs include TLR3, TLR7, TLR8, and TLR9. The interaction between TLRs with their cognate agonists instigates a cascade of cues which include recruitment of the adaptor molecules MyD88/TRIF and downstream phosphorylation of MAPK kinases and NF- κ B. These events culminate in the secretion of proinflammatory cytokines, including IL-12/23, IL-6 and TNF- α . TLR2 forms a heterodimer with TLR1 which recognizes bacterial triacylated lipopeptides, and a heterodimer with TLR6 which recognizes bacterial diacylated lipopeptides. TLR4 coupled to MD2 in complex with lipopolysaccharide-binding protein (LBP) and the co-receptor CD14 bind lipopolysaccharide (LPS) from gram negative bacteria.

[0007] Liver resident kupffer and hepatic stellate cells (HSC) express TLR2 which recognize triacylated lipopeptides from Gram-negative bacteria and mycoplasma and diacylated lipopeptides from Gram-negative bacteria and mycoplasma and TLR4 and its co-receptor CD14 which recognize lipopolysaccharide (LPS) from gram-negative bacteria. Both TLR2 and TLR4 can also bind to danger associated molecular patterns released from injured tissues. These TLR2 and TLR4 complexes mediate the production of pro-inflammatory cytokines and fibrogenic response by kupffer and stellate cells. Pre-clinical studies showed that nonalcoholic steatohepatitis and liver fibrosis are inhibited in TLR2 and TLR4 deficient mice, indicating its role in disease pathogenesis. In humans,

LPS plasma levels are elevated in NAFLD patients and alterations in TLR4 and CD14 genes are associated with risks of developing nonalcoholic steatohepatitis and fibrogenesis.

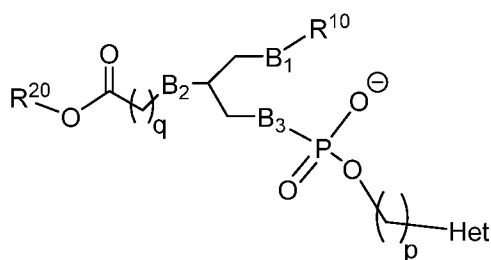
[0008] Monocytes are key players in the immune system, with critical roles in innate and adaptive immunity, immune surveillance and particle scavenging. Whereas a subset of monocytes is "resident" and recruited to tissues independently of inflammatory stimuli to assist in steady-state surveillance, wound-healing and resolution of inflammation, the absolute majority (80-90%) of human circulating monocytes is classified as "inflammatory". These monocytes can sense inflammatory stimuli and quickly migrate through the vascular or lymphatic endothelium to the periphery, where they can differentiate into macrophages and dendritic cells (DCs) which cooperate with additional cell subsets (such as Th1-cells) to promote inflammation. While playing a necessary role in host defense, monocytes were nonetheless identified as critical mediators of several inflammatory diseases, including atherosclerosis, rheumatoid arthritis (RA) and multiple sclerosis (MS). Suppressing the accumulation of unwanted monocytes/macrophages in a chronically inflamed tissue has therapeutic potential, and migration inhibitors have accordingly demonstrated promising anti-inflammatory results in animal models and clinical trials.

[0009] Renal fibrosis (kidney fibrosis) is a wound healing/scarring response following kidney injury that occurs in many forms of chronic kidney disease (CKD). Following kidney injury, resident fibroblasts are activated by various pro-inflammatory and pro-fibrotic stimuli. Activated fibroblasts, also called myofibroblasts, produce excessive ECM proteins that accumulate in the interstitium, and therefore are considered a mediator of renal fibrosis. Regardless of the primary insult leading to renal fibrosis, chronic inflammation appears to be a process heralding renal fibrogenesis. Elevated levels of inflammatory markers were associated with an increased risk of developing CKD. Induction of various pro-inflammatory cytokines interleukin (IL)-6, IL-8, IL-10, chemokine (C-C motif) ligand 2 (CCL2), tumor necrosis factor- α (TNF- α) and adhesion molecules (intercellular adhesion molecule-1 and vascular cell adhesion molecule-1) attracted the transmigration of macrophages and T cells from the circulation to the interstitium, thereby further enhancing the inflammatory state. Evidence suggests that TLRs and macrophages are associated with the pathogenesis of renal fibrosis.

[0010] Fibrosis or inflammatory diseases or disorders can cause severe morbidity and deleterious effects on patients' daily function, activity of daily living (ADL) and quality of life, and can lead to a poor prognosis. For example, idiopathic pulmonary fibrosis (IPF) is a chronic intractable disease associated with worsening and debilitating shortness of breath. IPF patients become oxygen dependent, and have an average median survival time of three years and a five year survival rate of 20% to 40% after diagnosis. Therefore, the development of new therapies for fibrosis and inflammatory diseases or disorders is needed.

SUMMARY OF THE INVENTION

[0011] In some embodiments, the present invention provides oxidized lipid compounds according to Formula 1,



Formula 1

[0012] or a stereoisomer, a stereoisomeric mixture, or a salt thereof,

[0013] wherein each of B₁, B₂, and B₃ is independently selected from the group consisting of oxygen, sulfur, nitrogen, phosphorus and silicon, wherein each of said nitrogen, phosphorus and silicon is optionally substituted by one or more substituents selected from the group consisting of alkyl, halo, cycloalkyl, aryl, hydroxy, thiohydroxy, alkoxy, aryloxy, thioaryloxy, thioalkoxy, and oxo;

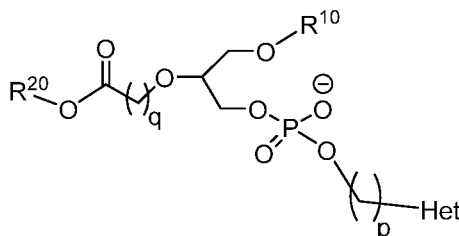
[0014] wherein R¹⁰ is a C₂₋₂₈ alkyl optionally substituted by one to five R¹¹ substituents, wherein each R¹¹ is independently selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, halo, trihalomethyl, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, phosphonate, phosphate, phosphinyl, sulfonyl, sulfinyl, sulfonamide, amide, carbonyl, thiocarbonyl, C-carboxy, O-carboxy, C-carbamate, N-carbamate, C-thiocarboxy, S-thiocarboxy, and amino;

[0015] wherein p is an integer selected from 1-10;

- [0016] wherein q is an integer selected from 1-26;
- [0017] wherein R²⁰ is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, and heteroaryl; and
- [0018] wherein Het is a heteroalicyclic ring or a heteroaryl.
- [0019] Suitable B₁, B₂, and B₃ for Formula 1 are defined herein. In some embodiments, B₁ is O. In some embodiments, B₂ is O. In some embodiments, B₃ is O. In some embodiments, at least two of B₁, B₂, and B₃ are O, e.g., B₁, B₂ are O and B₃ is O or S; B₁, B₃ are O and B₂ is O or S; or B₂, B₃ are O and B₁ is O or S. In some embodiments, B₁ is S. In some embodiments, B₂ is S. In some embodiments, B₃ is S. In some embodiments, at least two of B₁, B₂, and B₃ are S, e.g., B₁, B₂ are S and B₃ is O or S; B₁, B₃ are S and B₂ is O or S; or B₂, B₃ are S and B₁ is O or S. In some embodiments, all of B₁, B₂, and B₃ are O. In some embodiments, all of B₁, B₂, and B₃ are S.
- [0020] Suitable R¹⁰ for Formula 1 are defined herein. In some embodiments, R¹⁰ is a C₂₋₂₈ alkyl. In some embodiments, R¹⁰ is a straight chain C₂₋₂₈ alkyl, e.g., an alkyl chain having 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 carbons, substituted or unsubstituted. In some embodiments, R¹⁰ is a straight chain C₂₋₂₈ alkyl, e.g., an alkyl chain having 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 carbons, substituted by one to five R¹¹ substituents, wherein each R¹¹ is independently as defined herein, e.g., a halogen (e.g., F) or an alkyl (e.g., a C₁₋₁₀ alkyl). In some embodiments, R¹⁰ is selected from the group consisting of hexadecyl, dodecyl, octadecyl, octyl, eicosanyl, cis-9-hexadecenyl, (2'-octyl)dodecyl, and (15'-carboxy)pentadecyl. In some embodiments, R¹⁰ is hexadecyl. In some embodiments, R¹⁰ is (2'-octyl)dodecyl. In some embodiments, R¹⁰ is eicosanyl.
- [0021] Suitable R²⁰ for Formula 1 are defined herein. In some embodiments, R²⁰ is a hydrogen or an alkyl. In some embodiments, R²⁰ is hydrogen. In some embodiments, R²⁰ is an alkyl, e.g., a C₁₋₄ alkyl (e.g., methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, isobutyl, or tert-butyl). In some embodiments, R²⁰ is methyl.
- [0022] Suitable values for p and q in Formula 1 are defined herein. In some embodiments, q is an integer of 1-10, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, q is 4. In some embodiments, p is an integer of 1-7, e.g., 1, 2, 3, 4, 5, 6, or 7. In some embodiments, p is 2.
- [0023] Suitable Het for Formula 1 are defined herein. In some embodiments, Het is a heteroaryl. In some embodiments, Het is a monocyclic heteroaryl. In some

embodiments, Het is a nitrogen containing heteroaryl (e.g., monocyclic heteroaryl). In some embodiments, Het is a monocyclic heteroaryl containing 1, 2, 3, or 4 nitrogen atoms. In some embodiments, Het is a 6-member ring monocyclic heteroaryl, e.g., pyridine, pyrimidine, pyridazine, pyrazine, triazine, etc. In some embodiments, Het is a 5-member ring monocyclic heteroaryl, e.g., imidazole, thiazole, isothiazole, oxazole, isoxazole, oxidiazole, pyrazole, triazole, etc. In some embodiments, Het is a bicyclic heteroaryl containing, e.g., 1-3 nitrogen atoms, e.g., quinoline, isoquinoline, quinazoline, thienopyridine, thienopyrimidine, pyrrolopyridine, imidazopyridine, etc. In any of the embodiments described herein, Het can be a nitrogen containing heteroaryl, wherein a nitrogen atom of the heteroaryl is directly connected to the alkylene chain, i.e., $-(CH_2)_p-$ in Formula 1 to form a cation. In some embodiments, Het is pyridine, wherein the nitrogen atom of the pyridine is directly connected to the alkylene chain, i.e., $-(CH_2)_p-$ in Formula 1 to form a pyridinium salt (e.g., an internal salt or an external salt as described herein). In some embodiments, Het is an unsubstituted pyridine, wherein the nitrogen atom of the pyridine is directly connected to the alkylene chain, i.e., $-(CH_2)_p-$ in Formula 1. In some embodiments, Het is a substituted pyridine, wherein the nitrogen atom of the pyridine is directly connected to the alkylene chain, i.e., $-(CH_2)_p-$ in Formula 1, wherein the pyridine is substituted by one to five (e.g., 1, 2, 3, 4, or 5) R^{12} substituents, wherein each R^{12} is independently as defined herein, e.g., a halogen (e.g., F, Cl), a C_{6-10} aryl (e.g., phenyl), a heteroaryl, or an alkyl (e.g., a C_{1-10} alkyl, e.g., a C_{1-4} alkyl (e.g., methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, tert-butyl)). In some embodiments, Het is a substituted pyridine and the pyridine is substituted by one R^{12} substituent at the 2-, 3-, or 4-position of the pyridine, wherein R^{12} is as defined herein, for example, a halogen (e.g., F, Cl), a phenyl, or a methyl. In some embodiments, Het is 3-fluoro-pyridine or 3-phenyl-pyridine.

[0024] In some embodiments, an oxidized lipid of the invention is a compound having a structure according to Formula 2:

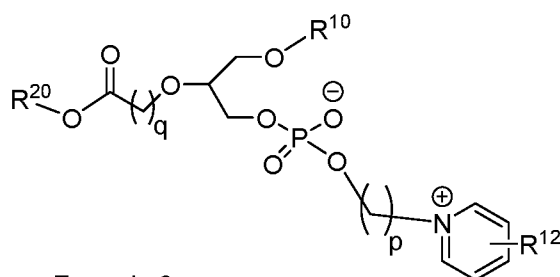


Formula 2

[0025] or a stereoisomer, a stereoisomeric mixture, or a salt thereof. Suitable R^{10} , R^{20} , p , q , and Het are those as defined herein for Formula 1.

[0026] In some embodiments according to Formula 2, R^{10} is selected from the group consisting of hexadecyl, dodecyl, octadecyl, octyl, eicosanyl, cis-9-hexadecenyl, (2'-octyl)dodecyl, and (15'-carboxy)pentadecyl; R^{20} is hydrogen or an alkyl, e.g., a C_{1-4} alkyl (e.g., methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, or tert-butyl); q is an integer of 1-10, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; p is an integer of 1-7, e.g., 1, 2, 3, 4, 5, 6, or 7; and Het is an unsubstituted pyridine, wherein the nitrogen atom of the pyridine is directly connected to the alkylene chain, i.e., $-(CH_2)_p-$ in Formula 1, or Het is a substituted pyridine, wherein the nitrogen atom of the pyridine is directly connected to the alkylene chain, i.e., $-(CH_2)_p-$ in Formula 1, wherein the pyridine is substituted by one to five (e.g., 1, 2, 3, 4, or 5) R^{12} substituents, wherein each R^{12} is independently as defined herein, e.g., a halogen (e.g., F, Cl), a C_{6-10} aryl (e.g., phenyl), a heteroaryl (e.g., monocyclic heteroaryl), or an alkyl (e.g., a C_{1-10} alkyl, e.g., a C_{1-4} alkyl (e.g., methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, tert-butyl)). In some embodiments, Het is a substituted pyridine and the pyridine is substituted by one R^{12} substituent at the 2-, 3-, or 4-position of the pyridine, wherein R^{12} is as defined herein, for example, a halogen (e.g., F, Cl), a phenyl, or a methyl. In some embodiments, Het is 3-fluoro-pyridine or 3-phenyl-pyridine.

[0027] In some embodiments, an oxidized lipid of the invention is a compound having a structure according to Formula 3:



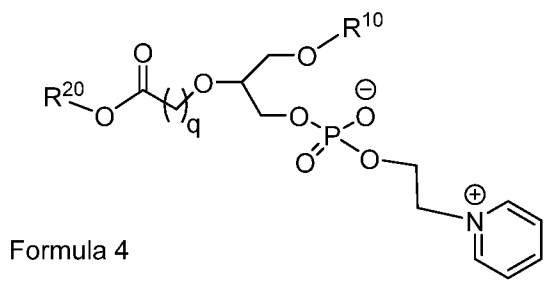
Formula 3

[0028] or a stereoisomer, a stereoisomeric mixture, or a salt thereof, wherein R^{10} , R^{11} , R^{12} , R^{20} , p , and q are as defined herein for Formula 1.

[0029] In some embodiments according to Formula 3, R^{10} is selected from the group consisting of hexadecyl, eicosanyl and (2'-octyl)dodecyl; R^{20} is hydrogen or a C_{1-4} alkyl (e.g., methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, or tert-butyl); q is an

integer of 2-6, e.g., 2, 3, 4, 5, or 6; p is an integer of 2-5, e.g., 2, 3, 4, or 5; and the pyridine is substituted by 0 to 3 (e.g., 0, 1, 2, or 3) R^{12} substituents, wherein each R^{12} is independently as defined herein, e.g., a halogen (e.g., F, Cl), a C_{6-10} aryl (e.g., phenyl), a heteroaryl, or a C_{1-4} alkyl (e.g., methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, tert-butyl). In some embodiments, R^{10} is selected from the group consisting of hexadecyl, eicosanyl and (2'-octyl)dodecyl; R^{20} is hydrogen or methyl; q is 2, 3, 4, 5, or 6; p is 2, 3, 4, or 5; and the pyridine is substituted by 0 to 3 (e.g., 0, 1, 2, or 3) R^{12} substituents, wherein each R^{12} is independently a halogen (e.g., F, Cl), a C_{6-10} aryl (e.g., phenyl), a heteroaryl, or a C_{1-4} alkyl (e.g., methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, tert-butyl). In some embodiments, R^{10} is selected from the group consisting of hexadecyl, eicosanyl and (2'-octyl)dodecyl; R^{20} is hydrogen or methyl; q is 4; p is 2; and the pyridine is substituted by 0, 1, or 2 R^{12} substituents, wherein each R^{12} is independently a halogen (e.g., F, Cl), a phenyl, or a methyl. In some embodiments, the pyridine is substituted by one R^{11} substituent at the 2-, 3-, or 4-position of the pyridine, wherein R^{11} is as defined herein, for example, a halogen (e.g., F, Cl), a phenyl, or a methyl. In some embodiments, the pyridine ring is substituted by one R^{12} substituent, wherein the one R^{12} substituent is fluorine or phenyl. In some embodiments, the pyridine ring is 3-fluoro-pyridine or 3-phenyl-pyridine.

[0030] In some embodiments, an oxidized lipid of the invention is a compound having a structure according to Formula 4:



[0031] or a stereoisomer, a stereoisomeric mixture, or a salt thereof. Suitable R^{10} , R^{20} , and q are those as defined herein for Formula 1.

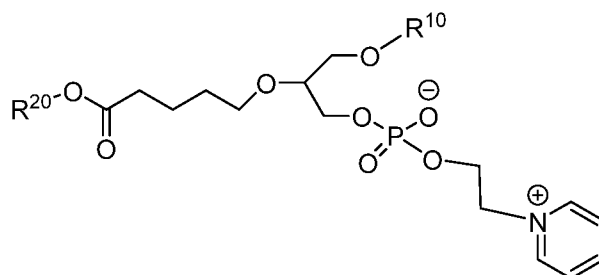
[0032] In some embodiments according to Formula 4, R^{10} is selected from the group consisting of hexadecyl, dodecyl, octadecyl, octyl, eicosanyl, cis-9-hexadecenyl, (2'-octyl)dodecyl, and (15'-carboxy)pentadecyl. In some embodiments, R^{10} is hexadecyl. In some embodiments, R^{10} is (2'-octyl)dodecyl. In some embodiments, R^{10} is eicosanyl.

[0033] In some embodiments, R^{20} is a hydrogen or an alkyl. In some embodiments, R^{20} is hydrogen. In some embodiments, R^{20} is an alkyl, e.g., a C_{1-4} alkyl (e.g., methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, or tert-butyl). In some embodiments, R^{20} is methyl.

[0034] In some embodiments, q is an integer of 1-10, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, q is 4.

[0035] In some embodiments, R^{10} is selected from the group consisting of hexadecyl, eicosanyl and (2'-octyl)dodecyl; R^{20} is hydrogen or a C_{1-4} alkyl (e.g., methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, or tert-butyl); and q is an integer of 2-6, e.g., 2, 3, 4, 5, or 6. In some embodiments, R^{10} is selected from the group consisting of hexadecyl, eicosanyl and (2'-octyl)dodecyl; R^{20} is hydrogen; and q is an integer of 2-6, e.g., 2, 3, 4, 5, or 6. In some embodiments, R^{10} is selected from the group consisting of hexadecyl, eicosanyl and (2'-octyl)dodecyl; R^{20} is methyl; and q is an integer of 2-6, e.g., 2, 3, 4, 5, or 6.

[0036] In some embodiments, an oxidized lipid of the invention is a compound having a structure according to Formula 5:



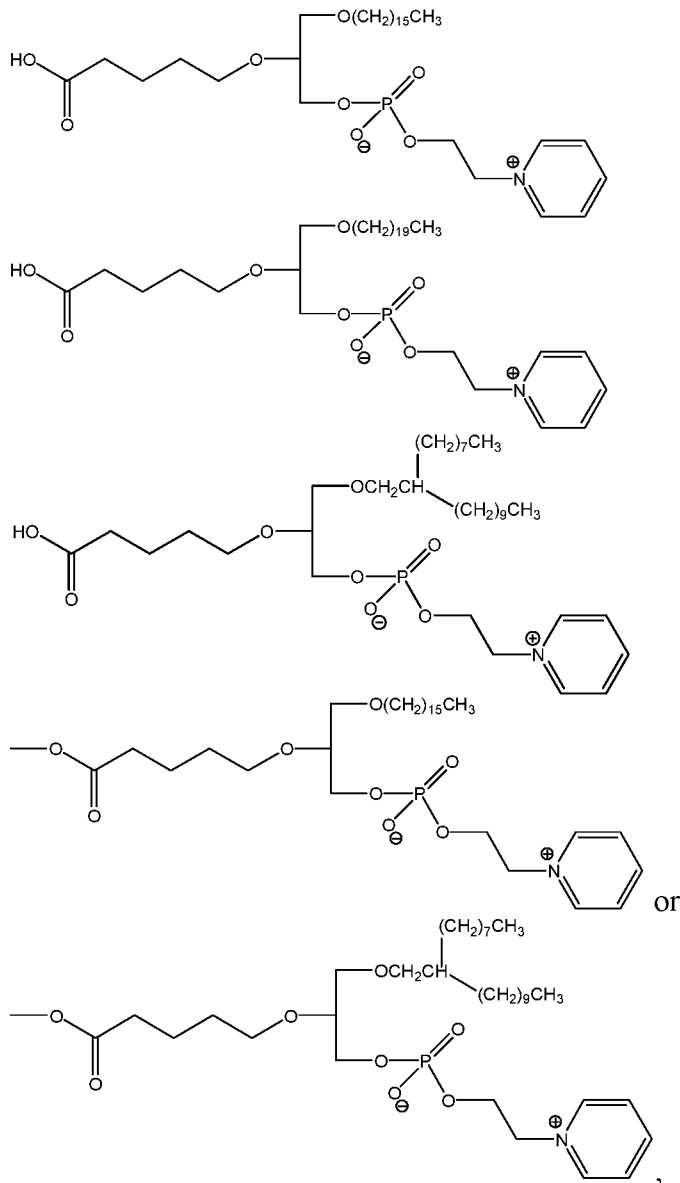
Formula 5

[0037] or a stereoisomer, a stereoisomeric mixture, or a salt thereof, wherein R^{10} and R^{20} are as defined herein for Formula 1.

[0038] In some embodiments according to Formula 5, R^{10} is selected from the group consisting of hexadecyl, dodecyl, octadecyl, octyl, eicosanyl, cis-9-hexadecenyl, (2'-octyl)dodecyl, and (15'-carboxy)pentadecyl. In some embodiments, R^{10} is hexadecyl. In some embodiments, R^{10} is (2'-octyl)dodecyl. In some embodiments, R^{10} is eicosanyl.

[0039] In some embodiments, R^{20} is a hydrogen or an alkyl. In some embodiments, R^{20} is hydrogen. In some embodiments, R^{20} is an alkyl, e.g., a C_{1-4} alkyl (e.g., methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, or tert-butyl). In some embodiments, R^{20} is methyl.

[0040] In some embodiments, an oxidized lipid of the invention is a compound having a structure of:

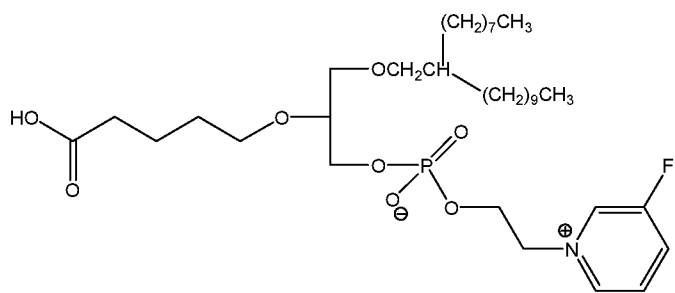


or a stereoisomer, a stereoisomeric mixture, or a salt thereof.

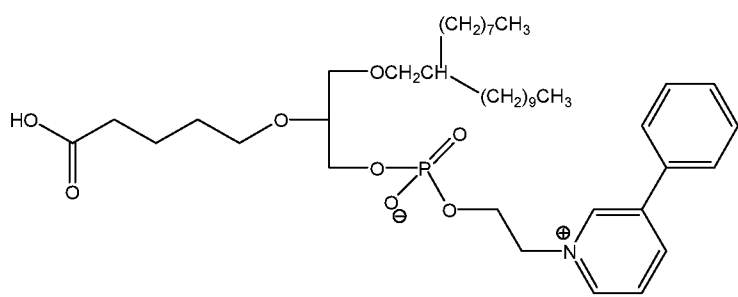
[0041] In some embodiments, the invention provides a compound selected from the group consisting of (R)-1-hexadecyl-2-(4'-carboxy)butyl-sn-glycero-3-phosphoric acid pyridiniummethyl ester (VB-701); (R)-1-eicosanyl-2-(4'-carboxy)butyl-sn-glycero-3-phosphoric acid pyridiniummethyl ester (VB-702); (R)-1-(2'-octyl)dodecyl-2-(4'-carboxy)butyl-sn-glycero-3-phosphoric acid pyridiniummethyl ester (VB-703); (R)-1-hexadecyl-2-(4'-carboxymethyl)butyl-sn-glycero-3-phosphoric acid pyridiniummethyl ester (VB-704); and (R)-1-(2'-octyl)dodecyl-2-(4'-carboxymethyl)butyl-sn-glycero-3-

phosphoric acid pyridiniummethyl ester (VB-705). The prefix "(R)-" refers to the configuration of the C-2 carbon of the glycerol backbone.

[0042] In some embodiments, the invention provides an oxidized lipid having a structure of:



or



[0043] or a stereoisomer, a stereoisomeric mixture, or a salt thereof.

[0044] In some embodiments, the invention provides a compound selected from the group consisting of (R)-1-(2'-octyl)dodecyl-2-(4'-carboxy)butyl-glycero-sn-3-phosphoric acid 3-(2-(4-fluorophenyl)ethyl)pyridiniummethyl ester (VB-706) and (R)-1-(2'-octyl)dodecyl-2-(4'-carboxy)butyl-glycero-sn-3-phosphoric acid 3-phenylpyridiniummethyl ester (VB-707). The prefix "(R)-" refers to the configuration of the C-2 carbon of the glycerol backbone.

[0045] In still other embodiments, the present invention provides pharmaceutical compositions comprising an oxidized lipid of the invention, methods of making an oxidized lipid of the invention, and methods of preventing or treating fibrosis or an inflammatory disease or disorder with an oxidized lipid or composition of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0046] Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention.

- [0047] FIG. 1A shows the structures of the VB-701, VB-702, VB-703, VB-704 and VB-705 oxidized lipid compounds. FIG. 1B shows the structures of the VB-706 and VB-707 oxidized lipid compounds.
- [0048] FIG. 2 shows VB-701 inhibits lipopolysaccharide (LPS) (TLR4)-induced signaling in human monocytes (primary CD14+).
- [0049] FIG. 3 shows VB-701 inhibits PGN (TLR2)-induced signaling in human monocytes (THP-1 cell line).
- [0050] FIG. 4 shows VB-701 inhibits MCP-1-induced signaling in human monocytes (THP-1 cell line).
- [0051] FIG. 5 shows VB-701 inhibits chemokine-induced migration of human monocytes (primary CD14+).
- [0052] FIG. 6 shows VB-702 inhibits LPS (TLR4)-induced signaling in human monocytes (primary CD14+).
- [0053] FIG. 7 shows VB-702 inhibits RANTES-induced signaling in human monocytes (primary CD14+).
- [0054] FIG. 8 shows VB-702 inhibits chemokine-induced migration of human monocytes (primary CD14+).
- [0055] FIGs. 9A-9B show VB-701 and VB-702 inhibit IL-12p40 levels in human monocytes (primary CD14+) that are LPS (TLR4)-stimulated (FIG. 9A) and Pam3CSK4 (TLR2)-stimulated (FIG. 9B).
- [0056] FIGs. 10A-10B show the effect of VB-703 and VB-201 on LPS-induced signaling in human monocytes (primary CD14+) and LPS binding inhibition assay. Human primary monocytes were pretreated with VB-201 or VB-703 at the indicated concentrations followed by activation with LPS. Samples were analyzed by western blotting for inhibition of phosphorylation (FIG. 10A). Heat shock protein HSP90 was used for loading control. FIG. 10B shows results from samples incubated with VB-201 or VB-703 at the indicated concentrations for 20 minutes before biotin-LPS (100 ng/ml) was added for an additional 15 minutes. Results are the mean fluorescence intensity (MFI) of triplicates.
- [0057] FIGs. 11A-11B show the effect of VB-703 on liver fibrosis. NASH was induced by injection of mice with 200 µg streptozotocin (STZ) two days after birth and by feeding a high fat diet (HFD) from 4 weeks of age. Mice were then either treated with vehicle (negative control), VB-703 (4 mg/kg), or telmisartan (10 mg/kg; positive control) at six

weeks of age for three weeks. Normal mice (not NASH-induced) were also used as a control. Mice were sacrificed at nine weeks of age. Staining of liver histological samples with Sirius red was used to determine the extent of fibrosis. FIG. 11A shows the mean fibrosis area following treatment (% from analyzed liver section; Mean \pm S.E; Normal - n=5, Vehicle - n=8, Telmisartan - n=6). FIG. 11B shows representative Sirius red staining of liver samples following treatment (200X magnification).

[0058] FIGs. 12A-12B show the effect of VB-703 on liver inflammation. Mice were treated and evaluated as explained in the examples. FIG. 12A shows the mean liver inflammation score following treatment (Mean \pm S.E; Normal - n=5, Vehicle- n=8, VB-703- n=8, Telmisartan - n=6). FIG. 12B shows representative H&E staining of liver samples following treatment (200X magnification).

[0059] FIG. 13 shows VB-703 inhibits PGN (TLR2)-induced signaling in human monocytes (THP-1 cell line).

[0060] FIG. 14 shows VB-703 inhibits IL-6 secretion in LPS (TLR4)-induced signaling in monocyte derived dendritic cells.

[0061] FIG. 15 shows VB-703 inhibits IL-12p40 secretion in LPS (TLR4)-induced signaling in monocyte derived dendritic cells.

[0062] FIG. 16 shows VB-704 does not inhibit LPS-biotin binding to human monocytes (primary CD14+).

[0063] FIG. 17 shows VB-704 inhibits chemokine-induced cell migration of human monocytes (primary CD14+).

[0064] FIG. 18 shows VB-705 and VB-201 inhibit TLR4-induced signaling in human monocytes (primary CD14+).

[0065] FIG. 19 shows VB-703 and VB-705 inhibit LPS binding in human monocytes (primary CD14+).

[0066] FIG. 20 shows VB-705 does not inhibit SDF1-induced cell migration in human monocytes (THP-1 cell line).

[0067] FIG. 21A-21C present bar graphs showing the expression levels of two pro-inflammatory cytokines, IL-12/23p40 (FIG. 21A) and IL-1 β (FIG. 21C) , and the chemokine, MCP-1 (FIG. 21B), in livers taken from NASH-induced mice. These data show that the expression of IL-12/23p40, MCP-1, and IL-1 β were significantly inhibited in livers taken from NASH-induced mice that were treated with VB-703.

[0068] FIG. 22 presents bar graphs showing the effect of VB-703 on albumin/creatinine (mg/mg/day). Albumin/Creatinine/Day in healthy rats (n=3) (white bar), sham operated rats (n=3) (white bar with stripes), nephrectomized rats treated with solvent control (0.5% ethanol/PBS) (black bar) (n=7), nephrectomized rats VB-703 4 mg/kg treated (n=7) (light gray bar) or nephrectomized rats telmisartan 10 mg/kg treated (n=8) (dark gray bar) were evaluated at 8 weeks. Statistical data vs. nephrectomized rats treated with solvent control (0.5% ethanol/PBS) is presented as follows: * represents $p=0.002$; and ** represents $p\leq 0.001$. Abbreviations are: Nx, nephrectomized; Eth, ethanol.

[0069] FIG. 23 presents bar graphs showing the effect of VB-703 in reducing the number of damaged glomeruli (%). Damaged glomeruli (%) in healthy rats (n=3) (white bar), sham operated rats (n=3) (white bar with stripes), nephrectomized rats treated with solvent control (0.5% ethanol/PBS) (black bar) (n=7), nephrectomized rats VB-703 4 mg/kg treated (n=7) (light gray bar) or nephrectomized rats telmisartan 10 mg/kg treated (n=8) (dark gray bar) were evaluated at 8 weeks. Statistical data vs. nephrectomized rats treated with solvent control (0.5% ethanol/PBS) is presented as follows: * represents $p\leq 0.005$; and ** represents $p\leq 0.001$. Abbreviations are: Nx, nephrectomized; Eth, ethanol.

[0070] FIG. 24 presents bar graphs showing the effect of VB-703 in reducing glomerular sclerosis (%). Glomerular sclerosis (%) in healthy rats (n=3) (white bar), sham operated rats (n=3) (white bar with stripes), nephrectomized rats treated with solvent control (0.5% ethanol/PBS) (black bar) (n=7), nephrectomized rats VB-703 4 mg/kg treated (n=7) (light gray bar) or nephrectomized rats telmisartan 10 mg/kg treated (n=8) (dark gray bar) were evaluated at 8 weeks. Statistical data vs. nephrectomized rats treated with solvent control (0.5% ethanol/PBS) is presented as follows: * represents $p\leq 0.005$; and ** represents $p\leq 0.001$. Abbreviations are: Nx, nephrectomized; Eth, ethanol.

[0071] FIG. 25 presents PAS staining images (x400) showing the effect of VB-703 in reducing glomerular sclerosis. Renal morphology was assessed by light microscope in PAS stained sections of healthy rats (Healthy x400), sham operated rats (Sham x400), nephrectomized rats treated with solvent control (0.5% ethanol/PBS) (Nx PBS 0.5% Eth x400), nephrectomized rats VB-703 4mg/kg treated (Nx VB-703 4mg/kg x400) or nephrectomized rats telmisartan 10 mg/kg treated (Nx Telmisartan 10 mg/kg x400) at 8 weeks following the first surgery. Abbreviations are: Nx, nephrectomized; Eth, ethanol, PAS, Periodic Acid-Schiff.

[0072] FIGs. 26A-26C present bar graphs showing the effect of VB-703 on pro-fibrotic markers. Collagen IV (FIG. 26A), fibronectin (FIG. 26B) and TGF- β (FIG. 26C) related expression in the kidney were evaluated in healthy rats (white bar), sham operated rats (white bar with stripes), nephrectomized rats treated with solvent control (0.5% ethanol/PBS) (black bar), nephrectomized rats VB-703 4 mg/kg treated (light gray bar), or nephrectomized rats telmisartan 10 mg/kg treated (dark gray bar) at 8 weeks. Abbreviations are: Nx, nephrectomized; Eth, ethanol. The p - values in FIGs. 26A-26C represent statistically-significant differences from nephrectomized rats treated with PBS.

[0073] FIGs. 27A-27B show the output of an experiment evaluating the effect of VB-703 on monocyte/macrophage cell infiltration in the glomeruli (FIG. 27A) or in the interstitium (FIG. 27B). CD68 positive cells in the glomeruli (cells/glomeruli) and in the interstitium (cells/mm²) were evaluated in healthy rats (n=3) (white bar), sham operated rats (n=3) (white bar with stripes), nephrectomized rats treated with solvent control (0.5% ethanol/PBS) (black bar) (n=7), nephrectomized rats VB-703 4 mg/kg treated (n=7) (light gray bar), or nephrectomized rats telmisartan 10 mg/kg treated (n=8) (dark gray bar) at 8 weeks. Abbreviations are: Nx, nephrectomized; Eth, ethanol.

DETAILED DESCRIPTION OF THE INVENTION

[0074] Before explaining embodiments of the invention in detail, it is to be understood that the invention is not limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

General Definitions

[0075] The terms "comprises", "comprising", "includes", "including", "having", and their conjugates mean "including but not limited to."

[0076] The word "optionally" is used herein to mean "is provided in some embodiments and not provided in other embodiments." Any particular embodiment of the invention can include a plurality of "optional" features unless such features conflict.

- [0077] As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.
- [0078] As used herein, the term "about" modifying an amount related to the invention refers to variation in the numerical quantity that can occur, for example, through routine testing and handling; through inadvertent error in such testing and handling; through differences in the manufacture, source, or purity of ingredients employed in the invention; and the like. Whether or not modified by the term "about", the claims include equivalents of the recited quantities. In one embodiment, the term "about" means within 10% of the reported numerical value.
- [0079] The term "therapeutically effective amount," as used herein, refers to that amount of a given therapeutic agent sufficient to result in amelioration of one or more symptoms of a disorder or condition, or prevent appearance or advancement of a disorder or condition, or cause regression of or cure from the disorder or condition. In some embodiments, a therapeutically effective amount of the compound described herein is about 5 mg to about 160 mg of the compound per day.
- [0080] As used herein throughout, the term "alkyl" refers to a saturated aliphatic hydrocarbon including straight chain and branched chain groups. In some embodiments, the alkyl group has 1 to 20 carbon atoms. Whenever a numerical range; *e.g.*, "1-20", is stated herein, it implies that the group, in this case the alkyl group, may contain 1 carbon atom, 2 carbon atoms, 3 carbon atoms, etc., up to and including 20 carbon atoms. In some embodiments, the alkyl is a medium size alkyl having 1 to 10 carbon atoms. In some embodiments, the alkyl is a lower alkyl having 1 to 4 carbon atoms. The alkyl group can be substituted (*e.g.*, with 1 to 5 substituent groups) or unsubstituted. In any of the embodiments described herein, the alkyl can be unsubstituted. In any of the embodiments described herein, the alkyl can also be substituted by one to five substituent groups, wherein the substituent group can be, for example, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azide, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, oxo, carbonyl, thiocarbonyl, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, and amino, as these terms are defined herein.

[0081] A "cycloalkyl" group refers to an all-carbon monocyclic or fused ring (*i.e.*, rings which share an adjacent pair of carbon atoms) group wherein one of more of the rings does not have a completely conjugated pi-electron system. Examples, without limitation, of cycloalkyl groups are cyclopropane, cyclobutane, cyclopentane, cyclopentene, cyclohexane, cyclohexadiene, cycloheptane, cycloheptatriene, and adamantane. A cycloalkyl group can be substituted (e.g., with 1 to 5 substituent groups) or unsubstituted. In any of the embodiments described herein, the cycloalkyl can be unsubstituted. In any of the embodiments described herein, the cycloalkyl can also be substituted by one to five substituent groups, wherein the substituent group can be, for example, alkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azide, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, oxo, carbonyl, thiocarbonyl, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, and amino, as these terms are defined herein.

[0082] An "alkenyl" group refers to an aliphatic hydrocarbon group which contains at least two carbon atoms and at least one carbon-carbon double bond, which can be straight or branched. An alkenyl group can be substituted or unsubstituted.

[0083] An "alkynyl" group refers to an aliphatic hydrocarbon group which contains at least two carbon atoms and at least one carbon-carbon triple bond. An alkynyl group can be substituted or unsubstituted.

[0084] An "aryl" group refers to an all-carbon monocyclic or fused-ring polycyclic (*i.e.*, rings which share adjacent pairs of carbon atoms) groups having a completely conjugated pi-electron system. In any of the embodiments described herein, aryl groups can have 6 to 14 carbons, e.g., 6 to 10 carbons. Examples, without limitation, of aryl groups are phenyl, naphthalenyl and anthracenyl. The aryl group can be substituted (e.g., with 1 to 5 substituent groups) or unsubstituted. When substituted, the substituent group can be, for example, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azide, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, oxo, carbonyl, thiocarbonyl, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, and amino, as these terms are defined herein. In any of the embodiments described herein, the aryl group can be a phenyl group, optionally substituted, for example, by one to five substituent such as

halogens (e.g., fluorine or chlorine), alkyl groups (e.g., a C₁₋₄ alkyl), or halogen substituted alkyls (e.g., trifluoromethyl).

