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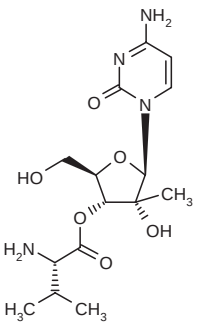
Valopicitabine

Prop INN; USAN

Anti-Hepatitis C Virus Drug
RNA-Directed RNA Polymerase (NS5B) Inhibitor

NM-283
val-mCyd

2'-*C*-Methyl-3'-*O*-(L-valyl)cytidine
L-Valine 3'-ester with 2'-*C*-methylcytidine



$C_{15}H_{24}N_4O_6$
Mol wt: 356.3744

CAS: 640281-90-9

EN: 359750

Abstract

Chronic hepatitis C is caused by infection with the hepatitis C virus (HCV), a member of the Flaviviridae family of viruses. Currently available treatment for HCV, including the standard combination therapy with interferon and ribavirin, is often unsuccessful at eradicating infection. In addition, the therapies now used to treat chronic hepatitis C are associated with substantial side effects. Therefore, new therapeutic strategies such as the use of antiviral drugs targeted to HCV-specific viral enzymes are being explored. One such option is the RNA-directed RNA polymerase (NS5B) inhibitor valopicitabine (NM-283), an orally bioavailable prodrug of the novel ribonucleoside analogue NM-107. This compound has shown *in vitro* activity against HCV-related bovine viral diarrhea virus (BVDV) polymerase. In patients with HCV-1 infection, valopicitabine produced reductions in HCV RNA viral load when administered either as monotherapy or in combination with pegylated interferon. When used together, valopicitabine and interferon appear to have synergistic antiviral effects both *in vitro* and *in vivo*. The compound is generally well tolerated, with gastrointestinal effects being the most commonly observed treatment-related adverse events.

Synthesis

Valopicitabine can be prepared as follows:

1) Treatment of D-fructose (I) with aqueous calcium oxide gives 2-methyl-D-ribonic-γ-lactone (II), which is esterified with benzoyl chloride in the presence of Et_3N/DMAP to give the tribenzoyl derivative (III). Lactone (III) reduction by means of Red-Al gives the lactol (IV), and further acylation of (IV) with benzoyl choride and Et_3N/DMAP affords 1,2,3,5-tetra-*O*-benzoyl-2-methyl-D-ribofuranose (V) (1). Condensation of (V) with cytosine (VI) in the presence of $SnCl_4$ and *N*,*O*-bis(trimethylsilyl)-acetamide (BSA) yields adduct (VII), from which the benzoate ester groups are removed by treatment with methanolic NaOMe to obtain the cytidine analogue (VIII). Coupling of (VIII) with *N*-Boc-L-valine (IX) in the presence of CDI provides the 3'-ester (X). The *N*-Boc protecting group in (X) is finally removed under acidic conditions to furnish valopicitabine (1) (Scheme 1).

2) In a related method, 1,2,3,5-tetra-*O*-benzoyl-2-methyl-D-ribofuranose (V) is coupled with benzoyl cytosine (XI), giving adduct (XII), from which the benzoyl groups are removed by treatment with methanolic ammonia to give (VIII). Protection of the cytosine amino group with dimethylformamide dimethylacetal, followed by silylation of the primary hydroxyl group, furnishes (XIII), which is coupled to *N*-Boc-L-valine (IX) to afford the ester (XIV). Deprotection of (XIV) to obtain the title compound is then achieved by refluxing in MeOH with ammonium fluoride, followed by treatment with HCl in EtOAc (1) (Scheme 1).

Background

The hepatitis C virus (HCV) belongs to the Flaviviridae family of viruses, a family that is only known to infect humans and chimpanzees. This blood-borne, enveloped, 9.5-kb positive-strand RNA virus mutates rapidly, making infection with HCV difficult to treat.

