EBOLA THERAPIES

[US2011027388](#) -- **Cobalt Hexammine as a Potential Therapeutic Against HIV and/or Ebola Virus**

[US2010021556](#) -- **Method for the production of an agent against an infectious disease**

[US2010016244](#) -- **D-GLUCOPYRANOSE 1-[3,5-BIS (1,1-DIMETHYLETHY)-4-HYDROXYBENZOATE] AND ITS DERIVATIVES, PREPARATION AND USE THEREOF**

[US2009203675](#) -- **Sulfonyl Semicarbazides, Semicarbazides and Ureas, Pharmaceutical Compositions Thereof, and Methods for Treating Hemorrhagic Fever Viruses, Including Infections Associated with Arena Viruses**

[WO2007100525](#) -- **VIRAL TREATMENT**

US2011027388
Cobalt Hexammine as a Potential Therapeutic Against HIV and/or Ebola Virus

BACKGROUND

[0002] In this specification where a document, act or item of knowledge is referred to or discussed, this reference or discussion is not an admission that the document, act or item of knowledge or any combination thereof was at the priority date, publicly available, known to the public, part of common general knowledge, or otherwise constitutes prior art under the applicable statutory provisions; or is known to be relevant to an attempt to solve any problem with which this specification is concerned.

[0003] Hexaamminecobalt(III) chloride, also called Cohex, is notable for its ability to "condense" dsDNA into toroidal-like superstructures under low salt conditions. The metal ion itself, Co(III), with its high positive charge density, is an ideal candidate for binding nucleotides with their high negative charge density. Although Co(III) is not stable by itself in aqueous solutions, it is stabilized by coordinating with donor atoms (usually N) that make strong contributions to the ligand field. These coordinating donors could either be monodentate ligands, e.g., NH_3, or polydentate chelators, such as cyclen, $C_8H_{20}N_4$. The Co(III)-chelator complexes (e.g., cobalt cyclen complexes) have been used for mechanistic studies of phosphodiester cleavage for both its efficient hydrolysis rates and kinetic inertness, whereby the kinetic inertness of Co(III) ions results in the continued binding of the complex to the hydrolyzed phosphate.

[0004] Due to the kinetic inertness of Co(III) ions, the Cohex complex sequesters the "inner-sphere" ammonia ligands from most exchange-reactions in solution; therefore, the usual interactions with solution molecules are by "outer-sphere" coordination via water bridges to the ammonia ligands and via the high charge-density of the Co(III) ion. These two characteristics play an important role in the strong attachment of Cohex to either DNA or RNA and in enabling Cohex to often substitute for hydrated $Mg^{2+}(aq)$ as a cofactor in nucleic acid biochemistry.

[0005] For example, Cohex complexation with 5S RNA-where Cohex was used in place of $Mg^{2+}(aq)$-was found to provide no significant shifts in the [lambda]max of the absorption bands of Cohex, indicating that Cohex interaction with RNA was through outer-sphere complexation (and, of course, opposing charge attraction). It has also been reported that the number of binding sites on RNA was similar for Cohex and $Mg^{2+}(aq)$ and that the number was greater than expected for simple charge neutralization of the RNA backbone. These observations demonstrate that Cohex has a great propensity to bind to nucleotides at sites similar to Mg^{2+}-binding sites and either inhibit or slow down the bio-functions of DNA and RNA.

[0006] While certain aspects of conventional technologies have been discussed to facilitate disclosure of the invention, Applicants in no way disclaim these technical aspects, and it is contemplated that the claimed invention may encompass one or more of the conventional technical aspects discussed herein.

BRIEF SUMMARY

[0007] Cohex can inhibit viral transcription/translation via interference with viral RNA. This interference can be either via general "blockade" of the nucleotide strands from transcription/translation or may be made more overt by attaching hybridizing oligonucleotide strands to the Cohex. It has been shown that Cohex does not hydrolyze nucleotides, but does show potent antiviral properties against the Sindbis virus and Adenovirus, which are positive single-stranded (ss) RNA, double-strand (ds) DNA, respectively, and furthermore can act as an antibiotic. See US Patent Application Publication Nos. 2008/0182835 and 2010/0004187, each of which is incorporated by reference in its entirety.

[0008] In one embodiment, a method for treating a viral infection comprises administering to a patient a hexaamminecobalt(III) compound (e.g., hexaamminecobalt(III) chloride) in an amount effective to reduce an extent of a viral infection.

[0009] In a further embodiment, a method for treating a viral infection comprises administering to a human patient a hexamminecobalt(III) compound in an amount effective to reduce an extent of an infection of the patient with Ebola virus or HIV.

[0010] In another embodiment, a kit for delivery of a hexamminecobalt(III) compound by injection comprises a hexamminecobalt(III) compound in a pharmaceutically acceptable carrier, and equipment for delivery thereof by injection, wherein the equipment comprises at least one of a container, injection tubing, or an injection needle.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] FIG. 1 is an illustration of hexacoordinated Co(III), hexamminecobalt(III) (chloride

counterions not shown), and magnesium(II) hexahydrate, $Mg(H_2O)_6^{2+}$, both form octahedral coordination geometry with their respective ligands.

[0012] FIG. 2 is a double-Y semi-log plot is shown of the decrease in RT activity (left), as a measure of viral activity, or uninfected cell viability (right) for HIV-1 NL4-3 isolate. "% VC" means "% Virus Control" and "% CC" means "% Cell Control."

[0013] FIG. 3 is a double-Y semi-log plot is shown of the decrease in RT activity (left), as a measure of viral activity, or uninfected cell viability (right) for HIV-1 Ba-L isolate. "% VC" means "% Virus Control" and "% CC" means "% Cell Control."

[0014] FIG. 4 plots levels of GFP expression in cells infected with Zaire Ebola GFP, normalized against infected cells with no therapeutic (+/-control). Left plot: Relative GFP levels for A549 cells as a function of Cohex concentration, from 2.5 [mu]M to 5 mM. Right plot: Relative GFP levels for HepG2 cells as a function of Cohex concentration

[0015] FIG. 5 plots of the levels of GFP expression in cells infected with Zaire Ebola GFP, normalized against infected cells with no therapeutic (+/-control). Left plot: Relative GFP levels for 293T cells as a function of Cohex concentration, from 2.5 [mu]M to 5 mM. Right plot: Relative GFP levels for VeroE6 cells as a function of Cohex concentration.

[0016] FIG. 6 shows semi-log plots of the % viable (live) cells as a function of Cohex concentration. Left plot: A549 cells. Right plot: HepG2 cells.

[0017] FIG. 7 shows linear plots of the same data as FIG. 6, showing the region of greatest cytotoxic effect. Left plot: A549 cells. Right plot: HepG2 cells.

[0018] FIG. 8 shows linear plots of the % viable (live) cells as a function of Cohex concentration. Left plot: VeroE6 cells. Right plot: 293T cells.

[0019] FIG. 9 shows results for flow cytometric assay using PI as a marker for dead cells show almost no change between 0 to ~1.2 mM Cohex.

[0020] FIG. 10 shows a curve fit of inhibition by Cohex. For purposes of fitting, the negative (-%) inhibitory % were turned into positive numbers; so 100%=100% inhibition. The IC50 for the fit was found to be 0.38 mM Cohex.
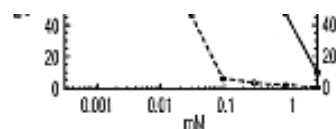

FIG. 1
PRIOR ART
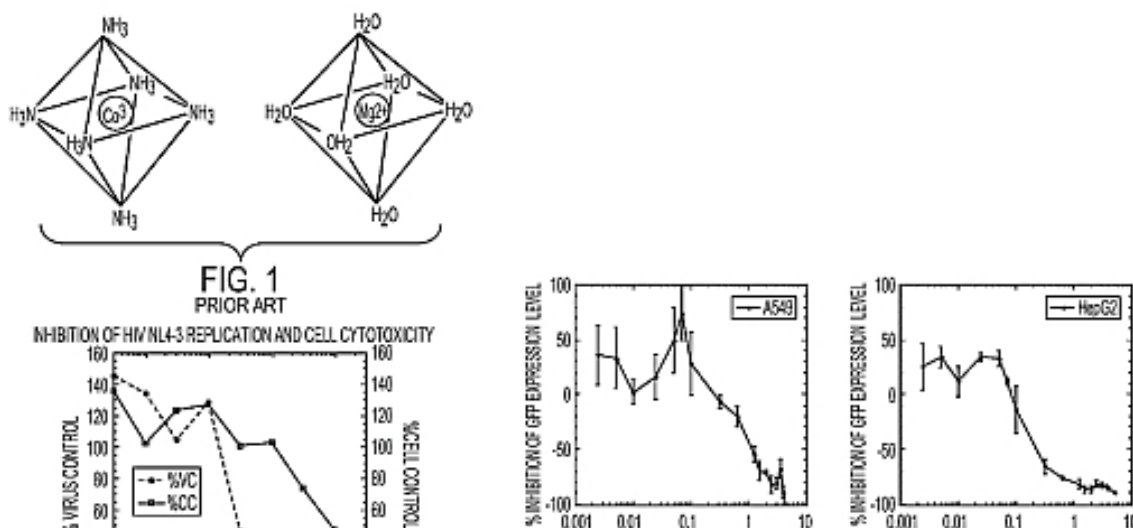
FIG. 4A
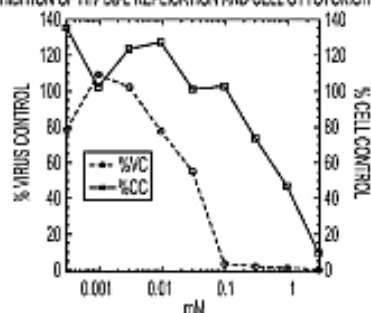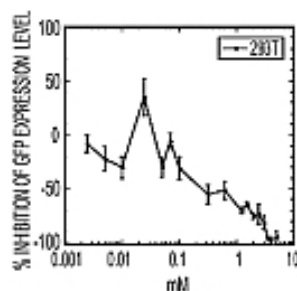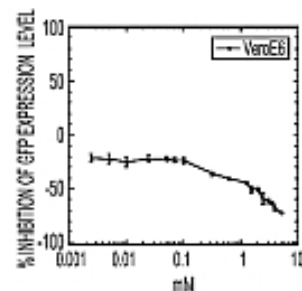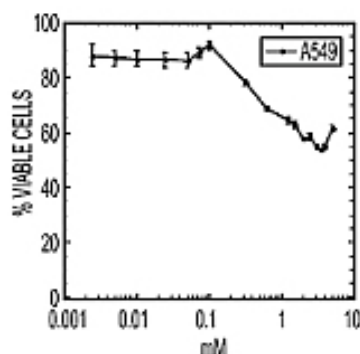
FIG. 4B

FIG. 2

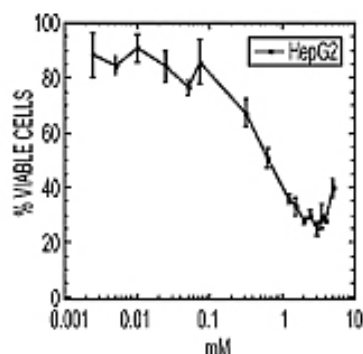INHIBITION OF HIV Ba-L REPLICATION AND CELL CYTOTOXICITY

FIG. 3

FIG. 5A

FIG. 5B

FIG. 6A

FIG. 6B

FIG. 7A

FIG. 7B

FIG. 8A

FIG. 8B

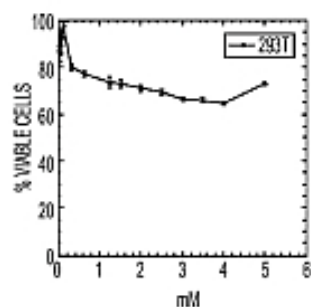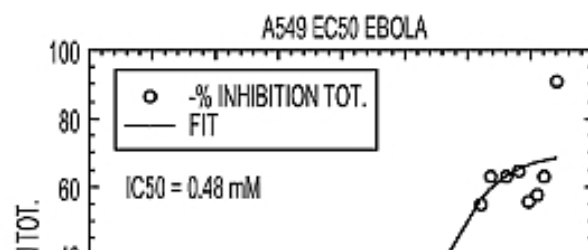A549 EC50 EBOLA

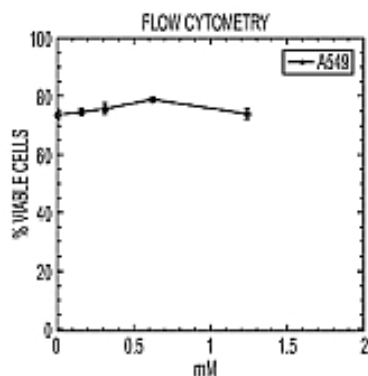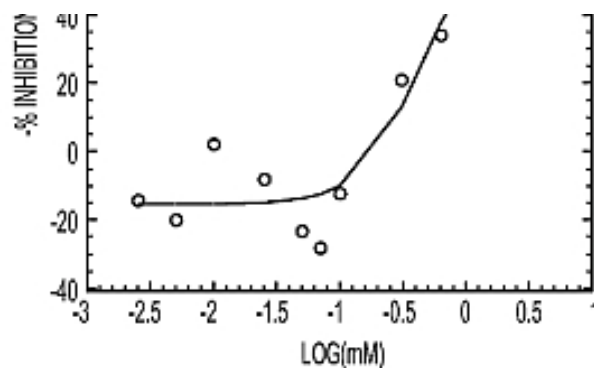○ -% INHIBITION TOT.
— FIT

IC50 = 0.48 mM
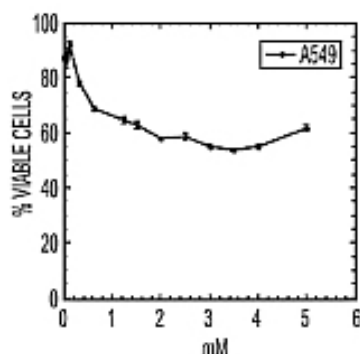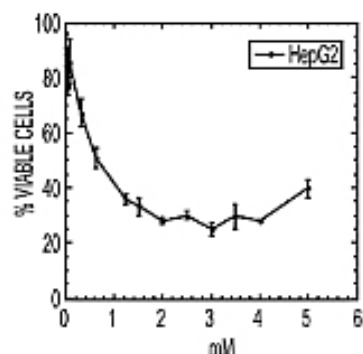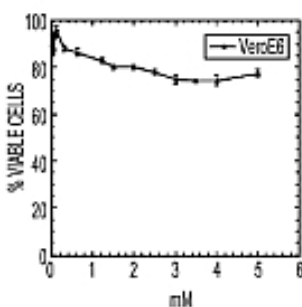
FIG. 9

FIG. 10

DETAILED DESCRIPTION

[0021] Hexaamminecobalt(III) (Cohex; FIG. 1), in particular the chloride salt thereof, is notable for its ability to "condense" dsDNA into toroidal-like superstructures under low salt conditions. The metal ion itself, Co(III), with its high (+)charge-density, is an ideal candidate for binding nucleotides with their high (-)charge density. Although Co(III) is not stable by itself in aqueous solutions, it is stabilized by coordinating with donor atoms (usually N) that make strong contributions to the ligand field. These coordinating donors could either be monodentate ligands, e.g., NH3, or polydentate chelators, such as cyclen, C8H20N4. The Co(III)-chelator complexes (e.g., cobalt cyclen complexes) have been used for mechanistic studies of phosphodiester cleavage for both its efficient hydrolysis rates and kinetic inertness, whereby the kinetic inertness of Co(III) ions results in the continued binding of the complex to the hydrolyzed phosphate.

[0022] Due to the kinetic inertness of Co(III) ions, the Cohex complex sequesters the "inner-sphere" ammonia ligands from most exchange-reactions in solution; therefore, the usual interactions with solution molecules are by "outer-sphere" coordination via water bridges to the ammonia ligands and via the high charge-density of the Co(III) ion. These two characteristics play an important role in the strong attachment of Cohex to either DNA or RNA[5] and in enabling Cohex to often substitute for hydrated Mg^{2+}(aq) as a cofactor in nucleic acid biochemistry. For example, Cohex complexation with 5S RNA-where Cohex was used in place of Mg^{2+}(aq)-was examined and found to provide no significant shifts in the [lambda]max of the absorption bands of Cohex, indicating that Cohex interaction with RNA was through outer-sphere complexation (and, of course, opposing charge attraction). It has also been reported that the number of binding sites on RNA was similar for Cohex and Mg^{2+}(aq) and that the number was greater than expected for simple charge neutralization of the RNA backbone. These observations demonstrate that Cohex has a great propensity to bind to nucleotides at sites similar to Mg^{2+}-binding sites and either inhibit or slow down the bio-functions of DNA and RNA.

[0023] Cohex may function as a new type of broad-spectrum antiviral compound. For example, Cohex can be effective in significantly enhancing cell viability and in depressing viral expression for Sindbis infected BHK cells, with similar significant effects of Cohex against adenovirus in A549. See US Patent Application Publication No. 2008/0182835. These observations point to the potential broad-spectrum nature of Cohex against viruses.

[0024] As disclosed herein, Cohex demonstrates antiviral properties against two additional viruses. Ebola virus is a negative-strand, filamentous, enveloped microorganism that belongs

to the filoviridae family of viruses. Cohex can decrease the viral expression levels in a dose-dependent manner, in a variety of cells infected with the Ebola virus. Cohex also demonstrates antiviral properties against human immunodeficiency virus (HIV). HIV is a member of the genus lentivirus and belongs to the Retroviridae family. It has a single-strand (-)RNA genome, which is transcribed into a complementary DNA (cDNA) inside the host cell by an RNA-dependent DNA polymerase. The sense cDNA serves as a template for DNA-dependent DNA polymerase to make an antisense DNA copy, which forms a double-stranded viral DNA (dsDNA). The dsDNA is then transported into the cell nucleus where it gets integrated into the host cell's genome. Virus replication is initiated when the integrated DNA provirus is transcribed into mRNA.

DEFINITIONS

[0025] As used herein, the term "reduce an extent of the viral infection" with regard to a patient means that the ability of viruses to multiply within a patient is at least partially reduced.

[0026] As used herein, a "patient" can be a human or other mammal.

Antiviral Uses of Cohex

[0027] It is contemplated that Cohex could be used to treat a viral infection in a patient. In one embodiment, an effective amount of Cohex is administered to a patient suspected or known to have a viral infection. Optionally, a method of treatment includes identifying a patient who is or may be in need of such treatment. The patient can be a human or other mammal, including without limitation a primate, dog, cat, cow, pig, or horse.

[0028] In an embodiment, Cohex is administered to a patient known or suspected of being infected by a virus. In a further embodiment, Cohex is administered prior to exposure of the patient to a virus. In another embodiment, Cohex is administered subsequent to exposure of the patent to a virus.

[0029] The Cohex may be administered by any of various means including orally or nasally, or by suppository, or by injection including intravenous, intramuscular, or intraperitoneal injection, or combinations of any of these.

[0030] In an embodiment, equipment for injection of Cohex in a pharmaceutically acceptable comprises at least one of a container for the compound (such as a tube, bottle, or bag), injection tubing, or an injection needle.

[0031] The quantity of Cohex effective to treat an infection can be ascertained by one of ordinary skill in the art. Exemplary amounts of Cohex include 0.5, 1, 2, 4, 8, 10, 12, 14, 16, 18, or 20 mg/kg, or more.

[0032] Viral infections that can be treated include, but are not limited to, those associated with human immunodeficiency virus (HIV), human T cell leukemia virus (HTLV), Papillomavirus (e.g., human papilloma virus), Polyomavirus (e.g., SV40, BK virus, DAR virus), orthopoxvirus (e.g., variola major virus (smallpox virus)), EBV, herpes simplex virus (HSV), hepatitis virus, Rhabdovirus (e.g., Ebola virus), alphavirus (e.g., Sindbis virus), adenovirus, and/or cytomegalovirus (CMV). In preferred embodiments, the viral infection is

by HIV or Ebola virus.

Preparation of Co(III) Hexammine

[0033] While Cohex is available commercially, its synthesis is fairly straight forward, using air to oxidize Co(II) to Co(III):

[0000]
$$CoCl_2+4NH_4Cl+20NH_3+O_2->4[Co(NH_3)_6]Cl_3+2H_2O$$
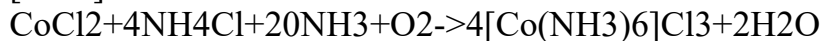
[0034] 9.6 g of CoCl2.6H2O (0.06 mol) and 6.4 g of NH4Cl (0.12 mol) were added to 40 ml of water in a 250 ml Erlenmeyer flask with a side arm and shaken until most of the salts are dissolved. Then 1 g of fresh activated decolorizing charcoal and 20 ml concentrated ammonia were added. Next the flask was connected to the aspirator or vacuum line and air drawn through the mixture until the red solution becomes yellowish brown (usually 2-3 hours). The air inlet tube if preferably of fairly large bore (~10 mm) to prevent clogging with the precipitated Co(NH3)6<3+> salt.

[0035] The crystals and charcoal were filtered on a Buchner funnel and then a solution of 6 ml of concentrated HCl in 75 ml of water was added. The mixture was heated on a hot plate to effect complete solution and filtered while hot. The hexamminecobalt (III) chloride was crystallized by cooling to 0[deg.] C. and by slowly adding 15 ml of concentrated HCl. The crystals were filtered, washed with 60% and then with 95% ethanol, and dried at 80-100[deg.] C.

Cohex Activity Against HIV

[0036] There are two known strains of HIV: HIV-1 and HIV-2, of which HIV-1 is the more virulent virus and is the major cause of HIV infections. The first clinically useful drugs developed for HIV-1 were the nucleoside reverse transcriptase (RT) inhibitors. AZT, or 3-azido-3-deoxythymidine, is a synthetic pyrimidine analog of thymidine was actually initially developed as an anticancer drug before it became known as a popular anti-HIV compound. The active form of AZT is its phosphorylated triphosphate (TP) form, which is a competitive inhibitor of RT because AZT-TP binds to the HIV-1 RT better than to the natural substrate deoxythymidine triphosphate (dTTP).

[0037] Cohex was tested in a standard PBMC cell-based microtiter anti-HIV assay against one CXCR4-tropic HIV-1 isolate and one CCR5-tropic HIV-1 isolate. For this study peripheral blood mononuclear cells (PBMCs) were pre-treated with the compound for two hours prior to infection.

[0038] Cohex was stored at 4[deg.] C. as a powder and solubilized for tests. The solubilized stock was stored at -20[deg.] C. until the day of the assay. Stocks were thawed at room temperature on each day of assay setup and were used to generate working drug dilutions used in the assays. Working dilutions were made fresh for each experiment and were not stored for re-use in subsequent experiments performed on different days. Cohex was evaluated using a 3 mM (3,000 [mu]M) high-test concentration with 8 additional serial half-log dilutions in the PBMC assays.

PBMC Assay

[0039] Freshly prepared PBMCs were centrifuged and suspended in RPMI 1640 with 15% FBS, L-glutamine, penicillin, streptomycin, non-essential amino acids (MEM/NEAA; Hyclone; catalog #SH30238.01), and 20 U/ml recombinant human IL-2. PBMCs were maintained in this medium at a concentration of $1-2*10^6$ cells/ml, with twice-weekly medium changes until they were used in the assay protocol. Monocyte-derived-macrophages were depleted from the culture as the result of adherence to the tissue culture flask.

[0040] For the standard PBMC assay, the cells were plated in the interior wells of a 96 well round bottom microplate at 50 [mu]L/well ($5*10^4$ cells/well) in a standard format developed by the Infectious Disease Research department of Southern Research Institute. Each plate contains virus control wells (cells plus virus) and experimental wells (drug plus cells plus virus). Test drug dilutions were prepared at a 2* concentration in microtiter tubes and 100 [mu]L of each concentration was placed in appropriate wells using the standard format. 50 [mu]L of a predetermined dilution of virus stock was placed in each test well (final MOI ~0.1). Separate plates were prepared identically without virus for drug cytotoxicity studies using an MTS assay system (described below; cytotoxicity plates also include compound control wells containing drug plus media without cells to control for colored compounds that affect the MTS assay). The PBMC cultures were maintained for seven days following infection at 37[deg.] C., 5% CO2. After this period, cell-free supernatant samples were collected for analysis of reverse transcriptase activity and compound cytotoxicity was measured by addition of MTS to the separate cytotoxicity plates for determination of cell viability. Wells were also examined microscopically and any abnormalities were noted.

Reverse Transcriptase Activity Assay

[0041] A microtiter plate-based reverse transcriptase (RT) reaction was utilized (detailed in Buckheit et al., AIDS Research and Human Retroviruses 7:295-302, 1991). Tritiated thymidine triphosphate (3H-TTP, 80 Ci/mmol, NEN) was received in 1:1 dH2O:Ethanol at 1 mCi/ml. Poly rA:oligo dT template:primer (Pharmacia) was prepared as a stock solution by combining 150 poly rA (20 mg/ml) with 0.5 ml oligo dT (20 units/ml) and 5.35 ml sterile dH2O followed by aliquoting (1.0 ml) and storage at -20[deg.] C. The RT reaction buffer was prepared fresh on a daily basis and consisted of 125 [mu]l 1.0 M EGTA, 125 [mu]l dH2O, 125 [mu]l 20% Triton X100, 50 [mu]l 1.0 M Tris (pH 7.4), 50 [mu]l 1.0 M DTT, and 40 [mu]l 1.0 M MgCl2. The final reaction mixture was prepared by combining 1 part 3H-TTP, 4 parts dH2O, 2.5 parts poly rA:oligo dT stock and 2.5 parts reaction buffer. Ten microliters of this reaction mixture was placed in a round bottom microtiter plate and 15 [mu]l of virus-containing supernatant was added and mixed. The plate was incubated at 37[deg.] C. for 60 minutes. Following incubation, the reaction volume was spotted onto DE81 filter-mats (Wallac), washed 5 times for 5 minutes each in a 5% sodium phosphate buffer or 2*SSC (Life Technologies), 2 times for 1 minute each in distilled water, 2 times for 1 minute each in 70% ethanol, and then dried. Incorporated radioactivity (counts per minute, CPM) was quantified using standard liquid scintillation techniques.

MTS Staining for PBMC Viability to Measure Cytotoxicity

[0042] At assay termination, the uninfected assay plates were stained with the soluble tetrazolium-based dye MTS (CellTiter 96 Reagent, Promega) to determine cell viability and quantify compound toxicity. MTS is metabolized by the mitochondria enzymes of metabolically active cells to yield a soluble formazan product, allowing the rapid quantitative

analysis of cell viability and compound cytotoxicity. This reagent is a stable, single solution that does not require preparation before use. At termination of the assay, 20-25 [mu]L of MTS reagent is added per well and the microtiter plates are then incubated for 4-6 hrs at 37[deg.] C., 5% CO2 to assess cell viability. Adhesive plate sealers were used in place of the lids, the sealed plate was inverted several times to mix the soluble formazan product and the plate was read spectrophotometrically at 490/650 nm with a Molecular Devices SPECTRAmax plate reader.

Assay Results

[0043] The PBMC data were normalized by dividing by either the average control, infected, untreated value for the infection measurements (% Viral Control) or by the control, uninfected, untreated value for the cytotoxicity measurements (% Cell Control). The normalized values were then analyzed for IC50 (50% inhibition of virus replication), CC50 (50% cytotoxicity), and therapeutic index values (TI=CC/IC; also referred to as Antiviral Index or AI).

[0044] Cohex was tested for antiviral efficacy against one CXCR4-tropic HIV-1 isolate and one CCR5-tropic HIV-1 isolate in PBMCs. For this study PBMCs were pre-treated with the compound for two hours prior to infection. FIG. 2 illustrates the decrease in RT activity (left), as a measure of viral activity, or uninfected cell viability (right) for HIV-1 NL4-3 isolate. FIG. 3 illustrates of the decrease in RT activity (left), as a measure of viral activity, or uninfected cell viability (right) for HIV-1 Ba-L isolate. In these Figures, "% VC" means "% Virus Control" and "% CC" means "% Cell Control." The results of the testing are summarized in Table 1.

[0045] Cohex displayed definite antiviral activity against the virus isolates evaluated in this study, with an average IC50 value of 31.2 [mu]M. There did not appear to be any difference in the activity of the compound based on co-receptor tropism, as the compound had approximately equal activity against both virus isolates tested. Cytotoxicity was observed with the compound at concentrations above 100 [mu]M (TC50=833 [mu]M), resulting in an average Therapeutic Index value of 26.7. These results can be summarized with IC50, CC50, and TI values given in Table 1.

[0000]

TABLE 1
Summary of Cohex Activity Against HIV-1 in PBMCs

Compound	HIV-1 Isolate	IC50	CC50	Therapeutic Index
Cohex	Ba-L	33.8 [mu]M	833 [mu]M	24.7
	NL4-3	28.6 [mu]M		29.1

[0046] The results show that Cohex displays very similar activity against HIV as against other types of viruses, attesting to the very broad-spectrum nature of the compound. The antiviral activity is not as high as specific antiviral drugs, like AZT, but there are situations where the use of Cohex can be an advantage.

