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[Continued on next page]

(54) Title: IMINOSUGARS AND METHODS OF TREATING BUNYAVIRAL AND TOGAVIRAL DISEASES

Fig. 5

Compound	Activity vs. viral families IC50, uM		
	Bunya	Toga	
	RVFV	VEEV	CHIKV
NB-DNJ	250	156	>500
NN-DNJ	58	12	56
N7-O-DNJ	220	>250	500
N9-DNJ	250	>250	500
NAP-DNJ	49	2	22

Virus abbreviations:

RVFV - Rift Valley fever

VEEV - Venezuelan equine encephalitis

CHIKV - Chikungunya

(57) Abstract: Provided are novel methods of treating and/or preventing a disease or condition caused by or associated with a virus belonging to the Bunyaviridae or Togaviridae family using iminosugars, such as DNJ derivatives.



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IMINOSUGARS AND METHODS OF TREATING BUNYAVIRAL AND TOGAVIRAL DISEASES

CROSS-REFERENCE TO RELATED APPLICATIONS

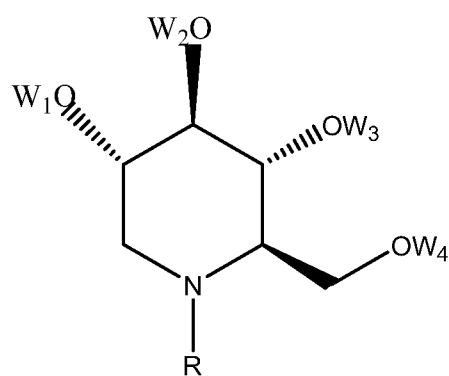
[0001] This application claims priority from U.S. Provisional Application No. 61/186,614, filed June 12, 2009, the entire contents of which are incorporated herein by reference.

FIELD

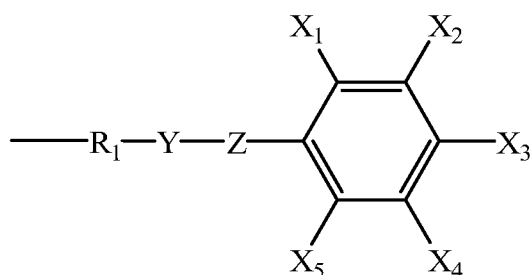
[0002] The present application relates to iminosugars and methods of treating viral diseases with iminosugars and, in particular, to the use of iminosugars for treatment and prevention of diseases caused by or associated with a virus that belongs to the Bunyaviridae or Togaviridae family.

SUMMARY

[0003] One embodiment provides a method of treating or preventing a disease or condition caused by or associated with a virus belonging to the Bunyaviridae family, the method comprising administering to a subject in need thereof a compound of the formula,



or a pharmaceutically acceptable salt thereof, wherein R is either selected from substituted or unsubstituted alkyl groups, substituted or unsubstituted cycloalkyl groups, substituted or unsubstituted aryl groups, or substituted or unsubstituted oxaalkyl groups; or wherein R is



R_1 is a substituted or unsubstituted alkyl group;

X_{1-5} are independently selected from H, NO_2 , N_3 , or NH_2 ;

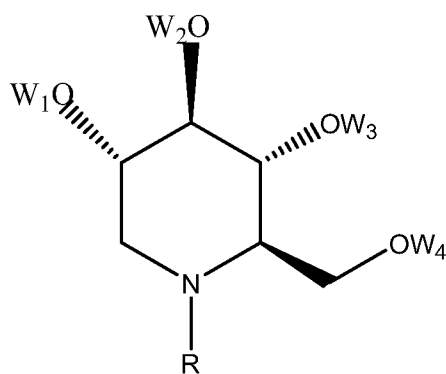
Y is absent or is a substituted or unsubstituted C_1 -alkyl group, other than carbonyl; and

Z is selected from a bond or NH; provided that when Z is a bond, Y is absent, and provided that when Z is NH, Y is a substituted or unsubstituted C_1 -alkyl group, other than carbonyl; and

wherein W_{1-4} are independently selected from hydrogen, substituted or unsubstituted alkyl groups, substituted or unsubstituted haloalkyl groups, substituted or unsubstituted alkanoyl groups, substituted or unsubstituted aroyl groups, or substituted or unsubstituted haloalkanoyl groups.

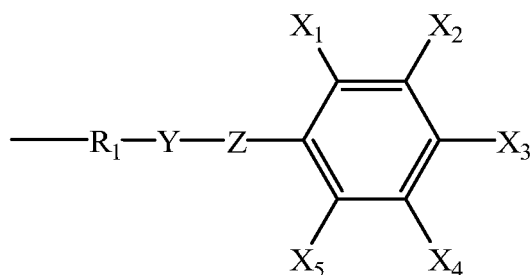
[0004] Another embodiment provides a method of treating or preventing a disease or condition caused by or associated with a virus belonging to the Togaviridae family, the method comprising

administering to a subject in need thereof a compound of the formula,



or a pharmaceutically acceptable salt thereof, wherein R is

either selected from substituted or unsubstituted alkyl groups, substituted or unsubstituted cycloalkyl groups, substituted or unsubstituted aryl groups, or substituted or unsubstituted oxaalkyl groups; or wherein R is



R_1 is a substituted or unsubstituted alkyl group;

X_{1-5} are independently selected from H, NO_2 , N_3 , or NH_2 ;

Y is absent or is a substituted or unsubstituted C_1 -alkyl group, other than carbonyl; and

Z is selected from a bond or NH; provided that when Z is a bond, Y is absent, and provided that when Z is NH, Y is a substituted or unsubstituted C_1 -alkyl group, other than carbonyl; and

wherein W_{1-4} are independently selected from hydrogen, substituted or unsubstituted alkyl groups, substituted or unsubstituted haloalkyl groups, substituted or unsubstituted alkanoyl groups, substituted or unsubstituted aroyl groups, or substituted or unsubstituted haloalkanoyl groups.

DRAWINGS

[0005] Figures 1(A)-(E) present chemical formulas of the following iminosugars: A) *N*-Butyl deoxynojirimycin (NB-DNJ or UV-1); B) *N*-Nonyl deoxynojirimycin (NN-DNJ or UV-2); C) *N*-(7-Oxadecyl)deoxynojirimycin (N7-O-DNJ or UV-3); D) *N*-(9-Methoxynonyl)deoxynojirimycin (N9-DNJ or UV-4); E) *N*-(*N*-{4'-azido-2'-nitrophenyl}-6-aminoethyl)deoxynojirimycin (NAP-DNJ or UV-5).

[0006] Figure 2 is a synthesis scheme for NN-DNJ.

[0007] Figures 3A-D illustrate synthesis of N7-O-DNJ. In particular, Figure 3A shows a sequence of reactions leading to N7-O-DNJ; Figure 3B illustrates preparation of 6-propyloxy-1-hexanol; Figure 3C illustrates preparation of 6-propyloxy-1-hexanal; Figure 3D illustrates synthesis of N7-O-DNJ.

[0008] Figures 4A-C relate to synthesis of *N*-(9-Methoxynonyl) deoxynojirimycin. In particular, Figure 4A illustrates preparation of 9-methoxy-1-nonanol; Figure 4B illustrates preparation of 9-methoxy-1-nonanal; Figure 4C illustrates synthesis of *N*-(9-Methoxynonyl) deoxynojirimycin.

[0009] Figure 5 presents a table with in vitro IC₅₀ (μm) data for NB-DNJ; NN-DNJ; N7-O-DNJ; N9-DNJ and NAP-DNJ against selected Bunyaviruses (Rift Valley Fever virus (RVFV)) and Togaviruses (Venezuelan equine encephalitis virus (VEEV)) and Chikungunya virus (CHIKV).

[0010] Figure 6 presents dose response curves for Rift Valley Fever virus (RVFV).

[0011] Figure 7 presents dose response curves for Venezuelan equine encephalitis virus (VEEV).

[0012] Figure 8 presents dose response curves for Chikungunya virus (CHIKV).

DETAILED DESCRIPTION

Definition of terms

[0013] Unless otherwise specified, “a” or “an” means “one or more.”

[0014] As used herein, the term “viral infection” describes a diseased state, in which a virus invades a healthy cell, uses the cell’s reproductive machinery to multiply or replicate and ultimately lyse the cell resulting in cell death, release of viral particles and the infection of other cells by the newly produced progeny viruses. Latent infection by certain viruses is also a possible result of viral infection.

[0015] As used herein, the term “treating or preventing viral infection” means to inhibit the replication of the particular virus, to inhibit viral transmission, or to prevent the virus from establishing itself in its host, and to ameliorate or alleviate the symptoms of the disease caused by the viral infection. The treatment is considered therapeutic if there is a reduction in viral load, decrease in mortality and/or morbidity.

[0016] IC₅₀ or IC₉₀ (inhibitory concentration 50 or 90) is a concentration of a therapeutic agent, such as an iminosugar, used to achieve 50% or 90% reduction of viral load, respectively.

Disclosure

[0017] The present inventors discovered that certain iminosugars, such as deoxynojirimycin derivatives, can be effective against viruses that belong to the Bunyaviridae or Togaviridae family and, thus, these iminosugars can be useful for treating or preventing a disease or

condition caused by or associated with a virus that belongs to the Bunyaviridae or Togaviridae family

[0018] The family Bunyaviridae contains the following genera: Genus *Hantavirus*; Genus *Nairovirus*; Genus *Orthobunyavirus*; Genus *Phlebovirus*; Genus *Tospovirus*; Genus *Tenuivirus*. Of these genera, all can infect vertebrates except Tospoviruses, which can only infect arthropods and plants.

