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(54) **Title:** DEOXYNOJIRIMYCIN DERIVATIVES AND METHODS OF THEIR USING

(57) **Abstract:** The present application provide novel iminosugars and their use in treatment of viral infection, such as Dengue infec-
tion and Influenza A infection.



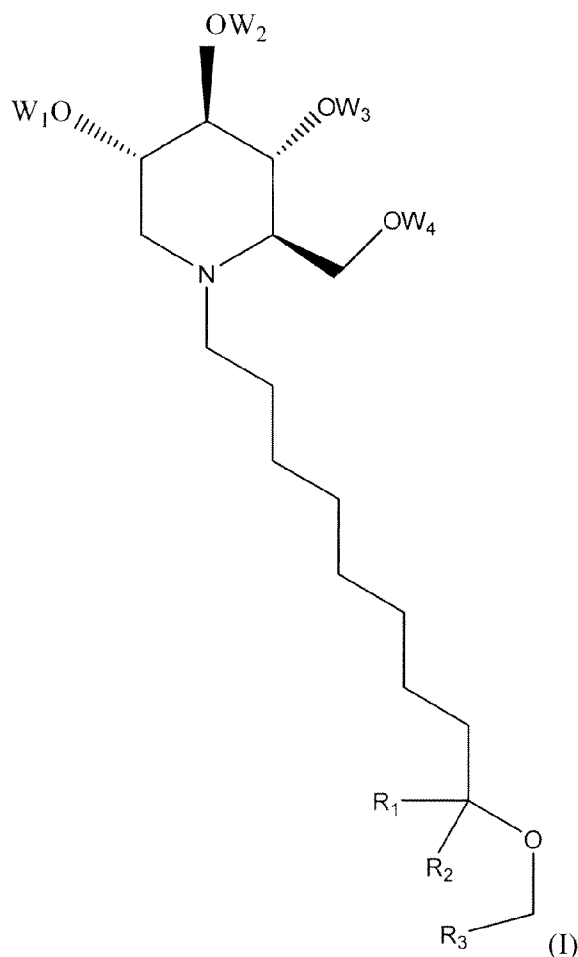
DEOXYNOJIRIMYCIN DERIVATIVES AND METHODS OF THEIR USING

FIELD

The present invention relates generally to iminosugars and their methods of use and in particular, to *N*-substituted deoxynojirimycin compounds and their use for treating and/or preventing viral infections.

SUMMARY

One embodiment is a compound of formula (I):



or a pharmaceutically acceptable salt thereof, wherein W_{1-4} and R_{1-3} are each independently selected from hydrogen and C_{1-3} alkyl groups and wherein at least one of R_{1-3} is not hydrogen.

Yet another embodiment is a method of treating a disease or condition caused by or associated with a virus belonging to the Orthomyxoviridae family comprising administering to a subject in need thereof the compound of formula (I) or a pharmaceutically acceptable salt thereof.

And yet another embodiment is a method of treating a disease or condition caused by or associated with Dengue virus comprising administering to a subject in need thereof the compound of formula (I) or a pharmaceutically acceptable salt thereof.

FIGURES

Figure 1: Survival of infected mice grouped by treatment. Groups of mice (n=10) received the treatment TID starting 1 hour prior to infection; Mice were infected intranasally with influenza at a dose of $\sim 1\text{LD}_{90}$. Survival data is plotted as percent survival against days post infection. Graph shows survival of animals in each group.

Figure 2: Weights of infected mice grouped by treatment. Groups of mice (n=10) received the treatment TID starting 1 hour prior to infection; Mice were infected intranasally with influenza at a dose of $\sim 1\text{LD}_{90}$. Weight data is plotted as percent of original weight against days post infection.

Figure 3: Temperatures of infected mice grouped by treatment. Groups of mice (n=10) received the treatment TID starting 1 hour prior to infection; Mice were infected intranasally with influenza at a dose of $\sim 1\text{LD}_{90}$. Temperature data is plotted as percent of original temperature against days post infection.

Figure 4: Health scores of infected mice grouped by treatment. Groups of mice (n=10) received the treatment TID starting 1 hour prior to infection; Mice were infected intranasally with influenza at a dose of $\sim 1\text{LD}_{90}$. Health data is plotted as health score vs days post infection.

Figure 5 presents a plot demonstrating *in vivo* efficacy of UV-12 against dengue virus.

Figure 6: Survival of mice grouped by treatment delivery route. Groups of mice (n=10) received the first treatment dose of compound in water 1 h before an intranasal infection with Influenza A/Texas/36/91 (H1N1) at a dose of $\sim 1\text{LD}_{90}$. Survival data is plotted as percent survival against days post infection. (A) Treatment of 100 mg/kg of UV-4B, UV-8, UV-9, UV-10, UV-11, and UV-12, assuming a starting weight of $\sim 20\text{g}$ per mouse. Graph includes survival curve of vehicle only control group.

Figure 7: Statistical analysis of survival of infected mice. The survival data plotted in Figure 6 were analyzed using the Mantel-Cox and Gehan-Breslow-Wilcoxon tests. (A) Statistical analysis is by comparing the treated groups to the H₂O vehicle control. Statistical significance is indicated by a p value < 0.05 .

Figures 8A-F: Analysis of Weights. Mice received the first dose of compound in H₂O at 1 hour prior to the intranasal infection with $\sim 1\text{LD}_{90}$ of Influenza A/Texas/36/91 (H1N1). The mean weights for each group are plotted as percent of the weight on day 0 (baseline) with the

standard deviations. (A) Treatment of infected mice with UV-4B or UV-8, (B) treatment of infected mice with UV-4B or UV-9, (C) treatment of infected mice with UV-4B or UV-10, (D) treatment of infected mice with UV-4B or UV-11, (E) treatment of infected mice with UV-4B or UV-12, and (F) treatment of uninfected mice with UV-8, -9, -10, -11, or -12, without standard deviations.

Figure 9: Statistical analysis of weight data. The weight data for the influenza infected mice plotted in Figure 8 were analyzed using a repeated-measures 2-way ANOVA (GraphPad Prism) against the vehicle control. Data was only analyzed through day 7 post-infection (p.i.) due to deaths at later time points. Compounds UV-9, UV-10, and UV-11 had both 0% survival and a MTD < 9 days and were thus omitted from further statistical analysis. Statistical significance is indicated by a p value lower than 0.05 ($p < 0.05$).

Figure 10: Analysis of Temperatures. Mice received the first dose of compound in H₂O at 1 hour prior to the intranasal infection with ~ 1LD₉₀ of Influenza A/Texas/36/91 (H1N1). The mean temperatures for each group are plotted as percent of the weight on day 0 (baseline) with the standard deviations. (A) Treatment of infected mice with UV-4B or UV-8, (B) treatment of infected mice with UV-4B or UV-9, (C) treatment of infected mice with UV-4B or UV-10, (D) treatment of infected mice with UV-4B or UV-11, (E) treatment of infected mice with UV-4B or UV-12, and (F) treatment of uninfected mice with UV-8, -9, -10, -11, or -12, without standard deviations.

Figure 11: Statistical analysis of temperature data. The temperature data for the influenza infected mice plotted in Figure 10 were analyzed using a repeated-measures 2-way ANOVA (GraphPad Prism) against the vehicle control. Data was only analyzed through day 7 post-infection (p.i.) due to deaths at later time points. Compounds UV-9, UV-10, and UV-11 had both 0% survival and a MTD < 9 days and were thus omitted from further statistical analysis. Statistical significance is indicated by a p value lower than 0.05 ($p < 0.05$).

Figures 12 present results of the study performed in Example 4. This study was determining survival of dengue virus infected mice. The survival data and animal body weight are plotted in Figure 12. All compounds were given in water by the oral route (3x per day intragastric via oral gavage – IG) for a total number of 7 days after the start of dosing. The treatment dose was 50 mg/kg of UV-4B, UV-8, UV-9, UV-10, UV-11, and UV-12. Groups of mice received the first treatment dose of compound 0.5-1 h before an intravenous infection with dengue

virus at a dose of $\sim 1LD_{90}$. Survival and body weights were measured until 3 days after dosing.

Figure 13 presents chemical formulae of UV-8, UV-9, UV-10, UV-11 and UV-12 compounds.

Figure 14 presents chemical formula of UV-28.

Figure 15 provides a synthesis scheme for UV-8.

Figure 16 provides a synthesis scheme for UV-9.

Figure 17 provides a synthesis scheme for UV-10.

Figure 18 provides a synthesis scheme for UV-11.

Figure 19 provides a synthesis scheme for UV-12.

Figure 20 provides a synthesis scheme for UV-28.

Figure 21 provides an alternative synthesis scheme for UV-28.

DETAILED DESCRIPTION

Related Documents

The following patent documents, each of which is incorporated herein by reference in its entirety, may be useful for understanding the present disclosure:

US patents nos. 6,545,021; 6,809,803; 6,689,759; 6,465,487; 5,622,972; 7,816,650; 7,256,005; 8,450,345; 7,612,093; and 8,426,445; US patent application publications nos. 20110184019; 20130150405; 20100222384; 20110065754; 20110065753; 20110065752; and 2007-0275998; and US patent application no. 13/870,341 filed April 25, 2013.

Definition of terms

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

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.

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.

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.

LD₉₀ stands for (lethal dose 90%) is an estimated dose of an agent at which 90% of the population is expected to die.

DENV stands for Dengue virus.

INFLV stands for influenza virus.

IV stands for intravenous.

IG stands for intragastric.

IP stands for intraperitoneal.

PFU stands for a plaque-forming unit.

PBS stands for phosphate buffered saline.

ANOVA stands for an analysis of variance.

Disclosure

The present inventors discovered certain deoxynojirimycin derivatives may be effective against one or more viruses, which may be, for example, a Dengue virus and/or a virus belonging to the Orthomyxoviridae family, such as an Influenza A virus.

In particular, such deoxynojirimycin derivatives may be useful for treating a disease or condition caused by or associated with one or more viruses, which may be, for example, a Dengue virus and/or a virus belonging to the Orthomyxoviridae family, such as an Influenza A virus. In certain embodiments, the deoxynojirimycin derivatives may increase a survival rate or probability for a subject infected with one or more viruses, which may be, for example, a Dengue virus and/or a virus belonging to the Orthomyxoviridae family, such as an Influenza A virus.

Dengue viruses

Dengue virus belongs to the genus *Flavivirus* of the *Flaviridae* family and causes dengue hemorrhagic fever (DHF). Dengue virus includes four closely related serotypes, usually referred to as Dengue 1, Dengue 2, Dengue 3 and Dengue 4. Recovery from infection by one provides lifelong immunity against that serotype but confers only partial and transient protection against infection by the other three. A good evidence exists that sequential infection increases the risk of more serious disease, resulting in DHF. Emerging DHF epidemics are causing increasing concern in the Americas and in Asia, where all four dengue viruses are endemic. DHF has become a leading cause of hospitalization and death among children in several countries. In 2007, there were more than 890,000 reported cases of dengue in the Americas, of which 26,000 cases were DHF.

Dengue is transmitted primarily by the *Aedes aegypti* mosquito and is the most common mosquito-borne viral disease of humans. Globally, 2.5 billion people – 40% of the world's population – live in the warm areas where *Aedes aegypti* is common and dengue can be transmitted. The rapid growth of tropical cities and their human and mosquito populations is bringing ever greater numbers of people into contact with this vector. The geographical spread of both the mosquito vectors and the virus has led to a global resurgence of epidemic dengue fever and the emergence of dengue hemorrhagic fever (DHF).

Orthomyxoviridae family

The Orthomyxoviridae family is a family of RNA viruses that includes five genera: Influenzavirus A, Influenzavirus B, Influenzavirus C, Isavirus and Thogotovirus. The first three genera contain viruses that can cause influenza in vertebrates, including birds, humans and other mammals.

The Influenzavirus A genus includes a single species, which can cause influenza in birds and certain mammals, including humans, pigs, felines, canines and equines.

Influenza A viruses are negative sense, single-stranded, segmented RNA viruses. Several subtypes of Influenza A virus exist, labeled according to an H number (for the type of hemagglutinin) and an N number (for the type of neuraminidase). Currently known 16 different H antigens (H1 to H16) and nine different N antigens (N1 to N9). Serotypes and

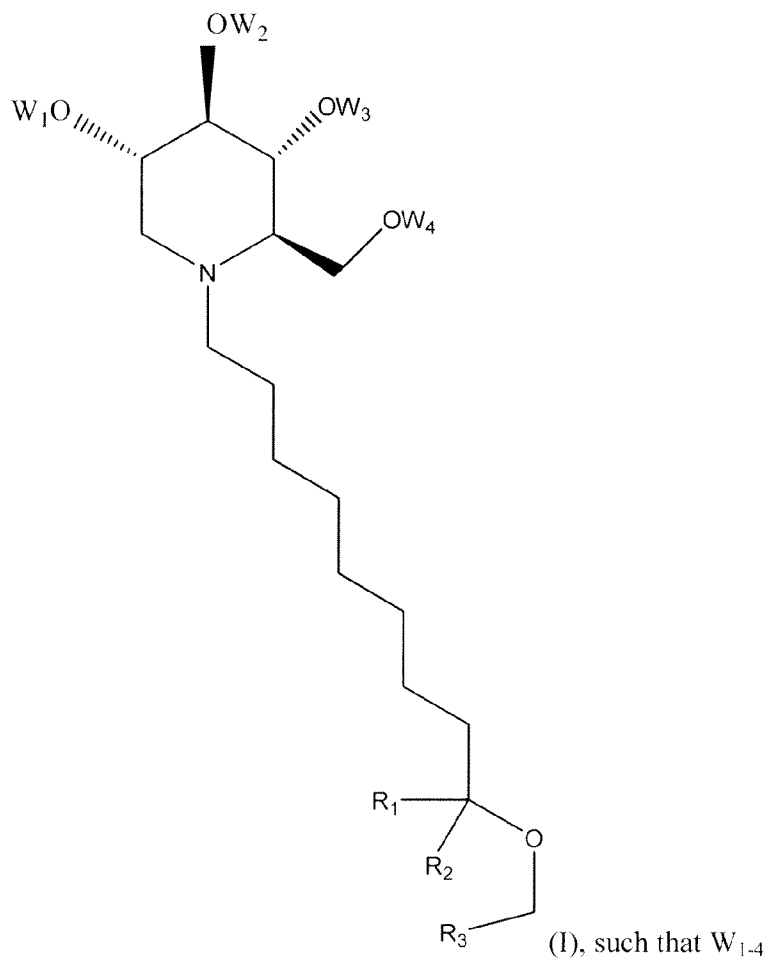
subtypes of Influenza A include H1N1 Influenza A; H1N2 Influenza A; H2N2 Influenza A; H3N1 Influenza A; H3N2 Influenza A; H3N8 Influenza A; H5N1 Influenza A; H5N2 Influenza A; H5N3 Influenza A; H5N8 Influenza A; H5N9 Influenza A; H5N9 Influenza A; H7N1 Influenza A; H7N2 Influenza A; H7N3 Influenza A; H7N4 Influenza A; H7N7 Influenza A; H9N2 Influenza A; H10N7 Influenza A.

The Influenzavirus B genus includes a single species, which can cause influenza in humans and seals.

The Influenzavirus C genus includes a single species, which can cause influenza in humans and pigs.

Deoxynojirimycin derivatives

In some embodiments, the deoxynojirimycin derivative may be a compound belonging to a



genus defined by formula (I):

and R_{1-3} are each independently selected from hydrogen and C_{1-3} alkyl groups, where at least one of R_{1-3} is not hydrogen. C_{1-3} alkyl groups include methyl, ethyl and propyl. In some embodiments, R_2 and R_3 may be such that they form together one of the following groups: $-CH_2-$, $-CH_2-CH_2-$ or $-CH_2-CH_2-CH_2-$.

