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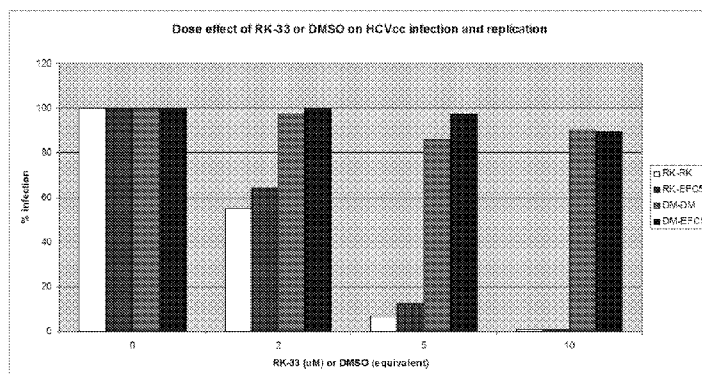
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FIGURE 3



(57) Abstract: In accordance with one or more embodiments, the present invention provides methods for treating a viral infection in a subject comprising administering to the subject a therapeutically effective amount of a fused benzodiazepine compound of Formulas I-V or a salt, solvate, stereoisomer or derivative thereof. In some embodiments, the viral infections are viruses from the family Flaviviridae, including Hepatitis C virus. Other embodiments include methods where the fused benzodiazepine compound of Formulas I-V are combined with other anti-viral agents. Methods for screening compounds for antiviral activity are also provided.

METHODS OF PREVENTION AND TREATMENT OF VIRAL DISEASE BY USE OF DDX3 INHIBITORS

REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/017,304, filed on June 26, 2014, which is hereby incorporated by reference for all purposes as if fully set forth herein.

BACKGROUND OF THE INVENTION

[0002] Hepatitis C virus (HCV) affects approximately 170 million people worldwide. Approximately 20-25% of patients with acute hepatitis C achieve spontaneous clearance of the virus but 75%-80% develop chronic infection. Approximately 20% of chronic hepatitis C patients develop cirrhosis and of these, 4% will develop hepatocellular carcinoma and 6% will develop end stage liver disease.

[0003] HCV is a small enveloped Flaviviridae family virus with a 9.6-kb single, positive-stranded RNA genome consisting of a 5' untranslated region (UTR), a large open reading frame encoding the virus-specific proteins, and a 3' UTR. The 5' UTR contains an internal ribosome entry site (IRES) that mediates translation of a single polyprotein of approximately 3000 amino acids.

[0004] The structural proteins of HCV are core and the envelope glycoproteins E1 and E2, while the non-structural proteins are p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B. HCV core is a highly conserved basic, RNA-binding protein that forms the viral nucleocapsid. It interacts specifically with the cellular DEAD-box RNA helicase DDX3. In mammalian cells, DDX3 is present throughout the cytoplasm and can also be found in the nucleus. Expression of HCV core in virus-infected cells results in redistribution of a proportion of the DDX3 to distinct cytoplasmic sites where it co-localizes with core. Furthermore, DDX3 is essential for virus replication in cultured cells, and this requirement is independent of its interaction with HCV core.

[0005] The exact cellular function of DDX3 has yet to be defined, but there is evidence for its involvement in splicing, translation initiation and repression, cell cycle regulation, nucleo-cytoplasmic RNA shuttling, interferon induction and apoptosis. Both up-regulation and down-regulation of DDX3 has been reported in various tumor tissues suggesting divergent roles of DDX3 in cancer-related pathogenesis.

[0006] The current standard of HCV therapy is IFN treatment with ribavirin, and more recently, protease inhibitors such as, for example, boceprevir, telaprevir, sofosbuvir, daclatasvir or ledipasvir. However, sustained viral response rates for infected individuals vary greatly from 30-100% depending on the therapy and the genotype of the HCV being treated.

[0007] Therefore, there exists a need for novel antiviral molecules which can prevent and/or inhibit viral infections.

SUMMARY OF THE INVENTION

[0008] In accordance with an embodiment, the present invention provides a method for treating a viral infection in a subject comprising administering to the subject a therapeutically effective amount of a compound of Formulas I-V or a salt, solvate, stereoisomer or derivative thereof.

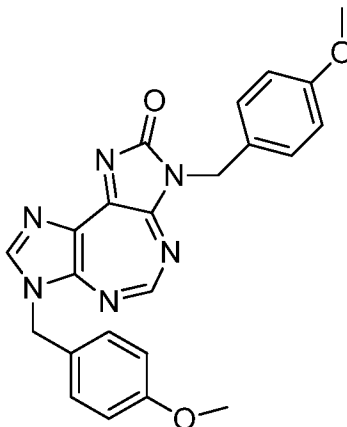
[0009] In accordance with another embodiment, the present invention provides a method for treating a Flaviviridae viral infection in a subject comprising administering to the subject a therapeutically effective amount of a compound of Formulas I-V or a salt, solvate, stereoisomer or derivative thereof.

[0010] In accordance with a further embodiment, the present invention provides a method for treating a HCV infection in a subject comprising administering to the subject a therapeutically effective amount of a compound of Formulas I-V or a salt, solvate, stereoisomer or derivative thereof.

[0011] In accordance with an embodiment, the present invention provides a method for treating a HCV infection in a subject comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a compound of Formulas I-V or a salt, solvate, stereoisomer or derivative thereof and a pharmaceutically acceptable carrier.

[0012] In accordance with another embodiment, the present invention provides a method for treating a HCV infection in a subject comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a compound of Formulas I-V or a salt, solvate, stereoisomer or derivative thereof, at least one additional anti-HCV compound and a pharmaceutically acceptable carrier.

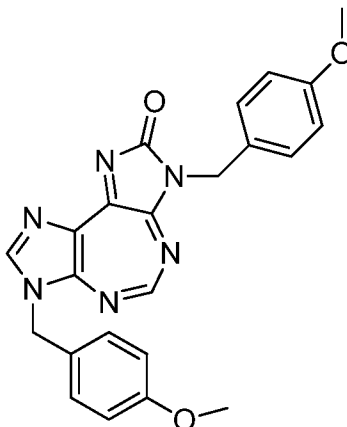
[0013] In accordance with an embodiment, the present invention provides a method for treating a HCV infection in a subject comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising RK-33:



RK-33

or a salt solvate or stereoisomer of derivative thereof, and a pharmaceutically acceptable carrier.

[0014] In accordance with an embodiment, the present invention provides a method for treating a HCV infection in a subject comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising RK-33:



RK-33

or a salt solvate or stereoisomer of derivative thereof, at least one additional anti-HCV compound and a pharmaceutically acceptable carrier.

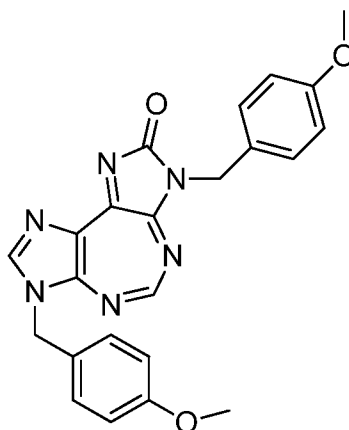
[0015] In accordance with an embodiment, the present invention provides a method of preventing or treating a viral infection susceptible to treatment by downregulation of DDX3 comprising administering to a subject in need thereof an effective amount of a composition or formulation comprising a compound that downregulates DDX3.

[0016] In accordance with another embodiment, the present invention provides a method of preventing or treating a viral infection susceptible to treatment by downregulation of DDX3 comprising administering to a subject in need thereof an effective amount of a composition or formulation comprising a compound of Formulas I-V or a salt, solvate, stereoisomer or derivative thereof.

[0017] In accordance with yet another embodiment, the present invention provides a method of preventing or treating a viral infection susceptible to treatment by downregulation of DDX3 comprising administering to a subject in need thereof an effective amount of a composition or formulation comprising a compound of Formulas I-V or a salt, solvate, stereoisomer or derivative thereof.

[0018] In accordance with a further embodiment, the present invention provides a method of preventing or treating a viral infection susceptible to treatment by downregulation of DDX3 comprising administering to a subject in need thereof an effective amount of a composition or formulation comprising a compound of Formulas I-V or a salt, solvate, stereoisomer or derivative thereof, at least one additional anti-HCV compound and a pharmaceutically acceptable carrier.

[0019] In accordance with still another embodiment, the present invention provides a method of preventing or treating a viral infection susceptible to treatment by downregulation of DDX3 comprising administering to a subject in need thereof an effective amount of a composition or formulation comprising RK-33:

**RK-33**

[0020] In accordance with an embodiment, the present invention provides a method for identifying a target compound which inhibits HCV infectivity comprising: a) providing a first and second human hepatoma cell line, wherein the first hepatoma cell line is infected with HCV JFH1 virus, and the second hepatoma cell line is a control; b) incubating the first and second cell lines with the target compound in a growth medium capable of sustaining growth of the hepatoma cell lines in culture for a specified period of time; c) comparing the amount of infectivity of the HCV JFH1 virus in the first and second hepatoma cell lines; and d) determining that the target compound inhibits HCV infectivity when the amount of infectivity in the first hepatoma cell line is significantly less than the amount of infectivity in the second hepatoma cell line.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] Figure 1 illustrates the effect of DDX3 knockdown on HCVcc infection. Huh7 cells were transduced or not (mock) with lentivirus vectors expressing shRNA to DDX3 (shDDX3), HCV (shHCV) or a scrambled sequence (shControl). At 2 days post-transduction, cells were infected with cell culture-derived JFH1 HCV (HCVcc). At 2 days post-infection, the virus released into the medium of infected cells was titrated on Huh7 cells to determine level of infectivity (presented as % infectivity relative to that of shControl-transduced cells). Western blot (left) shows the level of expressed SEAP in the experimental groups and control protein tubulin. Graph (right) shows % infectivity levels.

[0022] Figures 2A and 2B depict the determination of cell viability following incubation of RK-33 or DMSO with Huh-7 cells for either 3 or 96 hours respectively. Cell viability determined using the WST assay (Roche).

