

TITLE OF THE INVENTION

ANTIVIRAL COMPOUNDS AND METHOD FOR TREATING HEPATOTROPIC VIRAL INFECTION, PARTICULARLY HEPATITIS B AND HEPATITIS D

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Application Ser. Nos. 63/001,723, filed March 30, 2020, and 63/053,908, filed July 20, 2020, which are incorporated herein by reference in entirety.

FIELD OF THE INVENTION

[0002] The present invention pertains to antiviral compounds, which is capable of treating hepatotropic viral infection, particularly caused by hepatitis virus B and D (HBV and HDV).

BACKGROUND OF THE INVENTION

[0003] Viral hepatitis is liver inflammation due to a viral infection. The most common causes of viral hepatitis are the infections by five hepatotropic viruses, hepatitis virus A, B, C, D, and E (HAV, HBV, HCV, HDV and HEV). Among those five hepatotropic viruses, HAV and HEV predominantly cause acute infection and will be completely cleared by the immune system, whereas the infections by HBV, HCV and HDV often become chronic. For those chronic viral hepatitis, only hepatitis C can be cured by presently available treatments, and no currently available treatment can be applied to cure hepatitis B or hepatitis D.

[0004] Hepatitis B virus (HBV) causes acute and chronic viral hepatitis in humans. HBV infection is often associated with severe liver diseases, including cirrhosis and hepatocellular carcinoma (HCC). The prevalence of HBV infection in the world is very high. About 350 million individuals are chronically infected, despite the availability of an effective vaccine for more than 25 years. Approximately a 100-fold increase in the relative risk of HCC among HBV carriers compared to non-carriers. An increasing number of patients with HBV infection cannot use the currently approved anti-HBV drugs, including interferon alpha or nucleos(t)ide analogues that inhibit the viral reverse transcriptase, due to the adverse effects and the emergence of drug resistance.

[0005] Hepatitis D virus (HDV) infects humans with chronic hepatitis B. Although HDV can propagate only in the presence of the hepatitis B virus (HBV), simultaneous HDV and HBV infection is considered the most serious type of viral hepatitis due to its severity of complications. These complications include a greater likelihood of experiencing liver failure in acute infections and a rapid progression to liver cirrhosis, with an increased risk of developing liver cancer in chronic

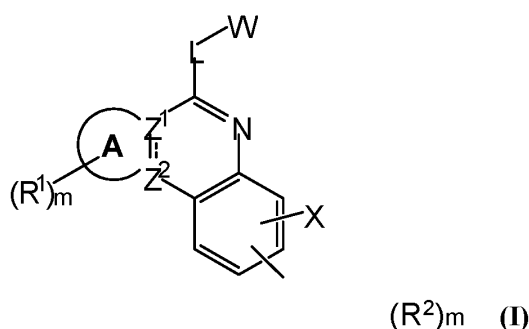
infections. In combination with hepatitis B virus, hepatitis D has the highest fatality rate of all the hepatitis infections, at 20%.

[0006] Although some treatments are available for hepatitis B and hepatitis D, none of the above treatments can completely clear the virus. Furthermore, viral resistance and cross drug resistance often result in ineffectiveness of the treatments. Therefore, it is desirable to develop effective, safe and affordable anti-viral agents against HBV and HDV to improve the treatment outcome.

SUMMARY OF THE INVENTION

[0007] Accordingly, the present invention provides methods and compounds for the treatment of hepatotropic viral infections, wherein the compounds are CK2 inhibitors. CK2 inhibitors is a dual-action drug, which are able to inhibit viral replication and control cytokines within homeostasis simultaneously.

[0008] In one aspect, the present invention provides a method for treating a hepatotropic viral infection in a human or an animal, which comprises administering to said human or animal a therapeutically effective amount of a compound of Formula (I):



wherein:

A is a saturated or partially saturated optionally substituted 5, 6 or 7 membered ring;

---- represents a single bond or a double bond;

Z¹ and Z² are independently N or C when ---- represents a single bond, provided Z¹ and Z² are not both N; and

Z¹ and Z² are C when ---- represents a double bond;

L is a linker selected from a bond, NR³, O, S, CR⁴R⁵, CR⁴R⁵-NR³, CR⁴R⁵-O-, and CR⁴R⁵-S;

each R¹, R², R³, R⁴ and R⁵ is independently H, or an optionally substituted member selected from the group consisting of C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C12 heteroaryl, C7-C12 arylalkyl, and C6-C12 heteroarylalkyl group, or halo, OR, NR₂, NROR, NRNR₂, SR, SOR,

SO₂R, SO₂NR₂, NRSO₂R, NRCONR₂, NRCSNR₂, NRC(=NR)NR₂, NRCOOR, NRCOR, CN, COOR, CONR₂, OOCR, COR, or NO₂,

wherein each R is independently H or C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl, and wherein two R on the same atom or on adjacent atoms can be linked to form a 3-8 membered ring, optionally containing one or more N, O or S;

and each R group, and each ring formed by linking two R groups together, is optionally substituted with one or more substituents selected from halo, =O, =N-CN, =N-OR', =NR', OR', NR'₂, SR', SO₂R', SO₂NR'₂, NR'SO₂R', NR'CONR'₂, NR'CSNR'₂, NR'C(=NR')NR'₂, NR'COOR', NR'COR', CN, COOR', CONR'₂, OOCR', COR', and NO₂,

wherein each R' is independently H, C1-C6 alkyl, C2-C6 heteroalkyl, C1-C6 acyl, C2-C6 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-12 arylalkyl, or C6-12 heteroarylalkyl, each of which is optionally substituted with one or more groups selected from halo, C1-C4 alkyl, C1-C4 heteroalkyl, C1-C6 acyl, C1-C6 heteroacyl, hydroxy, amino, and =O;

and wherein two R' on the same atom or on adjacent atoms can be linked to form a 3-7 membered ring optionally containing up to three heteroatoms selected from N, O and S;

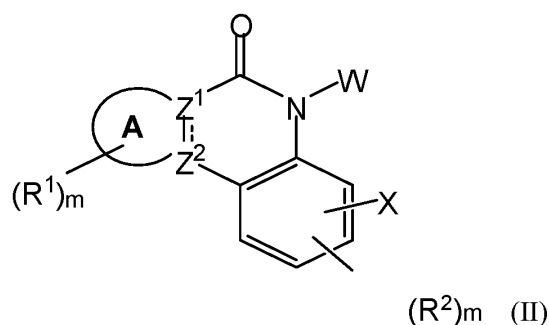
and R¹ can be =O, or two R¹ groups on the same atom or on adjacent connected atoms, can optionally be linked together to form a 3-8 membered cycloalkyl or heterocycloalkyl, which is optionally substituted; and R⁴ and R⁵, when on the same atom or on adjacent connected atoms, can optionally be linked together to form a 3-8 membered cycloalkyl or heterocycloalkyl, which is optionally substituted;

W is alkyl, heteroalkyl, aryl, heteroaryl, cycloalkyl, heterocyclyl, arylalkyl or heteroarylalkyl, each of which can be optionally substituted;

X is a polar substituent; and each m is independently 0-3;

or a pharmaceutically acceptable salt or ester thereof.

[0009] In one embodiment of the invention, the compound is the compound of Formula (II):



wherein:

A is a saturated or partially saturated optionally substituted 5, 6 or 7 membered ring;

----- represents a single bond or a double bond;

Z¹ and Z² are independently N or C when ----- represents a single bond, provided Z¹ and Z² are not both N; and

Z¹ and Z² are C when ----- represents a double bond; each of R¹ and R² is independently H, or an optionally substituted member selected from the group consisting of C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C12 heteroaryl, C7-C12 arylalkyl, and C6-C12 heteroarylalkyl group, or halo, OR, NR₂, NROR, NRNR₂, SR, SOR, SO₂R, SO₂NR₂, NRSO₂R, NRCONR₂, NRCSNR₂, NRC(=NR)NR₂, NRCOOR, NRCOR, CN, COOR, CONR₂, OOCR, COR, or NO₂, wherein each R is independently H or C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl, and wherein two R on the same atom or on adjacent atoms can be linked to form a 3-8 membered ring, optionally containing one or more N, O or S; and each R group, and each ring formed by linking two R groups together, is optionally substituted with one or more substituents selected from halo, =O, =N-CN, =N-OR', =NR', OR', NR'₂, SR', SO₂R', SO₂NR'₂, NR'SO₂R', NR'CONR'₂, NR'CSNR'₂, NR'C(=NR')NR'₂, NR'COOR', NR'COR', CN, COOR', CONR'₂, OOCR', COR', and NO₂, wherein each R' is independently H, C1-C6 alkyl, C2-C6 heteroalkyl, C1-C6 acyl, C2-C6 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-12 arylalkyl, or C6-12 heteroarylalkyl, each of which is optionally substituted with one or more groups selected from halo, C1-C4 alkyl, C1-C4 heteroalkyl, C1-C6 acyl, C1-C6 heteroacyl, hydroxy, amino, and =O; and wherein two R' on the same atom or on adjacent atoms can be linked to form a 3-7 membered ring optionally containing up to three heteroatoms selected from N, O and S;

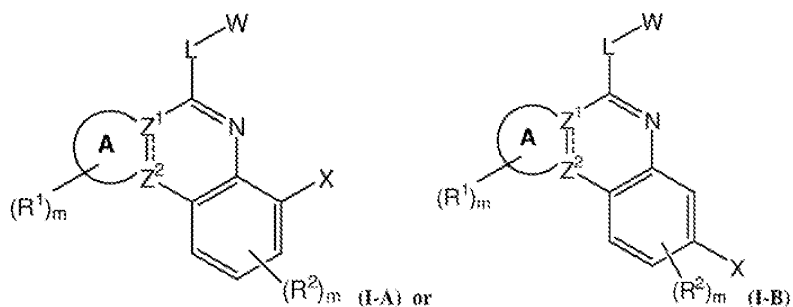
and R¹ can be =O, or two R¹ groups on the same atom or on adjacent connected atoms, can optionally be linked together to form a 3-8 membered cycloalkyl or heterocycloalkyl, which is optionally substituted;

W is alkyl, heteroalkyl, aryl, heteroaryl, cycloalkyl, heterocyclyl, arylalkyl or heteroarylalkyl, each of which can be optionally substituted;

X is a polar substituent; and each m is independently 0-3;

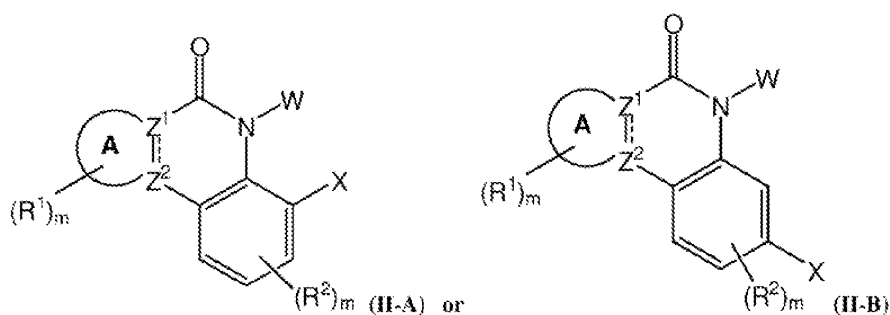
or a pharmaceutically acceptable salt or ester thereof.

[0010] In some embodiments of Formula (I), the compound has the structure of Formula (I-A) or (I-B):



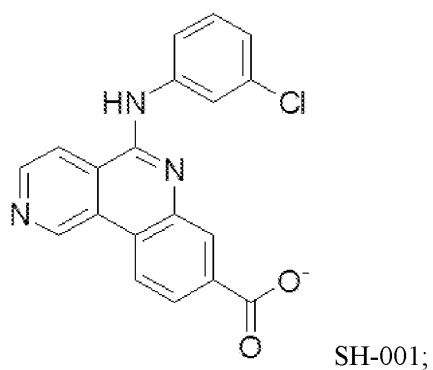
or a pharmaceutically acceptable salt or ester thereof, wherein A, Z¹, Z², L, W, X, R¹, R² and m are defined as in Formula (I).

