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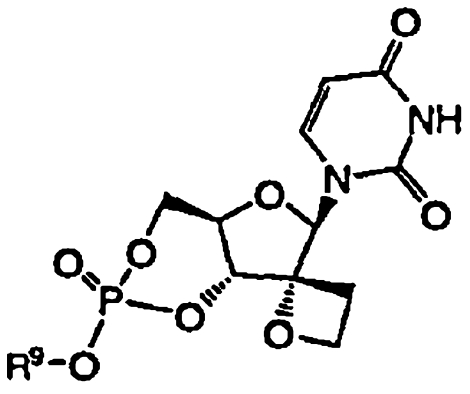
54 **Titre : Uracyl spirooxetane nucleosides.**

57 **Abrégé :**

The present invention relates to compounds of the formula I



(I)

including any possible stereoisomers thereof, wherein R9 has the meaning as defined herein, or a pharmaceutically acceptable salt or solvate thereof. The present invention also relates to processes for preparing said compounds, pharmaceutical compositions containing them and their use, alone or in combination with other HCV inhibitors, in HCV therapy.

Uracyl Spirooxetane Nucleosides

Background of the Invention

This invention relates to spirooxetane nucleosides and nucleotides that are inhibitors of the hepatitis C virus (HCV).

HCV is a single stranded, positive-sense RNA virus belonging to the *Flaviviridae* family of viruses in the hepacivirus genus. The NS5B region of the RNA polygene encodes a RNA dependent RNA polymerase (RdRp), which is essential to viral replication. Following the initial acute infection, a majority of infected individuals develop chronic hepatitis because HCV replicates preferentially in hepatocytes but is not directly cytopathic. In particular, the lack of a vigorous T-lymphocyte response and the high propensity of the virus to mutate appear to promote a high rate of chronic infection. Chronic hepatitis can progress to liver fibrosis, leading to cirrhosis, end-stage liver disease, and HCC (hepatocellular carcinoma), making it the leading cause of liver transplantations. There are six major HCV genotypes and more than 50 subtypes, which are differently distributed geographically. HCV genotype 1 is the predominant genotype in Europe and in the US. The extensive genetic heterogeneity of HCV has important diagnostic and clinical implications, perhaps explaining difficulties in vaccine development and the lack of response to current therapy.

Transmission of HCV can occur through contact with contaminated blood or blood products, for example following blood transfusion or intravenous drug use. The introduction of diagnostic tests used in blood screening has led to a downward trend in post-transfusion HCV incidence. However, given the slow progression to the end-stage liver disease, the existing infections will continue to present a serious medical and economic burden for decades.

Current HCV therapy is based on (pegylated) interferon-alpha (IFN-α) in combination with ribavirin. This combination therapy yields a sustained virologic response in more than 40% of patients infected by genotype 1 HCV and about 80% of those infected by genotypes 2 and 3. Beside the limited efficacy against HCV genotype 1, this combination therapy has significant side effects and is poorly tolerated in many patients. Major side effects include influenza-like symptoms, hematologic abnormalities, and neuropsychiatric symptoms. Hence there is a need for more effective, convenient and better-tolerated treatments.

Recently, therapy possibilities have extended towards the combination of a HCV protease inhibitor (e.g. Telaprevir or boceprevir) and (pegylated) interferon-alpha (IFN-α) / ribavirin.

Experience with HIV drugs, in particular with HIV protease inhibitors, has taught that sub-optimal pharmacokinetics and complex dosing regimes quickly result in inadvertent compliance failures. This in turn means that the 24 hour trough concentration (minimum plasma concentration) for the respective drugs in an HIV regime frequently falls below the IC_{90} or ED_{90} threshold for large parts of the day. It is considered that a 24 hour trough level of at least the IC_{50}, and more realistically, the IC_{90} or ED_{90}, is essential to slow down the development of drug escape mutants. Achieving the necessary pharmacokinetics and drug metabolism to allow such trough levels provides a stringent challenge to drug design.

The NS5B RdRp is essential for replication of the single-stranded, positive sense, HCV RNA genome. This enzyme has elicited significant interest among medicinal chemists. Both nucleoside and non-nucleoside inhibitors of NS5B are known. Nucleoside inhibitors can act as a chain terminator or as a competitive inhibitor, or as both. In order to be active, nucleoside inhibitors have to be taken up by the cell and converted *in vivo* to a triphosphate. This conversion to the triphosphate is commonly mediated by cellular kinases, which imparts additional structural requirements on a potential nucleoside polymerase inhibitor. In addition this limits the direct evaluation of nucleosides as inhibitors of HCV replication to cell-based assays capable of *in situ* phosphorylation.

Several attempts have been made to develop nucleosides as inhibitors of HCV RdRp, but while a handful of compounds have progressed into clinical development, none have proceeded to registration. Amongst the problems which HCV-targeted nucleosides have encountered to date are toxicity, mutagenicity, lack of selectivity, poor efficacy, poor bioavailability, sub-optimal dosage regimes and ensuing high pill burden and cost of goods.

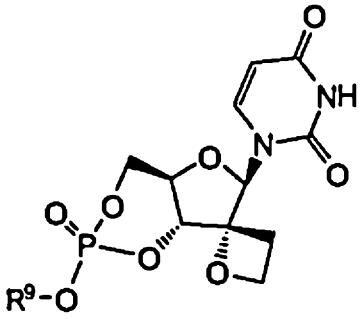
Spirooxetane nucleosides, in particular 1-(8-hydroxy-7-(hydroxy- methyl)-1,6-dioxaspiro[3.4]octan-5-yl)pyrimidine-2,4-dione derivatives and their use as HCV inhibitors are known from WO2010/130726, and WO2012/062869, including CAS-1375074-52-4.

There is a need for HCV inhibitors that may overcome at least one of the disadvantages of current HCV therapy such as side effects, limited efficacy, the emerging of resistance, and compliance failures, or improve the sustained viral response.

The present invention concerns a group of HCV-inhibiting uracyl spirooxetane derivatives with useful properties regarding one or more of the following parameters: antiviral efficacy towards at least one of the following genotypes 1a, 1b, 2a, 2b, 3,4 and 6, favorable profile of resistance development, lack of toxicity and genotoxicity, favorable pharmacokinetics and pharmacodynamics and ease of formulation and administration.

Description of the Invention

In one aspect the present invention provides compounds that can be represented by the formula I:

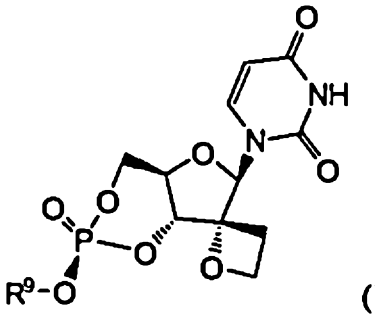


(I)

including any possible stereoisomer thereof, wherein:

R^9 is C_1-C_6alkyl, phenyl, C_3-C_7cycloalkyl or C_1-C_3alkyl substituted with 1, 2 or 3 substituents each independently selected from phenyl, naphtyl, C_3-C_6cycloalkyl, hydroxy, or C_1-C_6alkoxy;
or a pharmaceutically acceptable salt or solvate thereof.

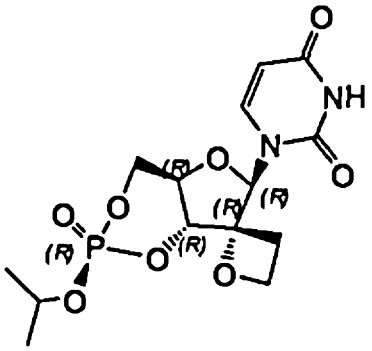
Of particular interest are compounds of formula I or subgroups thereof as defined herein, that have a structure according to formula Ia:



(Ia)

In one embodiment of the present invention, R^9 is C_1-C_6alkyl, phenyl, C_3-C_7cycloalkyl or C_1-C_3alkyl substituted with 1 substituent selected from phenyl, C_3-C_6cycloalkyl, hydroxy, or C_1-C_6alkoxy. In another embodiment of the present invention, R^9 in Formula I or Ia is C_1-C_6alkyl or C_1-C_2alkyl substituted with phenyl C_1-C_2alkoxy or C_3-C_6cycloalkyl. In a more preferred embodiment, R^9 is C_2-C_4alkyl and in a most preferred embodiment, R^9 is *i*-propyl.