[0023] Figure 3 depicts the dose effect of RK-33 on HCVcc infection and replication. RK-RK = Huh7-J20 cells throughout the course of infection incubated in the presence of RK-33; RK-EFC5 = Huh7-J20 cells infected in the presence of RK-33 for 3 hours and then inoculum replaced with fresh medium lacking the drug; DM-DM = Huh7-J20 cells throughout the course of infection incubated in the presence of equivalent DMSO; and DM-EFC5 = Huh7-J20 cells infected in the presence of DMSO for 3 hours and then inoculum replaced with fresh medium lacking DMSO. Virus infectivity levels were determined by SEAP reporter assay, as described previously (Iro et al., 2009 Antiviral Research 83, 148-155). Briefly, Huh7J-20 cells were plated out 24 hours prior to infection at a density of 3×10^3 per well in a 96-well plate. Cells were infected with the virus in the presence of RK-33 or DMSO as described. At 3 hours post-infection, the inoculum was replaced with fresh medium and incubated for 72 hours (with or without drug as described). The virus infectivity levels were determined by measurement of the SEAP activity released into the medium.

[0024] Figure 4A shows the viability of Huh7-J20 cells passaged 2X in the presence of RK-33 or DMSO.

[0025] Figure 4B depicts the effect of RK-33 or DMSO on HCV infection of Huh7-J20 cells propagated in the presence of these drugs (as per Figure 4A). R-cells = Cells that have been passaged 2X in the presence of 2 μ M RK-33; D-cells = Cells that have been passaged 2X in the presence of equivalent DMSO; These cells were seeded into dishes, infected or not, and then tested for cell viability following incubation in the presence of RK-33 or DMSO (top panel, Figure 4A) 96 hours post-incubation. At the same time the levels of virus infectivity were determined (bottom panel, Figure 4B) 96 hours post-infection as described in the legend to Figure 3 above. Supplemented = 2 μ M of RK-33 (or equivalent DMSO) added to medium every 24 hours.

DETAILED DESCRIPTION OF THE INVENTION

[0026] As described herein, the present inventors investigated whether the fused benzodiazepine, RK-33, had any effect on viral growth in cultured cells. Indeed,

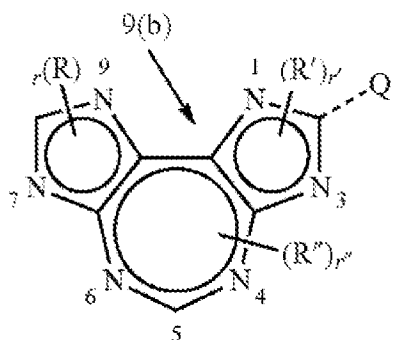
surprisingly, significant levels of inhibition (50%) of viral replication were observed when cells were infected with the flavivirus, HCV, in the presence of 2 μ M of RK-33, a concentration at which the cell viability was $\sim$ 95%. This novel inhibitory effect occurred regardless of whether the infected cells were incubated with the drug for 3 hours or 96 hours, or whether the cells had been passaged in the presence of 2 μ M of the drug or DMSO prior to infection.

[0027] These results indicate that a new class of small molecule drugs, the fused benzodiazepines, can be useful in the therapeutic development of novel drugs for the prevention and treatment of at least flaviviral infections, such as HCV, and other viruses such as Vaccinia, HIV, Norovirus, and HBV, and may also be useful in the treatment of its associated conditions such as cirrhosis and hepatocellular carcinoma. DDX3 is also involved in the life cycle of Dengue virus, another flavivirus, and the present invention may be useful in the prevention or treatment of Dengue viral infection, of which there is currently no known therapy.

[0028] In accordance with an embodiment, the present invention provides methods for treating a viral infection in a subject comprising administering to the subject a therapeutically effective amount of a compound of Formulas I-V or a salt, solvate, stereoisomer or derivative thereof.

[0029] The compounds of Formulas I-V and their exemplary embodiments are described in PCT/US2009/005273 and are incorporated herein by reference.

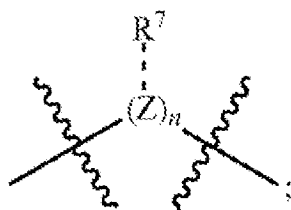
[0030] As used herein, in one or more embodiments, the invention encompasses compositions and formulations for use in the treatment of, or prevention of a viral infection in a subject comprising a compound of Formula (I):



(I);

or pharmaceutically acceptable salts and prodrugs thereof, wherein: R, R', and R'' are each independently a hydrogen, hydroxyl; substituted or unsubstituted: cyclic and acyclic alkyl group, cyclic and acyclic alkenyl group, cyclic and acyclic alkynyl group, aryl group, alkylaryl group, arylalkyl group, benzyl group, cyclic and acyclic heteroalkyl group, heteroaryl group; $-\text{C}(\text{O})\text{R}^3$; $-\text{C}(\text{S})\text{R}^3$; $-\text{S}(\text{O})\text{R}^3$; $-\text{S}(\text{O})_2\text{R}^3$; $-\text{C}(\text{O})\text{NR}^4\text{R}^5$; $-\text{C}(\text{S})\text{NR}^3\text{R}^4$; $-\text{C}(\text{X})\text{YR}^5\text{R}^6$; $-\beta\text{-D-ribose}$; $-\alpha\text{-D-ribose}$; $-\beta\text{-L-ribose}$; $-\alpha\text{-L-ribose}$; 2'-deoxy- $\beta\text{-D-ribose}$; 2'-deoxy- $\beta\text{-L-ribose}$; 2'-deoxy- $\alpha\text{-D-ribose}$; 2'-deoxy- $\alpha\text{-L-ribose}$; and ribose or deoxyribose sugars substituted with one or more halogens;

[0031] R, R', and R'' can also form a ring with one or more C, S, O, N atoms such that, for example, R and R' together include:



R^7 is a hydrogen; hydroxyl; substituted and unsubstituted: cyclic and acyclic alkyl group, group, alkenyl group, alkynyl group, aryl group, aryloxy group, alkylaryl group, arylalkyl group, heteroaryl group, heterocycloalkyl group; $-\text{C}(\text{O})\text{alkyl}$; $-\text{C}(\text{O})\text{alkenyl}$; $-\text{C}(\text{O})\text{alkynyl}$; $-\text{C}(\text{O})\text{aryl}$; $-\text{C}(\text{O})\text{benzyl}$; $-\text{C}(\text{O})\text{NR}^3\text{R}^4$; $-\text{C}(\text{S})\text{alkyl}$; $-\text{C}(\text{S})\text{alkenyl}$; $-\text{C}(\text{S})\text{alkynyl}$; $-\text{C}(\text{S})\text{aryl}$; $-\text{C}(\text{S})\text{benzyl}$; $-\text{C}(\text{S})\text{NR}^3\text{R}^4$; $-\text{C}(\text{X})\text{YR}^1\text{R}^2$; wherein

Q is O, NH, or S;

X is O, N, or S;

Y is O, CH_2 , NH, or S;

Z is CH, N, P, or C;

----- is a single bond or double bond; wherein if ----- is a double bond, R^2 or R^7 is independently O, S, or NH;

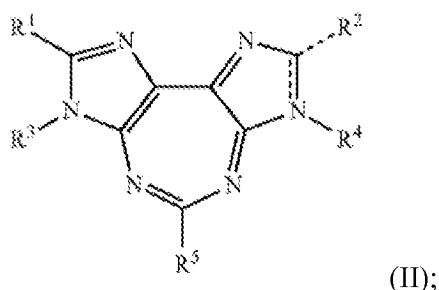
n is 1, 2, 3, or 4; and

r, r', and r'' are each independently an integer from 1 to about 3.

[0032] In certain illustrative embodiments, R, R', and R'' are each independently are a substituted benzyl, alkyl, aryl, cycloalkyl, heteroaryl, or heterocycloalkyl with one or more substituents, such as, but not limited to, $-\text{H}$, $-\text{F}$, $-\text{Cl}$, $-\text{Br}$, $-\text{I}$, $-\text{OH}$, azido, $-\text{SH}$,

alkyl, aryl, heteroalkyl, alkoxy, alkylthiol, amino, hydroxylamino, N-alkylamino, —N,N-dialkylamino, —N,N-dimethylamino, acyl, alkyloxycarbonyl, sulfonyl, urea, —NO₂, triazolyl.

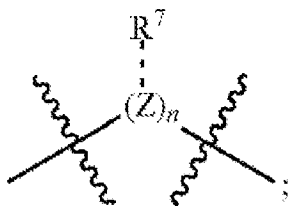
[0033] In another embodiment, the invention encompasses compositions and formulations for treatment of, or prevention of a viral infection in a subject comprising a compound of Formula (II):



or pharmaceutically acceptable salts and prodrugs thereof,

wherein

R¹, R², R³, R⁴, and R⁵ are each independently a hydrogen; hydroxyl; substituted or unsubstituted: cyclic and acyclic alkyl group, cyclic and acyclic alkenyl group, cyclic and acyclic alkynyl group, aryl group, alkylaryl group, arylalkyl group, benzyl group, cyclic and acyclic heteroalkyl group, heteroaryl group; —C(O)R³; —C(S)R³; —S(O)R³; —S(O)₂R³; —C(O)NR⁴R⁵; —C(S)NR³R⁴; —C(X)YR⁵R⁶; -β-D-ribosyl; -α-D-ribosyl; -β-L-ribosyl; -α-L-ribosyl; 2'-deoxy-β-D-ribosyl; 2'-deoxy-α-D-ribosyl; 2'-deoxy-α-L-ribosyl; and ribose or deoxyribose sugars substituted with one or more halogens; R¹ and R³ or R² and R⁴ can also form a ring with one or more C, S, O, N atoms such that R¹ and R³ or R² and R⁴ together include:



R⁷ is a hydrogen; hydroxyl; substituted and unsubstituted: cyclic and acyclic alkyl group, group, alkenyl group, alkynyl group, aryl group, aryloxy group, alkylaryl group, arylalkyl group, heteroaryl group, heterocycloalkyl group; —C(O)alkyl; —C(O)alkenyl; —

C(O)alkynyl; —C(O)aryl; —C(O)benzyl; —C(O)NR³R⁴; —C(S)alkyl; —C(S)alkenyl; —C(S)alkynyl; —C(S)aryl; —C(S)benzyl; —C(S)NR³R⁴; —C(X)YR¹R²;

wherein

X is O, N, or S;

Y is O, CH₂, NH, or S;

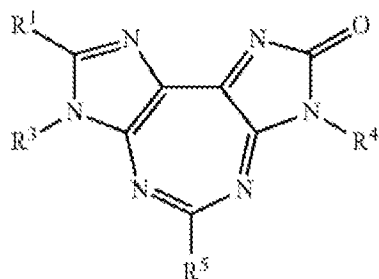
Z is CH, N, P, or C;

----- is a single bond or double bond; wherein if ----- is a double bond, R² or R⁷ is independently O, S, or NH;

n is 1, 2, 3, or 4.