[0085] A "heteroaryl" group refers to a monocyclic or fused ring (*i.e.*, rings which share an adjacent pair of atoms) group having in the ring(s) one or more atoms, such as, for example, nitrogen, oxygen and sulfur and, in addition, having a completely conjugated pi-electron system. In any of the embodiments described herein, heteroaryl groups can have 5 to 14 ring atoms, e.g., 5 to 10 ring atoms (e.g., 5 or 6 ring atoms). Examples, without limitation, of heteroaryl groups include pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrimidine, quinoline, isoquinoline and purine. The heteroaryl group can be substituted (e.g., with 1 to 5 substituent groups) or unsubstituted. When substituted, the substituent group can be, for example, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azide, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, oxo, carbonyl, thiocarbonyl, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, and amino, as these terms are defined herein.

[0086] A "heteroalicyclic" group refers to a monocyclic or fused ring group having in the ring(s) one or more heteroatoms such as nitrogen, oxygen and sulfur. The rings may also have one or more double bonds. However, the rings do not have a completely conjugated pi-electron system. In any of the embodiments described herein, heteroalicyclic groups can have 3 to 10 ring atoms, e.g., 5 to 10 ring atoms (e.g., 5 or 6 ring atoms). The heteroalicyclic can be substituted (e.g., with 1 to 5 substituent groups) or unsubstituted. When substituted, the substituted group can be, for example, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azide, sulfonyl, sulfinyl, sulfonamide, phosphonyl, phosphinyl, oxo, carbonyl, thiocarbonyl, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, and amino, as these terms are defined herein. Representative examples are piperidine, piperazine, tetrahydrofuran, tetrahydropyran, morpholine and the like.

[0087] An "alkoxy" group refers to both an -O-alkyl and an -O-cycloalkyl group, wherein the alkyl or cycloalkyl can be any of those as defined herein.

- [0088] An "aryloxy" group refers to both an -O-aryl and an -O-heteroaryl group, wherein the aryl or heteroaryl can be any of those as defined herein.
- [0089] A "thiohydroxy" group refers to a -SH group.
- [0090] A "thioalkoxy" group refers to both an -S-alkyl group, and an -S-cycloalkyl group, wherein the alkyl or cycloalkyl can be any of those as defined herein.
- [0091] A "thioaryloxy" group refers to both an -S-aryl and an -S-heteroaryl group, wherein the aryl or heteroaryl can be any of those as defined herein.
- [0092] A "carbonyl" group refers to a -C(=O)-R group, wherein R is hydrogen, alkyl, alkenyl, cycloalkyl, aryl, heteroaryl (bonded through a ring carbon) or heteroalicyclic (bonded through a ring carbon) as defined herein.
- [0093] An "aldehyde" group refers to a carbonyl group, wherein R is hydrogen.
- [0094] A "thiocarbonyl" group refers to a -C(=S)-R group, wherein R is as defined herein.
- [0095] A "C-carboxy" group refers to a -C(=O)-O-R groups, wherein R is as defined herein.
- [0096] An "O-carboxy" group refers to an RC(=O)-O- group, wherein R is as defined herein.
- [0097] An "oxo" group refers to a =O group.
- [0098] A "carboxylic acid" group refers to a C-carboxyl group in which R is hydrogen.
- [0100] A "halo" group or "halogen" refers to fluorine, chlorine, bromine or iodine.
- [0101] A "trihalomethyl" group refers to a -CX₃ group wherein X is a halo group as defined herein, e.g., a CF₃ group.
- [0102] A "sulfinyl" group refers to an -S(=O)-R group, wherein R is as defined herein.
- [0103] A "sulfonyl" group refers to an -S(=O)₂-R group, wherein R is as defined herein.
- [0104] An "S-sulfonamido" group refers to a -S(=O)₂-NR₂ group, with each of R as is defined herein.
- [0105] An "N-sulfonamido" group refers to an RS(=O)₂-NR group, wherein each of R is as defined herein.
- [0106] An "O-carbamyl" group refers to an -OC(=O)-NR₂ group, wherein each of R is as defined herein.
- [0107] An "N-carbamyl" group refers to an ROC(=O)-NR- group, wherein each of R is as defined herein.

- [0108] An "O-thiocarbamyl" group refers to an $-OC(=S)-NR_2$ group, wherein each of R is as defined herein.
- [0109] An "N-thiocarbamyl" group refers to an $ROC(=S)NR-$ group, wherein each of R is as defined herein.
- [0110] An "amino" group refers to an $-NR_2$ group wherein each of R is as defined herein.
- [0111] A "C-amido" group refers to a $-C(=O)-NR_2$ group, wherein each of R is as defined herein.
- [0112] An "N-amido" group refers to an $RC(=O)-NR-$ group, wherein each of R is as defined herein.
- [0113] An "urea" group refers to an $-NRC(=O)-NR_2$ group, wherein each of R is as defined herein.
- [0114] A "guanidino" group refers to an $-RNC(=N)-NR_2$ group, wherein each of R is as defined herein.
- [0115] A "guanyl" group refers to an $R_2NC(=N)-$ group, wherein each of R is as defined herein.
- [0116] The term "phosphonyl" or "phosphonate" describes a $-P(=O)(OR)_2$ group, with R as defined herein.
- [0117] The term "phosphate" describes an $-O-P(=O)(OR)_2$ group, with each of R as defined herein.
- [0118] A "phosphoric acid" is a phosphate group wherein each of R is hydrogen.
- [0119] The term "phosphinyl" describes a $-PR_2$ group, with each of R as defined herein.
- [0120] The term "thiourea" describes a $-NR-C(=S)-NR-$ group, with each of R as defined herein.
- [0121] The term "saccharide" refers to one or more sugar units, either an open-chain sugar unit or a cyclic sugar unit (e.g., pyranose- or furanose-based units), and encompasses any monosaccharide, disaccharide and oligosaccharide, unless otherwise indicated.
- [0122] The term "stereoisomer" includes geometric isomers, such as E or Z isomers, enantiomers, diastereomers, and the like.
- [0123] The term "stereoisomeric mixture" includes any mixture in any ratio of stereoisomers defined herein. In some embodiments, a stereoisomeric mixture includes a racemic mixture. In some embodiments, a stereoisomeric mixture includes an

enantiomerically enriched mixture. In some embodiments, a stereoisomeric mixture includes a mixture of diastereomers in any ratio.

[0124] The term "enantiomeric excess" or "ee" refers to a measure for how much of one enantiomer is present compared to the other. For a mixture of R and S enantiomers, the percent enantiomeric excess is defined as $|R - S| * 100$, where R and S are the respective mole or weight fractions of enantiomers in a mixture such that $R + S = 1$. With knowledge of the optical rotation of a chiral substance, the percent enantiomeric excess is defined as $([\alpha]_{\text{obs}}/[\alpha]_{\text{max}})*100$, where $[\alpha]_{\text{obs}}$ is the optical rotation of the mixture of enantiomers and $[\alpha]_{\text{max}}$ is the optical rotation of the pure enantiomer.

[0125] The term "salt" includes both internal salt or external salt. In some embodiments, the salt is an internal salt, i.e., a zwitterion structure. In some embodiments, the salt is an external salt. In some embodiments, the external salt is a pharmaceutically acceptable salt having a suitable counter ion. Suitable counterions for pharmaceutical use are known in the art.

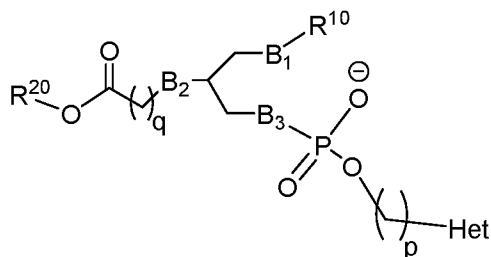
[0126] Throughout this application, various embodiments of this invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range, such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

[0127] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Oxidized Lipids

[0128] The present invention is directed, in part, to oxidized lipid compounds.

[0129] In some embodiments, an oxidized lipid of the invention is a compound according to Formula 1:



Formula 1

[0130] wherein each of B_1 , B_2 , and B_3 is independently selected from the group consisting of oxygen, sulfur, nitrogen, phosphorus and silicon, wherein each of said nitrogen, phosphorus and silicon is optionally substituted by one or more substituents selected from the group consisting of alkyl, halo, cycloalkyl, aryl, hydroxy, thiohydroxy, alkoxy, aryloxy, thioaryloxy, thioalkoxy and oxo;

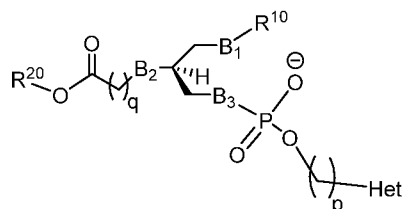
[0131] wherein R^{10} is a C_{2-28} alkyl optionally substituted by one to five R^{11} substituents, wherein each R^{11} is independently selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, halo, trihalomethyl, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, phosphonate, phosphate, phosphinyl, sulfonyl, sulfinyl, sulfonamide, amide, carbonyl, thiocarbonyl, C-carboxy, O-carboxy, C-carbamate, N-carbamate, C-thiocarboxy, S-thiocarboxy, and amino;

[0132] wherein p is an integer selected from 1-10;

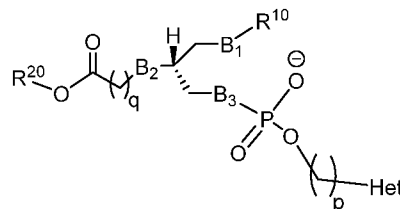
[0133] wherein q is an integer selected from 1-26;

[0134] wherein R^{20} is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, and heteroaryl; and

[0135] wherein Het is a heteroalicyclic ring or a heteroaryl. In other embodiments, the oxidized lipid is a compound according to Formula 1, or a stereoisomer, a stereoisomeric mixture, or a salt thereof. In some embodiments, the oxidized lipid having a structure according to Formula 1 has a zwitterionic structure. In some embodiments, the oxidized lipid is an external salt (e.g., a pharmaceutically acceptable salt) of the compound having a structure according to Formula 1. In some embodiments, the compound according to Formula 1 has a structure according to Formula 1a or Formula 1b:



Formula 1a



Formula 1b

[0136]

thereof.

[0137]

In some embodiments, the compound has a structure according to Formula 1a, wherein the compound has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more. In some embodiments, the compound has a structure according to Formula 1b, wherein the compound has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more. In other embodiments, the compound has an enantiomeric purity of from about 80% ee to about 100% ee, about 85% ee to about 100% ee, about 90% ee to about 100% ee, about 95% ee to about 100%, about 80% ee to about 99.5% ee, about 85% ee to about 99.5% ee, about 90% ee to about 99.5% ee, about 95% ee to about 99.5%, or any range thereof.

[0138]

Suitable B₁, B₂, and B₃ for Formula 1 are defined herein. In some embodiments, B₁ is O. In some embodiments, B₂ is O. In some embodiments, B₃ is O. In some embodiments, at least two of B₁, B₂, and B₃ are O, e.g., B₁, B₂ are O and B₃ is O or S; B₁, B₃ are O and B₂ is O or S; or B₂, B₃ are O and B₁ is O or S. In some embodiments, B₁ is S. In some embodiments, B₂ is S. In some embodiments, B₃ is S. In some embodiments, at least two of B₁, B₂, and B₃ are S, e.g., B₁, B₂ are S and B₃ is O or S; B₁, B₃ are S and B₂ is O or S; or B₂, B₃ are S and B₁ is O or S. In some embodiments, all of B₁, B₂, and B₃ are O. In some embodiments, all of B₁, B₂, and B₃ are S.

[0139]

Suitable R¹⁰ for Formula 1 are defined herein. In some embodiments, R¹⁰ is a C₂₋₂₈ alkyl. In some embodiments, R¹⁰ is a straight chain C₂₋₂₈ alkyl, e.g., an alkyl chain having 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 carbons, substituted or unsubstituted. In some embodiments, R¹⁰ is a straight chain C₂₋₂₈ alkyl, e.g., an alkyl chain having 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 carbons, substituted by one to five R¹¹ substituents,

wherein each R¹¹ is independently as defined herein, e.g., a halogen (e.g., F) or an alkyl (e.g., a C₁₋₁₀ alkyl). In some embodiments, R¹⁰ is selected from the group consisting of hexadecyl, dodecyl, octadecyl, octyl, eicosanyl, cis-9-hexadecenyl, (2'-octyl)dodecyl, and (15'-carboxy)pentadecyl. In some embodiments, R¹⁰ is hexadecyl. In some embodiments, R¹⁰ is (2'-octyl)dodecyl. In some embodiments, R¹⁰ is eicosanyl.

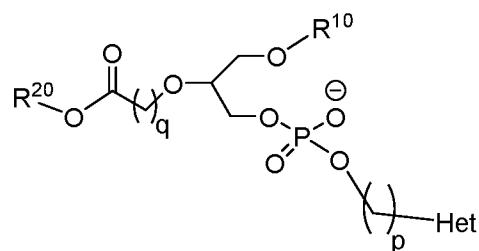
[0140] Suitable R²⁰ for Formula 1 are defined herein. In some embodiments, R²⁰ is a hydrogen or an alkyl. In some embodiments, R²⁰ is hydrogen. In some embodiments, R²⁰ is an alkyl, e.g., a C₁₋₄ alkyl (e.g., methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, or tert-butyl). In some embodiments, R²⁰ is methyl.

[0141] Suitable values for p and q in Formula 1 are defined herein. In some embodiments, q is an integer of 1-10, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, q is 4. In some embodiments, p is an integer of 1-7, e.g., 1, 2, 3, 4, 5, 6, or 7. In some embodiments, p is 2.

[0142] Suitable Het for Formula 1 are defined herein. In some embodiments, Het is a heteroaryl. In some embodiments, Het is a monocyclic heteroaryl. In some embodiments, Het is a nitrogen containing heteroaryl (e.g., monocyclic heteroaryl). In some embodiments, Het is a monocyclic heteroaryl containing 1, 2, 3, or 4 nitrogen atoms. In some embodiments, Het is a 6-member ring monocyclic heteroaryl, e.g., pyridine, pyrimidine, pyridazine, pyrazine, triazine, etc. In some embodiments, Het is a 5-member ring monocyclic heteroaryl, e.g., imidazole, thiazole, isothiazole, oxazole, isoxazole, oxidiazole, pyrazole, triazole, etc. In some embodiments, Het is a bicyclic heteroaryl containing nitrogen atoms, e.g., 1-3 nitrogen atoms, e.g., quinoline, isoquinoline, quinazoline, thienopyridine, thienopyrimidine, pyrrolopyridine, imidazopyridine, etc. In any of the embodiments described herein, Het can be a nitrogen containing heteroaryl, wherein a nitrogen atom of the heteroaryl is directly connected to the alkylene chain, i.e., -(CH₂)_p- in Formula 1 to form a cation. In some embodiments, Het is pyridine, wherein the nitrogen atom of the pyridine is directly connected to the alkylene chain, i.e., -(CH₂)_p- in Formula 1 form a pyridinium salt (e.g., an internal salt or an external salt as described herein). In some embodiments, Het is an unsubstituted pyridine, wherein the nitrogen atom of the pyridine is directly connected to the alkylene chain, i.e., -(CH₂)_p- in Formula 1. In some embodiments, Het is a substituted pyridine, wherein the nitrogen atom of the pyridine is directly connected to the alkylene chain, i.e., -(CH₂)_p- in Formula 1, wherein the pyridine is substituted by one to five (e.g., 1, 2, 3, 4,

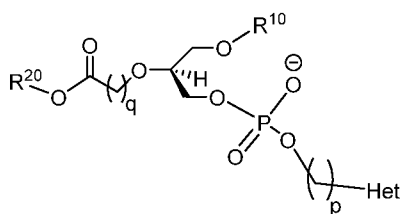
or 5) R^{12} substituents, wherein each R^{12} is independently selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, halo, trihalomethyl, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, phosphonate, phosphate, phosphinyl, sulfonyl, sulfinyl, sulfonamide, amide, carbonyl, thiocarbonyl, C-carboxy, O-carboxy, C-carbamate, N-carbamate, C-thiocarboxy, S-thiocarboxy, and amino as defined herein. In some embodiments, each R^{12} is independently, e.g., a halogen (e.g., F, Cl), a C_{6-10} aryl (e.g., phenyl), a heteroaryl, or an alkyl (e.g., a C_{1-10} alkyl, e.g., a C_{1-4} alkyl (e.g., methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, tert-butyl)). In some embodiments, Het is a substituted pyridine and the pyridine is substituted by one R^{12} substituent at the 2-, 3-, or 4-position of the pyridine, wherein R^{12} is as defined herein, for example, a halogen (e.g., F, Cl), a phenyl, or a methyl.

[0143] In some embodiments, an oxidized lipid of the invention is a compound having a structure according to Formula 2:

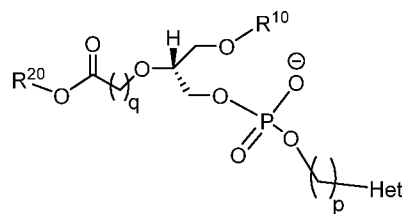


Formula 2

[0144] or a stereoisomer, a stereoisomeric mixture, or a salt thereof. Suitable R^{10} , R^{20} , p , q , and Het are those as defined herein for Formula 1. In some embodiments, the oxidized lipid having a structure according to Formula 2 has a zwitterionic structure. In some embodiments, the oxidized lipid is an external salt (e.g., a pharmaceutically acceptable salt) of the compound having a structure according to Formula 2. In some embodiments, the compound according to Formula 2 has a structure according to Formula 2a or Formula 2b:



Formula 2a



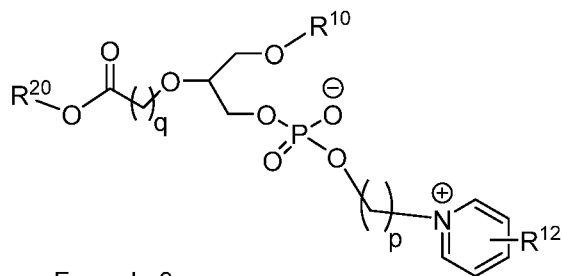
Formula 2b

[0145] , or a salt thereof.

[0146] In some embodiments, the compound is a S-isomer having a structure according to Formula 2a, wherein the compound has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more. In some embodiments, the compound is a R-isomer having a structure according to Formula 2b, wherein the compound has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more. In other embodiments, the compound has an enantiomeric purity of from about 80% ee to about 100% ee, about 85% ee to about 100% ee, about 90% ee to about 100% ee, about 95% ee to about 100%, about 80% ee to about 99.5% ee, about 85% ee to about 99.5% ee, about 90% ee to about 99.5% ee, about 95% ee to about 99.5%, or any range thereof.

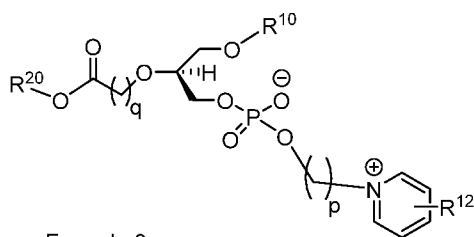
[0147] In some embodiments according to Formula 2, R¹⁰ is selected from the group consisting of hexadecyl, dodecyl, octadecyl, octyl, eicosanyl, cis-9-hexadecenyl, (2'-octyl)dodecyl, and (15'-carboxy)pentadecyl; R²⁰ is hydrogen or an alkyl, e.g., a C₁₋₄ alkyl (e.g., methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, or tert-butyl); q is an integer of 1-10, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; p is an integer of 1-7, e.g., 1, 2, 3, 4, 5, 6, or 7; and Het is an unsubstituted pyridine, wherein the nitrogen atom of the pyridine is directly connected to the alkylene chain, i.e., -(CH₂)_p- in Formula 2, or Het is a substituted pyridine, wherein the nitrogen atom of the pyridine is directly connected to the alkylene chain, i.e., -(CH₂)_p- in Formula 2, wherein the pyridine is substituted by one to five (e.g., 1, 2, 3, 4, or 5) R¹² substituents, wherein each R¹² is independently as defined herein, e.g., a halogen (e.g., F, Cl), a C₆₋₁₀ aryl (e.g., phenyl), a heteroaryl, or an alkyl (e.g., a C₁₋₁₀ alkyl, e.g., a C₁₋₄ alkyl (e.g., methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, tert-butyl)). In some embodiments, Het is a substituted pyridine and the pyridine is substituted by one R¹² substituent at the 2-, 3-, or 4-position of the pyridine, wherein R¹² is as defined herein, for example, a halogen (e.g., F, Cl), a phenyl, or a methyl.

[0148] In some embodiments, an oxidized lipid of the invention is a compound having a structure according to Formula 3:

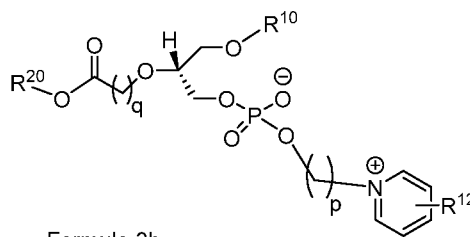


Formula 3

[0149] or a stereoisomer, a stereoisomeric mixture, or a salt thereof, wherein R^{10} , R^{12} , R^{20} , p , and q are as defined herein for Formula 1. In some embodiments, the oxidized lipid is a zwitterionic structure according to Formula 3. In some embodiments, the oxidized lipid is an external salt (e.g., a pharmaceutically acceptable salt) of the compound having a structure according to Formula 3. In some embodiments, the oxidized lipid according to Formula 3 has a structure according to Formula 3a or Formula 3b:



Formula 3a



Formula 3b

[0150]

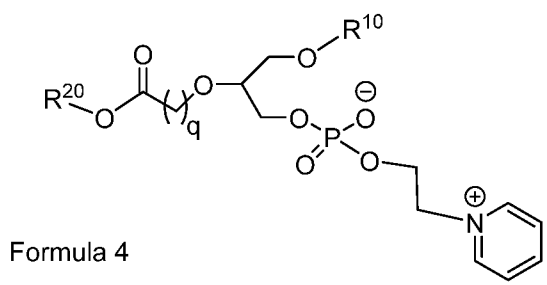
, or a salt thereof.

[0151]

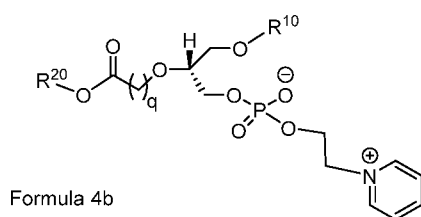
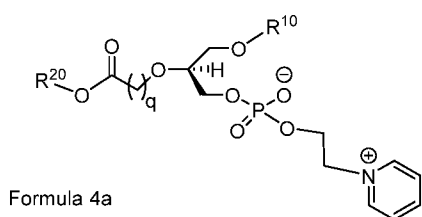
In some embodiments, the compound is a S-isomer having a structure according to Formula 3a, wherein the compound has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more. In some embodiments, the compound is a R-isomer having a structure according to Formula 3b, wherein the compound has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more. In other embodiments, the compound has an enantiomeric purity of from about 80% ee to about 100% ee, about 85% ee to about 100% ee, about 90% ee to about 100% ee, about 95% ee to about 100%, about 80% ee to about 99.5% ee, about 85% ee to about 99.5% ee, about 90% ee to about 99.5% ee, about 95% ee to about 99.5%, or any range thereof.

[0152] In some embodiments according to Formula 3, R^{10} is selected from the group consisting of hexadecyl, eicosanyl and (2'-octyl)dodecyl; R^{20} is hydrogen or a C_{1-4} alkyl (e.g., methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, or tert-butyl); q is an integer of 2-6, e.g., 2, 3, 4, 5, or 6; p is an integer of 2-5, e.g., 2, 3, 4, or 5; and the pyridine is substituted by 0 to 3 (e.g., 0, 1, 2, or 3) R^{12} substituents, wherein each R^{12} is independently as defined herein, e.g., a halogen (e.g., F, Cl), a C_{6-10} aryl (e.g., phenyl), a heteroaryl, or a C_{1-4} alkyl (e.g., methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, tert-butyl). In some embodiments, R^{10} is selected from the group consisting of hexadecyl, eicosanyl and (2'-octyl)dodecyl; R^{20} is hydrogen or methyl; q is 2, 3, 4, 5, or 6; p is 2, 3, 4, or 5; and the pyridine is substituted by 0 to 3 (e.g., 0, 1, 2, or 3) R^{12} substituents, wherein each R^{12} is independently is a halogen (e.g., F, Cl), a C_{6-10} aryl (e.g., phenyl), a heteroaryl, or a C_{1-4} alkyl (e.g., methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, tert-butyl). In some embodiments, R^{10} is selected from the group consisting of hexadecyl, eicosanyl and (2'-octyl)dodecyl; R^{20} is hydrogen or methyl; q is 4; p is 2; and the pyridine is substituted by 0, 1, or 2 R^{12} substituents, wherein each R^{12} is independently a halogen (e.g., F, Cl), a phenyl, or a methyl. In some embodiments according to Formulae 3, 3a, and 3b, R^{10} is selected from the group consisting of hexadecyl, eicosanyl, and (2'-octyl)dodecyl. In some embodiments according to Formulae 3, 3a, and 3b, R^{20} is a hydrogen or a C_{1-4} alkyl. In some embodiments according to Formulae 3, 3a, and 3b, q is an integer of 2-6. In some embodiments according to Formulae 3, 3a, and 3b, p is an integer of 2-5. In some embodiments according to Formulae 3, 3a, and 3b, the pyridine is substituted by 0 to 3 (e.g., 0, 1, 2, or 3) R^{12} substituents, wherein each R^{12} is independently as defined herein, e.g., a halogen (e.g., F, Cl), a C_{6-10} aryl (e.g., phenyl), a heteroaryl, or a C_{1-4} alkyl (e.g., methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, tert-butyl). In some embodiments according to Formulae 3, 3a, and 3b, the pyridine ring is substituted by one, two, or three R^{12} substituents. In some embodiments according to Formulae 3, 3a, and 3b, the pyridine ring is substituted by one R^{12} substituent, wherein the one R^{12} substituent is a fluorine or a phenyl, e.g., the pyridine ring is 3-fluoro-pyridine or 3-phenyl-pyridine. In some embodiments according to Formulae 3, 3a, and 3b, R^{10} is selected from the group consisting of hexadecyl, eicosanyl and (2'-octyl)dodecyl; R^{20} is hydrogen or methyl; q is 4; p is 2; and the pyridine is 3-fluoro-pyridine or 3-phenyl-pyridine.

[0153] In some embodiments, an oxidized lipid of the invention is a compound having a structure according to Formula 4:



[0154] or a stereoisomer, a stereoisomeric mixture, or a salt thereof. Suitable R^{10} , R^{20} , and q are those as defined herein for Formula 1. In some embodiments, the oxidized lipid has a zwitterionic structure according to Formula 4. In some embodiments, the oxidized lipid is an external salt (e.g., a pharmaceutically acceptable salt) of the compound having a structure according to Formula 4. In some embodiments, the oxidized lipid according to Formula 4 has a structure according to Formula 4a or Formula 4b:



[0155] salt thereof.

[0156] In some embodiments, the compound is a S-isomer having a structure according to Formula 4a, wherein the compound has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more. In some embodiments, the compound is a R-isomer having a structure according to Formula 4b, wherein the compound has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more. In other embodiments, the compound has an enantiomeric purity of from about 80% ee to about 100% ee, about 85% ee to about 100% ee, about 90% ee to about 100% ee, about 95% ee to about 100%, about 80% ee to about 99.5% ee, about 85% ee to about 99.5% ee, about 90% ee to about 99.5% ee, about 95% ee to about 99.5%, or any range thereof.

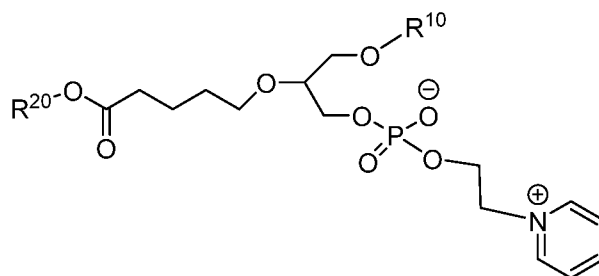
[0157] In some embodiments according to Formula 4, R^{10} is selected from the group consisting of hexadecyl, dodecyl, octadecyl, octyl, eicosanyl, cis-9-hexadecenyl, (2'-octyl)dodecyl, and (15'-carboxy)pentadecyl. In some embodiments, R^{10} is hexadecyl. In some embodiments, R^{10} is (2'-octyl)dodecyl. In some embodiments, R^{10} is eicosanyl.

[0158] In some embodiments, R^{20} is a hydrogen or an alkyl. In some embodiments, R^{20} is hydrogen. In some embodiments, R^{20} is an alkyl, e.g., a C_{1-4} alkyl (e.g., methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, or tert-butyl). In some embodiments, R^{20} is methyl.

[0159] In some embodiments, q is an integer of 1-10, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, q is 4.

[0160] In some embodiments, R^{10} is selected from the group consisting of hexadecyl, eicosanyl, and (2'-octyl)dodecyl; R^{20} is hydrogen or a C_{1-4} alkyl (e.g., methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, or tert-butyl); and q is an integer of 2-6, e.g., 2, 3, 4, 5, or 6. In some embodiments, R^{10} is selected from the group consisting of hexadecyl, eicosanyl and (2'-octyl)dodecyl; R^{20} is hydrogen; and q is an integer of 2-6, e.g., 2, 3, 4, 5, or 6. In some embodiments, R^{10} is selected from the group consisting of hexadecyl, eicosanyl, and (2'-octyl)dodecyl; R^{20} is methyl; and q is an integer of 2-6, e.g., 2, 3, 4, 5, or 6.

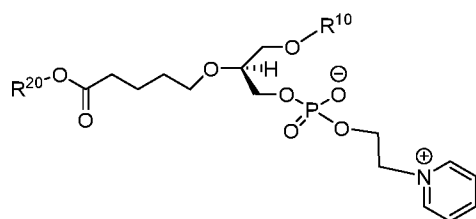
[0161] In some embodiments, an oxidized lipid of the invention is a compound having a structure according to Formula 5,



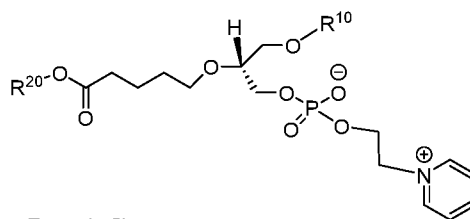
Formula 5

[0162] or a stereoisomer, a stereoisomeric mixture, or a salt thereof, wherein R^{10} and R^{20} are as defined herein for Formula 1. In some embodiments, the oxidized lipid has a zwitterionic structure according to Formula 5. In some embodiments, the oxidized lipid is an external salt (e.g., a pharmaceutically acceptable salt) of the compound having a structure according to Formula 5. In some embodiments, the oxidized lipid is a

compound according to Formula 5 has a structure according to Formula 5a or Formula 5b:



Formula 5a



Formula 5b

, or

[0163]

a salt thereof.

[0164]

In some embodiments, the compound is a S-isomer having a structure according to Formula 5a, wherein the compound has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more. In some embodiments, the compound is a R-isomer having a structure according to Formula 5b, wherein the compound has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more. In other embodiments, the compound has an enantiomeric purity of from about 80% ee to about 100% ee, about 85% ee to about 100% ee, about 90% ee to about 100% ee, about 95% ee to about 100%, about 80% ee to about 99.5% ee, about 85% ee to about 99.5% ee, about 90% ee to about 99.5% ee, about 95% ee to about 99.5%, or any range thereof

[0165]

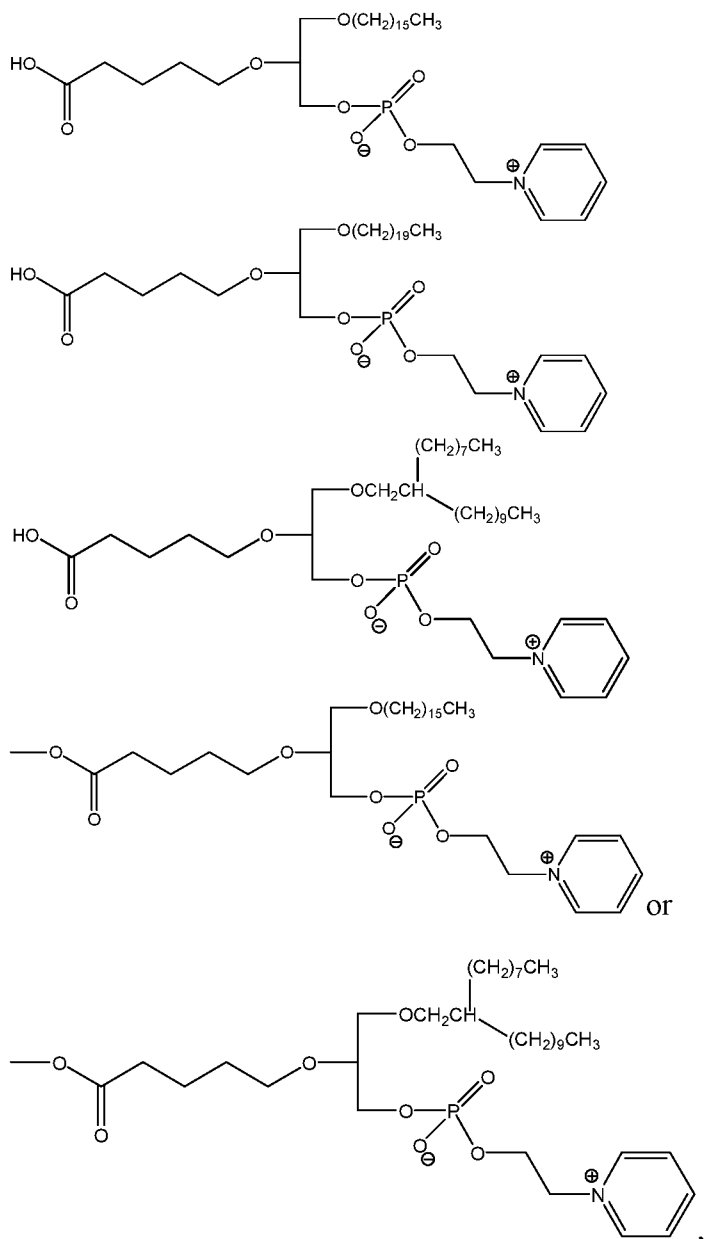
In some embodiments according to Formula 5, R^{10} is selected from the group consisting of hexadecyl, dodecyl, octadecyl, octyl, eicosanyl, cis-9-hexadecenyl, (2'-octyl)dodecyl, and (15'-carboxy)pentadecyl. In some embodiments, R^{10} is hexadecyl. In some embodiments, R^{10} is (2'-octyl)dodecyl. In some embodiments, R^{10} is eicosanyl.

[0166]

In some embodiments, R^{20} is a hydrogen or an alkyl. In some embodiments, R^{20} is hydrogen. In some embodiments, R^{20} is an alkyl, e.g., a C_{1-4} alkyl (e.g., methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, or tert-butyl). In some embodiments, R^{20} is methyl.

[0167]

In some embodiments, an oxidized lipid of the invention is a compound having a structure of:

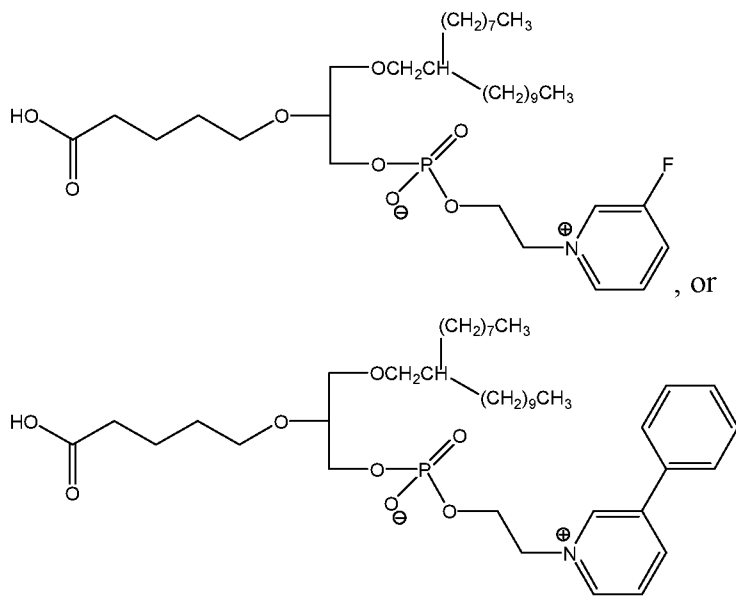


[0168] or a stereoisomer, a stereoisomeric mixture, or a salt thereof.

[0169] In some embodiments, the invention provides a compound selected from the group consisting of (R)-1-hexadecyl-2-(4'-carboxy)butyl-sn-glycero-3-phosphoric acid pyridiniummethyl ester (VB-701); (R)-1-eicosanyl-2-(4'-carboxy)butyl-sn-glycero-3-phosphoric acid pyridiniummethyl ester (VB-702); (R)-1-(2'-octyl)dodecyl-2-(4'-carboxy)butyl-sn-glycero-3-phosphoric acid pyridiniummethyl ester (VB-703); (R)-1-hexadecyl-2-(4'-carboxymethyl)butyl-sn-glycero-3-phosphoric acid pyridiniummethyl ester (VB-704); and (R)-1-(2'-octyl)dodecyl-2-(4'-carboxymethyl)butyl-sn-glycero-3-phosphoric acid pyridiniummethyl ester (VB-705). The prefix "(R)-" refers to the

configuration of the C-2 carbon of the glycerol backbone. In some embodiments, the compound has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more.

[0170] In some embodiments, an oxidized lipid of the invention is a compound having a structure of:



[0171] or a stereoisomer, a stereoisomeric mixture, or a salt thereof.

[0172] In some embodiments, the invention provides a compound selected from the group consisting of (R)-1-(2'-octyl)dodecyl-2-(4'-carboxy)butyl-glycero-sn-3-phosphoric acid 3-fluoro-pyridiniummethyl ester (VB-706) and (R)-1-(2'-octyl)dodecyl-2-(4'-carboxy)butyl-glycero-sn-3-phosphoric acid 3-phenyl-pyridiniummethyl ester (VB-707). The prefix "(R)-" refers to the configuration of the C-2 carbon of the glycerol backbone. In some embodiments, the compound has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more. In other embodiments, the compound has an enantiomeric purity of from about 80% ee to about 100% ee, about 85% ee to about 100% ee, about 90% ee to about 100% ee, about 95% ee to about 100%, about 80% ee to about 99.5% ee, about 85% ee to about 99.5% ee, about 90% ee to about 99.5% ee, about 95% ee to about 99.5%, or any range thereof.

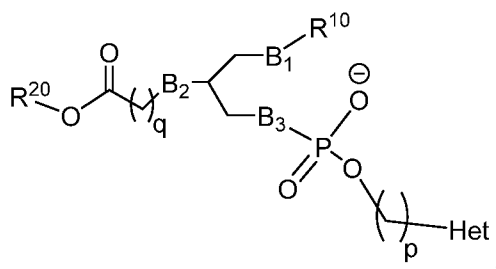
[0173] In other embodiments, an oxidized lipid compound of the invention treats or prevents fibrosis (e.g., liver fibrosis, kidney fibrosis, focal and segmental glomerulosclerosis, or any other fibrosis described herein) as well as, or better than, telmisartan. In other embodiments, an oxidized lipid compound of the invention reduces liver inflammation as well as, or better than, telmisartan. In other embodiments, an oxidized lipid compound of the invention reduces liver fibrosis as well as, or better than, telmisartan. In other embodiments, an oxidized lipid compound of the invention treats or prevents kidney fibrosis as well as, or better than, telmisartan. In other embodiments, an oxidized lipid compound of the invention treats or prevents focal and segmental glomerulosclerosis as well as, or better than, telmisartan.