Cohex Activity Against Ebola Virus

[0047] Ebola was first discovered simultaneously in 1976 in Sudan and in the Democratic

Republic of the Congo (formerly Zaire). While its origins are still not firmly established, Ebola likely came from the rain forests of Africa. The primary reservoir is likely not nonhuman primates, but rather that the virus is zoonotic, transmitted to humans from ongoing life cycles in animals or arthropods.

[0048] Ebola viruses belong to the filoviridae family and has five known strains (subtypes): Bundibugyo, Côte d'Ivoire, Sudan, Zaïre, and Reston. The Bundibugyo, Sudan, and Zaïre strains have caused outbreaks of Ebola hemorrhagic fever among humans in Africa, killing up to 90% of those infected. Of the Ebola viruses, the Zaire strain is the most virulent and the Reston strain is the least virulent.

[0049] The Ebola virus is transmitted via contact with bodily fluids of infected persons and can take from two days to three weeks for symptoms to appear. Disease symptoms start with fever, muscle aches and a cough before progressing to severe vomiting, diarrhea and rashes, along with kidney and liver problems. Death generally occurs as the result of either one or a combination of dehydration and/or massive bleeding from leaky blood vessels, kidney, and liver failure. The World Health Organization has documented 1,850 cases of Ebola (mostly in sub-Saharan Africa) since its discovery; only 600 (32 percent) of the victims survived. (32 percent) of the victims survived.

[0050] As with all viruses of the order Mononegavirales, filoviruses, such as Ebola, contain a single-stranded, negative-sense RNA molecule as their genome. The genomes of filoviruses are quite large at approximately 19,000 bases in length and contain seven sequentially arranged genes. Filovirus proteins can be subdivided into two categories, those that form the ribonucleoprotein (RNP) complex and those that are associated with the envelope. The proteins associated with the nucleocapsid are involved in the transcription and replication of the genome, whereas the envelope-associated proteins primarily have a role either in assembly of the virion or in receptor binding and virus entry.

[0051] There is no known cure for Ebola disease. Existing antiviral drugs do not work well against this virus and the best doctors can do is attempt to maintain the patient's body fluids and electrolytes levels under intensive care; while bleeding problems may require transfusions of platelets and/or fresh blood.

Activity of Cohex Against Ebola Virus in Cell Culture

[0052] For EC50 assays, cells were plated onto 96-well plates and incubated at 37[deg.] C. for 24 hours before adding compound followed by cell infection with Zaire Ebola GFP virus, a virus strain that contains a GFP gene. The infected cells were allowed to grow for an additional 48 hours before reading on a Molecular Devices spectrofluorometer (X=485 nm, M=515 nm). Controls were done for +virus/-compound and -virus/-compound. The -virus/+compound controls were part of the CC50 tests. Dosage of Cohex ranged from 2.5 [mu]M to 5 mM and were done in triplicates. Error bars for the figures are for standard error (SE) of the mean.

[0053] The results for A549 cells and HepG2 cells are shown in the left and right panels of FIG. 4, respectively. It is seen that there appears to be a general flat response from 2.5 [mu]M until around 0.1 mM Cohex, at which point, GFP expression drops until there is nearly 100% suppression (-100%) of viral expression at concentrations above 1 mM Cohex.

[0054] The results for 293T and VeroE6 cells are shown in the left and right panels of FIG. 5, respectively. For 293T cells, there is a monotonic decrease in GFP expression with increasing Cohex, even starting as low as 2.5 [mu]M Cohex. For VeroE6 cells, there is also a decrease in GFP expression with increasing Cohex, but the slope of the decrease is much less pronounced than for the other cells. There is another difference in the cells of FIG. 4 from FIG. 5. The values for concentrations below 0.1 mM in FIG. 1 fluctuate between 0 and +50 enhancement of GFP with large error bars, whereas the values in FIG. 2, for the same region of concentration, all show (except for 1 point) negative GFP enhancement (i.e., in the suppression of expression region). Thus, the behavior of Cohex for the different cell types exhibit differential amounts of viral expression decrease, but they all show decreasing levels of GFP fluorescence with increasing Cohex concentrations, especially above 0.1 mM.

[0055] In order to check whether the decreasing GFP levels were simply due to decreasing numbers of viable cells, in vitro cytotoxicity studies were performed for the same cell lines. That is, the same concentration ranges as used above were used in a CellTiter-Glo Luminescent Cell Viability Assay by Promega. This assay is based on quantitation of the ATP present in cells, which signals the presence of metabolically active cells, that is, a decrease in luminescence correlates with a decrease in the number of viable cells. The cells were plated out on 96-well plates, as above, and incubated at 37[deg.] C. for 24 hours before adding compound. The treated cells were then allowed to grow for an additional 48 hours before reading on the BMG Lumistar set on the ATP protocol.

[0056] In addition to the luminescence assay, a flow cytometry assay was performed using propidium iodide as a "dead" stain for A549 cells. The flow cytometry assay protocol for A549 cell line is similar to protocols known in the art, and is as follows. The cells were grown until confluent and reseeded at 100,000 cells/well in 1 ml in 24-well plates. The monolayers were allowed to form overnight at 37[deg.] C. under 5% CO2. The Cohex dilution series was added to appropriate wells and the plate incubated for 48 hours at 37[deg.] C. under 5% CO2. The cells were then washed, pelleted, resuspended in buffer, and transferred to BD falcon tubes for flow analysis. A BD FACSort cytometer and BD CellQuest software was used to quantify cell viability. Prior to flow analysis, 10 [mu]L of propidium iodide (PI) at 0.05 mg/ml was added to each tube to stain dead cells. Analysis was performed on $1*10^{4}$ events/well.

[0057] FIG. 6 shows the result of the cytotoxicity assay for A549 and HepG2 cells plotted on semi-log scale. There appears to be no toxic effect until about 0.1 mM, after which there is a decreasing % of viable cells. To better show the region from 2.5 [mu]M to 0.1 mM, FIG. 7 provides linear-scale plots to emphasize the concentration region that does affect cytotoxicity.

[0058] Both 293T and VerE6 cells lines show much less cytotoxic susceptibility to Cohex, leveling off between 70 to 80% viability, even at 5 mM Cohex. There is a variety of reactions to Cohex by different cell lines, but none of the cells were 100% killed, whereas suppression of GFP expression tends to bottom out close to -100% (except for VeroE6).

[0059] It is further notable that, in addition to variability between cell lines, different markers can also differ in their assessment of viability. As an example, the results of a flow cytometry measurement using propidium iodide (PI) as a marker for dead cells shown in FIG. 9. it can be seen that PI appears to measure a cell property (cell permeability) that is much less affected by Cohex than the luminescence study (ATP levels).

[0060] The IC50 for Cohex for the different cell lines can be estimated from FIGS. 1 and 2. By using a log concentration scale, the data can be fitted to the classic sigmoidal shape using a non-linear least-squares fitting program, seen in FIG. 10. The IC50 for the fit was found to be 0.38 mM Cohex.

[0061] The results with various cell types are shown in Table 2.

[0000]
TABLE 2
Summary of Cohex IC50 for Various Cell Types

	A549	HepG2	VeroE6	293T
IC50 (mM)	0.48	0.24	1.66	1.28

Cohex Animal Study Against Ebola

[0062] An efficacy study was conducted in mice to test whether Cohex would have a therapeutic affect against Ebola virus exposure. Initially, to determine whether the mice would tolerate the Cohex, they received intraperitoneal (IP) injections of Cohex once a day for 10 days at levels of 0.5, 1, 2, 4, and 8 mg/kg in this study. The mice tolerated the compound very well, with no adverse reactions reported.

[0063] To examine the efficacy of Cohex, mice were treated by IP injection with either phosphate buffered saline (PBS) or Cohex in PBS one hour before virus exposure, and further treated once a day for 9 more days. In comparing the results of the mice treated with PBS versus those treated with 8 mg/kg of Cohex, it was found to be statistically very likely (p=0.01 in a chi-squared test) that the 8 mg/kg treatment improved survival rates over the PBS treatment in mice infected with Ebola virus.

[0064] The general advantages of a broad-spectrum drug, such as Cohex, are its low-cost, stability, and, of course, ability to attack multiple microorganisms. When there is no treatment available, as in the case of Ebola virus, Cohex could be the only source of treatment. For viruses, such as HIV, where drugs with very high TI already exist, Cohex can be used in a combination drug therapy regime. There are several advantages to doing this: (1) as a broad-spectrum compound, Cohex can fight against opportunistic infections by other microorganisms; (2) Cohex may have a synergistic effect on existing anti-HIV drugs; (3) Cohex can significantly decrease the cost of anti-HIV treatment; (4) Cohex can slow the development of viral drug-resistance by presenting a very different mechanism that must be overcome.

[0065] All numbers expressing quantities of ingredients, constituents, reaction conditions, and so forth used in the specification are to be understood as being modified in all instances by the term "about." Notwithstanding that the numerical ranges and parameters set forth, the broad scope of the subject matter presented herein are approximations, the numerical values set forth are indicated as precisely as possible. Any numerical value, however, may inherently contain certain errors resulting, for example, from their respective measurement techniques, as evidenced by standard deviations associated therewith.

[0066] Although the present invention has been described in connection with preferred embodiments thereof, it will be appreciated by those skilled in the art that additions,

deletions, modifications, and substitutions not specifically described may be made without departing from the spirit and scope of the invention. Terminology used herein should not be construed in accordance with 35 U.S.C. $112, [paragraph]6 unless the term "means" is expressly used in association therewith.

US2010021556
Method for the production of an agent against an infectious disease

BACKGROUND OF THE INVENTION

[0001] The invention relates to a method for producing a composition against an infectious disease, in particular against HIV, Ebola or the like.

[0002] The treatment of HIV-infected people is one of the most urgent biomedical problems of recent times. It is as yet possible only to avoid an infection with the HIV virus by suitable measures, for example by using condoms during sexual intercourse. Once the HIV virus is present in the body, it is possible only to inhibit its effect and spread. Novel, promising therapies therefore relate to the inhibition of the rapid proliferation of the virus in human tissue. HIV prothease inhibitors block an important enzymatic metabolic pathway in the virus, leading to considerably reduced viral loads, thus slowing down the unremitting destruction of the immune system and the harmful effects, resulting therefrom, on human health.

[0003] A large number of chemical agents used for HIV injection treatment are known from the literature. These include for example azido derivatives of [beta]-L-2'-nucleosides as disclosed in DE 699 30 378 C2. DE 600 06 706 C2 describes N-acrylmethylthioamilite derivatives for inhibiting HIV replication. DE 602 04 967 T2 describes oversulfated polysaccharides as HIV inhibitors. All these chemical agents have undesired side effects which are to be avoided.

[0004] DE 693 27 236 T2 describes the use of dietetic whey proteins for the treatment of HIV-seropositive individuals. In this case, a denatured whey protein concentrate is described for the production of a medicament for the treatment of these individuals. The concentrate is to be designed so that the T-helper cell concentrations and the T-helper cell/T-suppressor cell ratio in an HIV-seropositive individual is increased.

[0005] The problem of the present invention is to provide a method and composition which serve to control infectious diseases, in particular HIV, Ebola or the like and show few side effects.

SUMMARY OF THE INVENTION

[0006] In accordance with the invention, medicinal oxygen is turbulently introduced under pressure into a solution which contains at least one plant constituent, in particular in the form of an extract, leading to the solution to the problem.

DETAILED DESCRIPTION

[0007] Medicinal oxygen is used for example in artificial respiration and in inhalation therapies. For this purpose, oxygen must be subjected to a special preliminary process in which this oxygen is specially purified and its aggressive effect is reduced.

[0008] In the present method, the medicinal oxygen is turbulently introduced over the course of about one hour with a superatmospheric pressure of about two atmospheres into the solution in such a way that the maximum amount of oxygen is introduced into the solution and also remains in the solution.

[0009] The solution preferably used is a physiological magnesium phosphoricum solution. However, this is to be understood as only exemplary, and other solutions are conceivable.

[0010] In a first exemplary embodiment of the invention, an extract from Afacimmune is to be used in the solution. Afacimmune means the fungus Agaricus Campestris which is normally grown on mineralized compost soil.

[0011] In a second exemplary embodiment of the invention, elder bark/flowers and/or Agaricus Blazei Murill is used as extract in the solution. The latter is the so-called almond fungus which originally comes from the Brazilian rainforest. Scarcely any fungus stimulates the immune system as effectively as the Agaricus. Its content of polysaccharides, especially of beta-glucans, are the highest by comparison with other medicinal fungi. For this reason, it is used for cancers. Its promoting effect on the production of blood in the bone marrow is also known. It is also suitable for use for alleviating liver disorders and assists the spleen in its purification of blood and defense functions.

[0012] In a further exemplary embodiment of the invention, the extract consists of St. John's wort and/or parsley juice in the solution. An extract of blue algae and/or buttercup also appears to be particularly effective. The blue algae extract is to contain about 80 g of lithium per gram of dry matter.

[0013] The buttercup extract is produced by pouring hot triple-distilled water over carefully dried buttercups and leaving the mixture to extract for seven minutes, with the above-mentioned medicinal oxygen being turbulently introduced in particular into the buttercup extract.

[0014] In a further preferred exemplary embodiment of the invention, a sugar is admixed with the solution apart from the solution with the Afacimmune extract. It is possible in this case for the sugar to have been specially treated, but normal granulated sugar is also possible.

[0015] The respective extract is preferably produced with hot triple-distilled water. The latter is water which has been distilled three times and is of very high purity.

[0016] Protection is also sought for the corresponding products produced by the above-mentioned methods.

US2010016244
D-GLUCOPYRANOSE 1-[3,5-BIS (1,1-DIMETHYLETHY)-4-HYDROXYBENZOATE] AND ITS DERIVATIVES, PREPARATION AND USE

THEREOF

[0001] The present invention relates to a compound: D-glucopyranose 1-[3,5-bis (1,1-dimethylethyl)-4-hydroxybenzoate] and its derivatives. It applies particularly but not exclusively, to the preparation and the use of these compounds for preparing medecine to treat and/or prevent infections by enveloped-viruses, particularly in humans, such as herpes, AIDS, influenza of the hepatitis B and C, virus of Dengue, Ebola and, in animals, Aujewsky's disease as for instance Aujewsky's in pigs.

[0002] The action of these derivatives is unique. They are not blocking viral replication as virustatics but they are shredding the viral lipid-protein membrane. These derivatives are virucide.

[0003] The herpes and AIDS viruses, like many others (influenza of the hepatitis B and & C, SARS, Ebola etc. . . .) are viruses surrounded by a lipid envelope unlike others-such as the virus of poliomyelitis who has no membrane-thus called naked-virus.

[0004] Enveloped-virus or naked-virus are non-cellular organism that are totally dependent of the cell they parasite for their survival. Viruses have no energy generating system (ATP) and no protein synthesis machinery. Although viral nucleic acids code for proteins, the synthesis of those proteins is performed on the host cell's ribosome. Hence, viruses must use the metabolic pathways of the cell as well as the capacity of these synthetic chemical factories that are the ribosome.

[0005] By rending impossible the access to viral metabolic pathways, virustatic (Tritherapy) disrupt metabolic pathways of the parasited molecules that the virus uses. This better reflects the poor tolerance of these biological therapies that block viral replication without killing the virus. Thus this limits significantly its effectiveness and use.

[0006] Taking into account the parasitic characteristics of the virus that makes it unable to survive outside a living eukaryotic cell, this invention seeks to prohibit its penetration into the living eukaryotic cell. Two methods are therefore possible:

Hiding the binding site of the host cell,

Eliminating the lipid envelope of the virus that contains the routing system and the protein adsorption on the membrane of the host cell.

[0009] In the first case, there is a risk of disruption of the metabolic external flux of the host cell, while lysing the viral envelope brings several benefits. It tends to annihilate skinned alive virus, making it unable to recognise the binding site and more importantly, it eliminates the proteins responsible for the adsorption of the virus on the membrane of the host cell. The virus and the cell can not merge, the virus left outside the cell dies.

[0010] It dies without any interference on the viral genome, in a way, by a mechanical action, limiting the risk of viral mutations which arise contrariwise to the mode of action of the virustatic.

[0011] This indifference towards virus' genetic heritage explains the effectiveness of these virucides on resistant mutant viruses to various new virucides available on the market.

(I)

OH

OH.

O

t-Bu

OH

OH

HO

OH

t-Bu
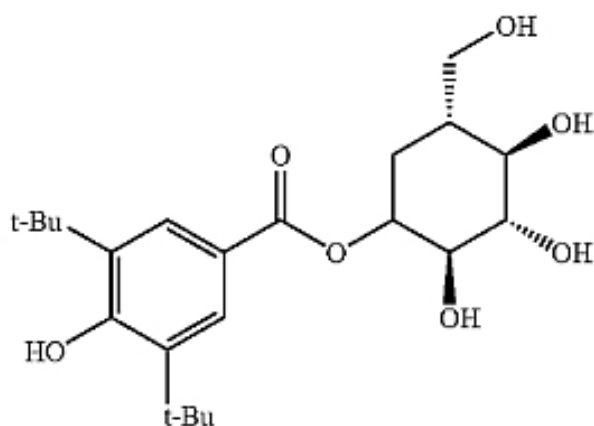
Mode of Action

[0012] The mode of action of virucides having a structure of di-tert-butyl such as BHT (butylhydroxytoluene) has been demonstrated in clinical trials against double-blind placebo in humans, by the disappearance or abortion of the herpes simply by application of a topical medicine from the onset.

[0013] Unlike the molecules acting on DNA, which induce a growth slow down, BHT is not involved in viral synthesis. One should seeks the origin of the properties of BHT elsewhere, in fact, Brugha M Jr, in an article published in "Science", demonstrated two points:

first, that chickens receiving food containing 200 ppm BHT were protected against infection inoculated by the virus responsible of the Newcastle disease (VMN). He noted a decrease in sero-conversion proportional to the administered BHT dose. Extending its experiment with cultures of pre-treated embryonic chicken cells with 25 [mu]g/ml BHT, he discovered that virus production is reduced by 65%.
second, that BHT inhibited the development of RNA virus (VMN) as well as that the development of DNA virus (VHS). He mentioned as a reason for this effect, a possible alteration of the envelope of the virus by the hydrophobic properties of BHT, although the effect of agonist VMN on the aggregation of chicken's erythrocytes-known characteristic of the membrane of this virion-is not changed, which seemed to him contradictory.

[0016] This hypothesis also proposed by Reimund and Cupp suggests that a modification of the geometry of the virus' lipid envelopes should prevent them to bind the membrane of the host cell.

[0017] Using electron microscopy, WINSTON, however, highlights the alteration, or even the break, of the virus' lipid envelopes, under the effect of treatment with BHT. BAMFORD demonstrates that the alteration of the viral envelope leads to the elimination of a protein (P3) responsible of the adsorption of the virus on the membrane of the host cell.

[0018] It remained to demonstrate the physico-chemical mechanism of these reactions.

[0019] Studying by electronic spin resonance, the composition of lipid envelopes, Aloia reveals the fluidity of enveloped-virus' membrane and in particular of HIV's membrane, under the effect of heat or BHT. By changing the composition of lipid envelopes and the

cholesterol/phospholipid ratio, the BHT reduces the membrane rigidity by disrupting its structure. This disruption, coupled with the loss of adsorption ability, prevents any recognition and any binding of the virus on the membrane of the host cell. ALOIA experimentally confirm that 30 minutes incubation at 37[deg.] C. in 320 [mu]g/ml BHT causes a decrease in viral infectivity on H9 lymphocytes, by a logarithmic factor of 4.

[0020] With AVF1 (3.5-di-tert-butyl-4-hydroxybenzoate octa-oxy-ethylene glycol), a substance derived from BHT, one manages to decrease HIV's infectivity by 7 log.

[0021] In summary, BHT's mode of action is complex:

virucidal, lysis of the protein-lipid envelope is explained by the hydrophobic properties of BHT. By promoting the binding with the transmembrane protein of the viral envelope they induce a modification of the cholesterol/phospholipid ratio responsible of the structural disruption of the envelope, its dehiscence and the expulsion of the viral adsorption protein. fusion-inhibitor through inability to identify and to merge on the cellular binding site.

[0024] Without cytopathic action on cells at effective doses, BHT is non-toxic for the organism, it only targets the membrane encoded by the virus and not the one of the host cell.

[0025] Through these complex reactions, viruses and membrane are no longer compatible. Key and lock being changed, the virus can not open the doors of the host cell for its reproduction. It dies being phagocyted.

[0026] The lipophilic properties of BHT and its specific mode of action, precise and limited, led to think that the group di-phenyl-tert-butyl may play a predominant role. It was therefore imperative for us to increase the availability of the molecule without altering its structure.

[0027] For this purpose, the invention proposes the preparation of compound D-glucopyranose 1-[3,5-bis (1,1-dimethylethyl)-4-hydroxybenzoate] defined by the following formula:

[0000]

[0028] The process of preparation of the compound D-glucopyranose 1-[3,5-bis (1,1-dimethylethyl)-4-hydroxybenzoate] comprises the following steps:

the production of the chloride of the 3,5-di-t-butyl-4-hydroxybenzoic acid,
a esterification by the reaction of the obtained chloride acid and the D-glucopyranose.

[0031] The compound according to the invention and its potential derivatives and additional salts to a mineral or organic acid pharmaceutically acceptable may be presented in a composition consisting of at least a pharmaceutically acceptable carrier.

[0032] This composition may arise for instance as tablets, capsules, dragees, drinkable solutions or suspensions, emulsions, suppositories.

[0033] In addition to non-toxic and pharmaceutically acceptable inert excipients, such as distilled water, glucose, lactose from starch, talc, vegetable oils, ethylene glycol . . . , the compositions thus obtained can also contain preservation agents.

[0034] Other active ingredients may be added to these compositions such as 3,5-di-t-butyl-4-hydroxybenoic acid (BG4) or 3.5-di-tert-butyl-4-hydroxybenzoate octa-oxy-ethylene glycol (AVF1) or a pharmaceutically acceptable derivatives.

[0035] The amount of compound according to the invention and any other active ingredients in such compositions will vary depending on the application, age and weight of the patient.

[0036] The synthesis of 3,5-di-t-butyl-4-hydroxybenoic acid (BG4), and its halides, such as chloride and bromide, was described in the application EP 0 269 981.

[0037] This acid has been proposed for the preparation of antiviral drugs for the treatment of diseases linked to infection of a person by viruses having a lipid envelope and especially the herpes virus, or AIDS.

[0038] The compound of the present invention has several advantages particularly with regard to the BHT and 3,5-di-t-butyl-4-hydroxybenoic acid (BG4):

Better solubility in water which facilitates the development of pharmaceutical preparations for a more suitable product,
Virucidal activity in lower concentrations,
A pro drugs effect.

[0042] An example of preparing a compound according to the invention will be described below, as a non-limiting example.

[0043] The process of preparing about one kilogramme of the compound D-glucopyranose 1-[3,5-bis (1,1-dimethylethyl)-4-hydroxybenzoate comprises the following steps:

[0044] The first step comprises the synthesis of acid chloride

[0045] In a flask, 700 grams of 3,5-di-t-butyl-4-hydroxybenoic acid are dissolved while stirring, in 1400 ml of dioxane. Then, 450 grams of thionyl chloride (3 equivalents) are introduced and the mixture is heated to 80[deg.] C. for 3 hours.

[0046] The progress of the reaction is monitored by thin layer chromatography (TLC). Once the reaction is completed, the excess of thionyl chloride is removed by evaporation under vacuum and then the mixture is incorporated in 1400 ml of dioxane.

[0047] The second step comprises an esterification

[0048] In a flask, 360 grams of D-glucopyranose are dissolved in 500 ml of dioxane, then 170 ml of pyridine are added.

[0049] The solution obtained during the first step is fed into the flask and then the mixture is shaken at 50[deg.] C. for 3 hours.

[0050] The progress of the reaction is monitored by thin layer chromatography (TLC), the reference front or RF is 0.05 using a mixture toluene/formic acid/acetone and phthalate para-anisidine as a developer,

[0051] Once the reaction is completed, solvents are eliminated by evaporation under vacuum.

[0052] Then the gross product is dissolved in a mixture of water/ethyl acetate (to a total of 10 liters). After settling and washing the organic phase with acidic water, the latter is concentrated. The product thus obtained is recrystallized by a mixture of ethanol/water mixture (20 liters) and then filtered on frit and dried.

[0053] The compound RDW031 of a molecular weight of 412.54 g.mol-1 is obtained with a purity of 98% controlled by liquid chromatography (HPLC) and further characterized by proton NMR at 400 MHz in deuterated chloroform.

[0054] The compound RDW031 of the present invention has several advantages over BHT and the 3,5-di-t-butyl-4-hydroxybenoic acid (BG4):

a better water solubility which facilitates the development of pharmaceutical preparations best suited for a drug.

[0000]
BG4 RDW031

Solubility [1/2] H 0.84 g/litre (no 1.08 g/litre (no at 100[deg.] C. desolubilization at desolubilization at room temperature room temperature

Solubilité [1/2] H No measurable 40 mg/litre à 23[deg.] c.

Test No 2

[0056]

[0000]
BG4 RDW031 batch RV 34
Solubility 12 H insoluble 1.2 g/litre at 23[deg.] C.
Materiel and   250 mg (slight excès) of RDW031 batch methode
RV41 + 100 ml H2O stirred for 48 hours.

This gives a suspension which is then filtered and concentrated under vacuum and weighted.

Test no 3
[0057]
[0000]

BG4 RDW031 batch RV 41
Solubility 0.13 g/litre 1.23 g/0litre after pH = 5.6 Note: formation of a fine white stirring for   suspension 48 H at   ->centrifugation 23[deg.] C.   pH = 5.9
Materiel 1 g (excès of BG4, 1 g (exces of RDW31 batch and originated from SIGMA-RV41) + 100 ml H2O stirred for methode ALDRICH) + 100 ml H2O 48 hours. This gives a stirred for 48 hours. This suspension which is then gives a suspension which is filtered, as the trouble then filtered. The filtrate is persists, the suspension is then evaporated under centrifuged and the supernatant vacuum and weighted is then evaporated and weighted

A virucidal activity at very low concentrations

A pro-drug effect: D-glucopyranose 1-[3,5-bis (1,1-dimethylethyl)-4-hydroxybenzoate] and 3,5-di-t-butyl-4-hydroxybenzoic acid, structure that decomposes, forming a highly active equilibrium, the two molecules having a strong virucidal power (reduced by 5 log the virulence of a HIV culture)

Virologic Studies on VHS (Herpes Simplex Virus)

[0060] Results of the tests conducted in the laboratory of Prof. Chiron, (Faculty of Pharmacy of Tours):

[0000]
Solution at 0.946 g/l dans l'eau
RDW 031 pur [1/2] [1/5]
 mg/ml 0.85140 0.42570 0.17028
Contact time test n[deg.] 4 15 min 1.25 - 0.00
code: 04/179 30 min 1.86 - 0.00
0.946 g/l (water) 60 min 2.15 0.00 0.00
120 min   2.32 1.48 0.00

Decrease expressed in log

[0061] In the above example, RDW031's virucidal activity on herpes begins of concentration much lower (0009%) than the one required for the effectiveness of BG 4 on VHS (0.5%) (FR 2 668 931)

RDW 031

Concentrations to Study

[0062] hypothesis: 10 mg/8 ml (solubility check)
Stock-solution: 33.47 mg/24.10 ml (x 1.11 C) i.e.: 11.11 mg/8 ml

[0000]
Dilutions Pur [1/2] [1/5]   1/20
Mg/ml 1.25000 0.62500 0.25000 0.06250
Contact time
15 min - 3.68 0.00 -
30 min - - - 0.21
60 min - - - -
120 min   - - - -

Dilutions   1/50   1/200   1/500   1/1000
Mg/ml 0.02500 0.00625 0.00250 0.00125
Contact time

15 min - - - -
30 min 0.31 - - -
60 min - 1.06 1.06 -

120 min   - - 1.15 0.31

Reduction expressed in log

[0063] Expressed in Mol, the comparisons are in favor of the new molecule RDW 031, which acts at concentrations inferior to a log for a substantially identical inhibitory activity:

BG 4: from 0.5% to 1%, i.e.: 0.04 to 0.02 Mol,
AVF1: from 0.5% to 1%, i.e.: 0.0083 at 0166 Mol ($8.3*10^{-3}$ to $1.66*10^{-2}$ Mol)
RDW031 active at concentration starting of 0.0625%, i.e.: 0.0015 Mol ($1.5*10^{-3}$ Mol)

[0067] It is worth recalling that the BHT, which has a very low toxicity, thus remaining a reference molecule, act on enveloped-viruses only at concentrations of 8 to 10%, that is to say at molars concentrations of 0.3 to 0.4 Mol that are 100 times stronger than RDW 031.