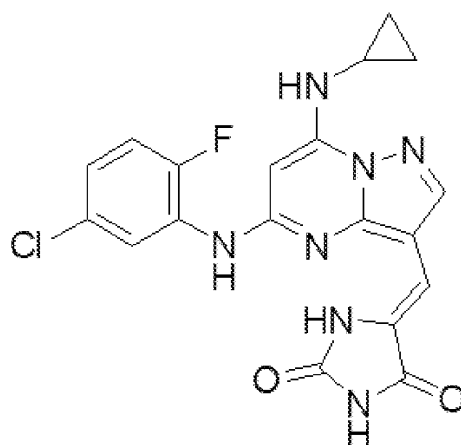
[0011] In some embodiments of Formula (II), the compound has the structure of Formula (II-A) or (II-B):



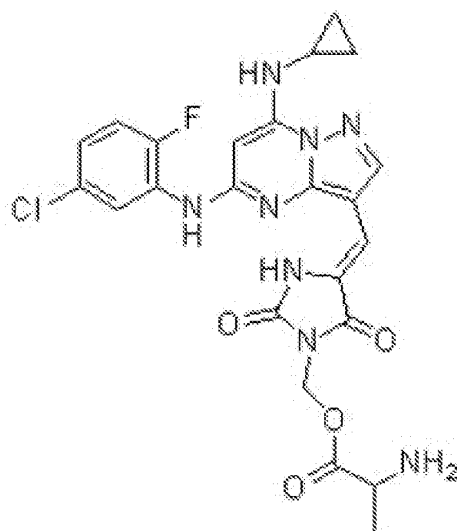
or a pharmaceutically acceptable salt or ester thereof, wherein A, Z¹, Z², W, X, R¹, R² and m are defined as in Formula (II).

[0012] In some embodiments of the compounds used in the invention, the compounds is one selected from the group consisting of the following compounds:





SH-002; and



SH-003.

[0013] In other aspects, the invention provides an anti-hepatotropic viral pharmaceutical composition in a human or an animal, comprising the compounds as mentioned above.

[0014] In the invention, the pharmaceutical composition of the invention comprises a compound described herein and at least one pharmaceutically acceptable carrier or excipient, or one or more pharmaceutically acceptable carriers and/or excipients.

[0015] In one further aspect, the invention provides a use of one or more of these compounds for manufacturing a medicament for treating a hepatotropic virus infection, particularly hepatitis B and hepatitis D.

[0016] In one particular embodiment of the invention, the virus infection is hepatitis B caused by Hepatitis B virus (HBV).

[0017] In another particular embodiment of the invention, the virus infection is hepatitis D caused by Hepatitis D virus (HDV).

[0018] Also provided are compositions comprising the above described molecules in combination with other agents, and methods for using such molecules in combination with one or more of other anti-virus agents.

[0019] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiment which is presently preferred. It should be understood, however, that the invention is not limited to this embodiment.

[0021] Figure 1 shows the effect of three compounds (SH-001, SH-002, and SH-003) on cell viability; wherein Vero E6 cells were treated with 0-320 μ M of the tested compounds as indicated for 48 h, then the MTT assay was performed to detect cell viability. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

[0022] Figure 2 shows the effect of two compounds (SH-001, and SH-002) on cell viability; wherein HepG2.2.15 cells were treated with 0-320 μ M of the tested compounds for 48 h, then the MTT assay was performed to detect cell viability. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

[0023] Figure 3 shows the effect of two compounds (SH-001, and SH-002) on cell viability; wherein HuS-E/2 cells were treated with 0-320 μ M of the tested compounds for 48 h, then the MTT assay was performed to detect cell viability. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

[0024] Figure 4 shows the inhibitory effect of SH-001 on HBV replication in HepG2.2.15 cells; wherein HepG2.2.15 cells were cultured with different concentrations of SH-001 for 48 h, then the culture medium was collected to measure HBV HBsAg (A), HBeAg (B) by ELISA, and HBV DNA (C) by real-time PCR. Plasmid p1.3HBcl, which contains a 1.3-fold HBV genome (ayw subtype), was used as standard in parallel PCR reactions. The results are expressed as a percentage of the non-drug-treated positive control (NT) and are shown as mean $\pm$ SD for three independent experiments. The data of IC₅₀ was shown in the lower panel. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

[0025] Figure 5 shows the inhibitory effect of SH-002 on HBV replication in HepG2.2.15 cells; wherein HepG2.2.15 cells were cultured with different concentrations of SH-002 for 48 h, then the culture medium was collected to measure HBV HBsAg (A), HBeAg (B) by ELISA, and HBV DNA (C) by real-time PCR. Plasmid p1.3HBcl, which contains a 1.3-fold HBV genome (ayw subtype), was used as standard in parallel PCR reactions. The results are expressed as a percentage of the non-drug-treated positive control (NT) and are shown as mean $\pm$ SD for three independent experiments. The data of IC₅₀ was shown in the lower panel. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

[0026] Figure 6 shows that SH-001 and SH-002 inhibited HDV infection with HuS-E/2 cells; wherein HuS-E/2 cells were exposed to HDV at a MOI of 20 for 18 h in the presence of the indicated concentration of SH-001 or SH-002, then the cells were washed to remove HDV and then treated with SH-001 or SH-002 for further 48 h. HDV genome was measured by RT-PCR and expressed as the percentage of the value for the non-drug-treated controls (NT). Control PCRs were performed for endogenous GAPDH mRNA as the loading control. The data of IC₅₀ was shown in the lower panel. The results are the mean $\pm$ SD for three independent experiments. The data of IC₅₀ was shown in the lower panel. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

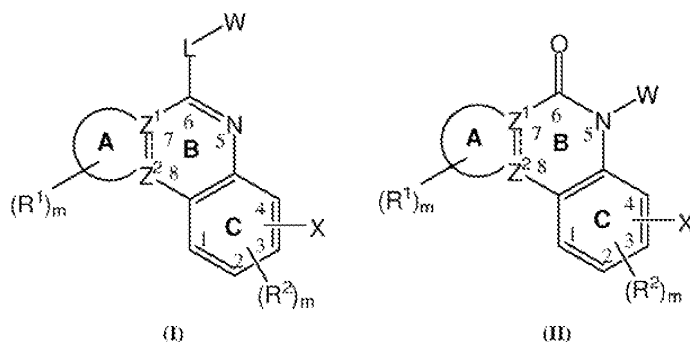
DETAILED DESCRIPTION OF THE INVENTION

[0027] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by a person skilled in the art to which this invention belongs.

[0028] These and other embodiments of the invention are described in the description that follows.

[0029] Modes of Carrying Out the Invention

[0030] For convenience, and without regard to standard nomenclature, when the position of groups on the bicyclic core portion of Formula (I) and Formula (II) need to be described, the ring positions will be identified by number using the following numbering scheme:



[0031] In this scheme, positions 1-4 are in the lower (phenyl) ring, and positions 5 (Nitrogen) through 8 are in the second ring. So, for example, the position of the polar substituent X on the phenyl ring may be described as position 4 if that group is attached to the unsubstituted carbon adjacent to the phenyl ring carbon attached to N in the second ring. Also for convenience, the phenyl ring is labeled as the C-ring in this structure and throughout the application, while the second ring containing N is referred to as the B-ring. The same relative numbering scheme will be used for other compounds that share the B and C ring bicyclic structure, while the additional ring containing Z¹-Z² fused to this bicyclic group will be referred to as the A-ring herein.

[0032] The term “optionally substituted” as used herein refers to the particular group or groups having no non-hydrogen substituents, or the group or groups having one or more non-hydrogen substituents. If not otherwise specified, the total number of such substituents that may be present is equal to the number of H atoms present on the unsubstituted form of the group being described. Where an optional substituent is attached via a double bond, such as a carbonyl oxygen (=O), the group takes up two available valences, so the total number of substituents that may be included is reduced according to the number of available valences.

[0033] The compounds of the invention often have ionizable groups so as to be capable of preparation as salts. In that case, wherever reference is made to the compound, it is understood in the art that a pharmaceutically acceptable salt may also be used. These salts may be acid addition salts involving inorganic or organic acids or the salts may, in the case of acidic forms of the compounds of the invention be prepared from inorganic or organic bases. Frequently, the compounds are prepared or used as pharmaceutically acceptable salts prepared as addition products of pharmaceutically acceptable acids or bases. Suitable pharmaceutically acceptable acids and bases are well-known in the art, such as hydrochloric, sulphuric, hydrobromic, acetic, lactic, citric, or tartaric acids for forming acid addition salts, and potassium hydroxide, sodium hydroxide, ammonium hydroxide, caffeine, various amines, and the like for forming basic salts. Methods for preparation of the appropriate salts are well-established in the art. In some cases, the compounds may contain both an acidic and a basic functional group, in which case they may have two ionized groups and yet have no net charge.

[0034] In some cases, the compounds of the invention contain one or more chiral centers. The invention includes each of the isolated stereoisomeric forms as well as mixtures of stereoisomers in varying degrees of chiral purity, including racemic mixtures. It also encompasses the various diastereomers and tautomers that can be formed. The compounds of the invention may also exist in more than one tautomeric form; the depiction herein of one tautomer is for convenience only, and is also understood to encompass other tautomers of the form shown.

[0035] As used herein, the terms “alkyl,” “alkenyl” and “alkynyl” include straight-chain, branched-chain and cyclic monovalent hydrocarbyl radicals, and combinations of these, which contain only C and H when they are unsubstituted. Examples include methyl, ethyl, isobutyl, cyclohexyl, cyclopentylethyl, 2-propenyl, 3-butenyl, and the like. The total number of carbon atoms in each such group is sometimes described herein, *e.g.*, when the group can contain up to ten carbon atoms it can be represented as 1-10C or as C1-C10 or C1-10. When heteroatoms (N, O and S typically) are allowed to replace carbon atoms as in heteroalkyl groups, for example, the numbers describing the group, though still written as *e.g.* C1-C6, represent the sum of the number of carbon atoms in the group plus the number of such heteroatoms that are included as replacements for carbon atoms in the backbone of the ring or chain being described.

[0036] Typically, the alkyl, alkenyl and alkynyl substituents of the invention contain 1-10C (alkyl) or 2-10C (alkenyl or alkynyl). Preferably they contain 1-8C (alkyl) or 2-8C (alkenyl or alkynyl). Sometimes they contain 1-4C (alkyl) or 2-4C (alkenyl or alkynyl). A single group can include more than one type of multiple bond, or more than one multiple bond; such groups are included within the definition of the term “alkenyl” when they contain at least one carbon-carbon double bond, and are included within the term “alkynyl” when they contain at least one carbon-carbon triple bond.

[0037] Alkyl, alkenyl and alkynyl groups are often optionally substituted to the extent that such substitution makes sense chemically. Typical substituents include, but are not limited to, halo, =O, =N-CN, =N-OR, =NR, OR, NR₂, SR, SO₂R, SO₂NR₂, NRSO₂R, NRCONR₂, NRCSNR₂, NRC(=NR)NR₂, NRCOOR, NRCOR, CN, C CR, COOR, CONR₂, OOCR, COR, and NO₂, wherein each R is independently H, C1-C8 alkyl, C2-C8 heteroalkyl, C1-C8 acyl, C2-C8 heteroacyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C6-C10 aryl, or C5-C10 heteroaryl, and each R is optionally substituted with halo, =O, =N-CN, =N-OR', =NR', OR', NR'₂, SR', SO₂R', SO₂NR'₂, NR'SO₂R', NR'CONR'₂, NR'CSNR'₂, NR'C(=NR')NR'₂, NR'COOR', NR'COR', CN, C CR', COOR', CONR'₂, OOCR', COR', and NO₂, wherein each R' is independently H, C1-C8 alkyl, C2-C8 heteroalkyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl or C5-C10 heteroaryl. Alkyl, alkenyl and alkynyl groups can also be substituted by C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl or C5-C10 heteroaryl, each of which can be substituted by the substituents that are appropriate for the particular group. Where two R or R' are present on the same atom (*e.g.*, NR₂), or on adjacent atoms that are bonded together (*e.g.*, -NR-C(O)R), the two R or R' groups can be taken together with the atoms they are connected to form a 5-8 membered ring, which can be substituted with C1-C4 alkyl, C1-C4 acyl, halo, C1-C4 alkoxy, and the like, and can contain an additional heteroatom selected from N, O and S as a ring member.

[0038] “Acetylene” substituents are C2-C10 alkynyl groups that are optionally substituted, and are of the formula $-C \equiv C-R^a$, wherein R^a is H or C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl, and each R^a group is optionally substituted with one or more substituents selected from halo, =O, =N-CN, =N-OR', =NR', OR', NR'₂, SR', SO₂R', SO₂NR'₂, NR'SO₂R', NR'CONR'₂, NR'CSNR'₂, NR'C(=NR')NR'₂, NR'COOR', NR'COR', CN, COOR', CONR'₂, OOCR', COR', and NO₂, wherein each R' is independently H, C1-C6 alkyl, C2-C6 heteroalkyl, C1-C6 acyl, C2-C6 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-12 arylalkyl, or C6-C12 heteroarylalkyl, each of which is optionally substituted with one or more groups selected from halo, C1-C4 alkyl, C1-C4 heteroalkyl, C1-C6 acyl, C1-C6 heteroacyl, hydroxy, amino, and =O; and wherein two R' can be linked to form a 3-7 membered ring optionally containing up to three heteroatoms selected from N, O and S. In some embodiments, R^a of $-C \equiv C-R^a$ is H or Me. Where two R or R' are present on the same atom (e.g., NR₂), or on adjacent atoms that are bonded together (e.g., -NR-C(O)R), the two R or R' groups can be taken together with the atoms they are connected to form a 5-8 membered ring, which can be substituted with C1-C4 alkyl, C1-C4 acyl, halo, C1-C4 alkoxy, and the like, and can contain an additional heteroatom selected from N, O and S as a ring member.

