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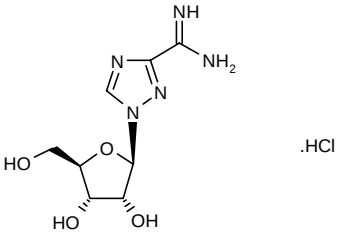
Viramidine Hydrochloride

IMPDH Inhibitor
Anti-Hepatitis C Virus Drug

Ribamidine hydrochloride
AVS-206
ICN-3142

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamidine hydrochloride



$C_8H_{13}N_5O_4$.HCl

Mol wt: 279.6826

CAS: 040372-00-7

CAS: 119567-79-2 (as free base)

EN: 146039

Abstract

Hepatitis C virus (HCV) is a major cause of both acute and chronic hepatitis. It affects around 3% of the global population, and is the single most important cause of chronic liver disease, cirrhosis and liver cancer in the Western world. The current standard of care for HCV is ribavirin in combination with pegylated interferon alfa, which improves the sustained virological response to around 55%. Although current anti-HCV therapies are effective, they are often associated with adverse effects, such as hemolytic anemia, and are poorly tolerated. Research efforts have focused on developing new therapeutic candidates with improved safety and tolerability profiles. Viramidine, a liver-targeted prodrug of ribavirin, is one such candidate. Like ribavirin, viramidine is an effective antiviral agent with immunomodulatory properties. However, viramidine exhibits a greater therapeutic index, a more favorable tissue distribution and an improved safety profile compared with ribavirin. Viramidine is also associated with significantly lower red blood cell (RBC) accumulation compared with ribavirin. Viramidine may therefore prove to be a suitable therapeutic candidate for the oral treatment of HCV infection, with the potential to reduce the hemolytic liability associated with ribavirin.

Synthesis

Viramidine can be synthesized by several related ways:

1) Reaction of triethyl orthoformate (I) with 1-cyanoformimidic acid hydrazide (II) by means of anhydrous HCl in dioxane gives 1,2,4-triazole-3-carbonitrile (III), which is condensed with tetra-*O*-acetyl-β-D-ribofuranose (IV) by means of di-*p*-nitrophenyl phosphate (BNPP) at 150 °C to yield a mixture of the regioisomeric ribonucleosides (V) and (VI), easily separated by chromatography. Finally, the suitable regiosomer 3-cyano-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole (V) is treated with anhydrous NH_3 and NH_4Cl at 85 °C in a pressure bomb (1, 2). Scheme 1.

2) Alternatively, condensation of 2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl bromide (VII) with 1-(trimethylsilyl)-1,2,4-triazole-3-carbonitrile (VIII) – prepared by treatment of triazole (III) with hexamethyldisilazane in dry acetonitrile – in acetonitrile also gives the regioisomeric mixture of nucleosides (V) and (VI) (1). Scheme 1.

3) Acylation of ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) (IX) with Ac_2O and pyridine gives the triacetate (X), which is dehydrated by means of $POCl_3$ and triethylamine in chloroform to yield 3-cyano-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole (V). Treatment of nucleoside (V) with NaOMe in methanol affords the methyl carboximidate (XI), which is finally treated with NH_4Cl and dry NH_3 gas in methanol (3). Scheme 1.

Introduction

There are approximately 170 million people, or 3% of the world's population, infected with the hepatitis C virus (HCV) (4). Over the past decade, significant advances have been made in the treatment of HCV infection, particularly with the development of ribavirin, a purine nucleoside analogue with a broad spectrum of activity against various DNA and RNA viral infections (5). Ribavirin

