22

Novel 4′-Azido-2′-Deoxy-Nucleoside Analogs are Potent Inhibitors of NS5B-Dependent HCV Replication

David Smith[1,*], Han Ma[1], Sophie Le Pogam[1], Vincent Leveque[1], Carl Brown[1], Nils Gunnar Johansson[2], Genadiy Kalayanov[2], Staffan Eriksson[3], Elena Usova[3], Christian Sund[2], Anna Winqist[2], Tatiana Maltseva[2], Mark Smith[1], Joseph Martin[1], Isabel Najera[1], Klaus Klumpp[1]

[1] Roche Palo Alto LLC, CA, USA; [2] Medivir AB, Huddinge, Sweden; [3] Swedish University of Agricultural Sciences, Department Mol. Biosciences, The Biomedical Centre, Uppsala, Sweden

Two novel deoxynucleoside analogs have been identified as potent inhibitors of HCV replication targeting HCV polymerase NS5B. Despite lacking the 2′-a-hydroxy moieties of ribonucleosides, which are generally recognized by RNA polymerases through direct hydrogen bonding interaction, the deoxycytidine triphosphate analogs RO-0622-TP and RO-9187-TP were efficiently incorporated into nascent RNA by HCV polymerase NS5B in a template base specific manner, causing chain termination. Both nucleosides were also excellent substrates for deoxycytidine kinase and were phosphorylated with efficiencies similar to or higher than deoxycytidine. Consistent with these findings, high levels of triphosphate were formed in human hepatocytes and both compounds were potent inhibitors of HCV replication in the replicon system ($IC_{50} = 171 \pm 12$ nM and 24 ± 3 nM for RO-9187 and RO-0622, respectively; $CC_{50} > 1$ mM). Both compounds inhibited RNA synthesis by HCV polymerases from either genotypes 1a and 1b or containing the S282T point mutation with similar potencies, suggesting no cross-resistance with 2′-C-methyl nucleosides. Pharmacokinetic studies with RO-9187 in rats, dogs and monkeys showed that plasma concentrations exceeding HCV replicon IC_{50} values up to 150-fold could be achieved by low dose (10 mg/kg) oral administration. Therefore, 4′-azido-2′-deoxy nucleosides are a new class of antiviral nucleosides with promising preclinical properties as potential medicines for the treatment of HCV infection.

doi:10.1016/j.antiviral.2007.01.030

23

Characterization of the Intracellular Metabolism of β-D-2′-Deoxy-2′-Fluoro-2′-C-Methyl-Cytidine and the Inhibition of HCV Polymerase NS5B by its 5′-Triphosphate Species

Han Ma*, Wen-Rong Jiang, Nicole Robledo, Vincent Leveque, Samir Ali, David Smith, Mohammad Masjedizadeh, Teresa Lara-Jaime, Nick Cammack, Klaus Klumpp, Julian Symons

Roche Palo Alto LLC, Palo Alto, CA, USA

β-D-2′-Deoxy-2′-fluoro-2′-C-methyl-cytidine (R1656, PSI-6130) is a potent inhibitor of hepatitis C virus (HCV) replication in the subgenomic HCV replicon system and its corresponding 5′-triphosphate is a potent inhibitor of the HCV RNA polymerase in vitro. In this study, the formation of R1656-triphosphate was characterized in primary human hepatocytes isolated from several independent donors. Intracellular concentrations of R1656 and its 5′-phosphorylated derivatives were determined, with R1656 parent compound being the major intracellular species. In addition, the deaminated derivative of R1656, β-D-2′-deoxy-2′-fluoro-2′-C-methyl-uridine (RO2433, PSI-6026) and its corresponding phosphorylated metabolites were identified in human hepatocytes after incubation with R1656. The formation of R1656-TP and RO2433-TP increased with time and reached steady state level at 48 h. R1656-TP and RO2433-TP were the major phosphorylated species at steady state. The formation of both R1656-TP and RO2433-TP demonstrated a linear relationship with the extracellular concentrations of R1656 up to 100 μM, suggesting a high capacity of human hepatocytes to generate the two triphosphates. The mean half lives of R1656-TP and RO2433-TP were 4.7 and 38 h, respectively. RO2433-TP also inhibited RNA synthesis by the native HCV replicase isolated from HCV replicon cells and the recombinant HCV polymerase NS5B with potencies comparable to those of R1656-TP. Incorporation of RO2433-MP into nascent RNA by NS5B led to chain termination, similar to that of R1656-MP. These results suggest that R1656 is metabolized to two pharmacologically active species in primary human hepatocytes. The long half life of RO2433-TP suggests the potential for investigating once daily dosing regimens of R1656 for the treatment of HCV infection.

doi:10.1016/j.antiviral.2007.01.031

24

Sub Micromolar Inhibitors of HCV Generated from Inactive Nucleosides by Application of ProTide Technology

Christopher McGuigan[1,*], Plinio Perrone[1], Giovanna Luoni[1], Mary Rose Kelleher[1], Felice Daverio[1], Annette Angell[1], Sinead Mulready[1], Costantino Congiatu[1], Sonal Rajyaguru[2], Joseph Martin[2], Vincent Lévêque[2], Sophie Le Pogam[2], Isabel Najera[2], Klaus Klumpp[2], David Smith[2]

[1] Welsh School of Pharmacy, Cardiff University, UK; [2] Roche Palo Alto, CA, USA