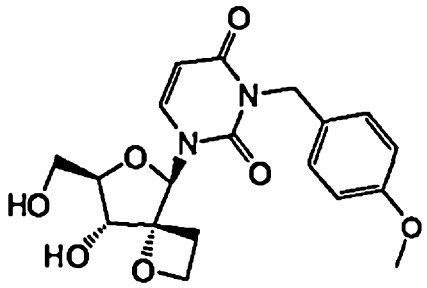
A preferred embodiment according to the invention is a compound according to formula Ib:



(Ib)

including any pharmaceutically acceptable salt or solvate thereof and the use of compound (V) in the synthesis of a compound according to Formula I, Ia or Ib.

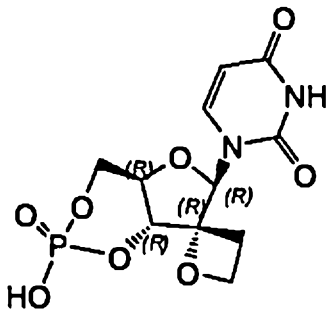
The invention further relates to a compound of formula V:



(V)

including any pharmaceutically acceptable salt or solvate thereof and the use of compound (V) in the synthesis of a compound according to Formula I, Ia or Ib.

In addition, the invention relates to a compound of formula VI:



(VI)

including any stereochemical form and/or pharmaceutically acceptable salt or solvate thereof.

Additionally, the invention relates to a pharmaceutical composition comprising a compound according to Formula I, Ia or Ib, and a pharmaceutically acceptable carrier. The invention also relates to a product containing (a) a compound of formula I, Ia or Ib a, and (b) another HCV inhibitor, as a combined preparation for simultaneous, separate or sequential use in the treatment of HCV infections

Yet another aspect of the invention relates to a compound according to Formula I, Ia or Ib or a pharmaceutical composition according to the present invention for use as a medicament, preferably for use in the prevention or treatment of an HCV infection in a mammal.

In a further aspect, the invention provides a compound of formula I Ia or Ib or a pharmaceutically acceptable salt, hydrate, or solvate thereof, for use in the treatment or prophylaxis (or the manufacture of a medicament for the treatment or prophylaxis) of HCV infection. Representative HCV genotypes in the context of treatment or prophylaxis in accordance with the invention include genotype 1b (prevalent in Europe) or 1a (prevalent in North America). The invention also provides a method for the treatment or prophylaxis of HCV infection, in particular of the genotype 1a or 1b.

Of particular interest is compound **8a** mentioned in the section "Examples" as well as the pharmaceutically acceptable acid addition salts of this compound.

The compounds of formula I have several centers of chirality, in particular at the carbon atoms 1', 2', 3', and 4'. Although the stereochemistry at these carbon atoms is fixed, the compounds may display at least 75%, preferably at least 90%, such as in excess of 95%, or of 98%, enantiomeric purity at each of the chiral centers.

The phosphorus center can be present as R_P or S_P, or a mixture of such stereoisomers, including racemates. Diastereoisomers resulting from the chiral phosphorus center and a chiral carbon atom may exist as well.

The compounds of formula I are represented as a defined stereoisomer, except for the stereoisomerism at the phosphorous atom. The absolute configuration of such compounds can be determined using art-known methods such as, for example, X-ray diffraction or NMR and/or implication from starting materials of known stereochemistry. Pharmaceutical compositions in accordance with the invention will preferably comprise stereoisomerically pure forms of the indicated stereoisomer of the particular compound of formula I.

Pure stereoisomeric forms of the compounds and intermediates as mentioned herein are defined as isomers substantially free of other enantiomeric or diastereomeric forms of the same basic molecular structure of said compounds or intermediates. In particular, the term "stereoisomerically pure" concerns compounds or intermediates having a stereoisomeric excess of at least 80% (i.e. minimum 90% of one isomer and maximum 10% of the other possible isomers) up to a stereoisomeric excess of 100% (i.e. 100% of one isomer and none of the other), more in particular, compounds or intermediates having a stereoisomeric excess of 90% up to 100%, even more in particular having a stereoisomeric excess of 94% up to 100% and most in particular having a stereoisomeric excess of 97% up to 100%, or of 98% up to 100%. The terms "enantiomerically pure" and "diastereomerically pure" should be understood in a similar way, but then having regard to the enantiomeric excess, and the diastereomeric excess, respectively, of the mixture in question.

Pure stereoisomeric forms of the compounds and intermediates of this invention may be obtained by the application of art-known procedures. For instance, enantiomers may be separated from each other by the selective crystallization of their diastereomeric salts with optically active acids or bases. Examples thereof are tartaric acid, dibenzoyl-tartaric acid, ditoluoyltartaric acid and camphorsulfonic acid. Alternatively, enantiomers may be separated by chromatographic techniques using chiral stationary layers. Said pure stereochemically isomeric forms may also be derived from the corresponding pure stereochemically isomeric forms of the appropriate starting materials, provided that the reaction occurs stereospecifically. Preferably, if a specific stereoisomer is desired, said compound is synthesized by stereospecific methods of preparation. These methods will advantageously employ enantiomerically pure starting materials.

The diastereomeric racemates of the compounds of formula I can be obtained separately by conventional methods. Appropriate physical separation methods that may advantageously be employed are, for example, selective crystallization and chromatography, e.g. column chromatography.

The pharmaceutically acceptable addition salts comprise the therapeutically active non-toxic acid and base addition salt forms of the compounds of formula I. Of interest are the free, i.e. non-salt forms of the compounds of formula I, or of any subgroup of compounds of formula I specified herein.

The pharmaceutically acceptable acid addition salts can conveniently be obtained by treating the base form with such appropriate acid. Appropriate acids comprise, for example, inorganic acids such as hydrohalic acids, e.g. hydrochloric or hydrobromic acid, sulfuric, nitric, phosphoric and the like acids; or organic acids such as, for example, acetic, propionic, hydroxyacetic, lactic, pyruvic, oxalic (i.e. ethanedioic), malonic, succinic (i.e. butanedioic acid), maleic, fumaric, malic (i.e. hydroxyl-butanedioic acid), tartaric, citric, methanesulfonic, ethanesulfonic, benzenesulfonic, *p*-toluenesulfonic, cyclamic, salicylic, *p*-aminosalicylic, palmoic and the like acids. Conversely said salt forms can be converted by treatment with an appropriate base into the free base form.

The compounds of formula I containing an acidic proton may also be converted into their non-toxic metal or amine addition salt forms by treatment with appropriate organic and inorganic bases. Appropriate base salt forms comprise, for example, the ammonium salts, the alkali and earth alkaline metal salts, e.g. the lithium, sodium, potassium, magnesium, calcium salts and the like, salts with organic bases, e.g. the benzathine, *N*-methyl-D-glucamine, hydrabamine salts, and salts with amino acids such as, for example, arginine, lysine and the like.

The term "solvates" covers any pharmaceutically acceptable solvates that the compounds of formula I as well as the salts thereof, are able to form. Such solvates are for example hydrates, alcoholates, e.g. ethanolates, propanolates, and the like.

Some of the compounds of formula I may also exist in their tautomeric form. For example, tautomeric forms of amide (-C(=O)-NH-) groups are iminoalcohols (-C(OH)=N-), which can become stabilized in rings with aromatic character. The uridine base is an example of such a form. Such forms, although not explicitly

indicated in the structural formulae represented herein, are intended to be included within the scope of the present invention.

