Title: MODIFIED NUCLEOSIDE DERIVATIVES FOR TREATING FLAVIVIRIDAE INFECTIONS

Abstract: The present invention relates to the use of compounds of Formula (I) either as alone or in combination with immunomodulators for preventing or treating West Nile virus as well as infections caused by other viruses of the Flaviviridae family either as alone or in combination with immunomodulators.
MODIFIED NUCLEOSIDE DERIVATIVES FOR TREATING FLAVIVIRIDAE INFECTIONS

The invention relates to the field of antiviral therapy and in particular to nucleoside derivatives for diseases mediated by viruses of the family *Flaviviridae*. The invention relates to the use of nucleoside derivatives of formula I either as alone or in combination with immunomodulators for treatment or prophylaxis of diseases mediated by viruses of the family *Flaviviridae*; to the use of such compounds either as alone or in combination with immunomodulators for the preparation of medicaments for the treatment or prophylaxis of such diseases and to pharmaceutical compositions for such treatment or prophylaxis.

The flaviviridae comprise over 60 viruses. Arthropod vectors including ticks and mosquitoes disseminate many virus of this family. Twenty-six flaviviridae are known to produce human disease. Among the common serious human diseases caused by this family are: dengue fever, west Nile virus, St. Louis encephalitis, Japanese encephalitis, and yellow fever. Human hepatitis C virus HCV is mediated by a virus which has similarities to the flaviviruses and the animal pestiviruses. Although it is a member of the Flavivirus genus of, there is 45-49% homology with the pestiviruses sequence in the 5'-untranslated region while the hydrophobicity profile of the HCV polyprotein is more closely reminiscent of the flaviviruses.

West Nile virus an arthropod-borne flavivirus, has emerged in recent years as a deadly health threat to not only humans, but also to other animal species such as horses and birds. New York was the first area in North American to report cases of West Nile virus infections. West Nile virus infection in humans has been found previously only in Africa, the Middle East and Eastern Europe.

Among infected humans, approximately one in every 150 to 300 become ill with fever, myalgia and possible rash. Among those who are symptomatic, approximately 10-15% will have evidence of meningitis (headache, stiff neck) or encephalitis (change of mental status, peripheral neurologic abnormalities, muscle weakness). However, almost all fatalities have occurred among humans over the age of 50. The fatality rate among patients with central nervous system infection was 11% in New York. Fatalities have been due to prolonged central nervous system dysfunction requiring ventilatory support and leading to secondary complications. Prolonged neurologic symptoms have occurred in survivors of encephalitis.
Denque fever is endemic to the tropics, especially the Caribbean, the Pacific and some areas of West Africa. Denque is endemic in tropical areas where Stegomyia species are constantly active. *Aedes aegypti* is probably the most common vector in urban areas.

Dengue fever typically is manifested by a sudden onset of fever which often becomes biphasic, severe headache, pain behind the eyes, backache, chilliness and generalized muscle and joint pains. Severe manifestations of dengue fever include dengue hemorrhagic fever which can lead to plasma leakage from the vascular system and severe shock which has a poor prognosis.

Treatment of dengue fever typically is supportive with fluid replacement therapy when appropriate. Mortality in simple cases of dengue fever is low; however convalescence can be last for weeks. Mortality in dengue hemorrhagic fever ranges from 6-30% and infants are most susceptible.

The Japanese encephalitis antigenic complex includes Japanese encephalitis, West Nile Virus, Saint Louis encephalitis and Murray Valley encephalitis. These viruses exhibit approximately 60% sequence homology.

Saint Louis Encephalitis is one of the most common arbovirus transmitted diseases in the US and has caused major epidemics. The disease is most common in the Mississippi-Ohio river basin, Texas and Florida. The disease is transmitted by *Culex* spp. and can appear whenever standing water produce optimal conditions for mosquito breeding. Common clinical symptoms include encephalitis, aseptic meningitis and febrile headache. The fatality rate ranges from 2% in the young to about 22% in the elderly. Treatment is symptomatic and convalescence can be prolonged. Japanese encephalitis is occurs throughout the eastern seaboard of Asia and through parts of India and Sri Lanka. It is spread by rice-field breeding *Culex* spp. and the virus is amplified in swine. Initial symptoms are similar to those of St. Louis encephalitis. Treatment is supportive and convalescence is often protracted and sequelae are common in children. Case fatality has ranged from 20% to 70% during epidemics but the high rate is probably indicative of a lack of quality medical care.

Murray Valley encephalitis occurs primarily in Australia although scattered cases have been reported in New Guinea. The disease is transmitted by *Culex* spp. and birds and mammals appear to reservoirs for infection. The disease begins with headache, fever and generalized malaise which become progressively more severe.

The incidence of yellow fever has been reduced by the existence of an effective vaccine. Outbreaks of yellow fever are still common in South American and Asian countries.
and it is an important cause of viral hemorrhagic fever. The disease is transmitted by 
*Aedes* spp. Mortality is low but treatment of the disease is symptomatic.

These arboviral diseases have no effective treatment once an outbreak occurs. Thus 
there exists an urgent need for new therapies to ameliorate the effect of these arboviral 
disease. West Nile virus also affects various animal species including horses and birds 
and therapy to control arboviral outbreaks in animals also is desirable.

