(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

PCT

(43) International Publication Date
14 July 2011 (14.07.2011)

(10) International Publication Number
WO 2011/083469 A1

(51) International Patent Classification:
A61K 45/00 (2006.01)

(21) International Application Number:
PCT/IL2011/000012

(22) International Filing Date:
5 January 2011 (05.01.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

61/292,226	5 January 2010 (05.01.2010)	US
61/282,250	7 January 2010 (07.01.2010)	US
61/351,975	7 June 2010 (07.06.2010)	US
61/389,318	4 October 2010 (04.10.2010)	US
61/423,112	15 December 2010 (15.12.2010)	US

(71) Applicant *(for all designated States except US)*: **VASCULAR BIOGENICS LTD.** [IL/IL]; 6 Yoni Netanyahu Street, 60376 Or Yehuda (IL).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **MENDEL, Itzhak** [IL/IL]; 20/7 Shpinoza Street, 76452 Rechovot (IL). **YACOV, Niva** [IL/IL]; 22/15 Hadar Yosef Street, 69705 Tel-Aviv (IL). **COHEN, Yael** [IL/IL]; 100 Stern Street, 55602 Kiryat-Ono (IL). **FEIGE, Erez** [IL/IL]; P.O. Box 170, 50295 Hemed (IL). **BREITBART, Eyal** [IL/IL]; 27 HaYitzhar Street, 73127 Hashmonaim (IL).

(74) Agents: **G.E. EHRLICH (1995) LTD.** et al.; 11 Menachem Begin Road, 52681 Ramat Gan (IL).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

WO 2011/083469 A1

(54) **Title**: TREATMENT WITH VB-201

(57) **Abstract**: Novel unit dosage forms, methods and treatment regimens, utilizing VB-201 are disclosed. In particular, disclosed are unit dosage forms comprising less than 1 mg VB-201 and a pharmaceutically acceptable carrier, and formulated for oral administration, as well as treatment regimens comprising oral administration of VB-201 once per two or more days, for treating an inflammatory disease or disorder. Further disclosed are methods of treating metabolic syndrome, by administration of VB-201.

TREATMENT WITH VB-201

FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to the field of pharmacology and more particularly, but not exclusively, to a novel dosage form and treatment regimen of the oxidized phospholipid VB-201, which can be efficiently utilized in treating and preventing inflammation associated diseases and disorders such as, for example, cardiovascular diseases and disorders, autoimmune diseases or disorders such as psoriasis, arthritis and multiple sclerosis, and inflammatory bowel diseases. The present invention, is some embodiments thereof, further relates to a novel method of treating metabolic syndrome.

Oxidized phospholipids have been previously described as useful in the treatment of medical conditions such as, for example, cardiovascular diseases, cerebrovascular diseases and inflammatory diseases and disorders.

International Patent Application No. PCT/IL2004/000453 (Publication No. WO 04/106486), by the present assignee, describes oxidized lipids for prevention and treatment of inflammation associated with endogenous oxidized lipids. An exemplary such compound is described and known as CI-201 (1-hexadecyl-2-(4'-carboxybutyl)-glycerol-3-phosphocholine; also referred to in the art as VB-201).

International Patent Application No. PCT/IL01/01080 (Publication No. WO 02/41827), by the present assignee, describes oxidized lipids for prevention and treatment of atherosclerosis and related diseases.

U.S. Provisional Patent Application Nos. 61/292,226 and 61/282,250, filed January 5, 2010, and January 7, 2010, respectively, by the present assignee, describe unit dosage forms comprising between 1 mg and 100 mg VB-201 for oral administration, and treatment regimens comprising oral administration of VB-201 once or twice daily.

Metabolic syndrome is a condition originally described as consisting of obesity, insulin resistance, hypertension, impaired glucose tolerance or diabetes, hyperinsulinemia and dyslipidemia characterized by elevated triglyceride, and low HDL concentrations.

Metabolic syndrome is discussed in detail by Paresh Dandona et al, [*Circulation* 2005;111;1448-1454], which is incorporated herein by reference.

Additional background art includes International Patent Application Nos. PCT/IL05/000735 (Publication No. WO 06/006161), PCT/IL02/00005 (Publication No. 5 WO 02/053092) and PCT/IL08/000013 (Publication No. WO 08/084472), all being also by the present assignee.

All of the above cited publications are incorporated by reference as if fully set forth herein.

10 SUMMARY OF THE INVENTION

While exploring pharmacological aspects of 1-hexadecyl-2-(4'-carboxybutyl)-glycerol-3-phosphocholine (VB-201) in order to gain further insight on its mechanism of action, the present inventors have surprisingly uncovered the following:

(i) that individual dosages of less than 1 mg VB-201 surprisingly exhibit 15 significant beneficial therapeutic effects;

(ii) that VB-201 surprisingly exhibits potent effects on immune system memory, and that such effects on memory can result in long-term therapeutic effects as a result of an individual dose of VB-201; and

(iii) that VB-201 can be used in the treatment of metabolic syndrome.

20 The beneficial effects of VB-201 at such low doses, the long-term beneficial effects of individual doses of VB-201, and the therapeutic efficacy of VB-201 in treating metabolic syndrome were previously unsuspected.

Based on the beneficial effects of individual low doses of VB-201 and the long-term beneficial effects of individual doses of VB-201, the present inventors have devised 25 novel and effective treatment regimens and unit dosage forms for administering VB-201 at low doses and/or infrequent intervals.

Such treatment regimens result in a low total amount of VB-201 administered to the subject, which is particularly useful for preventing adverse side effects, yet are highly effective due to the potency of low doses of VB-201 and to the long-term effects 30 of VB-201, as described herein.

According to an aspect of some embodiments of the present invention, there is provided a pharmaceutical composition unit dosage form comprising from 1 μg to 1 mg

VB-201 and a pharmaceutically acceptable carrier, the pharmaceutical composition unit dosage form being formulated for oral administration.

According to an aspect of some embodiments of the present invention, there is provided a use of VB-201 in the manufacture of a unit dosage form of a medicament for treating or preventing an inflammatory disease or disorder, the unit dosage form comprising from 1 μg to 1 mg VB-201 and being formulated for oral administration.

According to some embodiments of the invention, the unit dosage form comprises from 0.01 mg to 0.5 mg VB-201.

According to some embodiments of the invention, the pharmaceutical composition unit dosage form is packaged in a packaging material and identified in print, in or on the packaging material, for use in the treatment or prevention of an inflammatory disease or disorder.

According to some embodiments of the invention, the pharmaceutical composition unit dosage form is identified for use once per two or more days.

According to an aspect of some embodiments of the present invention, there is provided a method of treating or preventing an inflammatory disease or disorder, the method comprising orally administering to a subject in need thereof a therapeutically effective amount of VB-201, wherein the therapeutically effective amount ranges from 1 μg per day to 1 mg per day.

According to some embodiments of the invention, the therapeutically effective amount of VB-201 is administered once per two or more days.

According to some embodiments of the invention, the therapeutically effective amount ranges from 0.01 mg per day to 0.5 mg per day. According to an aspect of some embodiments of the present invention, there is provided a method of treating or preventing an inflammatory disease or disorder, the method comprising orally administering to a subject in need thereof a therapeutically effective amount of VB-201, wherein the administering is effected once per two or more days.

According to some embodiments of the invention, the therapeutically effective amount ranges from 1 μg per day to 1 mg per day.

According to some embodiments of the invention, the methods described herein comprise administering a unit of the pharmaceutical composition unit dosage form described herein.

According to some embodiments of the invention, the inflammatory disease or disorder is associated with an endogenous oxidized lipid.

According to some embodiments of the invention, the inflammatory disease or disorder is selected from the group consisting of an idiopathic inflammatory disease or disorder, a chronic inflammatory disease or disorder, an acute inflammatory disease or disorder, an autoimmune disease or disorder, an infectious disease or disorder, an inflammatory malignant disease or disorder, an inflammatory transplantation-related disease or disorder, an inflammatory degenerative disease or disorder, a disease or disorder associated with a hypersensitivity, an inflammatory cardiovascular disease or disorder, an inflammatory cerebrovascular disease or disorder, a peripheral vascular disease or disorder, an inflammatory glandular disease or disorder, an inflammatory gastrointestinal disease or disorder, an inflammatory cutaneous disease or disorder, an inflammatory hepatic disease or disorder, an inflammatory neurological disease or disorder, an inflammatory musculo-skeletal disease or disorder, an inflammatory renal disease or disorder, an inflammatory reproductive disease or disorder, an inflammatory systemic disease or disorder, an inflammatory connective tissue disease or disorder, an inflammatory tumor, necrosis, an inflammatory implant-related disease or disorder, an inflammatory aging process, an immunodeficiency disease or disorder, a proliferative disease or disorder and an inflammatory pulmonary disease or disorder.

According to an aspect of some embodiments of the present invention, there is provided a method of treating or preventing metabolic syndrome, the method comprising administering to a subject in need thereof a therapeutically effective amount of VB-201.

According to an aspect of some embodiments of the present invention, there is provided a use of VB-201 in the manufacture of a medicament for treating or preventing metabolic syndrome.

According to an aspect of some embodiments of the present invention, there is provided VB-201, for use in treating or preventing metabolic syndrome.

According to an aspect of some embodiments of the present invention, there is provided VB-201, for use in treating or preventing metabolic syndrome by oral administration.

According to some embodiments of the invention, the administering comprises oral administration.

According to some embodiments of the invention, the medicament is formulated for oral administration.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

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. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIG. 1 presents an image of a Western blot of phosphorylated p38 MAPK (p-p38), p38 MAPK, phosphorylated IKKα/β (p-IKKα/β), IκBα, phosphorylated ERK1/2 (p-ERK1/2), ERK1/2 and α-tubulin (αTub) in peritoneal macrophages stimulated with lipopolysaccharide (LPS) immediately after addition of 1, 2 or 5 μg/ml VB-201 or solvent (Sol) to the culture medium; control cells were unstimulated (left lane);

FIG. 2 presents an image of a Western blot of phosphorylated p38 MAPK (p-p38), phosphorylated IKKα/β (p-IKKα/β), IκBα and α-tubulin (αTub) in peritoneal macrophages pre-treated for 20 minutes with solvent (lanes 2 and 8) or with 5 μg/ml VB-201 (lanes 3-6 and 9), followed immediately by LPS-stimulation (lanes 2-3), or by washing followed by LPS-stimulation immediately (lane 4), 1 hour later (lane 5), 3 hours later (lane 6) or 24 hours later (lanes 8 and 9); control cells were untreated (lanes 1 and 7);

FIG. 3 presents an image of a Western blot of phosphorylated p38 MAPK (p-p38), phosphorylated IKKα/β (p-IKKα/β) and α-tubulin (αTub) in peritoneal macrophages pre-treated with solvent (lanes 2 and 10) or with 5 μg/ml VB-201 (lanes 3-8 and 11), with (lanes 4-7 and 12) or without (lanes 2-3, 8 and 10-11) subsequent washing after 20 minutes, followed by LPS-stimulation immediately (lanes 2-4 and 10), 1 hour later (lane 5), 3 hours later (lane 6), 6 hours later (lanes 7-8) or 24 hours later (lanes 11-12); control cells were untreated (lanes 1 and 9);

FIG. 4 is a graph showing VB-201 uptake by primary human monocyte-derived dendritic cells (DCs), primary human CD4+ T cells; primary human CD19+ B cells, and primary human CD14+ monocytes, with uptake by U937 monocytes and CD11c+ mouse bone marrow-derived dendritic cells (BMDCs) as positive controls;

FIG. 5 is a graph showing the mean clinical score of PLP 139-151 peptide-induced EAE (experimental autoimmune encephalomyelitis) and the incidence of EAE (number of mice exhibiting EAE / number of mice in treatment group) for SJL mice administered 0.004 mg/kg, 0.04 mg/kg, 0.4 mg/kg or 4 mg/kg VB-201 or PBS;

FIG. 6 is a graph showing the mean clinical score of MOG 35-55 peptide-induced chronic EAE and the incidence of EAE (number of mice exhibiting EAE / number of mice in treatment group) for C57BL/6 mice administered 0.004 mg/kg, 0.04 mg/kg, 0.4 mg/kg or 4 mg/kg VB-201 or PBS;

FIG. 7 is a graph showing the mean clinical score of PLP 139-151 peptide-induced EAE and the incidence of EAE (number of mice exhibiting EAE / number of mice in treatment group) for SJL mice administered 0.04 mg/kg, 0.4 mg/kg or 4 mg/kg VB-201 or PBS;

FIG. 8 is a graph showing the number of mice exhibiting severe psoriasis, partial recovery from psoriasis, or complete recovery from psoriasis, following administration of 0.04 mg/kg or 4 mg/kg VB-201, or PBS (solvent);

FIG. 9 is a graph showing the effect of administration of 0.04 mg/kg VB-201, anti-murine IL-12/23 p40 antibodies, or vehicle (saline) on body weight of mice with T-cell-induced colitis, as a function of time after induction of colitis by T cell transfer (body weight data of healthy control mice with no induced colitis are shown for comparison);

FIG. 10 is a graph showing the effect of administration of 0.04 mg/kg or 0.4 mg/kg VB-201, 10 mg/kg sulfasalazine, or vehicle (saline) on body weight of mice with TNBS (trinitrobenzenesulfonic acid)-induced colitis;

FIG. 11 is a graph showing the effect of administration of 0.04 mg/kg or 0.4 mg/kg VB-201, 10 mg/kg sulfasalazine, or vehicle (saline) on the mean clinical score of TNBS-induced colitis in mice;

FIG. 12 is a graph showing the effect of administration of 0.04 mg/kg or 0.4 mg/kg VB-201, 10 mg/kg sulfasalazine, or vehicle (saline) on the mean gross pathology score of colons of mice with TNBS-induced colitis (mean scores represent mean for 8 animals);

FIGs. 13A and 13B are photomicrographs showing the effect of administration of 0.04 mg/kg VB-201 or vehicle (saline) on colon inflammation in mice with TNBS-induced colitis;

FIG. 14 is a graph showing the effect of administration of 0.04 mg/kg, 0.4 mg/kg or 4 mg/kg VB-201, anti-IL-12 p40 antibody (αIL-12p40), or PBS with 0.5 % ethanol (PBS 0.5% EtOH) on the disease activity index of DSS (dextran sulfate sodium)-induced colitis in mice, as a function of time after induction of colitis;

FIG. 15 is a graph showing the effect of administration of 0.04 mg/kg, 0.4 mg/kg or 4 mg/kg VB-201, anti-IL-12 p40 antibody (αIL-12 100μgx3), or PBS with 0.5 % ethanol (PBS) on the survival of mice following induction of colitis;

FIG. 16 is a graph showing the effect of administration of 0.04 mg/kg, 0.4 mg/kg or 4 mg/kg VB-201, anti-IL-12 p40 antibody (αIL-12), or PBS with 0.5 % ethanol (PBS 0.5% EtOH) on the colon length in mice with DSS-induced colitis;

FIG. 17 is a graph showing the area of aortic sinus lesions in male Apo E KO mice administered daily PBS or 0.1 μg, 0.33 μg, 1 μg, 3.3 μg, 10 μg and 33 μg VB-201 per mouse;

FIGs. 18A-18B are graphs showing the effect of administration (15 doses during a 12-week period) of PBS or 0.004 mg/kg (0.1 μg per mouse; FIG. 18A) or 0.4 mg/kg (10 μg per mouse; FIG. 18B) VB-201 on the area of aortic sinus lesions in male (FIG. 18A) and female (FIG. 18B) Apo E KO mice, as compared to the area of aortic sinus lesions in control mice at the beginning of the treatment period (Base Line);

FIG. 19 is a graph showing the arthritis score over time for mice administered daily PBS or 0.004 mg/kg, 0.04 mg/kg, 0.4 mg/kg or 4 mg/kg VB-201, following induction of arthritis by collagen injections (on days 0 and 21);

FIG. 20 is a graph showing the pathological score of arthritis for mice administered daily PBS or 0.004 mg/kg or 0.04 mg/kg VB-201, following induction of arthritis by collagen injections;

FIGs. 21A-21C present photographs showing a representative image of a joint of a mouse with collagen-induced arthritis (FIG. 21A) which was treated daily with PBS, a healthy mouse (FIG. 21B), and a mouse with collagen-induced arthritis which was treated daily with 0.04 mg/kg VB-201 (FIG. 21C); the joint from PBS treated mice (FIG. 21A) exhibits inflammatory infiltrates, pannus formation and bone destruction, while the joint from VB-201 treated mice (FIG. 21C) exhibits minor to non-inflammatory infiltrates;

FIGs. 22A-22B are graphs showing the percentage of platelet aggregation as a function of time following addition of 0.01 μM VB-201 (FIG. 22A), 0.1 μM VB-201 (FIG. 22A), 1 μM VB-201 (FIG. 22A and 22B), 10 μM VB-201 (FIG. 22B), or 0.25 μM PAF (platelet activating factor) (FIG. 22B);

FIG. 23 is a graph showing the percentage of platelet aggregation as a function of time following addition of ADP (adenosine diphosphate) with and without pre-incubation of the platelets with 0.1 μM VB-201;

FIGs. 24A-24F are graphs showing mean C-reactive protein (CRP) levels (FIGs. 24A-24C) and individual C-reactive protein (CRP) levels (FIGs. 24D-24F) in human serum before (day 0) and after (day 14 or day 28) daily oral administration of 40 mg VB-201 for 14 days (FIGs. 24A and 24D), 80 mg VB-201 for 14 days (FIGs. 24B and 24E) or 80 mg VB-201 for 28 days (FIGs. 24C and 24F), as determined by high sensitivity CRP (hsCRP) assay (N indicates number of subjects tested, and % reduction in CRP levels are shown);

FIGs. 25A and 25B present images of Western blots of phosphorylated ERK1/2 (p-ERK1/2) and ERK1/2 (FIGs. 25A and 25B) and phosphorylated p38 MAPK (p-p38), p38 MAPK, IκBα, and phosphorylated Jnk1 (p-Jnk1) (FIG. 25B) in unstimulated (FIG. 25A) and LPS-stimulated or soluble CD40 ligand (sCD40L)-stimulated (FIG. 25B) human dendritic cells pre-treated with 1, 2.5 or 5 μg/ml VB-201 or with 5, 10, 20 or 40

μg/ml oxidized PAPC (Ox-PAPC); control cells were untreated (Unt) or treated with solvent alone (Sol);

FIG. 26 presents an image of a Western blot of phosphorylated p38 (p-p38), phosphorylated IKKα/β (p-IKKα/β) and α-tubulin (αTub) in unstimulated and LPS-stimulated human dendritic cells treated with 1 or 5 μg/ml VB-201 or with 10 or 40 μg/ml of oxidized PAPC (Ox-PAPC/72h) or partially oxidized PAPC (Ox-PAPC/24h); control cells were untreated (Unt) or treated with solvent alone (Sol);

FIG. 27 presents an image of a Western blot of phosphorylated p38 (p-p38), phosphorylated IKKα/β (p-IKKα/β), IκBα, phosphorylated ERK1/2 (p-ERK1/2) and α-tubulin (αTub) in mouse dendritic cells pre-treated with 1, 5 or 10 μg/ml VB-201 or with solvent (Sol) and stimulated with LPS (a TLR4 agonist), Poly I:C (a TLR3 agonist), Pam3CSK4 (PAM3) (a TLR1:2 agonist) or IMQ (a TLR7 agonist); control cells (Unt) were unstimulated and were not treated with VB-201;

FIG. 28 presents an image of a Western blot of phosphorylated TAK1 (p-TAK1), phosphorylated p38 (p-p38), phosphorylated IKKα/β (p-IKKα/β), IκBα, phosphorylated ERK1/2 (p-ERK1/2), ERK1/2 and α-tubulin (αTub) in mouse peritoneal macrophages cells pre-treated with 1, 5, 10 or 20 μg/ml VB-201 or with solvent (Sol) and stimulated with LPS (a TLR4 agonist) or Pam3CSK4 (Pam3) (a TLR1:2 agonist); control cells (Unt) were unstimulated and were not treated with VB-201;

FIGs. 29A and 29B present images of Western blots of phosphorylated TAK1 (p-TAK1), IκBα, and phosphorylated IKKα/β (p-IKKα/β) (FIG. 29A), or phosphorylated p38 (p-p38), p-38, phosphorylated ERK1/2 (p-ERK1/2), phosphorylated Jnk1/2 (p-Jnk1/2) and tyrosine-416 phosphorylated Src-family kinases (p-SFK416) (FIG. 29B), in mouse peritoneal macrophages pre-treated with 20 μg/ml VB-201 or with solvent alone (Solv) and stimulated with Pam3CSK4 (Pam3);

FIGs. 30A and 30B are graphs showing migration of human primary monocytes pre-treated with VB-201 (8 μM) or solvent towards MCP-1, MIP-1α, RANTES (FIGs. 30A and 30B) and MCP-3 (FIG. 30A only) (FIGs. 30A and 30B present results from separate experiments; * indicates $p<0.05$);

FIG. 31 is a graph showing migration of human primary monocytes pre-treated with VB-201 or Ox-PAPC as a function of concentration; monocytes pre-treated with

solvent alone (Sol) served as a control (* indicates $p<0.05$ relative to solvent-treated sample);

FIG. 32 is a graph showing migration of untreated human primary monocytes towards supernatant of HUVEC cells treated for 16 hours with 30 μg/ml Ox-PAPC or solvent alone (Sol) (* indicates $p<0.05$ relative to solvent-treated sample);

FIG. 33 is a graph showing migration of human primary monocytes pre-treated with 1, 2.5 or 5 μM VB-201 or with solvent alone (Sol) towards supernatant of HUVEC cells treated with 30 μg/ml Ox-PAPC for 16 hours (* indicates $p<0.05$ relative to solvent-treated sample);

FIG. 34 is a graph showing migration of monocytes/macrophages to the peritoneal cavity following a thioglycollate injection in mice administered 0.4 mg/kg or 4 mg/kg VB-201 or solvent (PBS with 0.5 % ethanol) alone (* indicates $p<0.05$, and ** indicates $p<0.01$, relative to PBS-treated mice);

FIG. 35 is a graph showing IL-12/23 p40 production in an exemplary sample of LPS-activated human monocyte-derived dendritic cells treated with 1.7 μM VB-201 or solvent alone;

FIGs. 36A-36C are graphs showing production of IL-12/23 p40 (FIG. 36A), IL-6 (FIG. 36B) and TNF-α (FIG. 36C) in LPS-activated human monocyte-derived dendritic cells treated with 1.7, 0.85 or 0.17 μM VB-201 or solvent alone (* indicates $p<0.05$, and ** indicates $p<0.001$, relative to solvent-treated sample);

FIGs. 37A-37C are graphs showing production of IL-12/23 p40 (FIG. 37A), IL-6 (FIG. 37B) and TNF-α (FIG. 37C) in peptidoglycan-activated human monocyte-derived dendritic cells treated with 1.7, 0.85 or 0.17 μM VB-201 or solvent alone (** indicates $p<0.001$ relative to solvent-treated sample);

FIGs. 38A-38C are graphs showing production of IL-12/23 p40 (FIG. 38A), IL-6 (FIG. 38B) and TNF-α (FIG. 38C) in Poly I:C-activated human monocyte-derived dendritic cells treated with 1.7, 0.85 or 0.17 μM VB-201 or solvent alone (* indicates $p<0.05$ relative to solvent-treated sample);

FIGs. 39A and 39B are graphs showing relative IL-12/23 p40 mRNA expression (normalized according to GAPDH expression) of human monocyte-derived dendritic cells stimulated with LPS for 2, 3, 4 or 24 hours, and treated with VB-201 (bricks) or

solvent alone (black bars) (red bar shows IL-12/23 p40 mRNA expression prior to LPS stimulation);

FIG. 40 is a graph showing IL-12/23 p40 production by mouse peritoneal macrophages treated with VB-201, as a function of VB-201 concentration (% reduction in IL-12/23 p40 levels are shown; * indicates $p<0.05$, and ** indicates $p<0.001$, relative to sample with 0 μM VB-201);

FIG. 41 is a graph showing relative IL-8 mRNA expression (normalized according to GAPDH expression) of HUVEC cells incubated for 4 hours with 4 μM or 9 μM VB-201, 40 μg/ml or 100 μg/ml Ox-PAPC, or 4 μM PGPC, or incubated without treatment (* indicates $p<0.001$ relative to cells with no treatment);

FIGs. 42A and 42B are graphs showing IL-8 protein levels in supernatant of HUVEC cells incubated for 4 hours (FIG. 42A) or 24 hours (FIG. 42B) with 4 μM or 8.5 μM VB-201, 40 μg/ml or 100 μg/ml Ox-PAPC, or 4 μM or 8.5 μM PGPC, or incubated with PBS (* indicates $p<0.05$, and ** indicates $p<0.001$, relative to cells treated with PBS);

FIGs. 43A-43D are graphs showing protein levels of MCP-1 (FIG. 43A), IL-8 (FIG. 43B), soluble VCAM-1 (FIG. 43C) and soluble ICAM-1 (FIG. 43D) in supernatant of HUVEC cells incubated for 24 hours with LPS, following pre-treatment with 4 μM (green bars) or 9 μM (violet bars) VB-201 or without VB-201 pre-treatment (black bars), or unstimulated HUVEC cells (incubated without LPS or VB-201; gray bars);

FIG. 44 is a graph showing the area of aortic lesions and mean total cholesterol levels (numbers presented within columns) in rabbits administered daily PBS (Control) or 1 or 4 mg/kg VB-201;

FIG. 45 presents photographs showing staining of aortic lesions with Sudan VI in exemplary aortas from rabbits administered daily PBS or 4 mg/kg VB-201;

FIGs. 46A and 46B present flow cytometry plots showing the levels of CXCR2 (y-axis, FIG. 46A), CXCR1 (x-axis, FIG. 46A), CCR1 (y-axis, FIG. 46B) and CCR5 (x-axis, FIG. 46B) in human CD14+ monocytes incubated with 1, 2.5 or 5 μg/ml VB-201 or with solvent alone;

FIG. 47 presents an image of a Western blot of phosphorylated ERK1/2 (p-ERK1/2) and α-tubulin in human CD14+ monocytes pre-treated for 10 minutes with 5

μg/ml VB-201 or with solvent and stimulated with 100 ng/ml MIP-1α for 2, 5, 15, 30 or 60 minutes; control cells (Sol) were treated with solvent without MIP-1α;

FIG. 48 presents an image of a Western blot of phosphorylated ERK1/2 (p-ERK-1/2), phosphorylated MEK1/2 (p-MEK-1/2) and total ERK 1/2 in human CD14+ monocytes pre-treated for 30 minutes with 2 μg/ml VB-201 or with solvent and stimulated with 50 ng/ml RANTES for 2, 5, 15, 30 or 60 minutes; control cells were treated with solvent without RANTES (Sol) or were untreated (Unt);

FIG. 49 presents an image of a Western blot of phosphorylated ERK1/2 (p-ERKs1/2), phosphorylated MEK1/2 (p-MEK-1/2) and α-tubulin in human CD14+ monocytes pre-treated for 30 minutes with 2 μg/ml VB-201 or with solvent and stimulated with 100 ng/ml MCP-1 for 2, 5, 15, 30 or 60 minutes; control cells were treated with solvent without MCP-1 (Sol) or were untreated (Unt);

FIG. 50 is a scheme (from Dandona et al. [*Circulation* 2005, 111:1448-1454]) depicting features of metabolic syndrome on the basis of effects of insulin; and

FIG. 51 is a scheme (from Dandona et al. [*Circulation* 2005, 111:1448-1454]) depicting the pathogenesis of metabolic syndrome.

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to the field of pharmacology and more particularly, but not exclusively, to a novel dosage form and treatment regimen of the oxidized phospholipid VB-201, which can be efficiently utilized in treating and preventing inflammation associated diseases and disorders such as, for example, cardiovascular diseases and disorders, autoimmune diseases or disorders such as psoriasis, arthritis and multiple sclerosis, and inflammatory bowl disease. The present invention, is some embodiments thereof, further relates to a use of the oxidized phospholipid VB-201 in the treatment of metabolic syndrome.

The principles and operation of the present invention may be better understood with reference to the figures and accompanying descriptions.

Before explaining at least one embodiment 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.

VB-201 (also referred to herein and in the art as CI-201) has shown considerable promise as a therapeutically active agent in various *in vitro* models and *in vivo* animal models of inflammatory conditions.

In an attempt to improve treatment of inflammatory diseases and disorders, the present inventors have studied in detail the effects and mechanism of action of VB-201 at various doses in *in vitro* and *in vivo* models. The protocols of these assays are described in detail in the Examples section that follows. Based on the data obtained in the studies conducted, the present inventors have developed improved treatment regimens and unit dosage forms.

Referring now to the drawings and tables, FIG. 1 shows that immediately after administration, VB-201 was capable of inhibiting lipopolysaccharide (LPS)-induced activation of the p38, ERK1/2 and NFκB pathways, whereas FIGs. 2 and 3 show decreased LPS-mediated activation of the p38 and NFκB pathways by VB-201 up to 24 hours after VB-201 was washed out of the samples. These results show that VB-201 affects immune cell memory for over 24 hours after being removed from the system.

FIG. 4 shows that VB-201 undergoes uptake in the human immune system primarily by dendritic cells.

FIGs. 5-7 show that VB-201 delays and reduces the severity of experimental autoimmune encephalomyelitis (EAE) in both PLP-induced (FIGs. 5 and 7) and MOG-induced (FIG. 6) EAE mouse models, and that doses as low as 0.004 mg/kg are effective.

FIG. 8 shows the effectiveness of various doses of VB-201 against psoriasis in a xenotransplant psoriasis mouse model.

FIGs. 9-16 show the effectiveness of various doses of VB-201 against colitis in a T cell-induced colitis mouse model (FIG. 9), in a TNBS-induced colitis mouse model (FIGs. 10-13B), and in a DSS-induced colitis mouse model (FIGs. 14-16).

FIGs. 17-18B show the effectiveness of VB-201 at doses as low as 0.004 mg/kg at inhibiting atherosclerotic lesion progression.

FIGs. 19-21C show that VB-201 at doses as low as 0.004 mg/kg are effective against arthritis in a mouse model, and that 0.04 mg/kg is even more effective than higher doses of VB-201.

FIG. 22A-23 show that VB-201 does not induce platelet aggregation (FIGs. 22A and 22B), but rather slightly inhibits platelet aggregation (FIG. 23).

FIGs. 24A-24F show that VB-201 reduces serum CRP levels.

FIGs. 25A-29B show that VB-201 inhibits the activation of p38, ERK1/2 and NFκB pathways by a variety of Toll-like receptors (e.g., TLR4 and TLR1:2 activation).

FIGs. 30A-31 and FIG. 33 show that VB-201 inhibits migration of immune cells towards a variety of chemoattractants *in vitro*. FIG. 34 shows that VB-201 inhibits immune cells migration *in vivo*. FIG. 32 shows that oxidized PAPC induces cells to produce chemoattractants.

FIGs. 35-39 show that VB-201 inhibits production of IL-12/23 p40 by immune cells induced by a variety of stimulating agents (e.g., LPS, peptidoglycan and Poly I:C). FIGs. 36A-37C also show that VB-201 inhibits production of IL-6 induced by some stimulating agents. FIG. 40 shows that VB-201 inhibits production of IL-12/23 p40 by non-stimulated immune cells.

FIG. 43A-43D show that VB-201 inhibits production of various chemokines and adhesion molecules in LPS-stimulated cells. FIGs. 41-42B show that VB-201 does not affect IL-8 production in non-stimulated cells, but that oxidized PAPC enhances IL-8 production.

FIGs. 44 and 45 show that VB-201 (1-4 mg/kg) is effective against atherosclerosis.

FIGs. 46A and 46B show that VB-201 does not affect expression of cytokine receptors. FIGs. 47-49 show that VB-201 inhibits cell signaling (ERK1/2 and MEK1/2 phosphorylation) downstream of cytokine receptors.