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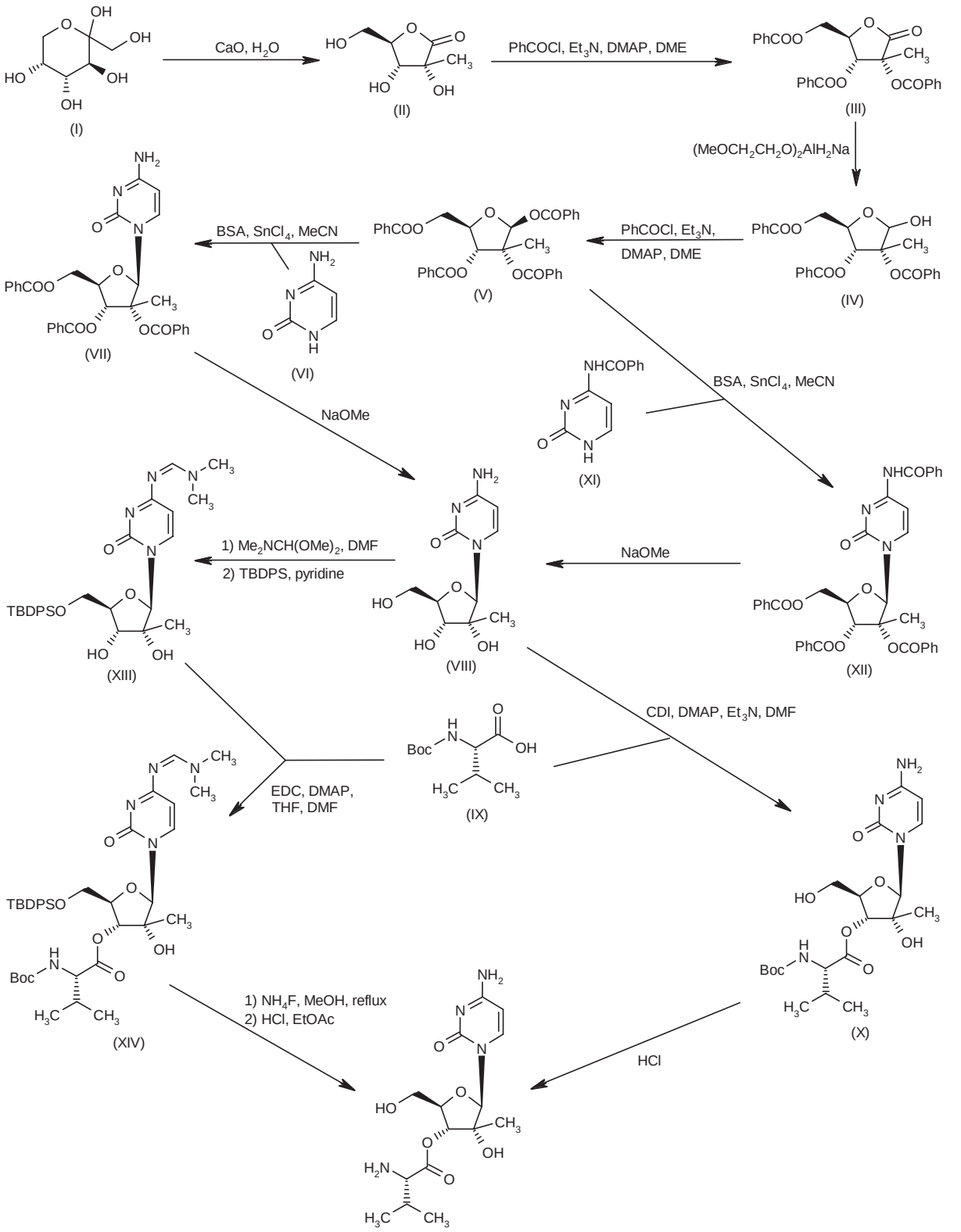
Scheme 1: Synthesis of Valopicitabine



Infection with HCV causes symptoms such as jaundice, fatigue, abdominal pain, loss of appetite, nausea and vomiting; however, these symptoms often do not become evident until the infection has become chronic. About 75-85% of individuals with acute HCV infection progress to chronic hepatitis, although the factors responsible for causing HCV infection to become chronic are not well understood. Infection with HCV can result in progressive liver damage that may include cirrhosis, hepatocellular carcinoma, liver failure and death. The World Health Organization (WHO) estimates that about 2% of the world's population currently has chronic hepatitis C, although the prevalence varies widely from one country to another. Furthermore, due to the large number of individuals who carry the virus, the number of deaths from hepatitis is expected to increase dramatically in coming decades if effective new therapies are not discovered soon (2).

Currently available treatments for HCV infection are unsuccessful in about 50% of patients with the genotype-1 strain of the hepatitis C virus (HCV-1), and re-treatment of patients who do not respond initially is rarely effective. Until recently, an 18-24-month course of monotherapy with interferon alfa was the standard therapy for hepatitis C. Now, treatment for chronic hepatitis C usually involves the combined use of pegylated interferon (peginterferon) and the antiviral agent ribavirin. This combination therapy leads to the loss of HCV RNA in 50-55% of patients and to sustained loss in 35-45%. However, this therapy is associated with significant side effects and cannot be administered to certain types of patients. The development of antiviral drugs targeted to HCV-specific viral enzymes is now regarded as a superior strategy for controlling and perhaps even eliminating HCV infection (2-5).