Therapies for most viral diseases are still relatively limited and in the absence of a 
prophylactic vaccine most therapy is symptomatic. Ribavirin and interferon alpha-2b 
are active against hepatitis C virus which is a member of the genus Flavivirus. West 
Nile virus is also a member of the genus Flavivirus. U. S. Patent Application No. 
2002/0061290 A1 (J. J. Rahal) describe the use of ribavirin, interferon-2b or 
combinations thereof for treatment of West Nile Virus and other Flavivirus-induced 
diseases.

Although ribavirin is used in the treatment of viral diseases, it unfortunately is has side 
effects which can limit its potential use. The major toxicity of ribavirin is hemolysis 
due to accumulation of ribavirin triphosphate within erythrocytes leading to a 
decreased life span. Such accumulation occurs due to inability of erythrocytes to 
deposphorylate the triphosphate. Inhibition of erythrocyte release from bone 
marrow occurs at high doses (30 mg/kg). Hemolysis is related to the dose and duration 
of therapy, and is reversible after discontinuation. Ribavirin is teratogenic and should 
not be given during, or within 6 months of pregnancy. Bioavailability is increased in 
patients with renal dysfunction. Nucleoside derivatives frequently exhibit high levels of 
biological activity; however, their practical utility is often limited by suboptimal 
physical properties and poor pharmacokinetics and the present invention relates also 
to nucleoside prodrugs with pharmacokinetic properties.

Thus a continuing need for to identify new antiviral therapies with high efficacy and 
reduced side effects.

Surprisingly a series of nucleic acid derivatives have now been identified which are 
efficacious against viruses in the family *Flaviviridae*. The utility of these compounds 
against Hepatitis C virus has been disclosed in U.S. Patent Application No. 10/167,106, 
filed June 11, 2002 which claims priority the United Kingdom application GB 
0114286.8 which was filed June 12, 2001. Prodrugs of an antiviral nucleoside have also 
been described in U.S. Patent Application 60/427,447 filed November 19, 2002. Both 
U.S. Patent Applications 10/167,106 and 60/427,447 are hereby incorporated in their 
totality by reference.
The present invention relates to the use of compounds of formula I

wherein:

R² and R³ are independently selected from the group consisting of hydrogen, COR⁵, CO₂R⁵ and COCH(R⁶)NHR⁷;

R⁴ independently of the other are selected from the group consisting of hydrogen, COR⁵, CO₂R⁵ and COCH(R⁶)NHR⁷, or R³ and R⁴ taken together are selected from the group consisting of CH₂, C(CH₃)₂, and CHPh;

R⁵ is independently selected from the group consisting of C₁₋₆ unbranched or branched alkyl, C₁₋₆ unbranched or branched alkenyl, C₁₋₆ unbranched or branched alkynyl, C₁₋₆ lower haloalkyl, C₃₋₈ cycloalkyl, alkyl substituted C₃₋₈ cycloalkyl, phenyl optionally independently substituted with one to three substituents selected from the group consisting of halo, lower alkyl, lower alkoxy, lower thioalkyl, lower alkyl sulfanyl, lower alkyl sulfonyl, nitro, and cyano, CH₂Ph wherein in phenyl ring is optionally substituted as described above and CH₂OPh wherein in phenyl ring is optionally substituted as described above;

R⁶ is selected from the group consisting of the side chains of naturally occurring amino acids and C₁₋₅ unbranched or branched alkyl;

R⁷ is selected from the group consisting of hydrogen, R⁴OOCO, and;

hydrates, solvates, clathrates and acid addition salts thereof; and,

pharmaceutical compositions comprising such compounds for the preparation of a medicament for the treating a viral infection mediated by a virus of family Flaviviridae;
with the proviso that the viral infection is not mediated by Hepatitis C Virus.

In one embodiment the invention relates to a compound according to formula I wherein \( R^1, R^2, R^3 \) and \( R^4 \) are as defined hereinabove as antiviral agent for the treatment of viral infections mediated by a virus of family \textit{Flaviviridae}; and to the use of such compound for the manufacture of a medicament for the treatment of viral infections mediated by a virus of family \textit{Flaviviridae}.

In another embodiment the present invention relates to a compound according to formula I wherein \( R^1, R^2, R^3 \) and \( R^4 \) are as defined hereinabove as antiviral agent for the treatment of viral infections mediated by dengue fever virus, West Nile virus, St. Louis encephalitis, Japanese encephalitis or Murray Valley encephalitis; and to the use of such compounds for the manufacture of a medicament for the treatment of viral infections mediated by dengue fever virus, West Nile virus, St. Louis encephalitis, Japanese encephalitis or Murray Valley encephalitis.

In another embodiment the present invention relates to a compound according to formula I wherein \( R^1, R^2, R^3 \) and \( R^4 \) are hydrogen as antiviral agent for the treatment of viral infections mediated by a virus of family \textit{Flaviviridae}; and to the use of such compounds for the manufacture of a medicament for the treatment of viral infections mediated by a virus of family \textit{Flaviviridae}.