[0039] “Heteroalkyl”, “heteroalkenyl”, and “heteroalkynyl” and the like are defined similarly to the corresponding hydrocarbyl (alkyl, alkenyl and alkynyl) groups, but the ‘hetero’ terms refer to groups that contain 1-3 O, S or N heteroatoms or combinations thereof within the backbone residue; thus at least one carbon atom of a corresponding alkyl, alkenyl, or alkynyl group is replaced by one of the specified heteroatoms to form a heteroalkyl, heteroalkenyl, or heteroalkynyl group. The typical and preferred sizes for heteroforms of alkyl, alkenyl and alkynyl groups are generally the same as for the corresponding hydrocarbyl groups, and the substituents that may be present on the heteroforms are the same as those described above for the hydrocarbyl groups. For reasons of chemical stability, it is also understood that, unless otherwise specified, such groups do not include more than two contiguous heteroatoms except where an oxo group is present on N or S as in a nitro or sulfonyl group.

[0040] While “alkyl” as used herein includes cycloalkyl and cycloalkylalkyl groups, the term “cycloalkyl” may be used herein to describe a carbocyclic non-aromatic group that is connected via a ring carbon atom, and “cycloalkylalkyl” may be used to describe a carbocyclic non-aromatic group that is connected to the molecule through an alkyl linker. Similarly, “heterocyclyl” may be used to describe a non-aromatic cyclic group that contains at least one heteroatom as a ring member and that is connected to the molecule via a ring atom, which may be C or N; and “heterocyclylalkyl” may be

used to describe such a group that is connected to another molecule through a linker. The sizes and substituents that are suitable for the cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclalkyl groups are the same as those described above for alkyl groups. As used herein, these terms also include rings that contain a double bond or two, as long as the ring is not aromatic.

[0041] As used herein, “acyl” encompasses groups comprising an alkyl, alkenyl, alkynyl, aryl or arylalkyl radical attached at one of the two available valence positions of a carbonyl carbon atom, and heteroacyl refers to the corresponding groups wherein at least one carbon other than the carbonyl carbon has been replaced by a heteroatom chosen from N, O and S. Thus heteroacyl includes, for example, $-C(=O)OR$ and $-C(=O)NR_2$ as well as $-C(=O)$ -heteroaryl.

[0042] Acyl and heteroacyl groups are bonded to any group or molecule to which they are attached through the open valence of the carbonyl carbon atom. Typically, they are C1-C8 acyl groups, which include formyl, acetyl, pivaloyl, and benzoyl, and C2-C8 heteroacyl groups, which include methoxyacetyl, ethoxycarbonyl, and 4-pyridinoyl. The hydrocarbyl groups, aryl groups, and heteroforms of such groups that comprise an acyl or heteroacyl group can be substituted with the substituents described herein as generally suitable substituents for each of the corresponding component of the acyl or heteroacyl group.

[0043] “Aromatic” moiety or “aryl” moiety refers to a monocyclic or fused bicyclic moiety having the well-known characteristics of aromaticity; examples include phenyl and naphthyl. Similarly, “heteroaromatic” and “heteroaryl” refer to such monocyclic or fused bicyclic ring systems which contain as ring members one or more heteroatoms selected from O, S and N. The inclusion of a heteroatom permits aromaticity in 5-membered rings as well as 6-membered rings. Typical heteroaromatic systems include monocyclic C5-C6 aromatic groups such as pyridyl, pyrimidyl, pyrazinyl, thienyl, furanyl, pyrrolyl, pyrazolyl, thiazolyl, oxazolyl, and imidazolyl and the fused bicyclic moieties formed by fusing one of these monocyclic groups with a phenyl ring or with any of the heteroaromatic monocyclic groups to form a C8-C10 bicyclic group such as indolyl, benzimidazolyl, indazolyl, benzotriazolyl, isoquinolyl, quinolyl, benzothiazolyl, benzofuranyl, pyrazolopyridyl, quinazolinyl, quinoxalinyl, cinnolinyl, and the like. Any monocyclic or fused ring bicyclic system which has the characteristics of aromaticity in terms of electron distribution throughout the ring system is included in this definition. It also includes bicyclic groups where at least the ring which is directly attached to the remainder of the molecule has the characteristics of aromaticity. Typically, the ring systems contain 5-12 ring member atoms. Preferably the monocyclic heteroaryls contain 5-6 ring members, and the bicyclic heteroaryls contain 8-10 ring members.

[0044] Aryl and heteroaryl moieties may be substituted with a variety of substituents including C1-C8 alkyl, C2-C8 alkenyl, C2-C8 alkynyl, C5-C12 aryl, C1-C8 acyl, and heteroforms of these, each of which can itself be further substituted; other substituents for aryl and heteroaryl moieties include halo, OR, NR₂, SR, SO₂R, SO₂NR₂, NRSO₂R, NRCONR₂, NRCSNR₂, NRC(=NR)NR₂, NRCOOR, NRCOR, CN, C CR, COOR, CONR₂, OOCR, COR, and NO₂, wherein each R is independently H, C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C6-C10 aryl, C5-C10 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl, and each R is optionally substituted as described above for alkyl groups. Where two R or R' are present on the same atom (e.g., NR₂), or on adjacent atoms that are bonded together (e.g., -NR-C(O)R), the two R or R' groups can be taken together with the atoms they are connected to form a 5-8 membered ring, which can be substituted with C1-C4 alkyl, C1-C4 acyl, halo, C1-C4 alkoxy, and the like, and can contain an additional heteroatom selected from N, O and S as a ring member.

[0045] The substituent groups on an aryl or heteroaryl group may of course be further substituted with the groups described herein as suitable for each type of such substituents or for each component of the substituent. Thus, for example, an arylalkyl substituent may be substituted on the aryl portion with substituents described herein as typical for aryl groups, and it may be further substituted on the alkyl portion with substituents described herein as typical or suitable for alkyl groups.

[0046] Similarly, "arylalkyl" and "heteroarylalkyl" refer to aromatic and heteroaromatic ring systems which are bonded to their attachment point through a linking group such as an alkylene, including substituted or unsubstituted, saturated or unsaturated, cyclic or acyclic linkers. Typically, the linker is C1-C8 alkyl or a hetero form thereof. These linkers may also include a carbonyl group, thus making them able to provide substituents as an acyl or heteroacyl moiety.

[0047] An aryl or heteroaryl ring in an arylalkyl or heteroarylalkyl group may be substituted with the same substituents described above for aryl groups. Preferably, an arylalkyl group includes a phenyl ring optionally substituted with the groups defined above for aryl groups and a C1-C4 alkylene that is unsubstituted or is substituted with one or two C1-C4 alkyl groups or heteroalkyl groups, where the alkyl or heteroalkyl groups can optionally cyclize to form a ring such as cyclopropane, dioxolane, or oxacyclopentane. Similarly, a heteroarylalkyl group preferably includes a C5-C6 monocyclic heteroaryl group that is optionally substituted with the groups described above as substituents typical on aryl groups and a C1-C4 alkylene that is unsubstituted or is substituted with one or two C1-C4 alkyl groups or heteroalkyl groups, or it includes an optionally substituted phenyl ring or C5-C6 monocyclic heteroaryl and a C1-C4 heteroalkylene that is

unsubstituted or is substituted with one or two C1-C4 alkyl or heteroalkyl groups, where the alkyl or heteroalkyl groups can optionally cyclize to form a ring such as cyclopropane, dioxolane, or oxacyclopentane.

[0048] Where an arylalkyl or heteroarylalkyl group is described as optionally substituted, the substituents may be on either the alkyl or heteroalkyl portion or on the aryl or heteroaryl portion of the group. The substituents optionally present on the alkyl or heteroalkyl portion are the same as those described above for alkyl groups generally; the substituents optionally present on the aryl or heteroaryl portion are the same as those described above for aryl groups generally.

[0049] “Arylalkyl” groups as used herein are hydrocarbyl groups if they are unsubstituted, and are described by the total number of carbon atoms in the ring and alkylene or similar linker. Thus, a benzyl group is a C7-arylalkyl group, and phenylethyl is a C8-arylalkyl.

[0050] “Heteroarylalkyl” as described above refers to a moiety comprising an aryl group that is attached through a linking group, and differs from “arylalkyl” in that at least one ring atom of the aryl moiety or one atom in the linking group is a heteroatom selected from N, O and S. The heteroarylalkyl groups are described herein according to the total number of atoms in the ring and linker combined, and they include aryl groups linked through a heteroalkyl linker; heteroaryl groups linked through a hydrocarbyl linker such as an alkylene; and heteroaryl groups linked through a heteroalkyl linker. Thus, for example, C7-heteroarylalkyl would include pyridylmethyl, phenoxy, and N-pyrrolylmethoxy.

[0051] “Alkylene” as used herein refers to a divalent hydrocarbyl group; because it is divalent, it can link two other groups together. Typically it refers to $-(CH_2)_n-$ where n is 1-8 and preferably n is 1-4, though where specified, an alkylene can also be substituted by other groups, and can be of other lengths, and the open valences need not be at opposite ends of a chain. Thus $-CH(Me)-$ and $-C(Me)_2-$ may also be referred to as alkenes, as can a cyclic group such as cyclopropan-1,1-diyl. Where an alkylene group is substituted, the substituents include those typically present on alkyl groups as described herein.

[0052] In general, any alkyl, alkenyl, alkynyl, acyl, or aryl or arylalkyl group or any heteroform of one of these groups that is contained in a substituent may itself optionally be substituted by additional substituents. The nature of these substituents is similar to those recited with regard to the primary substituents themselves if the substituents are not otherwise described. Thus, where an embodiment of, for example, R^7 is alkyl, this alkyl may optionally be substituted by the remaining substituents listed as embodiments for R^7 where this makes chemical sense, and where this does not undermine the size limit provided for the alkyl *per se*; e.g., alkyl substituted by alkyl or by alkenyl would simply extend the upper limit of carbon atoms for these embodiments, and is not included.

However, alkyl substituted by aryl, amino, alkoxy, =O, and the like would be included within the scope of the invention, and the atoms of these substituent groups are not counted in the number used to describe the alkyl, alkenyl, etc. group that is being described. Where no number of substituents is specified, each such alkyl, alkenyl, alkynyl, acyl, or aryl group may be substituted with a number of substituents according to its available valences; in particular, any of these groups may be substituted with fluorine atoms at any or all of its available valences, for example.

[0053] “Heteroform” as used herein refers to a derivative of a group such as an alkyl, aryl, or acyl, wherein at least one carbon atom of the designated carbocyclic group has been replaced by a heteroatom selected from N, O and S. Thus, the heteroforms of alkyl, alkenyl, alkynyl, acyl, aryl, and arylalkyl are heteroalkyl, heteroalkenyl, heteroalkynyl, heteroacyl, heteroaryl, and heteroarylalkyl, respectively. It is understood that no more than two N, O or S atoms are ordinarily connected sequentially, except where an oxo group is attached to N or S to form a nitro or sulfonyl group.

[0054] “Halo”, as used herein includes fluoro, chloro, bromo and iodo. Fluoro and chloro are often preferred.

[0055] “Amino” as used herein refers to NH₂, but where an amino is described as “substituted” or “optionally substituted”, the term includes NR'R” wherein each R' and R” is independently H, or is an alkyl, alkenyl, alkynyl, acyl, aryl, or arylalkyl group or a heteroform of one of these groups, and each of the alkyl, alkenyl, alkynyl, acyl, aryl, or arylalkyl groups or heteroforms of one of these groups is optionally substituted with the substituents described herein as suitable for the corresponding group. The term also includes forms wherein R' and R” are linked together to form a 3-8 membered ring which may be saturated, unsaturated or aromatic and which contains 1-3 heteroatoms independently selected from N, O and S as ring members, and which is optionally substituted with the substituents described as suitable for alkyl groups or, if NR'R” is an aromatic group, it is optionally substituted with the substituents described as typical for heteroaryl groups.

[0056] As used herein, the term “carbocycle” refers to a cyclic compound containing only carbon atoms in the ring, whereas a “heterocycle” refers to a cyclic compound comprising a heteroatom. The carbocyclic and heterocyclic structures encompass compounds having monocyclic, bicyclic or multiple ring systems. As used herein, these terms also include rings that contain a double bond or two, as long as the ring is not aromatic.