[0019] Genus *Hantavirus* includes the following viruses: Andes virus (ANDV); Bayou virus (BAYV); Black Creek Canal Virus (BCCV); Cano Delgadito virus (CADV); Choclo virus (CHOV); Dobrava-Belgrade virus (DOBV); Hantaan virus (HNTV); Isla Vista virus (ISLAV); Khabarovsk virus (KHAV); Laguna Negra virus (LANV); Muleshoe virus (MULV); New York virus (NYV); Prospect Hill Virus (PHV); Puumala virus (PMV); Rio Mamore virus (RIOMV); Rio Segundo virus (RIOSV); Seoul virus (SEOV); Sin Nombre virus (SNV); Thailand virus (THAIV); Thottapalayam (TPMV); Topografov virus (TOPV); Tula virus (TULV); Bakau virus.

[0020] Genus *Nairovirus* includes the following viruses: Crimean-Congo hemorrhagic fever virus; Dugbe Virus; Qalyub Virus; Sakhalin Virus; Dera Ghazi Khan; Thiafora Virus; and Hughes Virus.

[0021] Genus *Orthobunyavirus* includes La Crosse virus; California encephalitis virus and Jamestown Canyon virus.

[0022] Genus *Phlebovirus* includes Alenquer virus, Chandiru virus, Chagres virus, Sandfly Fever Naples virus, Sandfly Fever Sicilian virus, Sandfly Fever Toscana virus, Rift Valley Fever virus and Punta Toro virus.

[0023] Diseases and conditions that can be caused by or associated with viruses, that belong to the family Bunyaviridae, include, but not limited to, Hantavirus infection; hemorrhagic fever with renal syndrome (HFRS), which can be caused by a virus of Hantavirus Genus, such as Hantaan virus, Puumala virus, Seoul virus and Dobrava virus; Hantavirus cardiopulmonary syndrome (HCPS or HPS), which can be caused by a virus of Hantavirus Genus, such as Sin Nombre virus, Andes virus, New York virus, Bayou virus, and Black Creek Canal virus; Nephropathia epidemica, which can be caused by Puumala virus; hemorrhagic fever caused by Seoul virus; Sweating sickness; Crimean-Congo hemorrhagic fever; La Crosse encephalitis; California encephalitis, which can be caused by a virus of

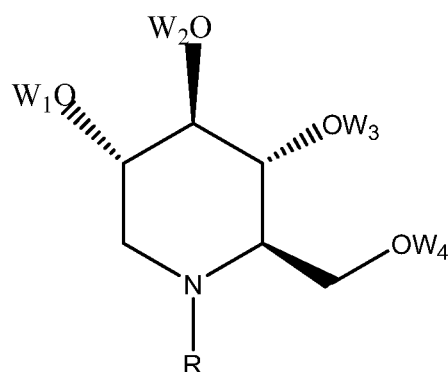
Genus *Orthobunyavirus*, such as La Crosse virus, California encephalitis virus and Jamestown Canyon virus; Phlebotomus fever; and Rift Valley fever.

[0024] The Togaviridae family includes Genus Alphavirus and Genus Rubivirus.

[0025] Genus Alphavirus includes the following viruses: Sindbis virus; Semliki Forest virus; O'nyong'nyong virus; Chikungunya virus; Mayaro virus; Ross River virus; Barmah Forest virus; Eastern equine encephalitis virus; Western equine encephalitis virus; and Venezuelan equine encephalitis virus. Genus Rubivirus includes Rubella viruses.

[0026] Diseases and conditions that can be caused by or associated with viruses, that belong to the family Togaviridae, include, but not limited to, sindbis fever; O'nyong'nyong fever; Chikungunya disease; Ross River fever; Barmah Forest virus infection; Eastern equine encephalitis; Western equine encephalitis; Venezuelan equine encephalitis and Rubella.

[0027] The iminosugar can be a compound of the following formula:



where W_{1-4} are independently selected from hydrogen, substituted or unsubstituted alkyl groups, substituted or unsubstituted haloalkyl groups, substituted or unsubstituted alkanoyl groups, substituted or unsubstituted aroyl groups, or substituted or unsubstituted haloalkanoyl groups.

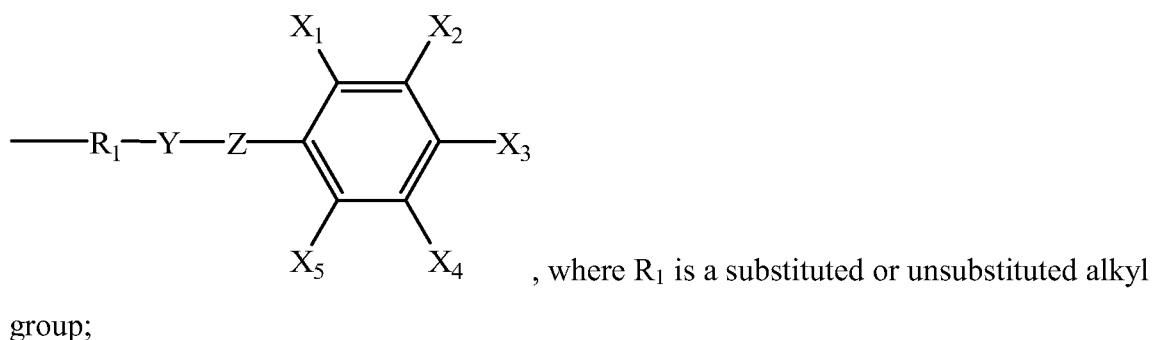
[0028] In some embodiments, R can be selected from substituted or unsubstituted alkyl groups, substituted or unsubstituted cycloalkyl groups, substituted or unsubstituted aryl groups, or substituted or unsubstituted oxaalkyl groups.

[0029] In some embodiments, R can be substituted or unsubstituted alkyl groups and/or substituted or unsubstituted oxaalkyl groups comprise from 1 to 16 carbon atoms, from 4 to 12 carbon atoms or from 8 to 10 carbon atoms. The term "oxaalkyl" refers to an alkyl derivative, which can contain from 1 to 5 or from 1 to 3 or from 1 to 2 oxygen atoms. The term "oxaalkyl" includes hydroxyterminated and methoxyterminated alkyl derivatives.

[0030] In some embodiments, R may be selected from, but is not limited to $-(\text{CH}_2)_6\text{OCH}_3$, $-(\text{CH}_2)_6\text{OCH}_2\text{CH}_3$, $-(\text{CH}_2)_6\text{O}(\text{CH}_2)_2\text{CH}_3$, $-(\text{CH}_2)_6\text{O}(\text{CH}_2)_3\text{CH}_3$, $-(\text{CH}_2)_2\text{O}(\text{CH}_2)_5\text{CH}_3$, $-(\text{CH}_2)_2\text{O}(\text{CH}_2)_6\text{CH}_3$, and $-(\text{CH}_2)_2\text{O}(\text{CH}_2)_7\text{CH}_3$.

[0031] In some embodiments, R may be an branched or unbranched, substituted or unsubstituted alkyl group. In certain embodiments, the alkyl group may be a long chain alkyl group, which may be C6-C20 alkyl group; C8-C16 alkyl group; or C8-C10 alkyl group.

[0032] In some embodiments, R can have the following formula



X₁₋₅ are independently selected from H, NO₂, N₃, or NH₂;

Y is absent or is a substituted or unsubstituted C₁-alkyl group, other than carbonyl; and

Z is selected from a bond or NH; provided that when Z is a bond, Y is absent, and provided that when Z is NH, Y is a substituted or unsubstituted C₁-alkyl group, other than carbonyl.

[0033] In some embodiments, Z is NH and R₁-Y is a substituted or unsubstituted alkyl group, such as C2-C20 alkyl group or C4-C12 alkyl group or C4-C10 alkyl group.

[0034] In some embodiments, X₁ is NO₂ and X₃ is N₃. In some embodiments, each of X₂, X₄ and X₅ is hydrogen.

[0035] In some embodiments, the iminosugar is a DNJ derivative disclosed in U.S. Patent application publication no. 2007/0275998, which is incorporated herein by reference.

[0036] In some embodiments, the deoxynojirimycin derivative can be one of the compounds presented in Figure 1.

[0037] Methods of synthesizing deoxynojirimycin derivatives are disclosed, for example, in U.S. Patent nos. 5,622,972, 5,200,523, 5,043,273, 4,994,572, 4,246,345, 4,266,025, 4,405,714, and 4,806,650 and U.S. Patent application publication no. 2007/0275998, which are all incorporated herein by reference.

[0038] In some embodiments, the iminosugar can be in a form of a salt derived from an inorganic or organic acid. Pharmaceutically acceptable salts and methods for preparing salt

forms are disclosed, for example, in Berge et al. (*J. Pharm. Sci.* 66:1-18, 1977). Examples of appropriate salts include but are not limited to the following salts: acetate, adipate, alginate, citrate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, camphorate, camphorsulfonate, digluconate, cyclopentanepropionate, dodecylsulfate, ethanesulfonate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, fumarate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, nicotinate, 2-naphthalenesulfonate, oxalate, palmoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, mesylate, and undecanoate.

[0039] In some embodiments, the iminosugar may also be used in a form of a prodrug. Prodrugs of DNJ derivatives, such as the 6-phosphorylated DNJ derivatives, are disclosed in U.S. Patents nos. 5,043,273 and 5,103,008.