Short description of the Figure

Figure 1: *In vivo* efficacy of compound 8a and CAS-1375074-52-4 as determined in a humanized hepatocyte mouse model.

Definitions

As used herein "C_1-$C_n$alkyl" as a group or part of a group defines saturated straight or branched chain hydrocarbon radicals having from 1 to n carbon atoms. Accordingly, "C_1-C_4alkyl" as a group or part of a group defines saturated straight or branched chain hydrocarbon radicals having from 1 to 4 carbon atoms such as for example methyl, ethyl, 1-propyl, 2-propyl, 1-butyl, 2-butyl, 2-methyl-1-propyl, 2-methyl-2-propyl. "C_1-C_6alkyl" encompasses C_1-C_4alkyl radicals and the higher homologues thereof having 5 or 6 carbon atoms such as, for example, 1-pentyl, 2-pentyl, 3-pentyl, 1-hexyl, 2-hexyl, 2-methyl-1-butyl, 2-methyl-1-pentyl, 2-ethyl-1-butyl, 3-methyl-2-pentyl, and the like. Of interest amongst C_1-C_6alkyl is C_1-C_4alkyl.

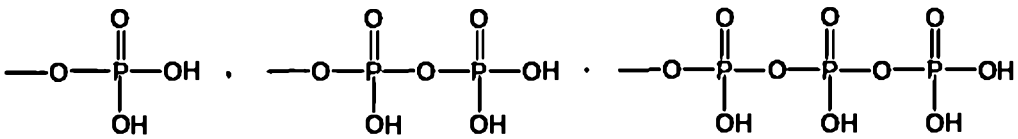
'C_1-$C_n$alkoxy' means a radical -O-C_1-$C_n$alkyl wherein C_1-$C_n$alkyl is as defined above. Accordingly, 'C_1-C_6alkoxy' means a radical -O-C_1-C_6alkyl wherein C_1-C_6alkyl is as defined above. Examples of C_1-C_6alkoxy are methoxy, ethoxy, n-propoxy, or isopropoxy. Of interest is 'C_1-C_2alkoxy', encompassing methoxy and ethoxy.

"C_3-C_6cycloalkyl" includes cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl.

In one embodiment, the term "phenyl-C_1-C_6alkyl" is benzyl.

As used herein, the term '(=O)' or 'oxo' forms a carbonyl moiety when attached to a carbon atom. It should be noted that an atom can only be substituted with an oxo group when the valency of that atom so permits.

The term "monophosphate, diphosphate or triphosphate ester" refers to groups:



Where the position of a radical on a molecular moiety is not specified (for example a substituent on phenyl) or is represented by a floating bond, such radical may be

positioned on any atom of such a moiety, as long as the resulting structure is chemically stable. When any variable is present more than once in the molecule, each definition is independent.

Whenever used herein, the term 'compounds of formula I', or 'the present compounds' or similar terms, it is meant to include the compounds of Formula I, Ia and Ib, including the possible stereochemically isomeric forms, and their pharmaceutically acceptable salts and solvates.

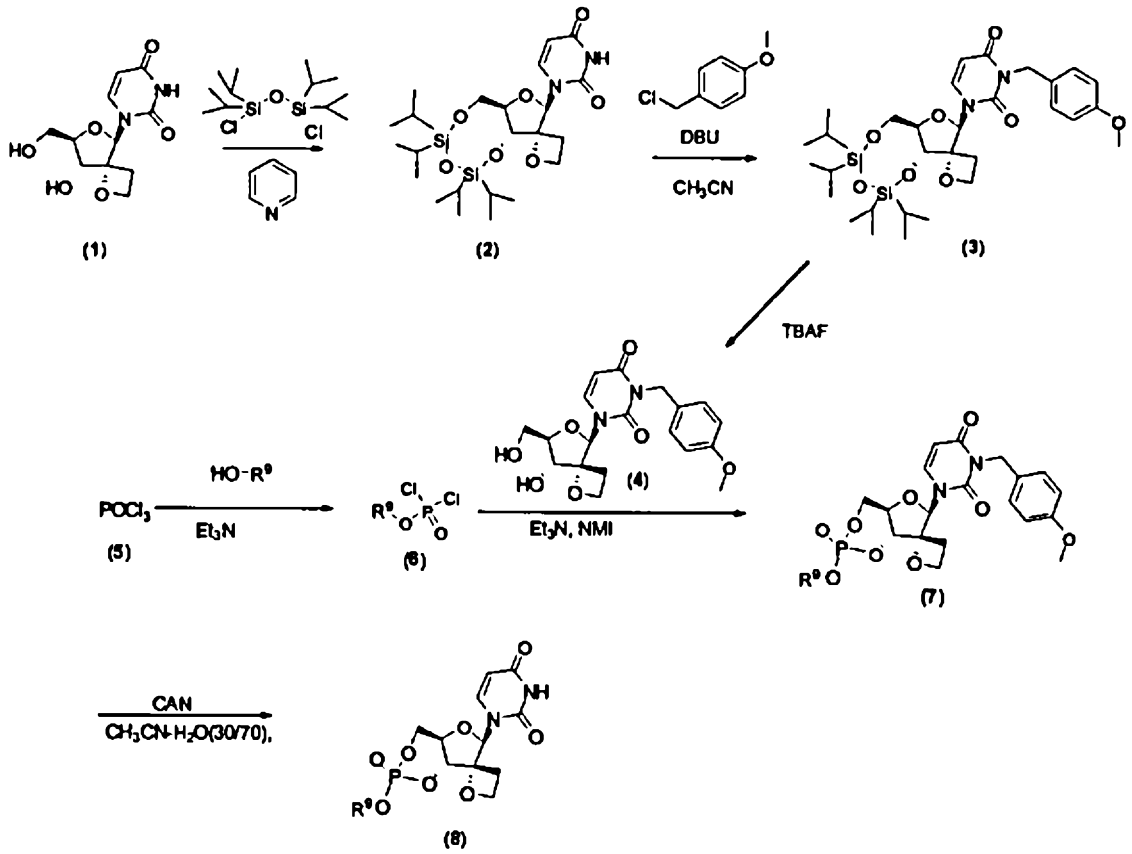
The present invention also includes isotope-labeled compounds of formula I or any subgroup of formula I, wherein one or more of the atoms is replaced by an isotope that differs from the one(s) typically found in nature. Examples of such isotopes include isotopes of hydrogen, such as ^{2}H and ^{3}H; carbon, such as ^{11}C, ^{13}C and ^{14}C; nitrogen, such as ^{13}N and ^{15}N; oxygen, such as ^{15}O, ^{17}O and ^{18}O; phosphorus, such as ^{31}P and ^{32}P, sulphur, such as ^{35}S; fluorine, such as ^{18}F; chlorine, such as ^{36}Cl; bromine such as ^{75}Br, ^{76}Br, ^{77}Br and ^{82}Br; and iodine, such as ^{123}I, ^{124}I, ^{125}I and ^{131}I. Isotope- labeled compounds of the invention can be prepared by processes analogous to those described herein by using the appropriate isotope-labeled reagents or starting materials, or by art-known techniques. The choice of the isotope included in an isotope-labeled compound depends on the specific application of that compound. For example, for tissue distribution assays, a radioactive isotope such as ^{3}H or ^{14}C is incorporated. For radio-imaging applications, a positron emitting isotope such as ^{11}C, ^{18}F, ^{13}N or ^{15}O will be useful. The incorporation of deuterium may provide greater metabolic stability, resulting in, e.g. an increased in vivo half life of the compound or reduced dosage requirements.

General Synthetic Procedures

The following schemes are just meant to be illustrative and are by no means limiting the scope.

The starting material 1-[(4R,5R,7R,8R)-8-hydroxy-7-(hydroxymethyl)-1,6-dioxa-spiro[3.4]octan-5-yl]pyrimidine-2,4(1*H*,3*H*)-dione (1) can be prepared as exemplified in WO2010/130726. Compound (1) is converted into compounds of the present invention via a p-methoxybenzyl protected derivative (4) as exemplified in the following Scheme 1.