FIGs. 50 and 51 depict processes involved with metabolic syndrome.

As demonstrated in the experimental results presented in the Examples section below, oral administration of VB-201 at doses (typically daily doses) of 0.04 mg/kg and 0.004 mg/kg were highly effective against a variety of pathologies in mice. As is known in the art, doses of 0.04 mg/kg and 0.004 mg/kg in mice are equivalent to doses of approximately 0.25 mg and 0.025 mg, respectively, in humans, based on the typical

body surface area, weight and pharmacokinetic parameters of humans vs. mice. The surprising potency of such low doses of VB-201 was previously unsuspected.

Hence, according to an aspect of some embodiments of the invention there is provided a method of treating or preventing an inflammatory disease or disorder, the method comprising orally administering to a subject in need thereof a therapeutically effective amount of VB-201.

In some embodiments, the therapeutically effective amount is lower than 1 mg per day, lower than 0.5 mg per day, lower than 0.1 mg per day and even lower than 0.05 mg per day. In some embodiments, the therapeutically effective amount ranges from 1 μg per day to 1 mg per day.

In some embodiments, the therapeutically effective amount is less than 0.75 mg per day, optionally less than 0.5 mg per day, and optionally less than 0.25 mg per day.

In some embodiments, the therapeutically effective amount is at least 3 μg per day, and optionally at least 10 μg (0.01 mg) per day.

Optionally, the therapeutically effective amount ranges from 0.01 mg per day to 0.5 mg per day.

The therapeutically effective amounts described herein refer to therapeutically effective amounts for administration to adult subjects. Optionally, amounts of VB-201 to be administered to a child are adjusted, for example, according to body weight of the child.

According to some embodiments, the therapeutically effective amounts described herein are absolute, and do not depend on the body weight of the subject, with the optional exception of subjects with a body weight far from the adult average (e.g., children).

According to alternative embodiments, the therapeutically effective amounts described herein are considered suitable for a subject of average body weight (e.g., 70 kg), and the amount to be administered is adjusted according to the body weight of the subject receiving VB-201. For example, a dose of 0.1 mg recited herein is optionally understood to refer to a dose of 0.1 mg per 70 kg body weight (i.e., 1.43 μg/kg).

In many embodiments, the therapeutically effective amount is administered repeatedly, for example, at pre-determined intervals.

In some embodiments, the administration of the therapeutically effective amount is effected by administering VB-201 once or twice per day, optionally twice per day. Administration of VB-201 once per day typically results in relatively stable VB-201 plasma concentrations, and plasma concentrations are typically even more stable when VB-201 is administered twice per day. Stable plasma concentrations are often desirable during treatment.

In some embodiments, the administration of the therapeutically effective amount is effected by administering VB-201 less frequently than once per day, such that on some days, no VB-201 is administered.

It is to be appreciated that herein, administration of a given amount "per day" refers to the average amount administered per day over a period of time, such that it is not necessary to administer VB-201 on each day.

As demonstrated herein, VB-201 exhibits long term effects on immune responses, such that an effect of VB-201 is observed even after VB-201 is no longer present. Consequently, a relatively consistent therapeutic effect of VB-201 may be obtained even if plasma concentrations drop considerably between doses of VB-201.

Without being bound by any particular theory, it is suggested that the observed long-term effect is a result of the efficient uptake of VB-201 by dendritic cells, which play a role in immune system memory.

Hence, in some embodiments, VB-201 is administered less than once per day. Optionally, the VB-201 is administered once per two or more days.

As used herein, the phrase "once per two or more days" encompasses frequencies of once per two days, once per three days, once per week, once per month, and the like. It is to be appreciated that administration intervals which are not defined by days *per se*, for example, twice per week, thrice per week, twice per month and so forth, are also encompassed by the phrase "once per two or more days". The administration intervals may be precise (e.g., such that administration is exactly once per two days, once per three days, etc.) or an average (e.g., such that over a period of time, administration is on average once per two days, once per three days, etc.).

Administration at intervals of less than once per day may also be performed with VB-201 amounts other than the amounts described hereinabove, for example, therapeutically effective amounts described in U.S. Provisional Patent Application Nos.

61/292,226 and 61/282,250, and in PCT International Patent Application entitled "TREATMENT WITH VB-201", having attorney's Docket No. 50377, which is co-filed with the instant application, the teachings of all of the above being incorporated by reference as if fully-set forth herein.

5 Thus, for example, is some embodiments of this aspect of embodiments of the invention, the administered dosage of VB-201 is, for example, 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg or 100 mg VB-201 per day.

Alternatively or additionally, in some embodiments of this aspect of embodiments of the invention, the method is effected by administering, for example, 10 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg or 100 mg VB-201 less than once per day. When such a dosage is administered less than once per day, the average daily dosage will be less than the administered dosage.

Hence, according to another aspect of embodiments of the present invention, there is provided a method of treating or preventing an inflammatory disease or disorder, 15 the method comprising orally administering to a subject in need thereof a therapeutically effective amount of VB-201, wherein administering is effected once per two or more days (as this phrase is described herein).

The range of doses according to embodiments of the present invention may be formulated as unit dosage forms of a medicament (e.g., a pharmaceutical composition) 20 designed for easy and convenient administration of a therapeutically effective amount of VB-201 described herein. It may be beneficial for the therapeutically effective amount to consist of no more than two unit dosage forms, as administration of more than two dosage forms at any one time may be more difficult. For example, it is commonly difficult to swallow more than two solid unit dosage forms (e.g., pills, tablets, capsules, 25 etc.). Moreover, administration of more than two unit dosage forms at any one time increases the likelihood of confusion and administration of an inappropriate dose.

Hence, according to optional embodiments, the method comprises administering one or two units of a unit dosage form at each administration. Optionally, one unit of the unit dosage form is administered per administration.

30 According to some embodiments, the method is effected by administering one or two units (optionally one unit) of a unit dosage form of VB-201.

Suitable unit dosage forms comprising VB-201 and therapeutically effective amounts of VB-201 are described herein, as well as in U.S. Provisional Patent Application Nos. 61/292,226 and 61/282,250, and in PCT International Patent Application entitled "TREATMENT WITH VB-201", having attorney's Docket No. 50377, which is co-filed with the instant application, the teachings of all of the above being incorporated by reference as if fully-set forth herein.

Thus, for example, is some embodiments of this aspect of embodiments of the invention, the method is effected by administering one or two units (optionally one unit) comprising, for example, 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg and/or 100 mg VB-201.

In view of the abovementioned benefits of the low doses of VB-201 described herein and of suitable unit dosage forms, according to another aspect of embodiments of the present invention, there is provided a use of VB-201 in the manufacture of a unit dosage form of a medicament (e.g., a pharmaceutical composition) for treating or preventing an inflammatory disease or disorder, the unit dosage form comprising from 1 μg to 1 mg VB-201, and being formulated for oral administration. Optionally, the unit dosage form comprises from 0.01 mg to 0.5 mg VB-201, and optionally any other therapeutically effective amount described herein.

In any of the methods and uses described herein, the VB-201 can be utilized either *per se* or as a part of a pharmaceutical composition, which further comprises a pharmaceutically acceptable carrier.

Hence, according to another aspect of embodiments of the present invention, there is provided a pharmaceutical composition unit dosage form comprising between 1 μg and 1 mg of VB-201 and a pharmaceutically acceptable carrier. The pharmaceutical composition unit dosage form is formulated for oral administration. Optionally, the pharmaceutical composition unit dosage form comprises between 0.01 mg and 0.5 mg of VB-201. Optionally, the pharmaceutical composition unit dosage form comprises an amount of VB-201 equal to any daily dose (e.g., such that a unit dosage form comprises a therapeutically effective amount described herein) described herein, or to half of a daily dose described herein (e.g., such that two unit dosage forms comprise a therapeutically effective amount described herein).

According to optional embodiments of the present invention, such a pharmaceutical composition unit dosage form is packaged in a packaging material and identified in print, in or on the packaging material, for use in the treatment or prevention of an inflammatory disease or disorder. In some embodiments, a plurality of unit dosage forms is packaged in a packaging material and identified in print, in or on the packaging material, for use in the treatment or prevention of an inflammatory disease or disorder. In some embodiments, the package insert comprises instructions of the number of administrations per day (or per more than 1 day, as described herein) and/or the number of unit dosage forms to be taken per each administration.

Suitable inflammatory diseases and disorders are discussed in more detail elsewhere herein.

As used herein, a "pharmaceutical composition" refers to a preparation of VB-201 (as active ingredient), or physiologically acceptable salts or prodrugs thereof, with other chemical components, including, but not limited to, physiologically suitable carriers, excipients, lubricants, buffering agents, antibacterial agents, bulking agents (e.g. mannitol), antioxidants (e.g., ascorbic acid or sodium bisulfite), and the like. The purpose of the pharmaceutical composition is to facilitate administration of VB-201 to a subject.

The term "unit dosage form", as used herein, describes physically discrete units, each unit containing a predetermined quantity of VB-201 calculated to produce the desired therapeutic effect, in association with at least one pharmaceutically acceptable carrier, diluent, excipient, or combination thereof.

Herein, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier", which are used interchangeably, describe a carrier or a diluent that does not cause significant irritation to the subject and does not abrogate the biological activity and properties of the VB-201.

As used herein, the term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered.

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient.

Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences" Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated for oral administration in a conventional manner using one or more pharmaceutically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the compounds into preparations which can be used pharmaceutically.

A pharmaceutical composition unit dosage form according to some embodiments can be formulated readily by combining VB-201 with pharmaceutically acceptable carriers well known in the art. Using such carriers the active ingredient (VB-201) is formulated, for example, as sachets, pills, caplets, capsules, tablets, dragee-cores or discrete (e.g., separately packaged) units of powder, granules, or suspensions or solutions in water or non-aqueous media. Thickeners, diluents, flavorings, dispersing aids, emulsifiers or binders may be desirable.

Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active doses.

Pharmaceutical compositions, which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active ingredient may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. Preferably, formulations for oral administration further include a protective coating, aimed at protecting or slowing enzymatic degradation of the preparation in the GI tract.

Composition unit dosage forms according to the present embodiments may, if desired, be presented in a pack or dispenser device, such as an FDA (the U.S. Food and Drug Administration) approved kit, which may contain one or more unit dosage forms containing VB-201. The pack or dispenser device may, for example, comprise metal or plastic foil, such as, but not limited to a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack may also be accompanied by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions for human administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Composition unit dosage forms comprising VB-201 formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an inflammatory disease or disorder, as defined herein.

As discussed herein, VB-201 may be advantageously administered according to some embodiments of the present invention less than once per day (e.g., once per two or more days).

Hence, according to another aspect of embodiments of the present invention, there is provided a pharmaceutical composition unit dosage form, formulated as described herein, and identified (e.g., in the abovementioned instructions for administration) for use once per two or more days, as described herein.

The unit dosage form according to this aspect optionally comprises VB-201 in an amount described herein, or in an amount described in U.S. Provisional Patent

Application Nos. 61/292,226 and/or 61/282,250 and in PCT International Patent Application entitled "TREATMENT WITH VB-201", having attorney's Docket No. 50377, which is co-filed with the instant application, the teachings of all of the above being incorporated by reference as if fully-set forth herein.

5 Thus, for example, is some embodiments of this aspect of embodiments of the invention, the unit dosage form can comprise less than 1 mg VB-201, as described herein, or alternatively, can comprise, 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg or 100 mg VB-201.

According to some embodiments of any of the unit dosage forms described 10 herein, the unit dosage form is identified (e.g., in the abovementioned instructions for administration) for use such that an individual dose consists of one or two units of the unit dosage form, and optionally one unit of the unit dosage form.

According to some embodiments, the unit dosage form is identified (e.g., in the abovementioned instructions for administration) for use during, or after a meal, as 15 described herein.

Optionally, a unit dosage form is designed so as to facilitate division of a unit dosage form into two half-unit dosage forms. For example, a pill, tablet or caplet may be scored so as to be readily broken in half.

According to further aspects of embodiments of the present invention, there are 20 provided pharmaceutical composition unit dosage forms, uses and methods as described herein, comprising an oxidized phospholipid other than VB-201. Suitable oxidized phospholipids, which are structurally related to VB-201 and thus exhibit similar activities, are described, for example, in International Patent Applications PCT/IL2004/000453 and PCT/IL2009/001049 (Publication Nos. WO 2004/106486 and 25 WO 2010/052718, respectively), which are incorporated by reference as if fully set forth herein.

The present embodiments further encompass any enantiomer, diastereomer, pharmaceutically acceptable salts, prodrugs, hydrates and solvates of the compounds (e.g., VB-201 and other oxidized phospholipids) described hereinabove.

30 VB-201 (1-hexadecyl-2-(4'-carboxybutyl)-glycerol-3-phosphocholine) according to embodiments of the present invention may be a chiral enantiomer of 1-hexadecyl-2-(4'-carboxybutyl)-glycerol-3-phosphocholine, i.e., either the *(R)*- enantiomer (*(R)*-1-

hexadecyl-2-(4'-carboxybutyl)-*sn*-glycerol-3-phosphocholine) or the *(S)*- enantiomer (*(S)*-1-hexadecyl-2-(4'-carboxybutyl)-glycerol-3-phosphocholine), or a mixture thereof (e.g., a racemate). According to exemplary embodiments, VB-201 is *(R)*-1-hexadecyl-2-(4'-carboxybutyl)-*sn*-glycerol-3-phosphocholine.

5 The term "prodrug" refers to an agent, which is converted into the active compound (the active parent drug) *in vivo*. Prodrugs are typically useful for facilitating the administration of the parent drug. They may, for instance, be bioavailable by oral administration whereas the parent drug is not. The prodrug may also have improved solubility as compared with the parent drug in pharmaceutical compositions. Prodrugs 10 are also often used to achieve a sustained release of the active compound *in vivo*. An example, without limitation, of a prodrug would be a compound as described herein, having one or more carboxylic acid moieties, which is administered as an ester (the "prodrug"). Such a prodrug is hydrolysed *in vivo*, to thereby provide the free compound (the parent drug). The selected ester may affect both the solubility characteristics and 15 the hydrolysis rate of the prodrug.

The phrase "pharmaceutically acceptable salt" refers to a charged species of the parent compound and its counter ion, which is typically used to modify the solubility characteristics of the parent compound and/or to reduce any significant irritation to an organism by the parent compound, while not abrogating the biological activity and 20 properties of the administered compound. An example, without limitation, of a pharmaceutically acceptable salt would be a carboxylate anion and a cation such as, but not limited to, ammonium, sodium, potassium and the like.

The term "solvate" refers to a complex of variable stoichiometry (e.g., di-, tri-, tetra-, penta-, hexa-, and so on), which is formed by a solute (the compound of present 25 embodiments) and a solvent, whereby the solvent does not interfere with the biological activity of the solute. Suitable solvents include, for example, ethanol, acetic acid and the like.

The term "hydrate" refers to a solvate, as defined hereinabove, where the solvent is water.

30 According to embodiments of the methods, uses and pharmaceutical composition unit dosage forms described herein, the inflammatory disease or disorder treatable

according to embodiments of the present invention is an inflammatory disease or disorder associated with an endogenous oxidized lipid.

As used herein, the phrase "an endogenous oxidized lipid" refers to one or more oxidized lipids that are present or formed *in vivo*, as a result of inflammatory and other cell- or humoral-mediated processes. Oxidized low-density lipoprotein (oxidized-LDL) is an example of an endogenous oxidized lipid associated with an inflammatory disease or disorder.

Inflammatory diseases or disorders treatable according to exemplary embodiments of the present invention include psoriasis (e.g., plaque psoriasis), rheumatoid arthritis, and atherosclerosis and related conditions, such as inflammation of an artery (e.g., inflammation of a carotid artery and/or inflammation of an aorta).

Additional examples of inflammatory diseases or disorders treatable according to exemplary embodiments of the present invention include multiple sclerosis and inflammatory bowel disease (e.g., chronic inflammatory bowel disease).

Representative inflammatory diseases and disorders treatable according to embodiments of the present invention include, for example, idiopathic inflammatory diseases or disorders, chronic inflammatory diseases or disorders, acute inflammatory diseases or disorders, autoimmune diseases or disorders, infectious diseases or disorders, inflammatory malignant diseases or disorders, inflammatory transplantation-related diseases or disorders, inflammatory degenerative diseases or disorders, diseases or disorders associated with a hypersensitivity, inflammatory cardiovascular diseases or disorders, inflammatory cerebrovascular diseases or disorders, peripheral vascular diseases or disorders, inflammatory glandular diseases or disorders, inflammatory gastrointestinal diseases or disorders, inflammatory cutaneous diseases or disorders, inflammatory hepatic diseases or disorders, inflammatory neurological diseases or disorders, inflammatory musculo-skeletal diseases or disorders, inflammatory renal diseases or disorders, inflammatory reproductive diseases or disorders, inflammatory systemic diseases or disorders, inflammatory connective tissue diseases or disorders, inflammatory tumors, necrosis, inflammatory implant-related diseases or disorders, inflammatory aging processes, immunodeficiency diseases or disorders, proliferative diseases and disorders and inflammatory pulmonary diseases or disorders, as is detailed hereinbelow.

Non-limiting examples of hypersensitivities include Type I hypersensitivity, Type II hypersensitivity, Type III hypersensitivity, Type IV hypersensitivity, immediate hypersensitivity, antibody mediated hypersensitivity, immune complex mediated hypersensitivity, T lymphocyte mediated hypersensitivity, delayed type hypersensitivity, helper T lymphocyte mediated hypersensitivity, cytotoxic T lymphocyte mediated hypersensitivity, TH1 lymphocyte mediated hypersensitivity, and TH2 lymphocyte mediated hypersensitivity.

Non-limiting examples of inflammatory cardiovascular disease or disorder include occlusive diseases or disorders, atherosclerosis, a 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 disease or disorder, 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.

Stenosis is an occlusive disease of the vasculature, commonly caused by atheromatous plaque and enhanced platelet activity, most critically affecting the coronary vasculature.

Restenosis is the progressive re-occlusion often following reduction of occlusions in stenotic vasculature. In cases where patency of the vasculature requires the mechanical support of a stent, in-stent-stenosis may occur, re-occluding the treated vessel.

Non-limiting examples of cerebrovascular diseases or disorders include stroke, cerebrovascular inflammation, cerebral hemorrhage and vertebral arterial insufficiency.

Non-limiting examples of peripheral vascular diseases or disorders include gangrene, diabetic vasculopathy, ischemic bowel disease, thrombosis, diabetic retinopathy and diabetic nephropathy.

Non-limiting examples of autoimmune diseases or disorders include all of the diseases caused by an immune response such as an autoantibody or cell-mediated

immunity to an autoantigen and the like. Representative examples are chronic rheumatoid arthritis, juvenile rheumatoid arthritis, systemic lupus erythematosus, scleroderma, mixed connective tissue disease, polyarteritis nodosa, polymyositis/dermatomyositis, Sjogren's syndrome, Bechet's disease, multiple sclerosis, autoimmune diabetes, Hashimoto's disease, psoriasis, primary myxedema, pernicious anemia, myasthenia gravis, chronic active hepatitis , autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura, uveitis, vasculitides and heparin induced thrombocytopenia.

Non-limiting examples of inflammatory glandular diseases or disorders include pancreatic diseases or disorders, Type I diabetes, thyroid diseases or disorders, Graves' disease, thyroiditis, spontaneous autoimmune thyroiditis, Hashimoto's thyroiditis, idiopathic myxedema, ovarian autoimmunity, autoimmune anti-sperm infertility, autoimmune prostatitis and Type I autoimmune polyglandular syndrome.

Non-limiting examples of inflammatory gastrointestinal diseases or disorders include colitis, ileitis, Crohn's disease, chronic inflammatory intestinal disease, inflammatory bowel syndrome, inflammatory bowel disease, celiac disease, ulcerative colitis, an ulcer, a skin ulcer, a bed sore, a gastric ulcer, a peptic ulcer, a buccal ulcer, a nasopharyngeal ulcer, an esophageal ulcer, a duodenal ulcer and a gastrointestinal ulcer.

Non-limiting examples of inflammatory cutaneous diseases or disorders include acne, an autoimmune bullous skin disease, pemphigus vulgaris, bullous pemphigoid, pemphigus foliaceus, contact dermatitis and drug eruption.

Non-limiting examples of inflammatory hepatic diseases or disorders include autoimmune hepatitis, hepatic cirrhosis, and biliary cirrhosis.

Non-limiting examples of inflammatory neurological diseases or disorders include multiple sclerosis, Alzheimer's disease, Parkinson's disease, myasthenia gravis, motor neuropathy, Guillain-Barre syndrome, autoimmune neuropathy, Lambert-Eaton myasthenic syndrome, paraneoplastic neurological disease or disorder, paraneoplastic cerebellar atrophy, non-paraneoplastic stiff man syndrome, progressive cerebellar atrophy, Rasmussen's encephalitis, amyotrophic lateral sclerosis, Sydeham chorea, Gilles de la Tourette syndrome, autoimmune polyendocrinopathy, dysimmune neuropathy, acquired neuromyotonia, arthrogryposis multiplex, Huntington's disease, AIDS associated dementia, amyotrophic lateral sclerosis (AML), multiple sclerosis,

stroke, an inflammatory retinal disease or disorder, an inflammatory ocular disease or disorder, optic neuritis, spongiform encephalopathy, migraine, headache, cluster headache, and stiff-man syndrome.

Non-limiting examples of inflammatory connective tissue diseases or disorders include autoimmune myositis, primary Sjogren's syndrome, smooth muscle autoimmune disease or disorder, myositis, tendinitis, a ligament inflammation, chondritis, a joint inflammation, a synovial inflammation, carpal tunnel syndrome, arthritis, rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, a skeletal inflammation, an autoimmune ear disease or disorder, and an autoimmune disease or disorder of the inner ear.

Non-limiting examples of inflammatory renal diseases or disorders include autoimmune interstitial nephritis and/or renal cancer.

Non-limiting examples of inflammatory reproductive diseases or disorders include repeated fetal loss, ovarian cyst, or a menstruation associated disease or disorder.

Non-limiting examples of inflammatory systemic diseases or disorders include systemic lupus erythematosus, systemic sclerosis, septic shock, toxic shock syndrome, and cachexia.

Non-limiting examples of infectious disease or disorder include chronic infectious diseases or disorders, a subacute infectious disease or disorder, an acute infectious disease or disorder, a viral disease or disorder, a bacterial disease or disorder, a protozoan disease or disorder, a parasitic disease or disorder, a fungal disease or disorder, a mycoplasma disease or disorder, gangrene, sepsis, a prion disease or disorder, influenza, tuberculosis, malaria, acquired immunodeficiency syndrome, and severe acute respiratory syndrome.

Non-limiting examples of inflammatory transplantation-related diseases or disorders include graft rejection, chronic graft rejection, subacute graft rejection, acute graft rejection hyperacute graft rejection, and graft versus host disease or disorder. Exemplary implants include a prosthetic implant, a breast implant, a silicone implant, a dental implant, a penile implant, a cardiac implant, an artificial joint, a bone fracture repair device, a bone replacement implant, a drug delivery implant, a catheter, a pacemaker, an artificial heart, an artificial heart valve, a drug release implant, an electrode, and a respirator tube.

Non-limiting examples of inflammatory tumors include a malignant tumor, a benign tumor, a solid tumor, a metastatic tumor and a non-solid tumor.

Non-limiting examples of inflammatory pulmonary diseases or disorders include asthma, allergic asthma, emphysema, chronic obstructive pulmonary disease or disorder, sarcoidosis and bronchitis.

An example of a proliferative disease or disorder is cancer.

As discussed herein, and demonstrated in the experimental results herein, the present inventors have surprisingly uncovered that VB-201 can be used in the treatment of metabolic syndrome.

According to the American Diabetic Association, an adult person has metabolic syndrome if he has 3 of the following 5 indications:

(i) BMI over 25 or if his/her waist circumference is 102 cm (in Europe $\geq$ 94) for men and $\geq$88 cm (in Europe $\geq$ 80) for women;

(ii) Blood triglycerides level that is equal to or higher than 150 mg/dl;

(iii) Blood HDL cholesterol level lower than 40 mg/dl (for men) or lower than 50 mg/dl (for women);

(iv) Blood glucose level that is equal to or higher than 100 mg/dl; and/or

(v) Blood pressure that is equal to or higher than 130/85 mmHg.

Metabolic syndrome constitutes a significant risk for coronary heart disease.

Several new features have been shown to be associated with the metabolic syndrome over time. These include elevated plasminogen activator inhibitor-1 (PAI-1) concentrations and now, elevated C-reactive protein (CRP) concentrations. Although several authors proposed their incorporation into the classic terminology of metabolic syndrome, this has not yet been agreed despite a large body of evidence from the literature suggesting the association.

Insulin has been shown to suppress several pro-inflammatory transcription factors, such as nuclear factor NF-κB, Egr-1, and activating protein-1 (AP-1) and the corresponding genes regulated by them, which mediate inflammation. An impairment of the action of insulin because of insulin resistance would thus result in the activation of these pro-inflammatory transcription factors and an increase in the expression of the corresponding genes.

Obesity is also associated with inflammatory processes: plasma concentrations of TNF-α, IL-6, CRP, MIF, and other inflammatory mediators have been shown to be increased in the obese. Adipose tissue has been shown to express most of these pro-inflammatory mediators. It has also been shown that macrophages residing in the adipose tissue may also be a source of pro-inflammatory factors and that they also may modulate the secretory activity of adipocytes. Tissue macrophages are derived from monocytes in blood. Recently, the mononuclear cells of the obese, of which monocytes are a fraction, have also been shown to be in an inflammatory state, expressing increased amounts of pro-inflammatory cytokines and related factors. In addition, these cells have been shown to have a significantly increased binding of NF-kB, the key pro-inflammatory transcription factor, and an increase in the intranuclear expression of p65 (Rel A), the major protein component of NF-kB. These cells also express diminished amounts of IkBβ, the inhibitor of NF-kB. Clearly, therefore, evidence of inflammation exist in various cells and in plasma in obesity.

In conclusion, the pro-inflammatory state of obesity and metabolic syndrome originates with excessive caloric intake and is probably a result of over nutrition in a majority of patients in the United States. The pro-inflammatory state induces insulin resistance, leading to clinical and biochemical manifestations of the metabolic syndrome. This resistance to insulin action promotes inflammation further through an increase in FFA concentration and interference with the anti-inflammatory effect of insulin.

The present inventors have now demonstrated that VB-201 is associated with the conversion of "bad fatty cells" into "good fatty cells".

As exemplified in the Examples section hereinbelow, VB-201 reverses certain inflammatory processes which are specifically involved in metabolic syndrome. Thus, for example, the present inventors have now uncovered that VB-201 inhibits the NF-kB pathway, raising IkBα levels and reducing IKKα/β phosphorylation, and reduces levels of C-reactive protein (CRP), which are increased in metabolic syndrome (see, for example, FIGs. 50 and 51). In addition, VB-201 inhibits platelet aggregation, thereby protecting against platelet hyperaggregability caused by metabolic syndrome (see, for example, FIG. 50).

Hence, according to another aspect of embodiments of the present invention, there is provided a method of treating or preventing metabolic syndrome, the method comprising administering (e.g., orally) to a subject in need thereof a therapeutically effective amount of VB-201. Optionally, the therapeutically effective amount of VB-201 is a therapeutic amount described herein (e.g., in a range of from 1 μg per day to 1 mg per day). Optionally, the therapeutically effective amount of VB-201 is a therapeutic amount described in U.S. Provisional Patent Application Nos. 61/292,226 and 61/282,250 and in PCT International Patent Application entitled "TREATMENT WITH VB-201", having attorney's Docket No. 50377, which is co-filed with the instant application, the teachings of all of the above being incorporated by reference as if fully-set forth herein.

Thus, for example, is some embodiments of this aspect of embodiments of the invention, the therapeutically effective amount is less than 1 mg VB-201 per day, as described herein, or alternatively, can comprise, 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg or 100 mg VB-201 per day.

According to another aspect of embodiments of the present invention, there is provided a use of VB-201 in the manufacture of a medicament for treating or preventing metabolic syndrome. Formulation of the medicament may be according to any method described herein. Optionally the medicament is formulated for oral administration.

According to another aspect of embodiments of the present invention, there is provided VB-201, or an oxidized phospholipid structurally related to VB-201 (as described hereinabove), for use in treating or preventing metabolic syndrome (e.g., by oral administration thereof).

As used herein the term “about” refers to ± 10 %.

The terms "comprises", "comprising", "includes", "including", “having” and their conjugates mean "including but not limited to".

The term “consisting of means “including and limited to”.

The word “exemplary” is used herein to mean “serving as an example, instance or illustration”. Any embodiment described as “exemplary” is not necessarily to be construed as preferred or advantageous over other embodiments and/or to exclude the incorporation of features from other embodiments.

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 may include a plurality of “optional” features unless such features conflict.

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.

Throughout this application, various embodiments of this invention may 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.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases “ranging/ranges between” a first indicate number and a second indicate number and “ranging/ranges from” a first indicate number “to” a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

As used herein, the term “treating” includes abrogating, substantially inhibiting, slowing or reversing the progression of a condition, substantially ameliorating clinical or aesthetical symptoms of a condition or substantially preventing the appearance of clinical or aesthetical symptoms of a condition.

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.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

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.

MATERIALS AND METHODS

Materials:

ADP (adenosine diphosphate) was obtained from DiaMed;

Anti-ERK1/2 antibody was obtained from Calbiochem or from Cell Signaling Technology;

Anti-IκBα antibody was obtained from Cell Signaling Technology;

Anti-p38 antibody was obtained from Santa Cruz Biotechnology;

Anti-phospho-p38, anti-phospho-IKKα/β, anti-phospho-ERK1/2, anti-phospho-MEK1/2, anti-phospho-Jnk1, anti-phospho-TAK1, and anti-phosphotyrosine-416-Src family kinase antibodies were obtained from Cell Signaling Technology;

Anti-α-tubulin antibody was obtained from Sigma;

Anti-mouse IL-12/23p40 monoclonal antibody was obtained from BioLegend, (San Diego, CA);

Anti-mouse CD4 and CD45Rb antibodies were obtained from eBioscience (San Diego, CA).

Anti-IL-12/23 p40, anti-IL-6, anti-IL-8, anti-TNF-α, anti-sVCAM-1, and anti-sICAM-1 antibodies were obtained from R&D Systems or eBioscience;

Anti-CCR5 and anti-CXCR1 antibodies, conjugated to phycoerythrin (PE), were obtained from Biolegend;

5 Anti-CCR1 and anti-CCR5 antibodies, conjugated to Alexa Fluor 647 dye, were obtained from BD Bioscience;

Anti-CD14 antibody conjugated to fluorescein isothiocyanate (FITC) was obtained from eBiosciences;

$CD4^{+}CD45Rb^{high}$ T-cells were isolated using FACSAria cell sorter

10 (BDBioscience, San Jose, CA);

Collagen was obtained from MDBiosciences;

Fetal calf serum was obtained from Biological Industries (Israel);

Granulocyte-macrophage colony-stimulating factor (human and mouse) was obtained from Peprotech (Israel);

15 HUVEC cells (human umbilical vein endothelial cells) were purified from human umbilical cord;

IL-4 (human) was obtained from Peprotech (Israel);

IMQ was obtained from InvivoGen (San Diego, CA);

LPS (lipopolysaccharide) was obtained from Sigma;

20 MCP-1 was obtained from Peprotech (Israel);

MCP-3 was obtained from Peprotech (Israel);

MIP1α was obtained from Peprotech (Israel);

Myelin oligodendrocyte glycoprotein (MOG) 35-55 peptide was obtained from Mercury (Israel);

25 RANTES was obtained from Peprotech (Israel);

RPMI 1640 medium was obtained from Biological Industries (Israel);

Pam3CSK4 was obtained from InvivoGen (San Diego, CA);

PAPC (1-palmitoyl-2-arachidonyl-phosphatidyl choline) was obtained from Avanti Polar Lipids (Alabaster, AL);

30 Peptidoglycan was obtained from InvivoGen (San Diego, CA);

Pertussis toxin was obtained from Sigma;

PGPC (1-palmitoyl-2-glutaryl-phosphatidyl choline) was obtained from Avanti Polar Lipids;

Phosphate buffer saline was obtained from Biological Industries (Israel);

Plasma was obtained from mouse and human peripheral blood;

5 Poly I:C was obtained from InvivoGen (San Diego, CA);

Proteolipid protein (PLP) 139-151 peptide was obtained from the Weizmann Institute (Israel);

Soluble CD40 ligand (sCD40L) was obtained from Peprotech (Israel); and

TNBS (trinitrobenzenesulfonic acid) was obtained from Sigma.