[0057] As used herein, the term “heteroatom” refers to any atom that is not carbon or hydrogen, such as nitrogen, oxygen or sulfur.

[0058] Illustrative examples of heterocycles include but are not limited to tetrahydrofuran, 1,3-dioxolane, 2,3-dihydrofuran, pyran, tetrahydropyran, benzofuran, isobenzofuran, 1,3dihydro-

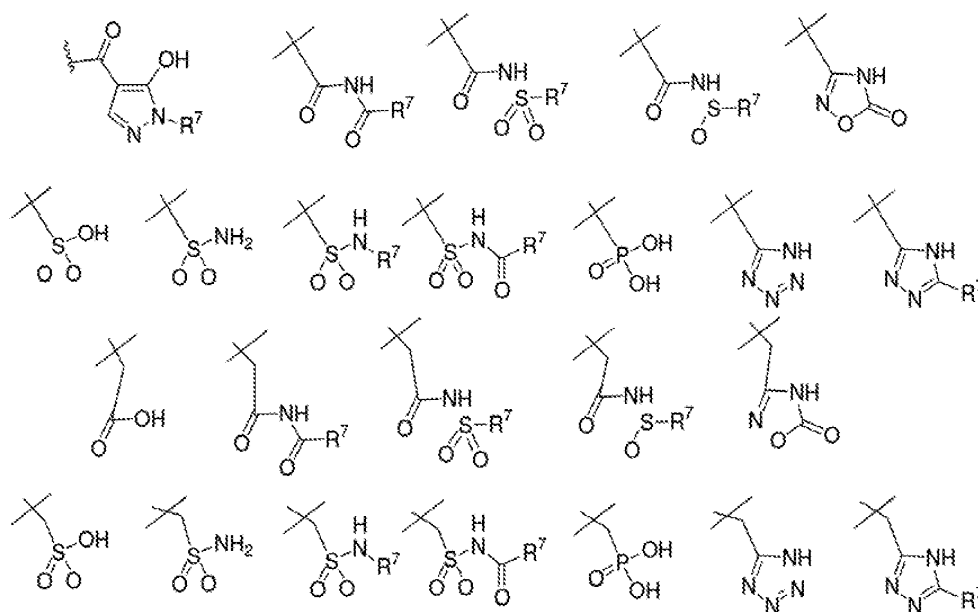
isobenzofuran, isoxazole, 4,5-dihydroisoxazole, piperidine, pyrrolidine, pyrrolidin-2-one, pyrrole, pyridine, pyrimidine, octahydro-pyrrolo[3,4 b]pyridine, piperazine, pyrazine, morpholine, thiomorpholine, imidazole, imidazolidine 2,4-dione, 1,3-dihydrobenzimidazol-2-one, indole, thiazole, benzothiazole, thiadiazole, thiophene, tetrahydro thiophene 1,1-dioxide, diazepine, triazole, guanidine, diazabicyclo[2.2.1]heptane, 2,5-diazabicyclo[2.2.1]heptane, 2,3,4,4a,9,9a-hexahydro-1H-carboline, oxirane, oxetane, tetrahydropyran, dioxane, lactones, aziridine, azetidine, piperidine, lactams, and may also encompass heteroaryls. Other illustrative examples of heteroaryls include but are not limited to furan, pyrrole, pyridine, pyrimidine, imidazole, benzimidazole and triazole.

[0059] As used herein, the term “inorganic substituent” refers to substituents that do not contain carbon or contain carbon bound to elements other than hydrogen (e.g., elemental carbon, carbon monoxide, carbon dioxide, and carbonate). Examples of inorganic substituents include but are not limited to nitro, halogen, azido, cyano, sulfonyls, sulfinyls, sulfonates, phosphates, etc.

[0060] The term “polar substituent” as used herein refers to any substituent having an electric dipole, and optionally a dipole moment (e.g., an asymmetrical polar substituent has a dipole moment and a symmetrical polar substituent does not have a dipole moment). Polar substituents include substituents that accept or donate a hydrogen bond, and groups that would carry at least a partial positive or negative charge in aqueous solution at physiological pH levels. In certain embodiments, a polar substituent is one that can accept or donate electrons in a noncovalent hydrogen bond with another chemical moiety.

[0061] In certain embodiments, a polar substituent is selected from a carboxy, a carboxy bioisostere or other acid-derived moiety that exists predominately as an anion at a pH of about 7 to 8 or higher. Other polar substituents include, but are not limited to, groups containing an OH or NH, an ether oxygen, an amine nitrogen, an oxidized sulfur or nitrogen, a carbonyl, a nitrile, and a nitrogen-containing or oxygen-containing heterocyclic ring whether aromatic or nonaromatic. In some embodiments, the polar substituent (represented by X) is a carboxylate or a carboxylate bioisostere.

[0062] “Carboxylate bioisostere” or “carboxy bioisostere” as used herein refers to a moiety that is expected to be negatively charged to a substantial degree at physiological pH. In certain embodiments, the carboxylate bioisostere is a moiety selected from the group consisting of:



and salts of the foregoing, wherein each R^7 is independently H or an optionally substituted member selected from the group consisting of C1-10 alkyl, C2-10 alkenyl, C2-10 heteroalkyl, C3-8 carbocyclic ring, and C3-8 heterocyclic ring optionally fused to an additional optionally substituted carbocyclic or heterocyclic ring; or R^7 is a C1-10 alkyl, C2-10 alkenyl, or C2-10 heteroalkyl substituted with an optionally substituted C3-8 carbocyclic ring or C3-8 heterocyclic ring.

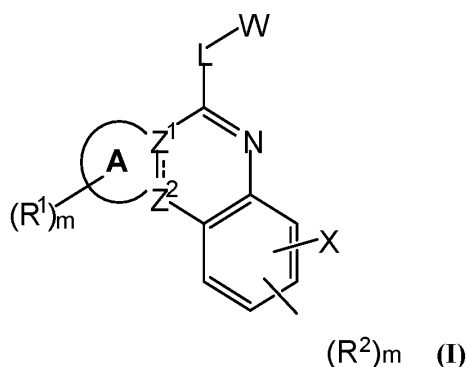
[0063] In certain embodiments, the polar substituent is selected from the group consisting of carboxylic acid, carboxylic ester, carboxamide, tetrazole, triazole, oxadiazole, oxothiadiazole, thiazole, aminothiazole, hydroxythiazole, and carboxymethanesulfonamide. In some embodiments of the compounds described herein, at least one polar substituent present is a carboxylic acid or a salt, or ester or a bioisostere thereof. In certain embodiments, at least one polar substituent present is a carboxylic acid-containing substituent or a salt, ester or bioisostere thereof. In the latter embodiments, the polar substituent may be a C1-C10 alkyl or C1-C10 alkenyl linked to a carboxylic acid (or salt, ester or bioisostere thereof), for example.

[0064] The term “solgroup” or “solubility-enhancing group” as used herein refers to a molecular fragment selected for its ability to enhance physiological solubility of a compound that has otherwise relatively low solubility. Any substituent that can facilitate the dissolution of any particular molecule in water or any biological media can serve as a solubility-enhancing group. Examples of solubilizing groups are, but not limited to: any substituent containing a group susceptible to being ionized in water at a pH range from 0 to 14; any ionizable group susceptible to form a salt; or any highly polar substituent, with a high dipolar moment and capable of forming strong interaction with molecules of water. Examples of solubilizing groups are, but are not limited to: substituted alkyl

amines, substituted alkyl alcohols, alkyl ethers, aryl amines, pyridines, phenols, carboxylic acids, tetrazoles, sulfonamides, amides, sulfonylamides, sulfonic acids, sulfinic acids, phosphates, sulfonylureas.

[0065] Suitable groups for this purpose include, for example, groups of the formula $-A(CH_2)_{0-4}-G$, where A is absent, O, or NR, where R is H or Me; and G can be a carboxy group, a carboxy bioisostere, hydroxy, phosphonate, sulfonate, or a group of the formula $-NR^y_2$ or $P(O)(OR^y)_2$, where each R^y is independently H or a C1-C4 alkyl that can be substituted with one or more (typically up to three) of these groups: NH_2 , OH, NHMe, NMe₂, OMe, halo, or =O (carbonyl oxygen); and two R^y in one such group can be linked together to form a 5-7 membered ring, optionally containing an additional heteroatom (N, O or S) as a ring member, and optionally substituted with a C1-C4 alkyl, which can itself be substituted with one or more (typically up to three) of these groups: NH_2 , OH, NHMe, NMe₂, OMe, halo, or =O (carbonyl oxygen).

[0066] In some embodiments of the invention, the compound is one of the compounds of Formula (I):



wherein:

A is a saturated or partially saturated optionally substituted 5, 6 or 7 membered ring;

----- represents a single bond or a double bond;

Z^1 and Z^2 are independently N or C when ----- represents a single bond, provided Z^1 and Z^2 are not both N; and

Z^1 and Z^2 are C when ----- represents a double bond;

L is a linker selected from a bond, NR^3 , O, S, CR^4R^5 , $CR^4R^5-NR^3$, CR^4R^5-O- , and CR^4R^5-S- ; each R^1 , R^2 , R^3 , R^4 and R^5 is independently H, or an optionally substituted member selected from the group consisting of C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C12 heteroaryl, C7-C12 arylalkyl, and C6-C12 heteroarylalkyl group, or halo, OR, NR_2 , $NROR$, $NRNR_2$, SR, SOR, SO_2R ,

SO₂NR₂, NRSO₂R, NRCONR₂, NRC₂SNR₂, NRC(=NR)NR₂, NR₂COOR, NRCOR, CN, COOR, CONR₂, OOCR, COR, or NO₂,

wherein each R is independently H or C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl,

and wherein two R on the same atom or on adjacent atoms can be linked to form a 3-8 membered ring, optionally containing one or more N, O or S; and each R group, and each ring formed by linking two R groups together, is optionally substituted with one or more substituents selected from halo, =O, =N-CN, =N-OR', =NR', OR', NR'₂, SR', SO₂R', SO₂NR'₂, NR'SO₂R', NR'CONR'₂, NR'CSNR'₂, NR'C(=NR')NR'₂, NR'COOR', NR'COR', CN, COOR', CONR'₂, OOCR', COR', and NO₂, wherein each R' is independently H, C1-C6 alkyl, C2-C6 heteroalkyl, C1-C6 acyl, C2-C6 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-12 arylalkyl, or C6-12 heteroarylalkyl, each of which is optionally substituted with one or more groups selected from halo, C1-C4 alkyl, C1-C4 heteroalkyl, C1-C6 acyl, C1-C6 heteroacyl, hydroxy, amino, and =O;

and wherein two R' on the same atom or on adjacent atoms can be linked to form a 3-7 membered ring optionally containing up to three heteroatoms selected from N, O and S;

and R¹ can be =O, or two R¹ groups on the same atom or on adjacent connected atoms, can optionally be linked together to form a 3-8 membered cycloalkyl or heterocycloalkyl, which is optionally substituted; and R⁴ and R⁵, when on the same atom or on adjacent connected atoms, can optionally be linked together to form a 3-8 membered cycloalkyl or heterocycloalkyl, which is optionally substituted;

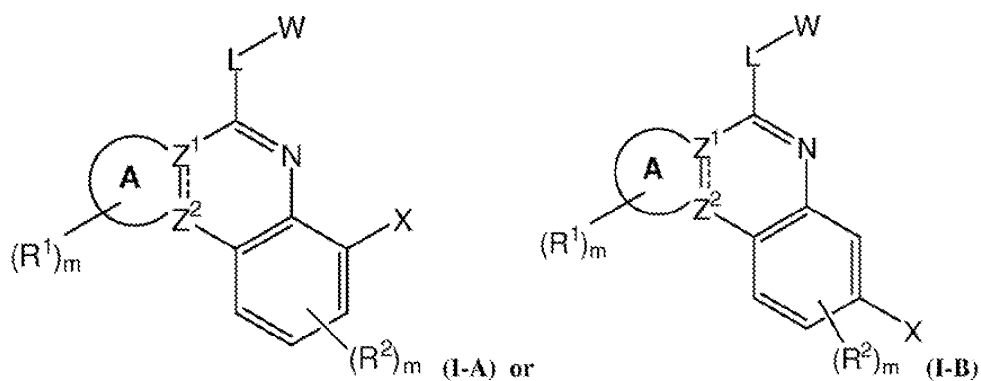
W is alkyl, heteroalkyl, aryl, heteroaryl, cycloalkyl, heterocyclyl, arylalkyl or heteroarylalkyl, each of which can be optionally substituted;

X is a polar substituent;

and each m is independently 0-3;

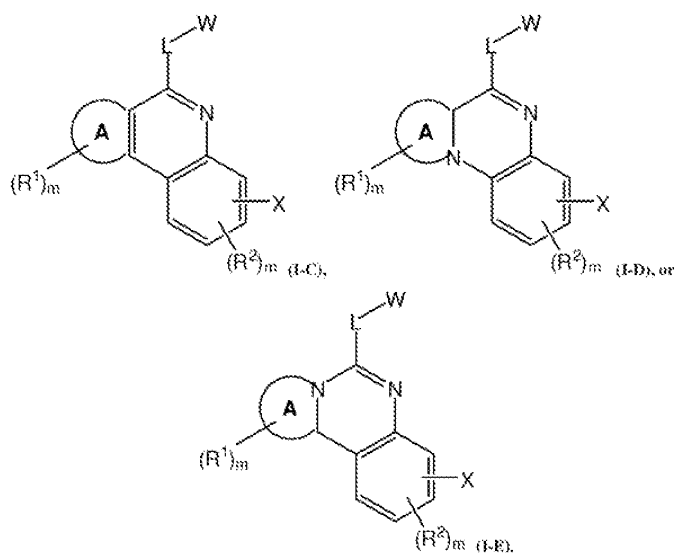
or a pharmaceutically acceptable salt or ester thereof.