A compound of formula (I) with each of W_{1-4} and R_{1-3} being hydrogen is *N*-(9-Methoxynonyl) deoxynojirimycin, which is also known as N9-DNJ or UV-4. The compounds of the defined above genus may be viewed as derivatives of UV-4. The compounds of the defined above genus, such as compounds UV-12 and UV-28 (see Figures 13-14) may have one or more advantages compared to other derivatives of UV-4, such as, for example, compounds UV-8, UV-9, UV-10 and UV-11 (see Figure 13). For example, the compounds of the defined above genus, such as compounds UV-12 and UV-28 may be more efficient compared to other derivatives of UV-4, such as compounds UV-8, UV-9, UV-10 and UV-11, against one or more viruses, which may be, for example, a Dengue virus and/or a virus belonging to the Orthomyxoviridae family, such as an Influenza A virus.

UV-4 derivatives, such as UV-8, UV-9, UV-10, UV-11, UV-12 and UV-28, may be synthesized as depicted in Figures 15-21.

Methods of synthesizing deoxynojirimycin derivatives are also 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.

In some embodiments, the deoxynojirimycin derivative may 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.

In some embodiments, the deoxynojirimycin derivative may be also 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.

In some embodiments, the deoxynojirimycin derivative 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.

In some embodiments, the pharmaceutical composition may consist essentially of *N*-substituted deoxynojirimycin, which may mean that the *N*-substituted deoxynojirimycin is the only active ingredient in the composition.

Yet in some embodiments, *N*-substituted deoxynojirimycin may be administered with one or more additional antiviral compounds.

In some embodiments, the deoxynojirimycin derivative may be used in a liposome composition, such as those disclosed in US publications nos. 2008/0138351, 2009/0252785 and 2010/0266678.

The DNJ derivative may be administered to a cell or an animal affected by a virus. The DNJ derivative may inhibit morphogenesis of the virus, or it may treat the individual. The treatment may reduce, abate, or diminish the virus infection in the animal.

In some embodiments, the animal may be an animal infected with a Dengue virus which may be a vertebrate, such as a mammal, which may be, for example, a rodent or a primate, such as a human.

In some embodiments, the amount of the DNJ derivative administered to an animal or to an animal cell to the methods of the invention may be an amount effective to inhibit the morphogenesis of Dengue virus from the cell. The term "inhibit" as used herein may refer to the detectable reduction and/or elimination of a biological activity exhibited in the absence of the iminosugar. The term "effective amount" may refer to that amount of the DNJ derivative necessary to achieve the indicated effect. The term "treatment" as used herein may 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 Dengue virus in a subject who is free therefrom.

In some embodiments, the animals may be an animal infected with a virus that belongs to the Orthomyxoviridae family, which may be a vertebrate, such as a bird or a mammal, including primates, such as humans; felines; equines, and canines.

In some embodiments, the amount of the DNJ derivative administered to an animal or to an animal cell to the methods of the invention may be an amount effective to inhibit the morphogenesis of a virus belonging to the Orthomyxoviridae family from the cell. The term "inhibit" as used herein may refer to the detectable reduction and/or elimination of a biological activity exhibited in the absence of the DNJ derivative. The term "effective amount" may refer to that amount of the DNJ derivative necessary to achieve the indicated effect. The term "treatment" as used herein may 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 belonging to the Orthomyxoviridae family in a subject who is free therefrom.

Treatment of the disease caused by or associated with a virus, which may be, for example, a Dengue virus or a virus belonging to the Orthomyxoviridae family, such as Influenza A virus, may 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 DNJ derivative, which may be administered to the cell or animal is preferably an amount that does not induce toxic effects which outweigh the advantages which accompany its administration.

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.

The selected dose level may depend on the activity of the DNJ derivative, 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 may 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 DNJ derivative per 10 kilogram body weight. In some embodiments, a total daily dose may be from 0.1 mg/kg body weight to 100 mg/kg body weight or from 1 mg/kg body weight to 60 mg/kg body weight or from 2 mg/kg body weight to 50 mg/kg body weight or from 3 mg/kg body weight to 30 mg/kg body weight. The daily dose may be administered over one or more administering events over day. For example, in some embodiments, the daily dose may be distributed over two (BID) administering events per day, three administering events per day (TID) or four administering events (QID). In certain embodiments, a single administering event dose ranging from 1 mg/kg body weight to 10 mg/kg body weight may be administered BID or TID to a human making a total daily dose from 2 mg/kg body weight to 20 mg/kg body weight or from 3 mg/kg body weight to 30 mg/kg body weight. Of course, the amount of the DNJ derivative which should be administered to a cell or animal may depend upon numerous factors well understood by one of skill in the art, such as the molecular weight of the DNJ derivative and the route of administration.

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 DNJ derivative, 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 DNJ derivative. 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.

These compositions may be administered a in a single dose or in multiple doses which are administered at different times. Because the inhibitory effect of the composition upon a virus 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.

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

Working examples

EXAMPLE 1

Efficacy of UV-12 and UV-28 against INFV A/Texas/36/91 (H1N1) Challenge in Mice

Study Summary: This study tested the ability of iminosugars UV-12 and UV-28 to protect mice from lethal influenza infection (~1 LD₉₀ of influenza A/Texas/36/91 (H1N1) administered intranasally). Compounds were delivered 30-60 minutes prior to viral challenge via the oral (IG) route at 100 or 40 mg/kg and continued three times daily for 10 days. The mice used were ~20 gram, 6-8 week old female BALB/c mice in groups of 10 for efficacy (a summary of the study groups is shown in Table 1). Temperature and weights were taken daily. Endpoint was day 14 post infection, death, or euthanasia. Animals displaying severe illness as determined by >30% weight loss, extreme lethargy, or paralysis were euthanized.

I. Introduction

Purposed: This study aimed to determine the efficacy of small molecules UV-12 and UV-28 *in vivo* against a lethal influenza A/Texas/36/91 (H1N1) infection.

II. Materials and Methods

Materials

Table 1: Test articles

Name	Amount (mg)	Solvent
UV-12	20 or 8 mg/ml	water
UV-28	20 or 8 mg/ml	water

Table 2: Viruses for challenge

Name	Strain	Stock titer	Additional Info
Influenza	A/Texas/36/91 (H1N1)	2.6×10^5 PFU/ml	100 ul of a 1:500 viral dilution was given to mice for ~52 PFU/mouse

Table 3. Animals used

Species	Strain	Age	Sex	Vendor	Additional Info
Mouse	BALB/c	6-8 weeks	F	Charles River	n=10 per group

Table 4: Equipment

Item	Vendor
Syringes	BD
Animal Housing	InnoVive
Biodata chips and scanner	Bio Medic Data Systems
Ohaus scale	Ohaus

Study design

This study tested the ability of UV-12 and UV-28 to protect mice from lethal influenza infection. The mice used were 6-8 week old female BALB/c mice in groups of 10 (see Table

5 below). Mice were treated with 100 or 40 mg/kg three times daily (TID) via IG route starting approximately 30-60 minutes prior to challenge. Mice were challenged with ~1 LD₉₀ of influenza A/Texas/36/91 (H1N1) administered intranasally (IN). Endpoint was day 14 post infection, death, or euthanasia. Animals displaying severe illness as determined by >30% weight loss, extreme lethargy, or paralysis were euthanized. Temperature and weights were taken daily.

Table 5: Mouse groups for the study. The time point of initial dosing (treatment start relative to the infection), dosing regimen and the dose levels/routes are listed.

Group (n=10)	Test Article	Dosage, TID	Readouts (After ~1 LD ₉₀ of influenza A/Texas/36/91 (H1N1) administered intranasally)
1	Water	Vehicle Only	• Endpoint is day 14 post infection, severe morbidity, death, or >30% weight loss. • Animals displaying severe illness (as determined by >30% weight loss, extreme lethargy, or paralysis) will be euthanized. • Health assessments, weights and temperature to be taken daily for 15 days total (days 0-14 post infection)
2	UV-12	100 mg/kg	
3		40 mg/kg	
4		100 mg/kg	
5	UV-28	40 mg/kg	

STANDARD PROTOCOLS

Standard Protocol for intranasal infection of mice

1. Female 6-8 week old BALB/c mice were housed in groups of 5 mice. Mice were quarantined at the study site (Noble Life Sciences, Gaithersburg, MD) for at least 3 days prior to the start of the study.
2. Food and water was provided *ad libitum*.
3. The groups of mice challenged with INFV were infected via intranasal (IN) inoculation with ~1xLD₉₀ in 100 µL of a 1:500 dilution of INFV in PBS under light anesthesia (Isoflurane).
4. After the infection, mice were placed back into their cages for observation and subsequent dosing.

Protocol for oral gavage of mice for test article delivery

1. Mice were treated with 100 or 40 mg/kg of test article given by the IG route in 100 μ L of water (see Table 5 for dosing regimens) three times daily for 10 days.
2. After dosing, mice were returned to their cages and monitored for any distress related to dosing.

Observation of mice

1. Mice were observed through 13 days post infection (14 days total, 0-13 days post infection).
2. Mice were weighed daily on an Ohaus scale and the weights were recorded.
3. All animals had chips implanted at least 3 days prior to virus challenge that monitored the body temperature. The temperatures were recorded daily.
4. Survival and health of each mouse was evaluated once time a day using a scoring system of 1-7.

Table 6. Scoring system

Score	Initials	Description	Appearance	Mobility	Attitude
1	H	Healthy	Smooth Coat. Bright Eyes.	Active, Scurrying, Burrowing	Alert
2	SR	Slightly Ruffled	Slightly Ruffled coat (usually only around head and neck)	Active, Scurrying, Burrowing	Alert
3	R	Ruffled	Ruffled Coat throughout body. A "wet" appearance.	Active, Scurrying, Burrowing	Alert
4	S	Sick	Very Ruffled coat. Slightly closed, inset eyes.	Walking, but no scurrying.	Mildly Lethargic
5	VS	Very Sick	Very Ruffled Coat. Closed, inset eyes.	Slow to no movement. Will return to upright	Extremely Lethargic

				position if put on its side.	
6	E	Euthanized	---	---	---
7	FD	Found Dead	---	---	---

III. RESULTS

Survival

Mice were infected with a $\sim 1\text{LD}_{50}$ of influenza A/Texas/36/91 (H1N1) and treated with 100 or 40 mg/kg of UV-12 or UV-28 three times daily for 10 days. Survival in each infected treatment group, calculated as percent survival versus days post-infection, is shown in Figure 1 and Table 7. The infected groups which were treated with 100 mg/kg of both UV-12 and -28 showed 100% survival. The infected groups that were treated with 40 mg/kg of UV-12 and -28 displayed 20 and 60% survival, respectively. The untreated control group showed 0% survival and was all 'found-dead' or 'euthanized' by day 7 post-infection.

Figure 1 is a plot presenting survival data of infected mice grouped by treatment. Table 7 presents results of analysis of survival of infected mice. The survival data plotted in Figure 1 were analyzed using the Mantel-Cox (Log rank) test in GraphPad Prism.

Table 7

Group	Mean Survival (Days)	% Survival	<i>P</i> value to water control
Water	7	0	N/A
UV-12 100 mg/kg	>13	100	<0.0001
UV-12 40 mg/kg	9	20	<0.0001
UV-28 100 mg/kg	>13	100	<0.0001
UV-28 40 mg/kg	>13	60	<0.0001

Biometric analysis

During the course of this study, individual weights, health scores, and temperatures were monitored daily for each group. The average weights for each group of mice are shown in

Figure 2. Every animal was tagged with a chip to perform daily temperature readings using a BMDS scanner; the average temperatures are shown in Figure 3. The health scores are shown in Figure 4.

CONCLUSIONS

Influenza-infected mice were treated with 100 or 40 mg/kg of UV-12 or UV-28 via oral gavage three times daily for 10 days. Both groups that were treated with 100 mg/kg showed 100% survival, and groups that were treated with 40 mg/kg showed 60 and 20% survival for UV-28 and UV-12, respectively. While UV-28 did appear to show better efficacy at 40 mg/kg, it appears to be more toxic than UV-12 (Figure 4). While mice that were treated with 100 mg/kg of UV-12 fully recovered from the infection and returned to normal, mice that were treated with 100 mg/kg of UV-28 never recovered their health score and remained 'ruffled' for the entire course of the study. In combination with a higher health score, indicating morbidity, the mice that were treated with UV-28 did not recover their weight as well, while mice treated with UV-12 almost completely recovered. Mice that were treated only with vehicle all succumbed to infection by day 7 post-infection, displaying 0% survival.

EXAMPLE 2

Survival Analysis of UV-12 in A129 ADE Model

Purpose: This study determined the efficacy of UV-12 in promoting survival of mice challenged with dengue virus. Compound were given by the oral route (3x per day intragastric via oral gavage – IG) for a total number of 7 days after the start of dosing. The experiment used the A129 ADE model of infection. (Prestwood TR, Morar MM, Zellweger RM, Miller R, May MM, Yauch LE, Lada SM, Shresta S. Gamma interferon (IFN- γ) receptor restricts systemic dengue virus replication and prevents paralysis in IFN- α/β receptor-deficient mice. J Virol. 2012 Dec;86(23):12561-70.) Animals received the virus challenge dose ~1 LD₉₀ on day 0. The first dose was given 0.5-1 hr pre-virus challenge. Survival was measured until 30 days after infection.

Iminosugar candidate: UV-12 .

Experimental Design for the Study:

Control, H₂O + DENV (S221) [10 mice]

UV-12, (100 mg/kg/dose) + DENV (S221) [10 mice]

Mice: Sex matched 5-6 weeks old A129 (129/SV IFN- α , - β receptor^{-/-})

Administration Route:

Iminosugar: Orally 3 x day, (gavage (IG)) every 8 hours

Antibody: IP

Virus: IV

Antibody and Iminosugar Compound were given simultaneously, then virus within 30 minutes

Virus Challenge:

Antibody: 100 μ g 2H2 (IgG2a anti-DENV1-4 prM) from ATCC, day 0 and day 1 in 40 μ l

Virus: DENV2 Strain S221 (v512) (Zellweger RM, Prestwood TR, Shrestha S.

Enhanced infection of liver sinusoidal endothelial cells in a mouse model of antibody-induced severe dengue disease. Cell Host Microbe. 2010 Feb 18;7(2):128-39)

Dose: 1E11 GE (genomic equivalents) per animal

Read-out:

Animal survival. Animals displaying severe illness (as determined by 20% weight loss, extreme lethargy, ruffled coat, or paralysis) are euthanized.

Resources:

A129 mice, 2H2 antibody, and S221 virus were supplied by Sujana Shrestha, La Jolla Institute for Allergy and Immunology

Supplied by Unither Virology: UV-12

Figure 5 presents results of this study. Mice were treated with UV-12 diluted in 50 μ L water orally, three times per day for a total of 7 days. Initiation of treatment was 1 hr before virus intravenous challenge. UV-12 treated A129 mice displayed an increased survival compared to control animals that was only orally administered 50 μ L of water three times per day.