Valopicitabine (NM-283) is an orally bioavailable prodrug of the novel ribonucleoside analogue NM-107 (2'-*C*-methylcytosine). As an RNA-directed RNA polymerase (NS5B) inhibitor, this compound appears to act by inhibiting the HCV viral polymerase directly and also by being incorporated into growing strands of viral RNA, thereby terminating RNA chain extension. NM-107 has been demonstrated to competitively inhibit purified HCV-related bovine viral diarrhea virus (BVDV) polymerase *in vitro* (K_i approximately 160 nM) and to act as a chain terminator of BVDV RNA synthesis. The compound has also shown antiviral activity in the HCV replicon system, as well as in chronically infected chimpanzees. In humans, valopicitabine produced a reduction in viral load, sometimes to below detectable levels, when administered at tolerated doses. Combination therapy with valopicitabine and interferon has also shown activity against HCV-1 infection in clinical trials (3-5).

Preclinical Pharmacology

An *in vitro* study assessed the antiviral activity of NM-107 in combination with recombinant human interferon alfa-2b (Intron® A) or interferon beta-1a (Avonex®). Results from studies using BVDV NS5B polymerase indicate that NM-107 is a specific chain terminator of BVDV RNA synthesis. In Madin-Darby bovine kidney (MDBK) cells infected with BVDV, NM-107 potently inhibited BVDV propagation (EC_{90} = 0.87 ± 0.18 μM) and eradicated persistent infection after 28 days. The EC_{90} value for interferon alfa-2b was 32.5 ± 18.2 IU/ml. Viral activity was inhibited in a synergistic manner by the combination of NM-107 (4 μM) and interferon alfa-2b (2000 IU/ml). Enhancements in antiviral activity with the combination were 2.4 $\log_{10}$ greater than additive in general, and over 4 $\log_{10}$ greater than additive against the NY1 strain of BVDV. No inhibitory activity against BVDV was observed for interferon beta (6).

Using an *in vitro* BVDV infection model in MDBK cells, researchers investigated the potential for the development of resistance to valopicitabine. The cytopathic BVDV strain NADL was repeatedly passaged with the active drug NM-107, and no resistant mutants were produced. Against noncytopathic BVDV biotypes, higher concentrations of NM-107 eradicated infection, whereas lower concentrations caused the selection of resistant virus after 3-5 cell passages. This resistance was caused by an S405T amino acid substitution near the start of the B-domain motif of the BVDV NS5B polymerase. The predicted homologous mutation in HCV NS5B polymerase is S282T. BVDV strains resistant to NM-107 show at least a 50-fold reduction in susceptibility to the compound, along with decreased replication fitness *in vitro* (*i.e.*, "small plaque" phenotype, slow growth kinetics and lower virus titers). The S405T mutant virus had a 38-fold increased susceptibility to interferon alfa-2b (Intron® A) compared to wild-type BVDV, and no viral resistance developed when NM-107 was tested *in vitro* in combination with Intron® A. No additional compensatory mutations that restore the phenotype of the S405T mutant BVDV to that of wild-type BVDV have been found (7).

In chimpanzees chronically infected with HCV-1, oral valopicitabine (8.3 or 16.6 mg/kg) showed potent antiviral activity when administered once daily for 1 week. Serum HCV RNA titers declined after 2 days of therapy, with mean viral load reductions of 1.05 and 0.83 $\log_{10}$ being observed at day 7 in the high- and low-dose groups, respectively (8).

Pharmacokinetics and Metabolism

The pharmacokinetics and pharmacodynamics of valopicitabine were determined in a randomized, placebo-controlled, dose-escalating phase I/II study in patients with chronic HCV-1 infection and compensated, noncirrhotic chronic liver disease (87% nonresponders to prior interferon-based therapies). In this study, valopicitabine (50-800 mg/day for 15 days) showed rapid absorption and conversion to the active drug NM-107 (80% of plasma exposure on a molar basis) and the metabolite NM-106 (15% of plasma exposure on a molar basis). The plasma half-life of valopicitabine was 1.4 h, and no predose or trough valopicitabine was detected. The metabolite NM-106 was only detected transiently at the 50- and