[0068] Thus, the D-glucopyranose 1-[3,5-bis (1, 1-dimethylethyl)-4-hydroxybenzoate] is a new molecule that combines a better solubility, a greater virucidal activity at lower doses than those of BHT and BG4.

[0069] As these molecules, the hydrophilic pole leads to the disintegration of the viral envelope of the virus herpes simplex (VHS) and has no effect on polio virus (naked-virus).

[0070] Its activity concerns all enveloped-viruses and particularly the AIDS virus for which promising studies are underway for various pharmaceutical packaging: film-coated tablets for oral administration in combination or in substitution of protease inhibitors when they are poorly supported.

[0071] The very low cyto-toxicity and high therapeutic scope eases the use with children. Without interference on the viral and human genome, it is possible first-line medication in pregnant women. All studies on rats have never shown any detectable effects on progeny nor on mutagenic effect, as this is expected with active virucide without interference on the viral or human genome.

[0072] The invention is a serious step forward in the battle against enveloped-viruses and especially against AIDS. One can hope viruses eradication by disappearance of viral loads which is not accessible to current virustatic that block partially the viral replication without killing the virus.

[0073] The therapeutic failures force the proliferation of drug combinations.

[0074] Only virucide can totally eliminate the virus colonies and allow the revival of the white line of CD 4 lymphocytes in particular and to restore the immune system of the body that HIV paralysis.

[0075] At the end of the regulatory pharmaco-toxicological tests, studies on humans will began.

[0076] From now on clinical trials on avian and porcine influenza will be undertaken. They will guide future studies.

Sulfonyl Semicarbazides, Semicarbazides and Ureas, Pharmaceutical Compositions Thereof, and Methods for Treating Hemorrhagic Fever Viruses, Including Infections Associated with Arena Viruses

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

FIELD OF THE INVENTION

[0003] The present invention relates to the use of sulfonyl semicarbazides, semicarbazides and ureas, as well as derivatives and analogs thereof, and pharmaceutical compositions containing the same, for the treatment or prophylaxis of viral infections and diseases associated therewith. In particular, those viral infections and associated diseases caused by hemorrhagic fever viruses, such as Arenaviruses may be treated.

BACKGROUND OF THE INVENTION

[0004] Hemorrhagic fever viruses have been discussed in the scientific literature. The following publications, patents and patent applications are cited in this application as superscript numbers:

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[0044] All of the publications, patents and patent applications cited in this application are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

[0045] The National Institute of Allergy and Infectious Diseases (NIAID) and the Centers for Disease Control and Prevention (CDC) have classified a number of viruses as potential agents of bioterrorism (www.bt.cdc.gov/agent/agentlist-category.asp). The highest threat agents, the Category A pathogens, have the greatest potential for adverse public health impact and mass casualties if used in ill-intentioned ways. Within the Category A pathogens, there are a number of viruses that can cause viral hemorrhagic fevers with high case fatality rates. The Category A hemorrhagic fever viruses pose serious threats as potential biological weapons because: 1) they can be disseminated through aerosols; 2) a low dose (1-10 plaque forming unit (pfu)) can cause disease; 3) they cause severe morbidity and mortality (case fatality rates of 15-30%); 4) they can cause fear and panic in the general public; 5) there are no U.S.-approved effective vaccines or specific antivirals available; 6) these pathogens are easily available and can be readily produced in large quantities; and 7) research on weaponizing various hemorrhagic fever viruses has been conducted.[1]

[0046] Arenaviruses are enveloped viruses with a genome that consists of two single-stranded RNA segments designated small (S, 3.5Kb) and large (L, 7.5Kb), both with an ambisense coding arrangement.[36] The S RNA segment encodes the major structural proteins, nucleocapsid protein (NP) and a precursor envelope protein (GPC) encoding two envelope glycoproteins (external GP1 and transmembrane GP2),[18, 24, 30, 31] and the L RNA segment encodes the RNA polymerase protein L and an 11 KDa protein, Z protein, with putative regulatory function.[19] GP1 and GP2, which form the tetrameric surface glycoprotein spike, are responsible for virus entry into targeted host cells.

[0047] The family Arenaviridae consists of a single genus (Arenavirus) that includes several viruses (currently 23 recognized viruses[1]) causing severe hemorrhagic fever diseases in humans.[2] The Arenaviridae family has been divided into two groups according to sequence-based phylogeny. The "Old World" group, originated from Africa, includes the human pathogens lymphocytic choriomeningitis (LCM) virus and Lassa virus. The "New World" group, originated from Latin America, is divided into 3 clades. Clade B includes in addition to Tacaribe and Amapari viruses, the Category A human pathogenic viruses Junín (Argentine hemorrhagic fever), Machupo (Bolivian hemorrhagic fever), Guanarito (Venezuelan hemorrhagic fever), and Sabiá (Brazilian hemorrhagic fever). These Category A viruses are capable of causing severe and often fatal hemorrhagic fever disease in humans.

[0048] Rodents are the natural host of arenaviruses, although Tacaribe virus is found in bats. The arenaviruses characteristically produce chronic viremic infections in their natural host, [15] which in turn shed virus in their urine and feces, ultimately infecting humans in close contact with these infected materials either by aerosol or direct contact with skin abrasions or cuts. The natural history of the human disease is determined by the pathogenicity of the virus, its geographical distribution, the habitat and the habits of the rodent reservoir host, and the nature of the human-rodent interaction.[21]

[0049] Several Arenaviruses are associated with severe hemorrhagic disease in human. Lassa virus (from the Old World group) is responsible for Lassa hemorrhagic fever, while 4 viruses from the New World group (all from Clade B) cause severe hemorrhagic fever in human. Those viruses are: Junin virus responsible for Argentine hemorrhagic fever, Machupo virus for Bolivian hemorrhagic fever and Guanarito virus for Venezuelan hemorrhagic fever. Sabia virus was isolated from a fatal case of hemorrhagic fever in Brazil. It is estimated that Lassa virus causes 100,000-300,000 infections and approximately 5,000 deaths annually.[5] So far an estimated 30,000 confirmed cases of Junin infections have been documented, while about

2,000 of Machupo, 200 of Guanarito and only 2 of Sabia.<1 >

[0050] Recent concerns over the use of Arenaviruses as biological weapons have underscored the necessity of developing small molecule therapeutics that target these viruses.<1 >The availability of antiviral drugs directed at these viruses would provide treatment and a strong deterrent against their use as biowarfare agents. Since antiviral drugs can be easily administered (oral pill or liquid) and exert their antiviral effect within hours of administration, they will serve to effectively treat diseased patients, protect those suspected of being exposed to the pathogen (post-exposure prophylaxis), and assist in the timely containment of an outbreak.

[0051] Currently, there are no virus-specific treatments approved for use against Arenavirus hemorrhagic fevers. Present disease management consists of general supportive care: monitoring and correcting fluid, electrolyte and osmotic imbalances and treating hemorrhaging with clotting factor or platelet replacement. Convalescent immune serum therapy may be effective in treating cases of Junin and Machupo virus disease, but the availability of such serum is extremely limited.

[0052] Ribavirin, a nucleoside analog, has been used with some success in Lassa fever patients. In small trials, intravenous ribavirin given to patients within the first 6 days after development of fever decreased mortality from 76% to 9%.<7-9 >A controlled trial of 18 patients with Argentine hemorrhagic fever resulted in 13% mortality in treated patients compared with 40% in untreated patients.<10 >Ribavirin therapy is associated with adverse effects including a dose-related, reversible hemolytic anemia an d also has demonstrated teratogenicity and embryo lethality in several animal species. It is therefore classified as a pregnancy category X drug, contraindicated during pregnancy. Intravenous ribavirin is available in limited supplies in the U.S. for compassionate use under an FND application. The dosing regimen for ribavirin therapy that has been used in cases of Lassa fever consists of an initial 30 mg/kg intravenous (IV) loading dose, followed by 16 mg/kg IV every 6 hours for 4 days; then 8 mg/kg IV every 8 hours for 6 days (total treatment time 10 days). The cost of treatment for an adult male is approximately $800. The attributes of ribavirin make it less than ideal for the treatment of Arenavirus hemorrhagic fevers.

[0053] A number of in vitro inhibitors of Arenavirus replication have been reported in the literature including phenothiazines, trifluoroperazine and chlorpromazine,<1 >amantadine, <12,13 >brassinosteroids<14 >and actinomycin D.<15 >The anti-Arenavirus activities of these compounds are generally weak and non-specific.

[0054] The only Arenavirus hemorrhagic fever for which studies have been undertaken toward development of a vaccine has been Argentine hemorrhagic fever (AHF) caused by Junin virus. A live-attenuated vaccine, called Candid 1, has been evaluated in controlled trials among agricultural workers in AHF-endemic areas, where it appeared to reduce the number of reported AHF cases with no serious side effects.<16 >It is not known if the Candid 1 vaccine would be useful against other Arenavirus hemorrhagic fevers and this vaccine is not available in the United States of America.

[0055] Tacaribe virus is a biosafety level 2 (BSL 2) New World arenavirus (NWA) that is found in clade B and phylogenetically related to the Category A NWA (Junín, Machupo, Guanarito and Sabiá). Tacaribe virus is 67% to 78% identical to Junín virus at the amino acid level for all four viral proteins. In order to screen for inhibitors of NWA a high-throughput

screening (HTS) assay for virus replication was developed using Tacaribe virus as a surrogate for Category A NWA. A 400,000 small molecule library was screened using this HTS assay. A lead series was chosen based on drug properties and this series was optimized through iterative chemistry resulting in the identity of a highly active and specific small molecule inhibitor of Tacaribe virus with selective activity against human pathogenic NWA (Junín, Machupo, Guanarito and Sabiá). This molecule demonstrates favorable pharmacodynamic properties which permitted the demonstration of in vivo anti-arenavirus activity in a newborn mouse model.

[0056] All human pathogens Arenaviruses from the New World group causing hemorrhagic fever are from the Clade B. These human pathogen viruses require manipulation under high-level containment (BSL-4). However, Amapari and Tacaribe viruses also from Clade B can be grown in tissue culture under BSL-2 (low-level) containment. Working under low-level containment makes experimentations easier and safer with these viruses. While Amapari virus produces low cytopathic effect, Tacaribe virus can be grown readily in cell culture and produce robust CPE in 4 to 6 days. Since this CPE is directly related to viral replication, compounds that inhibit virus replication in cell culture can be identified readily as conferring protection from virus-induced CPE (although it is theoretically possible to inhibit CPE without inhibiting virus replication). Moreover, compounds having identified activity against Tacaribe virus will also likely be active against Arenavirus human pathogen causing hemorrhagic fever (Junin, Machupo, Guanarito and Sabia) given the high degree of homology (around 70% identity for all 4 proteins of Tacaribe virus compared to Junin virus, with long stretch of protein with perfect identity) between these viruses.

[0057] What is needed in the art are new therapies and preventives for the treatment of viral infections and associated diseases, such as caused by hemorrhagic fever viruses like Arenaviruses.

SUMMARY OF THE INVENTION

[0058] The present invention provides compounds and compositions and/or methods for the treatment and prophylaxis of viral infections, as well as diseases associated with viral infections in living hosts. In particular, the present invention provides compounds and compositions and/or methods for the treatment and prophylaxis of hemorrhagic fever viruses, such as Arenaviruses.

[0059] In one embodiment, the invention relates to a method for the treatment or prophylaxis of a viral infection or disease associated therewith, comprising administering in a therapeutically effective amount to a mammal in need thereof, a compound of formula I or a pharmaceutically acceptable salt thereof. In another embodiment, the invention relates to a pharmaceutical composition that comprises a pharmaceutically effective amount of the compound or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier. In addition, the invention also relates to compounds of formula I, as well as pharmaceutically acceptable salts thereof.

[0060] Preferred compounds of formula I include:

[0000]
[0000] wherein
n is an integer from 0-6;

[0061] m is an integer from 0-1;

[0062] p is an integer from 0-1;

[0063] R1 is selected from the group consisting of H and alkyl;

[0064] R2 is selected from the group consisting of substituted or unsubstituted phenyl, substituted and unsubstituted aryl, substituted and unsubstituted heteroaryl, substituted or unsubstituted alkyl, substituted or unsubstituted branched alkyl, and substituted or unsubstituted unsaturated cycloheteroalkyls;

[0000] or where R1 and R2 combine together to form a substituted or unsubstituted C4-10 cyclic saturated heteroalkyl;
R3 is selected from the group consisting of H and alkyl;
or a pharmaceutically acceptable salt thereof.
Other compounds of formula I include:

[0000]
[0000] wherein
R2 is selected from the group consisting of substituted or unsubstituted phenyl, substituted and unsubstituted aryl, substituted and unsubstituted heteroaryl, substituted or unsubstituted alkyl, substituted or unsubstituted branched alkyl, and substituted or unsubstituted unsaturated cycloheteroalkyls
or a pharmaceutically acceptable salt thereof.
Further compounds of formula I include:

[0000]
[0000] wherein
R1 is selected from the group consisting of H and alkyl;
R2 is selected from the group consisting of substituted or unsubstituted phenyl, substituted and unsubstituted aryl, substituted and unsubstituted heteroaryl, substituted or unsubstituted alkyl, substituted or unsubstituted branched alkyl, and substituted or unsubstituted unsaturated cycloheteroalkyls;
or where R1 and R2 combine together to form a substituted or unsubstituted C4-10 cyclic saturated heteroalkyl;
or a pharmaceutically acceptable salt thereof.

[0065] In other embodiments, in the compound of formula I, n is 0 or 1. Also, in other embodiments, in the compound of formula I, m is 1 and p is 1 or alternatively, m is 0 and p is 0.

[0066] In further embodiments, in Formula I, R1 and R2 combine together to form a substituted or unsubstituted C4-10 cyclic saturated heteroalkyl selected from the group consisting of:

[0000]

[0067] In still further embodiments, in Formula I, R2 is selected from the group consisting of:

[0000]

[0000] wherein each of R5, R6, R7, R8 and R9 is independently selected from the group consisting of: hydrogen, acetyl, methoxy, trifluoromethyl, fluoro, chloro, bromo, iodo, acylamino, methyl, sulfonamide, trifluoromethoxy, carboxy, cyano and 1,1,2,2-tetrafluoroethoxy.

[0068] In particular, certain embodiments relate to a compound of formula I selected from the group consisting of:
N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(4-(phenyl)-phenylsulfonyl]hydrazine-1-carboxamide;
N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(4-(2-methyl-2-propyl)-phenylsulfonyljhydrazine-1-carboxamide;
N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[7-(4-methyl-3,4-dihydro-2H-benzo[1,4]oxazinyl)sulfonyl]hydrazine-1-carboxamide;
N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[5-(1-dimethylamino-naphthyl)sulfonyl]hydrazine-1-carboxamide;
N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(2,4,6-trimethylphenyl)sulfonyl]hydrazine-1-carboxamide;
N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(3-chloro-6-methoxyphenyl)sulfonyl]hydrazine-1-carboxamide;
N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(3,6-dimethoxyphenyl)sulfonyl]hydrazine-1-carboxamide;
N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(4-(4-[1,2,3]thiadiazolyl)phenyl)sulfonyl]hydrazine-1-carboxamide;
N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(3-bromophenyl)sulfonyl]hydrazine-1-carboxamide;
N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(4-bromophenyl)sulfonyl]hydrazine-1-carboxamide;
N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(4-methylphenyl)sulfonyl]hydrazine-1-carboxamide;
N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(4-methoxyphenyl)sulfonyl]hydrazine-1-carboxamide;
N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(4-difluoromethoxyphenyl)sulfonyl]hydrazine-1-carboxamide;
N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(3-fluoro-4-chloro-phenyl)sulfonyl]hydrazine-1-carboxamide;
N-2-(1,1,1,3,33-Hexafluoro-2-methylpropyl)-2-[(4-trifluoromethoxyphenyl)sulfonyl]hydrazine-1-carboxamide;
N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(4-fluoro-phenyl)sulfonyl]hydrazine-1-carboxamide;
N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(3-methoxyphenyl)sulfonyl]hydrazine-1-carboxamide;
N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(2-methylphenyl)sulfonyl]hydrazine-1-carboxamide;
N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(3-trifluoromethylphenyl)sulfonyl]hydrazine-1-carboxamide;
N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(2,4-dimethoxyphenyl)sulfonyl]hydrazine-1-carboxamide;
N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[2-(5-chloro-1,3-dimethyl-1H-pyrazolyl)sulfonyl]hydrazine-1-carboxamide;
N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(3-methylphenyl)sulfonyl]hydrazine-1-

carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(4-trifluoromethylphenyl)sulfonyl]hydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(2-trifluoromethylphenyl)sulfonyl]hydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[4-(pyrrolidin-1-sulfonyl)phenyl sulfonyl]hydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(2-chlorophenyl)sulfonyl]hydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[2-(5-morpholin-4-yl)pyridyl sulfonyl]hydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(2-trifluoromethoxyphenyl)sulfonyl]hydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(2,4-dichlorophenyl)sulfonyl]hydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[phenylsulfonyl]hydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(3-difluoromethoxyphenyl)sulfonyl]hydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(3-cyanophenyl)sulfonyl]hydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(4-cyanophenyl)sulfonyl]hydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[5-(2,3-dihydrobenzo[1,4]dioxinyl) sulfonyl]hydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(4-methylphenyl)sulfonyl]-1-methylhydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(3-fluorophenyl)sulfonyl]hydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(3,4-difluorophenyl)sulfonyl]hydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(2,4-dimethylthiazol-5-yl)sulfonyl]hydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(4-acetylphenyl)sulfonyl]hydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(2,6-difluorophenyl)sulfonyl]hydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(2-fluorophenyl)sulfonyl]hydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(2,5-difluorophenyl)sulfonyl]hydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(4-methylphenyl)sulfonyl]-2-methylhydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(2,6-dichlorophenyl)sulfonyl]hydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(2,6-ditrifluoromethylphenyl) sulfonyl]hydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(4-methylphenyl)sulfonyl]hydrazine-1-methylcarboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-memylpropyl)-2-[(3,5-dimethylisoxazol-5-yl)sulfonyl]hydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(4-nitrophenyl)sulfonyl]hydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(1-methylimidazol-4-yl)sulfonyl]hydrazine-1-carboxamide;

N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[methylsulfonyl]hydrazine-1-carboxamide;

4-Phenylpiperazine-1-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-carboxamide;

4-Morpholino-1-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-carboxamide;

1-(2-Acetylphenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

1-Piperidino-1-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-carboxamide;

1-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-3-(3,4,5-trimethoxyphenyl)-urea;

1-(4-Trifluoromethylphenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

4-Methylpiperazine-1-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-carboxamide;

1-Naphthalen-1-yl-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

1-(4-Chlorophenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

4-Phenylpiperidin-1-yl-1-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-carboxamide;

1-(2-Phenyl(phenyl))-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

1-(2,6-Difluorophenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

2-[3-(1,1-Bis-trifluoromethylethyl)-ureido]benzamide;

1-(2-Chloro-6-fluorophenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

1-(3-Trifluoromethylphenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

2-[3-(1,1-Bis-trifluoromethylethyl)-ureido]benzenesulfonamide;

1-(2,2,3,3-Tetrafluoro-2,3-dihydrobenzo[1,4]dioxin-5-yl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

1-(3-Trifluoromethoxyphenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

1-(4-Trifluoromethoxyphenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

4-Methyl-1-piperidine-1-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-carboxamide;

1-Naphthalen-2-yl-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

1-(2-fluorophenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

1-(2,6-Dimethoxyphenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

3-Trifluormethoxy-4-[3-(1,1-bis-trifluoromethylethyl)-ureido]benzoicacid;

1-Phenyl-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

1-(3-Cyanophenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

1-(3-Methoxyphenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

1-(2-(1,1,2,2-Tetrafluoroethoxy)phenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

3-[3-(1,1-Bis-trifluoromethylethyl)-ureido]benzenesulfonamide;

1-(3-fluorophenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

1-(4-Bromophenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

1-(2-Cyanophenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

1-(4-Cyanophenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

1-(2,2-Difluorobenzo[1,3]dioxol-4-yl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

1-(4-Chlorophenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

1-(3-Methylphenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

4-[3-(1,1-Bis-trifluoromethylethyl)-ureido]benzenesulfonamide;

1-(2,6-Dibromophenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

1-(2-Methylphenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

1-(4-Methylphenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

1-Pyrrolidinyl-1-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-carboxamide;

1-(4-Fluorophenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;

1-(2,4-Dibromophenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;
Azepane-1-carboxylic acid (2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-amide;
1-(4-Bromo-2-trifluoromethoxyphenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-
urea;
1-(2-Trifluoromethoxyphenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;
1-(2-Trifluoromethylphenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea;
1-(2-Methoxyphenyl)-3-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-urea; and
N-2-(1,1,1,3,3,3-hexafluoro-1-methylpropyl)-2-[(4-
difluoromethoxyphenyl)sulfonyl]hydrazine-1-carboxamide.

[0168] In one embodiment, the mammal being treated is a human. In particular embodiments, the viral infection being treated is a hemorrhagic fever virus, such as an Areanvirus. The Arenavirus may be selected from the group consisting of Junin, Machupo, Guanavito, Sabia, and Lassa.

[0169] These and other objects, advantages, and features of the invention will become apparent to those persons skilled in the art upon reading the details of the methods and formulations as more fully described below.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0170] FIG. 1 provides the chemical structure, formula, and molecular weight of ST-336.

[0171] FIG. 2 shows the effect of the time of addition of ST-336 on Tacaribe virus yield and plaque formation. In FIG. 2A, Vero cells were infected with Tacaribe virus at a MOI=0.01. ST-336 was added prior to or during Tacaribe infection (-1, 3, 6, 9, 12, 15, 18 or 21 hrs p.i.). At 24 hrs p.i., virus yields were determined by plaque assay. In FIG. 2B, Vero cells were infected with 400 pfu Tacaribe virus. ST-336 was added for 1 hour before the infection (-1), for 1 hour during adsorption (0), and for 1 hour after the infection (+1). Infected monolayers were washed with PBS and overlayed with medium containing agarose. Five days post-infection, cells were glutaraldehyde fixed and crystal violet stained prior to plaque counting.

[0172] FIG. 3 shows that ST-336 binds with slow Koff to intact Tacaribe virion in the absence of cells. In FIG. 3A, a diagram of the virus dilution scheme prior to plating is is provided. The virus mixed with ST-336 and diluted (left side) or virus diluted and ST-336 added after dilution (right side). In FIG. 3B, pictures of the plaques that resulted after plating each dilution shown in FIG. 3A on Vero cells is provided.

[0173] FIG. 4 shows the mapping of ST-336 drug resistant variants ("DRVs"). In FIG. 4A, a linear map of the glycoprotein precursor ("GPC") showing the location of the signal peptide ("SP"), transmembrane domain ("TM"), the cleavage site between GP1 and GP2 (K261-A262), the location of the four ST-336 resistant mutants ("DR #1-4"), and the amino acid change for each is provided. In FIG. 4B, the amino acid sequence alignment of GP2 from wild type NWA and ST 336 DRVs is shown. Shown is the amino acid sequence of the C-terminal portion of GP2 (amino acids 397 to 457) containing the transmembrane domain (marked by vertical lines), the location of the mutations for DR#1-4 (underlined), and the amino acid difference in Amapari (in bold).

[0174] FIG. 5 provides the chemical structure, formula, and molecular weight for ST-294.

[0175] FIG. 6 shows the effect of ST-294 in newborn mice challenged with Tacaribe virus. Four day old BALB/c mice were infected IP with 30*LD50 Tacarbide virus and treated daily for 10 days with vehicle (control), ribavarin at 25 mg/kg, ST-294 twice a day (BID) at 50 mg/kg or once a day (SID) at 100 mg/kg. Shown in FIG. 6 are the percent survivors in each treatment group on day 9 and day 10 after infection.

Figure 1
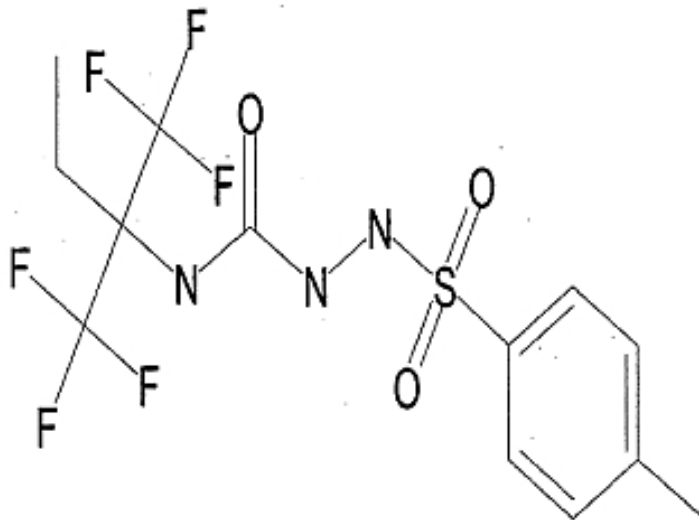
Formula: $C_{13}H_{15}N_3O_3F_6S_1$

MW: 407.33

Figure 2

Figure 3

1/10 dilution

400pfu
+ 0.5uM ST-336
(1B)

400pfu
+ DMSO
(2B)

400pfu and add
+ 0.5uM ST-336
(3B)

1/10 dilution

40pfu
+ 0.05uM ST-336
(1C)

40pfu
+ DMSO
(2C)

40pfu and add
+ 0.05uM ST-336
(3C)

1A   2A

1B   2B   3B

1C   2C   3C

GPC: 483aa

434   TM   453

SP
N-

S58-E59

K261-A262

T428N
DRV#2

I430T
DRV#1

F442I
DRV#4

S439I
DRV#3

-C

GP1

GP2

B

Tacaribe-WT#1	DFLISEILSKEYSERQGRTPITLVDICFWSTVFFTSTLFLHLIGFPTHRHIRGEGCPLPH
Tacaribe-WT#2	DFLISEILSKEYSERQGRTPITLVDICFWSTVFFTSTLFLHLIGFPTHRHIRGEGCPLPH
Tacaribe-WT#3	DFLISEILSKEYSERQGRTPITLVDICFWSTVFFTSTLFLHLIGFPTHRHIRGEGCPLPH
Tacaribe-WT#4	DFLISEILSKEYSERQGRTPITLVDICFWSTVFFTSTLFLHLIGFPTHRHIRGEGCPLPH
Tacaribe-DR#1.1	DFLISEILSKEYSERQGRTPTTLVDICFWSTVFFTSTLFLHLIGFPTHRHIRGEGCPLPH
Tacaribe-DR#2.1	DFLISEILSKEYSERQGRNPITLVDICFWSTVFFTSTLFLHLIGFPTHRHIRGEGCPLPH
Tacaribe-DR#3.1	DFLISEILSKEYSERQGRTPITLVDICFWSTVFFTITLFLHLIGFPTHRHIRGEGCPLPH
Tacaribe-DR#4.1	DFLISEILSKEYSERQGRTPITLVDICFWSTVFFTSTLILHLIGFPTHRHIRGEGCPLPH
Machupo	DHLISEMLSKEYAERQGKTPITLVDICFWSTIFFTASLFLHLVGIPTHRHLKGEACPLPH
Junin	DFLISRMLSKEYSDRQGKTPLTLVDICFWSTVFFTASLFLHLVGIPTHRHIRGEACPLPH
Candid 1	DFLISRMLSKEYSDRQGKTPLTLVDICIWSTVFFTASLFLHLVGIPSHRHIRGEACPLPH
Guanarito	DHLIAEMLSKEYQDRQGKTPLTLVDLCFWSAIFFTTSLFLHLVGFPTHRHIQGDPCPLPH
Sabia	DHLLSEMLNKEYIDRQGKTPLTLVDICFWSTLFFTTTLFLHLVGFPTHRHIRGEFCPLPH
Amapari	DHLIAEILSKEYQDRQGKTPITLVDMCFWSAIFFDNKSLLHLVGFPTHRHIVGEACPLPH

Transmembrane domain

Figure 4

Figure 5

Formula: $C_{12}H_{11}N_3O_4F_8S_1$

MW: 445.29

Figure 6

120

P=0.007

P=0.041

P=0.001

100
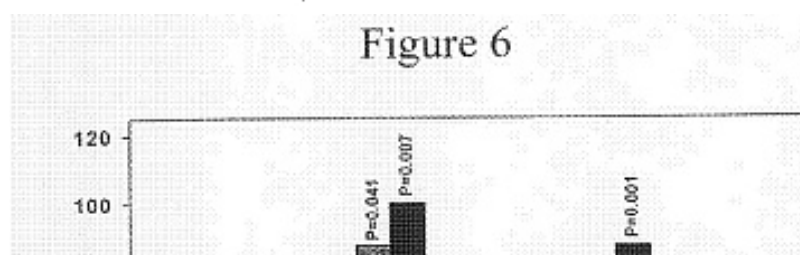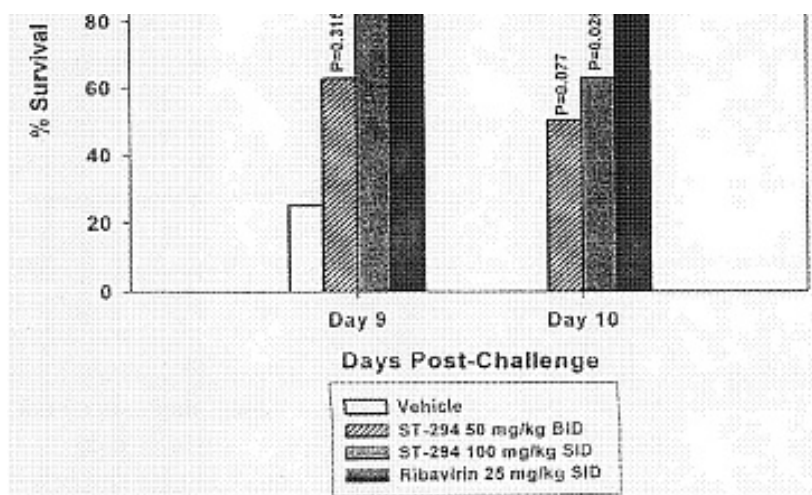
DETAILED DESCRIPTION OF THE INVENTION

[0176] As above, this invention relates to compounds which are useful for the treatment and prophylaxis of viral infections, as well as diseases associated with viral infections in living hosts. In particular, the present invention provides compounds and compositions and/or methods for the treatment and prophylaxis of hemorrhagic fever viruses, such as Arenaviruses. However, prior to describing this invention in further detail, the following terms will first be defined.