EXAMPLE 3

Efficacy of selected iminosugars in Mice; Influenza A/Texas/36/91 (H1N1) Challenge

Study Summary: This study analyzed the toxicity and efficacy of UV-8, UV-9, UV-10, UV-11, and UV-12 in mice during an H1N1 influenza infection. Previously, studies that were

performed with oral delivery of UV-4B *ter in die* (TID) at 100 mg/kg resulted in efficacy against H1N1 and did not show any discernible signs of toxicity. In the current study, we examined delivery doses of UV-4B, UV-8, UV-9, UV-10, UV-11, and UV-12 at 100 mg/kg, delivered *ter in die* by the oral route (intragastric via oral gavage or IG). Small groups (n=3) that were included to examine gross toxicity received 100 mg/kg of the iminosugars but without a viral challenge. UV-4B, UV-8, UV-9, UV-10, UV-11, or UV-12 was delivered to the animals starting at 1 hour before intranasal infection (IN) with ~1 LD₉₀ of influenza A/Texas/36/91 (H1N1). Animals were then treated TID for 10 days total (days 0-9 post infection). Efficacy was evaluated by comparing survival, temperature changes, and weight gain/loss to an infected untreated control group. Mice dosed with UV-8, -9, -10, or -11 did not show any improvement in survival over the untreated control group, while the group dosed with UV-12 and the positive control group dosed with UV-4B both showed a significant increase in survival.

I. Introduction

This study aimed to determine the efficacy of UV-8, UV-9, UV-10, UV-11, and UV-12 when administered orally at 100 mg/kg TID against a lethal intranasal infection with influenza virus A/Texas/36/91 (H1N1) in the BALB/c mouse model. In addition to the efficacy arm, each of the iminosugars was tested in a small group of animals with the same treatment regimen (100 mg/kg, oral gavage, TID) in the absence of a viral infection to examine gross toxicity of each analog (general health, weight, temperature, and mortality evaluations).

II. Materials and Methods

Materials

Table 8. Test articles

Name	Concentration	Solvent	Additional Info
UV-4B	100 mg/kg (2mg/dose)	H ₂ O	HCl salt
UV-8	100 mg/kg (2mg/dose)		
UV-9	100 mg/kg (2mg/dose)		

UV-10	100 mg/kg (2mg/dose)		
UV-11	100 mg/kg (2mg/dose)		
UV-12	100 mg/kg (2mg/dose)		

Table 9. Viruses for Challenge

Name	Strain	Stock titer
Influenza A virus	A/Texas/36/91 (H1N1)	2.8×10^5 PFU/mL

Table 10: Animals used

Species	Strain	Age	Sex	Vendor	Additional Info
Mouse	BALB/c	4-6 weeks	F	Charles River	7 groups n=10 5 groups n=3

Table 11: Equipment

Item	Vendor
Syringes	BD
Animal Housing	InnoVive
Plastic Feeding Tubes	Instech Solomon
Biodata chips and scanner	Bio Medic Data Systems
Ohaus scale	Ohaus

Study design

UV-8, -9, -10, -11, -12, and UV-4B were prepared in H₂O at 20 mg/ml for a delivery dose of 100 mg/kg, assuming ~20g mice given 0.1mL of compound. Groups of BALB/c mice were treated at one hour before (-1 h) intranasal infection with ~1 LD₅₀ of INFV A/Texas/36/91 (H1N1) and then compounds were administered three times daily for a total of 10 days (see Table 12 for study design summary). Weights, temperature, and survival were monitored and used for evaluation of protective efficacy and toxicity of each analog.

Table 12: Mouse groups for the study. The time point of initial dosing (treatment start relative to the infection), dosing regimen and the dose levels/routes are listed. Mice were dosed once or twice per day for total of 10 days.

Group	Mouse Strain	N	Treatment	Delivery route/frequency	Challenge	Readouts
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				on days 0-9 (10 days total starting at 1h before infection)		
1	Female	10	Vehicle	IG 100ul, TID	1 LD ₉₀ of Influenza A/Texas/36/91 (H1N1)	• Endpoint is day 14, death, or >30% weight loss. • Animals displaying severe illness (as determined by >30% weight loss, extreme lethargy, or paralysis) will be euthanized. • Temperature and weights to be taken daily for 14 days
2	BALB/C 4-6 weeks of age	10	100 mg/kg UV-4B			
3		10	100 mg/kg UV-8			
4		10	100 mg/kg UV-9			
5		10	100 mg/kg UV-10			
6		10	100 mg/kg UV-11			
7		10	100 mg/kg UV-12			
8		3	100 mg/kg UV-8		None (Toxicity assessment)	
9	3	100 mg/kg UV-9				
10	3	100				

			mg/kg UV-10			
11		3	100 mg/kg UV-11			
12		3	100 mg/kg UV-12			

STANDARD PROTOCOLS

Standard Protocol for intranasal infection of mice

1. Female 4-6 week old BALB/c mice were housed in groups of 3-5 mice. Mice were quarantined at the study site (Noble Life Sciences, Gaithersburg, MD) for at least 3 days prior to the start of the study.
2. Food and water was provided *ad libitum*.
3. The groups of mice challenged with influenza were anesthetized with 5% Isofluorene and maintained at 2.5% prior to intranasal inoculation with ~1 LD₉₀ of INFV in 100 µL PBS.
4. After the infection mice were placed back into their cages for observation and dosing.

Protocol for oral gavage or injection of mice for compound delivery

1. Mice were treated starting at 1 hour before infection with 100 µL of compound in H₂O (see Table 12 for dosing regimens) three times a day for 10 days total with 100 mg/kg of UV-4B, UV-8, UV-9, UV-10, UV-11, or UV-12 compound given by the oral route (intragastric via oral gavage).
2. After dosing, mice were returned to their cages and monitored for any distress related to dosing.

Observation of mice

1. Mice were observed through 13 days post infection (14 days total, 0-13 days post infection).
2. Mice were weighed daily on an Ohaus scale and the weights were recorded.
3. All animals had chips implanted that monitored the body temperature. The temperatures were recorded daily.
4. Survival and health of each mouse was evaluated three times a day using a scoring system of 1-7.
5. Mice were euthanized when scored at 5 or above (Very Sick; Very Ruffled Coat; Closed, inset eyes; Slow to no movement; Will return to upright position if put on its side; extremely lethargic).

III. RESULTS

Survival

Mice were infected with a $\sim 1LD_{50}$ of Influenza virus A/Texas/36/91 (H1N1) one hour after their first dose of UV-4B or UV-4 analogs, as outlined above. Survival tables, calculated as percent survival versus days post-infection, are shown in Figure 6. As expected based on previous studies, groups that were dosed orally TID with UV-4B at 100 mg/kg showed a survival rate of 100% and a MTD of >13 days (Figure 7). Mice that were dosed orally with UV-12 at 100 mg/kg showed a survival rate of 90%, and a MTD of >13 days (Figure 6, 7). Mice treated with UV-8, UV-9, UV-10, or UV-11 displayed a MTD of 10.5, 7, 7.5, and 8 days, respectively, and survival rates of 0% with the exception of UV-8 (30%) (Figure 6, 7). Negative control mice dosed with vehicle (H₂O) demonstrated 30% survival and a MTD of 9 days (Figures 6, 7).

Mice which received UV-8, -9, -10, -11, or -12 at 100 mg/kg without a viral challenge to examine gross toxicity displayed 100% survival (data not shown).

Biometric Analysis

During the course of this study, individual weights and temperatures were monitored daily for each group. The average weights for each group of mice are shown in Figure 8 with statistical analysis shown in Figure 9. The average temperatures are shown in Figure 10 with statistical analysis shown in Figure 11.

As a second biometrics the animals' temperatures were evaluated. Every animal was tagged with a chip to perform daily temperature readings using a scanner. The graphs in Figure 10 show the body temperatures for each test group. Significance shown against the vehicle control is indicated in Figure 11.

CONCLUSIONS

The group of infected mice dosed orally TID with 100 mg/kg of UV-4B exhibited 100% survival, where the groups which were orally dosed with 100 mg/kg of UV-9, UV-10, and UV-11 exhibited 0% survival. Statistical analysis on weights and temperatures for these groups was not performed due to a lower survival rate and MTD than the vehicle control. Mice dosed orally with UV-8 exhibited 30% survival, and thus no significant difference from the vehicle control group. Mice dosed orally with 100 mg/kg of UV-12 exhibited 90% survival, as well as significant increases in overall temperature and weight. UV-12 also showed mild toxicity with steady weight loss in the uninfected group, but uninfected mice dosed with UV-12 did not lose more than 10% weight overall and they were able to fully recover after the dosing regimen had been completed. Statistical analysis was not performed on any of the parameters examined for the uninfected groups of mice (gross toxicity) as the number of mice per group was limited (n=3) there was no uninfected, undosed control group for comparison.

EXAMPLE 4

Survival Analysis of UV-4 and UV-12 in ADE Model

Purpose: This study determined the efficacy of UV-4 and UV-12 in promoting survival of mice challenged with dengue virus. All compounds were given by the oral

route (3x per day intragastric via oral gavage – IG) for a total number of 7 days after the start of dosing. The experiment used the ADE model of infection developed in the lab (Zellweger *et al.* CellHostMicrob 7; pp1-12 (2010)). Animals received the virus challenge dose ~1 LD₉₀ on day 0. The first dose was given 0.5-1 hr pre-virus challenge. Survival was measured until 3 days after dosing was completed. UV-4 HCl salt was used in this study, which is equal to “UV-4B”.

Iminosugar candidates:

1. N-9-methoxynonyl-deoxynojirimycin (UV-4) (HCl Salt), UV-4B
2. UV-12
3. Control, H₂O

Experimental Design for the Study

1. Control, H₂O + DENV (S221) [7 mice]
2. UV-4, 1 mg (50 mg/kg/dose) + DENV (S221) [5 mice]
3. UV-12, 1 mg (50 mg/kg/dose) + DENV (S221) [5 mice]

Mice: Sex matched 5-6 weeks old

AG129 (129/SV IFN- α , β , and γ -receptor-/-) bred at LIAI by Sujan Shresta (also publically available by The Jackson Laboratory, Bar Harbor, Maine)

Route:

Iminosugar: Orally 3 x day, (gavage (IG)) every 8 hours

Antibody: IP (Intraperitoneal)

Virus: IV

Antibody and Compound were given simultaneously, then virus within 30 minutes

Virus Challenge:

Antibody: 5 μ g 2H2 (anti-prM) available from ATCC

Virus: DENV2 Strain S221 (v476) (Zellweger RM, Prestwood TR, Shresta S.

Enhanced infection of liver sinusoidal endothelial cells in a mouse model of antibody-induced severe dengue disease. *Cell Host Microbe*. 2010 Feb 18;7(2):128-39)

Dose: 1E9 GE (genomic equivalents) per animal

Read-out:

Animal survival. Animals displaying severe illness (as determined by 20% weight loss, extreme lethargy, ruffled coat, or paralysis) were euthanized.

Figure 12 presents results of this study. All groups were treated with the same dose of compounds:

Experimental Design for the Study

1. Control, H₂O + DENV (S221) [7 mice]
2. UV-4, 1 mg (50 mg/kg/dose) + DENV (S221) [5 mice]
3. UV-8, 1 mg (50 mg/kg/dose) + DENV (S221) [5 mice]
4. UV-9, 1 mg (50 mg/kg/dose) + DENV (S221) [5 mice]
5. UV-10, 1 mg (50 mg/kg/dose) + DENV (S221) [5 mice]
6. UV-11, 1 mg (50 mg/kg/dose) + DENV (S221) [5 mice]
7. UV-12, 1 mg (50 mg/kg/dose) + DENV (S221) [5 mice]

This study was to determine the efficacy of UV-4 and its analogues in promoting survival of mice challenged with dengue virus. All compounds were given by the oral route (3x per day intragastric via oral gavage – IG) for a total number of 7 days after the start of dosing. The experiment used the ADE model of infection (Zellweger et al. *CellHostMicrob* 7; pp1-12 (2010)). Animals received the virus challenge dose ~1 LD₉₀ on day 0. The first compound dose began 0.5-1 hr pre-virus challenge.

Survival were measured until 3 days after dosing.

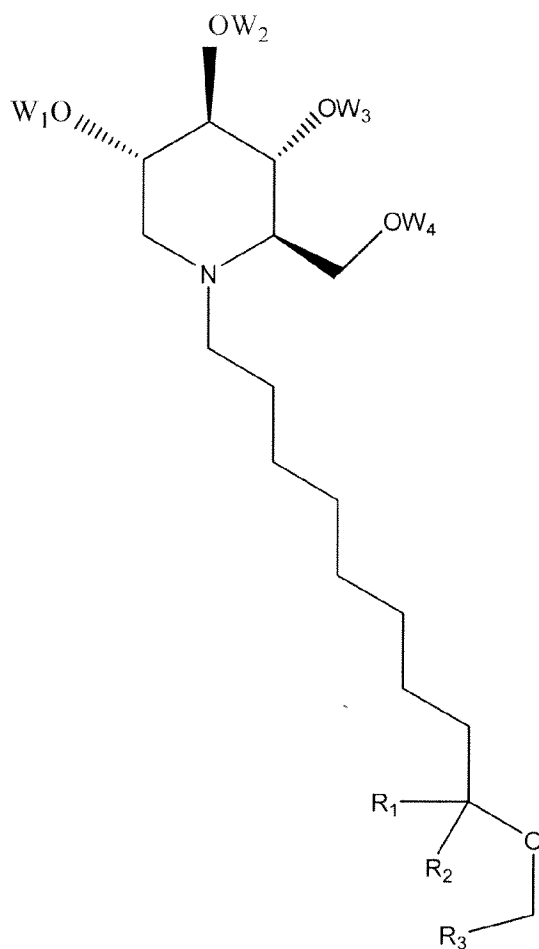
* * *

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.