Scheme 1



In Scheme 1, R^9 can be C_1-C_6alkyl, phenyl, naphtyl, C_3-C_7cycloalkyl or C_1-C_3alkyl substituted with 1,2 or 3 substituents each independently selected from phenyl, C_3-C_6cycloalkyl, hydroxy, or C_1-C_6alkoxy, preferably R^9 is C_1-C_6alkyl or C_1-C_2alkyl substituted with phenyl, C_1-C_2alkoxy or C_3-C_6cycloalkyl, even more preferably R^9 is C_2-C_4alkyl and most preferably R^9 is *i*-propyl.

In a further aspect, the present invention concerns a pharmaceutical composition comprising a therapeutically effective amount of a compound of formula I as specified herein, and a pharmaceutically acceptable carrier. Said composition may contain from 1% to 50%, or from 10% to 40% of a compound of formula I and the remainder of the composition is the said carrier. A therapeutically effective amount in this context is an amount sufficient to act in a prophylactic way against HCV infection, to inhibit HCV, to stabilize or to reduce HCV infection, in infected subjects or subjects being at risk of becoming infected. In still a further aspect, this invention relates to a process of preparing a pharmaceutical composition as specified herein, which comprises intimately mixing a pharmaceutically acceptable carrier with a therapeutically effective amount of a compound of formula I, as specified herein.

The compounds of formula I or of any subgroup thereof may be formulated into various pharmaceutical forms for administration purposes. As appropriate compositions there may be cited all compositions usually employed for systemically administering drugs. To prepare the pharmaceutical compositions of this invention, an effective amount of the particular compound, optionally in addition salt form or metal complex, as the active ingredient is combined in intimate admixture with a pharmaceutically acceptable carrier, which carrier may take a wide variety of forms depending on the form of preparation desired for administration. These pharmaceutical compositions are desirable in unitary dosage form suitable, particularly, for administration orally, rectally, percutaneously, or by parenteral injection. For example, in preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed such as, for example, water, glycols, oils, alcohols and the like in the case of oral liquid preparations such as suspensions, syrups, elixirs, emulsions and solutions; or solid carriers such as starches, sugars, kaolin, lubricants, binders, disintegrating agents and the like in the case of powders, pills, capsules, and tablets. Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit forms, in which case solid pharmaceutical carriers are obviously employed. For parenteral compositions, the carrier will usually comprise sterile water, at least in large part, though other ingredients, for example, to aid solubility, may be included. Injectable solutions, for example, may be prepared in which the carrier comprises saline solution, glucose solution or a mixture of saline and glucose solution. Injectable suspensions may also be prepared in which case appropriate liquid carriers, suspending agents and the like may be employed. Also included are solid form preparations intended to be converted, shortly before use, to liquid form preparations. In the compositions suitable for percutaneous administration, the carrier optionally comprises a penetration enhancing agent and/or a suitable wetting agent, optionally combined with suitable additives of any nature in minor proportions, which additives do not introduce a significant deleterious effect on the skin. The compounds of the present invention may also be administered via oral inhalation or insufflation in the form of a solution, a suspension or a dry powder using any art-known delivery system.

It is especially advantageous to formulate the aforementioned pharmaceutical compositions in unit dosage form for ease of administration and uniformity of dosage. Unit dosage form as used herein refers to physically discrete units suitable as unitary dosages, each unit containing a predetermined quantity of active ingredient calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. Examples of such unit dosage forms are tablets (including

scored or coated tablets), capsules, pills, suppositories, powder packets, wafers, injectable solutions or suspensions and the like, and segregated multiples thereof.

The compounds of formula I show activity against HCV and can be used in the treatment and/or prophylaxis of HCV infection or diseases associated with HCV. The latter include progressive liver fibrosis, inflammation and necrosis leading to cirrhosis, end-stage liver disease, and HCC. The compounds of this invention moreover are believed to be active against mutated strains of HCV and show a favorable pharmacokinetic profile and have attractive properties in terms of bioavailability, including an acceptable half-life, AUC (area under the curve) and peak values and lacking unfavorable phenomena such as insufficient quick onset and tissue retention.

The *in vitro* antiviral activity against HCV of the compounds of formula I can be tested in a cellular HCV replicon system based on Lohmann et al. (1999) Science 285:110-113, with the further modifications described by Krieger et al. (2001) Journal of Virology 75: 4614-4624 (incorporated herein by reference), which is further exemplified in the examples section. This model, while not a complete infection model for HCV, is widely accepted as the most robust and efficient model of autonomous HCV RNA replication currently available. It will be appreciated that it is important to distinguish between compounds that specifically interfere with HCV functions from those that exert cytotoxic or cytostatic effects in the HCV replicon model, and as a consequence cause a decrease in HCV RNA or linked reporter enzyme concentration. Assays are known in the field for the evaluation of cellular cytotoxicity based for example on the activity of mitochondrial enzymes using fluorogenic redox dyes such as resazurin. Furthermore, cellular counter screens exist for the evaluation of non-selective inhibition of linked reporter gene activity, such as firefly luciferase. Appropriate cell types can be equipped by stable transfection with a luciferase reporter gene whose expression is dependent on a constitutively active gene promoter, and such cells can be used as a counter-screen to eliminate non-selective inhibitors.

Due to their anti-HCV properties, the compounds of formula I, including any possible stereoisomers, the pharmaceutically acceptable addition salts or solvates thereof, are useful in the treatment of warm-blooded animals, in particular humans, infected with HCV, and in the prophylaxis of HCV infections. The compounds of the present invention may therefore be used as a medicine, in particular as an anti-HCV or a HCV-inhibiting medicine. The present invention also relates to the use of the present compounds in the manufacture of a medicament for the treatment or the prevention of HCV infection. In a further aspect, the present invention relates to a method of treating

a warm-blooded animal, in particular human, infected by HCV, or being at risk of becoming infected by HCV, said method comprising the administration of an anti-HCV effective amount of a compound of formula I, as specified herein. Said use as a medicine or method of treatment comprises the systemic administration to HCV-infected subjects or to subjects susceptible to HCV infection of an amount effective to combat the conditions associated with HCV infection.