[0174] In other embodiments, an oxidized lipid compound of the invention inhibits formation of ligand-induced phosphorylation of IKK, ERK, AKT, or p38 comparable to or more than VB-201 (1-hexadecyl-2-(4'-carboxy)butyl-glycero-3-phosphocholine or 1-hexadecyl-2-(4'-carboxybutyl)-glycerol-3-phosphocholine)) inhibits formation of ligand-induced phosphorylation of IKK, ERK, AKT, or p38. In other embodiments, an oxidized lipid compound of the invention inhibits ligand-induced cell migration comparable to or more than VB-201 inhibits ligand-induced cell migration. In other embodiments, the ligand is LPS, PGN, PAM3, or MCP1. In other embodiments, the cell migration is monocyte migration.

Methods of Synthesis

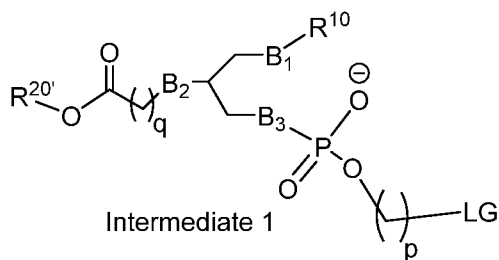
[0175] Other embodiments of the invention relate to methods of synthesizing oxidized lipids of the invention.

[0176] In some embodiments, the invention provides a method of synthesizing a compound of Formula 1,



Formula 1

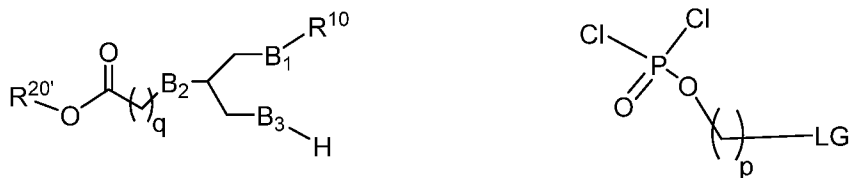
or a stereoisomer, a stereoisomeric mixture, or a salt thereof, wherein B_1 , B_2 , B_3 , R^{10} , R^{20} , Het, p, and q are defined herein above for Formula 1, the method comprising



[0177] a) reacting Intermediate 1 with Het to form the compound of Formula 1,

[0178] wherein B_1 , B_2 , B_3 , R^{10} , Het, p, and q are as defined above in Formula 1; and wherein LG is a leaving group. In some embodiments, $R^{20'}$ is the same as R^{20} . However, as described in the Examples section, when Intermediate 1 was heated in pyridine (e.g., at reflux temperature), conversion of $-\text{COOR}^{20'}$ into $-\text{COOH}$ was also observed. Thus, in some embodiments, R^{20} is H, $R^{20'}$ is selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl, and heteroaryl, the reacting of Intermediate 1 with Het (e.g., pyridine or substituted pyridine as defined herein) also converts $-\text{COOR}^{20'}$ into $-\text{COOH}$, or a salt thereof, to form the compound of Formula 1. In other embodiments, R^{20} is H, $R^{20'}$ is selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl, and heteroaryl, the reacting of Intermediate 1 with Het is followed by a step of hydrolysis to convert $-\text{COOR}^{20'}$ into $-\text{COOH}$, or a salt thereof, to form the compound of Formula 1. In preferred embodiments, R^{20} is H, $R^{20'}$ is methyl, the reacting of Intermediate 1 with Het (e.g., pyridine or substituted pyridine as defined herein) also converts $-\text{COOR}^{20'}$ into $-\text{COOH}$, or a salt thereof, to form the compound of Formula 1. In some embodiments, the LG is selected from the group consisting of halogen and oxygen containing leaving groups (e.g., as described herein).

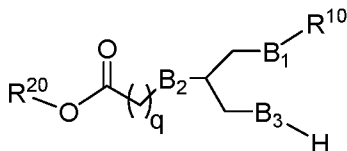
[0179] In some embodiments, the Intermediate 1 can be synthesized by



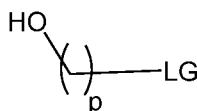
b) reacting Reactant 1 with Reactant 2 to form a monochloro reaction product; and

c) hydrolyzing the monochloro reaction product to form Intermediate 1, wherein B_1 , B_2 , B_3 , R^{10} , R^{20} , p , q , and LG are as defined above in Intermediate 1.

[0180] In other embodiments, the Intermediate 1 can be synthesized by



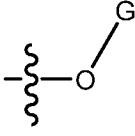
b') reacting Reactant 1 with $POCl_3$ to form a $POCl_3$ reaction product;

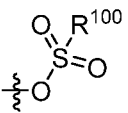


c') reacting Reactant 2A with the $POCl_3$ reaction product to form a second reaction product; and

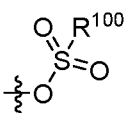
d') hydrolyzing the second reaction product to form Intermediate 1, wherein B_1 , B_2 , B_3 , R^{10} , R^{20} , p , q , and LG are as defined above in Intermediate 1. In some embodiments, steps b') and c') can be performed in a one-pot fashion. In some embodiments, the $POCl_3$ reaction product is not isolated before reacting with Reactant 2A.

[0181] Suitable LGs for the methods of synthesis described herein include any leaving group known in the art. In some embodiments, LG is a halogen, such as F, Cl, Br, or I. In some embodiments, LG is an oxygen containing leaving group, such as a tosylate, a mesylate, a triflate, etc. In some embodiments, LG is Br. Oxygen containing leaving

groups as used herein refer to leaving groups represented by formula , wherein

G is typically an electron withdrawing group, e.g., . Examples of oxygen containing leaving groups include sulfonates such as nonaflate, triflate, fluorosulfonate, tosylate, mesylate or besylate. Other oxygen containing leaving groups such as acyloxy and aryloxy groups are known in the art.

[0182] In some embodiments, the LG is a leaving group selected from the group

consisting of oxygen containing leaving groups represented by , wherein R¹⁰⁰ is selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, heteroalicycloalkyl, aryl, and heteroaryl, each of which is optionally substituted by one to five R¹³, wherein each R¹³ substituent is independently selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, halo, trihalomethyl, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, phosphonate, phosphate, phosphinyl, sulfonyl, sulfinyl, sulfonamide, amide, carbonyl, thiocarbonyl, C-carboxy, O-carboxy, C-carbamate, N-carbamate, C-thiocarboxy, S-thiocarboxy, and amino. In some embodiments, R¹⁰⁰ is p-tolyl, o-tolyl, phenyl, methyl, or trifluoromethyl. In some embodiments, R¹⁰⁰ is p-tolyl.

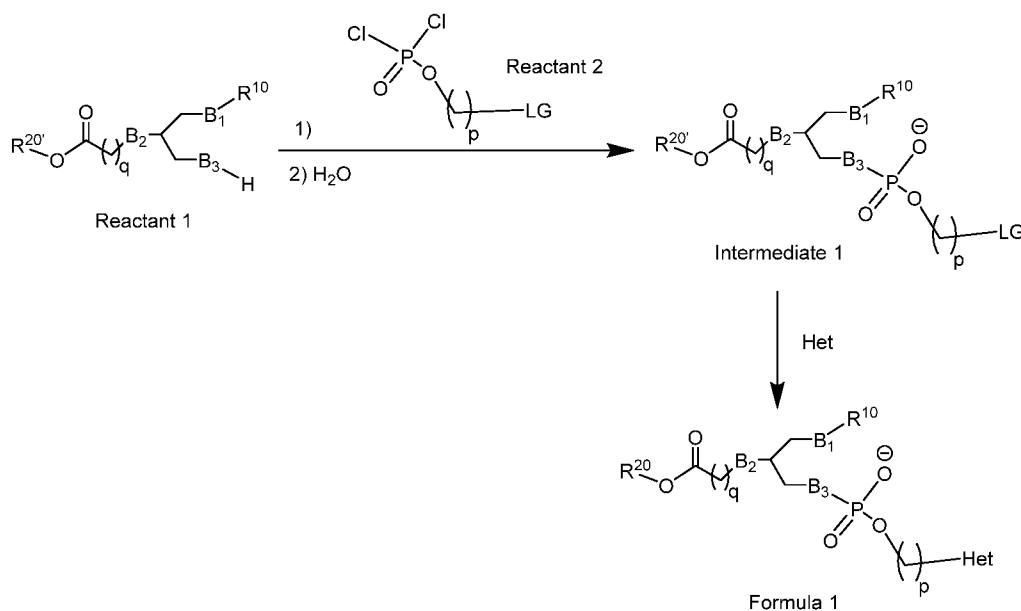
[0183] In some embodiments, all of B₁, B₂, and B₃ are O. In some embodiments, Het is an unsubstituted pyridine, wherein the nitrogen atom of the pyridine is directly connected to the alkylene chain, i.e., -(CH₂)_p- in Formula 1. In some embodiments, Het is 3-fluoropyridine or 3-phenyl-pyridine, wherein the nitrogen atom of the pyridine is directly connected to the alkylene chain, i.e., -(CH₂)_p- in Formula 1. In some embodiments, R¹⁰ is selected from the group consisting of hexadecyl, eicosanyl and (2'-octyl)dodecyl. In some embodiments, R²⁰ is hydrogen or a C₁₋₄ alkyl (e.g., methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, or tert-butyl). In some embodiments, p is 2. In some embodiments, q is 4.

[0184] In some embodiments, the method described above is directed to synthesizing compounds having a structure according to Formulae 2, 3, 4, or 5 (e.g., as described herein). In such embodiments, all of B₁, B₂, and B₃ are O, and suitable R¹⁰, R¹¹, R¹², R²⁰, Het, p, and q for the methods described above are those as defined herein for the respective Formulae 2, 3, 4, or 5. In some embodiments, R^{20'} is the same as R²⁰. In some embodiments, R²⁰ is H, R^{20'} is selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl, and heteroaryl, the reacting of Intermediate 1 with Het (e.g., pyridine or substituted pyridine as defined herein) also converts -COOR^{20'} into -COOH, or a salt thereof, to form the compound of Formulae 2, 3, 4, or 5. In other embodiments, R²⁰ is H, R^{20'} is selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl, and heteroaryl, the reacting of Intermediate 1 with Het is followed by a step of

hydrolysis to convert $-\text{COOR}^{20'}$ into $-\text{COOH}$, or a salt thereof, to form the compound of Formulae 2, 3, 4, or 5. In preferred embodiments, R^{20} is H in Formulae 2, 3, 4, or 5, $\text{R}^{20'}$ is methyl, the reacting of Intermediate 1 with Het (e.g., pyridine or substituted pyridine as defined herein) also converts $-\text{COOR}^{20'}$ into $-\text{COOH}$, or a salt thereof, to form the compound of Formulae 2, 3, 4, or 5.

[0185] Suitable solvents, reaction temperatures, as well as other reaction parameters for each of the process steps described herein can be ascertained by those skilled in the art based on the working Examples described herein.

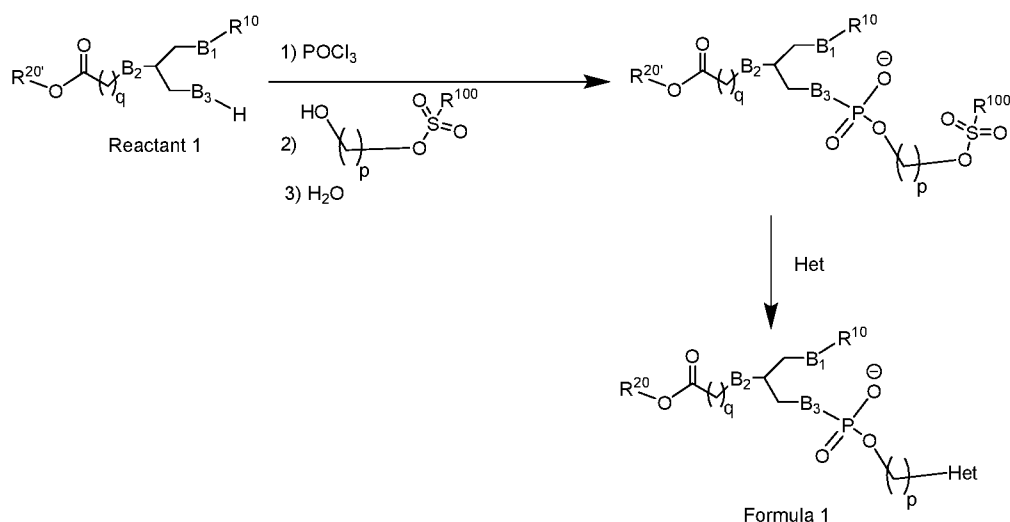
[0186] Thus, in some embodiments, a compound having a structure according to Formula 1 (e.g., as described herein) can be synthesized by a method comprising the steps in Method A:



[0187] Suitable LGs include any leaving group known in the art. In some embodiments, LG is a halogen, such as F, Cl, Br, or I. In some embodiments, LG is an oxygen containing leaving group, such as a tosylate, a mesylate, a triflate, etc. In some embodiments, LG is Br. In some embodiments, LG is tosylate.

[0188] In some embodiments, a compound having a structure according to Formula 1 can also be synthesized by a method comprising the steps in Method B:

Method B



[0189] Suitable R^{100} for Method B include alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl, optionally substituted by one to five R^{13} , wherein each R^{13} substituent is independently selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, halo, trihalomethyl, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, phosphonate, phosphate, phosphinyl, sulfonyl, sulfinyl, sulfonamide, amide, carbonyl, thiocarbonyl, C-carboxy, O-carboxy, C-carbamate, N-carbamate, C-thiocarboxy, S-thiocarboxy, and amino as described herein. In some embodiments, R^{100} is a p-tolyl, o-tolyl, phenyl, methyl, or trifluoromethyl moiety. In some embodiments, R^{100} is p-tolyl.

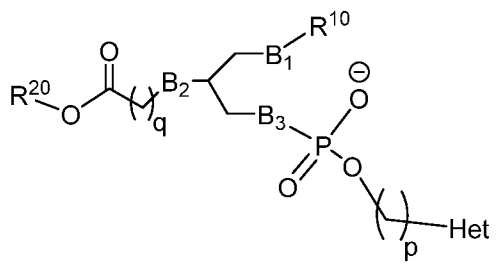
[0190] In some embodiments, the method for synthesizing a compound having a structure according to Formula 1 further comprises a step of hydrolysis to convert an ester into a carboxylic acid.

[0191] Suitable B_1 , B_2 , B_3 , R^{10} , R^{11} , R^{12} , R^{20} , Het, p, and q for Method A or B are those as defined herein for Formula 1. In some embodiments, all of B_1 , B_2 , and B_3 are O. In some embodiments, Het is an unsubstituted pyridine, wherein the nitrogen atom of the pyridine is directly connected to the alkylene chain, i.e., $-(\text{CH}_2)_p-$ in Formula 1. In some embodiments, R^{10} is selected from the group consisting of hexadecyl, eicosanyl and (2'-octyl)dodecyl. In some embodiments, R^{20} is hydrogen or a C_{1-4} alkyl (e.g., methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, iso-butyl, or tert-butyl). In some embodiments, p is 2. In some embodiments, q is 4. In some embodiments, $\text{R}^{20'}$ is the same as R^{20} . In some

embodiments, R^{20} is H, $R^{20'}$ is selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl, and heteroaryl, the reacting of Intermediate 1 with Het (e.g., pyridine or substituted pyridine as defined herein) also converts $-COOR^{20'}$ into $-COOH$, or a salt thereof, to form the compound of Formula 1. In other embodiments, R^{20} is H, $R^{20'}$ is selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl, and heteroaryl, the reacting of Intermediate 1 with Het is followed by a step of hydrolysis to convert $-COOR^{20'}$ into $-COOH$, or a salt thereof, to form the compound of Formula 1. In preferred embodiments, R^{20} is H, $R^{20'}$ is methyl, the reacting of Intermediate 1 with Het (e.g., pyridine or substituted pyridine as defined herein) also converts $-COOR^{20'}$ into $-COOH$, or a salt thereof, to form the compound of Formula 1.

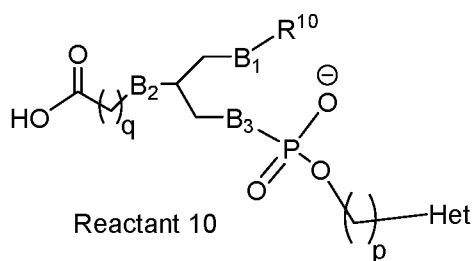
[0192] Similarly, compounds having a structure according to Formulae 2, 3, 4, or 5 (e.g., as described herein) can be synthesized by any of the methods described above, e.g., by a method comprising the steps in Method A or the steps in Method B. In the synthesis of compounds having a structure according to Formulae 2, 3, 4, or 5 (e.g., as described herein), all of B_1 , B_2 , and B_3 are O, and suitable R^{10} , R^{11} , R^{12} , R^{20} , Het, p, and q for Method A or B are those as defined herein for the respective Formulae 2, 3, 4, or 5. In some embodiments, $R^{20'}$ is the same as R^{20} . In some embodiments, R^{20} is H, $R^{20'}$ is selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl, and heteroaryl, the reacting of Intermediate 1 with Het (e.g., pyridine or substituted pyridine as defined herein) also converts $-COOR^{20'}$ into $-COOH$, or a salt thereof, to form the compound of Formulae 2, 3, 4, or 5. In other embodiments, R^{20} is H, $R^{20'}$ is selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl, and heteroaryl, the reacting of Intermediate 1 with Het is followed by a step of hydrolysis to convert $-COOR^{20'}$ into $-COOH$, or a salt thereof, to form the compound of Formulae 2, 3, 4, or 5. In preferred embodiments, R^{20} is H, $R^{20'}$ is methyl, the reacting of Intermediate 1 with Het (e.g., pyridine or substituted pyridine as defined herein) also converts $-COOR^{20'}$ into $-COOH$, or a salt thereof, to form the compound of Formulae 2, 3, 4, or 5.

[0193] In some embodiments, the invention provides a method of synthesizing a compound of Formula 1,



Formula 1

or a stereoisomer, a stereoisomeric mixture, or a salt thereof, the method comprising



a) reacting Reactant 10

with $R^{20}OH$ to form the

compound of Formula 1,

wherein B_1 , B_2 , B_3 , R^{10} , Het, p, and q are defined above; and wherein R^{20} is selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl, and heteroaryl. Preferably, R^{20} is an alkyl (e.g., C_{1-4} alkyl, e.g., methyl). In some embodiments, the reaction is catalyzed by an acid, e.g., HCl. Other known methods for forming an ester can also be used in step a).

[0194] In some embodiments, the method is directed to synthesizing compounds having a structure according to Formulae 2, 3, 4, or 5 (e.g., as described herein). In such embodiments, all of B_1 , B_2 , and B_3 are O, and suitable R^{10} , R^{11} , R^{12} , Het, p, and q for the methods described above are those as defined herein for the respective Formulae 2, 3, 4, or 5, wherein R^{20} is selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl, and heteroaryl. Preferably, R^{20} is an alkyl (e.g., C_{1-4} alkyl, e.g., methyl).

[0195] Oxidized lipids of the invention (e.g., Formulae 1a, 1b, 2a, 2b, 3a, 3b, 4a, 4b, 5a, 5b) having certain enantiomeric purity (e.g., as described herein) can be obtained from racemic mixtures by techniques known in the art. Examples include, but are not limited

to, the formation of chiral salts and the use of chiral or high performance liquid chromatography "HPLC" and the formation and crystallization of chiral salts. See, e.g., Jacques, J., et al., *Enantiomers, Racemates and Resolutions* (Wiley-Interscience, New York, 1981); Wilen, S. H., et al., *Tetrahedron* 33:2725 (1977); Eliel, E. L., *Stereochemistry of Carbon Compounds* (McGraw-Hill, NY, 1962); and Wilen, S. H., *Tables of Resolving Agents and Optical Resolutions* p. 268 (E. L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, Ind., 1972).

[0196] Oxidized lipids of the invention (e.g., Formulae 1a, 1b, 2a, 2b, 3a, 3b, 4a, 4b, 5a, 5b) having certain enantiomeric purity (e.g., as described herein) can also be synthesized by the general Methods A or B described herein using a starting material, or any synthetic intermediate, having certain enantiomeric purity, for example, (S)-1-(2'-octyl)dodecyl-2-(4'-carboxymethyl)butyl-glycerol, (S)-1-hexadecyl-2-(4'-carboxymethyl)butyl-glycerol, or (S)-1-eicosanyl-2-(4'-carboxymethyl)butyl-glycerol. The starting material, or any synthetic intermediate, having certain enantiomeric purity can be obtained by synthetic methods known in the art or by chiral separation from racemic mixtures as described herein.

[0197] In other embodiments, the present invention relates to a compound made by any of the methods of synthesis of the invention.

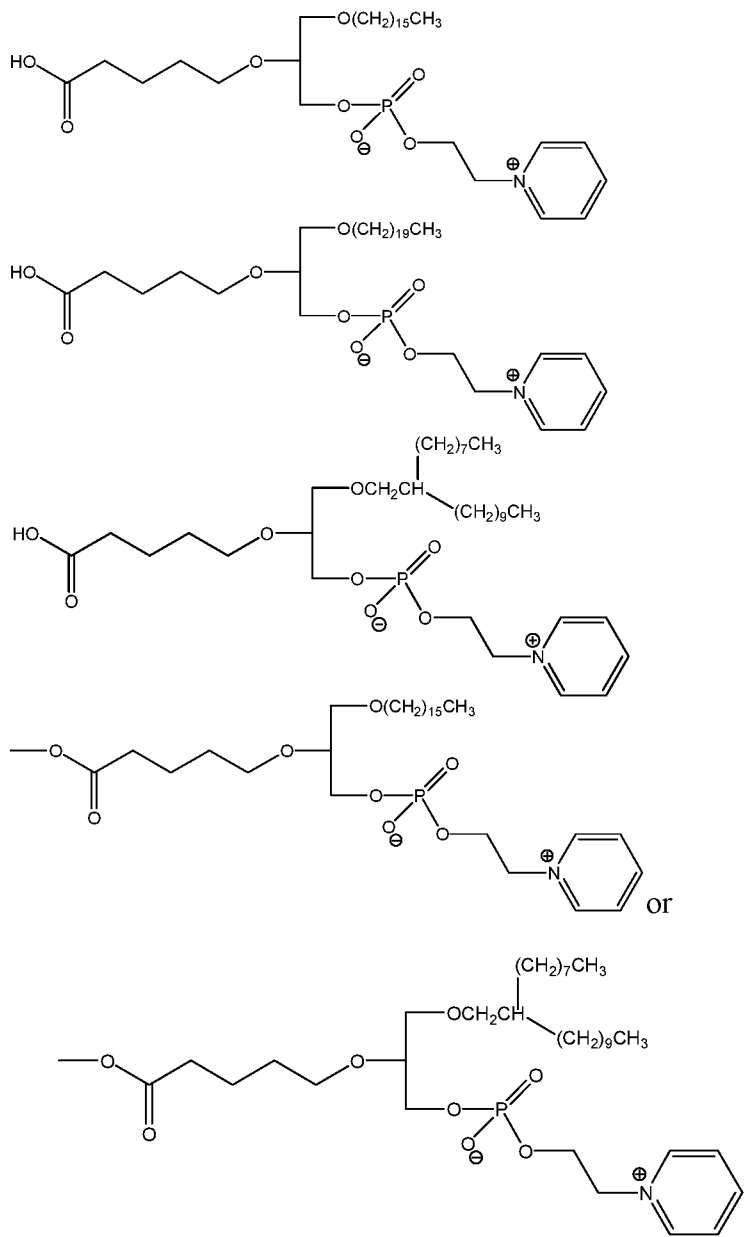
Pharmaceutical Compositions

[0198] Other embodiments of the invention relate to a pharmaceutical composition comprising an oxidized lipid of the invention. In some embodiments, the pharmaceutical composition comprises an oxidized lipid of the invention and a pharmaceutically acceptable vehicle. In other embodiments, the pharmaceutical composition comprises a therapeutically effective amount of the oxidized lipid. In some embodiments, the pharmaceutical composition comprises a therapeutically effective amount of the oxidized lipid and a pharmaceutically acceptable vehicle. As used herein, a therapeutically effective amount of an oxidized lipid is an amount effective to treat or prevent a disease or disorder of the present invention.

[0199] In other embodiments, the pharmaceutical compositions of the present invention can be orally administered.

[0200] In some embodiments, the pharmaceutical composition comprises a compound having a structure according to any of Formulae 1, 2, 3, 4, or 5 as described herein.

[0201] In other embodiments, the pharmaceutical composition comprises a compound having a structure of:



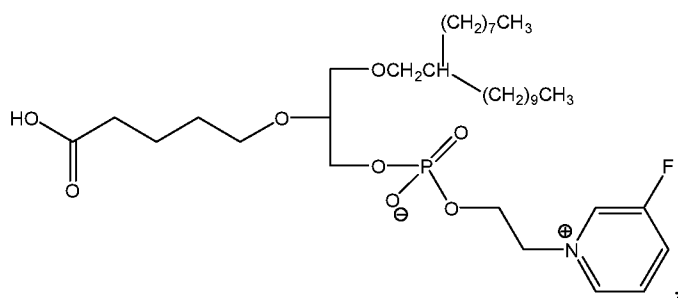
[0202] or a stereoisomer, a stereoisomeric mixture, or a salt thereof.

[0203] In some embodiments, the pharmaceutical composition comprises a compound selected from the group consisting of (R)-1-hexadecyl-2-(4'-carboxy)butyl-sn-glycero-3-

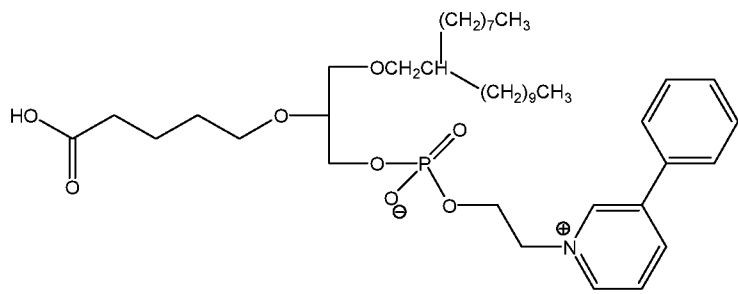
[0204] phosphoric acid pyridiniummethyl ester (VB-701); (R)-1-eicosanyl-2-(4'-carboxy)butyl-sn-glycero-3-phosphoric acid pyridiniummethyl ester (VB-702); (R)-1-(2'-octyl)dodecyl-2-(4'-carboxy)butyl-sn-glycero-3-phosphoric acid pyridiniummethyl ester (VB-703); (R)-1-hexadecyl-2-(4'-carboxymethyl)butyl-sn-glycero-3-phosphoric acid

pyridiniummethyl ester (VB-704); and (R)-1-(2'-octyl)dodecyl-2-(4'-carboxymethyl)butyl-sn-glycero-3-phosphoric acid pyridiniummethyl ester (VB-705). The prefix "(R)-" refers to the configuration of the C-2 carbon of the glycerol backbone. In some embodiments, the compound has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more. In other embodiments, the compound has an enantiomeric purity of from about 80% ee to about 100% ee, about 85% ee to about 100% ee, about 90% ee to about 100% ee, about 95% ee to about 100%, about 80% ee to about 99.5% ee, about 85% ee to about 99.5% ee, about 90% ee to about 99.5% ee, about 95% ee to about 99.5%, or any range thereof

[0205] In some embodiments, the pharmaceutical composition comprises a compound having a structure of:



or



[0206] or a stereoisomer, a stereoisomeric mixture, or a salt thereof.

[0207] In some embodiments, the pharmaceutical composition comprises a compound selected from the group consisting of (R)-1-(2'-octyl)dodecyl-2-(4'-carboxy)butyl-glycero-sn-3-phosphoric acid 3-fluoro-pyridiniummethyl ester (VB-706) and (R)-1-(2'-octyl)dodecyl-2-(4'-carboxy)butyl-glycero-sn-3-phosphoric acid 3-phenyl-pyridiniummethyl ester (VB-707). The prefix "(R)-" refers to the configuration of the C-2 carbon of the glycerol backbone. In some embodiments, the compound has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about

90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more. In other embodiments, the compound has an enantiomeric purity of from about 80% ee to about 100% ee, about 85% ee to about 100% ee, about 90% ee to about 100% ee, about 95% ee to about 100%, about 80% ee to about 99.5% ee, about 85% ee to about 99.5% ee, about 90% ee to about 99.5% ee, about 95% ee to about 99.5%, or any range thereof.

[0208] In other embodiments, the pharmaceutical composition treats or prevents fibrosis (e.g., liver fibrosis, kidney fibrosis, focal and segmental glomerulosclerosis, or any other fibrosis described herein) as well as, or better than, telmisartan. In other embodiments, the pharmaceutical composition reduces liver inflammation as well as, or better than, telmisartan. In other embodiments, the pharmaceutical composition reduces liver fibrosis as well as, or better than, telmisartan. In other embodiments, the pharmaceutical composition treats or prevents kidney fibrosis as well as, or better than, telmisartan. In other embodiments, the pharmaceutical composition treats or prevents focal and segmental glomerulosclerosis as well as, or better than, telmisartan.

[0209] In other embodiments, the pharmaceutical composition inhibits formation of ligand-induced phosphorylation of IKK, ERK, AKT or p38 comparable to or more than VB-201 (1-hexadecyl-2-(4'-carboxy)butyl-glycero-3-phosphocholine or 1-hexadecyl-2-(4'-carboxybutyl)-glycerol-3-phosphocholine)) inhibits formation of ligand-induced phosphorylation of IKK, ERK, AKT or p38. In other embodiments, the pharmaceutical composition inhibits ligand-induced cell migration comparable to or more than VB-201 inhibits ligand-induced cell migration. In other embodiments, the ligand is LPS, PGN, PAM3, or MCP1. In other embodiments, the cell migration is monocyte migration.

Methods of Treating or Preventing Fibrosis or Inflammatory Diseases or Disorders

[0210] Embodiments of the invention relate to a method for treating or preventing fibrosis or an inflammatory disease or disorder comprising administering an oxidized lipid of the invention. In other embodiments, the invention relates to a method for treating or preventing an inflammatory disease or disorder. In other embodiments, the method comprises administering a therapeutically effective amount of an oxidized lipid of the invention to a subject in need thereof. In other embodiments, the method comprises administering a pharmaceutical composition of the invention.

[0211] The methods described herein can be used for treating or preventing all types fibrosis. In some embodiments of the methods of the invention, the fibrosis is pulmonary fibrosis, liver fibrosis, skin fibrosis or kidney fibrosis. In some embodiments of the methods of the invention, the fibrosis is heart fibrosis, bone marrow fibrosis, intestine fibrosis, joint fibrosis (knee, shoulder, or other joints), hand fibrosis, finger fibrosis, skeletal muscle fibrosis, neurofibrosis, and penis fibrosis. In other embodiments, the fibrosis is idiopathic pulmonary fibrosis (IPF), cystic fibrosis, progressive massive fibrosis, cirrhosis, steatohepatitis (fatty liver disease), nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), endomyocardial fibrosis, myocardial infarction, atrial fibrosis, mediastinal fibrosis, myelofibrosis, retroperitoneal fibrosis, nephrogenic systemic fibrosis, keloid, Crohn's disease, scleroderma/systemic sclerosis, arthrofibrosis, Peyronie's disease, Dupuytren's contracture, adhesive capsulitis, or focal and segmental glomerulosclerosis. In some embodiments, the fibrosis is liver fibrosis. In some embodiments, the fibrosis is kidney fibrosis. In some embodiments, the subject in need of treatment or prevention of kidney fibrosis has a chronic kidney disease. In some embodiments, the fibrosis is focal and segmental glomerulosclerosis. In some embodiments, the subject in need of treatment or prevention of focal and segmental glomerulosclerosis has a chronic kidney disease.

[0212] In some embodiments, the fibrosis is a fibrosis that does not include idiopathic pulmonary fibrosis. In other embodiments, the fibrosis is a fibrosis that does not include cystic fibrosis. In other embodiments, the fibrosis is a fibrosis that does not include progressive massive fibrosis. In some embodiments, the fibrosis is a fibrosis that does not include cirrhosis. In some embodiments, the fibrosis is a fibrosis that does not include steatohepatitis (fatty liver disease). In some embodiments, the fibrosis is a fibrosis that does not include nonalcoholic fatty liver disease (NAFLD). In some embodiments, the fibrosis is a fibrosis that does not include nonalcoholic steatohepatitis (NASH). In some embodiments, the fibrosis is a fibrosis that does not include endomyocardial fibrosis. In some embodiments, the fibrosis is a fibrosis that does not include myocardial infarction. In some embodiments, the fibrosis is a fibrosis that does not include atrial fibrosis. In some embodiments, the fibrosis is a fibrosis that does not include mediastinal fibrosis. In some embodiments, the fibrosis is a fibrosis that does not include myelofibrosis. In some embodiments, the fibrosis is a fibrosis that does not include retroperitoneal fibrosis. In some embodiments, the fibrosis is a fibrosis that does not include nephrogenic systemic

fibrosis. In some embodiments, the fibrosis is a fibrosis that does not include keloid. In some embodiments, the fibrosis is a fibrosis that does not include Crohn's disease. In some embodiments, the fibrosis is a fibrosis that does not include scleroderma/systemic sclerosis. In some embodiments, the fibrosis is a fibrosis that does not include arthrofibrosis. In some embodiments, the fibrosis is a fibrosis that does not include Peyronie's disease. In some embodiments, the fibrosis is a fibrosis that does not include Dupuytren's contracture. In some embodiments, the fibrosis is a fibrosis that does not include adhesive capsulitis. In some embodiments, the fibrosis is a fibrosis that does not include focal and segmental glomerulosclerosis. In some embodiments, the fibrosis is a fibrosis that does not include fibrous lesions or plaques in the arteries.

[0213] In some embodiments, the oxidized lipid treats or prevents liver inflammation, but does not alter liver fibrosis. In other embodiments, the oxidized lipid treats or prevents liver fibrosis, but does not alter liver inflammation.

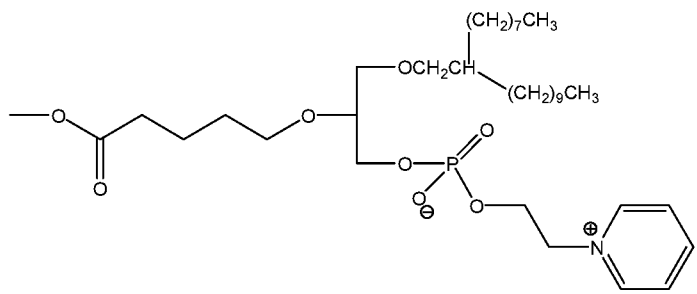
[0214] In other embodiments, the inflammatory disease or disorder is liver inflammation, atherosclerosis, rheumatoid arthritis, inflammatory bowel disease, ulcerative colitis, multiple sclerosis, or psoriasis.

[0215] In some embodiments, the inflammatory disease or disorder is an inflammatory cardiovascular disease or disorder, a cerebrovascular disease or disorder, or a peripheral vascular disease or disorder.

[0216] In some embodiments, the inflammatory disease or disorder is an inflammatory cardiovascular disease or disorder selected from the group consisting of occlusive diseases or disorders, atherosclerosis, cardiac valvular disease, stenosis, restenosis, in-stent-stenosis, myocardial infarction, coronary arterial disease, acute coronary syndromes, congestive heart failure, angina pectoris, myocardial ischemia, thrombosis, Wegener's granulomatosis, Takayasu's arteritis, Kawasaki syndrome, anti-factor VIII autoimmune diseases or disorders, necrotizing small vessel vasculitis, microscopic polyangiitis, Churg and Strauss syndrome, pauci-immune focal necrotizing glomerulonephritis, crescentic glomerulonephritis, antiphospholipid syndrome, antibody induced heart failure, thrombocytopenic purpura, autoimmune hemolytic anemia, cardiac autoimmunity, Chagas' disease or disorder, and anti-helper T lymphocyte autoimmunity.

[0217] In some embodiments, the inflammatory disease or disorder is a cerebrovascular disease or disorder selected from the group consisting of stroke, cerebrovascular inflammation, cerebral hemorrhage, and vertebral arterial insufficiency.

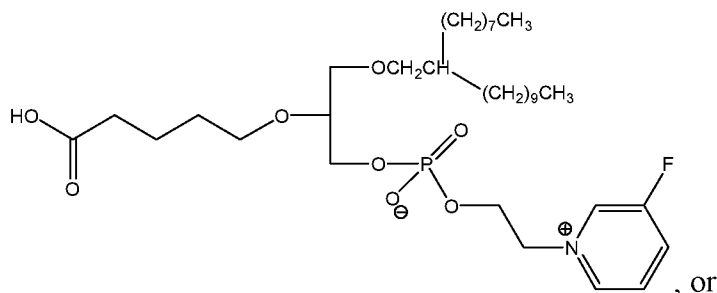
- [0218] In some embodiments, the inflammatory disease or disorder is a peripheral vascular disease or disorder is selected from the group consisting of gangrene, diabetic vasculopathy, ischemic bowel disease, thrombosis, diabetic retinopathy, and diabetic nephropathy.
- [0219] In other embodiments, activity of TLR2, TLR4 and/or CD14 is inhibited in a treated cell. In some embodiments, activity of TLR2 and TLR4 is inhibited; activity of TLR4 and CD14 is inhibited; activity of TLR2 and CD14 is inhibited; or activity of TLR2, TLR4 and CD14 is inhibited.
- [0220] In other embodiments, steatosis in a subject treated with an oxidized lipid of the invention is not reduced, compared to that in untreated or placebo-treated subjects. In other embodiments, liver lobular formation in a subject treated with an oxidized lipid of the invention is decreased, compared to that in untreated or placebo-treated subjects. In other embodiments, liver lobular formulation in a subject treated with an oxidized lipid of the invention is not decreased, compared to that in untreated or placebo-treated subjects. In other embodiments, steatosis in a subject treated with an oxidized lipid of the invention is not reduced and liver lobular formation in a subject treated with an oxidized lipid of the invention is decreased, compared to those in untreated or placebo-treated subjects, respectively. In other embodiments, steatosis in a subject treated with an oxidized lipid of the invention is not reduced and liver lobular formation in a subject treated with an oxidized lipid of the invention is not decreased, compared to those in untreated or placebo-treated subjects, respectively. In other embodiments, foam cell-like macrophages are decreased in a subject treated with an oxidized lipid of the invention, compared to that in untreated or placebo-treated subjects. In some embodiments, liver lobular formation and foam cell-like macrophages in a subject treated with an oxidized lipid of the invention are decreased, compared to those in untreated or placebo-treated subjects, respectively. In some embodiments, liver lobular inflammation in a subject treated with an oxidized lipid of the invention is decreased, compared to that in untreated or placebo-treated subjects. In some embodiments, liver lobular inflammation and foam cell-like macrophages in a subject treated with an oxidized lipid of the invention are decreased, compared to those in untreated or placebo-treated subjects, respectively. In some embodiments, liver lobular formation, liver lobular inflammation and foam cell-like macrophages in a subject treated with an oxidized lipid of the invention are decreased, compared to those in untreated or placebo-treated subjects, respectively. In some

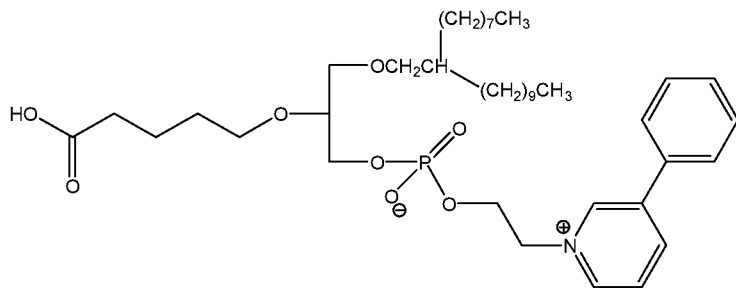


or a stereoisomer, a stereoisomeric mixture, or a salt thereof.