[0034] In certain illustrative embodiments, R¹, R², R³, R⁴, and R⁵ are each independently a substituted benzyl, alkyl, aryl, cycloalkyl, heteroaryl, or heterocycloalkyl with one or more substituents, such as, but not limited to, —H, —F, —Cl, —Br, —I, —OH, azido, —SH, alkyl, aryl, heteroalkyl, alkoxy, alkylthiol, amino, hydroxylamino, N-alkylamino, —N,N-dialkylamino, —N,N-dimethylamino, acyl, alkyloxycarbonyl, sulfonyl, urea, —NO₂, triazolyl.

[0035] In another embodiment, the invention encompasses compositions and formulations for treatment of, or prevention of a viral infection in a subject comprising a compound of Formula (III):



(III);

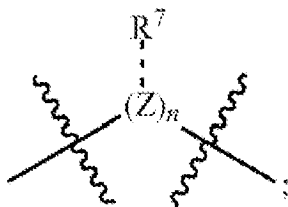
or pharmaceutically acceptable salts and prodrugs thereof,

wherein

R¹, R³, R⁴, and R⁵ are each independently a hydrogen; hydroxyl; substituted or unsubstituted: cyclic and acyclic alkyl group, cyclic and acyclic alkenyl group, cyclic and acyclic alkynyl group, aryl group, alkylaryl group, arylalkyl group, benzyl group, cyclic and acyclic heteroalkyl group, heteroaryl group; —C(O)R³; —C(S)R³; —S(O)R³; —S(O)₂R³; —C(O)NR⁴R⁵; —C(S)NR³R⁴; —C(X)YR⁵R⁶; -β-D-ribose; -α-D-ribose; -β-L-ribose; -α-L-

ribosyl; 2'-deoxy- β -D-ribosyl; 2'-deoxy- β -L-ribosyl; 2'-deoxy- α -D-ribosyl; 2'-deoxy- α -L-ribosyl; and ribose or deoxyribose sugars substituted with one or more halogens;

R^1 and R^3 can also form a ring with one or more C, S, O, N atoms such that R^1 and R^3 together include:



R^7 is a hydrogen; hydroxyl; substituted and unsubstituted: cyclic and acyclic alkyl group, group, alkenyl group, alkynyl group, aryl group, aryloxy group, alkylary group, aryalkyl group, heteroaryl group, heterocycloalkyl group; $—C(O)alkyl$; $—C(O)alkenyl$; $—C(O)alkynyl$; $—C(O)aryl$; $—C(O)benzyl$; $—C(O)NR^3R^4$; $—C(S)alkyl$; $—C(S)alkenyl$; $—C(S)alkynyl$; $—C(S)aryl$; $—C(S)benzyl$; $—C(S)NR^3R^4$; $—C(X)YR^1R^2$;

wherein

X is O, N, or S;

Y is O, CH_2 , NH, or S;

Z is CH, N, P, or C;

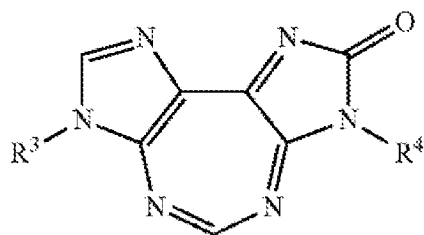
----- is a single bond or double bond; wherein if ----- is a double bond, R^2 or R^7 is independently O, S, or NH;

n is 1, 2, 3, or 4.

[0036] In certain illustrative embodiments, R^1 , R^3 , R^4 , and R^5 are not all hydrogen.

[0037] In certain illustrative embodiments, R^1 , R^3 , R^4 , and R^5 are each independently are a substituted benzyl, alkyl, aryl, cycloalkyl, heteroaryl, or heterocycloalkyl with one or more substituents, such as, but not limited to, $—H$, $—F$, $—Cl$, $—Br$, $—I$, $—OH$, azido, $—SH$, alkyl, aryl, heteroalkyl, alkoxy, alkylthiol, amino, hydroxylamino, N-alkylamino, $—N,N$ -dialkylamino, $—N,N$ -dimethylamino, acyl, alkyloxycarbonyl, sulfonyl, urea, $—NO_2$, triazolyl.

[0038] In another embodiment, the invention encompasses compositions and formulations for treatment of, or prevention of a viral infection in a subject comprising a compound of Formula (IV):



(IV);

or pharmaceutically acceptable salts and prodrugs thereof,

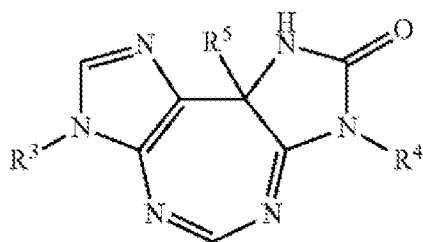
wherein

R³ and R⁴ are each independently a hydrogen; hydroxyl; substituted or unsubstituted: cyclic and acyclic alkyl group, cyclic and acyclic alkenyl group, cyclic and acyclic alkynyl group, aryl group, alkylaryl group, arylalkyl group, benzyl group, cyclic and acyclic heteroalkyl group, heteroaryl group; —C(O)R³; —C(S)R³; —S(O)R³; —S(O)₂R³; —C(O)NR⁴R⁵; —C(S)NR³R⁴; —C(X)YR⁵R⁶; -α-D-ribose; -β-L-ribose; -α-L-ribose; 2'-deoxy-β-D-ribose; 2'-deoxy-β-L-ribose; 2'-deoxy-α-D-ribose; 2'-deoxy-α-L-ribose; and ribose or deoxyribose sugars substituted with one or more halogens.

[0039] In certain illustrative embodiments, R³ and R⁴ are both not hydrogen.

[0040] In certain illustrative embodiments, R³ and R⁴ are each independently are a substituted benzyl, alkyl, aryl, cycloalkyl, heteroaryl, or heterocycloalkyl with one or more substituents, such as, but not limited to, —H, —F, —Cl, —Br, —I, —OH, azido, —SH, alkyl, aryl, heteroalkyl, alkoxy, alkylthiol, amino, hydroxylamino, N-alkylamino, —N,N-dialkylamino, —N,N-dimethylamino, acyl, alkyloxycarbonyl, sulfonyl, urea, —NO₂, triazolyl.

[0041] In another embodiment, the invention encompasses compositions and formulations for treatment of, or prevention of a viral infection in a subject comprising a compound of Formula (V):



(V);

or pharmaceutically acceptable salts and prodrugs thereof,

wherein

R^3 , R^4 , and R^5 are each independently a hydrogen; hydroxyl; substituted or unsubstituted: cyclic and acyclic alkyl group, cyclic and acyclic alkenyl group, cyclic and acyclic alkynyl group, aryl group, alkylaryl group, arylalkyl group, benzyl group, cyclic and acyclic heteroalkyl group, heteroaryl group; $-C(O)R^3$; $-C(S)R^3$; $-S(O)R^3$; $-S(O)_2R^3$; $-C(O)NR^4R^5$; $-C(S)NR^3R^4$; $-C(X)YR^5R^6$; $-\beta$ -D-ribosyl; $-\alpha$ -D-ribosyl; $-\beta$ -L-ribosyl; $-\alpha$ -L-ribosyl; 2'-deoxy- β -D-ribosyl; 2'-deoxy- β -L-ribosyl; 2'-deoxy- α -D-ribosyl; 2'-deoxy- α -L-ribosyl; and ribose or deoxyribose sugars substituted with one or more halogens.

[0042] In certain illustrative embodiments, R^3 , R^4 , and R^5 are not each hydrogen.

[0043] In certain illustrative embodiments, R^3 , R^4 , and R^5 are each independently are a substituted benzyl, alkyl, aryl, cycloalkyl, heteroaryl, or heterocycloalkyl with one or more substituents, such as, but not limited to, $-H$, $-F$, $-Cl$, $-Br$, $-I$, $-OH$, azido, $-SH$, alkyl, aryl, heteroalkyl, alkoxy, alkylthiol, amino, hydroxylamino, N-alkylamino, $-N,N$ -dialkylamino, $-N,N$ -dimethylamino, acyl, alkyloxycarbonyl, sulfonyl, urea, $-NO_2$, triazolyl.

[0044] As used herein and unless otherwise indicated, the term “alkoxy group” means an $-O$ -alkyl group, wherein alkyl is as defined herein. An alkoxy group can be unsubstituted or substituted with one or two suitable substituents. Preferably, the alkyl chain of an alkyloxy group is from 1 to 6 carbon atoms in length, referred to herein, for example, as “(C₁-C₁₀)alkoxy.”

[0045] As used herein and unless otherwise indicated, the term “alkenyl group” means a monovalent unbranched or branched hydrocarbon chain having one or more double bonds therein. The double bond of an alkenyl group can be unconjugated or conjugated to another unsaturated group. Suitable alkenyl groups include, but are not limited to (C₂-C₈)alkenyl groups, such as vinyl, allyl, butenyl, pentenyl, hexenyl, butadienyl, pentadienyl, hexadienyl, 2-ethylhexenyl, 2-propyl-2-butenyl, 4-(2-methyl-3-butene)-pentenyl. An alkenyl group can be unsubstituted or substituted with one or two suitable substituents.

[0046] As used herein and unless otherwise indicated, the term “alkylalkoxy” or “alkyloxyalkyl group” means a saturated, monovalent unbranched or branched hydrocarbon chain covalently bonded to an oxygen and covalently bonded to a second a saturated, monovalent unbranched or branched hydrocarbon chain (e.g., -alkyl-O-alkyl).