[0040] In some embodiments, the iminosugar may be used as a part of a composition, which further comprises a pharmaceutically acceptable carrier and/or a component useful for delivering the composition to an animal. Numerous pharmaceutically acceptable carriers useful for delivering the compositions to a human and components useful for delivering the composition to other animals such as cattle are known in the art. Addition of such carriers and components to the composition of the invention is well within the level of ordinary skill in the art.

[0041] In some embodiments, the iminosugar may be used in a liposome composition, such as those disclosed in US publication 2008/0138351; US application No. 12/410,750 filed March 25, 2009 and US provisional application No. 61/202,699 filed March 27, 2009.

[0042] The iminosugar, such as a DNJ derivative, can be administered to a cell or an animal affected by a virus. The iminosugar can inhibit morphogenesis of the virus, or it can treat the animal. The treatment can reduce, abate, or diminish the virus infection in the animal.

[0043] Animals that can be infected with a virus that belongs to the Bunyaviridae or Togaviridae family, include vertebrates, such as birds and mammals including primates, humans, rodents, livestock animals, such as sheep and goats, and equines such as horses, zebras and donkeys, as well as invertebrates.

[0044] The amount of iminosugar administered to a cell, or an animal can be an amount effective to inhibit the morphogenesis of a virus, that belongs to the Bunyaviridae or

Togaviridae family. The term "inhibit" as used herein can refer to the detectable reduction and/or elimination of a biological activity exhibited in the absence of the iminosugar. The term "effective amount" can refer to that amount of the iminosugar necessary to achieve the indicated effect. The term "treatment" as used herein can refer to reducing or alleviating symptoms in a subject, preventing symptoms from worsening or progressing, inhibition or elimination of the causative agent, or prevention of the infection or disorder related to the virus that belongs to the Bunyaviridae or Togaviridae family in a subject who is free therefrom.

[0045] Thus, for example, treatment of the disease caused by or associated with a virus can include destruction of the infecting agent, inhibition of or interference with its growth or maturation, and neutralization of its pathological effects. The amount of the iminosugar which can be administered to the cell or animal is preferably an amount that does not induce any toxic effects which outweigh the advantages which accompany its administration.

[0046] Actual dosage levels of active ingredients in the pharmaceutical compositions may vary so as to administer an amount of the active compound(s) that is effective to achieve the desired therapeutic response for a particular patient.

[0047] The selected dose level can depend on the activity of the iminosugar, the route of administration, the severity of the condition being treated, and the condition and prior medical history of the patient being treated. However, it is within the skill of the art to start doses of the compound(s) at levels lower than required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. If desired, the effective daily dose may be divided into multiple doses for purposes of administration, for example, two to four doses per day. It will be understood, however, that the specific dose level for any particular patient can depend on a variety of factors, including the body weight, general health, diet, time and route of administration and combination with other therapeutic agents and the severity of the condition or disease being treated. The adult human daily dosage may range from between about one microgram to about one gram, or from between about 10 mg and 100 mg, of the iminosugar per 10 kilogram body weight. Of course, the amount of the iminosugar which should be administered to a cell or animal can depend upon numerous factors well understood by one of skill in the art, such as the molecular weight of the iminosugar and the route of administration.

[0048] Pharmaceutical compositions that are useful in the methods of the invention may be administered systemically in oral solid formulations, ophthalmic, suppository, aerosol, topical or other similar formulations. For example, it may be in the physical form of a powder, tablet, capsule, lozenge, gel, solution, suspension, syrup, or the like. In addition to the iminosugar, such pharmaceutical compositions may contain pharmaceutically-acceptable carriers and other ingredients known to enhance and facilitate drug administration. Other possible formulations, such as nanoparticles, liposomes resealed erythrocytes, and immunologically based systems may also be used to administer the iminosugar. Such pharmaceutical compositions may be administered by a number of routes. The term "parenteral" used herein includes subcutaneous, intravenous, intraarterial, intrathecal, and injection and infusion techniques, without limitation. By way of example, the pharmaceutical compositions may be administered orally, topically, parenterally, systemically, or by a pulmonary route.

[0049] These compositions may be administered in a single dose or in multiple doses which are administered at different times. Because the inhibitory effect of the composition upon a virus, that belongs to the Bunyaviridae or Togaviridae family, may persist, the dosing regimen may be adjusted such that virus propagation is retarded while the host cell is minimally effected. By way of example, an animal may be administered a dose of the composition of the invention once per week, whereby virus propagation is retarded for the entire week, while host cell functions are inhibited only for a short period once per week.

[0050] Embodiments described herein are further illustrated by, though in no way limited to, the following working examples.

Working Examples

1. Synthesis of N-Nonyl DNJ

Table 1. Materials for NN-DNJ synthesis

Name	Amount
DNJ	500 mg
Nonanal	530 mg

Ethanol	100 mL
AcOH	0.5 mL
Pd/C	500 mg

[0051] Procedure: A 50-mL, one-necked, round-bottom flask equipped with a magnetic stirrer was charged with DNJ (500 mg), ethanol (100 mL), nonanal (530 mg), and acetic acid (0.5 mL) at room temperature. The reaction mixture was heated to 40-45 °C and stirred for 30-40 minutes under nitrogen. The reaction mixture was cooled to ambient temperature and Pd/C was added. The reaction flask was evacuated and replaced by hydrogen gas in a balloon. This process was repeated three times. Finally, the reaction mixture was stirred at ambient temperature overnight. The progress of reaction was monitored by TLC (Note 1). The reaction mixture was filtered through a pad of Celite and washed with ethanol. The filtrate was concentrated *in vacuo* to get the crude product. The crude product was purified by column chromatography (230-400 mesh silica gel). A solvent gradient of methanol in dichloromethane (10-25%) was used to elute the product from the column. All fractions containing the desired product were combined, and concentrated *in vacuo* to give the pure product (420mg). Completion of the reaction was monitored by thin layer chromatography (TLC) using a thin layer silica gel plate; eluent; methanol : dichloromethane = 1:2

2. Synthesis of N-7-Oxadecyl DNJ

2a. Synthesis of 6-propyloxy-1-hexanol

Table 2. Materials for synthesis of 6-propyloxy-1-hexanol

Name	Amount
1,6-hexanediol	6.00 g
1-Iodopropane	8.63 g
Potassium tert-butoxide	5.413 mg
THF	140 mL

[0052] Procedure: a 500-mL, one-necked, round-bottom flask equipped with a magnetic stirrer was charged with 1,6-hexanediol (6.00 g), potassium tert-butoxide (5.413 g) at room

temperature. The reaction mixture was stirred for one hour, and then 1-iodopropane (8.63 g) was added. The reaction mixture was heated to 70-80 °C and stirred overnight. The progress of reaction was monitored by TLC (Note 1). After completion of the reaction, water was added to the reaction mixture, and extracted with ethyl acetate (2 x 100 mL). The combined organic layers were concentrated *in vacuo* to get the crude product. The crude product was dissolved in dichloromethane and washed with water, and then brine, dried over sodium sulfate. The organic layer was concentrated *in vacuo* to get the crude product. The crude product was purified by column chromatography using 230-400 mesh silica gel. A solvent gradient of ethyl acetate in hexanes (10-45%) was used to elute the product from the column. All fractions containing the desired pure product were combined and concentrated *in vacuo* to give pure 6-propyloxy-1-hexanol (lot D-1029-048, 1.9 g, 25%) Completion of the reaction was monitored by thin layer chromatography (TLC); (eluent: 60% ethyl acetate in hexanes).

2b. Preparation of 6-propyloxy-1-hexanal

Table 3. Materials for preparation of 6-propyloxy-1-hexanal

Name	Amount
6-Propyloxy-1-hexanol	1.00 g
PDC	4.70 g
Celite	1.00 g
NaOAc	100 mg
CH ₂ Cl ₂	10 mL

[0053] Procedure: a 50-mL, one-necked, round-bottom flask equipped with a magnetic stirrer was charged with 6-propyloxy-1-hexanol (1.0 g), PDC (4.7 g), dichloromethane (10 mL), Celite (1.0 g), and sodium acetate (100 mg). The reaction mixture was stirred at room temperature under nitrogen for 5 minutes. PDC (4.70 g) was added to the reaction mixture, and stirred overnight. The progress of reaction was monitored by TLC (Note 1). After completion of the reaction, the reaction mixture was directly loaded on the column (230-400 mesh silica gel). A solvent gradient of dichloromethane in ethyl acetate (10-20%) was used to elute the product from the column. All fractions containing the desired pure product were

combined and concentrated *in vacuo* to give pure 6-propyloxy-1-hexanal (lot D-1029-050, 710 mg, 71%). Completion of the reaction was monitored by thin layer chromatography (TLC); (eluent: 60% ethyl acetate in hexanes).

2c Synthesis of N-7-Oxadecyl-DNJ

Table 4. Materials for Synthesis of N-7-Oxadecyl-DNJ

Name	Amount
DNJ	500 mg
6-Propyloxy-1-hexanal	585 mg
Pd/C	125 mg
Ethanol	15 mL
Acetic acid	mL

[0054] Procedure: a 50-mL, one-necked, round-bottom flask equipped with a magnetic stirrer was charged with DNJ (500 mg), ethanol (15 mL), 6-propyloxy-1-hexanal (585 mg), and acetic acid (0.1mL) t room temperature. The reaction mixture was heated to 40-45 °C and stirred for 30-40 minutes under nitrogen. The reaction mixture was cooled to ambient temperature and and Pd/C was added. The reaction flask was evacuated and replaced by hydrogen gas in a balloon. This process was repeated three times. Finally, the reaction mixture was stirred at ambient temperature overnight. The progress of reaction was monitored by TLC (Note 1). The reaction mixture was filtered through a pad of Celite and washed with ethanol. The filtrate was concentrated *in vacuo* to get the crude product. The crude product was purified by column chromatography (230-400 mesh silica gel). A solvent gradient of methanol in dichloromethane (10-40%) was used to elute the product from the column. All fractions containing the desired product were combined, and concentrated *in vacuo* to give the pure product. (Lot: D-1029-052 (840 mg). Completion of the reaction was monitored by thin layer chromatography (TLC); (eluent: 50% methanol in dichloromethane).