In another embodiment the present invention relates to a compound according to formula I wherein \( R^1, R^2, R^3 \) and \( R^4 \) are hydrogen as antiviral agent for the treatment of viral infections mediated by a virus of family \textit{Flaviviridae} wherein said viral infections are mediated by dengue fever virus, West Nile virus, St. Louis encephalitis, Japanese encephalitis or Murray Valley; and to the use of such compounds for the manufacture of a medicament for the treatment of viral infections mediated by a virus of family \textit{Flaviviridae} wherein said viral infections are mediated by dengue fever virus, West Nile virus, St. Louis encephalitis, Japanese encephalitis or Murray Valley.

In one embodiment the present invention relates to the use of a compound according to formula I wherein \( R^1, R^2, R^3 \) and \( R^4 \) are as defined hereinabove for the treatment of viral infections mediated by a virus of family \textit{Flaviviridae}; and to the use of such compounds for the manufacture of a medicament for the treatment of viral infections mediated by a virus of family \textit{Flaviviridae}.

In one embodiment the present invention relates to the use of of a compound according to formula I wherein \( R^1, R^2, R^3 \) and \( R^4 \) in combination with an immune system modulator as antiviral agents for the treatment of viral infections mediated by
a virus of family Flaviviridae; and to the use of such compounds for the manufacture of a medicament for the treatment of viral infections mediated by a virus of family Flaviviridae.

In one embodiment the present invention relates to the use of a compound according to formula I wherein \( R^1, R^2, R^3 \) and \( R^4 \) are as defined hereinabove, in combination with an interferon, interleukin, tumor necrosis factor, colony stimulating factor, or a further antiviral agent for the treatment of viral infections mediated by a virus of family Flaviviridae; and to the use of such compounds in combination with an interferon, interleukin, tumor necrosis factor, colony stimulating factor, or a further antiviral agent for the manufacture of a medicament for the treatment of viral infections mediated by a virus of family Flaviviridae.

In one embodiment the present invention relates to the use of a compound according to formula I wherein \( R^1, R^2, R^3 \) and \( R^4 \) in combination with an interferon or chemically derivatized interferon for the treatment of viral infections mediated by a virus of family Flaviviridae; and to the use of such compounds in combination with an interferon or chemically derivatized interferon for the manufacture of a medicament for the treatment of viral infections mediated by a virus of family Flaviviridae.

In one embodiment the present invention relates to the use of a compound according to formula I wherein \( R^1, R^2, R^3 \) and \( R^4 \) are as defined hereinabove in combination with an interferon-\( \alpha \) or chemically derivatized interferon-\( \alpha \) for the treatment of viral infections mediated by a virus of family Flaviviridae; and to the use of such compounds in combination with an interferon-\( \alpha \) or chemically derivatized interferon-\( \alpha \) for the manufacture of a medicament for the treatment of viral infections mediated by a virus of family Flaviviridae.

In one embodiment of the invention there is provided a method to treat viral infections mediated by a virus of family Flaviviridae by administering to a animal in need thereof a therapeutically effective amount of a compound according to formula I wherein \( R^1, R^2, R^3 \) and \( R^4 \) are as defined hereinabove.

In another embodiment of the present invention there is provided a method to treat viral infections mediated by dengue fever virus, West Nile virus, St. Louis encephalitis, Japanese encephalitis or Murray Valley encephalitis by administering to an animal in need thereof a therapeutically effective amount of a compound according to formula I wherein \( R^1, R^2, R^3 \) and \( R^4 \) are as defined hereinabove.
In one embodiment of the present invention there is provided a pharmaceutical composition for treating a viral infections mediated by a virus of family *Flaviviridae* comprising a therapeutically effective quantity of a compound of formula 1

\[
\begin{align*}
R^1 & \text{ are independently selected from the group consisting of hydrogen, } CO\text{R}^5, CO_2\text{R}^5 \text{ and } COCH(R^6)\text{NHR}^7; \\
R^3 & \text{ and } R^4 \text{ independently of the other are selected from the group consisting of hydrogen, } CO\text{R}^5, CO_2\text{R}^5 \text{ and } COCH(R^6)\text{NHR}^7, \\
& \text{or } R^3 \text{ and } R^4 \text{ taken together are selected from the group consisting of CH}_2, C(CH_3)_2 \text{ and CPhH;} \\
R^5 & \text{ is independently selected from the group consisting of } C_{1-6} \text{ unbranched or branched alkyl, } C_{1-6} \text{ unbranched or branched alkenyl, } C_{1-6} \text{ unbranched or branched alkynyl, } C_{1-6} \text{ lower haloalkyl, } C_{3-8} \text{ cycloalkyl, alkyl substituted } C_{3-8} \text{ cycloalkyl, phenyl optionally independently substituted with one to three substituents selected from the group consisting of halo, lower alkyl, lower alkoxy, lower thioalkyl, lower alkyl sulfanyl, lower alkyl sulfonyl, nitro, and cyano, CH}_2\text{Ph wherein in phenyl ring is optionally substituted as described above and CH}_2\text{OPh wherein in phenyl ring is optionally substituted as described above; } \\
R^6 & \text{ is selected from the group consisting of the side chains of naturally occurring amino acids and } C_{1-5} \text{ unbranched or branched alkyl;} \\
R^7 & \text{ is selected from the group consisting of hydrogen, } R^5\text{OCO, and;}
\end{align*}
\]
hydrates, solvates, clathrates and acid addition salts thereof; and, in combination
with one or more pharmaceutically acceptable carriers and excipients;
pharmaceutical compositions comprising such compounds; or, for the
preparation of medicaments for such treatment;

with the proviso that the viral infection is not mediated by Hepatitis C Virus.