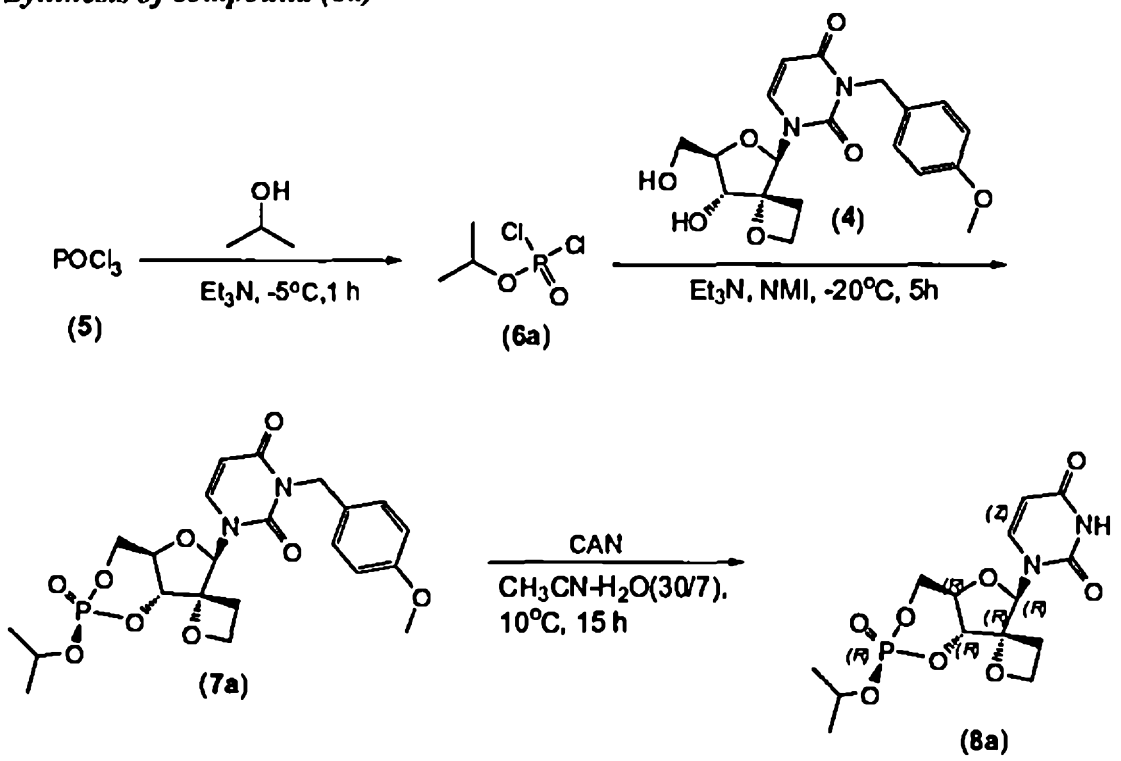
In general it is contemplated that an antiviral effective daily amount would be from about 1 to about 30 mg/kg, or about 2 to about 25 mg/kg, or about 5 to about 15 mg/kg, or about 8 to about 12 mg/kg body weight. Average daily doses can be obtained by multiplying these daily amounts by about 70. It may be appropriate to administer the required dose as two, three, four or more sub-doses at appropriate intervals throughout the day. Said sub-doses may be formulated as unit dosage forms, for example, containing about 1 to about 2000 mg, or about 50 to about 1500 mg, or about 100 to about 1000 mg, or about 150 to about 600 mg, or about 100 to about 400 mg of active ingredient per unit dosage form.

As used herein the term "about" has the meaning known to the person skilled in the art. In certain embodiments the term "about" may be left out and the exact amount is meant. In other embodiments the term "about" means that the numerical following the term "about" is in the range of ± 15%, or of ± 10%, or of ± 5%, or of ± 1%, of said numerical value.

Examples

Scheme 2

Synthesis of compound (8a)



Synthesis of compound (2)

Compound (2) can be prepared by dissolving compound (1) in pyridine and adding 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane. The reaction is stirred at room temperature until complete. The solvent is removed and the product redissolved in CH_2Cl_2 and washed with saturated $NaHCO_3$ solution. Drying on $MgSO_4$ and removal of the solvent gives compound (2).

Synthesis of compound (3)

Compound (3) is prepared by reacting compound (2) with p-methoxybenzylchloride in the presence of DBU as the base in CH_3CN.

Synthesis of compound (4)

Compound (4) is prepared by cleavage of the bis-silyl protecting group in compound (3) using TBAF as the fluoride source.

Synthesis of compound (6a)

A solution of isopropyl alcohol (3.86 mL,0.05mol) and triethylamine (6.983 mL, 0.05mol) in dichloromethane (50 mL) was added to a stirred solution of $POCl_3$ (5)

(5.0 mL, 0.0551mol) in DCM (50 mL) dropwise over a period of 25 min at -5°C. After the mixture stirred for 1h, the solvent was evaporated, and the residue was suspended in ether (100 mL). The triethylamine hydrochloride salt was filtered and washed with ether (20 mL). The filtrate was concentrated, and the residue was distilled to give the (**6**) as a colorless liquid (6.1g, 69 %yield).

Synthesis of compound (7a)

To a stirred suspension of (**4**) (2.0 g, 5.13 mmol) in dichloromethane (50 mL) was added triethylamine (2.07 g, 20.46 mmol) at room temperature. The reaction mixture was cooled to -20°C, and then (**6a**) (1.2 g, 6.78mmol) was added dropwise over a period of 10min. The mixture was stirred at this temperature for 15min and then NMI was added (0.84 g, 10.23 mmol), dropwise over a period of 15 min. The mixture was stirred at -15°C for 1h and then slowly warmed to room temperature in 20 h. The solvent was evaporated, the mixture was concentrated and purified by column chromatography using petroleum ether/EtOAc (10:1 to 5:1 as a gradient) to give (**7a**) as white solid (0.8 g, 32 % yield).

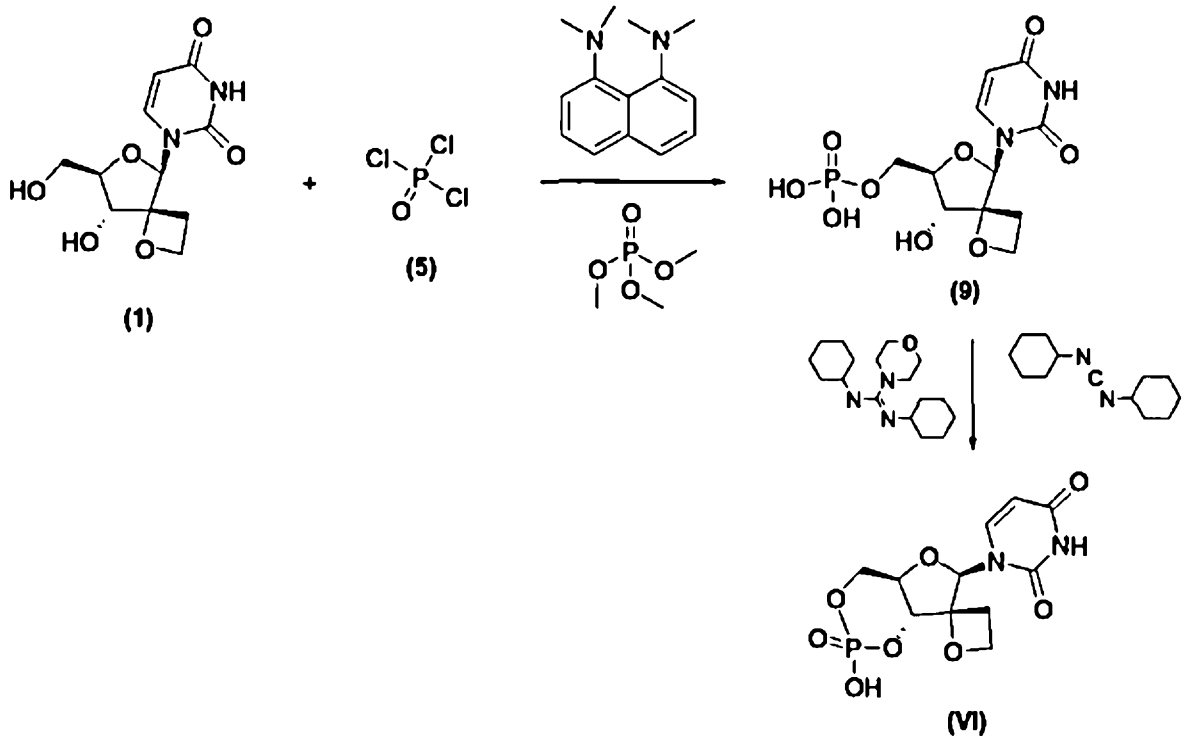
Synthesis of compound (8a)

To a solution of (**7a**) in CH_3CN (30 mL) and H_2O (7 mL) was add CAN portion wise below 20°C. The mixture was stirred at 15-20°C for 5h under N_2. Na_2SO_3 (370 mL) was added dropwise into the reaction mixture below 15°C, and then Na_2CO_3 (370 mL) was added. The mixture was filtered and the filtrate was extracted with CH_2Cl_2 (100 mL*3). The organic layer was dried and concentrated to give the residue. The residue was purified by column chromatography to give the target compound (**8a**) as white solid. (Yield: 55%)