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 compound of formula (I):

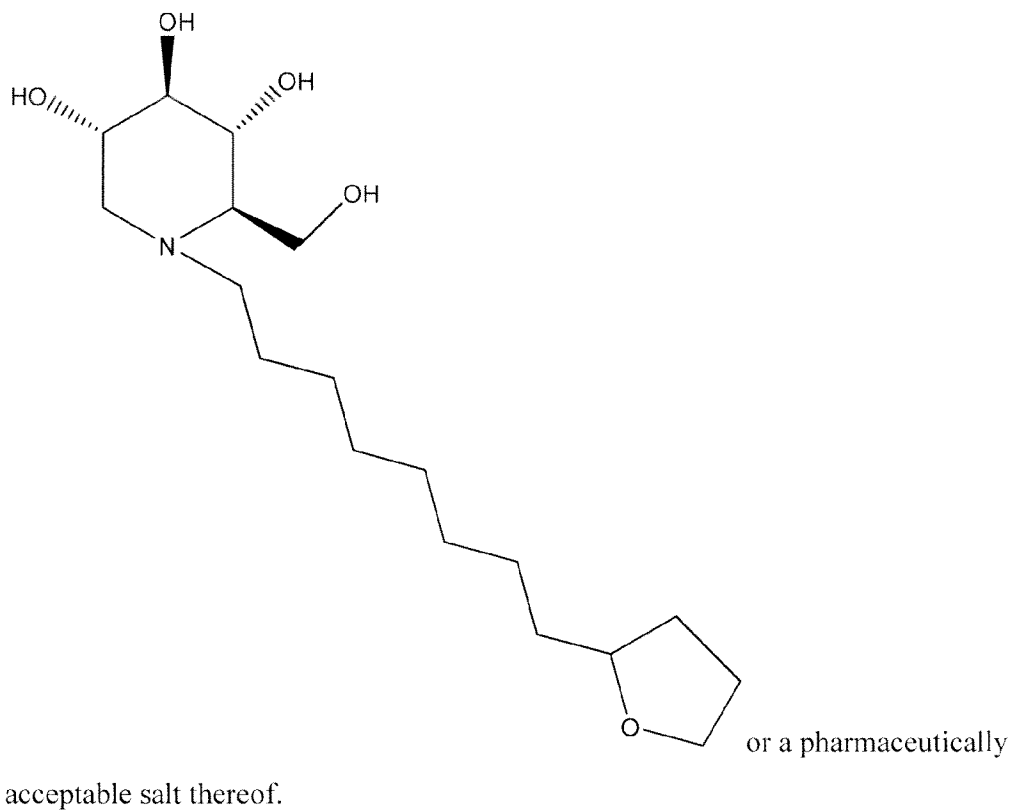


or a pharmaceutically acceptable salt thereof,

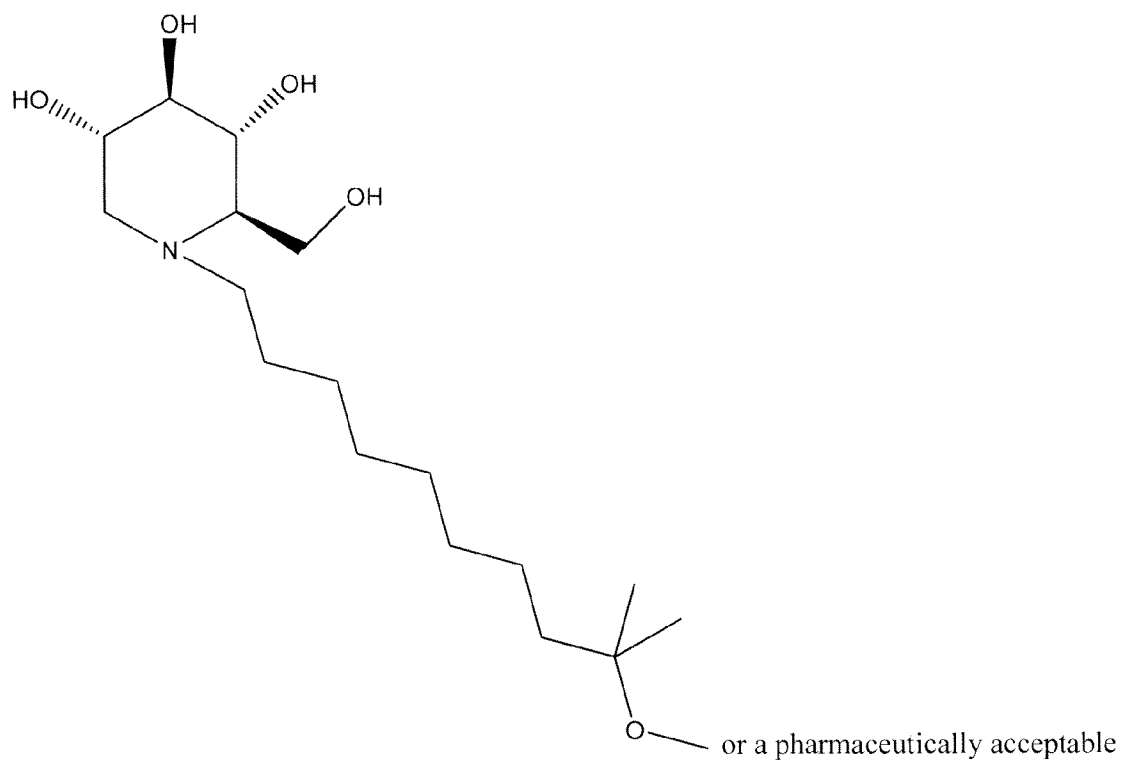
wherein W_{1-4} and R_{1-3} are each independently selected from hydrogen and C_{1-3} alkyl groups and wherein at least one of R_{1-3} is not hydrogen.

2. The compound of claim 1, wherein each of W_{1-4} is hydrogen.

3. The compound of claim 1 having formula

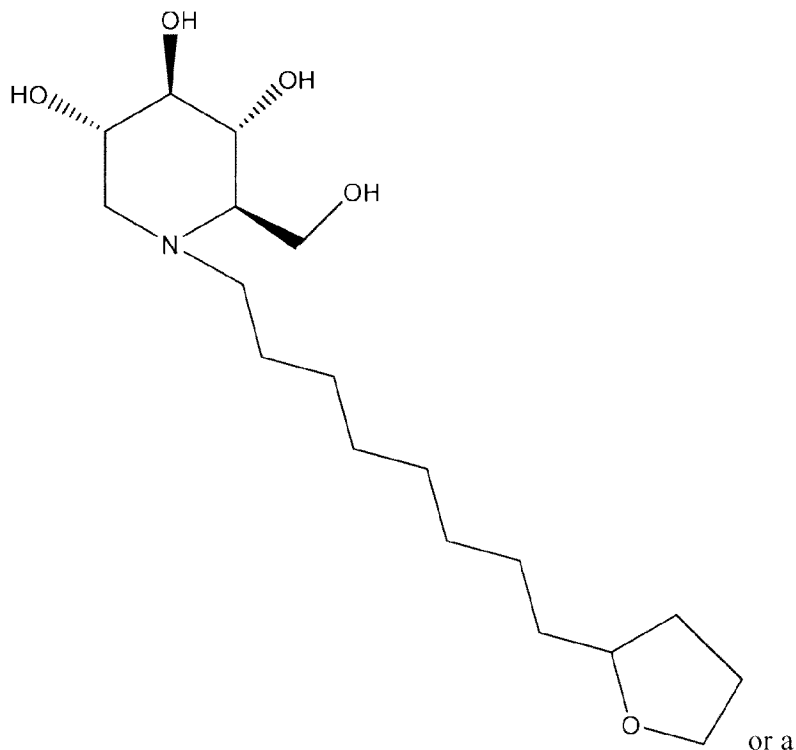


4. The compound of claim 1 having formula



salt thereof.

5. A pharmaceutical composition comprising a) a pharmaceutically effective amount of the compound of claim 1 and b) a pharmaceutically acceptable carrier.
6. A method of treating or preventing a Dengue viral infection comprising administering to a subject in need thereof the compound of claim 1 or a pharmaceutically acceptable salt thereof.
7. The method of claim 6, wherein the dengue viral infection is by or associated with a Dengue 2 virus.
8. The method of claim 5, wherein the compound is a compound

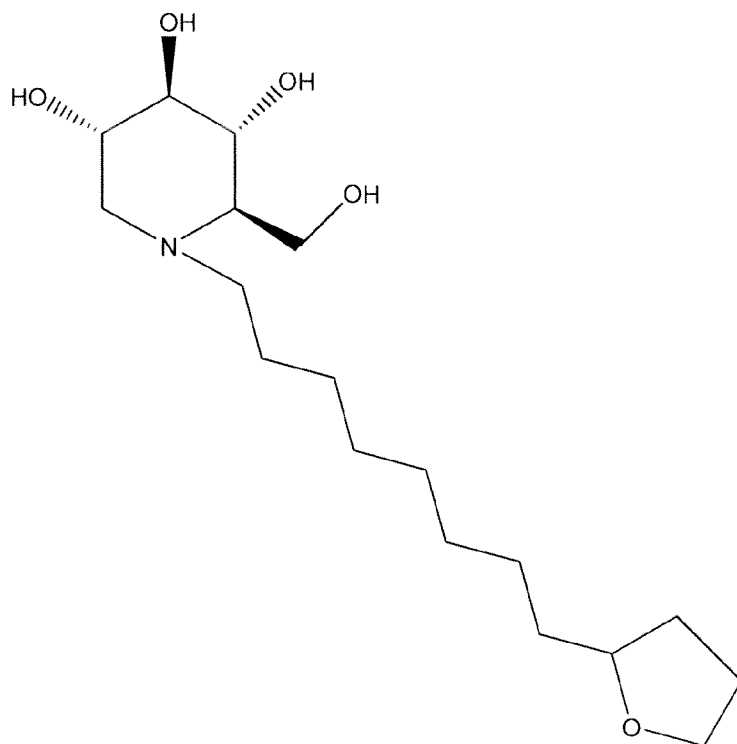


having formula

pharmaceutically acceptable salt thereof.

9. The method of claim 5, wherein the subject is a mammal.
10. The method of claim 9, wherein the subject is a human,
11. A method of treating a disease or condition caused by or associated with a virus belonging to the Orthomyxoviridae family comprising administering to a subject in need thereof the compound of claim 1 or a pharmaceutically acceptable salt thereof.
12. The method of claim 11, wherein the virus is an Influenza virus.
13. The method of claim 12, wherein the virus is an Influenza A virus.
14. The method of claim 13, wherein the virus is a H3N2 subtype of the Influenza A virus.
15. The method of claim 13, wherein the virus is a H1N1 subtype of the Influenza A virus.

16. The method of claim 11, wherein the compound is a compound having

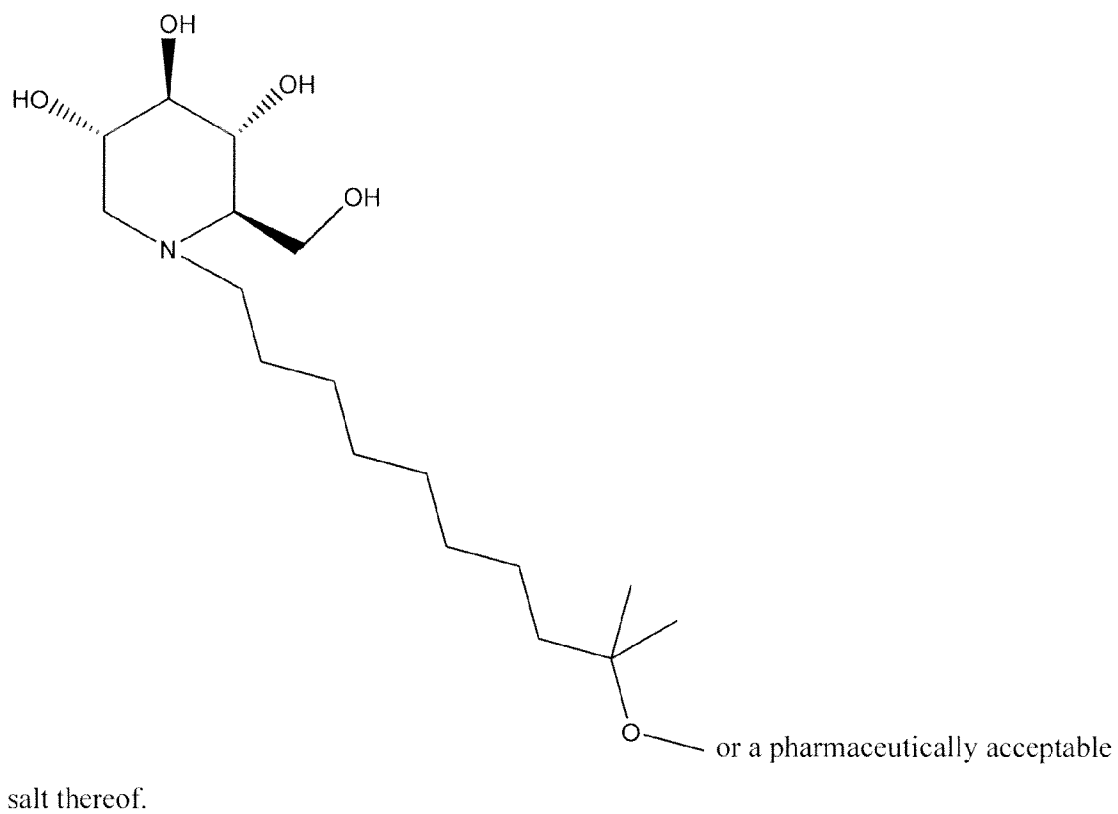


formula

acceptable salt thereof.

or a pharmaceutically

17. The method of claim 11, wherein the compound is a compound having



18. The method of claim 11, wherein the subject is a mammal.
19. The method of claim 18, wherein the subject is a human being.

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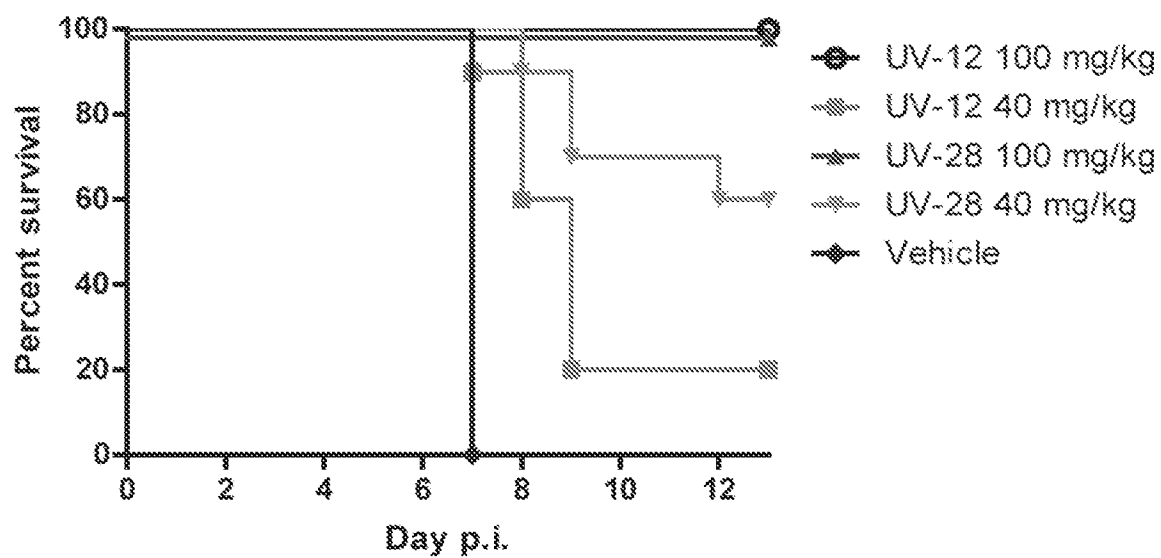