Compounds of the present invention include pro-drugs or bioprecursors of the parent
nucleoside and are converted in vivo to the compound of formula I wherein R¹, R², R³,
and R⁴ are hydrogen. Pro-drug derivatives include carboxylic esters in which the non-
carbonyl moiety of the ester group is selected from unbranched or branched alkyl (e.g.
methyl, n-propyl, n-butyl or t-butyl), alkoxyalkyl (e.g. methoxymethyl), aralkyl (e.g.
benzyl), aryloxyalkyl (e.g. phenoxyethyl), aryl (e.g. phenyl optionally substituted by
halogen, C₁₋₄ alkyl or C₁₋₄ alkoxy or amino); amino acid esters (e.g. L-valyl or L-
iso-leucyl) or pharmaceutically acceptable salts thereof. The preparation is carried out
according to known methods in the art, for example methods known from textbooks
on organic chemistry (e.g. from J. March (1992), "Advanced Organic Chemistry:

The phrase “a” or “an” entity as used herein refers to one or more of that entity; for
example, a compound refers to one or more compounds or at least one compound. As
such, the terms “a” (or “an”), “one or more”, and “at least one” can be used
interchangeably herein.

The phrase "as defined hereinabove" refers to the first definition provided in the
Detailed Description of the Invention.

The terms "optional" or "optionally" as used herein means that a described event or
circumstance may or may not occur, and that the description includes instances where
said event or circumstance occurs and instances in which it does not. For example,
"optionally substituted phenyl" means that the phenyl may or may not be substituted
and that the description includes both unsubstituted phenyl and phenyl wherein there
is substitution.

Compounds of the present invention may have asymmetric centers located on the side
chain of a carboxylic ester, amide or carbonate moiety that produce diastereomers
when linked to the nucleoside. All stereoisomers on the side chain of the compounds
of the instant invention are contemplated, either in admixture or in pure or
substantially pure form. The definition of the compounds according to the invention
embraces all possible stereoisomers and their mixtures. It also embraces the racemic
forms as well as the isolated optical isomers. The racemic forms can be resolved by
physical methods, such as, for example, fractional crystallization, separation or crystallization of diastereomERIC derivatives or separation by chiral column chromatography. The individual optical isomers can be obtained from the racemates by conventional methods, such as, for example, salt formation with an optically active acid followed by crystallization.

All configurational isomers of compounds of the present invention are contemplated, either in admixture or in pure or substantially pure form. The definition of compounds of the present invention embraces both cis and trans isomers of cycloalkyl rings.

The term “alkyl” as used herein denotes a unbranched or branched chain hydrocarbon residue containing 1 to 12 carbon atoms. The term “lower alkyl” denotes a unbranched or branched chain hydrocarbon residue containing 1 to 6 carbon atoms.

Representative lower alkyl groups include methyl, ethyl, propyl, i-propyl, n-butyl, i-butyl, t-butyl or pentyl.

The term “haloalkyl” as used herein denotes a unbranched or branched chain alkyl group as defined above wherein 1, 2, 3 or more hydrogen atoms are substituted by a halogen. Examples are 1-fluoromethyl, 1-chloromethyl, 1-bromomethyl, 1-iodomethyl, trifluoromethyl, trichloromethyl, tribromomethyl, triiodomethyl, 1-fluoroethyl, 1-chloroethyl, 1-bromoethyl, 1-idoethyl, 2-fluoroethyl, 2-chloroethyl, 2-bromoethyl, 2-idoethyl, 2,2-dichloroethyl, 3-bromopropyl or 2,2,2-trifluoroethyl.

The term “cycloalkyl” as used herein denotes a saturated carbocyclic ring containing 3 to 8 carbon atoms, i.e. cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl or cyclooctyl.

The term “alkenyl” as used herein denotes an unsubstituted [or substituted] hydrocarbon chain radical having from 2 to 7 carbon atoms, preferably from 2 to 4 carbon atoms, and having one or two olefinic double bonds, preferably one olefinic double bond. Examples are vinyl, 1-propenyl, 2-propenyl (allyl) or 2-butenyl (crotyl).

The term “alkynyl” as used herein denotes an unsubstituted hydrocarbon chain radical having from 2 to 7 carbon atoms, [preferably 2 to 4 carbon atoms], and having one or where possible two triple bonds[, preferably one triple bond]. Examples are ethynyl, 1-propynyl, 2-propynyl, 1-butylnyl, 2-butylnyl or 3-butylnyl.

The term “alkoxy” as used herein denotes an unsubstituted unbranched or branched chain alkoxy group, -O(alkyl), wherein the "alkyl" portion is as defined above such as methoxy, ethoxy, n-propoxyloxy, i-propoxy, n-butyloxy, i-butyloxy, t-butyloxy,
pentyloxy, hexyloxy, heptyloxy including their isomers. "Lower alkoxy" as used herein denotes an alkoxy group with a "lower alkyl" group as previously defined.