[0223] In some embodiments, the oxidized lipid is a compound selected from the group consisting of (R)-1-hexadecyl-2-(4'-carboxy)butyl-sn-glycero-3-phosphoric acid pyridiniummethyl ester (VB-701); (R)-1-eicosanyl-2-(4'-carboxy)butyl-sn-glycero-3-phosphoric acid pyridiniummethyl ester (VB-702); (R)-1-(2'-octyl)dodecyl-2-(4'-carboxy)butyl-sn-glycero-3-phosphoric acid pyridiniummethyl ester (VB-703); (R)-1-hexadecyl-2-(4'-carboxymethyl)butyl-sn-glycero-3-phosphoric acid pyridiniummethyl ester (VB-704); and (R)-1-(2'-octyl)dodecyl-2-(4'-carboxymethyl)butyl-sn-glycero-3-phosphoric acid pyridiniummethyl ester (VB-705). In some embodiments, the compound has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more. In other embodiments, the compound has an enantiomeric purity of from about 80% ee to about 100% ee, about 85% ee to about 100% ee, about 90% ee to about 100% ee, about 95% ee to about 100%, about 80% ee to about 99.5% ee, about 85% ee to about 99.5% ee, about 90% ee to about 99.5% ee, about 95% ee to about 99.5%, or any range thereof.

[0224] In some embodiments, the oxidized lipid is a compound having a structure of:





or a stereoisomer, a stereoisomeric mixture, or a salt thereof.

[0225] In some embodiments, the oxidized lipid is a compound selected from the group consisting of (R)-1-(2'-octyl)dodecyl-2-(4'-carboxy)butyl-glycero-sn-3-phosphoric acid 3-fluoro-pyridiniummethyl ester (VB-706) and (R)-1-(2'-octyl)dodecyl-2-(4'-carboxy)butyl-glycero-sn-3-phosphoric acid 3-phenyl-pyridiniummethyl ester (VB-707). The prefix "(R)-" refers to the configuration of the C-2 carbon of the glycerol backbone. In some embodiments, the compound has an enantiomeric purity of about 80% ee or more, e.g., about 80% ee, about 85% ee, about 90% ee, about 91% ee, about 92% ee, about 93% ee, about 94% ee, about 95% ee, about 96% ee, about 97% ee, about 98% ee, about 99% ee, about 99.5% ee or more. In other embodiments, the compound has an enantiomeric purity of from about 80% ee to about 100% ee, about 85% ee to about 100% ee, about 90% ee to about 100% ee, about 95% ee to about 100%, about 80% ee to about 99.5% ee, about 85% ee to about 99.5% ee, about 90% ee to about 99.5% ee, about 95% ee to about 99.5%, or any range thereof.

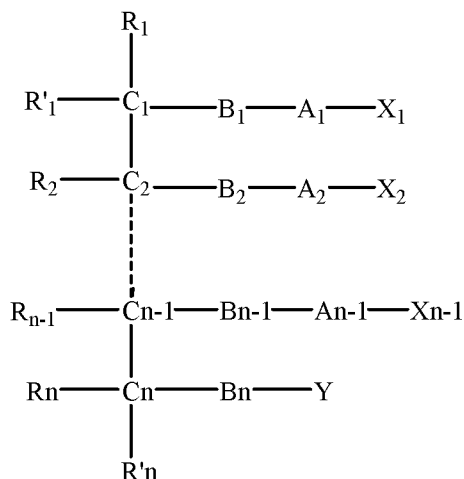
[0226] In other embodiments, the oxidized lipid compound treats or prevents fibrosis (e.g., liver fibrosis, kidney fibrosis, focal and segmental glomerulosclerosis, or any other fibrosis described herein) as well as, or better than, telmisartan. In other embodiments, the oxidized lipid compound reduces liver inflammation as well as, or better than, telmisartan. In other embodiments, the oxidized lipid compound reduces liver fibrosis as well as, or better than, telmisartan. In other embodiments, an oxidized lipid compound of the invention treats or prevents kidney fibrosis as well as, or better than, telmisartan. In other embodiments, an oxidized lipid compound of the invention treats or prevents focal and segmental glomerulosclerosis as well as, or better than, telmisartan.

[0227] In other embodiments, the oxidized lipid compound inhibits formation of ligand-induced phosphorylation of IKK, ERK, AKT, or p38 comparable to or more than VB-201 (1-hexadecyl-2-(4'-carboxy)butyl-glycero-3-phosphocholine or 1-hexadecyl-2-(4'-carboxybutyl)-glycerol-3-phosphocholine) inhibits formation of ligand-induced

phosphorylation of IKK, ERK, AKT, or p38. In other embodiments, the compound inhibits ligand-induced cell migration comparable to or more than VB-201 inhibits ligand-induced cell migration. In other embodiments, the ligand is LPS, PGN, MCP1, or PAM3. In other embodiments, the cell migration is monocyte migration.

[0228] In other embodiments, the subject is a mammal or a human. In other embodiments, the human is a female. In other embodiments, the human is a male.

[0229] In some embodiments, an oxidized lipid of the invention is a compound having a structure according to Formula 6:



Formula 6

[0230] or a stereoisomer, a stereoisomeric mixture, or a salt thereof,

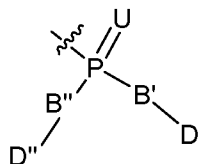
[0231] wherein:

[0232] n is an integer from 1 to 6, wherein when n is 1, C_n, B_n, R_n, and Y are absent, and C₁ is attached to R'_n;

[0233] each of B₁, B₂, ...B_{n-1} and B_n is independently selected from the group consisting of oxygen, sulfur, nitrogen, phosphorus and silicon, wherein each of said nitrogen, phosphorus and silicon is optionally substituted by one or more substituents selected from the group consisting of alkyl, halo, cycloalkyl, aryl, hydroxy, thiohydroxy, alkoxy, aryloxy, thioaryloxy, thioalkoxy, and oxo;

[0234] each of A₁, A₂, ...A_{n-1} and A_n is independently selected from the group consisting of CR''R''', C=O and C=S,

[0235] Y is a moiety having the general formula:

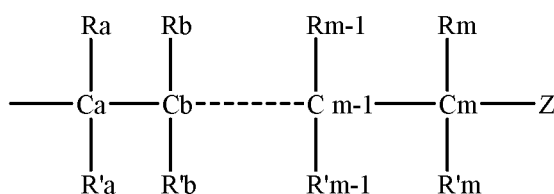


[0236] wherein:

[0237] each of U, B' and B'' is independently selected from the group consisting of sulfur and oxygen; and

[0238] each of D' and D'' is independently selected from the group consisting of hydrogen, a negative charge, alkyl, amino substituted alkyl, heteroalicyclic substituted alkyl, heteroaryl substituted alkyl, cycloalkyl, phosphonate, and thiophosphonate; and

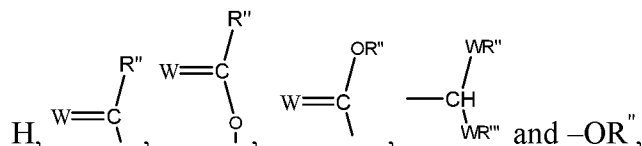
[0239] each of X₁, X₂, ... X_{n-1} is independently a saturated or unsaturated hydrocarbon having the general Formula 7:



Formula 7

[0240] wherein m is an integer from 1 to 26; and

[0241] Z is selected from the group consisting of:



[0242] wherein W is selected from the group consisting of oxygen and sulfur;

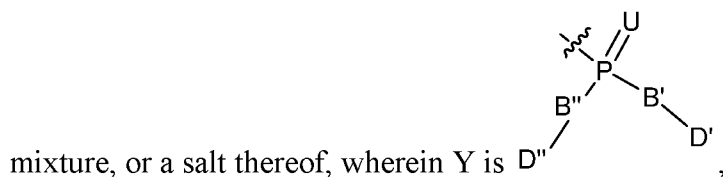
[0243] wherein at least one of X₁, X₂, ... X_{n-1} comprises a Z other than hydrogen,

[0244] and wherein:

[0245] each of R₁, R'₁, R₂, ... R_{n-1}, R_n, R'_n, each of R'' and R''' and each of Ra, R'a, Rb, R'b, ... R_{m-1}, R'm-1, R_m and R'm is independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, halo, trihalomethyl, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, phosphonate, phosphate, phosphinyl, sulfonyl, sulfinyl, sulfonamide, amide, carbonyl, thiocarbonyl, C-carboxy, O-carboxy, C-carbamate, N-carbamate, C-thiocarboxy, S-thiocarboxy and amino, or,

alternatively, at least two of R₁, R'₁, R₂, ...R_{n-1}, R_n and R'_n and/or at least two of R_a, R'_a, R_b, R'_b, ...R_{m-1}, R'_{m-1}, R_m and R'_m form at least one four-, five- or six-membered aromatic, heteroaromatic, alicyclic or heteroalicyclic ring.

[0246] In some embodiments, an oxidized lipid of the invention is a compound having a structure according to Formula 6 as defined herein, or a stereoisomer, a stereoisomeric



[0247] wherein:

[0248] n is 3;

[0249] each of U, B' and B'' is oxygen; and

[0250] wherein one of D' and D'' is a heteroalicyclic substituted alkyl or heteroaryl substituted alkyl, and the other of D' and D'' is hydrogen or a negative charge. In some embodiments, one of D' and D'' is a heteroaryl substituted alkyl, and the other of D' and D'' is hydrogen or a negative charge.

[0251] In some embodiments, the invention relates to deuterated analogs, prodrugs, hydrates, and solvates of any of the oxidized lipid described herein (e.g., having a structure according to Formulae 1-6).

EXAMPLES

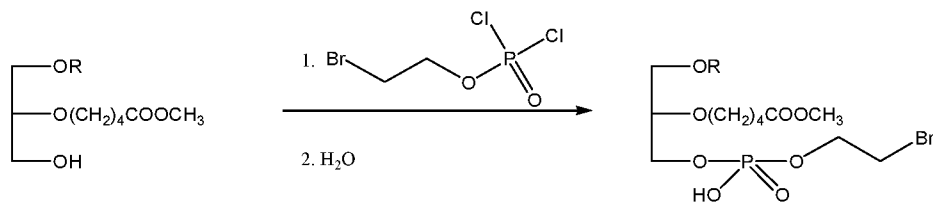
[0252] Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non-limiting fashion.

Example 1

General Synthetic Procedures

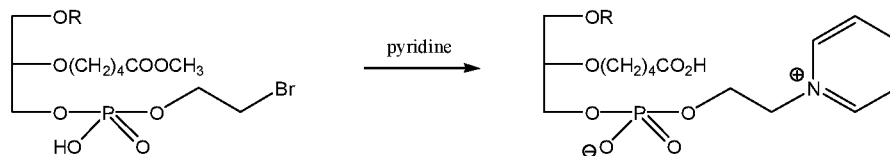
Procedure A

1-alkyl-2-(4'-carboxymethyl)butyl-glycero-sn-3-phosphoric acid bromoethyl ester

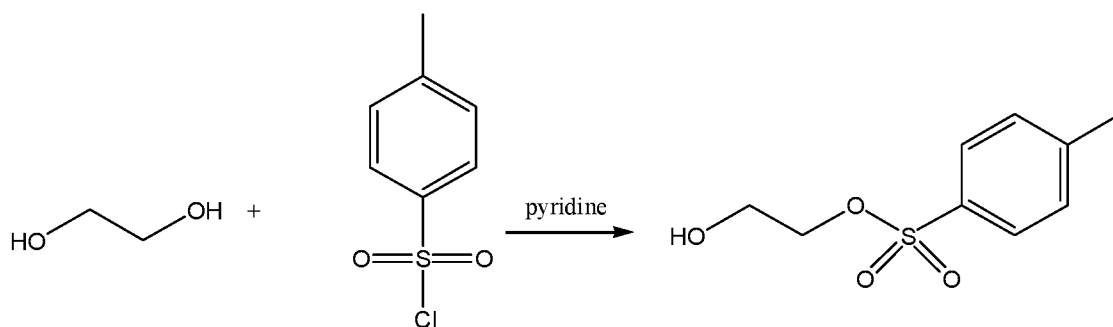


[0253] 1-alkyl-2-(4'-carboxymethyl)butyl-glycerol was dried by azeotropic distillation from benzene. After cooling to room temperature under nitrogen, (2-bromoethyl)-phosphorylchloride (1.5eq) was added and the mixture resulted was stirred at room temperature for 15 minutes. The reaction mixture was then cooled in an ice bath. Pyridine (anhydrous, 1.2eq) was then added dropwise. The reaction mixture was then allowed to stir at room temperature overnight. After which, the solvent was removed under reduced pressure. Water was then added and the reaction mixture was refluxed for 1 hr. After cooling to room temperature, the reaction mixture was extracted with ether. The combined organic phase was washed with water, dried over sodium sulfate, and then concentrated under reduced pressure to yield 1-alkyl-2-(4'-carboxymethyl)butyl-glycero-sn-3-phosphoric acid bromoethyl ester.

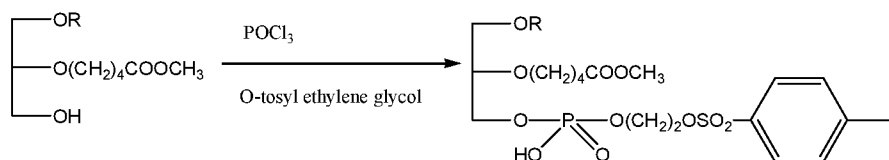
1-alkyl-2-(4'-carboxy)butyl-glycero-sn-3-phosphoric acid pyridiniummethyl ester



[0254] 1-alkyl-2-(4'-carboxymethyl)butyl-glycero-sn-3-phosphoric acid bromoethyl ester was dissolved in pyridine (anhydrous). The reaction mixture resulted was refluxed for 22 hrs. After removal of the pyridine under reduced pressure, the residue was dissolved in methanol. Sodium bicarbonate (4.5eq) was then added. The resulting mixture was refluxed for 1hr and hot filtered. The residue obtained after washing of the filtrate with methanol and removal of solvent under reduced pressure was purified by chromatography on a column of silica gel. Elution with mixtures of CHCl₃: MeOH (5-20%, v/v), followed by CHCl₃: MeOH: H₂O (70:26:4, v/v/v).

Procedure BO-tosyl ethylene glycol

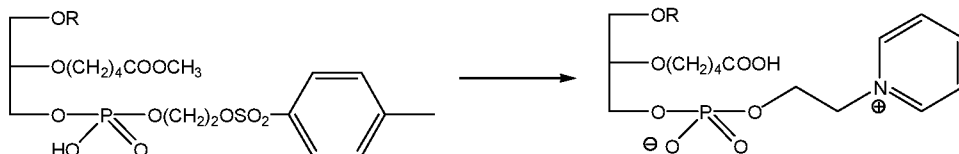
[0255] A solution of p-toluenesulfonyl chloride (7 g) in dichloromethane (anhydrous, 50 ml) was added dropwise at 0 °C to a stirred solution of ethylene glycol (anhydrous, 20 ml) in dichloromethane (anhydrous, 100 ml) and pyridine (anhydrous, 7 ml). The resulting mixture was stirred at room temperature overnight and then poured on ice and allowed to reach room temperature. The reaction mixture was extracted with dichloromethane (3 x 100 ml) and the combined organic phase was washed sequentially with water (150 ml), sulfuric acid (2%, 100 ml), water (150 ml), saturated sodium bicarbonate (150 ml) and again with water (150 ml). Removing the solvent under reduced pressure yielded O-tosyl ethylene glycol 7.74g as a colorless oil.

1-alkyl-2-(4'-carboxymethyl)butyl-glycero-sn-3-phosphoric acid tosyl ethyl ester

[0256] To a solution of 1-alkyl-2-(4'-carboxymethyl)butyl-glycerol (dried by azeotropic distillation with benzene) and triethylamine (3eq) in dry THF, an ice cooled solution of Phosphorus oxychloride (1.2eq) in dry THF was added dropwise (over 2hrs). The reaction mixture was stirred at 0 °C for an additional 15 minutes and then at room temperature of 45 minutes. After which, the reaction mixture was cooled again to 0 °C. A solution of O-tosyl ethylene glycol (1.1eq, dried by azeotropic distillation from benzene) in dry THF was added dropwise over 30 min. The resulting mixture was stirred at room temperature for overnight, and then filtered. The solvent was removed under reduced pressure. The residue obtained was dissolved in water and was refluxed for 1 hr. After being cooled to room temperature, the reaction mixture was extracted with ether.

The combined organic phase was washed with water and concentrated under reduced pressure. The residue resulted was purified by chromatography on silica gel. Elution with a mixture of CHCl_3 : MeOH (5-20%, v/v), followed by removal of solvent under reduced pressure yielded 1-alkyl-2-(4'-carboxymethyl)butyl-glycero-sn-3-phosphoric acid tosyl ethyl ester.

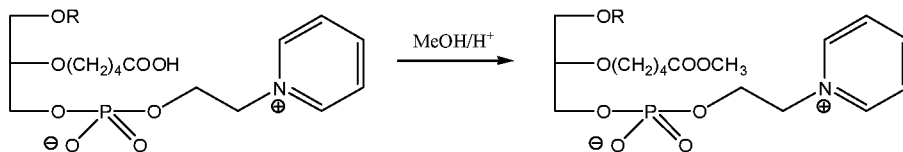
1-alkyl-2-(4'-carboxy)butyl-glycero-sn-3-phosphoric acid pyridinium methyl ester



[0257] A solution of 1-alkyl-2-(4'-carboxymethyl)butyl-glycero-sn-3-phosphoric acid tosyl ethyl ester in pyridine was stirred and heated to 40°C for 6 hrs. The solution was then cooled and stirred at room temperature overnight. After removing the solvent under reduced pressure, the residue obtained was purified by chromatography on silica gel. A mixture of CHCl_3 : MeOH (5-20%, v/v) was used as initial eluent, which was followed by a mixture of CHCl_3 : MeOH: H_2O (70:26:4, v/v/v). The fractions collected were concentrated under reduced pressure. The residue obtained was then dissolved in chloroform, which was dried over sodium sulfate. Removal of chloroform under reduced pressure then yielded purified 1-alkyl-2-(4'-carboxy)butyl-glycero-sn-3-phosphoric acid pyridinium methyl ester.

[0258] The general procedures above are applicable for synthesis of the oxidized lipid described herein. For methyl ester methylation, there is an additional step in the procedure: methylation in methanol and HCl.

General Procedure for Methyl Ester Formation



[0259] 1-alkyl-2-(4'-carboxy)butyl-glycero-sn-3-phosphoric acid pyridinium methyl ester was dissolved in methanol. Hydrochloric acid was added to the methanol solution and the mixture stirred at room temperature for 4 hrs. After which, water was added to the reaction mixture. The resulting mixture was extracted with CHCl_3 . The organic phase was washed sequentially with water, saturated sodium bicarbonate and again with water,

and then dried over sodium sulfate. Removal of the solvent under reduced pressure then yielded 1-alkyl-2-(4'-carboxymethyl)butyl-glycero-sn-3-phosphoric acid pyridiniummethyl ester.

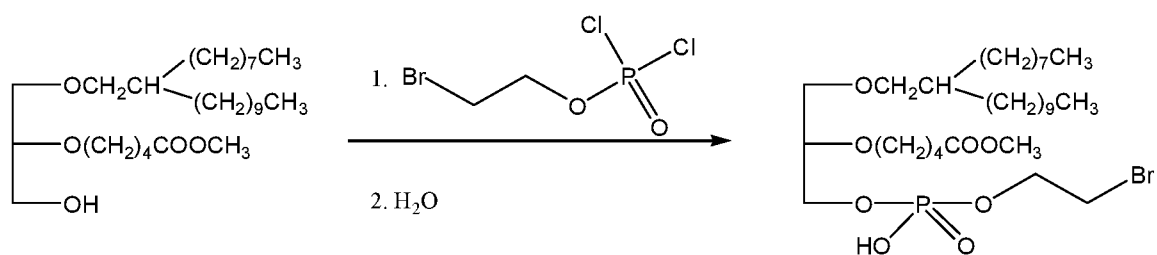
Example 2

Synthesis of VB-703

[0260] VB-703 was synthesized by two synthetic procedures according to the general procedures described above.

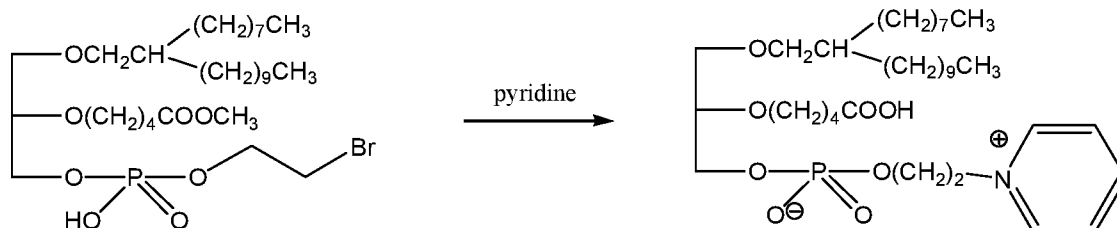
Procedure A

1-(2'-octyl)dodecyl-2-(4'-carboxymethyl)butyl-glycero-sn-3-phosphoric acid bromoethyl ester



[0261] 1-(2'-octyl)dodecyl-2-(4'-carboxymethyl)butyl-glycerol (13.75 g) was dissolved in benzene (100 ml) and dried by azeotropic distillation of 50 ml benzene. After cooling to room temperature under nitrogen, (2-bromoethyl)-phosphorylchloride (5.43 ml, 10.25 g) was added. The resulting mixture was stirred at room temperature for 15 minutes and then cooled to 0 °C. Pyridine (anhydrous, 2.73 ml, 2.68 g) was added dropwise. The reaction mixture was stirred at room temperature overnight and then the solvent removed under reduced pressure. Water (100 ml) was added and the mixture refluxed for 1 hr. After cooling to room temperature, the reaction mixture was extracted with ether (3 x 100 ml) and washed with water (100 ml). Drying over sodium sulfate and removal of the solvent under reduced pressure yielded 15.06 g of 1-(2'-octyl)dodecyl-2-(4'-carboxymethyl)butyl-glycero-sn-3-phosphoric acid bromoethyl ester.

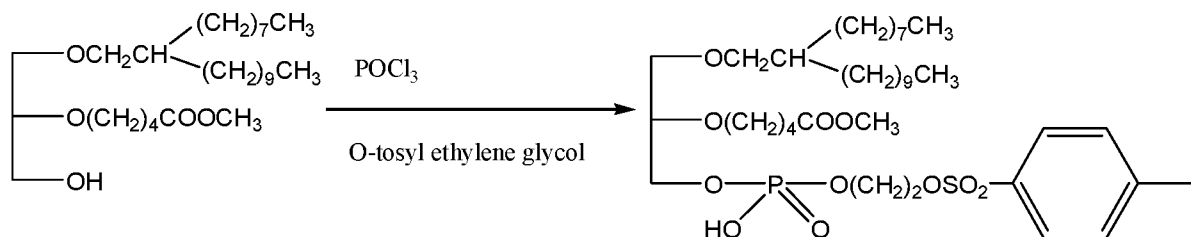
1-(2'-octyl)dodecyl-2-(4'-carboxy)butyl-glycero-sn-3-phosphoric acid pyridiniummethyl ester



[0262] 1-(2'-octyl)dodecyl-2-(4'-carboxymethyl)butyl-glycero-sn-3-phosphoric acid bromoethyl ester (15.06 g) was dissolved in pyridine (anhydrous, 50 ml) and the reaction mixture refluxed for 22 hrs. After removal of the solvent under reduced pressure, the residue was dissolved in methanol (100 ml) and sodium bicarbonate (10.68 g) was added. The resulting solution was refluxed for 1 hour (hr) and hot filtered. The residue obtained after washing of the filtrate with methanol (2 x 15 ml) and removal of solvent under reduced pressure was purified by chromatography on a column of silica gel (213 g). Elution with mixtures of CHCl_3 : MeOH (5-20%, v/v) followed by CHCl_3 : MeOH: H_2O (70:26:4, v/v/v). The solvent was removed under reduced pressure and the residue dissolved in chloroform. Drying of the solution over sodium sulfate, filtration and removal of the solvent under reduced pressure yielded 2.42 g 1-(2'-octyl)dodecyl-2-(4'-carboxy)butyl-glycero-sn-3-phosphoric acid pyridiniummethyl ester.

Procedure B

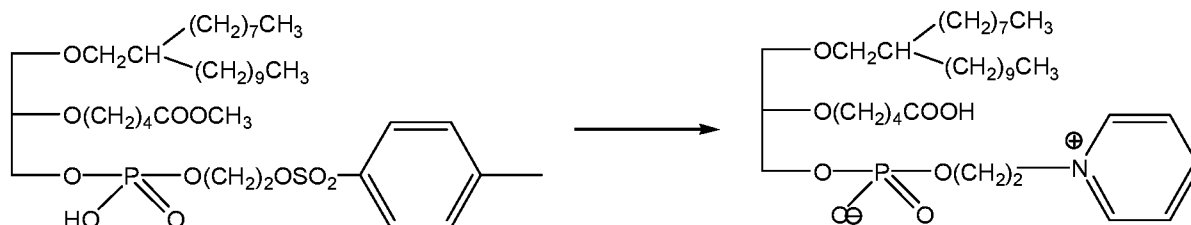
1-(2'-octyl)dodecyl-2-(4'-carboxymethyl)butyl-glycero-sn-3-phosphoric acid tosyl ethyl ester



[0263] A solution of 1-(2'-octyl)dodecyl-2-(4'-carboxymethyl)butyl-glycerol (7.47 g, dried by azeotropic distillation with benzene) and triethylamine (7 ml) in dry THF (80 ml)

was added dropwise for 2 hrs at 0 °C to a stirred solution of phosphorus oxychloride (2 ml) in dry THF (50 ml). The reaction mixture stirred at 0°C for an additional 15 minutes and at room temperature for an additional 45 minutes. The reaction mixture was cooled again to 0 °C and a solution of O-tosyl ethylene glycol (3.65 g, dried by azeotropic distillation with benzene) in dry THF (50 ml) was added dropwise for 30 min. The resulting mixture was stirred at room temperature overnight, then filtered, and the solvent removed under reduced pressure. The residue was dissolved in water (100 ml), refluxed for 1 hr, then cooled and extracted with ether (2 x 100 ml). The combined organic phase was washed with water (100 ml) and the solvent removed under reduced pressure. The residue (9.62 g) was purified by chromatography on a column of silica gel (250 g). Elution with mixtures of CHCl₃: MeOH (5-20%, v/v) and removal of solvent under reduced pressure yielded 6.90 g of 1-(2'-octyl)dodecyl-2-(4'-carboxymethyl)butyl-glycero-sn-3-phosphoric acid tosyl ethyl ester.

1-(2'-octyl)dodecyl-2-(4'-carboxy)butyl-glycero-sn-3-phosphoric acid pyridinium methyl ester



[0264] A solution of 1-(2'-octyl)dodecyl-2-(4'-carboxymethyl)butyl-glycero-sn-3-phosphoric acid tosyl ethyl ester (2.20 g) in pyridine (30 ml) was stirred and heated to 40 °C for 6 hrs, then cooled and stirred at room temperature overnight. After removing the solvent under reduced pressure, the residue was purified by chromatography on column of silica gel (95 g). Elution was done with mixtures of CHCl₃: MeOH (5-20%, v/v) followed by CHCl₃: MeOH: H₂O (70:26:4, v/v/v). The solvent was removed under reduced pressure and the residue dissolved in chloroform. Drying of the solution over sodium sulfate, filtration and removal of the solvent under reduced pressure yielded 0.4 g of 1-(2'-octyl)dodecyl-2-(4'-carboxy)butyl-glycero-sn-3-phosphoric acid tosyl ethyl ester.

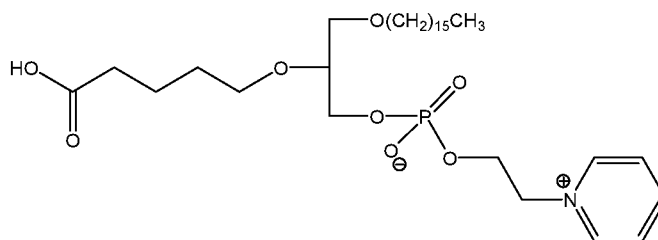
[0265] Mass spectrometry (MS): Calculated for $C_{35}H_{64}NO_8P$ 657. Found (ESI+) 658 $[M+H]^+$, 680 $[M+Na]^+$ and 696 $[M+K]^+$.

[0266] 1H -NMR (700MHz, $CDCl_3$, TMS ref.): δ 0.88 (t, 6H, 2 CH₃), 1.24-1.29 (m, 32H, 16 CH₂), 1.53 (m, 1H, CH), 1.59 (tt, 2H, CH₂), 1.69 (m, 1H, CH₂), 2.34 (t, 2H, CH₂), 3.29 (dt, 2H, CH₂), 3.41 (m, 1H, CH₂), 3.46 (m, 1H, CH₂), 3.55 (m, 1H, CH₂), 3.60 (m, 1H, CH), 3.66 (m, 1H, CH₂), 3.77 (m, 1H, CH₂), 3.88 (br s, 1H, CH₂), 4.33 ((br, 2H, CH₂), 4.87 ((br, 2H, CH₂), 8.07 (t, 2H, meta-aromatic), 8.49 (t, 1H, para-aromatic), 9.03 (d, 2H, ortho-aromatic).

[0267] ^{13}C -NMR: δ 14.17, 22.07, 22.85, 26.99, 27.01, 29.50, 29.54, 29.83, 29.84, 29.88, 30.29, 31.42, 31.46, 32.11, 34.09, 38.35, 62.41, 63.86, 65.99, 70.20, 71.16, 75.20, 78.29 (CH), 128.35 (meta aromatic), 145.66 (para aromatic), 145.75 (ortho aromatic), 176.67 (CO₂H).

Example 3

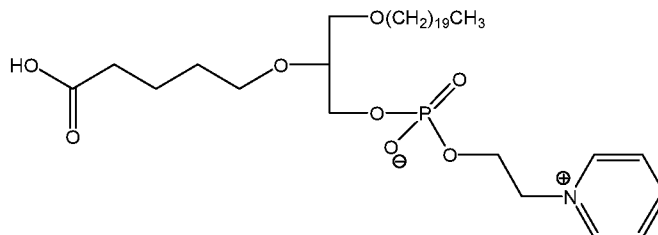
Synthesis of VB-701



[0268] 1-hexadecyl-2-(4'-carboxy)butyl-glycero-sn-3-phosphoric acid pyridiniummethyl ester (VB-701) was synthesized by following the general procedure A as described in Example 1. Mass spectrometry (MS): Calculated for $C_{31}H_{56}NO_8P$ 601 Found (ESI+) 602 $[M+H]^+$ and 624 $[M+Na]^+$.

Example 4

Synthesis of VB-702

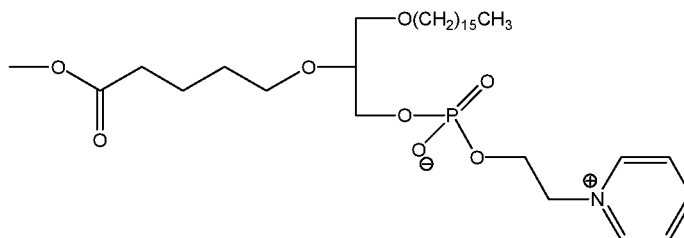


[0269] 1-eicosanyl-2-(4'-carboxy)butyl-glycero-sn-3-phosphoric acid pyridiniummethyl ester (VB-702) was synthesized by following the general procedure A as described in Example 1. Mass spectrometry (MS): Calculated for $C_{35}H_{64}NO_8P$ 657.4. Found (ESI+) 658.5 $[M+H]^+$ and 680.5 $[M+Na]^+$.

[0270] 1H -NMR (700MHz, $CDCl_3$, TMS ref.): δ 0.89 (t, 3H, CH₃), 1.27-1.32 (m, 34H, 17 CH₂), 1.54 (tt, 2H, CH₂), 1.60 (m, 2H, CH₂), 1.66 (m, 1H, CH₂), 1.73 (m, 1H, CH₂), 2.31 (br, t, 2H, CH₂), 3.41 (m, 1H, CH₂), 3.42 (m, 1H, CH₂), 3.47 (m, 1H, CH₂), 3.56 (m, 1H, CH), 3.58 (m, 1H, CH₂), 3.65 (m, 1H, CH₂), 3.80 (m, 1H, CH₂), 3.88 (m, 1H, CH₂), 4.38 ((br, 2H, CH₂), 4.95 ((br, 2H, CH₂), 8.10 (t, 2H, aromatic), 8.52 (t, 1H, aromatic), 9.10 (br, 2H, aromatic). ^{13}C -NMR: δ 14.22, 22.41, 22.99, 29.68, 29.85, 29.98-30.02, 32.26, 35.24, 62.51, 64.24, 66.02, 70.39, 70.56, 72.17, 78.27 (CH), 128.47 (aromatic), 145.72 (aromatic), 146.09 (aromatic), 179.08 (CO₂H).

Example 5

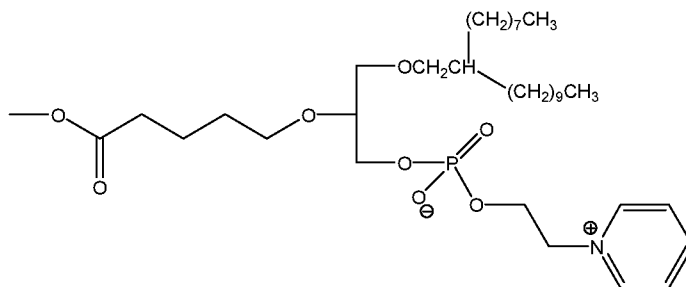
Synthesis of VB-704



[0271] 1-hexadecyl-2-(4'-carboxymethyl)butyl-glycero-sn-3-phosphoric acid pyridiniummethyl ester (VB-704) was synthesized by following the general procedure for methyl ester formation as described in Example 1. Mass spectrometry (MS): Calculated for $C_{32}H_{58}NO_8P$ 615; Found (ESI+) 616 $[M+H]^+$ and 638 $[M+Na]^+$.

Example 6

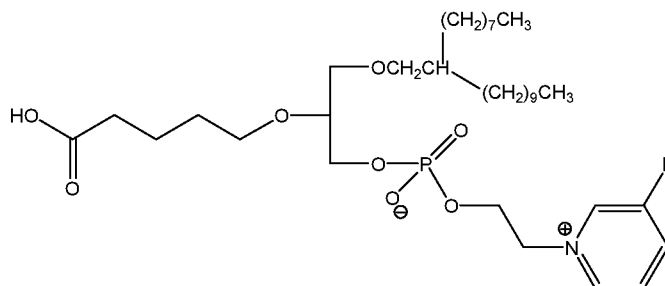
Synthesis of VB-705



[0272] 1-(2'-octyl)dodecyl-2-(4'-carboxymethyl)butyl-glycero-sn-3-phosphoric acid pyridiniummethyl ester (VB-705) was synthesized by following the general procedure for methyl ester formation as described in Example 1. Mass spectrometry (MS): Calculated for $C_{36}H_{66}NO_8P$ 671; Found (ESI+) 672 $[M+H]^+$ and 694 $[M+Na]^+$.

Example 7

Synthesis of VB-706



[0273] 1-(2'-octyl)dodecyl-2-(4'-carboxy)butyl-glycero-sn-3-phosphoric acid 3-fluoropyridiniummethyl ester (VB-706) was synthesized by following the general procedure B as described in Example 1. Mass spectrometry (MS): Calculated for $C_{35}H_{63}FNO_8P$ 675.43. Found (ESI+) 676.58 $[M+H]^+$ and 698.57 $[M+Na]^+$.

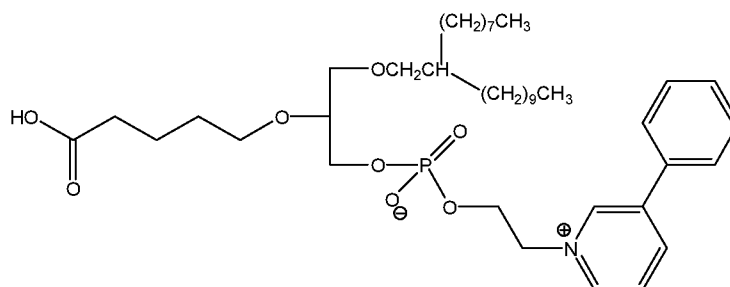
[0274] 1H -NMR (700MHz, $CDCl_3$, TMS ref.): δ 0.88 (t, 6H, 2 CH₃), 1.26-1.30 (m, 32H, 16 CH₂), 1.53 (m, 1H, CH), 1.60 (tt, 2H, CH₂), 1.69 (m, 1H, CH₂), 2.31 (t, 2H, CH₂), 3.30 (dt, 2H, CH₂), 3.42 (m, 1H, CH₂), 3.46 (m, 1H, CH₂), (3.55 (m, 1H, CH₂), 3.60 (m, 1H, CH₂), 3.68 (m, 1H, CH₂), 3.76 (m, 1H, CH₂), 3.89 (m, 1H, CH₂), 4.31 (br, 2H, CH₂), 4.88 ((br, 2H, CH₂), 8.14 (br, 1H, meta-aromatic), 8.35 (t, 1H, para-aromatic), 8.96 (d, 1H, ortho-aromatic), 9.25 (s, 1H, ortho-aromatic).

[0275] ^{13}C -NMR: δ 14.18, 22.39, 22.88, 27.02, 27.04, 29.50-30.30, 31.44, 31.48, 32.14, 35.00, 38.37, 63.08, 63.66, 66.08, 70.30, 75.24, 78.37 (CH), 129.65 (meta aromatic), 133.36 (para aromatic), 135.79 (ortho-aromatic), 143.05 (ortho aromatic), 160.81 (CF), 178.16 (CO₂H).

[0276] ^{19}F NMR (376.4 MHz) -111.55.

Example 8

Synthesis of VB-707



[0277] 1-(2'-octyl)dodecyl-2-(4'-carboxy)butyl-glycero-sn-3-phosphoric acid 3-phenylpyridiniummethyl ester (VB-707) was synthesized by following the general procedure B as described in Example 1. Mass spectrometry (MS): Calculated for C₄₁H₆₈NO₈P 733. Found (ESI+) 734 [M+H]⁺ and 756 [M+Na]⁺.

Example 9

VB-701 Inhibits LPS (TLR4)-Induced Signaling in Human Monocytes (primary CD14+)

Methods and Materials

Isolation of monocytes

[0278] Venous blood samples were obtained from healthy male donors in compliance with the Institutional Review Board at the Sheba Medical Center, Ramat Gan, Israel. PBMCs were isolated on Ficoll-Paque PLUS (GE Healthcare, Uppsala, Sweden) using 50 ml Leucosep tubes (Greiner Bio-One, Frickenhausen, Germany). Cells were washed in PBS (Kibbutz Beit Haemek, Israel) and incubated at 4 °C for 15 minutes in a buffer containing PBS and 0.5% bovine serum albumin (BSA) with human CD14 microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany).

Activation of cells and Western blotting

[0279] Cells (10^6 /ml) were pretreated for 20 min with VB-201 (1-hexadecyl-2-(4'-carboxy)butyl-glycero-3-phosphocholine or 1-hexadecyl-2-(4'-carboxybutyl)-glycerol-3-phosphocholine) or VB-701 at the doses indicated in Figure 2, or with solvent (Sol), followed by 15 min activation with 100 ng/ml lipopolysaccharide (LPS) or were untreated (Unt). Cells were washed and resuspended in lysis buffer containing 1:100 dithiothreitol (DTT), phosphatase and protease inhibitors (Thermo Scientific). Samples were loaded onto a precast Criterion TGX gel (Bio-Rad, Hemel Hempstead, UK) and transferred onto nitrocellulose membrane. Blots were blocked with 5% milk or BSA in Tris buffered saline and Tween 20 (TBST) for 1 h, followed by incubation with primary and secondary antibodies. Membranes were developed using an ECL kit (Thermo Scientific). The following antibodies were used for immunoblotting:

[0280] Primary antibodies: p-p38 (Cat. No. 4511; 1:1000) and p-IKK (Cat. No. 2697; 1:1000) were from Cell Signaling Technology (Danvers, MA, USA). p-ERK1/2 (Cat. No. M8159; 1:10 000) was purchased from Sigma (Israel). α Tubulin (Tub) served as a loading control.