[0047] As used herein and unless otherwise indicated, the term “alkyl” or “alkyl group” means a substituted or unsubstituted, saturated, monovalent unbranched or branched hydrocarbon chain. Examples of alkyl groups include, but are not limited to, (C₁-C₁₀)alkyl groups, such as methyl, ethyl, propyl, isopropyl, 2-methyl-1-propyl, 2-methyl-2-propyl, 2-methyl-1-butyl, 3-methyl-1-butyl, 2-methyl-3-butyl, 2,2-dimethyl-1-propyl, 2-methyl-1-pentyl, 3-methyl-1-pentyl, 4-methyl-1-pentyl, 2-methyl-2-pentyl, 3-methyl-2-pentyl, 4-methyl-2-pentyl, 2,2-dimethyl-1-butyl, 3,3-dimethyl-1-butyl, 2-ethyl-1-butyl, butyl, isobutyl, t-butyl, pentyl, isopentyl, neopentyl, and hexyl, and longer alkyl groups, such as heptyl, and octyl. An alkyl group can be unsubstituted or substituted with one or two suitable substituents.

[0048] As used herein and unless otherwise indicated, the term “alkynyl group” means monovalent unbranched or branched hydrocarbon chain having one or more triple bonds therein. The triple bond of an alkynyl group can be unconjugated or conjugated to another unsaturated group. Suitable alkynyl groups include, but are not limited to, (C₂-C₆) alkynyl groups, such as ethynyl, propynyl, butynyl, pentynyl, hexynyl, methylpropynyl, 4-methyl-1-butynyl, 4-propyl-2-pentynyl, and 4-butyl-2-hexynyl. An alkynyl group can be unsubstituted or substituted with one or two suitable substituents.

[0049] As used herein and unless otherwise indicated, the term “aryl group” means a monocyclic or polycyclic-aromatic radical comprising carbon and hydrogen atoms. Examples of suitable aryl groups include, but are not limited to, phenyl, tolyl, anthacenyl, fluorenyl, indenyl, azulenyl, and naphthyl, as well as benzo-fused carbocyclic moieties such as 5,6,7,8-tetrahydronaphthyl. An aryl group can be unsubstituted or substituted with one or two suitable substituents. Preferably, the aryl group is a monocyclic ring, wherein the ring comprises 6 carbon atoms, referred to herein as “(C₆) aryl.”

[0050] As used herein and unless otherwise indicated, the term “aryloxy group” means an —O-aryl group, wherein aryl is as defined herein. An aryloxy group can be unsubstituted or substituted with one or two suitable substituents. Preferably, the aryl ring of an aryloxy group is a monocyclic ring, wherein the ring comprises 6 carbon atoms, referred to herein as “(C₆) aryloxy.”

[0051] As used herein, the term “benzyl” means —CH₂-phenyl.

[0052] As used herein, the term “carbonyl” group is a divalent group of the formula —C(O)—.

[0053] As used herein and unless otherwise indicated, the term “compounds of the invention” means, collectively, the compounds of formulas I, II, III, IV, and V and pharmaceutically acceptable salts thereof as well as compounds depicted herein including Compounds 3, 11, 12, 13, 14, 21, 23, 25, 27, and 101-193, as described in PCT/US2009/005273, and are incorporated herein by reference. The compounds of the invention are identified herein by their chemical structure and/or chemical name. Where a compound is referred to by both a chemical structure and a chemical name, and that chemical structure and chemical name conflict, the chemical structure is determinative of the compound's identity. The compounds of the invention may contain one or more chiral centers and/or double bonds and, therefore, exist as stereoisomers, such as double-bond isomers (i.e., geometric isomers), enantiomers, or diastereomers. According to the invention, the chemical structures depicted herein, and therefore the compounds of the invention, encompass all of the corresponding compound's enantiomers and stereoisomers, that is, both the stereomerically pure form (e.g., geometrically pure, enantiomerically pure, or diastereomerically pure) and enantiomeric and stereoisomeric mixtures. Enantiomeric and stereoisomeric mixtures can be resolved into their component enantiomers or stereoisomers by well-known methods, such as chiral-phase gas chromatography, chiral-phase high performance liquid chromatography, crystallizing the compound as a chiral salt complex, or crystallizing the compound in a chiral solvent. Enantiomers and stereoisomers can also be obtained from stereomerically- or enantiomerically-pure intermediates, reagents, and catalysts by well-known asymmetric synthetic methods.

[0054] As used herein and unless otherwise indicated, the term “cyclic alkyl” and “cycloalkyl group” are used synonymously and each means a monocyclic or polycyclic saturated ring comprising carbon and hydrogen atoms and having no carbon-carbon multiple bonds. Examples of cycloalkyl groups include, but are not limited to, (C₃-C₇)cycloalkyl groups, such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cycloheptyl, and saturated cyclic and bicyclic terpenes. A cycloalkyl group can be unsubstituted or substituted by one or two suitable substituents. Preferably, the cycloalkyl group is a monocyclic ring or bicyclic ring.

[0055] As used herein and unless otherwise indicated, the term “halogen” means fluorine, chlorine, bromine, or iodine. Correspondingly, the meaning of the terms “halo” and “Hal” encompass fluoro, chloro, bromo, and iodo.

[0056] As used-herein and unless otherwise indicated, the term “formulation” refers to a composition comprising a compound of the invention that is described in a particular dosage form (e.g., tablet) or with a particular dosage amount (e.g., 30 mg/kg).

[0057] As used herein and unless otherwise indicated, the term “heteroaryl group” means a monocyclic- or polycyclic aromatic ring comprising carbon atoms, hydrogen atoms, and one or more heteroatoms, preferably 1 to 3 heteroatoms, independently selected from nitrogen, oxygen, and sulfur. Illustrative examples of heteroaryl groups include, but are not limited to, pyridinyl, pyridazinyl, pyrimidyl, pyrazyl, triazinyl, pyrrolyl, pyrazolyl, imidazolyl, (1,2,3)- and (1,2,4)-triazolyl, pyrazinyl, pyrimidinyl, tetrazolyl, furyl, thienyl, isoxazolyl, thiazolyl, furyl, phenyl, isoxazolyl, and oxazolyl. A heteroaryl group can be unsubstituted or substituted with one or two suitable substituents. Preferably, a heteroaryl group is a monocyclic ring, wherein the ring comprises 2 to 5 carbon atoms and 1 to 3 heteroatoms, referred to herein as “(C₂-C₅) heteroaryl.”

[0058] As used herein and unless otherwise indicated, the term “heterocycloalkyl group” means a monocyclic or polycyclic ring comprising carbon and hydrogen atoms and at least one heteroatom, preferably, 1 to 3 heteroatoms selected from nitrogen, oxygen, and sulfur, and having no unsaturation. Examples of heterocycloalkyl groups include pyrrolidinyl, pyrrolidino, piperidinyl, piperidino, piperazinyl, piperazino, morpholinyl, morpholino, thiomorpholinyl, thiomorpholino, and pyranlyl. A heterocycloalkyl group can be unsubstituted or substituted with one or two suitable substituents. Preferably, the heterocycloalkyl group is a monocyclic or bicyclic ring, more preferably, a monocyclic ring, wherein the ring comprises from 3 to 7 carbon atoms and from 1 to 3 heteroatoms, referred to herein as (C₁-C₇) heterocycloalkyl.

[0059] As used herein and unless otherwise indicated, the term “heterocyclic radical” or “heterocyclic ring” means a heterocycloalkyl group or a heteroaryl group.

[0060] As used herein and unless otherwise indicated, the term “hydrocarbyl group” means a monovalent group selected from (C₁-C₈) alkyl, (C₂-C₈) alkenyl, and (C₂-C₈) alkynyl, optionally substituted with one or two suitable substituents. Preferably, the hydrocarbon chain of a hydrocarbyl group is from 1 to 6 carbon atoms in length, referred to herein as “(C₁-C₆) hydrocarbyl.”

[0061] In accordance with an embodiment, the present invention provides the compounds of Formulas I-V, or a salt, solvate, stereoisomer, or derivative thereof, and a pharmaceutically acceptable carrier, for use as a medicament, preferably for use as a DDX3 inhibitor in a

mammalian cell or population of cells, more preferably for use as a treatment in a subject suffering from a viral infection, preferably a flaviviral infection, such as HCV.

[0062] Accordingly, included within the compounds and derivatives of the present invention are the tautomeric forms of the disclosed compounds, isomeric forms including enantiomers, stereoisomers, and diastereoisomers, and the pharmaceutically-acceptable salts thereof. The term "pharmaceutically acceptable salts" embraces salts commonly used to form alkali metal salts and to form addition salts of free acids or free bases, such as those used to improve water solubility. Examples of acids which may be employed to form pharmaceutically acceptable acid addition salts include such inorganic acids as hydrochloric acid, sulphuric acid and phosphoric acid, and such organic acids as maleic acid, succinic acid and citric acid. Other pharmaceutically acceptable salts include salts with alkali metals or alkaline earth metals, such as sodium, potassium, calcium and magnesium, or with organic bases, such as dicyclohexylamine. Suitable pharmaceutically acceptable salts of the compounds of the present invention include, for example, acid addition salts which may, for example, be formed by mixing a solution of the compound according to the invention with a solution of a pharmaceutically acceptable acid, such as hydrochloric acid, sulphuric acid, methanesulphonic acid, fumaric acid, maleic acid, succinic acid, acetic acid, benzoic acid, oxalic acid, citric acid, tartaric acid, carbonic acid or phosphoric acid. All of these salts may be prepared by conventional means by reacting, for example, the appropriate acid or base with the corresponding compounds of the present invention.

[0063] Salts formed from free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

[0064] For use in medicines, the salts of the compounds of the present invention should be pharmaceutically acceptable salts. Other salts may, however, be useful in the preparation of the compounds according to the invention or of their pharmaceutically acceptable salts.

[0065] In addition, embodiments of the invention include hydrates of the compounds of the present invention. The term "hydrate" includes but is not limited to hemihydrate, monohydrate, dihydrate, trihydrate and the like. Hydrates of the compounds of the present invention may be prepared by contacting the compounds with water under suitable conditions to produce the hydrate of choice.