The term "alkylthio" as used herein denotes a unbranched or branched chain (alkyl)S-group wherein the "alkyl" portion is as defined above. Examples are methylthio, ethylthio, n-propylthio, i-propylthio, n-butylthio, i-butylthio or t-butylthio.

The term "alkoxyalkyl" as used herein denotes an alkoxy group as defined above which is bonded to an alkyl group as defined above. Examples are methoxymethyl, methoxyethyl, methoxypropyl, ethoxymethyl, ethoxyethyl, ethoxypropyl, propoxypropyl, methoxybutyl, ethoxybutyl, propoxyoxybutyl, butyloxybutyl, t-butyloxybutyl, methoxypentyl, ethoxypentyl, and propoxyxbpentyl including their isomers.

The term "hydroxyalkyl" as used herein denotes a unbranched or branched chain alkyl group as defined above wherein 1, 2, 3 or more hydrogen atoms are substituted by a hydroxy group. Examples are hydroxymethyl, 1-hydroxyethyl, 2-hydroxyethyl, 1-hydroxypropyl, 2-hydroxypropyl, 3-hydroxypropyl, hydroxyisopropyl, hydroxybutyl and the like.

The term "aryl" as used herein denotes an optionally substituted monocyclic or polycyclic-aromatic group comprising carbon and hydrogen atoms. Examples of suitable aryl groups include, but are not limited to, phenyl and naphtyl (e.g. 1-napthyl or 2-napthyl). Suitable substituents for aryl are selected from the group consisting of alkyl, alkenyl, alkylnyl, arlyloxy, cycloalkyl, acyl, acylamino, alkoxy, amino, alkylamino, dialkylamino, halogen, haloalkyl, hydroxy, nitro and cyanoo.

The term "acyl" ("alkylcarbonyl") as used herein denotes a group of formula C(=O)R wherein R is hydrogen, unbranched or branched alkyl containing 1 to 7 carbon atoms or a phenyl group. Most preferred acyl groups are those wherein R is hydrogen, an branched or unbranched alkyl chain or containing 1 to 6 carbon atoms or an optionally substituted phenyl group.

The term halogen stands for fluorine, chlorine, bromine or iodine, preferably fluorine, chlorine, bromine.

The term "amino acid" as used herein refers to naturally occurring amino acids, as well as to optical isomers (enantiomers and diastereomers), synthetic analogs and derivatives thereof. α-Amino acids comprise a carbon atom bonded to a carboxyl group, an amino group, a hydrogen atom and a unique "side chain" group. The side chains of naturally occurring amino acids are well known and include hydrogen, alkyl,
hydroxyalkyl, thioalkyl, alkylthioalkyl, branched alkyl, carboxyalkyl, carboxamidoalkyl, aminoalkyl, arylalkyl, and heteroaryalkyl moieties. The term "naturally occurring amino acids" means the L-isomers of the naturally occurring amino acids. The naturally occurring amino acids are glycine, alanine, valine, leucine, isoleucine, serine, methionine, threonine, phenylalanine, tyrosine, tryptophan, cysteine, proline, histidine, aspartic acid, asparagine, glutamic acid, glutamine, γ-carboxyglutamic acid, arginine, ornithine and lysine.

The term "chemically-derivatized interferon" as used herein refers to an interferon molecule covalently linked to a polymer which alters the physical and/or pharmacokinetic properties of the interferon. A non-limiting list of such polymers include polyalkylene oxide homopolymers such as polyethylene glycol (PEG) or polypropylene glycol (PPG), polyoxyethyleneated polyols, copolymers thereof and block copolymers thereof, provided that the water solubility of the block copolymers is maintained. One skilled in the art will be aware of numerous approaches to linking the polymer and interferon (for example, see A. Kozlowski and J. M. Harris J. Control. Release 2001 72(1-3):217-24). A non-limiting list of chemically derivatized IFNα contemplated in the present patent includes peginterferon-α-2a (PEGASYS®) and peginterferon-α-2b (PEGINTRON®).

Compounds of formula I which are basic can form pharmaceutically acceptable salts with inorganic acids such as hydrohalic acids (e.g. hydrochloric acid and hydrobromic acid), sulphuric acid, nitric acid and phosphoric acid, and the like, and with organic acids (e.g. with acetic acid, tartaric acid, succinic acid, fumaric acid, maleic acid, malic acid, salicylic acid, citric acid, methanesulphonic acid and p-toluene sulphonylic acid, and the like). The formation and isolation of such salts can be carried out according to methods known in the art.

The term "solvate" as used herein means a compound of the invention or a salt, thereof, that further includes a stoichiometric or non-stoichiometric amount of a solvent bound by non-covalent intermolecular forces. Preferred solvents are volatile, non-toxic, and/or acceptable for administration to humans in trace amounts.

The term "hydrate" as used herein means a compound of the invention or a salt thereof, that further includes a stoichiometric or non-stoichiometric amount of water bound by non-covalent intermolecular forces.
The term "clathrate" as used herein means a compound of the invention or a salt thereof in the form of a crystal lattice that contains spaces (e.g., channels) that have a guest molecule (e.g., a solvent or water) trapped within.