[0281] Secondary antibodies: HRP donkey anti-rabbit (1:5000) and HRP goat anti-mouse (1:3000) were from Jackson ImmunoResearch (West Grove, PA, USA). HRP donkey anti-goat (1:5000) was from Santa Cruz Biotechnology.

Results

[0282] Figure 2 shows that VB-701 and VB-201 inhibit formation of p-IKK, p-ERK and p-p38 induced by LPS in human monocytes. Accordingly, VB-701 and VB-201 inhibit LPS (TLR4)-induced signaling in human monocytes (primary CD14+).

Example 10

VB-701 Inhibits PGN (TLR2)-Induced Signaling in Human Monocytes (THP-1 cell line)

*Methods and Materials*Activation of cells and Western blotting

[0283] The monocytic THP-1 cell line was purchased from the American Type Tissue Culture Collection (ATCC Cat. No. TIB-202). Cells (10^6 /ml) were pretreated for 20 min with VB-201 or VB-701 at the doses indicated in Figure 3, or with solvent, followed by activation with 20 μ g/ml peptidoglycan (PGN) (InvivoGen, San Diego, CA) for 15 minutes, or were untreated ("Unt"). Cells were washed and resuspended in lysis buffer containing 1:100 dithiothreitol (DTT), phosphatase and protease inhibitors (Thermo Scientific). Samples were loaded onto a precast Criterion TGX gel (Bio-Rad, Hemel Hempstead, UK) and transferred onto nitrocellulose membrane. Blots were blocked with 5% milk or BSA in Tris buffered saline and Tween 20 (TBST) for 1 h, followed by incubation with primary and secondary antibodies. Membranes were developed using an ECL kit (Thermo Scientific). The following antibodies were used for immunoblotting:

[0284] Primary antibodies: p-p38 (Cat. No. 4511; 1:1000) and p-IKK (Cat. No. 2697; 1:1000) were from Cell Signaling Technology (Danvers, MA, USA). p-ERK1/2 (Cat. No. M8159; 1:10000) was purchased from Sigma (Israel). α Tubulin served as a loading control.

[0285] Secondary antibodies: HRP donkey anti-rabbit (1:5000) and HRP goat anti-mouse (1:3000) were from Jackson ImmunoResearch (West Grove, PA, USA). HRP donkey anti-goat (1:5000) was from Santa Cruz Biotechnology.

Results

[0286] Figure 3 shows that VB-701 and VB-201 inhibit formation of p-IKK, p-ERK and p-p38 induced by PGN in THP-1 cells. Accordingly, VB-701 and VB-201 inhibit PGN (TLR2)-induced signaling.

Example 11

VB-701 Inhibits MCP-1-Induced Signaling in Human Monocytes (THP-1 cell line)

*Methods and Materials*Activation of cells and Western blotting

[0287] THP-1 cells (10^6 /ml) were pretreated for 20 min with VB-201 or VB-701 at the doses indicated in Figure 4, or with solvent, followed by activation with 50 ng/ml MCP1, or were untreated ("Unt"). Cells were washed and resuspended in lysis buffer containing 1:100 dithiothreitol (DTT), phosphatase and protease inhibitors (Thermo Scientific). Samples were loaded onto a precast Criterion TGX gel (Bio-Rad, Hemel Hempstead, UK) and transferred onto nitrocellulose membrane. Blots were blocked with 5% milk or BSA in Tris buffered saline and Tween 20 (TBST) for 1 h, followed by incubation with primary and secondary antibodies. Membranes were developed using an ECL kit (Thermo Scientific). The following antibodies were used for immunoblotting:

[0288] Primary antibodies: p-ERK1/2 (Cat. No. M8159; 1:10000) was purchased from Sigma (Israel). p-AKT (Cat. No. 4060; 1:1000) was purchased from Cell Signaling Technology (Danvers, MA). α Tubulin served as a loading control and was purchased from Sigma (Israel).

[0289] Secondary antibodies: HRP donkey anti-rabbit (1:5000) and HRP goat anti-mouse (1:3000) were from Jackson ImmunoResearch (West Grove, PA, USA). HRP donkey anti-goat (1:5000) was from Santa Cruz Biotechnology.

Results

[0290] Figure 4 shows that VB-701 and VB-201 inhibit formation of p-AKT and p-ERK induced by MCP1 in THP-1 cells. Accordingly, VB-701 and VB-201 inhibit MCP1-induced signaling.

Example 12

VB-701 Inhibits Chemokine-Induced Migration of Human Monocytes (primary CD14+)

*Methods and Materials*Isolation of monocytes

[0291] Venous blood samples were obtained from healthy male donors in compliance with the Institutional Review Board at the Sheba Medical Center, Ramat Gan, Israel. PBMCs were isolated on Ficoll-Paque PLUS (GE Healthcare, Uppsala, Sweden) using 50 ml Leucosep tubes (Greiner Bio-One, Frickenhausen, Germany). Cells were washed in PBS (Kibbutz Beit Haemek, Israel) and incubated at 4 °C for 15 minutes in a buffer containing PBS and 0.5% bovine serum albumin (BSA) with human CD14 microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany).

Activation of cells and cell migration trans-well assay

[0292] Cells (10^6 /ml) were pretreated for 20 min with VB-201 or VB-701 at the doses indicated in Figure 5, or with solvent (Sol).

[0293] To test for chemokine-induced cell migration, RANTES (100 ng/ml; Cat. No. 300-06, PeproTech, Israel) and MCP-1 (50 ng/ml; Cat. No. 300-04, PeproTech, Israel) were dissolved in RPMI-1640 medium supplemented with 0.5% fetal bovine serum (FBS) and placed at the lower chamber of QCM 24-well, 5 mm pore, migration assay plates (Corning-Costar, Corning, NY). Cells (3×10^5) were seeded in the upper chamber and incubated for 2-4 hours. Subsequently, the number of cells which migrated to the lower compartment was determined by fluorescence-activated cell sorting (FACS).

Results

[0294] Figure 5 shows that VB-701 and VB-201 inhibit chemokine-induced migration of human monocytes (primary CD14+).

Example 13

VB-702 Inhibits LPS (TLR4)-Induced Signaling in Human Monocytes (primary CD14+)

[0295] Human monocytes were obtained, treated and analyzed by western blot as described in Example 9 and Figure 6. Figure 6 shows that VB-702 and VB-201 inhibit formation of p-IKK, p-ERK and p-p38 induced by LPS in human monocytes. Accordingly, VB-702 and VB-201 inhibit LPS (TLR4)-induced signaling in human monocytes (primary CD14+).

Example 14

VB-702 Inhibits RANTES-Induced Signaling in Human Monocytes (primary CD14+)

[0296] Human monocytes were obtained, treated and analyzed by western blot as described in Example 9 and Figure 7, except that cells were induced with RANTES (100 ng/ml; Cat. No. 300-06, PeproTech, Israel) for 15 minutes. Figure 7 shows that VB-702 and VB-201 inhibit formation of p-ERK induced by RANTES in human monocytes. Accordingly, VB-702 and VB-201 inhibit RANTES-induced signaling in human monocytes (primary CD14+).

Example 15

VB-702 Inhibits Chemokine-Induced Migration of Human Monocytes (primary CD14+)

[0297] Human monocytes were obtained, treated and analyzed for cell migration by trans-well assay as described in Example 12 and Figure 8. Figure 8 shows that VB-702 and VB-201 inhibit chemokine-induced migration of human monocytes (primary CD14+).

Example 16

VB-701 and VB-702 Inhibit IL-12p40 Levels in Human Monocytes (primary CD14+) Stimulated with LPS (via TLR4) or Pam3CSK4 (TLR2-Stimulated)

Methods and Materials

[0298] Human monocytes were obtained as described in Example 9 and Figure 9A-9B. Human monocytes were seeded (10^6 /ml) and pretreated for 1 hour with VB-201, VB-701 or VB-702, followed by 24 hour activation with 100 ng/ml LPS from *Escherichia coli* strain 055:B5 (Sigma, Israel) (Figure 9A) or 300 ng/ml Pam3CSK4 (InvivoGen, San Diego, CA, USA) (Figure 9B) to induce cytokine production. IL-12/23p40 concentration in the supernatant was then measured by ELISA (R&D systems, Cat. No. DY1240). Cells activated with solvent (0.5% ethanol in PBS) were used as a control.

Results

[0299] Figures 9A-9B show that VB-201, VB-701 and VB-702 inhibit IL-12p40 levels in human monocytes (primary CD14+) LPS (TLR4)- and Pam3CSK4 (TLR2)-stimulated.

Example 17

VB-703 Inhibits LPS (TLR4)-Induced Signaling, LPS binding, Liver inflammation and Fibrosis

Methods and Materials

Isolation of monocytes

[0300] Venous blood samples were obtained from healthy male donors in compliance with the Institutional Review Board at the Sheba Medical Center, Ramat Gan, Israel. PBMCs were isolated on Ficoll-Paque PLUS (GE Healthcare, Uppsala, Sweden) using 50 ml Leucosep tubes (Greiner Bio-One, Frickenhausen, Germany). Cells were washed in PBS (Kibbutz Beit Haemek, Israel) and incubated at 4 °C for 15 minutes in a buffer containing PBS and 0.5% bovine serum albumin (BSA) with human CD14 microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany).

Activation of cells and Western blotting

[0301] Cells (10^6 /ml) were pretreated for 20 min with VB-201 or VB-703 followed by 15 min activation with 100 ng/ml LPS. Cells were washed and resuspended in lysis buffer containing 1:100 dithiothreitol (DTT), phosphatase and protease inhibitors (Thermo Scientific). Samples were loaded onto a precast Criterion TGX gel (Bio-Rad, Hemel Hempstead, UK) and transferred onto nitrocellulose membrane. Blots were blocked with 5% milk or BSA in Tris buffered saline and Tween 20 (TBST) for 1 h, followed by incubation with primary and secondary antibodies. Membranes were developed using an ECL kit (Thermo Scientific). The following antibodies were used for immunoblotting:

[0302] Primary antibodies: p-p38 (Cat. No. 4511; 1:1000) and p-IKK (Cat. No. 2697; 1:1000) were from Cell Signaling Technology (Danvers, MA, USA). p-ERK1/2 (Cat. No. M8159; 1:10 000) was purchased from Sigma (Israel). Heat shock protein (HSP) 90 (Cat. No. 13119; 1:500) was from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

[0303] Secondary antibodies: HRP donkey anti-rabbit (1:5000) and HRP goat anti-mouse (1:3000) were from Jackson ImmunoResearch (West Grove, PA, USA). HRP donkey anti-goat (1:5000) was from Santa Cruz Biotechnology.

LPS binding inhibition assay

[0304] To assess interference with lipopolysaccharide (LPS) binding, VB-201 or VB-703 were incubated for 20 min with cells (10^6 /ml) after which 100 ng/ml of biotin-LPS (InvivoGen) was added for an additional 15 minutes, all at 4 °C. Cells were washed, resuspended in FACS buffer and analyzed on a FACS-Calibur device.

Induction of NASH and liver fibrosis

[0305] Neonatal male mice exposed to low-dose streptozotocin (STZ) develop liver steatosis with diabetes. Continuous high fat diet (HFD) increases lobular inflammation with foam cell-like macrophages, showing nonalcoholic steatohepatitis (NASH) pathology. NASH was induced in 40 male mice by a single subcutaneous injection of 200 µg per mouse of STZ two days after birth and feeding HFD [57 kcal% fat]) from four weeks of age. Vehicle, VB-703 (4 mg/kg), or telmisartan (10 mg/kg) as positive control, were administered once daily for three weeks, starting from six weeks of age. Mice were sacrificed at nine weeks of age.

Steatohepatitis and fibrosis evaluation

[0306] Liver pathology was used to determine the effect of VB-703 on liver inflammation and fibrosis. Histology slides were stained with hematoxylin/eosin (H&E) to assess inflammation. The inflammation score was determined as follows:

- 0 - no inflammatory foci
- 1 - <2 inflammatory foci
- 2 - 2-4 inflammatory foci
- 3 - >4 inflammatory foci

[0307] Histology slides were stained with Sirius red to determine collagen content as a marker for the extent of fibrosis.

Q-PCR

[0308] RNA was prepared from livers from normal mice and NASH-induced mice treated with vehicle, VB-703, or telmisartan, using RNeasy mini kit (Qiagen). For cDNA preparation, 2 µg of RNA was combined with qScript reaction mix and qScript Reverse Transcriptase (Quanta BioSciences) for 5 min at 22° C and then for 30 min at 42° C. Reaction was ended by incubation for an additional 5 min at 85° C. All real time PCR reactions were performed using the 7300 Real Time PCR System (Applied Biosystems). Q-PCR with mouse ready set of probe with primer was used for IL-1β, IL-12/23p40 and MCP-1 (Applied Biosystems). GAPDH was used to normalize RNA levels.

Results

Effect of VB-703 and VB-201 on TLR4-mediated signaling events

[0309] To determine the effect of VB-201 and VB-703 on TLR4-mediated signaling pathways, isolated human primary monocytes were preincubated with VB-201 or VB-703 and then activated with LPS. Figure 10A shows that VB-703 inhibited inflammatory cell signaling molecules phosphorylated ERK kinase (p-ERK), phosphorylated p38 (p-p38) and phosphorylated IKK kinase (p-IKK) in a dose dependent manner. Moreover, VB-703 had at least a 10-fold higher efficacy in inhibiting TLR4-driven protein phosphorylation than VB-201. The effect of VB-703 on the binding of LPS to the TLR4 complex was then tested. Figure 10B shows that VB-703 inhibited the binding of LPS

with an IC₅₀ of ~0.5µg/ml, more than a 10-fold lower IC₅₀ than that of VB-201 (~7µg/ml).

VB-703 inhibits liver fibrosis

[0310] The effect of VB-703 on liver inflammation and fibrosis in a NASH mouse model was tested. Treatment with VB-703 significantly decreased fibrosis by 58% compared to vehicle treated mice (Figures 11A-11B). This effect was greater than of the positive control telmisartan (47%). However, VB-703 did not appear to significantly alter steatosis (Figures 12A-12B).

VB-703 inhibits expression of inflammation mediators

[0311] Figures 21A-21C show that the expression of two pro-inflammatory cytokines, IL-1β and IL-12/23p40, and the chemokine MCP-1 were significantly inhibited in livers taken from NASH-induced mice that were treated with VB-703.

Example 18

VB-703 Inhibits PGN (TLR2)-Induced Signaling in Human Monocytes (THP-1 cell line)

[0312] THP-1 cells were obtained, treated and analyzed by western blot as described in Example 10 and Figure 13. Figure 13 shows that VB-703 and VB-201 inhibit formation of p-IKK, p-ERK and p-p38 induced by PGN THP-1 cells. Accordingly, VB-703 and VB-201 inhibit PGN (TLR2)-induced signaling in human monocytes (THP-1 cell line).

Example 19

VB-703 Inhibits IL-6 Secretion in LPS (TLR4)-Stimulated Monocyte-Derived Dendritic Cells (Mo-Derived DCs)

Methods and Materials

[0313] To generate monocyte-derived DC (Mo-DCs), CD14⁺ monocytes were counted, washed and seeded (10⁶/ml) in medium containing RPMI-1640, L-glutamine, β-mercaptoethanol, 10% fetal calf serum (FCS), sodium pyruvate, non-essential amino acids, 0.01 M HEPES, antibiotics (penicillin, streptomycin), 50 ng/ml human

granulocyte-macrophage colony-stimulating factor (GM-CSF) and 20 ng/ml human IL-4 (both from PeproTech Asia, Israel). Medium was replaced every 2-3 days. Mo-DCs were collected 5-6 days post-culture, counted and seeded (10^6 /ml). Cells were pretreated for 1 hour with VB-201 or VB-703, followed by 24 hours activation with 100 ng/ml LPS from *Escherichia coli* strain 055:B5 (Sigma, Israel) to induce cytokine production. IL-6 concentration (Figure 14) and IL-12/23p40 (Figure 15) concentration in supernatant were measured by ELISA (R&D systems, Cat. No. DY1240 and Cat. No. DY206, respectively). Cells activated with solvent (0.5% ethanol in PBS) were used as a control.

Results

[0314] Figures 14 and 15 show VB-703 and VB-201 inhibit IL-6 (Figure 14) and IL-12p40 (Figure 15) secretion in LPS (TLR4) stimulated Mo-Derived DCs.

Example 20

VB-704 Does Not Inhibit LPS-Biotin Binding to Human Monocytes (primary CD14+)

[0315] Human monocytes were obtained, treated and analyzed by LPS binding inhibition assay as described in Example 17 and Figure 16. Figure 16 shows that VB-704 does not inhibit LPS-biotin binding to human monocytes (primary CD14+).

Example 21

VB-704 Inhibits Chemokine-Induced Migration in Human Monocytes (primary CD14+)

[0316] Human monocytes were obtained, treated and analyzed for cell migration by trans-well assay as described in Example 12 and Figure 17. Figure 17 shows that VB-704 inhibits chemokine-induced migration in human monocytes (primary CD14+).

Example 22

VB-705 Inhibits TLR4 Mediated Signaling in Human Monocytes (primary CD14+)

[0317] Human monocytes were obtained, treated and analyzed by western blot as described in Example 9 and Figure 18. Figure 18 shows that VB-705 and VB-201 inhibit formation of p-ERK and p-AKT induced by LPS in human monocytes. Accordingly, VB-705 and VB-201 inhibit LPS (TLR4)-induced signaling in human monocytes (primary CD14+).

Example 23

VB-705 Inhibits LPS Binding in Human Monocytes (primary CD14+)

[0318] Human monocytes were obtained, treated and analyzed with an LPS binding inhibition assay as described in Example 17 and Figure 19. Figure 19 shows that VB-705 inhibits LPS-biotin binding to human monocytes (primary CD14+).

Example 24

VB-705 Does Not Inhibit SDF1-Induced Migration of Human Monocyte (THP-1 cells)

Methods and Materials

[0319] THP-1 cells (10^6 /ml) were pretreated for 20 min with VB-201 or VB-705 at 5 μ g/ml, or with solvent (Sol). To test for chemokine-induced cell migration, RANTES (100 ng/ml, Cat. No. 300-06) (PeproTech, Israel) and MCP-1 (50 ng/ml, Cat. No. 300-04) (PeproTech, Israel) were dissolved in RPMI-1640 medium supplemented with 0.5% fetal bovine serum (FBS) and placed at the lower chamber of QCM 24-well, 5 mm pore, migration assay plates (Corning-Costar, Corning, NY). Cells (3×10^5) were seeded in the upper chamber and incubated for 2-4 hours. Subsequently, the number of cells which migrated to the lower compartment was determined by fluorescence-activated cell sorting (FACS).

Results

[0320] Figure 20 shows VB-705 does not inhibit SDF1-induced migration of human monocytes (THP-1 cell line).

Example 25

VB-703 and Focal and Segmental Glomerulosclerosis

Methods and Materials

Animals and Experimental Protocol

[0321] Male Sprague Dawley (SD) Rats (Harlan Laboratories, Israel) with an initial weight of 200 g were housed 2-3 per cage in IVC cages in dedicated HVAC (heat, ventilation, air conditioning animal facility at a temperature of $22\pm 2^\circ$ C and RH (relative humidity) of $55\pm 15\%$). Temperature and humidity were monitored continuously. The facility had no exposure to outside light and was maintained on automatic alternating cycles of 12 hours of light and 12 hours of dark. Animals were provided with a commercial rodent diet (Harlan Teklad TRM Rat/Mouse Diet) *ad libitum* and allowed free access to autoclaved water, supplied to each cage via polysulphone bottles with stainless steel sipper tubes. All animal work was approved by the Animal Care and Use Committee of Israel (IL-13-03-027).

Induction of Chronic Renal Disease by 5/6 Nephrectomy

[0322] Rats were divided into three groups: (1) Healthy rats (n=3) in group A, (2) Sham group – subjected to surgical process but without kidney mass reduction (n=3) in group B, and (3) the rest were induced with chronic renal failure (n=32). Chronic renal failure was induced by a two stage (5/6) nephrectomy (Nx), with subtraction firstly of about 2/3 of the left kidney by left flank incision and, one week later, complete removal of the right kidney. General anesthesia consisted of intraperitoneal injection of ketamine 100 mg/kg and xylazine 20 mg/kg (0.85 ml ketamine + 0.15 ml xylazine for each ml preparation; 1 μ l/g BW was injected I.P).

Experimental Groups

[0323] One week following the second surgery, rats were randomly assigned to the following experimental groups:

Healthy, orally administered with vehicle – PBS 0.5% Ethanol (n=3);

Sham-operated, orally administered with vehicle – PBS 0.5% Ethanol (n=3);

[0324] Nephrectomized, orally administered with vehicle – PBS 0.5% Ethanol (n=8);

Nephrectomized, orally administered with VB-703 4 mg/kg (n=8); and

Nephrectomized, orally administered with telmisartan 10 mg/kg as positive control (n=8).

[0325] Body weight (BW) was monitored throughout the study and rats were treated by oral gavage according to their body weight for 7 weeks. Rats were sacrificed by CO₂ inhalation 8 weeks from removal of the right kidney (2nd surgery).

Blood Analysis, Albuminuria, and Creatinine Clearance

[0326] Proteinuria and albuminuria was determined in urine specimens, collected during a 24-hour period, from animals housed in cages at 4 weeks (3w of treatment) and 8 weeks (7w of treatment) after the subtotal nephrectomy (2nd surgery). Urine samples were analyzed for: glucose, urea, sodium, potassium, creatinine, total protein, and albumin.

[0327] Serum was collected at 8 weeks after the subtotal nephrectomy (2nd surgery). The serum collected was also analyzed for glucose, urea, sodium, potassium, creatinine, total protein, albumin, and globulin.

Kidney Collection

[0328] Upon sacrifice, at 8 weeks, kidneys were collected, weighed and fixed in 4% formaldehyde.

Renal Morphology and Morphometric Analysis

[0329] For light microscopy, paraffin-embedded tissue slides of 4 µm were stained with Periodic Acid-Schiff (PAS) reagent, Masson's Trichrome and Hematoxylin & Eosin.

[0330] *Glomerular sclerosis index.* Glomerulosclerosis was assessed by PAS-stained sections using a semiquantitative scoring system. The extent of glomerulosclerosis was evaluated by examining mostly 100 randomly selected glomeruli at a magnification of x400 and applying a score system according to the percentage of sclerosed glomerular area. The score was graded from 0 to 4: (0=0% area; 1=1-25%; 2=26-50%, 3=51-75%,

4=76% and above). The mean of all scored glomeruli was presented. Moreover, the extent of global and segmental glomerulosclerosis was evaluated in the same glomeruli, where <80% sclerosis was referred to as segmental and >80% was referred to as global.

[0331] *Glomerular area.* The glomerular area of mostly 100 randomly selected glomeruli at a magnification of x100 was quantitated by counting squares covered by glomeruli area using a grid and the mean glomeruli area was calculated.

Immunohistochemistry

[0332] Renal tissues were fixed in 4% formaldehyde and embedded in paraffin. The paraffin-embedded tissues were then cut to form tissue slides of 4 μ m. Immunohistochemistry of the paraffin-embedded tissue slides was analyzed using antibodies in the following concentration: monoclonal mouse anti rat CD-68 (ED-1, Serotec MCA341) 1:25. For quantitation of interstitial CD68+ staining, the number of positive cells was counted in 20 randomly selected non-overlapping fields per animal, and the mean value was presented.

Real-time PCR

[0333] Kidney RNA was extracted with an RNeasy Fibrous Tissue Mini kit (Qiagen) and after DNase I treatment, single-stranded cDNA was synthesized from 2 μ g total RNA using the qScript cDNA Synthesis Kit (Quanta Biosciences) and diluted for real-time PCR. The expression of collagen 4 α , fibronectin and TGF β was quantified using the 7300 Real Time PCR System (Applied Biosystems). The assay was performed according to manufacturer instructions using the primers (Assay ID) represented at the table below supplied by Applied Biosystems. Data were normalized to the reference gene TATA box Binding Protein (TBP) and presented as relative mRNA levels compared with Sham PBS 0.5% Eth treatment (Table 1).

Table 1: Gene Expression references

Assay ID	Gene Symbol	Gene Name
Rn01482927_m1	Col IV α 1	Collagen; type IV; alpha 1
Rn00572010_m1	TGF β 1	Transforming growth factor; beta 1
Rn00569575_m1	Fn1	Fibronectin 1
Rn01455646_m1	TBP	TATA box binding protein

Statistics

[0334] Data are expressed as means ± SEM. Statistical significance was determined by one-way ANOVA or Student's t-test where appropriate. Statistical analyses were performed using Sigma Stat software.

Results

VB-703 Treatment Effect on Physiological Parameters

[0335] VB-703 treatment significantly improved urinary albumin/creatinine/day ratio at the termination of the study (FIG. 22).

VB-703 Treatment Effect on Glomerular Damage

[0336] Glomeruli were evaluated for their fibrosis extent by scoring and by calculation of the percent of glomeruli having segmental sclerosis, global sclerosis and the sum of global and segmental sclerotic glomeruli. Moreover, the area of the glomeruli was calculated and the percent of hypertrophied glomeruli was calculated. Damaged glomeruli included hypertrophied (at least x1.5 from normal area) and or sclerotic glomeruli.

[0337] VB-703 and telmisartan treatment significantly reduced the damaged glomeruli percent by 38% (p≤0.005) and 31% (p≤0.005) respectively (FIG. 23). This effect was partially contributed by the reduction in glomeruli hypertrophy. The major contribution to the reduction in glomeruli damage was due to the reduction in sclerotic glomeruli. VB-703 and telmisartan treatment resulted in a 49% (p≤0.005) and 57% (p≤0.005) reduction of sclerotic glomeruli, respectively (FIG. 24, Table 2).

Table 2: *Effect of VB-201 on Glomerular sclerosis (Mean±S.E)**

Treatment	Healthy PBS 0.5% Eth	Sham PBS 0.5% Eth	Nx PBS 0.5% Eth	Nx VB-703 4 mg/kg	Nx Telmisartan 10 mg/kg
Glomerular sclerosis					
Segmental %	1.0±0.58 (n=3) p≤0.001	1.3±0.88 (n=3) p≤0.001	41.0±4.81 (n=7)	22.9±2.83 (n=7) P<0.01	19.1±4.30 (n=8) P=0.005

Global %	0.0±0.00 (n=3) n.s	0.0±0.00 (n=3) n.s	7.1±4.3 9 (n=7)	1.9±1.55 (n=7) n.s	1.9±1.60 (n=8) n.s
Global & Segmental %	1.0±0.58 (n=3) p≤0.001	1.3±0.88 (n=3) p≤0.001	48.3±5.38 (n=7)	24.7±3.77 (n=7) P<0.005	21.0±5.45 (n=8) P<0.005

*Number of animals tested per group and p value versus Nx PBS 0.5% Eth group is presented.

[0338] FIG. 25 shows typical sclerotic changes in glomeruli (PAS staining) of vehicle treated nephrectomized animals in contrast with healthy or sham operated animals or with VB-703 treated animals, or telmisartan treated animal.

VB-703 Treatment Effect on Pro-fibrotic Markers

[0339] The mRNA expression of Collagen IV was increased by 7 or 8 fold respectively in vehicle treated nephrectomized rats (7.5±1.51) in contrast with healthy (1.1±0.12) or sham operated animals (1.0±0.32). VB-703 treatment significantly (p<0.05) reduced Collagen IV expression by 51% (3.7±0.52) compared to those observed for Nx PBS 0.5% Eth treatment. A 41% reduction in Collagen IV expression was observed in the telmisartan treated nephrectomized rats (4.4±0.23) compared to those observed for Nx PBS 0.5% Eth treatment, with marginal significance (p=0.064) (FIG. 26A).

[0340] The mRNA expression of fibronectin was increased by 16 or 13 fold respectively in vehicle treated nephrectomized rats (12.7±1.01) in contrast with healthy (0.8±0.08) or sham operated animals (1.0±0.31). VB-703 treatment significantly (p<0.005) reduced fibronectin expression by 47% (6.7±0.98) compared to those observed for Nx PBS 0.5% Eth treatment. Telmisartan treatment moderately reduced fibronectin expression by 23% (9.8±2.09); however this reduction was not statistically significant (FIG. 26B).

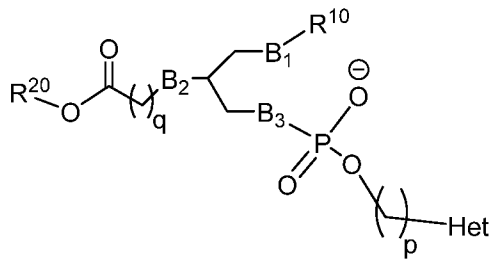
[0341] The mRNA expression of TGF-β was increased by 10 or 8 fold respectively in vehicle treated nephrectomized rats (8.4±0.49) in contrast with healthy (0.9±0.24) or sham operated animals (1.0±0.23) (p≤0.001). VB-703 and telmisartan treatment significantly (p≤0.001) reduced TGF-β expression by 42% (4.8±0.32), and by 44% (4.7±0.52), respectively, compared to those observed for Nx PBS 0.5% Eth treatment (FIG. 26C).

VB-703 Treatment Effect on Glomerular and Interstitial Monocyte/Macrophage Infiltration

- [0342]** FIGs. 27A-27B show the effect of VB-703 on monocyte/macrophage cell infiltration in the glomeruli (FIG. 27A) or in the interstitium (FIG. 27B). In this experiment, VB-703 and telmisartan did not produce a significant effect on reducing glomerular and interstitial monocyte/macrophage infiltration.
- [0343]** All publications, patents and patent applications mentioned in this application are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

WHAT IS CLAIMED IS:

1. A compound having a structure according to Formula 1,



Formula 1

wherein each of B₁, B₂, and B₃ is independently selected from the group consisting of oxygen, sulfur, nitrogen, phosphorus, and silicon; wherein each of said nitrogen, phosphorus and silicon is optionally substituted by one or more substituents selected from the group consisting of alkyl, halo, cycloalkyl, aryl, hydroxy, thiohydroxy, alkoxy, aryloxy, thioaryloxy, thioalkoxy, and oxo;

wherein R¹⁰ is a C₂₋₂₈ alkyl optionally substituted by one to five R¹¹ substituents, wherein each R¹¹ is independently selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, halo, trihalomethyl, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, phosphonate, phosphate, phosphinyl, sulfonyl, sulfinyl, sulfonamide, amide, carbonyl, thiocarbonyl, C-carboxy, O-carboxy, C-carbamate, N-carbamate, C-thiocarboxy, S-thiocarboxy, and amino;

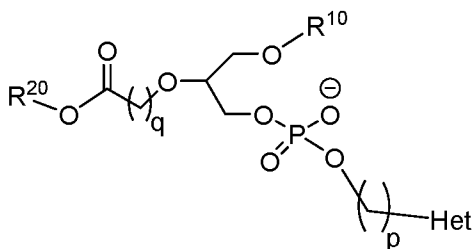
wherein p is an integer selected from 1-10;

wherein q is an integer selected from 1-26;

wherein R²⁰ is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, and heteroaryl; and

wherein Het is a heteroalicyclic ring or a heteroaryl.

2. The compound of claim 1, having a structure according to Formula 2,

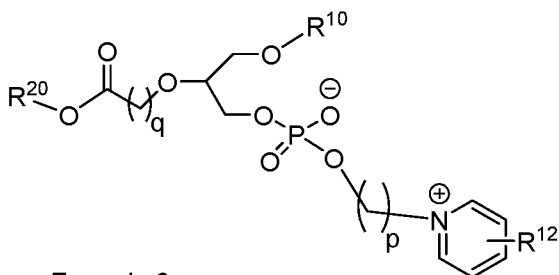


Formula 2

3. The compound of claim 1 or 2, wherein R^{10} is selected from the group consisting of hexadecyl, dodecyl, octadecyl, octyl, eicosanyl, cis-9-hexadecenyl, (2'-octyl)dodecyl, and (15'-carboxy)pentadecyl.

4. The compound of any one of claims 1-3, wherein R^{20} is a hydrogen or an alkyl.
5. The compound of any one of claims 1-4, wherein Het is a nitrogen containing heteroaryl.
6. The compound of claim 5, wherein one nitrogen atom of the heteroaryl is directly connected to the alkylene chain, $-(CH_2)_p-$.
7. The compound of any one of claims 1-6, wherein the Het is unsubstituted.
8. The compound of any one of claims 1-7, wherein the Het is substituted by one to five R^{12} .
9. The compound of claim 8, wherein each R^{12} is independently a halogen, an aryl, or an alkyl.

10. The compound of claim 1 or 2, having a structure according to Formula 3,



Formula 3

11. The compound of claim 10, wherein R^{10} is selected from the group consisting of hexadecyl, eicosanyl, and (2'-octyl)dodecyl.

12. The compound of claim 10 or 11, wherein R^{20} is a hydrogen or a C_{1-4} alkyl.

13. The compound of any one of claims 10-12, wherein q is an integer of 2-6.

14. The compound of any one of claims 10-13, wherein p is an integer of 2-5.

15. The compound of any one of claims 10-14, wherein the pyridine ring is not substituted.

16. The compound of any one of claims 10-14, wherein the pyridine ring is substituted by one, two, or three R^{12} substituents.

17. The compound of claim 16, wherein each R^{12} is independently a halogen, an aryl, or a C_{1-4} alkyl.

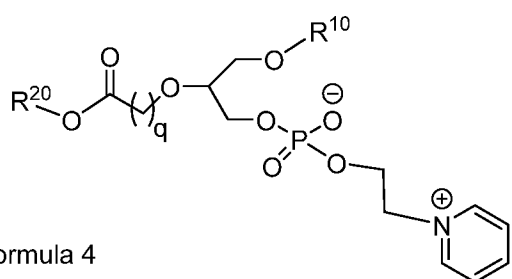
18. The compound of claim 17, wherein the pyridine ring is substituted by one R^{12} substituent, wherein the one R^{12} substituent is a fluorine or a phenyl.

19. The compound of claim 18, wherein the pyridine ring is 3-fluoro-pyridine or 3-phenyl-pyridine.

20. The compound of any one of claims 10-19, wherein p is 2.

21. The compound of any one of claims 10-20, wherein q is 4.

22. The compound of any one of claims 1, 2, or 10, having a structure according to Formula 4,



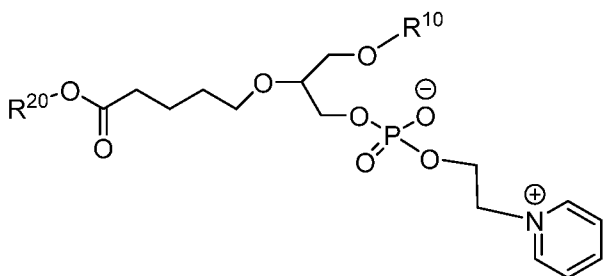
Formula 4

23. The compound of claim 22, wherein R^{10} is selected from the group consisting of hexadecyl, dodecyl, octadecyl, octyl, eicosanyl, cis-9-hexadecenyl, (2'-octyl)dodecyl, and (15'-carboxy)pentadecyl.

24. The compound of claim 22 or 23, wherein R^{20} is a hydrogen or an alkyl.

25. The compound of any one of claims 22-24, wherein q is an integer of 1-10.

26. The compound of claim 22, having a structure according to Formula 5,



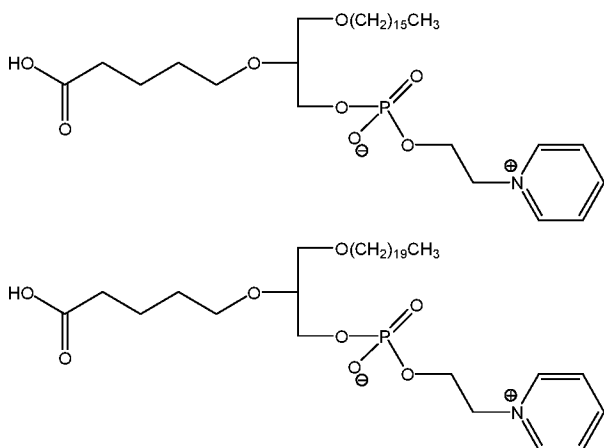
Formula 5

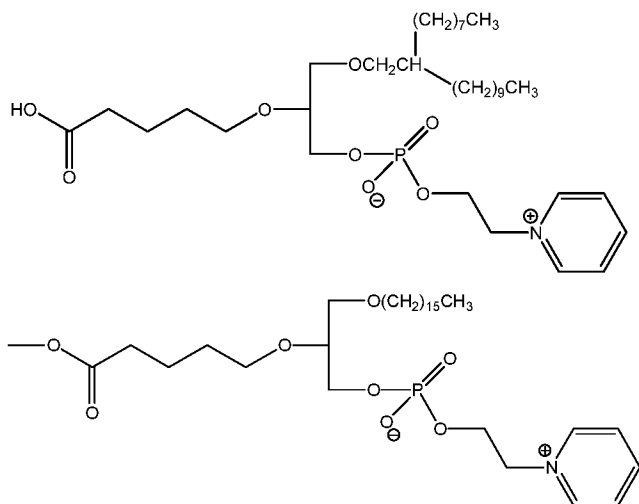
27. The compound of claim 26, wherein R^{10} is selected from the group consisting of hexadecyl, eicosanyl, and (2'-octyl)dodecyl.

28. The compound of claim 26 or 27, wherein R^{20} is a hydrogen or a C_{1-4} alkyl.

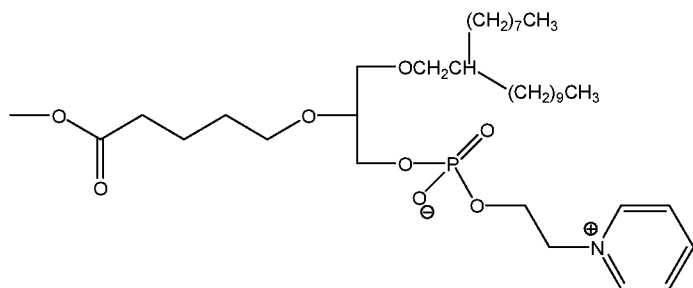
29. The compound of any one of claims 1-20 and 22-28, wherein R^{10} is selected from the group consisting of hexadecyl, eicosanyl, and (2'-octyl)dodecyl and R^{20} is a hydrogen or a methyl.

30. A compound having a structure selected from the group consisting of:





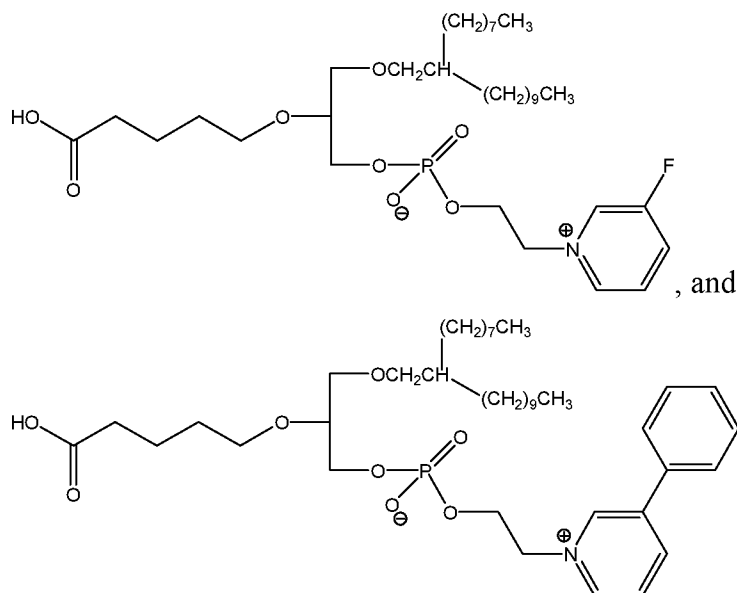
and



31. The compound of claim 30, selected from the group consisting of (R)-1-hexadecyl-2-(4'-carboxy)butyl-sn-glycero-3-phosphoric acid pyridiniummethyl ester (VB-701); (R)-1-eicosanyl-2-(4'-carboxy)butyl-sn-glycero-3-phosphoric acid pyridiniummethyl ester (VB-702); (R)-1-(2'-octyl)dodecyl-2-(4'-carboxy)butyl-sn-glycero-3-phosphoric acid pyridiniummethyl ester (VB-703); (R)-1-hexadecyl-2-(4'-carboxymethyl)butyl-sn-glycero-3-phosphoric acid pyridiniummethyl ester (VB-704); and (R)-1-(2'-octyl)dodecyl-2-(4'-carboxymethyl)butyl-sn-glycero-3-phosphoric acid pyridiniummethyl ester (VB-705).