3. Synthesis of N-(9-methoxy)-nonyl DNJ

3a Preparation of 9-methoxy-1-nonanol

Table 5. Materials for preparation of 9-methoxy-1-nonanol

Name	Amount
1,9-nonanediol	10.0 g
Dimethyl sulfate	41.39 g
Sodium hydroxide	5.0g
DMSO	100 mL

[0055] Procedure: a 500-mL, one-necked, round-bottom flask equipped with a magnetic stirrer and stir bar was charged with 1,9-nonanediol (10.00 g, 62.3 mmol) in dimethyl sulfoxide (100 mL) and H₂O (100 mL). To this was added slowly a solution of sodium hydroxide (5.0 g, 125.0 mmol) in H₂O (10 mL) at room temperature. During addition of sodium hydroxide the reaction mixture generated heat and the temperature rose to ~40 °C. The mixture was stirred for one hour, and then dimethyl sulfate (16.52 g, 131 mmol) was added in four portions while maintaining the temperature of the reaction mixture at ~ 40⁰C. The reaction mixture was stirred at room temperature overnight. Progress of the reaction was monitored by TLC (Note 1). TLC monitoring indicated that the reaction was 25 % conversion. At this stage additional dimethyl sulfate (24.78g, 196.44 mmol) was added and the resulting mixture was stirred at room temperature for an additional 24 h. After completion of the reaction, sodium hydroxide (10% solution in water) was added to the reaction mixture to adjust the pH of the solution to 11-13. The mixture was stirred at room temperature for 2 h and extracted with dichloromethane (3 x 100 mL). The combined organic layers were washed with H₂O (200 mL), brine (150 mL), dried over anhydrous sodium sulfate (20 g), filtered and concentrated *in vacuo* to obtain a crude product (14 g). The crude product was purified by column chromatography using 250-400 mesh silica gel. A solvent gradient of ethyl acetate in hexanes (10-50%) was used to elute the product from the column. All fractions containing the desired pure product were combined and concentrated *in vacuo* to give pure 9-methoxy-1-nonanol (lot D-1027-155, 2.38 g, 21.9 %). Completion of the reaction was monitored by thin

layer chromatography (TLC) using a thin layer silica gel plate; eluent: 60% ethyl acetate in hexanes.

3b Preparation of 9-methoxy-1-nonanal

Table 6. Materials for preparation of 9-methoxy-1-nonanal

Name	Amount
9-methoxy-1-nonanol	1.0 g
PDC	4.7 g
Molecular sieves, 3A	1.0 g
NaOAc	0.1 g
CH ₂ Cl ₂	10 mL

[0056] Procedure: a 50-mL, one-necked, round-bottom flask equipped with a magnetic stirrer and stir bar was charged with 9-methoxy-nonanol (1.0 g, 5.9 mmol), dichloromethane (10 mL), molecular sieves (1.0 g, 3A), sodium acetate (0.1 g) at room temperature. The reaction mixture was stirred at room temperature under nitrogen for 5 minutes. The reaction mixture was charged with pyridinium dichromate (4.7 g, 12.5 mmol) and stirred overnight. The progress of reaction was monitored by TLC (Note 1). After completion of the reaction, the reaction mixture was filtered through a bed of silica gel (~15 g). The filtrate was evaporated in *vacuo* to obtain a crude compound. This was purified by column chromatography using silica gel column (250-400 mesh, 40 g). A solvent gradient of ethyl acetate in hexane (10-50%) was used to elute the product from the column. All fractions containing the desired pure product were combined and concentrated *in vacuo* to give pure 9-methoxy-nonanal (lot D-1027-156, 553 mg, 54.4%). Completion of the reaction was monitored by thin layer chromatography (TLC) using a thin layer silica gel plate; eluent: 60% ethyl acetate in hexanes.

3c Synthesis of N-(9-methoxy)-nonyl DNJ

Table 7. Materials for synthesis of N-(9-methoxy)-nonyl DNJ

Name	Amount
DNJ	300 mg
9-methoxy-1-nonanal	476 mg
Pd/C	200 mg
Ethanol	20 mL

[0057] Procedure: a 50-mL, two-necked, round-bottom flask equipped with magnetic stirrer and a stir bar was charged with DNJ (300 mg, 1.84 mmol), ethanol (20 mL), 9-methoxy-1-nonanal (476 mg, 2.76 mmol) at room temperature. The reaction mixture was stirred for 5-10 minutes under nitrogen and Pd/C was added at room temperature. The reaction mixture was evacuated and was replaced by hydrogen gas using a balloon. This process was repeated three times and then reaction mixture was stirred under atmospheric hydrogen at room temperature. The progress of reaction was monitored by TLC (Note 1). The reaction mixture was filtered through a bed of Celite and was washed with ethanol (20 mL). The filtrate was concentrated *in vacuo* to get a crude product. The crude product was purified by column chromatography using 250-400 mesh silica gel (20 g). A solvent gradient of methanol in ethyl acetate (5-25%) was used to elute the product from the column. All fractions containing the desired pure product were combined, and concentrated *in vacuo* to give an off white solid. The solid was triturated in ethyl acetate (20 mL), filtered and dried in high vacuum to give a white solid [lot: D-1027-158 (165.3 mg, 28.1%). Completion of the reaction was monitored by thin layer chromatography (TLC) using a thin layer silica gel plate; eluent: 50% methanol in dichloromethane.

4. Inhibition of selected bunyaviruses and togaviruses

[0058] Figure 5 presents a table with *in vitro* IC₅₀ (μm) data for NB-DNJ; NN-DNJ; N7-O-DNJ; N9-DNJ and NAP-DNJ against Rift Valley Fever virus (RVFV)), which is a Bunyavirus, and Venezuelan equine encephalitis virus (VEEV)) and Chikungunya virus (CHIKV), which are Togaviruses.

[0059] Compounds. Base stocks of the following compounds were prepared in dimethylsulfoxide (DMSO) to a final maximal DMSO concentration of 0.5%: NB-DNJ, NN-

DNJ, N7-O-DNJ, N9-DNJ, and NAP-DNJ. All compounds were diluted from the base stocks to their experimental concentrations.

[0060] Viruses. The compounds were screened for inhibition against Rift Valley Fever Virus (Bunyavirus) MP12 strain, Chikungunya (Togaviridae) 181/25 strain, and the Venezuelan Equine Encephalitis (Togaviridae) TC-83 strain. Viral stocks were made by propagation in Vero cells using modified Eagle medium (MEM, Sigma), supplemented with 2% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 ug/ml streptomycin and titered using the standard plaque assay (method presented below). Viral stocks were stored at -80°C until used.

[0061] Virus Yield Reduction Assay. The virus yield assay were performed by standard plaque assay on supernatant samples generated from virus-infected cells incubated with different concentrations of the UV compound. 24-well cell culture plates were seeded with cells in 1mL MEM with 10% fetal bovine serum Vero cells (ATCC, Mannassas, VA; ATCC number CCL-81) in MEM with Earl's salts (Sigma, St Louis, MO) supplemented with 2mM L-glutamine, 100U/mL penicillin/streptomycin, and 2% heat-inactivated fetal bovine serum and incubated at 37°C for 24 hours or until ~80% confluency. Medium were replaced with medium supplemented with 2% fetal bovine serum and the compound concentrations to be used started at 250 uM (or 125 uM) and tested in triplicate using 8 dilutions. compounds starting at 250 or 125 uM dilution is added to appropriate well and incubated for 1hr at 37°C, 5% CO₂. After 1hr incubation virus is added to each well. Four days are required for the RVFV, three days for CHIKV, and two days for VEE virus infection. Upon completion of infection, supernatant were harvested and collected in 0.5mL MCF tubes for titering.

[0062] To titer RVFV MP12, CHIKV 181/25, and VEE TC-83, 12-well plates with 80% confluent Vero cells in growth medium were used. Viral supernatant were diluted from 10⁻³ to 10⁻⁸ and added (100uL) to the cells and incubated at 37°C for 1 hour with shaking every 5-10 minutes. Viral infection medium (100uL) were aspirated and replace with 1mL pre-warmed 2% low-melt agarose mixed 1:1 with 2X MEM (5% fetal calf serum) and incubated at 37°C, 5% CO₂ for 6 days followed by plaque visualization by neutral red staining. IC₅₀ was determined as concentration of compound resulting in 50% virus inhibition.

[0063] Figure 6 presents dose response curves for Rift Valley Fever virus (RVFV). The virus yield assay were performed as disclosed for Figure 5. RVFV MP12 virus inhibition

was found for compounds UV-2 (NN-DNJ), -3 (N7-O-DNJ), and -5 (NAP-DNJ) with EC50s of 58, 218, and 49 μ M. UV-2 was toxic to cells at the highest concentration (250 μ M).

Compounds UV-1 (NB-DNJ) and -4 (N9-DNJ) all have EC50s over 250 μ M.