^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 1.45 (dd, *J*=7.53, 6.27 Hz, 6 H), 2.65 - 2.84 (m, 2 H), 3.98 (td, *J*=10.29, 4.77 Hz, 1 H), 4.27 (t, *J*=9.66 Hz, 1 H), 4.43 (ddd, *J*=8.91, 5.77, 5.65 Hz, 1 H), 4.49 - 4.61 (m, 1 H), 4.65 (td, *J*=7.78, 5.77 Hz, 1 H), 4.73 (d, *J*=7.78 Hz, 1 H), 4.87 (dq, *J*=12.74, 6.30 Hz, 1 H), 5.55 (br. s., 1 H), 5.82 (d, *J*=8.03 Hz, 1 H), 7.20 (d, *J*=8.03 Hz, 1 H), 8.78 (br. s., 1 H); ^{31}P NMR (CHLOROFORM-*d*) δ ppm -7.13; LC-MS: 375 (M+1)+

Scheme 3

Synthesis of compound (VI)



Step 1: Synthesis of compound (9)

Compound (1), CAS 1255860-33-3 (1200 mg, 4.33 mmol) and 1,8-bis(dimethylamino)naphthalene (3707 mg, 17.3 mmol) were dissolved in 24.3 mL of trimethylphosphate. The solution was cooled to 0°C. Compound **(5)** (1.21 mL, 12.98 mmol) was added, and the mixture was stirred well maintaining the temperature at 0°C for 5 hours. The reaction was quenched by addition of 120 mL of tetraethylammonium bromide solution (1M) and extracted with CH_2Cl_2 (2x80 mL). Purification was done by preparative HPLC (Stationary phase: RP XBridge Prep C18 OBD-10μm, 30x150mm, mobile phase: 0.25% NH_4HCO_3 solution in water, CH_3CN), yielding two fractions. The purest fraction was dissolved in water (15 mL) and passed through a manually packed Dowex (H^+) column by elution with water. The end of the elution was determined by checking UV absorbance of eluting fractions. Combined fractions were frozen at -78°C and lyophilized. Compound **(9)** was obtained as a white fluffy solid (303 mg, (0.86 mmol, 20% yield), which was used immediately in the following reaction.

Step 2: Preparation of compound (VI)

Compound (9) (303 mg, 0.86 mmol) was dissolved in 8 mL water and to this solution was added N,N'-Dicyclohexyl-4-morpholine carboxamidine (253.8 mg, 0.86 mmol) dissolved in pyridine (8.4 mL). The mixture was kept for 5 minutes and then evaporated to dryness, dried overnight in vacuo overnight at 37°C. The residu was dissolved in pyridine (80 mL). This solution was added dropwise to vigorously stirred DCC (892.6 mg, 4.326 mmol) in pyridine (80 mL) at reflux temperature. The solution was kept refluxing for 1.5h during which some turbidity was observed in the solution. The reaction mixture was cooled and evaporated to dryness. Diethylether (50 mL) and water (50 mL) were added to the solid residu. N'N-dicyclohexylurea was filtered off, and the aqueous fraction was purified by preparative HPLC (Stationary phase: RP XBridge Prep C18 OBD-10μm, 30x150mm, mobile phase: 0.25% NH_4HCO_3 solution in water, CH_3CN), yielding a white solid which was dried overnight in vacuo at 38°C. (185 mg, 0.56 mmol, 65% yield). LC-MS: $(M+H)^+$: 333.
^{1}H NMR (400 MHz, DMSO-d_6) d ppm 2.44 - 2.59 (m, 2 H) signal falls under DMSO signal, 3.51 (td, J=9.90, 5.50 Hz, 1 H), 3.95 - 4.11 (m, 2 H), 4.16 (d, J=10.34 Hz, 1 H), 4.25 - 4.40 (m, 2 H), 5.65 (d, J=8.14 Hz, 1 H), 5.93 (br. s., 1 H), 7.46 (d, J=7.92 Hz, 1 H), 2H's not observed

Biological examples

Replicon Assays

The compounds of formula I were examined for activity in the inhibition of HCV-RNA replication in a cellular assay. The assay was used to demonstrate that the compounds of formula I inhibited a HCV functional cellular replicating cell line, also known as HCV replicons. The cellular assay was based on a bicistronic expression construct, as described by Lohmann et al. (1999) Science vol. 285 pp. 110-113 with modifications described by Krieger et al. (2001) Journal of Virology 75: 4614-4624, in a multi-target screening strategy.

Replicon assay (A)

In essence, the method was as follows. The assay utilized the stably transfected cell line Huh-7 luc/neo (hereafter referred to as Huh-Luc). This cell line harbors an RNA encoding a bicistronic expression construct comprising the wild type NS3-NS5B regions of HCV type 1b translated from an internal ribosome entry site (IRES) from encephalomyocarditis virus (EMCV), preceded by a reporter portion (FfL-luciferase), and a selectable marker portion (neo^R, neomycine phosphotransferase). The construct is bordered by 5' and 3' NTRs (non-translated regions) from HCV genotype 1b.

Continued culture of the replicon cells in the presence of G418 (neo^R) is dependent on the replication of the HCV-RNA. The stably transfected replicon cells that express HCV-RNA, which replicates autonomously and to high levels, encoding *inter alia* luciferase, were used for screening the antiviral compounds.

The replicon cells were plated in 384-well plates in the presence of the test and control compounds which were added in various concentrations. Following an incubation of three days, HCV replication was measured by assaying luciferase activity (using standard luciferase assay substrates and reagents and a Perkin Elmer ViewLux™ ultraHTS microplate imager). Replicon cells in the control cultures have high luciferase expression in the absence of any inhibitor. The inhibitory activity of the compound on luciferase activity was monitored on the Huh-Luc cells, enabling a dose-response curve for each test compound. EC_{50} values were then calculated, which value represents the amount of the compound required to decrease the level of detected luciferase activity by 50%, or more specifically, the ability of the genetically linked HCV replicon RNA to replicate.

Results (A)

Table 1 shows the replicon results (EC_{50}, replicon) and cytotoxicity results (CC_{50} (μM) (Huh-7)) obtained for the compound of the examples given above.

Table 1.

Compound number	EC_{50} (μM) (HCV)	CC_{50} (μM) (Huh-7)
8a	0.13 (n = 4)	> 100

Replicon assays (B)

Further replicon assays were performed with compound 8a of which the protocols and results are disclosed below.

Assay 1

The anti-HCV activity of compound 8a was tested in cell culture with replicon cells generated using reagents from the Bartenschlager laboratory (the HCV 1b bicistronic subgenomic luciferase reporter replicon clone ET). The protocol included a 3-day incubation of 2500 replicon cells in a 384-well format in a nine-point 1:4 dilution series of the compound. Dose response curves were generated based on the firefly luciferase

read-out. In a variation of this assay, a 3 day incubation of 3000 cells in a 96-well format in a nine-point dilution series was followed by qRT-PCR Taqman detection of HCV genome, and normalized to the cellular transcript, RPL13 (of the ribosomal subunit RPL13 gene) as a control for compound inhibition of cellular transcription.

Assay 2

The anti-HCV activity of compound 8a was tested in cell culture with replicon cells generated using reagents from the Bartenschlager laboratory (the HCV 1b bicistronic subgenomic luciferase reporter replicon clone ET or Huh-Luc-Neo). The protocol included a 3-day incubation of 2×10^4 replicon cells in a 96-well format in a six-point 1:5 dilution series of the compound. Dose response curves were generated based on the luciferase read-out.

Assay 3

The anti-HCV activity of compound 8a was tested in cell culture with replicon cells generated using reagents from the Bartenschlager laboratory (the HCV 1b bicistronic subgenomic luciferase reporter replicon clone ET or Huh-Luc-Neo). The protocol included either a 3-day incubation of 8×10^3 cells or 2×10^4 cells in a 96-well format in an eight-point 1:5 dilution series of the compound. Dose response curves were generated based on the luciferase read-out.

Results

Table 2 shows the average replicon results (EC_{50}, replicon) obtained for compound 8a following assays as given above.