32. The compound of claim 30, which is (R)-1-(2'-octyl)dodecyl-2-(4'-carboxy)butyl-sn-glycero-3-phosphoric acid pyridiniummethyl ester (VB-703).

33. A compound selected from the group consisting of



34. The compound of claim 32, selected from the group consisting of (R)-1-(2'-octyl)dodecyl-2-(4'-carboxy)butyl-glycero-sn-3-phosphoric acid 3-fluoro-pyridiniummethyl ester (VB-706) and (R)-1-(2'-octyl)dodecyl-2-(4'-carboxy)butyl-glycero-sn-3-phosphoric acid 3-phenyl-pyridiniummethyl ester (VB-707).

35. The compound of any one of claims 1-34, in the form of a stereoisomer, a stereoisomeric mixture, or a salt.

36. A pharmaceutical composition comprising the compound of any one of claims 1-35 and a pharmaceutically acceptable vehicle.

37. A method of treating or preventing fibrosis, comprising administering to a subject in need thereof a therapeutically effective amount of a compound of any one of claims 1-35.

38. A method of treating or preventing fibrosis, comprising administering to a subject in need thereof the pharmaceutical composition of claim 36.

39. The method of claim 37 or 38, wherein the fibrosis is pulmonary fibrosis, liver fibrosis, skin fibrosis, kidney fibrosis, idiopathic pulmonary fibrosis, cystic fibrosis, progressive massive fibrosis, cirrhosis, steatohepatitis (fatty liver disease), nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), endomyocardial fibrosis, myocardial infarction, atrial fibrosis, mediastinal fibrosis, myelofibrosis, retroperitoneal fibrosis, nephrogenic systemic

fibrosis, keloid, Crohn's disease, scleroderma/systemic sclerosis, arthrofibrosis, Peyronie's disease, Dupuytren's contracture, adhesive capsulitis, or focal and segmental glomerulosclerosis.

40. A method of treating or preventing an inflammatory disease or disorder, comprising administering to a subject in need thereof a therapeutically effective amount of a compound of any one of claims 1-35.

41. A method of treating or preventing an inflammatory disease or disorder, comprising administering to a subject in need thereof a pharmaceutical composition of claim 36.

42. The method of claim 40 or 41, wherein the inflammatory disease or disorder is atherosclerosis, rheumatoid arthritis, inflammatory bowel disease, ulcerative colitis, multiple sclerosis, or psoriasis.

43. The method of claim 40 or 41, wherein the inflammatory disease or disorder is an inflammatory cardiovascular disease or disorder, a cerebrovascular disease or disorder, or a peripheral vascular disease or disorder.

44. The method of claim 43, wherein the inflammatory cardiovascular disease or disorder is selected from the group consisting of occlusive diseases or disorders, atherosclerosis, cardiac valvular disease, stenosis, restenosis, in-stent-stenosis, myocardial infarction, coronary arterial disease, acute coronary syndromes, congestive heart failure, angina pectoris, myocardial ischemia, thrombosis, Wegener's granulomatosis, Takayasu's arteritis, Kawasaki syndrome, anti-factor VIII autoimmune diseases or disorders, necrotizing small vessel vasculitis, microscopic polyangiitis, Churg and Strauss syndrome, pauci-immune focal necrotizing glomerulonephritis, crescentic glomerulonephritis, antiphospholipid syndrome, antibody induced heart failure, thrombocytopenic purpura, autoimmune hemolytic anemia, cardiac autoimmunity, Chagas' disease or disorder, and anti-helper T lymphocyte autoimmunity.

45. The method of claim 43, wherein the cerebrovascular disease or disorder is selected from the group consisting of stroke, cerebrovascular inflammation, cerebral hemorrhage, and vertebral arterial insufficiency.

46. The method of claim 43, wherein the peripheral vascular disease or disorder is selected from the group consisting of gangrene, diabetic vasculopathy, ischemic bowel disease, thrombosis, diabetic retinopathy, and diabetic nephropathy.

47. The method of any one of claims 37-46, wherein activity of TLR2, TLR4, or CD14 is inhibited in a cell of the subject.

48. The method of any one of claims 37-46, wherein activity of TLR2 and TLR4; activity of TLR4 and CD14; activity of TLR2 and CD14; or activity of TLR2, TLR4, and CD14 is inhibited in a cell of the subject.

49. A method of treating or preventing liver fibrosis in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a compound of any one of claims 1-35.

50. A method of treating or preventing liver fibrosis in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of claim 36.

51. A method of treating or preventing kidney fibrosis in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a compound of any one of claims 1-35.

52. A method of treating or preventing kidney fibrosis in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of claim 36.

53. A method of treating or preventing focal and segmental glomerulosclerosis in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a compound of any one of claims 1-35.

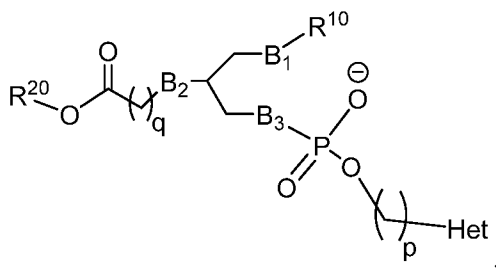
54. A method of treating or preventing focal and segmental glomerulosclerosis in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of claim 36.

55. The method of claim 49 or 54, wherein the compound is (R)-1-(2'-octyl)dodecyl-2-(4'-carboxy)butyl-sn-glycero-3-phosphoric acid pyridiniummethyl ester (VB-703).

56. The method of any one of claims 37-55, wherein the subject is a human.

57. The method of any one of claims 37-56, wherein the compound or pharmaceutical composition is administered orally.

58. A method of synthesizing a compound of Formula 1,



Formula 1

wherein each of B₁, B₂, and B₃ is independently selected from the group consisting of oxygen, sulfur, nitrogen, phosphorus, and silicon; wherein each of said nitrogen, phosphorus and silicon is optionally substituted by one or more substituents selected from the group consisting of alkyl, halo, cycloalkyl, aryl, hydroxy, thiohydroxy, alkoxy, aryloxy, thioaryloxy, thioalkoxy, and oxo;

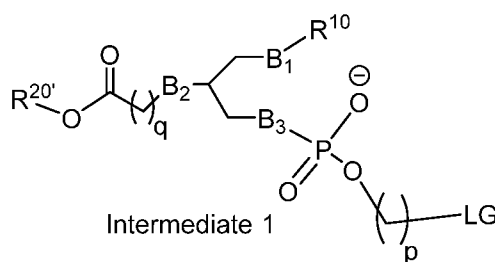
wherein R¹⁰ is a C₂₋₂₈ alkyl optionally substituted by one to five R¹¹ substituents, wherein each R¹¹ is independently selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, halo, trihalomethyl, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, phosphonate, phosphate, phosphinyl, sulfonyl, sulfinyl, sulfonamide, amide, carbonyl, thiocarbonyl, C-carboxy, O-carboxy, C-carbamate, N-carbamate, C-thiocarboxy, S-thiocarboxy, and amino;

wherein p is an integer selected from 1-10;

wherein q is an integer selected from 1-26;

wherein R^{20} is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, and heteroaryl; and wherein Het is a heteroalicyclic ring or a heteroaryl,

the method comprising:



a) reacting Intermediate 1

with Het to form the

compound of Formula 1,

wherein B_1 , B_2 , B_3 , R^{10} , Het, p , and q are as defined above in Formula 1;

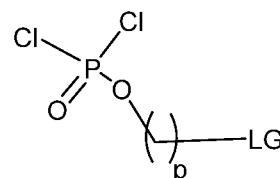
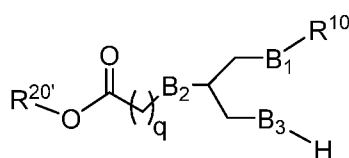
wherein LG is a leaving group selected from the group consisting of halogen and oxygen containing leaving groups;

wherein $R^{20'}$ is the same as R^{20} ;

R^{20} is H, $R^{20'}$ is selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl, and heteroaryl, the reacting of Intermediate 1 with Het is followed by a step of hydrolysis to convert $-\text{COOR}^{20'}$ into $-\text{COOH}$, or a salt thereof, to form the compound of Formula 1; or

R^{20} is H, $R^{20'}$ is selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl, and heteroaryl, the reacting of Intermediate 1 with Het converts $-\text{COOR}^{20'}$ into $-\text{COOH}$, or a salt thereof, to form the compound of Formula 1.

59. The method of claim 58, further comprising



b) reacting Reactant 1

Reactant 1

with Reactant 2

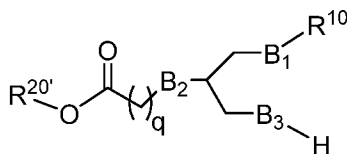
Reactant 2

to form a monochloro reaction product; and

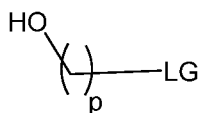
c) hydrolyzing the monochloro reaction product to form Intermediate 1,

wherein B_1 , B_2 , B_3 , R^{10} , $R^{20'}$, p , q , and LG are as defined above in Intermediate 1.

60. The method of claim 58, further comprising:



b') reacting Reactant 1 with $POCl_3$ to form a $POCl_3$ reaction product;

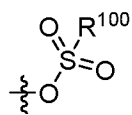


c') reacting Reactant 2A with the $POCl_3$ reaction product to form a second reaction product; and

d') hydrolyzing the second reaction product to form Intermediate 1,

wherein B_1 , B_2 , B_3 , R^{10} , $R^{20'}$, p , q , and LG are as defined above in Intermediate 1.

61. The method of any one of claims 58-60, wherein the LG is a leaving group selected from the group consisting of oxygen containing leaving groups represented by



, wherein R^{100} is selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, heteroalicycloalkyl, aryl, and heteroaryl, each of which is optionally substituted by one to five R^{13} , wherein each R^{13} substituent is independently selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, halo, trihalomethyl, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, phosphonate, phosphate, phosphinyl, sulfonyl, sulfinyl, sulfonamide, amide, carbonyl, thiocarbonyl, C-carboxy, O-carboxy, C-carbamate, N-carbamate, C-thiocarboxy, S-thiocarboxy, and amino.

62. The method of claim 61, wherein R^{100} is p-tolyl, o-tolyl, phenyl, methyl, or trifluoromethyl.

63. The method of claim 62, wherein R^{100} is p-tolyl.
64. The method of any one of claims 58-60, wherein the LG is a halogen.
65. The method of claim 64, wherein the LG is Br.
66. The method of any one of claims 58-65, wherein B_1 , B_2 , B_3 are each oxygen.
67. The method of any one of claims 58-66, wherein R^{10} is selected from the group consisting of hexadecyl, eicosanyl and (2'-octyl)dodecyl.
68. The method of any one of claims 58-67, wherein p is 2 and q is 4.
69. The method of any one of claims 58-68, wherein R^{20} is hydrogen and $R^{20'}$ is methyl.
70. The method of any one of claims 58-69, wherein Het is unsubstituted pyridine.
71. The method of any one of claims 58-70, wherein Het is 3-fluoro-pyridine or 3-phenyl-pyridine.
72. The method of any one of claims 58-71, wherein the compound of formula 1 is in the form of a stereoisomer, a stereoisomeric mixture, or a salt.

Fig 1A

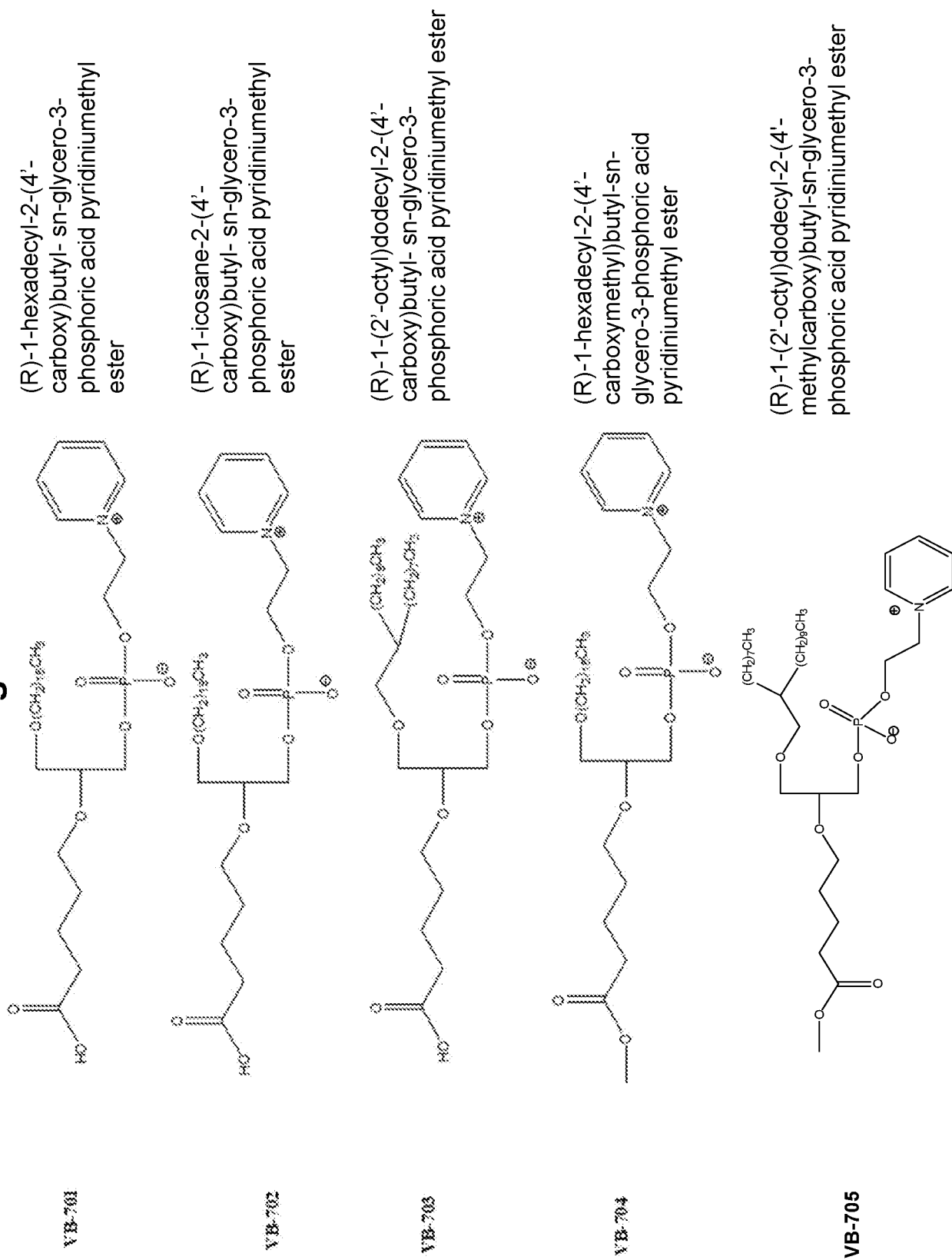


Fig 1B

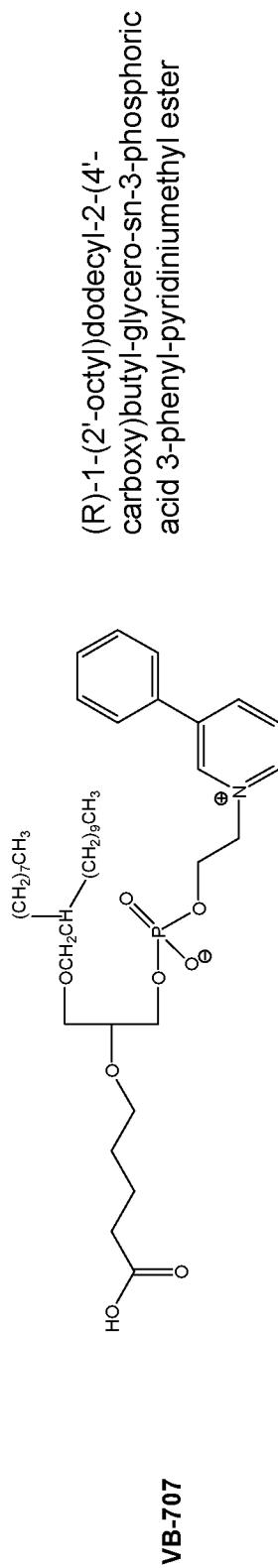
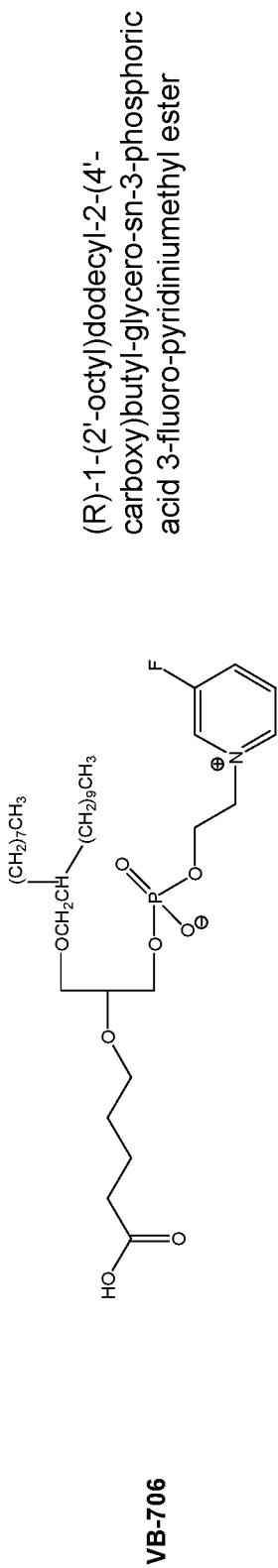
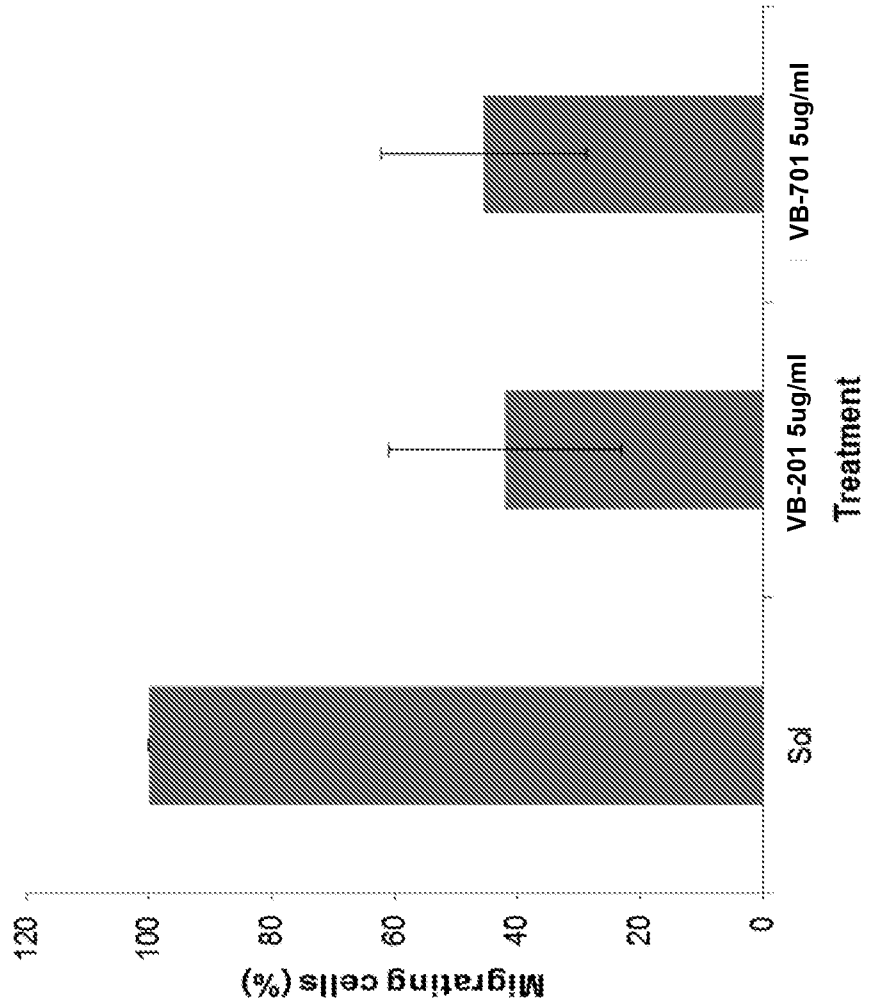