[0066] As used herein, the term "treat," as well as words stemming therefrom, includes preventative as well as disorder remitative treatment. The terms "reduce," "suppress," "prevent," and "inhibit," as well as words stemming therefrom, have their commonly understood meaning of lessening or decreasing. These words do not necessarily imply 100% or complete treatment, reduction, suppression, or inhibition.

[0067] With respect to pharmaceutical compositions described herein for use in the present invention, the pharmaceutically acceptable carrier can be any of those conventionally used, and is limited only by physico-chemical considerations, such as solubility and lack of reactivity with the active compound(s), and by the route of administration. The pharmaceutically acceptable carriers described herein, for example, vehicles, adjuvants, excipients, and diluents, are well-known to those skilled in the art and are readily available to the public. Examples of the pharmaceutically acceptable carriers include soluble carriers such as known buffers which can be physiologically acceptable (e.g., phosphate buffer) as well as solid compositions such as solid-state carriers or latex beads. It is preferred that the pharmaceutically acceptable carrier be one which is chemically inert to the active agent(s), and one which has little or no detrimental side effects or toxicity under the conditions of use.

[0068] The carriers or diluents used herein may be solid carriers or diluents for solid formulations, liquid carriers or diluents for liquid formulations, or mixtures thereof.

[0069] Solid carriers or diluents include, but are not limited to, gums, starches (e.g., corn starch, pregelatinized starch), sugars (e.g., lactose, mannitol, sucrose, dextrose), cellulosic materials (e.g., microcrystalline cellulose), acrylates (e.g., polymethylacrylate), calcium carbonate, magnesium oxide, talc, or mixtures thereof.

[0070] For liquid formulations, pharmaceutically acceptable carriers may be, for example, aqueous or non-aqueous solutions, suspensions, emulsions or oils. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, and injectable organic esters such as ethyl oleate. Aqueous carriers include, for example, water, alcoholic/aqueous solutions, cyclodextrins, emulsions or suspensions, including saline and buffered media.

[0071] Examples of oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, mineral oil, olive oil, sunflower oil, fish-liver oil, sesame oil, cottonseed oil, corn oil, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral formulations include, for example, oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters.

[0072] Parenteral vehicles (for subcutaneous, intravenous, intraarterial, or intramuscular injection) include, for example, sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's and fixed oils. Formulations suitable for parenteral administration include, for example, aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives.

[0073] Intravenous vehicles include, for example, fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Examples are sterile liquids such as water and oils, with or without the addition of a surfactant and other pharmaceutically acceptable adjuvants. In general, water, saline, aqueous dextrose and related sugar solutions, and glycols such as propylene glycols or polyethylene glycol are preferred liquid carriers, particularly for injectable solutions

[0074] In addition, in an embodiment, the compounds used in the methods of the present invention may further comprise, for example, binders (e.g., acacia, cornstarch, gelatin, carbomer, ethyl cellulose, guar gum, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, povidone), disintegrating agents (e.g., cornstarch, potato starch, alginic acid, silicon dioxide, croscarmellose sodium, crospovidone, guar gum, sodium starch glycolate), buffers (e.g., Tris-HCl, acetate, phosphate) of various pH and ionic strength, additives such as albumin or gelatin to prevent absorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile acid salts), protease inhibitors, surfactants (e.g. sodium lauryl sulfate), permeation enhancers, solubilizing agents (e.g., cremophor, glycerol, polyethylene glycerol, benzalkonium chloride, benzyl benzoate, cyclodextrins, sorbitan esters, stearic acids), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite, butylated hydroxyanisole), stabilizers (e.g., hydroxypropyl cellulose, hydroxypropylmethyl cellulose), viscosity increasing agents (e.g., carbomer, colloidal silicon dioxide, ethyl cellulose, guar gum), sweeteners (e.g., aspartame, citric acid), preservatives (e.g., thimerosal, benzyl alcohol, parabens), lubricants (e.g., stearic acid, magnesium stearate, polyethylene glycol, sodium lauryl sulfate), flow-aids (e.g., colloidal silicon dioxide), plasticizers (e.g., diethyl phthalate, triethyl citrate), emulsifiers (e.g., carbomer, hydroxypropyl cellulose, sodium lauryl sulfate), polymer coatings (e.g., poloxamers or poloxamines), coating and film forming agents (e.g., ethyl cellulose, acrylates, polymethacrylates), and/or adjuvants.

[0075] The choice of carrier will be determined, in part, by the particular compound, as well as by the particular method used to administer the compound. Accordingly, there are a variety of suitable formulations of the pharmaceutical composition of the invention. The following formulations for parenteral, subcutaneous, intravenous, intramuscular, intraarterial, intrathecal and interperitoneal administration are exemplary, and are in no way limiting. More than one route can be used to administer the compounds, and in certain instances, a particular route can provide a more immediate and more effective response than another route.

[0076] Suitable soaps for use in parenteral formulations include, for example, fatty alkali metal, ammonium, and triethanolamine salts, and suitable detergents include, for example, (a) cationic detergents such as, for example, dimethyl dialkyl ammonium halides, and alkyl pyridinium halides, (b) anionic detergents such as, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c) nonionic detergents such as, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylenepolypropylene copolymers, (d) amphoteric detergents such as, for example, alkyl- β -aminopropionates, and 2-alkyl-imidazoline quaternary ammonium salts, and (e) mixtures thereof.

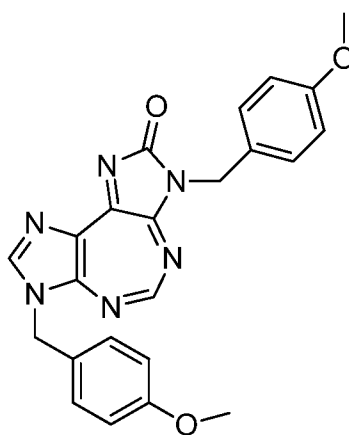
[0077] The parenteral formulations will typically contain from about 0.5% to about 25% by weight of the compounds in solution. Preservatives and buffers may be used. In order to minimize or eliminate irritation at the site of injection, such compositions may contain one or more nonionic surfactants, for example, having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulations will typically range from about 5% to about 15% by weight. Suitable surfactants include, for example, polyethylene glycol sorbitan fatty acid esters, such as sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol.

[0078] The parenteral formulations can be presented in unit-dose or multi-dose sealed containers, such as ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets.

[0079] Injectable formulations are in accordance with the invention. The requirements for effective pharmaceutical carriers for injectable compositions are well-known to those of

ordinary skill in the art (see, e.g., *Pharmaceutics and Pharmacy Practice*, J.B. Lippincott Company, Philadelphia, PA, Banker and Chalmers, eds., pages 238-250 (1982), and *ASHP Handbook on Injectable Drugs*, Trissel, 15th ed., pages 622-630 (2009)).

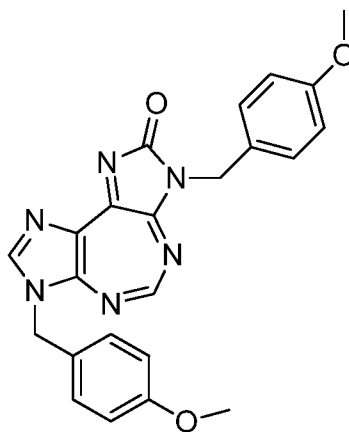
[0080] In accordance with an embodiment, the present invention provides a method for treating or preventing a HCV infection in a subject comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising RK-33 (compound 3):



RK-33

or a salt solvate or stereoisomer of derivative thereof, and a pharmaceutically acceptable carrier.

[0081] In accordance with an embodiment, the present invention the use of a pharmaceutical composition comprising RK-33 (compound 3):



RK-33

or a salt solvate or stereoisomer of derivative thereof, and a pharmaceutically acceptable carrier, in a therapeutically effective amount, for treatment of, or prevention of a HCV infection in a subject.

[0082] HCV is a member of the Flaviviridae family of viruses with a single-stranded positive-sense RNA genome. Following infection of host cells, the 9.6 Kb genome is translated into a polyprotein precursor of approximately 3,000 amino acids (reviewed in Lindenbach, B. D. and Rice, C. M. *Nature*. 2005, 436:933-938; Moradpour, D, Penin, F., and Rice, C. M. *Nature Reviews*. 2007, 5:453-463). Other examples of flaviviruses include, for example, Dengue virus, deer tick virus, tick borne encephalitis virus, Japanese encephalitis virus, West Nile virus, and Yellow fever virus.

[0083] In an embodiment, the term "administering" means that the compounds of the present invention are introduced into a subject, preferably a subject receiving treatment for a proliferative disease, and the compounds are allowed to come in contact with the one or more disease related cells or population of cells *in vivo*.

[0084] As defined herein, in another embodiment, the term "contacting" means that the one or more compounds of the present invention are introduced into a sample having at least one cancer cell and appropriate enzymes or reagents, in a test tube, flask, tissue culture, chip, array, plate, microplate, capillary, or the like, and incubated at a temperature and time sufficient to permit binding and uptake of the at least one compound to the cancer cell. Methods for contacting the samples with the compounds, and other specific binding components are known to those skilled in the art, and may be selected depending on the type of assay protocol to be run. Incubation methods are also standard and are known to those skilled in the art.

[0085] As used herein, the term "anti-HCV compound" means a compound having anti-HCV activity. Examples of such compounds include, for example, interferons, such as interferon alpha 2B, pegylated interferon alpha, consensus interferon, interferon alpha 2A, and lymphoblastoid interferon tau; cyclosporines, such as cyclosporine A; interleukin 2, interleukin 6, interleukin 12, interfering RNA, anti-sense RNA, Imiquimod, ribavirin, an inosine 5'-monophosphate dehydrogenase inhibitor, amantadine, rimantadine, ribavirin; and HCV protease inhibitors such as boceprevir, telaprevir, sofosbuvir, daclatasvir or ledipasvir.

[0086] As used herein, the term "subject" refers to any mammal, including, but not limited to, mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits. It is preferred that the mammals are from the order

Carnivora, including Felines (cats) and Canines (dogs). It is more preferred that the mammals are from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perssodactyla, including Equines (horses). It is most preferred that the mammals are of the order Primates, Ceboids, or Simoids (monkeys) or of the order Anthropoids (humans and apes). An especially preferred mammal is the human.