[0067] In some embodiments, the compound is the compound of Formula (I) having the structure of Formula (I-A) or (I-B), or a pharmaceutically acceptable salt or ester thereof:



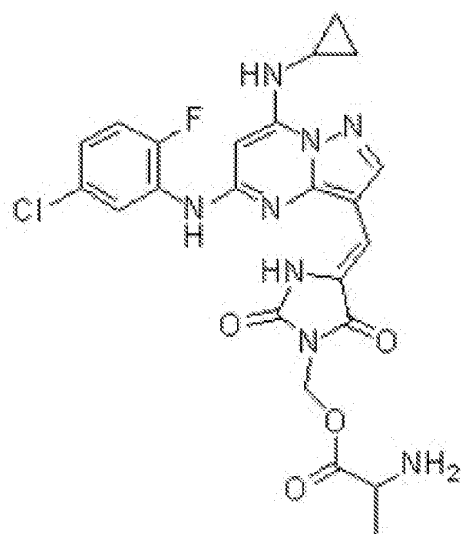
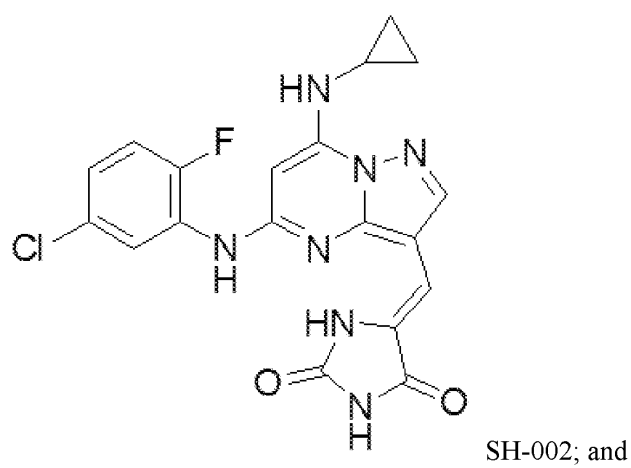
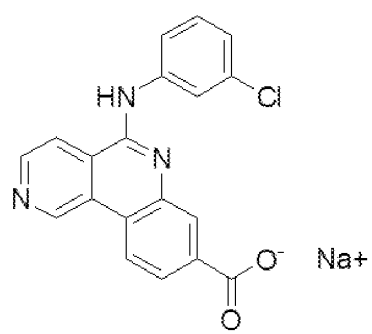
wherein A , Z^1 , Z^2 , L , W , X , R^1 , R^2 and m are defined as in Formula (I).

[0068] In other embodiments, the compound of Formula (I) has the structure of Formula (I-C), (I-D) or (I-E) or a pharmaceutically acceptable salt or ester thereof:

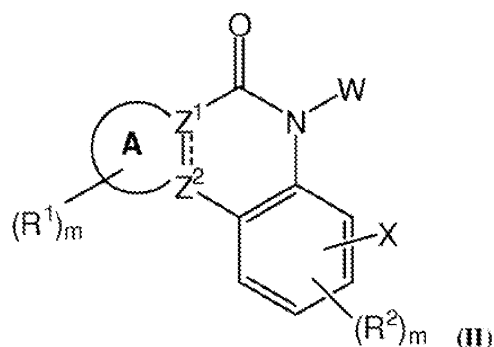


wherein A , L , W , X , R^1 , R^2 and m are defined as in Formula (I).

[0069] In the preferred embodiments of the invention, the compound is selected from the group consisting of the following compounds:



[0070] In another aspect, the invention provides compounds of Formula (II):



wherein:

A is a saturated or partially saturated optionally substituted 5, 6 or 7 membered ring;

----- represents a single bond or a double bond;

Z^1 and Z^2 are independently N or C when ----- represents a single bond, provided Z^1 and Z^2 are not both N; and

Z^1 and Z^2 are C when ----- represents a double bond;

each of R^1 and R^2 is independently H, or an optionally substituted member selected from the group consisting of C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C12 heteroaryl, C7-C12 arylalkyl, and C6-C12 heteroarylalkyl group, or halo, OR, NR_2 , $NROR$, $NRNR_2$, SR, SOR, SO_2R , SO_2NR_2 , $NRSO_2R$, $NRCONR_2$, $NRCSNR_2$, $NRC(=NR)NR_2$, $NRCOOR$, $NRCOR$, CN, COOR, $CONR_2$, OOCR, COR, or NO_2 ,

wherein each R is independently H or C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl,

and wherein two R on the same atom or on adjacent atoms can be linked to form a 3-8 membered ring, optionally containing one or more N, O or S;

and each R group, and each ring formed by linking two R groups together, is optionally substituted with one or more substituents selected from halo, =O, =N-CN, =N-OR', =NR', OR', NR'_2 , SR', SO_2R' , $SO_2NR'_2$, $NR'SO_2R'$, $NR'CONR'_2$, $NR'CSNR'_2$, $NR'C(=NR')NR'_2$, $NR'COOR'$, $NR'COR'$, CN, COOR', $CONR'_2$, OOCR', COR', and NO_2 , wherein each R' is independently H, C1-C6 alkyl, C2-C6 heteroalkyl, C1-C6 acyl, C2-C6 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-12 arylalkyl, or C6-12 heteroarylalkyl, each of which is optionally substituted with one or more groups selected from halo, C1-C4 alkyl, C1-C4 heteroalkyl, C1-C6 acyl, C1-C6 heteroacyl, hydroxy, amino, and =O;

and wherein two R¹ on the same atom or on adjacent atoms can be linked to form a 3-7 membered ring optionally containing up to three heteroatoms selected from N, O and S;

and R¹ can be =O, or two R¹ groups on the same atom or on adjacent connected atoms, can optionally be linked together to form a 3-8 membered cycloalkyl or heterocycloalkyl, which is optionally substituted;

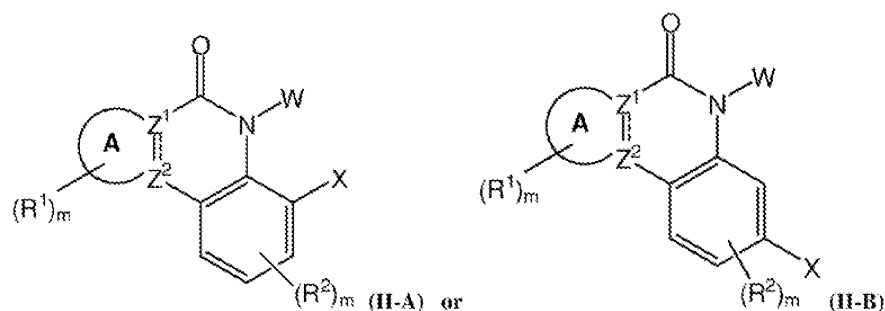
W is alkyl, heteroalkyl, aryl, heteroaryl, cycloalkyl, heterocyclyl, arylalkyl or heteroarylalkyl, each of which can be optionally substituted;

X is a polar substituent;

and each m is independently 0-3;

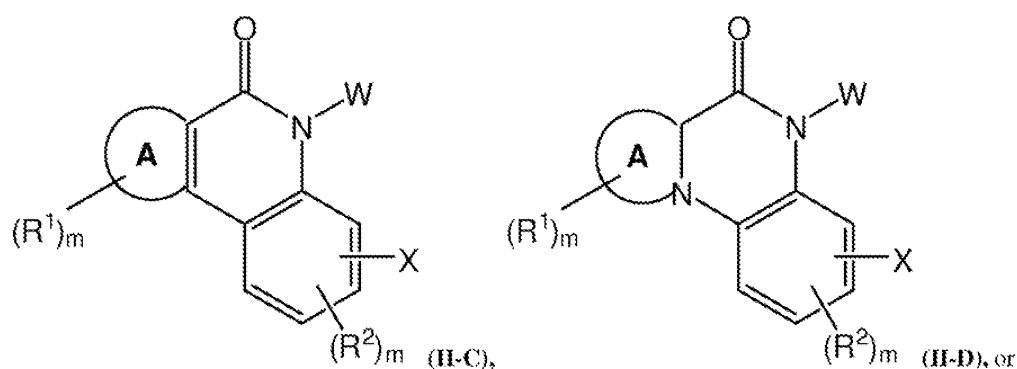
or a pharmaceutically acceptable salt or ester thereof.

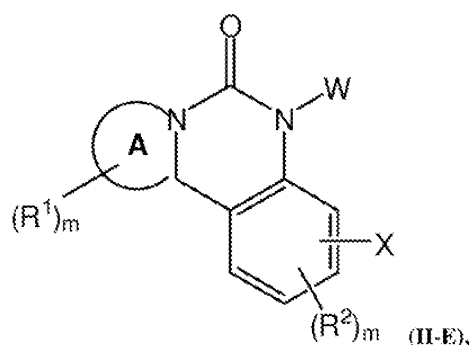
[0071] In some embodiments, the compound of Formula (II) has the structure of Formula (II-A) or (II-B):



or a pharmaceutically acceptable salt or ester thereof, wherein A, Z¹, Z², W, X, R¹, R² and m are defined as in Formula (II).

[0072] In other embodiments, the compound of Formula (II) has the structure of Formula (II-C), (II-D) or (II-E), or a pharmaceutically acceptable salt or ester thereof:





wherein A, W, X, R¹, R² and m are defined as in Formula (II).

[0073] It is understood that as described herein, compounds and embodiments of Formula (I) can include compounds of Formula (I-A), (I-B), (I-C), (I-D) or (I-E), and compounds of Formula (II) include compounds of Formula (II-A), (II-B), (II-C), (II-D) and (II-E).

[0074] In compounds of Formulae (I) and (II), A is a saturated or partially saturated optionally substituted 5-, 6- or 7-membered ring. The A-ring may be carbocyclic or heterocyclic ring that is saturated or partially saturated, and may be substituted by groups R¹ to the extent such groups make chemical sense.

[0075] In some embodiments of Formulae (I) and (II), Z¹ and Z² are independently N or C and --- represents a single bond, provided both of Z¹ and Z² are not N.

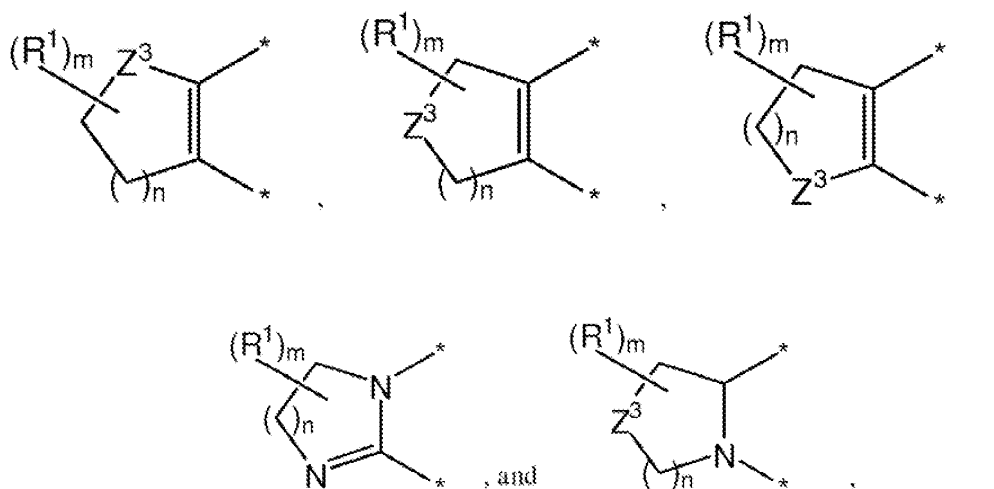
[0076] In other embodiments of Formulae (I) and (II), Z¹ and Z² are C and ----- represents a double bond.