Table 2:

Assay	Average EC_{50} value (8a):
1	57 μM (n = 8)
2	17.5 μM (n=4)
3	> 100 μM (n =1)

Primary human hepatocyte *in vitro* assay

The anti-HCV activity of compound 8a was determined in an in vitro primary human hepatocyte assay. Protocols and results are disclosed below.

Protocol

Hepatocyte isolation and culture

Primary human hepatocytes (PHH) were prepared from patients undergoing partial hepatectomy for metastases or benign tumors. Fresh human hepatocytes were isolated from encapsulated liver fragments using a modification of the two-step collagenase digestion method. Briefly, encapsulated liver tissue was placed in a custom-made perfusion apparatus and hepatic vessels were cannulated with tubing attached multichannel manifold. The liver fragment was initially perfused for 20 min with a prewarmed (37°C) calcium-free buffer supplemented with ethylene glycol tetraacetic acid (EGTA) followed by perfusion with a prewarmed (37°C) buffer containing calcium ($CaCl_2$, H_2O_2) and collagenase 0.05% for 10 min. Then, liver fragment was gently shaken to free liver cells in Hepatocyte Wash Medium. Cellular suspension was filtered through a gauze-lined funnel. Cells were centrifuged at low speed centrifugation. The supematant, containing damaged or dead hepatocytes, non parenchymal cells and debris was removed and pelleted hepatocytes were re-suspended in Hepatocyte Wash Medium. Viability and cell concentration were determined by trypan blue exclusion test.

Cells were resuspended in complete hepatocyte medium consisting of William's medium (Invitrogen) supplemented with 100 IU/L insulin (Novo Nordisk, France), and 10% heat inactivated fetal calf serum (Biowest, France), and seeded at a density 1,8x106 viable cells onto 6 well plates that had been precoated with a type I collagen from calf skin (Sigma-Aldrich, France) The medium was replaced 16–20 hours later with fresh complete hepatocyte medium supplemented with hydrocortisone hemisuccinate (SERB, Paris, France), and cells were left in this medium until HCV inoculation. The cultures were maintained at 37°C in a humidified 5% CO2 atmosphere.

The PHHs were inoculated 3 days after seeding. JFH1-HCVcc stocks were used to inoculate PHHs for 12 hours, at a multiplicity of infection (MOI) of 0.1 ffu per cell. After a 12-hours incubation at 37°C, the inoculum was removed, and monolayers were washed 3 times with phosphate-buffered saline and incubated in complete hepatocyte medium containing 0.1% dimethylsufoxide as carrier control, 100 IU/ml of IFNalpha as negative control or else increasing concentrations of compound 8a. The cultures then were maintained during 3 days.

Quantitation of HCV RNA

Total RNA was prepared from cultured cells or from filtered culture supernatants using the RNeasy or Qiamp viral RNA minikit respectively (Qiagen SA, Courtaboeuf, France) according to the manufacturer's recommendations. HCV RNA was quantified in cells and culture supernatants using a strand-specific reverse real-time PCR technique described previously (Carrière M and al 2007):

Reverse transcription was performed using primers described previously located in the 50 NCR region of HCV genome, tag-RC1 (5'-GGCCGTCATGGTGGCGAATAAGTCTAGCCATGGCGTTAGTA-3') and RC21 (5'-CTCCCGGGGCACTCGCAAGC-3') for the negative and positive strands, respectively. After a denaturation step performed at 70°C for 8 min, the RNA template was incubated at 4°C for 5 min in the presence of 200 ng of tag-RC1 primer and 1.25 mM of each deoxynucleoside triphosphate (dNTP) (Promega, Charbonnieres, France) in a total volume of 12 µl.
Reverse transcription was carried out for 60 min at 60°C in the presence of 20 U RNaseOutTM (Invitrogen,Cergy Pontoise, France) and 7.5 U ThermoscriptTM reverse transcriptase (Invitrogen), in the buffer recommended by the manufacturer. An additional treatment was applied by adding 1 µl (2U) RNaseH (Invitrogen) for 20 min at 37°C.

The first round of nested PCR was performed with 2 µl of the cDNA obtained in a total volume of 50 µl, containing 3 U Taq polymerase (Promega), 0.5 mM dNTP, and 0.5 µM RC1 (5'-GTCTAGCCATGGCGTTAGTA-3') and RC21 primers for positive-strand amplification, or Tag (5'-GGCCGTCATGGTGGCGAATAA-3') and RC21 primers for negative strand amplification. The PCR protocol consisted of 18 cycles of denaturation (94°C for 1 min), annealing (55°C for 45 sec), and extension (72°C for 2 min). The cDNA obtained was purified using the kit from Qiagen, according to the manufacturer's instructions.

The purified product was then subjected to real-time PCR. The reaction was carried out using the LightCycler 480 SYBR Green I Master (2x con) Kit (Roche, Grenoble, France), with LC480 instruments and technology (Roche Diagnostics). PCR amplifications were performed in a total volume of 10 µl, containing 5 µl of Sybrgreen I Master Mix (2x), and 25 ng of the 197R (5'-CTTTCGCGACCCAACACTAC-3') and 104 (5'-AGAGCCATAGTGGTCTGCGG-3') primers. The PCR protocol consisted of one step of initial denaturation for 10 min at 94°C, followed by 40 cycles of

denaturation (95°C for 15 sec), annealing (57°C for 5 sec), and extension (72°C for 8 sec).

The quantitation of 28Sr RNA by specific RT-PCR was used as an internal standard to express the results of HCV positive or negative strands per µg of total hepatocyte RNA. Specific primers for 28 S rRNA were designed using the Oligo6 software 5'-TTGAAAATCCGGGGGAGAG-3'(nt2717–2735) and 50-ACATTGTTCCAACATGCCAG-30 (nt 2816–2797). Reverse transcription was performed using AMV reverse transcriptase (Promega), and the PCR protocol consisted of one step of initial denaturation for 8 min at 95°C, followed by 40 cycles of denaturation (95°C for 15 sec), annealing (54°C for 5 sec), and extension (72°C for 5 sec).

Results

Table 3 shows the anti-HCV activity of compound 8a as determined in the in vitro primary human hepatocyte assay described above. The numbers are expressed as 10^6 HCV RNA copies/µg of total RNA. Results of two independent experiments (Exp 1 and Exp 2) are given. The data per experiment is the average of two measurements.

Table 3: Effect of compound 8a on positive strand HCV-RNA levels in primary human hepatocytes (expressed as 10^6 HCV RNA copies/µg of total RNA).

Table 3.

	Exp. 1	Exp. 2
No HCV	0	0
HCV control	3.56	5.53
IFNα (100IU/mL)	1.48	1.59
8a (0.195 µM)	2.18	1.12
8a (0.78 µM)	2.25	1.3
8a (3.12 µM)	1.09	0.94
8a (12.5 µM)	2.17	1.3
8a(50 µM)	0.94	1.33

***In vivo* efficacy assay**

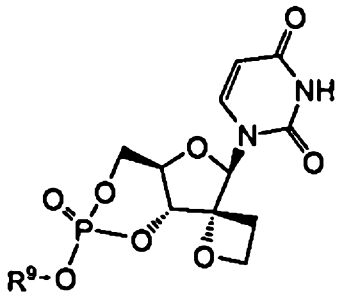
The *in vivo* efficacy of compound 8a and CAS-1375074-52-4 was determined in a humanized hepatocyte mouse model (PBX-mouse) as previously described in Inoue *et. al* (Hepatology. 2007 Apr;45(4):921-8) and Tenato *et. al.* (Am J Pathol 2004;165-901-912) with the following specification: Test animals: HCV G1a -infected PXB-mice, male or female, >70 % replacement index of human hepatocytes. Dosing was performed p.o for 7 days at doses indicated below wherein QD represents a single dose per day, BID represents two doses per day.

Efficacy of compound 8a was compared to CAS-1375074-52-4. Results are indicated in Figure 1. The Figure shows the log drop HCV viral RNA after dosing for a period of 7 days.