Fig 5



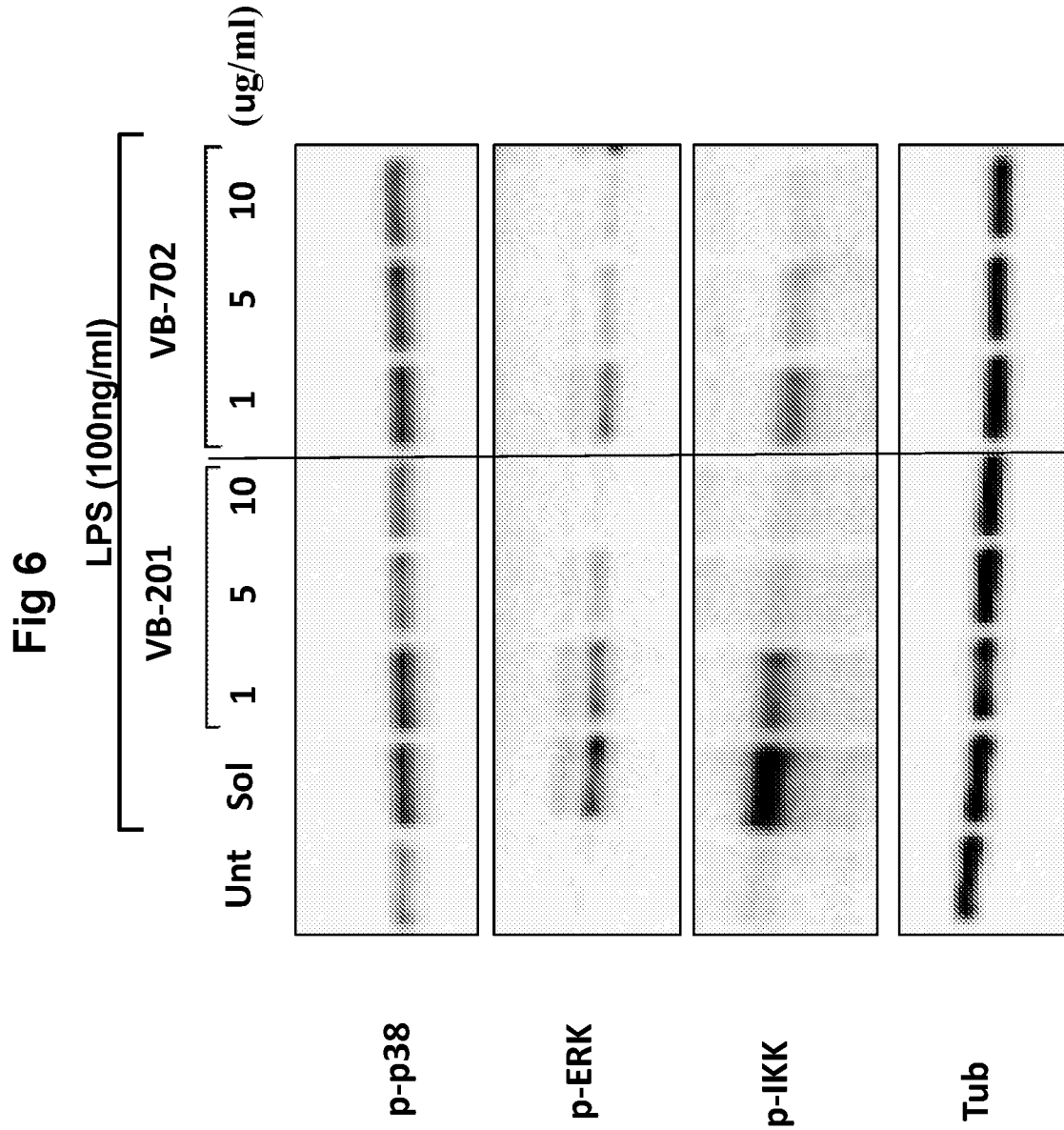


Fig 8

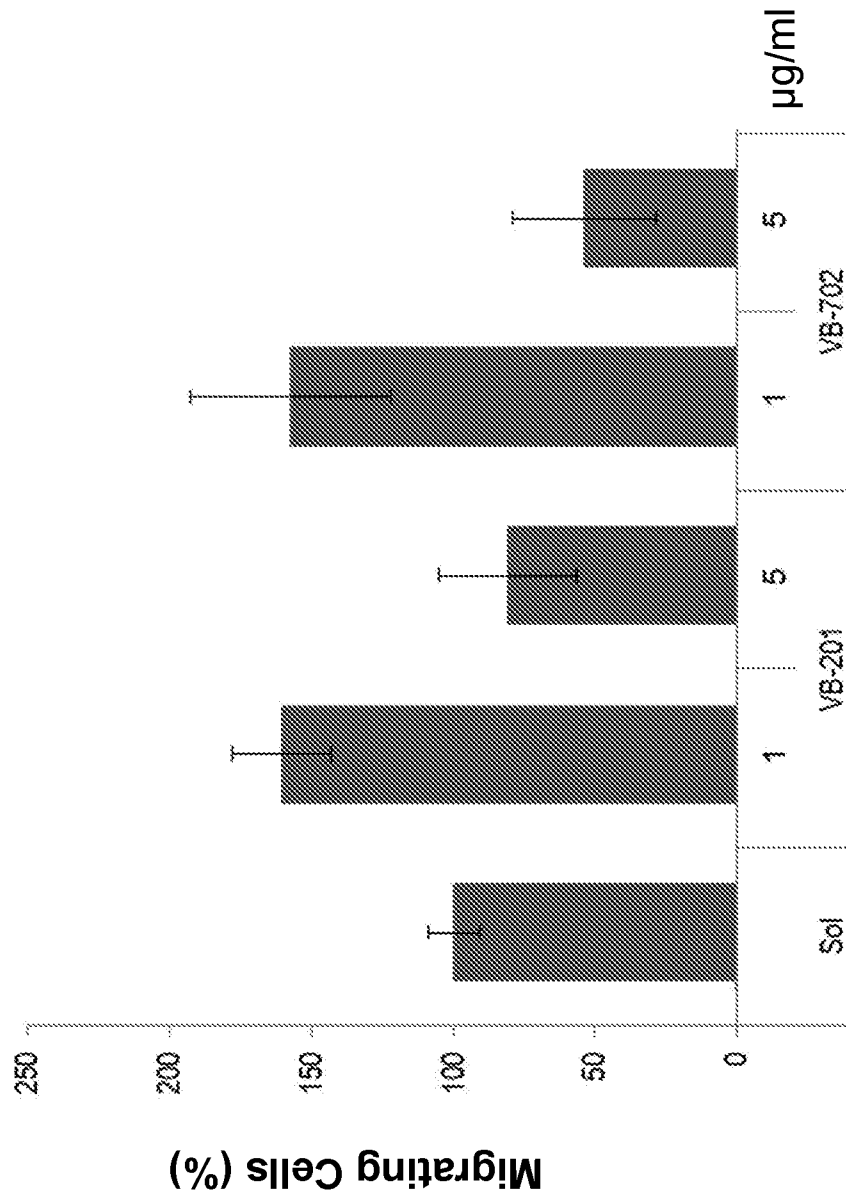


Fig 9A

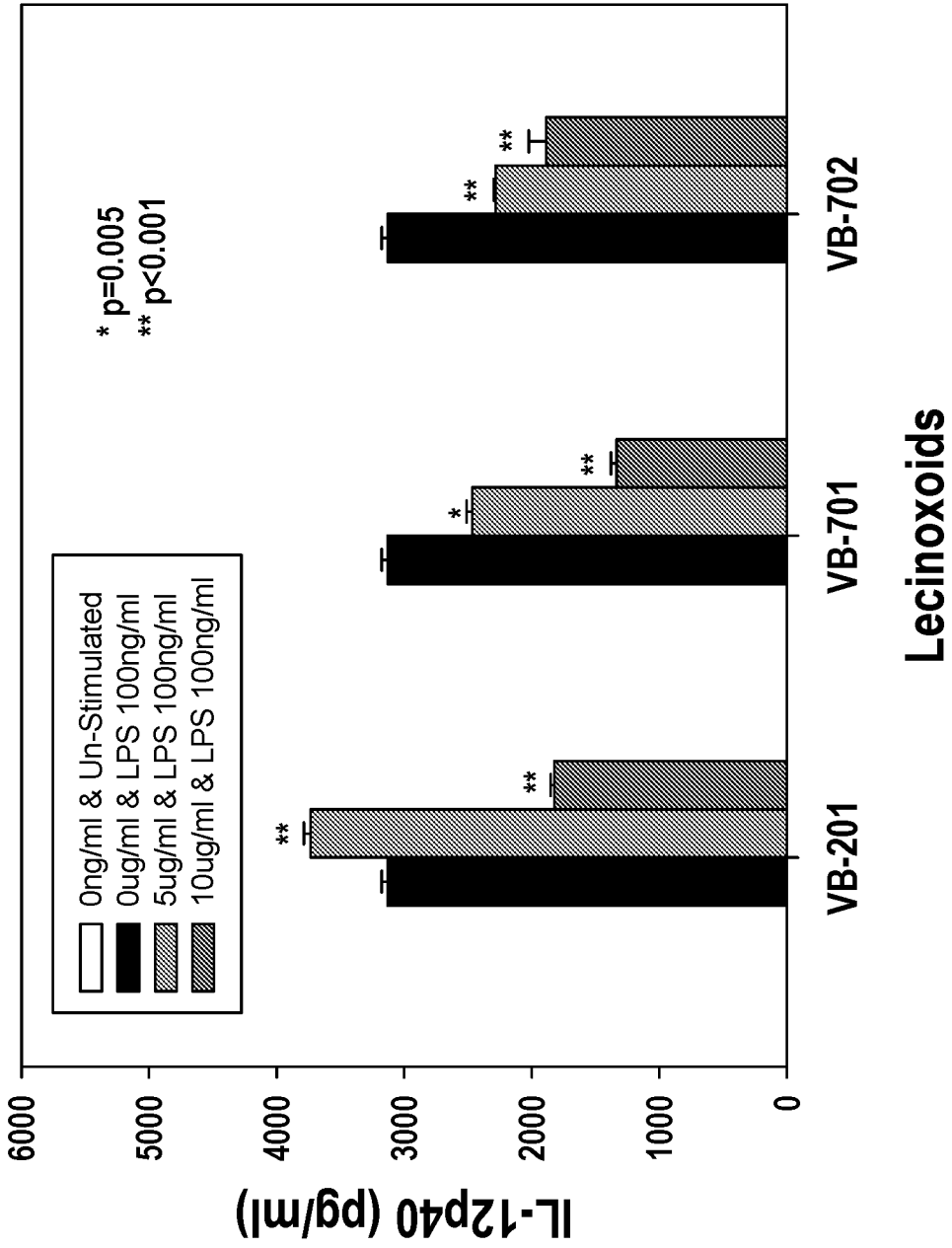


Fig 9B

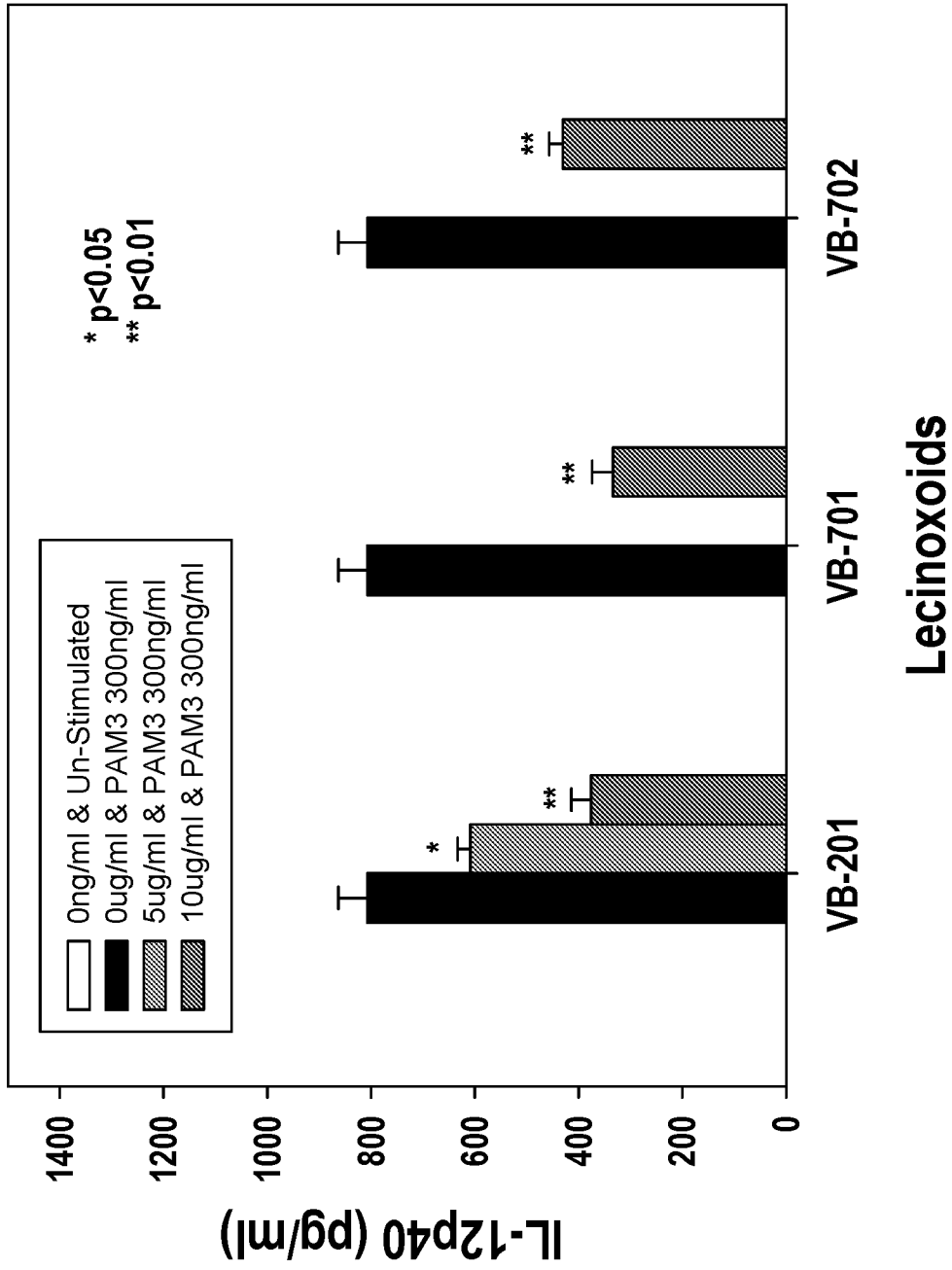


Fig 10A

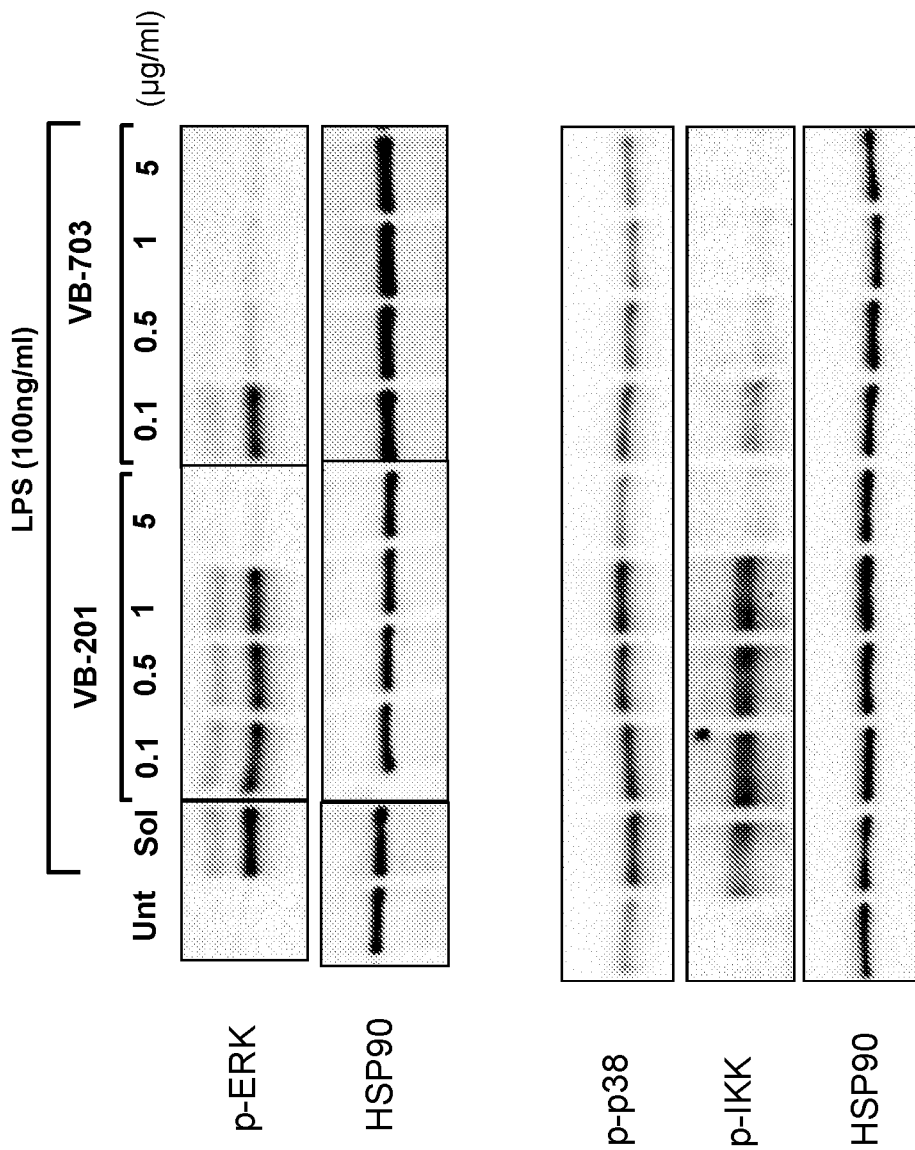


Fig 10B

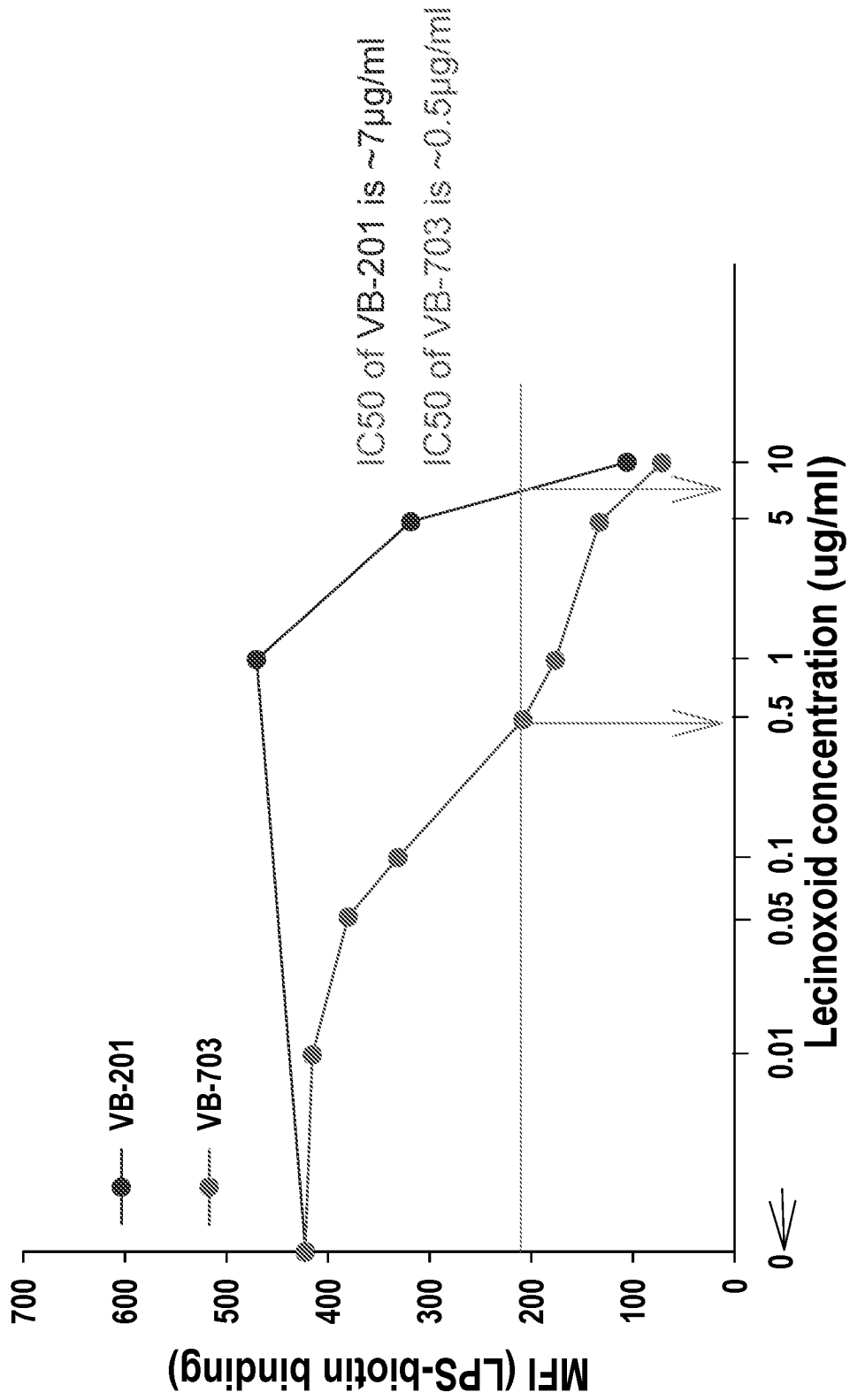


Fig 11A

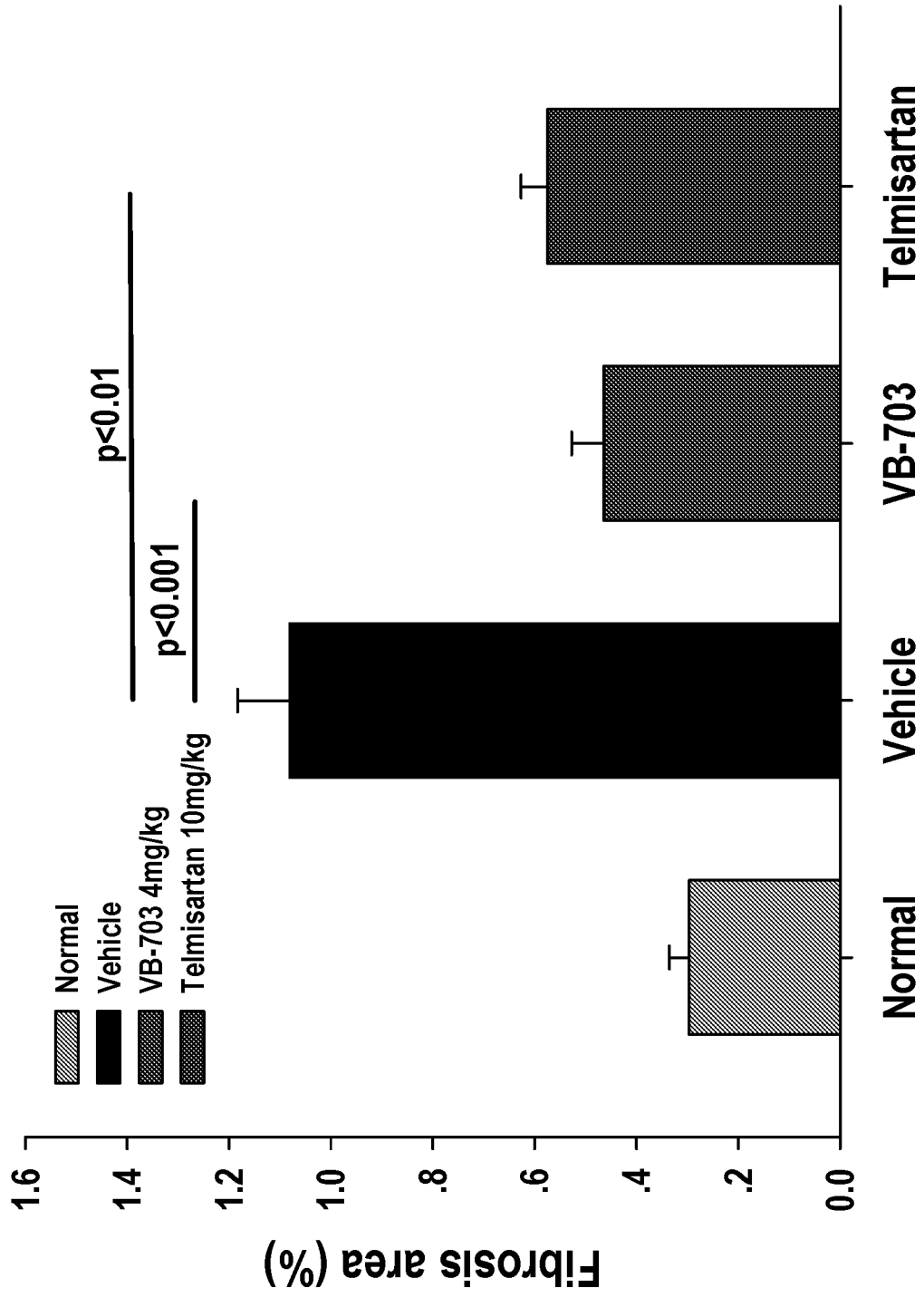


Fig 11B

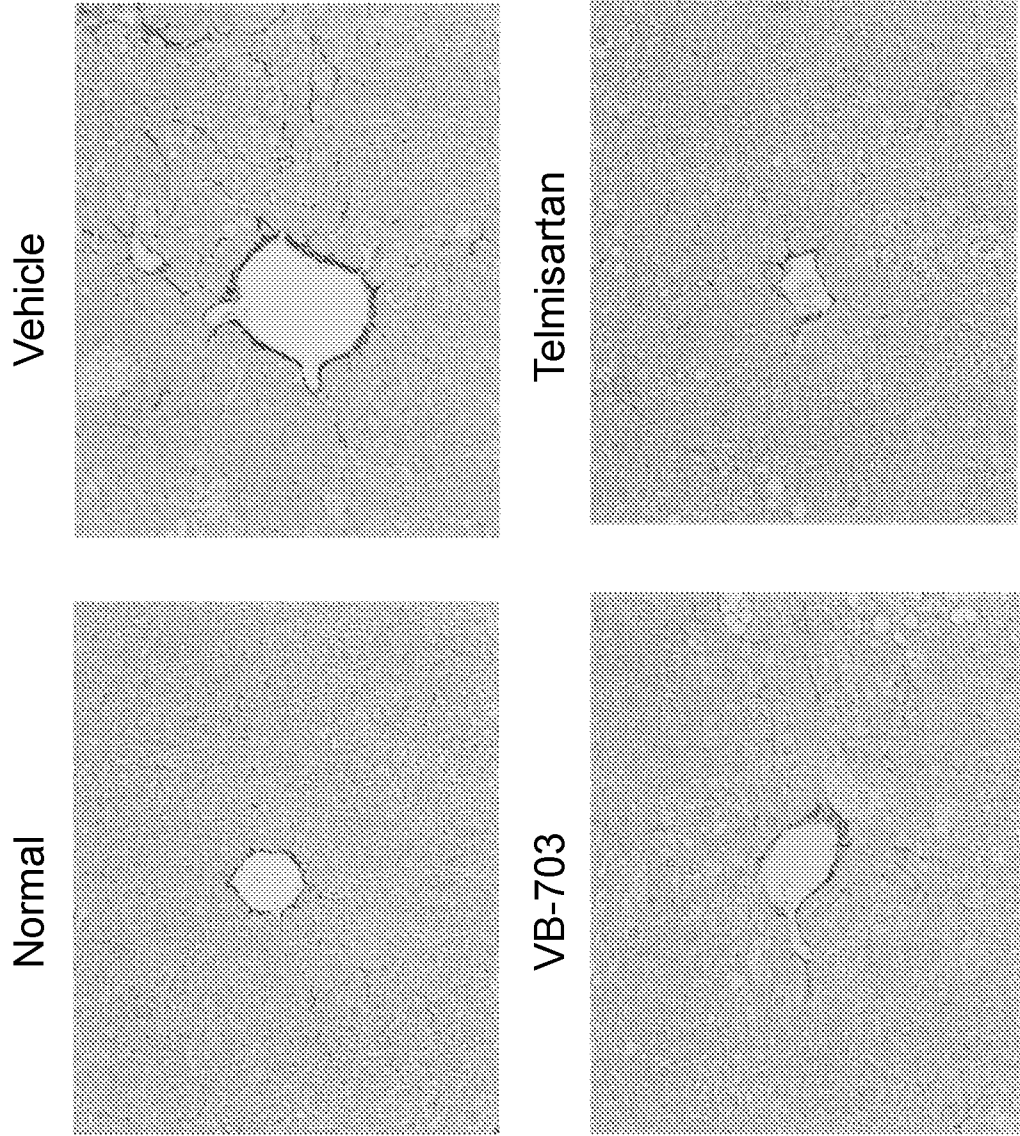


Fig 12A

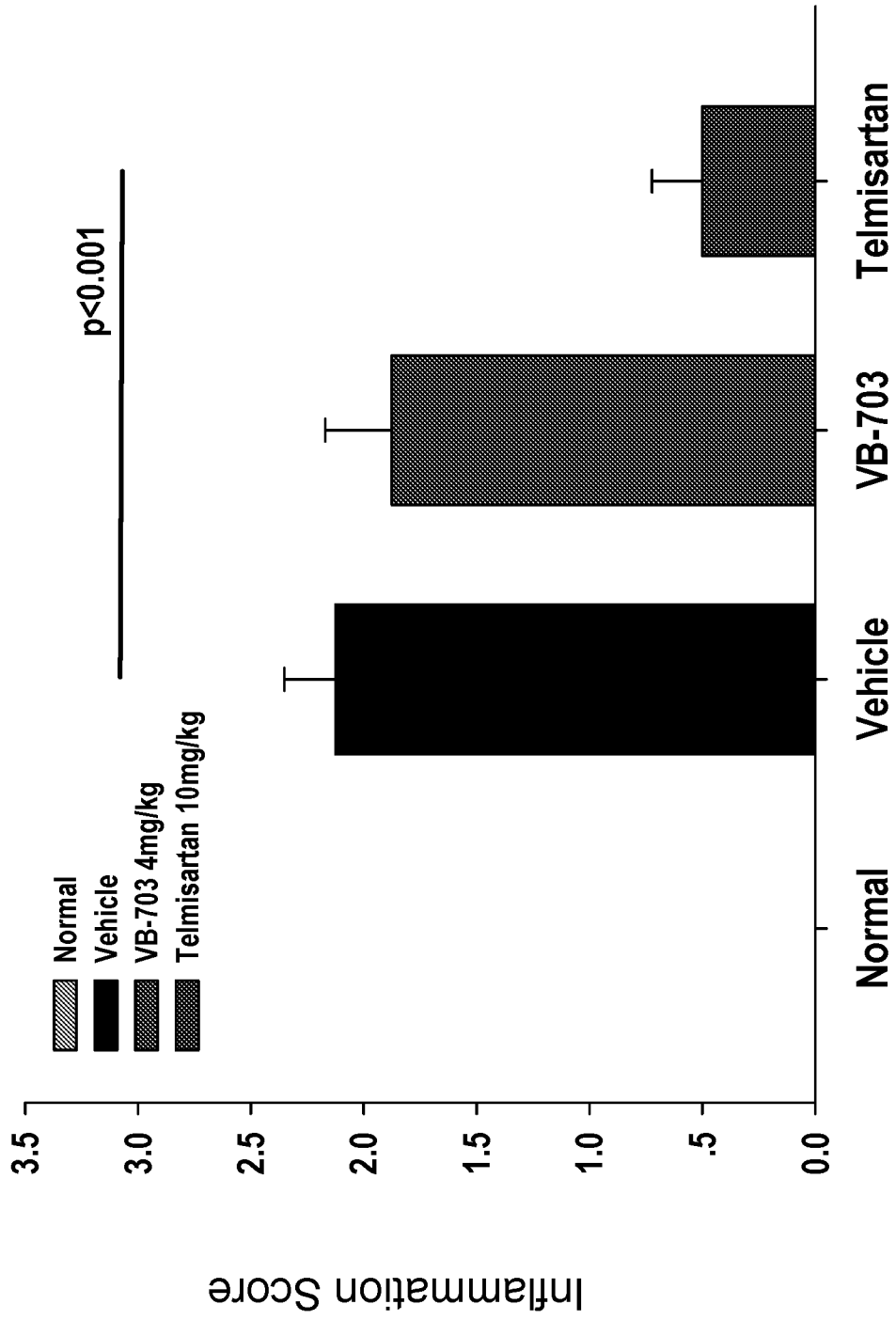
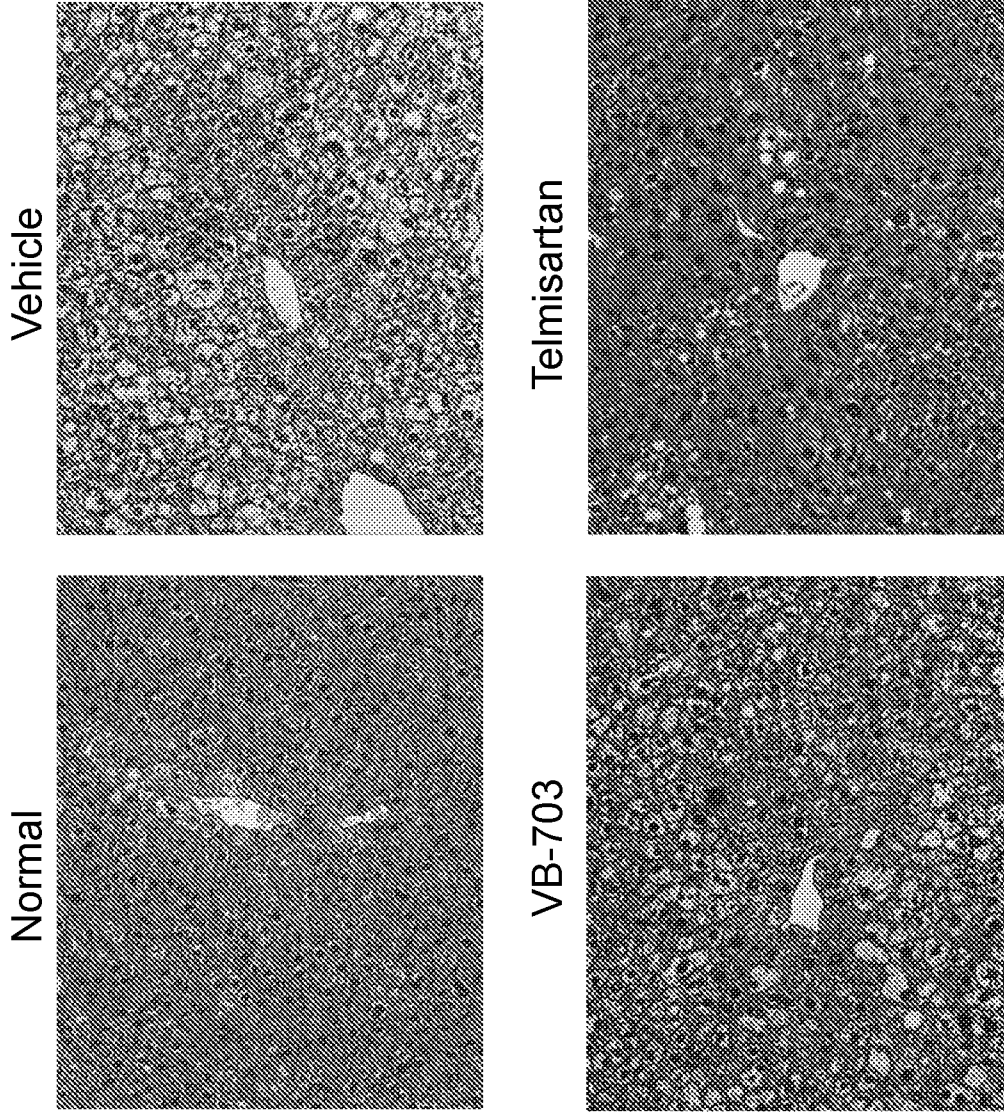


Fig 12B



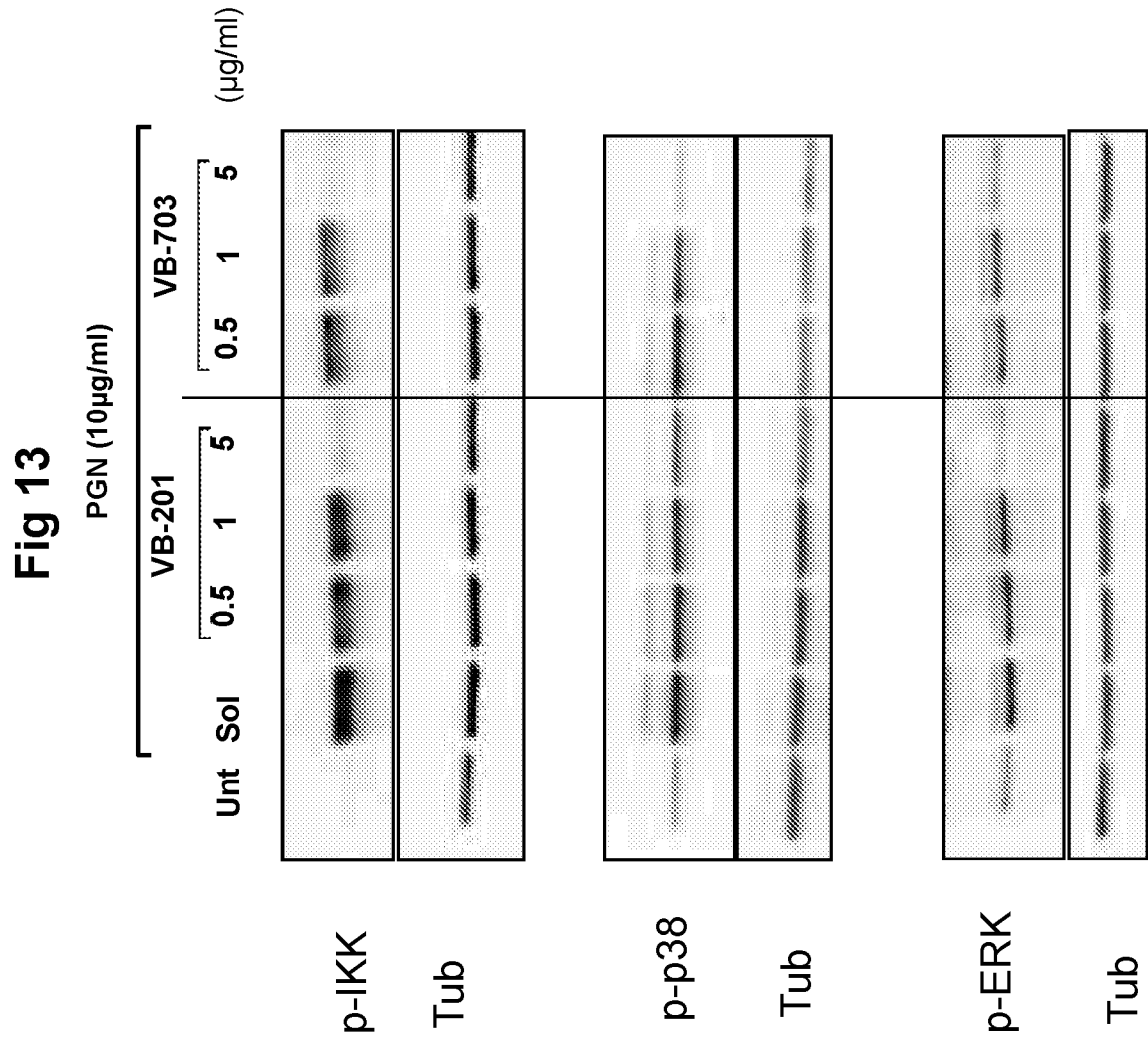


Fig 14
Mo-Derived DCs LPS (100 ng/ml) stimulated (O.N)

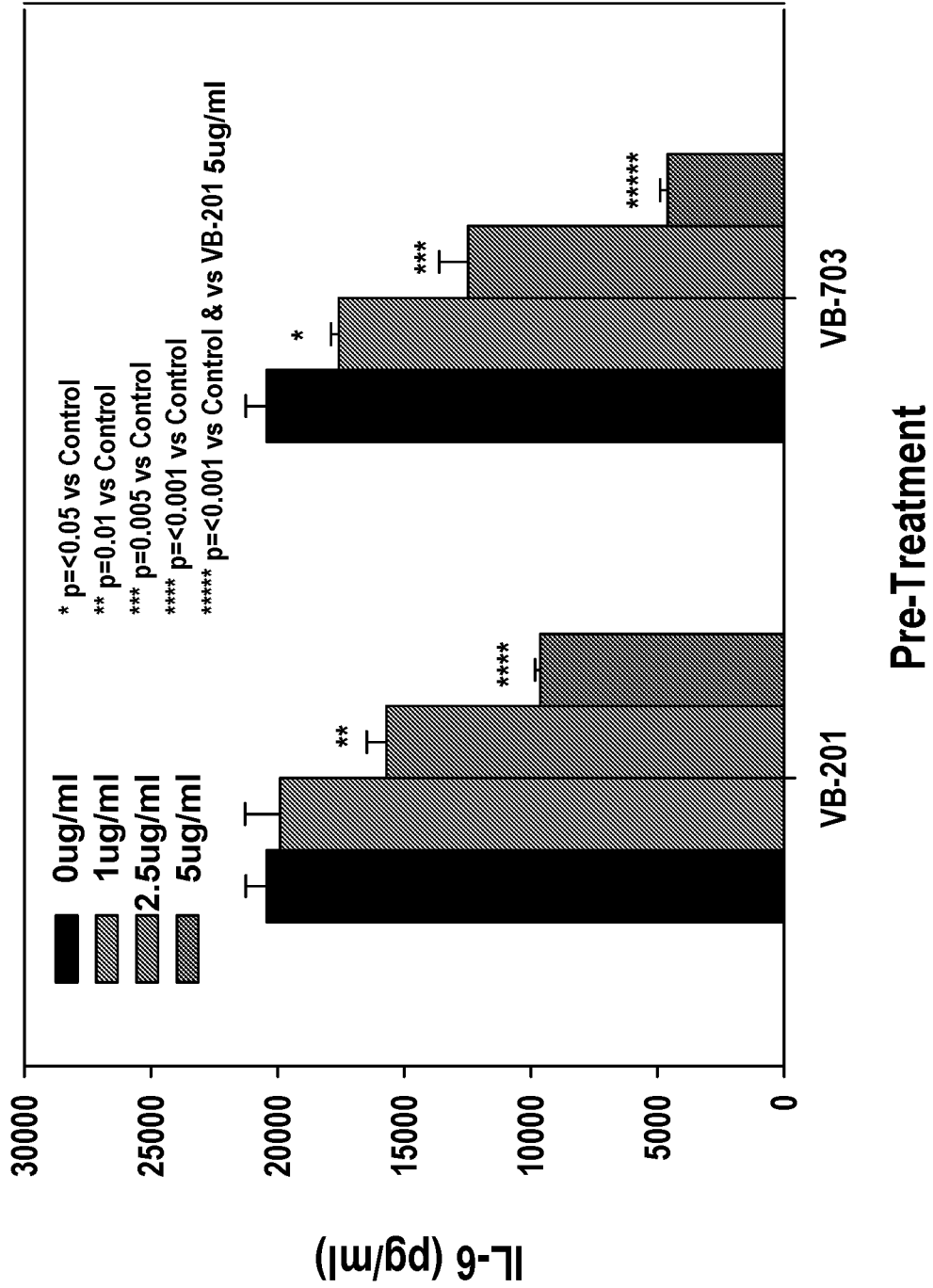


Fig 15
Mo-Derived DCs LPS (100ng/ml) stimulated (O.N)