10 ^{3}H-VB-201 was prepared by Amersham.

Cells were incubated at 37 °C in an atmosphere with 5 % CO_2.

Isolation of mouse CD11c+ bone marrow-derived cells:

Bone marrow was collected from femurs and tibias of 3-5 female C57 mice (8-10 weeks of age). B cells (B220+) and T cells (CD45+) were depleted by microbeads and
15 the rest of the cells were cultured for 5 days in RPMI 1640 medium supplemented with 20 ng/ml mGM-CSF (mouse granulocyte-macrophage colony-stimulating factor By the end of 5 days, cells were collected and CD11c+ cells were isolated with microbeads.

Isolation of human CD14+ monocytes:

Venous blood samples were obtained from healthy female and male donors who
20 signed an agreement to be tested, were not under steroid treatment, and had no viral or bacterial infection for at least one month before testing. PBMCs (peripheral blood mononuclear cells) were isolated on Ficoll-Paque PLUS density gradient centrifugation medium (GE Healthcare) using 50 ml Leucosep tubes (Greiner Bio One). Cells were washed in phosphate buffer saline (PBS), and incubated at 4 °C for 15 minutes in a
25 buffer containing PBS, 0.5 % bovine serum albumin (BSA) and EDTA (ethylenediaminetetraacetic acid) with human CD14 microbeads (Miltenyi Biotec). Cells were then washed and resuspended in the same buffer, and CD14+ monocytes were then isolated through LS columns (Miltenyi Biotec).

Isolation of human CD4+ T cells and CD19+ B cells:

30 T cells and B cells were isolated using the same procedures described above for isolating CD14+ monocytes, except that human CD4 microbeads (Miltenyi Biotec) were

used instead of CD14 microbeads to isolate T cells, and CD19 microbeads (Miltenyi Biotec) were used instead of CD14 microbeads to isolate B cells.

Human dendritic cells (DCs) derived from CD14+ monocytes:

To generate monocyte-derived dendritic cells, CD14+ monocytes (isolated as described above) were counted, washed and seeded at a concentration of 10^6/ml, for 5-7 days, in RPMI-1640 medium with L-glutamine, β-mercaptoethanol, 10 % FCS (fetal calf serum), sodium pyruvate, non-essential amino acids, 0.01 M HEPES, antibiotics (penicillin and streptomycin), and 50 ng/ml of human GM-CSF (granulocyte-macrophage colony-stimulating factor) and 20 ng/ml of human IL-4. The medium was replaced every other day.

T cell-induced colitis mouse model:

Treatment in all groups consisted of an oral administration of VB-201 (or solvent) once daily, at the doses indicated below. Administration of VB-201 (or solvent) was in a total volume of 200 μl per mouse.

Colitis was induced on day 0 by intraperitoneal administration of $CD4^+CD45Rb^{high}$ T-cells suspended in PBS (500 μl).

For testing prevention mode, day -5 was the day of the first administration of VB-201 or solvent. For testing treatment mode, day +7 was the day of the first administration of VB-201 or solvent.

Anti-mouse IL-12/23p40 monoclonal antibody (0.5mg/mouse I.P) given on days 7 and 35 was used as a positive control.

TNBS-induced colitis mouse model:

On day 0, all animals were treated with 80 μl of TNBS (trinitrobenzenesulfonic acid) dissolved in 50 % ethanol. Test substances were administered *per os* daily for a period of 13 days, from day -5 until day 7, at a volume of 5 ml/kg. Sulfasalazine, a positive control, was administered daily from day 0 until day 7. There were 16 mice in each treatment group.

DSS-induced colitis mouse model:

C57B6J mice received VB-201 (0.04, 0.4, or 4 mg/kg) once a day starting 5 days prior to disease induction. In order to induce colitis, DSS (dextran sulfate sodium) was supplemented in the drinking water in three cycles of 5 days each, on days 0-4, 19-23, and 32-36. Mice were sacrificed on day 39-40.

As a positive control, some mice with DSS-induced colitis were treated with anti-IL-12 antibody, by performing 3 intraperitoneal injections of 100 μg anti-IL-12 antibody per mouse on days 2, 5 and 8 after disease induction.

Upon sacrifice disease severity was evaluated by measurements of colon length.

5 ***Collagen-induced arthritis (CIA) mouse model:***

Each test group consisted of 10-11 male DBA/1 mice ages 11-12 week old. Vehicle (PBS with 0.5 % ethanol) was administered as a negative control, and another group of mice which were not induced with arthritis served as a healthy mouse control. Collagen-induced arthritis (CIA) was induced by two collagen injections, on days 0 and
10 21. Oral administration of the test and control substances began on day 22 and was carried out daily 6 times a week until the study end (days 41-42). Arthritis was evaluated from day 21 until the study end (days 41-42). Determination of individual arthritis score was made 2-3 times a week from day 21 (i.e., beginning on the day the second collagen injection was given).

15 The severity of arthritis was graded by an arthritis score as follows: Inflammation in each of the 4 paws was graded on a scale of 0-4, as described below, and scores of the 4 paws were added (maximum score per mouse 16).

0= normal

1= slight erythema and/or swelling of the ankle or wrist

20 2= moderate erythema and/or swelling of the ankle or wrist

3= severe erythema and/or swelling of the ankle or wrist

4= complete erythema and swelling of toes or fingers and ankle or wrist and inability to bend the ankle or wrist.

In addition, pathological scores were assessed by collecting hind paws from 5
25 mice per group (from the 0.04 mg/kg VB-201 and 0.004 mg/kg VB-201 groups and from the control groups). The severity of arthritis was assessed by the pathology department at Tel Hashomer Medical Center as follows:

Each hind paw was scored on a scale of 0-4:

0= normal

1= inflammatory infiltrates and synovial hyperplasia

2= pannus formation and cartilage erosion

5 3= important cartilage erosion and bone destruction

4= loss of joint integrity

Platelet aggregation assay:

Platelet aggregation was determined using a standard technique in which 225 µl platelet-rich plasma was incubated at 37 °C and stirred at 1,000 rpm in an AggRAM 4-
10 channel aggregometer (Helena Laboratories, Beaumont, TX). The change in light transmittance was recorded for 5 minutes. The platelet aggregometer was calibrated to yield 0 % light transmittance for platelet-rich plasma and 100 % transmittance for platelet-poor plasma. As a positive control, aggregation was induced by adding 25 µl of a 2.5 µM PAF (platelet-activating factor) solution in PBS. 25 µl of PBS was used as a
15 negative control.

In vitro migration assay:

Human CD14+ monocytes were isolated as described hereinabove. The purity of the CD14+ cells population was validated by FACS analysis using a specific monoclonal anti-CD14 antibody. Chemoattraction was induced by 100 ng/ml RANTES, 50 ng/ml
20 MIP1α, 50 ng/ml MCP-1 or 50 ng/ml MCP-3 in RPMI-1640 with 2 % fetal calf serum, or by medium collected from HUVEC cell cultures (passage 2-5) 24 hours after medium replacement. The chemoattractant medium was placed at the lower chamber of QCM^{TM} 24-well migration assay plate (Corning-Costar 5 µm pores). CD14+ cells were pre-incubated for 30 minutes with various concentrations of VB-201 (ranging between 1-10
25 µg/ml) or solvent (1 % ethanol in PBS). 300,000 cells were then seeded in the upper chamber, followed by incubation for 3-4 hours, after which the number of migrated cells was determined by FACS. For each chemoattractant, chemotaxis of VB-201-treated cells was compared to chemotaxis of solvent-treated control cells, which was defined as 100 %.

30 ***IL-12/23p40 mRNA measurement:***

Monocyte-derived dendritic cells were stimulated for 2, 3, 4 or 24 hours with 100 ng/ml LPS, alone or in the presence of 1 µM VB-201 added 1 hour before

stimulation. RNA was extracted from cells using RNeasy mini kit (Qiagen). For cDNA preparation, 1 μg of RNA was combined with Oligo dT for 10 minutes at 70 °C, 1st strand buffer. DTT and dNTP and super-script reverse transcriptase (SS-II) (Invitrogen) were added for 50 minutes at 42 °C, and the reaction was ended by incubation for an
5 additional 15 minutes at 70 °C. All real-time PCR reactions were performed using a LightCycler® Taqman® master (Roche Diagnostics) and run on the LightCycler® apparatus (Roche). Ready sets of probe with primers were used for IL12/23p40 and GAPDH assays (Applied Biosystems, assays #Hs99999905_m1 and Hs002233688_m1, respectively) with the latter serving to normalize RNA levels.

10

EXAMPLE 1

Education of immune cells by VB-201

Peritoneal macrophages were isolated from thioglycollate-elicited mice as described in International Patent Application PCT/IL2009/001049 (Publication No. WO
15 2010/052718). Cells were starved overnight in RPMI-1640 medium with 0.5 % FCS (fetal calf serum) and then treated with various doses of VB-201 (1-5 μg/ml; corresponding to 1.7-8.5 μM) in a solvent of 1 % ethanol in PBS (phosphate buffer saline), or with the solvent alone, followed immediately (in under 1 minute) by stimulation with 100 ng/ml LPS (lipopolysaccharide) for 15 minutes. Cells were then
20 washed twice with PBS, harvested in lysis buffer containing protease and phosphatase inhibitors, and resolved by Western blot. The lysates were resolved using a 10 % SDS/PAGE gel and transferred to nitrocellulose membrane, which was probed with specific antibodies. For assessment of LPS-induced signaling, phosphorylation of p38 MAPK and ERK1/2 and activation of the NFκB pathway were tested, using alpha-
25 tubulin levels as loading controls.

As shown in FIG. 1, VB-201 inhibited LPS signaling in peritoneal macrophages, particularly at doses of 2-5 μg/ml. LPS stimulation induced phosphorylation of p38 and ERK1/2 and activation of the NFκB pathway, as seen by increased phosphorylation of IKKα/β and degradation of IκBα, and this effect of LPS was decreased considerably by
30 VB-201.

The duration of VB-201 inhibition of LPS signaling was tested by treating peritoneal macrophages with 5 μg/ml VB-201 for 20 minutes, after which VB-201 was

washed away (or not) prior to stimulation with LPS. It was investigated for how long VB-201 can inhibit LPS-induced signaling after washout of VB-201.

As shown in FIG. 2, VB-201 inhibited LPS signaling in peritoneal macrophages even 24 hours after it was briefly applied to cells. In cells which received 5 μg/ml VB-201, LPS-induced activation of p38 and the NFκB pathway is dramatically decreased 1, 3 and 24 hours after VB-201 washout (compare lanes 5-6 to lane 2; lane 9 to lane 8).

The previously described experiment was repeated, testing VB-201 inhibitory effect on LPS-induced signaling 1, 3, 6 and 24 hours after VB-201 washout.

As shown in FIG. 3, VB-201 again inhibited LPS signaling in peritoneal macrophages 24 hours after it was briefly applied to cells. In cells which received 5 μg/ml VB-201, LPS-induced phosphorylation of p38 and the IKKα/β is dramatically decreased 1, 3, 6 and 24 hours after VB-201 washout (compare lanes 5-8 to lane 2; lane 12 to lane 10).

The results indicate a rapid but long-lasting effect of VB-201 on macrophage signaling and show that treatment with VB-201 can "educate" immune cells towards an anti-inflammatory phenotype.

EXAMPLE 2

Uptake of VB-201 by immune cells

In order to further characterize the effects of VB-201 on the immune system, the uptake of VB-201 by various types of immune cells was assayed using radiolabeled VB-201 (^{3}H-VB-201).

10^6 cells from the following sources were treated with ^{3}H-VB-201:

Human CD14+ monocytes;

Human dendritic cells (DCs) derived from CD14+ monocytes;

Human CD4+ T cells;

Human CD19+ B cells;

Mouse CD11c+ bone marrow dendritic cells (BMDCs) – as a positive control;

U937 monocytes (human cell line) – as a positive control.

The cells were obtained as described in the Materials and Methods section

Cells were cultured for 2 hours with 3.4 μM ^{3}H-VB-201 (hot and cold mix). At the end of 2 hours of incubation, cells were collected. For non-adherent cells, the cell suspension from each well was collected into an Eppendorf vial. Adherent cells were

collected by scraping. All cell suspensions were centrifuged and washed in PBS twice by adding 1 ml PBS. Cells were re-suspended in 100 μl of lysis buffer, and 20 μl of the lysis buffer was evaluated for radioactivity using a β-counter (DPM).

As shown in FIG. 4, uptake of VB-201 is considerably greater in human dendritic cells and monocytes than in T cells and B cells.

As dendritic cells are a major and potent determinant of immunological memory, these results further suggest that VB-201 is capable of inducing long-term anti-inflammatory effects by affecting immune cell education and memory.

EXAMPLE 3

Efficacy of VB-201 in an experimental autoimmune encephalomyelitis (EAE) mouse model

Experimental autoimmune encephalomyelitis (EAE), an established animal model for autoimmune conditions such as multiple sclerosis, and is induced by active immunization with myelin proteins and peptides thereof.

EAE was induced in female SJL mice (8-10 weeks of age) by subcutaneous injection of PLP (proteolipid protein) 139-151 peptide and intraperitoneal injections of pertussis toxin (PT). EAE was induced on day 0 by one subcutaneous injection (0.1 ml to each animal) of PLP 139-151 peptide and by two intraperitoneal injections of PT, one on day 0 and one on day 2.

4 doses of VB-201 (0.004, 0.04 and 0.4 and 4 mg/kg) or the control substance (PBS) were administered orally once daily, between days 10-14 following disease induction. The doses were at a total volume of 200 μl per mouse.

The mice were regularly monitored, and the effect of VB-201 was assessed using an EAE clinical score, as follows:

0= Normal

1= Limp tail

2= Weakness of hind limbs

3= Hind leg paralysis

4= Hind and fore leg paralysis

5= Moribund or death

All surviving animals were sacrificed on day 31 post-EAE induction.

As shown in FIG. 5 and in Table 1, VB-201 notably reduced the clinical manifestations of EAE in all groups. A lower disease incidence compared to the control group was observed at all doses of VB-201 and disease onset was delayed in correlation to the dose administered.

5

	PBS	**VB-201 (mg/kg)**			
		0.004	**0.04**	**0.4**	**4**
Incidence of diseased mice	7/7	4/6	4/6	4/6	4/6
Day of disease onset (each number describes one mouse)	15 15 16 18 18 21 22	15 15 16 27	15 20 22 22	18 18 21 23	14 18 24 29
Mean day of onset	17.8±2.6	18.2±5.1	19.7±2.9	20±2.1	21.2±5.7

Table 1: PLP-induced EAE manifestations in VB-201-treated mice

These results indicate that VB-201 is effective at treating autoimmune disorders at daily doses as low as 0.004 mg/kg (in mice).

The efficacy of VB-201 was also studied in a MOG-induced EAE model.

10 4 doses of VB-201 (0.004, 0.04 and 0.4 and 4 mg/kg) or the control substance (PBS) were administered orally once daily to C57BL/6 mice, beginning 5 days prior to EAE induction by myelin oligodendrocyte glycoprotein (MOG) 35-55 peptide. The doses were at a total volume of 200 μl per mouse.

EAE was induced on day 0 by two subcutaneous injections (0.2 ml to each 15 animal) of MOG 35-55 peptide, and by one intraperitoneal injection of PT on day 0 and one on day 2 or 3.

Mice were regularly monitored, and the effect of VB-201 was assessed using an EAE clinical score, as described above. Recording of clinical scores began on days 9-14 post-induction of EAE.

20 As shown in FIG. 6, all tested doses of VB-201 reduced disease severity, as measured by EAE clinical score.

In addition, all tested doses of VB-201 delayed disease onset. In control mice, mean day of disease onset was 16 ± 1.7, whereas the mean day of disease onset was 23.1 ± 5.1 in the 0.004 mg/kg group (P = 0.018 vs. control), 21.3 ± 3.6 in the 0.04 mg/kg group (P = 0.019 vs. control), 25.2 ± 4.2 in the 0.4 mg/kg group (P = 0.003 vs. 5 control), and 21.7 ± 7.1 in the 4 mg/kg group.

These results confirm that VB-201 is effective at treating autoimmune disorders at daily doses as low as 0.004 mg/kg (in mice).

An additional experiment was performed with a PLP-induced EAE mouse model as described above, except that the SJL mice were administered with various doses of 10 VB-201 (0.04, 0.4 and 4 mg/kg) prior to EAE induction by PLP 139-151 peptide.

EAE was induced on day 0 by one subcutaneous injection (0.2 ml to each animal) of PLP 139-151 peptide and by two intraperitoneal injections of PT, one on day 0 and one on day 2 or 3.

Doses of VB-201 (0.004, 0.04 and 0.4 and 4 mg/kg) or PBS were administered 15 orally once daily, beginning on day -5, and ending on day 4. The doses were at a total volume of 200 μl per mouse.

As shown in FIG. 7, VB-201 decreased EAE severity at all tested doses, with 0.04 mg/kg being even more effective than higher doses.

The above results indicate that low daily doses such as 0.04 and 0.004 mg/kg 20 VB-201 can be as effective (in mice) as, and even more effective than, higher doses of VB-201.

EXAMPLE 4

Efficacy of VB-201 in a xenotransplant psoriasis mouse model

25 Healthy human skin pieces with a width of 0.4 mm and surface area of 1.5 x 1.5 cm were provided from residual skin of routine plastic surgery procedures of 3 subjects. The skin was transplanted onto mice. In addition, blood samples from psoriatic patients were taken at a volume of 25 ml.

Mice of each group received natural killer (NK) cells from a psoriatic donor. 30 Four weeks following the engraftment (i.e., on day 28), the mice were injected with allogeneic NK-cells of psoriatic patients.

Two weeks following the injections (i.e., on day 42), the mice were divided and treated, twice a day for 14 days, as follows:

1. Group 1 Solvent (0.5 % ethanol in PBS)
2. Group 2 VB-201 - 4 mg/kg (in vehicle)
3. Group 3 VB-201 - 0.04 mg/kg (in vehicle)

VB-201 or solvent were given by oral gavage feeding needle (0.2 ml per day).

Two weeks after starting the treatment (i.e., on day 56), the skin was harvested. Grafts were analyzed by histology.

Histological assessment of the grafts was performed by light microscopy both before and after transplantation. Evaluation was performed by two blinded observers. Epidermal thickness was determined with an ocular micrometer, at a minimum of 50 points along the epidermis, selected to represent points of maximal and minimal thickness. Thickness of the suprapapillary plate was similarly measured at 50 points for each sample.

A shown in FIG. 8, 6 out of 10 grafts in mice treated with 4 mg/kg VB-201 showed recovery from the psoriasiform process, and 5 of 10 grafts in mice treated with 0.04 mg/kg VB-201 showed recovery. In contrast, in the control group, only 3 of 10 grafts showed recovery (partial recovery in 2 grafts and complete recovery in 1 graft). No effect was observed on skin thickness by VB-201 in any of the tested doses.

These results indicate that VB-201 is effective at treating psoriasis using the abovementioned doses.

EXAMPLE 5

Efficacy of VB-201 in a colitis mouse model

The efficacy of VB-201 was tested in a T cell-induced colitis mouse model, in a trinitrobenzenesulfonic acid (TNBS)-induced colitis mouse model and in a dextran sulfate sodium (DSS)-induced model as described in the Materials and Methods section.

The effect of VB-201 in a T cell-induced colitis model was assessed by monitoring the body weights of the mice. VB-201 was administered either in a prevention mode (5 days before induction of colitis) or in a treatment mode (7 days after induction).

As shown in FIG. 9, 0.04 mg/kg VB-201 administered before induction of the disease (prevention mode) reversed the weight decrease caused by colitis.

In addition, 0.04 mg/kg VB-201 was effective when administered in a treatment mode, as described above (data not shown).

These results indicate that 0.04 mg/kg (in mice) is an effective dose for treating a T cell-induced autoimmune condition.

In addition, the effect of 0.04 mg/kg and 0.4 mg/kg VB-201, administered beginning 5 days before induction of colitis, was assessed in a TNBS-induced colitis model by monitoring the body weights of the mice, and by using a daily clinical score as follows:

Score	Weight loss (%)	Stool consistency	Presence of blood in rectum
0	None	Normal	Negative
1	1-5	Loose stool	Negative
2	5-10	Loose stool	Minor bleeding
3	10-15	Diarrhea	Minor bleeding
4	>15	Diarrhea	Gross bleeding
5	Death		

Table 2: Clinical scoring of TNBS-induced colitis

On the last day of the study, immediately after animals were euthanized, the colon was removed and evaluated using histopathology and using a gross pathology score as follows:

Score	Signs
0	No abnormalities detected
1	Edema and redness on one location
2	Edema and redness in more than one location, or a very massive edema and redness capture more than 50% of the colon

3	One ulcer
4	More than one ulcer or a very long sever ulcer

Table 3: Gross pathology scoring of colitis

As shown in FIG. 10, both tested doses of VB-201 significantly decreased the weight loss caused by the colitis.

As shown in FIG. 11, both tested doses of VB-201 considerably reduced disease severity, as measured by the clinical score.

As shown in FIG. 12, both tested doses of VB-201 considerably reduced disease severity, as measured by the gross pathology score of the mouse colons.

As shown in FIGs. 13A and 13B, treatment with 0.04mg/kg of VB-201 markedly reduced colon inflammation, as observed by histopathological evaluation of the colons.

The effect of VB-201 administered in a prevention mode (5 days before induction of colitis) in a DSS-induced colitis model was assessed by measuring colon length and by using a disease activity index (DAI) based on daily clinical scoring as follows:

Score	Weight loss (%)	Stool consistency	Gross bleeding
0	None	Normal	Normal
1	1-5		
2	5-10	Loose	
3	10-15		
4	>15	Diarrhea	Gross bleeding

Table 4: Clinical scoring of DSS-induced colitis (normal stools = well formed pellets; loose stools = pasty stools that do not stick to the anus; diarrhea = liquid stools that stick to the anus)

The disease activity index (DAI) was calculated as the combined score of weight loss, stool consistency, and gross bleeding, divided by 3.

As shown in FIG. 14, administration of 0.04 mg/kg and 0.4 mg/kg VB-201 before induction of the disease (prevention mode) considerably reduced disease severity, as measured by the disease activity index.

As shown in FIG. 15, 0.04 mg/kg and 0.4 mg/kg VB-201 administered before induction of the disease (prevention mode) were effective in improving the survival of mice.

As shown in FIG. 16, administration of 0.04 mg/kg; 0.4 mg/kg and 4 mg/kg VB-201 before induction of the disease (prevention mode) considerably reduced disease severity, as measured by the reduction of colon length in mice.

As further shown in FIGs. 14-16, VB-201 administration was more effective than anti IL-12p40 treatment in reducing the severity of DSS-induced colitis.

These results indicate that VB-201 is effective for alleviating colitis in mice.

EXAMPLE 6

Efficacy of VB-201 in an atherosclerosis mouse model

The efficacy of VB-201 against atherosclerosis was tested using Apo E KO mouse models. VB-201 was tested against both early atherosclerotic plaque progression and advanced atherosclerotic plaque progression in Apo E KO mice.

Early atherosclerotic plaque progression in Apo E KO mice:

VB-201 was administered to male Apo E KO mice by oral gavage, at doses of 0.10 μg, 0.33 μg, 1 μg, 3.33 μg, 10 μg or 33.33 μg per mouse (corresponding to approximately 0.0045 mg/kg, 0.015 mg/kg, 0.045 mg/kg, 0.15 mg/kg, 0.45 mg/kg or 1.5 mg/kg, respectively). PBS was administered as a control. Dosage volumes were 0.2 ml. Administration was performed daily (5 times a week) for 8 weeks. Each treatment group contained 11 or 12 animals.

As shown in FIG. 17, all tested doses inhibited the progression of early atherosclerotic lesions in male mice.

The above results indicate that VB-201 is effective for inhibiting atherosclerosis progression, even at doses as low as 0.0045 mg/kg (in mice).

Advanced atherosclerotic plaque progression in Apo E KO mice:

VB-201 was administered by oral gavage to Apo E KO mice (male and female) at an advanced stage of atherosclerosis, at doses of 0.10 μg, 1 μg, or 10 μg or per mouse (corresponding to approximately 0.004 mg/kg, 0.04 mg/kg, or 0.4 mg/kg, respectively). PBS was administered as a control. Administration was performed in three sessions at four week intervals, with each session consisting of five doses administered every other

day. In all instances, doses were administered at a volume of 200 μl per animal. Atherosclerosis progression was evaluated at the end of a 12-week period in comparison to atherosclerotic lesions in mice from the base line group, which were sacrificed on day 0, the day when treatment began for the other groups. Each treatment group consisted of 11 female mice and 13 male mice (except for the group treated with 0.1 μg VB-201 per mouse, which contained 11 males and 11 females). The baseline group consisted of 10 males and 10 females.

As shown in FIGs. 18A and 18B, VB-201 was effective in both male and female mice in reducing atherosclerotic lesion areas, at doses as low as 0.004 mg/kg (FIG. 18A).

The above results indicate that VB-201 is effective for inhibiting atherosclerosis progression, even at doses as low as 0.004 mg/kg (in mice).

EXAMPLE 7

Efficacy of VB-201 in a collagen-induced arthritis (CIA) mouse model

The efficacy of VB-201 against arthritis was tested in a collagen-induced arthritis (CIA) mouse model, as described in the Materials and Methods section. Arthritis was induced by collagen injections on days 0 and 21. Daily administration *per os* of 4 mg/kg, 0.4 mg/kg, 0.04 mg/kg or 0.004 mg/kg VB-201 began on day 22.

As shown in FIG. 19, all tested doses of VB-201 except for 4 mg/mg reduced arthritis severity as determined by the arthritis score, with the lower doses (0.04 mg/kg and 0.004 mg/kg), particularly 0.04 mg/kg, being the most potent.

Similarly, as shown in FIG. 20, 0.04 mg/kg and 0.004 mg/kg VB-201 reduced arthritis severity as determined by the pathological score.

Arthritis incidence was also reduced along with the arthritis severity.

As shown in FIGs. 21A-21C, the joint of a PBS-treated mouse with collagen-induced arthritis (FIG. 21A) exhibits inflammatory infiltrates, pannus formation and bone destruction, whereas a joint from a VB-201-treated mouse (FIG. 21C) exhibits only minor to non-inflammatory infiltrates, and resembles a joint from a healthy mouse (FIG. 21B).

These results indicate that the abovementioned doses are effective for treating arthritis, with doses of approximately 0.04 mg/kg (in mice) being particularly effective.

EXAMPLE 8

Effect of VB-201 on platelet aggregation

Aggregation was platelets in plasma was determined as described in the Materials and Methods section.

5 As shown in FIGs. 22A and 22B, no aggregation of platelets in plasma was detected when VB-201 was added at concentrations of 0.01 μM, 0.1 μM, 1 μM or 10 μM.

The effect of VB-201 on platelet aggregation was further studied by pre-incubating platelets with 0.1 μM VB-201, and then inducing platelet aggregation by 10 adding 1.5 μM ADP (adenosine diphosphate).

As shown in FIG. 23, 0.1 μM VB-201 inhibited platelet aggregation induced by ADP.

These results indicate that VB-201 inhibits platelet aggregation, and does not induce platelet aggregation.

15

EXAMPLE 9

Effect of VB-201 on CRP (C-reactive protein) levels in humans

VB-201 was administered to healthy volunteers at doses of 40 mg per day for 14 days (6 subjects), 80 mg per day for 14 days (6 subjects), and 80 mg per day for 28 days 20 (6 subjects). Plasma samples were taken at day 0 (before VB-201 administration), at day 14, and at day 28 (when VB-201 was administered for 28 days). Aliquots of 100 μl human plasma were kept frozen (-70 °C) until assayed by a high-sensitivity C-reactive protein (hsCRP) test. C-reactive protein (CRP) levels in the plasma samples were measured using a CRP Latex assay kit (Olympus) and an AU400® immuno-analyzer 25 (Olympus). Statistical analysis was performed using two tailed student t-test.

As shown in FIG. 24A, administration of 40mg/day VB-201 reduced CRP levels on day 14 by 49 %.

As shown in FIGs. 24B and 24C, administration of 80 mg/day VB-201 reduced CRP levels on day 14 by 43 %., and on day 28 by 45 %.

30 These results indicate that VB-201 is effective at reducing plasma CRP levels *in vivo*.

EXAMPLE 10

Effect of VB-201 on Toll-like receptor (TLR) signaling

Human dendritic cells (DCs) were derived from monocytes obtained from a healthy donor, as described in the Materials and Methods section. In order to analyze the effect of VB-201 effect on DCs signaling, cells were plated at density of $2x10^6$ cells/well in 12-well plates and starved overnight in RPMI-1640 with 0.5 % FCS (fetal calf serum).

For comparison, oxidized PAPC (1-palmitoyl-2-arachidonyl phosphatidyl choline) was prepared, as inhibition of LPS-induced signaling by oxidized PAPC (Ox-PAPC) has been previously reported. Oxidized PAPC was prepared by exposure of PAPC to air for 72 hours. However, previous studies have shown by mass spectrometry that oxidation of PAPC results in a mixture of various oxidized species, and may yield different products depending on the extent of PAPC oxidation, which may result in different properties (e.g., pro/anti-inflammatory phenotypes). Partially oxidized PAPC was therefore prepared by exposure of PAPC to air for 24 hours, in order to compare the effects of partially oxidized PAPC with those of the fully oxidized PAPC (72 hour exposure to air).

For analysis of unstimulated cells, the cells were exposed to VB-201 (1 or 5 μg/ml) or Ox-PAPC (10 or 40 μg/ml) for 20 minutes, using untreated cells or solvent-treated cells as controls. For analysis of stimulated cells, the cells were stimulated for 15 minutes with 100 ng/ml lipopolysaccharide (LPS) or 1 μg/ml soluble CD40 ligand after the aforementioned treatment with VB-201 or Ox-PAPC. Following treatment, cells were washed twice with PBS, harvested in lysis buffer containing protease and phosphatase inhibitors, and then analyzed by Western blot. The lysates were resolved using a 10 % SDS/PAGE gel and transferred to nitrocellulose membrane, which was probed with specific antibodies. Total p38 and ERK1/2 levels or α-tubulin levels serve as loading controls.

As shown in FIG. 25A, unstimulated dendritic cells exhibit very low basal phosphorylation levels of ERK1/2, which are unaffected by VB-201, although phosphorylation can be induced by Ox-PAPC.

Stimulation with LPS resulted in phosphorylation of p38 and a decrease of IkBα levels. Similarly, stimulation with soluble CD40 ligand (sCD40L) resulted in phosphorylation of p38 and Jnk1, along with a decrease in IkBα levels.

As shown in FIG. 25B, pre-treatment with VB-201 inhibited the LPS-induced phosphorylation of p38 and the decrease of IkBα levels stimulation in a dose-dependent manner. As further shown therein, pre-treatment with VB-201 inhibited the sCD40L-induced phosphorylation of p38 and JNK1. VB-201 treatment additionally decreased ERK1/2 phosphorylation in LPS- and sCD40L-stimulated cells. In contrast, Ox-PAPC pre-treatment did not inhibit LPS- or sCD40L-stimulation in this experiment.