Figure 1 clearly shows that a dosing of 100mg/kg QD for CAS 1375074-52-4 (indicated as ✳, n=4) does not result in a significant log drop in HCV viral RNA. This in strong contrast to each of the indicated dose regimens for compound 8a, were a clear log drop is observed for 100 mg/kg QD (indicated as ◆, n=3), 200mg/kg QD (indicated as ●, n=4), 50mg/kg BID(indicated as ■, n=4). The most pronounced log drop effect in viral RNA is observed after a 7 day dosing of compound 8a at 100 mg/kg BID (indicated as ▲, n=4).

Claims

1. A compound of formula I:



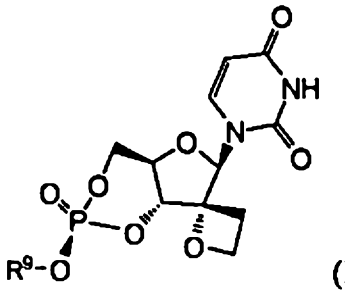
(I)

including any possible stereoisomer thereof, wherein:

R^9 is C_1-C_6alkyl, phenyl, C_3-C_7cycloalkyl or C_1-C_3alkyl substituted with 1, 2 or 3 substituents each independently selected from phenyl, naphtyl, C_3-C_6cycloalkyl, hydroxy, or C_1-C_6alkoxy;

or a pharmaceutically acceptable salt or solvate thereof.

2. A compound according to claim 1 which is of formula Ia:



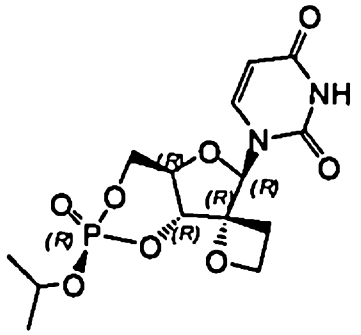
(Ia)

3. A compound according to claim 1 or 2, wherein R^9 is C_1-C_6alkyl or C_1-C_2alkyl substituted with phenyl C_1-C_2alkoxy or C_3-C_6cycloalkyl.

4. A compound according to any one of claims 1 to 3, wherein R^9 is C_2-C_4alkyl.

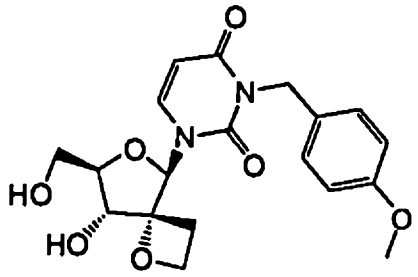
5. A compound according to any one of claims 1 to 4, wherein R^9 is *i*-propyl.

6. A compound according to any one of claims 1 to 5, which is of formula Ib:



(Ib)

7. A compound of formula V:

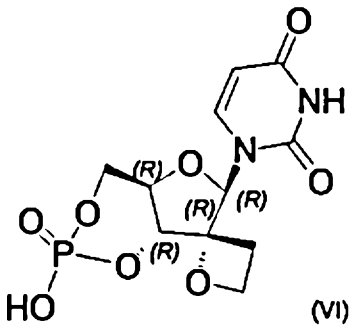


(V)

including pharmaceutically acceptable salt or solvate thereof.

8. The use of compound (V) in the synthesis of a compound according to any one of claims 1-6.

9. A compound of formula VI:



(VI)

including any stereochemical form and/or pharmaceutically acceptable salt or solvate thereof.

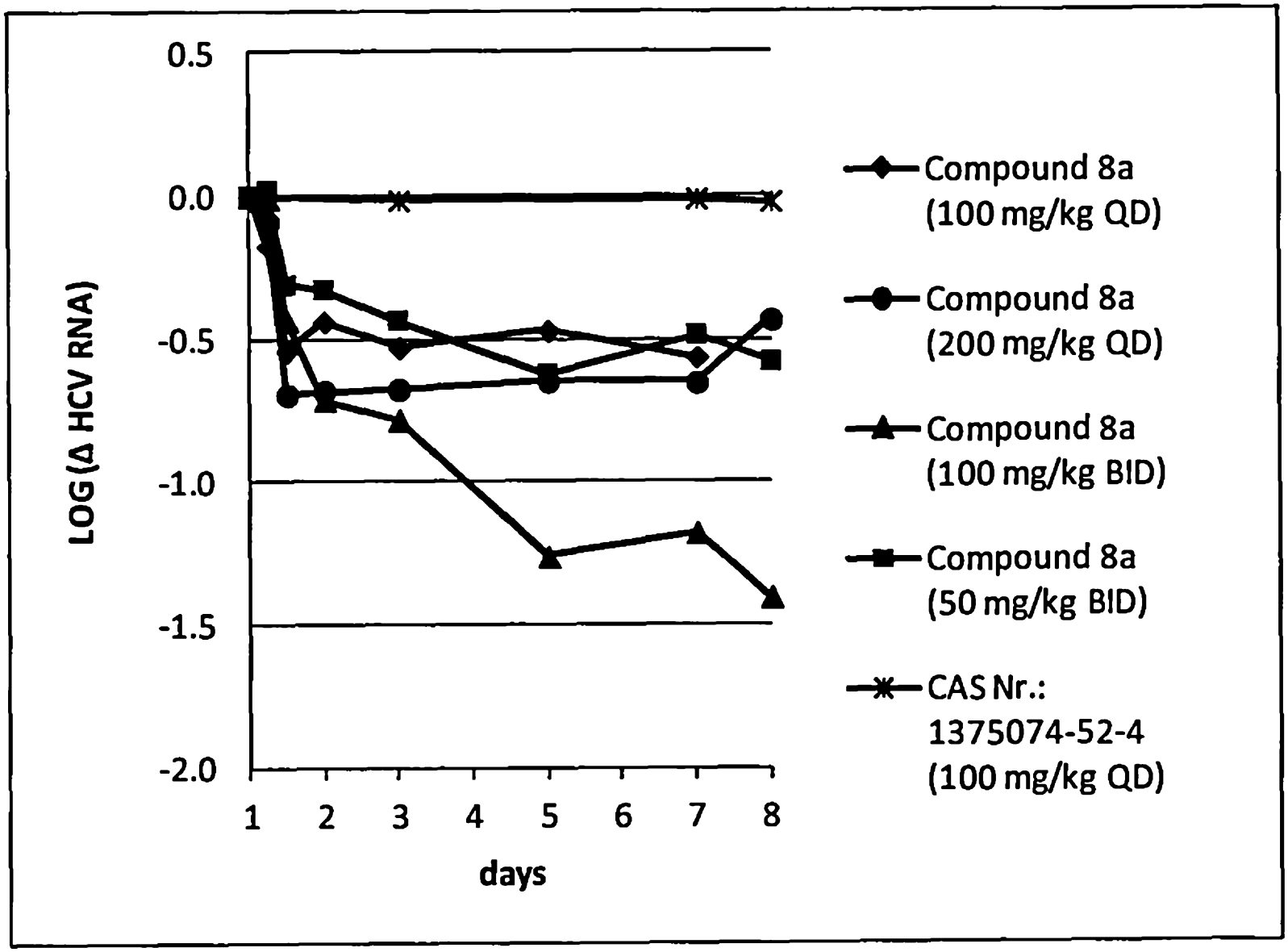
10. A pharmaceutical composition comprising a compound according to any of claims 1 to 6, and a pharmaceutically acceptable carrier.

11. A compound according to any of claims 1 to 6 or claim 9 or a pharmaceutical composition according to claim 10, for use as a medicament.

12. A compound according to any of claims 1 to 6 or claim 9, or a pharmaceutical composition according to claim 9, for use in the prevention or treatment of an HCV infection in a mammal.

13. A product containing (a) a compound of formula I as defined in any one of claims 1 to 6 or claim 9, and (b) another HCV inhibitor, as a combined preparation for simultaneous, separate or sequential use in the treatment of HCV infections.

Figure 1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Compound 8a (200 mg/kg QD)
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