DEFINITIONS

[0177] In accordance with this detailed description, the following abbreviations and definitions apply. It must be noted that as used herein, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise.

[0178] The publications discussed herein are provided solely for their disclosure. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates, which may need to be independently confirmed.
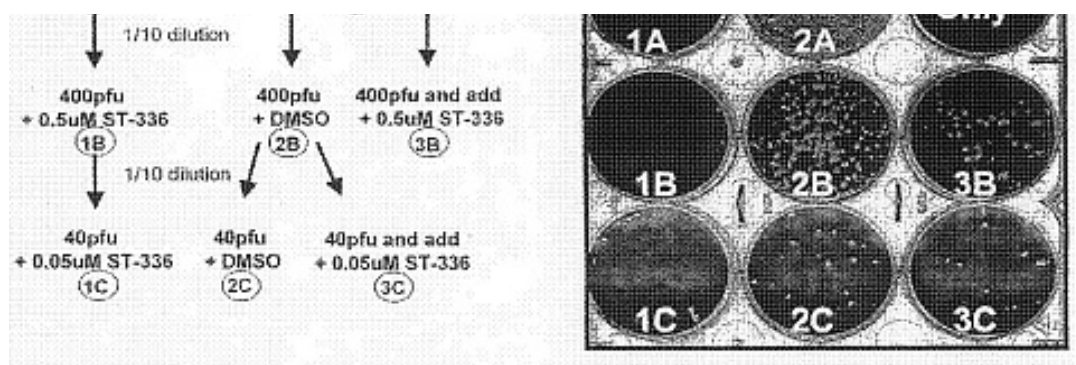
[0179] Where a range of values is provided, it is understood that each intervening value is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the invention. Also contemplated are any values that fall within the cited ranges.

[0180] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0181] By "patient" or "subject" is meant to include any mammal. A "mammal", for purposes

of treatment, refers to any animal classified as a mammal, including but not limited to humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, and the like. Preferably, the mammal is human.

[0182] The term "efficacy" as used herein in the context of a chronic dosage regime refers to the effectiveness of a particular treatment regime. Efficacy can be measured based on change the course of the disease in response to an agent of the present invention.

[0183] The term "success" as used herein in the context of a chronic treatment regime refers to the effectiveness of a particular treatment regime. This includes a balance of efficacy, toxicity (e.g., side effects and patient tolerance of a formulation or dosage unit), patient compliance, and the like. For a chronic administration regime to be considered "successful" it must balance different aspects of patient care and efficacy to produce the most favorable patient outcome.

[0184] The terms "treating", "treatment", and the like are used herein to refer to obtaining a desired pharmacological and physiological effect. The effect may be prophylactic in terms of preventing or partially preventing a disease, symptom or condition thereof and/or may be therapeutic in terms of a partial or complete cure of a disease, condition, symptom or adverse effect attributed to the disease. The term "treatment", as used herein, covers any treatment of a disease in a mammal, particularly a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it, i.e., causing the clinical symptoms of the disease not to develop in a subject that may be predisposed to the disease but does not yet experience or display symptoms of the disease; (b) inhibiting the disease, i.e., arresting or reducing the development of the disease or its clinical symptoms; or (c) relieving the disease, i.e., causing regression of the disease and/or its symptoms or conditions. The invention is directed towards treating a patient's suffering from disease related to pathological inflammation. The present invention is involved in preventing, inhibiting, or relieving adverse effects attributed to pathological inflammation over long periods of time and/or are such caused by the physiological responses to inappropriate inflammation present in a biological system over long periods of time.

[0185] As used herein, "acyl" refers to the groups H-C(O)-, alkyl-C(O)-, substituted alkyl-C(O)-, alkenyl-C(O)-, substituted alkenyl-C(O)-, alkynyl-C(O)-, substituted alkynyl-C(0)-cycloalkyl-C(O)-, substituted cycloalkyl-C(O)-, aryl-C(O)-, substituted aryl-C(O)-, heteroaryl-C(O)-, substituted heteroaryl-C(O), heterocyclic-C(O)-, and substituted heterocyclic-C(O)- wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic are as defined herein.

[0186] "Acylamino" refers to the group -C(O)NRR where each R is independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heteroaryl, substituted heteroaryl, heterocyclic, substituted heterocyclic and where each R is joined to form together with the nitrogen atom a heterocyclic or substituted heterocyclic ring wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic are as defined herein.

[0187] "Alkenyl" refers to alkenyl group preferably having from 2 to 10 carbon atoms and more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-2 sites of alkenyl unsaturation.

[0188] "Lower alkenyl" refers to an alkenyl group preferably having from 2 to 6 carbon atoms and having at least 1 site and preferably only 1 site of alkenyl unsaturation (i.e., >C-C<). This term is exemplified by groups such as allyl, ethenyl, propenyl, butenyl, and the like.

[0189] "Substituted alkenyl" refers to alkenyl groups having from 1 to 5 substituents independently selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, thiocarbonylamino, acyloxy, amino, amidino, alkylamidino, thioamidino, aminoacyl, aminocarbonylamino, aminothiocarbonylamino, aminocarbonyloxy, aryl, substituted aryl, aryloxy, substituted aryloxy, aryloxyaryl, substituted aryloxyaryl, halogen, hydroxyl, cyano, nitro, carboxyl, carboxylalkyl, carboxyl-substituted alkyl, carboxyl-cycloalkyl, carboxyl-substituted cycloalkyl, carboxylaryl, carboxyl-substituted aryl, carboxylheteroaryl, carboxyl-substituted heteroaryl, carboxylheterocyclic, carboxyl-substituted heterocyclic, cycloalkyl, substituted cycloalkyl, guanidino, guanidinosulfone, thiol, thioalkyl, substituted thioalkyl, thioaryl, substituted thioaryl, thiocycloalkyl, substituted thiocycloalkyl, thioheteroaryl, substituted thioheteroaryl, thioheterocyclic, substituted thioheterocyclic, heteroaryl, substituted heteroaryl, heterocyclic, substituted heterocyclic, cycloalkoxy, substituted cycloalkoxy, heteroaryloxy, substituted heteroaryloxy, heterocyclyloxy, substituted heterocyclyloxy, oxycarbonylamino, oxythiocarbonylamino, cycloalkyloxy, substituted cycloalkyloxy, heteroaryloxy, substituted heteroaryloxy, -OS(O)2-alkyl, -OS(O)2-substituted alkyl, -OS(O)2-aryl, -OS(O)2-substituted aryl, -OS(O)2-heteroaryl, -OS(O)2-substituted heteroaryl, -OS(O)2-heterocyclic, -OS(O)2-substituted heterocyclic, -OSO2-NRR where R is hydrogen or alkyl, -NRS(O)2-alkyl, -NRS(O)2-substituted alkyl, -NRS(O)2-aryl, -NRS(O)2-substituted aryl, -NRS(0)2-heteroaryl, -NRS(O)2-substituted heteroaryl, -NRS(O)2-heterocyclic, -NRS(O)2-substituted heterocyclic, -NRS(O)2-NR-alkyl, -NRS(O)2-NR-substituted alkyl, -NRS(O)2-NR-aryl, -NRS(O)2-NR-substituted aryl, -NRS(O)2-NR-heteroaryl, -NRS(O)2-NR-substituted heteroaryl, -NRS(O)2-NR-heterocyclic, -NRS(O)2-NR-substituted heterocyclic where R is hydrogen or alkyl, mono- and di-alkylamino, mono- and di-(substituted alkyl)amino, mono- and di-arylamino, mono- and di-substituted arylamino, mono- and di-heteroarylamino, mono- and di-substituted heteroarylamino, mono- and di-heterocyclic amino, mono- and di-substituted heterocyclic amino, unsymmetric di-substituted amines having different substituents independently selected from the group consisting of alkyl, substituted alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, substituted heterocyclic and substituted alkenyl groups having amino groups blocked by conventional blocking groups such as Boc, Cbz, formyl, and the like or alkenyl/substituted alkenyl groups substituted with -SO2-alkyl, -SO2-substituted alkyl, -SO2-alkenyl, -SO2-substituted alkenyl, -SO2-cycloalkyl, -SO2-substituted cycloalkyl, -SO2-aryl, -SO2-substituted aryl, -SO2-heteroaryl, -SO2-substituted heteroaryl, -SO2-heterocyclic, -SO2-substituted heterocyclic and -SO2NRR where R is hydrogen or alkyl.

[0190] Preferably, the substituents are independently selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminocarbonylamino, aminocarbonyloxy, aryl, substituted aryl, aryloxy, substituted aryloxy, carboxyl, carboxyl esters, cyano, cycloalkyl, substituted cycloalkyl, cycloalkyloxy, substituted cycloalkyloxy, halogen, heteroaryl, substituted heteroaryl, heteroaryloxy,

substituted heteroaryloxy, heterocyclic, substituted heterocyclic, hydroxyl, nitro, and oxycarbonylamino.

[0191] "Alkoxy" refers to the group "alkyl-O-" which includes, by way of example, methoxy, ethoxy, ra-propoxy, zso-propoxy, <<-butoxy, tert-butoxy, sec-butoxy, ra-pentoxy, n-hexoxy, 1,2-dimethylbutoxy, and the like.

[0192] "Substituted alkoxy" refers to the group "substituted alkyl-O-".

[0193] "Alkyl" refers to linear or branched alkyl groups preferably having from 1 to 10 carbon atoms and more preferably 1 to 6 carbon atoms. This term is exemplified by groups such as methyl, t-butyl, n-heptyl, octyl and the like.

[0194] "Lower alkyl" refers to monovalent alkyl groups having from 1 to 5 carbon atoms including straight and branched chain alkyl groups. This term is exemplified by groups such as methyl, ethyl, iso-propyl, ra-propyl, rc-butyl, wo-butyl, sec-butyl, ?-butyl, n-pentyl and the like. "Lower alkyl" may be optionally substituted with a halogen, such as chloro, fluoro, bromo and the like.

[0195] "Substituted alkyl" refers to an alkyl group, of from 1 to 10 carbon atoms, having from 1 to 5 substituents independently selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, thiocarbonylamino, acyloxy, amino, amidino, alkyl amidino, thioamidino, aminoacyl, aminocarbonylamino, aminothiocarbonylamino, aminocarbonyloxy, aryl, substituted aryl, aryloxy, substituted aryloxy, aryloxylaryl, substituted aryloxyaryl, cyano, halogen, hydroxyl, nitro, carboxyl, carboxylalkyl, carboxyl-substituted alkyl, carboxyl-cycloalkyl, carboxyl-substituted cycloalkyl, carboxylaryl, carboxyl-substituted aryl, carboxylheteroaryl, carboxyl-substituted heteroaryl, carboxylheterocyclic, carboxyl-substituted heterocyclic, cycloalkyl, substituted cycloalkyl, guanidino, guanidinosulfone, thiol, thioalkyl, substituted thioalkyl, thioaryl, substituted thioaryl, thiocycloalkyl, substituted thiocycloalkyl, thioheteroaryl, substituted thioheteroaryl, thioheterocyclic, substituted thioheterocyclic, heteroaryl, substituted aryl, substituted heteroaryl, heterocyclic, substituted heterocyclic, cycloalkoxy, substituted cycloalkoxy, heteroaryloxy, substituted heteroaryloxy, heterocyclyloxy, substituted heterocyclyloxy, oxycarbonylamino, oxythiocarbonylamino, cycloalkyloxy, substituted cycloalkyloxy, heteroaryloxy, substituted heteroaryloxy, -OS(O)2-alkyl, -OS(O)2-substituted alkyl, -OS(O)2-aryl, -OS(O)2-substituted aryl, -OS(O)2-heteroaryl, -OS(O)2-substituted heteroaryl, -OS(O)2-heterocyclic, -OS(O)2-substituted heterocyclic, -OSO2-NRR where R is hydrogen or alkyl, -NRS(O)2-alkyl, -NRS(O)2-substituted alkyl, -NRS(O)2-aryl, -NRS(O)2-substituted aryl, -NRS(O)2-heteroaryl, -NRS(O)2-substituted heteroaryl, -NRS(O)2-heterocyclic, -NRS(O)2-substituted heterocyclic, -NRS(O)2-NR-alkyl, -NRS(O)2-NR-substituted alkyl, -NRS(O)2-NR-aryl, -NRS(O)2-NR-substituted aryl, -NRS(O)2-NR-heteroaryl, -NRS(O)2-NR-substituted heteroaryl, -NRS(O)2-NR-heterocyclic, -NRS(O)2-NR-substituted heterocyclic where R is hydrogen or alkyl, mono- and di-alkylamino, mono- and di-(substituted alkyl)amino, mono- and di-arylamino, mono- and di-substituted arylamino, mono- and di-heteroarylamino, mono- and di-substituted heteroarylamino, mono- and di-heterocyclic amino, mono- and di-substituted heterocyclic amino, unsymmetric di-substituted amines having different substituents independently selected from the group consisting of alkyl, substituted alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic and substituted alkyl groups having amino groups blocked by conventional blocking groups such as Boc, Cbz, formyl, and the like or alkyl/substituted alkyl

groups substituted with -SO2-alkyl, -SO2-substituted alkyl, -SO2-alkenyl, -SO2-substituted alkenyl, -SO2-cycloalkyl, -SO2-substituted cycloalkyl, -SO2-aryl, -SO2-substituted aryl, -SO2-heteroaryl, -SO2-substituted heteroaryl, -SO2-heterocyclic, -SO2-substituted heterocyclic and -SO2NRR where R is hydrogen or alkyl.

[0196] Preferably, the substituents are independently selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminocarbonylamino, aminocarbonyloxy, aryl, substituted aryl, aryloxy, substituted aryloxy, carboxyl, carboxyl esters, cyano, cycloalkyl, substituted cycloalkyl, cycloalkyloxy, substituted cycloalkyloxy, halogen, heteroaryl, substituted heteroaryl, heteroaryloxy, substituted heteroaryloxy, heterocyclic, substituted heterocyclic, hydroxyl, nitro, and oxycarbonylamino.

[0197] "Amidino" refers to the group H2NC(-NH)- and the term "alkylamidino" refers to compounds having 1 to 3 alkyl groups (e.g., alkylHNC(-NH)-).

[0198] "Amino" refers to the group -NH2.

[0199] "Substituted amino" refers to the group -NRR, where each R group is independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, substituted heterocyclic, -SO2-alkyl, -SO2-substituted alkyl, -SO2-alkenyl, -SO2-substituted alkenyl, -SO2-cycloalkyl, -SO2-substituted cycloalkyl, -SO2-aryl, -SO2-substituted aryl, -SO2-heteroaryl, -SO2-substituted heteroaryl, -SO2-heterocyclic, -SO2-substituted heterocyclic, provided that both R groups are not hydrogen; or the R groups can be joined together with the nitrogen atom to form a heterocyclic or substituted heterocyclic ring.

[0200] "Aminoacyl" refers to the groups -NRC(O)alkyl, -NRC(O)substituted alkyl, -NRC(O)cycloalkyl, -NRC(O)substituted cycloalkyl, -NRC(O)alkenyl, -NRC(O)substituted alkenyl, -NRC(O)alkynyl, -NRC(O)substituted alkynyl, -NRC(O)aryl, -NRC(O)substituted aryl, -NRC(O)heteroaryl, -NRC(O)substituted heteroaryl, -NRC(O)heterocyclic, and -NRC(O)substituted heterocyclic where R is hydrogen or alkyl and wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic are as defined herein.

[0201] "Aryl" or "Ar" refers to an unsaturated aromatic carbocyclic group of from 6 to 14 carbon atoms having a single ring (e.g., phenyl) or multiple condensed rings (e.g., naphthyl or anthryl) which condensed rings may or may not be aromatic (e.g., 2-benzoxazolinone, 2H-1,4-benzoxazin-3(4H)-one-7yl, and the like) provided that the point of attachment is through an aromatic ring atom. Preferred aryls include phenyl, naphthyl and 5,6,7,8-tetrahydronaphth-2-yl.

[0202] "Substituted aryl" refers to aryl groups which are substituted with from 1 to 3 substituents selected from the group consisting of hydroxy, acyl, acylamino, thiocarbonylamino, acyloxy, alkyl, substituted alkyl, alkoxy, substituted alkoxy, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, amidino, alkylamidino, thioamidino, amino, aminoacyl, aminocarbonyloxy, aminocarbonylamino, aminothiocarbonylamino, aryl, substituted aryl, aryloxy, substituted aryloxy, cycloalkoxy, substituted cycloalkoxy,

heteroaryloxy, substituted heteroaryloxy, heterocyclyloxy, substituted heterocyclyloxy, carboxyl, carboxylalkyl, carboxyl-substituted alkyl, carboxyl-cycloalkyl, carboxyl-substituted cycloalkyl, carboxylaryl, carboxyl-substituted aryl, carboxylheteroaryl, carboxyl-substituted heteroaryl, carboxylheterocyclic, carboxyl-substituted heterocyclic, carboxylamido, cyano, thiol, thioalkyl, substituted thioalkyl, thioaryl, substituted thioaryl, thioheteroaryl, substituted thioheteroaryl, thiocycloalkyl, substituted thiocycloalkyl, thioheterocyclic, substituted thioheterocyclic, cycloalkyl, substituted cycloalkyl, guanidino, guanidinosulfone, halo, nitro, heteroaryl, substituted heteroaryl, heterocyclic, substituted heterocyclic, cycloalkoxy, substituted cycloalkoxy, heteroaryloxy, substituted heteroaryloxy, heterocyclyloxy, substituted heterocyclyloxy, oxycarbonylamino, oxythiocarbonylamino, -S(O)2-alkyl, -S(O)2-substituted alkyl, -S(O)2-cycloalkyl, -S(O)2-substituted cycloalkyl, -S(O)2-alkenyl, -S(O)2-substituted alkenyl, -S(O)2-aryl, -S(O)2-substituted aryl, -S(O)2-heteroaryl, -S(O)2-substituted heteroaryl, -S(O)2-heterocyclic, -S(O)2-substituted heterocyclic, -OS(O)2-alkyl, -OS(O)2-substituted alkyl, -OS(O)2-aryl, -OS(O)2-substituted aryl, -OS(O)2-heteroaryl, -OS(O)2-substituted heteroaryl, -OS(O)2-heterocyclic, -OS(O)2-substituted heterocyclic, -OSO2-NRR where R is hydrogen or alkyl, -NRS(O)2-alkyl, -NRS(O)2-substituted alkyl, -NRS(O)2-aryl, -NRS(O)2-substituted aryl, -NRS(O)2-heteroaryl, -NRS(O)2-substituted heteroaryl, -NRS(O)2-heterocyclic, -NRS(O)2-substituted heterocyclic, -NRS(O)2-NR-alkyl, -NRS(O)2-NR-substituted alkyl, -NRS(O)2-NR-aryl, -NRS(O)2-NR-substituted aryl, -NRS(O)2-NR-heteroaryl, -NRS(O)2-NR-substituted heteroaryl, -NRS(O)2-NR-heterocyclic, -NRS(O)2-NR-substituted heterocyclic where R is hydrogen or alkyl, mono- and di-alkylamino, mono- and di-(substituted alkyl)amino, mono- and di-arylamino, mono- and di-substituted arylamino, mono- and di-heteroarylamino, mono- and di-substituted heteroarylamino, mono- and di-heterocyclic amino, mono- and di-substituted heterocyclic amino, unsymmetric di-substituted amines having different substituents independently selected from the group consisting of alkyl, substituted alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic and amino groups on the substituted aryl blocked by conventional blocking groups such as Boc, Cbz, formyl, and the like or substituted with -SO2NRR where R is hydrogen or alkyl.

[0203] Preferred substituents are selected from the group consisting of hydroxy, acyl, acylamino, acyloxy, alkyl, substituted alkyl, alkoxy, substituted alkoxy, alkenyl, substituted alkenyl, amino, substituted amino, aminoacyl, aminocarbonyloxy, aminocarbonylamino, aryl, substituted aryl, aryloxy, substituted aryloxy, cycloalkoxy, substituted cycloalkoxy, heteroaryloxy, substituted heteroaryloxy, heterocyclyloxy, substituted heterocyclyloxy, carboxyl, carboxyl esters, cyano, cycloalkyl, substituted cycloalkyl, halo, nitro, heteroaryl, substituted heteroaryl, heterocyclic, substituted heterocyclic, and oxycarbonylamino.

[0204] "Cycloalkenyl" refers to cyclic alkenyl groups of from 3 to 8 carbon atoms having single or multiple unsaturation but which are not aromatic.

[0205] "Cycloalkoxy" refers to -O-cycloalkyl groups.

[0206] "Substituted cycloalkoxy" refers to -O-substituted cycloalkyl groups.

[0207] "Cycloalkyl", with regard to the compounds of Formulae I and II and the PEG derivatives, refers to cyclic alkyl groups of from 3 to 12 carbon atoms having a single or multiple condensed rings including, by way of example, adamantyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclooctyl and the like. Preferably "cycloalkyl" refers to cyclic alkyl groups of from 3 to 8 carbon atoms having a single cyclic ring.

[0208] "Cycloalkyl", with regards to the compounds of Formulae III-IX, refers to cyclic alkyl groups of from 3 to 8 carbon atoms having a single cyclic ring including, by way of example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclooctyl and the like. Excluded from this definition are multi-ring alkyl groups such as adamantanyl, etc.

[0209] "Lower cycloalkyl" refers to cyclic alkyl groups of from 3 to 6 carbon atoms having a single cyclic ring including, by way of example, cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

[0210] "Substituted cycloalkyl" and "substituted cycloalkenyl" refers to a cycloalkyl or cycloalkenyl group, preferably of from 3 to 8 carbon atoms, having from 1 to 5 substituents independently selected from the group consisting of oxo (=0), thioxo (-S), alkoxy, substituted alkoxy, acyl, acylamino, thiocarbonylamino, acyloxy, amino, amidino, alkylamidino, thioamidino, aminoacyl, aminocarbonylamino, aminothiocarbonylamino, aminocarbonyloxy, aryl, substituted aryl, aryloxy, substituted aryloxy, aryloxyaryl, substituted aryloxyaryl, halogen, hydroxyl, cyano, nitro, carboxyl, carboxylalkyl, carboxyl-substituted alkyl, carboxyl-cycloalkyl, carboxyl-substituted cycloalkyl, carboxylaryl, carboxyl-substituted aryl, carboxylheteroaryl, carboxyl-substituted heteroaryl, carboxylheterocyclic, carboxyl-substituted heterocyclic, cycloalkyl, substituted cycloalkyl, guanidino, guanidinosulfone, thiol, thioalkyl, substituted thioalkyl, thioaryl, substituted thioaryl, thiocycloalkyl, substituted thiocycloalkyl, thioheteroaryl, substituted thioheteroaryl, thioheterocyclic, substituted thioheterocyclic, heteroaryl, substituted heteroaryl, heterocyclic, substituted heterocyclic, cycloalkoxy, substituted cycloalkoxy, heteroaryloxy, substituted heteroaryloxy, heterocyclyloxy, substituted heterocyclyloxy, oxycarbonylamino, oxythiocarbonylamino, -OS(O)2-alkyl, -OS(O)2-substituted alkyl, -OS(O)2-aryl, -OS(O)2-substituted aryl, -OS(O)2-heteroaryl, -OS(O)2-substituted heteroaryl, -OS(O)2-heterocyclic, -OS(O)2-substituted heterocyclic, -OSO2-NRR where R is hydrogen or alkyl, -NRS(O)2-alkyl, -NRS(O)2-substituted alkyl, -NRS(O)2-aryl, -NRS(O)2-substituted aryl, -NRS(O)2-heteroaryl, -NRS(O)2-substituted heteroaryl, -NRS(O)2-heterocyclic, -NRS(O)2-substituted heterocyclic, -NRS(O)2-NR-alkyl, -NRS(O)2-NR-substituted alkyl, -NRS(O)2-NR-aryl, -NRS(O)2-NR-substituted aryl, -NRS(O)2-NR-heteroaryl, -NRS(O)2-NR-substituted heteroaryl, -NRS(O)2-NR-heterocyclic, -NRS(O)2-NR-substituted heterocyclic where R is hydrogen or alkyl, mono- and di-alkylamino, mono- and di-(substituted alkyl)amino, mono- and di-arylamino, mono- and di-substituted arylamino, mono- and di-heteroarylamino, mono- and di-substituted heteroarylamino, mono- and di-heterocyclic amino, mono- and di-substituted heterocyclic amino, unsymmetric di-substituted amines having different substituents independently selected from the group consisting of alkyl, substituted alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic and substituted alkynyl groups having amino groups blocked by conventional blocking groups such as Boc, Cbz, formyl, and the like or alkynyl/substituted alkynyl groups substituted with -SO2-alkyl, -SO2-substituted alkyl, -SO2-alkenyl, -SO2-substituted alkenyl, -SO2-cycloalkyl, -SO2-substituted cycloalkyl, -SO2-aryl, -SO2-substituted aryl, -SO2-heteroaryl, -SO2-substituted heteroaryl, -SO2-heterocyclic, -SO2-substituted heterocyclic and -SO2NRR where R is hydrogen or alkyl.

[0211] Preferred substituents are selected from the group consisting of oxo (=0), thioxo (-S), alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminocarbonylamino, aminocarbonyloxy, aryl, substituted aryl, aryloxy, substituted aryloxy, carboxyl, carboxyl esters, cyano, cycloalkyl, substituted cycloalkyl, cycloalkyloxy, substituted cycloalkyloxy, halogen, heteroaryl, substituted heteroaryl, heteroaryloxy,

substituted heteroaryloxy, heterocyclic, substituted heterocyclic, hydroxyl, nitro, and oxycarbonylamino.

[0212] "Halo" or "halogen" refers to fluoro, chloro, bromo and iodo and preferably is fluoro, chloro or bromo.

[0213] "Heteroaryl" refers to an aromatic carbocyclic group of from 2 to 10 carbon atoms and 1 to 4 heteroatoms selected from the group consisting of oxygen, nitrogen and sulfur within the ring or oxides thereof. Such heteroaryl groups can have a single ring (e.g., pyridyl or furyl) or multiple condensed rings (e.g., indolizinyl or benzothienyl) wherein one or more of the condensed rings may or may not be aromatic provided that the point of attachment is through an aromatic ring atom. Additionally, the heteroatoms of the heteroaryl group may be oxidized, i.e., to form pyridine N-oxides or 1,1-dioxo-1,2,5-thiadiazoles and the like. Additionally, the carbon atoms of the ring may be substituted with an oxo (=0). Preferred heteroaryls include pyridyl, pyrrolyl, indolyl, furyl, pyridazinyl, pyrimidinyl, pyrazinyl, 1-oxo-1,2,5-thiadiazolyl and 1,1-dioxo-1,2,5-thiadiazolyl. The term "heteroaryl having two nitrogen atoms in the heteroaryl ring" refers to a heteroaryl group having two, and only two, nitrogen atoms in the heteroaryl ring and optionally containing 1 or 2 other heteroatoms in the heteroaryl ring, such as oxygen or sulfur.