[0064] Figure 7 presents dose response curves for Venezuelan equine encephalitis virus (VEEV). The virus yield assay were performed as disclosed above for Figure 5. VEE virus inhibition was found for compounds UV-1 (NB-DNJ), -2 (NN-DNJ), and -5 (NAP-DNJ) with EC50s of 156, 12, and 2 μ M. UV-2 was toxic at the highest concentration (250 μ M).

Compounds UV-3 (N7-O-DNJ) and -4 (N9-DNJ) all had EC50s over 250 μ M.

[0065] Figure 8 presents dose response curves for Chikungunya virus (CHIKV). The virus yield assay were performed as in Figure 5. Chikungunya virus inhibition was found for compounds UV-5 (NAP-DNJ) with an EC50 of 22 μ M. UV-2 (NN-DNJ) showed protection with an EC50 of 56 μ M. Compounds UV-1 (NB-DNJ), -3 (N7-O-DNJ), -4 (N9-DNJ) all have EC50s over 500 μ M.

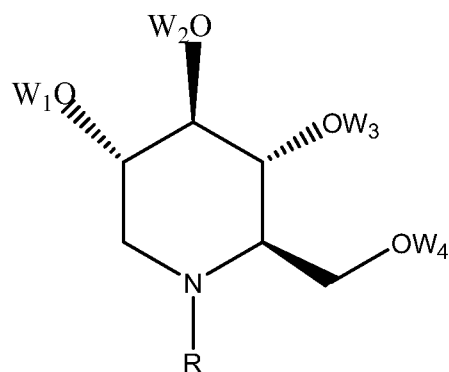
* * *

[0066] Although the foregoing refers to particular preferred embodiments, it will be understood that the present invention is not so limited. It will occur to those of ordinary skill in the art that various modifications may be made to the disclosed embodiments and that such modifications are intended to be within the scope of the present invention.

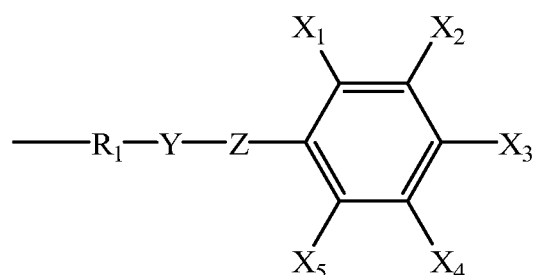
[0067] All of the publications, patent applications and patents cited in this specification are incorporated herein by reference in their entirety.

WHAT IS CLAIMED IS:

1. A method of treating or preventing a disease or condition caused by or associated with a virus belonging to the Bunyaviridae family, the method comprising administering to a subject in need thereof a compound of the formula,



or a pharmaceutically acceptable salt thereof, wherein R is either selected from substituted or unsubstituted alkyl groups, substituted or unsubstituted cycloalkyl groups, substituted or unsubstituted aryl groups, or substituted or unsubstituted oxaalkyl groups; or wherein R is



R₁ is a substituted or unsubstituted alkyl group;

X₁₋₅ are independently selected from H, NO₂, N₃, or NH₂;

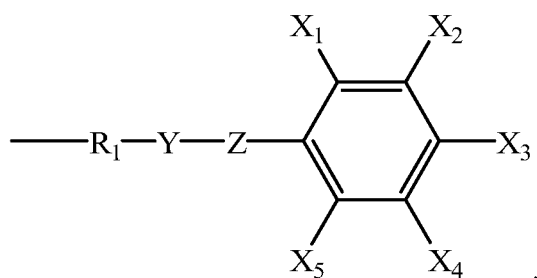
Y is absent or is a substituted or unsubstituted C₁-alkyl group, other than carbonyl; and

Z is selected from a bond or NH; provided that when Z is a bond, Y is absent, and provided that when Z is NH, Y is a substituted or unsubstituted C₁-alkyl group, other than carbonyl; and

wherein W₁₋₄ are independently selected from hydrogen, substituted or unsubstituted alkyl groups, substituted or unsubstituted haloalkyl groups, substituted or unsubstituted alkanoyl groups, substituted or unsubstituted aroyl groups, or substituted or unsubstituted haloalkanoyl groups.

2. The method of claim 1, wherein R is a C8-C16 alkyl group.
3. The method of claim 2, wherein each of W₁₋₄ is hydrogen.
4. The method of claim 3, wherein the compound is N-nonyl-deoxynojirimycin or a pharmaceutically acceptable salt thereof.

5. The method of claim 1, wherein R is

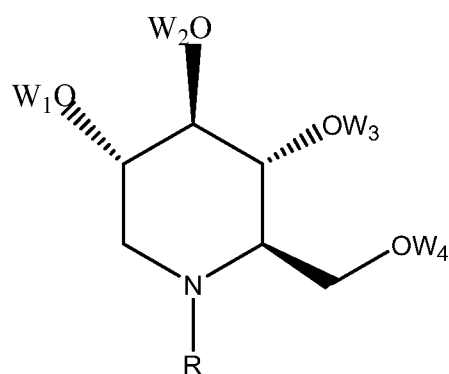


6. The method of claim 5, wherein each of W₁₋₄ is hydrogen.
7. The method of claim 5, wherein X₁ is NO₂ and X₃ is N₃.
8. The method of claim 5, wherein each of X₂, X₄ and X₅ is hydrogen.
9. The method of claim 5, wherein the compound is *N*-(*N*-(4'-azido-2'-nitrophenyl)-6-aminohexyl)deoxynojirimycin or a pharmaceutically acceptable salt thereof.
10. The method of claim 1, wherein the subject is a mammal.
11. The method of claim 1, wherein the subject is a human being.
12. The method of claim 1, wherein the virus is selected from Andes virus; Hantaan virus; Puumala virus; Seoul virus; Sin Nimbire virus; Dugbe virus; Crimean-Congo hemorrhagic fever virus; La Crosse virus and Raft Valley Fever virus.
13. The method of claim 1, wherein the virus belongs to Genus Phlebovirus.
14. The method of claim 1, wherein the virus is Rift Valley fever virus.

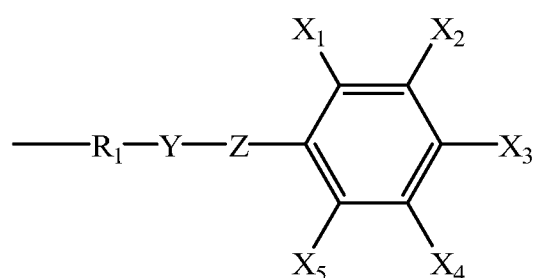
15. The method of claim 1, wherein the disease or condition is selected from hemorrhagic fever with renal syndrome; Hantavirus cardiopulmonary syndrome; Nephropathia epidemica; hemorrhagic fever caused by Seoul virus; Sweating sickness; Crimean-Congo hemorrhagic fever; La Crosse encephalitis; Phlebotomus fever; and Rift Valley fever.

16. The method of claim 1, wherein the disease or condition is Rift Valley fever.

17. A method of treating or preventing a disease or condition caused by or associated with a virus belonging to the Togaviridae family, the method comprising administering to a subject in need thereof a compound of the formula,



or a pharmaceutically acceptable salt thereof, wherein R is either selected from substituted or unsubstituted alkyl groups, substituted or unsubstituted cycloalkyl groups, substituted or unsubstituted aryl groups, or substituted or unsubstituted oxaalkyl groups; or wherein R is



R₁ is a substituted or unsubstituted alkyl group;

X₁₋₅ are independently selected from H, NO₂, N₃, or NH₂;

Y is absent or is a substituted or unsubstituted C₁-alkyl group, other than carbonyl; and

Z is selected from a bond or NH; provided that when Z is a bond, Y is absent, and provided that when Z is NH, Y is a substituted or unsubstituted C₁-alkyl group, other than carbonyl;

and

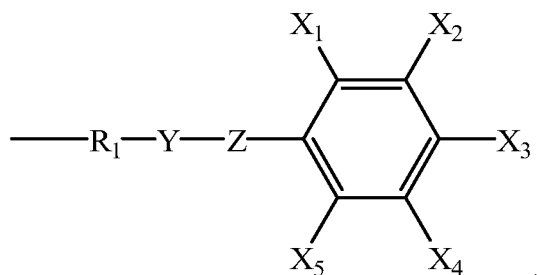
wherein W_{1-4} are independently selected from hydrogen, substituted or unsubstituted alkyl groups, substituted or unsubstituted haloalkyl groups, substituted or unsubstituted alkanoyl groups, substituted or unsubstituted aroyl groups, or substituted or unsubstituted haloalkanoyl groups.

18. The method of claim 17, wherein R is a C8-C16 alkyl group.

19. The method of claim 18, wherein each of W_{1-4} is hydrogen.

20. The method of claim 19, wherein the compound is N-nonyl-deoxynojirimycin or a pharmaceutically acceptable salt thereof.

21. The method of claim 17, wherein R is



22. The method of claim 21, wherein each of W_{1-4} is hydrogen.

23. The method of claim 21, wherein X_1 is NO_2 and X_3 is N_3 .

24. The method of claim 21, wherein each of X_2 , X_4 and X_5 is hydrogen.

25. The method of claim 21, wherein the compound is *N*-(*N*-(4'-azido-2'-nitrophenyl)-6-aminohexyl)deoxynojirimycin or a pharmaceutically acceptable salt thereof.

26. The method of claim 17, wherein the subject is a mammal.

27. The method of claim 17, wherein the subject is a human being.

28. The method of claim 17, wherein the virus is selected from Sindbis virus; Semliki Forest virus; O'nyong'nyong virus; Chikungunya virus; Mayaro virus; Ross River

virus; Barmah Forest virus; Eastern equine encephalitis virus; Western equine encephalitis virus; Venezuelan equine encephalitis virus and Rubella virus.