[0077] In compounds of Formulae (I) and (II), the A-ring comprises an optionally substituted 5-7 membered ring. In some embodiments, the A-ring is an optionally substituted 5-7 membered ring carbocyclic ring. For example, ring A is an optionally substituted cyclopentane, cyclopentene, cyclohexane, cyclohexene, cycloheptane or cycloheptene ring.

[0078] In other embodiments, the A-ring comprises an optionally substituted 5-7 membered heterocyclic ring, containing at least one heteroatom selected from N, O, and S. In some such embodiments, one of Z¹ and Z² is N, and there are no additional heteroatoms in the A-ring. In other such embodiments, one of Z¹ and Z² is N, and there is an additional heteroatom selected from O, N and S in the A-ring. In certain embodiments, ring A is an optionally substituted dihydrofuran, tetrahydrofuran, dihydrothiophene, tetrahydrothiophene, dihydropyrrole, pyrrolidine, dihydropyran, tetrahydropyran, pyran, dihydrothiopyran, tetrahydrothiopyran, thiopyran, piperidine, dihydropyridine, tetrahydropyridine, imidazoline, thiazolidine, oxazolidine, dihydrothiazole, dihydrooxazole, morpholine, thiomorpholine, piperazine, dihydropyrimidine, azepine,

dihydroazepine, tetrahydroazepine, hexahydroazepine ring, homomorpholine, homothiomorpholine, dizepine, dihydrodiazepine, tetrahydrodiazepine, hexahydrodiazepine ring, oxepane, or thiooxepane ring.

[0079] Sometimes, the A-ring containing is selected from the group consisting of:



wherein Z^3 is CR^{12} , NR^1 , $S(=O)_p$, or O; n is 1-3; and p is 0-2.

[0080] In compounds of Formula (I), L is a linker selected from a bond, NR^3 , O, S, CR^4R^5 , $CR^4R^5-NR^3$, CR^4R^5-O- , and CR^4R^5-S . Where L is a two-atom linker, it can be attached to the ring system through either end, i.e., either the carbon atom or the heteroatom of $CR^3R^4-NR^5$, CR^3R^4-O- , and CR^3R^4-S can be attached to the ring, and the other atom is attached to L. In some embodiments, L is a bond, or a 1-2 atom linker, including $-N(R^3)-$, $-O-$, $-S-$, $-CH_2-N(R^3)-$, $-N(R^3)-CH_2-$, $-O-CH_2-$, $-CH_2-O-$, $-CH_2-S-$, $-S-CH_2-$, $-CMe_2-N(R^3)-$, $-CMe_2-O-$, $-N(R^3)-CMe_2$, $-O-CMe_2-$, and the like. In certain embodiments, L is selected from a bond, NH, NMe, and $CH_2-N(R^3)-$ or $-N(R^3)-CH_2-$, where R^3 is H or Me.

[0081] In some embodiments of Formula (I), L is NH or NMe. In other embodiments, L can be NAc, where Ac represents a C1-C10 acyl group, i.e., L is a group of the formula $N-C(=O)-R^z$, where R^z is H or a C1-C9 optionally substituted alkyl group. These can serve as pro-drugs for compounds where L is NH. In still other embodiments, L is a bond; in these embodiments, W is often an aryl or heteroaryl, which is optionally substituted.

[0082] In some embodiments of Formulae (I) and (II), W is selected from optionally substituted aryl, optionally substituted heteroaryl, optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted arylalkyl, and optionally substituted heteroarylalkyl. For example, W can be an optionally substituted phenyl, pyridyl, pyrimidinyl, or pyrazinyl group; or a

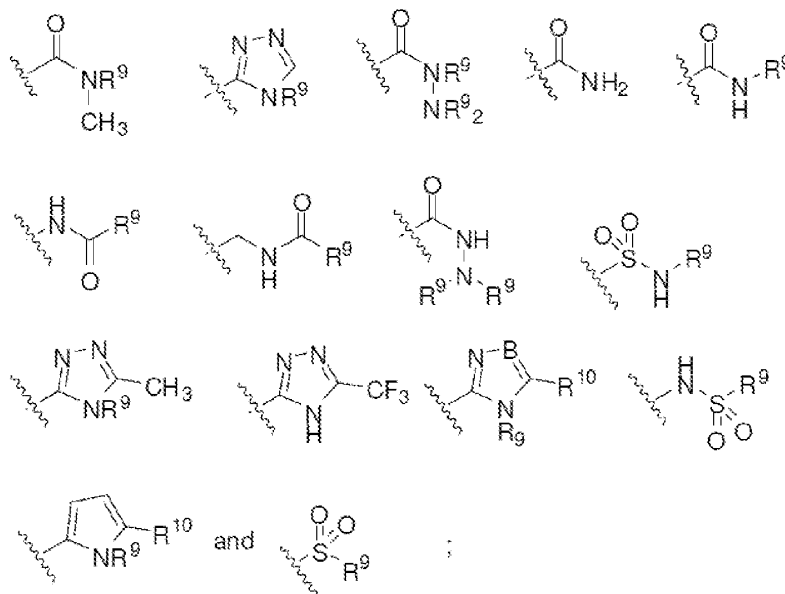
naphthyl, indole, benzofuran, benzopyrazole, benzothiazole, quinoline, isoquinoline, quinazoline or quinoxaline group. Suitable substituents for these groups include, but are not limited to, halo, C1-C4 alkyl, C2-C4 alkenyl or alkynyl, CN, OMe, COOMe, COOEt, CONH₂, CF₃, and the like, and typically the aryl group is substituted by up to 2 of these groups. In certain preferred embodiments, when W is aryl or heteroaryl, it is unsubstituted, or it is substituted by 1 or 2 substituents.

[0083] In some embodiments of Formulae (I) and (II), W is optionally substituted phenyl, optionally substituted heterocyclyl, or C1-C4 alkyl substituted with at least one member selected from the group consisting of optionally substituted phenyl, optionally substituted heteroalkyl, optionally substituted heteroaryl, halo, hydroxy and -NR''₂, where each R'' is independently H or optionally substituted C1-C6 alkyl; and two R'' taken together with the N to which they are attached can be linked together to form an optionally substituted 3-8 membered ring, which can contain another heteroatom selected from N, O and S as a ring member, and can be saturated, unsaturated or aromatic.

[0084] In some such compounds, W comprises at least one group of the formula -(CH₂)_p-NR^x₂, where p is 1-4, R^x is independently at each occurrence H or optionally substituted alkyl; and two R^x taken together with the N to which they are attached can be linked together to form an optionally substituted 3-8 membered ring, which can contain another heteroatom selected from N, O and S as a ring member, and can be saturated, unsaturated or aromatic.

[0085] In some embodiments, W can be aryl (e.g., phenyl), heterocyclic (e.g., pyrrolidine, piperidine, morpholine, piperazine, thiomorpholine), or heteroaryl (e.g., pyrrole, pyridine, pyrazine, pyrimidine, furan, thiophene, thiazole, isothiazole, thiadiazole, oxazole, isoxazole, imidazole, pyrazole, triazole, triazine, tetrazole and the like, each of which can be substituted. In some such embodiments, it is selected from phenyl, pyrrolidine, piperidine, piperazine, morpholine, and the like. In other embodiments, W can be arylalkyl or heteroarylalkyl, where the aryl and heteroaryl moieties of these groups are selected from the groups described above, attached to a C₁₋₆ and preferably a C₁₋₄ alkylene or heteroalkylene moiety. W can be substituted by a variety of substituents. In certain embodiments, W is an aryl ring substituted by a group of the formula -(CH₂)₀₋₄-NR^x₂, where each R^x can be H or C1-C4 alkyl, and can be substituted, and where two R^x can optionally cyclize into a ring. In some embodiments, this group is of the formula -(CH₂)₀₋₄-Az, where Az represents an azacyclic group such as pyrrolidine, piperidine, morpholine, piperazine, thiomorpholine, pyrrole, and the like. In some embodiments, this group is -(CH₂)₁₋₃-Az, where Az is 4-morpholinyl, 1-piperazinyl, 1-pyrrolidinyl, or 1-piperidinyl; -CH₂-CH₂-Az, where Az is 4-morpholinyl is one exemplary substituent for W, when W is substituted.

[0086] In some embodiments of Formulae (I) and (II), X is selected from the group consisting of COOR⁹, C(O)NR⁹-OR⁹, triazole, tetrazole (preferably linked to the phenyl ring via the carbon atom of the tetrazole ring), CN, imidazole, carboxylate, a carboxylate bioisostere,



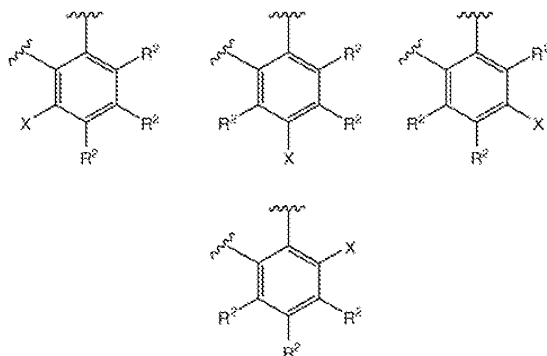
wherein each R⁹ is independently H or an optionally substituted member selected from the group consisting of alkyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, arylalkyl, cycloalkylalkyl, heterocycloalkylalkyl, and heteroarylalkyl,

and two R⁹ on the same or adjacent atoms can optionally be linked together to form an optionally substituted ring that can also contain an additional heteroatom selected from N, O and S as a ring member;

R¹⁰ is halo, CF₃, CN, SR, OR, NR₂, or R, where each R is independently H or optionally substituted C1-C6 alkyl, and two R on the same or adjacent atoms can optionally be linked together to form an optionally substituted ring that can also contain an additional heteroatom selected from N, O and S as a ring member; and B is N or CR¹⁰.

[0087] In compounds of Formulae (I) and (II), at least one polar substituent X may be at any position on the phenyl ring (C-ring), and the ring may include one, two, three or four polar substituents. In compounds of Formulae (I-A), (I-B), (II-A), and (II-B), the molecule contains at least one polar group, X, at the position indicated by the structure, and the ring may include one, two, three or four polar substituents. In certain embodiments, there is one polar group, X, and each R² is H, or up to two R² are substituents described herein other than H, such as, for example only, Me, Et, halo (especially F or Cl), MeO, CF₃, CONH₂, or CN. A polar group can be at any position on the

phenyl ring. In some embodiments, the phenyl ring is selected from the following options, which are oriented to match the orientation of Formula (I) herein, and depict the position of the polar substituent X:

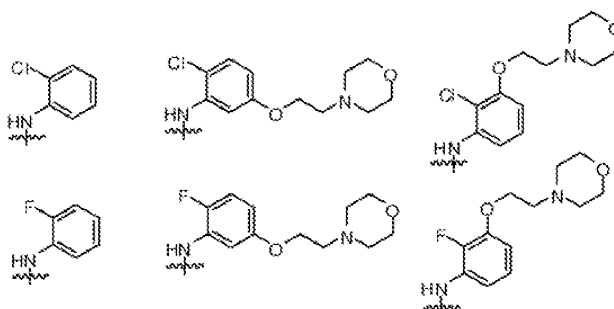


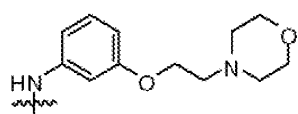
where X is a polar substituent and each R^2 is independently is selected from R^2 substituents, as defined above with respect to compounds of Formulae (I) and (II).

[0088] In some embodiments of the above-described compounds, the polar substituent X is located at position 4 on the phenyl ring. In alternative embodiments, the polar substituent X is located at position 3 on the phenyl ring. In certain embodiments, the polar substituent is a carboxylic acid or a tetrazole, and is at position 3 or 4 on the phenyl ring.