100-mg doses, increasing with higher doses or repeated daily dosing. The plasma half-lives of NM-106 and NM-107 were 12 and 4.5 h, respectively. Systemic exposure to all three compounds increased proportionally with the valopicitabine dose. For single valopicitabine doses of 50, 100, 200, 400 and 800 mg, the NM-107 C_{max} values were 0.43 ± 0.19, 0.94 ± 0.34, 1.65 ± 0.27, 2.90 ± 0.68 and 4.33 ± 0.68 μg/ml, respectively. The corresponding AUC values were 3.3 ± 1.4, 6.7 ± 1.8, 13.3 ± 3.3, 23.0 ± 3.9 and 38.9 ± 6.8 μg/ml.h, respectively. The AUC and C_{max} of NM-107 and NM-106 showed a significant correlation with serum HCV RNA viral load reduction at day 16 ($r > 0.7$; $p < 0.001$) (9, 10).

Clinical Studies

Efficacy data were also reported from 95 patients in the above trial. HCV RNA viral load reductions after 15 days of treatment were 0.15, 0.39, 0.30, 0.67 and 1.21 $\log_{10}$ copies/ml for valopicitabine doses of 50, 100, 200, 400 and 800 mg/day, respectively. In individual patients receiving the highest dose level (800 mg/day), reductions in viral load ranged from 0.41 $\log_{10}$ to 2.37 $\log_{10}$ (61-> 99% reduction), in contrast to the 0.03 $\log_{10}$ increase in HCV RNA levels observed in patients receiving placebo. Valopicitabine was generally well tolerated, with no serious adverse events or treatment-limiting toxicities observed. Gastrointestinal disturbances such as nausea or occasionally vomiting were the most common side effects and occurred more frequently in patients receiving valopicitabine at doses of 400 mg/day and above (9-13).

The antiviral activity of valopicitabine alone is being compared to that of valopicitabine plus peginterferon alfa-2b in treatment-naïve patients with chronic HCV-1 infection. The treatment regimen involves dose escalation of valopicitabine to 800 mg/day over the first week, followed by 800 mg/day thereafter, together with peginterferon alfa-2b at 1.0 μg/kg once weekly starting on day 8 in the patients assigned to combination therapy. In this multicenter, open-label phase IIa study, a total of 30 patients will be treated for 24 weeks. Interim results for 19 patients after 10 or 12 weeks of treatment showed mean HCV RNA reductions of 1.0 $\log_{10}$ IU/ml for patients receiving valopicitabine alone and 3.2 $\log_{10}$ IU/ml for patients receiving valopicitabine plus peginterferon alfa-2b. Of the 12 patients who have received combination treatment for at least 10 weeks, 11 showed a substantial reduction in HCV RNA viral load (1.2-6.2 $\log_{10}$ IU/ml). Four patients have achieved HCV levels in blood serum that are below detectable limits (< 10 IU/ml) (14, 15).

A randomized, open-label phase IIb trial is under way in 173 patients with chronic HCV-1 infection who previously failed to respond to therapy with peginterferon plus ribavirin. In this study, the efficacy of treatment with valopicitabine, alone or in combination with peginterferon, will be compared to that of re-treatment with peginterferon plus ribavirin. Four-week interim results in 97 patients found that HCV replication was suppressed more effectively with valopicitabine plus peginterferon than with peginterferon plus ribavirin. Among the patients receiving 800 mg/day valopicitabine plus 180 μg once weekly peginterferon, 55% achieved early virological response by week 4. Gastrointestinal side effects were common, but usually resolved spontaneously. Two patients discontinued treatment due to gastrointestinal side effects, 1 was discontinued because of lipase elevation, 1 patient required a reduction in treatment dose, and 5 patients refused treatment after randomization to peginterferon plus ribavirin (16).

Drug Interactions

A randomized, open-label trial assessed the pharmacokinetics of valopicitabine alone (n=12) and in combination with peginterferon alfa-2b (n=18) in treatment-naïve patients with HCV-1 infection. The steady-state pharmacokinetic parameters for the active drug NM-107 were comparable when valopicitabine was administered alone or in combination with peginterferon alfa-2b. The geometric mean ratios for combination *versus* monotherapy were 0.95 for C_{max}, 0.90 for AUC and 0.93 for $t_{1/2}$. Thus, coadministration with peginterferon does not alter the pharmacokinetics of valopicitabine (17).

Sources

Idenix Pharmaceuticals, Inc. (US); co-discovered with Universita degli Studi di Cagliari (IT); co-developed with Novartis Pharma AG (CH).

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