As shown in FIG. 26, LPS induced phosphorylation of p38 and IKKα/β, and pre-treatment with 5 μg/ml VB-201 potently inhibited this effect of LPS. Partially oxidized PAPC (24 hour oxidation) had no effect on the LPS-induced signaling, whereas fully oxidized PAPC (72 hour oxidation) almost completely blocked the effect of LPS at both concentrations tested.

The above results indicate that VB-201 can inhibit stimulation by LPS (a TLR4 agonist) or soluble CD40-ligand in human dendritic cells.

The effect of VB-201 on TLR signaling was studied further using mouse bone marrow-derived dendritic cells (BMDCs), which were obtained as described hereinabove. The BMDCs were pre-treated for 20 minutes with VB-201 or solvent, as described above for human dendritic cells. Stimulation was performed for 15 minutes using a variety of TLR agonists: 100 ng/ml LPS (TLR4 agonist), 50 μg/ml Poly I:C (TLR3 agonist), 300 ng/ml Pam3Csk4 (TLR1:2 agonist), or 10 μg/ml IMQ (TLR7 agonist). The activation status of p38, Erk1/2 and NFkB signaling pathways was analyzed by Western blot, as described above.

As shown in FIG. 27, LPS and Pam3Csk4 induced phosphorylation of p38 and Erk1/2 MAPK, and activation of the NFκB pathway, as seen by increased phosphorylation of IKKα/β and degradation of IκBα. As further shown therein, VB-201 inhibited activation of p38, Erk1/2 and the NFκB pathway in a dose-dependent manner. In IMQ-stimulated cells, VB-201 had no effect on p38 phosphorylation. Poly I:C had no observable effect in this experiment, although VB-201 increased IκBα levels in Poly I:C-treated cells.

These results indicate that VB-201 inhibits signaling by TLR1:2 and TLR4, which are surface receptors, and does not inhibit signaling by TLR7, an intracellular receptor. This suggests that VB-201 might preferentially act on surface TLRs and/or affect a signaling component/adaptor which is not shared by all TLRs.

5 The effect of VB-201 on TLR signaling was studied further using mouse peritoneal macrophages, which were isolated from thioglycollate-elicited mice as described in International Patent Application PCT/IL2009/001049 (Publication No. WO 2010/052718). The peritoneal macrophages were stimulated with LPS or Pam3Csk4 and analyzed by Western blot, as described hereinabove for dendritic cells.

10 As shown in FIG. 28, LPS and Pam3CSK4 induced phosphorylation of TAK1, a kinase involved in activation of the p38, JNK and NFκB pathways. Accordingly, downstream phosphorylations of p38, Erk1/2 and IKKα/β were observed, along with degradation of IκBα. As further shown therein, VB-201 inhibited activation of TAK1, p38, Erk1/2 and the NFκB pathway in a dose-dependent manner.

15 As shown in FIGs. 29A and 29B, Pam3CSK4 induced phosphorylation of TAK1 and downstream phosphorylation of p38, Jnk1, Erk1/2 and IKKα/β, along with degradation of IκBα, and tyrosine 416 phosphorylation of Src-family kinases (SFK). As further shown therein, pre-treatment with VB-201 effectively blocked all these phosphorylation events and rescued IκBα levels.

20 The above results indicate that VB-201 significantly inhibits both TLR4 and TLR1:2 signaling, as well as the p38, JNK and NFκB pathways, an effect which was reproducibly observed in dendritic cells from both human and mouse origins, as well as in mouse peritoneal macrophages. As the inhibitory effect was observed even for an upstream-acting kinase such as TAK1, it is possible that VB-201 inhibits early steps of

25 TLR stimulation.

EXAMPLE 11

Effect of VB-201 on chemotaxis

The effect of VB-201 on chemotaxis was determined using an *in vitro* migration

30 assay described in the Materials and Methods section, using MCP-1, MCP-3, MIP1α and RANTES as chemoattractants.

The results of two separate experiments are presented in FIGs. 30A and 30B. As shown in FIGs. 30A and 30B, VB-201 inhibited monocyte chemotaxis for each of the tested chemoattractants by 57-89 %. The difference between chemotaxis with and without VB-201 was statistically significant in each case ($p < 0.05$).

5 The migration assay was repeated using HUVEC cell medium as a chemoattractant, in order to determine the effect of VB-201 in the presence of a complex chemoattractant mix. In addition to the serum and growth factors present in the culture medium which may act as attractants, endothelial cells can secrete various chemoattractants capable of recruiting cells under normal and pathological conditions.

10 As shown in FIG. 31, VB-201 inhibited monocyte chemotaxis by 35-65 % in dose-dependent and statistically significant ($p < 0.05$) manner at concentrations of 1 to 10 μg/ml, whereas oxidized PAPC (Ox-PAPC) at concentrations of 5 to 80 μg/ml had no effect on chemotaxis.

An additional migration setting was tested, in which medium from HUVEC cells 15 treated for 16 hours with 30 μg/ml Ox-PAPC was used as the attractant. Medium from HUVEC cells treated with the solvent (PBS with 1 % ethanol) was used as a control.

As shown in FIG. 32, Ox-PAPC enhanced the chemoattractant efficacy of HUVEC cell medium in a statistically significant manner ($p < 0.05$).

In an additional experiment, monocytes were pre-treated with VB-201 as 20 described above before performing a migration assay towards medium from HUVEC cells treated for 16 hours with 30 μg/ml Ox-PAPC.

As shown in FIG. 33, VB-201 inhibited monocyte chemotaxis by approximately 35 % to 65 %, in a dose dependent manner, despite the enhancement of chemotaxis by Ox-PAPC (as shown in FIG. 32).

25 The effect of VB-201 on chemotaxis was also determined by an *in vivo* assay. 4 mg/kg or 0.4 mg/kg VB-201, or vehicle (PBS with 0.5 % ethanol) as a control, were administered to mice once a day for 8 days. Each treatment group contained 5 mice. On day 5, thioglycollate was injected into the peritoneal cavity in order to induce macrophage migration. On day 9, cells were collected by adding PBS into the 30 peritoneum, and migrating peritoneal macrophages were counted.

As shown in FIG. 34, VB-201 at doses of 4 mg/kg and 0.4 mg/kg significantly attenuated peritoneal macrophage migration by 54 % ($p < 0.05$) and 59 % ($p < 0.01$) respectively, relative to the solvent-fed control mice.

The possibility that the inhibition of migration results from monocyte cytotoxicity was ruled out by performing an apoptosis assay concomitantly with an *in vitro* migration assay. The apoptosis assay comprised staining with Annexin-V (which has an affinity for cells in early phases of apoptosis) and 7-amino-actinomycin (7-AAD) (a standard cell viability probe which stains cells which lack an intact membrane), as recommended by the manufacturer (BD Pharmingen), and inspection of the FACS data obtained during the process of cell counting.

The above results indicate that VB-201 considerably inhibits CD14+ cell migration towards various chemoattractants, including individual chemokines and biological mixtures such as HUVEC cell medium, and that inhibition of chemotaxis by VB-201 occurs *in vivo* as well as *in vitro*.

EXAMPLE 12

Effect of VB-201 on cytokine production

In order to measure the effect of VB-201 on cytokine production, human monocyte-derived dendritic cells were obtained as described hereinabove. 5-6 days post-culturing, cells were collected, counted and seeded (at a concentration of 10^6/ml) in RPMI-1640 medium with L-glutamine, β-mercaptoethanol, 10 % FCS, sodium pyruvate, non-essential amino acids, 0.01 M HEPES, antibiotics (penicillin and streptomycin), 50 ng/ml of human GM-CSF (granulocyte-macrophage colony-stimulating factor) and 20 ng/ml of human IL-4.

VB-201 was added for 1 hour at a concentration of 1.7, 0.85 or 0.17 μM and the cells were then activated for 24 hours with 100 ng/ml LPS, a TLR4 agonist, or 10 μg/ml peptidoglycan (PGN), a TLR2/6 agonist, or Poly I:C, a TLR3 agonist, in order to induce cytokine production. Cytokine levels in the supernatant were then measured by ELISA. Activated cells pre-treated with solvent (0.5 % ethanol in PBS) were used as a control.

As shown in FIGs. 35 and 36A-36C, VB-201 reduced levels of IL12/23p40 (FIGs. 35 and 36A) and IL-6 (FIG. 36B) in LPS-activated human monocyte-derived dendritic cells in a dose-dependent manner, but not levels of TNF-α (FIG. 36C).

As further shown in FIG. 35, VB-201 almost entirely eliminated IL12/23p40 in some samples.

Similarly, as shown in FIGs. 37A-37C, VB-201 reduced levels of IL12/23p40 (FIG. 37A) and IL-6 (FIG. 37B) in peptidoglycan-activated human monocyte-derived 5 dendritic cells in a dose-dependent manner, but not levels of TNF-α (FIG. 37C).

As shown in FIGs. 38A-38C, VB-201 reduced levels of IL12/23p40 (FIG. 38A) in Poly I:C-activated human monocyte-derived dendritic cells, but no effect of VB-201 on levels of IL-6 (FIG. 38B) or TNF-α (FIG. 38C) were observed.

The above results demonstrate reduction of IL12/23p40 protein levels by VB-10 201. The effect of VB-201 on the IL12/23p40 mRNA levels in monocyte-derived dendritic cells was then determined using the procedures described in the Materials and Methods section.

As shown in FIGs. 39A and 39B, VB-201 inhibited LPS-induced IL12/23p40 mRNA expression.

15 The above results indicate that treatment of human dendritic cells with VB-201 reduces IL12/23p40 production and mRNA expression induced by different TLR agonists. The reduction in IL-12/23p40 production was usually accompanied by reduced IL-6 production, but not by reduced TNF-α production.

The effect of VB-201 on IL-12/23p40 production was also tested in non-20 stimulated cells. Mouse peritoneal macrophages were isolated from C57B6J mice as described hereinabove. Following incubation for 24 hours with various concentrations of VB-201, the supernatant was collected and the level of IL-12/23p40 therein was measured by ELISA.

As shown in FIG. 40, VB-201 reduced levels of IL-12/23p40 in a dose 25 dependent manner, with concentrations of 9 to 34 μM VB-201 reducing the level of IL-12/23p40 by 40 to 49 %.

EXAMPLE 13

Effect of VB-201 on chemokine and adhesion molecule production

30 Metabolic syndrome is associated with alterations in the expression of proteins such as chemokines and adhesion molecules in the vascular endothelium, which can contribute to cardiovascular diseases. Leukocyte recruitment is a sequential multi-step

adhesion cascade involving leukocyte and endothelial cell adhesion molecules that support leukocyte tethering and rolling, firm adhesion and transmigration.

The effect of VB-201 on adhesion in endothelial cells was tested using HUVEC cells. HUVEC cells were plated in 24-well plates at a concentration of $0.1x10^6$ cells per well, or in 6-well plates at a concentration of $0.3x10^6$ cells per well, and incubated until they reached 80-90 % confluence (approximately two days). Fresh medium was then added to the cells, along with the tested substance.

The effect of VB-201 and other oxidized lipids on levels of the chemokine IL-8 was determined by measuring both IL-8 mRNA and protein levels.

For determining the effect on IL-8 mRNA expression, the HUVEC cells were incubated for 4 hours in 6-well plates with 4 or 9 μM VB-201, 40 or 100 μg/ml Ox-PAPC, prepared by exposing PAPC to air for 72 hours, or 4 μM PGPC (1-palmitoyl-2-glutaryl-phosphatidyl choline). The cells were then scraped from the plate, and the obtained cell pellet underwent RNA purification and qPCR analysis of IL-8 mRNA. IL-8 mRNA levels were normalized relative to GAPDH mRNA.

As shown in FIG. 41, Ox-PAPC considerably and dose-dependently increased IL-8 mRNA expression, whereas VB-201 and PGPC did not affect IL-8 mRNA levels.

For determining the effect on IL-8 protein levels, the HUVEC cells were incubated for 4 or 24 hours in 24-well plates with 4 or 8.5 μM VB-201, 40 or 100 μg/ml Ox-PAPC, or 4 or 8.5 μM PGPC. The supernatant was then collected, and IL-8 protein levels therein were determined by ELISA.

As shown in FIGs. 42A and 42B, Ox-PAPC considerably and dose-dependently increased IL-8 protein levels after both 4 and 24 hours of incubation, whereas VB-201 and PGPC did not affect IL-8 protein levels.

The effect of pre-treatment with VB-201 was assessed in cells stimulated with LPS. HUVEC cells were incubated with 4 or 9 μM VB-201 for 1-2 hours, followed by stimulation with 100 ng/ml LPS for 24 hours. The supernatant was then collected, and protein levels of MCP-1, IL-8, sVCAM-1 (soluble vascular cell adhesion molecule-1) or sICAM-1 (soluble intracellular adhesion molecule-1) therein were determined by ELISA.

As shown in FIGs. 43A-43D, pre-treatment with VB-201 reduced the levels of MCP-1, IL-8, sVCAM-1 and sICAM-1 in cells stimulated with LPS, in a dose-dependent manner.

The above results indicate that VB-201 is effective at reducing levels of a variety of chemokines and adhesion molecules in stimulated cells,, and that Ox-PAPC is effective at increasing IL-8 levels.

EXAMPLE 14

Effect of VB-201 on atherosclerosis

The efficacy of VB-201 against atherosclerosis was tested in New Zealand rabbits on a high-cholesterol diet.

VB-201 was administered daily by oral gavage, 5 times per week, for 14 weeks. One group of rabbits received 1 mg/kg VB-201 per administration, and another group received 4 mg/kg VB-201 per administration. Control rabbits were administered PBS by oral gavage. Each treatment group included 8 rabbits.

The total atherosclerotic lesion area was determined by Sudan IV staining of the aortas.

Blood chemistry was evaluated, although no major effects were observed. VB-201 blood levels were dose-dependent.

As shown in FIGs. 44 and 45, daily treatment with 1 mg/kg VB-201 reduced the total atherosclerotic lesion area in the aorta by 25 %, and daily treatment with 4 mg/kg VB-201 reduced the total atherosclerotic lesion area in the aorta by 50 %.

These results indicate that VB-201 effectively reduces atherosclerotic lesion progression.

EXAMPLE 15

Effect of VB-201 on chemotactic signaling

In order to determine the mechanism by which VB-201 affects cell signaling related to chemotaxis, the effect of VB-201 on expression of chemokine receptors was determined.

Human CD14+ monocytes isolated from a healthy donor were incubated for 3.5 hours with 1, 2.5 or 5 μg/ml VB-201 in a test tube. Surface expression of the

chemokine receptors CXCR1, CXCR2, CCR1 and CCR5 was tested by flow cytometry using receptor-specific antibodies conjugated to a fluorescent dye (phycoerythrin or Alexa Fluor 647).

As shown in FIGs. 46A and 46B, none of the tested doses of VB-201 had an effect on the expression of CXCR1 or CXCR2 (FIG. 46A), or CCR1 and CCR5 (FIG. 46B), in comparison with the solvent-treated control.

Furthermore, VB-201 did not alter the surface expression of the abovementioned receptors even in CD14+ cells subjected to trans-well migration under conditions in which VB-201 inhibited monocyte migration (data not shown).

These results indicate that VB-201 modulates chemotaxis by mechanisms other than modulation of chemokine receptor expression.

The effect of VB-201 on cell signaling downstream of chemokine receptors was also investigated. Human CD14+ monocytes were isolated from a healthy donor, and phosphorylation of ERK1/2 and MEK1/2 was determined by Western blot, following incubation with the chemokines MIP-1α, RANTES or MCP-1. α-Tubulin or total ERK1/2 levels were determined as a loading control.

In one experiment, human CD14+ monocytes were incubated with 5 μg/ml VB-201 or solvent for 10 minutes prior to being stimulated with 100 ng/ml of MIP-1α for 2-60 minutes.

As shown in FIG. 47, VB-201 blocked MIP-1α-induced ERK1/2 phosphorylation.

In another experiment, human CD14+ monocytes were incubated with 2 μg/ml VB-201 or solvent for 30 minutes prior to being stimulated with 50 ng/ml of RANTES for 2-60 minutes. Untreated cells were used as an additional negative control.

As shown in FIG. 48, VB-201 reduced the levels of the transient RANTES-induced MEK1/2 and ERK1/2 phosphorylation.

In another experiment, human CD14+ monocytes were incubated with 2 μg/ml VB-201 or solvent for 30 minutes prior to being stimulated with 100 ng/ml of MCP-1 for 2-60 minutes. Untreated cells were used as an additional negative control.

As shown in FIG. 49, VB-201 reduced the levels of the transient MCP-1-induced MEK1/2 and ERK1/2 phosphorylation.

These results indicate that VB-201 inhibits signaling downstream of chemokine receptors (but not their expression levels), thereby inhibiting monocyte migration.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

All publications, patents and patent applications mentioned in this specification 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 pharmaceutical composition unit dosage form comprising from 1 μg to 1 mg VB-201 and a pharmaceutically acceptable carrier, the pharmaceutical composition unit dosage form being formulated for oral administration.

2. Use of VB-201 in the manufacture of a unit dosage form of a medicament for treating or preventing an inflammatory disease or disorder, the unit dosage form comprising from 1 μg to 1 mg VB-201 and being formulated for oral administration.

3. The pharmaceutical composition unit dosage form or use of any of claims 1 to 2, wherein the unit dosage form comprises from 0.01 mg to 0.5 mg VB-201.

4. The pharmaceutical composition unit dosage form of any of claims 1 and 3, being packaged in a packaging material and identified in print, in or on said packaging material, for use in the treatment or prevention of an inflammatory disease or disorder.

5. The pharmaceutical composition unit dosage form of claim 4, being identified for use once per two or more days.

6. A method of treating or preventing an inflammatory disease or disorder, the method comprising orally administering to a subject in need thereof a therapeutically effective amount of VB-201, wherein said therapeutically effective amount ranges from 1 μg per day to 1 mg per day.

7. The method of treating or preventing metabolic syndrome, the method comprising orally administering to a subject in need thereof a therapeutically effective amount of VB-201, wherein said therapeutically effective amount ranges from 1 μg per day to 1 mg per day.

8. The method of any of claims 6 to 7, wherein said therapeutically effective amount of VB-201 is administered once per two or more days.

9. A method of treating or preventing an inflammatory disease or disorder, the method comprising orally administering to a subject in need thereof a therapeutically effective amount of VB-201, wherein said administering is effected once per two or more days.

10. The method of claim 9, wherein said therapeutically effective amount ranges from 1 μg per day to 1 mg per day.

11. A method of treating or preventing metabolic syndrome, the method comprising orally administering to a subject in need thereof a therapeutically effective amount of VB-201, wherein said administering is effected once per two or more days.

12. The method of claim 11, wherein said therapeutically effective amount ranges from 1 μg per day to 1 mg per day.

13. The method of any of claims 6 to 12, wherein said therapeutically effective amount ranges from 0.01 mg per day to 0.5 mg per day.

14. The method of any of claims 6 to 12, comprising administering a unit of the pharmaceutical composition unit dosage form of any of claims 1 and 3 to 5.

15. The pharmaceutical composition unit dosage form of any of claims 4 to 5, the use of claim 2, or the method of any of claims 6 and 9 to 10, wherein said inflammatory disease or disorder is associated with an endogenous oxidized lipid.

16. The pharmaceutical composition unit dosage form of any of claims 4 to 5, the use of claim 2, or the method of any of claims 6 and 9 to 10, wherein said inflammatory disease or disorder is selected from the group consisting of an idiopathic inflammatory disease or disorder, a chronic inflammatory disease or disorder, an acute

inflammatory disease or disorder, an autoimmune disease or disorder, an infectious disease or disorder, an inflammatory malignant disease or disorder, an inflammatory transplantation-related disease or disorder, an inflammatory degenerative disease or disorder, a disease or disorder associated with a hypersensitivity, an inflammatory cardiovascular disease or disorder, an inflammatory cerebrovascular disease or disorder, a peripheral vascular disease or disorder, an inflammatory glandular disease or disorder, an inflammatory gastrointestinal disease or disorder, an inflammatory cutaneous disease or disorder, an inflammatory hepatic disease or disorder, an inflammatory neurological disease or disorder, an inflammatory musculo-skeletal disease or disorder, an inflammatory renal disease or disorder, an inflammatory reproductive disease or disorder, an inflammatory systemic disease or disorder, an inflammatory connective tissue disease or disorder, an inflammatory tumor, necrosis, an inflammatory implant-related disease or disorder, an inflammatory aging process, an immunodeficiency disease or disorder, a proliferative disease or disorder and an inflammatory pulmonary disease or disorder.

17. The pharmaceutical composition unit dosage form, use or method of claim 16, wherein said disease or disorder is metabolic syndrome.

18. A method of treating or preventing metabolic syndrome, the method comprising administering to a subject in need thereof a therapeutically effective amount of VB-201.