29. The method of claim 17, wherein the virus belongs to Genus Alphavirus.
30. The method of claim 17, wherein the virus is Chikungunya virus.
31. The method of claim 17, wherein the virus is Venezuelan equine encephalitis virus.
32. The method of claim 17, wherein the disease or condition is selected from sindbis fever; O'nyong'nyong fever; Chikungunya disease; Ross River fever; Barmah Forest virus infection; Eastern equine encephalitis; Western equine encephalitis; Venezuelan equine encephalitis and Rubella.
33. The method of claim 17, wherein the disease or condition is Chikungunya disease.
34. The method of claim 17, wherein the disease or condition is equine encephalitis.
35. The method of claim 17, wherein the disease or condition is Venezuelan equine encephalitis.

FIGURES 1(A)-(E)

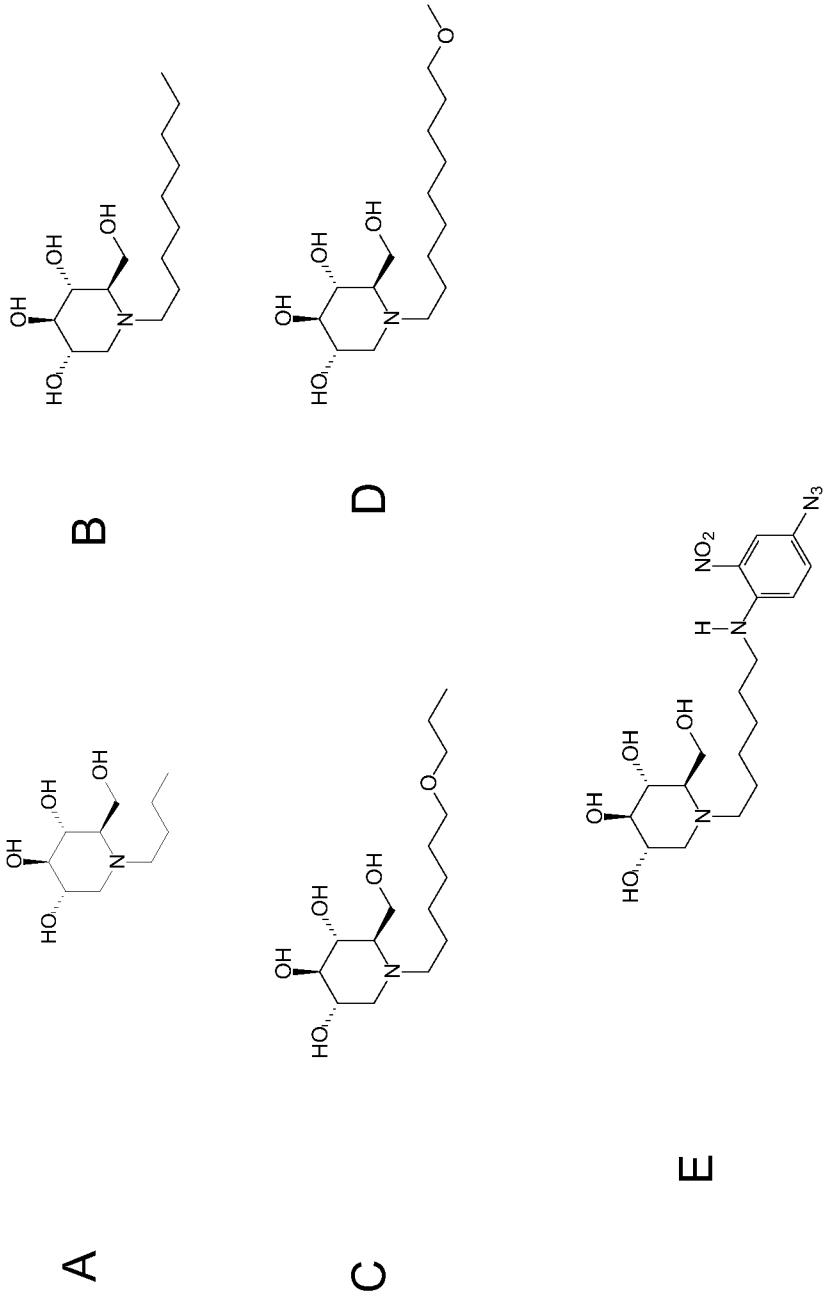


FIGURE 2

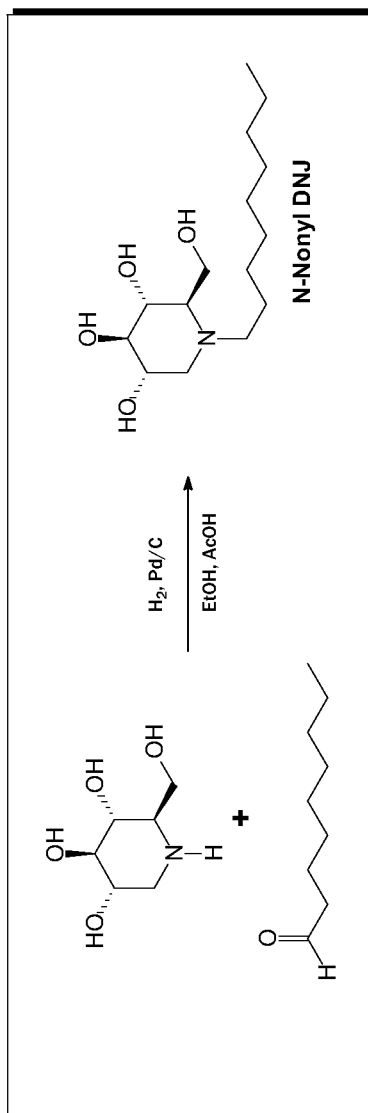
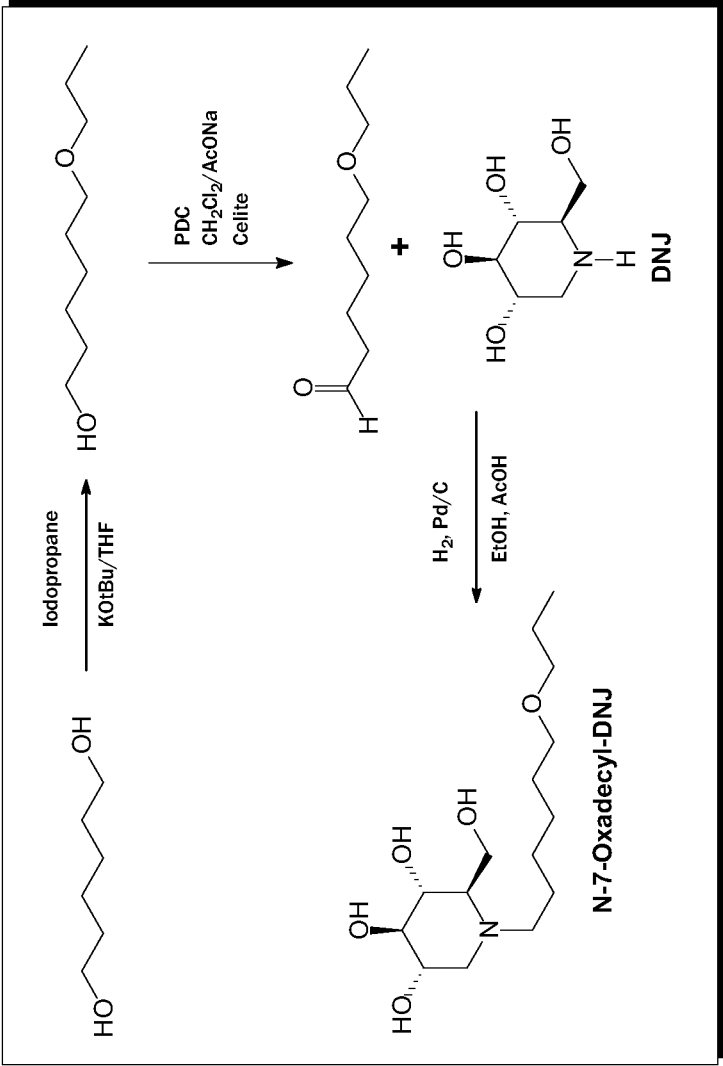
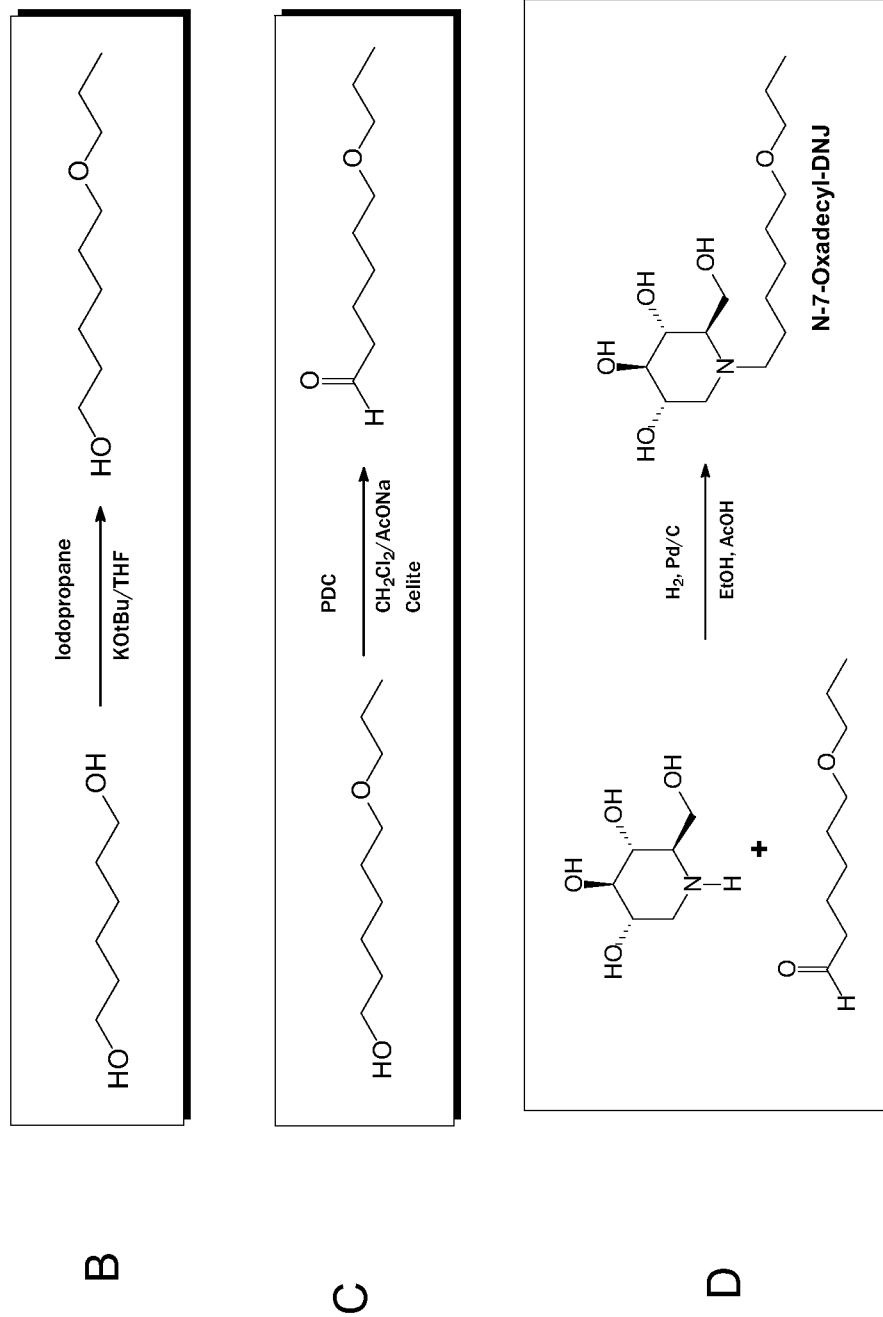