Figure 1

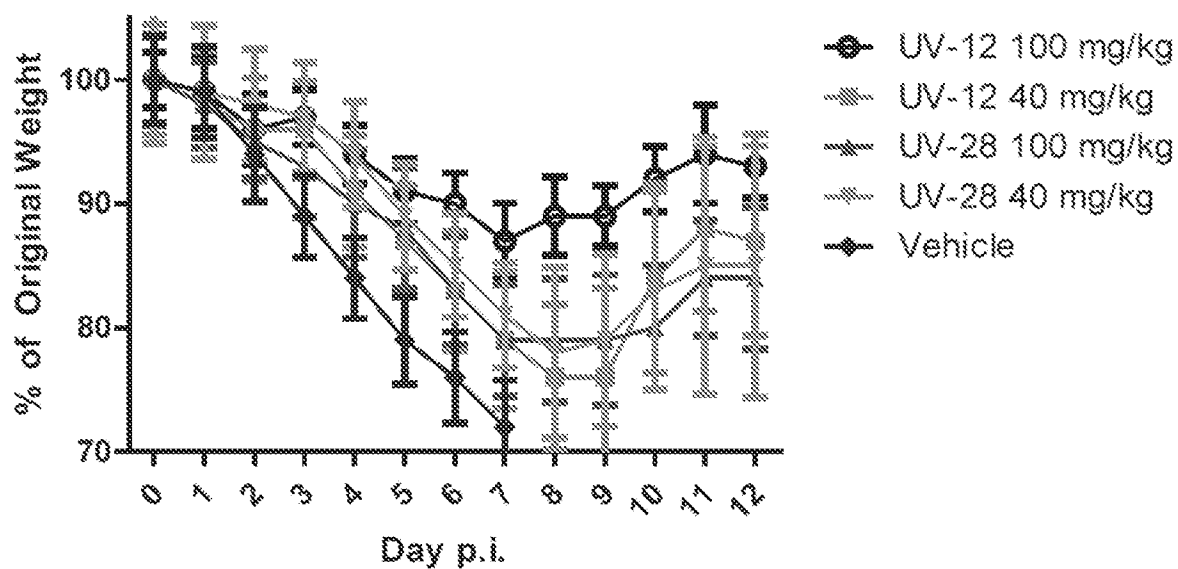


Figure 2

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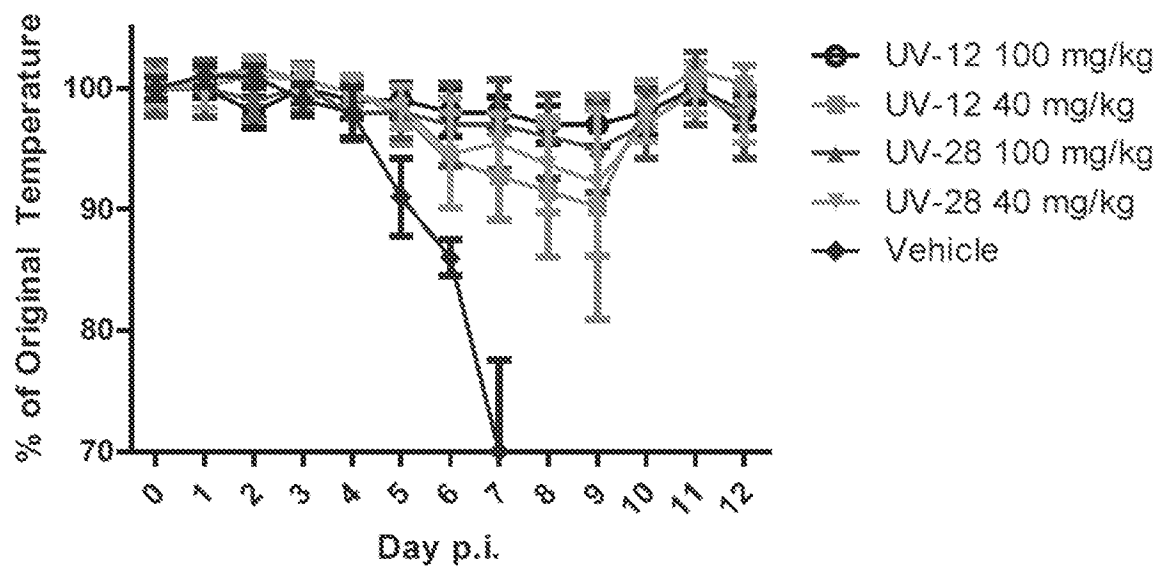


Figure 3

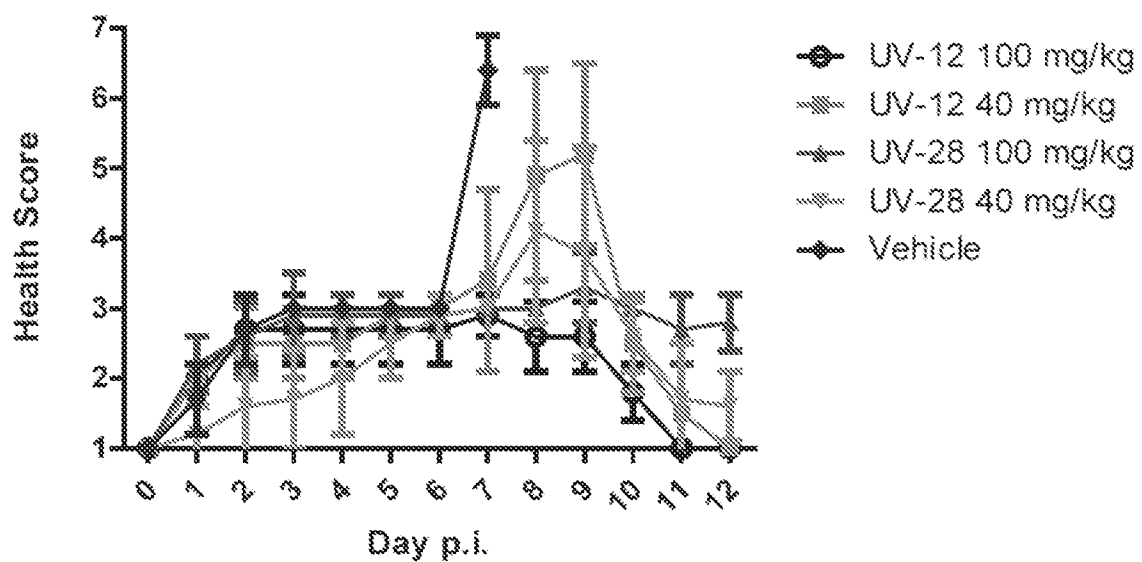


Figure 4

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In Vivo Efficacy against Dengue Virus
 - UV -12: Oral delivery, TID, 7 days

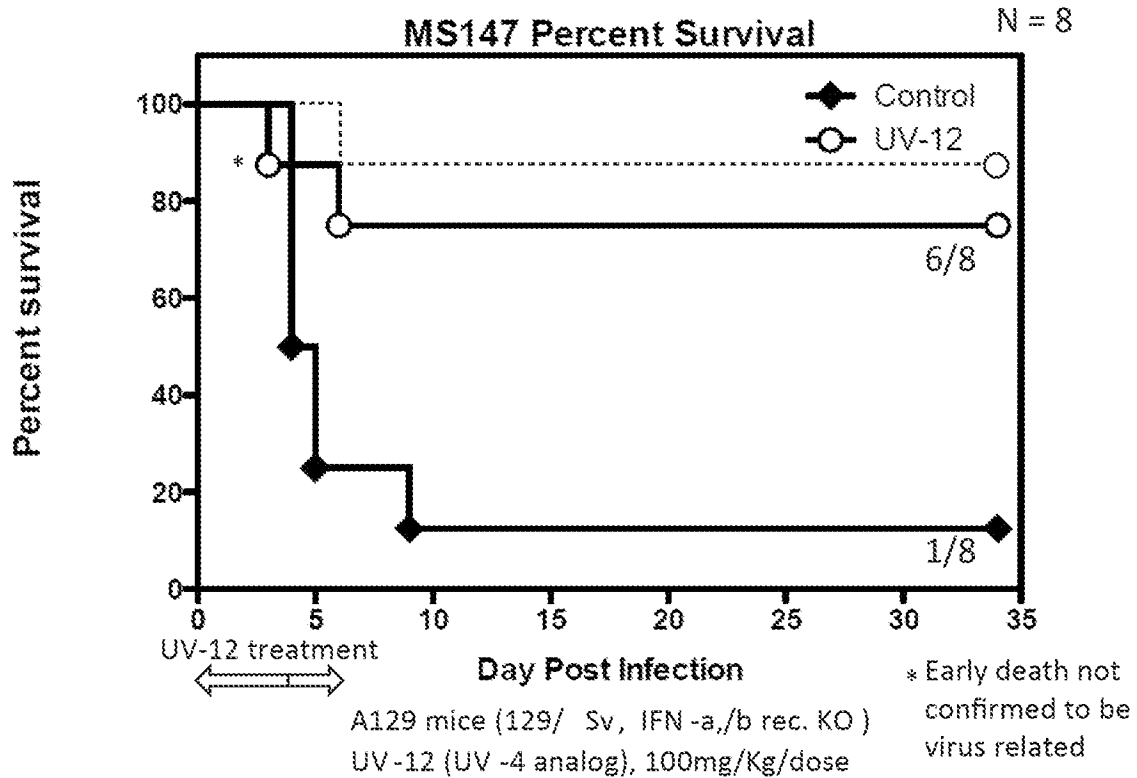


Figure 5

Survival of infected mice treated orally with 100 mg/kg of UV-4B, UV-8, UV-9, UV-10, UV-11, and UV-12. Infection with INFV A/Texas/36/91 (H1Ni)

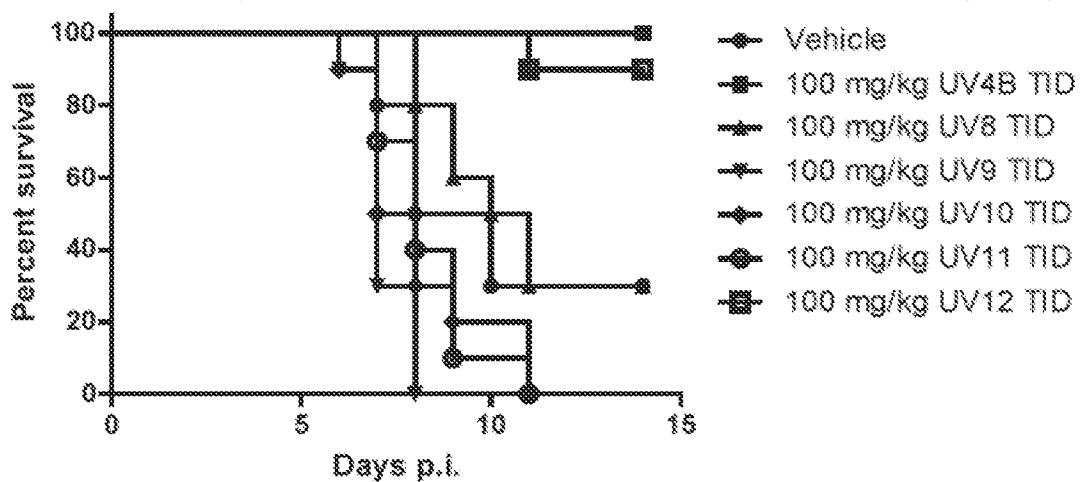


Figure 6

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Treated samples vs H2O Control Group						
	100 mg/kg UV-4B	100 mg/kg UV-8	100 mg/kg UV-9	100 mg/kg UV-10	100 mg/kg UV-11	100 mg/kg UV-12
Log-rank (Mantel-Cox) Test						
Chi square	9.429	0.2812	8.043	2.924	2.66	8.272
df	1	1	1	1	1	1
P value	0.0021	0.5959	0.0046	0.0873	0.1029	0.004
P value summary	**	ns	**	ns	ns	**
Significant?	Yes	No	Yes	No	No	Yes
Gehan-Breslow-Wilcoxon Test						
Chi square	8.978	0.9379	7.335	2.41	1.261	8.565
df	1	1	1	1	1	1
P value	0.0027	0.3328	0.0068	0.1206	0.2614	0.0034
P value summary	**	ns	**	ns	ns	**
Significant?	Yes	No	Yes	No	No	Yes
Median survival						
Control + H2O	9	9	9	9	9	9
Treated Group	Undefined	10.5	7	7.5	8	Undefined
Hazard Ratio						
Ratio	11.79	1.369	0.1389	0.3758	0.3845	8.977
95% CI of ratio	2.441 to 56.96	0.4286 to 4.373	0.03550 to 0.5435	0.1224 to 1.154	0.1219 to 1.213	2.012 to 40.05

Figure 7

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Weight development of infected mice treated with
100 mg/kg of UV-4B or UV-8

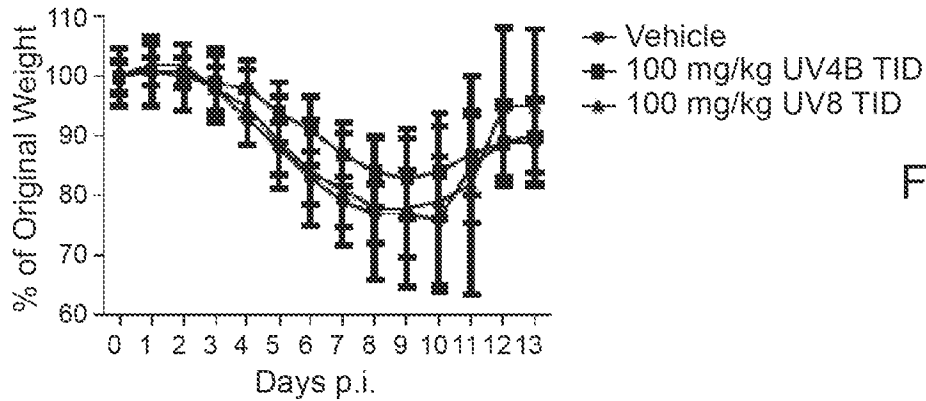


Figure 8A

Weight development of infected mice treated with
100 mg/kg of UV-4B or UV-9

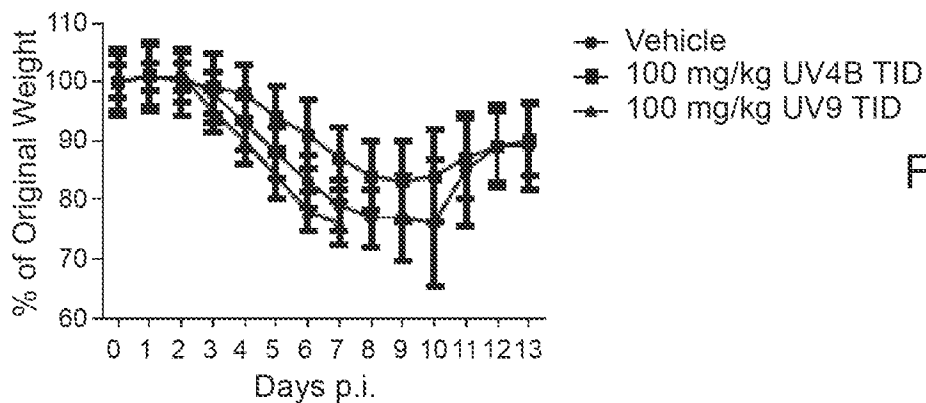


Figure 8B

Weight development of infected mice treated with
100 mg/kg of UV-4B or UV-10

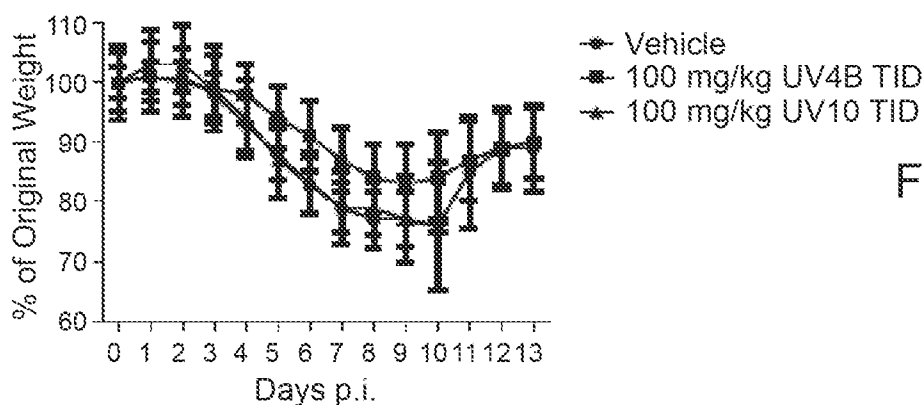


Figure 8C

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Weight development of infected mice treated with
100 mg/kg of UV-4B or UV-11

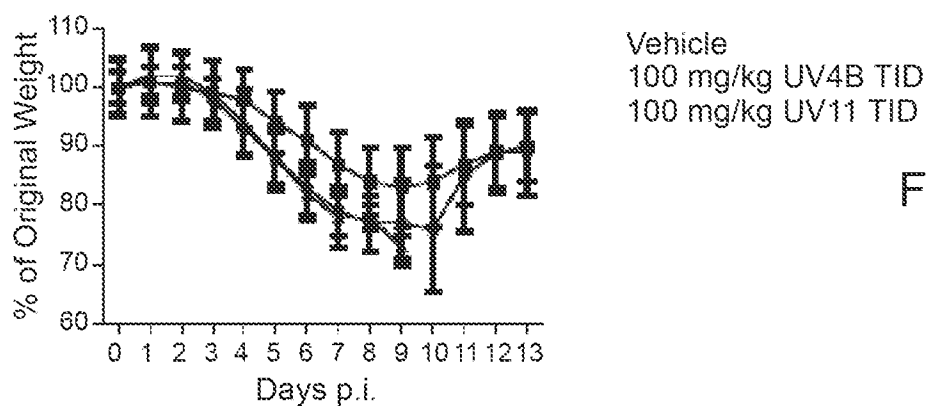


Figure 8D

Weight development of infected mice treated with
100 mg/kg of UV-4B or UV-12

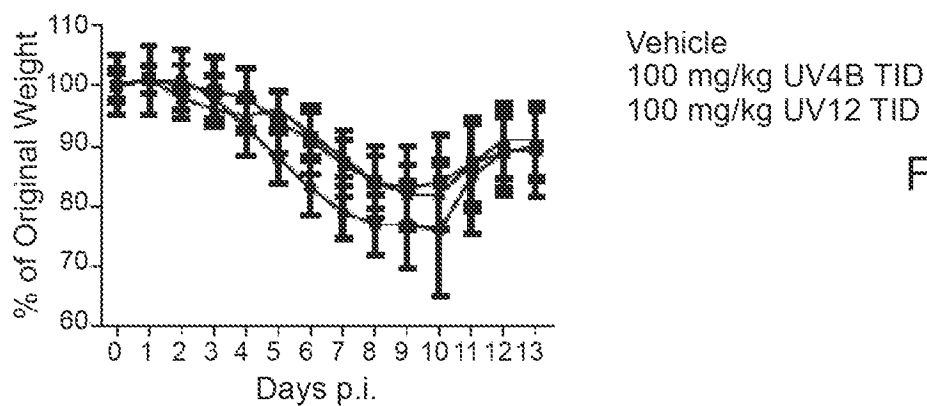


Figure 8E

Weight development of uninfected mice treated with
UV-8, -9, -10, -11, or -12.