While nucleoside derivatives of the present invention are optimized for delivery across the gastrointestinal mucosa, these compounds can be efficacious when administered by other routes of administration including continuous (intravenous drip) topical parenteral, intramuscular, intravenous, subcutaneous, transdermal (which may include a penetration enhancement agent), buccal, nasal and suppository administration, among other routes of administration. Oral administration can be in the form of tablets, coated tablets, dragées, hard and soft gelatine capsules, solutions, emulsions, syrups, or suspensions.

For the manufacture of pharmaceutical preparations, the nucleoside derivatives, as well as their pharmaceutically useable salts, can be formulated with a therapeutically inert, inorganic or organic excipient for the production of tablets, coated tablets, dragées, hard and soft gelatine capsules, solutions, emulsions or suspensions. The compounds of formula I can be formulated in admixture with a pharmaceutically acceptable carrier. For example, the compounds of the present invention can be administered orally as pharmacologically acceptable salts. Because the compounds of the present invention are mostly water soluble, they can be administered intravenously in physiological saline solution (e.g., buffered to a pH of about 7.2 to 7.5). Conventional buffers such as phosphates, bicarbonates or citrates can be used in the present compositions. Suitable excipients for tablets, coated tablets, dragées, and hard gelatin capsules are, for example, lactose, corn starch and derivatives thereof, talc, and stearic acid or its salts. If desired, the tablets or capsules may be enteric-coated or sustained release by standard techniques. Suitable excipients for soft gelatine capsules are, for example, vegetable oils, waxes, fats, semi-solid and liquid polyols. Suitable excipients for injection solutions are, for example, water, saline, alcohols, polyols, glycerine or vegetable oils. Suitable excipients for suppositories are, for example, natural and hardened oils, waxes, fats, semi-liquid or liquid polyols. Suitable excipients for solutions and syrups for enteral use are, for example, water, polyols, saccharose, invert sugar and glucose. The pharmaceutical preparations can also contain preservatives, solubilizers, stabilizers, wetting agents, emulsifiers, sweeteners, colorants, flavorants, salts for adjustment of the osmotic pressure, buffers, masking agents or antioxidants. The pharmaceutical preparations may also contain other therapeutically active agents known in the art.
Other suitable pharmaceutical carriers and their formulations are described in
pharmaceutical formulations containing a compound of the present invention are
described in Examples 6-8. A skilled formulation scientist may modify the
formulations within the teachings of the specification to provide numerous
formulations for a particular route of administration without rendering the
compositions of the present invention unstable or compromising their therapeutic
activity.

In particular, the modification of the present compounds to render them more soluble
in water or other vehicle, for example, may be easily accomplished by minor
modifications (salt formulation, esterification, etc.), which are well within the ordinary
skill in the art. It is also well within the ordinary skill of the art to modify the route of
administration and dosage regimen of a particular compound in order to manage the
pharmacokinetics of the present compounds for maximum beneficial effect in
patients.

The term "therapeutically effective amount" as used herein means an amount required
to reduce symptoms of the disease in an individual. That dosage can vary within wide
limits and will, of course, be adjusted to the individual requirements in each particular
case. For oral administration, a daily dosage of between about 0.01 and about 100
mg/kg body weight per day should be appropriate in monotherapy and/or in
combination therapy. A preferred daily dosage is between about 0.1 and about 500
mg/kg body weight, more preferred 0.1 and about 100 mg/kg body weight and most
preferred 1.0 and about 100 mg/kg body weight per day. A typical preparation will
contain from about 5% to about 95% active compound (w/w). The daily dosage can
be administered as a single dosage or in divided dosages, typically between 1 and 5
dosages per day.

The term "animals" as used herein include mammals, e.g., humans, companion
animals e.g., dogs and cats, laboratory animals, e.g., rats and mice, and farm animals,
e.g., swine, horses and cows. Animals include agronomically important avian species
e.g., chickens, ducks and turkeys, companion and wild avian species.

In another embodiment of the invention, the active compound or a salt can be
administered in combination with another antiviral agent, such as an anti-hepatitis
agent. When the active compound or its derivative or salt are administered in
combination with another antiviral agent the activity may be increased over the parent
compound. This can easily be assessed by preparing the derivative and testing its anti-HCV activity according to the method described herein.

It will be understood that references herein to treatment extend to prophylaxis as well as to the treatment of existing conditions, and that the treatment of animals includes the treatment of humans as well as other animals. Furthermore, treatment of a HCV infection, as used herein, also includes treatment or prophylaxis of a disease or a condition associated with or mediated by HCV infection, or the clinical symptoms thereof.

The pharmaceutical preparations are preferably in unit dosage forms. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form.

The nucleoside derivatives or the medicaments thereof may be used in monotherapy or combination therapy, i.e. the treatment may be in conjunction with the administration of one or more additional therapeutically active substance(s), for example, an immune system modulator such as an interferon, interleukin, tumor necrosis factor or colony stimulating factor; an antiviral agent or an anti-inflammatory agent. When the treatment is combination therapy, such administration may be concurrent or sequential with respect to that of the nucleoside derivatives. Concurrent administration, as used herein thus includes administration of the agents at the same time or at different times.

It will be understood that references herein to treatment extend to prophylaxis as well as to the treatment of existing conditions, and that the treatment of animals includes the treatment of humans as well as other animals. Furthermore, treatment of a Hepatitis C Virus (HCV) infection, as used herein, also includes treatment or prophylaxis of a disease or a condition associated with or mediated by Hepatitis C Virus (HCV) infection, or the clinical symptoms thereof.