19. The method of claim 18, wherein said administering comprises oral administration.

20. Use of VB-201 in the manufacture of a medicament for treating or preventing metabolic syndrome.

21. The use of claim 20, wherein said medicament is formulated for oral administration.

22. VB-201, for use in treating or preventing metabolic syndrome.

23. VB-201, for use in treating or preventing metabolic syndrome by oral administration.

\- LPS Stimulated

VB-201

Sol Sol 1 2 5 µg/ml

p-p38

p38

p-IKKα/β

IκBα

p-Erk1/2

Erk1/2

αTub

FIG. 1

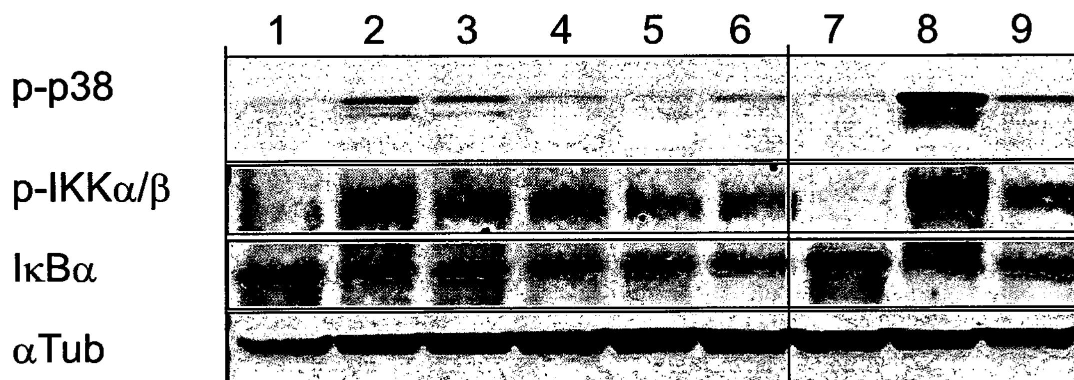


FIG. 2

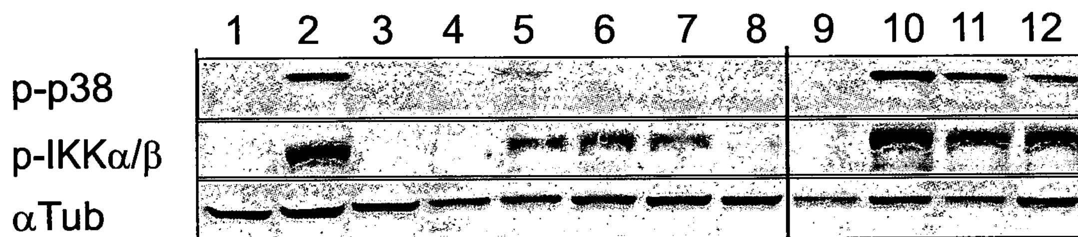


FIG. 3

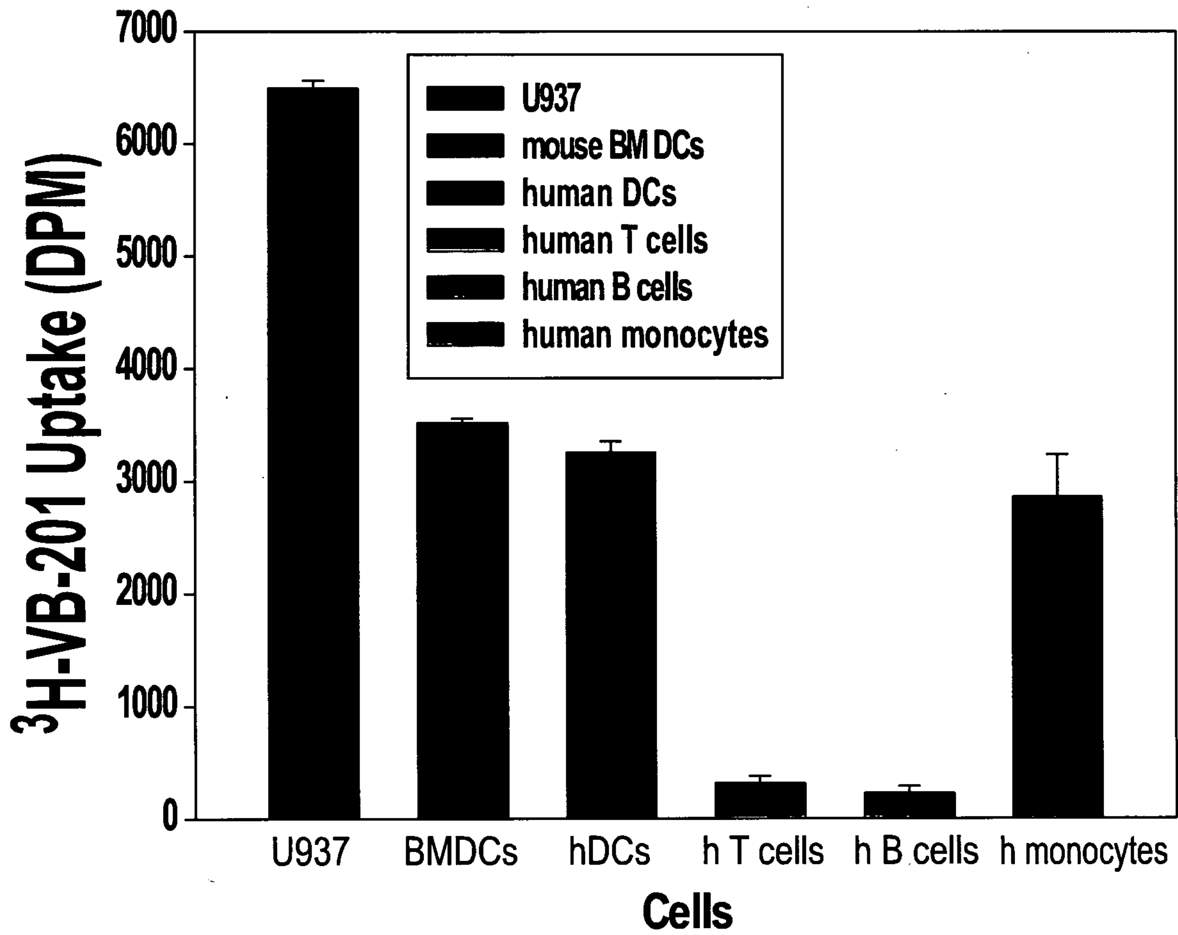


FIG. 4

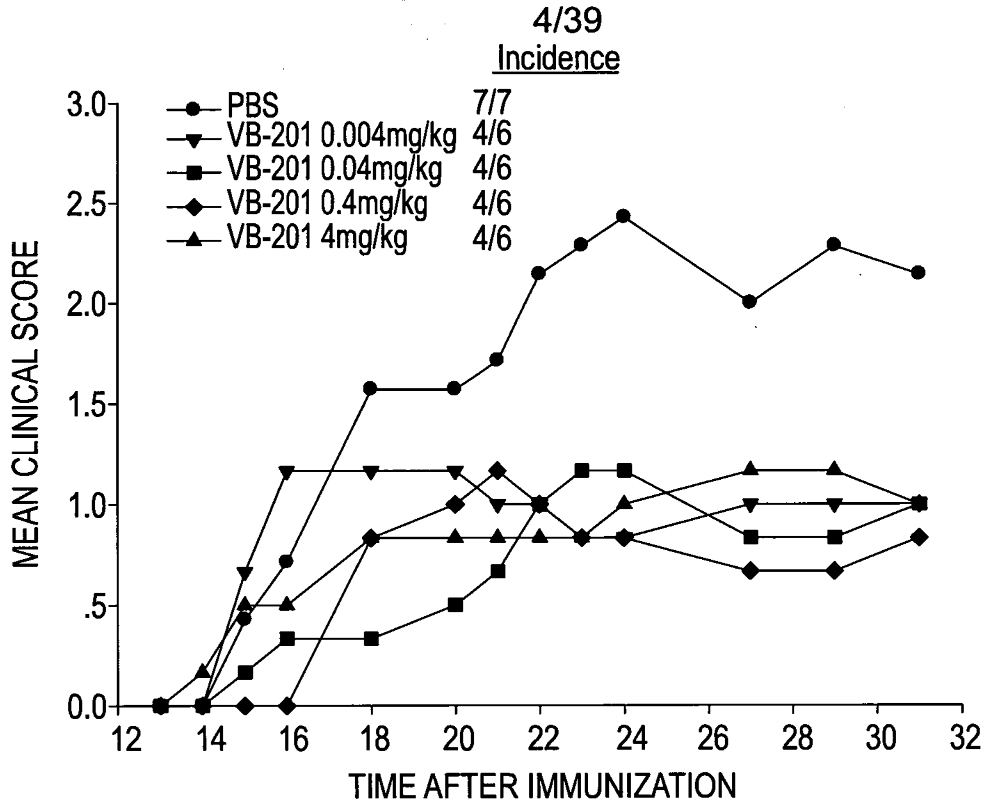


FIG. 5

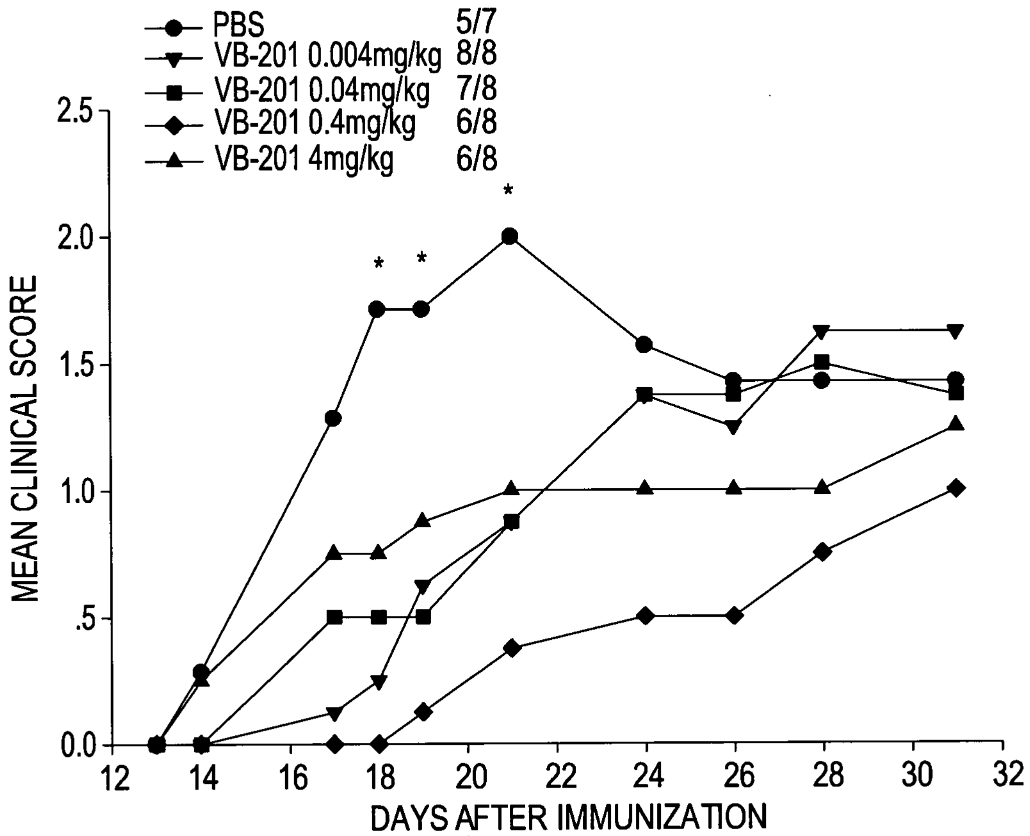


FIG. 6

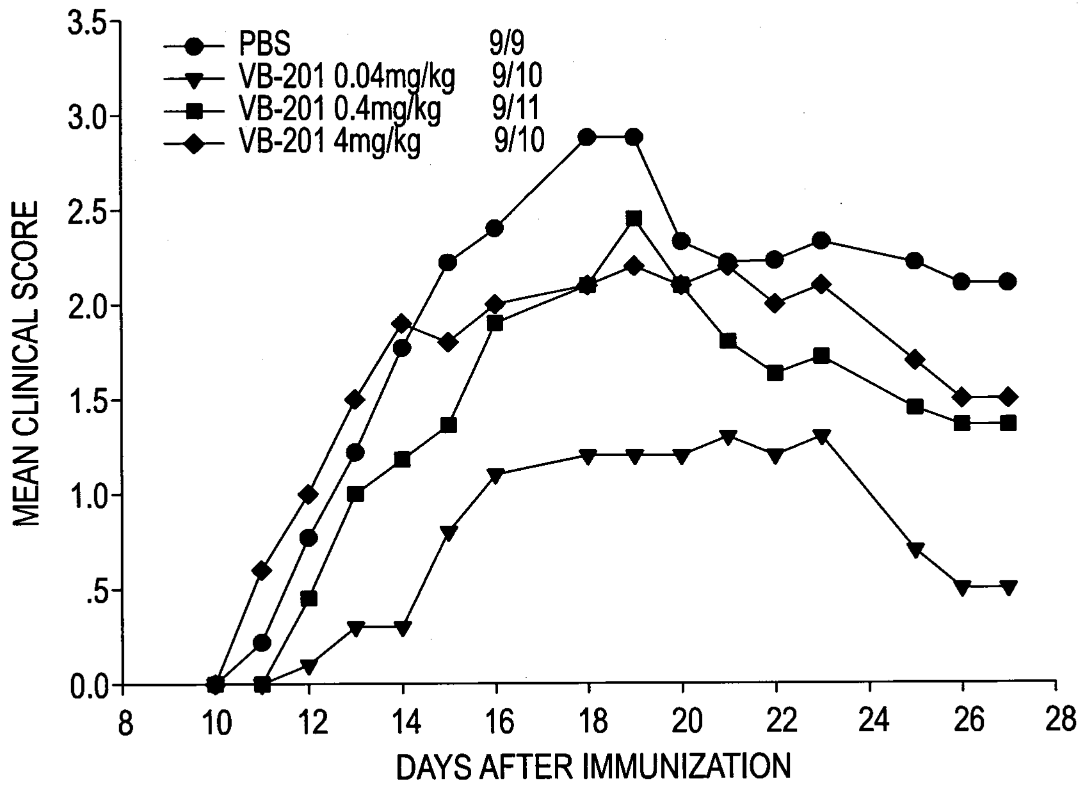


FIG. 7

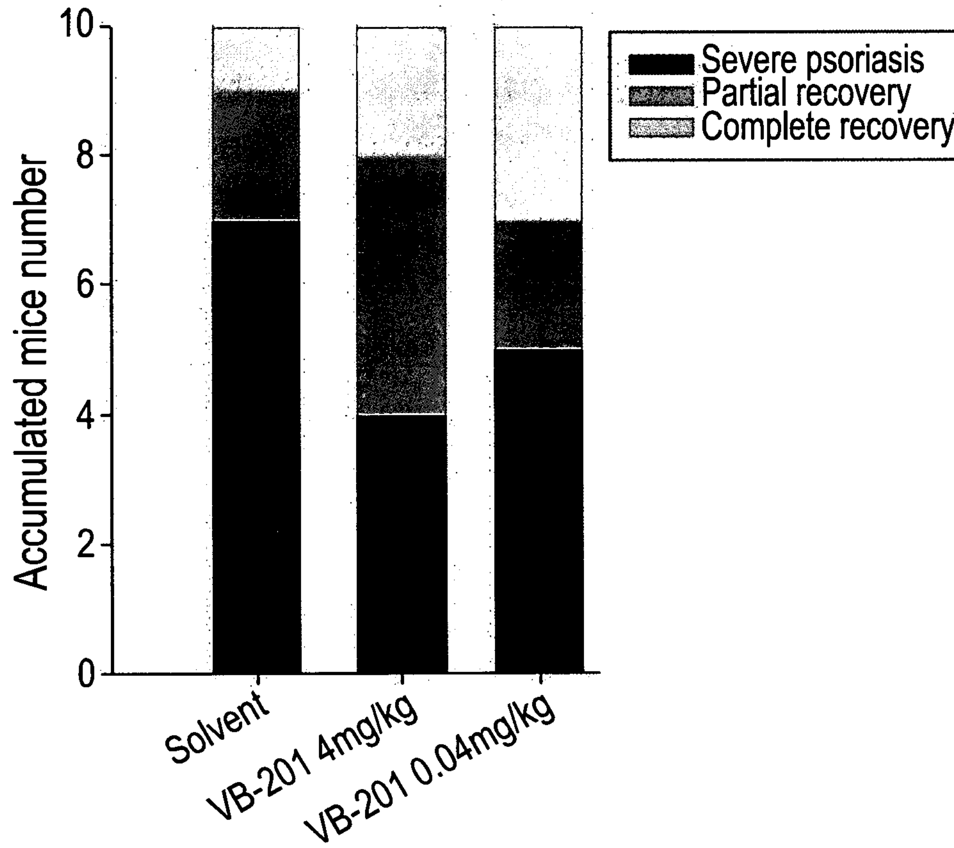


FIG. 8

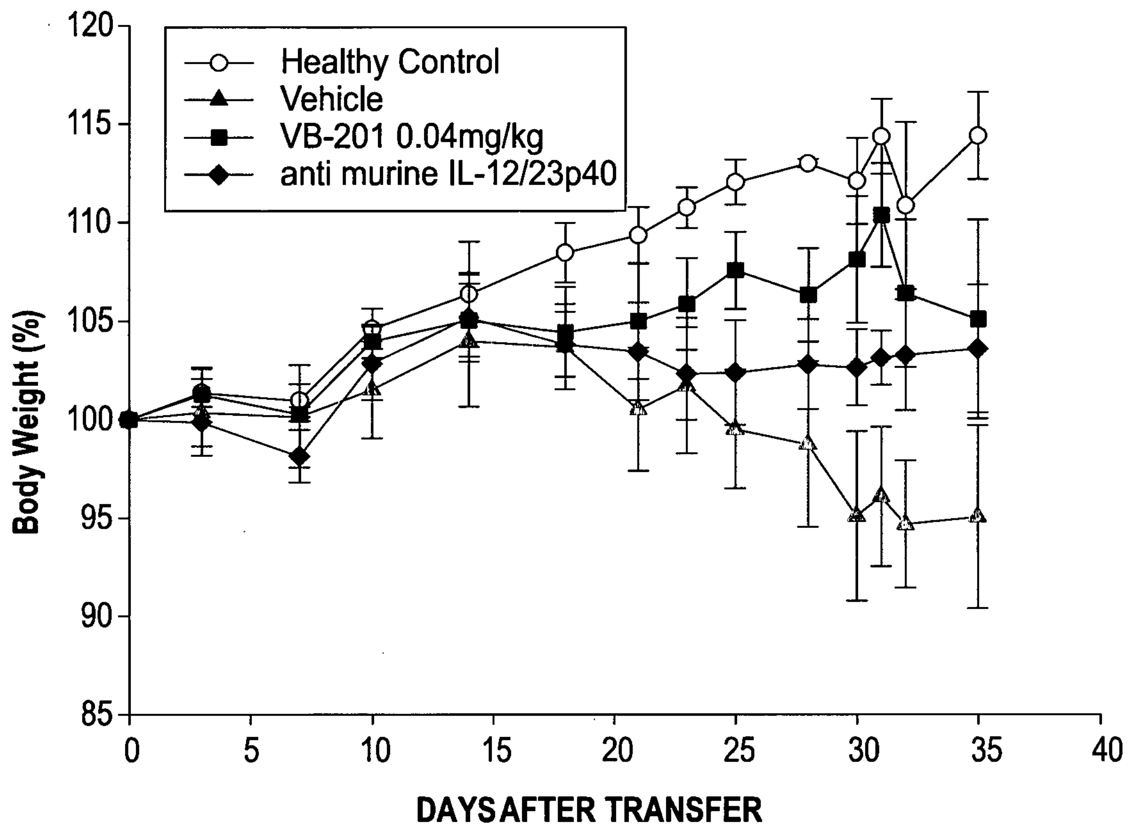


FIG. 9

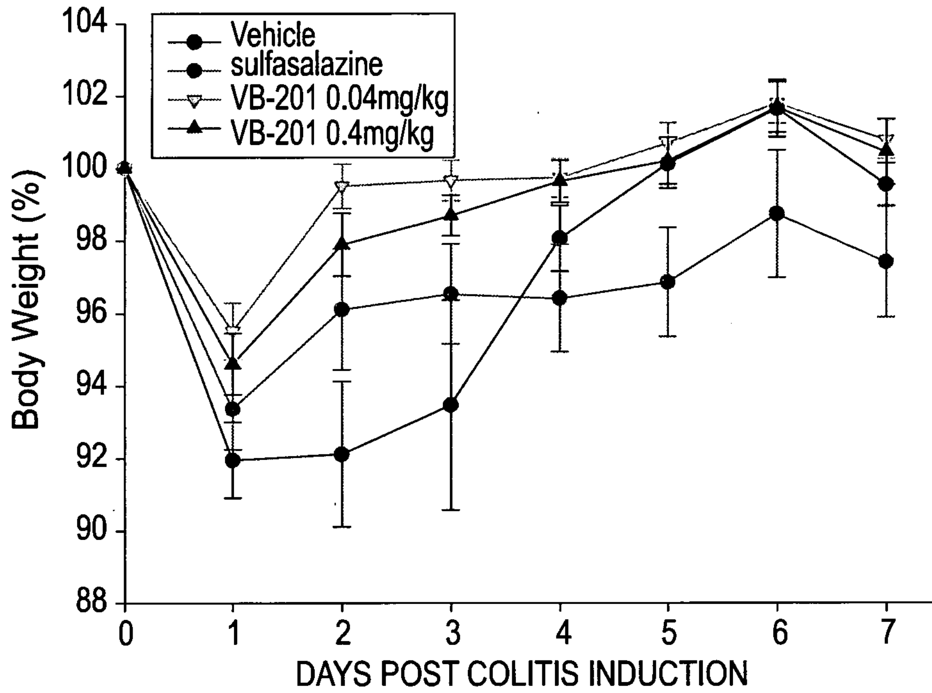


FIG. 10

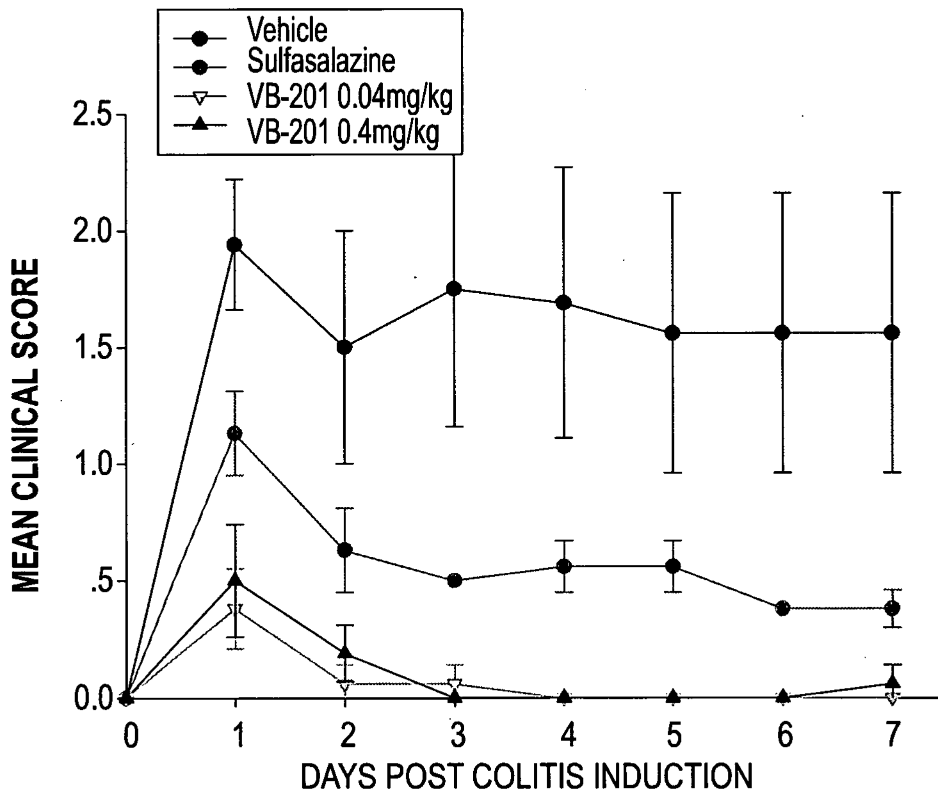


FIG. 11

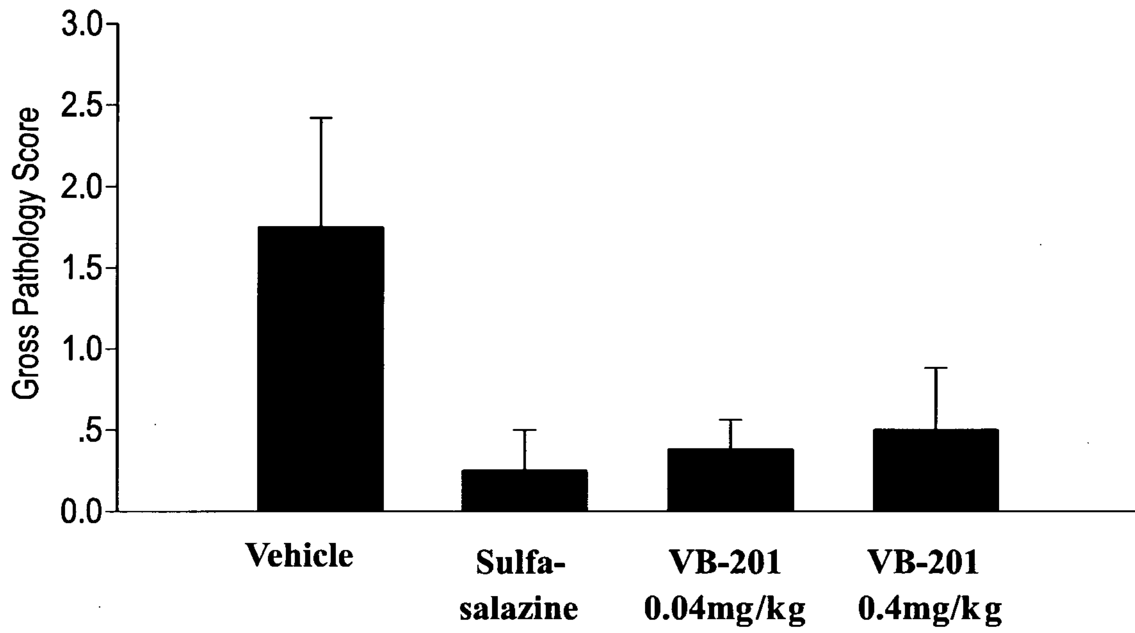


FIG. 12

Vehicle

FIG. 13A

VB-201 0.04mg/kg

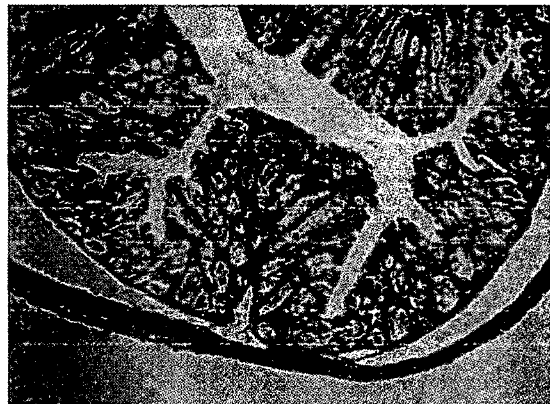


FIG. 13B

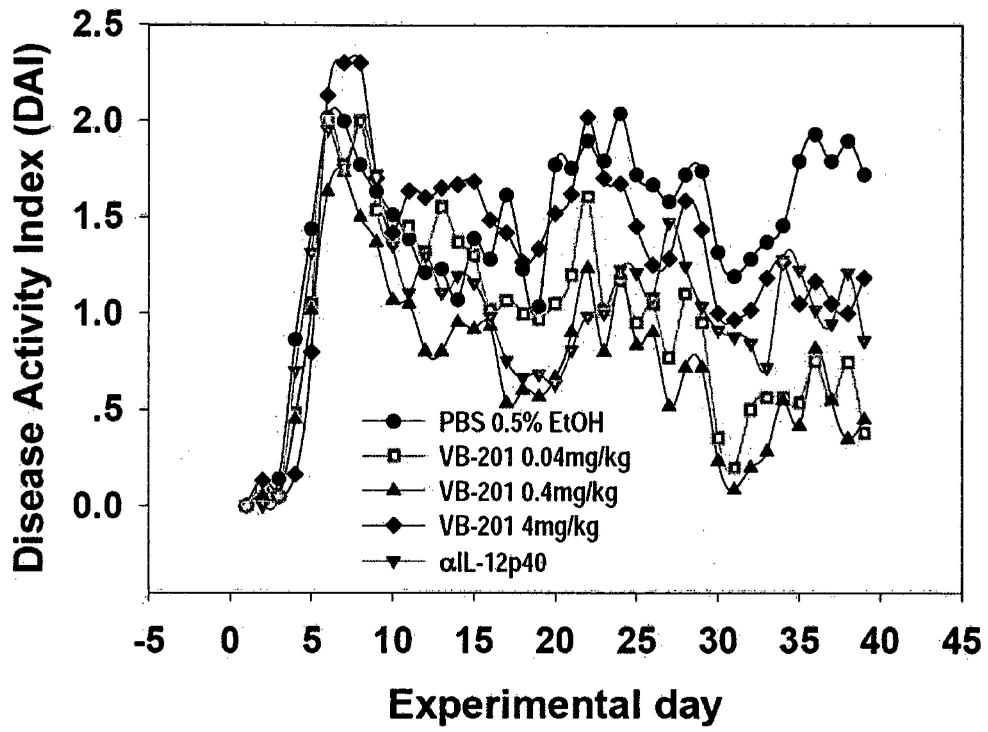