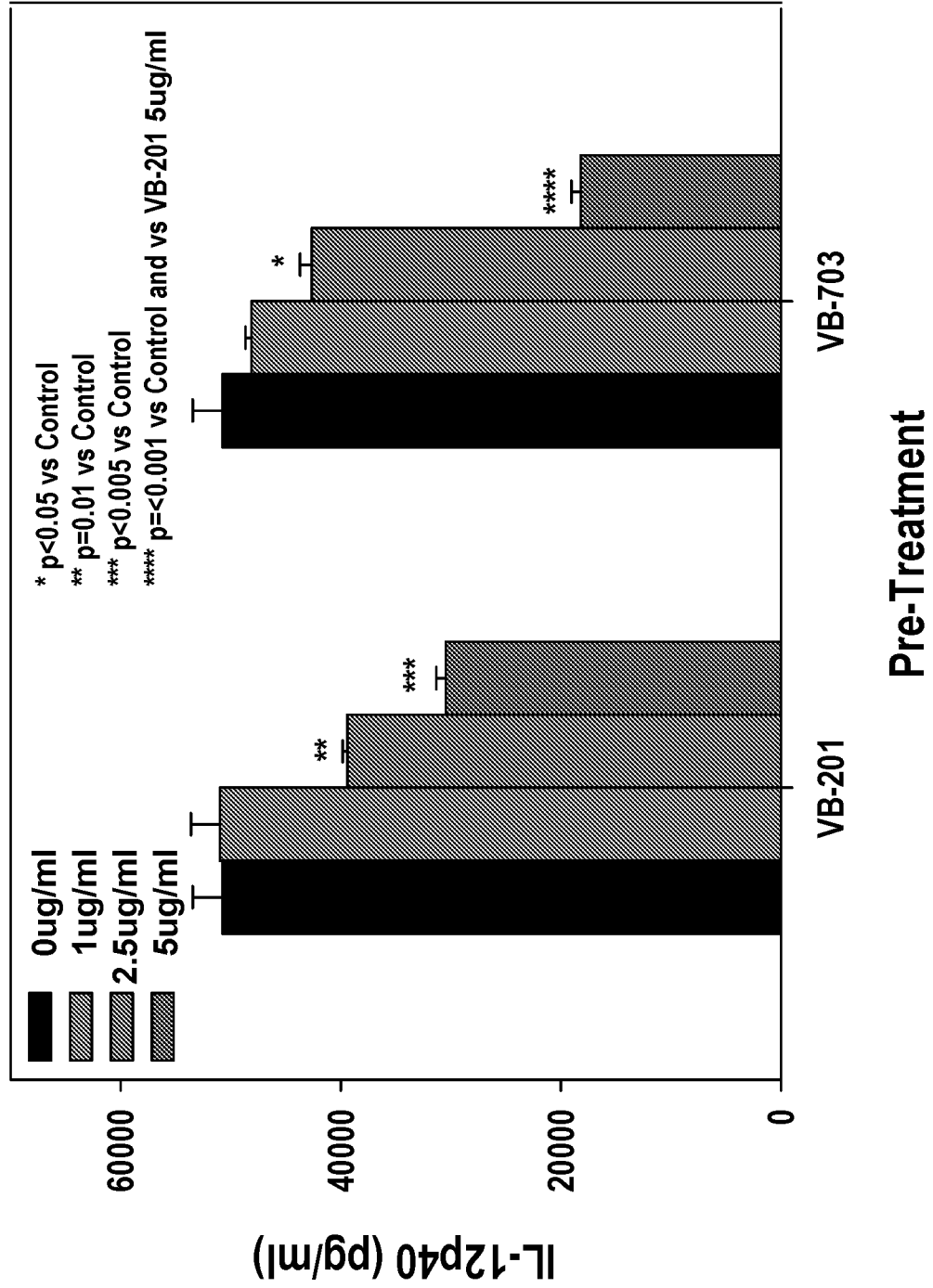


Fig 16

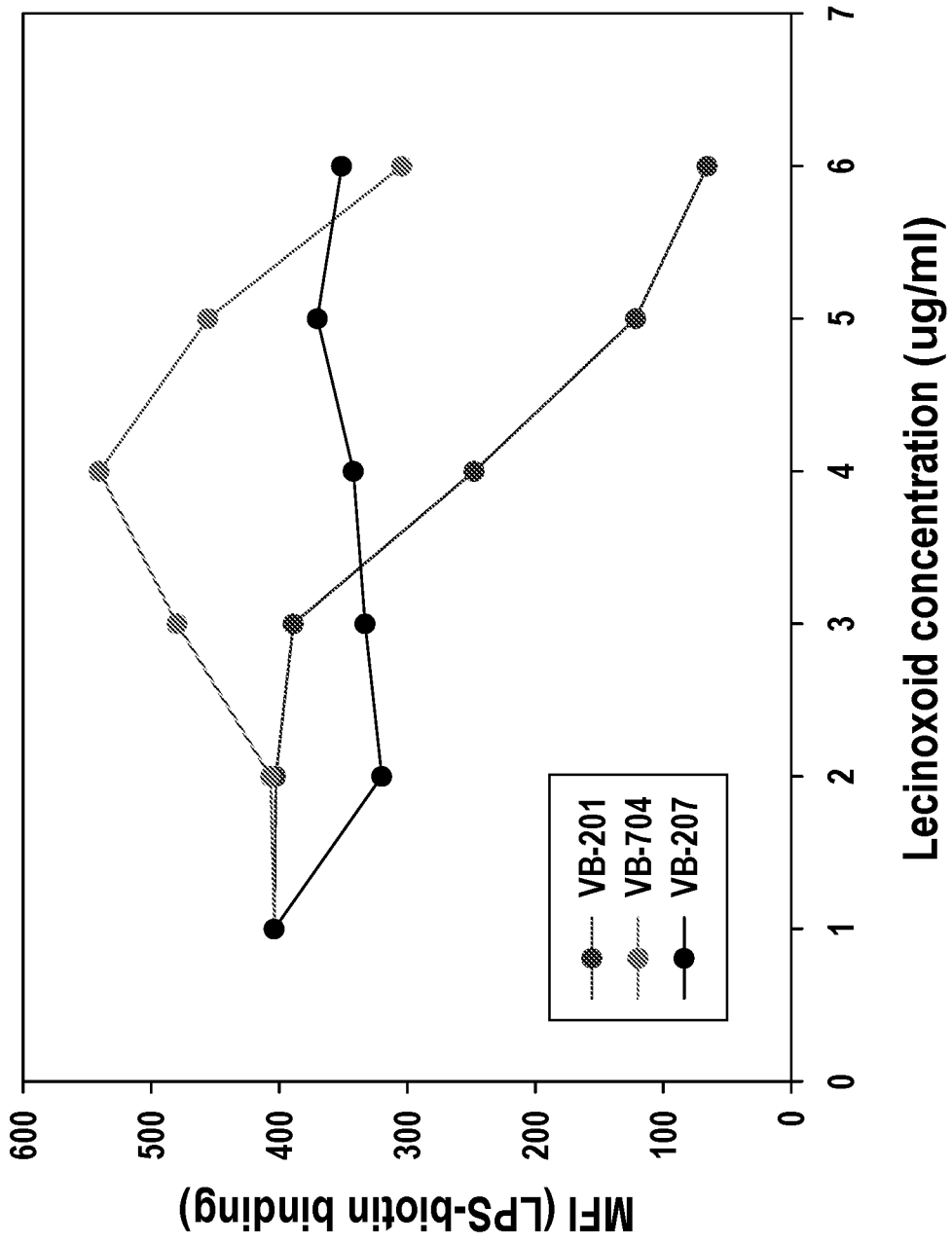


Fig 17

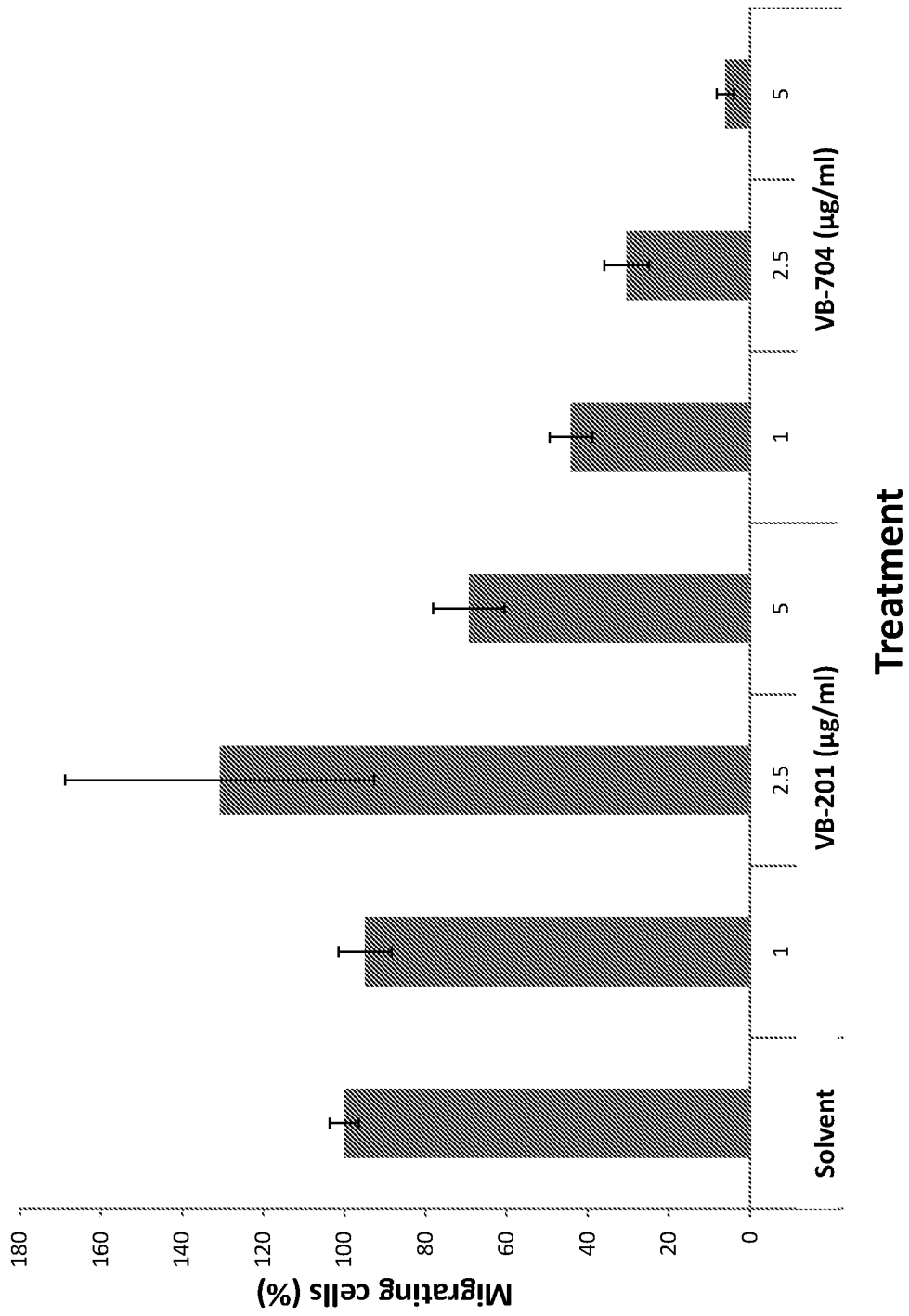


Fig 19

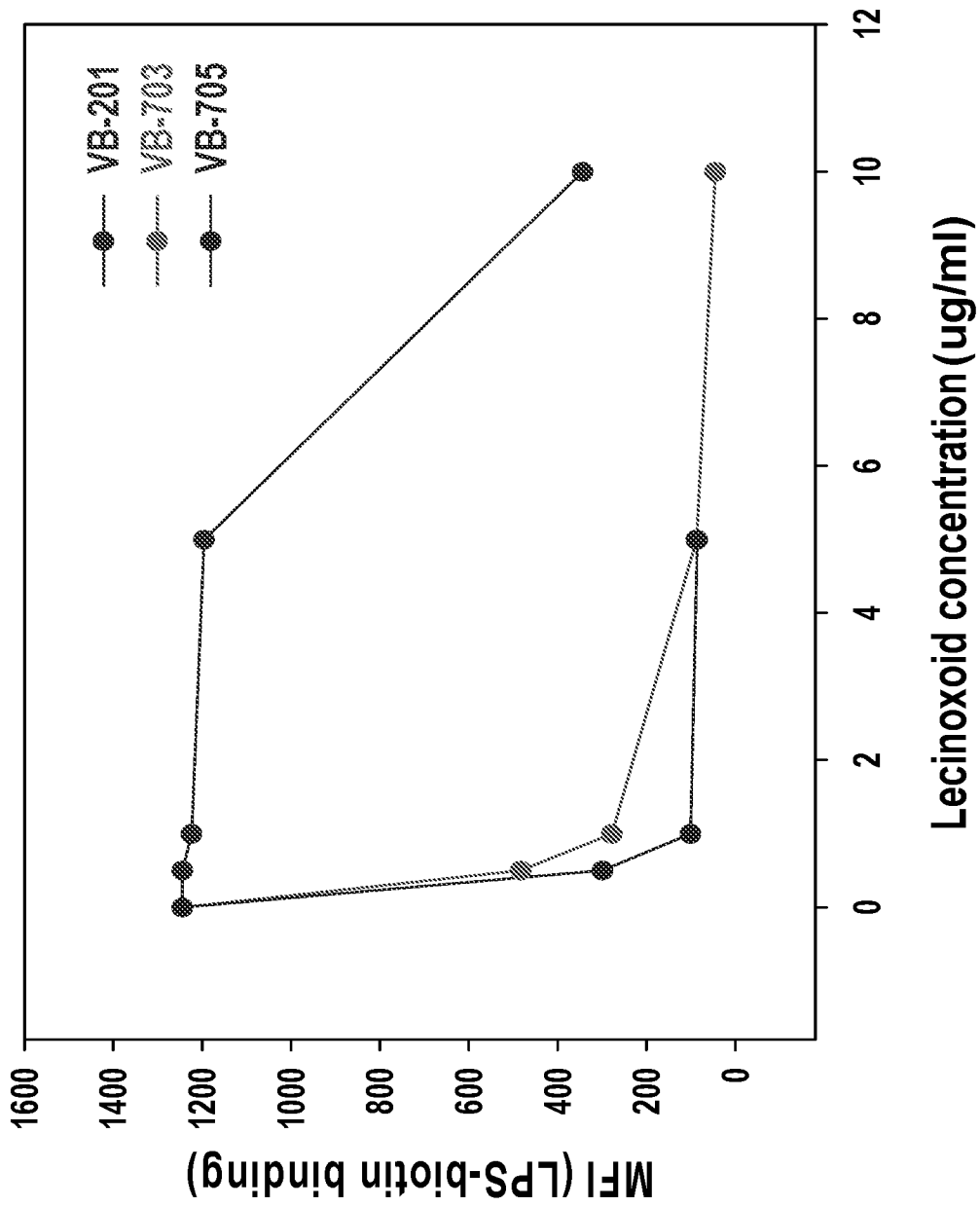


Fig 20

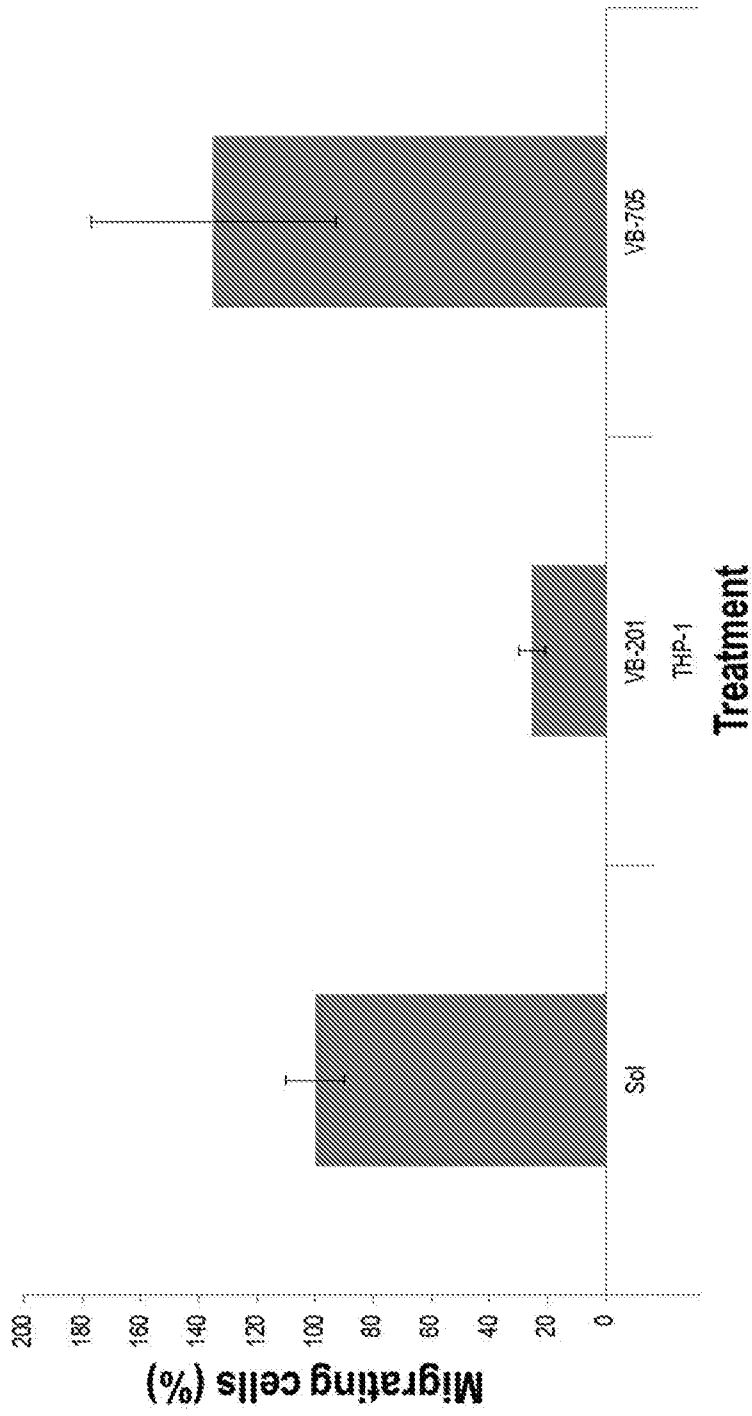


Fig 21A

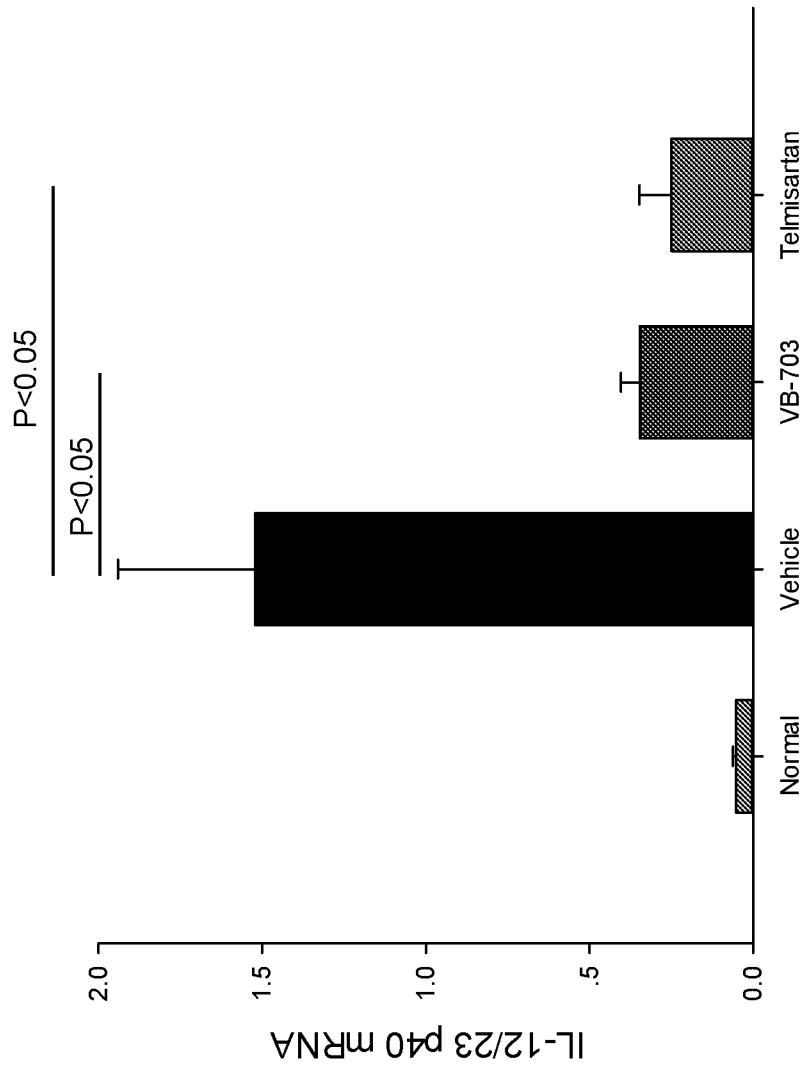


Fig 21B

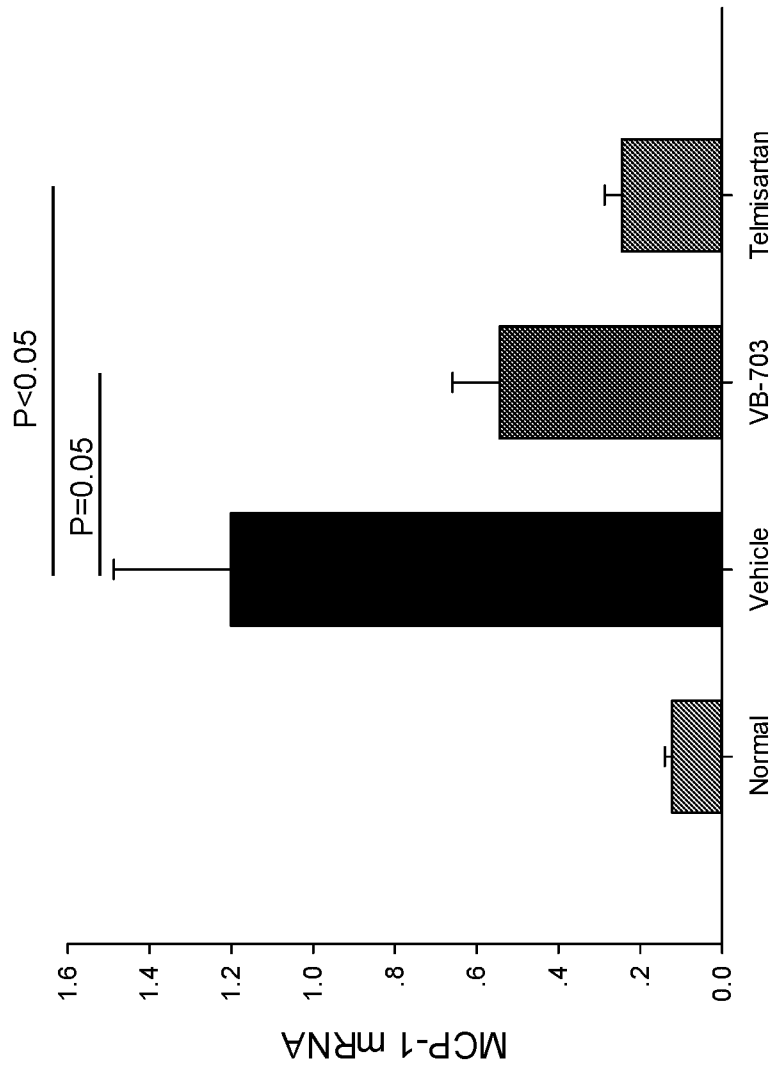


Fig 21C

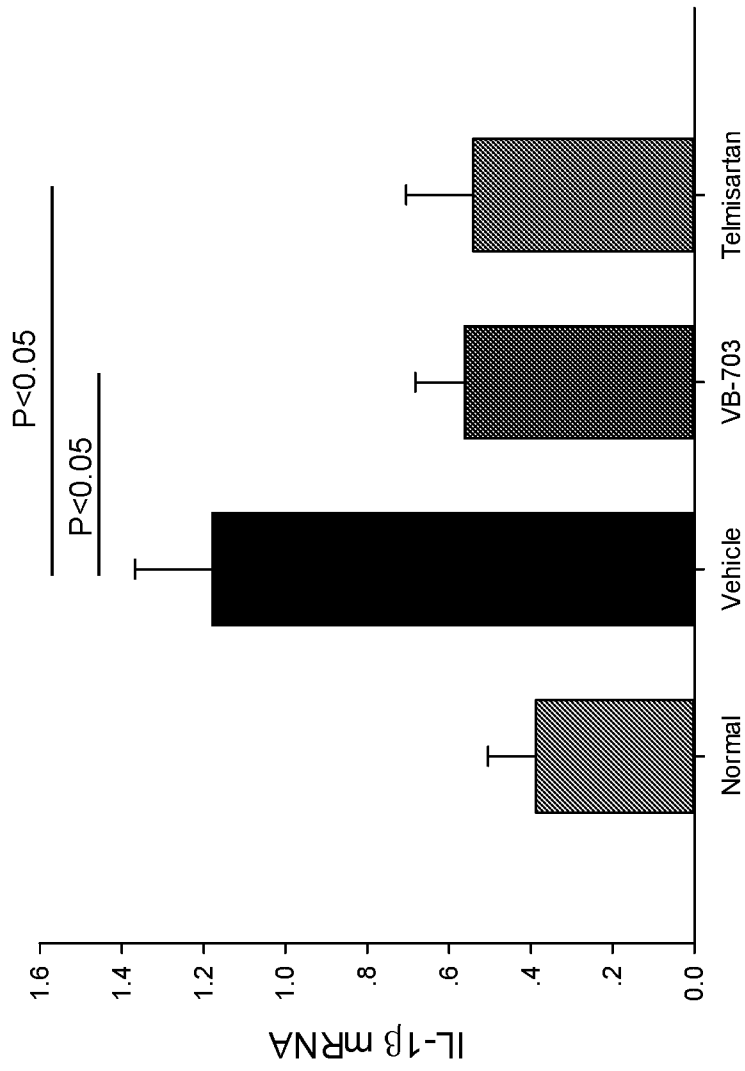


Fig 22

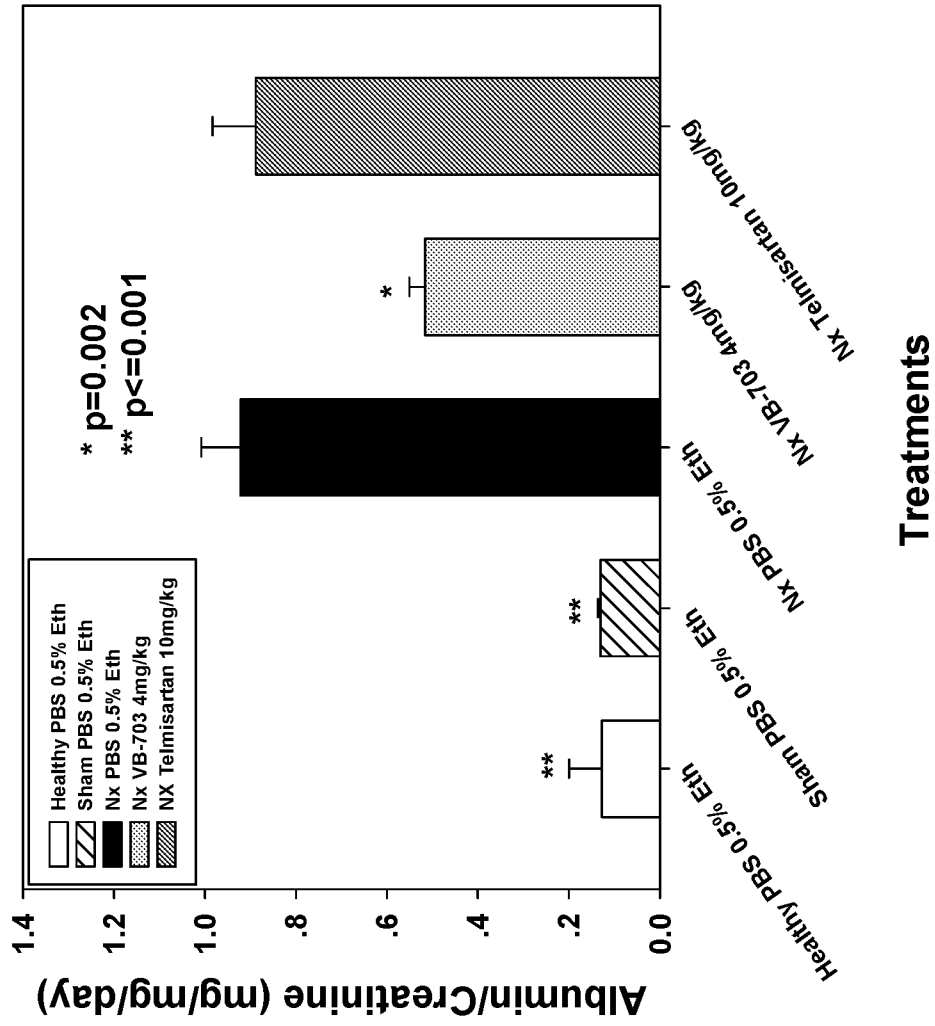


Fig 23

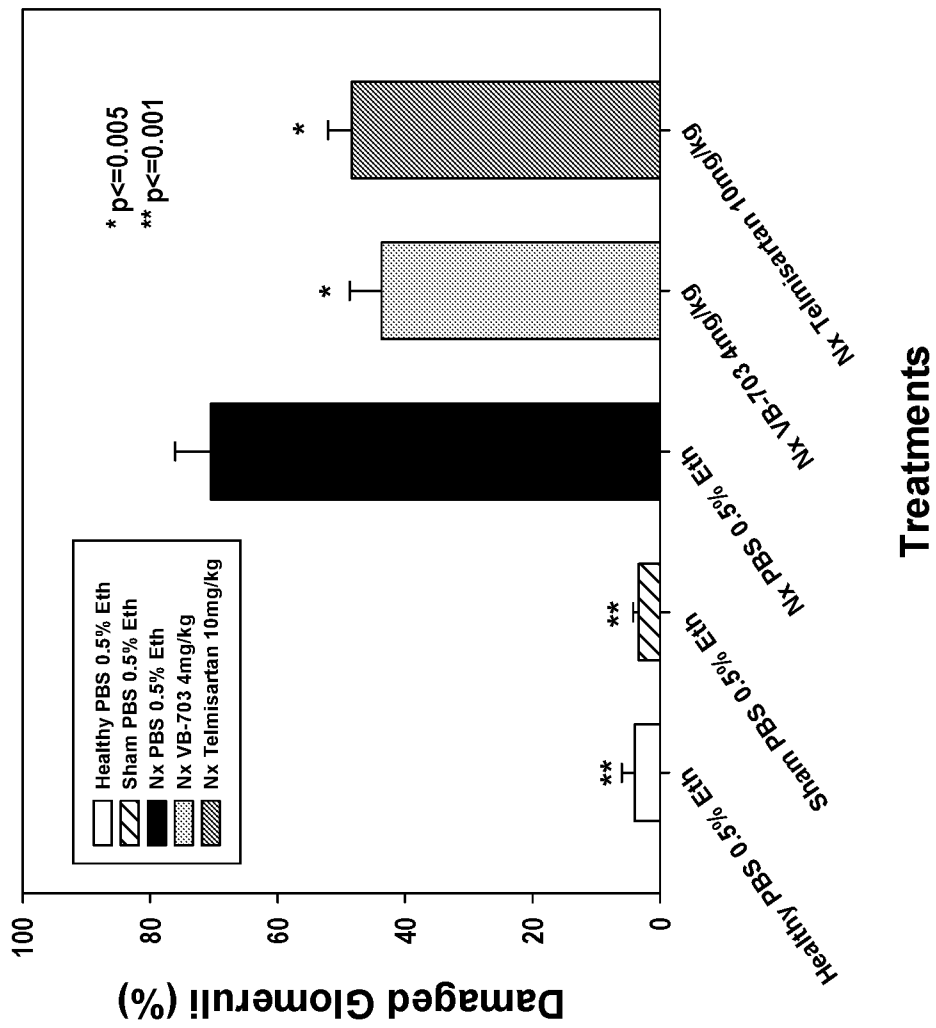


Fig 24

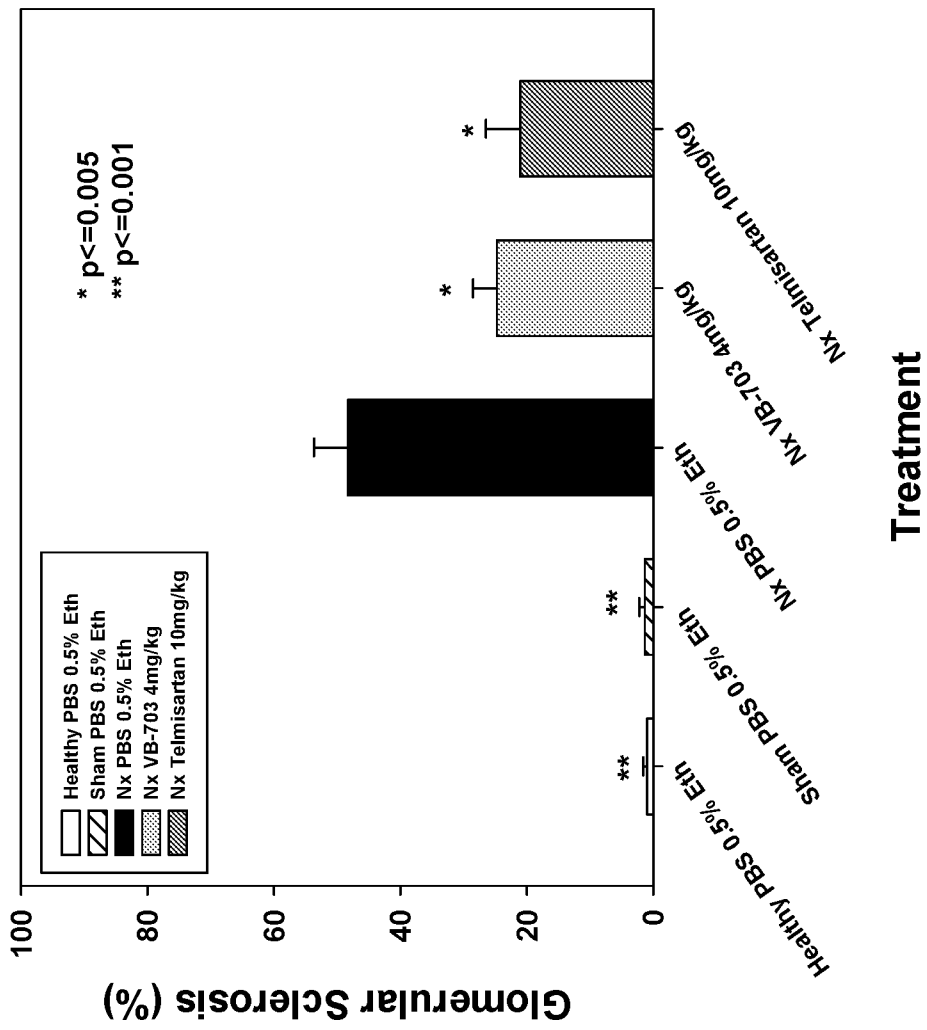
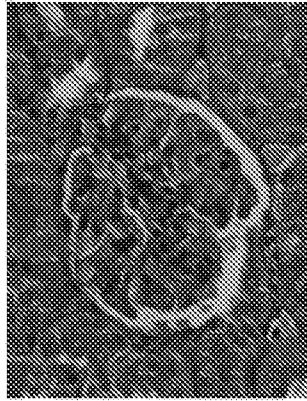


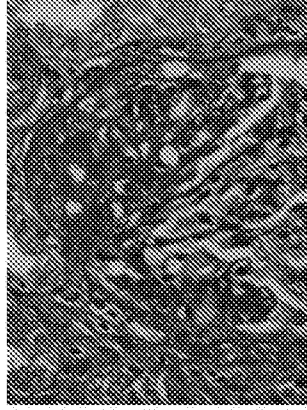
Fig 25



Healthy x400



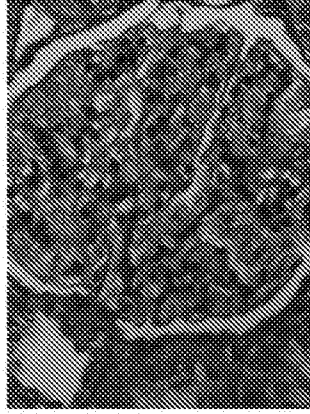
Sham x400



Nx PBS 0.5% Eth x400



Nx VB-703 4mg/kg
x400



Nx Telmisartan
10mg/kg x400

Fig 26A

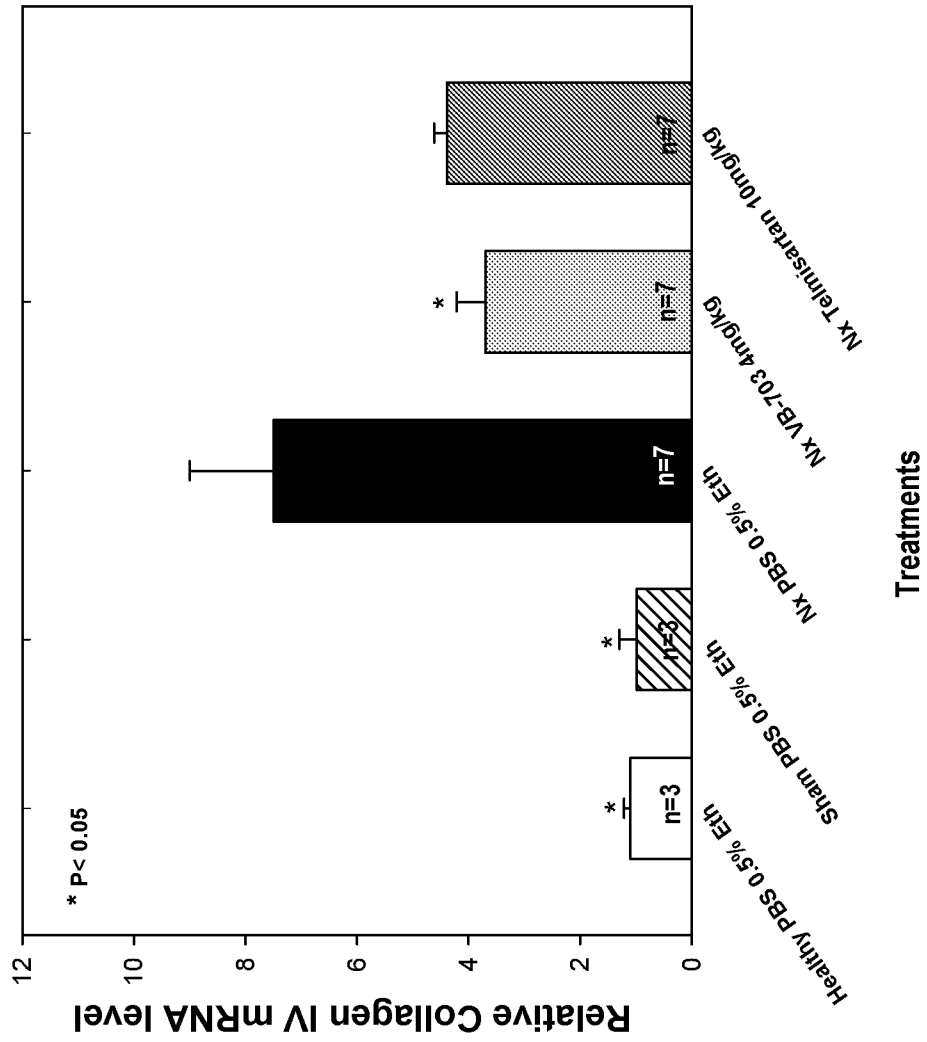


Fig 26B

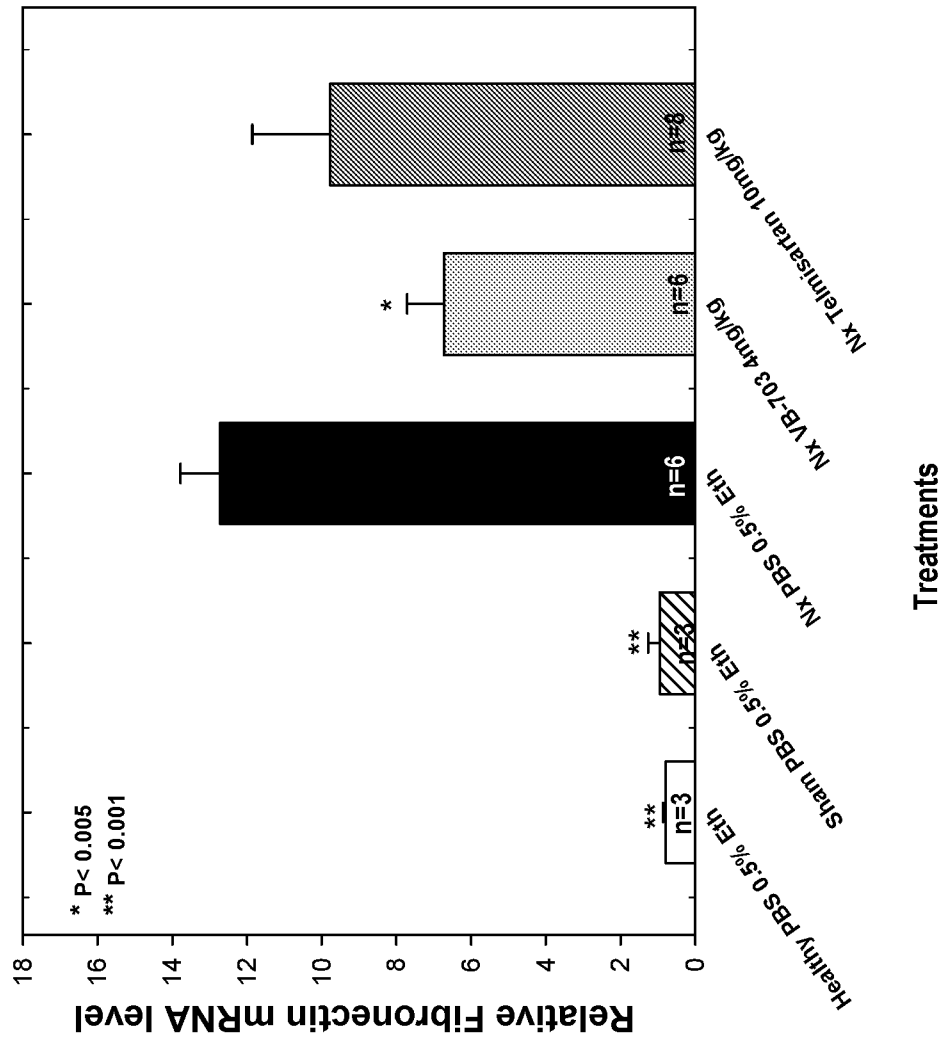


Fig 26C

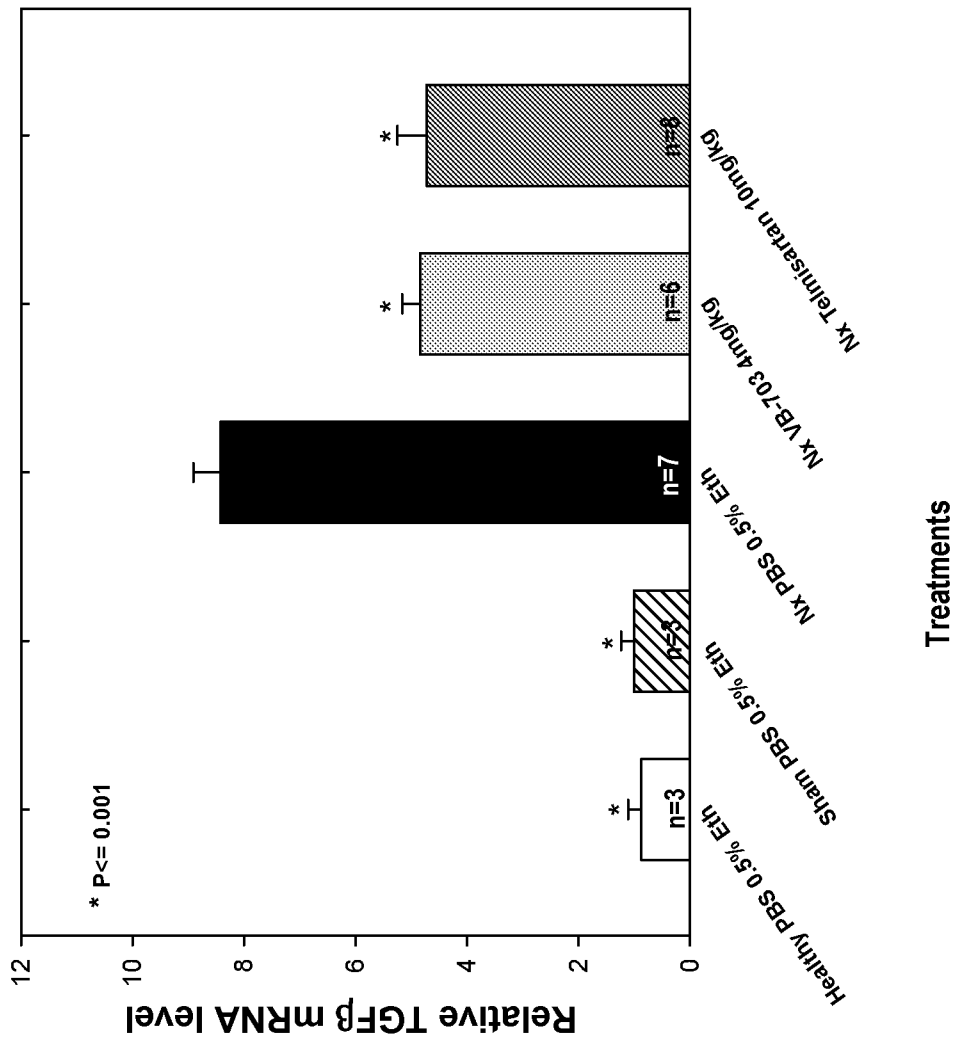


Fig 27A

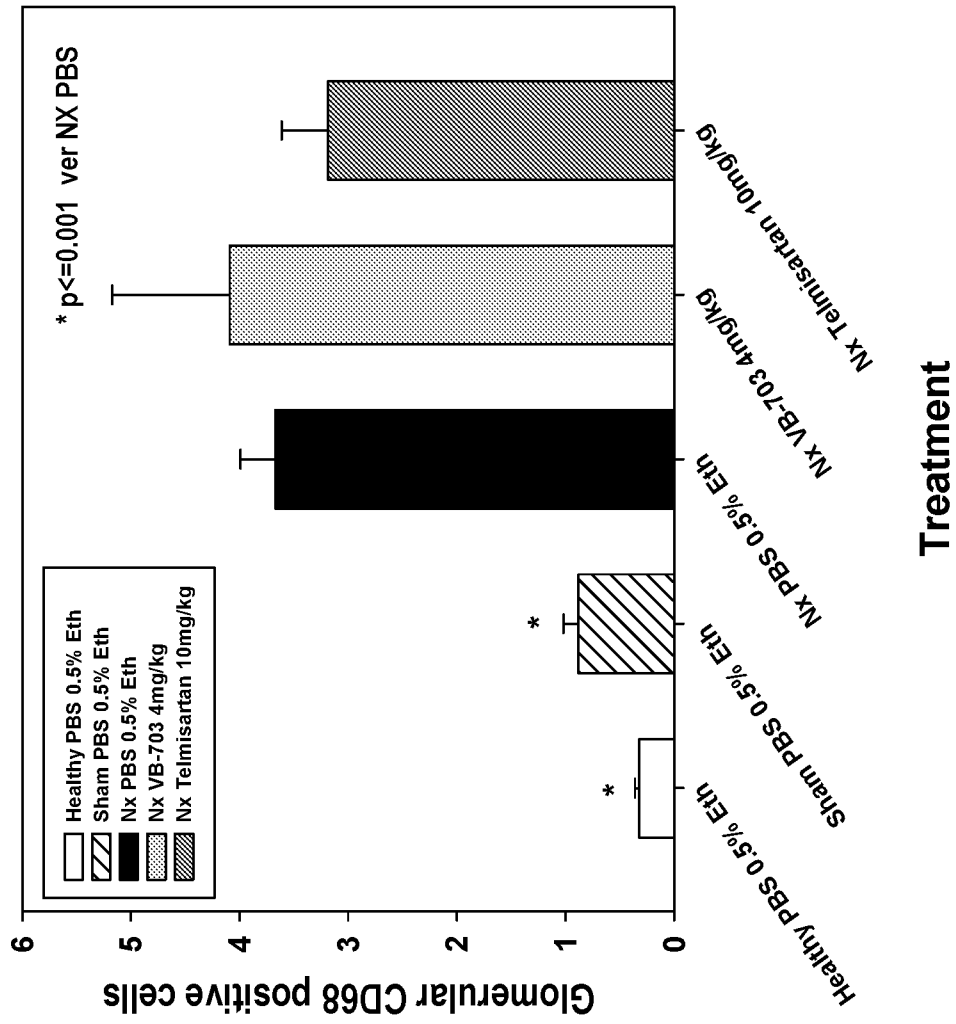
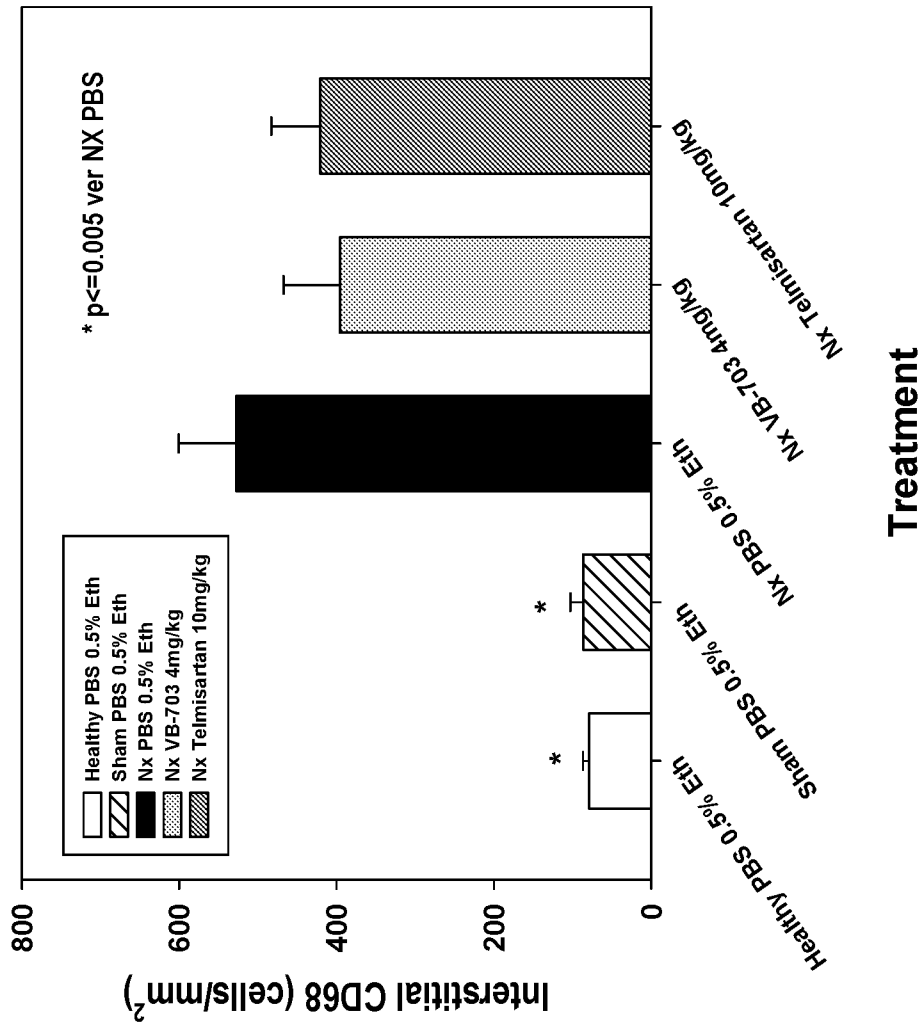


Fig 27B



INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB 15/59134

A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - A61K 31/075, C07C 43/13, C07F 9/02, A61K 31/24 (2016.01) CPC - A61K 31/19, C07C 43/1785, A61K 31/215, C07F 9/106, A61K 31/24 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) IPC(8) - A61K 31/075, C07C 43/13, C07F 9/02, A61K 31/24 (2016.01) CPC - A61K 31/19, C07C 43/1785, A61K 31/215, C07F 9/106, A61K 31/24 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched USPC - 514/114, 558/169, 558/170 Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Patbase, Google Patent, Google Web Search terms used - oxidized phospholipids fibrosis carboxylic acid pyridinium vb-201 lipids Pubchem substructure search		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y -- A	US 2013/0203707 A1 (Kovalevski-Ishai et al.) 08 August 2013 (08.08.2013); para [0430]-[0431], [0435], [0737], [0838]	1-3, 10-11, 30-32, 58-65 ----- 33-34
Y -- A	JP 58154512 A1 (Takeda Chem Ind Ltd) 14 September 1983 (14.09.1983); abstract, figure	1-3, 10-11, 30-32, 58-65 ----- 33-34
A	US 7,517,858 B1 (Hostetler et al.) 14 April 2009 (14.04.2009); entire document	1-3, 10-11, 30-34, 58-65
A	US 5,053,402 A1 (Masaki et al.) 01 October 1991 (01.10.1991); entire document	1-3, 10-11, 30-34, 58-65
A	US 5,614,548 A1 (Piantadosi et al.) 08 September 1997 (08.09.1997); entire document	1-3, 10-11, 30-34, 58-65
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/>		
* 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 "&" document member of the same patent family		
Date of the actual completion of the international search 11 February 2016		Date of mailing of the international search report 11 MAR 2016
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300		Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB 15/59134

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.: 4-9, 12-29, 35-57, 66-72
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.