Example 1

Antiviral Assays

Antiviral assays were run using the procedure of Sidwell and Hoffman (Appl Microbiol. 1971 22:797-801). These assays were previously utilized to establish the antiviral

EXAMPLE 2
Composition for Oral Administration

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<thead>
<tr>
<th>Ingredient</th>
<th>% wt./wt.</th>
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<tr>
<td>Active ingredient</td>
<td>20.0%</td>
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<tr>
<td>Lactose</td>
<td>79.5%</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>0.5%</td>
</tr>
</tbody>
</table>

The ingredients are mixed and dispensed into capsules containing about 100 mg each; one capsule would approximate a total daily dosage.

EXAMPLE 3
Composition for Oral Administration

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% wt./wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active ingredient</td>
<td>20.0%</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>0.5%</td>
</tr>
<tr>
<td>Crosscarmellose sodium</td>
<td>2.0%</td>
</tr>
<tr>
<td>Lactose</td>
<td>76.5%</td>
</tr>
<tr>
<td>PVP (polyvinylpyrrolidone)</td>
<td>1.0%</td>
</tr>
</tbody>
</table>

The ingredients are combined and granulated using a solvent such as methanol. The formulation is then dried and formed into tablets (containing about 20 mg of active compound) with an appropriate tablet machine.
EXAMPLE 4

Composition for Oral Administration

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
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<tbody>
<tr>
<td>Active compound</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Fumaric acid</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Methyl paraben</td>
<td>0.15 g</td>
</tr>
<tr>
<td>Propyl paraben</td>
<td>0.05 g</td>
</tr>
<tr>
<td>Granulated sugar</td>
<td>25.5 g</td>
</tr>
<tr>
<td>Sorbitol (70% solution)</td>
<td>12.85 g</td>
</tr>
<tr>
<td>Veegum K (Vanderbilt Co.)</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Flavoring</td>
<td>0.035 ml</td>
</tr>
<tr>
<td>Colorings</td>
<td>0.5 mg</td>
</tr>
<tr>
<td>Distilled water</td>
<td>q.s. to 100 ml</td>
</tr>
</tbody>
</table>

The ingredients are mixed to form a suspension for oral administration.

EXAMPLE 5

Composition for Parenteral Administration

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% wt./wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active ingredient</td>
<td>0.25 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>q.s. to make isotonic</td>
</tr>
<tr>
<td>Water for injection to</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

The active ingredient is dissolved in a portion of the water for injection. A sufficient quantity of sodium chloride is then added with stirring to make the solution isotonic. The solution is made up to weight with the remainder of the water for injection, filtered through a 0.2 micron membrane filter and packaged under sterile conditions.

EXAMPLE 6

Composition for Suppository

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% wt./wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active ingredient</td>
<td>1.0%</td>
</tr>
<tr>
<td>Polyethylene glycol 1000</td>
<td>74.5%</td>
</tr>
<tr>
<td>Polyethylene glycol 4000</td>
<td>24.5%</td>
</tr>
</tbody>
</table>
The ingredients are melted together and mixed on a steam bath, and poured into molds containing 2.5 g total weight.

**EXAMPLE 7**

**Topical Formulation**

<table>
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<tr>
<th>Ingredients</th>
<th>grams</th>
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<td>Active compound</td>
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</tr>
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<td>Span 60</td>
<td>2</td>
</tr>
<tr>
<td>Tween 60</td>
<td>2</td>
</tr>
<tr>
<td>Mineral oil</td>
<td>5</td>
</tr>
<tr>
<td>Petrolatum</td>
<td>10</td>
</tr>
<tr>
<td>Methyl paraben</td>
<td>0.15</td>
</tr>
<tr>
<td>Propyl paraben</td>
<td>0.05</td>
</tr>
<tr>
<td>BHA (butylated hydroxy anisole)</td>
<td>0.01</td>
</tr>
<tr>
<td>Water</td>
<td>q.s. 100</td>
</tr>
</tbody>
</table>

All of the ingredients, except water, are combined and heated to about 60°C with stirring. A sufficient quantity of water at about 60°C is then added with vigorous stirring to emulsify the ingredients, and water then added q.s. about 100 g.

**Nasal Spray Formulations**

Several aqueous suspensions containing from about 0.025-0.5 percent active compound are prepared as nasal spray formulations. The formulations optionally contain inactive ingredients such as, for example, microcrystalline cellulose, sodium carboxymethylcellulose, dextrose, and the like. Hydrochloric acid may be added to adjust pH. The nasal spray formulations may be delivered via a nasal spray metered pump typically delivering about 50-100 microliters of formulation per actuation. A typical dosing schedule is 2-4 sprays every 4-12 hours.

The features disclosed in the foregoing description, or the following claims, or the accompanying drawings, expressed in their specific forms or in terms of a means for performing the disclosed function, or a method or process for attaining the disclosed result, as appropriate, may, separately, or in any combination of such features, be utilized for realizing the invention in diverse forms thereof.