[0214] "Substituted heteroaryl" refers to heteroaryl groups which are substituted with from 1 to 3 substituents selected from the group consisting of hydroxy, acyl, acylamino, thiocarbonylamino, acyloxy, alkyl, substituted alkyl, alkoxy, substituted alkoxy, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, amidino, alkylamidino, thioamidino, amino, aminoacyl, aminocarbonyloxy, aminocarbonylamino, aminothiocarbonylamino, aryl, substituted aryl, aryloxy, substituted aryloxy, cycloalkoxy, substituted cycloalkoxy, heteroaryloxy, substituted heteroaryloxy, heterocyclyloxy, substituted heterocyclyloxy, carboxyl, carboxylalkyl, carboxyl-substituted alkyl, carboxyl-cycloalkyl, carboxyl-substituted cycloalkyl, carboxylaryl, carboxyl-substituted aryl, carboxylheteroaryl, carboxyl-substituted heteroaryl, carboxylheterocyclic, carboxyl-substituted heterocyclic, carboxylamido, cyano, thiol, thioalkyl, substituted thioalkyl, thioaryl, substituted thioaryl, thioheteroaryl, substituted thioheteroaryl, thiocycloalkyl, substituted thiocycloalkyl, thioheterocyclic, substituted thioheterocyclic, cycloalkyl, substituted cycloalkyl, guanidino, guanidinosulfone, halo, nitro, heteroaryl, substituted heteroaryl, heterocyclic, substituted heterocyclic, cycloalkoxy, substituted cycloalkoxy, heteroaryloxy, substituted heteroaryloxy, heterocyclyloxy, substituted heterocyclyloxy, oxycarbonylamino, oxythiocarbonylamino, -S(O)2-alkyl, -S(O)2-substituted alkyl, -S(O)2-cycloalkyl, -S(O)2-substituted cycloalkyl, -S(O)2-alkenyl, -S(O)2-substituted alkenyl, -S(O)2-aryl, -S(O)2-substituted aryl, -S(O)2-heteroaryl, -S(O)2-substituted heteroaryl, -S(O)2-heterocyclic, -S(O)2-substituted heterocyclic, -OS(O)2-alkyl, -OS(O)2-substituted alkyl, -OS(O)2-aryl, OS(O)2-substituted aryl, -OS(O)2-heteroaryl, -OS(0)2-substituted heteroaryl, -OS(O)2-heterocyclic, -OS(O)2-substituted heterocyclic, -OSO2-NRR where R is hydrogen or alkyl, -NRS(O)2-alkyl, -NRS(O)2-substituted alkyl, -NRS(O)2-aryl, -NRS(O)2-substituted aryl, -NRS(O)2-heteroaryl, -NRS(O)2-substituted heteroaryl, -NRS(O)2-heterocyclic, -NRS(O)2-substituted heterocyclic, -NRS(O)2-NR-alkyl, -NRS(O)2-NR-substituted alkyl, -NRS(O)2-NR-aryl, -NRS(O)2-NR-substituted aryl, -NRS(O)2-NR-heteroaryl, -NRS(O)2-NR-substituted heteroaryl, -NRS(O)2-NR-heterocyclic, -NRS(O)2-NR-substituted heterocyclic where R is hydrogen or alkyl, mono- and di-alkylamino, mono- and di-(substituted alkyl)amino, mono- and di-arylamino, mono- and di-substituted arylamino, mono- and di-heteroarylamino, mono- and di-substituted heteroarylamino, mono- and di-heterocyclic amino, mono- and di-substituted heterocyclic

amino, unsymmetric di-substituted amines having different substituents independently selected from the group consisting of alkyl, substituted alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic and amino groups on the substituted aryl blocked by conventional blocking groups such as Boc, Cbz, formyl, and the like or substituted with -SO2NRR where R is hydrogen or alkyl.

[0215] Preferably the substituents are selected from the group consisting of those defined above as preferred for substituted aryl.

[0216] "Heteroaryloxy" refers to the group -O-heteroaryl and "substituted heteroaryloxy" refers to the group -O-substituted heteroaryl.

[0217] "Heteroaralkoxy" refers to the group heteroaryl-alkylene-O-.

[0218] "Substituted heteroaralkoxy" refers to the group substituted heteroaryl-alkylene-O-.

[0219] "Heterocycle" or "heterocyclic" refers to a saturated or unsaturated group having a single ring or multiple condensed rings, from 1 to 10 carbon atoms and from 1 to 4 hetero atoms selected from the group consisting of nitrogen, sulfur or oxygen within the ring wherein, in fused ring systems, one or more the rings can be aryl or heteroaryl.

[0220] "Substituted heterocyclic" refers to heterocycle groups which are substituted with from 1 to 3 substituents selected from the group consisting of oxo (=0), thioxo (-S), alkoxy, substituted alkoxy, acyl, acylamino, thiocarbonylamino, acyloxy, amino, amidino, alkylamidino, thioamidino, aminoacyl, aminocarbonylamino, aminothiocarbonylamino, aminocarbonyloxy, aryl, substituted aryl, aryloxy, substituted aryloxy, aryloxyaryl, substituted aryloxyaryl, halogen, hydroxyl, cyano, nitro, carboxyl, carboxylalkyl, carboxyl-substituted alkyl, carboxyl-cycloalkyl, carboxyl-substituted cycloalkyl, carboxylaryl, carboxyl-substituted aryl, carboxylheteroaryl, carboxyl-substituted heteroaryl, carboxylheterocyclic, carboxyl-substituted heterocyclic, cycloalkyl, substituted cycloalkyl, guanidino, guanidinosulfone, thiol, thioalkyl, substituted thioalkyl, thioaryl, substituted thioaryl, thiocycloalkyl, substituted thiocycloalkyl, thioheteroaryl, substituted thioheteroaryl, thioheterocyclic, substituted thioheterocyclic, heteroaryl, substituted heteroaryl, heterocyclic, substituted heterocyclic, cycloalkoxy, substituted cycloalkoxy, heteroaryloxy, substituted heteroaryloxy, -C(O)O-aryl, -C(O)O-substituted aryl, heterocyclyloxy, substituted heterocyclyloxy, oxycarbonylamino, oxythiocarbonylamino, -0S(0)2-alkyl, -OS(O)2-substituted alkyl, -OS(O)2-aryl, -OS(O)2-substituted aryl, -OS(O)2-heteroaryl, -OS(O)2-substituted heteroaryl, -OS(O)2-heterocyclic, -OS(O)2-substituted heterocyclic, -OSO2-NRR where R is hydrogen or alkyl, -NRS(O)2-alkyl, -NRS(O)2-substituted alkyl, -NRS(O)2-aryl, -NRS(O)2-substituted aryl, -NRS(O)2-heteroaryl, -NRS(O)2-substituted heteroaryl, -NRS(O)2-heterocyclic, -NRS(O)2-substituted heterocyclic, -NRS(O)2-NR-alkyl, -NRS(O)2-NR-substituted alkyl, -NRS(O)2-NR-aryl, -NRS(O)2-NR-substituted aryl, -NRS(O)2-NR-heteroaryl, -NRS(O)2-NR-substituted heteroaryl, -NRS(O)2-NR-heterocyclic, -NRS(O)2-NR-substituted heterocyclic where R is hydrogen or alkyl, mono- and di-alkylamino, mono- and di-(substituted alkyl)amino, mono- and di-arylamino, mono- and di-substituted arylamino, mono- and di-heteroarylamino, mono- and di-substituted heteroarylamino, mono- and di-heterocyclic amino, mono- and di-substituted heterocyclic amino, unsymmetric di-substituted amines having different substituents independently selected from the group consisting of alkyl, substituted alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic and substituted alkynyl groups having amino groups

blocked by conventional blocking groups such as Boc, Cbz, formyl, and the like or alkynyl/substituted alkynyl groups substituted with -SO2-alkyl, -SO2-substituted alkyl, -SO2-alkenyl, -SO2-substituted alkenyl, -SO2-cycloalkyl, -SO2-substituted cycloalkyl, -SO2-aryl, -SO2-substituted aryl, -SO2-heteroaryl, -SO2-substituted heteroaryl, -SO2-heterocyclic, -SO2-substituted heterocyclic and -SO2NRR where R is hydrogen or alkyl.

[0221] Preferably, the substituents are selected from the group consisting of the preferred substitutents defined for substituted cycloalkyl.

[0222] Examples of heterocycles and heteroaryls include, but are not limited to, azetidine, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidin, pyridazine, indolizine, isoindole, indole, dihydroindole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, indoline, phthalimide, 1,2,3,4-tetrahydroisoquinoline, 4,5,6,7-tetrahydrobenzo[b]thiophene, thiazole, thiazolidine, thiophene, benzo[b]thiophene, morpholino, morpholinyl, thiomorpholino, thiomorpholinyl (also referred to as thiamorpholinyl), piperidinyl, pyrrolidine, tetrahydrofuranyl, and the like.

[0223] "Optionally substituted" means that the recited group may be unsubstituted or the recited group may be substituted.

[0224] "Pharmaceutically acceptable carrier" means a carrier that is useful in preparing a pharmaceutical composition or formulation that is generally safe, non-toxic and neither biologically nor otherwise undesirable, and includes a carrier that is acceptable for veterinary use as well as human pharmaceutical use. A pharmaceutically acceptable carrier or excipient as used in the specification and claims includes both one or more than one of such carriers.

[0225] "Pharmaceutically-acceptable cation" refers to the cation of a pharmaceutically-acceptable salt.

[0226] "Pharmaceutically acceptable salt" refers to salts which retain the biological effectiveness and properties of the compounds of this invention and which are not biologically or otherwise undesirable. Pharmaceutically acceptable salts refer to pharmaceutically acceptable salts of the compounds, which salts are derived from a variety of organic and inorganic counter ions well known in the art and include, by way of example only, sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium, and the like; and when the molecule contains a basic functionality, salts of organic or inorganic acids, such as hydrochloride, hydrobromide, tartrate, mesylate, acetate, maleate, oxalate and the like.

[0227] Pharmaceutically-acceptable base addition salts can be prepared from inorganic and organic bases. Salts derived from inorganic bases, include by way of example only, sodium, potassium, lithium, ammonium, calcium and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary and tertiary amines, such as alkyl amines, dialkyl amines, trialkyl amines, substituted alkyl amines, di(substituted alkyl) amines, tri(substituted alkyl) amines, alkenyl amines, dialkenyl amines, trialkenyl amines, substituted alkenyl amines, di(substituted alkenyl) amines, tri(substituted alkenyl) amines, cycloalkyl amines, di(cycloalkyl) amines, tri(cycloalkyl) amines, substituted cycloalkyl amines, disubstituted cycloalkyl amine, trisubstituted cycloalkyl amines, cycloalkenyl

amines, di(cycloalkenyl) amines, tri(cycloalkenyl) amines, substituted cycloalkenyl amines, disubstituted cycloalkenyl amine, trisubstituted cycloalkenyl amines, aryl amines, diaryl amines, triaryl amines, heteroaryl amines, diheteroaryl amines, triheteroaryl amines, heterocyclic amines, diheterocyclic amines, triheterocyclic amines, mixed di- and tri-amines where at least two of the substituents on the amine are different and are selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl, heterocyclic, and the like. Also included are amines where the two or three substituents, together with the amino nitrogen, form a heterocyclic or heteroaryl group.

[0228] Examples of suitable amines include, by way of example only, isopropylamine, trimethyl amine, diethyl amine, tri(iso-propyl) amine, tri(n-propyl) amine, ethanolamine, 2-dimethylaminoethanol, tromethamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, N-alkylglucamines, theobromine, purines, piperazine, piperidine, morpholine, N-ethylpiperidine, and the like. It should also be understood that other carboxylic acid derivatives would be useful in the practice of this invention, for example, carboxylic acid amides, including carboxamides, lower alkyl carboxamides, dialkyl carboxamides, and the like.

[0229] Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Salts derived from inorganic acids include hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Salts derived from organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluene-sulfonic acid, salicylic acid, and the like.

[0230] A compound of Formula (I) may act as a pro-drug. Prodrug means any compound which releases an active parent drug according to Formula (I) in vivo when such prodrug is administered to a mammalian subject. Prodrugs of a compound of Formula (I) are prepared by modifying functional groups present in the compound of Formula (I) in such a way that the modifications may be cleaved in vivo to release the parent compound. Prodrugs include compounds of Formula (I) wherein a hydroxy, amino, or sulfhydryl group in compound (I) is bonded to any group that may be cleaved in vivo to regenerate the free hydroxyl, amino, or sulfhydryl group, respectively. Examples of prodrugs include, but are not limited to esters (e.g., acetate, formate, and benzoate derivatives), carbamates (e.g., N,N-dimethylamino-carbonyl) of hydroxy functional groups in compounds of Formula (I), and the like.

[0231] "Treating" or "treatment" of a disease includes:

(1) preventing the disease, i.e. causing the clinical symptoms of the disease not to develop in a mammal that may be exposed to or predisposed to the disease but does not yet experience or display symptoms of the disease,
(2) inhibiting the disease, i.e., arresting or reducing the development of the disease or its clinical symptoms, or
(3) relieving the disease, i.e., causing regression of the disease or its clinical symptoms.

[0235] A "therapeutically effective amount" means the amount of a compound or antibody that, when administered to a mammal for treating a disease, is sufficient to effect such treatment for the disease. The "therapeutically effective amount" will vary depending on the

compound, the disease and its severity and the age, weight, etc., of the mammal to be treated.

Pharmaceutical Formulations of the Compounds

[0236] In general, the compounds of the subject invention will be administered in a therapeutically effective amount by any of the accepted modes of administration for these compounds. The compounds can be administered by a variety of routes, including, but not limited to, oral, parenteral (e.g., subcutaneous, subdural, intravenous, intramuscular, intrathecal, intraperitoneal, intracerebral, intraarterial, or intralesional routes of administration), topical, intranasal, localized (e.g., surgical application or surgical suppository), rectal, and pulmonary (e.g., aerosols, inhalation, or powder). Accordingly, these compounds are effective as both injectable and oral compositions. The compounds can be administered continuously by infusion or by bolus injection. Preferably, the compounds are administered by parenteral routes. More preferably, the compounds are administered by intravenous routes. Such compositions are prepared in a manner well known in the pharmaceutical art.

[0237] The actual amount of the compound of the subject invention, i.e., the active ingredient, will depend on a number of factors, such as the severity of the disease, i.e., the condition or disease to be treated, age and relative health of the subject, the potency of the compound used, the route and form of administration, and other factors.

[0238] Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds that exhibit large therapeutic indices are preferred.

[0239] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range which includes the IC50 (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography. The effective blood level of the compounds of the subject invention is preferably greater than or equal to 10 ng/ml.

[0240] The amount of the pharmaceutical composition administered to the patient will vary depending upon what is being administered, the purpose of the administration, such as prophylaxis or therapy, the state of the patient, the manner of administration, and the like, hi therapeutic applications, compositions are administered to a patient already suffering from a disease in an amount sufficient to cure or at least partially arrest the symptoms of the disease and its complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on the disease condition being

treated as well as by the judgment of the attending clinician depending upon factors such as the severity of the inflammation, the age, weight and general condition of the patient, and the like.

[0241] The compositions administered to a patient are in the form of pharmaceutical compositions described supra. These compositions may be sterilized by conventional sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous carrier prior to administration. The pH of the compound preparations typically will be between 3 and 11, more preferably from 5 to 9 and most preferably from 7 to 8. It will be understood that use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of pharmaceutical salts.

[0242] The active compound is effective over a wide dosage range and is generally administered in a pharmaceutically or therapeutically effective amount. The therapeutic dosage of the compounds of the present invention will vary according to, for example, the particular use for which the treatment is made, the manner of administration of the compound, the health and condition of the patient, and the judgment of the prescribing physician. For example, for intravenous administration, the dose will typically be in the range of about 0.5 mg to about 100 mg per kilogram body weight, preferably about 3 mg to about 50 mg per kilogram body weight. Effective doses can be extrapolated from dose-response curves derived from in vitro or animal model test systems. Typically, the clinician will administer the compound until a dosage is reached that achieves the desired effect.

[0243] When employed as pharmaceuticals, the compounds of the subject invention are usually administered in the form of pharmaceutical compositions. This invention also includes pharmaceutical compositions, which contain as the active ingredient, one or more of the compounds of the subject invention above, associated with one or more pharmaceutically acceptable carriers or excipients. The excipient employed is typically one suitable for administration to human subjects or other mammals. In making the compositions of this invention, the active ingredient is usually mixed with an excipient, diluted by an excipient or enclosed within a carrier which can be in the form of a capsule, sachet, paper or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

[0244] In preparing a formulation, it may be necessary to mill the active compound to provide the appropriate particle size prior to combining with the other ingredients. If the active compound is substantially insoluble, it ordinarily is milled to a particle size of less than 200 mesh. If the active compound is substantially water soluble, the particle size is normally adjusted by milling to provide a substantially uniform distribution in the formulation, e.g., about 40 mesh.

[0245] Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, sterile water, syrup, and

methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents. The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art.

[0246] The quantity of active compound in the pharmaceutical composition and unit dosage form thereof may be varied or adjusted widely depending upon the particular application, the manner or introduction, the potency of the particular compound, and the desired concentration. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient. The concentration of therapeutically active compound may vary from about 1 mg/ml to 250 g/ml.

[0247] Preferably, the compound can be formulated for parenteral administration in a suitable inert carrier, such as a sterile physiological saline solution. For example, the concentration of compound in the carrier solution is typically between about 1-100 mg/ml. The dose administered will be determined by route of administration. Preferred routes of administration include parenteral or intravenous administration. A therapeutically effective dose is a dose effective to produce a significant steroid tapering. Preferably, the amount is sufficient to produce a statistically significant amount of steroid tapering in a subject.

[0248] Administration of therapeutic agents by intravenous formulation is well known in the pharmaceutical industry. An intravenous formulation should possess certain qualities aside from being just a composition in which the therapeutic agent is soluble. For example, the formulation should promote the overall stability of the active ingredient(s), also, the manufacture of the formulation should be cost effective. All of these factors ultimately determine the overall success and usefulness of an intravenous formulation.

[0249] Other accessory additives that may be included in pharmaceutical formulations of compounds of the present invention as follow: solvents: ethanol, glycerol, propylene glycol; stabilizers: EDTA (ethylene diamine tetraacetic acid), citric acid; antimicrobial preservatives: benzyl alcohol, methyl paraben, propyl paraben; buffering agents: citric acid/sodium citrate, potassium hydrogen tartrate, sodium hydrogen tartrate, acetic acid/sodium acetate, maleic acid/sodium maleate, sodium hydrogen phthalate, phosphoric acid/potassium dihydrogen phosphate, phosphoric acid/disodium hydrogen phosphate; and tonicity modifiers: sodium chloride, mannitol, dextrose.

[0250] The presence of a buffer is necessary to maintain the aqueous pH in the range of from about 4 to about 8 and more preferably in a range of from about 4 to about 6. The buffer system is generally a mixture of a weak acid and a soluble salt thereof, e.g., sodium citrate/citric acid; or the monocation or dication salt of a dibasic acid, e.g., potassium hydrogen tartrate; sodium hydrogen tartrate, phosphoric acid/potassium dihydrogen phosphate, and phosphoric acid/disodium hydrogen phosphate.

[0251] The amount of buffer system used is dependent on (1) the desired pH; and (2) the amount of drug. Generally, the amount of buffer used is in a 0.5:1 to 50:1 mole ratio of buffenalendronate (where the moles of buffer are taken as the combined moles of the buffer

ingredients, e.g., sodium citrate and citric acid) of formulation to maintain a pH in the range of 4 to 8 and generally, a 1:1 to 10:1 mole ratio of buffer (combined) to drug present is used.

[0252] A useful buffer in the invention is sodium citrate/citric acid in the range of 5 to 50 mg per ml. sodium citrate to 1 to 15 mg per ml. citric acid, sufficient to maintain an aqueous pH of 4-6 of the composition.

[0253] The buffer agent may also be present to prevent the precipitation of the drug through soluble metal complex formation with dissolved metal ions, e.g., Ca, Mg, Fe, Al, Ba, which may leach out of glass containers or rubber stoppers or be present in ordinary tap water. The agent may act as a competitive complexing agent with the drug and produce a soluble metal complex leading to the presence of undesirable particulates.

[0254] In addition, the presence of an agent, e.g., sodium chloride in an amount of about of 1-8 mg/ml, to adjust the tonicity to the same value of human blood may be required to avoid the swelling or shrinkage of erythrocytes upon administration of the intravenous formulation leading to undesirable side effects such as nausea or diarrhea and possibly to associated blood disorders. In general, the tonicity of the formulation matches that of human blood which is in the range of 282 to 288 mOsm/kg, and in general is 285 mOsm/kg, which is equivalent to the osmotic pressure corresponding to a 0.9% solution of sodium chloride.

[0255] The intravenous formulation can be administered by direct intravenous injection, i.v. bolus, or can be administered by infusion by addition to an appropriate infusion solution such as 0.9% sodium chloride injection or other compatible infusion solution.

[0256] The compositions are preferably formulated in a unit dosage form, each dosage containing from about 5 to about 100 mg, more usually about 10 to about 30 mg, of the active ingredient. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient.

[0257] The active compound is effective over a wide dosage range and is generally administered in a pharmaceutically effective amount. It, will be understood, however, that the amount of the compound actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

[0258] For preparing solid compositions such as tablets, the principal active ingredient is mixed with a pharmaceutical excipient to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation is then subdivided into unit dosage forms of the type described above containing from, for example, 0.1 to about 500 mg of the active ingredient of the present invention.

[0259] The tablets or pills of the present invention may be coated or otherwise compounded

to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

[0260] The liquid forms in which the novel compositions of the present invention may be incorporated for administration orally or by injection include aqueous solutions suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

[0261] Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described supra. Preferably the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably pharmaceutically acceptable solvents may be nebulized by use of inert gases. Nebulized solutions may be breathed directly from the nebulizing device or the nebulizing device may be attached to a face masks tent, or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions may be administered, preferably orally or nasally, from devices which deliver the formulation in an appropriate manner.

[0262] The compounds of this invention can be administered in a sustained release form. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the protein, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (e.g., poly(2-hydroxyethyl-methacrylate) as described by Langer et al., J. Biomed. Mater. Res. 15: 167-277 (1981) and Langer, Chem. Tech. 12: 98-105 (1982) or poly(vinyl alcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al., Biopolymers 22: 547-556, 1983), non-degradable ethylene-vinyl acetate (Langer et ah, supra), degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT(TM) (i.e., injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid (EP-133,988).

[0263] The compounds of this invention can be administered in a sustained release form, for example a depot injection, implant preparation, or osmotic pump, which can be formulated in such a manner as to permit a sustained release of the active ingredient. Implants for sustained release formulations are well-known in the art. Implants may be formulated as, including but not limited to, microspheres, slabs, with biodegradable or non-biodegradable polymers. For example, polymers of lactic acid and/or glycolic acid form an erodible polymer that is well-tolerated by the host. The implant is placed in proximity to the site of protein deposits (e.g., the site of formation of amyloid deposits associated with neurodegenerative disorders), so that the local concentration of active agent is increased at that site relative to the rest of the body.

[0264] The following formulation examples illustrate pharmaceutical compositions of the present invention.

Formulation Example 1

[0265] Hard gelatin capsules containing the following ingredients are prepared:

[0000]

Ingredient	Quantity (mg/capsule)
Active Ingredient	30.0
Starch	305.0
Magnesium stearate	5.0

[0266] The above ingredients are mixed and filled into hard gelatin capsules in 340 mg quantities.

Formulation Example 2

[0267] A tablet formula is prepared using the ingredients below:

[0000]

Ingredient	Quantity (mg/capsule)
Active Ingredient	25.0
Cellulose, microcrystalline	200.0
Colloidal silicon dioxide	10.0
Stearic acid	5.0

[0268] The components are blended and compressed to form tablets, each weighing 240 mg.

Formulation Example 3

[0269] A dry powder inhaler formulation is prepared containing the following components:

[0000]

Ingredient	Weight %
Active Ingredient	5
Lactose	95

[0270] The active mixture is mixed with the lactose and the mixture is added to a dry powder inhaling appliance.

Formulation Example 4

[0271] Tablets, each containing 30 mg of active ingredient, are prepared as follows:

[0000]
    Quantity

Ingredient (mg/capsule)
Active Ingredient 30.0 mg
Starch 45.0 mg
Microcrystalline cellulose 35.0 mg
Polyvinylpyrrolidone 4.0 mg
(as 10% solution in water)
Sodium Carboxymethyl starch 4.5 mg
Magnesium stearate 0.5 mg
Talc 1.0 mg
Total 120 mg

[0272] The active ingredient, starch and cellulose are passed through a No. 20 mesh U.S. sieve and mixed thoroughly. The solution of polyvinyl-pyrrolidone is mixed with the resultant powders, which are then passed through a 16 mesh U.S. sieve. The granules so produced are dried at 50[deg.] to 60[deg.] C. and passed through a 16 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 30 mesh U.S. sieve, are then added to the granules, which after mixing, are compressed on a tablet machine to yield tablets each weighing 150 mg.

Formulation Example 5

[0273] Capsules, each containing 40 mg of medicament are made as

[0274] follows:

[0000]
  Quantity
Ingredient (mg/capsule
Active Ingredient   40.0 mg
Starch 109.0 mg
Magnesium stearate   1.0 mg
Total 150.0 mg

[0275] The active ingredient, cellulose, starch, an magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 150 mg quantities.

Formulation Example 6

[0276] Suppositories, each containing 25 mg of active ingredient are made as follows:

[0000]
Ingredient Amount
Active Ingredient 25 mg
Saturated fatty acids glycerides to 2,000 mg

[0277] The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2.0 g capacity and allowed to cool.

Formulation Example 7

[0278] Suspensions, each containing 50 mg of medicament per 5.0 ml dose are made as follows:

[0000]

Ingredient	Amount	
Active Ingredient	50.0	mg
Xanthan gum	4.0	mg
Sodium carboxymethyl cellose (11%)		
Microcrystalline cellulose (89%)	500	mg
Sucrose	1.75	g
Sodium benzoate	10.0	mg
Flavor and color	q.v.	
Purified water	to 5.0	ml

[0279] The medicament, sucrose and xanthan gum are blended, passed through a No. 10 mesh U.S. sieve, and then mixed with a previously made solution of the microcrystalline cellulose and sodium carboxymethyl cellulose in water. The sodium benzoate, flavor, and color are diluted with some of the water and added with stirring. Sufficient water is then added to produce the required volume.

Formulation Example 8

[0280] Hard gelatin tablets, each containing 15 mg of active ingredient are made as follows:

[0000]

Ingredient	Quantity (mg/capsule)
Active Ingredient	15.0 mg
Starch	407.0 mg
Magnesium stearate	3.0 mg
Total	425.0 mg

[0281] The active ingredient, cellulose, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 560 mg quantities.

Formulation Example 9

[0282] An intravenous formulation may be prepared as follows:

[0000]

Ingredient	Quantity	
Active Ingredient	250.0	mg
Isotonic saline	1000	ml

[0283] Therapeutic compound compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle or similar sharp instrument.

Formulation Example 10

[0284] A topical formulation may be prepared as follows:

[0000]
  Ingredient Quantity
  Active Ingredient 1-10 g
  Emulsifying Wax 30 g
  Liquid Paraffin 20 g
  White Soft Paraffin to 100 g

[0285] The white soft paraffin is heated until molten. The liquid paraffin and emulsifying wax are incorporated and stirred until dissolved. The active ingredient is added and stirring is continued until dispersed. The mixture is then cooled until solid.

Formulation Example 11

[0286] An aerosol formulation may be prepared as follows: A solution of the candidate compound in 0.5% sodium bicarbonate/saline (w/v) at a concentration of 30.0 mg/mL is prepared using the following procedure:

A. Preparation of 0.5% Sodium Bicarbonate/Saline Stock Solution: 100.0 mL

[0287]

[0000]
  Ingredient Gram/100.0 mL Final Concentration
  Sodium Bicarbonate 0.5 g 0.5%
  Saline q.s. ad 100.0 mL q.s. ad 100%

[0288] Procedure:

1. Add 0.5 g sodium bicarbonate into a 100 mL volumetric flask.
2. Add approximately 90.0 mL saline and sonicate until dissolved.
3. Q.S. to 100.0 mL with saline and mix thoroughly.

B. Preparation of 30.0 mg/mL Candidate Compound: 10.0 mL

[0292]

[0000]
Ingredient Gram/10.0 mL Final Concentration
Candidate Compound 0.300 g 30.0 mg/mL
0.5% Sodium Bicarbonate/ q.s. ad 10.0 mL q.s ad 100%
Saline Stock Solution

[0293] Procedure:

1. Add 0.300 g of the candidate compound into a 10.0 mL volumetric flask.
2. Add approximately 9.7 mL of 0.5% sodium bicarbonate/saline stock solution.