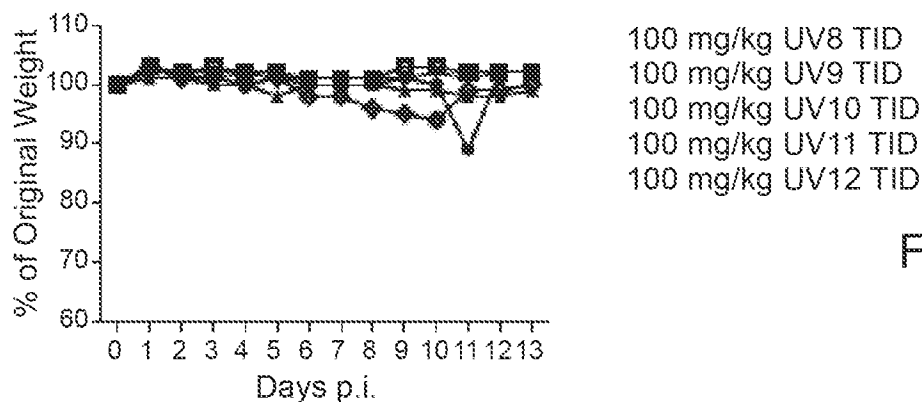


Figure 8F

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100 mg/kg UV-4B		
Source of Variation	% of Total Variation	P Value
Interaction	5.9	< 0.0001
Time	77.65	< 0.0001
Treatment	5.16	0.0008
Subjects (matching)	5.7963	< 0.0001

100 mg/kg UV-8		
Source of Variation	% of Total Variation	P Value
Interaction	0.19	0.8896
Time	81.29	< 0.0001
Treatment	0.26	0.502
Subjects (matching)	10.1209	< 0.0001

100 mg/kg UV-12		
Source of Variation	% of Total Variation	P Value
Interaction	12.31	< 0.0001
Time	71.47	< 0.0001
Treatment	5.25	0.0004
Subjects (matching)	5.1095	< 0.0001

Figure 9

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Temperature development of infected mice treated with
100 mg/kg of UV-4B or UV-8

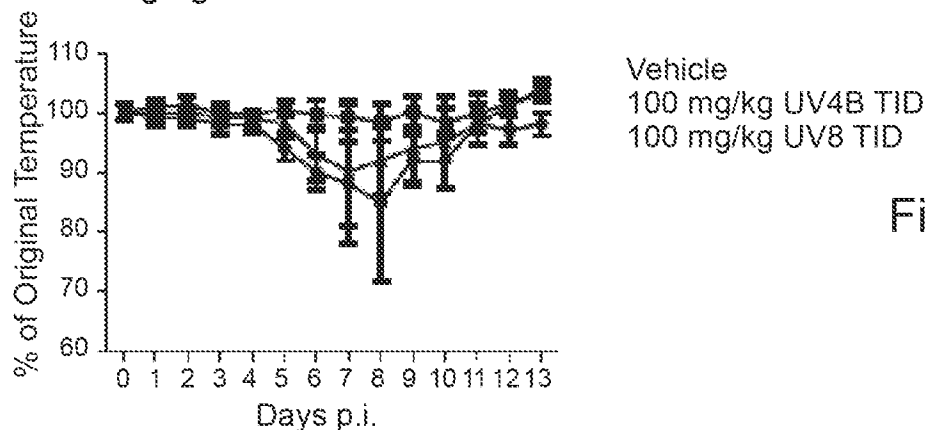


Figure 10A

Temperature development of infected mice treated with
100 mg/kg of UV-4B or UV-9

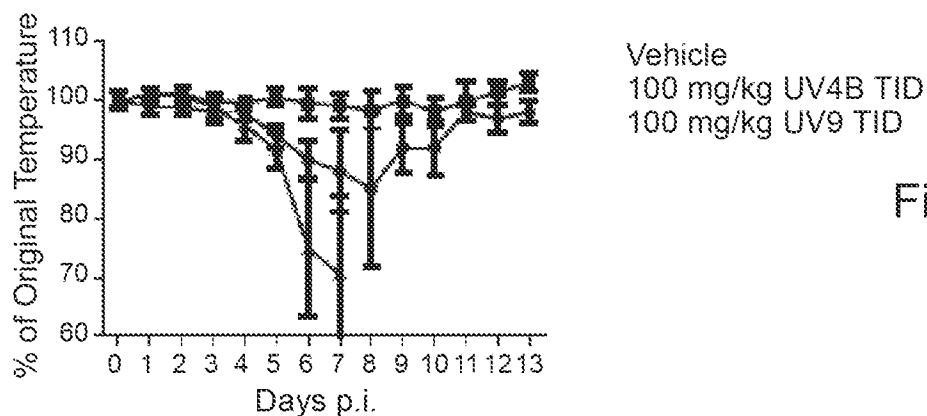


Figure 10B

Temperature development of infected mice treated with
100mg/kg of UV-4B or UV-10

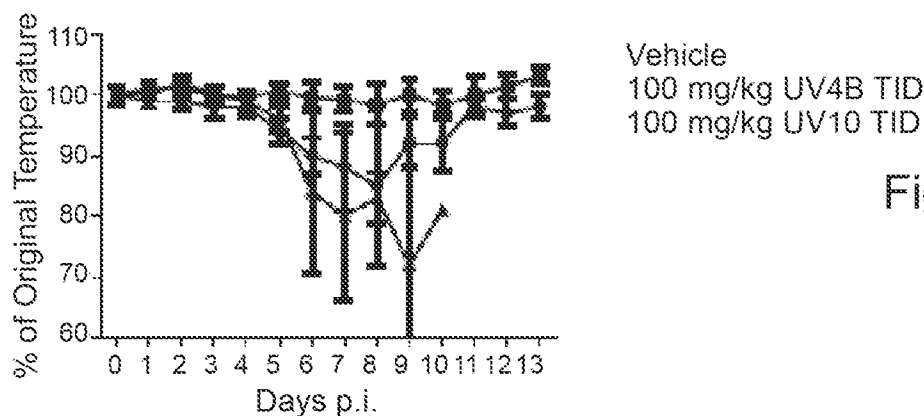


Figure 10C

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Temperature development of infected mice treated with
100 mg/kg of UV-4B or UV-11

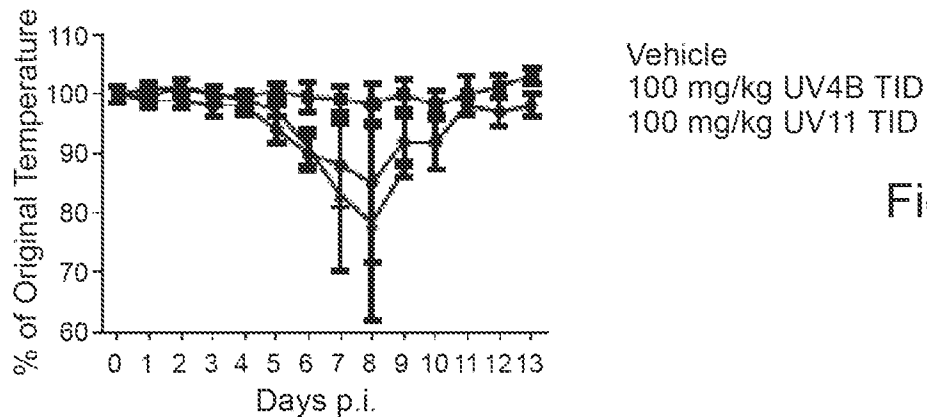


Figure 10D

Temperature development of infected mice treated with
100 mg/kg of UV-4B or UV-12

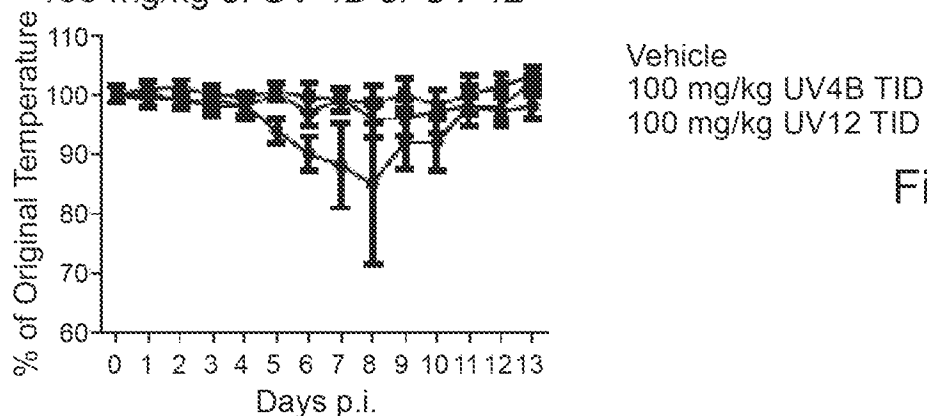


Figure 10E

Weight development of uninfected mice treated with
UV-8, -9, -10, -11, or -12.

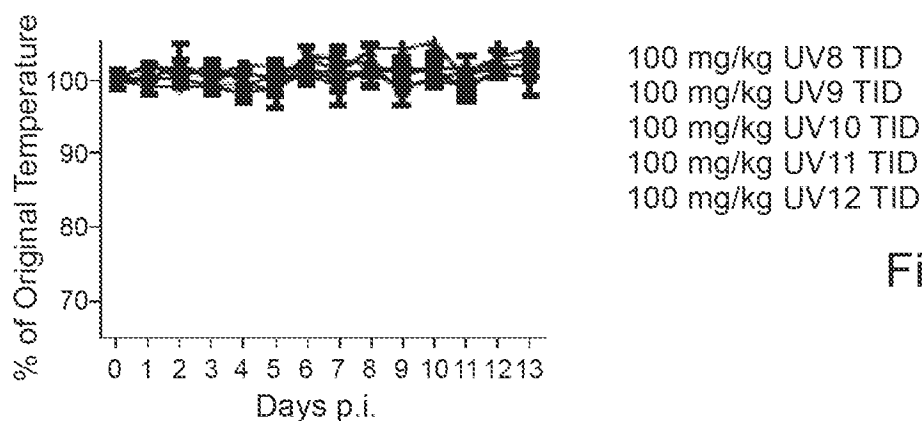


Figure 10F

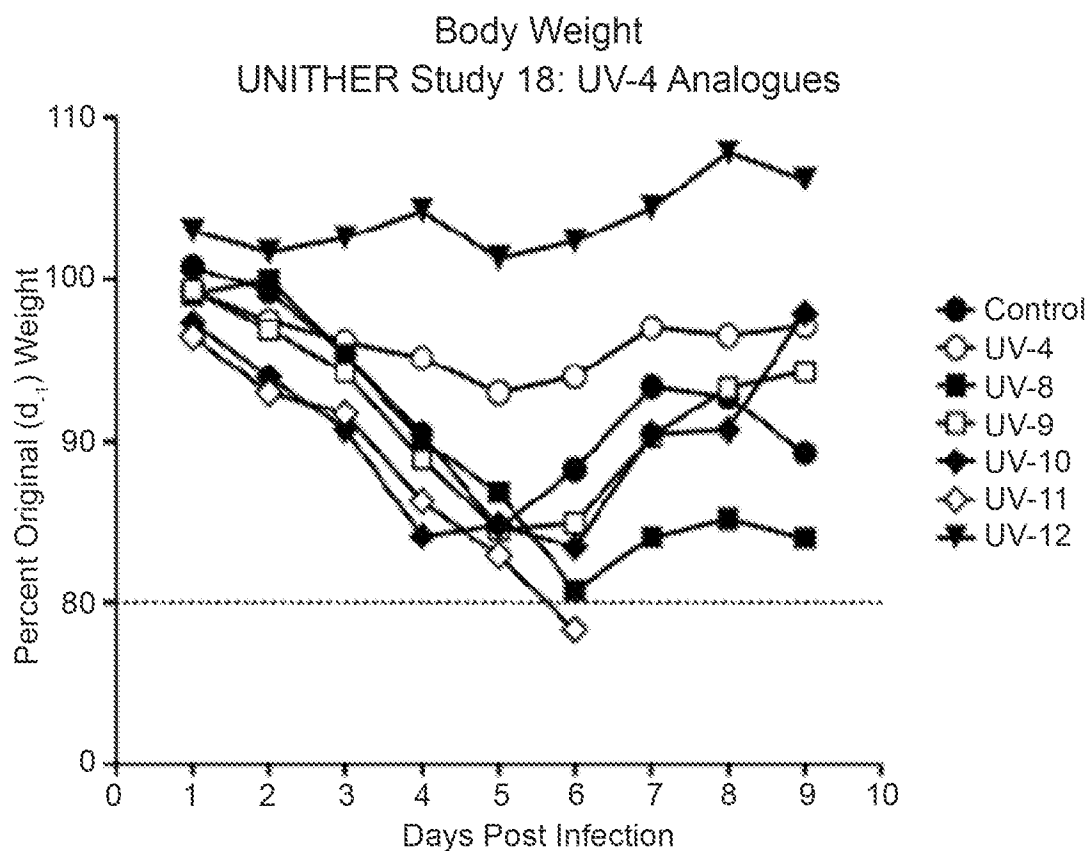
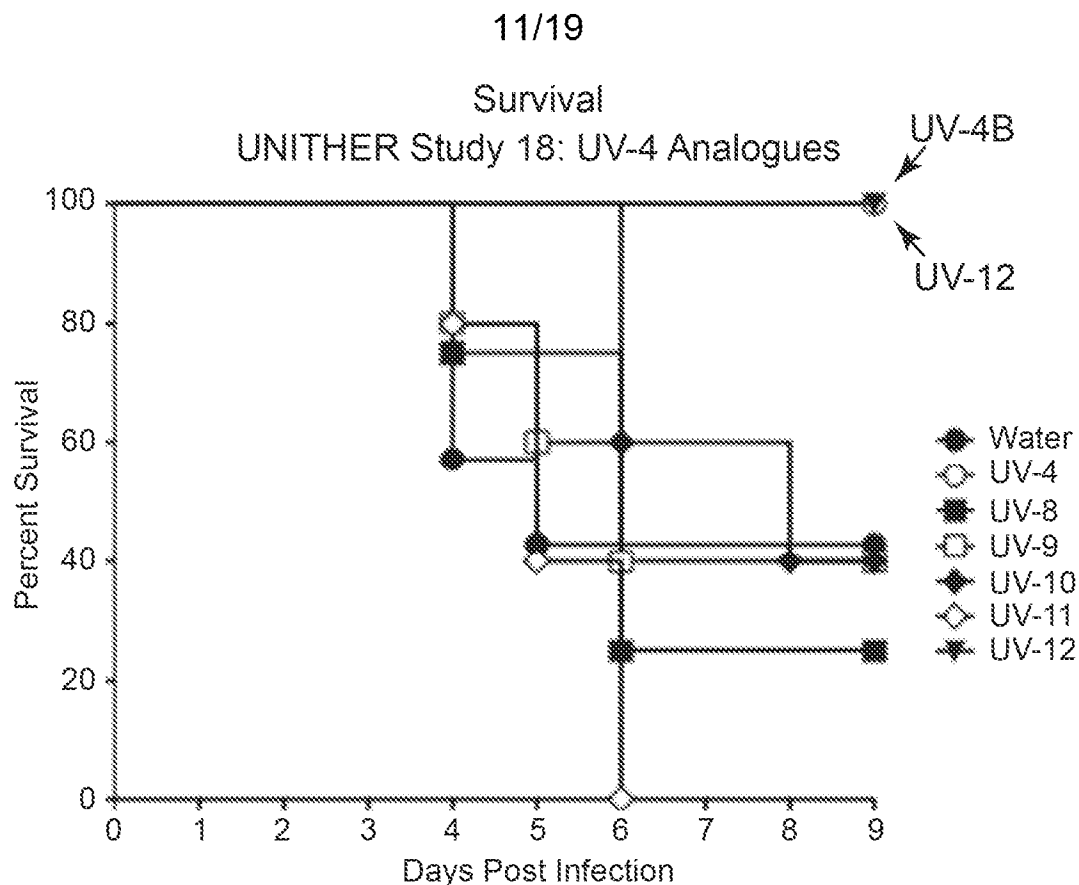
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100 mg/kg UV-4B		
Source of Variation	% of Total Variation	P Value
Interaction	19.46	< 0.0001
Time	29.22	< 0.0001
Treatment	22.78	< 0.0001
Subjects (matching)	7.9206	< 0.0001