We report the application of our phosphoramidate ProTide technology to various 4′-substituted ribonucleoside analogues, designed as potential inhibitors of hepatitis C virus (HCV) (Fig. 1). Thus, ProTides were prepared from 4′-azidouridine (AZU), -cytidine (AZC), -adenosine (AZA) and -5-methyluridine (AZMeU), besides other 4′-substituted uridines and cytidines. In each case, ProTide families included variations in the aryl, ester, and amino acid regions. A number of compounds showed potent inhibitory properties in cell culture without detectable cytotoxicity. These results confirm that phosphoramidate ProTides can deliver monophosphates of ribonucleoside analogues and suggest a potential path to the generation of novel antiviral agents against HCV infection. Of particular note was the sub-μM potency displayed by certain ProTides of AZU; a nucleoside analogue, which was itself inac-

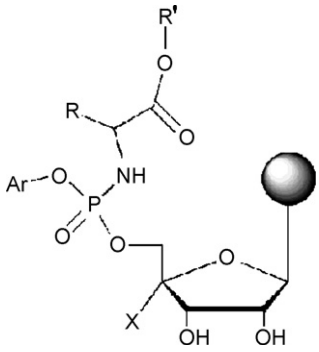

Fig. 1.

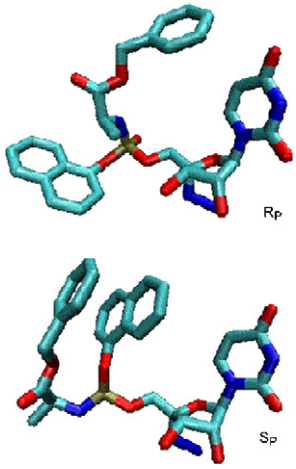

Fig. 2.

tive in the assay. In some cases, we were able to separate, and separately evaluate the phosphate stereoisomers generated in the synthesis; in a few cases the absolute phosphate stereochemistry was solved (Fig. 2). The generic message is that ProTide synthesis from inactive parent nucleosides may be a warranted drug discovery strategy.

doi:10.1016/j.antiviral.2007.01.032

25

GL59728: A Potent Allosteric Inhibitor of the HCV NS5b RNA Dependent RNA Polymerase with Excellent Pharmacokinetic Properties

Christopher Roberts*, Janos Botyanszki, Dong-fang Shi, Joshua Gralapp, Samantha Koo-McCoy, John Zhang, Kevin Fung, Mohan Sivaraja, Jeffrey Pouliot, Ting Wang, Kevin Dunlop, Wenbao Li, Lillian Lou, Uli Schmitz, Peter Young, Ron Griffith

Genelabs Technologies, Inc. 505 Penobscot Dr., Redwood City, CA 94063, USA

The HCV RNA dependent RNA polymerase NS5b is a virally encoded enzyme responsible for HCV RNA replication and is essential for viral replication. A class of non-nucleoside compounds that target an allosteric site in the finger-loop region of the polymerase was identified. Multiple rounds of optimization led to compounds with nanomolar potencies against both the purified NS5b enzyme and the subgenomic HCV replicon. In vivo DMPK profiling and optimization led to GL59728, which displays excellent cross-species pharmacokinetic properties. Finally, GL59728 displays outstanding in vitro antiviral efficacy when combined with other classes of potential anti-HCV agents.

doi:10.1016/j.antiviral.2007.01.033

26

Substituted Imidazopyridines as Potent Inhibitors of Hepatitis C Virus Replication that Target the Viral Polymerase

Inge Vliegen[1,*], Jan Paeshuyse[1], Laura S. Lehman[2], Weidong Zhong[2], Sofie Roofthooft[1], Hélène Dutartre[3], Barbara Selisko[3], Bruno Canard[3], Nina Boddeker[2], Steven Bondy[2], David Oare[2], Erik De Clercq[1], William A. Lee[2], Gerhard Pürstinger[4], Johan Neyts[1]

[1] Rega Institute, K.U.Leuven, Belgium; [2] Gilead Sciences, Foster City, CA, USA; [3] CNRS and Universités d'Aix-Marseille I et II, France; [4] Universität Innsbruck, Austria

Following lead optimization, a series of substituted imidazopyridines was identified as potent and selective inhibitors of in vitro HCV replication, as was determined using various HCV subgenomic replicon systems. The activity was also assessed in hepatoma cells infected with an infectious full-length chimeric HCV construct. The particular characteristics and mechanism of action of one of the most potent congeners in this series (GS-327073) was studied. Typically, 50% effective concentrations (EC_{50}) for inhibition of replicon or viral replication ranged between 2.4 and 22 nM; 50% cytostatic concentrations (CC_{50}) were $\geq$16 μM, thus resulting in selectivity indices of >800 to >8000. GS-327073 remained active against HCV replicons that were resistant to various HCV polymerase or protease inhibitors. When GS-327073 was combined with either interferon 2α or several polymerase or protease inhibitors, an additive antiviral activity was obtained. Several months of selective in vitro pressure with GS-327073 were required before drug resistant variants were obtained. Genotyping of the GS-327073res replicons resulted in the identification of mutations C316C/Y, C445F and Y452H in the polymerase gene. Transfection of naïve cells with RNA isolated from GS-327073res replicons transferred drug resistance, indicating that resistance is associated with the viral genome and not with the host cell. Reintroduction of the mutations in wild-type replicons resulted in a drug-resistant phenotype, whereby the total number of mutations correlates with the degree of resistance. Although the viral polymerase is obviously the target for the antiviral activity, GS327073 was not able to inhibit the activity of recombinant HCV polymerase in initiation or elongation assays. Further studies are ongoing to determine by which mechanism the compound inhibits the viral polymerase in infected cells.

doi:10.1016/j.antiviral.2007.01.034