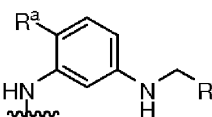
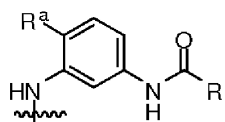
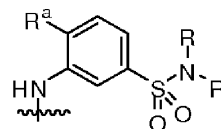
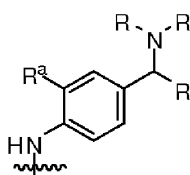
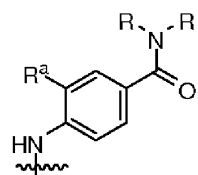
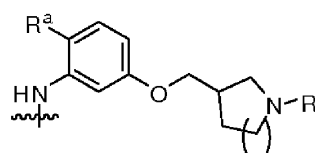
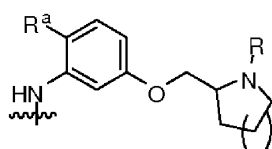
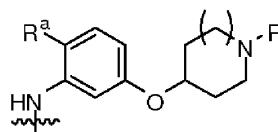
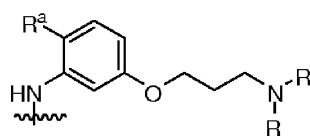
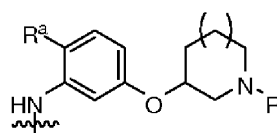
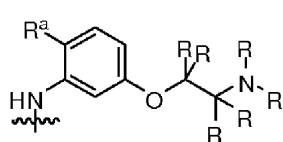
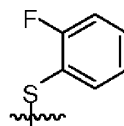
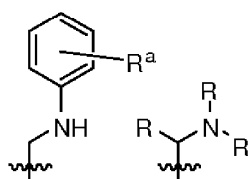
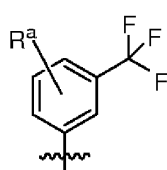
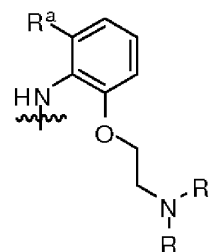
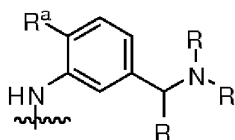
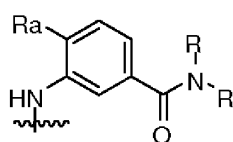
[0089] In some embodiments of these compounds, the phenyl ring (i.e., C-ring) is substituted by up to three additional substituents, in addition to the polar substituent X. Suitable substituents for the phenyl are described above. In some embodiments, these substituents are selected from halo, C1-C4 alkyl, C1-C4 haloalkyl, C1-C4 alkoxy, amino, C1-C4 alkylthio, and CN. In some embodiments, there is only one such substituent (i.e., m is 1), or there is no additional substituent besides the polar substituent X, i.e., m is 0.

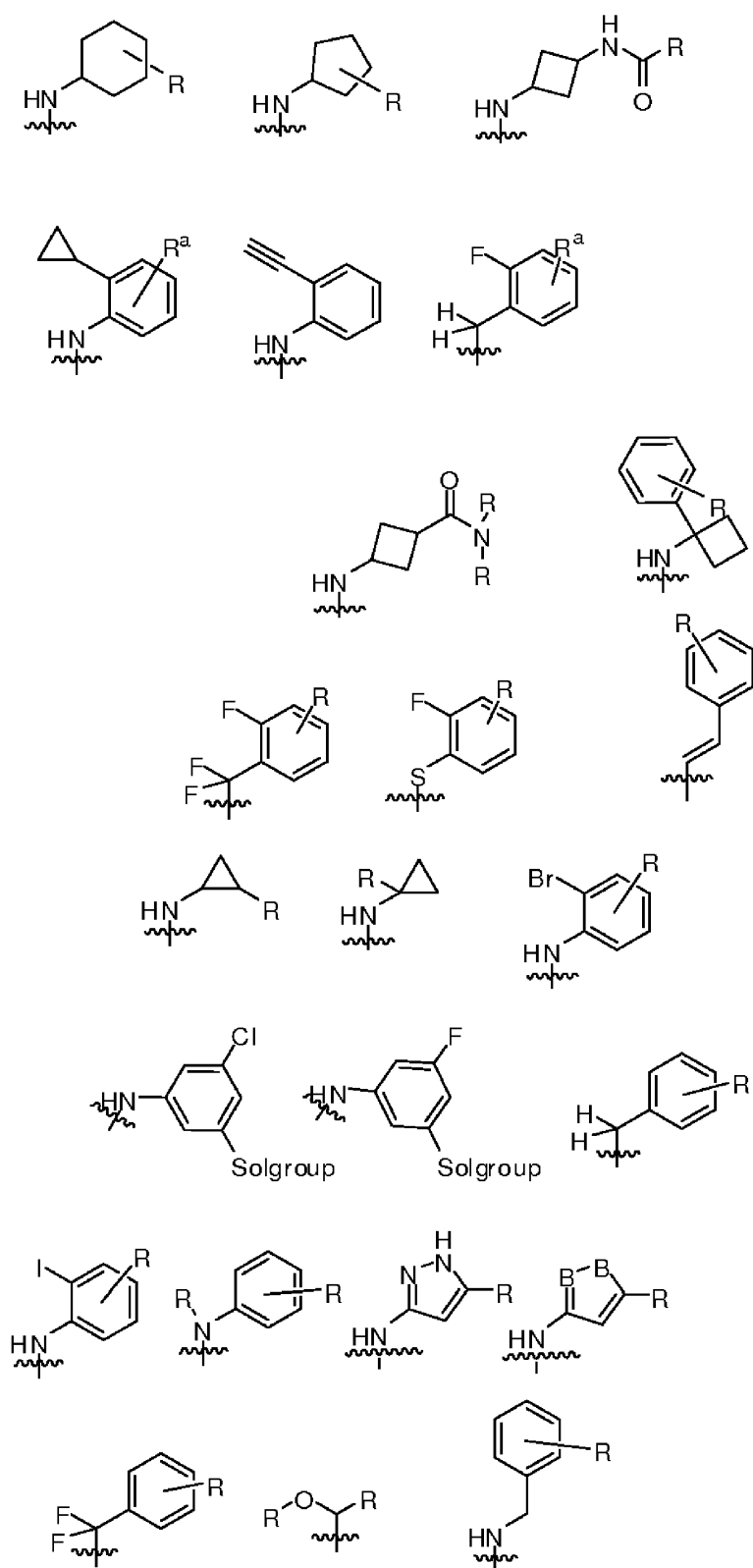
[0090] In some embodiments of Formula (I), $-L-W$ is selected from:

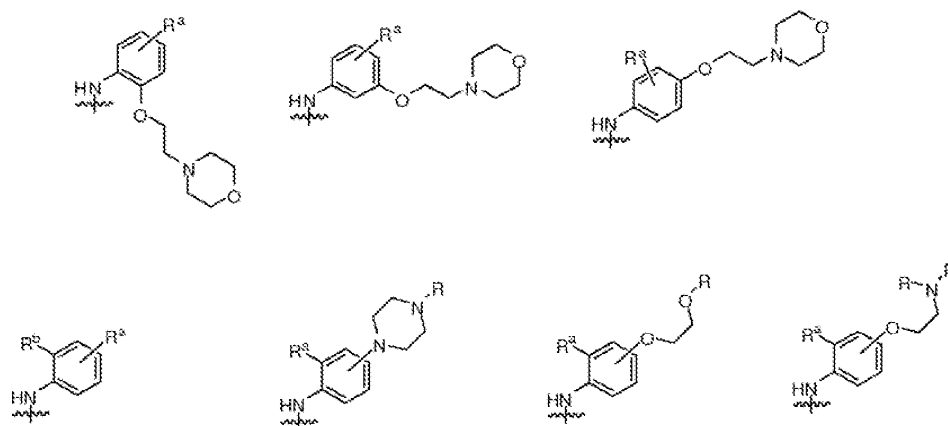




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wherein each R^a is independently H, Cl or F;

each R^b is independently Me, F, or Cl;

each R is independently selected from H, halo, C1-C4 alkyl, C1-C4 alkoxy, and C1-C4 haloalkyl, and two R groups on the same or adjacent connected atoms can optionally be linked together to form a 3-8 membered ring;

each B is N or CR;

and each Solgroup is a solubility-enhancing group.

[0091] In one aspect, the invention provides a method for treating hepatotropic virus infection in a human, which comprises administering to said human a therapeutically effective amount of one or combination thereof of these compounds.

[0092] Considering updated drugs in use today and their mechanism, it is found that anti-inflammatory drugs were also used to treat HBV. It is obvious that the need of dual action drug, anti-viral and anti-inflammation, is desirable. It is unexpectedly found that these compounds as a CK2 inhibitor, which is a dual-action drug, provide the efficacies in inhibition of viral replication and control of cytokines within homeostasis simultaneously.

[0093] In another aspect, the invention provides a pharmaceutical composition for treating hepatitis virus infection. The pharmaceutical compositions can comprise a compound of any of the formulae described herein, admixed with at least one pharmaceutically acceptable excipient or carrier. Frequently, the composition comprises at least two pharmaceutically acceptable excipients or carriers.

[0094] In a further aspect, the invention provides a use of one of these compounds for manufacturing a medicament for treating hepatitis virus infection in a human or an animal.

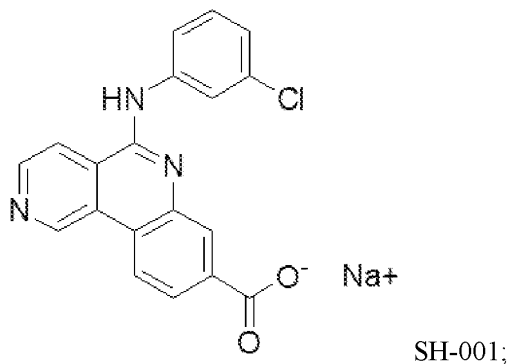
[0095] In the invention, it is ascertained that some virus infections can be treated using these compounds described herein.

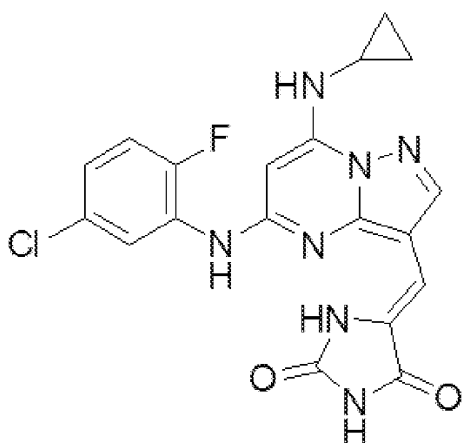
[0096] Particularly for hepatitis viruses, the virus infections are caused by hepatitis virus.

[0097] In the invention, the term “hepatitis virus” refers to hepatotropic viruses, which includes but not limited to Hepatitis A virus (HAV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Hepatitis D virus (HDV), and Hepatitis E virus (HEV). In one particular embodiment of the invention, the compounds of the invention are effective in treating an HBV infection. In another particular embodiment of the invention, the compounds of the invention are effective in treating an HDV infection.

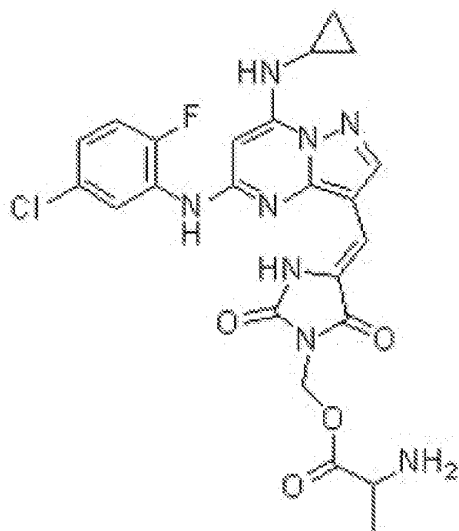
[0098] The terms “treat” and “treating” as used herein refer to ameliorating, alleviating, lessening, and removing symptoms of a disease or condition caused by a hepatitis virus infection. A candidate molecule or compound described herein may be in a therapeutically effective amount in a formulation or medicament, which is an amount that can lead to a biological effect, such as anti-virus effect, or lead to ameliorating, alleviating, lessening, or removing symptoms of a disease or condition, for example.

[0099] In preferred embodiments of the present invention, the compound is a compound of Formula (I) or (II) described in one of the lists of compounds provided herein, or a pharmaceutically acceptable salt of one of these compounds. The most preferred compounds are Silmitasertib also known as SH-001 (disclosed in US Patent No. 9,062,043), SH-002 (disclosed in US Patent No. 8,575,177), and SH-003 (disclosed in US Patent No. 8,575,177):





SH-002; and



SH-003.

[00100] Formulations and routes of administration

[00101] Any suitable formulation of a compound described above can be prepared for administration. Any suitable route of administration may be used, including, but not limited to, oral, parenteral, intravenous, intramuscular, transdermal, topical and subcutaneous routes. Depending on the subject to be treated, the mode of administration, and the type of treatment desired -- *e.g.*, prevention, prophylaxis, therapy; the compounds are formulated in ways consonant with these parameters. Preparation of suitable formulations for each route of administration are known in the art. A summary of such formulation methods and techniques is found in *Remington's Pharmaceutical Sciences*, latest edition, Mack Publishing Co., Easton, PA, which is incorporated herein by reference. The formulation of each substance or

of the combination of two substances will generally include a diluent as well as, in some cases, adjuvants, buffers, preservatives and the like. The substances to be administered can be administered also in liposomal compositions or as microemulsions.