100 mg/kg UV-8		
Source of Variation	% of Total Variation	P Value
Interaction	0.77	0.9137
Time	47.47	< 0.0001
Treatment	1.74	0.145
Subjects (matching)	13.5295	0.001

100 mg/kg UV-12		
Source of Variation	% of Total Variation	P Value
Interaction	23.23	< 0.0001
Time	32.8	< 0.0001
Treatment	13.33	< 0.0001
Subjects (matching)	5.2539	0.1205

Figure 11



AG129 DENVADE model

Figure 12

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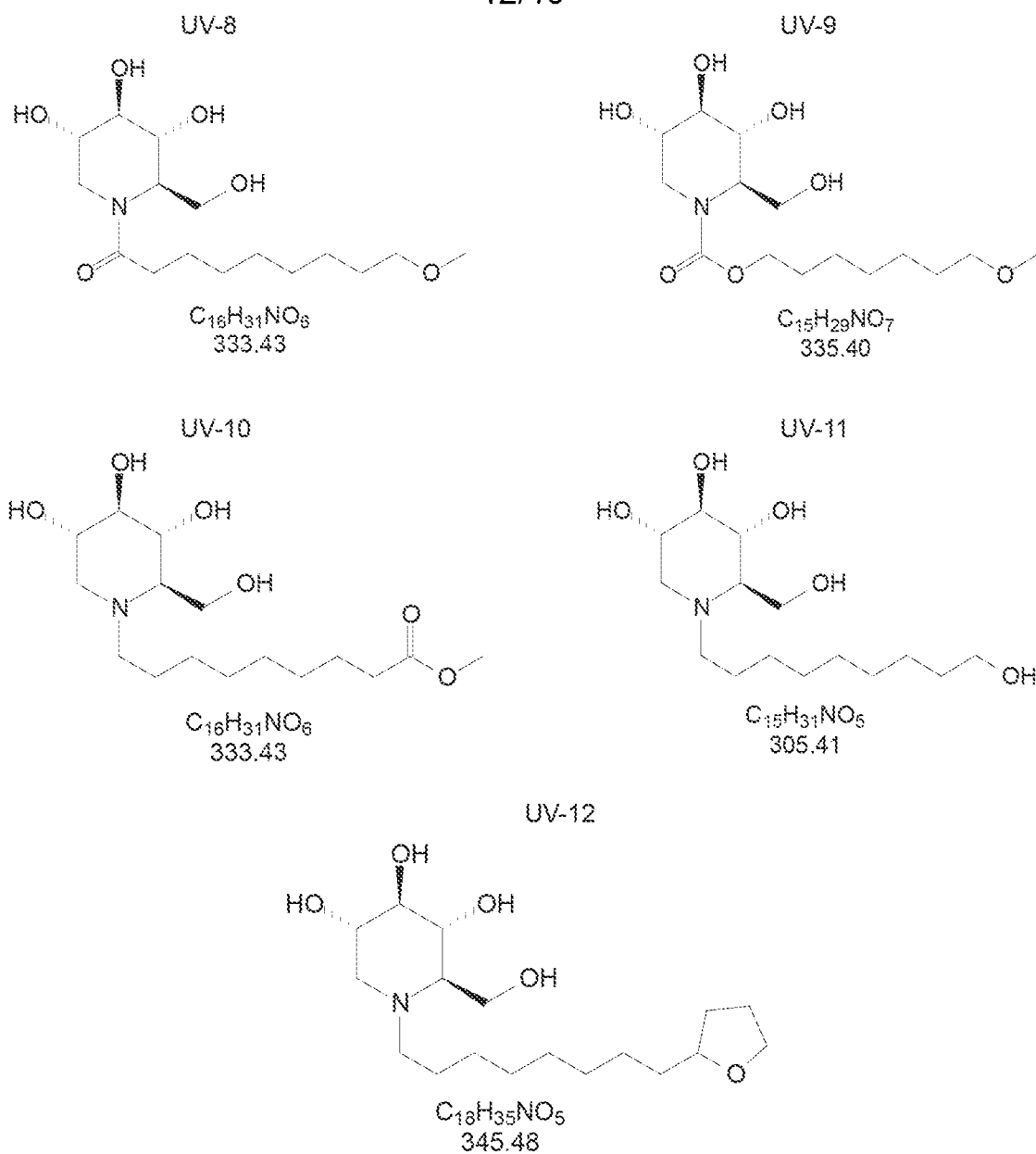


Figure 13

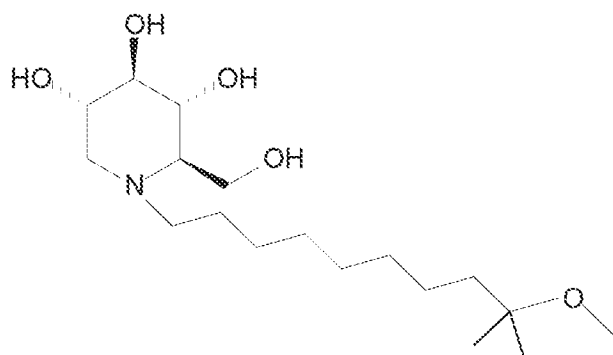


Figure 14

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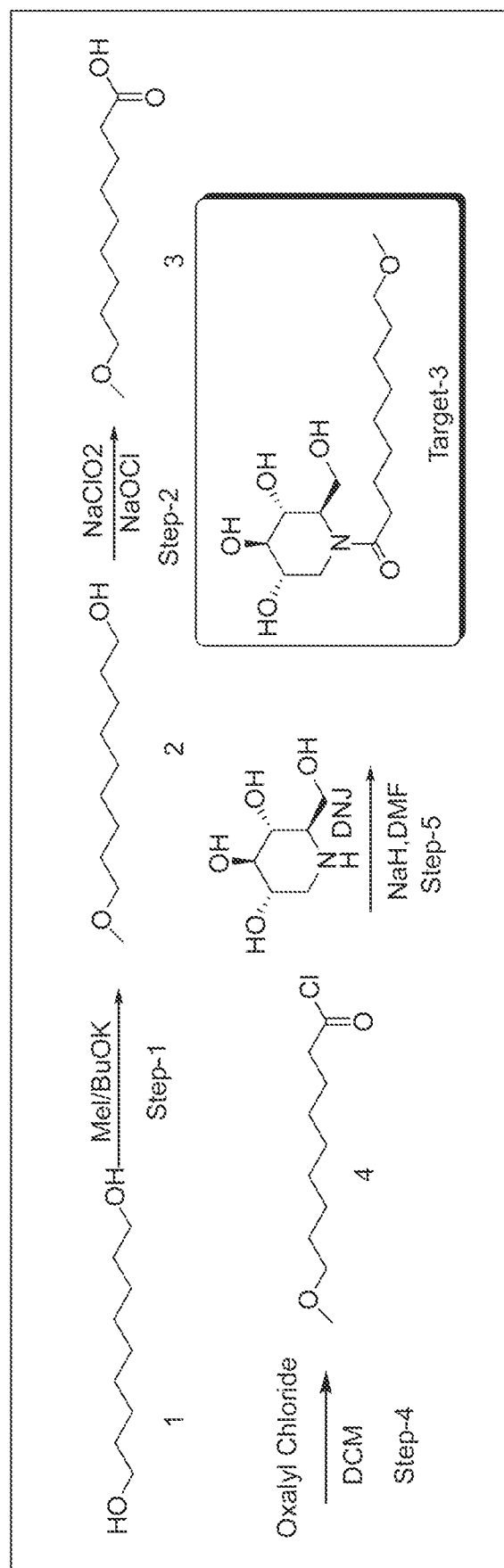
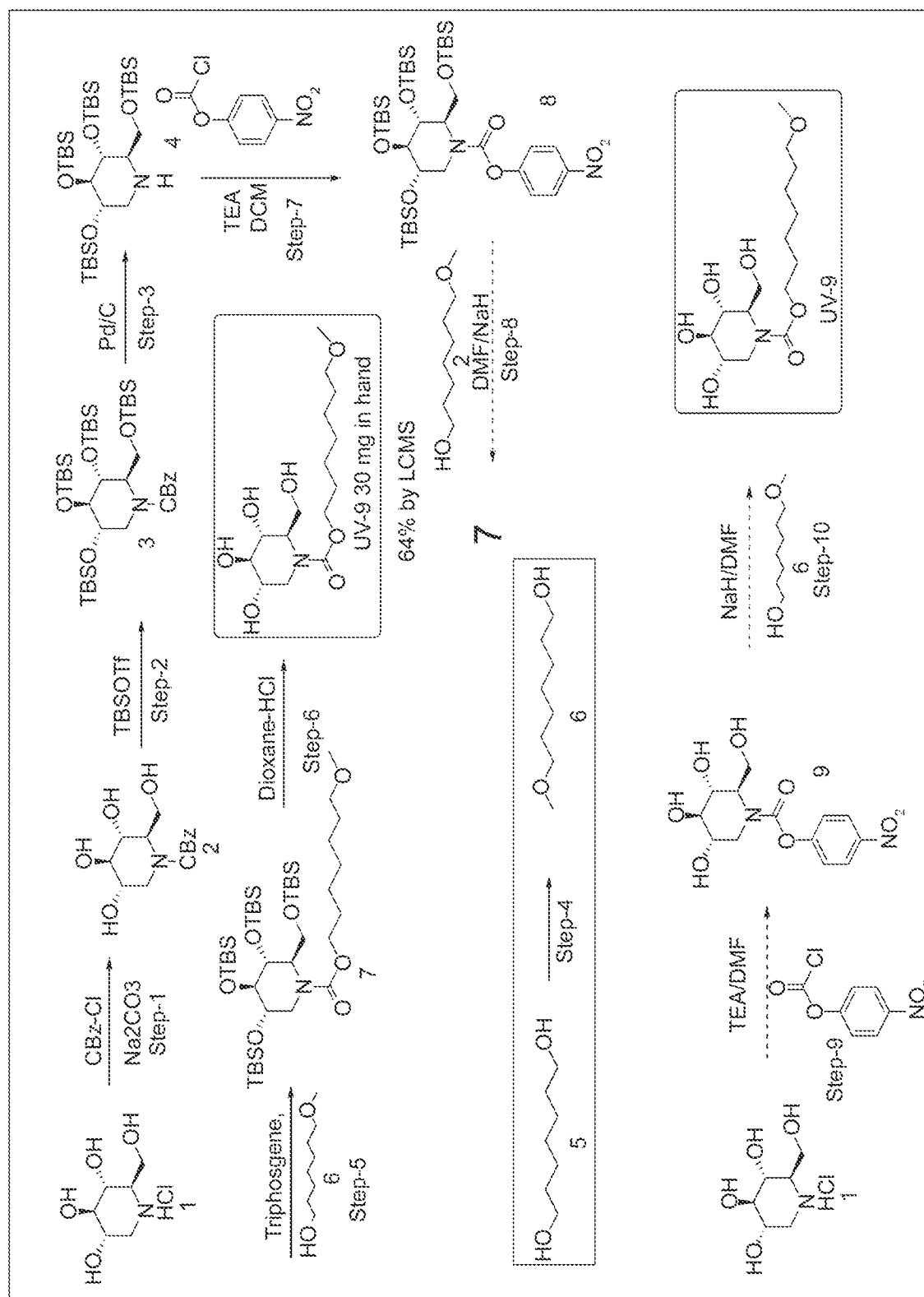
UV-8: C₁₆H₃₁NO₆ (Mol Wt 333.43)

Figure 15



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UV-10: C16H31NO6 (Mol Wt 333.43)