3. Sonicate until the candidate compound is completely dissolved.
4. Q.S. to 10.0 mL with 0.5% sodium bicarbonate/saline stock solution and mix

[0298] Another preferred formulation employed in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of the compounds of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. See, e.g., U.S. Pat. No. 5,023,252, issued Jun. 11, 1991, herein incorporated by reference. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

[0299] Direct or indirect placement techniques may be used when it is desirable or necessary to introduce the pharmaceutical composition to the brain. Direct techniques usually involve placement of a drug delivery catheter into the host's ventricular system to bypass the blood-brain barrier. One such implantable delivery system used for the transport of biological factors to specific anatomical regions of the body is described in U.S. Pat. No. 5,011,472, which is herein incorporated by reference.

[0300] Indirect techniques, which are generally preferred, usually involve formulating the compositions to provide for drug latentiation by the conversion of hydrophilic drugs into lipid-soluble drugs. Latentiation is generally achieved through blocking of the hydroxy, carbonyl, sulfate, and primary amine groups present on the drug to render the drug more lipid soluble and amenable to transportation across the blood-brain barrier. Alternatively, the delivery of hydrophilic drugs may be enhanced by intra-arterial infusion of hypertonic solutions which can transiently open the blood-brain barrier.

[0301] In order to enhance serum half-life, the compounds may be encapsulated, introduced into the lumen of liposomes, prepared as a colloid, or other conventional techniques may be employed which provide an extended serum half-life of the compounds. A variety of methods are available for preparing liposomes, as described in, e.g., Szoka et al., U.S. Pat. Nos. 4,235,871, 4,501,728 and 4,837,028 each of which is incorporated herein by reference.

[0302] Pharmaceutical compositions of the invention are suitable for use in a variety of drug delivery systems. Suitable formulations for use in the present invention are found in Remington's Pharmaceutical Sciences, Mace Publishing Company, Philadelphia, Pa., 17th ed. (1985).

Utility

[0303] The compounds and pharmaceutical compositions of the invention show biological activity in treating and preventing viral infections and associated diseases, and, accordingly, have utility in treating viral infections and associated diseases, such as Hemorrhagic fever viruses, in mammals including humans.

[0304] As noted above, the compounds described herein are suitable for use in a variety of drug delivery systems described above. Additionally, in order to enhance the in vivo serum half life of the administered compound, the compounds may be encapsulated, introduced into the lumen of liposomes, prepared as a colloid, or other conventional techniques may be employed which provide an extended serum half life of the compounds. A variety of methods are available for preparing liposomes, as described in, e.g., Szoka, et al, U.S. Pat. Nos.

4,235,871, 4,501,728 and 4,837,028, each of which is incorporated herein by reference.

[0305] The amount of compound administered to the patient will vary depending upon what is being administered, the purpose of the administration, such as prophylaxis or therapy, the state of the patient, the manner of administration, and the like. In therapeutic applications, compositions are administered to a patient already suffering from AD in an amount sufficient to at least partially arrest further onset of the symptoms of the disease and its complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on the judgment of the attending clinician depending upon factors such as the degree or severity of AD in the patient, the age, weight and general condition of the patient, and the like. Preferably, for use as therapeutics, the compounds described herein are administered at dosages ranging from about 0.1 to about 500 mg/kg/day.

[0306] In prophylactic applications, compositions are administered to a patient at risk of developing AD (determined for example by genetic screening or familial trait) in an amount sufficient to inhibit the onset of symptoms of the disease. An amount adequate to accomplish this is defined as "prophylactically effective dose." Amounts effective for this use will depend on the judgment of the attending clinician depending upon factors such as the age, weight and general condition of the patient, and the like. Preferably, for use as prophylactics, the compounds described herein are administered at dosages ranging from about 0.1 to about 500 mg/kg/day.

[0307] As noted above, the compounds administered to a patient are in the form of pharmaceutical compositions described above. These compositions may be sterilized by conventional sterilization techniques, or may be sterile filtered. When aqueous solutions are employed, these may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous carrier prior to administration. The pH of the compound preparations typically will be between 3 and 11, more preferably from 5-9 and most preferably from 7 and 8. It will be understood that use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of pharmaceutical salts.

[0308] Hemorrhagic fever viruses (HFVs) are RNA viruses that cause a variety of disease syndromes with similar clinical characteristics. HFVs that are of concern as potential biological weapons include but are not limited to: Arenaviridae (Junin, Machupo, Guanavito, Sabia and Lassa), Filoviridae (ebola and Marburg viruses), Flaviviridae (yellow fever, omsk hemorrhagic fever and Kyasanur Forest disease viruses), and Bunyaviridae (Rift Valley fever). The naturally occurring arenaviruses and potential engineered arenaviruses are included in the Category A Pathogen list according to the Center for Disease control and Prevention as being among those agents that have greatest potential for mass casualties.

[0309] Risk factors include: travel to Africa or Asia, handling of animal carcasses, contact with infected animals or people, and/or arthropod bites. Arenaviruses are highly infectious after direct contact with infected blood and/or bodily secretions. Humans usually become infected through contact with infected rodents, the bite of an infected arthropod, direct contact with animal carcasses, inhalation of infectious rodent excreta and/or injection of food contaminated with rodent excreta. The Tacaribe virus has been associated with bats. Airborne transmission of hemorrhagic fever is another mode, but somewhat less common. Person-to-person contact may also occur in some cases.

[0310] All of the hemorrhagic fevers exhibit similar clinical symptoms. However, in general the clinical manifestations are non-specific and variable. The incubation period is approximately 7-14 days. The onset is gradual with fever and malaise, tachypnea, relative bradycardia, hypotension, circulatory shock, conjeunctival injection, pharyngitis, lymphadenopathy, encephalitis, myalgia, back pain, headache and dizziness, as well as hyperesthesia of the skin. Some infected patients may not develop hemorrhagic manifestations.

[0311] Methods of diagnosis at specialized laboratories include antigen detection by antigen-capture enzyme-linked immunosorbent assay (ELISA), IgM antibody detection by antibody-capture enzyme-linked immunosorbent assay, reverse transcriptase polymerase chain reaction (RT-PCR), and viral isolation. Antigen detection (by enzyme-linked immunosorbent assay) and reverse transcriptase polymerase chain reaction are the most useful diagnostic techniques in the acute clinical setting. Viral isolation is of limited value because it requires a biosafety level 4 (BSL-4) laboratory.

[0312] The following synthetic and biological examples are offered to illustrate this invention and are not to be construed in any way as limiting the scope of this invention.

EXAMPLES

[0313] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and is not intended to limit the scope of what the inventors regard as their invention nor is it intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Synthesis of Compounds

[0314] The compounds of formula I, as well as IA and IB above are readily prepared via several divergent synthetic routes with the particular route selected relative to the ease of compound preparation, the commercial availability of starting materials, and the like.

[0315] The compounds of Formulae I and II can be prepared from readily available starting materials using the following general methods and procedures. It will be appreciated that where typical or preferred process conditions (i.e., reaction temperatures, times, mole ratios of reactants, solvents, pressures, etc.) are given, other process conditions can also be used unless otherwise stated. Optimum reaction conditions may vary with the particular reactants or solvent used, but such conditions can be determined by one skilled in the art by routine optimization procedures.

[0316] Additionally, as will be apparent to those skilled in the art, conventional protecting groups may be necessary to prevent certain functional groups from undergoing undesired reactions. Suitable protecting groups for various functional groups as well as suitable conditions for protecting and deprotecting particular functional groups are well known in the art. For example, numerous protecting groups are described in T. W. Greene and G. M. Wuts,

Protecting Groups in Organic Synthesis, Second Edition, Wiley, New York, 1991, and references cited therein.

[0317] Furthermore, the compounds of this invention will typically contain one or more chiral centers. Accordingly, if desired, such compounds can be prepared or isolated as pure stereoisomers, i.e., as individual enantiomers or diastereomers, or as stereoisomer-enriched mixtures. All such stereoisomers (and enriched mixtures) are included within the scope of this invention, unless otherwise indicated. Pure stereoisomers (or enriched mixtures) may be prepared using, for example, optically active starting materials or stereoselective reagents well-known in the art. Alternatively, racemic mixtures of such compounds can be separated using, for example, chiral column chromatography, chiral resolving agents and the like.

[0318] Unless otherwise indicated, the products of this invention are a mixture of R, S enantiomers. Preferably, however, when a chiral product is desired, the chiral product can be obtained via purification techniques which separates enantiomers from a R, S mixture to provide for one or the other stereoisomer. Such techniques are known in the art.

[0319] In another embodiment, the compounds can be provided as prodrugs which convert (e.g., hydrolyze, metabolize, etc.) in vivo to a compound of Formula I above. In a preferred example of such an embodiment, the carboxylic acid group of the compound of Formula I is modified into a group which, in vivo, will convert to a carboxylic acid group (including salts thereof).

[0320] In the examples below, if an abbreviation is not defined above, it has its generally accepted meaning. Further, all temperatures are in degrees Celsius (unless otherwise indicated). The following Methods were used to prepare the compounds set forth below as indicated.

[0321] The following examples are provided to describe the invention in further detail. These examples illustrate suitable methods for the synthesis of representative members of this invention. However, the methods of synthesis are intended to illustrate and not to limit the invention to those exemplified below. The starting materials for preparing the compounds of the invention are either commercially available or can be conveniently prepared by one of examples set forth below or otherwise using known chemistry procedures.

Examples 1-12, 14-45, 47-50

[0322] The compounds of Examples 1-50 were prepared following the below mentioned general procedure for Example 13 using compound 13 (a) and reacting it with the following benzenesulfonylhydrazines: 4-Phenylbenzenesulfonyl hydrazine, 4-t-butylbenzenesulfonyl hydrazine, 4-methyl-3,4-dihydro-2i7-benzo[1,4]oxazine-7-sulfonyl hydrazine, 5-(1-dimethylaminonaphthyl)sulfonyl hydrazine, 2,4,6-trimethylbenzenesulfonyl hydrazine, 3-chloro-6-methoxybenzenesulfonyl hydrazine, 2,5-dimethoxybenzenesulfonyl hydrazine, 4-(4-[1,2,3]thiadiazolyl)benzenesulfonyl hydrazine, 3-bromobenzenesulfonyl

[0000] hydrazine, 4-bromobenzenesulfonyl hydrazine, 4-methylbenzenesulfonyl hydrazine, 4-methoxybenzenesulfonyl hydrazine, 3-fluoro-4-chlorobenzenesulfonyl hydrazine, 4-trifluoromethoxybenzenesulfonyl hydrazine, 4-fluorobenzenesulfonyl hydrazine, 3-methoxybenzenesulfonyl hydrazine, 2-methylbenzenesulfonyl hydrazine, 3-trifluoromethylbenzenesulfonyl hydrazine, 2,4-dimethoxybenzenesulfonyl hydrazine, 5-

chloro-1,3-dimethyl-1H-pyrazolylsulfonyl hydrazine, 3-methylbenzenesulfonyl hydrazine, 4-trifluoromethylbenzenesulfonyl hydrazine, 2-trifluoromethylbenzenesulfonyl hydrazine, 4-(pyrrolidin-1-sulfonyl)benzenesulfonyl hydrazine, 2-chlorobenzenesulfonyl hydrazine, 5-(2-morpholin-4-yl)pyridylsulfonyl hydrazine, 2-trifluoromethoxybenzenesulfonyl hydrazine, 2,4-dichlorobenzenesulfonyl hydrazine, benzenesulfonyl hydrazine, 3-difluoromethylbenzenesulfonyl hydrazine, 3-cyanobenzenesulfonyl hydrazine, 4-cyanobenzenesulfonyl hydrazine, 5-(2,3-dihydrobenzo[1,4]dioxinyl)sulfonyl hydrazine, 2-(4-methylbenzenesulfonyl)-1-methyl hydrazine, 3-fluorobenzenesulfonyl hydrazine, 3,4-difluorobenzenesulfonyl hydrazine, 2,4-dimethylthiazol-5-ylsulfonyl hydrazine, 4-acetylbenzenesulfonyl hydrazine, 2,6-difluorobenzenesulfonyl hydrazine, 2-fluorobenzenesulfonyl hydrazine, 2,5-difluorobenzenesulfonyl hydrazine, 1-(4-methylbenzenesulfonyl)-1-methyl hydrazine, 2,6-dichlorobenzenesulfonyl hydrazine, 2,6-ditrifluoromethylbenzenesulfonyl hydrazine, 3,5-dimethylisoxazol-5-ylsulfonyl hydrazine, 4-nitrobenzenesulfonyl hydrazine, (1-methylimidazol-4-yl)sulfonyl hydrazine, and methylsulfonyl hydrazine.

Example 13

Preparation of N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(4-difluoromethoxyphenyl)sulfonyl]hydrazine-1-carboxamide

a. Preparation of 1,1,1,3,3,3-Hexafluoro-2-isocyanato-2-methylpropane, compound 13(a)

[0323]
[0324] A solution of trimethylsilylazide (26 mL, 180 mmol) was slowly added dropwise to a solution of 2,2-bis(trifluoromethyl)propionyl fluoride (38 g, 179 mmol) and benzyltriethylammonium chloride (0.065 g, 0.28 mmol) in xylenes (120 mL) at 0[deg.] C. Upon completion of the addition, the resulting mixture was heated at 110[deg.] C. After 4 h, the mixture was distilled at 760 mm Hg, and the fraction boiling at 40-50[deg.] C. contained 13 (a). Yield of the liquid product is 60%.

b. Preparation of N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(4-difluoromethoxyphenyl)sulfonyl]hydrazine-1-carboxamide

[0325]

[0326] To a solution of 4-difluorobenzenesulfonyl chloride (60 mg, 0.25 mmol) in tnethylamine (25 mg, 0.25 mmol) in 1 mL of dry THF was added anhydrous hydrazine (15 mg, 0.26 mmol) at room temperature. After stirring at room temperature for 2 h, a solution of 1,1,1,3,3,3-hexafluoro-2-isocyanato-2-methylpropane (13a) (54 mg, 0.26 mmol) in 1 mL of diethylether. The reaction mixture was stirred at room temperature for 12 h. The solvent was removed in vacuo, and the crude material subjected to reverse phase HPLC affording the product as a white, waxy solid (83 mg, 75%).

Example 46

Preparation of N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(4-methylphenyl)sulfonyl]hydrazine-1-methylcarboxamide

[0327]

[0328] To a solution of N-2-(1,1,1,3,3,3-Hexafluoro-2-methylpropyl)-2-[(4-methylphenyl)sulfonyl]hydrazine-1-carboxamide (100 mg, 0.254 mmol) prepared as described above, and cesium carbonate (165 mg, 0.51 mmol) in 1.6 mL of NMP was added iodomethane (17.5 yL, 0.28 mmol). The yellow mixture was stirred at room temperature for 2 h before adding 5 mL of water. The mixture was extracted with EtOAc, and the organic phase washed successively with water and brine. The organic phase was dried over MgSO4, and concentrated in vacuo. The crude product was chromatographed on silica gel with 10% EtOAc in hexanes.

Example 51

Preparation of 4-Phenylpiperazine-1-(2,2,2-trifluoro-1-methyl-1-trifluoromethylethyl)-carboxamide

[0329] To 1-phenylpiperazine (0.04 mL, 0.25 mmol) was added 1,1,1,3,3,3-hexafluoro-2-isocyanato-2-methylpropane (13a) (124 mg, 0.6 mmol) in 1 mL of diethylether. The mixture was stirred at room temperature in a tightly capped vial for 12 h. The reaction mixture was subjected to reverse phase HPLC(CH3CN/H2O) and the isolated product lyophilized to provide the product as a white solid.

Examples 52-99

[0330] The compounds of Examples 52-99 were prepared following the above mentioned general procedure for Example 51 using compound 13 (a) and reacting it with the following amines or anilines: morpholine, 2-acetylaniline, piperidine, 3,4,5-trimethoxyaniline, 4-trifluoromethylaniline, 4-methylpiperazine, 1-aminonaphthalene, 2-chloroaniline, 4-phenylpiperidine, 2-phenylaniline, 2,6-difluoroaniline, 2-aminobenzamide, 2-chloro-6-fluoroaniline, 3-trifluoromethylaniline, 2-aminobenzenesulfonamide, 5-amino(2,2,3,3-Tetrafluoro-2,3-dihydrobenzo[1,4]dioxane), 3-trifluoromethoxyaniline, 4-trifluoromethoxyaniline, 4-methylpiperidine, 2-aminonaphthalene, 2-fluoroaniline, 2,6-dimethoxyaniline, 4-amino-3-trifluoromethoxybenzoic acid, aniline, 3-cyanoaniline, 3-methoxyaniline, 2-(1,1,2,2-tetrafluoroethoxy)aniline, 3-aminobenzenesulfonamide, 3-fluoroaniline, 4-bromoaniline, 2-cyanoaniline, 4-cyanoaniline, 3-amino-2,2-difluorobenzo[1,3]dioxane, 4-chloroaniline, 3-methylaniline, 4-aminobenzenesulfonamide, 2,6-dibromoaniline, 2-methylaniline, 4-methylaniline, pyrrolidine, 4-fluoroaniline, 2,4-dibromoaniline, azepane, 4-bromo-2-trifluoromethoxyaniline, 2-trifluoromethoxyaniline, 2-trifluoromethylaniline, and 2-methoxyaniline.

[0000]

Example

Number Structure Name

1
  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(4-(phenyl)- phenylsulfonyl]hydrazine-1-carboxamide
2
  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(4-(2-methyl-2- propyl)-phenylsulfonyl]hydrazine- 1-carboxamide

3

N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[7-(4-methyl-3,4- dihydro-2H-benzo[1,4]oxazinyl)sulfonyl]hydrazine- 1-carboxamide

4

N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[5-(1- dimethylamino- naphthyl)sulfonyl]hydrazine-1- carboxamide

5

N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(2,4,6-trimethylphenyl)sulfonyl]hydrazine- 1-carboxamide

6

N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(3-chloro-6-methoxyphenyl)sulfonyl]hydrazine- 1-carboxamide

7

N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(3,6-dimethoxyphenyl)sulfonyl]hydrazine- 1-carboxamide

8

N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(4-(4- [1,2,3]thiadiazolyl)phenyl)sulfonyl]hydrazine-1-carboxamide

9

N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(3- bromophenyl)sulfonyl]hydrazine-1-carboxamide

10

N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(4- bromophenyl)sulfonyl]hydrazine-1-carboxamide

11

N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(4- methylphenyl)sulfonyl]hydrazine-1-carboxamide

12

N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(4- methoxyphenyl)sulfonyl]hydrazine- 1-carboxamide

13

N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(4-difluoromethoxyphenyl)sulfonyl]hydrazine- 1-carboxamide

14

N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(3-fluoro-4- chloro-phenyl)sulfonyl]hydrazine-1- carboxamide

15

N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(4-trifluoromethoxyphenyl)sulfonyl]hydrazine- 1-carboxamide

16

N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(4-fluoro- phenyl)sulfonyl]hydrazine-1-carboxamide

17

N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(3- methoxyphenyl)sulfonyl]hydrazine- 1-carboxamide

18

N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(2- methylphenyl)sulfonyl]hydrazine-1-carboxamide

19

N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(3-

trifluoromethylphenyl)sulfonyl]hydrazine- 1-carboxamide

20

  -2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(2,4- dimethoxyphenyl)sulfonyl]hydrazine-1-carboxamide

21

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[2-(5-chloro-1,3- dimethyl-1H-pyrazolyl)sulfonyl]hydrazine-1- carboxamide

22

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(3- methylphenyl)sulfonyl]hydrazine-1-carboxamide

23

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(4-trifluoromethylphenyl)sulfonyl]hydrazine- 1-carboxamide

24

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(2-trifluoromethylphenyl)sulfonyl]hydrazine- 1-carboxamide

25

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[4-(pyrrolidin-1-sulfonyl)phenylsulfonyl]hydrazine- 1-carboxamide

26

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(2- chlorophenyl)sulfonyl]hydrazine-1-carboxamide

27

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[2-(5-morpholin-4-yl)pyridylsulfonyl]hydrazine-1- carboxamide

28

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(2-trifluoromethoxyphenyl)sulfonyl]hydrazine- 1-carboxamide

29

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(2,4- dichlorophenyl)sulfonyl]hydrazine-1-carboxamide

30

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2- [phenylsulfonyl]hydrazine-1- carboxamide

31

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(3-difluoromethoxyphenyl)sulfonyl]hydrazine- 1-carboxamide

32

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(3- cyanophenyl)sulfonyl]hydrazine-1-carboxamide

33

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(4- cyanophenyl)sulfonyl]hydrazine-1-carboxamide

34

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[5-(2,3-dihydrobenzo[1,4]dioxinyl)sulfonyl] hydrazine-1-carboxamid

3 5

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(4- methylphenyl)sulfonyl]-1-methylhydrazine-1-carboxamide

36

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(3- fluorophenyl)sulfonyl]hydrazine-1-

carboxamide

37

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(3,4- difluorophenyl)sulfonyl]hydrazine-1-carboxamide

38

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(2,4- dimethylthiazol-5-yl)sulfonyl]hydrazine-1- carboxamide

39

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(4- acetylphenyl)sulfonyl]hydrazine-1-carboxamide

40

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(2,6- difluorophenyl)sulfonyl]hydrazine-1-carboxamide

41

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(2- fluorophenyl)sulfonyl]hydrazine-1-carboxamid

42

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(2,5- difluorophenyl)sulfonyl]hydrazine-1-carboxamide

43

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(4- methylphenyl)sulfonyl]-2-methylhydrazine-1-carboxamide

44

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(2,6- dichlorophenyl)sulfonyl]hydrazine-1-carboxamide

45

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(2,6-ditrifluoromethylphenyl)sulfonyl]hydrazine- 1-carboxamide

46

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(4- methylphenyl)sulfonyl]hydrazine-1-methylcarboxamide

47

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(3,5- dimethylisoxazol-5-yl)sulfonyl]hydrazine-1- carboxamide

48

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(4- nitrophenyl)sulfonyl]hydrazine-1-carboxamide

49

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2-[(1- methylimidazol-4-yl)sulfonyl]hydrazine-1- carboxamide

50

  N-2-(1,1,1,3,3,3-Hexafluoro-2- methylpropyl)-2- [methylsulfonyl]hydrazine-1-carboxamide

51

  4-Phenylpiperazine-1-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-carboxamide

52

  4-Morpholino-1-(2,2,2-trifluoro-1- methyl-1-trifluoromethylethyl)- carboxamide

53

  1-(2-Acetylphenyl)-3-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-urea

54

1-Piperidino-1-(2,2,2-trifluoro-1- methyl-1-trifluoromethylethyl)- carboxamide

55

1-(2,2,2-trifluoro-1-methyl-1- trifluoromethylethyl)-3-(3,4,5- trimethoxyphenyl)-urea

56

1-(4-Trifluoromethylphenyl)-3- (2,2,2-trifluoro-1-methyl-1- trifluoromethylethyl)-urea

57

4-Methylpiperazine-1-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-carboxamide

58

1-Naphthalen-1-yl-3-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-urea

59

1-(4-Chlorophenyl)-3-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-urea

60

4-Phenylpiperidin-1-yl-1-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-carboxamide

61

1-(2-Phenyl(phenyl))-3-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-urea

62

1-(2,6-Difluorophenyl)-3-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-urea

63

2-[3-(1,1-Bis-trifluoromethylethyl)- ureido]benzamide

64

1-(2-Chloro-6-fluorophenyl)-3- (2,2,2-trifluoro-1-methyl-1- trifluoromethylethyl)-urea

65

1-(3-Trifluoromethylphenyl)-3- (2,2,2-trifluoro-1-methyl-1- trifluoromethylethyl)-urea

66

2-[3-(1,1-Bis-trifluoromethylethyl)- ureido]benzenesulfonamide

67

1-(2,2,3,3-Tetrafluoro-2,3- dihydrobenzo[1,4]dioxin-5-yl)-3- (2,2,2-trifluoro-1-methyl-1- trifluoromethylethyl)-urea

68

1-(3-Trifluoromethoxyphenyl)-3- (2,2,2-trifluoro-1-methyl-1- trifluoromethylethyl)-urea

69

1-(4-Trifluoromethoxyphenyl)-3- (2,2,2-trifluoro-1-methyl-1- trifluoromethylethyl)-urea

70

4-Methyl-1-piperidine-1-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-carboxamide

71

1-Naphthalen-2-yl-3-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-urea

72

1-(2-fluorophenyl)-3-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-urea

73

1-(2,6-Dimethoxyphenyl)-3-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-urea

74

3-Trifluormethoxy-4-[3-(1,1-bis- trifluoromethylethyl)- ureido]benzoic acid

75

1-Phenyl-3-(2,2,2-trifluoro-1- methyl-1-trifluoromethylethyl)-urea

76

1-(3-Cyanophenyl)-3-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-urea

77

1-(3-Methoxyphenyl)-3-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-urea

78

1-(2-(1,1,2,2- Tetrafluoroethoxy)phenyl)-3-(2,2,2- trifluoro-1-methyl-1-

trifluoromethylethyl)-urea

79

  3-[3-(1,1-Bis-trifluoromethylethyl)- ureido]benzenesulfonamide

80

  1-(3-fluorophenyl)-3-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-urea

81

  1-(4-Bromophenyl)-3-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-urea

82

  1-(2-Cyanophenyl)-3-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-urea

83

  1-(4-Cyanophenyl)-3-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-urea

84

  1-(2,2-Difluorobenzo[1,3]dioxol-4- yl)-3-(2,2,2-trifluoro-1-methyl-1- trifluoromethylethyl)-urea

85

  1-(4-Chlorophenyl)-3-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-urea

86

  1-(3-Methylphenyl)-3-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-urea

87

  4-[3-(1,1-Bis-trifluoromethylethyl)- ureido]benzenesulfonamide

88

  1-(2,6-Dibromophenyl)-3-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-urea

89

  1-(2-Methylphenyl)-3-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-urea

90

  1-(4-Methylphenyl)-3-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-urea

91

  1-Pyrrolidinyl-1-(2,2,2-trifluoro-1- methyl-1-trifluoromethylethyl)- carboxamide

92

  1-(4-Fluorophenyl)-3-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-urea

93

  1-(2,4-Dibromophenyl)-3-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-urea

94

  Azepane-1-carboxylic acid (2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-amide

95

  1-(4-Bromo-2- trifluoromethoxyphenyl)-3-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-urea

96

  1-(2-Trifluoromethoxyphenyl)-3- (2,2,2-trifluoro-1-methyl-1- trifluoromethylethyl)-urea

97

  1-(2-Trifluoromethylphenyl)-3- (2,2,2-trifluoro-1-methyl-1- trifluoromethylethyl)-urea

98

  1-(2-Methoxyphenyl)-3-(2,2,2- trifluoro-1-methyl-1- trifluoromethylethyl)-urea

Assay 1

[0331] Approximately 400,000 compounds from the compound library were tested in this assay. Assay plates were set up as follows. Vero cells were plated at 80% confluency on 96-well plates. Test compounds (80 per plate) from the library were added to wells at a final concentration of 5 uM. Tacaribe virus (TRVL 11573) was then added at a virus dilution that

would result in 90% CPE after 5 days (pre-determined as an 800-fold dilution of the virus stock; multiplicity of infection [MOI] approximately 0.001). Plates were incubated at 37[deg.] C. and 5% CO2 for 5 days, then fixed with 5% glutaraldehyde and stained with 0.1% crystal violet. The extent of virus CPE was quantified spectrometrically at OD570 using a Molecular Devices VersaMax Tunable Microplate Reader. The inhibitory activity of each compound was calculated by subtracting from the OD570 of test compound well from the average OD570 of virus-infected cell wells, then dividing by the average OD570 of mock-infected cell wells. The result represents the percent protection against Tacaribe virus CPE activity conferred by the compound. "Hits" in this assay were defined as compounds that inhibited virus-induced CPE by greater than 50% at the test concentration (5 (J.M). Of the approximately 400,000 compounds screened in the Tacaribe virus HTS campaign, 2,347 hits were identified (0.58% hit rate).

[0332] Quality hits are defined as inhibitor compounds (hits) that exhibit acceptable chemical structures, antiviral potency and selectivity, and spectrum of antiviral activity. Specifically, compounds identified as hits in HTS assays (described above) were evaluated against four criteria: (i) chemical tractability, (ii) inhibitory potency, (iii) inhibitory selectivity and, (iv) antiviral specificity. Based on the HTS parameters, all hits have EC50 values <5 uM. The chemical structures of compounds that met this initial criterion were visually examined for chemical tractability. A chemically tractable compound is defined as an entity that is synthetically accessible using reasonable chemical methodology, and which possesses chemically stable functionalities and (potential) drug-like qualities. Hits that passed this medicinal chemistry filter were evaluated for their inhibitory potency. EC50 values were determined from a plot of the compound inhibitory activity typically across eight compound concentrations (50, 15, 5, 1.5, 0.5, 0.15, 0.05 and 0.015 uM). To assess whether the hit is a selective inhibitor, the effect on cellular functions was determined using a standard cell proliferation assay. A 50% cytotoxicity concentration (CC50) was determined using a tetrazolium-based colorimetric method, which measures the in situ reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to insoluble blue formazan crystals by mitochondrial enzymes in metabolically active cells. Solubilized crystals were quantified spectrometrically. Using the EC50 and CC50 values, a Selective Index (SI) was calculated (SI=CC50/EC50). Hits with SI values of at least 10 were considered further.