[00102] For injection, formulations can be prepared in conventional forms as liquid solutions or suspensions or as solid forms suitable for solution or suspension in liquid prior to injection or as emulsions. Suitable excipients include, for example, water, saline, dextrose, glycerol and the like. Such compositions may also contain amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, such as, for example, sodium acetate, sorbitan monolaurate, and so forth.

[00103] Various sustained release systems for drugs have also been devised, and can be applied to compounds of the invention. See, for example, U.S. patent No. 5,624,677, the methods of which are incorporated herein by reference.

[00104] Systemic administration may also include relatively noninvasive methods such as the use of suppositories, transdermal patches, transmucosal delivery and intranasal administration. Oral administration is also suitable for compounds of the invention. Suitable forms include syrups, capsules, tablets, as is understood in the art.

[00105] For administration to animal or human subjects, the appropriate dosage of a compound described above often is 0.01-15 mg/kg, and sometimes 0.1-10 mg/kg. Dosage levels are dependent on the nature of the condition, drug efficacy, the condition of the patient, the judgment of the practitioner, and the frequency and mode of administration; however, optimization of such parameters is within the ordinary level of skill in the art.

[00106] The amount of each of these materials to be administered will vary with the route of administration, the condition of the subject, other treatments being administered to the subject, and other parameters. The therapeutic agents of the invention may, of course, cause multiple desired effects; and the amount of modulator to be used in combination with the therapeutic agent should be an amount that increases one or more of these desired effects. An amount is "effective to enhance a desired effect of the therapeutic agent", as used herein, if it increases by at least about 25% at least one of the desired effects of the therapeutic agent alone. Preferably, it is an amount that increases a desired effect of the therapeutic agent by at least 50% or by at least 100% (i.e., it doubles the effective activity of the therapeutic agent.) In some embodiments, it is an amount that increases a desired effect of the therapeutic agent by at least 200%.

[00107] When a compound or composition of the invention is used in combination with another agent or therapeutic agent, the present invention provides, for example,

simultaneous, staggered, or alternating treatment. Thus, the compound of the invention may be administered at the same time as an anti-virus agent or additional therapeutic agent, in the same pharmaceutical composition; the compound of the invention may be administered at the same time as the other agent, in separate pharmaceutical compositions; the compound of the invention may be administered before the other agent, or the other agent may be administered before the compound of the invention, for example, with a time difference of seconds, minutes, hours, days, or weeks.

[00108] The compound of the invention and the additional therapeutic agent may be administered in the same dosage form, e.g., both administered as intravenous solutions, or they may be administered in different dosage forms, e.g., one compound may be administered topically and the other orally. A person of ordinary skill in the art would be able to discern which combinations of agents would be useful based on the particular characteristics of the drugs and the cancer involved.

[00109] Additional therapeutic agents useful for therapy in combination with the compounds of the invention include the following types of agents and inhibitors:

[00110] Compounds of the invention can be prepared using available methods and reagents, based on the ordinary level of skill in the art and methods. The preparation of the compounds as previously described in WO2009061131, US7,956,064, US9,062,043 and US8,575,177.

[00111] Experiments on Antiviral Activity

[00112] Materials and Methods

[00113] Compounds

[00114] The compounds below were tested:

SH-001

SH-002

SH-003

[00115] Cell lines and DNA transfection.

[00116] a) HepG2.2.15 cells

[00117] Continuous HBV proliferation can be achieved in HepG2.2.15 cells (RRID:CVCL_L855) stably transfected with the HBV genome of the ayw subtype. HepG2.2.15 cells were maintained in Dulbecco's modified Eagle medium (DMEM; Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Thermo) plus 100 units of penicillin and 100 µg of streptomycin per ml (both from Invitrogen).

[00118] To test the effect of the compounds on HBV replication, the compounds were added to the medium at the indicated concentration and cultured for 48 h. The viruses were then collected from the supernatant. Q-PCR was used to detect HBV DNA as an index of efficiency of HBV replication. Next, the HBV HBsAg and HBeAg were examined by ELISA assay.

[00119] b) HuS-E/2 cells

HuS-E/2 cells that retain primary hepatocyte characteristics after prolonged culture were utilized for HDV infection. For HDV infection, HuS-E/2 cells were differentiated with 1% DMSO for 5-7 days, and HDV virus particles were added to infect and replicate in HuS-E/2 cells (MOI=10) as described by Huang et al. (J Virol. 2012 Sep;86(17):9443-53. Epub 2012 Jun 27) The HuS-E/2 cells are useful to assay infectivity of HDV, and screening of anti-HDV agents.

[00120] The compounds were added to the medium at the indicated concentration during infection with HDV for 18 h, then the infected cells were incubated in medium containing the compounds for further 48 h. Real-time PCR was performed to detect HDV RNA as an efficiency index of HDV infection.

[00121] Assays

[00122] a) Cell viability assay.

[00123] HepG2.2.15 and HuS-E/2 cells were seeded at 1×10^4 cells/well in a 96-well plate for 20 h, then the cells were treated with the tested compounds at various concentration (0-500 μ M) for 48 h. After the treatment, cell viability was examined using MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] assay (Sigma Chemical Co.). Briefly, a final concentration of 0.5 mg/ml MTT was added to each well and the samples were incubated for 1 h at 37°C, and then the supernatant was aspirated. The MTT-formazan crystals formed by metabolically viable cells were dissolved in 70 μ l of DMSO, and the absorbance at 550 nm was measured on a microplate reader.

[00124] b) Collection of HBV particles for detection of HBV DNA, HBeAg and HBsAg.

[00125] The culture medium from compound-treated HepG2.2.15 cells was clarified by centrifugation at 1,000 X g at 4°C for 10 min, and then the supernatant was layered on top of a 20% sucrose cushion (20% sucrose, 20 mM HEPES, pH 7.4, 0.1% bovine serum albumin [BSA]) and centrifuged at 197,000 X g for 3 h at 4°C to pellet the HBV particles. The particles were then concentrated 100 fold to detect HBV DNA.

[00126] c) DNA and RNA isolation, reverse transcription and real-time PCR

[00127] Total DNA was extracted with a Genomic DNA isolation kit (Nexttec Biotechnologie, Germany). Total RNA was isolated from cultured cells using TRIzol® reagent (Invitrogen). Reverse transcription was performed with the RNA templates, AMV reverse transcriptase (Roche), and oligo-dT primer. The products were subjected to real-time PCR with primer sets of specific genes and SYBR Green PCR Master Mix (Bio-Rad). The primer sets used for HBV DNA and HDV RNA were as described by Huang et al. (J Virol. 2012 Sep;86(17):9443-53. Epub 2012 Jun 27) The results were analyzed with the iCycler iQ real-time PCR detection system (Bio-Rad). Plasmid p1.3HBcl was prepared at 10-fold dilutions (2×10^4 - 2×10^9 copies/ml) to generate a standard curve in parallel PCR reactions.

[00128] **d) Enzyme-linked immunosorbent assay (ELISA)**

[00129] The HBsAg and HBeAg ELISA Kit (General Biologicals Corp.) were used to detect hepatitis B surface antigen (HBsAg) and hepatitis B e-antigen (HBeAg) with the protocol suggested by the manufacturer.

[00130] **Statistical analysis**

[00131] All values are expressed as mean $\pm$ S.E. Each value is the mean of at least three experiments in each compound *in vitro* experiments. Student's *t*-test is used for statistical comparison. * indicates that the values are significantly different from the control (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

[00132] **Experiment 1: Cell viability test with the compounds**

[00133] The compounds used were SH-001, SH-002, and SH-003. Before testing their potential antiviral effect on HBV and HDV infection, we first examined their toxicity on VeroE6 cells, HepG2.2.15 cells and HuS-E/2 immortalized human primary hepatocytes cells at concentration from 0 to 320 μ M as indicated. Our results showed the CC50 > 204.7 μ M for SH-001, CC50 > 61.3 μ M for SH-002, and CC50 > 204.7 μ M for SH-003 in VeroE6 cells (Figure 1). In HepG2.2.15 cells, the CC50 > 194.9 μ M for SH-001 and the CC50 > 164.1 μ M for SH-002 (Figure 2). In HuS-E/2 cells, The CC50 > 194.9 μ M for SH-001 and the CC50 > 164.1 μ M for SH-002 (Figure 3).

[00134] **Experiment 2: The inhibitory effect of SH-001 and SH-002 on HBV replication in HepG2.2.15 cells.**

[00135] To test the effect of the compounds on HBV replication, SH-001 and SH-002 were added to the cell culture medium at indicated concentrations and cultured for 48 h, and then the viruses are collected from supernatant. Q-PCR was performed to detect HBV DNA as an efficiency index of HBV replication. Moreover, the HBV HBsAg and HBeAg were

examined by ELISA assay. The results from ELISA assays showed that levels of HBsAg (Figure 4A and 5A) and HBeAg (Figure 4B and 5B) in the culture supernatant, which reflects the quantity of secreted HBV particles, was significantly decreased in the presence of SH-001 and SH-002. In addition, as shown in Figure 4C and 5C, HBV DNA was also repressed in the treatment of SH-001 and SH-002 in a dose-dependent manner. Taken together, the results showed that both SH-001 and SH-002 suppress HBV replication in HepG2.2.15 cells.

[00136] After treated HepG2.2.15 cells with 10 μ M SH-001 and 10 μ M SH-002, HBsAg levels were reduced to $39.85 \pm 1.78\%$ and $52.01 \pm 3.05\%$, respectively (Figure 4A and 5A), and the half-maximal inhibitory concentration (IC₅₀) were estimated to be 6.63 and 11.82 μ M. As shown in Figure 4B and 5B, when using 10 μ M SH-001 and 10 μ M SH-002, HBeAg levels were reduced to $53.66 \pm 3.02\%$ and $61.18 \pm 6.43\%$, and the IC₅₀ were estimated to be 12.0 and 167.6 μ M, respectively. A remarkable dose-dependent reduction in HBV DNA levels in culture medium was also observed: in the presence of 10 μ M SH-001 and 10 μ M SH-002, the amount of HBV DNA in drug-treated cells were decreased from 3.43×10^5 copies/ml in untreated cells to 5.51×10^4 copies/ml and 3.13×10^4 copies/ml, respectively (Fig. 4C and 5C)..

[00137] Experiment 3: The inhibitory effect of SH-001 and SH-002 on HDV replication in HuS-E/2 cells

[00138] To investigate the effect of the compounds on HDV infection, SH-001 and SH-002 were added to the medium at indicated concentrations during HDV infection in HuS-E/2 cells for 18 h, respectively. The infected cells were then washed and incubated in medium containing the tested compounds for further 48 h, and real-time PCR was used to detect HDV mRNA as an efficiency index of HDV infection. The results showed that SH-001 and SH-002 significantly inhibited HDV replication in HuS-E/2 human hepatocytes.

[00139] Using 2.5 and 5 μ M SH-001, HDV RNA levels were reduced to $28.8 \pm 22.8\%$ and $8.7 \pm 1.3\%$, respectively, compared to in its absence (Figure 6A) and the half-maximal inhibitory concentration (IC₅₀) was estimated to be approximately 0.1457 μ M. Using 0.1 and 0.2 μ M SH-002, HDV RNA levels were reduced to $53.1 \pm 16.8\%$ and $40.8 \pm 18.6\%$, respectively, compared to in its absence, and the IC₅₀ was estimated to be approximately 0.069 μ M, respectively (Figure 6B).

[00140] It is expressly predicted from the results that by inhibiting virus through a multiple mode of actions, the compounds should be able to develop a broad spectrum antiviral drug to treat other hepatotropic virus, such as HAV, HCV and HEV.

[00141] The *in vitro* data showed that CK2 inhibitors, demonstrated by SH-001 and SH-002, has the IC₅₀ is as low as 0.005 uM, whereas Lamifudine (a commonly used HBV drug) has the IC₅₀ of 0.358 uM, with the same human liver cell line as the host. The potency difference is significant. In conclusion, both SH-001 and SH-002 had good anti-viral performance. It is also suggested that these compounds are potent to develop broad spectrum anti-viral drugs.

[00142] All publications, patents, and patent documents cited herein above are incorporated by reference herein, as though individually incorporated by reference.

[00143] The invention has been described with reference to various specific and preferred embodiments and techniques. However, one skilled in the art will understand that many variations and modifications may be made while remaining within the spirit and scope of the invention.