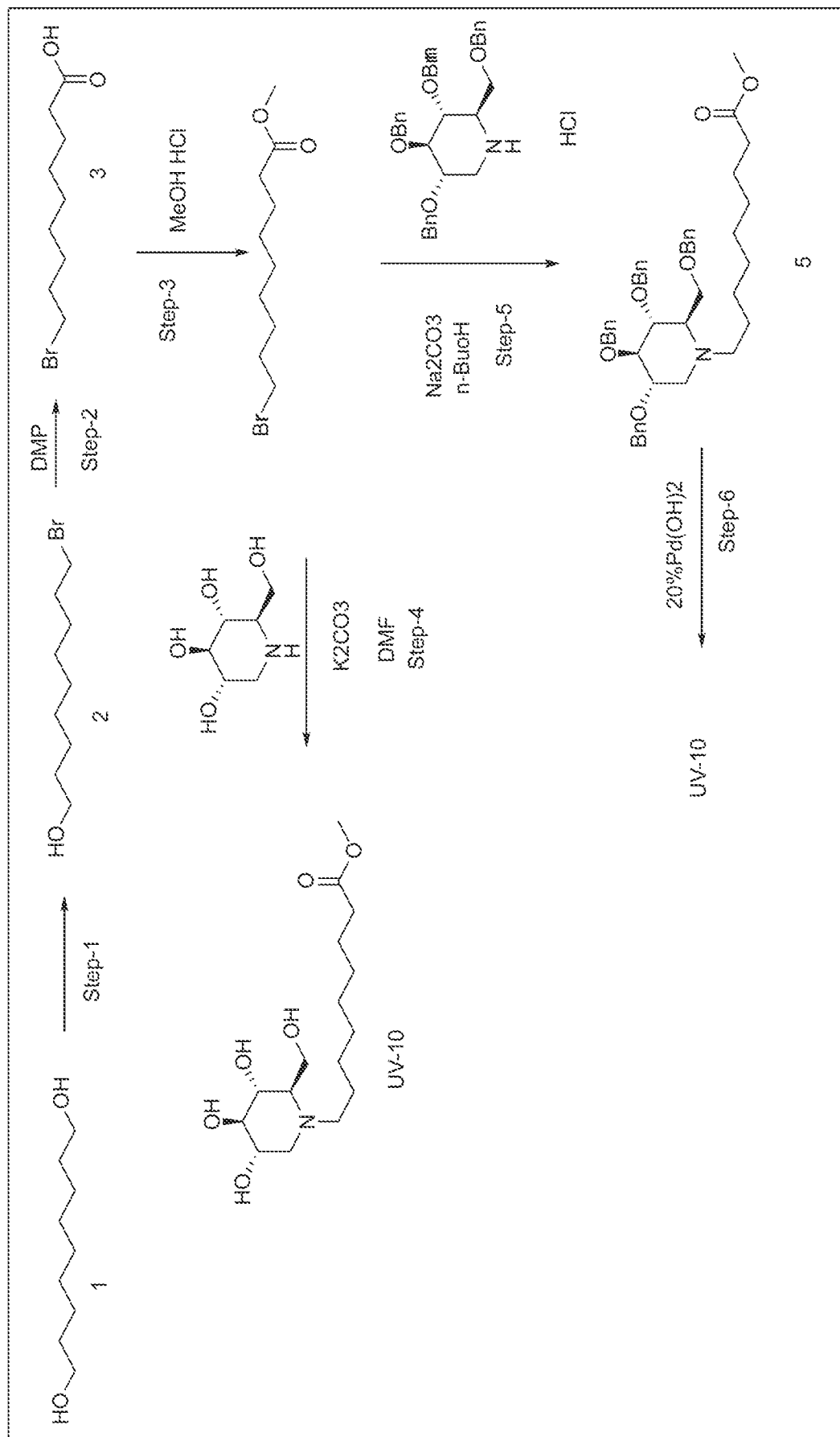


Figure 17

UV-11: C15H31NO5 (Mol Wt 305.41)

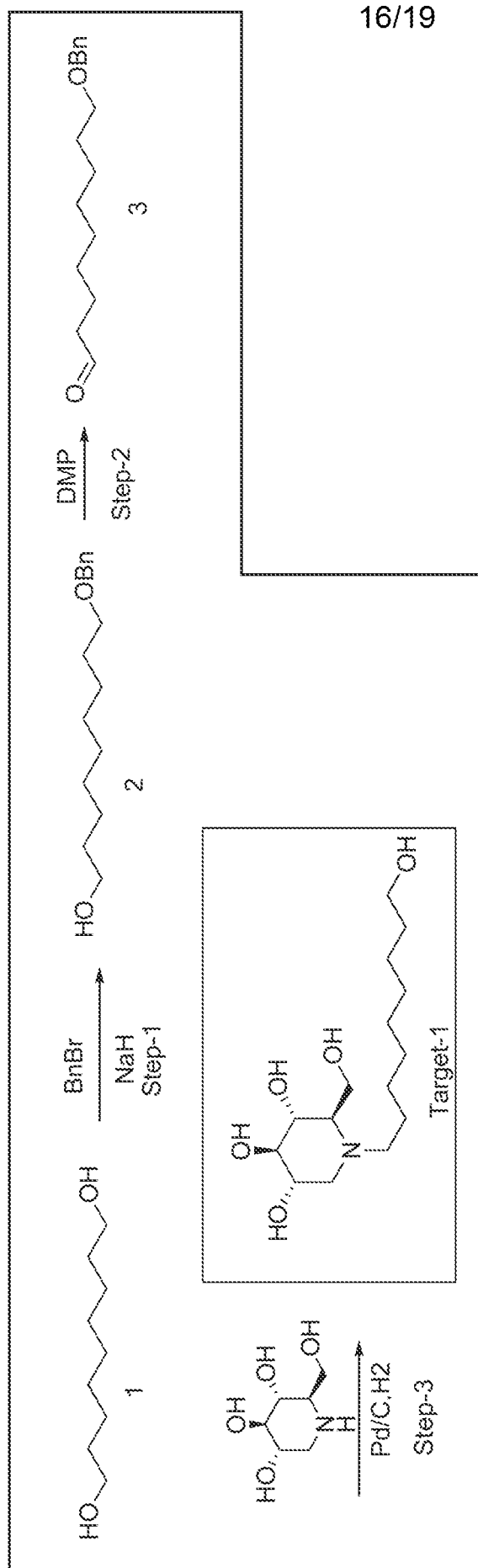


Figure 18

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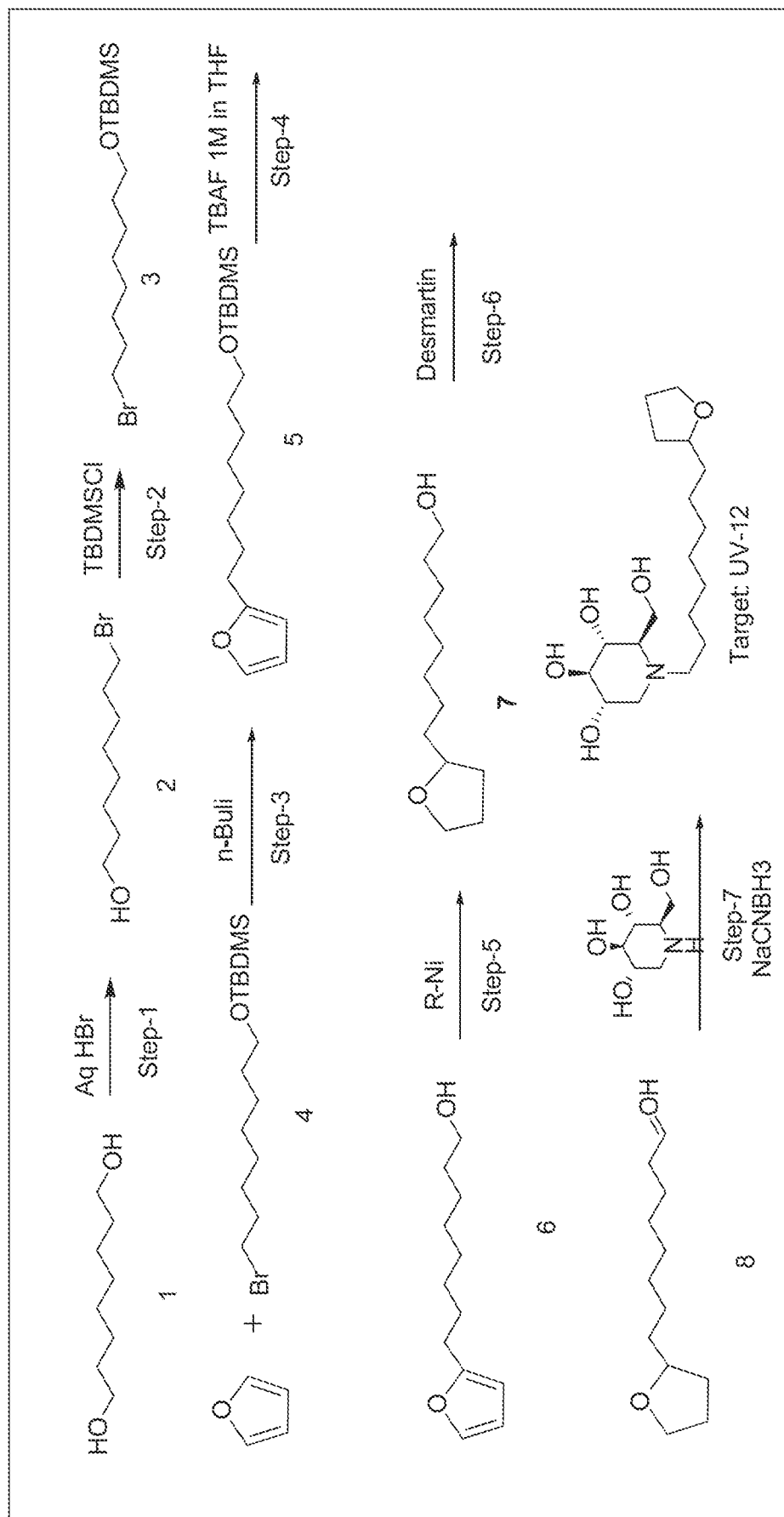
UV-12: C₁₈H₃₅NO₅ (Mol Wt 345.48)

Figure 19

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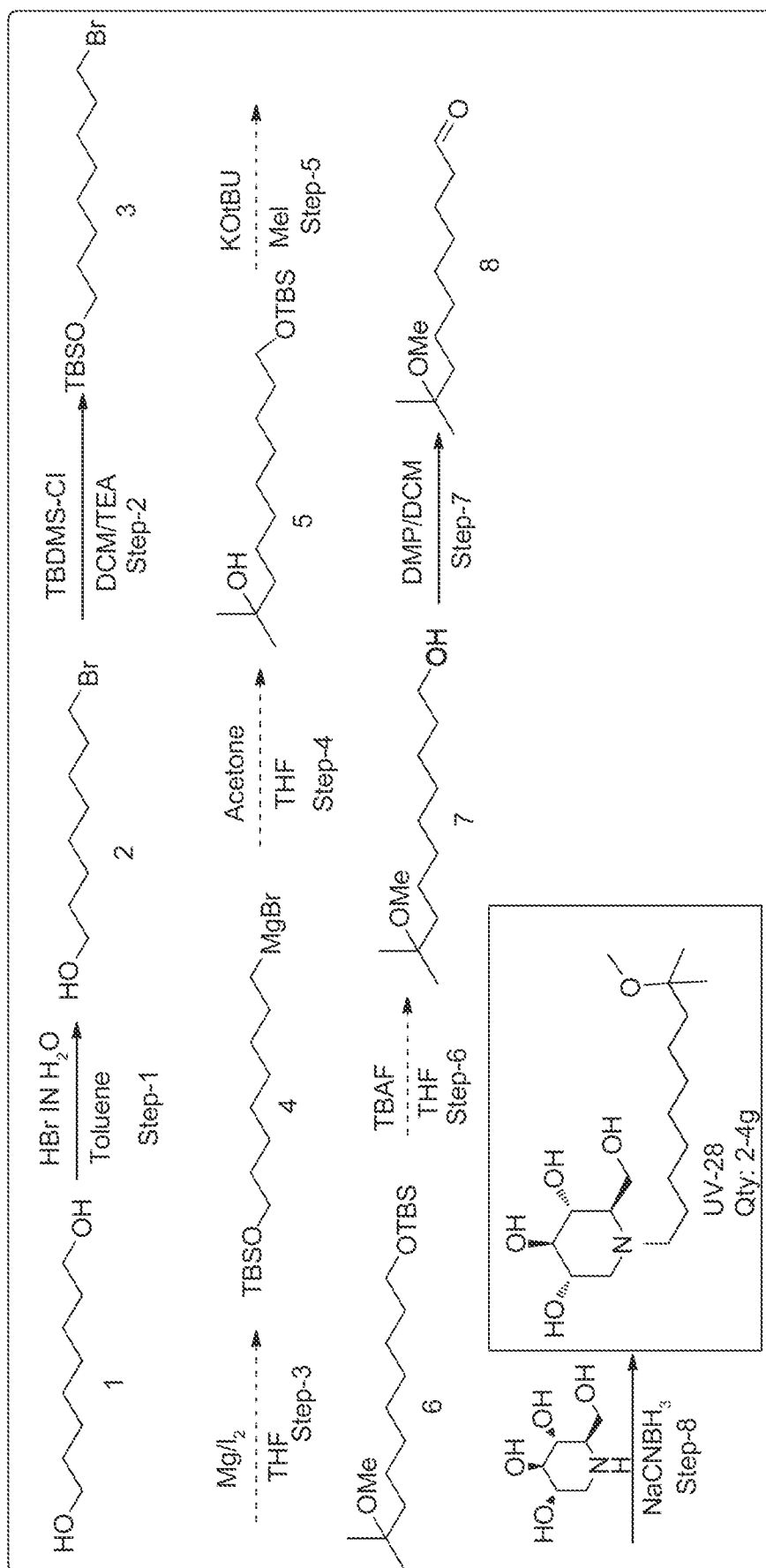
Synthesis of UV-28

Figure 20

Alternative scheme for UV-28

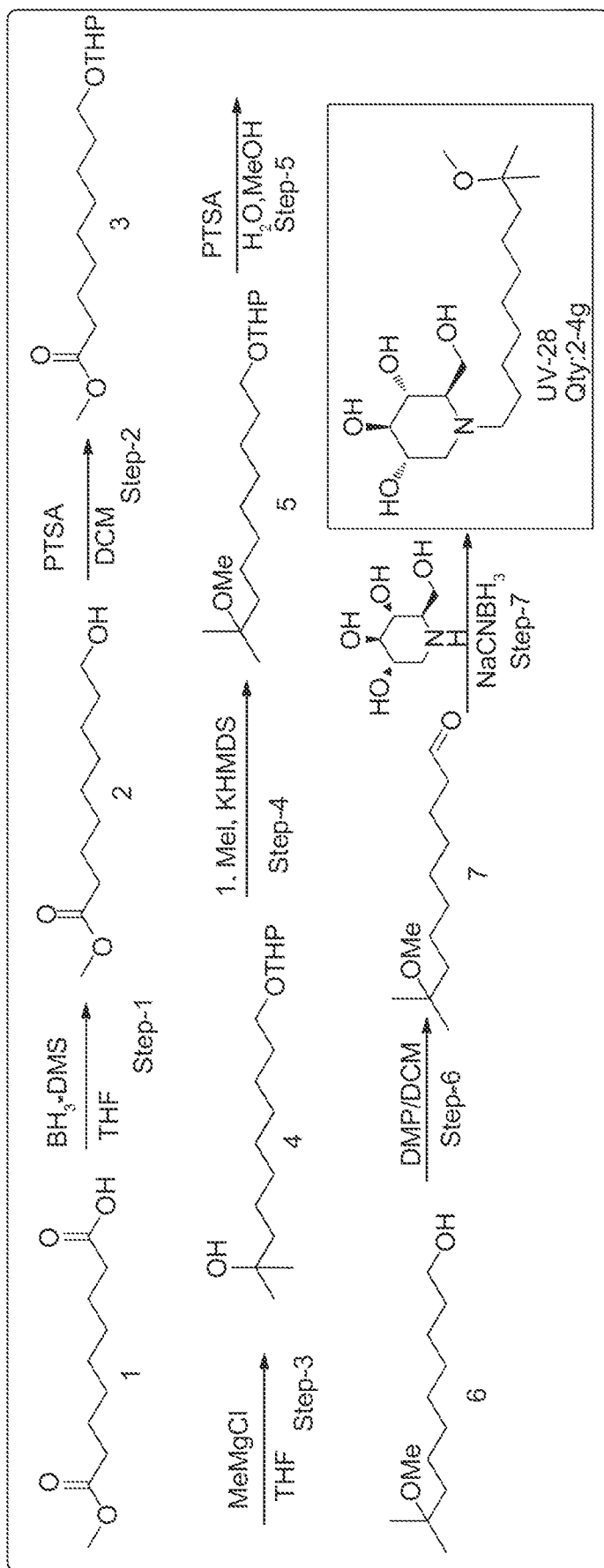


Figure 21