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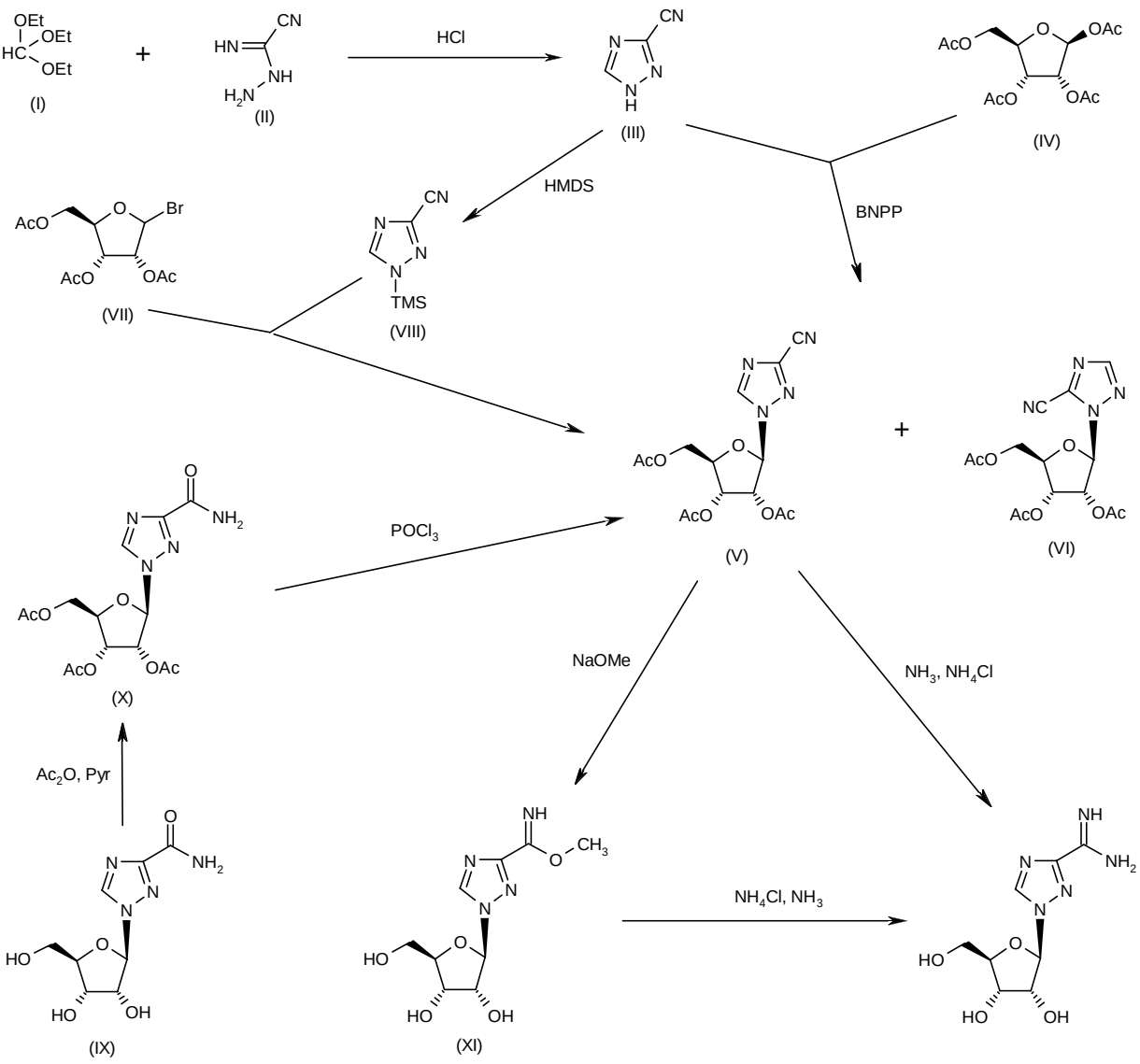
Scheme 1: Synthesis of Viramidine



together with pegylated interferon alfa-2b is currently the treatment of choice for the initial therapy of patients with chronic HCV infection, effective in achieving a sustained virological response in approximately half of the patients (6). Ribavirin, however, is also associated with dose-limiting hemolytic anemia. Following absorption, ribavirin is transported to red blood cells (RBCs) to a significant extent and phosphorylated to several metabolites, which accumulate intracellularly, causing the hemolytic anemia (7-10). In an attempt to reduce the accumulation of ribavirin metabolites in RBCs, and to deliver ribavirin more specifically to the liver, researchers have focused their efforts on several derivatives of ribavirin. The 3-carboxamidine derivative of ribavirin, viramidine, has emerged as one of the most promising (1, 3).

Viramidine is a liver-targeted prodrug of ribavirin that is currently being investigated in phase III trials for the oral treatment of patients with HCV infection. The phase III program consists of two global studies –VISER1 (VIramidine Safety and Efficacy vs. Ribavirin) and VISER2– at 80 sites with approximately 1,000 patients in each study. The studies will compare viramidine and ribavirin, each in conjunction with a pegylated interferon (11, 12).