[0087] In accordance with yet another embodiment, the present invention provides a method of preventing or treating a viral infection susceptible to treatment by downregulation of DDX3 comprising administering to a subject in need thereof an effective amount of a composition or formulation comprising a compound of Formulas I-V or a salt, solvate, stereoisomer of derivative thereof.

[0088] Typically, an attending physician will decide the dosage of the compositions described herein with which to treat each individual subject, taking into consideration a variety of factors, such as age, body weight, general health, diet, sex, compound to be administered, route of administration, and the severity of the condition being treated. By way of example, and not intending to limit the invention, the dose of the compositions of the present invention can be about 0.001 to about 1000 mg/kg body weight of the subject being treated, from about 0.01 to about 100 mg/kg body weight, from about 0.1 mg/kg to about 10 mg/kg, and from about 0.5 mg to about 5 mg/kg body weight. In another embodiment, the dose of the compositions of the present invention can be at a concentration from about 1 nM to about 100 mM, preferably from about 100 nM to about 50 μ M, more preferably from about 1 μ M to about 25 μ M.

[0089] In accordance with an embodiment, the present invention provides a method for identifying a target compound which inhibits HCV infectivity comprising: a) providing a first and second human hepatoma cell line, wherein the first hepatoma cell line is infected with HCV JFH1 virus, and the second hepatoma cell line is a control; b) incubating the first and second cell lines with the target compound in a growth medium capable of sustaining growth of the hepatoma cell lines in culture for a specified period of time; c) comparing the amount of infectivity of the HCV JFH1 virus in the first and second hepatoma cell lines; and d) determining that the target compound inhibits HCV infectivity when the amount of infectivity in the first hepatoma cell line is significantly less than the amount of infectivity in the second hepatoma cell line. It will be understood by those of ordinary skill in the art that other hepatic cell lines, such as HepG2 and others can be used with the inventive methods.

[0090] In some embodiments, the compounds of the present invention can be tested for their anti-viral effect using methods known in the art. In an embodiment, human liver cell lines, such as Huh-7 can be grown in culture using methods known in the art. The cells are then divided into experimental and control groups where the compounds of the present invention are introduced into the growth media, and then after specific periods of time, the cells are then exposed to HCV viral infection. In some embodiments, the HCV virus used is the JFH1 subtype of HCV. After a period of exposure to the virus, the media is changed and after another period of time, the number of infected cells can be compared between experimental and control groups. A lowering of the number of infected cells in the experimental group compared to controls indicates that the compound or target molecule inhibits viral infection.

EXAMPLES

[0091] Cell lines: Human hepatoma Huh-7 cells (Nakabayashi et al., 1982. Growth of human hepatoma cell lines with differentiated functions in chemically defined medium. Cancer Res. 42, 3858–3863) and the secreted alkaline phosphatase (SEAP) reporter Huh7-J20 cells (Iro et al., 2009 Antiviral Research 83; 148-155) were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 5% nonessential amino acids, and 200 mM L-glutamine (Invitrogen). The Huh7-J20 cell line stably expresses the enhanced green fluorescent protein fused in frame to SEAP via a recognition sequence of the viral NS3/4A serine protease. During HCV replication, the SEAP reporter is released from the fusion protein and secreted into the extracellular culture medium following cleavage by NS3/4A. The level of SEAP activity in the culture medium directly correlates with the level of intracellular viral RNA replication (Iro et al., 2009).

[0092] Infectivity: HCVcc were generated essentially as described previously (Wakita et al., 2005 Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. Nat. Med. 11, 791–796). Briefly, linearized plasmids carrying the full-length HCV genomic cDNA were used as a template to generate viral genomic RNA by in vitro transcription. Ten microgram of this RNA was electroporated into Huh7 cells. Culture medium of electroporated cells was harvested 4 days post-transfection, filtered through a 0.45 µm-pore membrane, and used as virus stock for infection assays. The effect of drug on virus infectivity was tested as described in the figure legends. Cell viability was determined using the WST assay kit (Roche) as per the manufacturers' instructions.

[0093] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

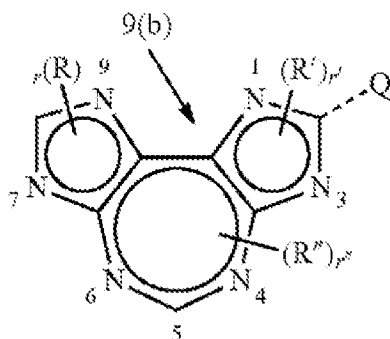
[0094] The use of the terms “a” and “an” and “the” and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to,”) unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0095] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

Claims:

1. Use of a compound of Formula I:

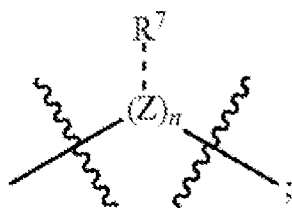
Formula (I):



(I);

or pharmaceutically acceptable salts and prodrugs thereof, wherein: R, R', and R'' are each independently a hydrogen, hydroxyl; substituted or unsubstituted: cyclic and acyclic alkyl group, cyclic and acyclic alkenyl group, cyclic and acyclic alkynyl group, aryl group, alkylaryl group, arylalkyl group, benzyl group, cyclic and acyclic heteroalkyl group, heteroaryl group; $-\text{C}(\text{O})\text{R}^3$; $-\text{C}(\text{S})\text{R}^3$; $-\text{S}(\text{O})\text{R}^3$; $-\text{S}(\text{O})_2\text{R}^3$; $-\text{C}(\text{O})\text{NR}^4\text{R}^5$; $-\text{C}(\text{S})\text{NR}^3\text{R}^4$; $-\text{C}(\text{X})\text{YR}^5\text{R}^6$; $-\beta\text{-D-ribose}$; $-\alpha\text{-D-ribose}$; $-\beta\text{-L-ribose}$; $-\alpha\text{-L-ribose}$; $2'\text{-deoxy-}\beta\text{-D-ribose}$; $2'\text{-deoxy-}\beta\text{-L-ribose}$; $2'\text{-deoxy-}\alpha\text{-D-ribose}$; $2'\text{-deoxy-}\alpha\text{-L-ribose}$; and ribose or deoxyribose sugars substituted with one or more halogens;

alternatively, R, R', and R'' can form a ring with one or more C, S, O, N atoms such that, for example, R and R' together include:



R⁷ is a hydrogen; hydroxyl; substituted and unsubstituted: cyclic and acyclic alkyl group, group, alkenyl group, alkynyl group, aryl group, aryloxy group, alkylaryl group,

aryalkyl group, heteroaryl group, heterocycloalkyl group; —C(O)alkyl; —C(O)alkenyl; —C(O)alkynyl; —C(O)aryl; —C(O)benzyl; —C(O)NR³R⁴; —C(S)alkyl; —C(S)alkenyl; —C(S)alkynyl; —C(S)aryl; —C(S)benzyl; —C(S)NR³R⁴; —C(X)YR¹R²;

wherein

Q is O, NH, or S;

X is O, N, or S;

Y is O, CH₂, NH, or S;

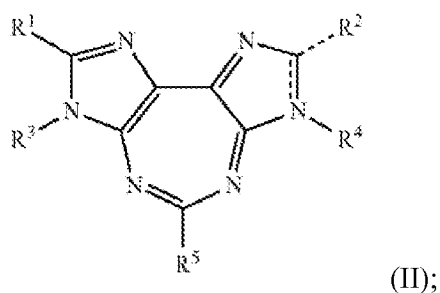
Z is CH, N, P, or C;

----- is a single bond or double bond; wherein if ----- is a double bond, R² or R⁷ is independently O, S, or NH;

n is 1, 2, 3, or 4; and

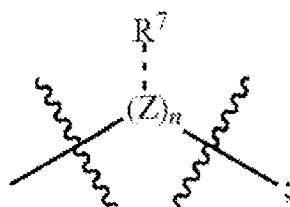
r, r', and r'' are each independently an integer from 1 to about 3, or a salt, solvate or stereoisomer thereof, in a therapeutically effective amount for prevention or treatment a viral infection in a subject.

2. Use of a compound of formula II:



or pharmaceutically acceptable salts and prodrugs thereof,
wherein

R^1 , R^2 , R^3 , R^4 , and R^5 are each independently a hydrogen; hydroxyl; substituted or unsubstituted: cyclic and acyclic alkyl group, cyclic and acyclic alkenyl group, cyclic and acyclic alkynyl group, aryl group, alkylaryl group, arylalkyl group, benzyl group, cyclic and acyclic heteroalkyl group, heteroaryl group; $—C(O)R^3$; $—C(S)R^3$; $—S(O)R^3$; $—S(O)_2R^3$; $—C(O)NR^4R^5$; $—C(S)NR^3R^4$; $—C(X)YR^5R^6$; $-\beta$ -D-ribosyl; $-\alpha$ -D-ribosyl; $-\beta$ -L-ribosyl; $-\alpha$ -L-ribosyl; 2'-deoxy- β -D-ribosyl; 2'-deoxy- α -D-ribosyl; 2'-deoxy- α -L-ribosyl; and ribose or deoxyribose sugars substituted with one or more halogens; R^1 and R^3 or R^2 and R^4 can also form a ring with one or more C, S, O, N atoms such that R^1 and R^3 or R^2 and R^4 together include:



R^7 is a hydrogen; hydroxyl; substituted and unsubstituted: cyclic and acyclic alkyl group, group, alkenyl group, alkynyl group, aryl group, aryloxy group, alkylary group, aryalkyl group, heteroaryl group, heterocycloalkyl group; $—C(O)$ alkyl; $—C(O)$ alkenyl; $—C(O)$ alkynyl; $—C(O)$ aryl; $—C(O)$ benzyl; $—C(O)NR^3R^4$; $—C(S)$ alkyl; $—C(S)$ alkenyl; $—C(S)$ alkynyl; $—C(S)$ aryl; $—C(S)$ benzyl; $—C(S)NR^3R^4$; $—C(X)YR^1R^2$;

wherein

X is O, N, or S;

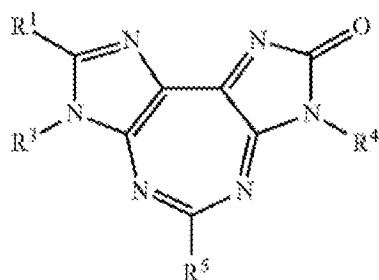
Y is O, CH_2 , NH, or S;

Z is CH, N, P, or C;

----- is a single bond or double bond; wherein if ----- is a double bond, R^2 or R^7 is independently O, S, or NH;

n is 1, 2, 3, or 4, or a salt, solvate or stereoisomer thereof, in a therapeutically effective amount for prevention or treatment a viral infection in a subject.