FIG. 14

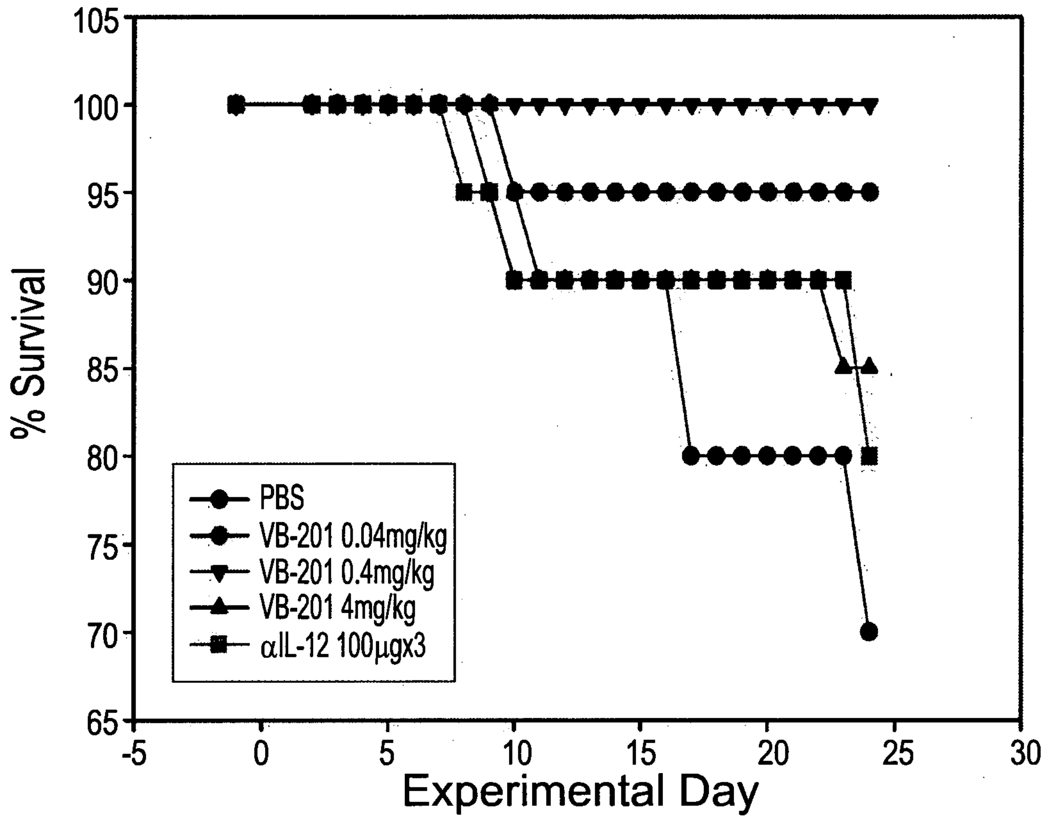


FIG. 15

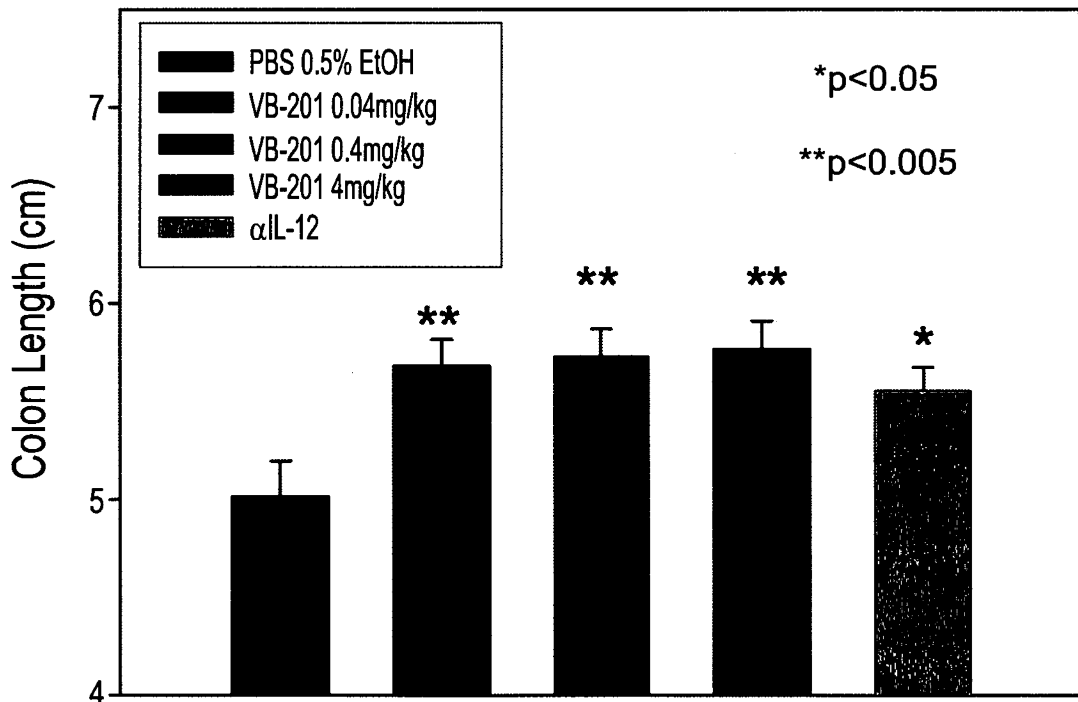


FIG. 16

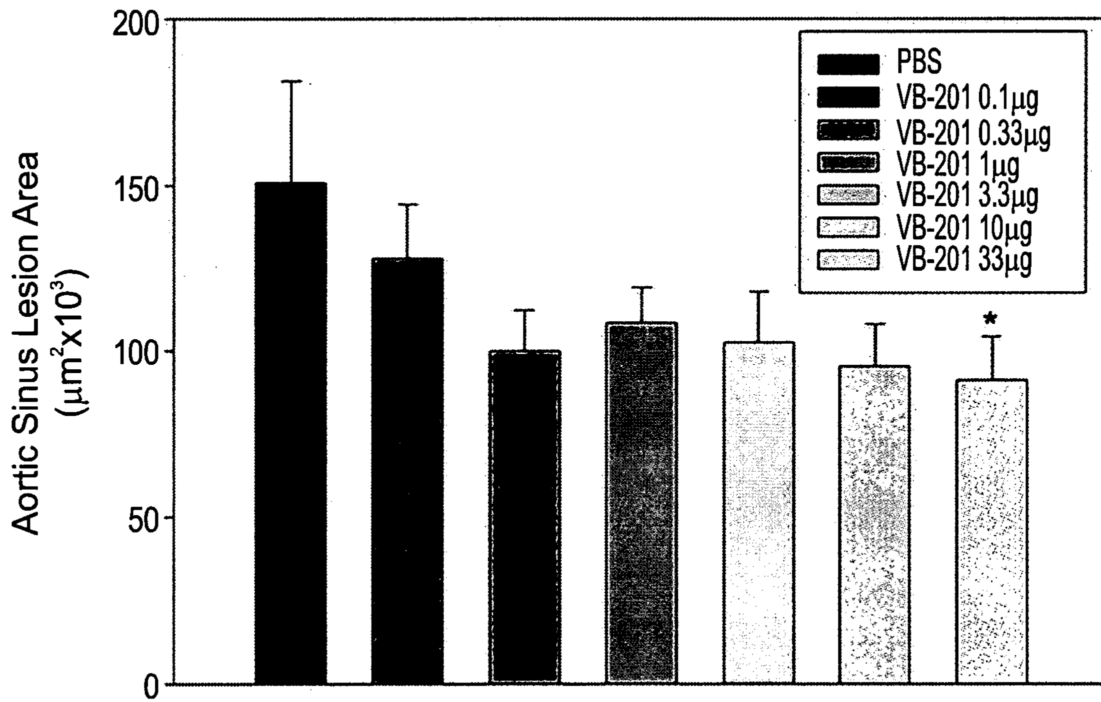


FIG. 17

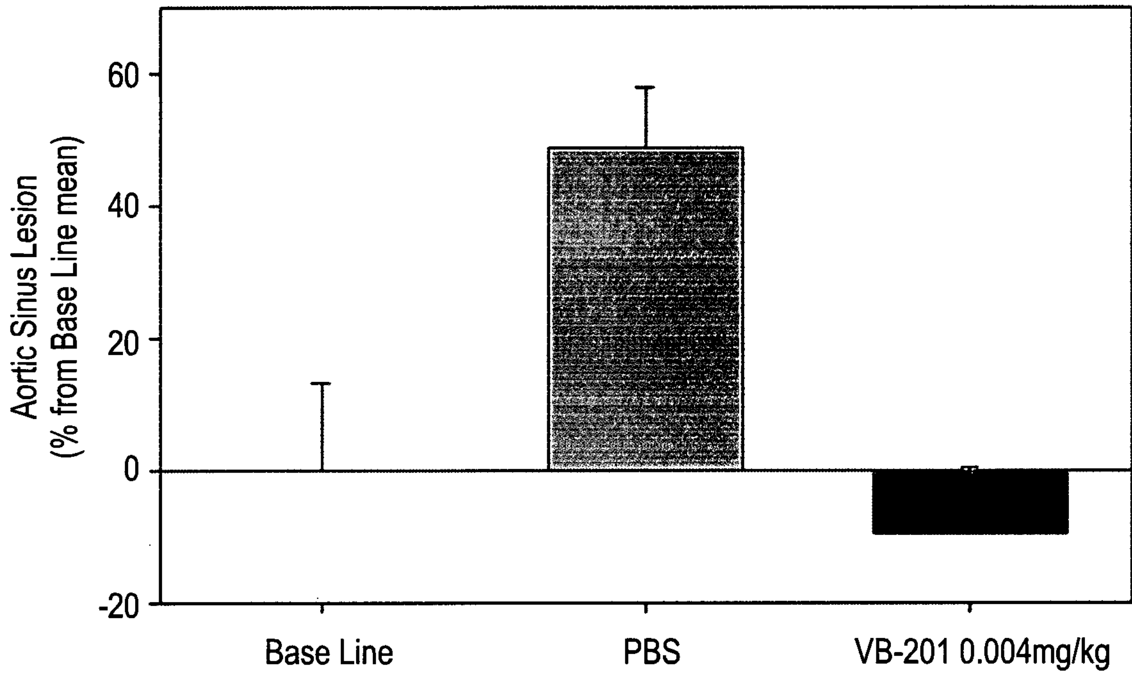


FIG. 18A

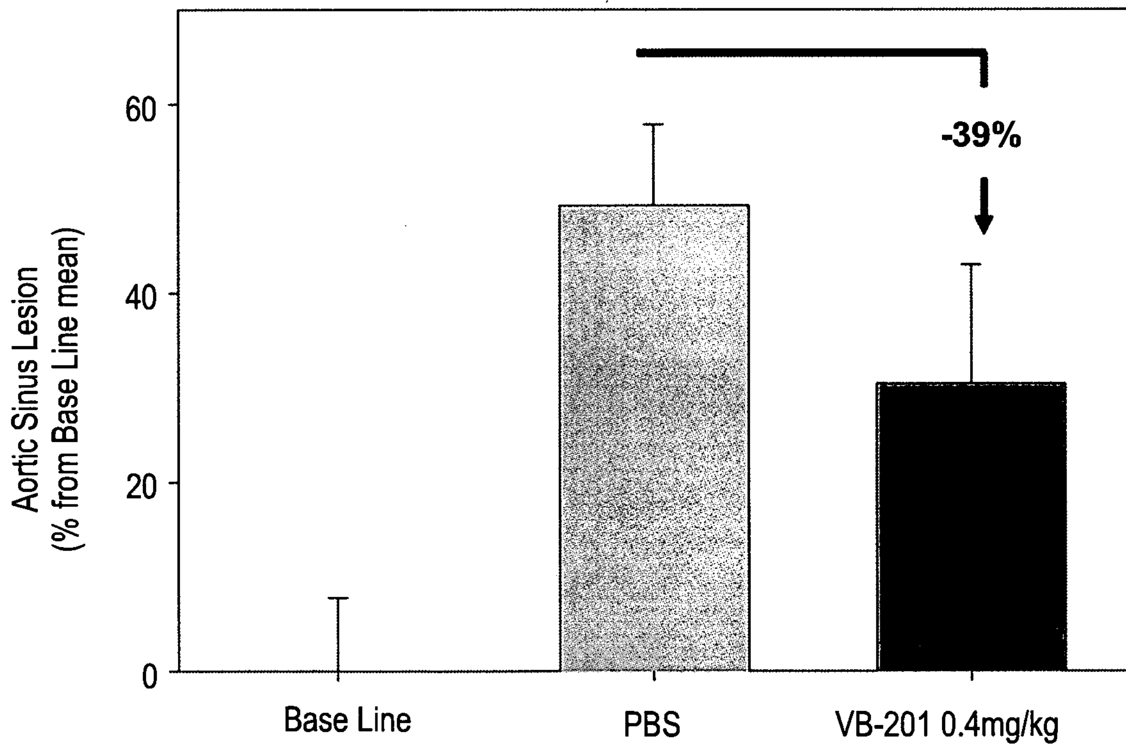


FIG. 18B

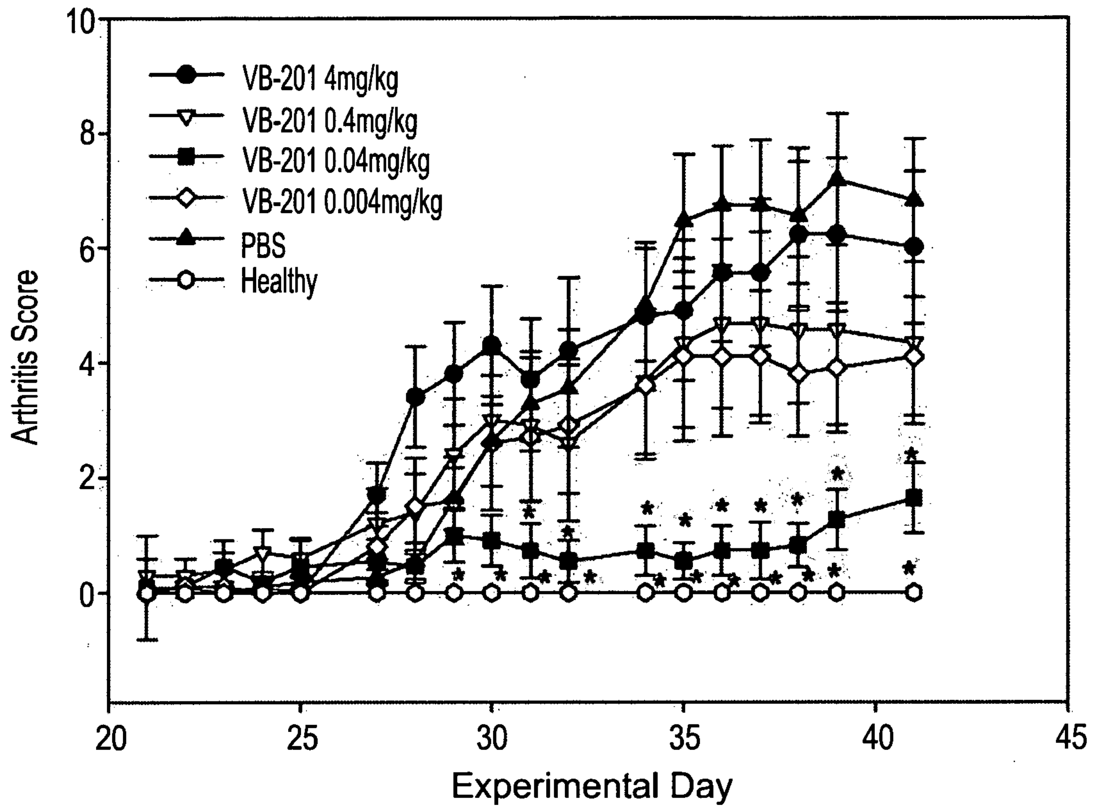


FIG. 19

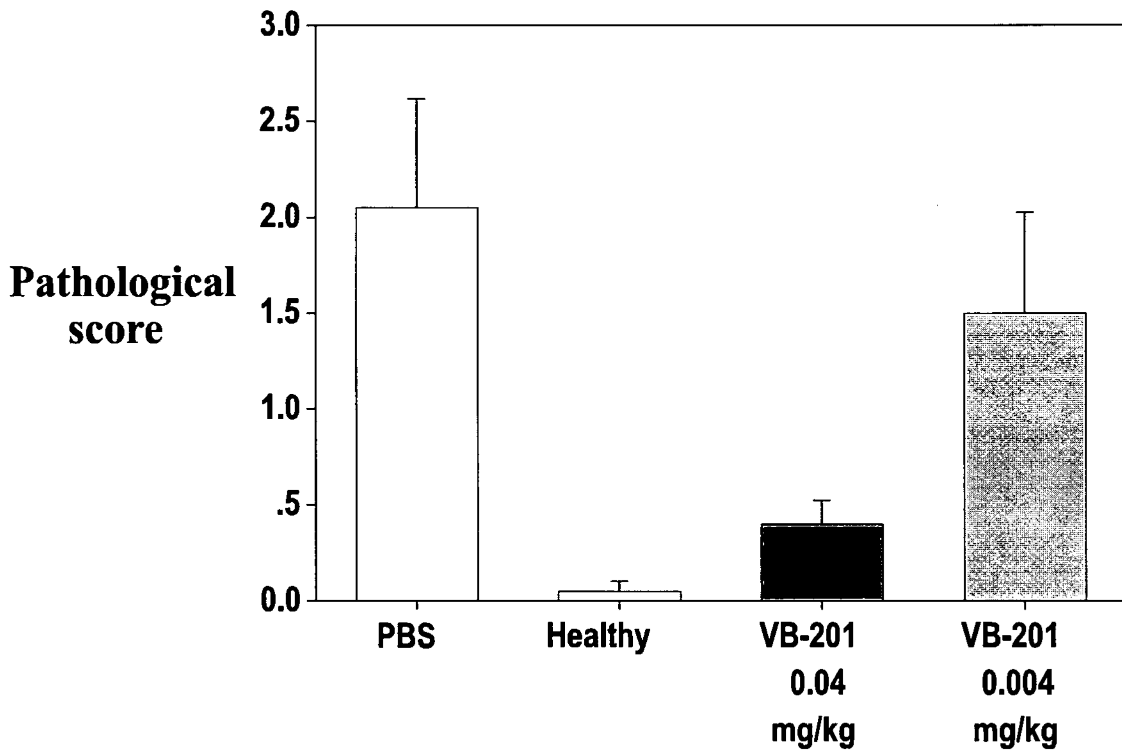


FIG. 20

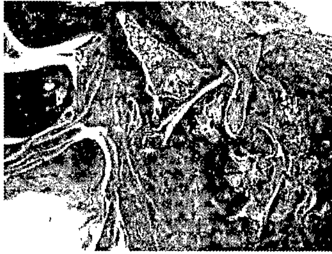


FIG. 21A

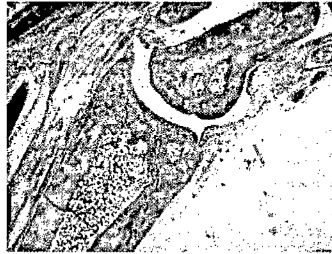


FIG. 21B

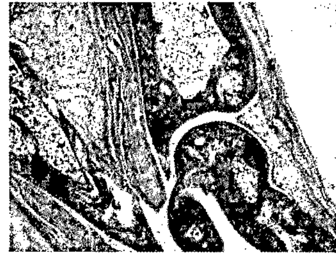


FIG. 21C

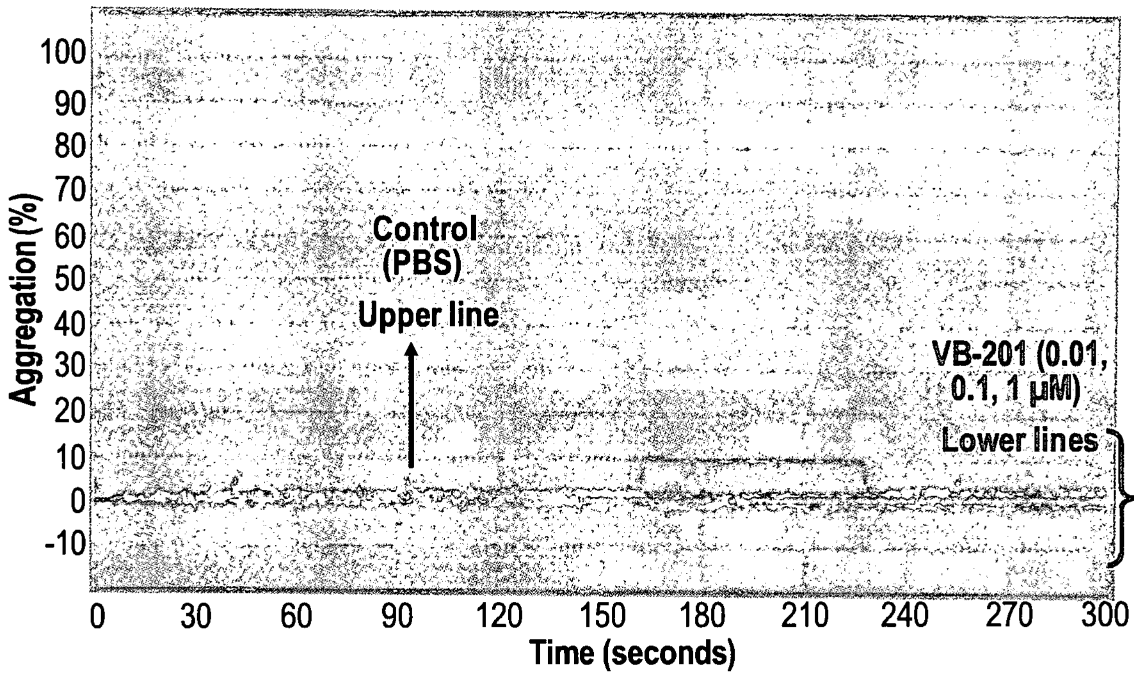


FIG. 22A

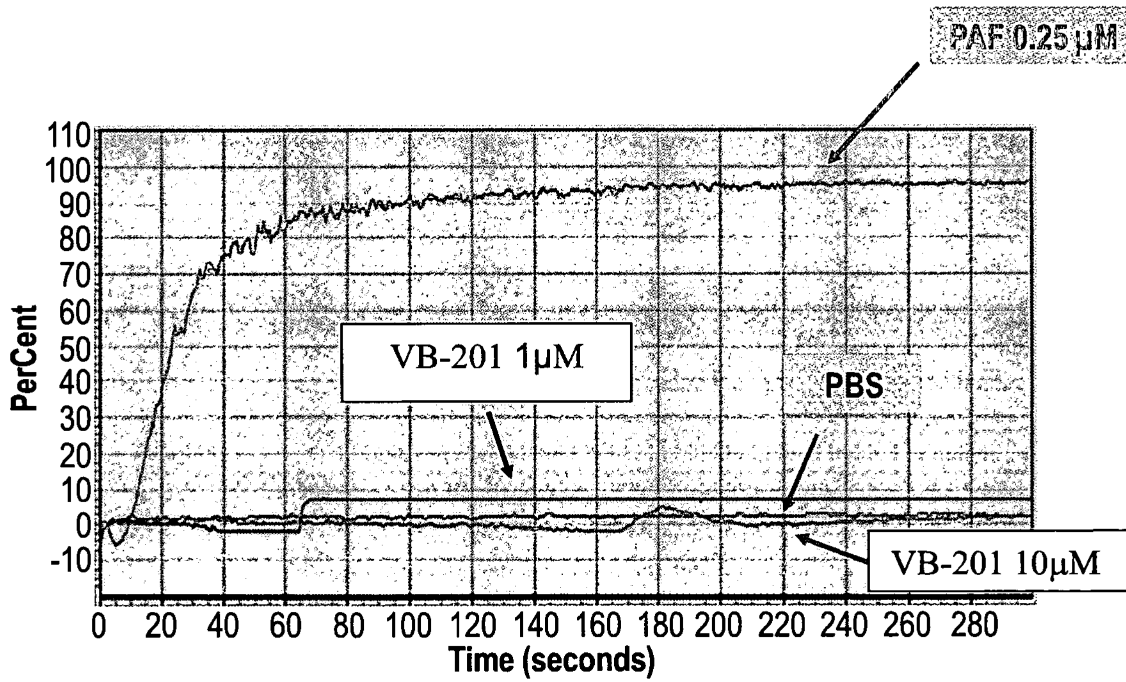
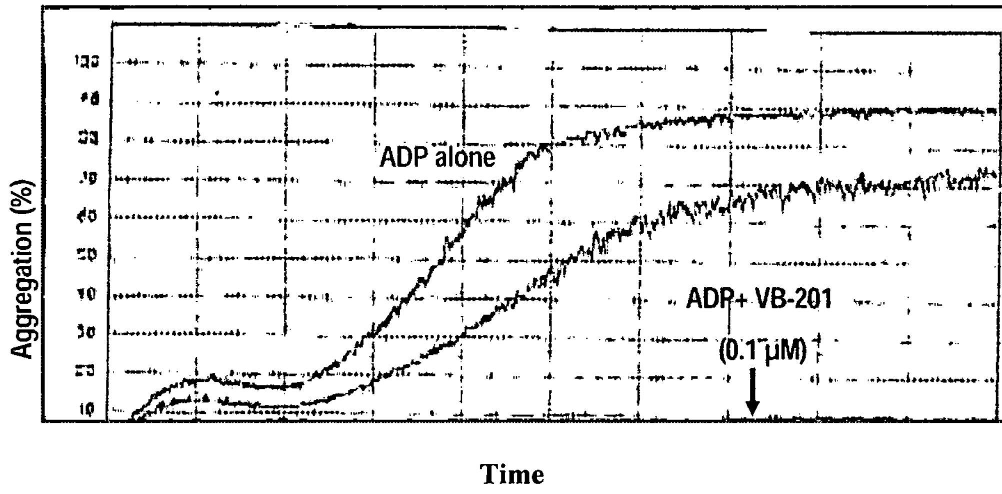


FIG. 22B



Time

FIG. 23

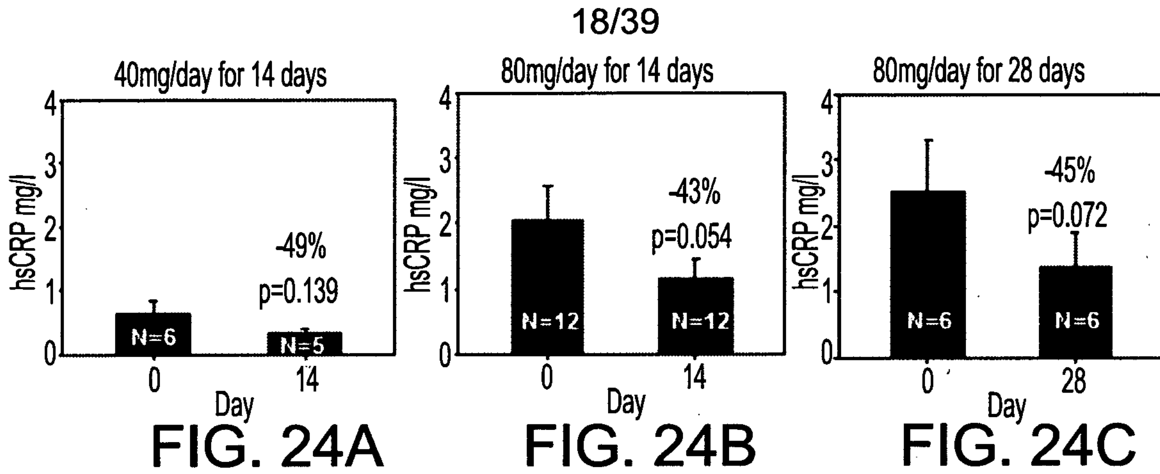


FIG. 24A FIG. 24B FIG. 24C

FIG. 24D

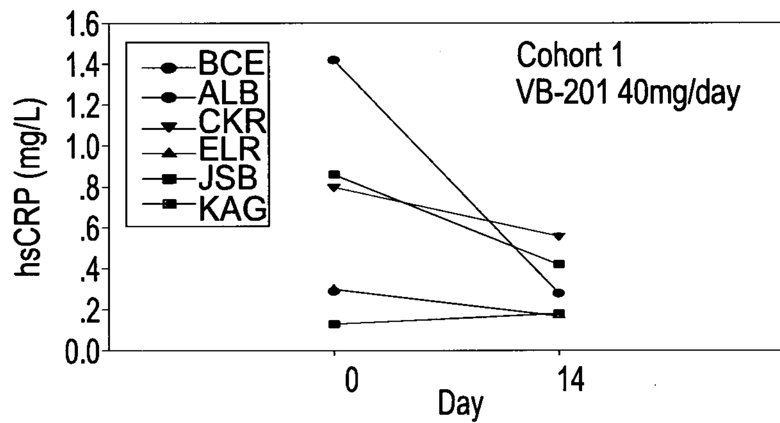


FIG. 24E

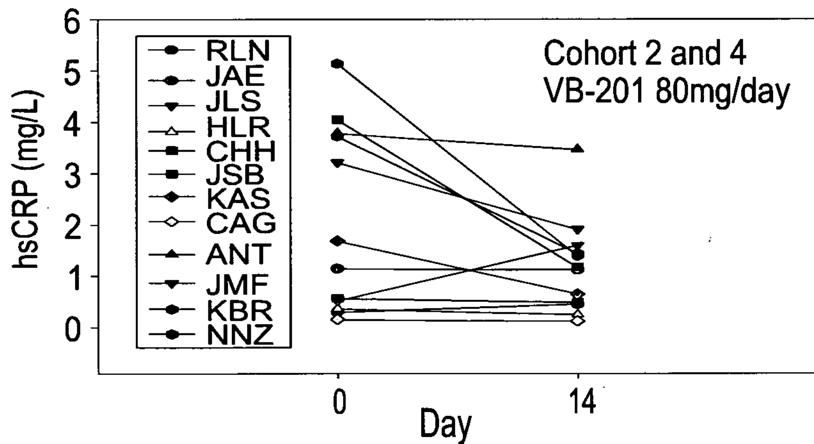
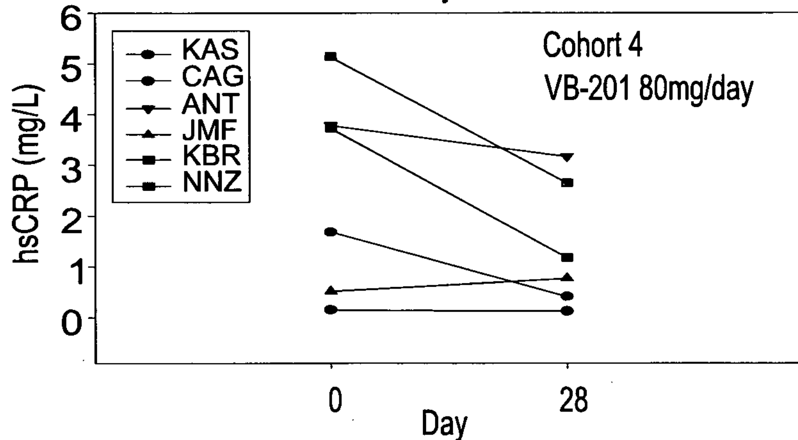


FIG. 24F



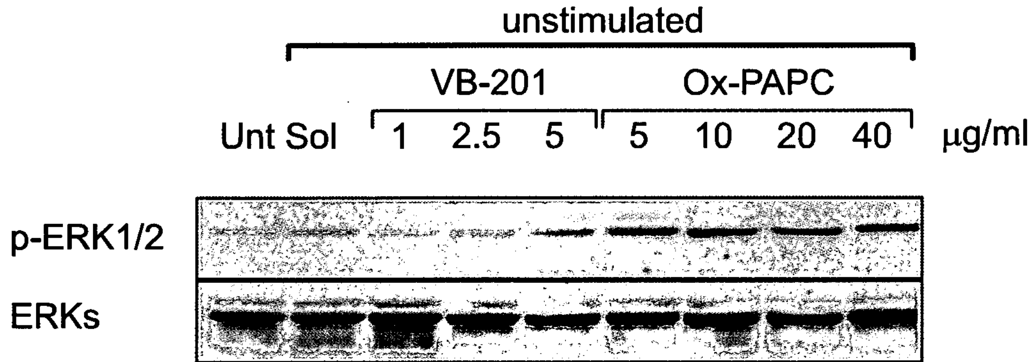


FIG. 25A

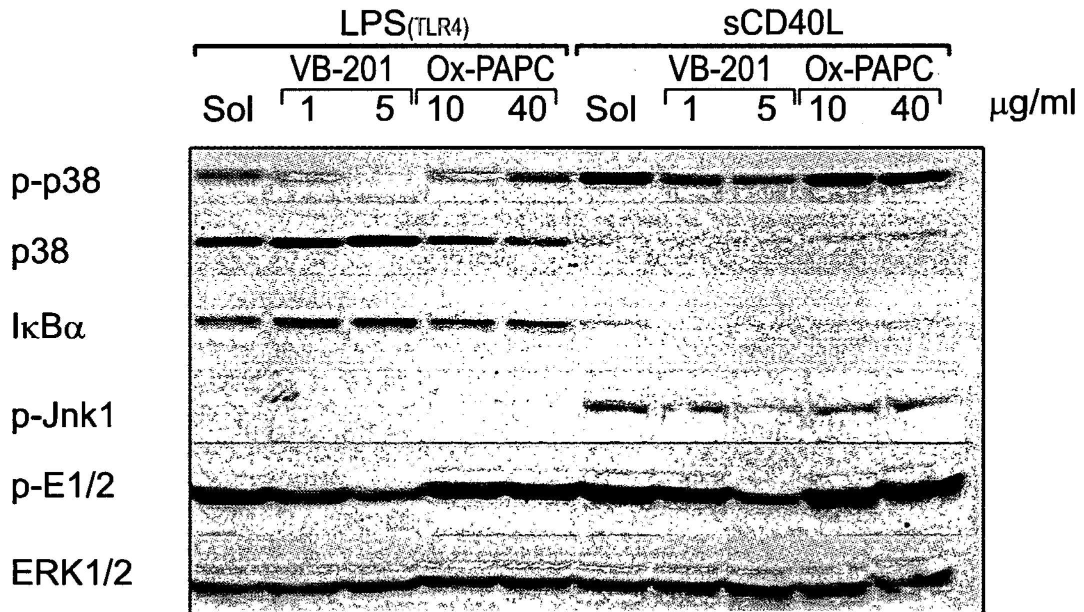


FIG. 25B

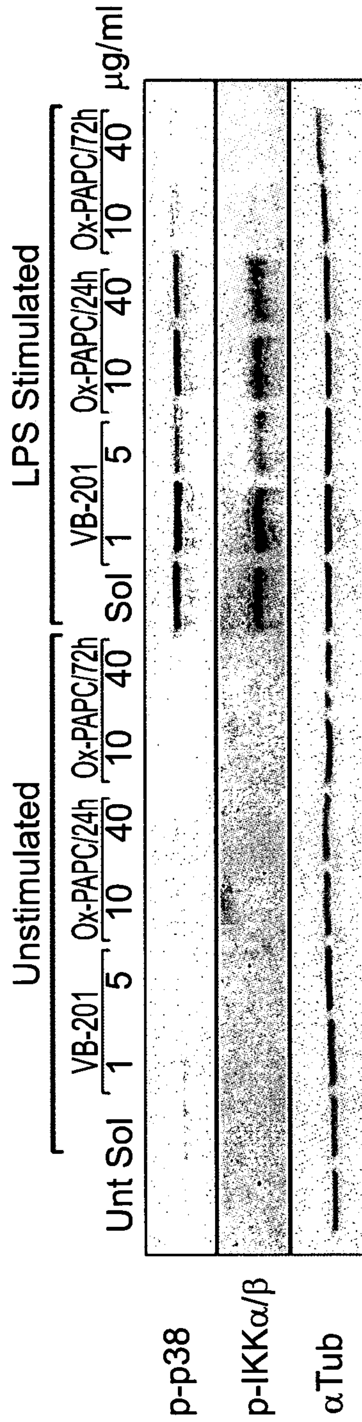


FIG. 26

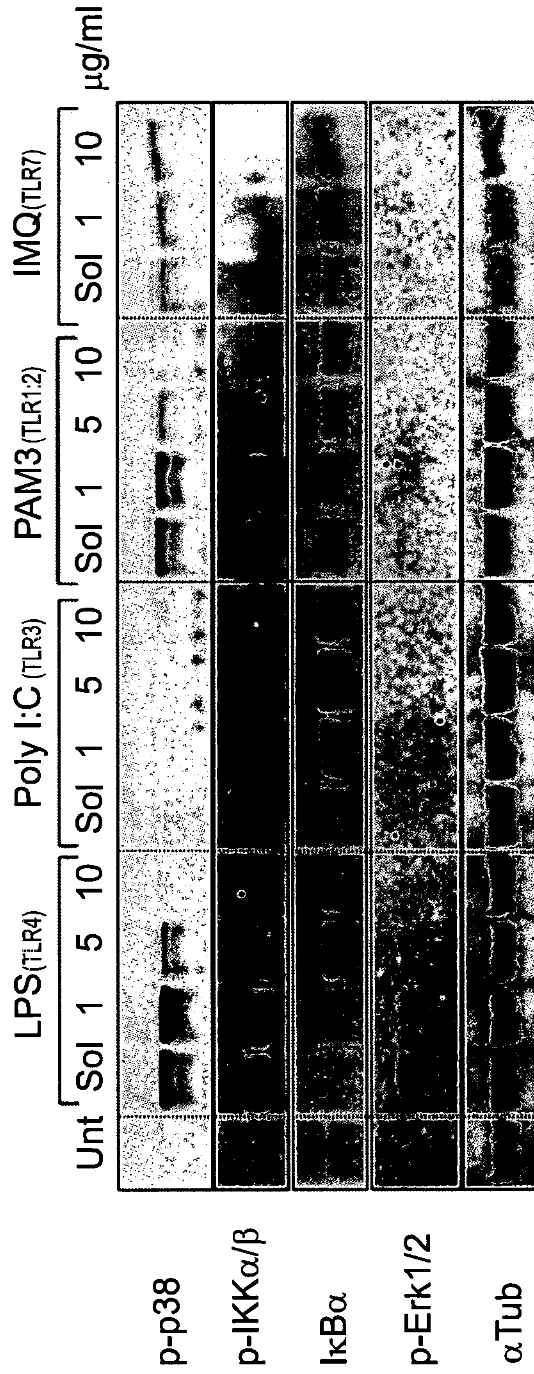


FIG. 27

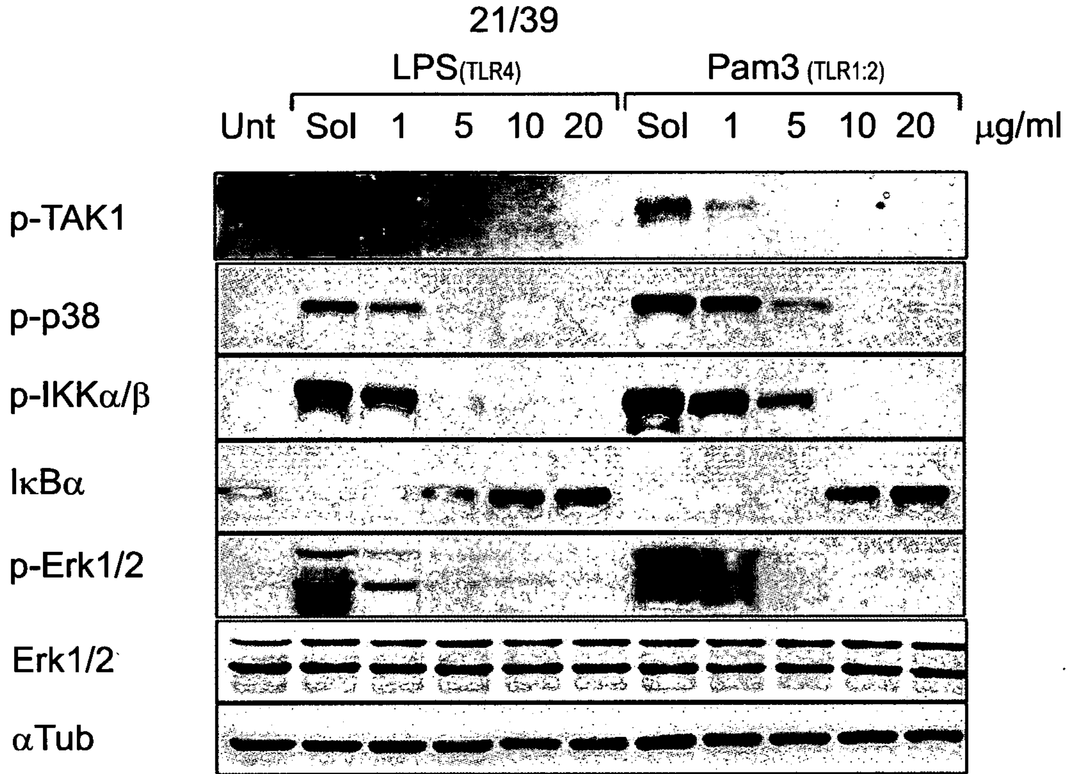
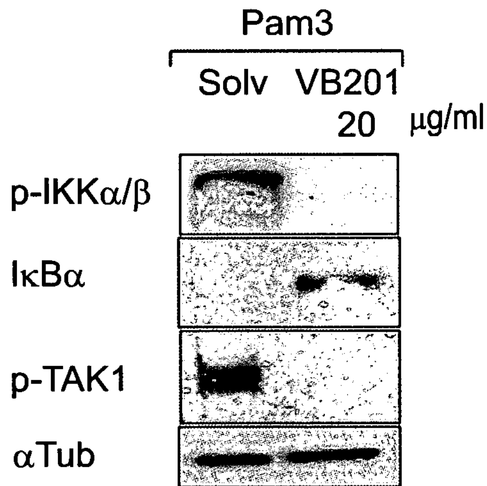