Pharmacological Actions

The antiviral activity of viramidine has been examined against several RNA and DNA viruses (1, 13-15). Viramidine shows a similar spectrum of activity to ribavirin *in vitro*, with good inhibitory activity against vaccinia virus (IC_{50} = 59 μg/ml), respiratory syncytial virus (IC_{50} = 16 μg/ml), influenza A and B virus (IC_{50} = 48 μg/ml), sandfly fever virus (IC_{50} = 36 μg/ml), Punta Toro virus (IC_{50} = 83 μg/ml) (13), herpes simplex virus type 1 (HSV-1), type 13 rhinovirus and parainfluenza virus type 3 (1, 13). Viramidine is marginally effective against Japanese encephalitis, yellow fever and Venezuelan equine encephalomyelitis viruses, inhibiting viral cytopathicity by 25-40% at a concentration of 320 μg/ml (13). Viramidine is also active against dengue-4 virus (IC_{50} = 100 μg/ml), producing 99% plaque reduction at concentrations of 100 and 250 μg/ml (13). Viramidine was cytotoxic to Vero cells at 1000 μg/ml and to LLC-MK2 cells at a concentration of 250 μg/ml. No activity was detected against adenovirus type 5 (Ad5), HIV-1 and measles viruses in LLC-MK2 cells (13) or against the New York and Uganda isolates of West Nile virus in Vero cells (16).

Viramidine also has *in vivo* efficacy against experimentally induced viral infections in mice, including influenza A2 virus (1), Punta Toro virus (13-15, 17-20), the Crimean-Congo hemorrhagic fever virus (15, 21), and Rift Valley and Rauscher leukemia viruses (15). Viramidine is also effective in reducing virus titers and improving survival of Pichinde virus-infected hamsters, although its effect is slightly less than that of ribavirin (15, 22).

The immunomodulatory properties of viramidine have been studied both *in vitro* and *in vivo*, and proved to be similar to those of ribavirin (17, 23). In activated human T-cells, viramidine concentration-dependently (0.67-10 μM) increased staphylococcal enterotoxin B-stimulated type 1 cytokine expression (IL-2, interferon gamma and TNF-α), similar to ribavirin. In a mouse model of a type 1 cytokine-mediated immune response, i.p. administration of viramidine (0.28, 0.56 and 112 mg/kg) increased type 1 cytokine-mediated contact hypersensitivity responses to dinitrofluorobenzene (DNFB) in a dose-dependent manner, with efficacy similar to ribavirin (23). In the Hantaan-infected immunodeficient SCID mouse, viramidine at a dose of 75 mg/day delayed viral replication and provided protection against death similar to ribavirin (24).

Pharmacokinetics and Metabolism

In human liver microsomes, viramidine has no inhibitory or inductive effect on several cytochrome P-450 enzymes, suggesting little potential for drug interactions; at concentrations of 5, 10 and 50 μM, viramidine did not inhibit the enzyme activity of CYP3A4, CYP2D6, CYP2C9, CYP2C19, CYP2E1 and CYP1A2 (25). In male rats, oral administration of viramidine at a dose of 120 mg/kg/day for 8 days did not cause induction of CYP1A, CYP2B1/B2, CYP3A1/A2 or CYP4A1/A3 at the protein level, and had no significant effect on the mRNA expression of liver toxicological genes (25, 26).

Whole-body autoradiography has been performed to examine the tissue distribution of viramidine and ribavirin in rats. Following equivalent (30 mg/kg) oral doses of [^{14}C]-viramidine and [^{14}C]-ribavirin, over 30% higher radioactivity in the liver was detected after viramidine compared to ribavirin. High levels of radioactivity were found in small intestine, stomach wall, spleen, kidney, liver, small intestine wall and stomach at 2-4 h after [^{14}C]-viramidine administration, and high levels of radioactivity were detected for ribavirin in stomach and small intestine at these time points. In monkeys, radioactivity retention in the liver was about 3 times higher with viramidine than with ribavirin following single and multiple oral doses, and the liver was also shown to be the main site of conversion of viramidine to ribavirin (27, 28).

On the other hand, *in vitro* studies of [^{3}H]-viramidine and [^{3}H]-ribavirin accumulation in RBCs from rats, mice, dogs, monkeys and humans demonstrated greater RBC-associated radioactivity with ribavirin than with viramidine (28, 29). Viramidine also displayed less association with rat, monkey and human RBCs *in vivo*. Ribavirin showed at least twice the RBC radioactivity levels as its prodrug (25, 28, 30, 31).