3. Use of a compound of Formula (III):

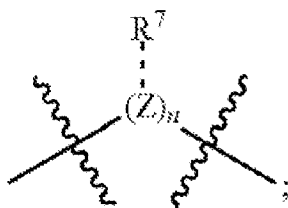


(III);

or pharmaceutically acceptable salts and prodrugs thereof,
wherein

R^1 , R^3 , R^4 , and R^5 are each independently a hydrogen; hydroxyl; substituted or unsubstituted: cyclic and acyclic alkyl group, cyclic and acyclic alkenyl group, cyclic and acyclic alkynyl group, aryl group, alkylaryl group, arylalkyl group, benzyl group, cyclic and acyclic heteroalkyl group, heteroaryl group; $-\text{C}(\text{O})\text{R}^3$; $-\text{C}(\text{S})\text{R}^3$; $-\text{S}(\text{O})\text{R}^3$; $-\text{S}(\text{O})_2\text{R}^3$; $-\text{C}(\text{O})\text{NR}^4\text{R}^5$; $-\text{C}(\text{S})\text{NR}^3\text{R}^4$; $-\text{C}(\text{X})\text{YR}^5\text{R}^6$; $-\beta\text{-D-ribose}$; $-\alpha\text{-D-ribose}$; $-\beta\text{-L-ribose}$; $-\alpha\text{-L-ribose}$; $2'\text{-deoxy-}\beta\text{-D-ribose}$; $2'\text{-deoxy-}\beta\text{-L-ribose}$; $2'\text{-deoxy-}\alpha\text{-D-ribose}$; $2'\text{-deoxy-}\alpha\text{-L-ribose}$; and ribose or deoxyribose sugars substituted with one or more halogens;

R^1 and R^3 can also form a ring with one or more C, S, O, N atoms such that R^1 and R^3 together include:



R^7 is a hydrogen; hydroxyl; substituted and unsubstituted: cyclic and acyclic alkyl group, group, alkenyl group, alkynyl group, aryl group, aryloxy group, alkylaryl group,

aryalkyl group, heteroaryl group, heterocycloalkyl group; —C(O)alkyl; —C(O)alkenyl; —C(O)alkynyl; —C(O)aryl; —C(O)benzyl; —C(O)NR³R⁴; —C(S)alkyl; —C(S)alkenyl; —C(S)alkynyl; —C(S)aryl; —C(S)benzyl; —C(S)NR³R⁴; —C(X)YR¹R²;

wherein

X is O, N, or S;

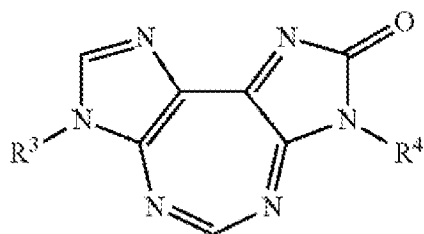
Y is O, CH₂, NH, or S;

Z is CH, N, P, or C;

----- is a single bond or double bond; wherein if ----- is a double bond, R² or R⁷ is independently O, S, or NH;

n is 1, 2, 3, or 4, or a salt, solvate or stereoisomer thereof, in a therapeutically effective amount for prevention or treatment a viral infection in a subject.

4. Use of a compound of Formula (IV):



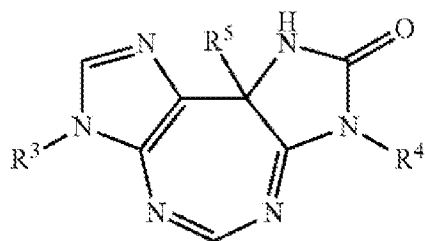
(IV);

or pharmaceutically acceptable salts and prodrugs thereof,
wherein

R³ and R⁴ are each independently a hydrogen; hydroxyl; substituted or unsubstituted: cyclic and acyclic alkyl group, cyclic and acyclic alkenyl group, cyclic and acyclic alkynyl group, aryl group, alkylaryl group, arylalkyl group, benzyl group, cyclic and acyclic heteroalkyl group, heteroaryl group; —C(O)R³; —C(S)R³; —S(O)R³; —S(O)₂R³; —C(O)NR⁴R⁵; —C(S)NR³R⁴; —C(X)YR⁵R⁶; -α-D-riboseyl; -β-L-riboseyl; -α-L-riboseyl; 2'-deoxy-β-D-riboseyl; 2'-deoxy-β-L-riboseyl; 2'-deoxy-α-D-riboseyl; 2'-deoxy-α-L-riboseyl; and

ribose or deoxyribose sugars substituted with one or more halogens, or a salt, solvate or stereoisomer thereof, in a therapeutically effective amount for prevention or treatment a viral infection in a subject.

5. Use of a compound of Formula (V):



(V);

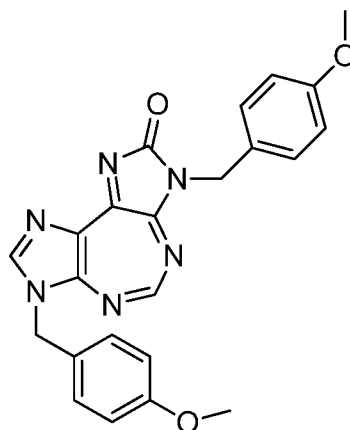
or pharmaceutically acceptable salts and prodrugs thereof,
wherein

R³, R⁴, and R⁵ are each independently a hydrogen; hydroxyl; substituted or unsubstituted: cyclic and acyclic alkyl group, cyclic and acyclic alkenyl group, cyclic and acyclic alkynyl group, aryl group, alkylaryl group, arylalkyl group, benzyl group, cyclic and acyclic heteroalkyl group, heteroaryl group; —C(O)R³; —C(S)R³; —S(O)R³; —S(O)₂R³; —C(O)NR⁴R⁵; —C(S)NR³R⁴; —C(X)YR⁵R⁶; -β-D-ribosyl; -α-D-ribosyl; -β-L-ribosyl; -α-L-ribosyl; 2'-deoxy-β-D-ribosyl; 2'-deoxy-β-L-ribosyl; 2'-deoxy-α-D-ribosyl; 2'-deoxy-α-L-ribosyl; and ribose or deoxyribose sugars substituted with one or more halogens, or a salt, solvate or stereoisomer thereof, in a therapeutically effective amount for prevention or treatment a viral infection in a subject.

6. The use of any of claims 1 to 5, wherein the viral infection is a virus of the Flaviviridae family.

7. The use of any of claims 1 to 5, wherein the viral infection is by the Hepatitis C virus.

8. The use of claim 1, wherein the compound of Formula I is RK-33:

**RK-33**

or a salt solvate or stereoisomer or derivative thereof.

9. The use of any of claims 1 to 5, wherein the compound of Formulas I-V or a salt, solvate, stereoisomer or derivative thereof is in a composition with a pharmaceutically acceptable carrier.

10. The use of any of claims 1 to 5, wherein the use further comprises the use of an additional compound having anti-HCV activity.

11. A method for identifying a target compound which inhibits HCV infectivity comprising:

- a) providing a first and second human hepatoma cell line, wherein the first hepatoma cell line is infected with HCV JFH1 virus, and the second hepatoma cell line is a control;
- b) incubating the first and second cell lines with the target compound in a growth medium capable of sustaining growth of the hepatoma cell lines in culture for a specified period of time;
- c) comparing the amount of infectivity of the HCV JFH1 virus in the first and second hepatoma cell lines; and
- d) determining that the target compound inhibits HCV infectivity when the amount of infectivity in the first hepatoma cell line is significantly less than the amount of infectivity in the second hepatoma cell line.