The foregoing invention has been described in some detail by way of illustration and example, with reference to the specific embodiments for purposes of clarity and understanding. It will be obvious to one of skill in the art that changes and
modifications may be made and equivalents substituted without departing from the true spirit and scope of the invention. Therefore, it is to be understood that the above description is intended to be illustrative and not restrictive. Many modifications may be made to adapt a particular situation, material, composition of matter, process, or process step or steps, to the objective spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

All patents, patent applications and publications cited in this application are hereby incorporated by reference in their entirety for all purposes to the same extent as if each individual patent, patent application or publication were so individually denoted.
Claims

1. The use of compounds of formula I

![Chemical Structure Diagram]

wherein:

5. $R^1$ and $R^2$ are independently selected from the group consisting of hydrogen, COR, CO$_2$R, and COCH(R$^6$)NR$^7$;

$R^3$ and $R^4$ independently of the other are selected from the group consisting of hydrogen, COR, CO$_2$R, and COCH(R$^6$)NR$^7$, or $R^3$ and $R^4$ taken together are selected from the group consisting of CH$_2$, C(CH$_3$)$_2$, and CHPh;

$R^5$ is independently selected from the group consisting of C$_{1-6}$ unbranched or branched alkyl, C$_{1-6}$ unbranched or branched alkenyl, C$_{1-6}$ unbranched or branched alkynyl, C$_{1-6}$ lower haloalkyl, C$_{3-8}$ cycloalkyl, alkyl substituted C$_{3-8}$ cycloalkyl, phenyl optionally independently substituted with one to three substituents selected from the group consisting of halo, lower alkyl, lower alkoxy, lower thioalkyl, lower alkyl sulfanyl, lower alkyl sulfonyl, nitro, and cyano, CH$_2$Ph wherein in phenyl ring is optionally substituted as described above and CH$_2$OPh wherein in phenyl ring is optionally substituted as described above;

$R^6$ is selected from the group consisting of the side chains of naturally occurring amino acids and C$_{1-5}$ unbranched or branched alkyl;

$R^7$ is selected from the group consisting of hydrogen, R$^5$OCO, and;

hydrates, solvates, clathrates and acid addition salts thereof;
for the preparation of a medicament for the treating a viral infection mediated by a virus of family Flaviviridae;

with the proviso that the viral infection is not mediated by Hepatitis C Virus.

2. The use according to claim 1, wherein said viral infections are mediated by dengue fever virus, West Nile virus, St. Louis encephalitis virus, Japanese encephalitis virus or Murray Valley encephalitis virus.

3. The use according to claim 1 wherein R1, R2, R3 and R4 are hydrogen.

4. The use according to claim 1 wherein said viral infections are mediated by dengue fever virus, West Nile virus, St. Louis encephalitis virus, Japanese encephalitis virus or Murray Valley encephalitis virus.

5. The use of a compound of formula I as defined in claim 1 for the preparation of a medicament according to claim 1 further comprising an immune system modulator.

6. The use according to claim 5 wherein the immune system modulator is an interferon, interleukin, tumor necrosis factor or colony stimulating factor, an antiviral agent or an anti-inflammatory agent.

7. The use according to claim 6 wherein the immune system modulator is an interferon or chemically derivatized interferon.

8. The use according to claim 7 wherein the immune system modulator is interferon-α or chemically derivatized interferon-α.

9. A pharmaceutical composition for treating a viral infection mediated by a virus of family Flaviviridae comprising a therapeutically effective quantity of a compound of formula I as defined in claim 1.

10. A method according to treating a viral infection mediated by a virus of family Flaviviridae by administering to an animal in need thereof a therapeutically effective amount of a compound according to formula I as defined in claim 1.

* * * * * * *
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/7068 A61P31/14

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tbody>
<tr>
<td>X</td>
<td>WO 02/100415 A (HOFFMANN LA ROCHE) 19 December 2002 (2002-12-19) cited in the application</td>
<td>9</td>
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<td>Y</td>
<td>WO 02/32920 A (PHARMASSET LTD; STUYVER LIEVEN (US); WATANABE KYOICHI A (US)) 25 April 2002 (2002-04-25) claim 27</td>
<td>1-8, 10</td>
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<td>A</td>
<td>EP 0 371 366 A (SYNTEX INC) 6 June 1990 (1990-06-06) claims 1-22</td>
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</table>

[X] Patient family members are listed in annex.

Further documents are listed in the continuation of box C.

Special categories of cited documents:

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

*E* earlier document but published on or after the international filing date

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

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

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

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

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

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

*Y* document member of the same patent family

Date of the actual completion of the International search: 28 May 2004

Date of mailing of the International search report: 09/06/2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentboulevard 2 NL - 2280 HV Rijswijk
Tel. (+31-70) 340-3049, Tx. 31 651 epo nl, Fax (+31-70) 340-3016

Authorized officer

Siatou, E
# INTERNATIONAL SEARCH REPORT

**Box I** Observations where certain claims were found unsearable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. **X** Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
   
   Although claim 10 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. **☐** Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. **☐** Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II** Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. **☐** As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. **☐** As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. **☐** As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. **☐** No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

1. **☐** The additional search fees were accompanied by the applicant's protest.

2. **☐** No protest accompanied the payment of additional search fees.
<table>
<thead>
<tr>
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<td>WO 02100415 A</td>
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<td>US 2003124512 A1</td>
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<td>DK 582489 A</td>
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