The pharmacokinetic and metabolic profiles of viramidine have been investigated in rats, mice, dogs, monkeys (27, 28, 30, 32) and humans (33, 34). Metabolic studies with viramidine show that it is orally absorbed and efficiently and rapidly converted to ribavirin by adenosine deaminase, followed by further metabolism to ribofuranosyl-triazole-carboxylic acid and triazole-carboxamide (27, 30, 32, 33, 35). Following oral administration of viramidine to healthy volunteers, peak plasma concentrations occur within about 1.5-3 h, and the AUC is lower than that for ribavirin (33, 34). Dose proportionality has been demonstrated for the peak plasma concentration (C_{max}) and the AUC with viramidine, and for the AUC with ribavirin. Viramidine undergoes extensive metabolism and very little drug is excreted unchanged in the urine. Absorption is influenced by the presence of food in the gastrointestinal tract; the mean AUC and C_{max} of viramidine are increased by 44% and 20%, respectively, following a high-fat meal (33). The mean plasma protein binding of viramidine is around 7% in dogs, rats, monkeys and humans (25). In human volunteers, the estimated half-life for 200-1200 mg oral viramidine is variable, and in the range of 3.8-35.6 h (33).

Toxicity

Toxicity studies with viramidine have been performed in several animal species. Viramidine exhibited a similar toxicity profile to ribavirin in rats, but a better safety profile in monkeys (28, 36, 37). Treatment of monkeys with viramidine at doses of up to 600 mg/kg/day p.o. for 28

days was not associated with any significant changes in hematological parameters (hematocrit, hemoglobin or RBC), or in the number of platelets and reticulocytes. However, ribavirin (100-600 mg/kg/day) was associated with significant decreases in hematological parameters, increased numbers of platelets and reticulocytosis. In rats, doses of 60 mg/kg/day viramidine for 14 or 28 days were not associated with any clinical side effects, although a higher dose of 120 mg/kg/day resulted in transient decreases in body weight and food consumption. Significant changes were observed in white blood cells, reticulocytes, platelets, RBCs, hemoglobin and hematocrit with the highest dose of viramidine. However, these parameters returned to normal following the 28-day recovery phase. Similar findings were observed with ribavirin 120 mg/kg/day.

Clinical Studies

The safety profile of viramidine has been evaluated in healthy individuals. Viramidine at doses of 200, 600 or 1200 mg/day was well tolerated and had a favorable safety profile (33, 34). There were no reports of serious adverse events, and most were mild, and they included headache, upper respiratory tract infections, sore throat, nasal congestion, nausea and cough. Treatment-related events were reported in 50% of the group receiving 1200 mg, 26% of the 600-mg group and 0% of the 200-mg group (33).

An ongoing randomized, dose-ranging phase II study conducted in 180 treatment-naïve hepatitis C-infected patients examined the antiviral activity of viramidine and its effect on hemoglobin concentrations. Patients were randomized to receive peginterferon alfa-2a 180 μg/week s.c. in combination with either viramidine 400-800 mg b.i.d. or ribavirin 1000-1200 mg/day. An interim analysis at 24 weeks showed comparable antiviral activity for viramidine and ribavirin, but a significantly lower incidence of anemia in viramidine-treated patients. There were fewer reports of hemoglobin reductions with viramidine than with ribavirin (< 10 g/dl: 0% *vs.* 24%: decrease of at least 2.5 g/dl: 48% *vs.* 82%) (38-43). Overall, 13% of patients in the ribavirin-treated group *versus* 0% of the viramidine-treated group required dose modification due to a significant decline in hemoglobin (38, 43). Ribavirin, but not viramidine, was associated with significant gender differences in the occurrence of a decrease in hemoglobin of at least 2.5 g/dl (39, 43). However, viramidine and ribavirin were equally effective in reducing HCV RNA levels. Sustained reductions in HCV RNA of about 2.51 $\log_{10}$ were obtained on all doses of viramidine, and both drugs reduced HCV RNA by at least 2 $\log_{10}$ or to undetectable levels in 83% of patients at 24 weeks (38-43).

Source

Valeant Pharmaceuticals International (US).

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