FIGURE 3A



A

FIGURES 3B-D



FIGURES 4A-C

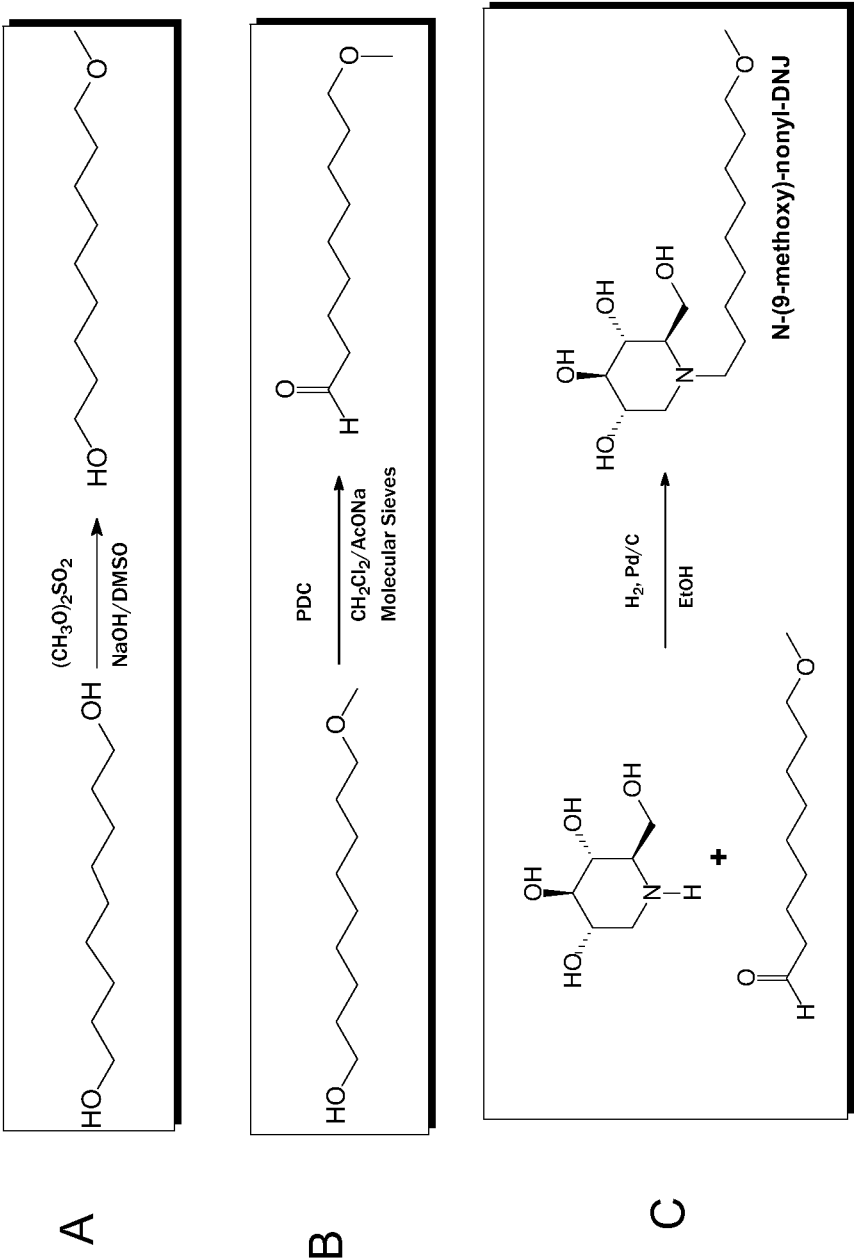


Fig. 5

Compound	Activity vs. viral families IC50, μ M		
	Bunya	Toga	
	RVFV	VEEV	CHIKV
NB-DNJ	250	156	>500
NN-DNJ	58	12	56
N7-0-DNJ	220	>250	500
N9-DNJ	250	>250	500
NAP-DNJ	49	2	22

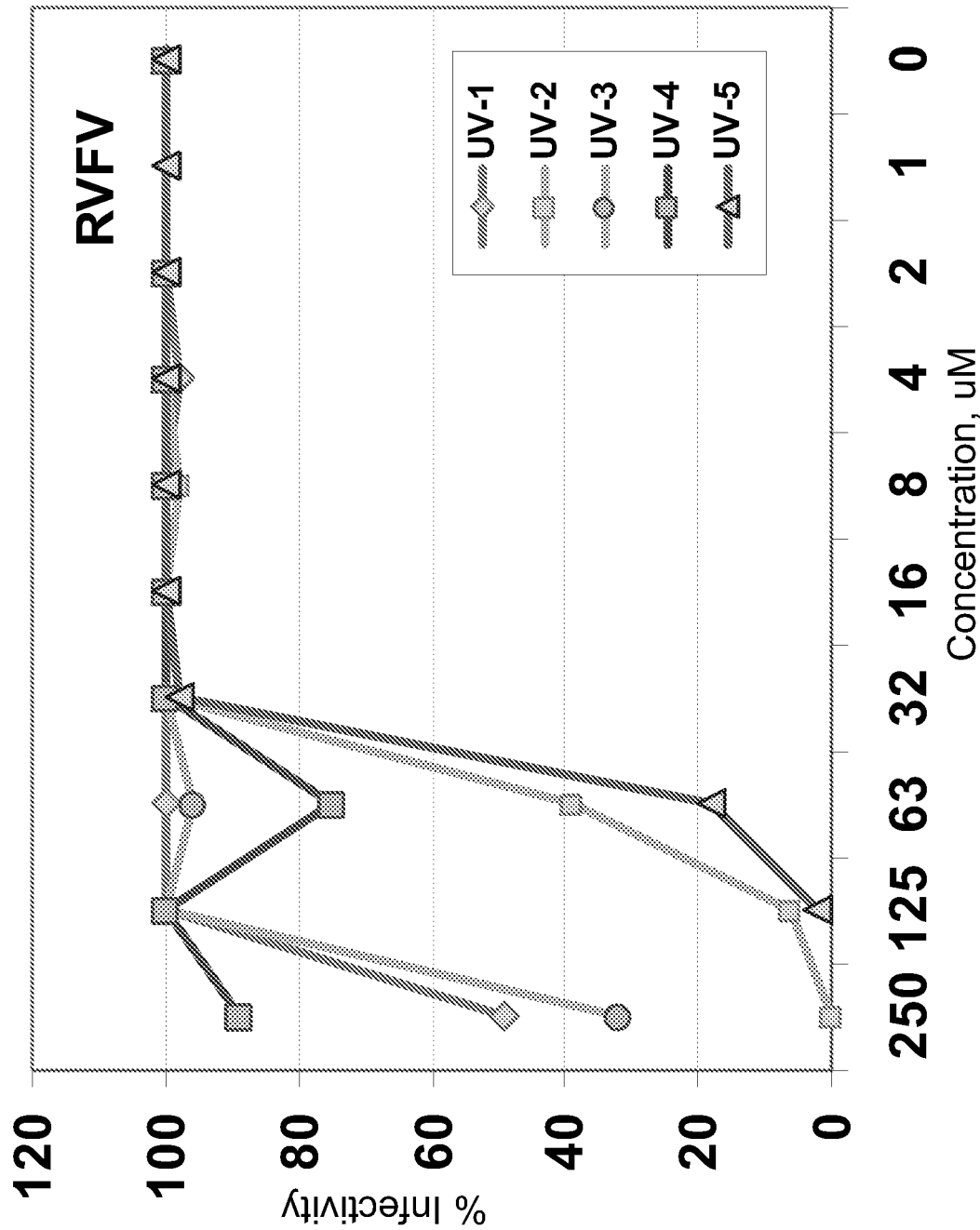
Virus abbreviations:

RVFV - Rift Valley fever

VEEV - Venezuelan equine encephalitis

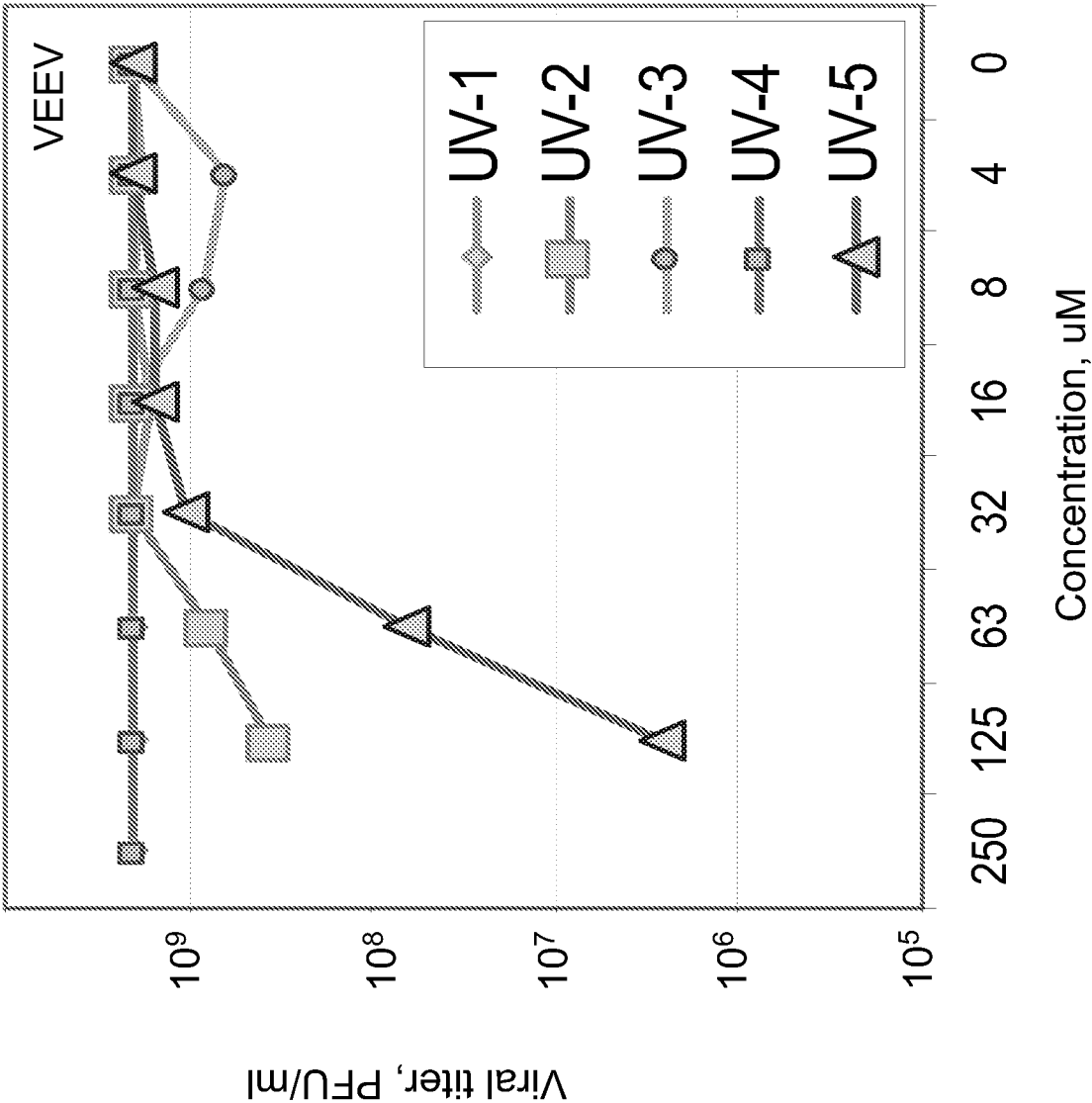
CHIKV - Chikungunya

FIGURE 6



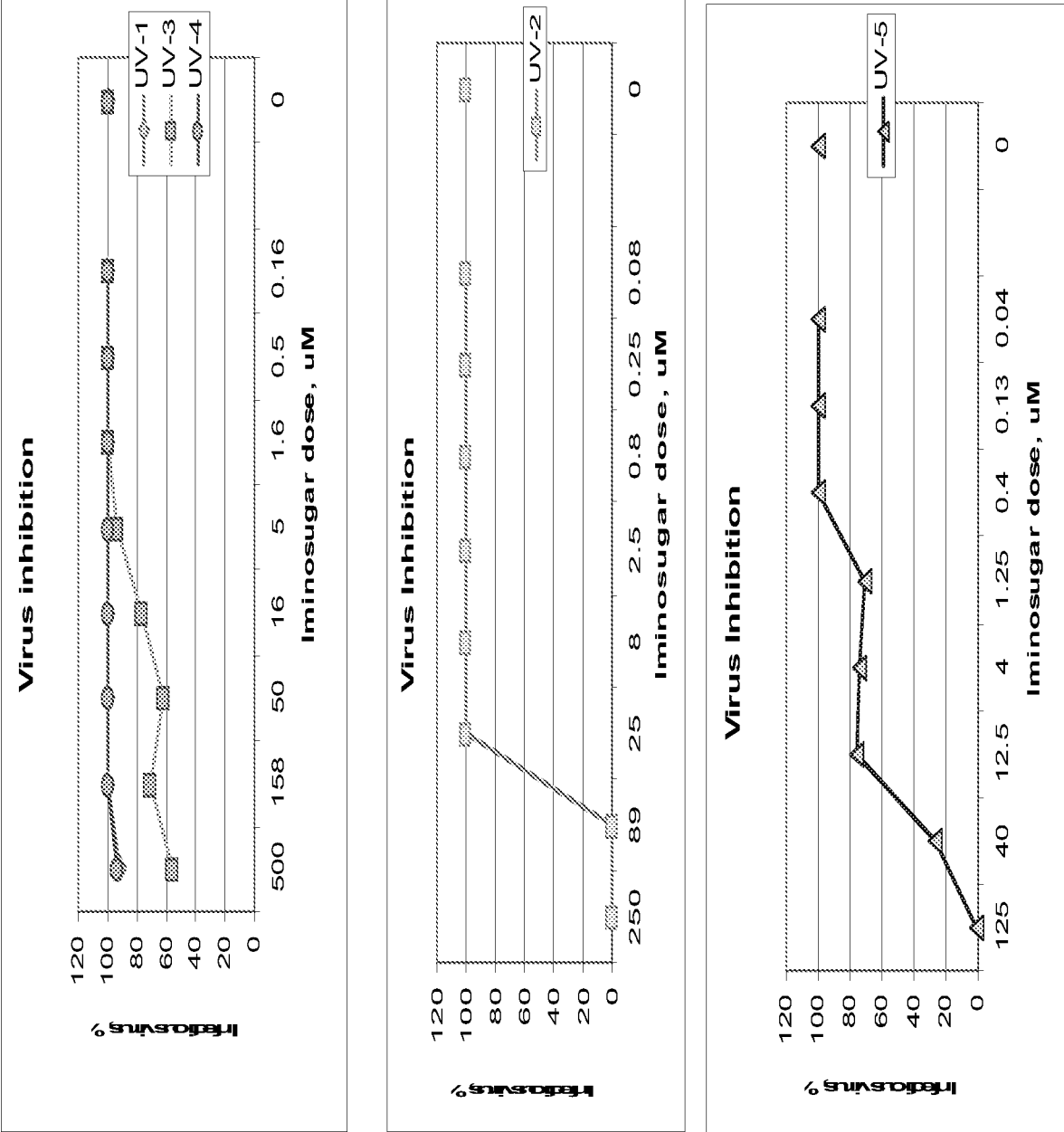
RVFV - Rift Valley fever virus

FIGURE 7



VEEV – Venezuelan equine encephalitis virus
Source: Unither Virology

FIGURE 8



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 10/38247

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01N 43/40; A61K 31/445 (2010.01)

USPC - 514/327-328

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

USPC - 514/327-328

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 514/225.5, 315, 317, 319 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PubWest (PGPB,USPT,USOC,EPAB,JPAB); Google

Search Terms Used:

bunyaviridae, n-nonyl-deoxynojirimycin, togavirus, piperocaine, genus phlebovirus, chikungunya, venezuelan equine encephalitis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---	WO 2006/077427 A2 (NASH et al.) 27 July 2006 (27.07.2006) pg 1, 46-47	1-4, 12-20, 28-35 ---
Y		5-11, 21-27
Y	US 2007/0275998 A1 (BUTTERS et al.) 29 November 2007 (29.11.2007) para [0008]-[0019]	5-11, 21-27

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

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

27 July 2010 (27.07.2010)

Date of mailing of the international search report

15 NOV 2010

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