[0333] The specificity of the antiviral activity exhibited by hit compounds was determined by testing the compounds against a number of related and unrelated viruses. Compounds are tested against a variety of unrelated DNA (HSV, CMV, vaccinia virus) and RNA (RSV, rotavirus, Rift Valley fever, Ebola virus, Ebola GP-pseudotype, Lassa GP-pseudotype, HIV env-pseudotype) viruses. Compounds that will be selected for further development are thosejha are selective against the selected original target virus and inactive against unrelated viruses.

[0000]

Tacaribe EC50 Candide I

A = <0.5 [mu]M A = <0.5 [mu]M

B = 0.5 to <1.0 [mu]M B = 0.5 to <1.0 [mu]M

Example C = 1.0 to <5 [mu]M C = 1.0 to <5 [mu]M

Number	D = >=5 [mu]M	D = >=5 [mu]M
1	A	
2	A	
3	A	
4	A	
5	A	
6	A	
7	A	
8	A	
9	A	
10	A	
11	A	
12	A	
13	A	
14	B	
15	B	
16	B	
17	B	
18	B	
19	B	
20	B	
21	B	C
22	B	
23	B	
24	C	
25	C	
26	C	
27	C	
28	C	
29	C	
30	C	
31	C	
32	C	
33	C	
34	C	
35	C	
36	C	
37	C	
38	C	
39	C	D
40	C	
41	C	
42	C	
43	C	
44	C	
45	C	
46	D	
47	D	
48	D	
49	D	

50 D
51 A
52 A
53 B
54 B
55 B
56 B
57 C
58 C
59 C
60 C
61 C
62 C
63 C
64 C
65 C
66 C
67 C
68 C
69 C
70 C
71 C
72 C
73 C
74 C
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77 C
78 C
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80 C
81 C
82 C
83 C
84 C
85 D
86 D
87 D
88 D
89 D
90 D
91 D
92 D
93 D
94 D
95 D
96 D
97 D
98 D

Assay 2

[0334] A chemical library was created and screened that represents a broad and well-balanced collection of 400,000 compounds accumulated over a number of years from a variety of distinct sources. The library achieves broad coverage across property space involving the following chemical descriptors: calculated logarithm of n-octanol/water partition coefficient (ClogP), polar (water-accessible surface area (PSA), globularity (three dimensional structure) and molecular weight (average: 394.5 daltons).

Cells and Viruses

[0335] Vero (African green monkey kidney epithelial, ATCC #CCL-81) cells were grown in Eagle's minimum essential medium (MEM, Gibco) supplemented with 2 mM L-glutamine, 25 [mu]g/ml gentamicin, and 10% heat-inactivated fetal bovine serum (FBS). For infection medium (IM), the serum concentration was reduced to 2%. HEp-2 cells (human carcinoma of the larynx epithelial; ATCC #CCL-23) were cultured in MEM containing 10% heat-inactivated FBS and 1% penicillin/streptomycin. MRC-5 cells (human normal lung fibroblast; ATCC #CCL-171) were cultured in MEM containing 10% heat-inactivated FBS, 1% penicillin/streptomycin, 1% L-glutamine (Invitrogen 25030-081), 1% Non-Essential Amino Acids (Invitrogen #11140-050), 1% sodium pyruvate (Invitrogen #11360-070), and 2% sodium bicarbonate. MA104 cells (epithelial African green monkey kidney, ATCC CRL-2378.1) were cultured in MEM with 1% penicillin/streptomycin, 1% L-glutamine, 1% Non-Essential Amino Acids, 1% sodium pyruvate, and 2% sodium bicarbonate and 62.5 ug/ml trypsin and no serum during virus infection. All cell lines were incubated at 37[deg.] C. and 5% CO2. Respiratory syncytial virus (RSV; A isolate), lymphocytic choriomeningitis virus (LCMV; Armstrong E350 isolate), cytomegalovirus (CMV; AD-169 isolate), herpes simplex virus 1 (HSV-1; KOS isolate), Vaccinia virus (Strain WR), Tacaribe virus (strain TRVL 11573) and rotavirus (strain WA) were obtained from ATCC (#VR-1422, #VR-1540, #VR-134, #VR-538, #VR-1493, #VR-1354, #VR-114, and #VR-2018 respectively). Candid 1 and Amapari BeAn 70563 were obtained from Dr. Robert Tesh at the University of Texas Medical Branch (Galveston, Tex.). Work done with BSL 4 viruses (Lassa, Machupo, Guanarito, and Junín) as well as severe acute respiratory syndrome-associated coronavirus (SARS-CoV) was conducted by collaborators at USAMRIID (Fort Detrick, Md.).

Antiviral Assays for Specificity Screening: Cytopathic Effect ("CPE") Assay, Virus Plaque Reduction Assay, and ELISA

[0336] A viral CPE assay was used to evaluate the antiviral effect of compounds against Tacaribe virus (Vero cells), Candid-1 vaccine virus (Vero cells), Amapari virus (Vero cells), SARS-CoV (Vero cells), HSV-1 (Vero cells), RSV (HEp-2 cells), vaccinia virus (Vero cells), and Rotavirus (MA104). An enzyme-linked immunosorbent assay ("ELISA") was used to evaluate the antiviral effect of compounds against CMV (MRC-5 cells) and LCMV (Vero cells). All of these assays were carried out in the appropriate media containing 2% heat-inactivated FBS. Ninety-six-well cell culture plates were seeded 24 hours before use with $1.5*10^4$ (Vero), $2.2*10^4$ (HEp-2 and MA104), and $4.5*10^4$ (MRC-5) cells per well. For compound susceptibility testing, compounds (solubilized with 100% DMSO) were added to duplicate wells at final concentrations of 50, 15.8, 5, 1.6, 0.5, 0.16, 0.05, 0.016 and 0 [mu]M. The final concentration of DMSO in the assays was 0.5%. Virus stocks were titrated in a separate experiment to determine the concentration that resulted in 90% destruction of the cell monolayer (CPE assay) after 3 days (HSV-1, Rotavirus and vaccinia) or 4 days

(SARS-CoV, RSV, Tacaribe virus, Candid 1 vaccine virus and Amapari virus) or the concentration that generated an ELISA signal of 2.5 at an optical density of 650 nm (OD650) after 3 days (LCMV) or 4 days (CMV). These pre-established dilutions of virus were added to wells containing serial dilutions of compound. Uninfected cells and cells receiving virus without compound were included on each assay plate. In addition, reference agents, when available, were included on each assay plate (gancyclovir for HSV-1 and CMV, Sigma #G2536; ribavirin for LCMV and RSV, Sigma #R9644; and rifampicin for vaccinia virus, Sigma #R3501). Plates were incubated at 37[deg.] C. and 5% CO2 for either 3 days (HSV-1, Rotavirus, LCMV, Vaccinia virus) or 4 days (Tacaribe virus, Amapari virus, Candid 1 virus, SARS-CoV, RSV, and CMV). HSV-1, SARS-CoV, Rotavirus, Vaccinia virus, RSV, Tacaribe virus, Amapari virus, Candid 1 vaccine virus infected plates were processed for crystal violet staining while plates infected with CMV and LCMV were processed for ELISA analysis. For crystal violet staining, the plates were fixed with 5% glutaraldehyde and stained with 0.1% crystal violet. After rinsing and drying, the optical density at 570 nm (OD570) was measured using a Microplate Reader. For ELISA analysis, the medium from the LCMV and CMV-infected plates was removed and the cells were fixed with 100% methanol (Fisher, CAS #67-56-1, HPLC grade) for 20 minutes at room temperature. The methanol solution was removed and the plates were washed 3 times with PBS. Non-specific binding sites were blocked by the addition of 130 [mu]L of Superblock Blocking Buffer (Pierce #37515) for 1 hour at 37[deg.] C. The blocking agent was removed and the wells were washed 3 times with PBS. Thirty [mu]L of a 1:20 dilution of LCMV Nuclear Protein (NP) specific monoclonal antibody (generous gift of Juan Carlos de la Torre, The Scripps Research Institute, La Jolla Calif.) or 30 [mu]L of a 1:200 dilution of CMV (protein 52 and unique long gene 44 product) specific cocktail monoclonal antibodies (Dako, #M0854) in Superblock Blocking Buffer containing 0.1% Tween-20 was added. Following 1 hour incubation at 37[deg.] C., the primary antibody solution was removed and the wells were washed 3 times with PBS containing 0.1% Tween-20. Forty [mu]L of goat anti-mouse horseradish peroxidase conjugated monoclonal antibody (Bio-Rad #172-1011) diluted 1:4000 (LCMV) or 1:400 (CMV) in Superblock Blocking Buffer containing 0.1% Tween-20 was added to the wells and the plates were incubated for 1 hour at 37[deg.] C. The secondary antibody solution was removed and the wells were washed 5 times with PBS. The assay was developed for 15 minutes by the addition of 130 [mu]L of 3,3',5,5-tetramethylbenzidine substrate (Sigma #T0440) to quantify peroxidase activity. The OD650 of the resulting reaction product was measured using a Molecular Devices Kinetic Microplate Reader with a 650 nm filter.

[0337] Antiviral activity against Tacaribe virus was evaluated by three methods: CPE Assay, Plaque Reduction, and Virus Yield Inhibition Assay. For the HTS CPE Assay, Vero cells were plated at 80% confluency on 96-well plates. Test compounds (80 per plate) from the library were added to wells at a final concentration of 5 [mu]M. Tacaribe virus was then added at a virus dilution that would result in 90% CPE after 5 days (multiplicity of infection ("MOI") approximately 0.001). Plates were incubated at 37[deg.] C. and 5% CO2 for 5 days, then fixed with 5% glutaraldehyde and stained with 0.1% crystal violet. The extent of virus CPE was quantified spectrometrically at OD570 using an Envision Microplate Reader. The inhibitory activity of each compound was calculated by subtracting from the OD570 of test compound well from the average OD570 of virus-infected cell wells, then dividing by the average OD570 of mock-infected cell wells. The result represents the percent protection against Tacaribe virus CPE activity conferred by each compound. "Hits" in this assay were defined as compound that inhibited virus-induced CPE by greater than 50% at the test concentration (5 [mu]M). Hits that possessed chemical tractability were further evaluated for their inhibitory potency. The inhibitory concentration 50% (EC50) values were determined

from a plot of the compound inhibitory activity following the CPE assay across eight compound concentrations (50, 15, 5, 1.5, 0.5, 0.15, 0.05 and 0.015 [mu]M). All determinations were performed in duplicate.

[0338] In the Plaque Reduction Assay, Vero cell monolayers grown in 6-well plates were infected with about 50 PFU/well in the absence or presence of various concentrations of the compounds. After 1 h of virus adsorption at 37[deg.] C., residual inoculum was replaced by a 50:50 mix of 1% seaplaque agarose (in de-ionized water) and 2*MEM. Plaques were counted after 5-7 days of incubation at 37[deg.] C. The EC50 was calculated as the compound concentration required to reduce virus plaque numbers by 50%. Under BSL 4 conditions at USAMRIID the plaque reduction assays (with Lassa, Machupo, Guanarito, and Junín viruses) were performed as follows: 200 PFU of each virus was used to infect Vero cells. After virus adsorption, cell monolayers were rinsed and overlaid with complete medium containing 1% agarose and either lacking test compound or with different concentrations ranging from 15 [mu]M to 0.05 [mu]M. After 5 days incubation at 37[deg.] C., the monolayers were stained with neutral red and the numbers of plaques were counted.

[0339] In Virus Yield Reduction Assays, Vero cells grown in 24-well plates were infected with Tacaribe virus at a multiplicity of infection ("MOI") of 0.1 in the presence of different concentrations of the compounds, two wells per concentration. After 48 h of incubation at 37[deg.] C. virus was harvested and the virus yields were determined by plaque formation in Vero cells. The EC50 values were calculated as indicated above and similar calculations were performed to determine EC90 and EC99.

Cytotoxicity Assay

[0340] Cell viability was measured by a cell proliferation assay to determine a compound's effect on cellular functions so that a 50% cytotoxicity concentration (CC50) could be calculated; the ratio of this value to the EC50 is referred to as the selective index (S.I.=CC50/EC50). Two types of assays were used to determine cytotoxicity. One was a colorimetric method that measures the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), and the other uses fluorimetry to measure the reduction of resazurin (Alamar Blue). Both methods produced similar data. Confluent cultures in 96-well plates were exposed to different concentrations of the compounds, with two wells for each concentration, using incubation conditions equivalent to those used in the antiviral assays.

Medicinal Chemistry

[0341] Several potent compounds were identified by the Tacaribe HTS and were grouped into several clusters of structure type. One cluster of compounds, with ST-336 (FW=407.3) representing the prototype based on antiviral activity and chemical tractability, was chosen for further development. Through retrosynthetic analysis of ST-336, it was determined that a library of analogues could be prepared convergently in a single synthetic step by combining an isocyanate with an acyl hydrazide. Using this chemistry, 165 analogues were prepared and the most potent examined for in vitro metabolism (S9).

Time of Addition Experiment

[0342] This experiment was designed to characterize the mechanism of action of the anti-

viral compounds. Vero cells were grown in 24 well culture plates. The medium was removed when the cells reached 70-80% confluency and replaced with infection medium. Cells were infected with Tacaribe virus at MOI=0.1. After 1 hour adsorption, the viral inoculum was removed and replaced with fresh infection medium. Duplicate wells were treated with 3 [mu]M ST-336 h prior to infection, at the time of infection or at specific times post infection (from 1 to 20 h p.i.). Control infected cell cultures were treated with drug vehicle (DMSO) only. ST-336 was removed 1 hour post absorption and the monolayer was washed twice with cold PBS-M and replaced with fresh infection medium. The cells were harvested at 24 h p.i. and were titrated as described above.

[0343] In a separated experiment, Vero cells plated in a 6 well dish were infected with Tacaribe virus at MOI=4. Absorption was carried out for 1 hour. Three [mu]M of ST-336 was added for 1 hour at 1 hour before infection, during infection, and 1 hour following infection. Following drug addition and virus infection, monolayers were washed 3 times with complete media. Four hours following last drug addition, monolayers were overlaid with 1% agarose without compound until plaques developed. At 5 to 7 days post infection, monolayers were fixed, crystal violet stained and plaque numbers counted.

Assay for Compound Binding to Intact Virus

[0344] This experiment was designed to test the binding/fusion inhibitory properties of ST-336 towards Tacaribe virus. Vero cells were grown in MEM with 2% fetal calf serum. For this experiment, cells were grown to 70-80% confluency in 24-well culture plates. In one set of tubes Tacaribe virus (4000 pfu) was treated with 1% DMSO, serially diluted tenfold in infection medium and treated with the specific concentrations of ST-336 (400 pfu+0.5 [mu]M ST-336, 40 pfu+0.05 [mu]M ST-336) or DMSO only (400 pfu or 40 pfu+DMSO). In another set of tubes Tacaribe virus (4000 pfu) was treated with 5 [mu]M ST-336 then serially diluted tenfold in infection medium. The suspensions were plated in wells and after adsorption for one hour inocula were removed and overlaid with 0.5% Seaplaque agarose in MEM. The plate was incubated at 37[deg.] C. until cytopathic effect was observed in the DMSO control well. The cells were fixed with 5% gluteraldehyde and stained with 0.1% crystal violet for plaque visualization.

[0345] Another assay employed to test the binding properties of ST-336 to pre-fusion F-proteins on virions was a dialysis experiment. Purified Tacaribe virus (1000 pfu) was incubated with 5 [mu]M of ST-336 or 0.5% DMSO. The suspensions were dialyzed overnight at 4[deg.] C. in a dialysis chamber. Twenty four hours post dialysis viral suspensions were titrated on Vero cells. Post one hour adsorption, inocula were removed and a 0.5% Seaplaque agarose in MEM overlay was applied. The plate was incubated at 37[deg.] C. until cytopathic effect was observed. The cells were fixed with 5% gluteraldehyde and stained with 0.1% crystal violet. To confirm absence of free drug in dialysed virus-drug sample, virus was spiked in dialysed mixture at time of infection and plaques developed as described above.

Isolation of Drug Resistant Variant Viruses

[0346] Initially, single plaques of WT Tacaribe virus was isolated. For this plaque-purification Vero cells in a 6-well plate were infected with 50 pfu/well of WT Tacaribe virus for 1 hour at 37[deg.] C. Following virus adsorption the inoculum was removed and each well was overlaid with 0.5% Seaplaque agarose in MEM and incubated at 37[deg.] C. until plaques were visible (5-7 days). Four plaques were picked and further amplified in Vero cells

in a 24-well plate until CPE developed (5-7 days). Virus-infected cell extracts were harvested by scraping cells into the media and then collected in 1.5-ml microcentrifuge tubes. Each plaque-purified isolate was further amplified in 150 mm plates, and then each virus stock that originated from one virus plaque was titrated.

[0347] For the isolation of compound-resistant Tacaribe virus variants, each wild type plaque-purified isolate was titrated in the presence of 3 [mu]M ST-336 as described. Vero cells in a 6-well plate were infected with 10^4-10^6 pfu/well in media containing 3 [mu]M ST-336 for 1 hour, then the cells were overlaid with 0.5% seaplaque agarose in MEM containing 3 [mu]M ST-336 and incubated until plaques formed. Plaques were picked and used to infect Vero cells in a 24-well plate without compound. When CPE developed the infected wells were harvested. Each drug-resistant isolate was then titrated on a 96-well plate in 0.5 log dilutions, starting with 25 [mu]L of pure virus stock, without compound and with 1 [mu]M and 3 [mu]M ST-336. Each mutant went through several rounds of plaque purification before final virus stocks were made.

Sequencing

[0348] RNA was extracted from each of the Tacaribe WT isolates (1-4) and four of the drug resistant isolates (DR#1-4) and used for reverse transcription PCR. Primers specific to the GPC (Tac-forward: 5' GCCTAACTGAACCAGGTGAATC (SEQ ID NO:1) and Tac-reverse: 5' TAAGACTTCCGCACCACAGG (SEQ ID NO:2)) from Tacaribe were used for amplification and sequencing.

Solubility

[0349] Two tests were used to assess compound solubility: solubility in cell culture medium with and without various concentrations of serum and solubility in aqueous buffer at pH 7.4. The solutions were stirred overnight and then filtered through an Amicon Centrifree YM-30 column with a 30,000 MW cut off to remove potentially precipitated compound and compound bound to protein. The compound was quantified by LC/MS or UV spectrometry.

Stability

[0350] In vitro metabolic stability was determined by Absorption Systems (Exton, Pa.) using the 9000*g supernatant (S9) of homogenized liver from various species as a source of oxidative conjugation enzymes (e.g., cytochromes P450, UdP-glucuronosyl transferase) that are known to be the primary pathways of biotransformation for most drugs. The metabolic stability was measured as the persistence of parent compound over incubation time in the S9 fractions by mass spectrometry. Briefly, human, rat, mouse and guinea pig S9 fractions were obtained from Xenotech (Lenexa, Kans.). The reaction mixture, minus cofactor cocktails, was prepared (1 mg/ml liver S9 fractions, 1 mM NADPH, 1 mM UDPGA, 1 mM PAPS, 1 mM GSH, 100 mM potassium phosphate pH 7.4, 10 mM magnesium chloride, 10 [mu]M test article) and equilibrated at 37[deg.] C. for 3 min. An aliquot of reaction mixture was taken as a negative control. The reaction was initiated by the addition of cofactor cocktails to the reaction mixture only, and then the reaction mixture and negative control were incubated in a shaking water bath at 37[deg.] C. Aliquots (100 [mu]l) were withdrawn in triplicate at 0, 15, 30, and 60 minutes and combined with 900 [mu]l of ice-cold 50/50 acetonitrile/dH2O to terminate the reaction. Each sample was analyzed via LC/MS/MS. The natural log of the percent remaining was plotted versus time. A linear fit was used to determine the rate

constant. The fit was truncated when percent remaining of test article was less than 10%. The elimination half-lives associated with the disappearance of test and control articles were determined to compare their relative metabolic stability.

Genotoxicity

[0351] An exploratory bacterial mutagenicity assay (Ames test) was used to assess the potential of the compound genotoxicity. This assay utilized S. typhimurium tester strains TA7007 and TA7006 (single base pair mutations) and TA98 (frame shift mutation) with and without metabolic activation (Arochlor-induced rat liver S9) as described previously.<32 >

Pharmacokinetic ("PK") Assessments in Rats and Newborn Mice

[0352] Analysis of the oral pharmacokinetics of selected compounds was performed in Sprague Dawley rats in a single dose study with serum samples taken over a 24 h period. For the newborn mice PK evaluation, 4 day old BALB/c mice were dosed intraperitoneally (IP) and serum samples were taken over a 24 hour period. A 50 [mu]l aliquot of plasma was combined with 150 [mu]l of 100% acetonitrile containing an internal standard (100 ng/ml tolbutamide) in a 1.5 ml centrifuge tube. Samples were vortexed and centrifuged at 13,000 rpm for ten minutes. An 80 [mu]l aliquot of the resulting supernatant was then transferred to an HPLC for vial analysis. Plasma levels of each compound were determined by LC/MS/MS, and pharmacokinetic parameters were determined using WinNolin software.

Efficacy in Newborn Mouse Model

[0353] To determine tolerability of ST-294, newborn (4 days old) BALB/c mice were given IP dosages of 0 (vehicle), 10, 25, or 100 mg/kg/day of ST-294 for 5 days with assessment of clinical status daily.

[0354] To test the efficacy of ST-294 in the Tacaribe newborn mouse model, four day old BALB/c mice (8 per dose group) were challenged with 3*10<3 >PFU (30XLD50) of Tacaribe virus per mouse by IP injection with death as the end point. Mice were either treated with placebo (vehicle), ribavirin (MP Biomedical) administered IP at 25 mg/kg once a day for 10 days, or ST-294 administered IP at 100 mg/kg once a day or at 50 mg/kg twice a day for 10 days. Mice were monitored daily and weighed every other day throughout the study. Any mice showing signs of morbidity were euthanized by CO2 asphyxiation. All animal studies conformed to the Institute for Laboratory Animal Research and were approved through appropriate IACUC review.

Results

Homology Between Tacaribe and Other BSL 4 NWA

[0355] There are currently 23 recognized viral species of the Arenaviridae family.<4 >These viruses have been classified into two groups: the Old World (Lassa/LCM) arenaviruses and the New World (Tacaribe complex) group. The New World Tacaribe complex comprises three phylogenetic lineages, designated clades A, B, and C. Clade B includes the prototypic Tacaribe virus, Amapari virus and the four South American Category A pathogens (Junín, Machupo, Guanarito and Sabiá). Tacaribe virus is 67% to 78% identical to Junín virus at the amino acid level for all four viral proteins.<23 >Working with authentic Category A

arenaviruses requires maximum laboratory containment (BSL-4), and therefore presents significant logistical and safety issues. Since Tacaribe virus is closely related to the Category A pathogens it was chosen as a surrogate BSL 2 NWA for the development of a HTS assay to screen for inhibitors of virus replication.

Tacaribe HTS Assay

[0356] Since Tacaribe virus grows well in cell culture and causes clear virus-induced cytopathic effect (CPE) a robust HTS CPE assay was developed in a 96-well plate. The CPE assay is a whole cell assay which allows for calculation of the selective index of the compounds and identification of inhibitors of any essential steps in the virus life cycle. Of the 400,000 compounds screened in the Tacaribe virus HTS assay, 2,347 hits were identified (0.58% hit rate). All of these hits had EC50 values <=5 [mu]M. The 2,347 hits were then qualified based on four criteria: i) chemical tractability, ii) inhibitory potency, iii) inhibitory selectivity, and iv) antiviral specificity. A chemically tractable compound is defined as an entity that is synthetically accessible using reasonable chemical methodology, and which possesses chemically stable functionalities and potential drug-like qualities. Hits that passed this medicinal chemistry filter were evaluated for their inhibitory potency. EC50, CC50, and selective index (SI) values were determined to assess whether the hit was a selective inhibitor. Hits with SI values of at least 10 were considered further. Of the 2,347 hits identified, 36 compounds exhibited all the characteristics of quality hits. These compounds were chemically tractable, had EC50 values <=5 [mu]M and SI values >=10. Among the 36 quality hits, there were several clusters of structure type. One structure type was chosen for further development and ST-336 is the representative prototype for this series. ST-336 is a 407.33 dalton compound and its structure is shown in FIG. 1.

[0000]

TABLE 1

Specificity of ST-336

Virus (assay)	ST-336 ([mu]M)
NWA	
Tacaribe	
(CPE) EC50	0.055
(CPE) EC90	0.125
(Virus yield) EC90	0.068
(Virus yield) EC99	0.085
(Plaque reduction) EC50	0.100
Candid1 (CPE) EC50	0.062
Amapari (CPE) EC50	>20*
Machupo (Plaque reduction) EC50	0.150
Guanarito (Plaque reduction) EC50	0.300
Junin (Plaque reduction) EC50	0.150
OWA	
Lassa (plaque reduction) EC50	>20
LCMV (Elisa) EC50	>20

Results represent the average of at least two independent determinations.
*20 [mu]M represents limit of compound solubility

Characterization of ST-336

[0357] As seen in Table 1, ST-336 has submicromolar potency, good selectivity, and antiviral specificity against Tacaribe virus as well as the Category A NWA. Evaluation of ST-336 in a virus yield reduction assay against Tacaribe virus produced EC90 and EC99 values of 0.068 [mu]M and 0.085 [mu]M respectively. The CC50 value for ST-336 on Vero cells is >20 [mu]M, which represents the solubility limit of this compound in cell culture media, giving it a selective index of >363. The activity of ST-336 against Tacaribe virus was tested on multiple cell lines and all the EC50 values were similar to those achieved on Vero cells (data not shown). When tested against several arenaviruses, ST-336 showed no inhibitory activity against OWA, either LCM virus or authentic Lassa virus (Table 1). This drug also lacked activity against the NWA Amapari virus. This was a surprising result given the close phylogenetic relationship between Amapari and Tacaribe viruses.<23, 19 >This discrepancy is later discussed following sequencing of GP2 of all NWA. However, importantly ST-336 showed potent antiviral activity against the vaccine strain of Junín virus (Candid 1) as well as Machupo, Guanarito, and Junín (Table 1).

[0000]

TABLE 2
Selectivity of ST-336

Virus (assay)	ST-336 EC50([mu]M)
DNA viruses	
HSV-1 (CPE)	>20*
CMV (Elisa)	>20
Vaccinia (CPE)	>20
RNA viruses	
RSV-A (CPE)	>20
Rotavirus (CPE)	>20
SARS (CPE)	>20
Ebola (CPE)	>20

Results represent the average of at least two independent determinations.

*20 [mu]M represents limit of compound solubility

[0358] The specificity of the antiviral activity exhibited by ST-336 was determined by testing against a number of related and unrelated viruses. As shown in Table 2, ST-336 showed no activity against a variety of unrelated DNA (HSV, CMV, vaccinia virus) and RNA (RSV, Rotavirus, SARS and Ebola virus) viruses.

Mechanism of Action of ST-336

[0359] A single cycle (24 h) time of addition experiment was done to determine when during the virus replication cycle ST-336 exerts its antiviral activity. The effect of ST-336 on Tacaribe virus yield was determined following addition of compound to Vero cell cultures at various times before or after infection. ST-336 was added at one hour before infection (-1 h), during virus adsorption (0 h), and at several times post-infection. Drug was kept, following sequential addition, on infected cell cultures for the entire time of the experiment. Control infected cultures were treated with drug vehicle (DMSO) only. At 24 hours post-infection,

samples were collected, and virus yields were determined by plaque assay. As shown in FIG. 2A, ST-336 exerted its inhibitory effect only at the very early stage in the virus life cycle. Addition of ST-336 at any time points post-infection had no effect on virus yield. These data suggest that ST-336 is an early stage inhibitor of virus replication.

[0360] These results were confirmed in a second type of time addition experiment. In this experiment, compound was spiked in the culture medium for only 1 hour, at 1 hour before infection (-1 h), during infection (0) and at 1 hour post infection (+1 h), and then removed. The cultures were washed to remove any residual compound and overlaid with agarose. Virus plaque numbers were then determined at 5 days post-infection. Data in FIG. 2B showed that while compound added before and after virus adsorption for 1 hour had no effect on plaque formation, compound added during the 1 h adsorption/entry process dramatically reduced Tacaribe plaque formation. These data are consistent with ST-336 being an adsorption/entry inhibitor.