FIG. 28



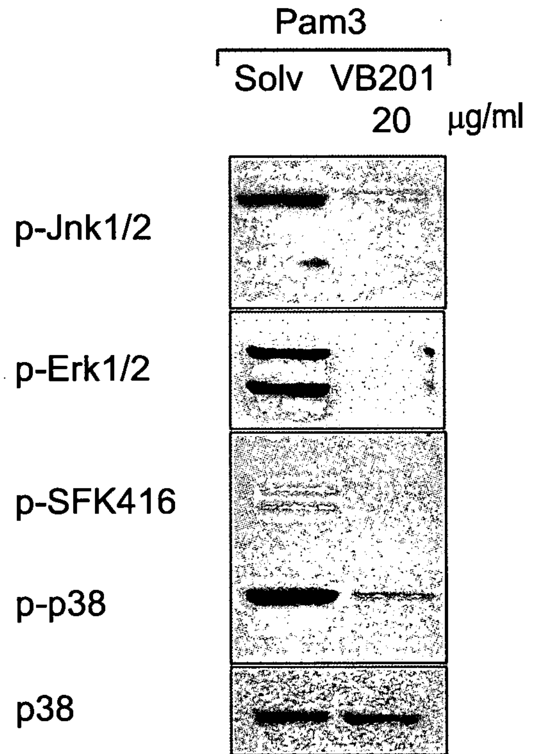


FIG. 29A

FIG. 29B

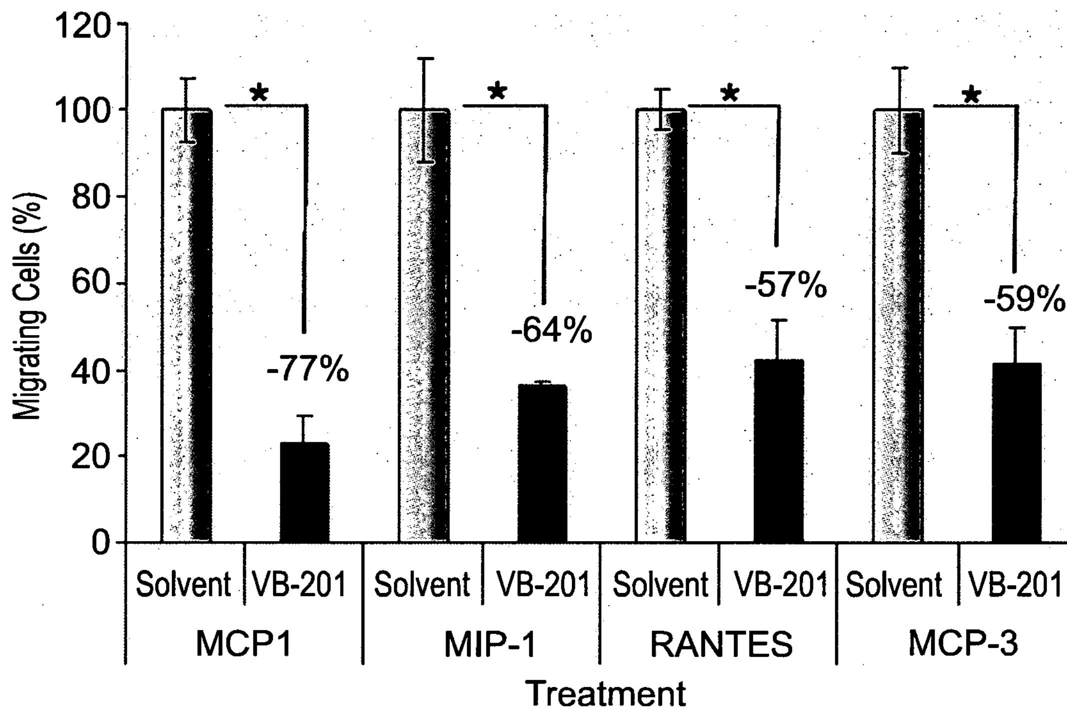


FIG. 30A

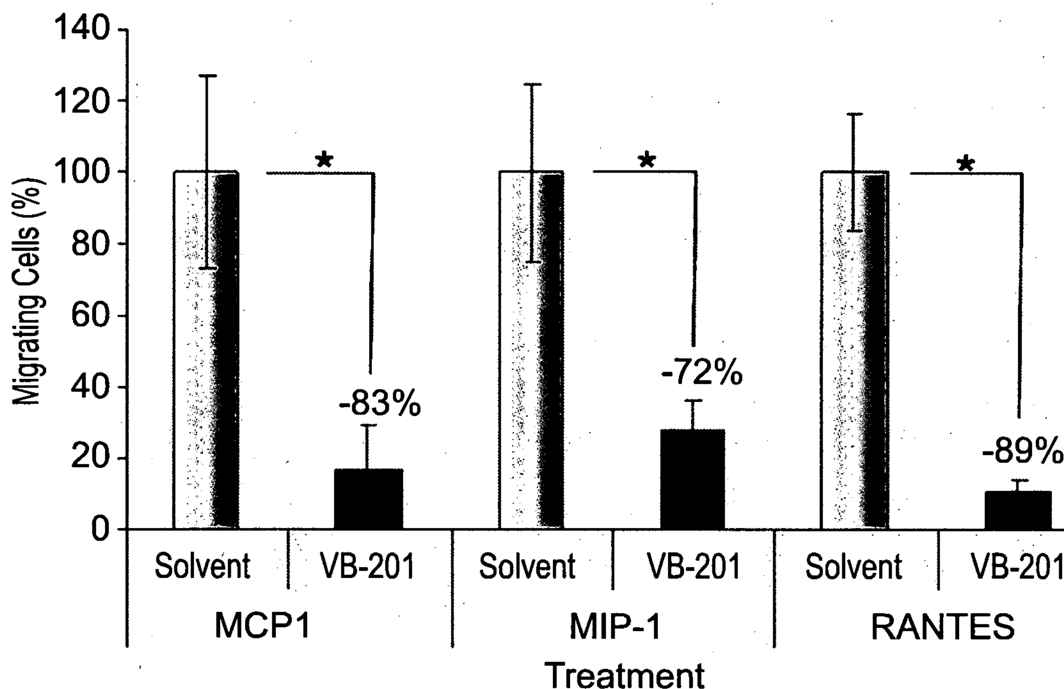


FIG. 30B

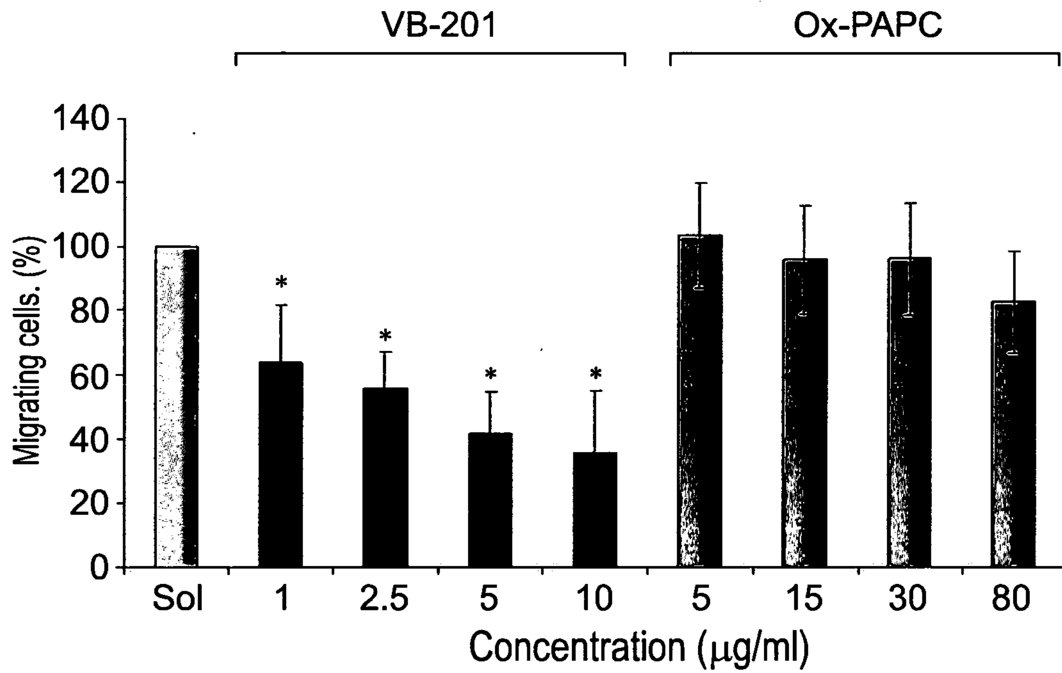


FIG. 31

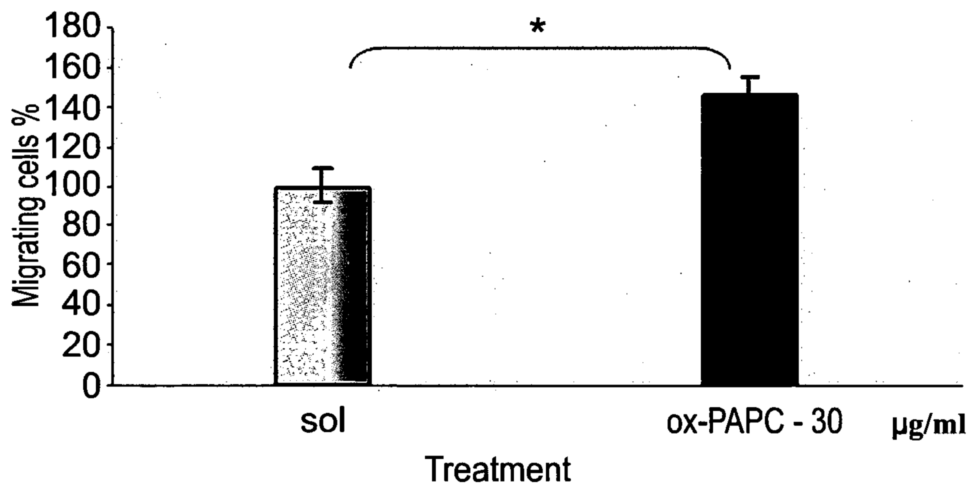


FIG. 32

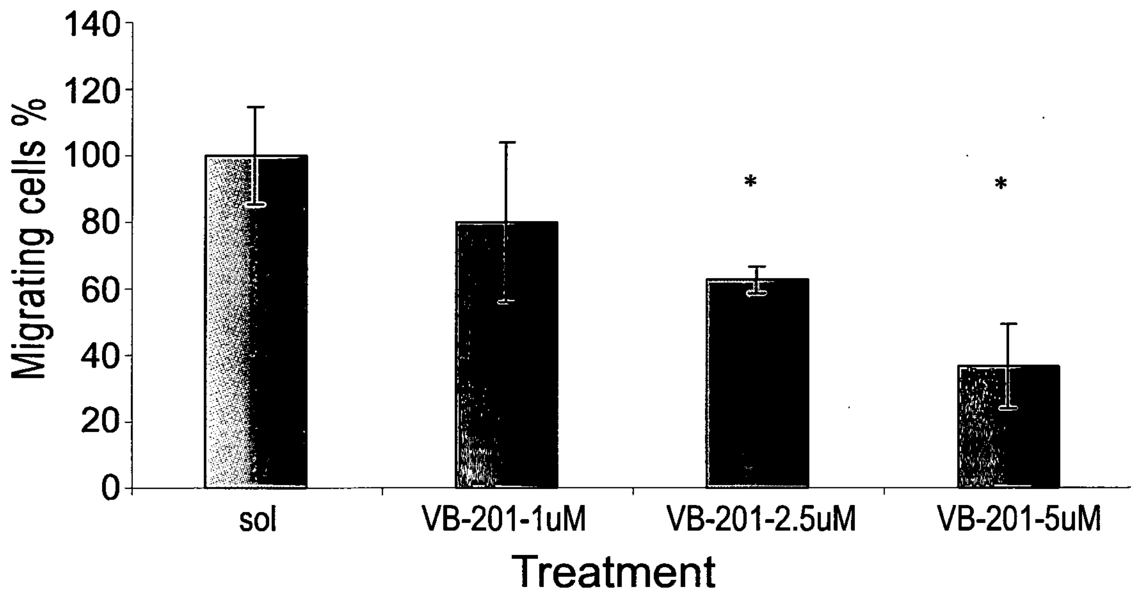


FIG. 33

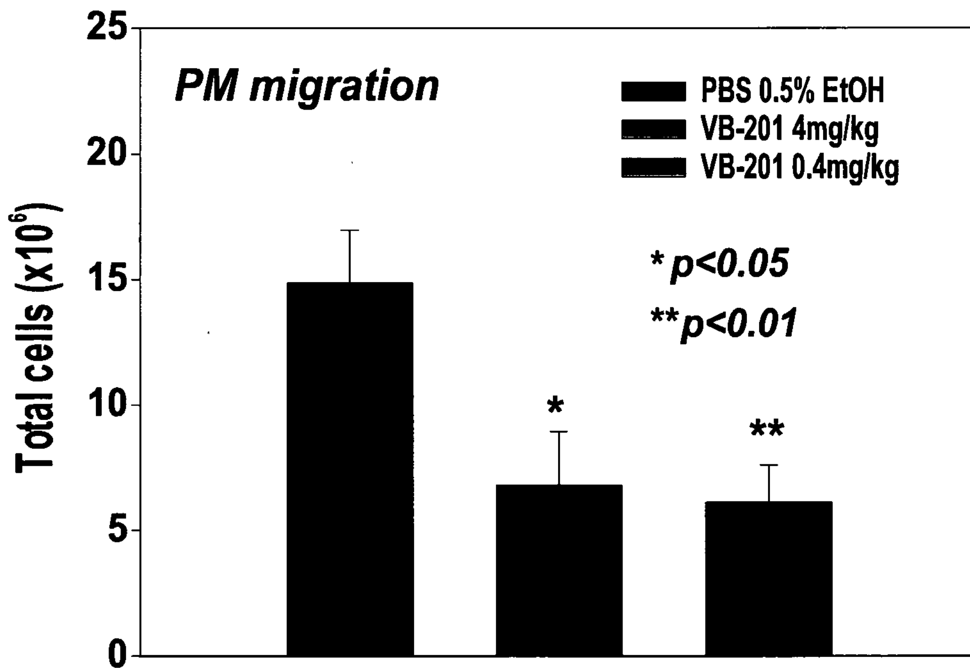


FIG. 34

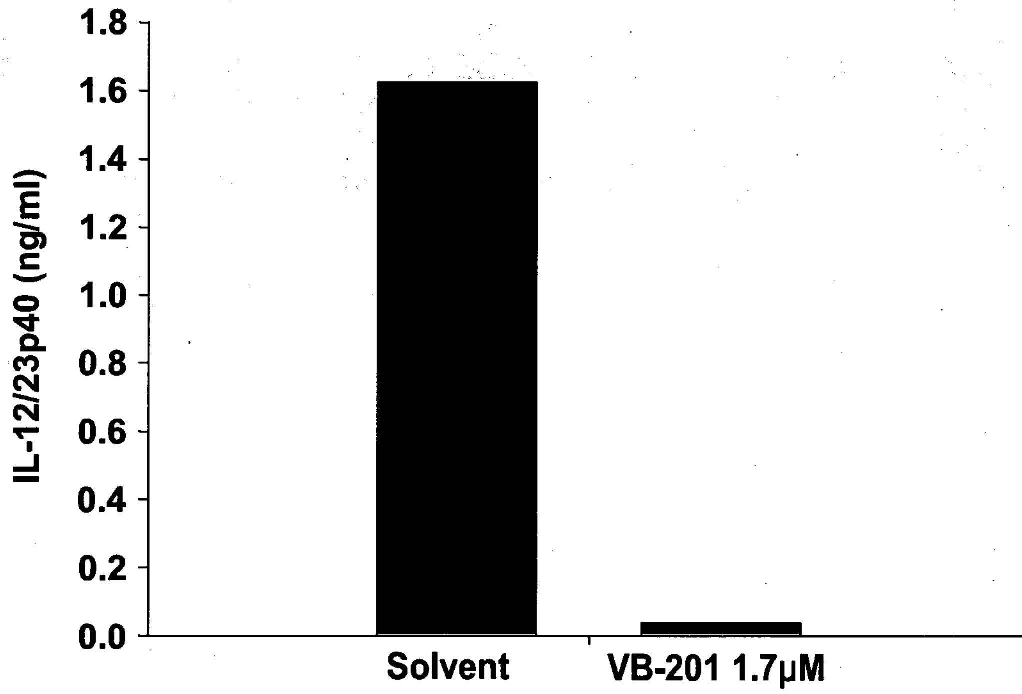


FIG. 35

FIG. 36A

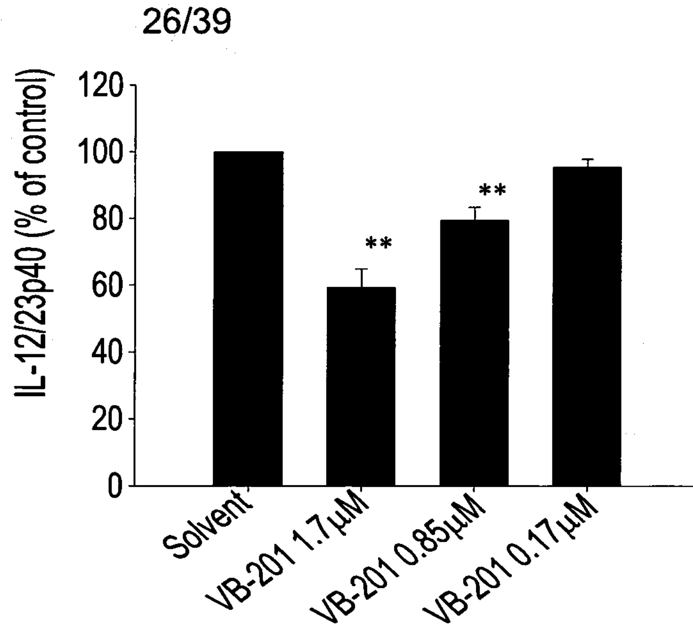


FIG. 36B

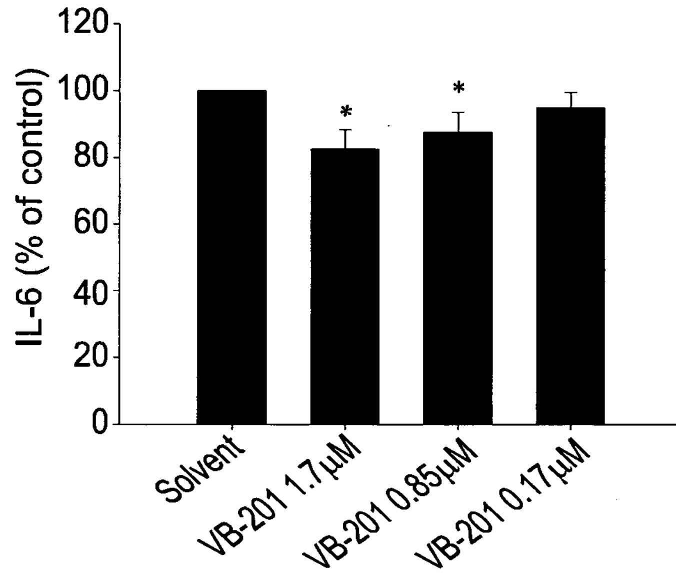


FIG. 36C

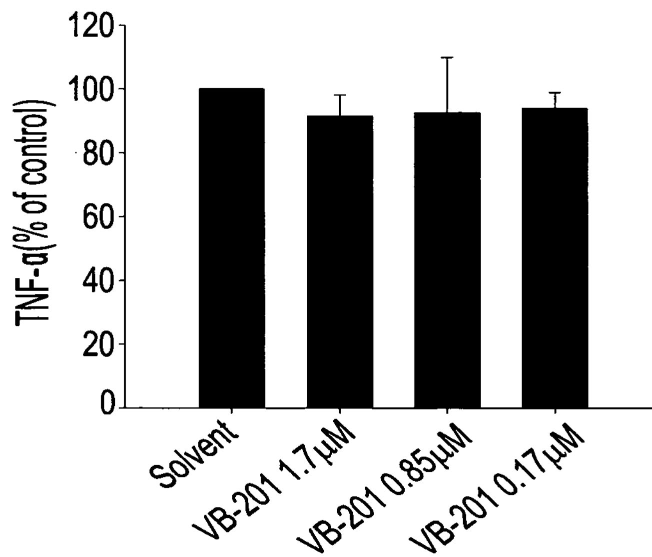


FIG. 37A

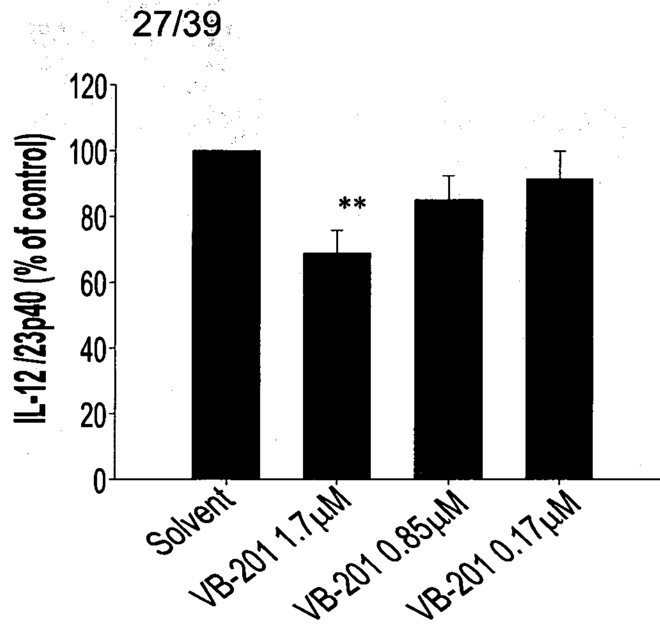


FIG. 37B

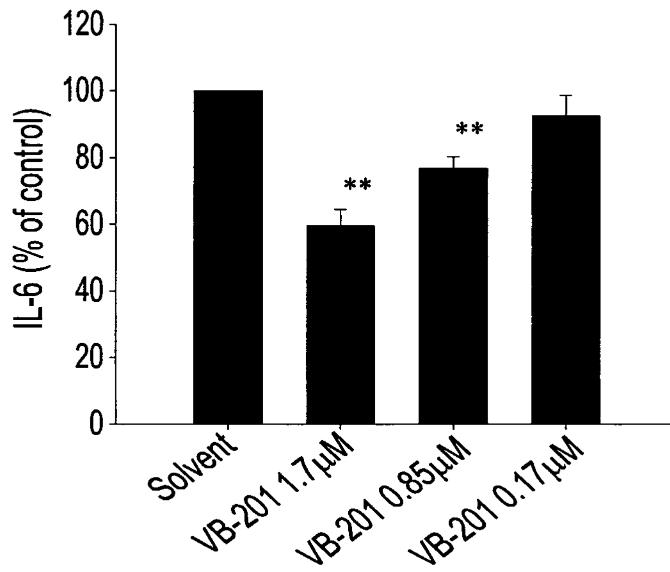


FIG. 37C

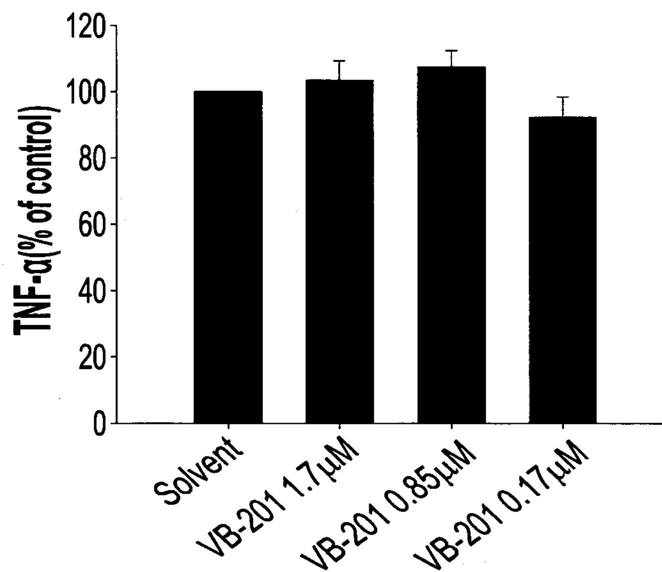


FIG. 38A

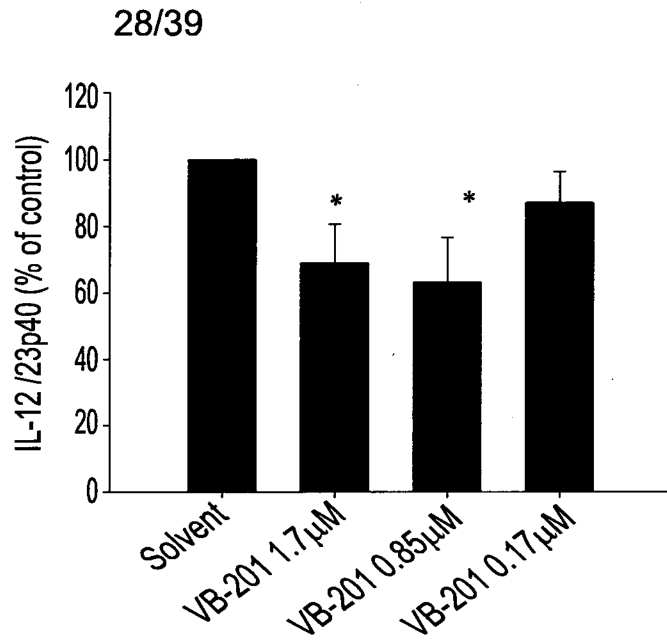


FIG. 38B

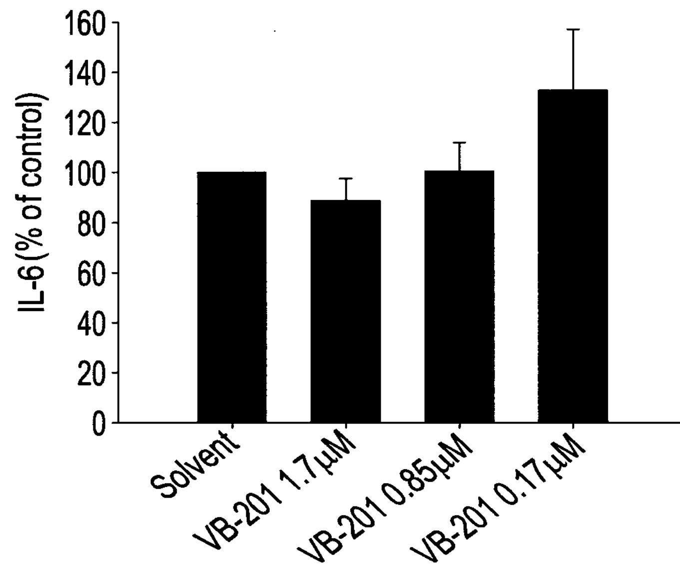
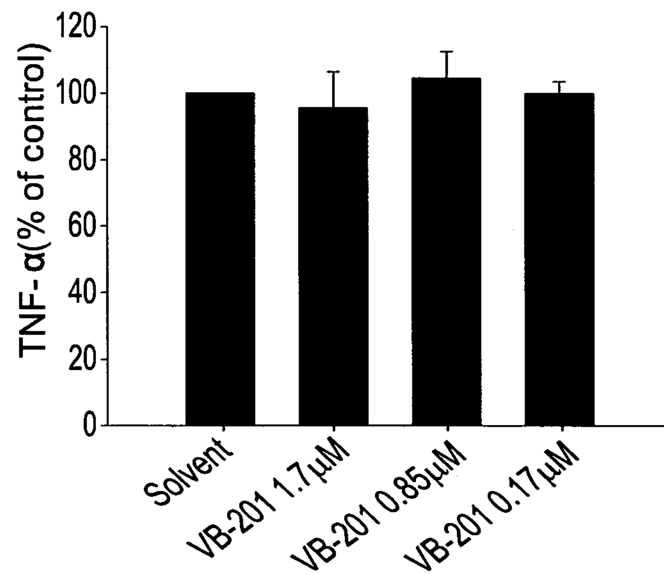