FIGURE 1

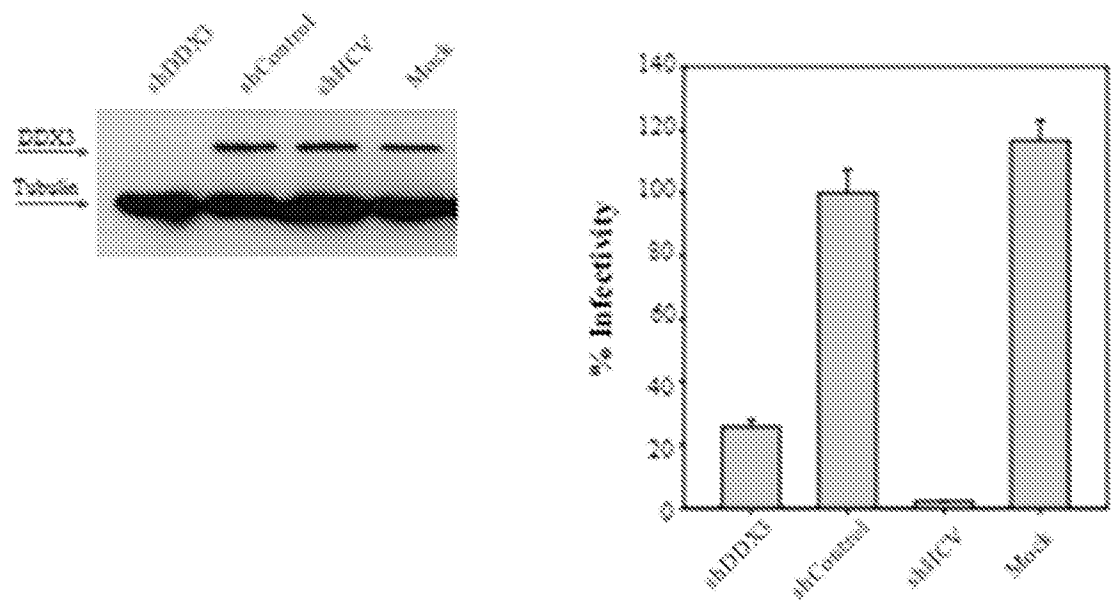


FIGURE 2A

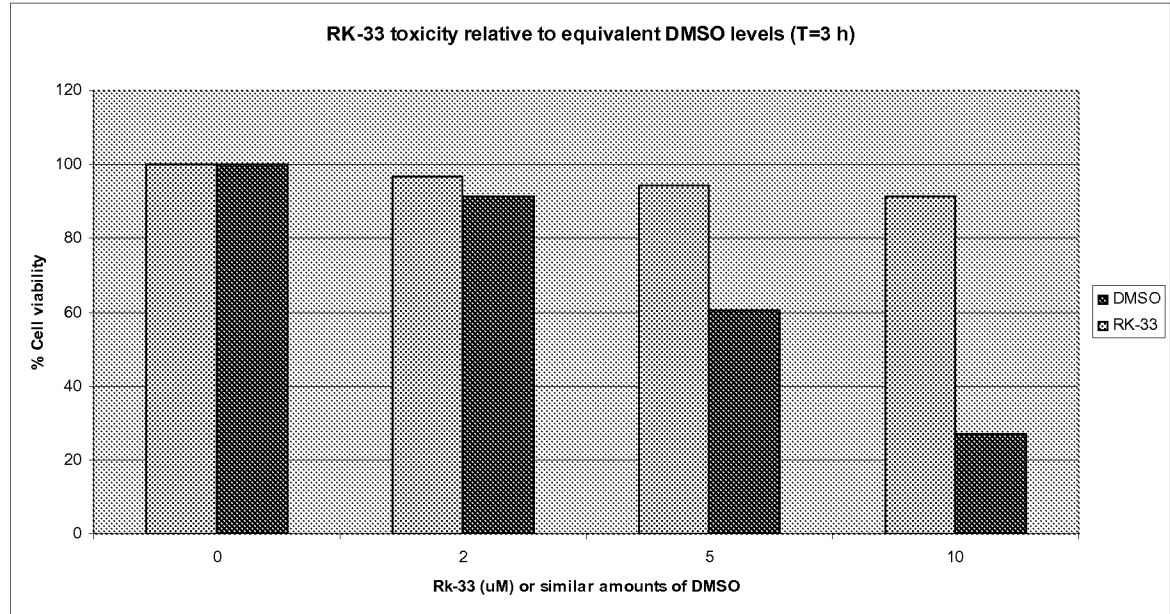


FIGURE 2B

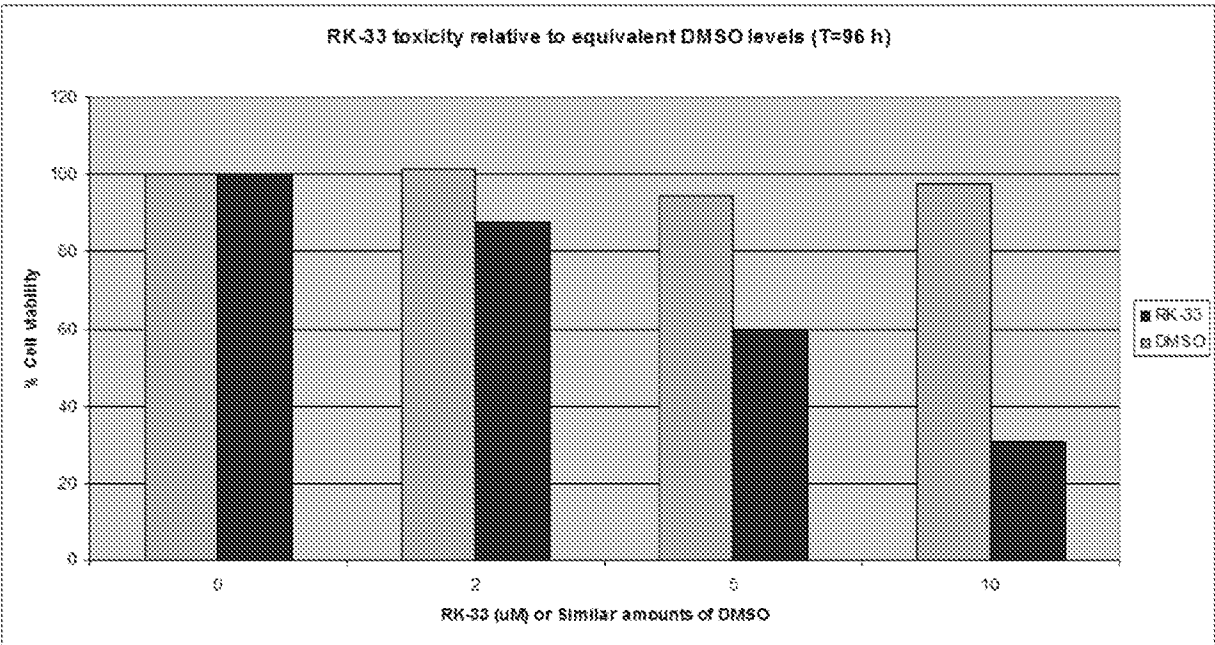


FIGURE 3

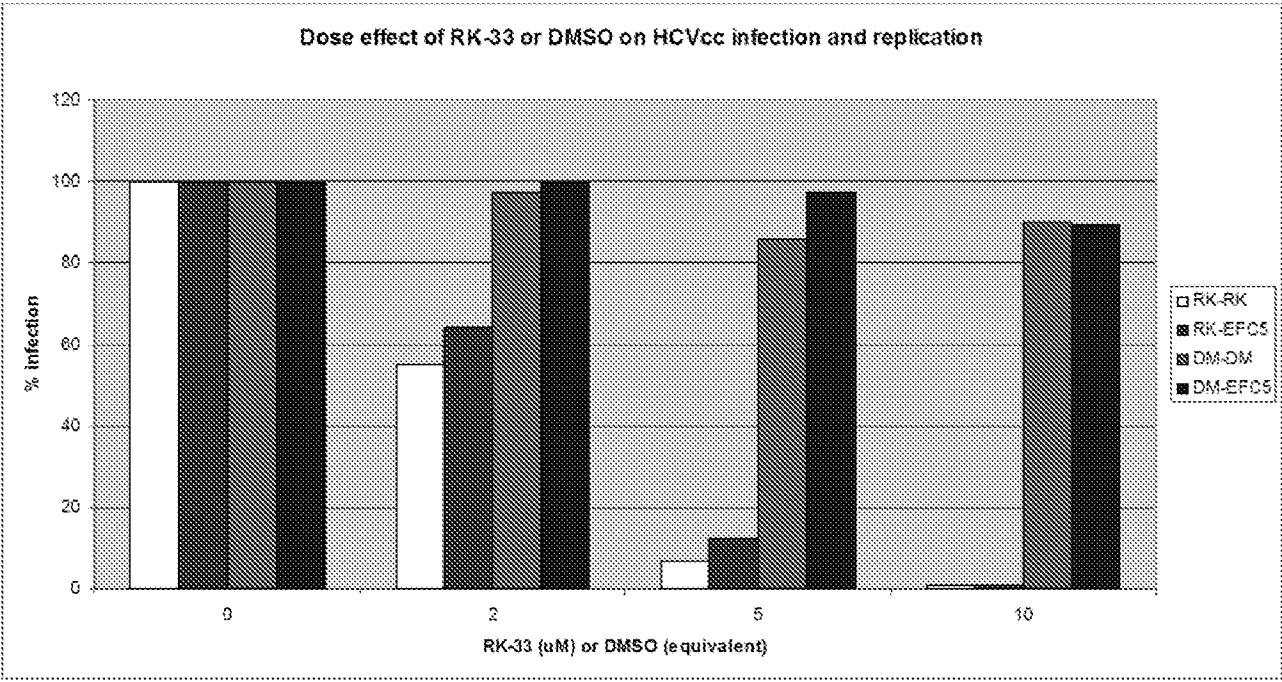


FIGURE 4A

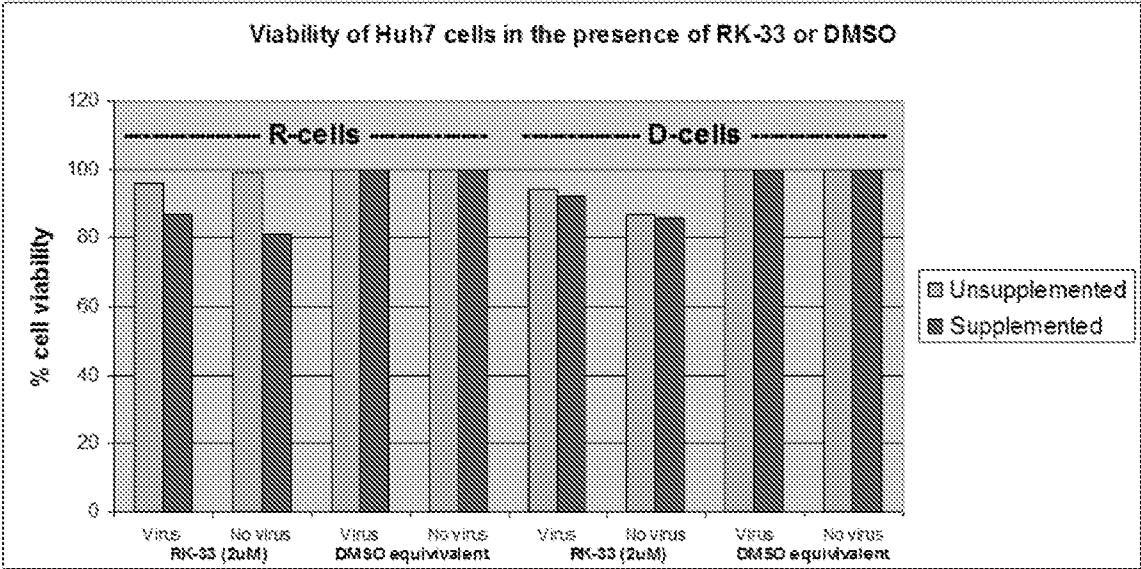


FIGURE 4B