[0361] Two approaches were taken to determine if ST-336 is binding to intact virions. In the first experiment, 1000 PFU of purified Tacaribe virus was incubated with ST-336 or DMSO and dialyzed overnight at 4[deg.] C. and titrated. While no virus was titrated from the dialyzed bag originally incubated with drug, more than 300 PFU of virus was titrated from the DMSO vehicle dialyzed bag (data not shown). No drug was biologically detected in the dialysis bag originally containing 5 [mu]M of drug as measured by the incapability of the virus plus drug dialyzed mixture to inhibit freshly added Tacaribe virus (300 PFU). These data suggested that ST-336 binds intact virions with a very slow dissociation constant. In the second experiment (FIG. 3), Tacaribe virus was incubated in a test tube with 5 [mu]M of ST-336 or DMSO. Serial 1:10 dilutions were performed and for some samples ST-336 was added as a specified dilution representing the concentration of drug expected following sample dilution. As virus and compound are diluted with media, the compound concentration will reach a concentration without an inhibitory effect, unless the compound was capable of binding to virus. Test virus without compound in the initial tube was also diluted in media and compound concentrations corresponding to that found in the tubes where virus and compound were diluted together was added to each virus dilution. Titration on Vero cells showed that ST-336 present in excess in the initial tube was carried over for two additional 1:10 dilutions through specific virus binding and inhibits virus infection. Whereas when drug was added at a specified dilution virus was not inhibited to the same degree as virus diluted with drug (data not shown). These data suggest that ST-336 binds with at least a slow Koff to intact protein present on Tacaribe virus.

Isolation of Drug Resistant Variants

[0362] The expected mutation rate of RNA viruses is very high (1 mutant in 10,000) and a common approach to determining the target of an antiviral is to isolate virus resistance to the antiviral and then map the site of resistance. Virus variants with reduced susceptibility to ST-336 were isolated from wild type Tacaribe virus stocks plated in the presence of ST-336. The observed frequency of ST-336 drug resistant (ST-336<DR>) variants was as expected for RNA viruses. Sixteen ST-336<DR >isolates from four independent wild type Tacaribe virus stocks were isolated and plaque purified three times. All ST-336<DR >isolates were tested for their ability to grow in the presence of ST-336. The growth of ST-336<DR >isolates was unaffected by the presence of ST-336 at concentrations that completely inhibited wild type Tacaribe virus replication (data not shown). The isolation and confirmation of drug resistant virus variants strongly suggest that ST-336 acts as a direct antiviral inhibitor.

[0363] To determine the genetic basis for resistance and the molecular target of ST-336, RNA was isolated from the wild type and ST-336<DR >isolates. Based on the time of addition experiments, it was suspected that the viral glycoproteins might be the target of ST-336. The entire glycoprotein precursor GPC region of the S segment was sequenced. Sequence analysis was performed on four wild type isolates (WT#1-4) and four ST-336<DR >isolates derived from drug selection applied to each corresponding parental wild type isolate (DR#1.1 from WT#1, DR#2.1 from WT#2, DR#3.1 from WT#3 and DR#4.1 from WT#4). The sequence analysis showed that the GPC gene from the four parental wild type isolates had identical sequences. When compared to the GPC sequences of four drug resistant variants, each possessed a single nucleotide change that in all cases resulted in an amino acid change. FIG. 4A shows the location of each of the mutations which are located in or around the transmembrane domain of GP2. The sequence alignments of the region of the GP2 containing the changes is presented in FIG. 4B. The single change in DR#1.1 was at amino acid position 418 (I418T), in DR#2.1 at amino acid 416 (T416N), in DR#3.1 at amino acid 433 (S433I) and in DR#4.1 at amino acid 436 (F436I). I418 is similarly conserved (I or L, but never a T) in all clade B New World arenavirus, while T416 is conserved among all clade B NWA. F436 is similarly conserved with one exception; Amapari virus encodes a leucine at position 436. This change in Amapari virus may explain its lack of susceptibility to ST-336 (Table 2). I418, T416, S433 and F436 lie near the N-terminal and C-terminal limits of the putative transmembrane domain of GP2, a region known to play a vital role in enveloped virus fusion. <17, 27, 28, 38, 39 >Taken together, these data suggest that amino acid changes in arenavirus GP2 at either position 416, 418, 433 or 436 are sufficient to confer reduced susceptibility to ST-336 and are consistent with the proposed fusion inhibition mechanism suggested by virological experiments.

Hit-to-Lead Optimization

[0364] Preliminary data showed that ST-336, while demonstrating interesting antiviral activity and specificity, had poor pharmacokinetic (PK) properties in rodents (mouse and rats, data not shown). In order to improve the PK properties of ST-336, a lead optimization chemistry campaign was initiated. The objective of the optimization program was to develop compounds that possess attributes consistent with the ultimate drug product profile. Lead optimization activities comprised a series of iterations involving design and chemical synthesis of analogs of the lead structure, followed by a series of biological, physiochemical, and pharmacological evaluations of the new compounds. Chemical analogs flowed through a compound evaluation paradigm that involved first in vitro virological and cytotoxicity assessments, followed by a series of evaluations as listed: in vitro metabolic stability (S9), solubility, exploratory bacterial mutagenesis and pharmacokinetic assessments. 165 analogues were prepared and the most potent were examined for in vitro metabolism in S9 liver extracts. The most stable were dosed in rats, and ST-294 emerged as a potent, orally bioavailable representative of the compounds.

Characterization of ST-294

[0365] The structure of ST-294 (N-2-(1,1,1,3,3,3-hexafluoro-1-methylpropyl)-2-[(4-difluoromethoxyphenyl)sulfonyl]hydrazine-1-carboxamide) is show in FIG. 5. ST-294 was tested against the drug resistant Tacaribe mutants generated with ST-336 (DR#1-4) and all of the mutants elicited cross-resistance to ST-294 suggesting that this compound is targeting the same area of GP2 as ST-336 (data not shown). The activity of ST-294 against Tacaribe, Machupo, Guanarito, and Junín viruses was similar to that seen with ST-336 (Table 3). The

CC50 of ST-294 on Vero cells is >50 [mu]M yielding a selective index of >416. Further characterization of ST-294 showed that this compound is soluble up to 23 [mu]M in media containing 10% fetal calf serum and up to 480 [mu]M in buffer at pH 7.4 (Table 3). The metabolic stability of ST-294 was tested in S9 liver extracts from rat, mouse, human, and guinea pigs and was found to be most stable in human S9 followed by mouse, rat and guinea pig respectively (Table 3). Analysis of the oral pharmacokinetics of ST-294 was initially performed in the rat as this species is well characterized for this type of study. The rats were dosed with ST-294 by oral gavage and samples were taken over a 24 h period. Serum levels were very high (Cmax=6670 ng/ml) and ST-294 has good oral bioavailability (68.2%) (Table 3).

[0000]

TABLE 3
Characterization of ST-294
  Virus (assay) ST-294
  Tacaribe
  (CPE) EC50 0.120 [mu]M
  (Plaque reduction) EC50 0.100 [mu]M
  Machupo
  (Plaque reduction) EC50 0.300 [mu]M
  Guanarito
  (Plaque reduction) EC50 1.0 [mu]M
  Junin
  (Plaque reduction) EC50 0.300 [mu]m
  Properties
  Solubility (0%, 2%, 10% FBS) 18, 21 and 23 uM
  Solubility (pIon, pH 7.4) 480 [mu]M
  Stability (S9) rat/mouse/human/g.p 26/74/100/23 min
  Genotoxicity (Ames test) negative
  PK (rat/oral)
  [1/2] life 2 hours
  bioavailability (F) 68.2%
  PK (newborn mouse/IP)
  [1/2] life 3 hours
  Cmax 2910 ng/ml

Efficacy Study with ST-294 in Newborn Mouse Model

[0366] ST-294 has potent antiviral activity against NWA and good drug-like properties, so the next step was to test the ability of ST-294 to inhibit NWA-induced disease in an animal model. For the Category A agents, the experiments require BSL 4 containment. However, in an effort to obtain an initial readout, a Tacaribe virus challenge model in newborn mice was established. In preparation for this study, PK and tolerability experiments were performed with ST-294 in newborn mice prior to conducting an efficacy trial. Newborn (4 day old) BALB/c mice were dosed IP with 10 mg/kg of ST-294 and blood samples were collected for analysis. Relative to in vitro antiviral concentrations required to inhibit Tacaribe virus CPE (EC50=66 ng/ml), mean plasma concentrations in newborn mice were well above this level for prolonged periods of time (>15* through 8 h and 6* at 24 h after dosing, data not shown). In this model the drug is delivered via the IP route due to the difficulty of performing

multiple oral gavages on newborn mice. To test tolerability, newborn mice were given IP dosages ranging from 0-100 mg/kg/day of ST-294 for 5 days. Dosages of 100 mg/kg/day for 5 days were well tolerated by the newborn mice as there were no clinical signs of toxicity and the mice gained weight at the same rate as the control mice (data not shown). This highest tested concentration of ST-294 of 100 mg/kg/day was used in a Tacaribe animal efficacy study.

[0367] The drug levels and half-life shown in the PK study in the newborn mice was not equivalent to that seen in the rats, but the serum levels seemed sufficient to perform a proof-of-concept animal study in the Tacaribe animal model. Four day old mice were challenged with 30*LD50 of Tacaribe virus and treated with placebo, ribavirin as a control or ST-294. As the results in FIG. 6 demonstrate, ST-294 showed efficacy in the Tacaribe infected newborn mice with both survival and a delay in death similar to the drug control (ribavirin). Taken together these data suggest that ST-294 is a promising and appropriate drug candidate to advance into definitive animal studies where guinea pigs and primates will be challenged with authentic NWA (Junín and Guanarito viruses) and treated at various times post infection and prophylatically with ST-294.

Discussion

[0368] Through a successful HTS and medicinal chemistry program, a NWA antiviral drug candidate, ST-294, has been identified. This drug potently and selectively inhibits NWA viruses in vitro including the 3 NIAID/CDC Category A viruses (Junín, Machupo, and Guanarito viruses). This compound was also evaluated for stability in S9 liver extracts and for it's pharmacokinetic properties and was found to be metabolically stable and orally bioavailable. In a preliminary animal efficacy study, ST-294 showed significant protection against Tacaribe virus induced disease in newborn mice. Through mechanism of action studies it is apparent that this series of compounds targets GP2 and are viral entry inhibitors.

[0369] From the dialysis and dilution experiments (FIG. 3) it is apparent that the drug binds to virus and is carried over during dilutions. This phenomenon could potentially have an effect when titrating virus samples during other experiments. However, in the time of addition experiment, there was not enough drug carry over due to high dilution to affect the titers when added 1 hour or more after infection (FIG. 2).

[0370] Since ST-294 has better S9 stability than ST-336 does, it is thought that metabolism occurs at the methyl group on the aromatic ring (FIG. 1). The benzylic position is susceptible to oxidation. When there is no benzylic hydrogen present as in ST-294 (FIG. 2), the oxidation is blocked and thus eliminates the fastest metabolism pathway. The addition of the difluoromethoxy group in ST-294 gave this compound increased S9 stability, but did not reduce antiviral activity.

[0371] In the Tacaribe newborn mouse model the mice appear to die of a neurological disease (indicated by hind quarter paralysis) and it is not known whether ST-294 can cross the blood brain barrier. Also the drug levels and half-life of this drug candidate given IP in newborn mice is not as good as oral dosing in rats so serum levels and compound getting to the brain may have compromised the ability to obtain complete protection in this model. The more appropriate animal models for hemorrhagic fever caused by arenaviruses are in guinea pigs and non-human primates where the virus replicates predominantly in the spleen, lymph nodes and bone marrow causing hemorrhagic diathesis. Guinea pig models are well established for

Junín, Machupo, and Guanarito virus diseases, and represent the best small animal model for evaluation during preclinical studies.<26, 34 >Guinea pigs infected with pathogenic strains of Junín virus develop a fatal disease akin to human AHF.<37 >

[0372] There are many reports of the role of transmembrane in the function of viral fusion proteins. In the case of influenza virus hemagglutinin, it is clear that a transmembrane anchor is required for full fusion activity.<27 >In contrast, specific sequence requirements within the transmembrane domain have been identified, for example, in human immunodeficiency virus (HIV) type 1, murine leukemia virus, foamy viruses, coronavirus, Newcastle disease virus and measles virus.<27 >Based on the drug resistant variants generated during these studies, the ST-336 class of compounds targets the GP2 envelope protein, with mutations eliciting reduced susceptibility to the drug arising in or around the transmembrane region (FIG. 4).

[0373] Drugs that target the interactions between the virus envelope and the cellular receptor represent a new class of antiviral drugs. For HIV therapy, entry inhibitors have recently raised great interest because of their activity against multi-drug resistant viruses. A new antiviral against HIV was recently approved by the FDA called enfuvirtide. Enfuvirtide (Fuzeon) is a potent fusion inhibitor that blocks formation of the six-helix bundle and thus prevents membrane fusion.<29 >Enfuvirtide has been successful in improving the virological and immunological response in treatment-experienced HIV-infected patients.<33 >There are several other compounds that counter HIV entry that are in different developmental stages, among them: 1) the attachment inhibitor dextrin-2-sulfate; 2) the inhibitors of the glycoprotein (gp) 120/CD4 interaction PRO 542, TNX 355 and BMS 488043; and 3) the co-receptor inhibitors subdivided in those targeting CCR5 or CXCR4.<20 >The success of enfuvirtide and others in the development pathway are proof that virus entry inhibitors can be used to treat viral diseases in humans.

[0374] ST-294 also has the potential for prophylactic use since this drug appears to bind to the virus (FIG. 3) and would prevent infection. Other virus entry inhibitors have demonstrated protection when given prophylactically.<22 >This is an indication that can be pursued to determine its feasibility.

[0375] The results presented here show that ST-294 is a potent specific inhibitor of New World arenaviruses including the Category A hemorrhagic fever viruses (Junín, Machupo, and Guanarito). More importantly, the target of ST-294 (virus entry into the cell) serves as a viable target for antiviral development. Since virus infection can be completely inhibited at concentrations in the nanomolar range, the target for ST-294 would seem to be both accessible and extremely sensitive to reagents that disrupt its role in the infection process. Therefore, it will be important to further define the mechanism involved in ST-294 mediated inhibition.

[0376] While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the invention.

[0377] All references cited herein are herein incorporated by reference in their entirety for all

purposes.

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VIRAL TREATMENT

Background of the Invention

[02] The present invention provides a method of treating various diseases caused by viruses. Such diseases include Severe Acute Respiratory Syndrome Pneumonia (SARS Coronavirus or SARS-CoV), Ebola Hemorrhagic Fever (Ebola Virus), Marburg Hemorrhagic Fever (Marburg Virus), West Nile Fever or Encephalitis (West Nile virus), German Measles (Rubella), Yellow Fever (Yellow Fever Virus), Saint Louis Encephalitis (Saint Louis Encephalitis Virus), Japanese Encephalitis (Japanese Encephalitis Virus), California Encephalitis (California Encephalitis Virus), Human T-cell Leukemia (HTLV-I), Newcastle Disease (Newcastle Disease Virus), respiratory tract infection and bronchitis (Respiratory Syncytial Virus), Lymphocytic Choriomeningitis (Lymphocytic Choriomeningitis Virus), Lassa

Hemorrhagic Fever (Lassa Virus), and Hanta Hemorrhagic Fever (Hantavirus). The present invention represents an ongoing effort to find effective treatments (either to cure or to ameliorate symptoms) against these diseases.

Summary of the Invention

[03] SARS, Ebola, Marburg, West Nile, German Measles, Yellow Fever, Saint Louis

Encephalitis, Japanese Encephalitis, California Encephalitis, Human T-cell Leukemia, Newcastle Disease, respiratory tract infection and bronchitis, Lymphocytic Choriomeningitis, Lassa Hemorrhagic Fever, and Hanta Hemorrhagic Fever are treated by intramuscular (IM) injection of a composition comprising a first ingredient selected from the group consisting of procaine, chloroprocaine, tetracaine, chlorotetracaine, bromoprocaine, proparacaine, fluoroprocaine and benzocaine, and a second ingredient selected from the group consisting of dexamethasone, flumethasone and betamethasone. The treatment further comprises administration of an electrolyte solution such as Hydrite, PEDIALYTE, etc. In an embodiment, the hydration solution is about half a tablet of Hydrite in approximately 500 ml water. In addition, the patient is also treated by administration of an antipyretic, such as calpol, paracetamol, aspirin, acetaminophen, ibuprofen, etc.

Detailed Description of the Invention

[04] The present invention is directed to the treatment of SARS, Ebola, Marburg, West

Nile, German Measles, Yellow Fever, Saint Louis Encephalitis, Japanese Encephalitis, California Encephalitis, Human T-cell Leukemia, Newcastle Disease, respiratory tract infection and bronchitis, Lymphocytic Choriomeningitis, Lassa Hemorrhagic Fever, and Hanta Hemorrhagic Fever by IM injection of a mixture comprising a first ingredient selected from the group consisting of procaine, chloroprocaine, tetracaine, chlorotetracaine, bromoprocaine, proparacaine, fmoroprocaine and benzocaine, and a second ingredient

selected from the group consisting of dexamethasone, flumethasone and betamethasone. In the context of the present disclosure, the named ingredients also include therapeutically effective salts and hydrates, thereof. [05] The scope of the present invention includes variants of viruses that cause the above-referenced diseases. For example, the Hantavirus includes numerous variants, including the Hantaan virus, the Puumala virus, the SEO virus, the Dobrava virus, the Sin Nombre virus, etc. Conversely, some of the above-referenced diseases are variants of the same genus. For example West Nile Disease and Japanese encephalitis are caused by variants of the Flavivirus genus, which also includes the Murray Valley encephalitis virus. The present disclosure encompasses all variants of the specifically referenced diseases that are capable of being treated by the method described herein.

[06] The treatment further comprises administration of an electrolyte solution such as Hydrite, PEDIALYTE, etc. Electrolytes can be administered orally or intravenously. In addition, the patient is also treated by administration of an antipyretic, such as calpol, paracetamol, aspirin, acetaminophen, ibuprofen, etc.

[07] The term "therapeutically effective salts or hydrates," as use herein, represents those salts or hydrates which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like and correspond to a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well-known in the art. The salts can be prepared in-situ during the final isolation and purification of the compounds of the invention or separately by reacting the free base group with a suitable organic acid. Representative acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphersulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate salts and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methyl amine, dimethyl amine, trimethylamine, triethylamine, ethylamine and the like.

[08] Injectable mixtures of this invention comprise pharmaceutically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol and the like), vegetable oils (such as olive oil), injectable organic esters (such as ethyl oleate) and suitable mixtures thereof. Proper fluidity may be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants.

[09] These compositions may also contain adjuvants such as preservative, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben,

chlorobutanol, phenol sorbic acid and the like. It may also be desirable to include isotonic agents such as sugars, sodium chloride and the like. Prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

[10] The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium just prior to use.

[HJ A SARS patient is IM injected with a 2 ml dose of a 9:1 mixture of chloroprocaine (20 mg/ml) and dexamethasone (4 mg/ml) mixture followed by a second dose 60 minutes later. In addition, the patient is treated with electrolyte solution (about 500 ml per day) and aspirin, as needed. The treatment is repeated a second day and a third day (each with a 90 minute interval).

[12] A West Nile infant patient is IM injected with a 1 ml dose of a mixture of procaine and betamethasone mixture followed by a second dose 60 minutes later. In addition, the patient is treated with electrolyte solution and paracetamol. The treatment is repeated a second and a third day.

[13] An Ebola patient is IM injected with a 2 ml dose of a 9:1 mixture of tetracaine (20 mg/ml) and flumethasone (4 mg/ml) mixture. In addition, the patient is treated with electrolyte solution. The treatment is repeated daily for four days.

[14] An elderly Human T-cell Leukemia patient is IM injected with a 2 ml dose of a mixture of chloroprocaine and flumethasone mixture. In addition, the patient is treated with oral electrolyte solution. The treatment is repeated daily for 30 days. Thereafter, a maintenance dose is provided once weekly.

[15] An adult Marburg patient is treated with between about 1 -10 ml IM injection of a mixture comprising between about 0.6-9.5 ml of about 0.5-13% chloroprocaine and between about 0.1-8.7 ml of about 4 mg/ml dexamethasone sodium phosphate. In another embodiment, an adult Marburg patient is treated with between about 0.1-10 ml IM injection of a mixture comprising between about 0.1 -9.9 ml of about 0.5- 11 % proparacaine and between about 0.1 -8.4 ml of about 2-10 mg/ml betamethasone. A further embodiment treats an adult Marburg patient with between about 1-10 ml IM injection of between about 0.1-9.6 ml of about 0.5-30% benzocaine and between about 0.1-7.9 ml of about 2-10 mg/ml dexamethasone. Yet another embodiment treats an adult Marburg patient with between about 1-10 ml IM injection of between about 0.4-9.6 ml of about 0.5-20% chlorotetracaine and between about 0.1-8.0 ml of about 2-10 mg/ml flumethasone. In yet another embodiment, an adult Marburg patient is treated with between about 1-10 ml IM injection of amixture comprising between about 0.1-8.5 ml of about 0.5-18% tetracaine and between about 0.01-7.7 ml of about 4 mg/ml dexamethasone. Yet another embodiment treats an adult Marburg patient with between about 1-10 ml IM injection of a mixture comprising between about 0.6-9.5 ml of about 0.5-13% fluoroprocaine and between about 0.1-8.7 ml of about 4 mg/ml dexamethasone sodium phosphate. In a further embodiment, the patient is further treated with an oral hydration solution during treatment.

[16] In a Lymphocytic Choriomeningitis treatment, two injections of between about 0.1-9.6

ml of about 0.5-30% benzocaine and between about 0.1-7.9 ml of about 2-10 mg/ml betamethasone are administered daily over an about 60-120 minute interval for between about 3- 5 days. As an example, an adult Lymphocytic Choriomeningitis patient may be treated with two about 2 ml doses at about 60-90 minute intervals for about 11-18 days. During the IM treatment, the patient is further treated with an oral hydration solution. A child may be treated with about 1 ml doses.

[17] A Newcastle Disease patient is treated with between about 1-10 ml IM injection of a mixture comprising between about 0.3-8.7 ml of about 1-12% bromoprocaine and between about 0.1-7.9 ml of about 2-10 mg/ml flumethasone. In another embodiment, an adult Newcastle Disease patient is treated with between about 1-10 ml IM injection of a mixture comprising between about 0.1-9.0ml of about 0.5-15% chloroprocaine and between about 0.1-8.4 ml of about 4 mg/ml dexamethasone. A further embodiment treats an adult Newcastle Disease patient with between about 1-10 ml IM injection of a mixture comprising between about 0.3-8.6 ml of about 0.5-14% tetracaine and between about 0.1-8.8 ml of about 2-10 mg/ml betamethasone.

Yet another embodiment treats an adult Newcastle Disease patient with between about 1-5 ml IM injection of a mixture comprising between about 0.2-8.9 ml of about 1-17% chloroprocaine and between about 0.1-7.8 ml of about 4 mg/ml betamethasone. In yet another embodiment, an adult Newcastle Disease patient is treated with between about 0.5-10 ml IM injection of a mixture comprising between about 0.2-7.9 ml of about 0.5-22% proparacaine and between about 0.01 -9.2 ml of about 4 mg/ml flumethasone. In another embodiment, an adult Newcastle Disease patient is treated with between about 1-10 ml IM injection of a mixture comprising between about 0.1- 9.0 ml of about 0.5-15% fluoroprocaine and between about 0.1-8.4 ml of about 4 mg/ml dexamethasone. During the IM treatment, the patient is further treated with an oral hydration solution. [18] In a Hanta Hemorrhagic Fever embodiment, one or two injections of chloroprocaine and dexamethasone are administered daily for between about 1-3 days. When two injections are made, they are administered over an about 60-120 minute interval. As an example, an adult Hanta Hemorrhagic Fever patient is treated with about two 2 ml doses at about 60-90 minute intervals for 1-5 days. The patient is further treated with an oral hydration solution (bottled water 500 ml mixed with Vi electrolyte tablet). A child may be treated with 1 ml doses.

[19] More generally, an adult Encephalitis patient is treated with between about 1-10 ml IM injection of a mixture comprising between about 0.2-7.9 ml of about 0.5-17% tetracaine and between about 0.1-8.5 ml of about 2-10 mg/ml flumethasone. In another embodiment, an adult Encephalitis patient is treated with between about 0.1-10 ml IM injection of a mixture comprising between about 0.1-8.8 ml of about 0.5-14% chloroprocaine and between about 0.1- 9.3 ml of about 4 mg/ml flumethasone. A further embodiment treats an adult Encephalitis patient with between about 1-10 ml IM injection of a mixture comprising between about 0.2-7.9 ml of about 1-15% bromoprocaine and between about 0.2-9.9 ml of about 4 mg/ml dexamethasone. Yet another embodiment treats an adult Encephalitis patient with between about 1-10 ml IM injection of a mixture comprising between about 0.1-9.2 ml of about 0.5-15% proparacaine and between about 0.3-9.6 ml of about 4 mg/ml betamethasone. In yet another embodiment, an adult Encephalitis patient is treated with between about 0.5-10 ml IM injection of a mixture comprising between about 0.1-9.4 ml of about 0.5-33% benzocaine and between about 0.1-8.1 ml of about 2-10 mg/ml betamethasone. A further embodiment treats an adult Encephalitis patient with between about 1-10 ml IM injection of a mixture comprising between about 0.2-7.9 ml of about 1-15%

fluoroprocaine and between about 0.2-9.9 ml of about 4 mg/ml dexamethasone. In a further Encephalitis embodiment, the patient is further treated with an oral hydration solution during the treatment.

[20] In a Yellow Fever embodiment, two injections of between about 0.1-9.2 ml of about 0.5-15% proparacaine and between about 0.3-9.6 ml of about 4 mg/ml betamethasone are administered daily for between about 3-5 days. The two injections are administered over an about 60-90 minute interval. As an example, an adult is treated with two about 2 ml doses daily for 3-5 days. The patient is further treated with an oral hydration solution during the treatment. A child may be treated with 1 ml doses. [21] More generally, patients having any of SARS, Ebola, Marburg, West Nile,

German Measles, Yellow Fever, Saint Louis Encephalitis, Japanese Encephalitis, California Encephalitis, Human T-cell Leukemia, Newcastle Disease, respiratory tract infection and bronchitis, Lymphocytic Choriomeningitis, Lassa Hemorrhagic Fever, and Hanta Hemorrhagic Fever are treated with an IM injection of a mixture comprising between about 0.2-7.9 ml of about 0.5-17% a first ingredient select from the group consisting of procaine, chloroprocaine, tetracaine, chlorotetracaine, bromoprocaine, proparacaine, fluoroprocaine and benzocaine, and between about 0.1-8.5 ml of about 4 mg/ml of a second ingredient selected from the group consisting of dexamethasone, flumethasone and betamethasone. The treatment plans for SARS, Ebola, Marburg, West Nile, German Measles, Yellow Fever, Saint Louis Encephalitis, Japanese Encephalitis, California Encephalitis, Newcastle Disease, respiratory tract infection and bronchitis, Lymphocytic Choriomeningitis, Lassa Hemorrhagic Fever, and Hanta Hemorrhagic Fever generally involve two injections at about 60-120 minute intervals for between about 1-5 days. For Human T-cell Leukemia, the treatment plan is similar except for the 15-30 day treatment duration. In a further embodiment, the Human T-cell Leukemia patient is treated for 5 days, and then treated daily under lab testing until favorable results are found. The patients are further treated with an electrolyte solution during the treatment.

[22] Generally, for all treatments, children may be treated by IM injections of between about 0.5-7 ml of the above-described mixtures in the above-described time intervals. In particular embodiments, an adult is injected with about 2 ml of the mixture, while children under 13 are injected with about 1 ml of the mixture. The treatment interval for all treatments can vary, depending on virus, from once every 3 days up to 3-4 per day.

[23] In an embodiment, a composition according to the present invention is made by mixing 2% chloroprocaine and dexamethasone Sodium Phosphate. Around 30 mg of chloroprocaine in 1.5 ml of a 20 mg/ml solution is mixed with around 2 mg of dexamethasone in 0.5 ml of a 4 mg/ml solution. The total volume of 2 ml comprising the two mixed formulations are gently mixed and aseptically transferred into a sterile 2 ml syringe. Empirical observation indicated that treatment by chloroprocaine and dexamethasone took less time and produced improved results over procaine and dexamethasone. In an alternative embodiment, 3 ml is taken out of a 30 ml bottle of chloroprocaine (20 mg/ml). Then 3 ml of dexamethasone (4 mg/ml) is added into the above-recited chloroprocaine bottle. This provides a 9:1 mixture of chloroprocaine and dexamethasone. The bottle is gently mixed (shaken) and the solution is ready to be aseptically transferred into a sterile syringe for IM administration.