FIG. 38C



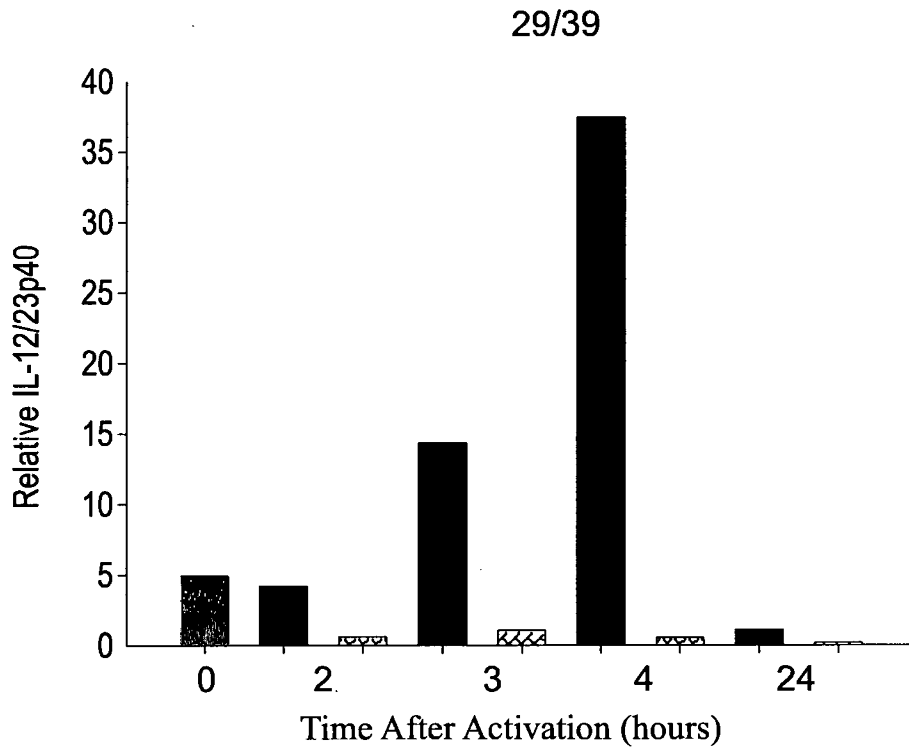


FIG. 39A

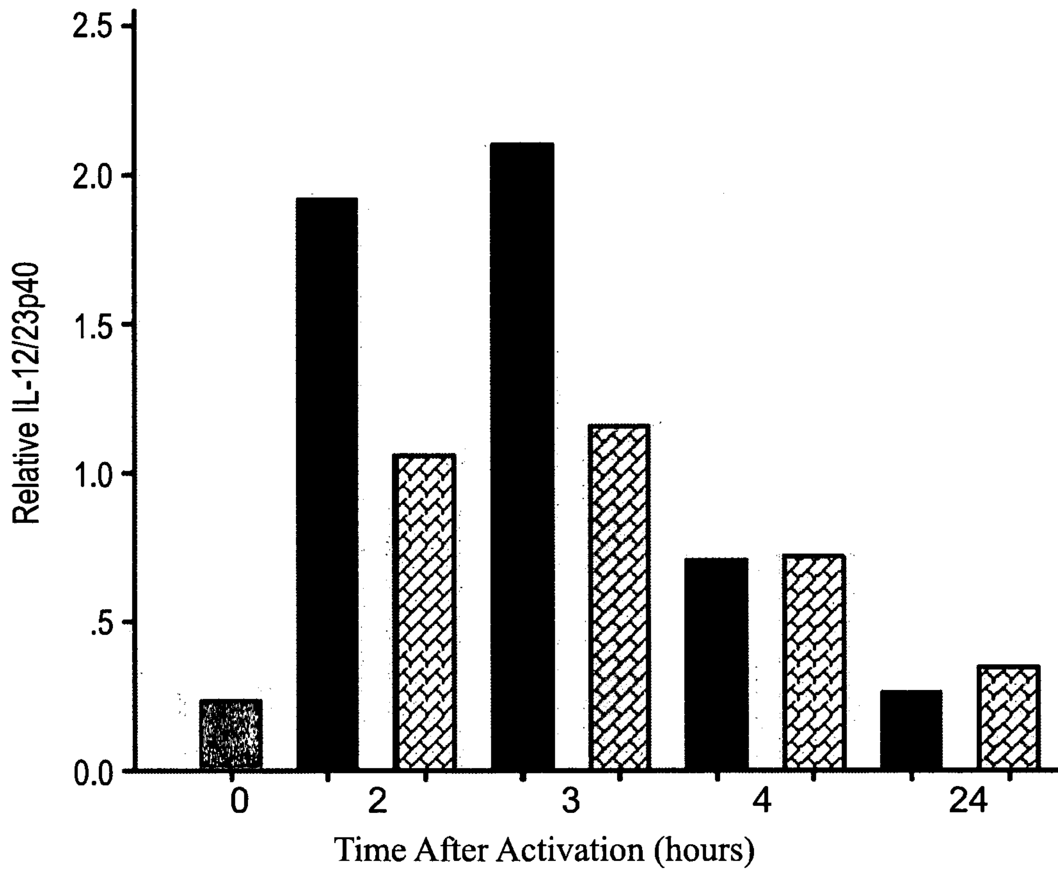


FIG. 39B

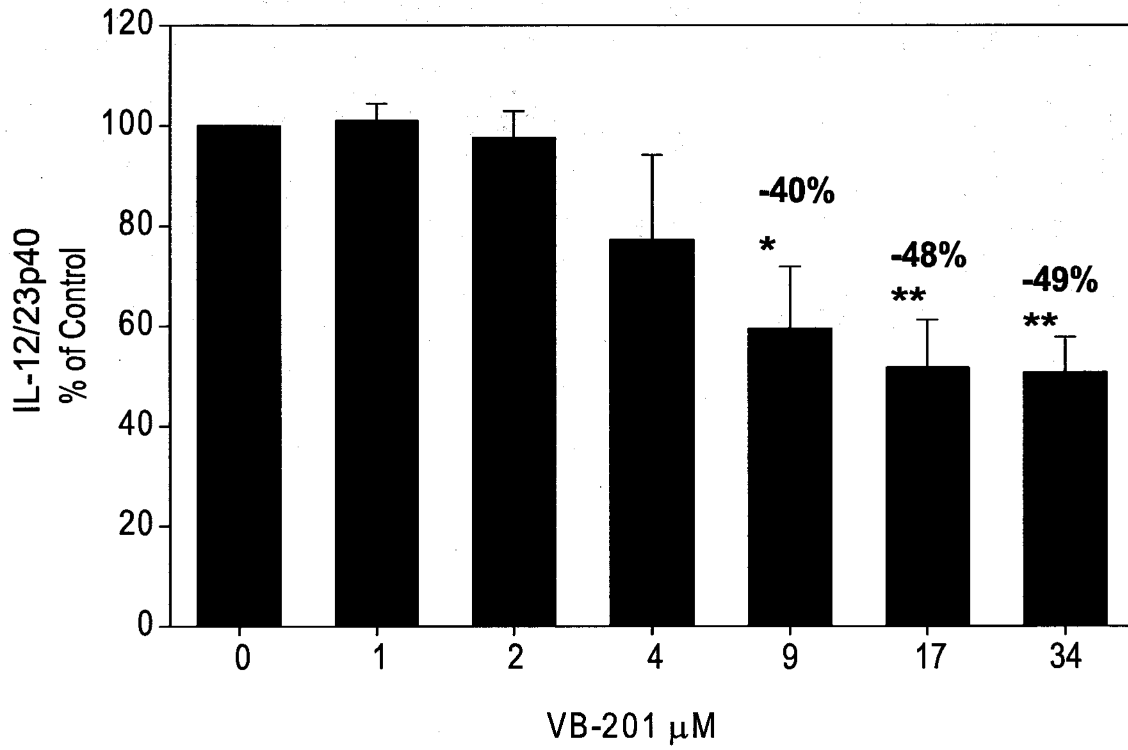


FIG. 40

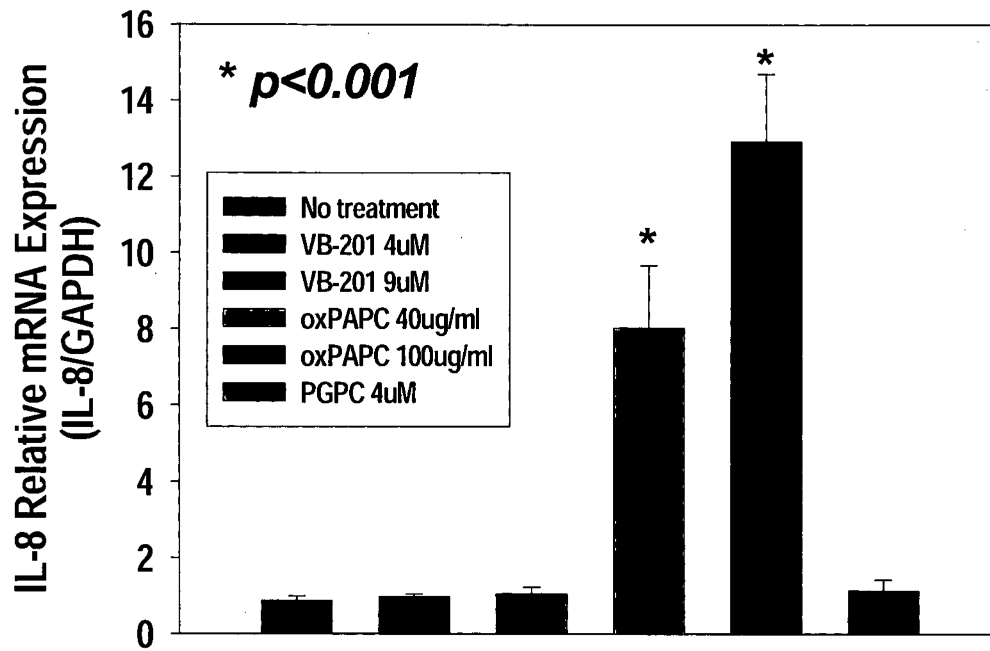


FIG. 41

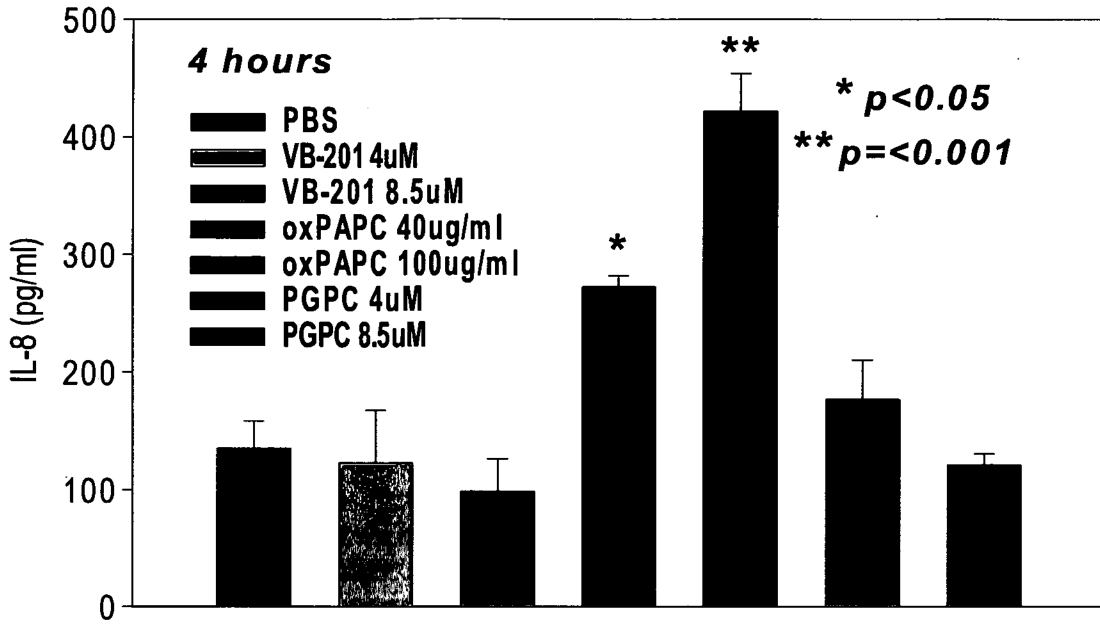


FIG. 42A

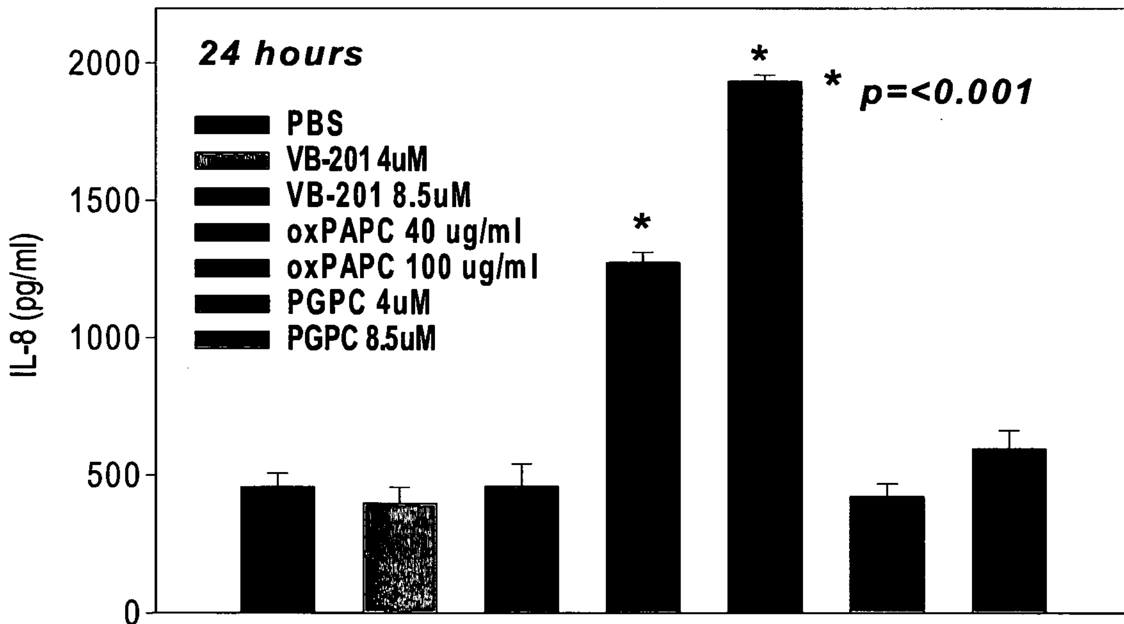


FIG. 42B

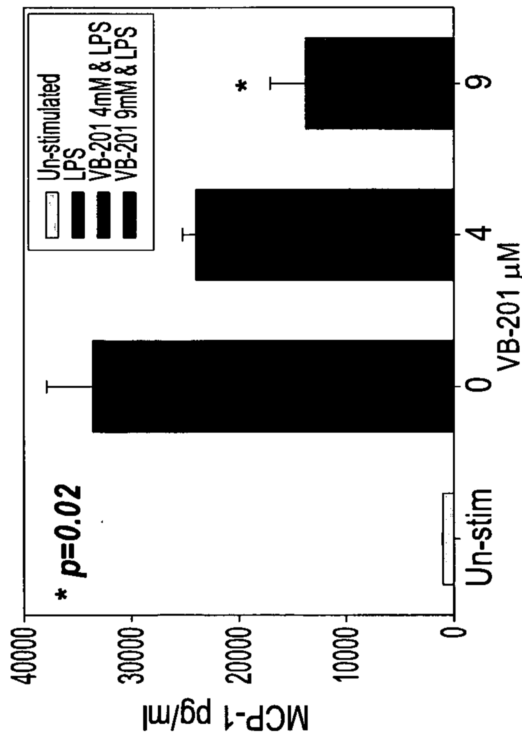


FIG. 43A

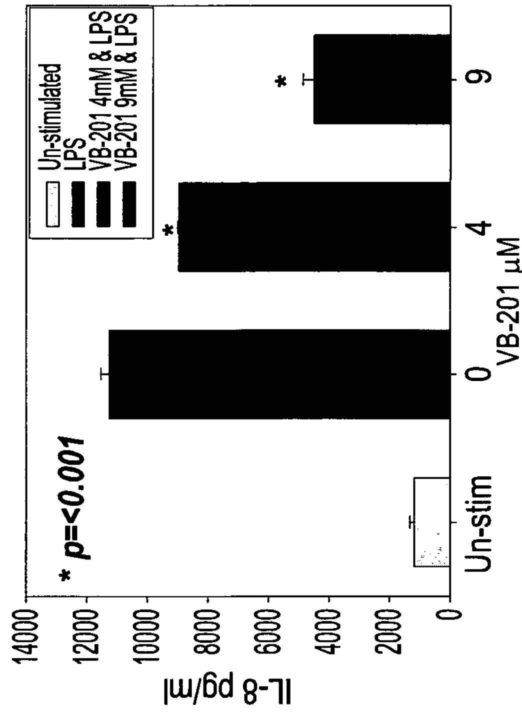


FIG. 43B

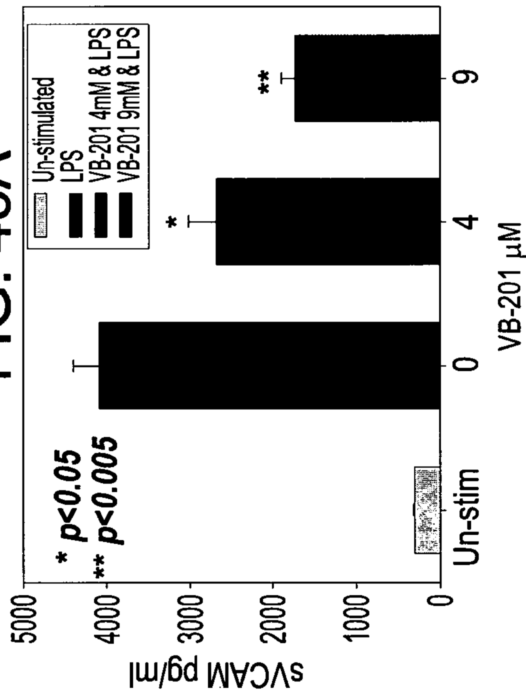


FIG. 43C

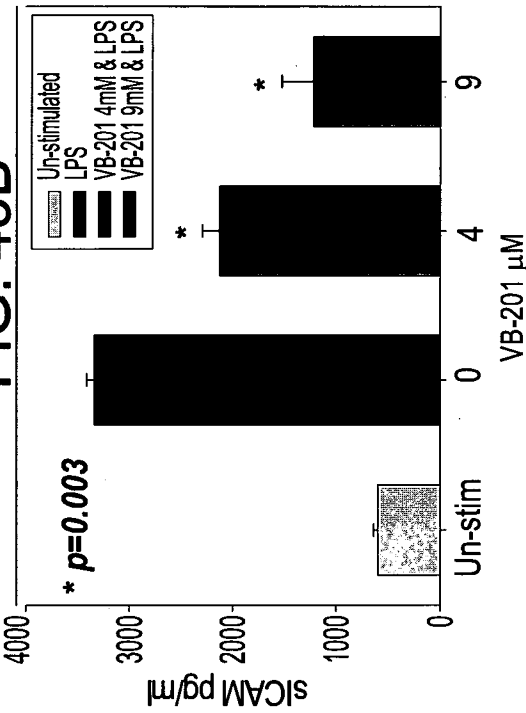


FIG. 43D

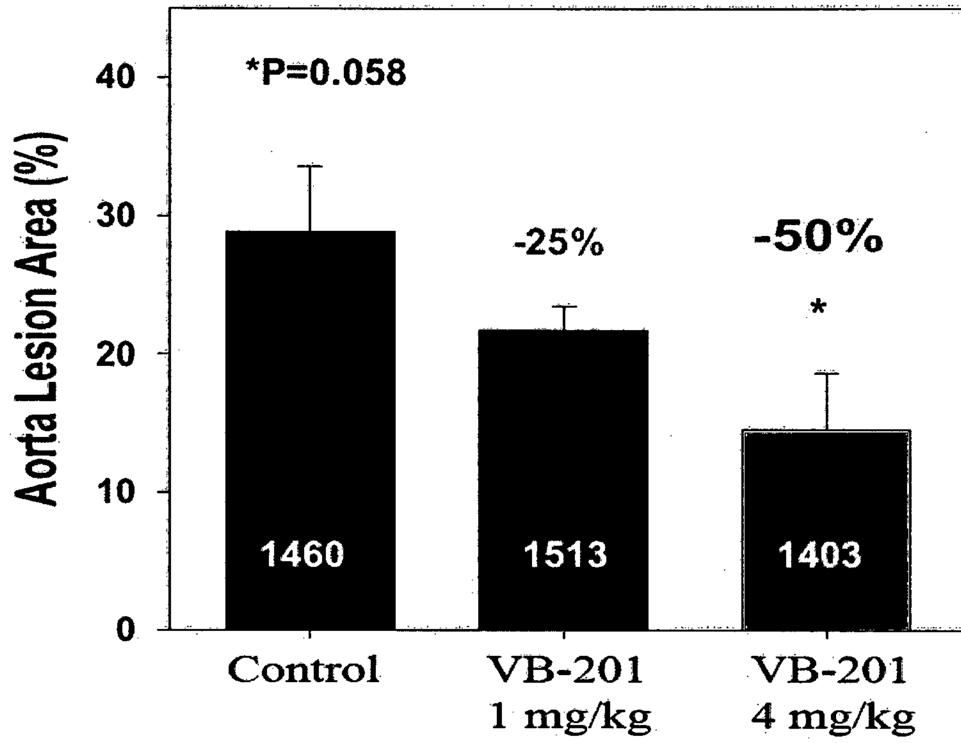


FIG. 44

FIG. 45

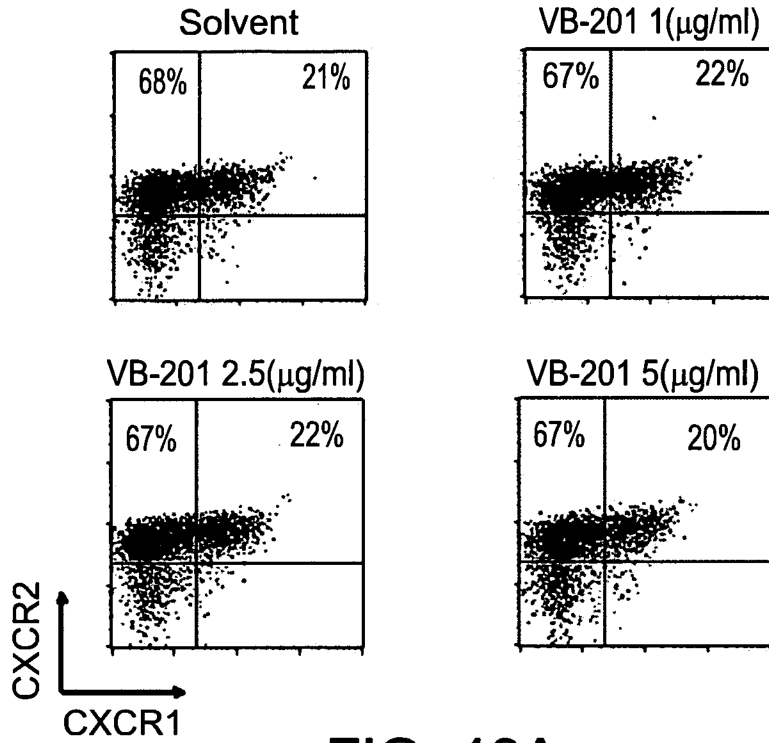


FIG. 46A

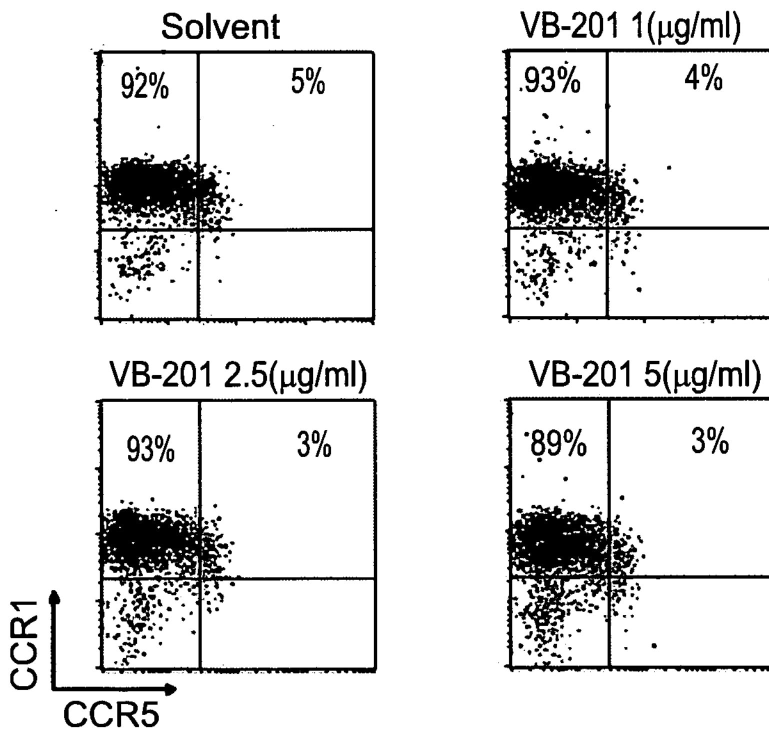


FIG. 46B

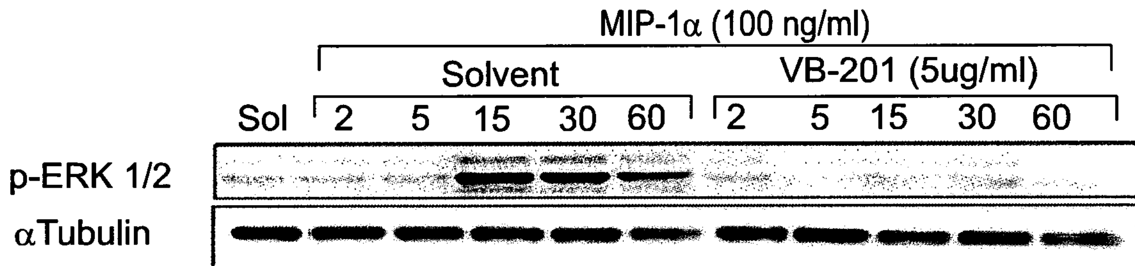


FIG. 47

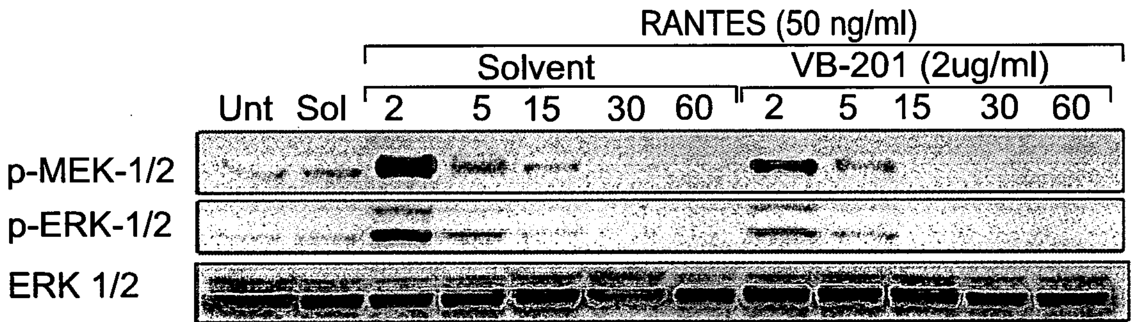


FIG. 48

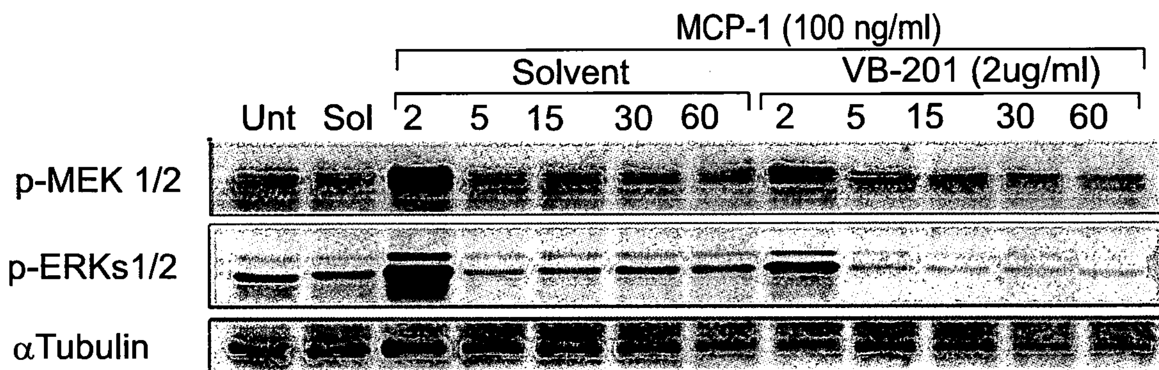


FIG. 49

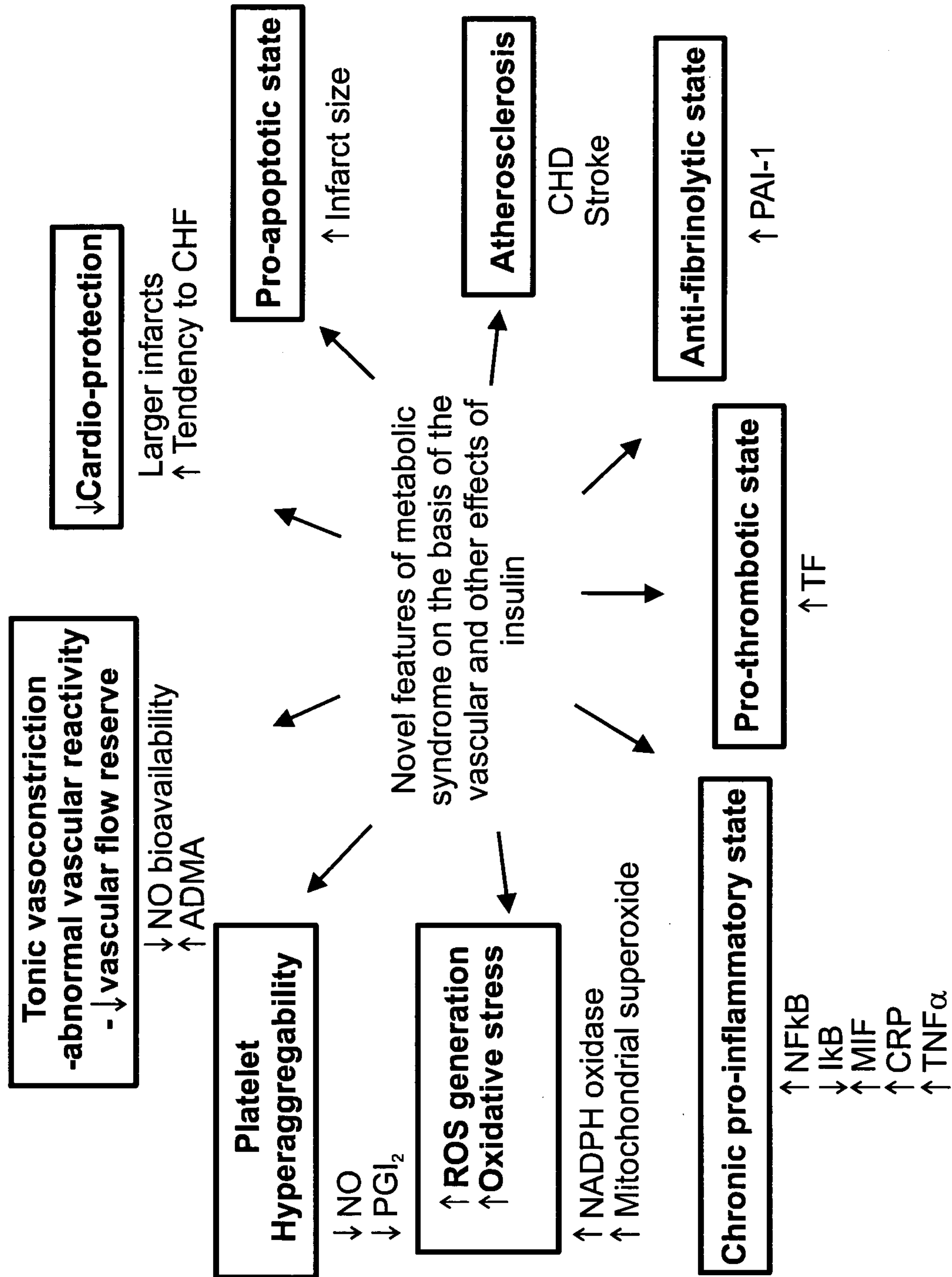


FIG. 50

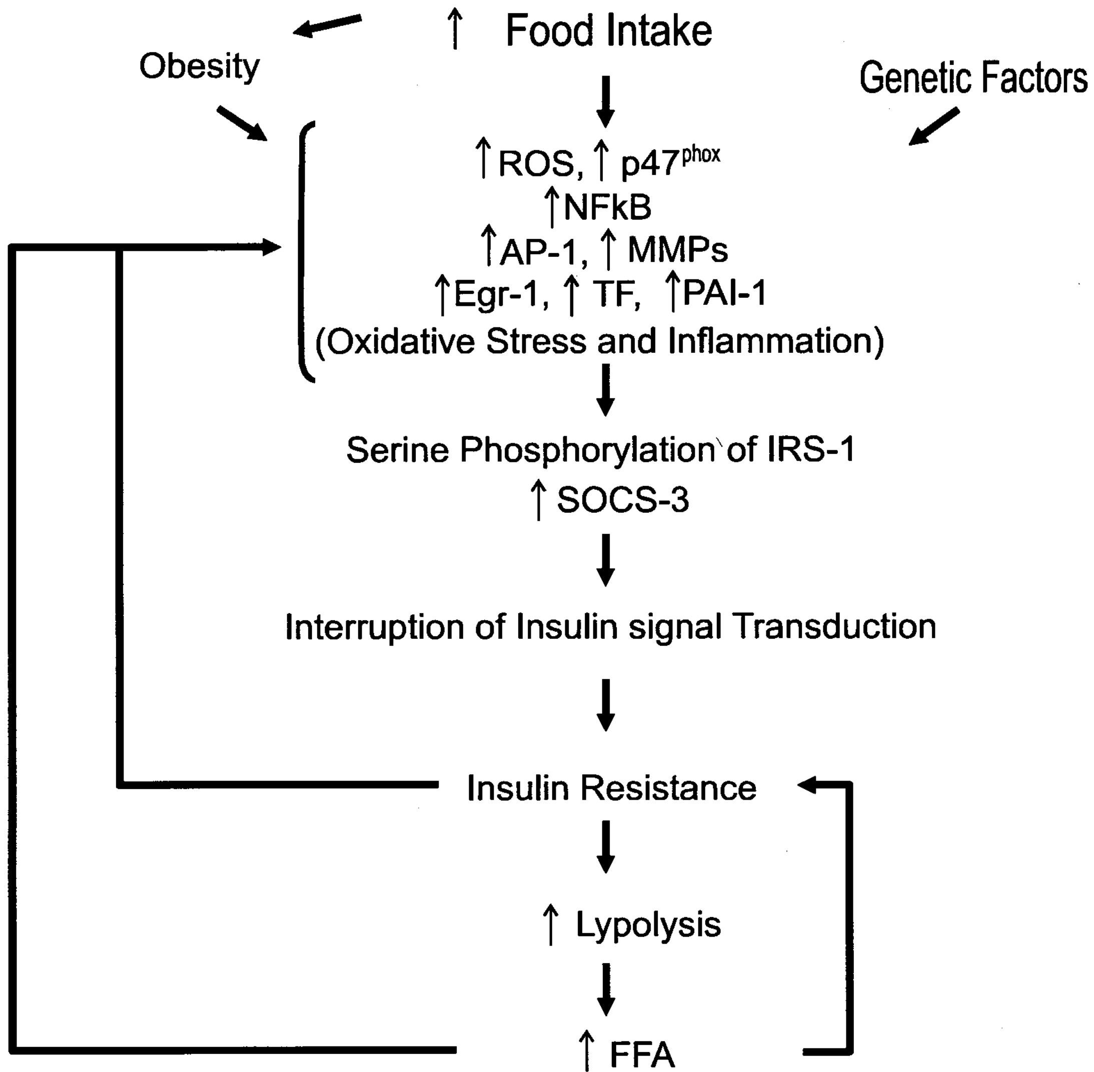


FIG. 51

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL 11/00012

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 45/00 (2011.01)
USPC - 424/85.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC - 424/85.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 514/16.4; 530/350 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (PGPB, USPT, EPAB, JPAB); Google (Google Scholar, Google Patents)
Search Terms Used: VB-201, VB201, VB 201, CI-201, CI 201, CI201, 1-hexadecyl-2-(4'-carboxybutyl)-glycerol-3-phosphocholine, 1-hexadecyl-2-(4'-carboxybutyl)-sn-glycerol-3-phosphocholine, 1-Hexadecyl-2-(5'-Carboxy-butyl)-sn-glycero-3-phosphocholine

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2008/0261865 A1 (HARATS et al.) 23 October 2008 (23.10.2008) para [0080], [0084], [0224], [0226], [0238], [0240], [0252], [0254], [0305]; fig 14a; claims 140, 162-163, 197	1-3, 6-12, 18-23

☐ Further documents are listed in the continuation of Box C. ☐

* 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	Date of mailing of the international search report
08 April 2011 (08.04.2011)	18 APR 2011

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. 571-273-3201

Authorized officer:
Lee W. Young
PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL 11/00012

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.: 4-5 and 13-17
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.